EVALUATION AND COMPARISON OF THE EFFICACY OF LOCALY DELIVERED OCIMUM SANCTUM GEL AND 8-HYDROXYDEOXYGUANOSINE LEVELS IN GINGIVAL CREVICULAR FLUID IN PATIENTS WITH CHRONIC PERIODONTITIS BEFORE AND AFTER PHASE I THERAPY.

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LIST OF ABBREVIATIONS

Sr. No.	Short Form	Full Form
1	ROS	Reactive Oxygen Species
2	DNA	Deoxyribonucleic acid
3	8-OHdG	8- hydroxydeoxyguanosine
4	GCF	Gingival crevicular fluid
5	LDD	Local drug delivery
6	O.S	Ocimum sanctum
7	O. Sanctum	Ocimum sanctum
8	*C. albicans*	Candida *albicans*
9	AO	Antioxidant
10	PPD	Probing pocket depth
11	PI	Plaque index
12	GI	Gingival index
13	CEJ	Cementoenamel junction
14	CAL	Clinical attachment level
15	PD	Probing depth
16	SD	Standard deviation
17	LDD	Local drug delivery
18	SRP	Scaling and root planning
19	CP	Chronic periodontitis
20	AgP	Aggressive periodontitis
21	HA	Hydroxyappatite
22	UNC	University of North Carolina

INTRODUCTION

Periodontal diseases refers to inflammation and destruction of the elements of the periodontium as a result of complex interaction between the pathogenic bacteria and host immune response.[1] Accumulation of plaque and calculus, the proliferation of pathogenic organisms residing subgingival crevices leading to progressive attachment loss and bone loss.[2] It is characterized by periodontal pocket formation and recession of gingiva. These inflammatory periodontal diseases are widely accepted as being caused by local factors, such as microbial plaque accumulation, in otherwise healthy individuals[2]. More than 300 million people worldwide are estimated to be affected by this disease.[3]

Reactive oxygen species (ROS) and its role on periodontal diseases

Oxidative stress is caused by imbalance between the ROS and antioxidant defense mechanism via damaging cellular micro-molecules which is susceptible to oxidative damage.[4]

ROS are important for homeostasis and cell signaling pathways. They are a product of normal metabolic processes and are important for normal physiology. They exist as reactive oxygen radicals or nonreactive radicals. For example, $-O_2$ is needed to end the effect of nitric oxide, which is a major compound involved in vascular functions, including regulation of smooth muscle tone and blood pressure, activation of platelets, and vascular signaling. Cells have several endogenous antioxidants that keep the ROS at controlled levels. These compounds include vitamin E, ascorbic acid, and glutathione. In addition, several enzymes such as catalase and superoxide dismutase are involved in reactions and lead to breakdown of these reactive species, most often resulting in water as the byproduct. As a consequence of these mechanisms, the levels of ROS are fairly low. However, damage can occur when levels of ROS increase.[5] This excessive production of ROS cause various cellular and extracellular tissue damage by DNA oxidation, lipid peroxidation, protein disruption and proinflammatory cytokine induction.[6] In periodontium, neutrophil infiltration, fibroblast, osteoclasts & endothelial cells predominantly lead to an increase ROS level resulting break down of epithelium and damage to connective tissue in the nearby area[1].

Mechanism of DNA damage by ROS includes

1. Strand breaks

2. Base pair mutations

3. Conversion of guanine to 8- hydroxyguanine which is measured as marker of DNA damage.

4. Deletions

5. Insertions

6. Nicking

7. Sequence amplification.

In the oral cavity, free radicals can result from external sources, such as nicotine, hydrogen peroxide and alcohol as well as dental procedures and materials, including veneers, implants, and crowns. Infection due to gingivitis, periodontitis, or even root caries also generates free radicals as part of the inflammatory response.[7]

Effect of ROS on periodontal tissue[6]

Gingival cell - ROS generated using a neutrophil myeloperoxidase, chloride, glucose, and glucose oxidase system caused lysis of epithelial cells that could be inhibited by azide and catalase.[8]

Bone – Some ROS such as superoxide and hydrogen peroxide activate osteoclasts and stimulate osteoclast formation.[9]This Osteoclasts produce by ROS at the ruffle border/bone interface are responsible for resorption.[10] Moseley et al. studies reported direct role in bone resorption in periodontitis by hydroxyl radicals and hydrogen peroxide can degrade alveolar bone proteoglycans in vitro.[11]

Ground substance- More recent studies have reported the effects of ROS on glycosaminoglycans and proteoglycans present in the soft and calcified tissues of the periodontium. All glycosaminoglycans undergo a variable degree of chain depolymerization and residue modification in the presence of hydroxyl radicals. Hydroxyl radicals were mostly susceptible to damage Chondroitin sulfate proteoglycans from alveolar bone, which caused degradation of both the core proteins and

glycosaminoglycan chains. On other hand hydrogen peroxide lead to more selective damage with core proteins being more vulnerable than glycosaminoglycan chains. A similar type of ROS damage can occur with proteoglycans isolated from gingival soft tissue.[12]Additionally the impact of ROS is consistent with a role for oxidative damage to non-collagenous hard and delicate tissues of the periodontium.

Collagen: Type I collagen has been affected by ROS in vitro including direct fragmentation and polymerization as well as producing oxidative modifications, interpreting the molecule which is more prone to proteolysis. Presence of high prolineor hydroxyproline content in collagen structure is mostly susceptible to damage by ROS. Superoxide anions and hydroxyl radicals cleave collagen into small peptides at proline and hydroxyproline residues, releasing hydroxyproline-containing peptides. While periodontal disease is associated with increased levels of superoxide dismutase-1 present in the cytoplasm and nuclei of cells in gingival extracts. Modification of collagen and serum proteins indirectly by ROS, via interaction with lipid peroxidation products such as malondialdehyde, can significantly alter fibroblast functions such as adhesion, proliferation, and longevity.[13]Suchadjustments of in vivo fibroblast function are normal in periodontal disease because of increase in lipid peroxidation inside the gingival tissues. While the presence of the collagen metabolites in gingival crevicular fluid is probably going to be the consequence of of proteolysis by host and bacterial collagenases, oxidative damage may make an immediate or aberrant contribution to their production. The difference of metalloproteinases and their tissue inhibitors in gingival crevicular fluid and in tissues related with disease could be the result of a immediate damage of tissue inhibitor of matrix metalloproteinases by ROS.

DNA damage various authors reported, DNA damage in gingival tissues in periodontal health and disease. PCR found deletions within mitochondrial DNA only just in tests

from periodontitis patients. When harmed, oxidative stress inside the cell can be amplified because of decreased expression of proteins critical for electron transport.[4]

Cigarette smoking is considered to be one of the most important environmental risk factors that is closely related not only with the risk but also the prognosis of periodontitis. And smoking is second most common risk factor after microbial dental plaque.[14] Insmoker, prevalence of periodontal pathogen, clinical attachment loss and bone loss is significantly higher.[15]Cigarette smoke contains many oxidants and free radicals and organic compounds, such as superoxide andnitric oxide. In addition, inhalation of cigarette smoke into the lung stimulates some endogenous mechanisms, for example accumulation of neutrophils and macrophages, which furtherincrease the oxidant injury.Nicotine is main component of tobacco smoke.It has the potential to inhibit neutrophil, ROS release in low doses, impairing the elimination of periodontal pathogens and in high dose nicotine stimulates ROS release that causes oxidative stress mediated tissue damage in gingiva periodontal tissue. Thus, cigarette smokeexaggerate the severity of periodontal destruction[4]

An excess of ROS or free radicals results in oxidative stress. Prolonged oxidative stress can initiate a chronic inflammatory state including systemic inflammatory disease.[16] However, oxidative stress is reversed as free radicals are neutralized through the presence of antioxidants.

Antioxidants are defined as those substances which when present at low concentrations, compared to those of an oxidizable substrate, will significantly delay or inhibit oxidation of that substrate.[17] Research over the past few years indicates that antioxidants are an important factor in dealing with free radicals and oxidative stress in the oral cavity.[4]

Antioxidant has potential to decrease the level of ROS. And reduce the concentration of various markers of oxidative stress such as 8-Hydroxydeoxyguanosine(8-OHdG) and it is the best documented biomarkers of oxidative stress which is involving in various diseases including periodontitis. So increased 8-OHdG level can be consider a sign of periodontal damage.[1]

An increase in 8-OHdG levels which is the most common output of oxidative DNA damage has been found in the body fluids andtissues of humans in various disorders such as neurodegenerative diseases, diabetes mellitus, cancer, chronic renal failure, atherosclerotic cardiovascular disease, rheumatoid arthritis and has also shown increase with age, obese and chronic periodontitis patients.[18]

Synonyms of 8-OHdG–

8-oxo-2- deoxyguanosine (oxo8dG), 7,8-dihydro-8-oxo-2'deoxyguanosine (8-oxo-dG) and 8-oxo-7,8-dihydro- 2'-deoxyguanosine(8-oxodG)[19].

Mechanism of formation of 8-OHdG:

Reactive oxygen species can attack the DNA replication procedure, particularly the guanine, thymine and deoxyribose level with consequence over the replication process. One of the first modifications that the DNA suffers through the radical attack is its transformation due to the oxidation of deoxyguanosine (constituent of the DNA) and the formation of 8-OHdG. The cells possess enzymes that can remove the radicals of 8-OHdG, thus repairing the DNA. The continuous attack by reactive oxygen species cause permanent modification of the DNA with severe consequences on the physiology or even death of the target cells.

Gingival crevicular fluid (GCF) is a serum derived exudate that originates from periodontal connective tissues and exit by mean of the gingival crevice into the oral

cavity. Various enzymatic and non-enzymatic biomarkers of both host and bacterial origin are present in GCF which makes it a potential diagnostic tool in assessing the biological state of the periodontium in both health and disease.[12]

Nonsurgical therapy remains the foundation of periodontal treatment. This involves treatment of gingival and periodontal inflammation through scaling and root planning to the degree that the nearby soft tissues maintain or return to a sound, non-inflamed state.[20]However, only mechanical therapy may not enough to reduce or eliminate plaque and calculus and anaerobic infection at the base of the deep periodontal pocket. To overcome this, local drug delivery with antimicrobials was initiated to improve nonsurgical therapy by aiding as an adjunct to scaling and root planing. A local drug delivery(LDD) can attain 100 fold higher concentration of antimicrobial agent in subgingival sites compared with a systemic drug regimen.[21]

Various studies reported the efficacy of the tetracycline, doxycycline minocycline, penicillin, metronidazole and clindamycin drugs as local drug delivery (LDD) systems and found to be effective in the treatment of periodontitis.[22] But most of these LDD systems are difficult to procure and expensive. Also certainly unwanted side effect such as gastrointestinal disturbances and development of antibiotic resistance. India as a developing country, the cost of the treatment is a matter of concern for patients. Hence, the use of indigenous herbal drugs has been an area of significant interest in the treatment of periodontal diseases. Several research studies have shown herbal drugs like Aloe vera, Neem, Propolis, Cranberry, Turmeric, Tulsi etc in the treatment of periodontal diseases.[23-25]Among these herbal plant ocimum sanctum is known as Queen of herbs. In ancient literature it is consider as sacred medicinal plant and frequently mentioned as one of the main pillars of medicine.

Scientific classification:[26]

Kingdom: Plantae

(unranked) Angiosperms

(unranked) Eudicots

(unranked) Asterids

Order: Lamiales

Family: Lamiaceae

Genus: Ocimum

Species: O. tenuiflorum

Binomial name: Ocimum tenuiflorumor Ocimum sanctum L.

Chemical Constituents of Ocimum sanctum:[26]

The different parts of *Ocimum sanctum* have different types of constituents in variable amounts.

In leaves - Toluene, Camphene, Octane, Benzene, Citronellel, Sabinene, Limocene, Ledol, Dimethylbenzene, Ethyl-2- methylbutyrate, Eugenol, Terpiniolene, β-elemene, Isocaryophyllene, Iso-eugenol, α-amorphene, α-guaiene, α-humulene, α-terpeneol, Borneol, Calamine, Nerolidol, Carvacrol, Geraneol, Humulene oxide, Elemol, Tetradecanal, (EZ)-famesol, Cissesquisainenehydrate,α-bisbolol, Selin-11-en-4-α-ol,α-murolene, 14-hydroxy-α-humulene .(essential oil)

In Seeds - Oleic acid, Stearic acid,Hexourenic acid, Palmitic acid, Linodilinolin and Linolenic acid. (Fixed oil)

In extraction of fresh leavesand stem - Apigenin, Circimaritin, Isothymusin, Eugenol and Rosameric acid (phenolic compounds) are present as well as *O. sanctum* is also a source of monoterpenes and ssesquiterpenes like Neral, Campene. Cholesterol and

stigma sterol. It is also a reservoir of Vitamin A and Vitamin C which works on to stimulates antibody production up to 20% to provide protection against diseases.

Pharmacological Activities:

In the ancient Ayurvedic text, the CharakaSamhita, Tulsi has been accepted to be of massive use in the treatment of headaches, rhinitis, stomach disorders, inflammation, heart diseases, various forms of poisoning and malaria.[26]

Each part of the plant has beneficial effect against various diseases; the aqueous and alcoholic extract from the leaves have various pharmacological activities such as anti-inflammatory, antipyretic, analgesic, antiasthmatic, antiemetic, antidiabetic, hepatoprotective, hypotensive, hypolipidemic, and antistress agents.

Further, distillation of the leaves yields oil of the plant which is known to possess antibacterial, antioxidant, and anti-inflammatory properties and is used extensively in the pharmaceutical industry mainly for skin cream preparations.

Role of Tulsi (O. Sanctum) in General medicine:[27]

- Respiratory disorders: Boiled tulsi leaves water is effective for sore throat. When leaves are taken with cloves and common salt gives immediate relief of influenza. Also the leaves of tulsi are very helpful in common cold and fever.
- Ocular disorders: The leaf juice of tulsi together with triphala is helping to cure, glaucoma, cataract and conjunctivitis.
- Cardiovascular disorders: Tulsi leaves act as a vasodilator due to the present of eugenol extracted and also reduces the blood cholesterol level.
- Renal disorders: Tulsi leaves if taken along with honey helps in treatment of renal calculi. Seeds of the tulsi plants has been reported to reduce blood and urinary uric acid levels.

- Dermatological disorders: Topical application of *Ocimum sanctum L.* leaves juice is effective in treatment of ringworm. And cream is useful in treatment of leukoderma.
- Gastrointestinal disorders: Aqueous decocation of *Ocimum sanctum L.* leaves is work as mild laxative hence it is given to patients suffering from gastric and hepatic disorders and has been show to possess anthelmintic properties.

Application of Tulsi in Dentistry:[27]

Oral infections

Chewing a few tulsi leaves help in maintaining oral hygiene. Carracrol andTetpene, Sesquiterpene b-caryophyllene are antibacterial agents present in this plant. This constituent in FDA approved food additive which is naturally present in Tulsi.

Tooth Ache

Tulsi can act as COX-2 inhibitor, like modern analgesics due to its significant amount of eugenol (1-hydroxyl-2methoxy-4 allyl benzene) *Ocimum sanctum* leaves contain 0.7% volatile oil encompassing about 71% eugenol and 20% methyl eugenol.

Anticariogenic agent

*Streptococcus mutans*is a microorganism which has been well known to causing dental caries. Various vitro study performed on different concentrations of the Tulsi extracts. These have been assessed against *Streptococcus mutans*and concluded that the composition of tulsi extract 4%, 6%, 10% has maximum antimicrobial potential.

Candidiasis

Some investigation reported that the essential oil contain eugenol and linalool have antifungal activity against *C. albicans*and *C. tropicalis*and concluded thatlinalool is more promising and effective against candida.

Lichen planus

Ocimum sanctum have the unique property of acting on the skin and blood tissue and also bring about the desired immunomodulation and it is one of the treatment options in Ayurveda for treating lichen planus.

Leukoplakia and oral submucous fibrosis

Polyphenol rosmarinic acid present in tulsi can act as a powerful antioxidant property hence it can therapeutically utilized in treating common oral precancerous lesions and conditions.

Pemphigus

Ayurvedic treatment aims at boosting the immune system and promotes healing of blisters and sores. Due to its immunomodulating property *Ocimum sanctum* may find its potential use in treating immunologically mediated mucosal condition called pemphigus.

Aphthous ulcerations

Anti-ulcer effect of *Ocimum sanctum* may be due to its cytoprotective effect rather than antisecretory activity. Conclusively *Ocimum sanctum* is found to possess potent antiulcerogenicas well as ulcer-healing properties and could act as a potent therapeutic agent against peptic ulcer disease.

Nutrient

Tulsi contains Vitamin A and C, calcium, zinc and iron. It also has chlorophyll and many other phytonutrients. Deficiency of these nutrients has been associated with variety of oral diseases.

Periodontal disease[29]

Tulsi leaves are mostly effective in oral infections. Carracrol and Tetpene, bcaryophyllene and Sesquiterpene are naturally present antibacterial agents. This constituent is approved by FDA. Tulsi has proven to be very active in

neutralizinghalitosis. Also has anti-inflammatory property makes it a suitable remedy for gingivitis and periodontitis, and it can be used for massaging the gingiva. Tulsi contains Vitamin A and C, calcium, zinc and iron. It also has chlorophyll and many other phytonutrients. These are very effective on oxidative stress.

Side effects or interactions

Some studies performed on animal suggested that large amounts of holy basil might negatively affect fertility[30] but no adverse reactions have been reported in human clinical trials.[31]

There is paucity of literature on its use in the treatment of chronic periodontitis. Therefore, the present study was hypothesized that the antioxidant potential of Tulsi by assessing the 8-OhdG level in gingival crevicular fluid. These are strong oxidative stress biomarker associated with chronic periodontitis in smoker and non-smoker patients.

AIM AND OBJECTIVES

The aim of the present study is to evaluate and compare the efficacy of locally delivered ocimum sanctum gel and 8-OHdG level in gingival crevicular fluid in smokers and nonsmokers with chronic periodontitis patients.

Also glued to this aim were certain objectives

1. To evaluate and compare the efficacy of locally delivered ocimum sanctum gel in smokers and non-smokers pre and post phase I periodontal therapy in chronic periodontitis patients.
2. To evaluate the 8-OHdG level in gingival crevicular fluid in smoker patients.
3. To evaluate the 8-OHdG level in gingival crevicular fluid in non-smoker patients.
4. To compare the 8-OHdG level pre and post phase I periodontal therapy.

REVIEW OF LITERATURE

Periodontal disease is chronic inflammatory disorder that affect the supporting tissue of the teeth. The prevalence of periodontal disease most commonly found in adult population in both developing and developed countries. Plaque and calculus are the primary etiological factors for periodontal diseases but apart from that according to WHO in 2002 reported, there are some risk factors such as lack of dental awareness, poor oral hygiene, socioeconomic status and the tobacco consumption and smoking. Numerous studies till date have proved the direct association between oxidative stress and tobacco smoker on chronic periodontal diseases. 8-OHdG is most stable biomarker produced of oxidative DNA damage caused from ROS. And nonsurgical periodontal

therapy is first treatment of choice to treat periodontal disease either in smoker and non-smoker patient.

For the sake of better understanding, the review of literature has been divided into following parts-

A. Review of studies on association of 8 -OHdG level and chronic periodontitis
B. Review of studies on association of smoking, oxidative stress marker and chronic periodontitis.
C. Review of studies on association of smoking and chronic periodontitis
D. Review of studies on effect of herbal drugs as local drug delivery adjunct with scaling and root planning in patient with chronic periodontitis.
E. Review of studies on ocimum sanctum in general health and dentistry**.**

A. Review of studies on association of 8-OHdG level and chronic periodontitis

M Takane et al (2002)[32] - This study performed to evaluate the 8-OHdG level in saliva of the PT Of chronic inflammatory diseases. In this 78 untreated periodontitis patients of mean age 49.4 year were recruited and 17 healthy subjects were considered in control group .The clinical examination included BOP, CAL, PD at pretreatment and posttreatment by three experienced examiner. Also whole saliva sample were collected at pre and post therapy and stored at -80 degree Celsius until 8-OHdG analyzed. All collected saliva sample were centrifuged at 10.000 for 10 min and the supernatant was used to determine 8- OHdG level with ELISA kit with the determination range was 0.125-200 ng/ml. Mean value of 8-OHdG in saliva of periodontally diseased subject 4.28±0.10ng/ml was significantly higher ($p<0.01$) than that of clinically healthy subjects(1.56±0.10 ng/ml/). After periodontal therapy significantly reduced the value of

salivary 8-OHdG level. Hence study indicated that 8-OHdG in saliva appear to reflect the status of periodontal health.

Sawamoto et al (2005)[33] - Assessed the periodontopathic bacteria and 8-OHdG level in saliva of chronic periodontitis patient. The salivary levels of Actinobacillusactinomycetemcomitans, Porphyromonasgingivalis, and Tannerella forsythia were calculated using real-time polymerase chain reaction. The 8-OHdG levels were measured using an enzyme-linked immunosorbent assay The salivary levels of 8-OHdG, P. gingivalis, and T. forsythia in the periodontitis patients were significantly higher than those in healthy subjects.in other side, the A. actinomycetemcomitans level in healthy subjects was higher than in periodontitis patients. 8-OHdG was significantly correlated with P. gingivalis. Statistically significant decreases in the levels of P. gingivalis, probing depth, bleeding on probing, and 8-OHdG were observed after initial periodontal treatment. These results suggest that the 8-OHdG levels in saliva reflect the load of periodontal pathogens. 8-OHdG could be a useful biomarker for assessing periodontal status accurately, and for evaluating the efficacy of periodontal treatment.

T. Konopka et al (2007)[34] - Determined 8-hydroxy-2'-deoxyguanosine (8-OHdG) concentrations and total antioxidant status (TAS) in peripheral and gingival blood serum of periodontitis patients. The author recruited total 56 untreated periodontitis patient on which 26 patient were aggressive periodontitis and 30 patients were chronic periodontitis and 25 healthy volunteers were included in their study. ELISA kit was used to measure 8- OHdG level and a calorimetric method was used to measure total antioxidant status (TAS). Significantly higher 8-OHdG concentrations were identified in

the gingival blood in both groups of patients with periodontitis than in the control group. There were statistically significant level of TAS found in peripheral blood as well as gingival blood in aggressive and chronic periodontitis group compare with healthy group. Thus the author concluded the oxidative burst in periodontitis may lead to significant local damage to nucleic acids. The significantly decreased TAS level in the gingival blood of CP patients compared with the healthy subjects suggests the possibility of a significant decrease in local antioxidant system capacity during the course of periodontitis. The decreased TAS level in the peripheral blood in the group of all patients with periodontitis may be one of the pathogenic mechanisms underlying the links between periodontal disease and several systemic diseases for which periodontitis is regarded as a independent risk factor.

C. F. Çanakçi et al (2009)[35] - Reported early oxidative DNA damage markers such as 8-hydroxydeoxyguanosine (8-OHdG) and mitochondrial DNA (mtDNA) deletion in gingival tissue and saliva of 32 chronic periodontitis patient and 32 healthy individuals. The clinical condition of each subject, the plaque index (PI), gingival index (GI), clinical attachment level (CAL), and probing depth (PD) were measured. the salivary 8-OHdG levels and the 7.4-kbp and 5-kbp mtDNA deletions were examined using the ELISA and polymerase chain reaction methods and the author found mtDNA deletion was more significant I e. 20 out of 30 in chronic periodontitis patient. And The mean value of 8-OHdG in the saliva of the periodontitis patients was significantly higher.so the author conclude Increased oxidative stress may lead to premature oxidative DNA damage in the gingival tissue of periodontitis patients and the salivary 8-OHdG level may indicate premature oxidative mtDNA damage in diseased gingival tissue.

U. Sezer et al. (2012)[36]- This study hypothesized that Increased salivary levels of 8-hydroxydeoxyguanosine may be found in periodontal disease so total 60 subjects divided in to 3 groups , Group I - 20 chronic periodontitis, Group II- 20 chronic gingivitis and Group III healthy individuals. Clinical parameter such as PI, GI, PPD, CAL were measure using clinical and radiographic criteria stated and described on the 1999 Consensus Classification of Periodontal Diseases and Determination of salivary 8-hydroxydeoxyguanosine level by enzyme-linked immunosorbent assay (ELISA) There was no significant difference between the mean ages of the three groups ($p > 0.05$). PI, GI scores of the CP and CG groups were significantly higher than the control group. In laboratory finding The mean 8-OHdG level in the saliva of the periodontally diseased patients in the CP group (3.13 ± 0.22 ng/ml) was significantly higher ($p < 0.001$) than control (1.56 ± 0.12 ng/ml) and CG (1.58 ± 0.13 ng/ml) groups. Suggestive of that elevated salivary levels of 8-OHdG may be a marker for disease activity and it reflects indirectly disease severity parameters such as CAL.

Dede F et al (2013)[37] - Evaluated the effects of phase 1 periodontal treatment in the gingival crevicular fluid (GCF) and salivary levels of 8-hydroxy-deoxyguanosine (8-OHdG) in chronic periodontitis patients. GCF and saliva samples were obtained from 24 patients with CP and 24 individuals with clinically healthy periodontium. GCF, saliva samples, and clinical periodontal measurements were repeated at day 10, 1 month, and 3 months following initial periodontal therapy in patients with CP. 8-OHdG levels of GCF and saliva samples were investigated by using an enzyme- linked immunosorbent assay. and the results obtain higher Statistically significant 8-OHdG levels in GCF and a significant decrease after initial periodontal therapy were determined in the CP group. A significant positive correlation was found between 8-

OHdG levels in GCF and clinical periodontal measurements. However, salivary levels of 8-OHdG did not differ between groups or during initial periodontal therapy hence this study concluded that DNA injury and oxidative stress increase in tissue cells and especially in periodontal pockets in patients with CP, and the periodontal treatment results in a significant decrease of 8-OHdG levels in the GCF samples.

R Arunachalam et al (2014)[38] - To evaluate the Salivary 8 OHdG level as a valuable indicator for oxidative DNA damage in periodontal diseasedpatients this is case control study performed on 30 healthy volunteers and 30 CP PT. Diagnosis was based on radiographic and clinical examination according to 1999 Consensus Classification of Periodontal Diseases. In CP patient consisted CAL 1-3 mm and in healthy group CAL were < 1 mm. H/O Smoking, any systemic diseases or pregnant or lactating women were not included in this study. PI, GI, PPD, CAL, were performed using UNC-15 periodontal probe at baseline and 1 month after periodontal treatment.8-OHdG levels were calculated at baseline and one month following phase one periodontal therapy. 8-OHdG levels in saliva were inspected by using an enzyme linked immunosorbent assay [ELISA]. A significant decrease of 8-OHdG levels after initial periodontal therapy were determined in CP group. The mean 8-OHdG level in the saliva of the CP group(645.18±84.91) was significantly higher than Healthy groups(527.23±62.19) ($p< 0.001$). Statistically significant correlation was only detected between the salivary levels of 8-OHdG(532.18±91.37) and GI ($p< 0.05$) in CP(1.39±0.18) group. Therefore this study concluded elevated salivary levels of 8-OHdG may be a marker for disease activity which may reflect indirectly disease severity parameters which aids in the diagnosis and monitorisation of the treatment in periodontal disease.

Kurgan et al.(2015)[39] – In this study saliva sample were collected from 23 patient with generalized chronic periodontitis (8 female and 15 male , mean 46 year of age) 25 healthy volunteers (15 female and 10 male , mean 44.9 year of age). Clinical parameter such as PI, GI, CAL,BOP,GR,PPD were measure at baseline and 6 week and saliva sample were collected at baseline and 6 week post nonsurgical therapy. ELISA kit were used to measure Salivary 8-OHdG levels in chronic periodontitis patients. And author found 8-OHdG level in saliva significantly decreased after phase 1 therapy and Statistically significant positive correlations were observed between PI, GI, PPD, CAL, BOP values and LC-MS/MS and ELISA levels of 8-OHdG ($p < 0.001$). Hence concluded LC-MS/MS is a reliable and sensitive method for evaluating salivary 8-OHdG levels to monitor the treatment response of periodontitis.

F Bulut, et al. (2015)[40] – Determined the 8-OHdG level as an oxidative DNA damage marker and malondialdehyde (MDA) in serum level in PT with chronic periodontitis and hyperlipidemia. In this study 74 subjects divide in to 4 groups, 18 patients with hyperlipidemia and CP, 18 periodontally healthy patients with hyperlipidemia, 19 systemically healthy individuals with CP, and 19 systemically and periodontally healthy controls. Clinical periodontal parameters such as PPD, CAL, BOP,PI, GI, % BOP were measured, and serum lipids, MDA, and 8-OHdG levels were calculated in blood samples using ELISA kit. According to this study clinical parameter were significantly higher in hyperlipidemia group than the CP group and in serum levels of the MDA and 8-OHdG were significantly higher in the hyperlipidemia with CP group compared with the CP group. It has been suggested that not only bacterial pathogens, but also the hyperlipidemic condition may induce oxidative damage in tissues by increasing lipid peroxidation.

Shin et al (2016)[41] – The cross-sectional study performedto evaluated the association between salivary 8-hydroxydeoxyguanosine (8-OHdG) and periodontitis among community-dwelling Korean adults. In this study, total 211 volunteers of age range between 48-93 (80 men and 131 women) were selected. Periodontitis selection criteria based on defined of Periodontitis having at least 30% of teeth with proximal attachment loss ≥5 mm and the salivary 8-OHdG level was categorized into : low (<0.916 ng/ml), medium (0.916 to <2.675 ng/ml) and high (≥2.675 ng/ml). volunteer for control group based on Sociodemographic, habitual and systemic health-related factors were considered Periodontal status was examined by two experienced dentist including clinical attachment loss measured using UNC 15 Probe measurement made at six site such as mid-buccal, distobuccal, distolingual, midlingual and mesiolingual on twelve index teeth: the second molar and second premolar from the upper right sextant, the right lateral incisor and left central incisor from the upper anterior sextant, the first premolar and first molar from the upper left sextant, the second molar and second premolar from the lower left sextant, the left lateral incisor and right central incisor from the lower anterior sextant and the first premolar and first molar from the lower right sextant. Logistic regression analysis was performed for the outcome of severe periodontitis. Analysis of covariance in general linear model was performed for the outcome of 8-OHdG. The 8-OHdG level showed a significantly higher association with periodontitis group.

B. Review of studies on association of smoking and oxidative stress marker.

Chandra et al (2012)[1] – Evaluated the clinical efficacy of lycopene gel in the treatment of chronic periodontitis patients compared with placebo gel .in this study total 110 patients recruited and divided in to 3 groups , 50 smokers with chronic periodontitis group , 50 nonsmoker with chronic periodontitis group and 10 control. Patients with smoker and non-smoker groups one site treated with lycopene gel and another treated with placebo. Clinical parameter such as PI, GI, PPD, CAL were measured at baseline 1 and 3 month. and GCF 8- OHdG level were measured at baseline and 3 month after scaling and root planing. In this study showed that the lycopene treated site showed significant reduction in probing depth and 8-OHdG level in GCF and gain clinical attachment level compared with placebo in chronic periodontitis patients. Conclusion is the use of lycopene adjunct to SRP produce effective gain in clinical attachment level and reduce gingival inflammation, PPD and oxidative stress injury compare with placebo in smoking and non-smoking patients.

Reddy S et al.(2012)[42-] Determined the influence of smoking on the blood and salivary superoxide dismutase (SOD) levels among smokers, and demonstrated the significance in alterations in the levels of SOD in association with patient age, periodontal disease status, and duration and smoking frequency. 60 healthy male patients of age range 20–60 year were included. Blood and saliva samples were collected, and the periodontal index was measured using a Community Periodontal Index probe. Blood and salivary SOD levels were calculated with the reagents prepared, and values were measured in a UV spectrophotometer. There was a significant decrease in mean SOD levels with an increase in age and smoking duration and frequency (0.23–0.05 U/mL). There was a range of 0.31–0.06 U/mL variation in the mean SOD levels as the periodontal disease

condition deteriorated. There was a significant reduction in the SOD levels in the blood and saliva of smokers with increased duration and frequency of smoking and as the periodontal disease condition worsened.

Hendek M et al (2014)[43] - Performed study on 47 patients with chronic periodontitis 24 smoker and 23 nonsmoker patients and 46 periodontally healthy individuals on which 23 smokers and 23 non-smokers individuals. Gingival crevicular fluid (GCF), serum and saliva samples were obtained and clinical periodontal measurements were recorded at baseline and at the 1st and 3rd months after periodontal therapy. 8-OHdG, 4-hydroxynonenal (HNE) levels and glutathione peroxidase (GSH-Px) enzyme activity were analyzed with enzyme-linked immunosorbent assay. Result obtained 8-OHdG in GCF was found significantly higher in both periodontitis groups compared to both periodontally healthy groups. 8-OHdG level and GSH-Px enzyme activity in saliva in both periodontitis groups were significantly increased compared to nonsmoker healthy group and after initial periodontal treatment, the level of 8-OHdG in GCF and saliva were significantly decreased in both periodontitis groups. Hence the author concluded that initial periodontal therapy may be helpful for diminishing oxidative stress in periodontitis.

C. Review of studies on association of smoking and chronic periodontitis

Stoltenberg J et al.(1993)[44]– This study associated between cigarette smoking and signs of periodontal disease after controlling for the confounding variables of age, sex, plaque, and calculus. In this study total 615 subject were selected. Plaque, Calculus, Gingivitis, and PPD were measured at the proximal surfaces of all teeth in one randomly selected posterior dental sextant. Subgingival plaque sample was collected from the same sites and assayed for the presence of P. gingivalis, A.

actinomycetemcomitans, P. intermedia, Eikenellacorrodens, and F.nucleatum. A subsample of non-smokers (n = 126), who were similar to smokers (n = 63) with respect to age, sex, plaque, and calculus, was randomly drawn from the original sample. These two groups were then compared on the basis of clinical and microbial parameters. The results indicated that the odds of having a mean PPD > 3.5 mm were 5 times greater for smokers than the non-smoker subsample. There was no statistically significant difference found in the prevalence of any bacteria between smokers and the non-smoker subsample. Based on logistic regression analyses of each of the 5 bacteria and smoking, mean PPD> 3.5 mm was significantly associated with the presence of A. actinomycetemcomitans, P. intermedia, E. corrodens, and smoking ($P < 0.05$). They concluded that cigarette smoking is a stronger risk indicator for periodontal disease.

Pucher J et al.(1997)[45] – In this study87 adult patients were selected in which 54 non-smokers and 33 smokers with moderate to advanced periodontitis were treated with 1-hour full-mouth subgingival scaling and root planing, with no maintenance recalls, during this 9-month study. Clinical parameters such as PPD, CAL, BOP, GI and PI were assesed. Data were collected at baseline, 3, 6, and 9 months. Baseline PPD for non-smokers was 5.46 ± 0.46 mm and for smokers 5.70 ± 0.66 mm. Data analysis shown that both non-smokersand smokers had a statistically significant decrease in PPD at 3months which was continued throughout the study. At 9 months non-smokers maintained a mean decrease in PPD of 0.60 mm and smokers a mean decrease of 0.65 mm. Both smokers and non-smokers showed a significant gain ($P < 0.05$) in CAL after SRP when compared to baseline value. At 9 months the mean gain in CAL for non-smokers was 0.47 mm and 0.59 mm for smokers. PI scores remained consistent for smokers and non-smokers for the duration of the study. The GI at baseline was

significantly (P < 0.05) lower in smokers (1.32 ± 0.45) than non-smokers (1.45 ± 0.40).By 9 months only the GI of non-smokers decreased significantly compared to baseline (1.26 ± 0.37). Bleeding on probing was a prerequisite for target sites at baseline. At 9 months both smokers (0.67 ± 0.39) and non-smokers (0.78 ± 0.30) had a significant decrease in BOP compared to baseline. At 9 months there were no significant differences between smokers and non-smokers comparing PPD, CAL, PI, BOP, and GI. The data have shown that smokers and non-smokers responded similarly after 9 months to the limited amount of initial therapy provided.

Kinane DF et al. (1997)[46]- Evaluated the effect of smoking on the outcome of periodontal therapy. The study consisted of 54 patients who participated in a 4-group parallel-arm clinical trial on the efficacy of three locally delivered antimicrobial systems as adjuncts to scaling and root planing in the treatment of sites with persistent pocketing after a course of scaling and root planing. These groups included scaling and root planing either alone (S) (n = 3), or in conjunction with the application of 25% tetracycline fibers (n = 13), 2% minocycline gel (n = 14), or 25% metronidazole gel (n = 14). In each patient four pockets > 5 mm with BOP and/or suppuration were studied. The PPD, attachment level and other clinical parameters were assessed at baseline and 6 weeks after treatments. Regardless of the type of treatment, the change in the probing depth and attachment gain were greater in non-smoker subjects than smoker subjects. PPD was 1.14 mm versus 0.76 mm (P = 0.019), and attachment gain was 0.52 mm versus 0.50 mm at (P = 0.845) for non-smokers and smokers respectively. There was a significant interaction between the "smoking" and the "baseline PD." Further analysis using linear regression indicated that, while there was a significant relationship between the baseline PD and the probing depth or attachment gain among the non-smokers, weak

and insignificant relationship existed among the smoker subjects. Thus, smoking may have an important role in determining the prognosis of periodontal treatment, particularly in persistent and deep pockets.

Haffajee AD and Socransky SS (2001)[47] - Investigated and examined clinical features of periodontal disease and patterns of attachment loss in 289 adult periodontitis subjects who were current, past or never smokers. A questionnaire was used to obtain smoking history. Measures of plaque accumulation, overt gingivitis, bleeding on probing, suppuration, probing pocket depth and probing attachment level were taken. Subjects were subset according to smoking history into never, past and current smokers and for certain analyses into age categories <41, 41–49, >49. They found that current smokers had significantly more attachment loss, missing teeth, deeper pockets and fewer sites exhibiting bleeding on probing than past or never smokers. Current smokers had greater attachment loss than past or never smokers whether the subjects had mild, moderate or severe initial attachment loss. Increasing age and smoking status were independently significantly related to mean attachment level and the effect of these parameters was additive. Mean attachment level in non-smokers <41 years and current smokers >49 years was 2.49 and 4.10 mm respectively. Stepwise multiple linear regression indicated that age, pack years and being a current smoker were strongly associated with mean attachment level. Full mouth attachment level profiles indicated that smokers had more attachment loss than never smokers particularly at maxillary lingual sites and at lower anterior teeth suggesting the possibility of a local effect of cigarette smoking.

Darby et al. (2005)[48] - Compared the effects of scaling and root planing (SRP) on clinical and microbiological parameters at selected sites in smoker and non-smoker

chronic and generalized aggressive periodontitis patients. Clinical parameters including PPD, relative attachment level (RAL), and BOP, and subgingival plaque samples were taken from four sites in 28 chronic periodontitis (GCP) and 17 generalized aggressive periodontitis (GAgP) patients before and after SRP. Polymerase chain reaction assays were used to determine the presence of A. actinomycetemcomitans, P. gingivalis, Tannerellaforsythensis (T. forsythensis), P. intermedia and Treponemadenticola.(T. denticola) Both GCP and GAgP non-smokers had significantly greater reduction in pocket depth (1.0 ± 1.3mm in GCP smokers versus 1.7 ± 1.4mm in non-smokers, p =0.007 and 1.3 ± 1.0 in GAgP smokers versus 2.4 ± 1.2mm in GAgP nonsmokers, p<0.001) than respective non-smokers, with a significant decrease in T. forsythensis in GCP sites (smokers 25% increase and non-smokers 36.3% decrease, p<0.001) and P. intermedia at GAgP sites (smokers 25% reduction versus 46.9% in non-smokers, p=0.028). SRP was effective in reducing clinical parameters in both groups. The inferior improvement in PPD following therapy for smokers may reflect the systemic effects of smoking on the host response and the healing process. The lesser reduction in microflora and greater post-therapy prevalence of organisms may reflect the deeper pockets seen in smokers and poorer clearance of the organisms. These detrimental consequences for smokers appear consistent in both aggressive and GCP.

Suzuki et al. (2016)[49] - Investigated the relationships among salivary stress biomarkers, cigarette smoking, and mood states. A total of 49 healthy sixth-year dental students was the study population and Lifetime exposure to smoking was calculated using the Brinkman index (BI).Resting saliva samples were collected, and concentrations of cortisol, secretory immunoglobulin A (SIgA), interleukin (IL)-1β, interleukin-6, and tumor necrosis factor (TNF)-α were determined. Mood states (tension-anxiety,

depression-dejection, anger-hostility, fatigue, confusion, and vigor) over the previous week were assessed using the Profile of Mood States - Brief Japanese Version. Salivary IL-1β levels were significantly higher in smokers than non-smokers (P = 0.044), regardless of the BI or mood state. Higher fatigue scores and lower vigor scores were observed in smokers. They concluded that IL-1β has strong association with the smoking status.

D. K. Gautam et al (2018)[50] - Performed the study to evaluated the periodontal health status among cigarette smokers and non cigarette smokers, and oral hygiene measures in 400 male which includes 200 cigarette smokers and 200 non smokers of age range between 18-65 years. Inclusion criteria were presence of more than 10 natural teeth in oral cavity. Periodontally healthy and H/O chronic systemic diseased patients were excluded. The patients were randomly selected from the patients attending dental out-patient department of civil hospital and Himachal Dental College, Sundernagar. Community Periodontal Index (CPI) score was recording for each patient and a questionnaire was ask to each patient. There were statistically significant differences between cigarette smokers and nonsmokers for gingival bleeding (*P*=0.007; non smokers more likely to have gingival bleeding),and periodontal pocket (*P*=0.045; cigarette smokers more likely to have deep pockets than non smoker.This study proved that smoking is a major environmental factor associated with accelerated periodontal destruction.

D. Review of studies on effect of herbal drugs as local drug delivery adjunct with scaling and root planing in patient with chronic periodontitis

The concept of local delivery to periodontal pocket was first given by Max Goodson at Forsyth Dental Infirmary in Boston(1979). He used devices which contain hollow fibres of cellulose acetate filled with tetracycline.[51] these fibres released tetracycline in an exponential fashion with 95% drug released in first 2 hours; they were primary local drug delivery devices with minimal control on drug release. The concept of controlled drug delivery system was thus developed as it involves the phenomenon in which the antimicrobial agent is protected from local removal mechanisms after placement, enabling zero order kinetics, which maintains consistently elevated pocket concentrations of an agent during its application as an adequate drug microbial contact time must be attained for an antibacterial agent to exert its bactericidal or bacteriostatic effects against targeted microbes.[52]

Recently Various local drug delivery systems used for treating periodontitis are-

- Fibres
- Films
- Injectable systems
- Gels
- Strips and compacts
- Vesicular systems
- Microparticle system
- Nanoparticle system etc.

Gel formulation indeed have some advantages such as faster release of drug, It can be easily prepared and administered. They possess a higher biocompatibility and bioadhesivity, allowing adhesion to the mucosa in the periodontal pocket and finally they can be rapidly eliminated through normal catabolic pathways, decreasing the risk of irritative or allergic host reactions at the application site.[53]

local drug delivery as an adjunct with antimicrobial agent like tetracycline, minocycline, azithromycin, clarithromycin, doxycycline etcare produce several disadvantage such as systemic side effect, development of antibiotic resistance, patient compliance, expensive etc. to overcome this problem herbal drugs such as Aloevera, Neem, Propolis, Turmeric,Cranberry, Pomegranate, cucumber cocoa husk,Sesbaniagrandiflora[54-61] etc. could be used as adjunct with SRP in deep periodontal pocket and have shown significant improvement in PI,GI,PPD,CAL in gingivitis and chronic periodontitis patients.

A Moghaddam et al. (2017)[62] -To evaluate the effects of local application of aloe vera gel as an adjunct to scaling and root planning in the treatment of patients with chronic periodontitis. This is split mouth single blind clinical trial was performed on 20 patient with moderate to severe chronic periodontitis under the supervision of Committee of Research, and Ethics Committee of Guilan University of Medical Sciences. Patient having probing pocket depth of 4-5 mm and presence of bleeding on probing were included in this study patients were candidates for nonsurgical treatment phase. history of allergy to aloe vera or its products, tobacco use, habits like mouth breathing or tongue trusting, endo-dontic and periodontal combined lesions, periapical lesion, severe decay of teeth, partially impacted teeth, patients undergoing orthodontic treatment, systemic diseases, systemic or oral topical antibiotics use six months prior to the study,

any periodontal treatment six months prior to the study, and pocket depth more than 5mm were excluded from study. 98% aloe-vera gel were prepared. Clinical parameter such as PI, GI and PD were measured at baseline before SRP, 30th and 60th day after SRP.

M Jain et al (2018)[63] - To evaluated the clinical effectiveness of locally delivered xanthan-based Chlosite® gel as an adjunctive therapy to scaling and root planing in treatment of chronic periodontitis. It is a randomized controlled clinical study, total 30 patients were selected.in which 30 control site and 30 test site were selected on same side of the mouth. Pocket depth between 5 and 7 mm was selected. In control side plane SRP were performed and in test side SRP with application of Chlosite® gel was added. The clinical parameters, probing depth (PD), CAL, PI and BOP, GI were recorded at baseline, 6 weeks, 3 months and 6 months. Statistical Analysis: Paired/unpaired t-test was used, significance was placed at 5% level of significance, i.e., $P < 0.05$ was considered as a significant. Results: From baseline to a period of 6 months, significant difference was found between test and control group for PD, CAL, PI and BOP, P value being PD ($P = 0.002$), CAL index ($P = 0.014$), respectively. Therefore this study conclude Subgingival injection of xanthan-based Chlosite® gel adjunct with scaling and root planing enhance significant improvement compared with scaling and root planing alone in patients with chronic periodontitis.

E. Review of studies on ocimum sanctum in general health and Dentistry.

S Godhwani, et al (1987)[64] – Performed an experimental study to evaluating Ocimum sanctum anti-inflammatory, analgesic and antipyretic Activity in animals fresh leaves of 0. ***sanctum***were collected from the nursery of the Sardar Patel Medical College Campus, Bikaner, Rajasthan, and it identified from authenticated resident botanist and a pharmacognocist. And extract of the 0. ***sanctum***were prepared such that Suspension doses contain 2 ml/100 g body weight then Inbred albino mice weighing 20-35 g recruited and divided into 6-8 animals. Acute inflammation was induced by injecting 0.1 ml of freshly prepared 1% carrageenan in normal saline underneath the plantar aponeurosis of pows of rats One group attended as control and delivered equal volume of distilled water while the other groups received an ocimum sanctum extract drug 30 min before the plantar injection.

Ramesh et al (2010)[65] - Analyze the antioxidant both enzymic and nonenzymic activities of leaves of Ocimum sanctum hydroalcoholic extract against cadmiuminduced damage in albino rats. Fresh leaves of Ocimum sanctum were Collected and dried and powdered with a mechanical grinder to obtain a coarse powder. 1 kg of powdered material was then subjected to cold maceration with 50% alcohol for 3 days with intermittent shaking, filtered, evaporated and vacuum dried. And hyrdroalcoholic extract prepared. 36 Male albino rats, weighing 150–200 g were obtained from animal breeding center, PSG Institute of Medical Sciences & Research, Coimbatore, Tamilnadu, India. And divided into six group Group I: Normal control, Group II: Cadmium ($CdCl_2$) induced (6 mg/kg body weight/day) orally for a period of 30 days , Group III: Cadmium induced plus Ocimum sanctum extract (100 mg/kg body weight)

10 consecutive days before CdCl2 administration and until 30 days of CdCl2 administration , Group IV: Cadmium induced plus Ocimum sanctum extract (200 mg/kg body weight, po) 10 consecutive days before CdCl2 administration and until 30 days of CdCl2 administration, Group V, Ocimum sanctum extract (100 mg/kg body weight) and Group VI, Ocimum sanctum extract (200 mg/kg body weight).6 rats included in each group various antioxidant enzyme like Lipid peroxidation , SOD ,CAT , GPx GSH and Ascorbate were analyzed before and after cadmium intoxication . Group II rats showed a significant increase in the liver LPO levels when compared to Group I rats were p value is 0.01. Group III and Group IV rats administered with low and high doses of Ocimum sanctum extract showed a significant (P\0.01) decrease in the liver Lipid peroxidation levels when compared to Group II rats. Group III and Group IV rats showed a significant (P\0.01) increase in the levels of SOD, CAT, GSH, Ascorbate levels and significant (P\0.05) increase in the GPx levels when compared to Group II rats. This shows that the Ocimum sanctum extract can reduce reactive free radicals that might lessen oxidative damage to the liver and improve the activities of the hepatic antioxidant enzymes like SOD and CAT, protecting the liver from cadmium intoxication. Hence they concluded hydroalcoholic extract of Ocimum sanctum has antioxidant activity based on free radical scavenging or modulation of antioxidant status and tissue regeneration in the liver of cadmium intoxicated rats.

M Hosamane et al (2014)[66] – Performed study to evaluate the antibacterial efficacy of holy basil in vitro against some periodontopathogens and its antiplaque effect in vivo. 30 Volunteers, 17 males and 13 females were randomly allocated and divided into three groups, ten volunteers in each group. Group 1 [positive control] was the chlorhexidine group, Group 2 [test] was the holy basil group and Group 3 [negative control] was the

sterile water group. In zero to four days, all volunteers had to refrain from carrying out all mechanical oral hygiene measures. And ask to rinsed for 1min twice daily with 10 ml groupwiseallocated mouthwash solution. The PI was assessed at days 0 and 5. And other side according to Minimum Inhibitory Concentration of the holy basil extract was evaluated by broth dilution method against two periodontopathic organisms: *F. nucleatum*and *P. intermedia*, to determine its antibacterial activity. Various dilutions of the holy basil extract i.e. 500 mg/mL, 250 mg/mL, 125 mg/mL, 62.5 mg/mL, 31.25 mg/mL,16.125 mg/mL, 8 mg/mL, 4 mg/mL, 2 mg/ mL, and 1 mg/mL were tested against F. nucleatum and P. intermedia organisms. Various dilutions of the holy basil extract and the organism to be tested was incubated in brain heart infusion agar for 24 hours and observed for turbidity. Study showed that both the tested periodontopathogens [*P. intermedia*and *F. nucleatum*] were susceptible to holy basil extract at all the concentrations tested ranging from 10^{-1} to 10 suggesting a very high antibacterial activity. At baseline there were no significant difference found between mean of PI scores in the three groups [with $p \leq 0.05$] but there were found a significant difference between mean of PI scores in the three groups on day 5 [$p = 0.01$]. Differences between the individual rinse solutions and the placebo solution, determined via the Tukey Post hoc test, demonstrated significantly less plaque regrowth with respect to both chlorhexidine [$p = 0.007$] and holy basil [$p = 0.041$] as compared to the placebo. The difference between chlorhexidine and holy basil was not statistically significant [$p = 0.187$] suggesting the holy basil mouthwash has an antiplaque effect and is efficacious against *P. intermedia*and *F. nucleatum*strains in vitro. Hence holy basil mouthwash may have potential as an antiplaque mouthwash with prophylactic benefits.

Gaur J et al (2015)[67]- Conducted a study to determine the effectiveness of ocimum sanctum on bacterial plaque and its comparison with Chlorhexidinegluconate. Total 30 patient were recruited and divided into two groups. Group A, 15 PTreceived scaling and root planing plus intra-pocket irrigation of Ocimum sanctum and Group B 15 PT received scaling and root planing plus intra-pocket irrigation with Chlorhexidinegluconate . Clinical parameters containing the plaque index, gingival index, pocket probing depth and clinical attachment level were assessed at baseline and 30 days. And clinical parameter compare in both group analyzed using SPSS 17 softwere the author found Ocimum sanctum was equally effective as Chlorhexidinegluconate to reduce clinical parameter as well as periodontal indices. And there were no any side effect when compare with chlorhexidine.

R. Hosadurga et al (2015)[68] - Evaluate the anti-inflammatory activity; assess duration of the action and the efficacy of 2% O. sanctum gel in the treatment of experimental periodontitis in Wistar Albino rat model.36 Wistar albino rats were randomly selected and divided in to 3 groups. Group 1-control; Group 2-Plain gel (standard)and Group 3-2% tulsi (O. sanctum) gel. Tulsi gel and control gel prepared according to the formulation given in NSGM institute of Pharmaceutical sciences, NITTE university, Mangalore and properties checked like physical evaluation, homogeneity, spreadabilty, grittiness, extrudability, and pH measurement. Evaluation of anti-inflammatory activity in 18 rats were assessed by Carrageenan induced Paw edema method. These divide in to three groups first test group in which 2% *O. sanctum gel* administer , second , standard in which 1% VoveronEmulgel gel was administer and third control group were plain gel introduced with six animals (n = 6) in each group. And percentage inflammation measured using formula [Percentage inflammation = (V−Vi÷Vi) × 100.]

and Percent inhibition of the edema was calculated using the formula Percent inhibition of the edema = (Vt÷Vc) × 100 .And Duration of anti-inflammatory activity of 2% *Ocimum sanctum* gel measuredat 0, 2, 4, 6, 12, 24, and 48 h prior to administration of Carrageenan. **.** Gingival index (GI) and probing pocket depth were measured before and after treatment. The rats were sacrificed. Morphometric analysis was done using Stereomicroscope and Image J software mean percentage of inflammation shown in control, standard and test groups were 68.387±25.05 15.74±4.634 45.36±23.22 respectively. 2% tulsi gel showed 33.66% inhibition of edema and peak activity was noted at 24 h. There was statistically significant change in the GI and probing pocket depth. No toxic effects were seen on oral administration of 2000 mg/kg of Tulsi extract not produce any toxic effect.Conclude that 2% tulsi gel was effective in the treatment of experimental periodontitis.

Ayesha et al.(2015)[69] –Evaluated the use of Ocimum sanctum leaf extract for its anti-ulcerproperty by inducing stress ulcers on rats. In this study Albino rats were randomly allocated and divided into 10 experimental groups in which group I and group VI is control group (administered plane distilled water) while other Group I-V (Restraint stress model) and group VII – IX X (Ethanol induced Stress model) have received 50mg/kg, 100mg/kg, 200 mg/kg ocimum sanctum extract and group V (Restraint stress model) and X (Ethanol induced Stress model) were received 10 mg/kg ranitidine. , 6 rat included in each group .and aqueous leaf extract of Ocimum sanctum was given for once a day for 7 days orally. Stress ulcers were induced by restraint and ethanol administered methods and results were compared with other groups (standard) were drug ranitidine administered orally once a day for 7 days. After that animal was sacrificed and stomach was dissected out and stomachs were observed for the ulceration

with the help of magnifying lens and studied its external, internal surface and ulcer index was evaluated according to the severity of ulcers. The stomach were stored and fixed in 5% formalin and studied for histopathological changes. The ulcer index was high in control group i.e. 7.33 ± 2.07, 5.88 ± 1.04 respectively. whereas Animal pretreated with Ocimum sanctum at the dose 100 & 200 mg/kg showed 1.83 ± 0.75, 2.50 ± 0.98 in restraint model and 1.38 ± 0.88, 1.63 ± 0.21 in ethanol model shows significantly less mucosal injury and the percentage of damage (84.65 and 79.86)were less compared to control group. Thus the study concluded pretreatment with Ocimum sanctum at dose 100-200 mg/kg caused a significant anti-ulcer effect in rats in comparison with control group and its effect is comparable to the standard drug ranitidine

S. Mallikarjun, et al (2016)[70] – Performed a study on Antimicrobial efficacy of Tulsi leaf (*Ocimum sanctum*) extract on periodontal pathogens it is an *in vitro* study Ethanolic extract of Tulsi was prepared by cold extraction method. Extract was diluted with an inert solvent, dimethyl formamide, to obtain five different concentrations 0.5%, 1%, 2%, 5%, and 10%. These extracts were collected in sterile containers and transported for microbiological assays. In this study, doxycycline used as a positive control and dimethyl formamide solvent used in the extract preparation, was used as a negative control. Both were used in the treatment of aggressive periodontitis. In microbiological assay blood agar used to culture different microorganism such as aerobic and anaerobic bacteria these Colonies of microorganisms were transferred to the agar plates using a swab, and their turbidity was visually adjusted with the broth to equal that of a 0.5 McFarland turbidity standard that had been vortexed. The entire surface of agar plate was then swabbed 3 times with the cotton swab, transferring the inoculum, while the

plates were rotated by approximately 60° between streaks to ensure even distribution. The overall procedure of inoculum preparation and inoculation of culture media remained the same for all three bacteria. Each bacterium was inoculated on five agar plates for five respective concentrations (0.5%, 1%, 2%, 5%, and 10%) of the Tulsi extract. Therefore, a total of 15 plates were inoculated to test all the three bacteria. Incubation was done for 48 h for both aerobic (*A. actinomycetemcomitans*) and anaerobic (*P. gingivalis*and *P. intermedia*) bacteria. For anaerobic organisms, plates were incubated in the McIntosh and Filde's anaerobic jar, while aerobic micro-organism was cultured in the incubator at 37°C for 48 h. After the incubation period, plates were read only if the lawn of growth was confluent or nearly confluent. The microbiological procedure was repeated 4 times for each bacterium, and corresponding four values of zones of inhibition for each concentration of Tulsi extract along with doxycycline and dimethyl formamide were obtained for each of the three bacteria. The values so obtained were compared within the group (same concentration of extract) and with different groups (different concentrations of extract) and also with the positive control (doxycycline) for different bacteria Statistical analysis was done using the software Statistical Package for Social Sciences Descriptive statistics was retrieved, and data were analyzed using one-way analysis of variance (ANOVA), and Tukeypost-hoctest was used for comparison within the group and with different groups. Statistical significance level was established at $P < 0.05$. Result obtain at 5% and 10% concentrations, Tulsi extracts demonstrated antimicrobial activity against *A.*actinomycetemcomitans, similar to doxycycline with similar inhibition zones ($P > 0.05$). *P.* gingivalisand P. intermedia, however, exhibited resistance to Tulsi extract that showed significantly smaller inhibition zones ($P < 0.05$). Conclude Tulsi has antimicrobial property against A. actinomycetemcomitans, suggesting it is use as an

effective and affordable" along with the standard care in the management of periodontal conditions.

EswarP et al. (2016)[71] - This study determined *Ocimum sanctum* has an anti-microbial activity against Actinobacillusactinomycetemcomitansin human dental plaque and to compare the antimicrobial activity of *Ocimum sanctum* extract with 0.2% chlorhexidine as the positive control and dimethyl sulfoxide as the negative control. This is invitro experimental study design Five male subjects aged between 50-52 years, diagnosed with periodontal disease based on clinical examination were selected from the Department of Periodontology and Implantology of the dental college and hospital. written informed consent was obtained from all the selected subjects prior to the start of the study. Diagnosis of periodontal disease was made based on the criteria of Community Periodontal Index were periodontal pocket depth of 4-5mm ,determined using the Community Periodontal Index probe. All the patients selected were using tooth brush and toothpaste without fluoride and anti-plaque agents once daily in the morning as a primary oral self care. None of the patients were using mouthwash, dental floss or inter-dental cleaning aids. Patient did not suffer from any cognitive deficiencies, chronic medical conditions, systemic diseases or infectious diseases, no history of drug, alcohol or tobacco addiction, patient did not have any other oral diseases, periodontal disease or any prosthesis or oral appliances were not a part of the study. Patients were asked rinse mouth to reduce the contamination of plaque with soft debris. The area of plaque collection was isolated and subgingival and supragingival plaque sample were collected using Sterile stainless steel jaquettescaler and sterile Gracey curette. This collected plaque sample were transported to the laboratory in 200µl peptone water as the transport medium. In other side. Ethanolic extract of *Ocimum sanctum*was prepared

by the cold extraction method. The extract was diluted with an inert solvent, dimethyl sulfoxide, to obtain ten different concentrations such as 1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, 10% of extract. Isolation of *Actinobacillusactinomycetemcomitans*from plaque samples was done using Tryptic Soy Serum Bacitracin Vancomycin agar (TSBV) medium. Identification of *Actinobacillusactinomycetemcomitans*was done based on cultural, microscopic, biochemical characterization and multiple drug resistance patterns. Then Anti-microbial activity of *Ocimum sanctum* extract was tested by agar well-diffusion method against 0.2% chlorhexidine as a positive control and dimethyl sulfoxide as a negative control. The zone of inhibition was measured in millimeters using Verniercallipers. At the 6% of concentration of *Ocimum sanctum* extract, a zone of inhibition of 22mm was found. Among all the 10 different concentration, 6% of concentration of *Ocimum sanctum* extract was observed the widest zone of inhibition . The zone of inhibition for positive control was 25mm and no zone of inhibition was observed around the negative control hence author concluded 6% concentration of ocimum sanctum extract has maximum antimicrobial potential therefore it is effective against Actinobacillusactinomycetemcomitans .

MATERIALS AND METHODS

Periodontal diseases are common infectious disease characterized by the inflammatory breakdown of tooth supporting hard and soft tissues. The initiation and progression of periodontal disease depend on complex interactions between periodontopathic bacteria and the host immune system. The antigen, lipopolysaccharide, and other virulent factors of microorganisms are known to be potent stimulators of host inflammatory mediators. The host response is a major determinant of the severity of the disease. Smoking is known risk factor for periodontal disease. Cigarette smoke increases the oxidative burden, as it contains a large number of free radicals, and it has been suggested that it may increase the susceptibility to periodontal pathogens. As smoking increases the risk

of periodontitis, the present study evaluated the effect of smoking on oxidative stress in chronic periodontitis patients, The present study was aimed to evaluate the levels of 8-OHdG in GCF in smokers and non-smokers with chronic periodontitis

Study Design

This was a randomized controlled trial with a split mouth study design a total number of 50 patients between the age group of 30-55 years visiting the Department of Periodontology, of our institute were selected in this study. The study design was reviewed and approved by the Institutional Ethics Committee and is in accordance with the Helsinki Declaration. Prior to the initiation of the study an informed consent was obtained from all the patients willing to participate in the study.

Smoking status was determined by verbal questioning. Smokers were enrolled if they regularly smoked 10 cigarettes/day, and non-smokers were consider as not having smoked cigarettes in their lifetime. The same number of smokers and non-smokers were present in the periodontitis groups. Complete case history, clinical and radiographic examination was evaluated of all participants. Supra and subgingival scaling was carried out in all the patients. Contralateral pairs of teeth in maxilla or mandible were randomized to receive test treatment or serve as controls. Randomization was done by coin toss method. The intraoral examination was conducted by a single examiner which included Probing pocket depth (PPD), Clinical attachment level (CAL), Plaque index (PI) [Silness and Loe 1964], Gingival index (GI) [Loe and Silness, 1963].

All 50 participants were divide into 3 groups.

Group I –10 Periodontally healthy patients. .

Group II -20 Smokers with chronic periodontitis.

Group III –20 Nonsmokers with chronic periodontitis.

After selection of smoker and non-smoker chronic periodontitis patients were further divided in to Test and Control site. Test and control site were contralateral with each other in which exhibited probing depth was ≥ 5mm and clinical attachment level ≥ 5 mm in each site.

Group II – Total 20 patients of smoker with chronic periodontitis and in each patient split mouth study was performed thus 20 sites were considered as test and 20 as control sites.

Test Site: (n = 20) which received subgingival 10% Ocimum sanctum (O.S) gel immediately after scaling and root planing.

Control Site: (n = 20) which received only scaling and root planing.

Group III - Total 20 patients of non-smoker with chronic periodontitis and in each patient split mouth study was performed thus 20 sites were considered as test and 20 as control sites.

Test Site: (n = 20) which received subgingival 10% Ocimum sanctum (O.S) gel immediately after scaling and root planing.

Control Site: (n = 20) which received only scaling and root planing.

Inclusion criteria

Group I

a) Periodontally healthy patients with no signs of periodontal disease were considered as Healthy controls.

b) Patients with no history of smoking.

Group II

a) Current smokers with untreated moderate to severe chronic periodontitis, as assessed by clinical finding of PPD ≥ 5 mm and CAL ≥3mm, and with radiographic evidence of bone loss.

b) Patients with history of smoking at least 10 cigarettes per day for the last 3 years

Group III

a) Non-smokers with untreated moderate to severe chronic periodontitis, as assessed by clinical finding of PPD ≥ 5mm and CAL≥3mm. And with radiographic evidence of bone loss.

b) Patients with no history of smoking.

Males and females were randomly selected in each group.

Exclusion criteria

1. Patients with self reported psychiatric disorders or psychotic medications.
2. Patients with any systemic disease.
3. Pregnant, post menopausal or lactating women.
4. Patient with history of any acute illness
5. Patient with history of any antibiotic therapy , steroid therapy , immunosuppressive therapy within 6 weeks
6. Patients who had undergone any type of periodontal therapy or oral prophylaxis in past 6 months.

Armamentarium

Following material and armamentarium was used for the assessment of clinical parameters and for the collection of GCF -

For examination of the patient:

1. Mouth mirror
2. UNC-15 (Hu-Freidy) periodontal probe.
3. Tweezer
4. Dental Explorer
5. Disposable gloves
6. Disposable face mask
7. Kidney tray
8. Weight measuring machine
9. Height measuring tape.
10. Waist circumference measuring tape.
11. Cotton swab.
12. Sphygmomanometer and stethoscope.

For collection of GCF sample

1. 5µl micro capillary pipette
2. Eppendorf tube
3. Sterilized cotton rolls.

Assessment of periodontal and clinical parameters

1. Probing Pocket Depth (PPD)

It was measured using Hu Friedy UNC-15 graduated periodontal probe on 4 sites of all present teeth. Patients were considered healthy if they exhibited probing depth < 3mm & there was no clinical attachment loss. Patients were diagnosed with chronic periodontitis if they exhibited PPD ≥ 5mm and Clinical attachment loss ≥ 5mm at multiple sites.

2. Clinical Attachment Level (CAL)

It was measured using Hu Friedy UNC-15 graduated periodontal probe on 4 sites from the cementoenamel junction to the base of the pocket of all the present teeth. Patients were considered healthy if they exhibited no clinical attachment loss. Patients were diagnosed with chronic periodontitis if they exhibited clinical attachment level ≥ 5mm at multiple sites.

3. Plaque index (PI): (Silness and Loe, 1964)[72]

PI was examined in the scoring units of teeth: distofacial, facial, mesiofacial and lingual surfaces. A mouth mirror and dental explorer were used to assess plaque index.

The criteria for scoring were as follows:

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

A plaque index per person was obtained by adding all of the plaque scores and dividing by the number of surfaces examined.

$$\text{PI} = \frac{\text{Total plaque score}}{\text{No of surfaces examined}}$$

The following suggested nominal scale was used for patient evaluation.

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

4. Gingival index (GI): (Loe and Silness, 1963)[73]

This is a system for assessing the severity of gingivitis in four possible areas. The tissues surrounding each tooth were divided into four gingival scoring units: the distofacial papilla, the facial margin, mesiofacial papilla and the entire lingual gingival margin. A blunt periodontal probe was used UNC-15 graduated) to assess the bleeding potential of the gingival margin.

According to the following criteria-

SCORE	**CRITERIA**
0	Normal gingival
1	Mild inflammation, slight change in color, slight edema, no bleeding on palpation
2	Moderate inflammation, redness, edema and glazing, bleeding on palpation
3	Severe inflammation, marked redness and edema, ulcerations, tendency of spontaneous bleeding

The scores of all the surfaces were added and divided by number of surfaces examined which provided the gingival index score per person.

$$\textbf{GI} = \frac{\textbf{Total GI scores per tooth}}{\textbf{No. of surfaces}}$$

The numerical score of the gingival index taken into consideration for varying degrees of clinical gingivitis were as follows-

Gingival scores	**Condition**
0.1 to 1.0	Mild gingivitis
1.1 to 2.0	Moderate gingivitis
2.1 to 3.0	Severe gingivitis

Laboratory armamentarium for assessment of biochemical parameters

- Calibrated, volumetric transfer pipettes with 0-5 µl range, 5-50 µl range, 50-200 µl range and 200-1000 µl range
- Sterilized test tubes with test tube stand
- Distilled water
- Beakers, Measuring cylinder
- Sterile gloves

Human 8-Hydroxy-desoxyguanosine(8-OHdG) ELISA kit (kinesisdx) which contains

Laboratory equipment

- -30°C deep freezer (REMI EquipmentsPvt. Ltd.)
- Lab Centrifuge machine (R-8C, REMI EquipmentsPvt. Ltd.)
- Vortex mixer (CM 101, REMI EquipmentsPvt. Ltd.)
- ELISA reader (LISA Microplate reader, REMI EquipmentsPvt. Ltd.)

Assessment of Biochemical parameters

GCF sample collection:

GCF samples were collected using microcapillary pipettes in the morning following an overnight fast of 8 h, during which patients were requested not to drink (except water) or eat. GCF sample was collected from a site with maximum attachment loss for CP in Group II and Group III and from a nondiseased site in healthy individuals. After isolating the site with cotton rolls, plaque along with the supragingival calculus was removed using area-specific Gracey curette and cotton gauze, to avoid contamination and blocking of microcapillary pipette by plaque. The sulcular areas were gently air-dried. A color-coded 1–5 µl, calibrated volumetric microcapillary pipette was placed at the entrance of the gingival crevice until a standardized volume of 2 µl of GCF was collected. The GCF samples which were contaminated with blood or saliva and air bubbles were discarded and fresh samples were collected. Collected GCF samples were immediately transferred to airtight plastic vials (eppendorf tubes) and stored at -30°C until assayed[74].

Estimation of GCF, 8-Hydroxy-desoxyguanosine(8-OHdG) levels using human 8-OHdG detection kit

Samples were assayed for 8-OHdG levels using commercially available ELISA (Enzyme linked immune-sorbent assay) Kinesis Dx Human 8-OHdG ELISA Kit. Samples were analyzed according to the instruction manual at a Department of Biochemistry, LataMangeshkar Hospital and Research Centre, Nagpur, India. Briefly GCF samples were diluted with dilution buffer in the kit and the amount of 8-OHdG determined. All samples have been run in duplication.

Reagents

1. Microtiter Coated Plate (2 x 96 wells)

2. **(8-OHdG)** Biotin Conjugate - 1 ml

3. Standard, 128 ng/ml – 0.5 ml

4. Streptavidin:HRP Conjugate - 6 ml

5. Wash Buffer (30X) – 20 ml

6. Standard Diluent – 3 ml

7. Substrate A – 6 ml

8. Substrate B – 6 ml

9. Stop Solution – 6 ml

Additional materials required

1. Microplate Reader able to measure absorbance at 450 nm.

2. Adjustable pipettes to measure volumes ranging from 50 µl to 1000 µl.

3. Deionized (DI) water.

4. Wash bottle or automated microplate washer.

5. Semi-log graph paper or software for data analysis.

6. Tubes to prepare standard/sample dilutions.

7. Timer.

8. Absorbent paper.

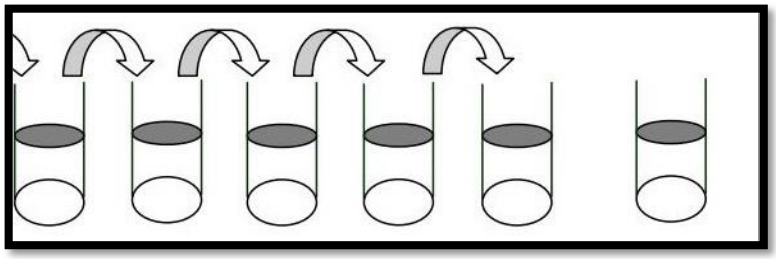
9. Incubator

Reagent preparation for GCF samples

1. All reagents and samples are brought to room temperature (18 - 25ºC) before use.

2. To make 1X Wash Solution, add 10 ml of 30X Wash Buffer in 290 ml of DI water.

3. For standard preparation: a vial of 120µl Original Standard was briefly spun to which 120µl Standard diluent was added to prepare a 32 ng/ml standard. The solution was thoroughly dissolved by a gentle mix. Pipetting of 120µl standard into each tube was done. The stock standard solution was used to produce a dilution series (shown below). Each tube was thoroughly mixed before the next transfer.



		Std5	**Std4**	**Std3**	**Std2**	**Std1**
Diluent Volume	Original Standard +Standard diluent	240 µl	240 µl	240 µl	240 µl	240 µl
Conc.	64ng/ml	32ng/ml	16ng/ml	8ng/ml	4ng/ml	2ng/ml

Assay procedure

1. All reagents and samples were brought to room temperature (18 - 25ºC) before use.
2. Remove the number of strips required for the assay.
3. Pipette out 50 µl of Standards and 40 µlSamples into the respective wells as mentioned in the work list. Note do not add the sample, Biotin Conjugate and Streptavidin-HRP to the blank well.
4. Pipette out 10 µl of (8-OHdG) Biotin Conjugate into each sample well. Do not pipette into the blank and standards wells.
5. Pipette out 50 µl of Streptavidin-HRP Conjugate into each sample and standards well. Do not pipette into the Blank well.
6. Cover the plate and incubate for 1 hour at 37 ℃ in the incubator.
7. Aspirate and wash plate 4 times with 1X Wash Buffer and blot residual buffer by firmly tapping plate upside down on absorbent paper. Wipe off any liquid from the bottom outside of the microtiter wells as any residue can interfere in the reading step. All the washes should be performed similarly. Then add Substrate A50 µl, then Substrate B 50 µl to each well including Blank well. Gently mixed, incubate for 10 min at 37 ℃ in dark.
8. Pipette out 50 µl of Stop Solution. Wells should turn from blue to yellow in colour.
9. Read the absorbance at 450 nm within 15 minutes after adding the Stop Solution blanking on the zero standards.

Assay Procedure Summary

All reagents and samples were brought to room temperature (18 - 25ºC) before use.

↓

Pipette out 50 µl of Standards and 40 µlSamples into the respective wells

↓

Pipette out 10 µl of Biotin Conjugate into each sample well.

↓

Pipette out 50 µl of Streptavidin-HRP Conjugate into each sample and standards well.

↓

Cover the plate and incubate for 1 hour at 37 °C in the incubator.

↓

Aspirate and wash plate 4 times with 1X Wash Buffer.

↓

Add Substrate A50 µl to each well.

↓

Add Substrate B 50 µl to each well.

↓

Gently mixed, incubate for 10 min at 37 ℃ in dark.

↓

Pipette out 50 µl of Stop Solution.

↓

Read the absorbance at 450 nm within 15 minutes after adding the Stop Solution

↓

blanking on the zero standards.

All the clinical parameters were recorded and the GCF samples were collected at the baseline and at 3 month.

Scaling and root planning along with application of 10 % Tulsi gel (Test site) will be performed in group II and group III on one side and on the other side only SRP (control site) will be performed. A re-evaluation examination will be performed at 1 and 3 months following the completion of initial therapy.

Preparation of 10% ocimum sanctum gel

Ingredients	**Quantity**
Tulsi	10%
Carbopol	1000 mg
Sodium Benzoate	25 mg
Methyl Paraben	10 mg
Propyl Paraben	10 mg
Distilled water	q.s

Procedure-

Carbopol is soaked with 50 ml of water in a beaker and kept for day. Next day the beaker is placed on magnetic stirrer, set the RPM of 1000, add magnetic bead and stirr for two hours. Further, add calculated quantity of Tulsi extract to the gel, followed by addition of sodiumbenzoate, methyl paraben, propyl paraben and a little quantity of water. Change the RPM to 1500 and stir the contents for eight hours with addition of water intermittently as and when required. Check the gel consistency, transfer and dispense in a plastic container.

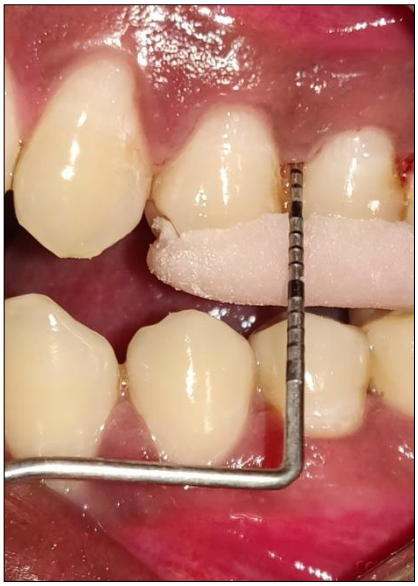
Sterilization:

Prepared gel was exposed under Ultra Violet light in UV chamber for a period of 30 minutes. The gel integrity was checked. Methyl paraben and propyl paraben were added as preservatives which prevent the growth of bacteria and keeps the gel stable

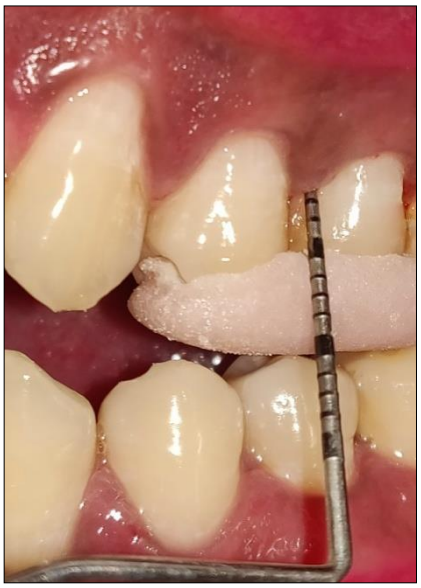
After the baseline clinical recording, GCF sampling, phase I periodontal therapy was initiated. All patients underwent therapy including oral hygiene instruction. Scaling and root planing were performed by ultrasonic instruments. Mechanical periodontal therapy was not accompanied by any medications like antibiotics or non-steroidal anti-inflammatory drugs mouthrinse etc.

Color Plate I

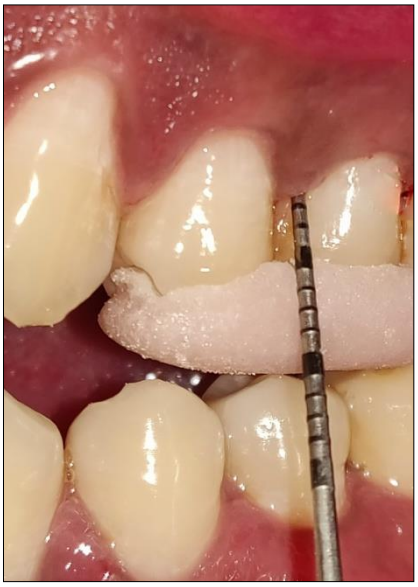
Group I (Healthy Group)



Probing depth at baseline



Probing depth at 1 month



Probing depth at 3 months

Color Plate II

Group II (Smoker + Chronic Periodontitis Group)

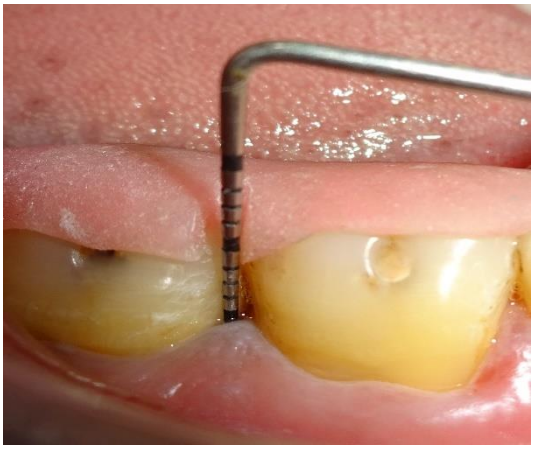
TEST Sites – Treated with SRP + 10% OS Gel

Probing depth at baseline [Test]

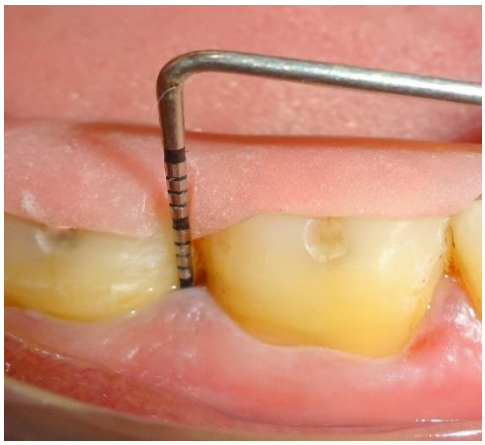
Probing depth at 1 months [Test]

Probing depth at 3 months [Test]

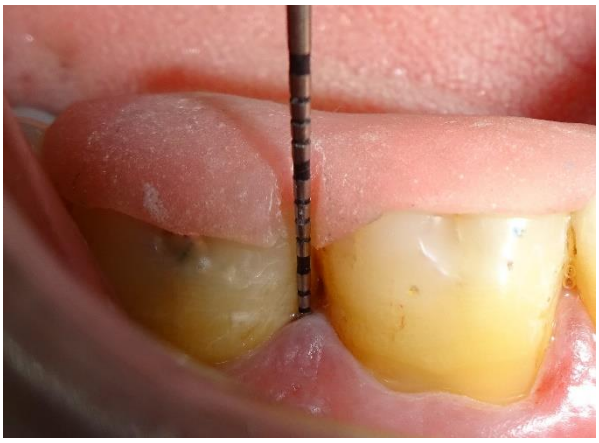
CONTROL Sites – Treated with SRP



Probing depth at baseline [Control]



Probing depth at 1 Month [Control]

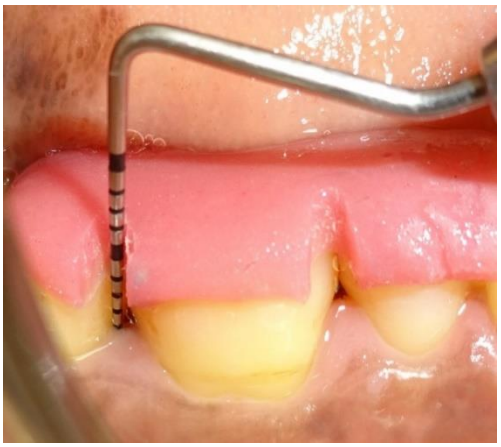


Probing depth at 3 Month [Control]

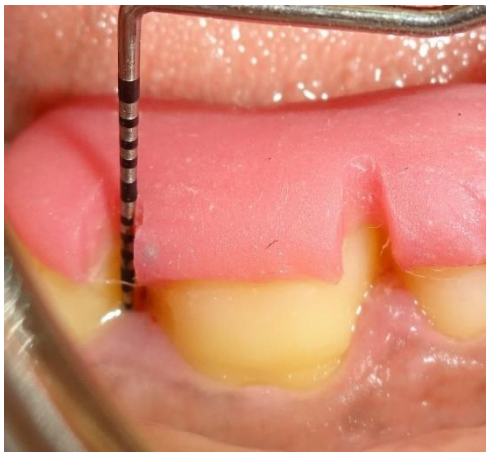
Color Plate III

Group III(Non smoker +Chronic Periodontitis)

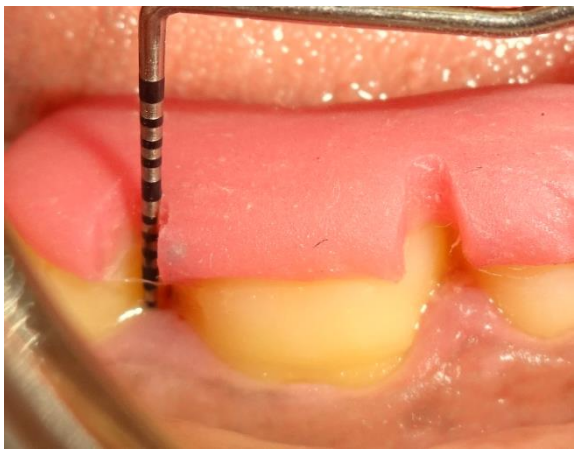
Test Site – Application of 10% tulsi (OS) Gel



Probing depth at baseline [Test]



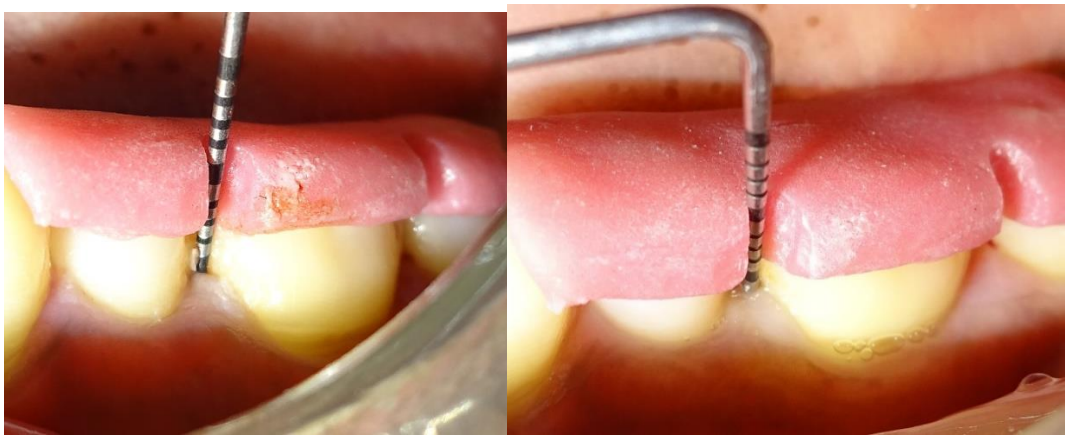
Probing depth at 1 month [Test]



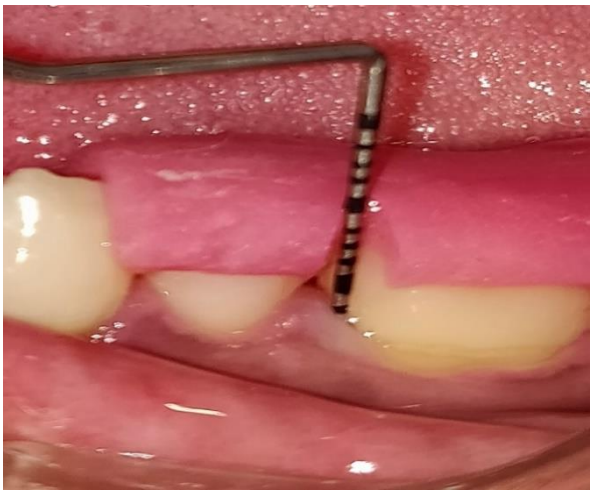
Probing depth at 3 months [Test]

Group III(Non smoker +Chronic Periodontitis)

Control site – Plain SRP Site

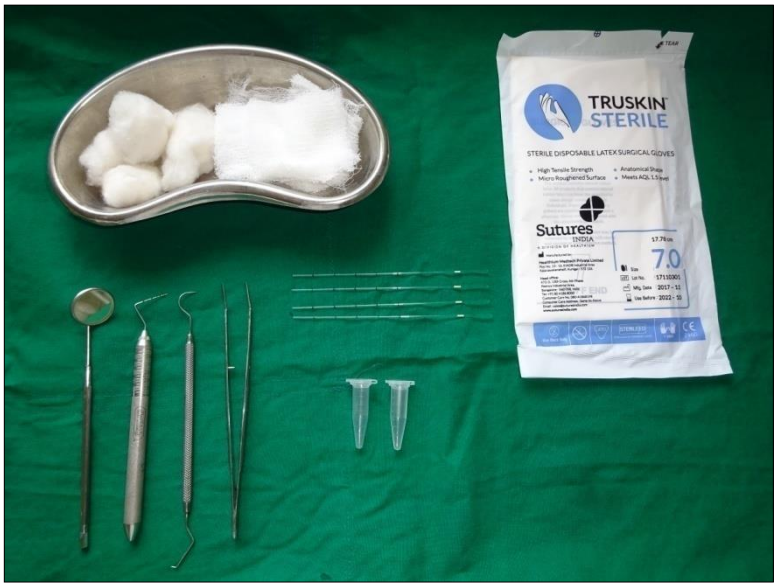


Probing depth at baseline in [control]Probing depth at 1 month [control]

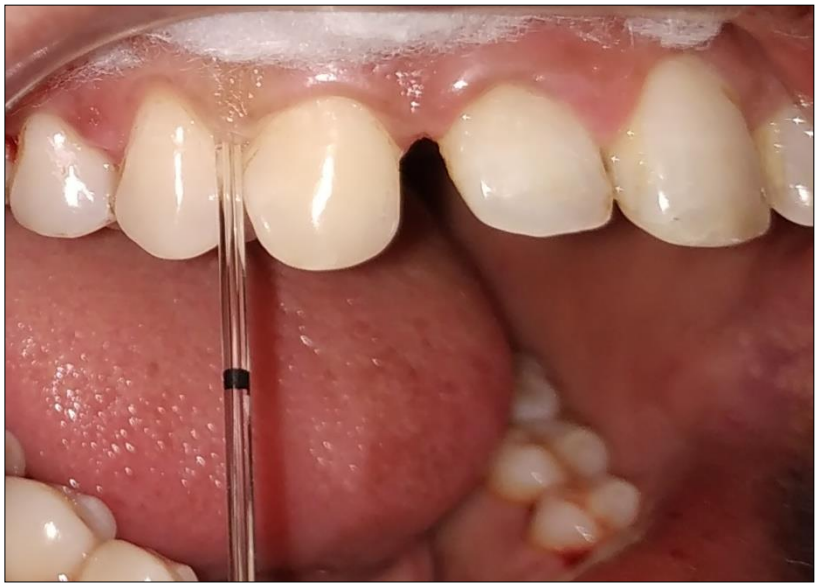


Probing depth at 3 Month [control]

Color Plate IV



Armamentarium for clinical examination and GCF collection



Collection of GCF

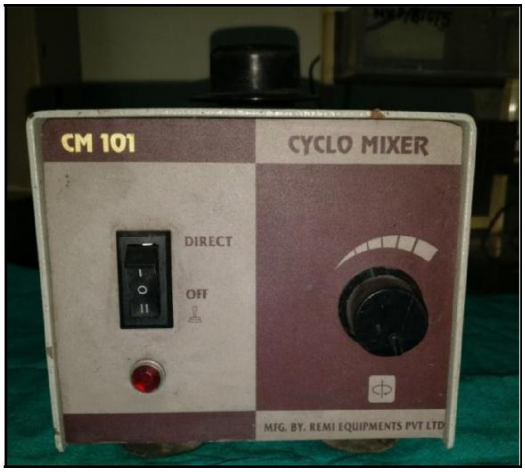
Color Plate V

Deep Freezer

Kinesis Dx Human 8-OHdG ELISA Kit

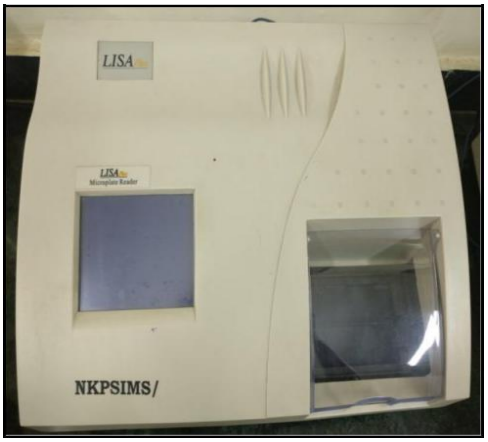
Color Plate VI

Centrifuge Machine



Vortex Mixer

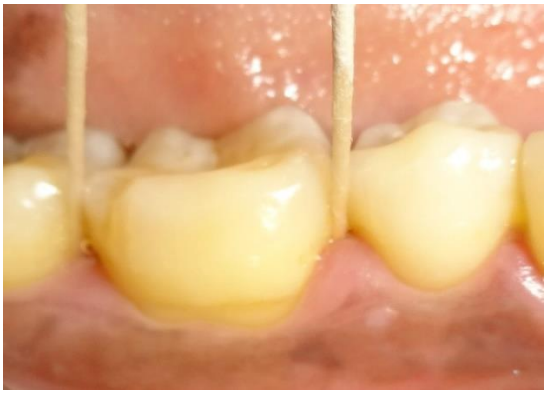
Micro-plate Washer



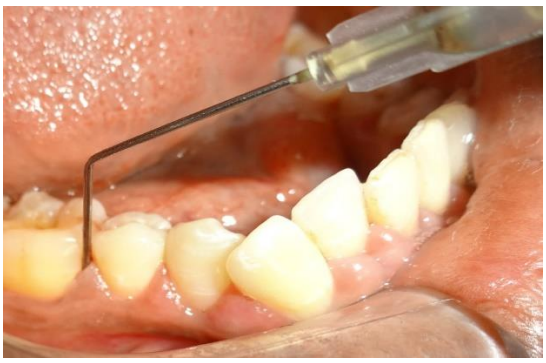
Micro-plate Reader Machine

COLOUR PLATE VII

10 % Ocimum sanctum gel



Isolation



10% ocimum sanctum gel Being delivered in to a periodontal pocket using blunt end precision tip.

RESULTS

The present experimental, split-mouth study evaluate and compare the efficacy of locally delivered ocimum sanctum gel and 8-OHdG level in gingival crevicular fluid in smokers and nonsmokers with chronic periodontitis patients pre and post phase I periodontal therapy.

A total of 50 patients were selected from those visiting the Department of Periodontology of our institute. These patients were assessed clinically and biochemically.

The study comprised of three groups-

1. **Group I -** 10 Periodontally healthy patients.

2. **Group II –** 20 Smokers with chronic periodontitis patients.

3. **Group III** - 20 Nonsmokers with chronic periodontitis patients.

After selection of smokers and non-smokers, chronic periodontitis patients were further divided into test and control site. Test and control sites were contralateral with each other in which exhibited probing depth was ≥ 5mm and clinical attachment level ≥ 5 mm in each site.

Group II – Included total 20 patients (40 site)of smoker with chronic periodontitis on which split mouth study was performed thus 20 sites were considered as test and 20 as control sites.

Test Site: (n = 20) which received subgingival 10 % Ocimum sanctum Gel (O.S) immediately after scaling and root planning.

Control Site: (n = 20) which received only scaling and root planing.

Group III - Included total 20 patients (40 site)of non-smoker with chronic periodontitis on which split mouth study was performed thus 20 sites were considered as test and 20 as control sites.

Test Site: (n = 20) which received subgingival 10% Ocimum sanctum Gel (O.S) immediately after scaling and root planing.

Control Site: (n = 20) which received only scaling and root planing.

Clinical parameters such as Plaque index (PI), gingival index (GI), periodontal probing depth (PPD) and Clinical attachment levels (CAL) were recorded at baseline, 1 month

and 3 month and The estimation of gingival crevicular fluid 8-OHdG levels was performed using human 8-OHdG ELISA kit.(kenesisDx)

Statistical analysis

Statistical software STATA version 24.0 was used for data analysis.Clinical parameters (PPD, CAL, GI and PI) were presented as Mean± SD. Mean GI, PI, PPD, and CAL were compared before and after 3 month of therapy in Group I, Group II and Group III by performing repeated measures analysis of variance (ANOVA) followed by multiple comparison test Bonferroni correction was done. And GCF 8-OHdG level measured at baseline and 3 month after phase 1 periodontal therapy (SRP) using paired t- test with P value <0.05 was considered as statistically significant.

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.

Plaque index

The plaque index at baseline, 1 and 3 months in Group I, Group II and Group III was obtained using repeated measures ANOVA test

Group I (Healthy) - The mean plaque index at baseline was 0.61±0.24 which reduced to 0.41 ±0.16 and 0.58 ±0.50 at 1 month and 3 month post-treatment follow-ups respectively. (Table 1and Graph 1)

Group II (Smoker + CP) – **At Test site,** the mean plaque index at baseline was 2.14 ±0.37 which reduced to 1.55 ±0.36, 0.72 ±0.28 at 1 month and 3 month post-treatment follow-ups respectively. (Table 1 and Graph 1) and

At Control site, the mean plaque index at baseline was 2.17 ±0.40 which reduced to 1.62 ±0.36, 0.82 ±0.23 at 1 month and 3 month post treatment follow-ups respectively. (Table 1 and Graph 1)

Group III(Nonsmokers + CP**) -** At Test site , the mean plaque index at baseline was 2.05 ±0.29 which reduced to 1.32 ±0.24 and 0.56 ±0.21 at 1 month and 3 month post-treatment follow-ups respectively. (Table 1 and Graph 1) and

At Control site,the mean plaque index at baseline was 2.25 ±0.40which reduced to 1.32 ±0.24 and 0.98 ±0.28 at 1 month and 3 month post-treatment follow-ups respectively. (Table 1 and Graph 1)

The mean difference in plaque index from baseline to 1 month and 3 month in Group I ,Group II and group III was found to be statistically highly significant .($p<0.005$). (Table 1 and Graph 1)

Intergroup comparison of Mean PI performed using pair t test between Test site and Control site in Group II showed nonsignificant difference at 1 month and 3 month post periodontal therapy while group III showed significant difference between Test site and Control site at 3 month post periodontal therapy. (Table 4)

Intergroup comparison of Mean PI was performed to evaluate the effect of Test site between smoker and non-smoker with chronic periodontitis using paired t test.

Nonsignificant difference was found in mean PI value among group II patients between Test site vs Control site while significant difference found in group III at 3 month ($P=0.000$) .(Table 5)

Gingival Index (GI)

The Gingival index at baseline, 1 and 3 months in Group I, Group II and Group III was obtained using repeated measures ANOVA test.

Group I - The mean GI at baseline was 0.36 ±0.24 which reduced to 0.27 ±0.13 and 0.03 ±0.04 at 1 month and 3 month post-treatment follow-ups respectively. (Table 1and Graph 1)

Group II (Smoker + CP) – At Test site, the mean GI at baseline was 1.91 ±0.38 which reduced to 1.36 ±0.35, 0.68 ±0.28 at 1 month and 3 month post-treatment follow-ups respectively. (Table 1 and Graph 2) and

At Control site, the mean GI at baseline was 1.91 ±0.38 which reduced to 1.46 ±0.25, 0.88 ±0.37 at 1 month and 3 month post-treatment follow-ups respectively. (Table1 & Graph 2)

Group III(Nonsmokers + CP**) -** At **Test site** , the GI at baseline was 2.28 ±0.44 which reduced to 1.11 ±0.45 and 0.45 ±0.17 at 1 month and 3 month post-treatment follow-ups respectively. (Table 1 and Graph 2) and

At Control site, the mean GI at baseline was 2.27 ±0.44which reduced to 1.32 ±0.32 and 0.58 ±0.24 at 1 month and 3 month post-treatment follow-ups respectively. (Table 1 and Graph 2)

The mean difference in Gingival index from baseline to 1 month and 3 month in Group II and Group III was found to be statistically highly significant .($p < 0.005$). (Table 1 and Graph 2)

Intergroup comparison of Mean GI performed using paired t test between Test site and Control site in Group II showed nonsignificant difference at 1 month (P=1.00) and 3 month (P=1.00) post periodontal therapy while group III showed significant difference between Test site and p Control site at 3 month post periodontal therapy. (Table 4)

Intergroup comparison of Mean GI was performed to evaluate Test site between smoker and non-smoker with chronic periodontitis using paired t test. significant difference was found in mean GI at baseline and 3 month post therapy while Control site of GI value among smoker with chronic periodontitis and non-smoker with chronic periodontitis showed significant association at baseline(P= 0.052) while 1st month (p=0.357) and 3rd month(P=0.144) was showed nonsignificant difference. (Table 5)

Periodontal pocket depth (PPD)

The PPDat baseline, 1 and 3 months in Group I, Group II and Group III was obtained using repeated measures ANOVA test

Group I (Healthy) - The mean PPD at baseline was 2.10 ±0.99 which reduced to 1.20 ±0.91 and 0.40 ±0.51 at 1 month and 3 month post-treatment follow-ups respectively. (Table 2 and Graph3)

Group II (Smoker + CP) – At Test site, the mean PPD at baseline was 5.50 ±1.00 which reduced to 4.10 ±0.72, 2.75 ±0.64 at 1 month and 3 month post-treatment follow-ups respectively. (Table 1 and Graph 3) and

At Control site, the mean PPD at baseline was 5.30 ±0.73 which reduced to 5.30 ±0.73, 5.10 ±0.78 at 1 month and 3 month post-treatment follow-ups respectively. (Table 2 and Graph 3)

Group III(Nonsmokers + CP**)** - At Test site , the PPD at baseline was 5.30 ±0.57which reduced to 4.00 ±0.56 and 2.50 ±0.68 at 1 month and 3 month post-treatment follow-ups respectively. (Table 2 and Graph 3) and

At Control site, the mean PPD at baseline was 5.25 ±0.91which reduced to 4.75 ±0.97 and4.20 ±0.95 at 1 month and 3 month post-treatment follow-ups respectively. (Table 2 and Graph 3)

The mean difference in PPD from baseline to 1 month and 3 month in Group II and group III was found to be statistically significant .($p < 0.005$). (Table 2 and Graph 3)

Intergroup comparison of Mean PPD performed using pair t test between Test site and Control site in Group II showed nonsignificant difference at baseline (0.414) while 1 month(P=0.021) and 3 month (P=0**.**000) showed significant association. And In group III showed nonsignificant difference between Test site and Control site at baseline (P= 1.000) while at1 month(P=.000) and 3 month (P= 0.000) showed highly significant difference . (Table 4)

Intergroup comparison of Mean PPD was performed to evaluate Test site between smoker and non-smoker with chronic periodontitis using paired t test. significant difference was found in mean PPD at 1 month(P=0.028) and 3 month (P=0.003**)** post therapy when compare from baseline(P =0.442) while Control site of mean PPD value among smoker with chronic periodontitis and non-smoker with chronic periodontitis showed nonsignificant association at baseline(P= 0.471) while 1st month (P=0.627) and 3rd month (P=0.241) was showed significant difference. (Table 5)

Clinical Attachment Level (CAL):

The Clinical attachment levelat baseline, 1 and 3 months in Group I, Group II and Group III was obtained using repeated measures ANOVA test.

Group I (Healthy) - The mean CAL at baseline was 2.10 ±0.99 which reduced to 1.90 ±0.73 and 0.20 ±0.78 at 1 month and 3 month post-treatment follow-ups respectively. (Table 2 and Graph 4)

Group II (Smoker + CP) – At Test site, the mean CAL at baseline was 5.50 ±1.00 which reduced to 4.20 ±0.77, 3.15 ±0.81 at 1 month and 3 month post-treatment follow-ups respectively. (Table 2 and Graph 4) and

At Control site, the mean CAL at baseline was 5.30 ±0.73 which reduced to 5.25 ±0.71, 5.10 ±0.85at 1 month and 3 month post-treatment follow-ups respectively. (Table 2 and Graph 4)

Group III(Nonsmokers + CP**)** - At Test site , the CAL at baseline was 5.30 ±0.57which reduced to 4.15 ±0.50 and 2.85 ±0.93 at 1 month and 3 month post-treatment follow-ups respectively. (Table 2 and Graph 4) and

At Control site, the mean CAL at baseline was 5.30 ±0.92which reduced to 5.00 ±0.85 and4.70 ±1.08 at 1 month and 3 month post-treatment follow-ups respectively. (Table 3 and Graph 4)

The mean difference in CAL from baseline to 1 month and 3 month in group I and group III was found to be statistically significant except group II.($p < 0.005$). (Table 3 and Graph 4)

Intergroup comparison of Mean CAL performed using pair t test between Test site and Control site in Group II showed nonsignificant association at baseline while 1 month(P=0.004) and 3 month (P=0.000)showed highly significant difference. And In group III showed nonsignificant difference between Test site and Control site at

baseline (P= 1.000) while at1 month(P=0.000) and 3 month (P= 0.000) showed highly significant difference . (Table 4)

Intergroup comparison of Mean CAL was performed to evaluate the effect Test site between smoker and non-smoker with chronic periodontitis using paired t test. nonsignificant association was found in mean CAL at baseline(P=0.442),1 month(P=0.807) and 3 month (P=0.285). And Control site of mean CAL value among smoker with chronic periodontitis and non-smoker with chronic periodontitis showed nonsignificant association at baseline (P= 0.605),1st month (P=0.235) and 3rdmonth(P=0.137). (Table 5)

Estimation of 8-OHdG Level :

The 8-OHdG level in gingival crevicular fluid at baseline, and 3 months in Group I, Group II and Group III was obtained using paired t test .

Group I (Healthy) - The mean 8-OHdG Level at baseline was 4.44 ±0.48 ng/ml which reduced 4.27 ±0.33 ng/ml at 3 month post-treatment follow-ups respectively

Group II (Smoker + CP) – At Test site, the mean 8-OHdG Level at baseline was 10.40 ±1.94 ng/ml which reduced to 6.06 ±1.67 ng/ml at 3 month post-treatment follow-ups respectively. (Table 3 and Graph 5) and

At Control site, the mean 8-OHdG Level at baseline was 10.25 ±1.59ng/ml which reduced to 8.29 ±1.38ng/mlat 3 month post-treatment follow-ups respectively. (Table 3 and Graph 5)

Group III(Nonsmokers + CP**) -** At Test site , the mean 8-OHdG Level at baseline was 8.20 ±1.63ng/ml which reduced to 4.42 ±0.57ng/ml at 3 month post-treatment follow-ups respectively. (Table 3 and Graph 5) and

At Control site, the mean 8-OHdG Level at baseline was 8.14 ±2.13 ng/mlwhich reduced to 7.13 ±1.97 ng/ml at 3 month post-treatment follow-ups respectively. (Table 3 and Graph 5)The mean difference in 8-OHdG Level from baseline to 3 month in Group II Group III was found to be statistically significant.($p < 0.005$). (Table 3 and Graph 5)

Intergroup comparison of Mean 8-OHdG Level performed using paired t test between Test site and Control site in Group II showed nonsignificant association at baseline (P=0.266) while at 3 month (P=0.000) showed highly significant difference.

In group III showed nonsignificant difference between Test site and Control site at baseline (P= 0.921) while at 3 month (P= 0.000) was showed highly significant difference . (Table 4)

Intergroup comparison of Mean 8-OHdG level was performed to evaluate the effect on Test site between smoker and non-smoker with chronic periodontitis using paired t test. significant association was found in mean 8-OHdG level at baseline(P=0.000) and at 3 month (P=0.000).and Control site of mean 8-OHdG level among smoker with chronic periodontitis and non-smoker with chronic periodontitis showed significant association at baseline(P= 0.004) and 3rd month(P=0.046). (Table 5)

DISCUSSION

Periodontitis is chronic inflammatory disease characterized by destruction of supporting tissue and loss of alveolar bone. This multifactorial disease is influenced by a variety of risk factors such as bacterial plaque calculus, tobacco smoking and various systemic diseases that may affect the onset, severity and progression of disease. Microbial plaque has key role for the initiation and progression of periodontitis and it comprises of mostly gram negative anaerobic or facultative bacteria[75-77]. However these bacteria residing in crevices leading to most of the tissue destruction by impairment of the inflammatory and immune response causing the release of neutrophils, reactive oxygen species, and enzymes.This increased level of ROS by PMNs would lead to tissue degeneration and a worse status of periodontal disease. Oxidative stress which is comprised as an imbalance between free oxygen radicals and antioxidant defense

system is capable of causing damage to various cellular and extracellular components. Various studies reported that oxidative stress has been associated with number of systemic disorders and periodontitis as well.

Various studies have reported that both oxidative stress and the individual's total antioxidant capacity are disturbed in patients with periodontal disease, showing the existence of a direct association between the rise in reactive oxygen species and the fall in total antioxidant capacity in the pathogenesis of periodontal diseases.[78-81] The imbalance between oxidants and antioxidants have been related with the destruction of the periodontium during inflammation. The use of some antioxidants like lycopene and tulsi, vitamin E, vitamin C as periodontal treatment has the potential to improve periodontal clinical parameters[82] still, the role of antioxidant/oxidative stress parameters need to further investigation.

The primary goal of any periodontal therapy is to preserve the natural dentition by achieving and maintaining a healthy periodontium. Mechanical nonsurgical periodontal therapy has long been documented as part of periodontal therapy. In the 1950s, disease progression was understood to be associated with the amount of plaque or calculus, which was thought to be a physical irritant to the gingival tissues. Therefore, removal of all tooth deposits was considered the first step of therapy directed at inflammation and pocket depth reduction. In 1984, Badersten et al. concluded that non-surgical mechanical debridement of the periodontal pocket will, in the majority of cases, result in improvement of gingival health, arrest disease progression and therefore reduce the risk of tooth loss.[83-85]Recent advances in local delivery technology have resulted in control release of drugs that are successful in maintaining effective drug concentration at a low dosage in periodontal pocket.

In dentistry, herbal medicines are most widely used to treat oral lesions such as lichen planus, oral submucous fibrosis, leukoplakia, pemphigus vulgaris, aphthous ulcer, candidiasis, herpes virus , oral infections, dental pain and tooth decay also the Herbal medicine has an active role in reducing periodontitis. These can be used as a mouthwash, local drug delivery system in treating gingivitis, halitosis, for surgical wound healing, even in reducing inflammation around dental implants.[86]Among all the medicinal plant ocimum sanctum known as "Queen of Herbs" because of its varied medicinal properties as well as mythological value. Various investigations reported that ocimum sanctum possess significant antioxidant[87] , antistress activity against oxidative stress[88] analgesic ,antifungal, antimicrobial, antispasmodic, anticancer, antidiabetic, hepatoprotective, cardioprotective , arthritis , skin diseases and chronic fever etc.[89]

Smoking is well-known environmental risk factor for periodontal diseases. Nicotincontaint in tobacco smoke increases oxidative stress by increased free radicals and thus accentuates the periodontal destruction.[1]

In the literature, there is paucity of studies depicting the antioxidant potential of Tulsi (O.S) by assessing the 8-OhdG level in gingival crevicular fluid in smokers and non-smokers with chronic periodontitis after phase I therapy.

The study comprised of three groups-

Group I - 10 Periodontally healthy patients.

Group II – 20 Smokers with chronic periodontitis.

Group III - 20 Nonsmokers with chronic periodontitis.

After selection of Group II and Group III Patients were diagnosed with chronic periodontitis if they exhibited probing depth ≥ 5mm and clinical attachment level ≥ 5 mm in two contralateral site where one site consider as test and other as control .

Test site- Total 20 site in each group received subgingival 10% Ocimum Sanctum gel immediately after scaling and root planing.

Control site- Total 20 site in each group received only scaling and root planing.

All periodontal parameters i.e. PI, GI, PPD and CAL were recorded using UNC # 15 periodontal probe. The GCF 8- OHdG levels were assessed using human OHdG detection kit (ELISA) at baseline and 3 month after phase I therapy because most of the inflammation subsides and healing generally occurs by the 6 week mark; though, repair may continue for a longer period of time.[86]

In present study clinical parameters (PI, GI, PPD and CAL) were recorded at baseline, 1 month and 3 months in Group I, Group II and Group III. No significant differences were observed in mean values of PI, GI, PPD and CAL at baseline in these groups except healthy group showing the homogeneity of the patients.

To the best of our knowledge there have been no studies evaluating the efficacy of 10% ocimum sanctum gel in chronic periodontitis hence a direct comparison with other studies is quiet challenging. There is paucity of literature on its use in the treatment of chronic periodontitis. Therefore, the present study was hypothesized that the antioxidant potential of Tulsi by assessing the 8-OhdG level in gingival crevicular fluid.

In the present study, Group, Group II and Group III showed significant reduction in mean plaque index (PI) and gingival index from baseline to 3 month in both sites i.e. Test and Control. However reduction in Mean PI in 3 month was 2.14 ±0.37 to 0.72 ±0.28 in Test siteand Control site 2.17 ±0.40 to 0.82 ±0.23 in Group II while in Group

III 2.05 ±0.29 to 0.56 ±0.21in Test site and control site was 2.25 ±0.40 to 0.98 ±0.28. After phase I therapy statistical significant differences were observed in all groups at different interval. Also, mean gingival index showed significant reduction after periodontal therapy in Group I, Group II and Group IIIwhen compare at baseline to 3 month. This result accordance with study done by **Chandra et al (2012)[1] found** significant reduction in PI and GI observed after periodontal therapy in smoker and non-smoker group and there was no significant difference found in lycopene and placebo groups. Similar in **Dede et al (2013)[37]** study significant reduction found in PI and GI after initial periodontal therapy. **S Kurgan et al (2015)[39]** reported Statistically significant positive correlations between plaque index, gingival index, probing pocket depth, clinical attachment level, bleeding on probing values in CP patient and healthy patients after initial periodontal therapy (SRP). **Hendek M et al (2014)[43]** observed that after initial periodontaltherapy, periodontal clinical parameters i.e PI, GI, PPD and CAL significantly decreased from baseline to the 1^{s} and the 3rd months in both smoker with CP and non-smoker with CP groups ($p<0.001$)

The mean probing pocket depth (PPD) was significantly reduced in the 1 month and 3 month compared to the baseline in both Test & Control site in group II and group III but in Test site, pocket depth reduction was significantly higher. It could be due 10 % ocimum sanctum gel acts anti-inflammatory activity due to variable amount of linoleic acid present which has capacity to block both cyclooxygenase and lipoxygenace pathway of archidonate metabolism. **kelam MA, et al (2000), Singh S et al (1997**)[90-91] also inhibiting the bacterial growth thus decreasing the formation of plaque.**J Gaur et al (2015)[67]**revealed that subgingival irrigation with 4% Ocimum sanctum may prove to be

effective owing to its ability in reducing plaque accumulation, gingival inflammation and bleeding and has no side effects as compared to chlorhexidine.

Sirisha P et al (2017)[92]Detected antimicrobial effect of silver nanoparticles synthesised with Ocimum Sanctum leaf extract on periodontal pathogens such as Fusobacteriumnucleatum (Fn), Porphyromonasgingivalis (Pg), Aggregatibacteractinomycetemcomitens (Aa) and Prevotellaintermedia (Pi) and results suggest that, all the test solutions reveal their sensitivity to these microorganism.

M hosamaneetal(2014)[66]Evaluated the antimicrobial efficacy of holy basil in vitro against some periodontopathogens (p. intermedia and F. nucleatum) and its antiplaque effect in vivo study (30 Valunteers). Author reported holy basil extract showed inhibition of both pathogen and in vivo studies showed no statistical difference between holy basil and chlorhexidinemouthrinse with respect to PI hence concluded that holybasil mouthwash may have potential as an antiplaque mouthwash with prophylactic benefits.

P Eswar et al (2016)[71] Performed invitro study of different concentration of ocimum sanctum extract (1%-10%) 0n Actinobacillusactinomycetemcomitance and reported 6 % ocimum sanctum extract has maximum antimicrobial potential against Actinobacillusactinomycetemcomitance. simillar In vitro studies done by **S Mallikarjun et al (2016)[70]**showed 5 % and 10 % concentration of tulsi extracts demonstrated antimicrobial activity against A. actinomycetecomitans similar to doxycycline. **Takane et al. (2002)[32]**conducted a study to evaluate the effect of SRP on salivary 8-OHdG levels and showed significant probing depth reduction at 2 to 4 months after SRP. The same author conducted a study in **2005**[93] to evaluated the effect of SRP on GCF and salivary 8-OHdG levels which showed significant probing depth reduction at 2-6 months after initial periodontal therapy which is similar to the reduction

observed in our study. **Dedeet al. (2013)**[37]showed a probing depth reduction of 0.18 mm, 3 months after SRP. **Hendek et al. (2015)**[43]showed a significant reduction in probing depth of 1.04 mm in chronic periodontitis patients, 3-months after SRP. **Arunachalam et al. 2015**[38]in his study showed a probing depth reduction of 0.91 mm, 1 month after SRP.

When intergroup comparison was done significant reduction in PPD and CAL found in group III compared to group II The results are in accordance with the study performed by **Ah M et al.(1994)**[94] who reported that non-smokers demonstrated significantly more reduction of probing depth and more reduction in clinical attachment level when compared to smoker after initial periodontal therapy. The findings of present study are consistent with those of **Preber& Bergstrom et al.(1985)**[95]who reported less probing depth reduction in smokers following non-surgical therapy. **Jin et al. (2000)**[96]reported significantly more reductions of mean PPD in non-smokers compared with smokers at 1 and 3 months following non-surgical therapy.

In contrast with the finding by **Zuabi et al. (1999)**[97]reported no difference in post-treatment probing depth and clinical attachment level between smokers and non-smokers. However, smokers had significantly greater probing depths at baseline compared with the non-smokers. Subsequently, the more probing depth reduction in smokers compared with non-smokers will itself have been a direct consequence of the greater depth of pockets in smokers at baseline. The severity of periodontal destruction and inferior clinical response could be attributed to the smoking induced host response alteration. Smoking may alter both the local and systemic immune response and the healing response, which would aggravate the periodontal destruction and suppress the

maximal beneficial effects of scaling and root planing. This would, in turn, lead to greater attachment loss and poorer clinical response.

It is well documented that various components of the host response and immune system could be impaired by smoking. These effects include the inhibition of neutrophil chemotaxis and phagocytosis [98] inhibition of cellular immunity, and suppression of local antibody production.[99] Cigarette smoke products such as nicotine can also incorporate into root surfaces and into fibroblasts which may inhibit the reattachment of fibroblasts to the root surface as part of the healing process.[100]Smokers may demonstrate a diminished gingival defense against bacterial attack due to an impairment of neutrophil function.

In the present study, oxidative stress markers were evaluated in gingival crevicular fluids in smokers and non-smokers with chronic periodontitis groups than healthy volunteers. Following scaling and root planning, 8- OHdG level in GCF significantly decreased in both periodontitis groups .This is in accordance with the study done by **Ramanarayana Boyapati1 (2017)[101]** which showed that 8- OHdG levels in saliva were significantly higher in smoker with CP and nonsmoker with CP groups compared with periodontally healthy nonsmokers after initial periodontal therapy significantly reduced 8-OHdG level in both group and ROS were significantly higher in smokers than in non-smokers groups. **Hendek et al (2015)[43]** reported that the level of 8-OHdD in GCF and saliva were significantly higher in smokers and non-smokers periodontitis groups compared to smoker and nonsmoker periodontally healthy groups after initial periodontal treatment, the level of 8-OHdG in GCF and saliva were significantly decreased in both periodontitis groups.**Dede et al.(2013)**[37] reported that the effects of SRP as periodontal treatment on the GCF and salivary levels of 8-OHdG in

periodontitis and they was showed that 8-OHdG level in GCF was significantly decreased after SRP periodontal therapy. also other study done by **U. Sezer et al.(2012)[36]** they found the mean 8-OHdG level in the saliva of the CP group was significantly higher than healthy and chronic gingivitis groups ($p < 0.001$) also statistically significant correlation was observed between the salivary levels of 8-OHdG and probing depth (PD) and CAL with ($p < 0.001$) in CP group. Hence he suggest that elevated salivary levels of 8-OHdG may be a marker for disease activity and it may reflect indirectly disease severity parameters.

T. Konopka et al. (2007)[34] observed an increased mean concentration of 8-OHdG in the gingival blood of both untreated chronic periodontitis and aggressive periodontitis patient groups compared with the healthy control group.

Muthuraj MSA et al (2017)[102] observed in study GCF 8-OHdG level and clinical parameters were significantly reduced in CP and CP with diabetes patient after periodontal therapy this result accordance with our result.

When intergroup comparison was made there was a significant reduction in 8-OHdG level in Group II as compared to Group III with a P value <0.05. This suggested that it may be due to improved oral hygine and antioxidant and anti-inflammatory effect of ocimum sanctum gel directly on diseased site. **Teruo Inoue et al (2003)[103]** study showed the 8-OHdG level was significantly higher in smokers than non-smokers, which were significantly reduced by the smoking abstention for 4 week as well as smoking abstention with vitamin C supplementation (2g/day) group. **Fraga*et al*.(1991)[104]**reported that a high intake of vitamin C protected against 8-OHdG formation in human seminal DNA.

T Konapka (2007)[34] concluded in his study the oxidative burst in periodontitis may lead to significant local damage to nucleic acids and the significantly decreased total

antioxidant status level in the gingival blood of CP patients compared with the healthy subjects suggests the possibility of a significant decrease in local antioxidant system capacity during the course of periodontitis. The decreased total antioxidant status level in the peripheral blood in the group of all patients with periodontitis may be one of the pathogenic mechanisms underlying the links between periodontal disease and several systemic diseases for which periodontitis is regarded as a independent risk factor

Koji Suzuki, (2002)[105] study suggested that higher serum 8-OHdG levels in smoker male as compared to nonsmoker and lower serum levels of oxidized LDL antibodies and carotenoids, such as A-carotene, A-cryptoxanthin, and zeaxanthin and lutein, compared to non-smokers. May cause difference in nutrient intake and increase oxidative stress in smoker. The result contradict with **Jothi V (2017)[106]** who reported that the smokers patients with and without chronic periodontitis had higher levels of salivary 8 OHdG compared to other groups which were of statistical significance ($p<0.05$) and initial periodontal therapy did not exhibit significantalterations in the values.

8-OHdG were significantly reduced after periodontal therapy from baseline to 3 month in both test and control site but in test site the value was highly significant in smoker and nonsmoker chronic periodontitis groups. It might be due to the chemical composition of ocimum sanctum has not only antioxidant property but also antiallergic, anticeptic anti-inflammatory, antimicrobial properties. It also promote the wound healing. The results are in accordance with study done by **Chandra et al(2012)[1]** who reported that lycopene treated site showed significant improvement in 8-OHdG level compare to placebo group in smoker and nonsmoker with chronic periodontitis .

CONCLUSION

The present experimental study was carried out to estimate the levels of 8-OHdG in gingival crevicular fluid in smokers and non-smokers with chronic periodontitis and healthy subjects. And also to scrutinise the effects of 10% Ocimum sanctum gel with a SRPand the only SRP in periodontal pocket on these levels. A total 50 subjects were selected and assessed clinically and biochemically. They were divided into Three groups i.e. Group I(Healthy) Group II (Smokers with chronic periodontitis) and Group III (Nonsmokers with Chronic periodontitis). The clinical examination of the study population included the assessment of the plaque index (PI), gingival index (GI probing pocket depth (PPD), clinical attachment level (CAL),). GCF 8-OHdG levels were determined by human 8-OHdg ELISA kit **(kinesis Dx)** all the groups were treated with

non-surgical approach. Clinical parameters along with GCF samples were evaluated 1month and 3 month after the treatment. All the clinical parameter were same before the therapy which shows homogeneity at baseline except healthy group.

The results of this study showed that all the clinical parameters showed statistically significant improvement after the Phase I therapy in all the groups but non-smokers with chronic periodontitis responded better than smoker with chronic periodontitis. And Test site showed significant improvement than control site in both smoker and non-smoker with CP groups .

The levels of GCF 8-OHdG were found to be greater in smoker with chronic periodontitis as compared to that of non-smokers with chronic periodontitis and healthy subjects.

Levels of GCF 8-OHdG were decreased after Phase I therapy in both the groups (Group II& III) but the reduction was higher in non-smokers with chronic periodontitis as compared to smoker with chronic periodontitis. GCF 8-OHdG levels were positively correlated with the clinical parameters in the present study.

From the analysis of the results, following conclusions can be drawn

1. Gingival crevicular fluid 8- OHdG level are higher at baseline in smokers with chronic periodontitis as compared to nonsmokers with chronic periodontitis and healthy subjects.

2. After Phase I therapy there is significant reduction in GCF 8-OHdG level in both Smoker and non-smoker with chronic periodontitis groups but the reduction is higher in Test site than Control site.

3. The levels of GCF 8-OHdG are positively correlated with the clinical parameters (PPD, CAL, PI, GI) in all the groups.

4. All the clinical parameter reduced in Test site when compare with Control site in Smoker and Nonsmoker with chronic periodontitis groups.

Within the limits of our study, it can be concluded that, GCF 8- OHdG levels can be utilized as a good indicator of the inflammatory status of the periodontium. More destructive nature of periodontal disease in smokers leads to an increase in the concentrations of 8-OHdG level as compared to nonsmokers with chronic periodontitis. When all smoker and nonsmoker with chronic periodontitis patient were one quadrant treated by 10 % Ocimum Sanctum gel adjunct with SRP and other with only SRP. The mean GCF 8- OHdG concentrations reduced significantly in both site in smoker and nonsmoker with chronic periodontitis group but the reduction was higher in Test site as compared to Control site in both chronic periodontitis groups.However, further longitudinal studies are needed to evaluate gingival crevicular fluid levels of 8-OHdG in smokers with chronic periodontitis and also to evaluate the effect of different concentration of Ocimum Sanctum gel adjunct with non-surgical periodontal therapy on these levels & underlying mechanism. It will be beneficial in clarifying their role in the pathogenesis of periodontitis and to validate 8-OHdG as "Novel Biomarker" of periodontal disease.

This study had the following limitations:

1. As this study was comprise limited number of samples, multi-centered longitudinal studies with large sample size are required to validate these results.

2. Selection of the subjects was made on the basis of clinical indicators such as PPD & CAL, which do not necessarily reflect active periodontal destruction.

3. In the present study, female smokers were not evaluated because of their low prevalence in India

4. Smoking status was recorded based on self-reporting by the participants. It has been suggested that the estimation of serum or saliva or GCF cotinine assays is more reliable for the evaluation of smoking status.

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Table 1. Intra group Comparison of Plaque index (PI) and Gingival index (GI) among the study groups at different time interval.

	Plaque Index Mean± SD			**Gingival index Mean±SD**		
Groups	Base line	1month	3month	Base line	1month	3month
Group I (Healthy)	0.61 ±0.24	0.41 ±0.16	0.58 ±0.50	0.36 ±0.24	0.27 ±0.13	0.03 ±0.04
	P = 0.003*			P= 0.001*		
Group II (Smokers with chronic periodontitis)						
LDD Site	2.14 ±0.37	1.55 ±0.36	0.72 ±0.28	1.91 ±0.38	1.36 ±0.35	0.68 ±0.28
	P=0.000*			P=0.000*		
Control Site	2.17 ±0.40	1.62 ±0.36	0.82 ±0.23	1.91 ±0.30	1.46 ±0.25	0.88 ±0.37
	P=0.000*			P=0.000*		
Group III (Nonsmokers with chronic periodontitis**)**						
Test Site	2.05 ±0.29	1.32 ±0.24	0.56 ±0.21	2.28 ±0.44	1.11 ±0.45	0.45 ±0.17
	P=0.000*			P=0.000*		
Control Site	2.25 ±0.40	1.32 ±0.24	0.98 ±0.28	2.27 ±0.44	1.32 ±0.32	0.58 ±0.24
	P=0.000*			P=0.000*		

*Mean value Significant measured by Repeated measures ANOVA

Table 2. Intra group Comparison of PPD and CAL among the study groups at different time interval.

	PPD Mean± SD			**CALMean±SD**		
Group	Base line	1month	3month	Base line	1month	3month
Group I (Healthy)	2.10 ±0.99	1.20 ±0.91	0.40 ±0.51	2.10 ±0.99	1.90 ±0.73	0.20 ±0.78
		P= 0.027*			P=0.008*	
Group II (Smokers with chronic periodontitis)						
Test Site	5.50 ±1.00	4.10 ±0.72	2.75 ±0.64	5.50 ±1.00	4.20 ±0.77	3.15 ±0.81
	P=0.000*			P=0.000*		
Control Site	5.30 ±0.73	5.30 ±0.73	5.10 ±0.78	5.30 ±0.73	5.25 ±0.71	5.10 ±0.85
	P=0.042*			P=0.232		
Group III (Nonsmokers with chronic periodontitis**)**						
Test Site	5.30 ±0.57	4.00 ±0.56	2.50 ±0.68	5.30 ±0.57	4.15 ±0.50	2.85 ±0.93
	P=0.000*			P=0.000*		
Control Site	5.25±0.91	4.75 ±0.97	4.20 ±0.95	5.30 ±0.92	5.00 ±0.85	4.70 ±1.08
	P=0.000*			P=0.004*		

*Comparison of Mean value Significant measured by Repeated measures ANOVA

Table 3. Intra group Comparison of GCF8-OHdGlevels among the study groups at baseline and 3 month.

	GCF 8-OHdG levels (Mean± SD)	
Group	Base line	3month
Group I (Healthy)	4.44 ±0.48	4.27 ±0.33
	P= 0.222	
Group II (Smokers with chronic periodontitis)		
Test Site	10.40 ±1.94	6.06 ±1.67
	P=0.000*	
Control Site	10.25 ±1.59	8.29 ±1.38
	P=0.000*	
Group III (Nonsmokers with chronic periodontitis**)**		
Test Site	8.20 ±1.63	4.42 ±0.57
	P=0.000*	
Control Site	8.14 ±2.13	7.13 ±1.97
	P=0.000*	

*Statistical significance measured by paired T test

Table 4- Inter group comparisons of PI, GI, PPD, CAL and 8-OHdG levels between LDD+SRP and Plain SRPgroups of smoker and nonsmoker

	Group II (Test site vs control site)		**Group III** (Test site vs control site)	
Clinical Parameter	t	Sig.	t	Sig .
PI Baseline	-.207	.837	-1.795	.081
PI 1 Month	-.659	.514	.000	1.000
PI 3 Month	-1.350	.185	-5.414	**.000**
GI Baseline	.000	1.000	.000	1.000
GI1Month	.000	1.000	-1.695	.098
GI 3Month	.000	1.000	-2.080	**.044**
PPD Baseline	.827	.414	.000	1.000
PPD1 Month	-2.414	**.021**	-6.296	**.000**
PPD 3 Month	-5.659	**.000**	-11.113	**0.000**
CAL Baseline	.657	.515	0.000	1.000
CAL1 Month	-3.107	**.004**	-5.670	**.000**
CAL3 Month	-5.126	**.000**	-7.962	0.000
8-OHdG Baseline	.266	.791	0.100	0.921
8-OHdG 3 Month	-4.592	**.000**	-5.880	**0.000**

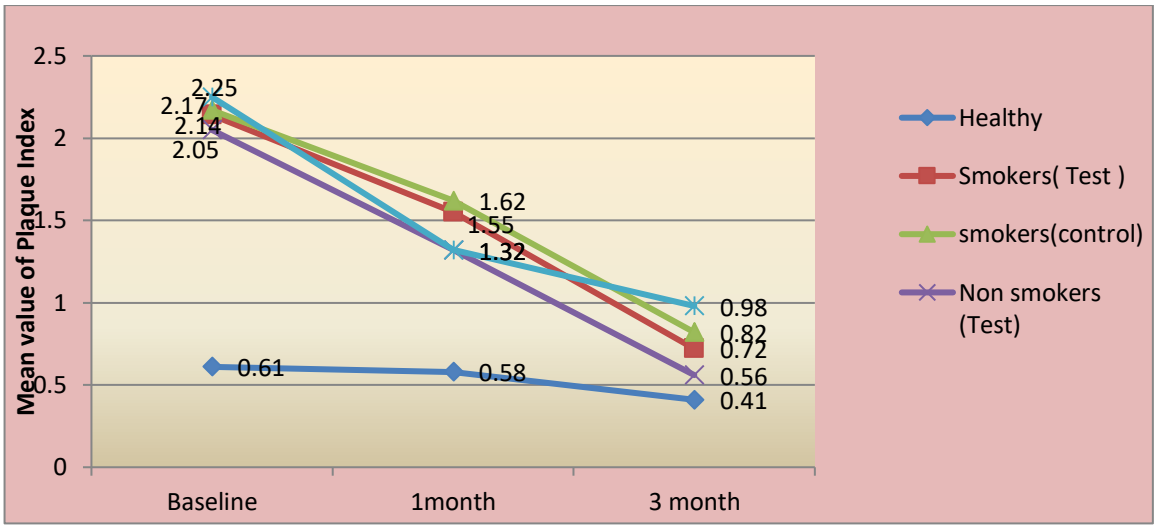
- Statistical significance measured by paired T test

Table 5- Inter group comparisons of PI, GI, PPD, CAL and 8-OHdG levels betweensmoker and nonsmoker with chronic periodontitis in Test site and Control Site.

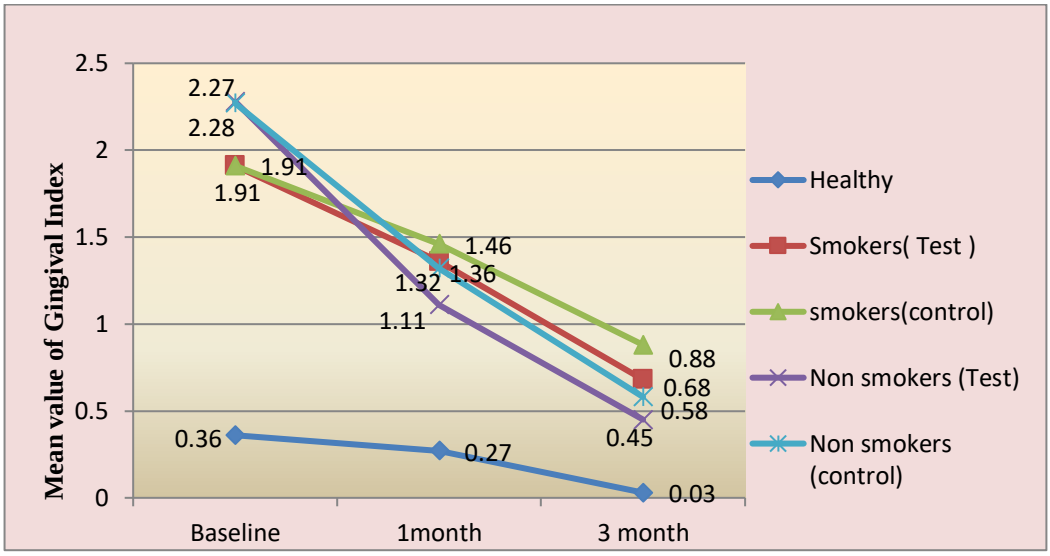
	Test site (Smoker Vs Non Smokers)		**Control site** (Smokers Vs non Smokers)	
Clinical Parameter	t	Sig.	t	Sig.
PI Baseline	0.908	0.369	-0.834	0.410
PI 1 Month	2.416	**0.021**	2.548	**0.015**
PI 3 Month	2.032	0.**049**	-1.744	0.090
GI Baseline	-2.832	0.**007**	-2.006	**0.052**
GI1Month	1.920	0.062	0.934	0.357
GI 3Month	3.222	0.**003**	1.494	0.144
PPD Baseline	0.777	0.442	-0.728	0.471
PPD 1 Month	-2.280	**0.028**	0.490	**0.627**
PPD 3 Month	-3.231	**0.003**	1.191	**0.241**
CAL Baseline	0.777	0.442	-0.522	0.605
CAL1 Month	0.246	0.807	-1.206	0.235
CAL3 Month	1.084	0.285	-1.520	0.137
8-OHdG Baseline	3.869	**0.000**	3.116	**0.004**
8-OHdG 3 Month	4.135	**0.000**	2.063	**0.046**

- Statistical significance measured by paired T test

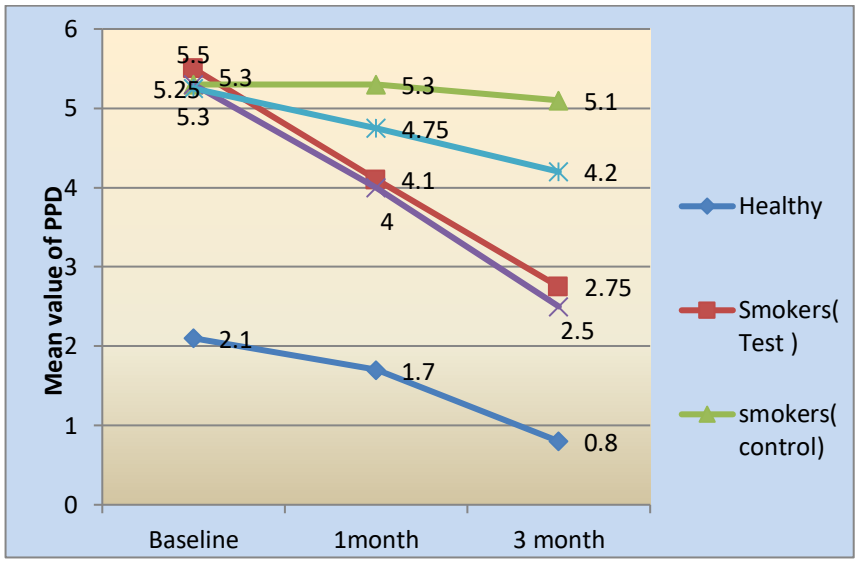
Graph 1: Intra group Comparison of plaque index among the study groups at different time interval.



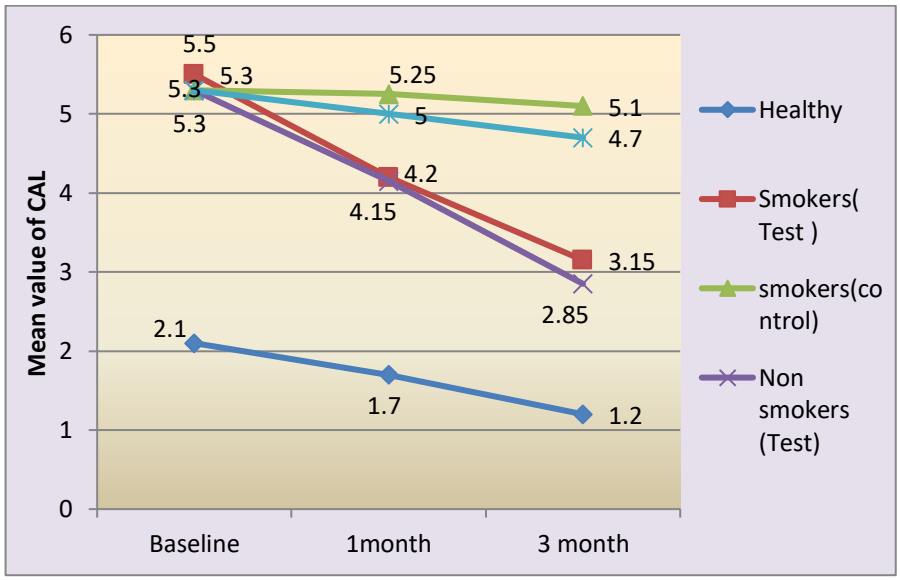
Graph 2: Intra group Comparison of Gingival index among the study groups at different time interval.



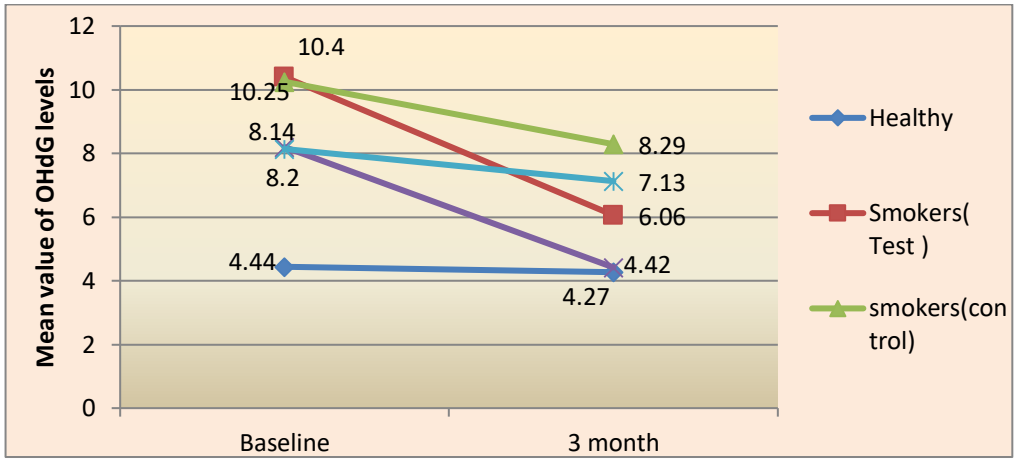
Graph 3: Intra group Comparison ofmean PPD among the study groups at different time interval.

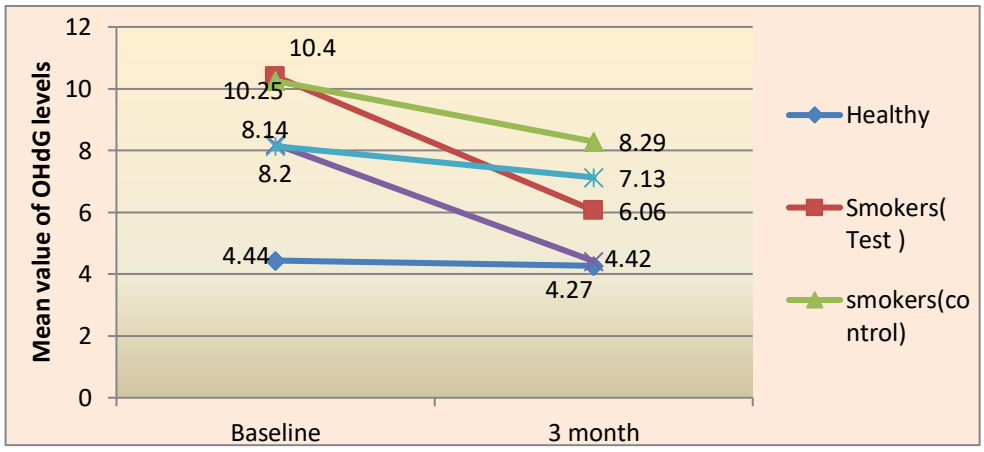


Graph 4: Intra group Comparison of mean CAL among the study groups at different time interval.



Graph 5: Intra group Comparison of GCF8-OHdG Levels among the study groups at baseline and 3 month**.**





GROUP I- HEALTHY GROUP

Sr. No	AGE	SEX	PI			GI			PPD (mm)			CAL (mm)		
			PRE	1 m	3 m	PRE	1 m	3 m	PRE	1m	3 m	PRE	1m	3m
1	22	Male	0.5	0.3	0.3	0.4	0.4	0	1	0	0	1	1	1
2	34	Male	0.4	0.2	0	0.3	0.3	0	1	1	1	1	1	1
3	30	Female	0.3	0.3	0.3	0.2	0.2	0	2	1	0	2	2	1
4	36	Female	0.4	0.2	0	0.1	0.1	0	1	0	0	1	1	0
5	26	Male	0.7	0.4	0.2	0.3	0.3	0.1	2	1	1	2	2	1
6	40	Male	0.7	0.5	0.3	0.4	0.4	0.1	3	2	0	3	3	3
7	21	Male	0.8	0.5	0.2	0.4	0.2	0	2	1	0	2	2	1
8	28	Female	0.9	0.6	0.3	0.3	0.1	0	4	3	1	4	3	2
9	30	Female	0.4	0.4	0	0.2	0.2	0	2	1	0	2	2	1
10	31	Male	1	0.7	0.2	1	0.5	0.1	3	2	1	3	1	1

GROUP I- HEALTHY GROUP

Sr. No	**GCF 8-OHdG LEVEL (ng/µl)**	
	PRE	**POST**
1	4.01	4.21
2	3.98	4.01
3	5.07	5.17
4	4.27	4.17
5	4.72	4.22
6	4.21	4.21
7	5.12	4.12
8	5.01	4.41
9	4.03	4.03
10	4	4.21

GROUP II- SMOKER WITH CHRONIC PERIODONTITIS (Test site)

Sr. No	site	AGE	SEX	PI			GI			PPD (mm)			CAL (mm)		
				PRE	1 m	3 m	PRE	1 m	3 m	PRE	1 m	3 m	PRE	1 m	3m
1	TEST	21	Male	1.9	1.4	0.9	1.4	0.9	0.3	5	5	4	5	5	4
2	TEST	30	Male	2.5	1.7	0.8	2.5	1.9	1.1	5	4	3	5	5	4
3	TEST	30	Male	1.8	1.2	0.5	1.8	1.2	0.7	6	4	3	6	4	3
4	TEST	34	Male	1.7	1.1	0.4	1.7	1.3	0.6	4	3	2	4	3	2
5	TEST	35	Male	2.5	1.5	0.9	2.2	1.8	0.4	6	4	2	6	5	3
6	TEST	33	Male	2.6	1.7	0.8	2.2	1.3	0.9	6	4	3	6	4	4
7	TEST	21	Male	1.7	1.2	0.4	1.7	1.4	0.5	5	4	2	5	4	2
8	TEST	22	Male	2.7	1.9	0.9	2.7	1.9	1.2	6	4	3	6	4	3
9	TEST	45	Male	2.4	1.8	1.1	2.4	1.7	0.7	4	3	2	4	3	2
10	TEST	48	Male	2.6	2.1	0.8	1.9	1.4	0.4	5	5	3	5	5	4
11	TEST	37	Male	2	1.6	0.4	1.4	0.9	0.4	6	5	3	6	5	3
12	TEST	45	Male	1.9	1.1	0.3	1.9	1.2	0.6	4	3	2	4	3	2
13	TEST	46	Male	1.8	1.2	0.6	1.8	1.4	0.5	7	4	3	7	4	3
14	TEST	38	Male	1.7	1.3	0.4	1.7	1.1	0.4	6	5	3	6	5	4
15	TEST	37	Male	2	1.6	0.4	2	1.6	0.8	4	3	2	4	3	2
16	TEST	28	Male	2.2	1.8	0.8	2.2	1.6	1.2	5	4	2	5	4	3
17	TEST	29	Male	2.5	2.2	1.3	2.1	1.7	0.9	7	5	3	7	5	4
18	TEST	27	Male	2.4	1.9	0.7	1.6	1.1	0.9	6	5	4	6	5	4
19	TEST	31	Male	1.6	0.9	0.3	1.6	0.9	0.7	6	4	3	6	4	3
20	TEST	30	Male	2.3	1.8	0.4	1.3	0.8	0.4	7	4	3	7	4	4

GROUP II- SMOKER WITH CHRONIC PERIODONTITIS (Control Site)

Sr. No	site	Age	Sex	PI			GI			PPD (mm)			CAL (mm)		
				PRE	1 m	3M	PRE	1 M	3M	PRE	1 M	3M	PRE	1M	3M
1	CONTROL	30	Male	1.7	1	0.4	1.9	1.4	0.6	5	4	3	5	4	4
2	CONTROL	34	Female	1.7	1.2	0.8	2.9	1.6	0.7	6	4	3	6	4	4
3	CONTROL	35	Female	1.6	1.4	0.7	1.9	1.2	0.4	5	5	2	5	4	3
4	CONTROL	45	Female	1.9	1.5	0.4	2.2	1.6	0.9	6	4	3	6	4	3
5	CONTROL	45	Male	1.9	1.2	0.4	2.1	1.4	0.3	5	4	2	5	4	2
6	CONTROL	46	Male	2.7	1.4	0.7	2.9	1.7	0.8	5	4	2	5	4	4
7	CONTROL	50	Male	2.2	1.3	0.8	1.8	1.2	0.7	5	4	2	5	4	2
8	CONTROL	49	Female	1.8	1.1	0.5	1.6	1.2	0.4	4	3	1	4	4	2
9	CONTROL	38	Female	2.5	1.7	1.1	2.8	1.8	0.7	5	4	3	5	4	3
10	CONTROL	38	Female	2.9	1.5	0.5	2.7	1.8	0.8	6	5	3	6	5	5
11	CONTROL	27	Female	2.4	2	0.8	2.5	0.9	0.3	6	5	4	6	5	4
12	CONTROL	27	Male	2.3	1.2	0.4	2.1	1.2	0.3	5	4	3	5	4	3
13	CONTROL	23	Male	2.1	1.2	0.4	2.9	1.7	0.4	5	4	2	5	4	2
14	CONTROL	23	Female	1.8	1.1	0.3	1.9	0.9	0.3	5	3	2	5	4	2
15	CONTROL	31	Male	2.7	1.2	0.5	2.9	1.3	0.4	6	4	2	6	4	3
16	CONTROL	23	Female	2.2	1.5	0.3	1.8	0.8	0.3	5	4	2	5	4	2
17	CONTROL	21	Male	2.3	1.1	0.5	1.9	o.9	0.6	5	3	2	5	4	3
18	CONTROL	21	Female	2.2	1.1	0.7	2.2	1	0.4	6	4	3	6	5	3
19	CONTROL	31	Female	2	1.4	0.5	2	1.3	0.7	6	4	3	6	4	3
20	CONTROL	30	Female	2.2	1.2	0.4	2.5	1.5	0.6	5	4	2	5	5	2

GROUP II- SMOKER WITH CHRONIC PERIODONTITIS GROUP

Sr. No	GROUP II Test site	GCF 8-OHdG level (ng/ml)		GROUP II Control site	GCF 8-OHdG level (ng/ml)	
		PRE	POST		PRE	POST
1	Test	11.34	5.24	Control	11.34	9.34
2	Test	10.67	4.78	Control	9.77	9.21
3	Test	9.42	4.09	Control	9.42	7.49
4	Test	11.23	6.21	Control	11.28	8.23
5	Test	12.11	4.03	Control	10.11	7.98
6	Test	13.12	5.21	Control	11.12	9.79
7	Test	10.23	5.12	Control	11.23	8.62
8	Test	10.64	4.31	Control	10.64	7.72
9	Test	9.23	4.37	Control	10.23	8.72
10	Test	9.24	4.67	Control	8.72	8.34
11	Test	10.11	4.92	Control	10.11	9.21
12	Test	7.68	4.02	Control	7.68	5.67
13	Test	9.42	4.72	Control	9.42	6.34
14	Test	11.21	5.39	Control	11.21	10.21
15	Test	6.07	4.29	Control	7.77	6.96
16	Test	7.42	4.79	Control	7.46	6.12
17	Test	14.21	6.91	Control	14.21	11.03
18	Test	11.27	4.28	Control	11.27	8.84
19	Test	11.12	5.03	Control	10.25	7.21
20	Test	12.28	7.89	Control	11.78	8.89

GROUP III- NONSMOKER WITH CHRONIC PERIODONTITIS GROUP

Sr. No	GROUP III Test site	GCF 8-OHdG level (ng/ml)		GROUP III Control site	GCF 8-OHdG level (ng/ml)	
		PRE	POST		PRE	POST
1	Test	9.01	4.07	Control	9.02	8.07
2	Test	6.37	4.02	Control	6.32	5.27
3	Test	11.03	5.23	Control	11.03	9.23
4	Test	7.37	5.01	Control	7.37	5.01
5	Test	7.92	4.01	Control	12.92	11.41
6	Test	6.42	4	Control	6.42	5.21
7	Test	9.04	5.02	Control	9.04	9.02
8	Test	9.27	6.01	Control	9.27	9.08
9	Test	8.47	4.01	Control	8.47	7.01
10	Test	6.37	4.27	Control	6.37	6.27
11	Test	4.37	4.01	Control	6.37	6.01
12	Test	8.37	4.04	Control	5.02	5.00
13	Test	9.41	4.71	Control	6.41	5.91
14	Test	8.52	4.11	Control	9.52	8.11
15	Test	7.52	4.32	Control	10.52	9.82
16	Test	10.37	4.01	Control	6.37	6.01
17	Test	6.23	4.27	Control	6.23	6.27
18	Test	9.03	4.03	Control	6.03	4.03
19	Test	8.92	4.19	Control	9.92	6.89
20	Test	10.02	5.21	Control	10.21	9.12

Evaluation and comparison of the efficacy of locally delivered ocimum sanctum gel and 8-Hydroxydeoxyguanosine levels in gingival crevicular fluid in patients with chronic periodontitis before and after phase I therapy.

CASE HISTORY PROFORMA

NAME: **OPD NO:**

AGE/SEX: **DATE:**

ADDRESS: **PHONE NO:**

OCCUPATION:

CHIEF COMPLAINT:

PAST DENTAL HISTORY:

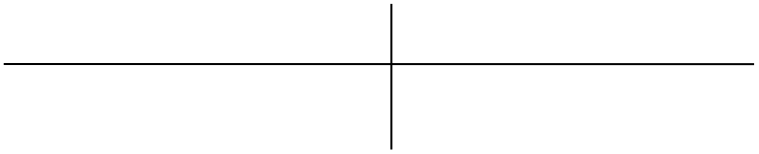
PAST MEDICAL HISTORY:

ORAL HYGIENE HABIT:

HISTORY OF CIGARETTE SMOKING: YES/NO

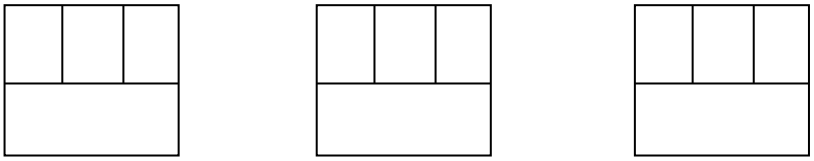
DURATION OF SMOKING:

TEETH PRESENT:

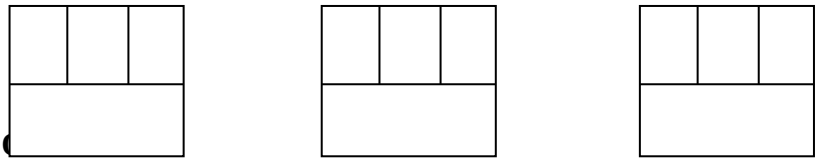


Baseline investigation -

GINGIVAL INDEX *(Loe&Sillness 1963*)

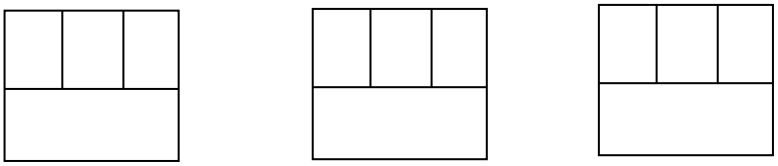


16 12 24



44 32 36

PLAQUE INDEX *(Sillness and Loe 1964)*Baseline



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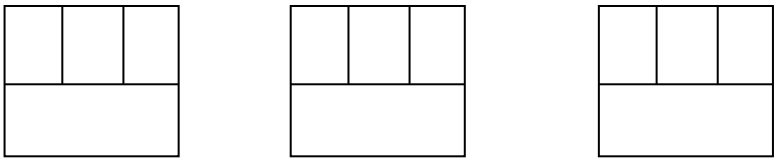
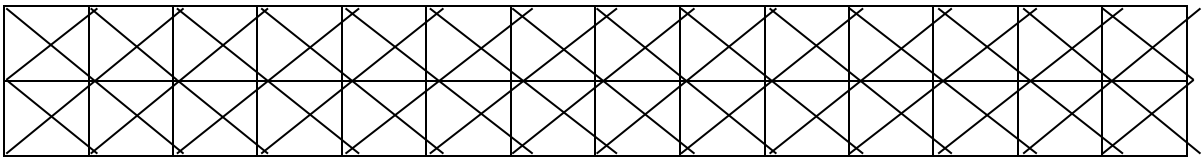


44 32 36

Score: $\frac{\text{Total scores of all teeth}}{\text{Total number of teeth examined}}$ **SCORE:**

PROBING POCKET DEPTH(mm**):**Baseline

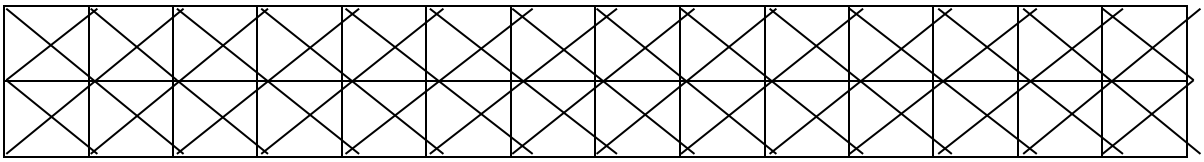
17 16 15 14 13 12 11 21 22 23 24 25 26 27



47 46 45 44 43 42 41 3132 33 34 35 36 37

CLINICAL ATTACHMENT LEVELS(mm):Baseline

17 16 15 14 13 12 11 21 22 23 24 25 26 27



47 46 45 44 43 42 41 31 32 33 34 35 36 37

Biochemical analysis

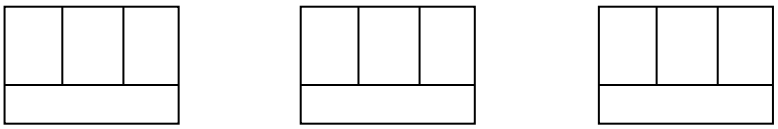
1. 8-**Hydroxydeoxyguanosine levels** =

Baseline					
Parameters	**Group I**	**Group II (Test side)**	**Group III (Control Site)**	**Group II (Test side)**	**Group III (Control Site)**
8-OHdG level					

1 Month

GINGIVAL INDEX (After 1 months)

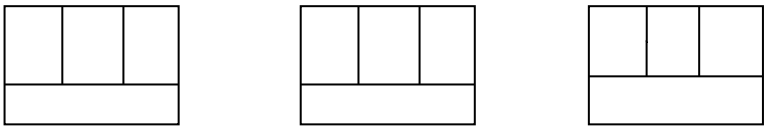
16 12 24



16 12 24

Score: = $\frac{\text{Total scores of all teeth}}{\text{Total number of teeth examined}}$ **SCORE:**

PLAQUE INDEX(After 1 months)



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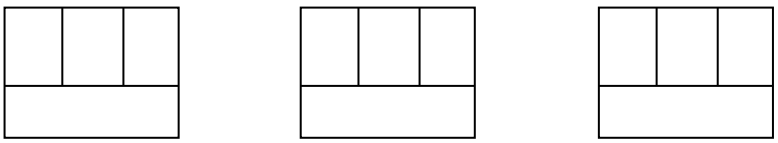
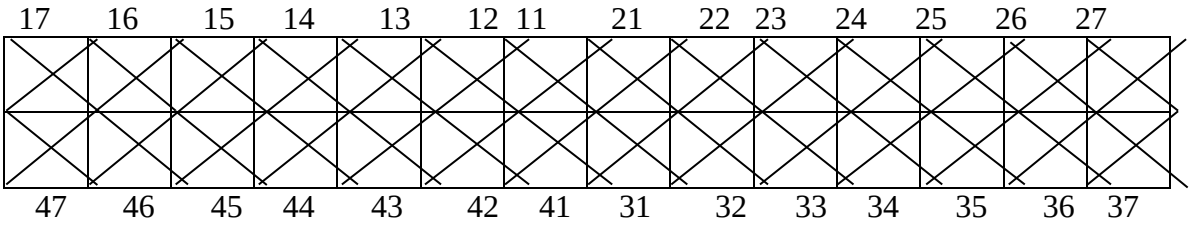


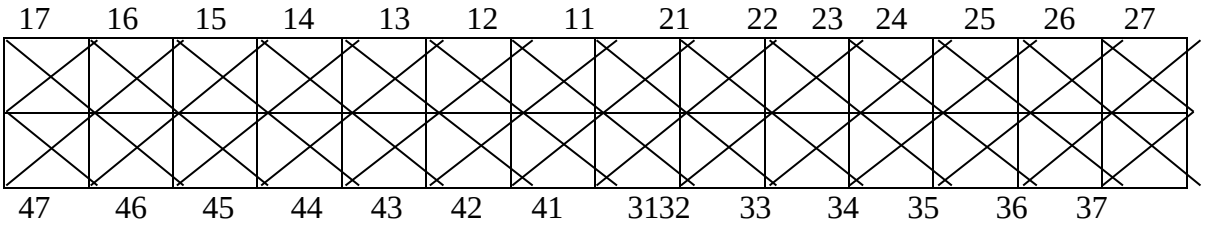
44 32 36

Score: $\frac{\text{Total scores of all teeth}}{\text{Total number of teeth examined}}$ **SCORE:**

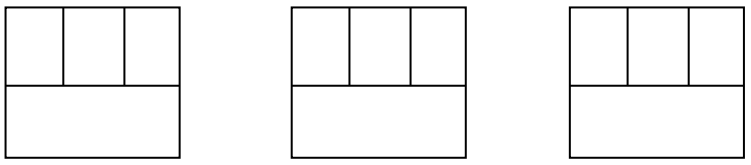
PROBING POCKET DEPTH (mm): After 1 months



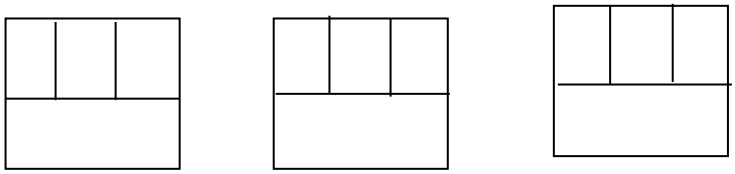
CLINICAL ATTACHMENT LEVELS (mm): After 1 months



GINGIVALINDEX(After 3 months)



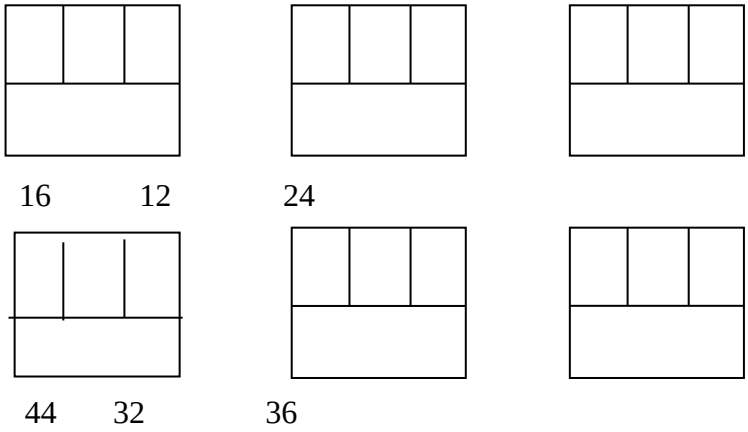
16 12 24



44 32 36

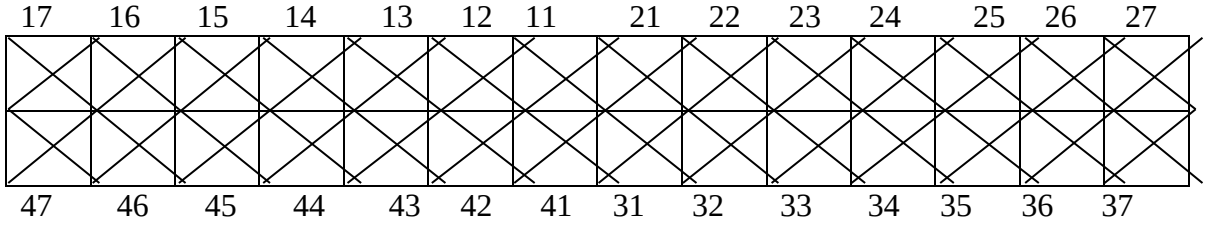
Score: $\frac{\text{Total scores of all teeth}}{\text{Total number of teeth examined}}$ **SCORE:**

PLAQUE INDEX(After 3 months)

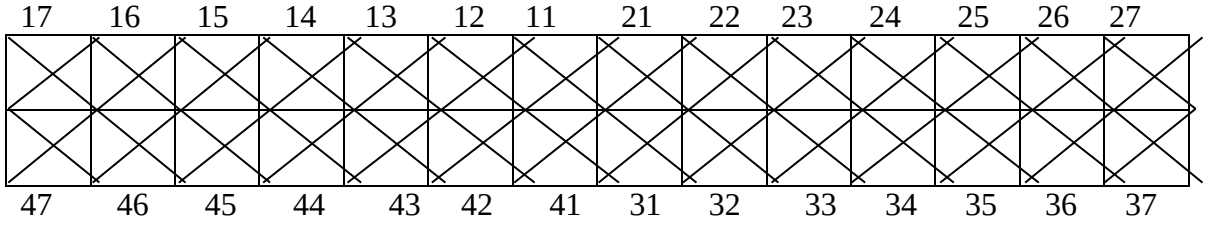


Score: $\frac{\text{Total scores of all teeth}}{\text{Total number of teeth examined}}$ **SCORE:**

PROBING POCKET DEPTH (mm): After 3 months



CLINICAL ATTACHMENT LEVELS (mm): After 3 months



3 Month					
Parameters	**Group I**	**Group II (Test side)**	**Group III (Control Site)**	**Group II (Test side)**	**Group III (Control Site)**
8-OHdG level					

(Confidential)
Informed Consent Form

"Evaluation and comparison of the efficacy of locally delivered ocimum sanctum gel and 8-Hydroxydeoxyguanosine levels in gingival crevicular fluid in patients with chronic periodontitis before and after phase I therapy"

"

Mr./Master/Mrs./Miss._____
Resident of: _____
_____ _____aged _____ years,
Exercising my free will/choice, without any pressure/lure of incentive in any form, hereby give my consent for the project to be conducted.

I acknowledge the receipt of "patient's information sheet", and also that the doctor has informed me about this research project suitably and sufficiently to my satisfaction. I agree to take part in this project and will not mix any other projects during the period of this trial. I permit to publishing the results of my participation in this study. I shall not be given any reimbursement or compensation. I hereby record my consent for participation in the said questionnaire.

_____	_____	_____	_____
Patient's name	Signature/thumbprint	Date	Time

	_____	_____	_____
Principal Investigator	Signature	Date	Time