Research Square

Preprints are preliminary reports that have not undergone peer review.
They should not be considered conclusive, used to inform clinical practice,
or referenced by the media as validated information.

Evaluation of Inflammatory Biomarkers in Chronic Periodontitis with and without Diabetes Mellitus (Type II) and to assess the effect of *Momordica Charantia* (Bitter Gourd) Oil as an Antioxidant Adjuvant in Periodontal Treatment

Hemalatha Ramakrishnan
Karpaga Vinayaga Institute of Dental Sciences
Vidyashree Nandini Venugopal
SRM Dental College and Hospital, SRM Institute of Science and Technology
Mathan Mohan Ayyadurai
Karpaga Vinayaga Institute of Dental Sciences
Asim K. Duttaroy
a.k.duttaroy@medisin.uio.no

University of Oslo

Research Article

Keywords: Chronic periodontitis, Diabetes mellitus, Momordica charantia, α-ESA, MMP9, ICTP

Posted Date: September 3rd, 2025

DOI: https://doi.org/10.21203/rs.3.rs-7371643/v1

License: This work is licensed under a Creative Commons Attribution 4.0 International License.
Read Full License

Additional Declarations: No competing interests reported.

Abstract

Background: Type II diabetes mellitus (T2DM) can have an impact on chronic periodontitis, a prevalent inflammatory disease. The interplay between oxidative stress, inflammatory biomarkers, and periodontal tissue destruction is well-documented. Antioxidants derived from plants are becoming popular as periodontal therapy adjuncts.

Objective: This study was aimed to measure the levels of important inflammatory biomarkers in patients with and without T2DM who had chronic periodontitis. Additionally, the study sought to determine whether *Momordica charantia* (bitter gourd) oil, recognized for its antioxidant qualities, might be used as an adjuvant in periodontal therapy.

Methods: 120 subjects were divided into four groups. Groups II and IV B (only Scaling and Root Planing (SRP)), and Group IV A (SRP + adjunct topical bitter gourd oil application) were the test groups. Groups I and III were the control groups. Clinical parameters were recorded at baseline and after 15 days. Gingival crevicular fluid (GCF) samples were analyzed for antioxidant levels, and inflammatory markers. Molecular docking and network-based analysis (STRING and STITCH) was also carried out.

Results: Post-treatment, Group IV A showed a notable reduction in biomarker levels (MMP9 and ICTP) and improved clinical outcomes compared to other groups, suggesting an enhanced therapeutic response with the use of *M. charantia* oil which was further validated by molecular docking and network-based analysis.

Conclusion: Elevated systemic and local indicators indicate that T2DM exacerbates periodontal inflammation. By reducing inflammatory reactions and improving clinical recovery, *Momordica charantia* oil shows encouraging supplemental advantages in periodontal therapy, especially in diabetes patients.

1 Introduction

Diabetes mellitus is a metabolic disorder that involves chronic hyperglycemia, which includes either reduced insulin secretion, action or both resulting in hyperglycemia or excretion of glucose in urine (1). One of the major healthcare concerns worldwide is the rising burden of Type II diabetes mellitus (T2DM). The rate of crude death due to diabetes in India has seen a steep surge from 1990 to 2016 by 131% (2). Chronic inflammation is one of the main characteristic of periodontal inflammation and T2DM. There exists a bidirectional relationship between diabetes and periodontal disease (3). Gram-negative anaerobic and facultative gram-positive oral bacteria are the important causative microorganisms of periodontitis that are manifested by inflammation of gingiva, alveolar bone destruction, Clinical Attachment Loss (CAL) and eventually tooth loss (4). The immune cells secrete inflammatory mediators like Matrix Metalloproteinases (MMPs), prostaglandins and cytokines, which are involved in disease progression (5). C telopeptide is a member of a family of pyridinoline crosslinks, a breakdown product of Type I collagen secreted in the Gingival Crevicular Fluid (GCF). About 90% of the organic matrix of the alveolar bone is made up of Type I collagen. Type I collagen degradation product (ICTP) levels are

directly proportional to collagen breakdown in the progression of periodontal disease. The components of GCF will be a valuable diagnostic aid in evaluating periodontal treatment outcomes and the severity of periodontal tissue destruction (6).

MMPs, also called matrixins, are a group of calcium-dependent endopeptidases containing zinc ions. MMPs play a pivotal role in the degradation of the Extracellular Matrix (ECM) components, namely collagen, elastin, laminin, fibronectin, and proteoglycans. They have a key role in pathological and physiological processes. Tissue inhibitors of metalloproteinases (TIMPs) are specific endogenous protease inhibitors to the MMPs. In health, there exists a balance between MMP and TIMP. An imbalance between MMP and TIMP leads to unwanted and excessive tissue breakdown. One of the important mediators in the progression of inflammation in periodontal disease is MMP 9, among the various isoforms of MMPs (7).

Momordica charantia, (bitter gourd) is a tropical vine that belongs to the gourd family and is one of the versatile plants, popular as a folklore antidiabetic medicine (8). *Momordica charantia* Polysaccharide treatment in diabetic rats have shown significant improvement in the levels of Superoxide Dismutase (SOD), catalase levels, Glutathione Peroxidase (GPx) (9). Bitter melon is a nutrient rich plant that is composed of a variety of beneficial compounds (10). The bioactive compounds present in the bitter gourd, namely the phenols, terpenoids, anthraquinones, and flavonoids have antioxidant property that contribute to the medicinal value of the plant (11).

Bitter gourd seed oil is shown to be a good source of α-eleostearic acid (α-ESA), a type of conjugated linolenic acid (CLnA), which usually makes up approximately 30–60% of the total fatty acids in the oil. α-ESA is a long-chain polyunsaturated fatty acid, with a chemical structure of C18:3 (9c,11t,13t), and its conjugated triene system is unique. The structure gives rise to a number of biological properties of α-ESA, which have raised interest because of their potential health benefits. α-ESA has been associated with antioxidant, anti-inflammatory, anti-atherosclerotic, and anti-tumor activities when considering a variety of disease models, suggesting therapeutic potential. While several natural products contain α-ESA, tung oil derived from *Vernicia fordii* also contains α-ESA at an even higher percentage (as high as 82%). Regardless, bitter gourd seed oil may be an important and more available dietary source of the bioactive fatty acid, especially in areas where tung oil is not well accepted or consumed as food. α-ESA has exhibited multiple beneficial actions in both *in vitro* and *in vivo* models. These beneficial properties include strong antioxidant and anti-inflammatory properties derived from its ability to scavenge reactive oxygen species and alter inflammatory pathways. There is also evidence suggesting α-ESA has both anti-atherosclerotic and anti-tumor action, which could mean improvement of cardiovascular health and inhibition of the growth of certain cancer cells. α-ESA is of particular interest for inflammatory and metabolic syndromes, and its associated health benefits. It could potentially limit inflammation in conditions, such as periodontitis, while providing protective effects in type 2 diabetes by activating PPARα, enhancing glucose and lipid metabolism, reducing oxidative stress, and preserving pancreatic β-cell function.

2 Materials and Methods

2.1 Study Design

This is an interventional study to evaluate the inflammatory biomarkers in chronic periodontitis with and without T2DM and to assess the effect of topical bitter gourd oil as an antioxidant adjuvant in periodontal treatment. Selection of subjects were done from the Department of Periodontics, Out Patient Department, Karpaga Vinayaka Institute of Dental Sciences. After obtaining approval from the Institutional Ethics Committee (IEC), the study was commenced (IEC NO: KIDS/IEC/015/2021/IV). A detailed explanation of the study protocol and information on the potential benefits were provided to all the participants. Following screening, before the subjects were enrolled in the study, informed consent was obtained to confirm their willingness in participation.

Our study was designed based on prior clinical evidence demonstrating the antioxidant and therapeutic effects of bitter gourd oil in patients with diabetes. The primary objective of our work was to evaluate the effect of bitter gourd oil supplementation (already established as beneficial in diabetes) on chronic periodontitis in diabetic patients. Therefore, the study was not conceptualized or conducted as a clinical trial in the conventional sense. Rather, it was an exploratory investigation aimed at assessing the impact of a known intervention (bitter gourd oil) on an additional parameter (chronic periodontitis) within an already validated clinical context.

2.2 Determination of periodontal status and eligibility criteria

Patients were selected based on the following criteria: individuals indicated for crown lengthening procedure; dentition with minimum of 20 teeth with exclusion of third molars; subjects with gingival inflammation, Bleeding on Probing (BOP), Probing Pocket Depth (PPD) ≥ 5 mm and CAL ≥ 3 mm, in the test groups; subjects with no signs of gingival inflammation, no BOP, PPD ≤ 3 mm, with no evidence of CAL, in the control groups, and known diabetic patients, with fasting blood glucose levels above 126mg/dl, well controlled diabetes with glycated Hemoglobin (HbA1C) ≤ 8 known Type II diabetic patients, under treatment for more than 2 years, for groups III and IV individuals. Exclusion criteria included systemic conditions other than Type II Diabetes Mellitus, pregnant and lactating women, smoking, active infection, if present, other than Chronic Periodontitis, and patients who received periodontal debridement /antibiotic/ anti-inflammatory/any other drug intake in the recent 6 months.

2.3 Intervention, periodontal treatment, randomization, and allocation concealment

The study includes 120 subjects in age range of 30–60 years. The study population was divided into the following groups:

a) Group I: Healthy Periodontium (30 subjects)

- Non-diabetic individuals with a healthy periodontium

b) Group II: Chronic Periodontitis (CP) without Diabetes Mellitus (30 subjects)

- Non-diabetic individuals with CP patients

c) Group III: Diabetes mellitus without Chronic Periodontitis (30 subjects)

- Known Type II Diabetes individuals with healthy periodontium

d) Group IV: Diabetic mellitus with Chronic Periodontitis (30 subjects)

- Known Type II Diabetes individuals who were further sub-classified into two sub-groups, Group IV A and Group IV B, each containing 15 subjects

Group II, Group IV A, and Group IV B are the test groups. Group I and Group III are the control groups. Treatment for test groups were given as:

- Groups II: only Scaling and Root Planing (SRP)
- Group IV A: SRP + adjunct topical bitter gourd oil application
- Group IV B: only SRP

2.4 Clinical Parameters and Collection of Samples

All study subjects were assessed for baseline clinical parameters, namely BOP, Plaque Index (PI), Gingival Index (GI), PPD, and Clinical Attachment Level. This was done by a single examiner to rule out bias. Following this, GCF was collected at baseline from subjects of all the groups, namely I, II, III, IVA, and IVB. The collected GCF was subjected to ELISA to evaluate and compare the levels of inflammatory markers namely MMP9, TIMP1, ICTP. At baseline, the GCF of group IVA and IVB was additionally analyzed for the levels of antioxidant enzyme namely the SOD. At baseline, in the groups IVA and IVB, in addition to the GCF samples, plaque samples were also collected and subjected to microbial analysis to find the Colony Forming Units (CFU). Following this, SRP was done for the subjects of the test groups, namely II, IVA, and IVB. Only Group IVA subjects were advised to use topical bitter gourd oil as an adjunct to SRP. Crude bitter gourd oil extracted from the seeds was dispensed to the subjects. They were advised to take a drop of bitter gourd oil in the index finger and apply it on the inflamed gingiva in a circular motion for about 2 to 3 minutes and to abstain from taking water or food for the next one hour after application. The topical application was advised to be done for about 3 to 4 times a day for about 7 days. The patients were asked to report after 15 days for maintenance therapy. A thorough oral prophylaxis was done during this visit. The subjects of the test groups were re-evaluated postoperatively after one month.

2.4.1 Collection of GCF Samples

To avoid salivary contamination, the site of sample collection was isolated with cotton rolls. Sterile paper points were placed in the gingival sulcus/periodontal pocket, for about 30 seconds. Eppendorf tubes containing Phosphate Buffered Saline (PBS) were used to store GCF until sent for biochemical analysis.

2.4.2 Collection of Plaque Samples

Samples were collected after drying and isolation at the collection site. Sterile cotton rolls were used to gently remove supragingival plaque. Plaque samples from deep periodontal pockets were collected with sterile curettes. The collected plaque sample was then transferred to a sterile cover containing thioglycolate broth until sent for microbial analysis.

2.4.3 ELISA for MMP9, TIMP1, and ICTP

The collected GCF samples were subjected to the Sandwich ELISA technique for inflammatory markers, MMP9, TIMP1, and ICTP. ELISA is performed in 96-well plates containing microtiter wells, having capture antibody-coated surface. To the pre-coated ELISA microwells, the antigen-containing sample is added and incubated. This is followed by the addition of primary and secondary antibodies and incubation. Using a wash buffer, the wells are washed to remove the unbound antigens. A coloured product is formed following the addition of an enzyme to the substrate, which confirms the presence of an antigen.

2.4.4 Microbial Culture

Following the collection of plaque samples from the deep periodontal pockets, they are then transferred to a sterile test tube containing reduced thioglycolate broth and transported for microbial analysis within 2 hours. To identify the bacterial isolates, and to grow bacterial colonies, the plaque samples are streaked onto nutrient agar plates using a sterile inoculating loop.

The blood agar plates contain 1.5% agar-which gives the mixture solidity, 0.3% beef extract/yeast extract-which confers the required nutrients, 0.5% peptone-which provides organic nitrogen. The inoculated blood agar plates were then subjected to incubation under aerobic conditions at 37˚C for 24 hours.

At the end of incubation, the bacterial colonies from the glass jar are taken aseptically using a sterilised inoculating loop, the culture is dragged across the plates and inoculated into new agar plate and incubated in aerobic environment at 37˚C for about 24 hours. Isolated colonies were picked and subjected to biochemical analysis after incubation.

Biochemical tests revealed the presence of cocci belonging to *Streptococcus viridans* group namely *Streptococcus pyogenes*, *Streptococcus mitis*. The colony forming units are evaluated for all the samples pre and postoperatively.

2.5 Statistical Analysis

Data was collected and tabulated in Microsoft Excel and later transferred into SPSS version 24 for analysis. The normality of the observation was tested using Shapiro-Wilk's test. The observed data were

not normally distributed. Mean and standard deviation was used to evaluate continuous data. Frequency and percentages were used to express categorical data such as gender were analysed using the chi-square test. Post hoc Tukey's test was done for comparison of more than two different groups after doing Kruskal-Wallis Test. Comparison of pre-treatment and post-treatment values among the groups was done by Wilcoxon's signed rank test. The association between HbA1c and MMP9 was done by Pearson correlation. $p \leq 0.05$ was considered statistically significant.

2.6 *In Silico* analysis

A molecular docking study was performed to investigate the molecular interactions of the major bioactive compound of the *Momordica charantia* seed oil with periodontal inflammatory biomarkers. The main active compound identified in the literature was α-ESA (a conjugated linolenic acid), which may display antioxidant and anti-inflammatory effects (12).

2.6.1 Ligand Preparation

The 3D structure of α-ESA (PubChem CID: 5281115) was downloaded from the PubChem database in 3D SDF format. It was processed into PDBQT file format using Open Babel that is integrated into the PyRx software for docking.

2.6.2 Target Protein Retrieval and Preparation

Two proteins of interest, Matrix Metalloproteinase-9 (MMP9) and Tissue Inhibitor of Metalloproteinases-1 (TIMP1), were selected based on their relevance to periodontal inflammation and extracellular matrix degradation. Their 3D structures were obtained from the Protein Data Bank (PDB): MMP9: PDB ID 1L6J, TIMP1: PDB ID 2MA2. Using BIOVIA Discovery Studio, the protein structures were prepared by removing water molecules, heteroatoms, ligands, and impurities. Hydrogen atoms were added to polar residues to stabilize the structure.

2.6.3 Active Site Identification

Active site residues of MMP9 and TIMP1 were identified using CASTp 3.0 (Computed Atlas of Surface Topography of proteins), which predicts the binding pockets based on surface topology (13).

2.6.4 Molecular Docking

The ligand and proteins were docked using AutoDock Vina in PyRx 0.8 as previously discussed. The grid box was created to capture the predicted active site residues from CASTp. The docking parameters were kept as default. The docking scores, or binding affinity, were noted in kcal/mol (14).

2.6.5 Visualization

The docked conformations were visualized using BIOVIA Discovery Studio Visualizer to analyse molecular interactions. Both 2D interaction diagrams and 3D binding poses were generated for further interpretation.

2.6.6 Protein-Protein Interaction (PPI) Network Construction Using STRING

A protein-protein interaction (PPI) network was constructed to visualize the functional relationships of the key proteins regulated by α-ESA. We used the STRING database (https://string-db.org/) to construct the PPI network, as previously described. The target proteins, MMP9 and TIMP1, were entered as query proteins and used Homo sapiens as the organism of interest. The interaction network was built using a combined score that integrated known and predicted associations based on associations extracted from experiments, text mining, co-expression, and curated databases. The interaction network was analyzed to assess direct and indirect associations among the proteins, which better contextualized the biological relevance of the proteins in response to α-ESA treatment.

2.6.7 Chemical-Protein Interaction Prediction Using STITCH

To further explore the potential interactions of α-ESA with human proteins and compare with experimental data, we used the STITCH database (http://stitch.embl.de/). In STITCH, α-ESA was investigated with its chemical name and chemical structure with Homo sapiens selected as the reference organism. The analysis did not find the direct interactions between α-ESA and MMP9 and TIMP1. A network-based strategy was utilized by returning the predicted or known protein target of α-ESA. Then considered those target proteins and how they directly connected with the proteins, MMP9 and TIMP1 in the same shared network environment, which could infer indirect regulatory pathways based on the STITCH results.

3 Results

3.1 Demographic Results

Table 1 shows that distribution of patients based on age the results shows that the mean age of group 1 was 44.17 ± 4.44, the mean age of group II was 43.90 ± 4.88, the mean age of group III was 43.50 ± 5.15, the mean age of group IV was 42.97 ± 4.42. The mean age was similar in all groups there was no statistical differences between the groups (P = 0.84) (Fig. 1(a)).

Table 1
Distribution of Study Participants based on Age.

GROUPS	AGE	F	p
GROUP I	44.17 ± 4.44	0.820	0.84
GROUP II	43.90 ± 4.88		
GROUP III	43.50 ± 5.15		
GROUP IV	42.97 ± 4.42		
*F Kruskal Wallis Test (P ≤ 0.05 is statistically significant)			

Table 2 shows that the distribution of study patients based on gender in which the results show that; among group I there were (53.3%) males and (46.7%) females, among group II there were (56.7%) males and (43.3%) females, among group III there were (53.3%) males and (46.7%) females and among group IV there were (63.3%) males and (36.7%) females, number of males were comparatively higher than the females in all the groups which was not statistically significant (P = 0.84) (Fig. 1(b)).

Table 2
Distribution of Study Participants Based on Gender.

GROUPS	GENDER		X^2	p
	MALE	FEMALE		
GROUP I	16 (53.3%)	14 (46.7%)	0.814	0.84
GROUP II	17 (56.7%)	13 (43.3%)		
GROUP III	16 (53.3%)	14 (46.7%)		
GROUP IV	19 (63.3%)	11 (36.7%)		
*X^2 CHI SQUARE Test (P ≤ 0.05 is statistically significant)				

Table 3 shows the baseline data of blood sugar levels among the groups which shows that the mean fasting blood sugar levels among the groups were 87.13 ± 1.22, 89.13 ± 1.10, 138.03 ± 1.22, 142.6 ± 1.15 and 142.7 ± 1.14 respectively. The mean HbA1C levels among the groups were 4.97 ± 0.05, 5.00 ± 0.10, 6.94 ± 0.06, 7.15 ± 0.09 and 7.15 ± 0.10 respectively. The mean FBS and HbA1C was lesser in the Group I (healthy controls) and Group II (chronic periodontitis without diabetics) when compared to the other groups which was statistically significant (p < 0.0001, p < 0.0001) (Fig. 1 (c)).

Table 3
Association of Blood Sugar Levels among Groups.

GROUPS	BLOOD INVESTIGATION	
	FBS (mg/dL)	HbA1C (%)
GROUP I	87.13 ± 1.22	4.97 ± 0.05
GROUP II	89.13 ± 1.10	5.00 ± 0.10
GROUP III	138.03 ± 1.22	6.94 ± 0.06
GROUP IV-A	142.6 ± 1.15	7.15 ± 0.09
GROUP IV-B	142.7 ± 1.14	7.15 ± 0.10
F	105.8	101.3
p	**< 0.0001**	**< 0.0001**

3.2 Clinical and Biochemical Findings

Table 4 shows evaluation of inflammatory markers before and after treatment in chronic periodontitis with and without diabetes mellitus. The pre-treatment value MMP 9 in Group II was 839.8 ± 6.85, group IV-A was 958.7 ± 27.48 and Group IV-B was 939.8 ± 6.97; MMP9 was highest in Group IV-A followed by Group IV-B and Group II. The post-treatment value MMP9 in Group II was 582.1 ± 36.37, group IV-A was 452.8 ± 13.74and Group IV-B was 605.8 ± 8.89; MMP9 was lowest in Group IV-A followed by Group II and Group IV-B. The values of MMP9 gradually reduced in post treatment when compared to the pre-treatment in all the test groups showing statistically significant difference (P = 0.002, 0.00, 0.003 respectively).

Table 4
Evaluation of Inflammatory markers before and after treatment in Chronic Periodontitis with and without Diabetes Mellitus.

INFLAMMATORY MEDIATORS		GROUP II	GROUP IV-A	GROUP IV-B
MMP 9 (ng/mL)	PRE	839.8 ± 6.85	958.7 ± 27.48	939.8 ± 6.97
	POST	582.1 ± 36.37	452.8 ± 13.74	605.8 ± 8.89
t		35.56	41.59	16.18
p		**0.002***	**0.00***	**0.003***
TIMP 1 (ng/mL)	PRE	110.2 ± 8.70	88.85 ± 2.32	89.4 ± 2.23
	POST	366.8 ± 9.57	413.2 ± 1.67	392.3 ± 5.72
t		25.69	-71.79	18.98
p		**0.00***	**0.00***	0.06*
ICTP (µg /mL)	PRE	3.73 ± 0.15	4.24 ± 0.11	4.25 ± 0.09
	POST	2.47 ± 0.13	2.62 ± 0.10	3.21 ± 0.06
t		55.69	-17.79	42.98
p		**0.00***	**0.00***	0.06*
*wilcoxon's signed rank test (P ≤ 0.05 is statistically significant)				

The pre-treatment value TIMP 1 in Group II was 110.2 ± 8.70, group IV-A was 88.85 ± 2.32 and Group IV-B was 89.4 ± 2.23; TIMP 1was highest in Group II followed by Group IV-B and Group IV-A. The post-treatment value TIMP 1 in Group II was 366.8 ± 9.57, group IV-A was 413.2 ± 1.67 and Group IV-B was 392.3 ± 5.72; TIMP 1was highest in Group IV-A followed by Group IV-B and Group II (postoperatively) The values of TIMP 1 gradually increased in post treatment when compared to the pre-treatment in all the test groups showing statistically significant difference among Group II and Group IV-A and not significant difference in Group IV-B (P = 0.00, 0.00, 0.06 respectively).

The pre-treatment value ICTP in Group II was 3.73 ± 0.15, group IV-A was 4.24 ± 0.11 and Group IV-B was 4.25 ± 0.09; ICTP was highest in both the Groups; Group IV-A and Group IV-B than Group II. The post-treatment value ICTP in Group II was 2.47 ± 0.13, group IV-A was 2.62 ± 0.10 and Group IV-B was 3.21 ± 0.06; ICTP was lowest in Group IV-A followed by Group IV-B than Group II (postoperatively). The values of ICTP gradually reduced in post treatment when compared to the pre-treatment in all the test groups showing statistically significant difference among Group II and Group IV-A and not significant difference in Group IV-B (P = 0.00, 0.00, 0.06 respectively) (Fig. 2(a)).

Table 5 shows the evaluation of antioxidant enzyme SOD before and after bitter gourd application; the results shows that SOD in the pre-treatment among Group IV-A was 43.34 ± 1.19 and SOD in the post-

treatment among Group IV-A was 53.47 ± 1.18. Antioxidant enzyme SOD was increased in the post treatment, after bitter gourd application which was statistically significant ($p < 0.0001$). SOD in the pre-treatment among Group IV-B was 43.34 ± 1.22 and SOD in the post-treatment among Group IV-B was 48.55 ± 1.09. Antioxidant enzyme SOD was increased in the post treatment, after scaling and root planning which was statistically significant ($p < 0.0001$). While comparing the post treatment between Group IV-A and Group IV-B, the values were higher in Group IV-A when compared to Group IV-B (Fig. 2(b)).

Table 5
Evaluation of SOD within and among the groups.

Parameters	GROUP IVA		Statistical Inference	GROUP IVB		Statistical Inference
	Pre	Post		Pre	Post	
SOD (Pg/mL)	43.34 ± 1.19	53.47 ± 1.18	< 0.0001*	43.34 ± 1.22	48.55 ± 1.09	< 0.0001*
*wilcoxon's signed rank test (P ≤ 0.05 is statistically significant)						

Table 6 shows the evaluation of Colony Forming Units (CFU) before and after Bitter gourd treatment; the results show that CFU in the pre-treatment among Group IV-A was 57.80 ± 3.38 and CFU in the post-treatment among Group IV-A was 10.80 ± 4.95. Colony Forming Units (CFU) was reduced in the post treatment, after Bitter gourd application which was statistically significant ($p < 0.0001$). CFU in the pre-treatment among Group IV-B was 58.67 ± 3.22 and CFU in the post-treatment among Group IV-B was 30.27 ± 1.28. Colony Forming Units (CFU) was reduced in the post treatment, after scaling and root planning which was statistically significant ($p < 0.0001$). While comparing the post treatment between Group IV-A and Group IV-B, the values were markedly lower in Group IV-A when compared to Group IV-B (Fig. 2(c)).

Table 6
Evaluation of CFU within and among the groups.

Parameters	GROUP IVA		Statistical Inference	GROUP IVB		Statistical Inference
	Pre	Post		Pre	Post	
CFU	57.80 ± 3.38	10.80 ± 4.95	< 0.0001*	58.67 ± 3.22	30.27 ± 1.28	< 0.0001*
*wilcoxon's signed rank test (P ≤ 0.05 is statistically significant)						

Figure 2(d) shows the comparison of GI among the test groups which shows that the GI in the pre-treatment in Group II was 1.75 ± 0.14, Group IV-A was 1.77 ± 0.11, and Group IV-B was 1.76 ± 0.14. GI in the post-treatment in Group II was 1.29 ± 0.07, Group IV-A was 1.00 ± 0.10, and Group IV-B was 1.18 ± 0.06. While comparing the GI before and after the treatment, the GI gradually reduced in the post

treatment which showed a statistically significant difference within the Group II, IV-A and IV-B ($p < 0.0001$).

Figure 2(e) shows the comparison of BOP among the test groups which shows that the BOP in the pre-treatment in Group II was 70.20 ± 1.42, Group IV-A was 73.93 ± 3.97and Group IV-B was 75.87 ± 3.02. BOP in the post-treatment in Group II was 46.00 ± 1.55, Group IV-A was 37.53 ± 1.60 and Group IV-B was 43.60 ± 1.50. While comparing the BOP before and after the treatment, the BOP gradually reduced in the post treatment which showed a statistically significant difference among the Group II, IV-A and IV-B ($p < 0.0001$).

Figure 2(f) shows the comparison of PI among the test groups which shows that the PI in the pre-treatment in Group II was 2.18 ± 0.10, Group IV-A was 2.34 ± 0.05, and Group IV-B was 2.30 ± 0.05. PI in the post-treatment in Group II was 1.71 ± 0.08, Group IV-A was 1.31 ± 0.07 and Group IV-B was 1.52 ± 0.06. While comparing the PI before and after the treatment, the PI gradually reduced in the post treatment among Group IV-A and Group IV-B which showed a statistically significant difference ($p < 0.0001$).

Figure 2(g) shows the comparison of periodontal probing depth among the test groups which shows that the periodontal probing depth in the pre-treatment in Group II was 5.83 ± 0.70, Group IV-A was 5.80 ± 0.68 and Group IV-B was 5.80 ± 0.68. Periodontal probing depth in the post-treatment in Group II was 3.90 ± 0.31, Group IV-A was 3.53 ± 0.52 and Group IV-B was 3.93 ± 0.26. While comparing the periodontal probing depth before and after the treatment, the periodontal probing depth gradually reduced in the post treatment which showed a statistically significant difference among the Group II, IV-A and IV-B ($p < 0.0001$).

Figure 2(h) shows the comparison of clinical attachment loss among the test groups which shows that the clinical attachment loss in the pre-treatment in Group II was 3.27 ± 0.45, Group IV-A was 3.27 ± 0.46, and Group IV-B was 3.27 ± 0.46. Clinical attachment loss in the post-treatment in Group II was 1.87 ± 0.35, Group IV-A was 1.40 ± 0.51 and Group IV-B was 1.73 ± 0.46. While comparing the clinical attachment loss before and after the treatment, the clinical attachment loss gradually reduced in the post treatment which showed a statistically significant difference among the Group II, IV-A and IV-B ($p < 0.0001$).

Table 8 shows the correlation of HbA1C and inflammatory mediators of MMP9. The result shows that there was no correlation between the MMP9 and HbA1C in Group III ($r = -0.109$, $P = 0.564$), Group-IVA ($r = -0.102$, $P = 0.715$) and Group-IVB ($r = -0.094$, $P = 0.737$). There is a negative correlation between MMP9 Group III and Group IV A with HbA1C. There is no correlation MMP9 Group-IVB with HbA1C. There is no statistically significant correlation between inflammatory mediators of MMP9 and HbA1C ($P = 0.564$, 0.715, 0.737 respectively).

Table 7
Evaluation of Clinical parameters before and after treatment in Chronic Periodontitis with and without Diabetes Mellitus.

CLINICAL PARAMETERS		GROUP II	GROUP IV-A	GROUP IV-B
GINGIVAL INDEX	PRE	1.75 ± 0.14	1.77 ± 0.11	1.76 ± 0.14
	POST	1.29 ± 0.07	1.00 ± 0.10	1.18 ± 0.06
p		< 0.0001*	< 0.0001*	< 0.0001*
BOP (%)	PRE	70.20 ± 1.42	73.93 ± 3.97	75.87 ± 3.02
	POST	46.00 ± 1.55	37.53 ± 1.60	43.60 ± 1.50
p		< 0.0001*	< 0.0001*	< 0.0001*
PLAQUE INDEX	PRE	2.18 ± 0.10	2.34 ± 0.05	2.30 ± 0.05
	POST	1.71 ± 0.08	1.31 ± 0.07	1.52 ± 0.06
p		< 0.0001*	0.0001*	< 0.0001*
PPD (mm)	PRE	5.83 ± 0.70	5.80 ± 0.68	5.80 ± 0.68
	POST	3.90 ± 0.31	3.53 ± 0.52	3.93 ± 0.26
p		< 0.0001*	< 0.0001*	< 0.0001*
CAL (mm)	PRE	3.27 ± 0.45	3.27 ± 0.46	3.27 ± 0.46
	POST	1.87 ± 0.35	1.40 ± 0.51	1.73 ± 0.46
p		< 0.0001*	< 0.0001*	< 0.0001*
*wilcoxon's signed rank test (P ≤ 0.05 is statistically significant)				

Table 8
Correlation of HbA1c with MMP 9 value.

GROUPS	HbA1c and MMP9 Correlation	p Value
GROUP III	r = -0.109	p = 0.564
GROUP IVA	r = -0.102	p = 0.715
GROUP IVB	r= -0.094	p = 0.737
* r- Pearson correlation (P ≤ 0.05 is statistically significant)		

3.3 Bioinformatics results

3.3.1 Molecular Docking

Table 9 shows significant interaction of α-ESA with both MMP9 and TIMP1 in docking study, showing negative binding affinities, which indicates stable binding. Table 10 reveals the specific amino acid residues of MMP9 and TIMP1 involved in interactions with α-ESA based on molecular docking analysis. Figure 3 shows the molecular docking interaction of α-ESA with inflammatory proteins.

Table 9
Binding affinity of α-eleostearic acid with MMP9 and TIMP1 proteins in Kcal/mol.

S.No	Ligand	Protein	PDB ID	Binding affinity (Kcal/mol)
1	α-eleostearic acid	MMP9	1L6J	-6.5
2		TIMP1	2MA2	-6.1

Table 10
Amino acid residues of MMP9 and TIMP1 involved in interactions with α-eleostearic acid based on molecular docking analysis.

S.No	Ligand	Protein	Interacting Amino Acid Residues
1	α-eleostearic acid	MMP9	Glutamic acid, Histidine, Proline, Alanine, Glycine, Leucine, Asparagine, Tyrosine
2		TIMP1	Lysine, Glutamic acid, Serine, Alanine, Tyrosine, Cysteine, Threonine, Leucine, Glycine, Proline

3.3.2 Protein-Protein Interaction Network

The STRING-based PPI network (Fig. 4(a)) showed a direct interaction between MMP9 and multiple functionally associated proteins, including TIMP1, CTSV, RECK, MMP20, MMP10, and TIMP4. Similarly, TIMP1 also interacted with the same group of proteins, further suggesting a dense interconnectivity among extracellular matrix regulators. MMP9 and TIMP1 also shared edges with each of the other five proteins, suggesting a coordinated regulation or a potential co-functionality in the response to α-ESA.

3.3.3 Chemical-Protein Interaction and Indirect Network Connectivity

The STITCH analysis (Fig. 4(b)) indicated that α-ESA directly binds to a number of the proteins identified, including PDAT, AT1G32200, AT4G24160, FAD2, LACS2, OLEO4, NIA1, NIA2, CBR, and AT3G16780, and that it had no direct binding with MMP9 or TIMP1. This suggests that α-ESA was not directly regulating MMP9 or TIMP1, but rather could be influencing both indirectly, through other proteins or components of their shared network. Further confirming this direction, the adjacent protein interaction network (Fig. 4(c)), showed that UBC was a centre protein that connects TIMP1, MMP9, MMP3, and CBR1 together. UBC interacts with ACSL1 as well, and is also part of a very large network of proteins that all clustered together surrounding DNA damage response proteins ATM, BRCA1, BRCA2, FANCI, FANCD2,

FANCL, FANCE, FANCF and FANCC. In conclusion, these data suggest that α-ESA may be influencing MMP9 and TIMP1 expression or levels indirectly through context-driven regulations by UBC and its associated regulatory network.

4 Discussion

In the current study, a significant reduction in the mean concentration of the inflammatory markers, namely MMP 9, ICTP with elevation of TIMP1 in all the test groups namely groups II, IVA and IVB after Scaling and Root Planning (SRP) with p value ≤ 0.05 was observed when compared to the baseline values. Mechanical periodontal therapy reduces bacterial load, thereby reducing the inflammatory reaction provoked by the periodontal pathogens. The results of the current study is concurrent with the findings of the previous studies, where a marked reduction of the inflammatory markers was evident in the diabetic groups namely IVA and IVB after mechanical periodontal treatment (Table 4, Fig. 2(a)).Concomitantly, a significant improvement in the clinical parameters was also observed postoperatively in all the treatment groups when compared to the pre-treatment values. Postoperatively, a marked reduction in the GI, PPD, PI, BOP, CAL was observed in all the treatment groups (Table 7, Figs. 2(d-h)).Postoperatively the mean concentration of MMP 9 (Table 4, Fig. 2(a)), ICTP (Table 4, Fig. 2(a)) in the group that received adjunct topical bitter gourd application namely group IVA was lowest when compared to the other test groups namely groups II and IVB with p value ≤ 0.05. Similarly, mean concentration of TIMP1 was highest in group IVA when compared to other test groups namely groups II and IVB with p-value ≤ 0.05 (Table 4, Fig. 2(a)).

Though both the groups namely IVA and IV B showed a statistically significant increase in SOD levels postoperatively, on comparison of the mean concentration of SOD, the values were higher in group IVA than IVB. (Table 5, Fig. 2(b))Both the groups namely IV A and IV B showed a statistically significant reduction in Colony Forming Unit (CFU). While comparing the post treatment mean values between group IV-A and group IV-B, the values were markedly lower in group IV-A when compared to group IV-B (Table 6, Fig. 2(c)).

In the current study, at baseline, the inflammatory markers were higher in the test groups compared to the control groups. SRP caused a marked reduction in the inflammatory markers in the test groups. The test group which received an adjunct bitter gourd oil application in addition to SRP, showed an increased antioxidant enzyme level namely SOD with a significant reduction in the bacterial colony count when compared to the group which did not receive the topical bitter gourd application. A marked reduction in the bacterial colony count in this group, i.e., diabetic individuals with chronic periodontitis could be due to the antibacterial properties in bitter gourd.

Though periodontitis is a risk factor which could influence the glycemic status of a diabetic individual, the current study shows there is no correlation between MMP 9 and HbA1C, when these parameters were compared in diabetic individuals without chronic periodontitis, that is group III and diabetic individuals without chronic periodontitis namely group IVA and IVB (Table 8). In diabetes, other

important factors like food habits, life style, co-morbidities like cardiac, renal disorders and the following pathologic events could have a major influence on the glycemic status than periodontitis.

The in silico docking experiments showed that α-ESA from *Momordica charantia* seed oil exhibited a moderate to strong level of binding affinity with MMP9 and TIMP1, both of which are prominently involved in the inflammatory destruction and regulation of the extracellular matrix for periodontal tissues (15). The binding affinity of -6.5 kcal/mol demonstrated potential inhibition of proteolytic activity from MMP9, which should decrease collagen degradation and periodontal destruction. Additionally, the binding with TIMP1 at -6.1 kcal/mol suggests possible modulation of the natural inhibition of MMPs. The current findings further substantiate the clinical observations of the study when adjunct bitter gourd oil lead to a significant reduction of inflammatory markers (MMP9, ICTP), a reduction in microbial colonies, and improved antioxidant capacity. In conclusion, computational docking has provided a molecular basis for the anti-inflammatory and antioxidant effects observed with the bitter gourd oil.

In addition to molecular docking, the network-based analyses performed by STRING and STITCH elucidated the mechanistic framework for the biological effects of α-ESA. The PPI network derived from STRING demonstrated that MMP9 and TIMP1 functionally connected with matrices regulatory proteins (e.g., MMP10, MMP20, RECK, CTSV, and TIMP4), supporting their role as central proteins for remodelling extracellular matrix. The STITCH analysis (Fig. 4(b)) indicated that α-ESA interacted directly with several proteins, including PDAT, FAD2, and CBR, but did not show direct interaction with MMP9 or TIMP1. That said, the extended STRING network, suggested an indirect interaction through UBC, a protein that connects α-ESA associated proteins (e.g., CBR1) to MMP9 and TIMP1 through shared nodes. Overall, analysis support that α-ESA may modulate extracellular matrix homeostasis and inflammation, coupling direct interactions via molecular binding as shown in docking studies, but also indirectly through larger networks interactions that affect expression or activity of the main regulators in the inflammatory pathway.

5 Conclusion

This study suggests that individuals with chronic periodontitis have higher levels of inflammatory biomarkers, and having T2DM aggravates this inflammatory response, suggesting a detrimental synergistic effect on periodontal health. When used as an antioxidant in conjunction with periodontal therapy, *Momordica charantia* (bitter gourd) oil showed a promising modulatory effect on inflammation. In particular, its administration was linked to a quantifiable decrease in important inflammatory biomarkers as TIMP1 and ICTP. According to these results, bitter gourd oil may help reduce oxidative stress and inflammatory burden in periodontal tissues, especially in diabetic individuals who have weakened systems. To confirm the therapeutic effectiveness and to optimise the dosage and distribution of *Momordica charantia* oil in clinical periodontal therapy, further extensive and long-term research is needed. Even though the available data is promising, well-planned randomised controlled clinical trials with bigger sample sizes are necessary to confirm its effectiveness, optimise dosing schedules, and prove long-term safety. New avenues for individualised, supplementary periodontal

therapy may be opened by including phytotherapeutics like *Momordica charantia* oil into periodontal care, especially in high-risk groups with systemic disorders like diabetes mellitus.

Declarations

Ethics statement

The studies involving humans were approved by the Institutional Ethics Committee of Karpaga Vinayaga Institute of Dental Sciences (IEC NO: KIDS/IEC/015/2021/IV). The studies were conducted in accordance with the local legislation and institutional requirements. The participants provided their written informed consent to participate in this study.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Generative AI statement

The author(s) declare that no Generative AI was used in the creation of this manuscript.

Funding

Not Applicable

Author Contribution

HR: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Resources, Software, Validation, Visualization, Writing-original draft, Writing-review & editing. VNV: Writing-review & editing. MMA: Writing-review & editing. AKDR: Bioinformatics, Writing-review & editing

Acknowledgement

The authors express gratitude to Karpaga Vinayaga Institute of Dental Sciences for their support and resources in conducting this study.

References

1. Piero MN, Nzaro GM, Njagi JM (2015) Diabetes mellitus-a devastating metabolic disorder. Asian J Biomed Pharma Sci 5(40):1. 10.15272/ajbps.v4i40.645
2. Vos T, Abajobir AA, Abate KH, Abbafati C, Abbas KM, Abd-Allah F et al (2017) Global, regional, and national incidence, prevalence, and years lived with disability for 328 diseases and injuries for 195 countries, 1990–2016: a systematic analysis for the Global Burden of Disease Study 2016. Lancet 390(10100):1211–1259. 10.1016/S0140-6736(17)32647-8
3. Llambés F, Arias-Herrera S, Caffesse R (2015) Relationship between diabetes and periodontal infection. World J Diabetes 6(7):927–935. 10.4239/wjd.v6.i7.927
4. Page RC (1991) The role of inflammatory mediators in the pathogenesis of periodontal disease. J Periodontal Res 26(3):230–242. 10.1111/j.1600-0765.1991.tb01649.x
5. Checchi V, Maravic T, Bellini P, Generali L, Consolo U, Breschi L et al (2020) The role of matrix metalloproteinases in periodontal disease. Int J Environ Res Public Health 17(14):4923. 10.3390/ijerph17144923
6. Taba M, Kinney J, Kim AS, Giannobile WV (2005) Diagnostic biomarkers for oral and periodontal diseases. Dent Clin North Am 49(3):551–571. 10.1016/j.cden.2005.03.009
7. Sapna G, Gokul S, Bagri-Manjrekar K (2014) Matrix metalloproteinases and periodontal diseases. Oral Dis 20(6):538–550. 10.1111/odi.12159
8. Poolperm S, Jiraungkoorskul W (2017) An update review on the anthelmintic activity of bitter gourd, *Momordica charantia*. Pharmacogn Rev 11(21):31–34. 10.4103/phrev.phrev_52_16
9. Raish M, Ahmad A, Jan BL, Alkharfy KM, Ansari MA, Mohsin K et al (2016) *Momordica charantia* polysaccharides mitigate the progression of STZ induced diabetic nephropathy in rats. Int J Biol Macromol 91:394–399. 10.1016/j.ijbiomac.2016.05.090
10. Tan SP, Kha TC, Parks SE, Roach PD (2016) Bitter melon (*Momordica charantia* L.) bioactive composition and health benefits: A review. Food Reviews Int 32(2):181–202. 10.1080/87559129.2015.1057843
11. Aeri V, Raj R (2020) Medicinal Properties of Bitter Gourd: Bioactives and Their Actions. In: Kole, C., Matsumura, H., Behera, T. (eds) *The Bitter Gourd Genome. Compendium of Plant Genomes. Springer, Cham*. :33–44. 10.1007/978-3-030-15062-4_3
12. Yoshime LT, de Melo ILP, Sattler JAG, de Carvalho EBT, Mancini-Filho J (2016) Bitter gourd (*Momordica charantia* L.) seed oil as a naturally rich source of bioactive compounds for nutraceutical purposes. Nutrire 41(12). 10.1186/s41110-016-0013-y
13. Tian W, Chen C, Lei X, Zhao J, Liang J (2018) CASTp 3.0: computed atlas of surface topography of proteins. Nucleic Acids Res 46(W1):W363–W367. 10.1093/nar/gky473
14. Dallakyan S, Olson AJ (2015) Small-Molecule Library Screening by Docking with PyRx. Methods in Molecular Biology. *Methods Mol Biol*. 1263:243 – 50. 10.1007/978-1-4939-2269-7_19
15. Luchian I, Goriuc A, Sandu D, Covasa M (2022) The Role of Matrix Metalloproteinases (MMP-8, MMP-9, MMP-13) in Periodontal and Peri-Implant Pathological Processes. Int J Mol Sci 23(3):1806. 10.3390/ijms23031806

Figures

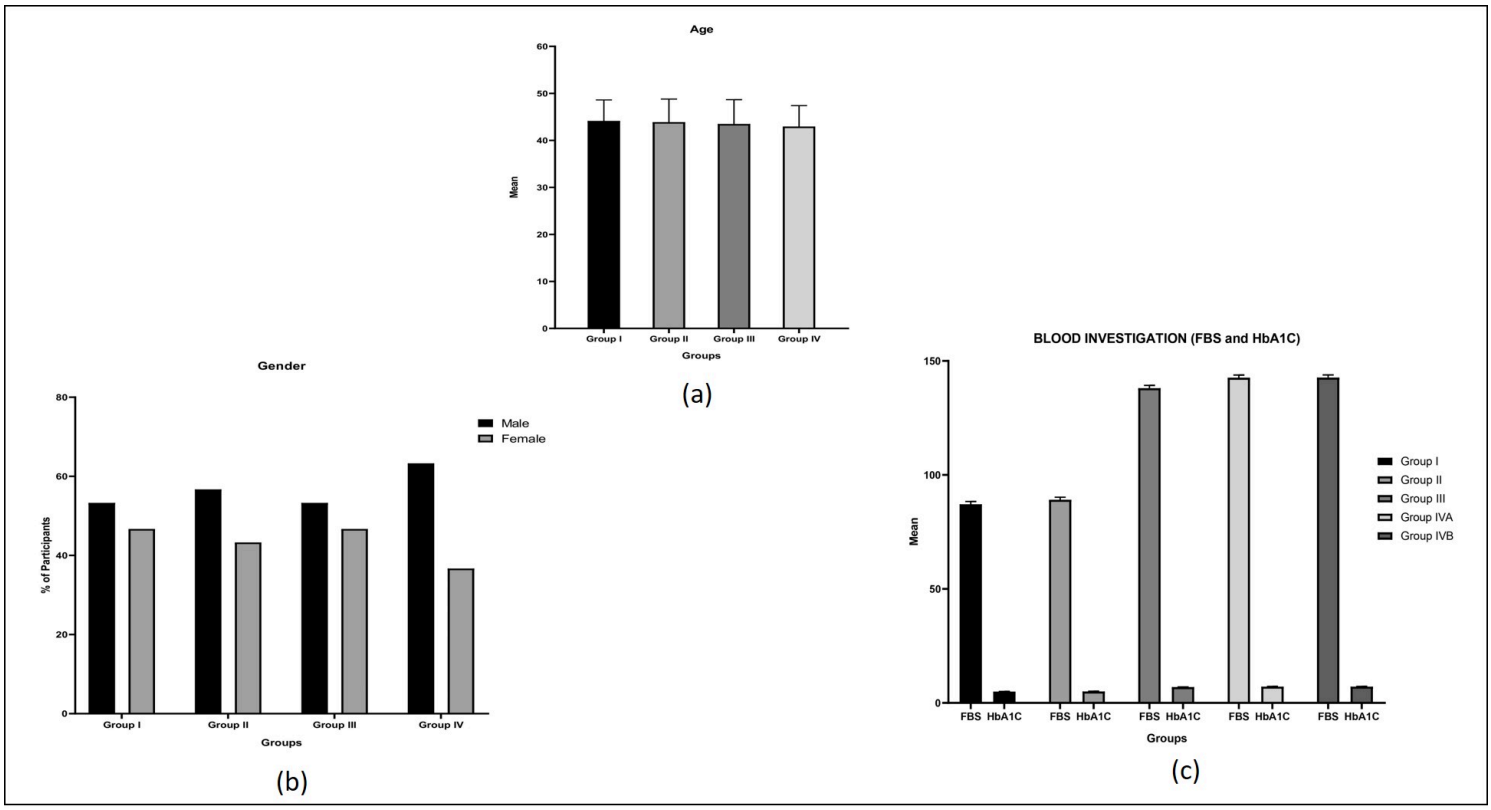


Figure 1

Demographic Results. (a) Distribution of Study Participants Based on Age, (b) Distribution of Study Participants Based on Gender, (c) Association of Blood Sugar Levels among Groups.

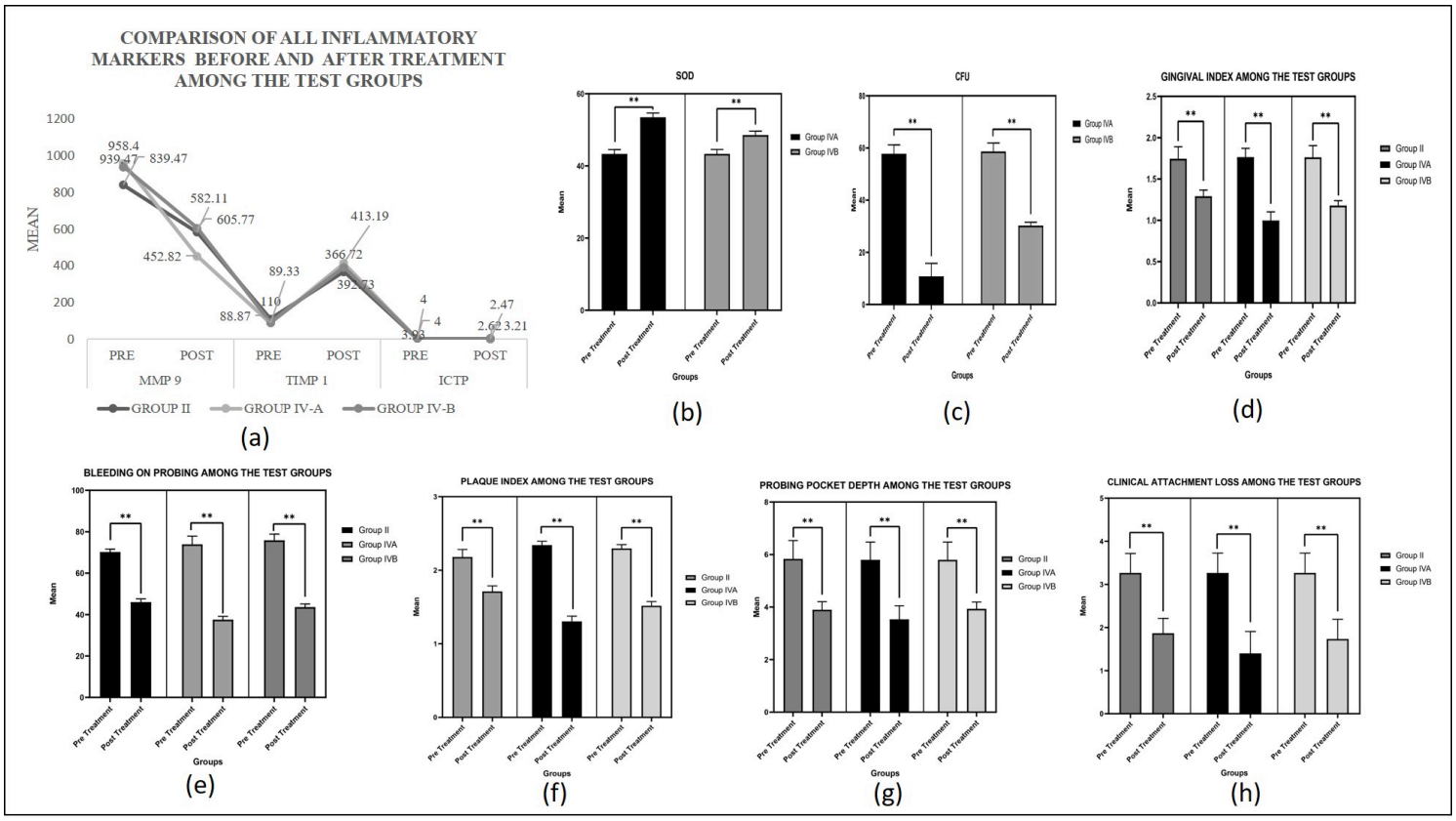


Figure 2

Clinical and Biochemical Findings. (a) Evaluation of Inflammatory markers before and after treatment in Chronic Periodontitis with and without Diabetes Mellitus, (b) Evaluation of SOD within and among the groups, (c) Evaluation of CFU within and among the groups, (d) Evaluation of Gingival Index (GI) before and after treatment in chronic periodontitis with and without Diabetes Mellitus, (e) Evaluation of Bleeding on Probing (BOP) before and after treatment in chronic periodontitis with and without Diabetes Mellitus, (f) Evaluation of Plaque Index (PI) before and after treatment in chronic periodontitis with and without Diabetes Mellitus, (g) Evaluation of Periodontal Probing Depth (PPD) before and after treatment in chronic periodontitis with and without Diabetes Mellitus, (h) Evaluation of Clinical Attachment Loss (CAL) before and after treatment in Chronic Periodontitis with and without Diabetes Mellitus.

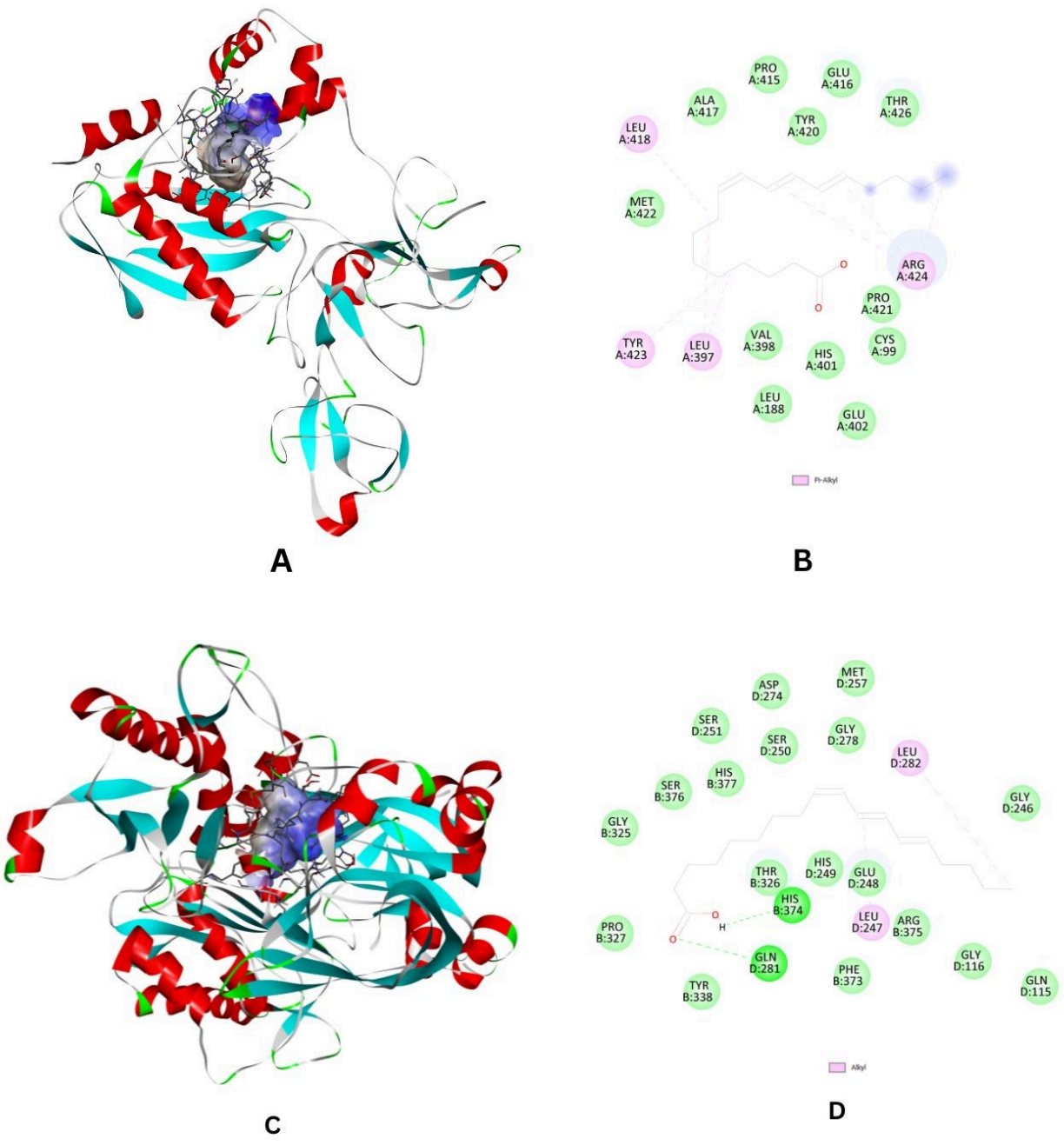


Figure 3

Molecular docking interaction of α-eleostearic acid with inflammatory proteins. (a) 3D binding pose of α-eleostearic acid within the active site of MMP9 (PDB ID: 1L6J), (b) 2D interaction diagram showing hydrogen bonds and hydrophobic contacts between α-eleostearic acid and MMP9, (c) 3D binding pose of α-eleostearic acid within the active site of TIMP1 (PDB ID: 2MA2), (d) 2D interaction diagram showing key interactions between α-eleostearic acid.

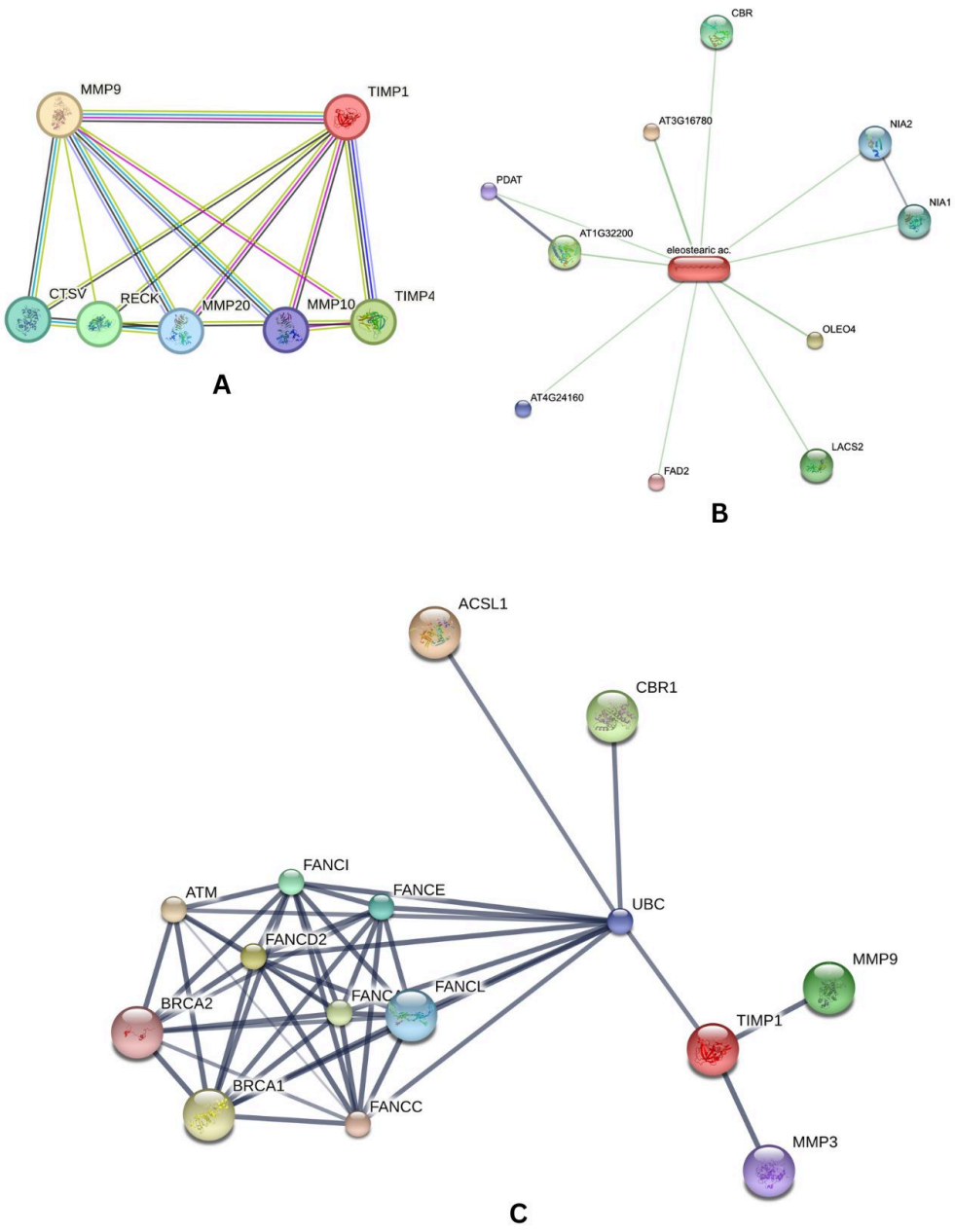


Figure 4

Network analysis of protein–protein and chemical–protein interactions related to α-eleostearic acid. (a) STRING PPI network showing interactions of MMP9 and TIMP1 with CTSV, RECK, MMP10, MMP20, and TIMP4, (b) STITCH network illustrating direct predicted interactions of α-eleostearic acid with proteins such as PDAT, FAD2, and CBR; no direct interaction with MMP9 or TIMP1 was observed, (c) Extended PPI

network revealing an indirect link between α-eleostearic acid and MMP9/TIMP1 through UBC, connecting with CBR1, DNA repair proteins, and matrix regulators and TIMP1.