Texila International Journal of Public Health
ISSN: 2520-3134
DOI: 10.21522/TIJPH.2013.12.01.Art004

Impact of Fasting Intervention on Inflammatory Markers and Mitochondrial Enzyme Complex Activity of Periodontal Tissue and Systemic Inflammation

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Abstract

Periodontitis (PD) is a severe oral inflammation that tends to decay alveolar bone and loss of periodontal ligament. Also, ruptures of gingival tissues and pocket formation take place which can also impact various systematic health conditions. The present study was carried out to evaluate the impact of fasting and other treatment interventions in the prevention of ligature-induced periodontitis as well as the impact of these interventions in systemic inflammation in mice. Periodontitis was induced in mice through 3.0 silk ligature wire by passing between incisor teeth. In this study, bacterial accumulation, bone loss, inflammatory markers such as TNF-α, IL-6, IL-1β, and C-reactive protein as well as oxidative stress i.e., TBARS, and antioxidant enzymes viz. GSH and catalase in dental tissue were measured. Additionally, mitochondrial enzyme complexes activity was significantly decreased in the periodontitis model which is significantly restored by the interventions applied in the study. Further histopathological analysis was also carried out in dental tissue. In periodontitis, there was a rise in bacterial accumulation and bone loss, as well as an increase in inflammatory markers, oxidative stress, and a decrease in mitochondrial enzyme complexes in gum tissue and antioxidative enzymes. Further, in ligature-induced periodontitis, infiltration of inflammatory cells along with the destruction of the cementum and the alveolar process was observed. However, these parameters were reversed after fasting and other treatment interventions. The current study reveals the following ligature removal and subsequent interventions including the impact of fasting to regenerate periodontal tissue.

Keywords*: Antioxidant Enzymes, Fasting, Inflammatory Markers, Mitochondrial Enzyme Complexes, Oxidative Stress, Periodontitis.*

Introduction

Periodontitis (PD) is a severe oral inflammation that tends to decay alveolar bone and loss of periodontal ligament. Also, ruptures of gingival tissues and pocket formation take place which can also impact various systematic health conditions [1, 2]. The major cause of periodontitis begins with plaque formation (a sticky film coating primarily made up of bacteria) and is due to inadequate oral hygiene. Periodontitis manifests clinically as gum inflammation and swollenness, pus between the gums and teeth, poor breath, uncomfortable biting, and newly formed gaps between teeth. Inadequate diet, poor dental hygiene practices, smoking, chewing tobacco, menopause-related hormonal changes, rheumatoid arthritis, and Crohn's disease are some factors that might increase the risk of developing periodontitis [3].

Ninety-five per cent of all-age and all sexes suffered from dental problems in 2017. In 2020,

Received: 15.12.2023 ***Accepted: 18.12.2023*** ***Published on: 29.3.2024***
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the situation of periodontitis in India was 47.2% and gingivitis was 49% [4]. The prevalence of periodontal diseases in both developed and developing nations is approximately 20 to 50 per cent of the worldwide population [5]. In the research carried out in 2012, over 47% of United States adults were diagnosed with aggressive inflammatory degradation of the tendons and ligaments and alveolar bone that surround and hold the tooth [6].

Severe periodontitis, a chronic non-communicable disease with a high prevalence that affects 11.2% of the world's population, is the sixth most common disease in people [7]. Information on patients who visited the University Clinic of Gandra between April 2021 and April 2022 was gathered, 941 patients were enrolled in total; 457 (48.6%) had periodontitis, 253 (26.9%) had gingivitis, and 231 (24.5%) were healthy individuals. Men in the 60-70 age group were facing Stage III chronic periodontal disease with a frequency of 51.2% [8].

Although inflammation occurs locally in the oral cavity, there is much evidence that many bacterial species are present in the oral cavity and the interaction of bacterial infection and host response results in periodontitis. Periodontitis results in the progression of various systemic diseases such as cardiovascular disease, rheumatoid arthritis, cancer, diabetes mellitus, chronic obstructive pulmonary disease, obesity, adverse pregnancy outcomes, and chronic kidney disease [9, 10]. There is compelling evidence connecting periodontal diseases to systemic conditions like diabetes, adverse pregnancy outcomes, and cardiovascular disease (CVD). Cardiovascular disease risk is predicted to increase by 19% as a result of periodontal disease, and this relative risk rises to 44% for people ≥65 years. As compared to people with minimal or mild periodontitis, the mortality risk for type 2 diabetics with the severe form of the condition is 3.2 times higher. In type 2 diabetes individuals, periodontal treatment has been proven to enhance glycemic control. Preterm birth, low birth weight, hypertension, and maternal illness are all linked to periodontitis.[5)]

Previous research demonstrated that the serum levels of pro-inflammatory cytokines such as tumours necrosis factor-α (TNF-α), interleukin (IL)-1β, or IL-6 were higher in patients with periodontitis than in healthy controls. Additionally, aspiration pneumonia, cancer, CVD, rheumatoid arthritis, and type 2 diabetes mellitus are all linked to periodontitis. Amongst systemic diseases, CVD occurs most frequently, because various clinical and epidemiological studies have revealed a strong connection between periodontitis and CVD [11]. Elevated levels of inflammatory markers viz. IL-6 and C-reactive Protein (CRP) are linked to periodontitis [12].

Different animal models are used to study the pathophysiology of periodontitis and the scope of its treatment. The effectiveness of the ligature-induced periodontitis model in mice has allowed researchers to better understand the causes of the condition and assess the efficacy of novel treatment substances. The so-called ligature-induced periodontitis model has the critical benefit of allowing the illness to start at a known time and progress in a predictable manner, leading to alveolar bone loss within a few days [13-15].

Management of periodontitis is done by using antibiotics as standard treatment some choices of antibiotics are Metronidazole, Tetracycline, Amoxycillin, and Ciprofloxacin, and their serial combinations are also used with anti-inflammatory drugs such as indomethacin some therapies like full mouth disinfection and host modulation. Many of these drugs are associated with several adverse effects [16]. Therefore, safe and effective intervention is required for the management of oral cavity disorders.

Calorie restriction, which involves intermittent fasting or a constant reduction in calorie intake without leading to starvation, is one of the reliable non-genetic, non-pharmacological experimental interventions

used to improve health and lengthen lifespan in numerous animal models [17].

Reactive oxygen species (ROS) and inflammatory cytokines are diminished by calorie restriction. Additionally, calorie restriction might enhance the levels of immune-suppressive mediators like transforming growth factor- β (TGF-β), while decreasing the expression of inflammatory cytokines including TNF-α, IL-6, IL-10, IL-12, and interferon-γ (INF-γ) [17]. Although fasting has been performed for thousands of years, only a few studies have demonstrated its contribution to responsive cellular functions that lower oxidative stress and inflammation, enhance energy metabolism, and strengthen cellular defence. The scientific evidence proved that intermittent fasting is the apt strategy for targeting realistic contributing aspects of different inflammatory and lifestyle-associated conditions.

Therefore, the current study has been conducted to evaluate the impact of fasting and other interventions on local inflammation and related systemic diseases associated with periodontitis induced by ligature wire in albino mice.

Materials and Methods

Animals

Thirty-five adult Swiss albino mice of either sex (6-8 weeks, old), with an average body weight of 25-30 g, were procured from the National Institute of Biologicals, NOIDA, UP (India), in the present study. The Institutional Animal Ethics Committee (IAEC) of the KIET School of Pharmacy, Ghaziabad, registered with CPCSEA (Regd. No. 1099/PO/Re/S/07/CPCSEA), authorized the animal protocol (IAEC/KSOP/2021/09). Mice were housed in each polypropylene cage as per conventional laboratory procedures. Water and standard chow pellets were always supplied, and the animal house's temperature and relative humidity were maintained at 22±2 ˚C and 50±15%, respectively.

Five groups with 7 animals in each group were used to get the statistical results. Except in the animals of the normal control group, periodontitis was induced through ligature silk wire in all the animals. All mice had ligatures in place for the duration of the 14-day experiment. Animals were grouped as follows: Group 1-Normal Control group; Group 2-Ligature wire induced periodontitis; Group 3-Periodontitis (14 days) + Fasting regimen (16:8 fasting condition i.e., 16-hour fasting with an 8-hour eating window); Group 4 – Periodontitis (14 days) + Amoxicillin and Metronidazole; Group 5 – Periodontitis (14 days) + Fasting regimen + Amoxicillin and Metronidazole.

Bacterial Accumulation

The ligatures were taken off, and the food particles and other undesired detritus were eliminated by washing the ligatures in a phosphate buffer solution (PBS). The sutures were put into Eppendorf tubes with 1 ml of PBS in them. Vortexing was used to remove the bacteria for two minutes at 3000 rpm. Accordingly, bacteria were taken from sutures procured on the 14th day after ligation and the 21st day after treatment and subjected to anaerobic growth for CFU evaluation to identify the local bacterial accumulation in the ligature animal model. The bacterial suspensions were diluted before being plated onto an agar plate, and after seven days of growth of bacteria at 37 ˚C, a colony-forming unit (CFU) was counted. CFU was divided by the matching suture's length (mm) to get the findings [18].

Anesthesia Protocol

As per Institutional Animal Care and Use Committee (IUCAC) guidelines, anaesthesia was performed intraperitoneal (I.P.) injection with the cocktail of ketamine 10% and xylazine 2% (10:2) with a dose of 0.02 ml/20 gm of mice with 1 ml syringe with a 26 G detachable needle. The correct dose was calculated according to weight measurements.

Ligature-Induced Periodontitis

After anaesthesia, mice were positioned on the operating table, and then with the help of a thread, the upper jaw and lower jaw were opened so that the mouth could be opened for ligating in lower incisor/molar teeth. A 3.0 silk suture with a 3.0 needle was taken, and then with the help of a suture holder the ligature silk was passed between the gum and one side of the incisor teeth was tied then by-passing silk on either side other teeth were also tied making an encircling shape.

Bone Loss Analysis

A radiological examination of periodontal tissues was carried out for the analysis of bone loss.

Inflammatory Markers (TNF-α, CRP, IL-1β, and IL-6) Determination

C-reactive protein (CRP) levels were evaluated by an automated assay named an immunoturbidimetric high-sensitive assay. The levels of TNF-α, IL-1β, IL-6, and CRP were measured in periodontal and heart tissues through ELISA kits (Thermo Fisher Scientific and Sigma-Aldrich) by the manufacturer's recommendations.

Measurement of Antioxidant Parameters and Lipid Peroxidation

All antioxidant enzyme activities were assessed after tissue homogenization with phosphate-buffered saline (PBS) at a pH of 7. Glutathione was estimated as per the Ellman Method (1959) [19], superoxide dismutase (SOD) activity according to the method of Marklund and Marklund (1974) [20], and catalase activity as per the method described by Claiborne (1985) [21]. By the method of Ohkawa et al. (1979), malondialdehyde equivalents with thiobarbituric acid were measured spectrophotometrically to determine the extent of lipid peroxidation, and the results were expressed as thiobarbituric acid reactive compounds (TBARS; nmol malondialdehyde/mg protein) [22].

Isolation of Gum Mitochondria and Measurement of Mitochondrial Enzyme Complex Activity

The Berman and Hastings (1999) approach was used to isolate the mitochondria from mouse gum. A buffer for isolation was used to homogenize the gum tissues. The homogenates were centrifuged for 5 min at 4 °C at 13,000 ×g. The pellets were re-suspended in the isolation buffer and spun again at 13,000 ×g for 5 min. The resultant supernatant was poured into fresh tubes, topped off with isolation buffer, and spun once more at 13,000 ×g for 10 min. The isolated buffer was used to resuspend the pellets of pure mitochondria [23].

NADH Dehydrogenase Activity (Complex-I)

The King and Howard (1967) method was used to measure the spectrophotometric NADH Dehydrogenase activity (Complex-I). The process involves reducing cytochrome C after catalytically oxidizing NADH to NAD^+. Adding the necessary quantity of solubilized mitochondrial material started the process. For two minutes, the change in absorbance at 550 nm was observed [24].

Succinate Dehydrogenase (Complex-II)

Succinate Dehydrogenase (SDH) was estimated spectrophotometrically as per the method described by King (1967). The process involves oxidizing succinate with potassium ferricyanide, a synthetic electron acceptor. The mitochondrial sample was added to start the reaction, and the change in absorbance at 420 nm was monitored for two minutes [25].

Cytochrome Oxidase (Complex-IV)

The Sottocasa et al. (1967) method was used to measure the activity of cytochrome oxidase in mitochondria. In 75 mM phosphate buffer, 0.3 mM reduced cytochrome C was present in the assay mixture. The solubilized mitochondrial sample was added to start the

process, and an absorbance change at 550 nm was monitored for two minutes [26].

Histopathology of Periodontal Tissue

The entire periodontal tissue was removed and preserved in a 10% formalin solution. The periodontal tissues were then dehydrated in alcohol and embedded in paraffin wax after being rinsed with 0.1 M PBS (pH 7.4) for 1 h. The whole periodontal tissues were then be cut into sequential coronal slices, with 5 µm thickness, and stained with eosin and hematoxylin.

Statistical Analysis

The observations were expressed as the mean ± standard error of the mean (SEM). To calculate each P value, a one-way analysis of variance (ANOVA) was performed. $P \leq 0.05$ was considered statistically significant. Statistical analysis was performed using GraphPad Prism software (version 5.00; GraphPad Software).

Results

Effect of Interventions on Various Bacterial Accumulation

It was found that some bacterial accumulation was present in the dental tissues of the normal control group while this bacterial accumulation was significantly ($P<0.05$) increased in the pathogenic control group as compared to the normal control group. Further, the findings of the bacterial accumulation revealed a time-dependent build-up of bacteria that seemed to peak on Day 14th and Day 21st deposition of bacteria gradually diminished significantly ($P>0.05$ compared with Day 14) due to the application of different interventions (Figure 1).

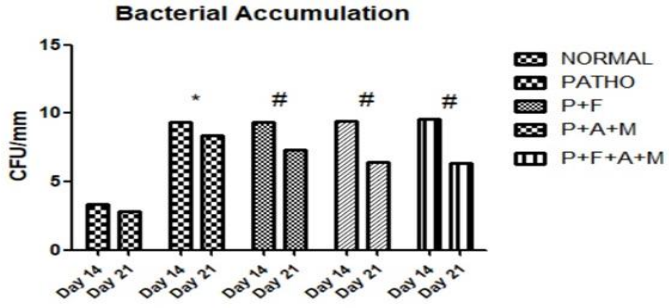

Figure 1. Measurement of Bacteria Accumulation After the Application of Ligatures

Values were analyzed as mean ± S.E.M, n=7; *P<0.05 vs normal control group; #P>0.05 (nonsignificant) vs Pathogenic control group. P=Patho, F=Fasting, A=Amoxicillin, M=Metronidazole

Effect on Bone Loss

Radiological examination of periodontal tissues showed a clear bone loss /bone resorption in mice (ligature-induced periodontitis) compared to normal control mice (Figure 2, A vs B) and this bone loss was recovered in the mice treated with different interventions. These findings are visible in (Figure 2 C-E).

Effect on Inflammatory Markers in Dental Tissue

There was a significant ($P<0.05$) increase in the levels of TNF-α, IL-1β, IL-6, and CRP in the periodontitis group as compared to the normal group but when these animals were treated with different interventions (indomethacin 5 mg/kg, amoxicillin 50 mg/kg, metronidazole 25 mg/kg, and intermittent fasting), the levels of the inflammatory markers were decreased significantly ($P<0.001$) (Table 1).

Effect on Oxidative Stress and Antioxidant Enzymes in Dental Tissue

Oxidative stress i.e. (TBARS and Nitrite levels) was significantly (P<0.05) elevated in the periodontitis group as compared to the normal group but when these animals were treated with different interventions, the levels of the these levels were suppressed significantly (P<0.001).

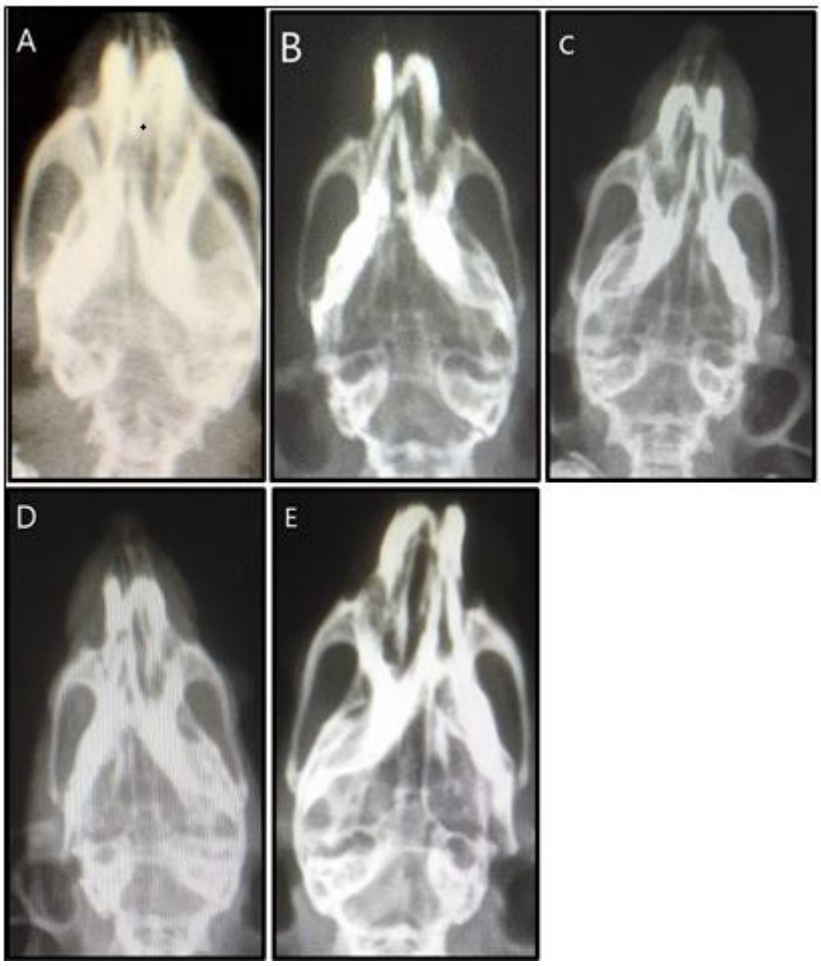

Figure 2. Effect on Bone Loss Through X-ray Examination

The levels of antioxidant enzymes i.e. catalase and GSH levels were signifcantly (P<0.05) decreased as compared to the normal control group and the levels of these enzymes were reveresed after application of various interventions (Table 1)

Table 1. Effect of Interventions on Inflammatory Markers and Antioxidant Enzymes in Dental Tissue

Parameters	**NC**	**P**	**P+ F**	**P+A+M**	**P+F+A+M**
CRP (µg/ml)	2.52±0.05	11.40±0.05*	8.41±0.06#	7.36±0.03#	6.32±0.08#
TNFα (pg/ml)	39.34±0.14	66.21±0.43*	61.2±0.31#	52.14±0.43#	48.44±0.19#
IL-1β (pg/ml)	35.40±0.12	60.55±0.32*	40.45±0.08#	39.55±0.09#	37.40±0.09#
IL-6 (pg/ml)	5.40±0.08	30.47±0.09*	20.44±0.09#	15.57±0.09#	10.57±0.08#

Parameters	NC	P	P+ F	P+A+M	P+F+A+M
TBRAS (nmol of MDA/mg protein)	0.138±0.005	0.934±0.011*	0.732±0.011#	0.534±0.007#	0.341±0.009#
CAT (nmol of H_2O_2/min/mg protein)	40.52±0.09	15.52±0.10*	18.54±0.07#	23.57±0.07#	35.48±0.11#
GSH (µmol of GSH/mg protein)	9.77±0.04	2.55±0.08*	6.41±0.05#	7.57±0.08#	9.17±0.04#
Nitrite (mM)	0.657±0.06	5.35±0.08*	4.44±0.09#	3.37±0.08#	1.57±0.10#

Results were analyzed by using one-way ANOVA multiple comparison tests. Values are expressed as Mean ± SEM (n=7); *P<0.01 vs normal control group; #P<0.01 vs Periodontitis group. NC- Normal Control group; P-Pathogenic (Periodontitis group); A- amoxicillin, F-Fasting, M- Metronidazole; CRP- C-reactive protein; TNFα- Tumor necrosis factor-alpha; IL- Interleukin; TBARS- Thiobarbituric acid reactive substances; CAT- Catalase; GSH- Glutathione

Effect on Inflammatory Markers in Cardiac Tissue

As shown in Table 2, there was a significant increase in the levels of TNF-α, IL-1β, IL-6, and CRP (P<0.05) in the periodontitis group as compared to the normal group but when these animals were treated with different interventions, the levels of the inflammatory markers were decreased significantly (P<0.001).

Effect on Oxidative Stress and Antioxidant Enzymes in Cardiac Tissue

Oxidative stress i.e. (TBARS and Nitrite levels) in cardiac tissue was significantly (P<0.05) elevated in the periodontitis group as compared to the normal group but when these animals were treated with different interventions, the levels of these levels were decreased significantly (P<0.001). The levels of antioxidant enzymes i.e. catalase and GSH levels were significantly (P<0.05) suppressed in comparison to the normal control group and the levels of these enzymes were reversed after application of various interventions (Table 2).

Table 2. Effect of İnterventions on İnflammatory Markers and Antioxidant Enzymes in Cardiac Tissue

Parameters	NC	P	P+ F	P+A+M	P+F+A+M
CRP (µg/ml)	3.61±0.03	9.15±0.10*	7.41±0.05#	6.98±0.04#	6.43±0.06#
TNFα (pg/ml)	40.42±0.49	64.21±0.68*	56.37±0.48#	53.57±0.45#	48.04±0.31#
IL-1β (pg/ml)	37.54±0.24	58.92±0.15*	41.74±0.29#	40.12±0.18#	39.11±0.25#
IL-6 (pg/ml)	6.82±0.19	29.47±0.68*	22.11±0.52#	19.42±0.38#	14.71±0.28#
TBRAS (nmol of MDA/mg protein)	0.154±0.006	1.017±0.023*	0.757±0.017#	0.588±0.018#	0.487±0.014#

CAT (nmol of H2O2/min/mg protein)	42.67±0.37	16.81±0.43*	20.11±0.51#	24.14±0.24#	32.77±0.58#
GSH (µmol of GSH/mg protein)	11.34±0.28	4.27±0.26*	7.55±0.15#	8.71±0.17#	9.25±0.21#
Nitrite (mM)	0.728±0.05	6.07±0.23*	4.54±0.10#	3.31±0.05#	2.27±0.18#

Results were analyzed by using one-way ANOVA multiple comparison tests. Values are expressed as Mean ± SEM (n=7); *P<0.01 vs normal control group; #P<0.01 vs Periodontitis group. NC- Normal Control group; P-Pathogenic (Periodontitis group); A- amoxicillin, F-Fasting, M- Metronidazole; CRP- C-reactive protein; TNFα- Tumor necrosis factor-alpha; IL- Interleukin; TBARS- Thiobarbituric acid reactive substances; CAT- Catalase; GSH- Glutathione

Effect on Mitochondrial Enzymes Complex in Gum Tissue

Mitochondrial enzyme complexes (NADH dehydrogenase, Succinate dehydrogenase and cytochrome oxidase levels) in gum tissue were significantly (P<0.05) reduced in the periodontitis group as compared to the normal group but when these animals were treated with different interventions, the levels of these levels were restored significantly (P<0.05) after application of various interventions (Figure 3).

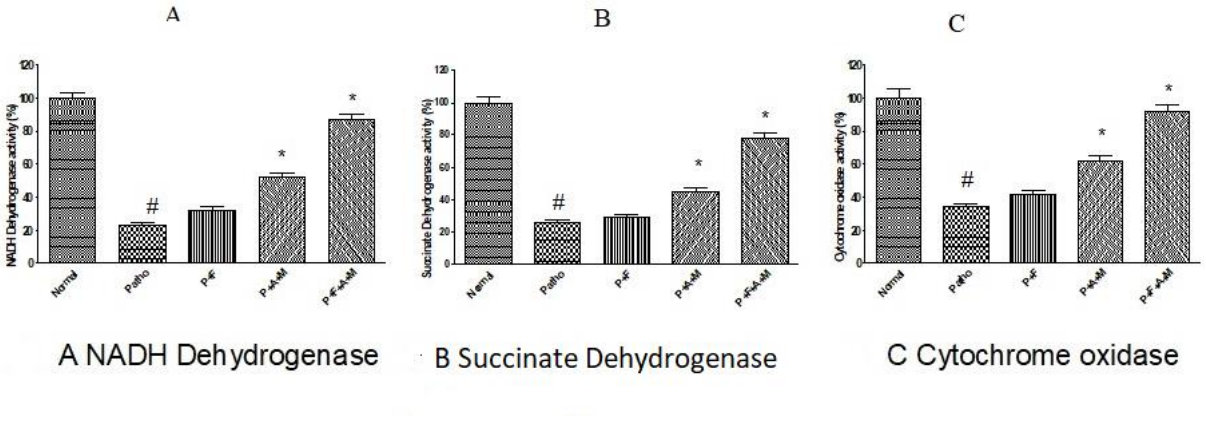

Figure 3. Effect of İnterventions on Mitochondrial Enzyme Complex in Gum Tissue

A: NADH Dehydrogenase, B: Succinate Dehydrogenase, C: Cytochrome Oxidase. Results were analyzed by using one-way ANOVA multiple comparison tests. Values are expressed as mean ± SEM; *P<0.05 vs normal control group; #P<0.01 vs pathogenic group. P-Pathogenic (Periodontitis group); A- amoxicillin, F-Fasting, M- Metronidazole

Histopathology

Histopathological examination of periodontal tissues revealed that all the structures of the periodontal tissue viz. ligament, cementum, and alveolar bone were well-maintained in the normal group (Figure 4A). While in ligature-induced periodontitis, infiltration of inflammatory cells along with the destruction of the alveolar and cementum processes were observed (Figure 4B). All these changes (reduced cellular infiltration, alveolar bone, and cementum) were reversed in animals by all the interventions applied in the study as compared to ligature-induced periodontitis (Figure 4C-E).

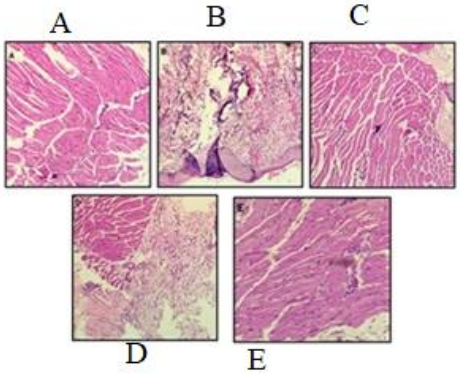

Figure 4. Effect on Tissue Histopathological Examination

Discussion

Worldwide, periodontitis is a chronic inflammatory illness characterized by inflammatory infiltration and progressive loss of alveolar bone. Periodontitis is the leading reason for bone loss, loss of periodontal attachment, and loss of teeth in adults. It is also linked to a higher risk of developing life-threatening systemic diseases like atherosclerosis, hypertension, rheumatoid arthritis, and diabetes [27].

Numerous studies on periodontal disease have been conducted in the past using various therapies and induction techniques. In the current protocol, periodontitis was induced with the help of silk ligature wire in mice which terms to be the best animal model based on the previous study [28]. In animals with periodontal disease, there was a rise in bacterial accumulation, bone loss, and increased levels of inflammatory markers like CRP, IL-1β, IL-6, and TNF-α. During the period of the increased inflammatory biomarkers, the oxidative stress seems to be increased with a decrease in antioxidant enzymes in dental and cardiac tissues. Similar types of findings have been reported during periodontitis by the previous researchers [11, 12].

In this study, the bacterial accumulation was done by recovering the ligature on the 14th day in pathogenic conditions and on the 21st day after treatment with different interventions. Bacterial accumulation was considerably increased in the pathogenic group compared to the control group on Day 14 and there was a decrease in bacterial accumulation on Day 21. Previous research shows an increase in bacterial accumulation concentration in periodontitis animals.[18)] The gingival epithelium releases additional cytokines such as IL-1, IL-8, and TNF-α in addition to the inflammatory mediators, which in turn encourages the attraction of macrophages [1].

The radiological examination of animals revealed in the pathogenic major loss in the periodontal bone and this bone loss was restored after treatment protocols. Previous findings also showed significant bone resorption in periodontic animals, and the bone loss was recovered after different interventions. The findings of the current study are in corroboration with previous findings [29, 30]. The possibility of mechanical damage due to the ligatures cannot be ruled out in small animals, which could lead to bone loss. Besides this, bacterial accumulation also plays a critical factor in the initiation of bone loss in the ligature-induced periodontitis in mice [18].

Inflammatory biomarkers viz. CRP, IL-1β, IL-6, and TNF-α were increased in the pathogenic conditions and were cured after various treatment interventions. These inflammatory biomarkers were also found to be significantly increased in previous studies.[11)] Additionally, proinflammatory pathways were outlined in

animals treated with ligature wire. Proinflammatory molecules like IL-6, IL-8, IL-1β, and TNF-α are expressed more frequently when such pathways are engaged by mono- or polymicrobial illnesses. Further, periodontitis increases the expression of genes involved in transport, immunological and inflammatory responses, cell proliferation, cell cycle, and apoptosis [1].

Inflammation in tissues causes an increase in oxidative stress in the form of increased levels of TBARS along with Nitrite and a marked reduction in anti-oxidative enzymes GSH along with Catalase. In the current study, it was noticed increments and decrements in oxidative stress and antioxidant enzymes, respectively. The results of the present study showed significance to previous findings [31].

According to various previous researchers [11, 12], mitochondrial dysfunction has a role in the aetiology of periodontitis. Multiple biochemical changes in oral cavity tissue, including decreased complex I activity and decreased complex II and IV activity, were seen in biochemical analyses of periodontitis patients' gum tissue [31]. Additionally, mitochondrial ultrastructural anomalies in infectious illnesses have been reported [1]. Similar to previous studies, the activity of mitochondrial complexes I, II, and IV in gum tissues has been reduced in the present study [27,28,32]. According to studies, anaerobic circumstances have been linked to ATP depletion, which in turn affects mitochondria's capacity to sequester Ca^{2+} activates caspase-9 and caspase-3 and results in bone damage from apoptosis.

Microscopic and histopathological images of mice showed no alteration in the normal group, but massive destruction was seen in pathogenic tissues and after giving treatment, the pathogenic condition was recovered in the cementum and alveolar tissues region. Previous studies have shown similar findings compared with the current protocol [27, 28, 32].

Conclusion

In conclusion, periodontitis was successfully induced in mice through ligature wire. Periodontitis was characterized by increased bacterial accumulation, inflammatory biomarkers, oxidative stress, and histopathological changes. The results of the current study reveal the following ligature removal and subsequent interventions including intermittent fasting to regenerate periodontal tissue. Intermittent fasting improved local inflammation as well as systemic inflammation in mice. Therefore, intermittent fasting could be one of the most prominent nonpharmacological interventions for the treatment of periodontitis in mice.

Conflicts of Interest

The authors declare no conflict of interest.

Acknowledgement

The author is thankful to KIET School of Pharmacy, Ghaziabad (UP), for carrying out the experimental work in the laboratory. There was no external funding source available to carry out the study.

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