EFFECTS OF THE SYSTEMIC ADMINISTRATION OF OMEGA-3 POLYUNSATURATED FATTY ACID ON EXPERIMENTAL PERIODONTITIS: A histopathological study for rat’s periodontal tissue

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ABSTRACT

Periodontitis is a common inflammatory disease characterized by the formation of periodontal pocket and can lead to bone and tooth loss. The aim of this study was to evaluate the effect of omega-3 fatty acids (Eicosapentaenoic acid) as a treatment for ligation induced periodontitis using an animal model.

Periodontitis was induced for the studied animals by ligation around the upper central incisor. Twenty five animals were used for the pilot study, and the eighty studied animal with the induced periodontitis were divided into four equal groups according to the treatment used: Water, scaling/root planing, 60mg/kg eicosapentaenoic acid, and scaling/root planing with eicosapentaenoic acid. After three days, two, four, and six weeks treatment, five animals from each group were sacrificed, and the maxillae were collected, processed, and analyzed.

The treatment by scaling and root planing together with the eicosapentaenoic acid can significantly reduce the periodontal damage index after four weeks, but when these treatment was used each one alone, it causes significant reduction in damage score after six weeks.

For short duration of periodontal treatment, eicosapentaenoic acid can be used with scaling and root planing as a good treatment for periodontal disease.

Keywords: Periodontitis, Omega-3, Eicosapentaenoic acid, Alveolar bone resorption

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INTRODUCTION

Periodontitis is inflammation that extends to periodontal structures beyond the gingiva, and characterized by apical migration of the junctional epithelium producing a loss of the connective tissue attachment of the teeth and loss of alveolar bone

Omega-3 polyunsaturated fatty acid (PUFA) is the bioactive lipids which consist of three major omega-3 fatty acids, eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) from marine sources, and alpha-linolenic acid (ALA) which is from plant sources. Omega-3 exhibited a strong antimicrobial...
activity against various oral pathogens\textsuperscript{5,6} and the supplementation of the diet with omega-3 fatty acid would exert anti-inflammatory effects in the gingival tissues of Porphyromonas gingivalis infected rats which impacts on the alveolar bone resorption in rats\textsuperscript{7}. Naqvi et al\textsuperscript{8} found that higher dietary intake of omega-3 fatty acids was inversely associated with the prevalence of periodontitis, and they concluded that fish dietary supplements could have potential benefits as host modulatory agents in the prevention and/or adjunctive management of periodontitis.

Periodontal disease is an immunoinflammatory condition that affects almost 90\% of the population\textsuperscript{9} and continue to increase with the growing ageing population\textsuperscript{10,11}. Till date, the most common periodontal treatment is still the mechanical removal of plaque and calculus deposits and local antibiotic application. Nowadays, researchers are focusing on exploring the pharmacological compounds from the natural origin for the treatment of periodontitis. For this reason, the present study was aimed to investigate and evaluate the effects of 60 mg/kg EPA on ligature induced periodontitis in rats through the histopathological analysis using hematoxylin and eosin staining.

MATERIALS AND METHODS

Rats and housing: All the Wister-albino rats used in the study were aged about 8-10 weeks, weighing 200-300 g and cared in the animal house of College of Medicine, Hawler Medical University, Erbil/ Iraq. They were allowed to adapt to the housing conditions for one week prior to the commencement of the study. Five rats were housed in each wire cage and maintained on a 12-hour light/dark cycle at 20± 5°C and 20%-30% humidity. The animals were kept in standard room conditions and fed with a standard rat chow and allowed to drink water \textit{ad libitum}. The research project was approved by the Research Ethics Committee at College of Dentistry, Hawler Medical University under protocol.

Pilot study for induction of experimental periodontitis: Five animals were used in this pilot study. They were anesthetized by intraperitoneal administration of ketamine (0.5 ml/kg b.w.) and placed on a proper operating table, which allowed open-mouth maintenance of the rats to facilitate access to the teeth. After that a (3.0) sterile black braided silk threads were placed around the cervix of maxillary right incisor for each animal and kept for two weeks. The ligatures are knotted on the labial side of the tooth, resulting in subgingival positioning on the palatal side and supragingival position on the labial side. Daily we perform ligatures control and checking, and if any had been lost or become loose, it was replaced (Figure -1).

At day fourteen, all the animals were sacrificed and the maxilla from each animal was taken, fixed in 10\% neutral buffered formalin, processed, sectioned in bucco-lingual direction, and stained with hematoxylin and eosin. Two representative tissue sections from each block were analyzed to examine the gingiva, bone, periodontal ligament and cementum on the palatal side of the tooth. The results showed that two weeks ligation caused active bone resorption and a large number of osteoclasts seen bordering the bone trabeculae. Inflammatory cell infiltration and congestion of blood vessels were also seen (Figure-2).
Figure 1: (A). Sterile black silk threads around the cervix of maxillary right incisors. (B). Development of periodontal disease after ligature removal. The gingiva appears red and edematous.

Figure 2: Periodontitis in rat’s maxillary right incisor after 14 days ligation shows: (A1) Active alveolar bone resorption. The bone shows irregular contour and with thin bone trabeculae. AB, alveolar bone and T, tooth (H&Ex100). (A2) Loss of epithelium in some areas with the presence of a large number of inflammatory cells in gingival connective tissue, arrow (H&Ex100). (A3) The osteoclast cells were seen bordering the bone trabeculae, inflammatory cells infiltration, and disorientation of periodontal collagen fiber are also seen (H&Ex400).

Pilot study for selection of the suitable dose of EPA: Twenty animals were used in this pilot study to find the effect of different doses of EPA (Eicosapentaenoic Acid, 100 mg, Solarbio, China) on the ligation induced periodontitis in rat’s maxillary right incisor after six weeks treatment. The animals were divided into four groups (five animals each). The first group was the Periodontitis/Water treatment group; they were received intragastric gavage of distilled water, while the other three were Periodontitis/EPA treatment groups, they were received intragastric gavage of EPA (20, 40, and 60 mg/kg b.w. respectively). The treatment for all groups by distilled water or by different concentrations of EPA was starting at the time of ligation removal (day zero), once time daily using gavage needle.

All the animals were sacrificed after six weeks treatment and the maxilla from the animals was taken, fixed, processed, sectioned in bucco-lingual direction, and stained for hematoxylin and eosin staining. Two representative tissue sections from each block were analyzed to examine the gingiva, bone, periodontal ligament and cementum on the palatal side of the tooth. A minimum of three fields were examined in each area of interest for every section. Results from two sections were averaged to provide a value for a given specimen. Parameters, such as inflammatory cell influx, alveolar bone and cementum integrity were analyzed by a single-blind fashion under 400x magnification and the periodontal scores were graded.
The results showed that the treatment by 20 mg/kg or by 40 mg/kg of EPA cause non-significant (p>0.05) decrease in the periodontal damage scores in comparison with the Periodontitis/Water treatment group, but a significant decrease (p<0.05) was seen in rats treated with 60 mg/kg (Table-1).

**Table 1:** Periodontal damage scores in rats after using different doses of EPA treatment plan for the ligation induced periodontitis in the pilot study.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Periodontal damage score after six weeks treatment (Mean±SD)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Periodontitis/Water</td>
<td>1.264±0.147</td>
<td>0.674 NS</td>
</tr>
<tr>
<td>Periodontitis/EPA (20 mg/kg)</td>
<td>1.198±0.180</td>
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</tr>
<tr>
<td>Periodontitis/Water</td>
<td>1.264±0.147</td>
<td>0.603 NS</td>
</tr>
<tr>
<td>Periodontitis/EPA (40 mg/kg)</td>
<td>1.164±0.234</td>
<td></td>
</tr>
<tr>
<td>Periodontitis/Water</td>
<td>1.264±0.147</td>
<td>0.012 S</td>
</tr>
<tr>
<td>Periodontitis/EPA (60 mg/kg)</td>
<td>0.699±0.074</td>
<td></td>
</tr>
<tr>
<td>Periodontitis/EPA (20 mg/kg)</td>
<td>1.198±0.180</td>
<td>0.920 NS</td>
</tr>
<tr>
<td>Periodontitis/EPA (40 mg/kg)</td>
<td>1.164±0.234</td>
<td></td>
</tr>
<tr>
<td>Periodontitis/EPA (20 mg/kg)</td>
<td>1.198±0.180</td>
<td>0.012 S</td>
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<tr>
<td>Periodontitis/EPA (40 mg/kg)</td>
<td>0.699±0.074</td>
<td></td>
</tr>
<tr>
<td>Periodontitis/EPA (60 mg/kg)</td>
<td>1.164±0.234</td>
<td>0.016 S</td>
</tr>
<tr>
<td>Periodontitis/EPA (60 mg/kg)</td>
<td>0.699±0.074</td>
<td></td>
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SD: Standard deviation, S: Significant, NS: Non-Significant.

**Experimental design for histological analysis:** Eighty animals with induced experimental periodontitis were randomly assigned into four experimental groups (20 animals each): Periodontitis/Water treatment group, Periodontitis /SRP treatment group (The right incisors were subjected to scaling and root planing with manual #1–2 mini-five curettes through 10 distal–mesial traction movements in the labial and palatal aspects, and the interproximal areas were scaled with the same curettes using cervico–incisal traction movements)\(^{13}\), Periodontitis / EPA (60 mg/kg) treatment group, and Periodontitis/SRP+ EPA treatment group.

They were sacrificed after three days, two, four and six weeks (5 animals each). The maxillae were collected, processed, and the periodontal damage scores were graded\(^{12}\).

**Statistical analysis:** Data were analyzed using SPSS software version 23. The data represent quantitative observations and were summarized using means and standard deviations. Statistical analysis with one-way analysis of variance (ANOVA) was performed to compare the differences in the means among groups, and when it revealed that there was a statistically significant difference; Mann-Whitney U test was performed to assess an individual pair of groups for statistically significant finding. A P value less than or equal to 0.05 was considered statistically significant.
RESULTS

After three days treatment, the histological features of rat’s upper incisors periodontal tissue for Periodontitis/Water treatment group (Figure 3 as shown The P/SRP/EPA treatment group shows less inflammatory cells infiltrations, blood vessels congestion, and bone resorption than the other three groups (B4, H&Ex400)) revealed an intense mixed inflammatory infiltrate predominantly of neutrophils (that includes gingival tissue, periodontal ligament and alveolar bone), blood vessels congestion, and alveolar bone resorption with increase in the number of osteoclasts cells. Histological features of rat’s upper incisors periodontal tissue for Periodontitis /SRP, Periodontitis/EPA, and Periodontitis/SRP/EPA treatment groups also showed severe inflammatory infiltration and bone resorption. Statistical analysis showed a non-significant decrease (p>0.05) in the damage scores after different types of treatments in comparison with Periodontitis/Water treatment group (Table-2).

After two weeks treatment, the histological features of rat’s upper incisors periodontal tissue for Periodontitis/Water treatment groups also revealed severe inflammatory cells infiltrations (mainly mononucleated cells and neutrophils), congestion, and bone resorption. In the Periodontitis/SRP, Periodontitis/EPA and Periodontitis/SRP/EPA treatment groups, the inflammatory cells infiltrations, blood vessels congestion and bone resorption are also still present with a non-significant decrease (p>0.05) in the damage scores in comparison with Periodontitis/Water treatment group.

After four weeks treatment, the histological features of rat’s upper incisors periodontal tissue for Periodontitis/Water treatment group (Figure 4 as shown The Periodontitis/Water treatment group still shows inflammatory infiltration and congestion in some areas (B1, H&Ex100). Bone formation and mild inflammatory cells infiltration are seen associated with Periodontitis/SRP (B2, H&Ex400), and P/EPA treatment groups (B3, H&Ex100). The P/SRP/EPA treatment group also shows new bone formation (arrows), increase in the number of osteoblasts bordering the bone trabeculae, and a decrease in the number of inflammatory cells. The osteoblasts become osteocytes by becoming trapped within a bony matrix and seen within large lacunae in newly formed bone (B4, H&Ex400)) also showed inflammatory cells infiltrations, congestion, and bone resorption. The treatment by SRP, EPA, or by SRP/EPA causes significant decrease in damage scores, and the bone formation can be seen started in some areas. Only the Periodontitis/SRP/EPA treatment group showed a significant decrease (p<0.05) in damage score in comparison with the Periodontitis/Water, Periodontitis/SRP, and Periodontitis/EPA treatment groups.

After six weeks treatment, the histological features of rat’s upper incisors periodontal tissue for Periodontitis/Water treatment group showed that the inflammation was still present, although compared with the results at first week there was a gradual reduction of the volume occupied by inflammatory infiltrate. The treatment by SRP, EPA, or by SRP/EPA causes significant decrease (p<0.05) in damage scores in comparison with the Periodontitis/Water treatment group. Non-significant differences (p>0.05) was present between Periodontitis/SRP and Periodontitis/EPA, Periodontitis/SRP and Periodontitis/SRP/EPA, Periodontitis/EPA and Periodontitis/SRP/EPA treatment groups regarding the damage scores.
Figure 3: Histological features of rat’s upper incisors periodontal tissue after two weeks treatment.

Figure 4: Histological features of rat’s upper incisors periodontal tissue after six weeks treatment.
Table 2: Periodontal damage score (mean± SD) in all studied groups.

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<tr>
<td>3 days</td>
<td>2.39±0.093</td>
<td>2.26±0.190</td>
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<td>2.23±0.148</td>
<td>2.22±0.093</td>
<td>2.26±0.190</td>
<td>2.23±0.148</td>
<td>2.22±0.093</td>
<td>2.26±0.190</td>
<td>2.23±0.148</td>
<td>2.22±0.093</td>
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<tr>
<td>P-value</td>
<td>0.298NS</td>
<td>0.116</td>
<td>0.060</td>
<td>NS</td>
<td>0.298NS</td>
<td>0.833</td>
<td>0.756</td>
<td>0.920</td>
<td>0.798NS</td>
<td>0.711</td>
<td>0.920</td>
</tr>
<tr>
<td>2 weeks</td>
<td>1.83±0.576</td>
<td>1.63±0.532</td>
<td>1.83±0.576</td>
<td>1.44±0.611</td>
<td>1.399±0.063</td>
<td>1.63±0.532</td>
<td>1.44±0.611</td>
<td>1.399±0.063</td>
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<tr>
<td>P-value</td>
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<td>NS</td>
<td>0.104</td>
<td>NS</td>
<td>0.711</td>
<td>0.711</td>
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<tr>
<td>4 weeks</td>
<td>1.46±0.138</td>
<td>1.13±0.167</td>
<td>1.46±0.138</td>
<td>1.08±0.091</td>
<td>0.766±0.182</td>
<td>1.13±0.167</td>
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<td>1.13±0.167</td>
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<tr>
<td>P-value</td>
<td>0.060</td>
<td>NS</td>
<td>0.104</td>
<td>NS</td>
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<td>0.603</td>
<td>NS</td>
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<tr>
<td>6 weeks</td>
<td>1.13±0.180</td>
<td>0.699±0.247</td>
<td>1.13±0.180</td>
<td>0.632±0.182</td>
<td>0.399±0.148</td>
<td>1.13±0.180</td>
<td>0.632±0.182</td>
<td>0.399±0.148</td>
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<td>0.399±0.148</td>
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<tr>
<td>P-value</td>
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<td>0.012</td>
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<td>0.012</td>
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SD: Standard deviation, S: Significant, NS: Non-Significant.

DISCUSSION

The use of animals like rats in research has increased because of the difficulty of studying the host response to therapeutic conditions and disease pathogenesis in humans. The histopathological analysis demonstrated a severe tissue breakdown was seen when rats received distilled water treatment for the induced periodontitis. This result confirms the report of Kara et al., Ionel et al. and Graves et al., which observed that ligature favors plaque accumulation and invasion of periodontal tissue by bacteria. Cytokines induced by the host response to bacterial product provoke the inflammatory reaction in periodontal tissue.

The present study showed that the Periodontitis/EPA and Periodontitis/SRP treatment groups showed significant decrease (p<0.05) in the damage scores after six weeks treatment in comparison with Periodontitis/Water treatment group, but the Periodontitis/SRP/EPA treatment groupshowed asignificant decrease (p<0.05) after four weeks treatment. The present study also showed that the treatment with EPA alone showed similar results when this procedure was compared with SRP use alone. In addition to the action of EPA, treatment with EPA caused less tissue trauma than scaling and root planing.

Vardar-Şengül et al. found that when the rats was gavaged daily with omega-3, it was effective in the treatment of the induced gingival inflammation. Araghizadeh et al. results suggest that omega-3 may have a modulating effect on destruction of gingival connective tissue, this may be due to decreasing in the levels of IL-1β and TNF-α, which can induce the production of matrix metalloproteinase (MMPs) as well as the destruction of connective tissue, or due to modulation in the inflammatory reactions which can lead to periodontal disease in infected rats.
In human, Requirand et al, Rosenstein et al, and El-Sharkawy et al, found that the higher dietary intake of omega-3 fatty acids was inversely associated with the prevalence of periodontitis. Calder found that PUFAs may influence cytokine gene expression by altering membrane fluidity, cell to cell signaling, and mobility of cells.

EPA can increase collagen synthesis, decrease prostaglandin E2 production, had antibacterial activity against various oral pathogens, anti-inflammatory and immunoregulatory actions, and decrease osteoclast activation. Al-Hashemi et al, Attia et al, and Abdou et al found that the total alkaline phosphatase in rat’s serum was significantly elevated after treatment with omega-3. The present study disagrees with that of the Vardar- Şengület study, they found that omega-3 cannot be used for periodontal treatment.

**CONCLUSION**

The treatment by SRP and EPA together can cause a significant reduction in damage score after four weeks.

**ETHICAL CLEARANCE**

The Research Ethical Committee at scientific research by ethical approval of both environmental and health and higher education and scientific research ministries in Iraq

**CONFLICT OF INTEREST**

The authors declare that they have no conflict of interest.

**FUNDING:** Self-funding

**REFERENCES**


