Effects of the systemic administration of omega-3 polyunsaturated fatty acid on experimental periodontitis (immunological analysis for rat’s serum)

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Abstract

Previous studies had been reported that IL-1, IL-6, and TNF-α were associated with various bacterial infections, including periodontitis. The present study was aimed to investigate and evaluate the effects of eicosapentaenoic acid (EPA) on ligature induced periodontitis in rats through the immunological analysis for rat’s serum level of IL-1β, IL-6, and TNF-α. The periodontitis was induced for the studied animals by ligation around the upper central incisor. Twenty-five animals were used as a control and gavaged by water only, and one hundred animals with the induced periodontitis were divided into four equal groups according to the treatment used: Water, scaling/root planing (SRP), 60mg/kg EPA, and SRP together with EPA. Blood was taken by cardiac puncture, three hours after ligation removal (day zero), 24 hours, three days, one week and two weeks for immunological analysis. The result showed that the treatment of the induced periodontitis by SRP together with 60mg/kg EPA cause a significant decrease in serum IL-1β after 24 hours from the treatment, but a significant decrease in IL-6 and TNF-α were seen after three hours in comparison with the periodontitis group treated by water only. As a conclusion from this study, the treatment of periodontitis by SRP together with EPA is better than SRP or EPA each one alone.

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

Introduction

Periodontitis is a common chronic inflammatory disease characterized by the formation of a periodontal pocket and resorption of alveolar bone [1, 2]. The pathogenesis of periodontitis involves both innate and acquired immune responses. The initial response to bacterial infection is a local inflammatory reaction that activates the innate immune system [3]. The inflammatory response results in the release of an array of cytokines and other mediators and propagation of inflammation and recruitment of inflammatory cells into the gingival tissue. The spread of inflammation to the adjacent connective tissue drives the destruction of connective tissue and alveolar bone, which is the cardinal sign of periodontal disease [4, 5].

Interleukin-1β (IL-1β), Interleukin-6 (IL-6), and tumor necrosis factor-alpha (TNF-α) have been associated with the presence of various bacterial infections, including periodontitis [6]. Rogers et al. [7] found that induction of periodontitis in an animal model can be induced by an increased inflammatory infiltrate, significantly increased immunostaining for IL-1β, and more TRAP-positive osteoclasts and led to increased bone loss. They also found that IL-6, which is pro-inflammatory, also contributed to periodontitis induced bone resorption. Decreased inflammatory response and bone destruction were also observed when TNF-α was inhibited using a soluble antagonist [8].

Polyunsaturated fatty acids (PUFAs) are fatty acids with more than 1 carbon-carbon double bond. Both omega-3 and omega-6 have been found to have anti-inflammatory effects through the production of nuclear transcription factors, enzymes, and cytokines in human cells [9]. Omega-3 from marine sources, such as eicosapentaenoic acid (EPA) and
docosahexaenoic acid (DHA), and vegetable sources, such as linolenic acid (LNA), which includes alpha-linolenic acid (ALA) have all been shown to have anti-inflammatory properties\cite{10,11}. In experimental models, topical application of bioactive products derived from omega-3 fatty acids (including EPA and DHA) confer protection against inflammation and bone loss associated with periodontitis \cite{12}. In humans study, Rosenstein et al found that when 30 subjects with periodontitis received a systemic therapy of EPA, they showed a trend towards a decrease in probing depth \cite{13}.

Periodontal disease is an immuno-inflammatory condition that affects almost 90% of the population \cite{14} and will continue to increase with the growing aging population \cite{15,16}. The dietary therapy, if effective, might be a less expensive and safer method for the treatment of periodontitis. Few natural interventions are of proven efficacy in the treatment of periodontitis. For this reason, the present study was aimed to investigate and evaluate the effects of omega-3 polyunsaturated fatty acid (60 mg/kg EPA) \cite{17} on ligation induced periodontitis in rats through the immunological analysis for serum level of IL-1β, IL-6, and TNF-α.

**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 the 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 standard rat chow and allowed to drink water ad libitum. The research project was approved by the Research Ethics Committee at the College of Dentistry, Hawler Medical University under the protocol.

**Induction of experimental periodontitis:** The upper incisor was chosen because the induced periodontal disease occurs more rapidly in that location due to the porosity of the spongy bone in the maxilla. The rats were anesthetized by intraperitoneal administration of ketamine (0.5 ml/kg b.w.) and the animals were placed on a proper operating table, which allowed open-mouth maintenance of the rats to facilitate access to the teeth. After that, 3.0 sterile black braided silk threads were placed around the cervix of the 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. This ligature acts as a gingival irritant for 14 days and promoted the accumulation of plaque and subsequently the development of periodontal disease \cite{18}.

**Experimental design for biochemical analysis:** One hundred and twenty five animals were used in the study. Twenty five animals (normal control), and one hundred animals with the induced periodontitis were randomly assigned into four experimental groups (25 animals each) according to the treatment used:

- **NC/W:** Normal control+ Distilled water treatment group.
- **P/W:** Ligature-induced periodontitis + Distilled water treatment group.
- **P/SRP:** Ligature-induced periodontitis + Scaling and root planing treatment group.
- **P/EPA:** Ligature-induced periodontitis + 60 mg /kg EPA treatment group.
- **P/SRP+EPA:** Ligature-induced periodontitis + Scaling and root planing +EPA treatment group.

The ligatures were removed on day 14 (day zero) and the different types of treatment were restarted directly. The treatment by intragastric gavage of distilled water or EPA were done once time daily for two weeks.

**Blood sampling for immunological assays:** Blood was taken by cardiac puncture, three hours after ligation removal (day zero), 24 hours, three days, one week and two weeks for immunological analysis. First, by anesthetizing the rat, placed on its back, the left index finger was placed at the level of the lowest ribs without applying any pressure, and the heart is located one cm above this point, slightly to the right \cite{20}. The five ml syringe was held at a 45-degree angle and the needle was inserted between two ribs, the plunger was pulled on slowly to fill the syringe. Then the samples were placed in non-heparinized tubes, allowed to clot for two hours at room temperature and centrifuged at 3000 rpm for 10 min at 4°C, and then the supernatant was collected. The serum was frozen at -20°C and used within one month to avoid loss of bioactivity and contamination. Serum IL-1β, IL-6, and

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TNF-α concentrations were measured using rat-specific enzyme-linked immunosorbent assay kits (K0331212 Koma Biotech INC, K0331229 MY Biosource, K0331196 Koma Biotech INC, USA respectively) according to the manufacturer’s instructions.

**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; the Mann-Whitney U test was performed to assess individual pair of groups for statistically significant finding. A p-value less than or equal to 0.05 was considered statistically significant.

**Results**

**Serum IL-1β**

Statistical analysis showed significant increase in serum IL-1β level (p<0.05) in the P/W, P/SRP, and P/EPA treatment groups in relation with the NC/W treatment groups in all durations studied, but the P/SRP+EPA treatment group showed significant difference (p<0.05) only after three and 24 hours. A non-significant difference (p>0.05) between the P/SRP+EPA treatment group and the NC/W treatment group regarding the serum IL-1β level was seen after three days, one and two weeks duration (Table-1).

The P/SRP treatment group showed a significant decrease (p<0.05) in serum IL-1β in the first and second weeks in comparison with the P/W treatment group. The P/EPA treatment group showed a significant decrease (p<0.05) in serum IL-1β after three days, one week, and two weeks in comparison with the P/W treatment group. The P/SRP+EPA treatment group showed a significant decrease (p<0.05) in serum IL-1β after 24 hours, three days, one week and two weeks in comparison with the P/W treatment group. No significant difference was seen between P/SRP and P/EPA treatment groups in all the durations studied.

The P/SRP+EPA treatment group showed a non-significant decrease (p>0.05) in serum IL-1β after three hours in comparison with the P/SRP treatment groups, but this difference was significant (p<0.05) after 24 hours and three days, and become non-significant after one and two weeks (p>0.05). The P/SRP+EPA treatment group showed non-significant decrease (p>0.05) in serum IL-1β after three hours, but this difference was significant (p<0.05) after 24 hours, and become non-significant (p>0.05) after three days, one and two weeks in comparison with the P/EPA treatment group (Table -2).

<table>
<thead>
<tr>
<th>Groups</th>
<th>IL-1β (pg/ml)</th>
<th>P-Value</th>
<th>P-Value</th>
<th>P-Value</th>
<th>P-Value</th>
<th>P-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>NC/W</strong></td>
<td><strong>P/W</strong></td>
<td>2.69±1.07</td>
<td>6.1±1.24</td>
<td>0.021</td>
<td>2.6±0.380</td>
<td>6.2±1.82</td>
</tr>
<tr>
<td>NC/W</td>
<td>P/SRP</td>
<td>2.69±1.07</td>
<td>5.9±1.14</td>
<td>0.021</td>
<td>2.6±0.380</td>
<td>6.1±1.252</td>
</tr>
<tr>
<td>NC/W</td>
<td>P/EPA</td>
<td>2.69±1.07</td>
<td>6.02±1.22</td>
<td>0.021</td>
<td>2.6±0.380</td>
<td>5.82±1.10</td>
</tr>
</tbody>
</table>

http://doi.org/10.36295/ASRO.2020.231127
Table 1: Serum interleukin-1β (mean± standard deviation) in Normal Control/Water treatment group in relation with all studied groups.

<table>
<thead>
<tr>
<th>Groups</th>
<th>IL-1β (pg/ml)</th>
<th>3 hours</th>
<th>P-value</th>
<th>24 hours</th>
<th>P-value</th>
<th>3 days</th>
<th>P-value</th>
<th>One Week</th>
<th>P-value</th>
<th>Two Weeks</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>P/W</td>
<td>6.1±1.24</td>
<td>0.67</td>
<td>6.2±1.82</td>
<td>0.104</td>
<td>6.34±0.96</td>
<td>0.528</td>
<td>5.94±0.563</td>
<td>0.028</td>
<td>5.86±0.581</td>
<td>0.021</td>
<td></td>
</tr>
<tr>
<td>P/SRP</td>
<td>5.9±1.14</td>
<td>0.75</td>
<td>5.1±1.252</td>
<td>0.603</td>
<td>5.74±0.31</td>
<td>0.173</td>
<td>4.6±0.547</td>
<td>0.674</td>
<td>4.2±0.836</td>
<td>0.920</td>
<td></td>
</tr>
<tr>
<td>P/EPA</td>
<td>6.02±1.22</td>
<td>0.833</td>
<td>6.1±1.252</td>
<td>0.036</td>
<td>5.74±0.31</td>
<td>0.047</td>
<td>4.6±0.547</td>
<td>0.360</td>
<td>4.2±0.836</td>
<td>0.144</td>
<td></td>
</tr>
<tr>
<td>P/SRP+EPA</td>
<td>5.8±1.09</td>
<td>0.528</td>
<td>5.8±1.10</td>
<td>0.036</td>
<td>4.6±0.54</td>
<td>0.173</td>
<td>4.4±0.547</td>
<td>0.060</td>
<td>4.1±0.835</td>
<td>0.144</td>
<td></td>
</tr>
</tbody>
</table>

Table 2: The relation of serum IL-1β (mean± standard deviation) between different studied treatments groups

Serum IL-6:
Statistical analysis showed significant increase in serum IL-6 (p<0.05) in the P/W, P/SRP, and P/EPA treatment groups in relation with the NC/W treatment group in all durations studied, but the P/SRP+EPA treatment group showed a significant difference after three hours, 24 hours, and three days. A non-significant difference (p>0.05) between the P/SRP+EPA treatment group and the NC/W treatment group regarding the serum IL-1 6 level was seen after one and two weeks duration (Table-3).

The P/SRP and the P/EPA treatment groups showed a significant decrease (p<0.05) in serum IL-6 in the second week in comparison with the P/W treatment group. The P/SRP+EPA treatment group showed a significant decrease (p<0.05) in serum IL-6 in all duration studied in comparison with the P/W treatment group. No significant difference (p>0.05) was seen between P/SRP and P/EPA treatment groups in all the durations studied. The P/SRP+EPA treatment group showed a significant decrease (p<0.05) in serum IL-6 in all durations studied in comparison with the P/SRP and P/EPA treatment groups (Table -4).

Table 3: Serum interleukin-6 (mean± standard deviation) in Normal Control/Water treatment group in relation with all studied groups.

<table>
<thead>
<tr>
<th>Groups</th>
<th>IL-6 (pg/ml)</th>
<th>3 hours</th>
<th>P-value</th>
<th>24 hours</th>
<th>P-value</th>
<th>3 days</th>
<th>P-value</th>
<th>One Week</th>
<th>P-value</th>
<th>Two Weeks</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>P/W</td>
<td>6.1±1.24</td>
<td>0.67</td>
<td>6.2±1.82</td>
<td>0.104</td>
<td>6.34±0.96</td>
<td>0.528</td>
<td>5.94±0.563</td>
<td>0.028</td>
<td>5.86±0.581</td>
<td>0.021</td>
<td></td>
</tr>
<tr>
<td>P/SRP</td>
<td>5.9±1.14</td>
<td>0.75</td>
<td>5.1±1.252</td>
<td>0.603</td>
<td>5.74±0.31</td>
<td>0.173</td>
<td>4.6±0.547</td>
<td>0.674</td>
<td>4.2±0.836</td>
<td>0.920</td>
<td></td>
</tr>
<tr>
<td>P/EPA</td>
<td>6.02±1.22</td>
<td>0.833</td>
<td>6.1±1.252</td>
<td>0.036</td>
<td>5.74±0.31</td>
<td>0.047</td>
<td>4.6±0.547</td>
<td>0.360</td>
<td>4.2±0.836</td>
<td>0.144</td>
<td></td>
</tr>
<tr>
<td>P/SRP+EPA</td>
<td>5.8±1.09</td>
<td>0.528</td>
<td>5.8±1.10</td>
<td>0.036</td>
<td>4.6±0.54</td>
<td>0.173</td>
<td>4.4±0.547</td>
<td>0.060</td>
<td>4.1±0.835</td>
<td>0.144</td>
<td></td>
</tr>
</tbody>
</table>

Statistical analysis showed significant increase in serum TNF-α (p<0.05) in the P/W, P/SRP, and P/EPA treatment groups in relation with the NC/W treatment group in all durations studied, but the P/SRP+EPA treatment group showed a significant difference (p<0.05) only after three and 24 hours. A non-significant difference (p>0.05) between the P/SRP+EPA treatment group and the NC/W treatment group regarding the serum TNF-α level was seen after three days, one and two weeks duration (Table-5).

The P/SRP, P/EPA, and P/SRP+EPA treatment groups showed a significant decrease (p<0.05) in TNF-α in all duration studied in comparison with the P/W treatment group. No significant difference (p>0.05) was seen between P/SRP and P/EPA treatment groups in all the durations studied. The P/SRP+EPA treatment group showed a significant decrease (p<0.05) in serum TNF-α in all duration studied in comparison with the P/SRP treatment group.

Table -4: The relation of serum IL-6 (mean± standard deviation) between different studied treatments groups.

<table>
<thead>
<tr>
<th>Groups</th>
<th>3 hours</th>
<th>P-value</th>
<th>24 hours</th>
<th>P-value</th>
<th>3 days</th>
<th>P-value</th>
<th>One Week</th>
<th>P-value</th>
<th>Two Weeks</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>P/W</td>
<td>24±3.162</td>
<td>0.528</td>
<td>23.8±2.863</td>
<td>0.528</td>
<td>23.4±2.408</td>
<td>0.674</td>
<td>21.2±0.836</td>
<td>0.528</td>
<td>20.4±1.140</td>
<td>0.021</td>
</tr>
<tr>
<td>P/SRP</td>
<td>25.6±2.607</td>
<td>0.313</td>
<td>25.2±1.788</td>
<td>0.016</td>
<td>25.6±3.577</td>
<td>1.0</td>
<td>21.2±0.836</td>
<td>0.116</td>
<td>20.4±1.140</td>
<td>0.021</td>
</tr>
<tr>
<td>P/EPA</td>
<td>23.2±2.280</td>
<td>0.250</td>
<td>25.2±1.788</td>
<td>1.0</td>
<td>23.8±0.447</td>
<td>0.400</td>
<td>20.8±0.836</td>
<td>0.298</td>
<td>17.8±1.303</td>
<td>1.0</td>
</tr>
<tr>
<td>P/SRP/EPA</td>
<td>25.6±2.607</td>
<td>0.021</td>
<td>25.2±1.788</td>
<td>0.012</td>
<td>25.6±3.577</td>
<td>0.012</td>
<td>20.8±0.836</td>
<td>0.012</td>
<td>17.8±1.303</td>
<td>0.012</td>
</tr>
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<td>25.6±2.607</td>
<td>0.016</td>
<td>25.2±1.788</td>
<td>0.012</td>
<td>25.6±3.577</td>
<td>0.012</td>
<td>20.8±0.836</td>
<td>0.012</td>
<td>17.8±1.303</td>
<td>0.012</td>
</tr>
<tr>
<td>P/EPA</td>
<td>23.2±2.280</td>
<td>0.021</td>
<td>25.2±1.788</td>
<td>0.012</td>
<td>23.8±0.447</td>
<td>0.012</td>
<td>19.8±1.303</td>
<td>0.012</td>
<td>17.6±1.673</td>
<td>0.016</td>
</tr>
<tr>
<td>P/SRP/EPA</td>
<td>25.6±2.607</td>
<td>0.016</td>
<td>25.2±1.788</td>
<td>0.012</td>
<td>23.8±0.447</td>
<td>0.012</td>
<td>19.8±1.303</td>
<td>0.012</td>
<td>17.6±1.673</td>
<td>0.016</td>
</tr>
</tbody>
</table>

Serum TNF-α
Statistical analysis showed significant increase in serum TNF-α (p<0.05) in the P/W, P/SRP, and P/EPA treatment groups in relation with the NC/W treatment group in all durations studied, but the P/SRP+EPA treatment group showed a significant difference (p<0.05) only after three and 24 hours. A non-significant difference (p>0.05) between the P/SRP+EPA treatment group and the NC/W treatment group regarding the serum TNF-α level was seen after three days, one and two weeks duration (Table-5).

The P/SRP, P/EPA, and P/SRP+EPA treatment groups showed a significant decrease (p<0.05) in TNF-α in all duration studied in comparison with the P/W treatment group. No significant difference (p>0.05) was seen between P/SRP and P/EPA treatment groups in all the durations studied. The P/SRP+EPA treatment group showed a significant decrease (p<0.05) in serum TNF-α in all duration studied in comparison with the P/SRP treatment group.
But the P/SRP+EPA treatment group showed a non-significant decrease (p>0.05) in serum TNF-α after three hours, and a significant decrease in the other durations studied in comparison with the P/EPA treatment groups (Table -6).

Table -5: Serum TNF-α (mean± standard deviation) in Normal Control/Water treatment group in relation with all studied groups.

<table>
<thead>
<tr>
<th>Groups</th>
<th>TNF-α (pg/ml)</th>
<th>3 hours</th>
<th>P-value</th>
<th>24 hours</th>
<th>P-value</th>
<th>3 days</th>
<th>P-value</th>
<th>One Week</th>
<th>P-value</th>
<th>Two Weeks</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>NC/W P/W</td>
<td>11.66±0.499</td>
<td>0.012</td>
<td>11.466±0.795</td>
<td>0.012</td>
<td>10.92±0.759</td>
<td>0.012</td>
<td>11.12±1.347</td>
<td>0.012</td>
<td>10.82±1.028</td>
<td>0.012</td>
<td></td>
</tr>
<tr>
<td>NC/W P/SRP</td>
<td>11.66±0.499</td>
<td>0.012</td>
<td>11.466±0.795</td>
<td>0.012</td>
<td>10.92±0.759</td>
<td>0.012</td>
<td>11.12±1.347</td>
<td>0.012</td>
<td>10.82±1.028</td>
<td>0.012</td>
<td></td>
</tr>
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<td>10.92±0.759</td>
<td>0.012</td>
<td>11.12±1.347</td>
<td>0.012</td>
<td>10.82±1.028</td>
<td>0.012</td>
<td></td>
</tr>
<tr>
<td>NC/W P/SRP+EPA</td>
<td>11.66±0.499</td>
<td>0.012</td>
<td>11.466±0.795</td>
<td>0.012</td>
<td>10.92±0.759</td>
<td>0.012</td>
<td>11.12±1.347</td>
<td>0.012</td>
<td>10.82±1.028</td>
<td>0.012</td>
<td></td>
</tr>
</tbody>
</table>

Table -6: The relation of serum TNF-α (mean± standard deviation) between different studied treatments groups.

<table>
<thead>
<tr>
<th>Groups</th>
<th>TNF-α (pg/ml)</th>
<th>3 hours</th>
<th>P-value</th>
<th>24 hours</th>
<th>P-value</th>
<th>3 days</th>
<th>P-value</th>
<th>One Week</th>
<th>P-value</th>
<th>Two Weeks</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>P/W P/SRP</td>
<td>18.3±0.393</td>
<td>0.012</td>
<td>17.9±0.552</td>
<td>0.012</td>
<td>17.7±0.839</td>
<td>0.012</td>
<td>17.5±0.595</td>
<td>0.012</td>
<td>17.1±0.981</td>
<td>14.5±1.027</td>
<td></td>
</tr>
<tr>
<td>P/W P/EPA</td>
<td>18.3±0.393</td>
<td>0.012</td>
<td>17.9±0.552</td>
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<td>17.7±0.839</td>
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<td>17.5±0.595</td>
<td>0.012</td>
<td>17.1±0.981</td>
<td>14.0±0.663</td>
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<tr>
<td>P/W P/SRP/EPA</td>
<td>18.3±0.393</td>
<td>0.012</td>
<td>17.9±0.552</td>
<td>0.012</td>
<td>17.7±0.839</td>
<td>0.012</td>
<td>17.5±0.595</td>
<td>0.012</td>
<td>17.1±0.981</td>
<td>15.8±0.825</td>
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<td>0.211</td>
<td>15.1±0.710</td>
<td>0.012</td>
<td>14.9±1.002</td>
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<td>14.7±1.206</td>
<td>0.465</td>
<td>14.5±1.027</td>
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<tr>
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<td>14.5±1.027</td>
<td>11.5±0.825</td>
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<td>0.600</td>
<td>14.7±1.002</td>
<td>0.012</td>
<td>14.4±0.897</td>
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<td>14.0±0.663</td>
<td>11.5±0.825</td>
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Discussion
Periodontitis is an inflammatory reaction that is initiated as a response to the presence of periodontal pathogens and their products which stimulate the cellular immune system to release cytokines and other mediators leading to the extinction of the inflammation throughout the gingival tissues reaching the alveolar bone [21, 22].

The effect of periodontitis on rat’s serum IL-1β, IL-6, and TNF-α
The present study showed that periodontitis causes a significant increase in IL-1β, IL-6, and TNF-α in all durations studied in comparison with the NC/W treatment group. Koseet al, found that the serum IL-1β, IL-6, and TNF-α levels in normal control rats were (4.5 ± 2.1, 13.4 ± 4.6, and 44.4 ± 8.6 pg/ml respectively), and these levels were increased significantly in the experimental periodontitis group to (7.9 ± 3.6, 22.2 ± 8.1, and 61.1 ± 10.2 pg/ml respectively) after 28 days from ligation removal compared to the normal control group [23]. Görskia and his colleagues observed a clear relationship between clinical parameters and IL-1β, and TNF-α concentration within serum samples of severe chronic periodontitis patients and suggested that they have an important role in the initiation and progression of periodontal disease [21]. Raunio et al, and Haba et al, had demonstrated high levels of serum IL-6 in patients with periodontitis when compared with unaffected control populations [24, 25]. Andrukhov et al 26 also observed the presence of a positive association between serum TNF-α level and periodontal inflammation [26].

These results disagree with that of Al-Rassam et al, study, they found no significant differences in serum IL-1β, IL-6, and TNF-α level between periodontitis and control groups [27], and Buduneliet al, study, in which they found that the serum IL-1β levels were very close to or below the minimum detection limit of the assay kit in the saline control and LPS injected rats and explained by the fact that the effect of E. coli LPS was localized to the gingiva rather than being reflected in circulation, or that serum levels of this proinflammatory cytokine had subsided [28]. Vahabi et al found that there were no correlations between cytokine concentrations and clinical parameters [29].

**The effect of SRP on serum IL-1β, IL-6, and TNF-α in rats with periodontitis**
The present study showed a significant (p<0.05) differences present between the serum IL-1β, IL-6, and TNF-α of the P/SRP group and that of NC/W in all durations studied. SRP causes a significant decrease in IL-1β in the first and second weeks, IL-6 in the second weeks, and TNF-α in all durations studied in comparison with the P/W treatment group. Wang et al found that after the commencement of the initial therapy by SRP, the serum concentration of the cytokines may be reduced significantly [30]. Erdemiret al, also found that the establishment of initial periodontal therapy by mechanical treatment leads to improved clinical status and the TNF-α values were lower than those recorded on initial examination [31]. Ide et al found that periodontitis patients undergoing an episode of subgingival scaling show a significant elevation in circulating TNF-α and IL-6 within the first two hours and this may be significant in terms of the relationship between periodontal disease and bacteremia. They also found a significant correlation between levels of IL-6 and TNF—α and periodontitis [32].

These results disagree with that of Buduneliet al, and Ide et al, studies, they found that the treatment by SRP can improve plaque and bleeding scores and reduce probing depth significantly in subjects with periodontitis, but no significant changes in the levels of IL-1B, IL-6, and TNF--α were seen [28,33].

**The effect of EPA on serum IL-1β, IL-6, and TNF-α in rats with periodontitis**
The present study showed a significant (p<0.05) differences present between the serum IL-1β, IL-6, and TNF-α of the P/EPA treatment group and that of NC/W in all durations studied. EPA causes a significant decrease in IL-1β after three days, first and second weeks, IL-6 after two weeks, and TNF-α in all durations studied in comparison with the P/W treatment group. The treatment by SRP with the EPA causes a significant decrease in serum IL-1β after 24 hours, three days, one and two weeks, and a significant decrease in serum IL-6, and TNF-α in all durations studied in comparison with the P/W treatment group.

Araghizadeh et al study showed significantly lower levels of IL-1β and TNF-α, in the treatment group gavaged by omega-3 compared with the LPS injected group which was treated oral gavage of saline [17]. Moreover, the previous study has shown a decreased production of inflammatory cytokines, such as IL-6, and TNF-α is due to reduced leukocyte chemotaxis in rats given EPA [34]. Rosenberg et al trial showed a significant decrease in probing depth in patients receiving EPA alone [35]. Naqvi et al also found that omega-3 intake, particularly EPA, is inversely associated with periodontitis in the US population [36]. The beneficial effect of EPA may be due to its antibacterial activity against various oral pathogens, including Streptococcus mutans, Candida albicans, Aggregatibacter actinomycetemcomitans, Fusobacterium nucleatum, and Porphyromonas gingivalis and anti-
inflammatory effects, they can form several potent anti-inflammatory lipid mediators like Resolvins and protectins[37, 38].

Kang and Weylandt found that omega-3 fatty acids inhibit the formation of omega-6 fatty acids-derived pro-inflammatory eicosanoids (e.g. PGE2 and LTB4) [39]. Omega-3 has been found to have anti-inflammatory effects through the production of nuclear transcription factors, enzymes and cytokines in human cells [40]. EPA increased levels of peroxisome proliferator-activated receptor-gamma (PPAR-γ) and reduced production of the pro-inflammatory cytokine interleukin-6 [40]. Furthermore, Omega-3s have been found in animal models of periodontitis to be substrates for neutrophil production of resolvins and protectins, which appear central to the resolution of inflammation [41]. Omega-3 may have a stabilizing effect on collagen fiber, and a modulating effect on destruction of gingival connective tissue, by decreasing in levels of IL-1β and TNF-α which can induce the production of matrix metalloproteinase (MMPs) as well as the destruction of connective tissue [42], and lead to a decreased production of PGE2 metabolites [43] and decrease osteoclastic activity [44].

These results disagree with that of Araghizadeh et al study; they found that the level of IL-1β in the treatment group was similar to that in the positive control group which was orally gavage by saline [17]. Conversely, the results of Vardar - Şengüçil et al indicated a significant increase in the level of IL-1β in rats that consumed omega-3 fish oil compared with those in the positive control group [38]. This discrepancy might be due to the different methods of determining IL-1β in tissue and serum, and differences in omega-3 dosage in the two studies. In conclusion, data from our study showing that the treatment by SRP with the EPA may have a potent immune modulator effect and can regulate inflammatory and immunologic reactions.

References


