An in vitro study on compatibility action of flaxseed oil on periodontal ligament cells

Vidya Shri. S1, R. Gayathri2*, V. Vishnu Priya2

INTRODUCTION

Flax or Linum usitatissimum is grown for both dietary purposes and extraction of its oil. Flaxseed oil is a rich source of fatty acids. It contains unsaturated fatty acids, essential omega-3 fatty acids, and the essential omega-6 fatty acid-linolenic acid. Nothing flaxseeds and flax oil contain an abundant amount of omega-3 fatty acids. Thus, for vegetarians who strictly adhere to plant food, flaxseeds, and flax oils are a good source of omega-3.

The periodontal ligament occupies the periodontal space, which is located between the cementum and the periodontal surface of the alveolar bone and extends coronally to the most apical part of the lamina propria of the gingiva. Periodontitis is the inflammatory disease of the periodontium caused by specific microorganisms, which results in progressive destruction of the periodontal ligament and alveolar bone with periodontal pocket formation, gingival recession, or both. Periodontitis occurs as a result of the immunoinflammatory response. Inflammation can be seen as a normal defense mechanism that protects the host from infection and other harmful occurrences.

Proper dietary intake of omega-3 polyunsaturated fatty acid increases tissue concentrations of fatty acids that reduce the inflammation. Use of omega-3 in periodontal inflammations can be seen as a viable treatment.

MATERIALS AND METHODS

Sources of Chemicals

Flaxseed oil was purchased from Hakim Chichi Pharma, Surat, Gujarat. Analytical grade chemicals and reagents of Qualigens and S.D. Fine Chemicals were used in the analysis.

Cell Culture

The periodontal ligament (PDL) cells were cultured in a humidified atmosphere at 37°C in the cell growth Dulbecco’s Modified Eagle Medium (DMEM) medium with 10% fetal bovine serum, L-glutamine, 1% penicillin

ABSTRACT

Introduction: Flaxseed oil is being heavily promoted as an alternative to fish oil. Flaxseed oil contains plant-based omega-3 fats. A diet low in carbohydrates, rich in omega-3 fatty acids, rich in Vitamins C and D and rich in fibers can significantly reduce gingival and periodontal inflammation. Materials and Methods: (1) Source of chemicals: Flaxseed oil was purchased from Hakim Chichi Pharma, Surat, Gujarat. (2) Cell culture: The periodontal ligament (PDL) cells were cultured in a humidified atmosphere at 37°C in the appropriate medium. (3) Bromodeoxyuridine proliferation assay: The bromodeoxyuridine proliferation assay was done using the appropriate chemicals. Results: Proliferation of periodontal ligament cells increased with increasing concentration of flaxseed oil in a dose-dependent manner. Integrin was taken a positive control. Conclusion: PDL cell proliferation is essential for wound healing by inducing the growth of immature PDL cells and that, in turn, accelerates periodontal regeneration. Thus, induction of proliferation of PDL cells by flaxseed oil was analyzed.

KEY WORDS: Compatibility, Flaxseed oil, Periodontal ligament cell proliferation
(100 U/ml), and streptomycin (100 μg/ml) at 37°C in a humidified CO₂ (5%) chamber, and 95% air. The cells were detached using 0.25% ethylenediaminetetraacetic acid trypsin. Neutralization of the trypsin was achieved using DMEM containing 10% FBS and PSGF, and cells were mechanically separated using a pipette. There were 96-well plastic culture plates filled with 200 μl of medium containing in each well. The plates were then incubated at 37°C in a humidified atmosphere containing 5% CO₂ and 95% air for 24 h to permit attachment of the cells to the plates.

**Bromodeoxyuridine Proliferation Assay**

The cells were plated in a 96 well plate and incubated with respective test substances. The typical seed cell number is 2500–100,000 cells per well depending on the cell growth rate and the typical incubation time is 1–72 h. It is cultured in 150 μl of medium supplemented with 10% FBS. After 24 h, flaxseed oil based on varying concentration was added into the culture medium and cultured for 2 more days. Then, prepared ×10 BrdU solution was added to the plate wells for a final ×1 concentration. The cells were then placed in the incubator. The typical incubation time is 1–24 h.

After incubation, the medium was removed. For suspension of cells, the plate was centrifuged at 300 g for 10 min. Then, fixing solution was added, and the plates were kept at room temperature for 30 min. 100 μl/well of detection antibody solution was added and the plate was kept at room temperature for 1 h. Then, the solution was removed, and the plate was washed 3 times with ×1 wash buffer.

100 μl of TMB substrate was added and incubation was done for 30 min at room temperature. Finally, 100 μl STOP solution was added. Absorbance was read at 400 nm.

**RESULTS**

This graph depicts the cell proliferation changes in treated cells.

**DISCUSSION**

The proliferation of periodontal ligament cells was not seen in cells which were not treated with flaxseed oil [Table 1 and Figure 1]. On treating with flaxseed oil, proliferation was observed in a dose-dependent manner, i.e., percentage proliferation increases with the increase of the concentration of flaxseed oil.

Integrins are transmembrane receptors that facilitate cell-extracellular matrix adhesion. Here, the flaxseed oil is seen as the extracellular matrix. Thus, in addition of integrity’s in a 1:500 concentration, maximum proliferation is observed which is around 256%. Here, integrity is taken as a positive control.

**Table 1: Bromodeoxyuridine proliferation assay**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Concentration (µM)</th>
<th>% Proliferation</th>
</tr>
</thead>
<tbody>
<tr>
<td>PDL untreated cells</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Flaxseed oil 25</td>
<td></td>
<td>105</td>
</tr>
<tr>
<td>Flaxseed oil 50</td>
<td></td>
<td>116</td>
</tr>
<tr>
<td>Flaxseed oil 100</td>
<td></td>
<td>149</td>
</tr>
<tr>
<td>Flaxseed oil 200</td>
<td></td>
<td>229</td>
</tr>
<tr>
<td>Integrin 1:500</td>
<td></td>
<td>256</td>
</tr>
</tbody>
</table>

**CONCLUSION**

PDL cell proliferation is essential for wound healing by inducing growth of immature PDL cells and that, in turn, accelerates periodontal regeneration. Dietary control using fatty acids in the form of flaxseeds and its oil can help in the reduction of periodontal inflammation. The subject of plant-based omega-3 for periodontal therapy is still in its nascent stage. Thus, by this study, induction of proliferation of PDL cells by flaxseed oil was analyzed.

**REFERENCES**


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