Effect of 10% kepok banana peel extract gel (musa paradisiaca linn. kepok) on periodontal regeneration process of Wistar rat

Suryono, Rezmelia Sari, Felia R. Wulandari, Hefy Andini, Jeanette Widjaja, Trisna D. Nugraheni

Abstract

Objective: The aim of our study was to determine the effect of application of 10% Kepok banana peel extract gel (Musa paradisiaca linn. Kepok) on the periodontal regeneration process in periodontal healing of Wistar rat (Rattus norvegicus).

Material and Methods: Forty-eight Wistar rats were divided into 3 groups, namely 16 rats as the positive control group (Aloclair gel), 16 rats as the negative control group (CMC-Na gel 2%) and 16 rats as the treatment group (10% Kepok banana peel extract gel). The induction of periodontitis was performed using a modified ligation technique for 7 days with an additional injection of Aggregatibacter actinomycetemcomitans bacteria on the first day. Wistar rats were euthanized on days 1, 3, 5, 7, and 14 for histological analysis to evaluate the number of macrophages, lymphocytes, osteoclasts, osteoblasts and fibroblasts.

Results: Data analysis showed a significant difference in the cell numbers among the groups. A decreasing number of lymphocytes, macrophages, and osteoclasts could already be observed since the 1st day. The fibroblasts and osteoblasts of the treatment group already reached their peaks on the 7th day, faster than the negative control and positive control groups that reached their peaks on the 5th day.

Conclusion: The application of 10% Kepok banana peel extract gel (Musa paradisiaca linn. Kepok) caused a significant acceleration of the periodontal regeneration process in the periodontal healing of Wistar rat (Rattus norvegicus).

Keywords: Periodontal regeneration, Periodontitis, Wound healing, Wistar rats

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Introduction

Periodontitis is an inflammation of the teeth’ supporting tissues, resulting in progressive damage to the periodontal ligament and alveolar bone. Anaerobic gram-negative bacteria such as Aggregatibacter actinomycetemcomitans, Porphyromonas gingivalis, and Prevotella intermedia are the three most common microorganisms involved in periodontitis. Periodontitis shows histological changes in the form of infiltration of inflammatory cells such as macrophages, neutrophils, mast cells, and lymphocytes. Other than that, collagen damage in the periodontal ligament, apical movement of junctional epithelium, and osteoclastic alveolar resorption can also be found in periodontitis. The healing process of periodontitis is characterized by mitotic activity in the gingival epithelium and connective tissue in the periodontal ligament, new alveolar bone formation, and cementum deposition. This process involves several cells which are delivered by fibroblasts and osteoblasts.

The treatment of periodontitis includes surgical and non-surgical therapies followed by antimicrobial and anti-inflammatory drug administration to support post-operative periodontal regeneration. Plant-based treatments have high potential to work as adjunctive therapy to accelerate periodontal regeneration after periodontal therapy of periodontitis. Kepok banana peel (Musa paradisiaca linn. Kepok) contains active ingredients such as flavonoids, tannins, saponins, and gallic acid. These active ingredients have antioxidant, antibacterial, anti-inflammatory and analgesic activities. The amount of active ingredients and antioxidant activity in green (unripe) banana peel is more abundant than yellow (ripe) banana peel. This study was intended to determine the effect of application of 10% Kepok banana peel extract gel (Musa paradisiaca linn. Kepok) on the process of regenerating periodontal tissue on periodontal healing.

Material and Methods

Experimental Design

The study obtained an ethical clearance No. 001501/K-KEP/FKG-UGM/EC/2018. The study was conducted at LPPT Unit IV Universitas Gadjah Mada Yogyakarta. Forty-eight male Wistar rats (Rattus norvegicus) aged 2 months with an average weight of 150 - 200 grams were induced with periodontitis. The subjects were divided into 3 groups, namely 16 rats as a positive control group (Aloclair gel), 16 rats as a negative control group (CMC-Na gel 2%) and 16 rats as a treatment group (10% Kepok banana peel extract gel).

Preparation of 10% kepok banana peel extract gel

10% Kepok banana peel extract gel was made by mixing 10 grams of unripe kepok banana peel...
Table 1. Average number of cells in the treatment, positive control, and negative control groups

<table>
<thead>
<tr>
<th>Day</th>
<th>Macrophages</th>
<th>Lymphocytes</th>
<th>Osteoclasts</th>
<th>Osteoblasts</th>
<th>Fibroblasts</th>
</tr>
</thead>
<tbody>
<tr>
<td>Treatment Group</td>
<td>1</td>
<td>7.89±0.33</td>
<td>19.37±1.17</td>
<td>7.22±0.78</td>
<td>14.11±1.20</td>
</tr>
<tr>
<td>2</td>
<td>9.18±0.81</td>
<td>16.70±0.44</td>
<td>7.92±1.14</td>
<td>16.33±1.44</td>
<td>13.63±1.91</td>
</tr>
<tr>
<td>3</td>
<td>8.29±1.06</td>
<td>10.56±0.59</td>
<td>5.37±1.12</td>
<td>17.91±1.66</td>
<td>19.62±3.05</td>
</tr>
<tr>
<td>5</td>
<td>5.19±0.34</td>
<td>6.11±0.40</td>
<td>5.00±0.73</td>
<td>16.85±1.19</td>
<td>17.78±1.00</td>
</tr>
<tr>
<td>7</td>
<td>-</td>
<td>3.56±1.10</td>
<td>15.37±1.28</td>
<td>8.00±0.80</td>
<td></td>
</tr>
<tr>
<td>Positive Control Group</td>
<td>1</td>
<td>7.00±1.40</td>
<td>20.33±0.97</td>
<td>8.15±0.84</td>
<td>11.70±1.84</td>
</tr>
<tr>
<td>3</td>
<td>8.33±1.50</td>
<td>19.26±0.17</td>
<td>9.48±0.61</td>
<td>15.41±1.90</td>
<td>15.37±0.81</td>
</tr>
<tr>
<td>5</td>
<td>5.19±0.71</td>
<td>12.48±1.00</td>
<td>6.52±1.23</td>
<td>15.45±0.73</td>
<td>21.33±2.72</td>
</tr>
<tr>
<td>7</td>
<td>4.78±0.77</td>
<td>7.33±1.17</td>
<td>5.89±0.62</td>
<td>16.95±1.76</td>
<td>15.07±2.13</td>
</tr>
<tr>
<td>Negative Control Group</td>
<td>1</td>
<td>8.41±0.65</td>
<td>18.81±1.67</td>
<td>8.70±0.68</td>
<td>11.22±1.46</td>
</tr>
<tr>
<td>3</td>
<td>8.52±0.86</td>
<td>19.33±0.29</td>
<td>9.87±0.87</td>
<td>13.15±1.32</td>
<td>10.15±1.59</td>
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<tr>
<td>5</td>
<td>10.40±0.70</td>
<td>20.59±1.00</td>
<td>8.81±0.97</td>
<td>14.22±2.28</td>
<td>16.22±0.51</td>
</tr>
<tr>
<td>7</td>
<td>9.70±0.28</td>
<td>20.89±1.56</td>
<td>7.74±0.32</td>
<td>14.59±1.32</td>
<td>17.81±1.26</td>
</tr>
<tr>
<td>14</td>
<td>-</td>
<td>5.52±0.97</td>
<td>13.33±0.45</td>
<td>7.11±0.59</td>
<td></td>
</tr>
</tbody>
</table>

Sig.: Statistical significance (p<0.05).

Table 2. Result of Post-Hoc LSD test

<table>
<thead>
<tr>
<th>Day</th>
<th>Group</th>
<th>Macrophages</th>
<th>Lymphocytes</th>
<th>Osteoclasts</th>
<th>Osteoblasts</th>
<th>Fibroblasts</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 Negative-treatment group</td>
<td>0.476</td>
<td>0.505</td>
<td>0.042*</td>
<td>0.028*</td>
<td>0.004*</td>
<td></td>
</tr>
<tr>
<td>Positive-treatment group</td>
<td>0.227</td>
<td>0.253</td>
<td>0.193</td>
<td>0.050*</td>
<td>0.435</td>
<td></td>
</tr>
<tr>
<td>3 Negative-treatment group</td>
<td>0.365</td>
<td>0.004*</td>
<td>0.008*</td>
<td>0.011*</td>
<td>0.009*</td>
<td></td>
</tr>
<tr>
<td>Positive-treatment group</td>
<td>0.248</td>
<td>0.005*</td>
<td>0.032*</td>
<td>0.438</td>
<td>0.244</td>
<td></td>
</tr>
<tr>
<td>5 Negative-treatment group</td>
<td>0.007*</td>
<td>0.000*</td>
<td>0.000*</td>
<td>0.004*</td>
<td>0.005</td>
<td></td>
</tr>
<tr>
<td>Positive-treatment group</td>
<td>0.009*</td>
<td>0.028*</td>
<td>0.109</td>
<td>0.045*</td>
<td>0.420</td>
<td></td>
</tr>
<tr>
<td>7 Negative-treatment group</td>
<td>0.000*</td>
<td>0.000*</td>
<td>0.000*</td>
<td>0.000*</td>
<td>0.000*</td>
<td></td>
</tr>
<tr>
<td>Positive-treatment group</td>
<td>0.574</td>
<td>0.151</td>
<td>0.211</td>
<td>0.049</td>
<td>0.116</td>
<td></td>
</tr>
<tr>
<td>14 Negative-treatment group</td>
<td>0.008*</td>
<td>0.094</td>
<td>0.279</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Positive-treatment group</td>
<td>0.065</td>
<td>0.403</td>
<td>0.346</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Statistical significance (p<0.050)
Results

Figure 1 shows a histological specimen of Wistar rats’ mandibular specimen after hematoxylin eosin staining under 400x magnification. The application of 10% Kepok banana peel extract gel in the treatment group significantly decreased the inflammation of periodontal tissue in the treatment group compared to the negative control group. Fewer macrophages and lymphocytes were observed in the positive control and treatment group compared to the negative control group in table 1. Decreased inflammation was shown by significantly less number of macrophages on days 5 (p=0.007) and 7 (p=0.000), and lymphocytes on days 3 (p=0.004), 5 (p=0.000), and 7 (p=0.000) in the treatment group compared to the negative control group table 2.

The bone damage process had gradually subsided since the 1st day of 10% Kepok banana peel extract gel application. There were fewer osteoclasts observed in the positive control and treatment group compared to the negative control group table 1. The number of osteoclasts in the treatment group significantly decreased compared to the negative control group on days 1 (p=0.042), 3 (p=0.008), 5 (p=0.000), 7 (p=0.000), and 14 (p=0.008) table 2.

An increased rate of periodontal ligament regeneration and osteoblastic bone formation could be observed since the 1st day of 10% Kepok banana peel extract gel application. More fibroblasts and osteoblasts were observed in the positive control and treatment groups compared to the negative control group table 1. The number of osteoblasts in the treatment group significantly increased compared to that in the negative control group on days 1 (p=0.004) and 3 (p=0.003) table 2. On the other hand, the number of osteoblasts in the treatment group significantly increased compared to that in the negative control group on days 1 (p=0.020), 3 (p=0.011), and 5 (p=0.004) table 2. The fibroblasts and osteoblasts of the treatment group already reached their peak on the 5th day, faster than the negative control and positive control groups.

Figure 2. Proliferation of MC3T3 E1 cells on the first day, third day and seventh day at a concentration of 2 mg/ml; 4 mg/ml; 8 mg/ml; and control.

Figure 3. The average number of lymphocytes in the negative control, treatment and positive control groups.

Figure 4. The average number of osteoclast in the negative control, treatment and positive control groups.

Figure 5. The average number of osteoblasts in the negative control, treatment and positive control groups.

Statistical analysis

The statistical analysis was performed first with a normality test (Shapiro Wilk) to verify the normality of the data, followed by a homogeneity test (Levene’s Test) to confirm the homogeneity of the data. Two Way ANOVA test followed by Post-Hoc LSD test was used to analyze the significance of the number of macrophage, lymphocyte, osteoclast, osteoblast, and fibroblast values. Value of p< 0.05 was considered statistically significant.

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groups that reached their peak on the 7th day.

Figure 2 presents a decreasing number of macrophages on day 1 in the positive control and treatment groups. They reached their peak number on day 3. A decreasing number of macrophages from both groups could be observed starting on day 5. Meanwhile, the average number of macrophages in the negative control group showed an increasing number on day 3 and reached its highest number on day 5. A decreasing number could be observed starting day 7 onwards.

Figure 3 presents a decreasing number of lymphocytes in the positive control and treatment groups on days 1 and 7. Meanwhile, the lymphocytes in the negative control group showed an increasing number on days 1 and 5 and stayed constant until day 7.

Figure 4 presents an increasing number of osteoclasts that could be observed from days 1 and 3. Meanwhile, a decreasing number of osteoclasts could be observed from day 5 until 14. The average number of osteoclasts in the treatment group was the lowest among the 3 groups.

Figure 5 presents an increasing number of osteoblasts in the negative control and positive control groups from day 1 until day 5; they reached their peak on day 7 and decreased on day 14. Meanwhile, the osteoblasts in the treatment group increased on days 1 and 3, peaked on day 5, and decreased from day 7 onwards. Osteoblasts of alveolar bone were higher in the treatment group (10% Kepok banana peel extract gel) than in the positive and negative control groups.

Discussion

Kepok banana peel extract gel contains active ingredients such as flavonoid, saponin, tannin, and gallicatechin which act as antioxidants and anti-inflammation. Gallicatechin is a type of catechin flavonoid which is abundant in banana peels. Flavonoids can inhibit some pro-inflammatory enzymes, including cyclooxygenase, so the production of prostaglandin E2 (PGE2) reduces. Prostaglandin production causes vasodilation and increases vascular permeability.9 Vasoconstriction and a decrease in vascular permeability decrease the rate of leukocyte (macrophages and lymphocytes) migration.3 Flavonoids can reduce the expression of certain types of proinflammatory cytokines and chemokines such as tumour necrosis factor-alpha (TNFα), interleukin-1β (IL-1β), interleukin-6 (IL-6), interleukin-8 (IL-8) and monocyte-chemoattractant proteins-1 (MCP-1).6

The result of this study showed that the osteoblasts in the treatment group reached their peak number on day 5, faster than the osteoblasts in negative and positive control groups (day 7). A previous study showed that osteoblasts reached their highest number normally on day 7 and stayed constant until day 14. Meanwhile, the average number of osteoclasts was the lowest in the treatment group. This means 10% Kepok banana peel extract can reduce the process of osteoclastogenesis and increase osteoblastogenesis. A decreased activity of reactive oxygen species (ROS) and PGE2 and an increased expression of transforming growth factor-β (TGF-β) result in a decreased expression of the receptor activator of nuclear factor kappa-β ligand (RANKL) and macrophage-colony stimulating factor (MCSF) that play a role in osteoclast differentiation.11,12 Flavonoid enables the activation of bone morphogenetic protein (BMP) signalling pathway, which could further increase the expression of Runx-related main transcription factor 2 (Runx2). An increased Runx2 expression results in increased mesenchymal cells for bone formation, resulting in increased proliferation and differentiation of osteoblasts.13

This study showed that the fibroblasts in the treatment and positive control groups reached their peak on day 5, faster than the fibroblasts in the negative control group, which reached their peak on day 7. The average number of fibroblasts in the treatment group was higher than that in the positive control group. A previous study showed that on normal conditions, fibroblasts can be observed since day 3 and reached their peak on day 7.14 On the other hand, the macrophages and lymphocytes in the treatment and positive control groups decreased faster than in the negative control group. A previous study showed that inflammatory cells would decrease starting on day 7.15,16

Kepok banana peel consists of flavonoids, phenol, and tannin, which have antioxidant properties. They work by binding to unstable free radicals in order to reduce tissue damage caused by free radicals. Faster reduction in tissue damage causes faster activation of cells’ proliferative phase. Flavonoids act as an anti-inflammatory agent and affects fibroblast cell proliferation.17 Phenol is an antioxidant that can control and reduce lipid peroxidation, which results in a reduction in tissue damage. Membrane permeability and the amount of secretion of growth factors by macrophage increase due to the presence of saponin.18-20 Macrophage will secrete growth factors such as fibroblast growth factor (FGF), platelet-derived growth factor (PDGF), TGF-β, and epidermal growth factor (EGF) that can attract and stimulate more fibroblast proliferation into the lesion area.9

Conclusion

In this study, the application of 10% kepok
banana peel extract gel (Musa paradisiaca linn: Kepok) caused a significant acceleration of the periodontal regeneration process in the periodontal healing of Wistar rat (Rattus norvegicus) by decreasing the number of inflammatory cells (lymphocytes and macrophages), decreasing osteoclastic bone resorption, increasing osteo/hyalin proliferation.

Acknowledgement

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Conflicts of Interests

The authors report no conflict of interest.

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