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BANANA PEEL EXTRACT: HISTOMETRICAL AND MORPHOMETRICAL EVALUATION IN THE TREATMENT OF PERIODONTITIS USING ANIMAL MODELS

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Abstract: Periodontitis, characterized by inflammation in the periodontal tissues, is primarily driven by the interplay between bacterial pathogens and the host tissue. Key bacterial culprits in periodontal diseases include Porphyromonas gingivalis and Aggregatibacter actinomycetemcommitans, which release lipopolysaccharides (LPS) as toxins. LPS-induced destruction of periodontal tissues triggers the release of inflammatory cytokines like IL-1, IL-6, and TNF-α. The consequential tissue damage is a leading cause of tooth loss in adults, potentially impacting oral health-related quality of life.

The initial treatment for periodontitis typically involves scaling and root planning, often complemented by adjuvant therapies like local hyaluronic acid gel application. These adjuvant therapies play a crucial role in promoting healing processes by exhibiting anti-inflammatory and antibacterial properties, while also stimulating cell growth.

Periodontal tissue regeneration primarily relies on the migration of nearby stem cells to the damaged site, where they differentiate into specialized cells, ultimately replacing the damaged tissue. Understanding these mechanisms and therapies is vital for enhancing periodontitis treatment outcomes.

Keywords: Periodontitis, Inflammation, Bacterial pathogens, Adjuvant therapy, Tissue regeneration

Introduction

Periodontitis is inflammation located on periodontal tissue. Periodontitis occurs because of the interaction between the periodontal tissue and bacteria[1, 2]. The most bacteria for periodontal diseases are Porphyromonasgingivalis and Aggregatibacteractinomycetemcommitans, which produce toxin called lipopolysaccharide. Periodontal tissue destruction induced by lipopolysaccharide and activated the inflammatory cytokine such as IL-1, IL-6 and TNF-α[3]. Periodontal tissue destruction on periodontitis is the major cause of tooth loss on adult, this can be a problem because tooth loss may have bad implication on oral health-related quality of life[4]. The initial treatment performed for periodontitis are scaling and root planning, which followed by adjuvant or additional therapy such as local application of hyaluronic acid gel. Adjuvant therapy support and promote healing process which by its action as antinflammation, antibacterial and inducing cell growth.[5]. Periodontal tissue healing occurs when nearby stem cells migrate to the destructed tissue and differentiate into new specific cells and replace the destructed cells**.**

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Challenge in adjuvant the raphy availability

The obstacles in hyaluronic acid gel as adjuvant therapy for periodontitis is the unavailability in some country and high in price, thus make some scientist looking for new local natural resources which available with lower price.

Banana (Musa paradisiaca L.) peel waste

Banana (Musa paradisiaca L.) peel wastes usually either proceed into livestock feed or just thrown away. Banana peel waste is proposed as new local resource to substitute the hyaluronic acid gel as adjuvant therapy for periodontitis because banana peel extract contain active substances such as gallic acid, gallocatechin, flavonoid and saponin, thus make banana peel have beneficial properties such as ant inflammation, antibacterial and inducing cells growth[8, 9].

Material and Method

Materials

10% banana peel extract gel was obtained by maceration method then extracted using 96% ethanol. Sodium Carboxymethyl Cellulose (CMC-Na) was used as gelation agent, propyl paraben and aquadest added until reached the concentration of 10%. Hyaluronic acid gel was obtained from the Gengigel® which contain 0,2% hyaluronic acid. CMC-Na gel was obtained by mixing CMC-Na powder with aquadest until it reach the concentration 2%.

Experimental Design

Gingiva of maxillary molar was injected by 10^9 cfu/ml A. actinomycetemcomitans suspension in phosphat buffer saline once a day for five days to induce periodontitis. Fifty-seven of male Rattus norvegicus were divided in three groups. CMC-Na gel was given for the first group as negative control. Banana peel extract gel was applied for the second group, and 0,2% hyaluronic acid gel was applied for third group as positive control. Gel was applied topically on maxillary molar twice a day.

The rats were sacrificed on day 3, 5, 7, 14, 28Samples in the form of periodontal tissue was carried out on 3rd, 5th, 7th, 14th, and 28th in each group. Angiogenesis and collagen density were observe histologically using binocular microscope and Optilab® in 400x magnification. Alveolar crest height was observed histologically and morphometrically.

Data of angiogenesis, alveolar bone height and collagen density were taken from histopathologic counting and measuring under light microscope and Optilab®. To get angiogenesis data, we counted alveolar bone's blood vessel in haematoxillin eosin-dyed slide with Image Raster® software. Alveolar bone height were measured by drawing out a line from cemento enamel junction of first and second molar to apical with Image Raster® software. Besides, alveolar bone height were also measured morphometrically using Digimizer®software.The density of periodontal ligament's collagen was measured on Mallory trichrome dye with Image J programme.

The significance level establised for all analyses was 5%. To see influence of those three gels to angiogenesis, alveolar bone height, and collagen density, we performed Two Way Anova analysis and continued by Post Hoc Test with Least Significant Difference (LSD) method.

Results

The results of the study showed application of 10% banana peel extract gel increased angiogenesis, alveolar bone height in histometric and morphmetric method and collagen density.

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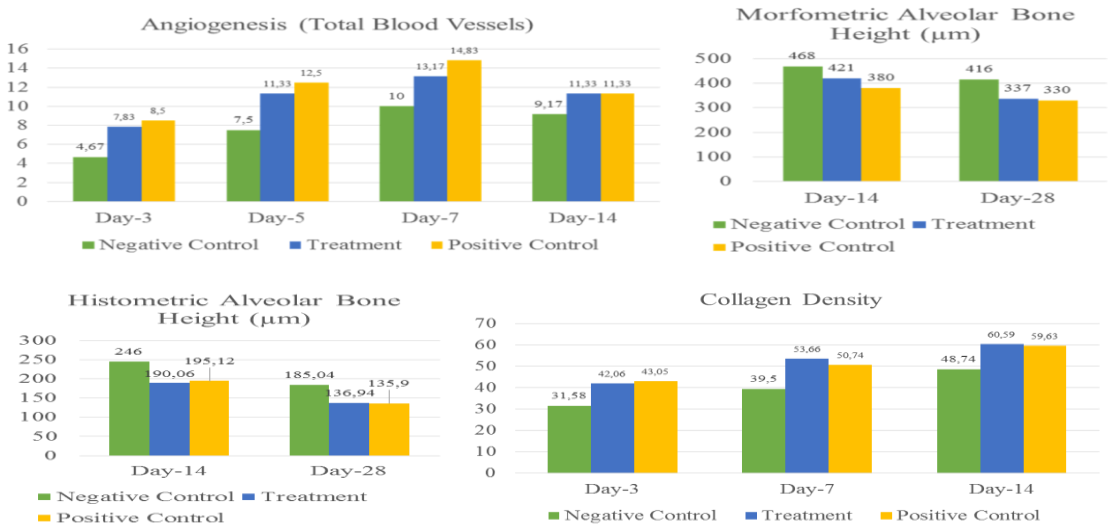
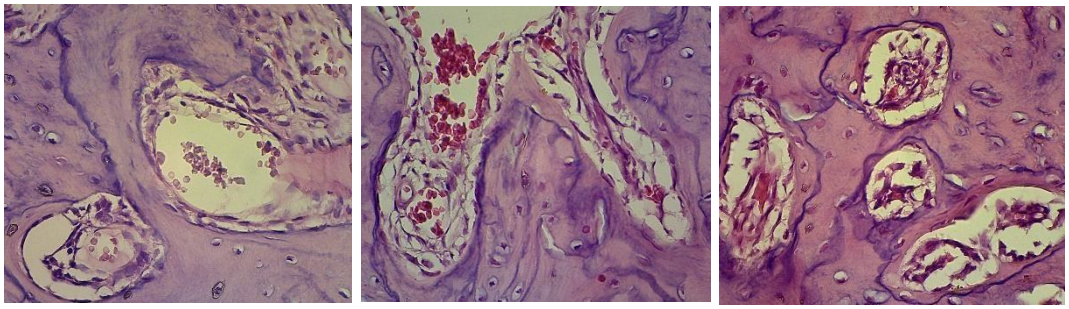


Picture 1. Diagrams showed means of angiogenesis (A), morphometric alveolar bone height (B), histometric alveolar bone height (C) and collagen density (D).

Angiogenesis

Picture 1(A) showed amount of blood vessels in all groups increased from day 3 to 7 and decreased at day 14. The amount of blood vessels in rats given CMC-Na on day 3, 5, 7 and 14 were 4,67; 7,5; 10 and 9,17; while in rats given 10% banana peel extract were 7,83; 11,33; 13,17 and 11,33. Rats which given hyaluronat acid gel showed highest

amount of blood vessels on day 3, 5, 7 and 14 (8,5; 12,5; 14,33 and 11,33).



(A)Negative Control (B) Treatment (C) Positive Control

Two Ways Anova indicates significance difference ($p<0,05$) of total blood vessels between three groups, which mean the three gels can influenced total blood vessels. Altough in positive control groups showed highest amount of blood vessels in all day, based on LSD test, there is no significance different ($p>0,05$) between treatment and positive control groups.

Alveolar Bone Height

According to morphometric and histometric method, in all groups from day 14 to 28 showed reduction of the distance between CEJ and alveolar bone crest (picture 1 (B and C)). It means the healing process was conducted and the alveolar bone crest got higher. The longest distance was owned by negative control group (morphometric: day 14 (468 µm) and day 28 (416 µm); histometric day 14(246 µm) and day 28 (185,04 µm)) while the shortest by positive control group (morphometric: day 14 was 380 µm and 28 was 330µm; histometric day 14 (195,12 µm) and day 28(135,9 µm)). However, the distance between positive control and treatment (morphometric: day 14 (421 µm) and day 28 (337 µm); histometric day 14(190,06 µm) and day 28 (136,94 µm) almost equal. The two way anova analysis showed significance difference ($p<0,05$) between three groups and LSD test showing no significance different ($p>0,05$) between treatment and positive control groups.

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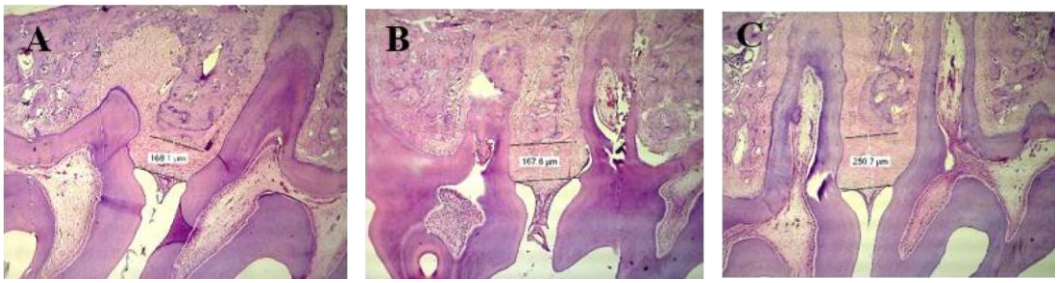


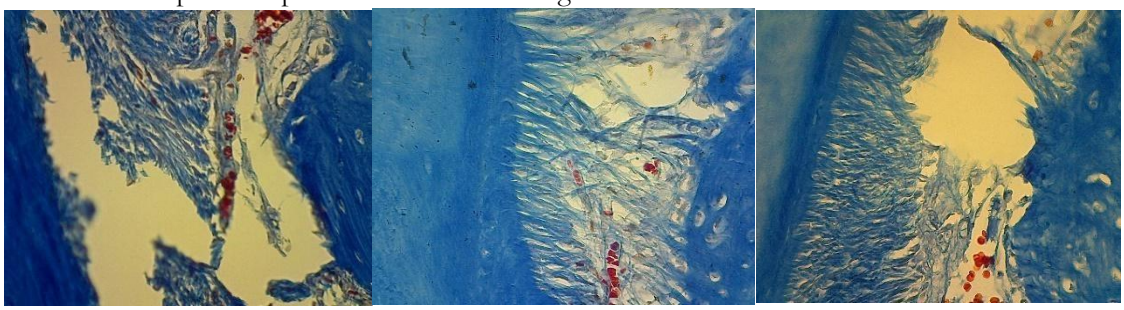
Picture 2. Positive control group (A), treatment group (B), negative control group (C)

Collagen Density

Based on picture 1(D), the density of periodontal ligament's collagen increased day by day. on day 7 and 14, application of 10% banana peel extract gel showed the most dense collagen (53,66% and 60,59%) rather than negative group (39,5% and 48,74%) and positive group (50,74% and 59,63%). Two Way Anova analysis indicates significance difference ($p<0,05$) of collagen density between three groups and LSD test showing no significance different ($p>0,05$) between treatment and positive control groups.

Discussion

Experimental results showed significant different between negative control group and positive control group either the treatment group. Topical aplication of hyaluronic acid gel (Gengigel®) and 10% banana peel extract gel could improve angiogenesis, collagen density, and alveolar bone height in periodontitis healing. These were caused by variable content among those gel. Bioactive components of yaluronic acid gel and banana peel extract gel have terapeutic effect that could promote periodontal tissue healing, while 2% CMC-Na gel does not have bioactive components that could promote periodontal tissue healing.



Negative control group Treatment group Positive control group

The amount of blood vessel on control positive and treatment group showed unsignificant difference. According to that data, it could be told that banana peel extract gel could induce blood vessel proliferation as well as hyaluronic acid gel. Tannin in banana peel extract could promote VEGF expression that later could promote endotelproliferation[10]. Hyaluronic acid containslow weight molecular hyaluronic acid that could induce endotel proliferation as tanin in banana peel extract[11].

Flavonoid in banana peel extract could inhibit matrix metalloproteinase activity by bonding metal component of MMP[12, 13].Flavonoid was also could promote ILGF-1, PDGF, and TGF-β1 expression were later could promote fibroblast proliferation and collagen synthesis[10, 14] .Gallocathecin in banana peel extract could increase tissue hidroxyproline[15] . Hidroxyproline was one of collagen matrix component[16] .Hyaluronic acid gel also could promote fibroblast proliferation and inhibit MMP ekspresion as well as banana peel extract gel, so that both of them could increase collagen density in periodontitis healing

Banana peel extract contain galat acid and gallocathecin that could inhibit oxidative stress reaction[15] . Inhibition of oxidative stress reaction could inhibit osteoclastogenesis by decreasing RANK expression and increasing osteoprotegrin production. Osteoprotegrin could bind RANK so that osteoprotegrin-RANK binding could prevent

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RANK and RANKL binding and prevent activation of osteoclast precursor become osteoclast[18]. Flavonoid of banana peel extract as antiinflammation could inhibit IL-1, IL-8, and TNF-α activity. Hyaluronic acid gel also has antioxidant and antiiflammation effect as well as banana peels extract[11]. The difference between hyaluronic acid gel and banana peel extract gel are banana peel extract contain saponin that could induce bone morphogenetic protein expression. Increasing of BMP expression could inhibit osteoclast formation and induce osteoblast proliferation so that bone matrix production could increased[19].

Summary

Banana (Musa paradisiaca L.) peel extract significantly improve periodontal healing by increasing alveolar bone height, collagen density on periodontal ligament and angiogenesis of alveolar bone, thus can be concluded that banana (Musa paradisiaca L.) peel extract gel can be used as adjuvant therapy candidate for periodontitis.

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