The effect of Binahong Gel (Anredera cordifolia (Ten.) Steenis) in accelerating the escalation expression of HIF-1α and FGF-2

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Abstract
Tooth extraction is one of treatment acts which is done by dentists in clinics, hospital, and even private practices. One thing that is needed to be observed after the treatment is the speed of wound recovery process post the tooth extraction. Binahong is commonly used as medicinal treatments in Asia, some of them to heal wounds, but there had never been research of the use of Binahong leaf in wound recovery after tooth extraction.

The aim of this study was to investigate the effect of Binahong Gel in accelerating the expression of HIF-1α and FGF-2 post tooth extraction on Wistar Rats. This study was used post test only control group design. 48 male Wistar Rats weight between 150-200 grams, 3 months of age are being used. Tooth extraction is being done on lower left incisor. The 48 rats are divided into four groups. The data were analyzed statistically using One-Way ANOVA and LSD 0.05. The result of every tested group showed p>0.05, therefore all the data had a normal distribution. Therefore, a One-Way Anova test with 5% significant rate was done and continued by LSD test to find a significant difference in each groups. Examination showed there was significant difference in expression of HIF-1α and FGF-2 between Binahong gel and two other groups (p<0.05).

The application of Binahong gel can accelerate the expression of HIF-1α and FGF-2 post tooth extraction on Wistar Rats.


Keywords: HIF-1α, fibroblast, Binahong gel.

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Introduction
Indonesia is one of the tropical countries which is rich in many different kinds of plants that useful for health. One of the kinds of plants which is known for its many usefulness for health is Binahong leaf (Anredera cordifolia (Ten.) Steenis). Binahong leaf has a complex chemical contents, some of them are: quercetin, saponin,and terpenoid compound. Saponin and terpenoid acts as antimicrobial, especially bacteria, fungus, and it also has an important role in taking care of inflammation and allergy, as well as one of the important nutrition which is needed for wound recovery.1-4

Wound recovery process from tooth extracted sometimes have an experiences disturbance so that complication may happen. Some researchers claim that the use of medicine post the tooth extraction can reduce the possibility of complication and it’s often expected to be able to accelerate the process of coagulating for blood, so that it will also accelerate the process of wound recovery.4 Healing or recovery of damaged tissue basically is a replacement of the damaged tissue with new normal tissue. The process of tissue recovery is the first stage of dynamic processes. Cellular oxygen mechanisms play major roles in wound healing post extraction socket. HIF-1α was ubiquitously expressed transcription factors that modulate gene expression to mediate cellular responses and adaptation in hypoxic environments.5-6

The healing process is important for normal structure maintenance, function, and life perpetuity of an individual, two of which have important role are HIF-1α and fibroblast. HIF-1α
could inducing angiogenesis by inducing VEGF-A expression as a direct target of HIF-1α. Fibroblast synthesize collagen, elastin, glycoaminoglycans, proteoglycans and multiadhesive glycoproteins. Fibroblast are the most common cells in connective tissue and are responsible for the synthesis of extracellular component such as collagen fiber. Collagens constitute the most abundant proteins found in the body. All collagens are composed of three polypeptide alpha chains coiled around each other to form the typical collagen triple helix configuration. Common features include the presence of the amino acid glycine in every third position, a high proportion of proline residues. Hydroxylation of proline and lysine occurs after these amino acids are incorporated into polypeptide chains, hydroxylation begins after the peptide chain has reached a certain minimum length and is still bound to ribosomes. The two enzymes involved are peptidyl proline hydroxylase and peptidyl lysine hydroxylase.7-10

Cells with intense synthetic activity are morphologically distinc from the quiescent fibroblasts that are scattered within the matrix they have already synthesized. The quiescent fibroblast is smaller and tends to be spindle shaped, it has fewer processes, a smaller, darker, elongated nucleus. Fibroblast, particularly those activated and responding to some type of stimulation, such as inflammation or mechanical forces, secrete a number of growth factor, cytokines, and inflammatory mediators. The repertoire of factors varies depending on the location and type of fibroblast but may include interleukin-1, interleukin-6, interleukin-8, tumor necrosis factorα, prostaglandin E2, platelet-derived growth factor, insulin-like growth factor, transforming growth factor β, vascular endothelial growth factor, basic fibroblast growth factor, hepatocyte growth factor, and keratinocyte growth factor.6-11

In response of damage tissue, mucosa damages integrity of tissues can disturbs the oxygen supply. Oxygen is an essential element for successful wound healing. Hypoxia has been shown to induce critical factors that stimulate proliferation and migration of endothelial cells, and fibroblasts in wounds. Cellular responses to hypoxia are mediated by hypoxia-inducible factor (HIF)-1 signaling. Fibroblast growth factor-2 proliferates and actively synthesizes matrix components and upon more specific observation on cellular level, the active fibroblast has an abundant and irregularly branched cytoplasm and also appears bigger and more basophilic. Its nucleus is ovoid, large, and pale staining, with fine chromatin and a prominent nucleolus. The cytoplasm is rich in rough endoplasmic reticulum, and the golgi complex is well developed. Fibroblast starts to appear on the wounded area three days after the laceration happens. Wound is a damage on body tissue which is caused by several kinds of factors. Wound recovery is an attempt to fix the damage. The main component in wound recovery process is fibroblast. Fibroblast is the cell which responsible for collagen synthesis. Fibroblast is a cell which comes from a mesenchymal tissue which is also embryonic tissue for connective tissue, bone tissue, cartilage, etc. fibroblast produce extracellular component from growing connective tissue. Fibroblast exist in all fibrous connective tissue in the body and responsible to synthesize precursors from collagen, reticular and elastic fibres.12-15

Binahong is commonly used as medicinal treatments, some of them to heal wounds, but there had never been research of the use of Binahong leaf in wound recovery after tooth extraction. The purpose of this research is to know the effect of Binahong Gel in accelerating the escalation expression of HIF-1α and FGF-2 post tooth extraction on Wistar Rats.

Materials and methods

This study is an experimental laboratory research using The Post-Test Only Control Group Design. 48 male Wistar Rats weigh between 150-200 grams, 3 months of age are being used. Have well condition, food and drink water given ad libitum. All animal procedures were approved by the University of Airlangga Surabaya Animal Care and Use Committee.

This animal is used because tooth extraction on Wistar Rats is easier with sufficiently wide socket extraction wound for applying Binahong Gel. Tooth extraction is being done on lower left incisor. The choosing of lower incisor is based on the structure and anatomical form of Rat’s teeth which enable extraction to be done. The 48 rats are divided into four groups. On the first and the second groups, after the extraction is done, Binahong Gel 10% and 20%
is applied on the extraction wound. On the third group, bone graft is applied on the extraction wound as positive control group, and on the last group, HPMC is applied on extraction wound as negative control group.

Binahong leaf which is made into gel form will be easier to be put into the extraction wound socket because of its solid, soft and elastic characteristics. This gel forms makes the substance durable in extraction wound socket, so that it helps the body in wound recovery process. The making of Binahong leaf gel is uses the mixture of HPMC and distillation of Binahong leaf. The characteristics of HPMC are for thickening, stabilizer, gel maker and in some things as emulsifiers. In hydrocolloid emulsion system it doesn’t function as emulsifiers, but more as substance which gives stabilization. This HPMC is easily soluble in hot or cold water, so it is easy to use. It is used as stabilizer because it’s easily obtainable and also reasonably priced.10

Animal’s mandible were decaputated at intervals of 3 and 7 days after extraction by median-sagittal cut, samples that had been detached from the body then fixated. Buffered isotonic solution of 10% formaldehyde was used for fixatives. 96% ethanol was used to extracted the water from the fragment. The ethanol then replaced with a solvent miscible with the embedding medium. In paraffin embedding, the solvent used is xylene. Once the tissue is impregnated with the solvent, it is placed in melted paraffin in the oven at 58-60°C. The heat causes the solvent to evaporate, and the space within the tissue become filled with paraffin.

The tissue together within its impregnating paraffin hardens taken out of the oven. Tissues embedded with plastic resin dehydrated in ethanol. The hard blocks containing the tissues are then taken to a microtome, and sliced into thin sections 4-5µm. The sections are floated on water and transferred to glass slide to be stained. Staining tissue with an anti-HIF-1α antibody and FGF-2 antibody (Novusbio, USA) was done to make the various tissue component conspicuous. Under the light microscope (Olympus, JAPAN) tissue are examined via a light beam that is transmitted through the tissue using image magnified 400 times.4

Results

The mean and standard deviation of the expression of HIF-1α and FGF-2 post extraction on Dawley Rats is shown in the table 1 and table 2. A Kolmogorof Smirnov test was carried out on the data to determine the normality of distribution.

<table>
<thead>
<tr>
<th>Group</th>
<th>X±SD</th>
<th>X±SD</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Day 3</td>
<td>Day 7</td>
</tr>
<tr>
<td>10%</td>
<td>36.56±3.32</td>
<td>43.76±2.53</td>
</tr>
<tr>
<td>20%</td>
<td>37.18±3.35</td>
<td>43.20±3.21</td>
</tr>
<tr>
<td>K+</td>
<td>26.26±2.75</td>
<td>32.10±3.74</td>
</tr>
<tr>
<td>K-</td>
<td>28.53±2.54</td>
<td>31.20±3.28</td>
</tr>
</tbody>
</table>

Table 1. Mean expression of HIF-1α in treatment group and control group.

<table>
<thead>
<tr>
<th>Group</th>
<th>X±SD</th>
<th>X±SD</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Day 3</td>
<td>Day 7</td>
</tr>
<tr>
<td>10%</td>
<td>39.06±3.28</td>
<td>24.26±2.52</td>
</tr>
<tr>
<td>20%</td>
<td>37.80±4.02</td>
<td>24.23±2.46</td>
</tr>
<tr>
<td>K+</td>
<td>26.23±2.54</td>
<td>20.63±2.26</td>
</tr>
<tr>
<td>K-</td>
<td>26.43±3.02</td>
<td>20.76±3.11</td>
</tr>
</tbody>
</table>

Table 2. Mean amount of FGF-2 in treatment group and control group.

Discussion

After an injury to either oral mucosa or socket post extraction, a clot from blood products forms in the area and the inflammatory response is triggered by its white blood cells. If the source
of injury is removed, tissue repair can begin within the next few days. The epithelial cells at the periphery of the injury will lose their desmosomal intercellular junction and migrate to form a new epithelial surface layer beneath the clot. It is very important in repair of the connective tissue and must be retained in the first day of repair because it acts as a guide to form a new surface. After the epithelial surface is repaired, the clot is broken down by enzymes because it is no longer needed. Repair of the epithelium is tied to the repair taking place in the deeper connective tissue.16-17

The result showed decreasing in amount of fibroblast by the 7th day because fibroblast synthesize proteins, such as collagen and elastin, that form collagen, reticular and elastic fibers by the 7th day. They also involved in the production of growth factors that influence cell growth and differentiation such as FGF-2. The experiment result showed fibroblast more active, so it has an abundant and irregularly branched cytoplasm and also appears bigger and more basophilic. Its nucleus become ovoid, large, and pale staining, with fine chromatin and a prominent nucleolus. It is also participate in the remodeling of connective tissue and bone through the degradation of collagen and other matrix molecules and their replacement by newly synthesized molecules.8-9 It is believed that some proteins that are the products of genes that play active roles in bone regeneration process.18

The significant escalation of expression HIF-1α and FGF-2 on the use of Binahong gel is caused by the existence of substance in Binahong, one of them is Quercetin. Quercetin is the main component in Binahong, the antiinflammation characteristic from Quercetin helps body to avoid infection, fever and all bacterial-related disease. Quercetin increases the biosynthesis regulation from type I collagen, polypeptide α chains are assembled on polyribosomes bound to rough endoplasmic reticulum membranes and injected into the cisternae as procollagen molecules. Collagens constitute the most abundant proteins found in the body, that plays important role in wound healing process.19

The essence of Binahong also helps the availability of terpenoid in body. This enzyme is really needed in every metabolism activity in human body, that’s why this leaf can strengthen body immunity system and also useful as adaptogen, to balance the work of cells in human body. Sorbic acid in Binahong leaf is the abundant source of vitamin C, sorbic acid is essentially needed by fibroblast to produce collagen. Vitamin C has a crucial role in wound recovery process as first antioxidants defence in plasma against Reactive Oxygen Species (ROS) and free radicals, which can heavily damage cell and interfere with wound recovery process. The escalation of ROS will cause damage in DNA which will cause cell death.11 Growth factors play an important role to in promote promoting the healing process.20

Another contents such as terpenoid and saponin have been known in their role as astringent to help wound recovery process, which is actively seen in wound contraction and increasing of epithelization process.21-22

In conclusion, the application of Binahong gel can accelerating the escalation expression of HIF-1α and FGF-2 post tooth extraction on Wistar Rat. It is showed that Binahong gel was a better modulator to recovery from damaged tissue especially in extraction socket.

Declaration of Interest

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References