Potential of *Jatropha multifida* sap against traumatic ulcer

Basri A. Gani,1 Abdillah Imron Nasution,1 Nazaruddin,2 Lidya Sartika,1 and Rahmat Kurniawan Alam1

1Department of Oral Biology, Faculty of Dentistry, Universitas Syiah Kuala, Banda Aceh-Indonesia
2Department of Pathology, Faculty of Veterinary, Universitas Syiah Kuala, Banda Aceh-Indonesia

**ABSTRACT**

**Background:** Traumatic ulcer is a lesion in oral mucosa as a result of physical and mechanical trauma, as well as changes in salivary pH. *Jatropha multifida* sap can act as antimicrobial, anti-inflammatory and re-epithelialization, and can also trigger the healing process of ulcers. **Purpose:** Research was aimed to determine the potential of *Jatropha multifida* sap against traumatic ulcer base on clinical and histopathological healing process. **Method:** This research was conducted laboratory experimental model, with rats (*Rattus norvegicus*) as the subject as well as *Jatropha multifida* sap for ulcer healing. Those subjects were divided into four groups: two treatment groups administrated with pellet and *Jatropha multifida* sap, one group as the positive control group administrated with 0.1% triamcinolone acetonide, and one group as the negative control group administrated with 0.9% NaCl. Ulcer manipulation was used 30% H2O2, and evaluation of ulcer healing was used clinical and histopathological approach. **Result:** Clinically, the healing process of ulcers in the treatment group with *Jatropha multifida* sap was faster than that in the positive control group with 0.1% triamcinolone acetonide, indicated with the reduction of the ulcer size until the missing of the ulcers started from the third day to the seventh one (p ≤ 0.05). Histopathologically inflammatory cells (lymphocytes, and plasma cells) declined started from the third day, and the formation of collagen and re-epithelialization then occurred. On the seventh day, the epithelial cells thickened, and the inflammatory cells infiltrated. Statistically, those groups were significant (p ≤ 0.05). **Conclusion:** *Jatropha multifida* sap has a significant potential to cure traumatic ulcers on oral mucosa clinically and histopathologically.

**Keywords:** traumatic ulcer; *Jatropha multifida*; oral mucosa

**INTRODUCTION**

Ulcer is a lesion on soft tissues of oral mucosa caused by physical trauma, thermal, chemical and also triggered by infectious agents (bacteria, viruses, and fungi), systemic diseases, cytotoxic drugs, immunological system disorders, neoplasm, radiotherapy, smoking, alcohol and allergy.1-3 Ulcer on oral cavity can cause damage to oral mucosa epithelial cells, interfering the secretion of secretory immunoglobulin A (sIgA) and extracellular matrix proteins as mucosal defenses against the antigen. Additionally, ulcer can reduce comfort and masticatory function, affecting nutritional intake, consequently, disrupting the healing process of ulcer.4

Ulcer would be classified into two phases, acute and chronic phases.5 The acute phase is characterized by pain as a result of early trauma and will heal itself in 7-10 days. The chronic phase is characterized by irritation as the effect of dental friction such as the adaptation failure of restoration and prosthesis materials.5,6 Some diseases manifested commonly in the oral cavity as chronic ulcers are HIV, syphilis, tuberculosis, squamous cell carcinoma, and deep fungal infection.7

Healing ulcers in the acute phase is generally facilitated by saliva, secretory immunoglobulin A (sIgA) and growth factors. Chronic phase is often become the trigger of infectious diseases in oral cavity.8-10 Martin,11 reported that ulcer healing process in the chronic phase normally takes a long time (30-45 days) to go through several phases, including homeostasis, inflammatory, proliferative, and maturation phase considered as a refinement phase of new tissue formation into permanent tissue.12,13

Generally, sIgA, collagen binding protein, IgM, IgG and polymorphonuclear (PMN) can accelerate the healing process of ulcer.
process of ulcer and also improve the tissue suffering from trauma. Some of those antibody proteins have a working phase based on the development level of ulcers or wounds. In the chronic phase, potential of those antibodies will decrease their effectiveness in healing ulcers, thus, requiring a trigger or therapeutic agents to accelerate the healing process of ulcers and improve the function of the body’s defense system against ulcers.

The 0.1% triamcinolone acetonide is a topical corticosteroid often used in ulcer treatment, and can trigger the intensity of immune system during ulcer healing process. However, the use of this synthetic drug in long term could be triggered the resistance of immune system, epithelial cell atrophy, skin hypopigmentation, and adrenal suppression. In addition, it can lead to increasing blood glucose, osteoporosis, and hypertension.

Alternative medicine derived from herbal ingredients is needed as ulcer treatment option since it is also more economical. Jatropha multifida is one of the herbs that can be used as an alternative medicine for healing traumatic ulcers and skin wound. Some researchers claim that it contains several chemical substances, namely alkaloids and saponins acting as antioxidant and anti-bacterial; tannin playing a role in granulation process, stopping bleeding, and acting as anti-inflammatory and anti-microbial; and flavonoids acting as anti-oxidant and contributing in collagen fiber formation by preventing elastin degradation and improving vascularization. Thus, this research was aimed to test the potential of Jatropha multifida against traumatic ulcers clinically and histopathologically.

MATERIALS AND METHODS

This research was a laboratory experimental research conducted at Biology Laboratory, Faculty of Mathematics and Natural Sciences, as well as at Pathology Laboratory and Laboratory of Experimental Animal Model in Veterinary Faculty of Universitas Syiah Kuala in 2013. The subjects of this research were forty-eight male Wistar rats at the age of 2-3 months.

Those subjects were divided into four groups: two treatment groups administrated with pellet and Jatropha multifida sap, one group as the positive control group administrated with 0.1% triamcinolone acetonide, and one group as the negative control group administrated with 0.9% NaCl. This research passed ethical clearance for animal models from the ethics committee of the Faculty of Medicine, University of Syiah Kuala 175/KE/FK/2013 dated May 24, 2013.

Jatropha multifida sap was obtained from its stems. It was collected and put into 30 ml test tubes, and then centrifuged for 15 minutes at 3000 rpm at 4°C. Pellet and supernantant obtained from the centrifugation were separated through decantation technique, and each fragment was then tested with phytochemical test for tannins, flavonoids, alkaloids and saponins contained.

Rats (Rattus norvegicus) that had been acclimatized for 7 days were given light-dark cycle treatment for 12/12 hours with standard food and water ad libitum. Modification of ulcer was then conducted by using 0.25 ml of 30% H₂O₂ smeared 1.5 cm on the right side of the lower jaw mucosa using a disposable micro-applicator sized 1.5 mm twice a day, morning and evening with a gap of 6 hours per day every 5 minutes during three days. Observation was conducted from day 0, day 3 to day 7 to measure the size of the ulcer area.

Subjects were divided into four groups, each of which consisted of twelve rats. Those rats in the negative control group were administrated with 0.25 ml of 0.9% NaCl, while those in the positive control group were administrated with 0.25 mg of 0.1% triamcinolone acetonide. In addition, those in the treatment groups were administrated with 0.25 mg of Jatropha multifida sap.

After the size of the ulcer area was measured, the subjects were sacrificed. Excision on mandibular labial mucosa was done and tissue was subsequently fixed in 10% formalin solution for making histopathological preparations. Clinical assessment was then conducted by measuring the size of the ulcer area using a periodontal probe vertically, horizontally and diagonally on both sides. For getting an average diameter, a formula was used as follow:

\[ L = \frac{1}{4} \pi \cdot d^2 \]

where:
- \( L \) is the area of ulceration in mm²
- \( \pi \) is 3.14 (provisions)
- \( d \) is average diameter in mm

Afterwards, mucosal healing ulcers were histopathologically measured with haematoxylin and eosin (HE) technique. This technique involves several phases, ie. tissue fixation, dehydrating, clearing, embedding, sectionin, and mounting the tissue with cover glass, then observed under a microscope with 4x and 10x magnifications. Confirmation of the ulcer healing process was assessed on the basis of scoring. Score 1 for total healing process of epithelium with fibrosis occurred on the underlying connective tissue and no inflammatory cells; score 2 for total healing process of epithelium with fibrosis occurred on the underlying connective tissue and inflammatory cells, such as macrophages, plasma cells and lymphocytes; score 3 for ulcers with 2/3 of the width of the ulcer covered by epithelium, mild fibrosis occurred on the underlying connective tissue and inflammatory cells, such as macrophages, plasma cells and lymphocytes; Score 4 for ulcers with 1/3 of the width of the ulcer covered by epithelium, moderate fibrosis occurred on the underlying connective tissue and inflammatory cells, such as macrophages, plasma cells and lymphocytes; and score 5 for ulcers with less than 1/3 of the width of the ulcer improved and high number of connective tissue and inflammatory cells.
Finally, data obtained were analyzed by Anova split plot test followed by least significant difference (LSD) test and the results of the histopathological observation were then analyzed with the Kruskal Wallis test followed by Mann-Whitney test.

RESULTS

Based on the phytochemical test, the supernatant and pellet of the positive (+) *Jatropha multifida* sap contains flavonoids, alkaloids, and tannins. Thus, *Jatropha multifida* sap was used as a material for healing traumatic ulcers in *Rattus norvegicus* rats. The healing process of the ulcer was clinically assessed by measuring the ulcer area after the administration of *Jatropha multifida* sap, whereas histopathologically assessed by observing the picture of cellular activity based on collagen tissue and epithelial cell repair, inflammatory response and infiltration with HE staining. On day 0, there were similar histopathological features on the ulcers in those four groups. The ulcers were formed, therefore, the healing process still did not occur. The connective tissue and inflammatory cells even were still dominated with granulocytes, neutrophils, macrophages, plasma and lymphocytes (Figure 1). On day 3, however, there were different histopathological features in those four groups. In the treatment groups with supernatant and pellet derived from *Jatropha multifida* sap, there were two thirds of the ulcers covered by epithelial cells of connective tissues, containing the moderate number of fibrosis. In those two treatment groups, moreover, the number of macrophages, lymphocytes and plasma cells were lower than on day 0 as well as in the positive control group (Figure 2). Meanwhile, in the negative control group, one-third of the ulcers were covered by epithelial cells of connective tissues, containing the moderate number of fibrosis. In this group, the number of inflammatory cells was still the same as on day 0, ie neutrophils, macrophages, plasma cells and lymphocytes. Nevertheless, on day 7, the ulcers in this group had different histopathological features from the treatment groups and the control positive group. In the treatment groups and the control positive group, the ulcers were totally covered by

Table 1. The assessment of the ulcer healing process based on histopathological description evaluated with traumatic ulcer healing score

<table>
<thead>
<tr>
<th>Day</th>
<th>Treatments</th>
<th>Number of preparations</th>
<th>Score</th>
<th>Histopathological description of the traumatic ulcers</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>Supernatant and pellet derived from <em>Jatropha multifida</em> Sap</td>
<td>3</td>
<td>5</td>
<td>Less than a third of the traumatic ulcers were covered by epithelial cells of connective tissue containing the moderate number of fibrosis, and the number of inflammatory cells was high, such as neutrophil granulocytes, macrophages, plasma and lymphocytes.</td>
</tr>
<tr>
<td></td>
<td>0.1% Triamcinolone acetonide</td>
<td>3</td>
<td></td>
<td>Two-thirds of the traumatic ulcers were covered by epithelial cells of connective tissue containing the moderate number of fibrosis, and the number of inflammatory cells was also moderate, such as macrophages, plasma cells and lymphocytes.</td>
</tr>
<tr>
<td></td>
<td>0.9% NaCl</td>
<td>3</td>
<td></td>
<td>One-third of the traumatic ulcers was covered by epithelial cells of connective tissue containing the moderate number of fibrosis, and the number of inflammatory cells was emerged, such as macrophages, plasma cells and lymphocytes.</td>
</tr>
<tr>
<td>3</td>
<td>Supernatant and pellet derived from <em>Jatropha multifida</em> Sap</td>
<td>3</td>
<td>3</td>
<td>The epithelial cells of connective tissue were totally improved based on the emergence of fibrosis and none or few of inflammatory cells.</td>
</tr>
<tr>
<td></td>
<td>0.1% Triamcinolone acetonide</td>
<td>3</td>
<td>4</td>
<td>One-third of the traumatic ulcers were covered by epithelial cells of connective tissue containing the moderate number of fibrosis, and the number of inflammatory cells emerged, such as macrophages, plasma cells and lymphocytes.</td>
</tr>
<tr>
<td></td>
<td>0.9% NaCl</td>
<td>3</td>
<td></td>
<td>The epithelial cells of connective tissue were totally improved based on the emergence of fibrosis and the moderate number of inflammatory cells, such as macrophages, plasma cells and lymphocytes.</td>
</tr>
<tr>
<td>7</td>
<td>Supernatant and pellet derived from <em>Jatropha multifida</em> Sap</td>
<td>3</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.1% Triamcinolone acetonide</td>
<td>3</td>
<td>3</td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.9% NaCl</td>
<td>1</td>
<td>2</td>
<td></td>
</tr>
</tbody>
</table>
Table 2. The average of the diameter size of the traumatic ulcers on day 0, day 3 and day 7

<table>
<thead>
<tr>
<th>Groups</th>
<th>Day 0 (mm)</th>
<th>Day 3 (mm)</th>
<th>Day 7 (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Supernatant derived from <em>Jatropha multifida</em> Sap</td>
<td>11.1</td>
<td>5.20</td>
<td>0</td>
</tr>
<tr>
<td>Pellet derived from <em>Jatropha multifida</em> Sap</td>
<td>12.3</td>
<td>6.32</td>
<td>0</td>
</tr>
<tr>
<td>Possitive Control (0.1 % <em>Triamcinolon acetonide</em>)</td>
<td>10.12</td>
<td>6.32</td>
<td>0</td>
</tr>
<tr>
<td>Negative Control (0.9% NaCl)</td>
<td>13.3</td>
<td>8.7</td>
<td>1.05</td>
</tr>
</tbody>
</table>

epithelial cells of the connective tissues, and infiltration of inflammatory cells was occurred (Figure 3). Meanwhile, in the negative control group, the ulcers were still not totally covered by epithelial cells of connective tissue, and inflammatory cells, such as macrophages, plasma cells and lymphocytes were still found (Table 1).

Clinically after three days, the size of the ulcers was various between 11 to 13 mm (Table 2). There was also a reddish color on the mucosa, yellow-gray on the center of the ulcers, and mucosal erythema was formed.

Based on statistical analysis using Anova split plot, the scoring of healing ulcers in the supernatant and pellet fractions derived from *Jatropha multifida* sap clinically had a significant effect on ulcer healing process (p≤0.05) as well as on ulcer healing time on days 0, 3, and 7 (p≤0.05). Based on the results of LSD test, furthermore, there was significant difference between the treatment groups, supernatant and pellet, and the negative control group (p≤0.05). But, there was no significant difference between the treatment groups, supernatant and pellet, and the positive control group (p≥0.05). Finally, there was significant difference between the negative control group and the positive control group.
Figure 3. Histopathological description of the traumatic ulcers on the rats on day 7. (A) supernatant: epithelial layer, that had already thickened (black arrow) and connective tissue; (B) pellets: Re-epithelialization (yellow arrow) and connective tissue; (C) control (+): epithelial layer, getting thicker (black arrow), connective tissue (yellow arrow); (D) control (-): thin epithelial layer (black arrow), infiltration of inflammatory cells (blue arrow). HE staining with an electric microscope magnification 400x.

DISCUSSION

This research used 30% H$_2$O$_2$ for making artificial traumatic ulcers due to chemical trauma with the normal size of ulcer, 0.3-1 cm.$^{27}$ Besides less toxic to the body, H$_2$O$_2$ has strong oxidizing properties against tissues and leaves no residue as one of the requirements for mucosa irritant.$^{30}$ The residue of H$_2$O$_2$ then can be broken down by catalase enzyme into water (H$_2$O) and oxygen (O$_2$) that are not dangerous.$^{31}$ *Jatropha multifida* sap can stimulate catalase enzyme on mucosa membranes characterized by the emergence of bubbles on the surface of the ulcers with clinical symptoms and redness on the mucosa with greyish-yellow erythema.$^{32}$

The results in Table 1 showed that *Jatropha multifida* sap was able to reduce the diameter of the ulcers from day 3 to 7. On day 0, the clinical features of the edge of the ulcers showed reddish color, while histopathologically this group still showed inflammatory reaction (Figure 1). This condition is as a result of increased blood flow to damaged tissue at the commencement of the inflammatory process, while a few hours after the formation of the ulcers, epithelial cells will be formed and grown from the wound edges, and then will migrate into the live connective tissue.$^{33}$ Consequently, thickening of the epidermis of the ulcers will occur within 24 hours, and perfect re-epithelialization then will occur less than 48 hours after the formation of the ulcers.$^{33,34}$ On day 3, histopathologically 2/3 of the ulcers were covered by epithelial cells (Table 2), and cell proliferation (Figure 2) occurred, facilitated by fibroblasts from mesenchyme cells. In this phase, fibroblasts will produce collagen fibers connecting the edges of the ulcer to provide strength and integrity, resulting in better healing process.$^{35}$ The increasing of fibroblasts then can trigger the increasing of collagen fibers, as a result, the wound healing process and the first emergence of fibroblasts can significantly be accelerated on day 3, and reach the peak on day 7.$^{36}$ *Jatropha multifida* sap contains saponin, in addition to a role in epithelialization, also can activate the function of TGF-$eta$ by fibroblast receptors stimulating migration and proliferation of fibroblasts further.$^{37}$ On day 3, neutrophils will be replaced by macrophages to activate the function of T cells and the differentiation of B cells as a specific defense system.$^{11}$

Clinically, the results of this research showed those three conditions, consequently, the size of the ulcers was reduced. Based on the theory, the wound healing process of the ulcers consists of inflammatory phase, proliferative phase and maturation phase.$^{38}$ Inflammatory reaction is usually started from day 1 to day 3 characterized by the occurrence of blood vessel vasodilatation with infiltration of inflammatory cells into the ulcer area, such as neutrophils, macrophages, plasma cells and lymphocytes.$^{39}$ After the trauma, inflammatory cells present in the injured tissue to destroy bacteria and remove debris from dead cells and broken matrix, so the healing process can be continued.$^{40}$

The inflammatory phase is characterized by cell infiltration of neutrophils, macrophages and lymphocytes, while the proliferative phase occurs simultaneously with the inflammatory phase by showing epithelial proliferation, angiogenesis, collagen synthesis and extracellular matrix formation followed by tissue remodeling and scarring formation.$^{3}$ This proliferation phase occurs from day 3 to day 14, which is characterized by formation of granulation tissue in the wound.$^{41}$ Granulation tissue is a combination of cellular elements, including fibroblasts and inflammatory cells, along with the growth of new capillaries from the matrix of collagen, fibronectin and hyaluronic acid.$^{42}$ In the maturation phase, the number of fibroblasts decreases periodically, and then the re-formation of new collagen fibers and vascular maturation occur.$^{43}$

On day seven, the ulcers had completely been cured significantly ($p<0.05$), and epithelial cells of the connective tissue containing solid collagen were totally improved (Figure 3 and Table 2). The increasing of blood flow to the area of infection and the decreasing of inflammatory cells are actually in proportion to the reduction of the infection on the ulcer.$^{44}$ Consequently, collagen fibers quickly will become a major factor forming a matrix to support tissue healing process and increase the rigidity and strength of the wound area tension.$^{45}$ Based on clinical assessment, fragments of the supernatant and pellet from *Jatropha multifida* sap have significant potential to cure ulcers.

The existence of flavonoids, alkaloids, and tannins in the second fragment may accelerate the inflammatory phase and trigger the proliferation phase, as a result, the formation of collagen fibers can be accelerated to the granulation
process on the edge of the ulcers. A study conducted by Jin et al., based on clinical observation and histopathological examination on traumatic ulcers up to 10 days, the healing process was directly proportional to the length of day needed, meaning that the longer the day needed, the more decreasing the measure of the ulcer, where wound healing is influenced by connective tissue.

The decreasing of the infection and the total healing process of the ulcers were triggered by active agents contained in Jatropha multifida sap, named tannins and flavonoid. Both active agents always play significant roles as astringents during epithelialization by increasing the activities of extracellular proteins in the epithelial cells to increase production of collagen fibers as the initial phase of wound healing process. Jatropha glandulifera Roxb still considered as a family of Jatropha multifida was also containing tannins and flavonoids that has antibacterial effect for wound infections and ulcers.

A research about bioactive compounds (tannin and flavonoid) that derived from Areca catheca shows that it’s bioactive could be trigger re-epithelialization and accelerate the wound healing process. Saponin contained in Jatropha gossypifolia Linn sap can also play a role in re-epithelialization process and inhibit bacterial infection. Revascularization of injuries can occur simultaneously with fibroplasias and blood capillary formation derived from blood vessels adjacent to wound. Several investigators have reported that cytokines, acidic fibroblast growth factor, epidermal fibroblast growth factor, bFGF and TGF- β are components of the body’s immune playing significant roles as stimulators for re-epithelialization and revascularization of wound healing.

In conclusion, pellet and supernatant of Jatropha multifida sap has been played a role on the healing process of traumatic ulcer, clinically and histopathologically which indicated by shrinking the diameter of the ulcers, decreasing the number of inflammatory cells and re-epithelialization and to step up the collagen tissues.

ACKNOWLEDGEMENT

The research was funded by Ministry of Education and Cultural, Indonesia, through Universitas Syiah Kuala, contract number: 187/UN11/S/LK-PNBP/2013.

REFERENCES


