Formulation and Effectivity of Noni Fruit (Morinda citrifolia) and Aloe vera Extract Gel (GEMOCA) for Burn Wounds in White Mouse (Mus musculus)

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Abstract. Burn wound is an anatomical defect on the skin that occurs due to contact with high-temperature objects. Noni fruit and Aloe vera are known to have various therapeutical activities, including burn wound healing. The research aims to determine the formulation and effects of noni fruit (Morinda citrifolia) and Aloe vera extract gel (GEMOCA) for burn wounds in white mice (Mus musculus). The method of this research was quantitative. Fifteen (n=15) male mice, weighing 40-50 grams were divided into five groups (three treatment groups and two control groups), which consisted of three mice for each group. The burn wounds were made by shaving a 3cm wide area on the back using a 20mm heated metal plate for three seconds. Three treatment groups were treated with Morinda citrifolia and Aloe vera extract (GEMOCA) gel with the following distinct concentration: 1% : 0.5% (F1), 3% : 0.5% (F2), and 5% : 0.5% (F3). Positive control (KP) group was administered with Bioplancenton® gel, whilst negative control (KN) received no treatment. All treatments were conducted every 6 hours for 15 days. Burn wound observation was carried out on day 1, 4, 8 and 15 by measuring the diameter of the wound. The obtained data were analyzed using one-way ANOVA. The result showed that the burn wound diameter in white mice applied with GEMOCA gel in F3 (4.04 cm) had no significant difference with KP group (3.94 cm) and was the fastest recovery time in wound healing among the other treatment groups. This signifies that GEMOCA gel could be an alternative treatment for burn wound healing. The lower result of wound diameter in treatment group compared to positive control group is presumably because of the low active agent content due to manual method for extraction. Therefore, further studies to find a better extraction method to obtain higher concentration of active agent were needed.

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1 Introduction

Burn wound is an anatomical defect on the skin that occurs due to contact with high-temperature objects, such as fire, hot water, electricity, radiation, or chemicals. The severity degree of burn wound depends on the duration and depth of the exposure to the injurious objects [1]. Wound healing occurs immediately after the injury, which involves three steps, namely inflammation, re-epithelialization, and contraction [2]. Inflammation is the first stage of wound healing characterized by migration of neutrophils, macrophages, platelets, and other inflammatory mediators to initiate the healing process. Re-epithelialization follows when new epithelial cells are formed to replace the dead cells and cover the surface of the wound. Simultaneously, angiogenic factors take places to form new blood vessels. The contraction phase ends the wound healing by pulling and closing the edge of the wound. Factors such as burn wound degree, site, and intervention play a significant role in burn wound healing.

In terms of accelerating the healing process, various herbal remedies are potential to be the candidate due to its beneficial effects, such as Morinda citrifolia (noni) and Aloe vera.

Noni, which is also called Morinda citrifolia belongs to Rubiaceae family. The plant is widely distributed throughout the world, particularly in tropical areas. Noni common characteristics is a tree that can reach the height of 4 – 6 meters. The stem is twisted, has stiff branches, and its bark is colored brownish gray. The leaves are green, measuring 15 – 50 x 5 -17 cm, with veined structure. Noni fruit is oval-shaped, sized 7.5 to 10 cm and has spots on its surface. Young noni fruit is green and will turn yellow overtime. The ripened fruit is whitish and soft. The scent of noni fruit is rancid, resembles rotten cheese due to capric and caprylic acid presence. Aloe vera is included to Asphodelaceae family [3], [4]. Its flower is red in color while its root is shallow. The stem is relatively short, measuring about 10 cm. The leaves are thick, rosette-shaped, which has thorns on its edge. Noni and Aloe vera are known to various therapeutical actions, such as anti-free radicals, anticarcinoma and particularly anti-inflammatory [5]. Based on previous in-vitro research by Oryan et al, Aloe vera was evidenced the remarkable outcome in terms of increasing the morphologic characteristic of cutaneous wound in white rats [6]. The statement was supported by Teplicki et al and Chelu et al, which stated that Aloe vera accelerates wound healing by promoting proliferation and migration of fibroblast, thanks to its acemannan contents [7], [8]. On the other hand, a study by Sinambela et al concluded that the antioxidant activities of flavonoids in noni fruit (Morinda citrifolia) could be potential for wound healing [9]. However, the research that reports the therapeutic effects of both plants as a combination is still lacking. Therefore, this study aimed to determine the curative impact of Morinda citrifolia and Aloe vera extract gel (GEMOCA) in treating burn wounds in mouse.

2 Research Methods

2.1. Samples and Grouping Protocols

The method of this research was quantitative research. Fifteen (n=15) white male mice (Mus musculus), weighing 40-45 grams, 2 months in age were collected and acclimatized for 5 days prior to research to adjust for the new environment. The mice then grouped into 5 groups (3 treatment groups, 2 control groups), each group consisted of 3 mice. Three treatment groups were administered with topical Morinda citrifolia and Aloe vera extract gel (GEMOCA) with the respective concentration: 1% : 0.5% (F1), 3% : 0.5% (F2), 5% : 0.5% (F3). Meanwhile, positive control group (KP) was treated with Bioplacenton® gel and
negative control group (KN) received no treatment. All treatments were carried out every 6 hours for 15 days.

2.2. Gemoca Gel Preparation

Firstly, 0.5 g Carbopol 940 was dissolved in hot water until a gel base (mucilage) is formed. Then, three sets of 0.5 g (gram) of Aloe vera and noni fruit (Morinda citrifolia) extract with corresponding concentration for each treatment group: 1 g (F1), 3 g (F2) and 5 g (F3) were added and mixed into gel base evenly. Benzoic acid (0.5 g), glycerin (15 g) and propylene glycol (25 g) were mixed and stirred in mortar. Finally, distilled water was poured until the gel mass was 100 g. The formulation for GEMOCA gel is shown in table 1.

<table>
<thead>
<tr>
<th>Substance</th>
<th>Function</th>
<th>Concentration (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Carbopol 940</td>
<td>Gel base</td>
<td>F1 0.5</td>
</tr>
<tr>
<td>Aloe vera extract</td>
<td>Active agent</td>
<td>F2 0.5</td>
</tr>
<tr>
<td>Morinda citrifolia extract</td>
<td>Active agent</td>
<td>F3 0.5</td>
</tr>
<tr>
<td>Benzoic Acid</td>
<td>Preservative</td>
<td>1</td>
</tr>
<tr>
<td>Glycerin</td>
<td>Humectant</td>
<td>15</td>
</tr>
<tr>
<td>Propylene glycol</td>
<td>Cosolvent</td>
<td>25</td>
</tr>
</tbody>
</table>

2.3. Burn Wound Application and Observation

The process was started by shaving an area on the mouse’s back. The area was then rubbed evenly with 70% alcohol solvent. Subsequently, a 20 cm wide iron plate was heated over a Bunsen burner for 1 minute and applied on the mouse’s back for 3 seconds. The wound healing observation was performed on day 1, 4, 8 and 15 by measuring the diameter of the wound using a vernier caliper.

2.4. Statistical Analysis

This research was conducted at UIN Raden Intan Lampung Integrated Laboratory. The reason to pick white mouse (Mus musculus) as the subject of research lies on the fact that the animal has analogous physiology as in human, particularly in integumentary and immune system [10].

Burn wounds are classified into three degrees based on their severity. The first-degree or superficial burn involves only epidermis layer. Morphologically, the wound appears to be pink to red, and dry with no blisters. This degree of wound will recover in 5 to 10 days with no scarring. The second-degree burn wound can reach superficial dermis. It appears red with blisters and is wet. The wound will resolve in 3 to 8 weeks with scarring. In addition, third-degree burn involves all layers of skin including subcutaneous layer. The color is white or black/brown and is dry. This wound requires more than 8 weeks to recover, with minimum to marked contracture [11]. In this study, burn wound was made by applying a 20 cm wide hot iron plate on white mouse’s back in 3 seconds. As a result, the wound appeared red with blister and was wet (figure 1). Therefore, burn wound degree in this research was classified into second degree burn.
In order to speed up the burn wound healing process, GEMOCA gel with different concentrations was applied on three treatment groups, namely F1, F2 and F3. On the other hand, KP (positive control group) was administered by Bioplacenton® and KN (negative control group) attained no treatment. The effect of GEMOCA gel for burn wound on white mouse (Mus musculus) was depicted in table 2. Based on one-way ANOVA test continued by Duncan test with P value = 0.05, there were significant differences within treatment and control groups.

Table 2. Burn wound average diameter of mouse after 15 days treatment.

<table>
<thead>
<tr>
<th>Group</th>
<th>N</th>
<th>Wound diameter (cm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>KP</td>
<td>3</td>
<td>3.91\textsuperscript{a}</td>
</tr>
<tr>
<td>F1</td>
<td>3</td>
<td>5.46\textsuperscript{b}</td>
</tr>
<tr>
<td>F2</td>
<td>3</td>
<td>4.95\textsuperscript{b}</td>
</tr>
<tr>
<td>F3</td>
<td>3</td>
<td>4.04\textsuperscript{a}</td>
</tr>
<tr>
<td>KN</td>
<td>3</td>
<td>6.53\textsuperscript{c}</td>
</tr>
</tbody>
</table>

Note: Means for group in homogenous subsets are displayed

To begin, KN group was significantly different from all other groups. This is due to the fact KN has no treatment whatsoever (placebo). Previous studies suggest that the natural duration of second-degree burn wound healing was 3 to 8 weeks [11]. Moreover, the healing process was determined by various factors, such as oxygen availability, status of immunity, and growth factors [12]–[14]. On the other hand, there was no significant difference between the F3 (treated with GEMOCA (5 : 0.5 %) group and KP group. As a positive control, Bioplacenton® is a topical gel that has been widely used for superficial wound medications, including burn [15]. Its active agents, placenta extract and neomycin sulphate, play a major role in wound healing [16]. Placenta extract accelerates burn closure by increasing the production of growth factors, including vascular endothelial growth factor (VEGF), while neomycin sulphate is an antibiotic that prevents secondary infections on wound surface [17]. This was also consistent with wound closure progress on day 15 (figure 2) and burn wound diameter (figure 3), which showed a positive outcome in both KP and F3.
Fig. 1. Burn wound diameter after treatment. The graph showed that both KP and F3 had the best outcome in terms of reducing the wound diameter.

Fig. 2. Burn wound closure progress on day-15. The picture indicated that burn wound on KP and F3 were on contraction phase, which is marked by wound closure compared to the other groups.

Similarly, the positive result of GEMOCA gel application on burn wound, particularly on F3 was inseparable from its active agents, namely mannose-6-phosphate and noni ethanol extract. Mannose-6-phosphate is a polysaccharide that predominantly present in Aloe vera gel [18]. Previous studies revealed that the substance actively promotes fibroblast and collagen synthesis, which are vital for re-epithelialization phase during wound healing [19], [20]. Noni ethanol extract contains numerous antioxidants, such as tannin, alkaloids, flavonoids, triterpenes, and polyphenol that have strong astringent and antimicrobial activities [21]. Deng et al also showed that noni (Morinda citrifolia) has robust anti-inflammatory activities in wound healing [22]. Moreover, Wasfy et al suggested that the polysaccharide in Aloe vera acts as an immunomodulator, which enhances skin healing [23]. As a unity, Aloe vera and Morinda citrifolia have been proven to work synergistically in the process of burn wound healing. This was consistent with the study conducted by Royani et al and Purwantiningsih et al, which concluded that active ingredients in Aloe vera and Morinda citrifolia had worked in synergy as a strong anti-inflammatory and anti-bacterial agent [24]. On top of that, this study signifies that Aloe vera and Morinda citrifolia could be a potential alternative treatment for burn wound healing. Nonetheless, the lower result of wound diameter in treatment group compared to positive control group, as described in table 2 was presumably because of the low active agent content due to manual method for extraction. Therefore, further studies to find a better extraction method to obtain higher concentration of active agent were needed.
3 Conclusion

The combination of 5% noni fruit (Morinda citrifolia) and 0.5% of Aloe vera gel extract showed the best activity in burn wound healing in white mouse (Mus musculus), resulted 4.04 cm wound diameter. This indicates that the plants could be the potential alternative treatment for burn wounds. The lower result of wound diameter in treatment group compared to positive control group (3.94 cm) was presumably because of the low active agent content due to manual method for extraction. Therefore, further studies to find a better extraction method to obtain higher concentration of active agent were needed.

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