The Effect of Psychotria malayana Extract on Wound Healing in Male White Rats (Rattus Norvegicus) Sprague Dawley Strain

Pengaruh Salep Ekstrak Daun Psychotria malayana terhadap Penyembuhan Luka pada Tikus Putih (Rattus Norvegicus) Jantan Galur Sprague Dawley

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ABSTRACT

Normal wounds are a complex and dynamic process, but they have a predictable pattern, and untreated open lesions are potentially infectious. According to the CDC (Centers for Disease Control), the incidence of surgical site infection (SSI) in the United States in 2018 was 157,500, with an estimated mortality rate of 8,205. Psychotria malayana plant contains flavonoids, alkaloids, and tannins, which play a role in the inflammatory phase and proliferation of wound healing. Many pharmacology effects of alkaloid and flavonoid activities include anti-inflammatory properties. The aim of this study was to determine the effect of Psychotria malayana extract ointment on wound healing in male white rats (Rattus norvegicus) of the Sprague-Dowley strain. This study is a laboratory experiment using the randomized post-test control only group design. Subjects were 25 male white rats (Rattus norvegicus) of the Sprague-Dowley strain. The rats were divided into five groups. The treatment was carried out for 14 days, and the wounds were shaved under anesthesia, and the skin was taken for histopathological evaluation after it had been examined macroscopically. An independent test showed that there is no significant difference macroscopically between control groups and treatment groups with a p-value >0.05. The Mann Whitney test showed a significant comparison microscopically at the level of angiogenesis, fibroblasts, and collagen in group negative control with group I (10% Psychotria malayana extract ointment) with a P value <0.05%. The conclusion is that 10% Psychotria malayana extract ointment increases fibroblast levels, angiogenesis, and collagen production in wound healing.

Keywords: Histopathology, Psychotria malayana, wound healing, white rat (Rattus norvegicus)

ABSTRAK


Kata Kunci: Histopatologi, penyembuhan luka, Psychotria malayana, Rattus norvegicus

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DOI: http://dx.doi.org/10.21776/ub.jkb.2023.032.04.3
INTRODUCTION

Wounds are defined as a loss of skin epithelial integrity. Normal wound healing is a complex and dynamic process with a predictable pattern. When the skin is injured, multiple epidermis, dermis, and subcutaneous fat layer cell types must coordinate at precise intervals to achieve healing. These phases of hemostasis, inflammation, angiogenesis, growth, re-epithelialization, and remodeling occur chronologically but also overlap (1,2). According to the CDC (Centers for Disease Control), the incidence of surgical site infection (SSI) in the United States in 2018 was 157,500, with an estimated mortality rate of 8,205. 11% of all ICU deaths are associated with SSIs. Each SSI requires 11 additional hospital days, a burden on patients, and has a combined annual cost of $3.2 billion (3). The discomfort experienced during the inflammatory phase is the basis for the development of anti-inflammatory drugs. Treatment with natural ingredients has been done long ago and is starting to grow again (4,5). Psychotria malayana jack is a plant in the Rubiaceae family that grows in Flora malesiana areas such as Malaysia, Indonesia, the Philippines, and Papua New Guinea (6). Rubiaceae family members are known as traditional medicinal plants in Indonesia. Several types of plants had been used as traditional medicine for free radical scavengers, reactive oxygen species, and superoxide, which are natural free radicals that have one unpaired electron. Interest in the Psychotria plant has increased in recent years. Psychotria contains alkaloids, coumarins, flavonoids, terpenoids, tannins, and cyclic peptides. Alkaloid and flavonoid activities have many pharmacological effects, such as anti-inflammatory, antioxidant, anticancer, anti-diabetic, antibacterial, antimalarial, antitumor, antimicrobial, antifungal, anti-inflammatory, and antiseptic. Tannins have antibacterial and biological antioxidant properties (7-10). Some studies show that Psychotria viridiflora has an effect as a wound healer and skin disease medicine in Suku Anak Dalam (SAD) when the plant is boiled, and then the boiled water is used for bathing (11). The aim of this study is to determine the effect of Psychotria malayana leaf extract on wound healing in the white rat.

METHOD

The randomized post-test control-only group design was used in this study, with experimental animals (Rattus norvegicus) as research objects. The criteria were: adult male Sprague Dawley strain, 2-3 months old, weight 250-300 grams, obtained from the Biomedical Laboratory FKIK UNJA. The sample is taken using simple random sampling, with 25 rats chosen at random and divided into 5 groups of 5 rats each. The negative control group was given Vaseline album; the positive control group was given 10% album; the test group 1 was given 10% Psychotria malayana extract; test group 2 was given 15% Psychotria malayana extract ointment; and test group 3 was given 20% Psychotria malayana extract ointment. The data were analyzed by the T-Independent and Mann-Whitney tests. This study was approved by the Ethics Committee of the Faculty of Medicine and Health Science, Jambi University, No. B/679/UN21.8/PT.01.04/2019.

Psychotria malayana Extract

Psychotria malayana leaves were collected in the Sarolangun district of Jambi, dried without exposure to sunlight, and dried in an oven at 500°C before being mashed, filtered, and weighed to yield 400 g of dry leaf powder. Maceration was carried out with 2000ml of 96% ethanol and stirring for 5-10 minutes every hour for 3 days. Filtered leaf soak with filter paper until free of coarse particles; obtained a solution of 1500 ml of Psychotria malayana extract. Then it is evaporated using a rotary evaporator at 60°C to get a thick extract from Psychotria malayana.

Ointment

The formula recipe for ointment were: Psychotria malayana extract 10% for group test 1, Psychotria malayana extract 15% for group test 2, Psychotria malayana extract 20% for group test 3 (Table 1).

<table>
<thead>
<tr>
<th>Formula Recipe</th>
<th>10%</th>
<th>15%</th>
<th>20%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Psychotria malayana extract</td>
<td>2gr</td>
<td>3gr</td>
<td>4gr</td>
</tr>
<tr>
<td>Ointment based (Vaseline album)</td>
<td>14gr</td>
<td>17gr</td>
<td>16gr</td>
</tr>
<tr>
<td>m.l zaff</td>
<td>20gr</td>
<td>20gr</td>
<td>20gr</td>
</tr>
</tbody>
</table>

Wound Making

Rats are anesthetized before being injured, and a 1.5cm ruler-measured wound is made in the rats’ back. Lifting the skin with tweezers and cutting until the dermis subcutis with surgical scissors.

Observation

Observations were done by measuring the average diameter of the wound in the vertical and horizontal directions by being photographed, then quantified by using the Image-J application and calculating the percentage of healing using the formula.

Microscopic Observation and Excision

Skin tissue samples were taken circularly on day 14, the fur is cleaned before the skin tissue to be taken, ±3 mm thick skin tissue was taken and then the sample is observed under a microscope. The following histological processes and structures were assessed using a semi-quantitative method: re-epithelization, inflammation cells (polymorphonuclear leucocytes), fibroblasts, new vessels, and new collagen (12). On a scale of 0 to 4 (Table 2 and Figure 1), sections were rated. For statistical comparison, we used the mean value.

<table>
<thead>
<tr>
<th>Histological observation</th>
<th>0</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Epithelialization</td>
<td>None</td>
<td>&lt;50%</td>
<td>&gt;50%</td>
<td>Moderate</td>
<td>Severe</td>
</tr>
<tr>
<td>Inflammation cells</td>
<td>None</td>
<td>ST minimal</td>
<td>DL/GT minimal</td>
<td>Moderate</td>
<td>Severe</td>
</tr>
<tr>
<td>Fibroblast</td>
<td>None</td>
<td>ST minimal</td>
<td>DL/GT minimal</td>
<td>Moderate</td>
<td>Severe</td>
</tr>
<tr>
<td>Angiogenesis</td>
<td>None</td>
<td>SCT minimal</td>
<td>DL/GT minimal</td>
<td>Moderate</td>
<td>Severe</td>
</tr>
<tr>
<td>Collagen</td>
<td>None</td>
<td>ST minimal</td>
<td>DL/GT minimal</td>
<td>Moderate</td>
<td>Severe</td>
</tr>
</tbody>
</table>

Note: ST = surrounding tissue; DL = demarcation line; SCT = subcutaneous tissue; GT = granulation tissue.
RESULT

Macroscopic Assessment

When performing a macroscopic assessment, wound closure is evaluated. This is done by calculating the percentage of the wound that has healed based on the results of the wound diameter during observation (Figure 2). The highest percentage of the wound healing process on day 7 happened in the positive control group (povidone-iodine 10% group), followed by group test 1 (10% Psychotria malayana extract ointment group) 59.16%, the lowest percentage wound healing process in group test 3 (20% Psychotria malayana extract ointment group) 38.71%. The highest percentage of the wound healing process on day 13 happened in group test 1 (10% Psychotria malayana extract ointment group) 94.71%, whereas the lowest percentage of the wound healing process is the negative control group which is 83.30% (Figure 3).

Microscopic Assessment

Figure 1. Histopathological picture of skin tissue from each group. Re-epithelialization is denoted by red arrows (⁎), inflammation by dark green arrows (⁎), fibroblasts by blue arrows (⁎), angiogenesis by light green arrows (⁎), and collagen by black arrows (⁎)

Note:
A. Epithelialization; A1: Vaseline album; A2: povidone iodin; A3: 10% ointment; A4: 15% ointment; A5: 20% ointment (Olympus Cx21,10X)
B. Inflammatory cells; B1: Vaseline album; B2: povidone iodin; B3: 10% ointment; B4: 15% ointment; B5: 20% ointment (Olympus Cx21,10X)
C. Fibroblast; C1: Vaseline album; C2: povidone iodin; C3: 10% ointment; C4: 15% ointment; C5: 20% ointment (Olympus Cx21,40X)
D. Angiogenesis; D1: Vaseline album; D2: povidone iodin; D3: 10% ointment; D4: 15% ointment; D5: 20% ointment (Olympus Cx21,40X)
E. Collagen; E1: Vaseline album; E2: povidone iodin; E3: 10% ointment; E4: 15% ointment; E5: 20% ointment (Olympus Cx21,40X)

Table 3. Mean percentage of wound healing in each group

<table>
<thead>
<tr>
<th>Group</th>
<th>Mean percentage of wound healing ± SD</th>
<th>Day 1</th>
<th>Day 7</th>
<th>Day 13</th>
</tr>
</thead>
<tbody>
<tr>
<td>Negative control (KN)</td>
<td>0.00±0.00</td>
<td>41.04±13.88</td>
<td>83.30±9.33</td>
<td></td>
</tr>
<tr>
<td>Positive control (KP)</td>
<td>0.00±0.00</td>
<td>69.90±4.44</td>
<td>88.92±5.67</td>
<td></td>
</tr>
<tr>
<td>Group test 1 (KU 1)</td>
<td>0.00±0.00</td>
<td>59.16±14.32</td>
<td>94.17±5.47</td>
<td></td>
</tr>
<tr>
<td>Group test 2 (KU 2)</td>
<td>0.00±0.00</td>
<td>49.03±24.68</td>
<td>89.34±11.31</td>
<td></td>
</tr>
<tr>
<td>Group test 3 (KU 3)</td>
<td>0.00±0.00</td>
<td>38.71±28.00</td>
<td>88.95±7.26</td>
<td></td>
</tr>
</tbody>
</table>

Note: KN=Vaseline album; KP= povidon iodine 10%; KU 1= Psychotria malayana 10% extract ointment; KU 2= Psychotria malayana 15% extract ointment; KU 3= Psychotria malayana 20% extract ointment
Figure 2. Macroscopic assessment of each group on days 1, 7, and 13

Note: KN = Vaseline album; KP = povidon iodine 10%; KU 1 = Psychotria malayana 10% extract ointment; KU 2 = Psychotria malayana 15% extract ointment; KU 3 = Psychotria malayana 20% extract ointment

Histological processes and structures were assessed using a semi-quantitative method: re-epithelization, inflammation cells (polymorphonuclear leucocytes), fibroblasts, new vessels, and new collagen. On a scale of 0 to 4 (Table 2 and Figure 3), sections were rated (Table 4). For statistical comparison, we used the mean value. The group that had the highest epithelialization—measured by the development of new epithelium and the presence of keratin formation and who had received the 10% Psychotria malayana leaf extract ointment of 3.4. The highest mean number of inflammatory cells (polymorphonuclear cells) was when Psychotria malayana leaf extract 20% ointment was applied, which was 2.2.

The extent of angiogenesis was assessed by counting the

Table 4. Histopathological evaluation of the average wound healing in each group

<table>
<thead>
<tr>
<th></th>
<th>Negative control (KN)</th>
<th>Positive control (KP)</th>
<th>Group test 1 (KU 1)</th>
<th>Group test 2 (KU 2)</th>
<th>Group test 3 (KU 3)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Epithelialization</td>
<td>1.6 ± 2.19</td>
<td>2.4 ± 1.51</td>
<td>3.4 ± 0.89</td>
<td>2.4 ± 1.67</td>
<td>2.2 ± 2.04</td>
</tr>
<tr>
<td>Inflammatory cells</td>
<td>1.8 ± 0.83</td>
<td>1.8 ± 0.83</td>
<td>1.2 ± 0.44</td>
<td>2.0 ± 1.40</td>
<td>2.2 ± 1.09</td>
</tr>
<tr>
<td>Fibroblast</td>
<td>2.0 ± 1.00</td>
<td>2.6 ± 1.14</td>
<td>3.6 ± 0.89</td>
<td>2.6 ± 0.89</td>
<td>3.0 ± 0.70</td>
</tr>
<tr>
<td>Angiogenesis</td>
<td>1.2 ± 0.83</td>
<td>2.2 ± 1.34</td>
<td>3.2 ± 1.09</td>
<td>3.2 ± 0.83</td>
<td>3.0 ± 1.00</td>
</tr>
<tr>
<td>Collagen</td>
<td>1.8 ± 1.09</td>
<td>2.2 ± 0.83</td>
<td>3.6 ± 0.89</td>
<td>3.2 ± 0.83</td>
<td>3.0 ± 1.00</td>
</tr>
</tbody>
</table>

Note: KN = Vaseline album; KP = povidon iodine 10%; KU 1 = Psychotria malayana 10% extract ointment; KU 2 = Psychotria malayana 15% extract ointment; KU 3 = Psychotria malayana 20% extract ointment

Figure 3. Percentage of wound healing on days 7 and 13

KN (Vaseline album) 41.04% 69.90% 59.16% 49.04% 38.72%
KP (povidon iodine 10%) 83.30% 88.92% 94.17% 89.35% 88.95%
KU 1 (Psychotria malayana 10% extract ointment) 41.04% 69.90% 59.16% 49.04% 38.72%
KU 2 (Psychotria malayana 15% extract ointment) 83.30% 88.92% 94.17% 89.35% 88.95%
KU 3 (Psychotria malayana 20% extract ointment) 41.04% 69.90% 59.16% 49.04% 38.72%
number of newly formed blood vessels, with the highest angiogenesis of 3.2 obtained by application of 10% and 15% *Psychotria malayana* leaf extract ointment. The highest number of fibroblasts and collagen was given 10% *Psychotria malayana* extract ointment, which was 3.6. The negative control group who were given Vaseline *album* had an average of epithelialization, fibroblast count, angiogenesis, and collagen that were the lowest compared to other groups, while the 10% *Psychotria malayana* extract ointment had a lower average number of inflammatory cells than the other groups. Based Mann-Whitney test to fibroblast level, there is a significant difference between the negative control group and group test 1 with a P value of 0.030. Angiogenesis levels show a significant difference too, between the negative control group and group tests 1, 2, and 3 with P values in sequence 0.021, 0.014, and 0.023. Collagen levels show a significant difference between the negative control group and group test 1 with a P value of 0.021.

**DISCUSSION**

*Psychotria malayana* is a Rubicaceae family plant that contains alkaloids, flavonoids, and tannins. Flavonoids, as strong antioxidants, can reduce lipid peroxidation, which can help the re-epithelialization process and act as antimicrobials. A decrease in lipid peroxidation in flavonoids can prevent wound necrosis, repair the vascularization of wounds, and increase the viability of collagen fibers. Tannins are antimicrobial and increase epithelialization. Tannins are also suspected of playing a role in regulating vascular endothelial growth factor (VEGF) transcription and translation. VEGF acts in a paracrine manner not only in the vascular endothelium of the skin but also in keratinocytes and immune cells; this shows the re-epithelialization effect and at the same time restores angiogenesis and oxygen perfusion (13,14).

Re-epithelialization is the phase of wound healing that involves mobilization, migration, mitosis, and differentiation of epithelial cells. These steps will restore skin integrity. On the surface of the skin, re-epithelialization will occur through the movement of epithelial cells from the edge of the free tissue to the damaged tissue. 10% *Psychotria Malaya* extract showed better epithelialization than other groups. This could be a flavonoid found in leaf extract that increases mitosis, cell interaction, and molecular adhesion, all of which play important roles in the proliferation and epithelialization phases of the wound healing process. Flavonoid has responsibility for increased contraction and epithelialization of the wound. (15) However, neither the control group nor the treatment group showed significant differences in each group. This is presumably because the process of mitosis is still at the stage of fibroblast cell proliferation and collagen formation and has not yet reached the stage of complete epithelialization. When fibroblasts are protected, they can migrate a lot to the wound area, and there can be attachment between collagen and fibroblasts at the edges of the wound so that the epithelium can thicken. It is possible that this is the case because the samples were only collected on day 14, when the proliferative phase was still in progress. This phase lasts from the end of the inflammatory phase until about the end of the third week. Therefore, it is necessary to observe the preparations at several time intervals that reflect the phases of inflammation, proliferation, and remodeling. During the first 14 days post-wound, inflammatory cells were dominated by macrophages and lymphocytes rather than neutrophils. Macrophages become active, and their number will double when inflammation occurs after neutrophils work to phagocytize foreign particles.

In this study, the group with 20% *Psychotria malayana* extract ointment was identified as having the highest number of inflammatory cells, indicating a high level of inflammation. This is because a scab that is too thick, dry, and peeling will leave new wounds. While the group with the 10% *Psychotria malayana* extract ointment had a lower number of inflammatory cells than the other groups, this is because there are active substances that can be well absorbed to help eliminate foreign particles (16). Angiogenesis in the group given the *Psychotria malayana* extract ointment showed significant differences at concentrations of 10%, 15%, and 20% compared to the negative control group. There is no statistically significant difference between the positive control group and the group given povidone iodine. The formation of new blood vessels may be the cause of wound healing. Blood vessels have an important role in providing nutritional intake for tissue regeneration. The number of neocapillaries increasing is one sign of wound healing.

Fibroblast growth occurs from day 7 until day 14 after injury and always continues until skin structure is normal. The density of fibroblasts in the administration of *Psychotria malayana* extract ointment with a concentration of 10% showed significant results compared to the negative control group, which was given Vaseline. There was no difference with the positive control group given 10% povidone iodine. This may be caused by flavonoid active substances in *Psychotria malayana* extract ointment, which work to activate T cells, differentiate, and proliferate into TH1, TH2, and TH3. Transforming Growth Factor (TGF-) is produced by TH3 cells and can stimulate fibroblast proliferation. Flavonoids can prevent or delay the onset of cell death, particularly in excess fibroblasts, as well as increase vascularity in the wound to reduce fat peroxidation (16,17). The function of the fibroblast is to help with vitamin B, C, and amino acid synthesis. Collagen is a protein substance that increases the tension on the wound surface. The increased amount of collagen increases the strength of the wound surface, making it less likely that the wound will open. This study shows increased collagen density in line with increased fibroblasts. The 10% *Psychotria malayana* extract group differed significantly from both the negative and positive control groups. The study on *areca nut* extract and fibroblast and collagen levels in wound healing shows that there is an increase in collagen level at 15% and 30% extract *areca nut* groups, but no increase in fibroblast level. (16,18) This study shows that there is no macroscopic difference between the group control and the *Psychotria malayana* extract group. Microscopically, there are significant differences in fibroblast, angiogenesis, and collagen levels in the 10% *Psychotria malayana* extract group. As a result, giving *Psychotria malayana* leaf extract ointment influences wound healing, especially at a concentration of 10%.
Psychotria malayana leaf extract has an effect on fibroblast level, angiogenesis, and collagen in the wound healing process in male white rats (Rattus norvegicus) of the Sparague Dawley strain.

REFERENCES


ACKNOWLEDGMENTS

We would like to thank the Faculty of Medicine and Health Science, Universitas Jambi, for the research funding under contract No: B/1445/UN21.18/PT.01.03/2019.