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In Vivo Histopathological Wound Healing in Mice (*Mus Musculus*) of Suruhan Extract (*Peperomia Pellucida L. Kunth*)

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ABSTRACT

A wound is a condition where the continuity of tissue is broken, which disrupts the function and anatomical structure of the body from the outermost surface to the deepest layer. When an injury occurs, the body will carry out a healing process consisting of four stages: haemostasis, inflammation, proliferation, and maturation. The wound healing process can be accelerated, one of which is using a messenger plant (*Peperomia pellucida*) which has a pharmacological function. The purpose of the study was to prove the difference between the administration of extracts and 10% povidone-iodine on the histopathological score of wound healing in mice (Mus musculus) 3,5,7 and 14 days. The type of research was true experimental with a post-test control group design. The sampling technique used is simple random sampling. The research sample was 32 mice (Mus musculus) male sex, healthy, 2-3 months old with a bodyweight of 20-40 g, divided into two groups, namely the treatment group and the control group where each group consisted of 4 subgroups. The treatment group was the group that was given 10% messenger extract topically once a day for 14 days. The control group was the group that was given 10% povidone-iodine once a day for 14 days. Skin tissue was taken and prepared with hematoxylin-eosin staining for histopathological examination, including epithelialization, granulation, inflammatory cells, and angiogenesis. The results of the study were analyzed by using the Mann-Whitney test. The results of the Mann-Whitney test showed p values on day 3 (p=0,200), day 5 (p=0,057), day 7 (p=0,029) and day 14 (p=0,343). In general, there is no significant difference in histopathological scores between the control group and the treatment group.

Keywords: *Wound Healing, Wound, Histopathology Scoring, Suruhan (Peperomia Pellucida)*

1. BACKGROUND

The wound is a state of loss of integrity of body tissues caused by trauma and chemicals. One of the most common are cuts caused by sharp objects such as knives and razors.[1] The wound healing mechanism is divided into four stages: hemostasis, inflammation, proliferation, and maturation (remodeling). The inflammatory phase begins immediately after the wound is characterized by redness, heat, swelling, pain, and impaired function.[2,3] The second phase is proliferation which begins on days 4 to 14 after the wound, where there is an increase in fibroblasts and extracellular matrix.[4] The proliferative phase consists of angiogenesis, granulation tissue formation, and epithelialization.[5] Angiogenesis is the process of growing new blood vessels influenced by growth factors such as FGF and VEGF.[5,6] The proliferative phase is strongly influenced by fibroblast cells found in the dermis layer to synthesize collagen tissue as the basis for the formation of granulation tissue.[7]

Granulation tissue comprises fibroblasts, inflammatory cells, new blood vessels, hyaluronic acid, and fibronectin.Macrophages produce growth factors for proliferation, migration, and extracellular matrix formation by fibroblasts.[6,7] The next stage is epithelialization, which migrates keratinocytes from the surrounding epithelial tissue to cover the wound.[8] The third phase is maturation (remodeling) which takes place on the 21st day to 2 years after the wound, and this phase aims to maximize the integrity and strength of the newlyorganized tissue structure in the wound.[9]

One of the plants often used in wound healing is suruhan (*Peperomia pellucida*). The plant has fibrous roots embedded in a shallow surface. The stems are succulent or watery and can live upright with a 15-45 cm height.[10] The leaves are single, shiny, heart-shaped, green with a characteristic odor, 1-4 cm long, 0.5-2 cm wide, and have a pointed tip.[11,12]

These plants contain chemical compounds belonging to glycosides, flavonoids, saponins, tannins, steroids, and triterpenoids.[13]-[14] Flavonoids function as antioxidants and can trigger fibroblast cells which later play a role in wound re-epithelialization.[15] Flavonoidswith triterpenoids have astringent effects that can help reduce inflammatory cells in wounds.[16] Tannins and flavonoids have antiseptic and antibacterial activity, the content of saponins triggers collagen synthesis, and steroids can act as an anti-inflammatory.[6] [17]

The research of Suciyati et al. in 2018 explained that the compounds in the messenger plants are antibacterial and anti-inflammatory to accelerate the wound healing process.[18] Based on the description above, the researcher is interested in researching the benefits of suruhan extract (*Peperomia pellucida*) on histopathological scores, including epithelialization, granulation tissue, inflammatory cell infiltration, and angiogenesis in the process of wound healing in mice.

2. METHOD

Study Design

This type of research is true experimental with a post- test only control group design. This study aims to calculate epithelialization, granulation, inflammation, angiogenesis with *Mehbarani* histological scoring on days 3, 5, 7, and 14.

Sample and Settings

The research is multidisciplinary, involving the disciplines of histology, pharmacy, and pharmacology. The research was carried out at the Pharmacology Laboratory of the Faculty of Pharmacy, Andalas University, and the Pathology and Anatomy Laboratory, Faculty of Medicine, Andalas University. The study was conducted from August 2021 to September 2021. The population in this study was mice (Mus musculus). Therefore, the sample is a population that meets the inclusion criteria, namely mice with male sex, healthy condition, weighing 20-40 grams, 2-3 months old, and the exclusion criteria being no anatomical abnormalities.

This study consisted of 2 groups, namely the treatment and control groups, divided into four subgroups for histopathological observations. Overall, there were eight experimental animals with a total sample of 32 mice, and each group contained four mice. The sampling technique in this study was simple random sampling. The treatment group was the group that was given a topical extract of 10% 0.4 ml/day for 14 days, and the control group was the group that was given povidone- iodine 10% 0.4 ml/day for 14 days topically.
Histological preparations were made on days 3, 5, 7, and 14.

Instruments

Histopathological scores (table 1) included epithelialization, granulation quality, cell infiltration included and angiogenesis under a microscope with 400x magnification, observations were made in a different field of view.

The data obtained were recorded, tabulated, and analyzed descriptive analysis, normality test, homogeneity test and comparison test with *Mann-Whitney*.

Table 1. Histopathology Scoring Mehbarani[19]

Score	Re-epithelialization	Granulation	Inflammatory cells	Angiogenesis
0	Absence of epithelial proliferation in ≥ 70% of tissue	Immature and inflammatory tissue in ≥ 70% of tissue	15-15 inflammatory cells per histological field	Absence of angiogenesis, presence of congestion, hemorrhage, edema
1	Poor epidermal organization in ≥ 60% of tissue	Thin immature and inflammatory tissue in ≥ 60% of tissue	10-13 inflammatory cells per histological field	1-2 vessels per site, edema, hemorrhage, congestion
2	Incomplete epidermal organization in ≥ 40% of tissue	Moderate remodeling in ≥ 40% of tissue	7-10 inflammatory cells per histological field	3-4 vessels per site, moderate edema, congestion
3	Moderate epithelial proliferation in ≥ 60% of tissue	Thick granulation layer and well formed collagen matrix in ≥ 60% of tissue	4-7 inflammatory cells per histological field	5-6 vessels per site, slight edema, congestion
4	Complete epidermal	Complete tissue	1-4 inflammatory	More than 7 vessels per

Scoring system of the histological changes in burn wound healing

3. RESULT AND DISCUSSION

Based on the data obtained, the histopathological scoresbetween the two groups were as follows:

Table 2. Differences in histopathological scores of wound healing in mice between the extract group and povidone-iodine on day 3

Groups	N	Mean±Std.Dev	Pvalue
Control D3	4	3,25±0,500	0,200
Treatment D3	4	4,75±1,500	

Table 2 obtained on day three the histopathological score of the control group was 3.25, and the treatment

group was 4.75. Therefore, statistically, the value of p = 0.200 (p> 0.05) means there is no difference in histopathological scores between the extract group and povidone-iodine on day

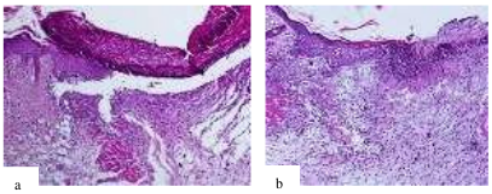

Figure 1. Histology of experimental animal skin tissue at 10x objective magnification, showing the epidermis. Control group (a) and treatment group (b) Day 3. Incomplete epithelialization was seen in both groups. (HE scale h; 200 m)

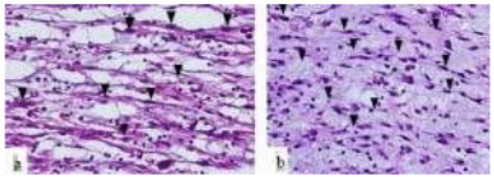

Figure 2. Histology of experimental animal skin tissue at 40x objective magnification, showing granulation tissue under the scar. The control group (a) and the treatment group (b) Day 3. The control group showed loose granulation with low angiogenesis, some partially dilated vessels, areas of edema, and bleeding. The angiogenesis treatment group showed better angiogenesis with more small capillaries without being accompanied by hyperemia, bleeding, and mild tissue edema. (HE scale h; 100 m)

Table 3. Differences in histopathological scores for wound healing in mice between the extract group and povidone-iodine on day 5

Group	N	Mean±Std.Dev	Pvalue
Control D5	4	5,00±0,816	0,057
Treatment D5	4	6,50±0,577	

Table 3 obtained on day three the histopathological score of the control group was 5.00, and the treatment group was 6.50. Therefore statistically, the value of p = 0.057 (p>0.05) means there is no difference in histopathological scores between the ordered extract group and povidone-iodine on day 5.

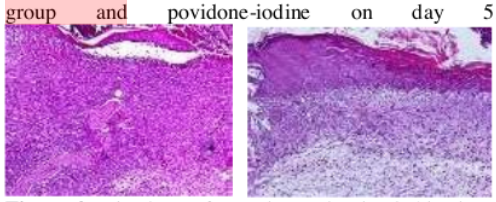

Figure 3. Histology of experimental animal skin tissue at 10x objective magnification, showing the epidermis. Control group (a) and treatment group (b) Day 5. Incomplete epithelialization was seen in both groups. (HE scale h; 200 m)

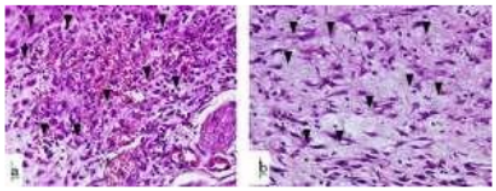

Figure 4. Histology of experimental animal skin tissue at 40x objective magnification, showing granulation tissue under the scar. The control group (a) and the treatment group (b) Day 5. The control group showed loose granulation with low angiogenesis, some partially dilated vessels, areas of edema, and bleeding. In the angiogenesis treatment group, the angiogenesis was better with more small capillaries, without being accompanied by hyperemia, bleeding, and mild tissue edema. (HE scale h; 100 m)

Table 4. Differences in histopathological scores for wound healing in mice between the extract group and povidone-iodine on day 7

Group	N	Mean±Std.Dev	Pvalue
Control D7	4	5,75±1,258	0,029
Treatment D7	4	9,75±0,957	

Table 4 obtained on day three the histopathological score of the control group was 5.75, and the treatment group was 9.75. Therefore, statistically, the value of p = 0.029 (p>0.05) means Ha is accepted or there is a difference in histopathological scores between the ordered extract group and povidone-iodine on the seventh day.

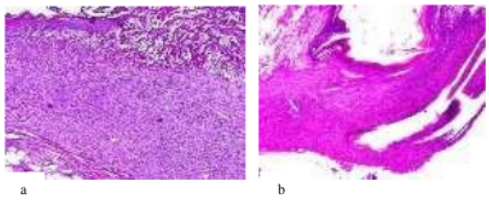

Figure 5. Histology of experimental animal skin tissue at 10x objective magnification, showing the epidermis. Control group (a) and treatment group (b) Day 3. Incomplete epithelialization was seen in both groups. (HE scale h; 200 m)

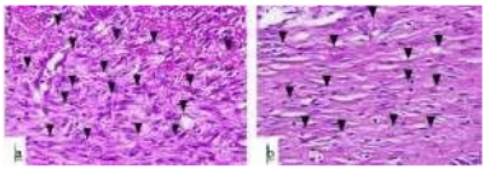

Figure 6. Histology of experimental animal skin tissue at 40x objective magnification, showing granulation tissue under the scar. The control group (a) and the treatment group (b) Day 7. The control group showed loose granulation with low angiogenesis, some partially dilated vessels, areas of edema, and bleeding. In the angiogenesis treatment group, it was better with smaller capillaries. Accompanied by hyperemia, bleeding, and mild tissue edema. (HE scale h; 100 m)

Table 5. Differences in histopathological scores for wound healing in mice between the extract group and povidone-iodine on day 14

Group	N	Mean±Std.Dev	Pvalue
Control D14	4	11,50±0,577	0,343
Treatment D14	4	12,00±0,000	

Table 5 obtained on day 14 the histopathological score of the control group was 11.50, and the treatment group was 12.00. Statistically, the value of p=0.343 (p>0.05) means Ha is rejected, or there is no difference in histopathological scores between the extract group and povidone-iodine on the 14th day.

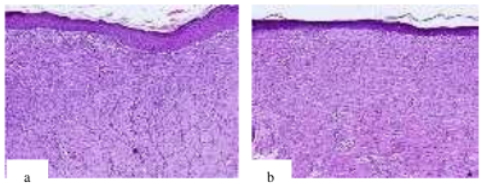

Figure 7. Histology of experimental animal skin tissue at 10x objective magnification, showing the epidermis. Control group (a) and treatment group (b) Day 14. Complete epithelialization on day 14, the epithelialization of the treatment group appeared thinner and of regular thickness than the control. (HE scale h; 200 m)

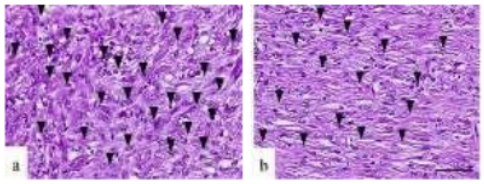

Figure 8. Histology of experimental animal skin tissue at 40x objective magnification, showing granulation tissue under the scar. Control group (a) and treatment group (b) Day 14. The treatment group showed denser granulation, more angiogenesis, less inflammatory cells than the control group. (HE scale h; 100 m)

Histopathological assessment of wound healing with a scoring system between the povidone-iodine control group and the extract treatment group showed a difference, but statistically significant differences were obtained on day 7. There were scars with incomplete epithelial healing in the control group on days 3 to 7. The wound surface was covered with crusts. Underneath, there was granulation tissue with fibroblasts, new blood vessels, and dense distribution of inflammatory cells—complete epithelialization on day 14. In the treatment group, scars appeared with incomplete epithelialization healing on days 3 to 7. The wound surface was covered with crusts. Underneath, there was granulation tissue with fibroblasts, new blood vessels, and scattered inflammatory cells. The distribution of inflammatory cells in the treatment group in the granulation tissue was lower than the control group on days 3, 5, 7, and 14. The granulation tissue on day 14 contained denser collagen fibers, but the population of fibroblasts and inflammatory cells was lower than the control group on the same day. Complete epithelialization on day 14 epithelialization appeared to be thinner and of regular thickness than the control group.

The results showed that the best use of Suruhan extract was from the time the wound started until day 7. Continuous use until day 14 did not show significant results on wound healing. When assessed by a global scoring system for wound healing assessment involving epithelialization,

granulation quality, inflammatory cell infiltration, and angiogenesis, it can be seen that the histopathological score of the treatment group gave a higher wound healing score than the control group. According to research by Majumder and Arum Kumar in 2011, there are many ingredients found in Suruhan plants, such as saponins, flavonoids, alkaloids, steroids, triterpenoids, and carbohydrates.[10]

Steroids as an anti- inflammatory. Tannins and flavonoids have antiseptic and antibacterial properties that inhibit and kill bacteria that infect wounds. The content of saponins can increase collagen formation in wound healing and help the re- epithelialization process, increasing the number offibroblast cells, which triggers the synthesis of fibronectin, which plays an essential role as an extracellular matrix mediator in the process of increasing cell linkage in the wound area.[20,21]

4. CONCLUSION

Based on the results of the research that has been carried out, it can be concluded that there is no difference between the administration of extracts and 10% povidone-iodine on the histopathological score of wound healing in mice (Mus musculus) on days 3, 5, and 14 but on day seven the analysis showed a significant difference. Significant between the two groups. Overall, the extract treatment group showed better wound healing than the 10% povidone-iodine control group. Suggestions for further research are regarding the dose and effect of the extract on humans.

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REFERENCES

[1] P. Sabale , B. Bhimani , C. Prajapati , V. Sabale , An overview of medicinal plants as wound healers 2012;2:143–50. https://doi.org/10.7324/JAPS.2012.21127.

[2] N. Primadina . A. Basori , Perdanakusuma DS. Proses Penyembuhan Luka Ditinjau dari Aspek Mekanisme Seluler dan Molekuler. Qanun Med - Med J Fac Med Muhammadiyah Surabaya 2019;3:31–5. https://doi.org/10.30651/jqm.v3i1.2198.

[3] H.N. Wilkinson, M.J. Hardman . Wound healing: cellular mechanisms and pathological outcomes. Open Biol 2020;10. https://doi.org/10.1098/RSOB.200223.

[4] R.M. Novyana. Susanti. Lidah Buaya (Aloe vera)untuk Penyembuhan Luka. J Kedokt Univ Lampung 2016;5:149–53.

[5] P. Handi, Sriwidodo, S. Ratnawulan, Review Sistematik : Proses Penyembuhan Dan Perawatan Luka. Junal Farmaka 2017;15:251–6. https://doi.org/10.24198/JF.V15I2.13366.

[6] N. Fitri. Penggunaan Krim Ekstrak Batang dan Daun Suruhan (Peperomia pellucida L.H.B.K) dalam Proses Penyembuhan Luka Bakar pada Tikus Putih (Rattus norvegicus). Biopendix 2015;1:83–91.

[7] H. Sorg, D.J. Tilkorn,S. Hager, J. Hauser, U. Mirastschijski. Skin Wound Healing: An Update on the Current Knowledge and Concepts. Eur Surg Res 2017;58:81–94. https://doi.org/10.1159/000454919.

[8] I.M.S. Wijaya, Perawatan Luka dengan Pendekatan Multidisiplin. 2018.

[9] F. Fatimatuzzahroh, N.K. Firani, H. Kristianto, Efektifitas Ekstrak Bunga Cengkeh (Syzygium aromaticum) terhadap Jumlah Pembuluh Darah Kapiler pada Proses Penyembuhan Luka Insisi Fase Proliferasi. Maj Kesehat FKUB 2016;2:92–8.

[10] P. Majumder, K.V. Arun Kumar, Establishment of quality parameters and pharmacognostic evaluation of leaves of Peperomia pellucida (L.) HBK. Int J Pharm Pharm Sci 2011;3:375–8.

[11] A. Badrunasar , H.B. Santoso, Tumbuhan Liar Berkhasiat Obat. 2016.

[12] E. Hanani, V. Ladeska, A. Citra Astuti, PHARMACOGNOSTICAL AND PHYTOCHEMICAL EVALUATION OF INDONESIAN Peperomia pellucida (PIPERACEAE). Int J Biol Pharm Res 2017;8:10–7. https://doi.org/10.21276/ijbpr.2017.8.1.3.

[13] I.M. Tarigan, S. Bahri, S. Awaluddin, Aktivitas Antihiperurisemia Ekstrak Etanol Herba Suruhan (Peperomia pellucida (L.) Kunth) Pada Mencit Jantan. J Pharm Pharmacol 2012;1:37– 43.

[14] A.D. Ardhany, Y. Puspitasari, Y. Meydawati, S. Novaryatiin, Review: Analisis Kualitatif dan Kuantitatif Kandungan Senyawa Kimia Herba Sasaladaan (Peperomia pellucida (L) H.B.K). JSains Dan Kesehat 2019;2:122–8.

[15] Studi P, Dokter P, Fakultas H, Hewan K, Syiah U, Patologi L, et al. 1 , 2 , 3 1 2017;01:584–91.

[16] T. Luka, B. Pada, K. Oryctolagus, T. Mappa, H.J. Edy, N Kojong, FORMULASI GEL EKSTRAK DAUN SASALADAHAN (Peperomia pellucida (L .) H . B . K) DAN UJI EFEKTIVITASNYA 2013;2:49–56.

[17] E. Asetat, D.A.N. Metanol, T. Suruhan 1* , 1 , 1 *2020;16:105–11.

[18] S., A.C. Wullur, W. Bodhi, Formulasi dan Uji Gel Ekstrak Etanol Herba Suruhan (Peperomia pellucida [L.] Kunth) terhadap LukaBakar pada kelinci (Oryctolagus cuniculus). NJurnal Ilm Farm 2018;7:10–21. https://doi.org/10.35799/pha.7.2018.18724.

[19] M. Hazrati, D. Mehrabani, A. Japoni, A. Montasery, N. Azarpira, A.R. Hamidian-Shirazi et al. Effect of honey on healing of Pseudomonas aeruginosa infected burn wounds in rat. J Appl Anim Res 2010;37:161–5. https://doi.org/10.1080/09712119.2010.9707117 .

[20] A.A. Alhasyimi INDUKSI RE-EPITELISASI PADA PROSES PENYEMBUHAN LUKA GINGIVA OLEH APLIKASI TOPIKAL EKSTRAK DAUN SAGE (Salvia officinalis L.) KONSENTRASI 50% (Kajian In Vivo Pada Tikus Sprague Dawley). B-Dent, J Kedokt Gigi Univ Baiturrahmah 2018;3:31–8. https://doi.org/10.33854/jbdjbd.35.

[21] A. Frankova, L. Vistejnova, T. Merinas-Amo, A. Leheckova, I. Doskocil, J. Wong Soon et al. In vitro antibacterial activity of extracts from Samoan medicinal plants and their effect on proliferation and migration of human fibroblasts. J Ethnopharmacol 2021;264. https://doi.org/10.1016/J.JEP.2020.113220.

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