

Betel Leaf Extract's Effect (Piper Batle Linn) on The Healing Process of Incision Wounds in Rats (Rattus Norvegicus)

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ABSTRACT

Introduction: An incision wound is a wound that is intentionally made by a clean-cut using a sharp object. Betel leaf is a natural ingredient that helps the wound healing process because it has active ingredients such as saponins, tannins, flavonoids, essential oils, and alkaloids. **Objectives:** The study aimed to determine the effect of betel leaf extract on the healing process of incision wounds in white rats. **Methods:** This study uses a true experimental research design conducted on white rats. Samples were taken by random sampling and divided into four groups, namely control group (KK) (n = 6), treatment group with a dose of 10% (P1) (n = 6), treatment group with a dose of 15% (P2) (n = 6) and treatment group with a dose of 20% (P3) (n = 6). **Results:** The mean of wound's length in KK=0.78 cm, P1=0.67 cm, P2=0.57 cm, and P3=0.15. Based on the Kruskal Wallis test, p value=0.000($\alpha < 0.05$), there is a significant difference in the wound healing process between the control and treatment groups. **Discussion:** Betel leaf contains active substances, namely flavonoids, alkaloids, saponins, tannins, and essential oils. The active substance causes the wound's edges to close faster, so the wound heals faster. **Keywords:** Betel leaf extract, wound healing, incision

ABSTRAK

Pendahuluan: Luka sayatan adalah luka yang sengaja dibuat dengan sayatan bersih menggunakan benda tajam. Daun sirih merupakan bahan alami yang membantu proses penyembuhan luka, karena memiliki bahan aktif seperti saponin, tanin, flavonoid, minyak atsiri dan alkaloid. **Tujuan:** Penelitian ini bertujuan untuk mengetahui pengaruh ekstrak daun sirih terhadap proses penyembuhan luka sayatan pada tikus putih. **Metode:** Penelitian ini menggunakan rancangan penelitian true eksperimental yang dilakukan pada tikus putih. Sampel diambil secara random sampling dan dibagi menjadi empat kelompok yaitu kelompok kontrol (KK) (n = 6), kelompok perlakuan dengan dosis 10% (P1) (n = 6), kelompok perlakuan dengan dosis 15% (P2) (n = 6) dan kelompok perlakuan dengan dosis 20% (P3) (n = 6). **Hasil:** Hasil penelitian menunjukkan bahwa rerata panjang luka pada KK=0,78 cm, P1=0,67 cm, P2=0,57 cm, dan P3=0,15. Berdasarkan uji Kruskal Wallis didapatkan nilai p=0,000($\alpha < 0,05$), yang berarti terdapat perbedaan yang signifikan dalam proses penyembuhan luka antara kelompok kontrol dan perlakuan. **Pembahasan:** Daun sirih mengandung zat aktif yaitu flavonoid, alkaloid, saponin, tanin, dan minyak atsiri. Zat aktif tersebut menyebabkan tepi luka lebih cepat menutup, sehingga luka lebih cepat sembuh.

Kata kunci: Ekstrak daun sirih, penyembuhan luka, sayatan

INTRODUCTION

The skin is the outermost and limiting organ of the human environment (Gilaberte *et al.*, 2016). The skin area in adults is about 1.5 m² and weighs about 15% of body weight (Liu, K *et al.*, 2020). Skin is an essential organ that reflects health (Prescott *et al.*, 2017). The skin is also very complex, elastic, and sensitive, varies according to climate, age, race, and the body's location (Berardesca, Farage, and Maibach, 2013). The skin has a vital role in protecting the body from foreign objects such as parasites, microorganisms, sharp objects, and fungi, regulating water and electrolyte balance, thermoregulation, and protection from ultraviolet rays (Bauer, 2013).

Skin injury caused by a sharp object. Skin injuries often occur, which cause damage to the epithelial tissue and the dissolution of the normal anatomical structure of the skin (Sulistyoning Suharto *et al.*, 2021). A wound is a condition where the anatomical structure of the skin is damaged, causing damage to the integrity of the skin (Suharto and Etika, 2019).

According to contamination and injury, wounds consisted of abrasions, contusions, stab wounds, and incision wounds (Agrawal, 2018). An incision wound is a wound that is intentionally made by a clean-cut using a sharp object (Payne-James, 2015). If not appropriately managed, wounds will cause complications such as infection and prolong wound healing time (Misiakos *et al.*, 2014). This will harm the patient because it will interfere with their activities. Prolonged wound healing time will also cause financial losses to the patient (Jenks *et al.*, 2014).

Wound healing is a complex process divided into three stages: inflammation, proliferation, and maturation (Mohanty and Sahoo, 2017). Wound healing is also related to cell regeneration until organ function recovers (Feng *et al.*, 2019). Wound management can be carried out with pharmacological and non-pharmacological methods. One of the non-pharmacological therapies is herbal therapy (Sulistyoning Suharto *et al.*, 2021). Herbal therapy is particularly regarded as an alternative/complement to conventional

pharmaceuticals (Anheyer *et al.*, 2018).

The content in herbal plants is usually balanced and neutralizes each other (Suharto and Etika, 2019). One of the herbal therapies that have been widely developed to treat wounds is betel leaf (Peddapalli *et al.*, 2020). Betel leaf is one of the plants known to be efficacious as an antiseptic (Roy and Guha, 2018). Betel leaf can be used as an antibacterial because it contains 4.2% essential oil, which mainly consists of betlephenol, which is an isomer of Euganol allypyrocatechine, Cineol methyl, eugenol, Caryophyllene (sesquiterpenes), kavikol, caphybetol, estragole, and terpinen-4-ol (Tirayo, Munir and Hutasoit, 2016). Betel leaf contains saponins, flavonoids, tannins, saponins, flavonoids, and tannins can also help wound healing and accelerate epithelialization (Mazumder, Roychowdhury and Banerjee, 2016).

As a nurse, one of the tasks carried out is to carry out wound care actions both independently and collaboratively. The use of betel leaf in wound care will be an innovation. Based on the description above, the researchers were interested in examining the effect of betel leaf extract (*Piper Betel Linn*) on the healing process of incisional wounds in rats (*Rattus norvegicus*).

METHOD

Study design

This study used a true experimental research design using a post-test-only control group design.

Population and Samples

This study had four groups. The number of samples for each group is calculated based on the Federrer formula (Sulistyoning Suharto *et al.*, 2021). The result from the procedure is each group consisted of 6 animals, so this study used 24 rats. The rats were divided into four groups, that are control group, the treatment group 10%(P1), the treatment group 15%(P2), and the treatment group 20%(P3).

Instruments

Instruments used include instruments for making incisions and instruments for wound care.

Instruments for making incisions include a scalpel, ruler and marker, scissors, cotton swab, clean gloves, sterile gauze, plaster, 3ml syringe, and hook. Instruments for wound care include sterile wound care sets, sterile gloves, bandages, swabs, plasters, cotton swabs, scissors, and combs. The device used to measure the wound length between the control and treatment groups was a ruler.

Research Prosedure

A total of 24 rats had an incision with a length of 1 cm on the right back. The mice were randomized and divided into four groups. The groups were the control group (KK), treatment group 10%(P1), treatment group 15%(P2), and treatment group 20%(P3). The control group is the group whose wound care is only done by cleaning the wound with distilled water. The treatment group was the group whose wound treatment used betel leaf extract (*Piper Betel Linn*) topically. P1 uses a 10% dose, P2 uses a 15% dose, and P3 uses a 20% dose. Wound care was carried out for seven days.

Data Collection

Data was collected by evaluating wound closure. Evaluation of wound closure is carried out during wound care by measuring the length of the wound with a ruler.

Data Analysis

The data analysis used in this study was the Kruskal Wallis test to compare the mean wound length in all groups.

Ethical consideration

The ethical consideration had been approved by the ethical committee, Faculty of Health Science, Kadiri University, before the data collection process with ethical clearance number 19/KE/FIK-UNIK/V/2021.

RESULT

Table 1 shows that the mean wound length in each group. Mean in post intervention is as

follows KK was 0.78 cm, P1 was 0.67 cm, P2 was 0.57 cm, and P3 was 0.15 cm. Based on the table above, it can be seen that the P3 group had the shortest average wound length, which was 0.15 cm. This shows that P3 (the 20% dose) is the most effective dose.

Table 1. Mean and Median wound length Pre and Post Intervention

Groups	N	Mean pre-intervention (cm)	Mean post-intervention (cm)	Median
KK	6	1	0.78	0.21
P1	6	1	0.67	0.73
P2	6	1	0.57	0.52
P3	6	1	0.15	0.15

Table 2. Kruskall Wallis Test Results

Wound length	
Chi-Square	18.82
Asymp. Sig. (p-value)	0.00

Table 2 shows p-value = 0.00($\alpha < 0.05$), which means that there was a significant difference in the wound healing process between the control and treatment groups.

Table 3. Mann Whitney Test Results

Group	p-value
KK vs. P1	0.00
KK vs. P2	0.01
KK vs. P2	0.03

Based on the results of the Mann Whitney test between the KK with P1, KK with P2, and KK with P3, there was a significant difference with the p-values sequentially are 0.00, 0.01, 0.03.

DISCUSSION

The Kruskal-Wallis test showed that there was an effect ($p < 0.05$) on the wound closure process with a p-value=0.00 between the control and treatment groups. This shows the effect of giving betel leaf extract on the healing process of incision wounds in rats.

The wound began to dry on macroscopic observation on the second and third days. This is in accordance with the inflammatory theory, which

explains that the inflammatory phase occurs within 24 hours after the wound occurs, fibroblast cells and vascular endothelial cells begin to proliferate to form granulation tissue, a significant sign of healing. The term granulation tissue comes from its soft, granular, pink appearance on the wound surface. On day 7, the wounds in the P3 group were almost closed with a length of 0.15 cm. This group had a faster wound closure time when compared to other groups.

The wound will trigger the metabolism of arachidonic acid. Arachidonic acid metabolism releases the inflammatory mediator prostaglandins in the cyclooxygenase pathway. The impact is the occurrence of vasodilation of blood vessels. The inflammatory phase continues within 24-48 hours after injury, monocytes replace neutrophils and become the main leukocytes (Sulistiyoning Suharto *et al.*, 2021). Monocytes undergo phenotypic changes to become tissue macrophages. Macrophages phagocytose and kill bacteria and scavenge tissue debris. Macrophages release several growth factors, including PDGF, fibroblast growth factor (FGF), epidermal growth factor (EGF), TGF- β , and TGF- α . These factors stimulate the migration and proliferation of fibroblasts and the production and modulation of the extracellular matrix. Macrophages phagocytize the remaining neutrophils within 1-2 days after injury (Suharto, Rahayu, and Etika, 2017).

In the proliferative phase, fibroblasts migrate into the wound 48 hours after injury. Fibroblasts move along the fibroblast matrix – fibronectin that settles in the early clot and produces fibronectin that facilitates its movement. Fibroblasts produce components of the extracellular matrix, including collagen types I and III, elastin, glycosaminoglycans, and proteoglycans. Type III collagen is the dominant type of collagen during early wound repair. Due to the formation of new connective tissue, fibroblasts undergo a phenotypic change to become actin-rich myofibroblasts. Myofibroblasts play an important role in wound contraction and are predominantly found in granulation tissue. Fibroblasts also play a role in stimulating the proliferation of

keratinocytes. The migration of keratinocytes plays an important role in resurrecting epidermal defects (reepithelialization process) (Etika, Nurrahayu, and Suharto, 2017).

Betel leaf contains active substances, namely flavonoids, alkaloids, saponins, tannins, and essential oils (Madhumita, Guha, and Nag, 2020). The active substance causes the edges of the wound to close faster because each of these active substances has functions that support the wound healing process, so the wound heals faster. Flavonoids have an effect as anti-inflammatory, antioxidant, and also antimicrobial. Flavonoids also function as antibacterial capable of forming complex compounds on extracellular proteins that disrupt skin integrity (Górniak, Bartoszewski and Króliczewski, 2019). Alkaloids have the ability to an antibacterial. By interfering with the constituent components of prostaglandins in bacterial cells, so that the layer on the cell wall is not fully formed, which will later cause death in these cells (Liu Y *et al.*, 2020). Saponins have the ability to clean, and antiseptics that function as a killer and prevent the growth of microorganisms (Zhao *et al.*, 2020). In addition, saponins also function to stimulate the formation of collagen (Yang, Wu and Wang, 2019). Tannins function as antibacterial by interfering with the permeability of bacterial cells (Herryawan and Sabirin, 2018). Tannins also function as astringents that can cause the closure of pores on the skin, stop exudates, harden the skin and stop light bleeding (Alamgir, 2017). Essential oils contain kavikol and phenol, which are useful as antimicrobial, antibacterial and disinfectants (Madhumita, Guha and Nag, 2020). Essential oils are also resistant to bleeding, heal wounds on the skin, and digestive tract disorders (Shedoeva *et al.*, 2019). Previously, Awal Darmawan (2021) conducted a systematic review of betel leaf. The systematic review aims to identify the potential of betel leaf extract in the inflammatory phase, proliferation, and remodeling of wound healing phases. Based on the systematic review, it was concluded that Betel leaf extract has the potential to heal wounds both acute wounds, infection wounds, and diabetic ulcers (Darmawan *et al.*,

2021). This research has limitations. The limitation lies in the basic nature of rats which are rodents that habitually bite wound dressings so that the bandage is open, but this doesn't really affect the result. Therefore, researchers could not control the cleanliness of the wound.

CONCLUSION

There is an effect of betel leaf extract on the healing process of the incision wound. The optimal dose of betel leaf extract is a dose of 20%. It is recommended to study with higher doses for future research until the toxic dose is found.

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