The Effect of Topical Gel Ethanol Extract of Gotu Kola Leaf (*Centella Asiatica* (L.) Urban) on Wound Healing in a White Male Rat (*Rattus Norvegicus*) Induced by Streptozotocin

I Dewa Ayu Sudiari Dewi[1], Nurmawati Fatimah[1], Sutji Kuswarini[1]
[1]*Faculty of Medicine, Universitas Airlangga, Surabaya, Indonesia*

Correspondent:
I Dewa Ayu Sudiari Dewi, Faculty of Medicine, Universitas Airlangga, Surabaya, East Java, Indonesia
Email address: dewaayu.sudiaridewi@gmail.com

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ABSTRACT

Background: Diabetes mellitus (DM) can have a number of consequences, one of which is diabetic neuropathy, which can cause sores, notably in patients' feet. Wound therapy with synthetic drugs in diabetes is still not functioning as well as expected. Centella Asiatica (L.) Urban has been demonstrated to promote wound healing through a variety of mechanisms, including collagen synthesis stimulation, fibroblast proliferation stimulation, antiulcer, antibacterial, and antioxidant activity. Pharmaceutical treatments for wound healing are routinely utilized upon the assumption that a moist wound is a gel.
Aims: The purpose of this study was to see how a topical gel ethanol extract of gotu kola (Centella Asiatica (L.) Urban) promoted wound healing in streptozotocin (STZ)-induced white male rats (Rattus Norvegicus).
Methods: This is an experimental study using twenty white male rats (Rattus Norvegicus) caused by STZ. The rats were placed into four groups, each with five rats. These four groups are the control group, the treatment group P1 (ethanol gel gotu kola leaf at 2.5% concentration), the treatment group P2 (ethanol gel gotu kola leaf at 5% concentration), and the treatment group P3 (ethanol gel gotu kola leaf at 10% concentration). STZ was used to promote blood sugar elevation in the rats. The wound healing rate was estimated by comparing the wound healing rates of each group using macroscopic observation.
Result: The findings of this study revealed that extract gel gotu kola leaf concentration applied to the wound on days 4, 7, 11, and 14 has a statistically significant influence on wound healing (p=0.000).
Conclusion: Based on research conducted, it can be concluded that gotu kola extract gel has a significant influence on the rats' wound healing.

Keywords : *Gel ethanol extract, Gotu kola (Centella asiatica (L.) Urban), Wound healing, White male rat* (Rattus Norvegicus), *STZ (Streptozotocin)*

INTRODUCTION

Diabetes mellitus is a chronic condition that arises when the pancreas fails to produce sufficient amounts of insulin or when the body can not use the insulin that is produced adequately. High blood sugar levels, also known as hyperglycemia, are a typical symptom of untreated diabetes and can cause significant damage to many systems within the body, including the brain system and blood vessels.[1] According to World Health Organization population estimates conducted in 2000,[1] Indonesia's general population ranks fourth in the world, trailing India (31.7 million), China (20.8 million), and the United States (17.7 million). The Indonesian Ministry of Health in Riskesdas 2007 found that the proportion of causes of death from diabetes in the age group 45-54 years in urban areas ranks 2nd at 14.7%, while in rural areas, DM ranks 6th at 5.8%.[2]

DM can cause all sorts of complications, one of which is diabetic neuropathy, which causes sores on the feet, especially in patients with DM. The greatest impact of foot injuries on DM patients was amputation.[3] Besides, DM wound therapy with synthetic drugs is still not as expected.

Synthetic drugs, commonly used topically, that contain antiseptics and antibiotics only solve the problem of infection.[4] Therefore, much research has been done on herbal remedies that can be used for wound healing. Research on plants that can accelerate wound healing includes *Garcinia mangostana* Linn,[5] *Centella asiatica* (L.) Urban,[6] *Piper betle* Linn,[7] and *Piper cr. Fragile*, Benth.[8]

Gotu kola (*Centella asiatica* (L.) Urban) has been observed to have activity in wound healing. The activity occurs through multiple mechanisms, such as stimulating the synthesis of collagen, increasing the secretion of collagen,[9] stimulating the proliferation of fibroblasts,[6] and antiulcer activities.[10] In addition, gotu kola also has antibacterial activity[11] and antioxidants.[12]

Based on preliminary studies that have been carried out using oil stocks, it is known that oil stocks are less effective because the oil that has been spread on the wound seeps into other areas around the wound, so the bandages used to be difficult to attach to cover the wound. Wound care is designed to create an atmosphere to support moist wound healing. Pharmaceutical preparations are often used for wound healing, with the concept that a moist wound is a gel. Mechanism: humid conditions help the wound heal through fibrinolysis, angiogenesis, the formation of growth factors, and stimulation of the active cell (Bryan, 2004). Seeing these conditions, researchers modified the oil into a gel formulation preparation. Gel formulations are known to be more efficiently used for wounds because they are not sticky, easy to spread, and easy to clean (Mohamed, 2004).

MATERIALS AND METHODS

The research was an experimental study to assess the effect of the topical gel ethanol extract of gotu kola leaf (*Centella asiatica* (L.) Urban) on wound healing of twenty white male rat (*Rattus norvegicus*) with the following inclusion criteria: 3-4 month-old; male; weighing 200-250 g; and healthy. The rats were divided into four groups and induced with STZ to raise their blood sugar levels. The rats were divided into four groups with five rats in each group. Those four groups are control (untreated) group and the treatment groups using ethanol gel gotu kola leaf extract at a concentration of 2.5% (treatment group P1), 5% (treatment group P2) and 10% (treatment group P3). Rate healing wounds using macroscopic observation by comparing the healing of wounds between groups. The research design can be seen in Figure 1.

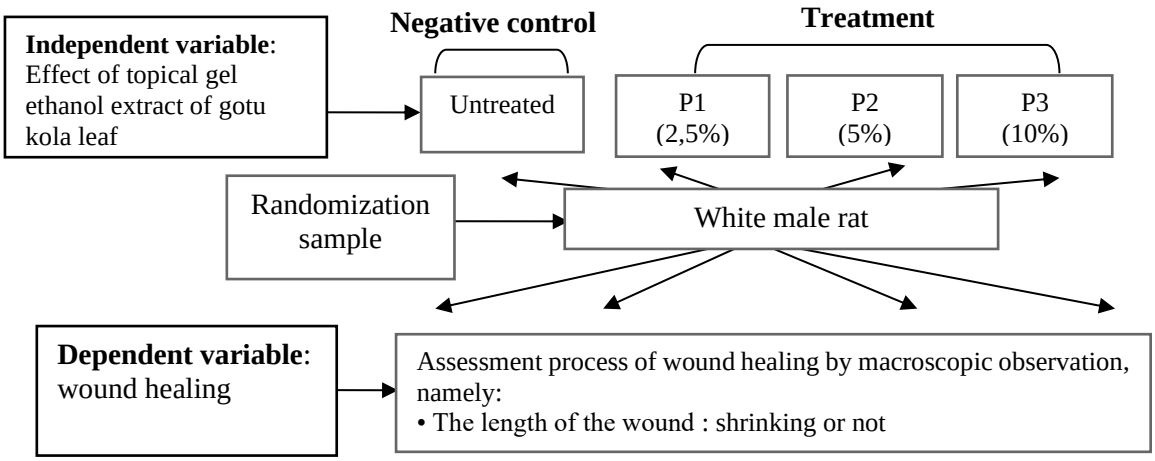

***Figure 1**: Research Design The Effect of Topical Gel Ethanol Extract Gotu Kola Leaf On The Wound Healing Of White Male Rat That Induced By STZ (Streptozotocin)*

The equipments and materials required in this research includes the equipments for extracting gotu kola leaf gel, for rat treatment, for the blood sugar measurements, and for wound care. Equipments for extracting the gotu kola leaf gel were an analytical scale, measuring cups, funnels, filter paper, a stir bar, an Erlenmeyer, vacuum pump, and a thermostatic water bath DHH-S4, funnel, stir bar, gel pot, petri dishes, pipettes, and gloves. Equipments for rat treatment were cage, a drink water, a scale to weigh the rat, rubber gloves, handscoon. Equipments for measuring blood sugar were glucose meter (glucose-DR), stick glucosure, Nesco glucose strips, and alcohol swabs. Equipments for the wound care were scissors, tweezers cirurgis, razors, bath sterile, cotton buds, and syringes.

The materials required in this research were simplicia gotu kola leaf, CMC-Na, propilenglikol, glycerin, and extract gel gotu kola (*Centella asiatica* (L.) Urban) with the concentrations of 2.5%, 5% and 10%. Chemicals Consumables used in this research was 96% alcohol, STZ, 0.9% NaCl solution, *Cleo* mineral water and ether.

The data were analyzed using a statistical program. The homogeneity of variance test is used to determine the sample taken from the same population for the research. The test of homogeneity of variance is calculated using the Levene test. If the test criteria for significance are >0.05, the data is expressed homogeneously; otherwise, if significance is 0.05, the data is not expressed homogeneously. Parametric tests with Oneway-ANOVA were used to determine the significance of the whole groups and determine the effects of the ethanol extract gel of gotu kola leaf on wound healing time and wound length change.

RESULTS

On day 4 post-treatment, there were changes in the length of the wound with male rats; each treatment group suffered alteration, effectively shrinking the wound in extract gel with gotu kola leaf concentration of 5%, whereas the control group had no change; the length of the wound is still the same as the initial size of 1 cm. On day 7, 11, and 14 of treatment using gotu kola extract gel, a the P2 treatment group changed very fast compared to the control group, the P1 treatment group, and the P3 treatment group.

In this research, the test result revealed homogeneity (p=0.204). The statistical Oneway-ANOVA effect of

concentration extract gel gotu kola leaf on wound length on days 4, 7, 11, and 14 indicated a very significant influence of 0.000 due to a significant value of <0.01.

The significance value was <0.05, which showed a significant effect, and >0.05, which showed no significant effect.

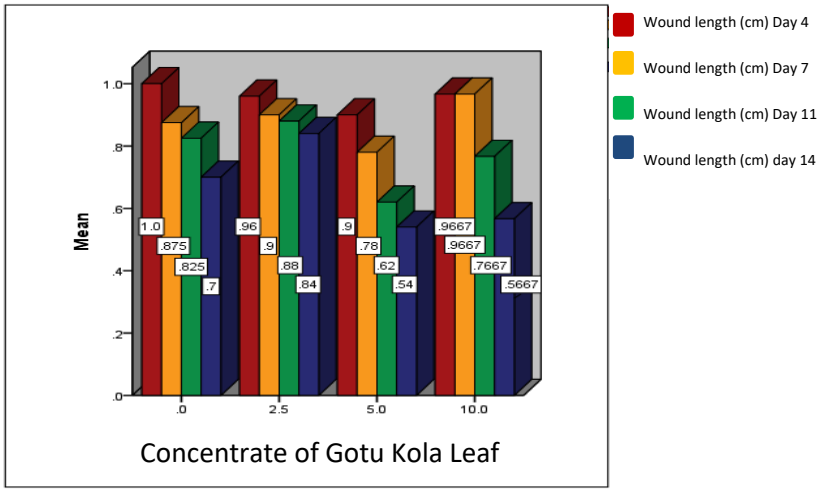

Figure 2: Graph Changes Wound Length Rat

DISCUSSION

The research results showed macroscopic signs of inflammation. According to Smeltzer (2002) the inflammatory phase started a few minutes after the injury and lasts several days, involving leukocytes (polymorphonuclear leukocytes or neutrophils), and showing signs of inflammation. Signs of inflammation have four stages: rubor (redness), calor (heat), tumor (swelling), dolor (pain), and function laesa (loss of function Excess fluid in the interstitial tissue or physical cavity may cause edema, which can be exudate or transudate. The gotu kola leaf content is *madecasoside,* which serves as an anti-inflammatory (Jie Liu, *et al*., 2012) and *asiatic acid* which acts as an antiseptic and antibacterial.

Changes in the length of the wound in the white male rat on day 4 post-treatment showed that each treatment group had suffered alteration, effectively shrinking the wound in extract gel gotu kola leaf concentration of 5%, whereas the control group had no change; the length of the wound is still the same as the initial size of 1 cm. On day 7, 11, and 14, the long wounds of the P2 treatment groups using gotu kola extract gel concentrations of 5% changed very fast compared to the control group, the P1 treatment group, and the P3 treatment group.

Test result statistics Oneway-ANOVA effect of concentration extract gel gotu kola leaf on the wound length on day 4, 7, 11, and 14 had a very significant influence of 0.000 due to the significant value of <0.01. This was because in diminution wounds that were in phase wound contraction in conjunction with the synthesis of collagen, the result of contraction would seem to be a smaller size of the wound. Based on the reference related to the ability of the active compound gotu kola (*asiaticoside, madecasoside,* and *asiatic acid*) to contribute to wound healing through a variety of mechanisms, first increase fibroblast proliferation.

Fibroblasts play an important role in wound healing because of their ability to produce the base substance that forms collagen fibers (Kusumawati, 2007). Second, it stimulates collagen synthesis and increases the secretion of collagen (Zheng,

2007). The content of gotu kola also has antibacterial activity (Oyedeji and Afolayan, 2005) and antiulcer activity (Abdulla, 2010).

Collagen is a white protein that can increase the tensile strength of the wound. When the amount of collagen increases, the wound tensile strength will also increase; therefore, the opportunities for more open wounds become increasingly rare (Alimul, 2006). The content of vitamin C in gotu kola plays an important role in collagen synthesis. Without the presence of vitamin C, stricken young collagen is excreted by fibroblasts, and injuries are few. If the reduced intake of vitamin C disrupts collagen formation, the cells can not stick together. Colagenitation occurs in the proliferative phase of the process of healing wounds, which takes 3 to 21 days after an injury. The formation of collagen will trigger the regeneration of new cells and replace damaged tissue (necrotic) under normal circumstances.

The wound length change in the control group (untreated) on the 4th day does not change; the length of the wound is still the same as the initial size of 1 cm. During the research, only NaCl 0.9% was used as a solvent in the control group (untreated). Normal saline was not able to give a good prognosis for healing wounds due to the fact that in the control group, normal saline was only capable of producing a low percentage of wound contraction in the proliferative phase. Normal saline liquid is a kind of liquid that is isotonic with a NaCl content of 0.9%. Normal saline fluid is less able to function when wounds retain moisture or are in dry conditions, so the injury that occurred is still susceptible to the infection process. Most wound care is designed to create a moist atmosphere to support wound healing. Pharmaceutical preparations are often used for wound healing with the concept that moist wound is gel. Mechanism moist conditions help the wound heal through fibrinolysis, angiogenesis, the formation of growth factors, and stimulation of the active cell (Bryan, 2004).

CONCLUSION

Gotu kola extract gel had a very significant influence (significance value <0.01) on the length of wound healing in white male rats that were induced by STZ on day 4, 7, 11, and 14. Normal saline can not give a good prognosis for healing wounds because in the control group given normal saline only capable of producing a low percentage of wound contraction in the proliferative phase.

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