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Test the effectiveness of clove leaf ethanol extract on wound healing in rats

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***Abstract*---**Clove leaves (Syzygium aromaticum (L.) Merr. & Perry) contain alkaloid compounds, flavonoids, tannins and essential oils that play a role in wound healing. This study used 20 male mice as samples divided into 4 treatment groups, namely group 1 (negative control group), group 2 (positive control group), group 3 (15% clove leaf extract ointment), and group 4 (leaf extract ointment). clove concentration 20%). All mice were injured 2 cm long. The results showed that there was a difference between the control and the clove leaf extract ointment at concentrations of 15% and 20%. In the Wilcoxon test the results showed a p value <0.05 where there was a treatment group. And for the Mann Whitney test the results showed that there were differences between the control group and the clove extract ointment. The p value for the three comparisons was only negative control with clove leaf extract ointment with a concentration of 15% and a concentration of 20% which was smaller than 0.05. So the hypothesis Ho is rejected, there is a difference between the negative control with clove leaf extract ointment with a concentration of 15% and 20%.

***Keywords*---**clove leaf extract, healing, ointment.

Introduction

In their daily work, humans are always faced with certain hazards, such as infectious hazards, toxic reagents and electrical equipment and glasses that are used daily so that they are potentially at risk of injury. Wounds are classified into two parts, namely acute wounds and chronic wounds. Acute wounds have a fast attack and heal according to the estimated time, while chronic wounds fail to heal in the expected time (Perdanakusuma, 2007). Wound healing is a form of business process to repair the damage that has occurred. Wound healing is highly dependent on the health of the patient. Likewise, the mechanism of wound healing is highly dependent on the cause and condition of the wound. Wound healing physiology naturally undergoes three phases, namely the inflammatory phase, the proliferation phase, and the wound healing phase.DE Stala, 2013, N. Mayet. et al. 2014)).

Indonesia is a country that has a lot of plant diversity spread throughout Indonesia. One of the plants that is often used as traditional medicine is Clove (Syzygium aromaticum L.) (. Batiha et al.) 2020 The results of the phytochemical test on clove leaf extract contain active compounds such as terpenoids, flavonoids, alkaloids, phenolics, tannins, saponins and glycosides, oils such aseugenol, -caryophyllene, and -humulene (Gonzales , et al. 2021).. It is suspected that the content of these compounds can help the wound healing process.

Materials and Methods

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A. Materials

The tools used are beaker glass, filter paper, digital scale, blender, glass jar, erlenmeyer, stirring rod, aluminum foil, scalpel, evaporator, razor, sterile gauze, oven, wound care kit, cage, place to eat and drink for mice, ointment pot, analytical balance. The materials used were clove leaf powder, 96% ethanol, vaseline album, adeps lanae, and betadine ointment.

B. Ointment Making

Clove leaf extract ointment was made with 2 concentrations, namely 15% and 20%. The use of these two types of concentration was chosen based on the trial and error method.

The formulation of the clove leaf extract ointment is as follows:

a. Clove leaf extract ointment formulation with a concentration of 15%

R/ Clove leaf extract 3 grams
Adeps lanae 2.55 grams
Vaseline alba 14.45 grams
mf ointment 20 grams

b. Clove leaf extract ointment formulation with a concentration of 15%

R/ Clove leaf extract 4 grams
Adeps lanae 2.4 grams
Vaseline alba 13.6 grams
mf ointment 20 grams

C. Procedure

Weighed 400 grams of clove leaf powder using an analytical balance, then crushed. Added 1.5 L of 96% ethanol solvent and put into a container, cover and leave for 72 hours protected from light while stirring. It was filtered using filter paper so that the maserate was obtained and stored in a clean bottle. The pulp was macerated with 96% ethanol using the same procedure, maceration was carried out until a clear maserate was obtained. The sample used as the object of this study was male white mice. Selected 32 male mice and then adapted for 7 days. After 7 days, 32 mice were randomly divided into 4 treatment groups and cuts were made. This study uses an experimental method with a pre-test and post-test only control group design

Before making the wound, the white mice were first anesthetized using ketamine and xylantine. The incision was made on the back of a white mouse with a scalpel. The incision is made in the back area with a length of 2 mm and a depth to the subcutaneous tissue

Results and Discussion

The study used 20 mice which were then divided into 4 treatment groups, namely the negative control group by giving the ointment base, the positive control group by betadine ointment, the treatment group by giving clove leaf extract ointment with a concentration of 15% and 20% by giving 1 time per day.
The following is a table for measuring wound healing:

Table 4.1 Measurement of Cuts in Mice

Treatment Group	Repetition	Pretest	Posttest
Negative control	1	2.00 mm	0.30 mm
	2	2.00 mm	0.72 mm
	3	2.00 mm	0.40 mm
	4	2.00 mm	0.20 mm
	5	2.00 mm	0.20 mm
positive control	1	2.00 mm	0.20 mm
	2	2.00 mm	0.25 mm
	3	2.00 mm	0.40 mm
	4	2.00 mm	0.00 mm
	5	2.00 mm	0.00 mm
Clove leaf extract ointment concentration 15%	1	2.00 mm	0.40 mm
	2	2.00 mm	0.00 mm
	3	2.00 mm	0.00 mm
	4	2.00 mm	0.00 mm
	5	2.00 mm	0.00 mm
20% concentration of clove leaf extract	1	2.00 mm	0.30 mm
	2	2.00 mm	0.00 mm

ointment	3	2.00 mm	0.00 mm
	4	2.00 mm	0.00 mm
	5	2.00 mm	0.00 mm

Based on table 4.1, the results show the amount of time (days) needed to heal cuts in mice was the most in the negative control group with more than 10 days. While the minimum amount of time (days) was indicated by the 20% concentration ointment treatment group for 6 days, and the 15% concentration ointment treatment group for 8 days, while the positive control group took 10 days to heal wounds. The results of the calculation of the difference in wound length for each group on day 1 to day 10, where the difference in wound length in the 15% concentration ointment treatment group and 20% ointment concentration had closed faster on the eighth day, then followed by the positive control group while the control group Negative results indicate the difference in length of the wound that has not closed on day 10.

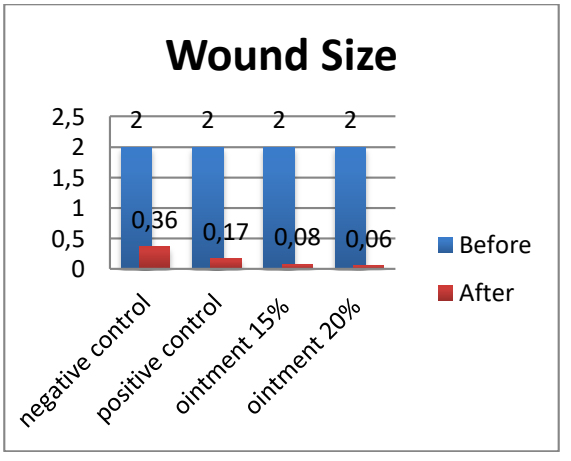

Figure 4.1 graph of the average wound healing based on Figure 4.1 it was found that there was a decrease in the graph from the negative control group to the treatment group giving clove leaf extract ointment with a concentration of 20%. This shows that the longest average for wound healing is in the negative control, which is 0.36 mm and the fastest is found in the 20% clove leaf extract ointment treatment group, which is 0.06 mm.

Table 4.2.1 the results of the calculation of the normality test

Tests of Normality b, c, d, e

	Kolmogorov-Smirnova			Shapiro-Wilk		
treatment	Statistics	df	Sig.	Statistics	df	Sig.
afte control -	.234	5	.200*	.834	5	.150

r	control +	.239	5	.200*	.902	5	.419
	15% ointment	.473	5	.001	.552	5	.000
	20% ointment	.473	5	.001	.552	5	.000

From the results of this test, it shows that the sig value is less than 0.05. The conclusion drawn is that H0 is rejected and the data is not normally distributed.

Table 4.2.2 Wilcoxon test results

Ranks

		N	Mean Rank	Sum of Ranks
after before	Negative Ranks	20a	10.50	210.00
	Positive Ranks	0b	.00	.00
	Ties	0c		
	Total	20		

a. after < before

b. after > before

c.after = before

Test Statisticsb

	after before
Z	-3.981a
asymp. Sig. (2-tailed)	.000

a. Based on positive ranks.

b. Wilcoxon Signed Ranks Test

In the results of this test obtained sig = 0.000 (sig < 0.05), then the conclusion drawn is H0 is rejected, then there are differences in wound healing before and after being given treatment

Table 4.2.3 Man Whitney Test

Treatment	Sig	Information
Negative control and positive control	0.202	There is no difference between negative control and positive control

Negative control and 15% concentration ointment	0.051	There is a difference between negative control and 15% concentration ointment
Negative control and 20% concentration ointment	0.030	There is a difference between negative control and 20% concentration ointment
Positive control and 15% concentration ointment	0.345	There is no difference between positive control and 15% concentration
Positive control and 20% concentration ointment	0.290	There was no difference between positive control and 20% concentration
15% concentration ointment and 20% concentration ointment	0.881	There is no difference between 15% concentration ointment and 20% concentration ointment

From the results of Man Whitney in table 4.2.3 it is stated that in the negative control with 15% and 20% concentration ointment there is a difference while for the others there is no difference, while seen from graph 4.1 it states that the clove leaf extract ointment with a concentration of 20% is more effective in healing wounds. it sucks compared to the others.

The presence of chemicals such as flavonoids and trepenoid saponins has a wound healing effect. (A. Mahmood et al. 2016) The mechanism of action of flavonoids is to improve blood circulation throughout the body and prevent blockages in blood vessels, contain anti-inflammatory properties, also function as antioxidants, and help reduce pain in the event of bleeding or swelling (Wahyuningsih, S., et al, 2006). Saponins work as antimicrobials (source of anti-bacterial and anti-viral), boost the immune system, increase vitality, blood sugar levels, reduce blood clotting, and saponins also affect collagen (early stage of tissue repair) (Kim et al.2011) namely by inhibiting the production of excessive wound tissue. Triterpenoid saponins are saponins that have extraordinary wound healing effects, including Asiatic acid, Madecassic acid Asiatocoside, Madecassoside, Asiaticoside which function to improve repair and strengthening of skin cells, stimulate growth of nails, hair and connective tissue (Kurniati, 2008). Tannin compounds also play a role in the wound healing processwhite mice (Su, et al. 2017 because tannin is useful as an astrigen where astrigen will reduce mucosal permeability and the bonds between mucosa become strong so that microorganisms and irritant chemicals cannot enter the wound (Suprapto, 2012; Pires et al 2020).). Tannins play a role in inhibiting hypersecretion of mucosal fluid and neutralizing inflammatory proteins (Fadilah and Santosa, 2019).Stating that tannin compounds contain anti-bacterial compounds where these compounds help shrink cell walls or cell membranes so as to inhibit bacterial permeability to develop Ajizah (2004 and Liu et al, 2020).

Based on the observations during the study, 20 white mice with cut wounds appeared to heal on the 8th to the 10th day after treatment with 20%

concentration ointment, 15% ointment concentration and positive control, tannin compounds capable of inhibiting hypersecretion of mucosal fluid and neutralize inflammatory proteins. Tannins have an affinity for proteins so they can be concentrated in the wound area (Suprapto, 2012 and sw. kang et al. 2014)).The wound closes after the wound undergoes the process of removing the scab. This indicates that the growth of new cells has occurred with the closer the wound edges. The scab is released where the underlying tissue is dry and the edges of the wound begin to be pulled to the center (Argamula, 2008).

Based on the results of this study, the administration of clove leaf extract ointment was treated by applying 2 times a day on the backs of white mice with negative control namely ointment base, positive control namely betadine ointment, clove leaf extract ointment concentration 1 5% and leaf extract ointment concentration 20% . The results of this study showed that the 20% concentration of clove leaf extract ointment (Banerjee. et al, 2020) was able to accelerate the healing of cuts in white mice. This is because clove leaf extract contains essential oils that can reduce pain if bleeding or swelling occurs. In addition to flavonoids, there are also tannin compounds that are able to have an effect on wound healing which function as astringents in the wound healing process.

Conclusion

Based on the results of the study concluded

1. That the ointment containing Syzygium aromaticum (L.) Merr. & Perry clove leaf extract can accelerate the process of wound healing in mice
2. And at a concentration of 20% it was more effective than a concentration of 15% in accelerating the healing of cuts in white mice while the positive control, namely betadine ointment, required a longer time interval for wound healing in mice.

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