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EFFECTIVENESS OF SNAIL MUCUS GEL (*Achatina fulica* Ferr) ON MICE (*Mus musculus* L.) BURNS

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Abstract: This study aimed to determine the effectiveness of snail mucus gel (*Achatina fulica*) on the length of time for burns healing, burns diameter, and the percentage of burns healing in mice (*Mus musculus*). The type of this research was experimental with a completely randomized design (CRD) method with 20 male mice divided into five treatment groups, namely NC (Negative Control; gel basis), PC (Positive Control; Bioplacenton 10%), P1 (3% gel), P2 (4% gel) and P3 (5% gel), with four replications for each treatment. The making of burns on the back of mice was done by using metal coins that were heated to become coals with an initial diameter of 20 mm, which are affixed for 1 second. The parameters observed were the length of time for burns healing, burns diameter, and the percentage of burns healing for 16 days. The data were analyzed by using Way ANOVA (Analysis Of Variance) ($\alpha = 0.05\%$) and continued with the LSD (Least Significant Different) test. The results showed that snail mucus gel (*A. fulica*) with a gel concentration of 5% was more effective in accelerating burns healing with an average healing time of 14 days, and positive controls healed within 14. In 25 days, 4% gel recovered in 14.5 days, 3% in 15 days, and negative controls recovered in 16 days. The fastest closure of burns diameter was in gel with a concentration of 5% with a mean of burns diameter of 0.33 mm and an average percentage of burn healing of 98.35%, followed by the Bioplacenton treatment group of 10%, gel with a concentration of 4%, 3%, and the longest burns closure was in the ointment base gel group.

Keywords: *Achatinafulica*, *Mus musculus* L., Burns Healing

INTRODUCTION

Snails are soft animals (mollusks) that belong to the gastropod class. People think that the snail (*Achatina fulica*) is only a pest on plants, but only a few know that the snail has many benefits, starting from its meat which has a high protein content, and mucus, which is rich in benefits [1]. Over time, the snail (*A. fulica*) has been widely cultivated because its meat can be consumed, and its mucus content can be used to treat various diseases, one of which can be used in wound healing [2].

The chemical constituents of snail slime (*Achatina fulica*), which can heal wounds, are achasin isolate, heparan sulfate, and acharan sulfate. Achasin isolate is a protein molecule that is active as an antibacterial [3]. Achasin protein can help the wound healing process because it reduces bacterial infection. Heparan sulfate affects the increase in fibroblasts as an extracellular matrix, accelerating wound healing [4]. Acharan sulfate contained in mucus is a glycosaminoglycan that, in the proliferation phase, will form a complex that plays an important role in wound healing.

Healing with snail mucus can be an alternative because it is easy to use, has good spreading power on the skin, does not clog skin pores, and also has an antibacterial effect. In the community, the extract of snail meat and its mucus is very useful for treating various diseases, including inflammation of the lining of the eye, toothache, itching, heart disease, diabetes, and medicine for external injuries such as wounds. Cuts and burns [5].

Burns is tissue damage or loss due to contact with heat sources such as fire, hot water, chemicals, electricity, and radiation. Burns are a type of trauma with high morbidity and mortality [6]. Burns are expressed with a degree that is determined by the depth of the burn, namely degree I only injures the epidermis layer, degree II A injures up to the epidermis layer, and part of the upper layer of the dermis, degree II B burns include the epidermis and deep layers of the dermis and burns Grade III damage includes skin, subcutis fat to the muscles and bones [7].

Treating burn patients by treating the wound using topical preparations because the hardened tissue from burns cannot be penetrated by administering drugs in oral or parenteral dosage forms [8]. The topical gel dosage form was chosen because it has several advantages, namely good dispersion on the skin, a cooling effect caused by slow evaporation of water on the skin, not inhibiting the physiological functions of the skin does not clog skin pores, and allowing use on hairy parts of the body [9].

Bioplacenton is a drug that contains placenta extract and neomycin sulfate. This drug is commonly used to treat burns, wounds with infections, chronic wounds, and other types of wounds. Placenta extract works by triggering the

formation of new tissue and for wound healing, while neomycin works by preventing or treating Gram-negative bacterial infections in the wound area. Research on healing burns using snail mucus in gel dosage form has yet to be widely carried out, so in this study; snail mucus was formulated as a gel for healing second-degree burns. The reason for using snail mucus is that snail mucus has moist properties on the skin, is beneficial in healing, and inhibits the inflammatory process [10].

RESEARCH METHODS

Preparation of Gel Base

The gel base is prepared by; putting 0.2 grams of Carbopol 940 into the mortar and then adding 10 mL of distilled water, which has been heated to 90ºC and stirred until it melts. Add 0.05 g of triethanolamine (TEA) and stir until a gel is formed, then add 0.05 g of methylparaben and 7.5 g of propylene glycone, then add distilled water and stir at constant speed until homogeneous. A gel base was used for the Negative Control.

Preparation of Snail Slime Gel (*Achatina fulica*)

Mucus gel was prepared as much as 50 grams in three concentrations, namely 3%, 4%, and 5%, for use for 16 days to treat burns in mice. The formulation for making snail slime gel is presented in Table 1.

Table 1. The formulation for Making Snail Mucus Gel (*Achatina fulica*)

Material	PI(3%)	P2(4%)	P3(5%)
Mucus (*A. fulica*)	1.5gr	2gr	2.5gr
Carbopol 940	0.2gr	0.2gr	0.2gr
Thetonolamin	0.05gr	0.05gr	0.05gr
Propilenglikol	7.5gr	7.5gr	7.5gr
Metyl paraben	0.05gr	0.05gr	0.05gr
Aquades	40.7mL	40.2 mL	39.7 mL

Preparation of Mice with Burns

Mice (*Mus musculus* L.) were first acclimated for seven days to avoid stress. Feed is given as much as 10 grams per day, and drink ad libitum. Burns were made by attaching a 20 mm hot metal coin to the back of the mouse for 1 second to form a category II A burn, marked with a reddish color and formed bullae [11]. Treatment for each group was applied three times a day for 5 hours.

Data Analysis

Data collection was obtained from observations of burns in mice after treatment with snail gel until healing occurred. These data include the diameter of the burn and the percentage of burn healing.

1. Duration of Healing Burns

Observation of the duration of healing of burns aims to determine the length of time healing of burns after treatment with snail mucus gel (*Achatina fulica*) and reduction in burn wound diameter in mice (*Mus musculus* L.) for 16 days of observation

2. Measuring the diameter of the burn

The measurement of wound diameter was carried out using the Morton method, namely measuring four wound diameters from various directions, then calculating the average diameter value for each measurement[12].

$$dx = \frac{dx\,(1) + dx\,(2) + dx\,(3) + dx\,(4)}{dx}$$

Note:
dx =Burn diameter on day x (mm)
$dx_{(1)}$ = Horizontal diameters (mm)
$dx_{(2)}$ = Vertical diameter (mm)
$dx_{(3)}$ = Diagonal diameters (mm)
$dx_{(4)}$ = Diagonal diameters (mm)

3. Percentage of Healing Burns

Calculation of the percentage of burn healing is calculated by the formula[13]:

$$P = \frac{L1 - Ln}{L1} \times 100\%$$

Note:
L_1 = First-day burn diameter
L*n* = diameter of burn day n

The data analysis technique used the Statistical Product Services Solution (SPSS) version 20.0 application program. The data obtained in wound healing time, burn diameter, and burn healing percentage were tested statistically by one-way ANOVA with a confidence level of 95%, $\alpha = 0.05$. If there is a significant difference, proceed with the LSD (Least Significant Different) test to see which treatment has a different effect.

RESULT AND DISCUSSION

Duration of Healing Burns

Observation of the duration of healing of burns aims to determine the length of healing after treatment with snail mucus gel (*Achatina fulica*) and reduction in burn wound diameter in mice (*Mus musculus* L.) for 16 days of observation. The percentage value is the result of a comparison between the diameter of the wound on one day minus the diameter of the wound on the nth day divided by the diameter of the wound on one day.

Based on Table 2, it can be seen that group P1 (3% gel) required an average wound healing time of 15 days or the equivalent of 348 hours. Group P2 (4% gel) needed an average wound healing time of 14.5 days or 360 hours. The P3 group (5% gel) needed an average wound healing

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time of 14 days or 336 hours. The PC group (Bioplacenton 10%) needed an average wound healing time of 14.25 days or 342 hours, and the NC group (gel base) needed an average wound healing time of 16 days or 382 hours.

Table 2. Average Duration of Healing Burns

Treatment	Healing Burns (Days)				Mean±SD	Healing Burns (Hours)
	1	2	3	4		
KN	16	16	16	16	16±0[c]	382
KP	14	14	14	15	14.25±0,5[a]	342
P1	15	15	15	15	15±0.57[ab]	348
P2	14	14	15	15	14.5±0[b]	360
P3	14	14	14	14	14±1[a]	336

Note: Numbers followed by the same letter in the same column show results that are not significantly different

Table 2 shows that the slowest wound healing occurred in the NC group (gel basis), while the snail mucus gel treatment at concentrations of 3% and 4% showed healing times that were not much different. The slow wound healing process in the negative control (gel base) is because the components in the gel base do not contain bioactive compounds that can help the wound healing process, so the process runs slower than other treatments. The slowest wound healing process occurs in wounds that are given a gel base because the gel base does not contain ingredients or substances that are productive for covering wounds and are antibacterial [14].

Diameter of Burns

The measurement of wound diameter was carried out using the Morton method, namely measuring four wound diameters from various directions, then calculating the average diameter value for each measurement. The result of the measurement of burn diameter is presented in Tabe 3.

Table 3. Average Diameter of Burns in Mice (*Mus musculus* L.) After Treatment with Snail Mucus Gel (*Achatina fulica*) For 16 Days

Treatment	Mean Burn Diameter ± SD			
	1st day	Day 5	12th day	16th day
NC	20	17.12±0.06	7.50±0.12	2.13±0.08[d]
PC	20	16.90±0.05	6.39±0.08	0.48±0.13[ab]
P1	20	17.04±0.10	7.05±0.04	0.94±0.10[c]
P2	20	16.96±0.10	6.76±0.13	0.57±0.11[b]
P3	20	16.83±0.06	6.33±0.07	0.33±0.05[a]

Note: Numbers followed by the same letter in the same column show results that are not significantly different

Table 3 shows that snail slime gel (*Achatina fulica*) is effective in reducing the diameter of burns. It can be seen in the gel treatment group with a concentration of 5% with a final mean diameter of the burn wound, namely 0.33 mm, the gel treatment group with a concentration of 4%, namely 0.57 mm, the gel treatment group with a concentration of 3%, namely 0.94 mm, the PC treatment group (10% Bioplacenton) obtained an average final burn diameter of 0.48 mm and the NC treatment group (gel basis) obtained an average final burn wound diameter measurement of 2.13 mm.

Treatment with the snail mucus gel causes a reduction in diameter in burns which is influenced by the content contained in snail mucus which acts as an antibacterial and anti-inflammatory so that, in the process, it can accelerate wound diameter reduction. This statement is supported by the research of [15], which states that the more active compound content in the preparations made, the faster wound healing will occur.

Diameter closure in burns occurs due to a reduced inflammatory reaction and the occurrence of a granulation process in the wound area, which causes closure of the skin so that, visually, a reduction in the diameter of the wound is seen [16]. The appearance of the stages of healing burns after treatment with snail slime gel is presented in Figure 1.

Figure 1 shows the stages of healing of burns starting from the initial stage of healing, namely the inflammatory phase, until the wound heals. Based on the data obtained, the fastest inflammatory phase occurred in the gel group with a concentration of 5%. This phase starts from day 1 to day 5. Compared to the other treatment groups, the inflammatory phase occurred until days 5, 5, and 6. The inflammatory phase could proceed quickly in the gel with a concentration of 5% due to the presence of a compound in the snail's mucus (*Achatina fulica*). Namely, Achasin isolates can coagulate and eradicate bacteria. [17], stated that the components in snail mucus include analgesic substances, antimicrobial peptides (again), and antiseptic substances, which function in wound healing. [18] stated that snail mucus can clean bacteria the first time it is applied to the wound; when the inflammatory phase of the wound can be passed properly without any infection, the fibroblast proliferation process will run well too.

From day 6 to day 12 is the proliferative phase. Based on the data obtained, the proliferative phase that occurred the fastest for all treatment groups was the gel group with a concentration of 5%, whereas, in this group, the proliferative phase occurred on days 6 to 12 compared to other treatment groups occurring

until day 13. Parameters observed in this phase were the formation of granulation tissue and fibroblasts as scabs on the wound.

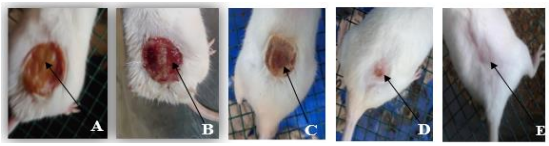

Figure 1. The appearance of Mice Burn Wound Healing Stages. A (blister), B (erythema), C (scab), D (wound closed), E (healed)

The 16th day is the remodeling phase, the last and longest phase in the wound healing process, with the goal of perfecting the formation of new tissue into strong tissue. This phase begins at the end of the proliferative phase and can last for months [19]. The fastest remodeling phase occurred for all gel treatment groups with a concentration of 5%, where the wound had healed on the 14th day compared to the other groups whose wounds had healed on the 15th and 16th day. The parameters observed in this phase have wound that close and healed, marked by hair growth.

Percentage of Healing of Burns

The percentage of wound healing was observed for 16 days (Table 4)

Table 4. Average Percentage of Healing Burns

Treatment	Percentage of Healing of Burns ±SD			
	1st day	Day 5	12th day	16th day
NC	0	14.40±1.19	62.50±1.19	89.32±1.61[a]
PC	0	15.50±0.72	68.05±1.19	97.56±1.25[cd]
P1	0	14.80±1.19	64.75±1.76	95.28±1.57[b]
P2	0	15.20±1.02	66.20±4.62	97.15±1.61[c]
P3	0	15.85±1.87	68.35±2.77	98.35±0.72[d]

Note: Numbers followed by the same letter in the same column show results that are similar.

Based on Table 4 shows that the highest average percentage of burn healing was in the K3 group (5% gel), with an average percentage of burn healing of 98.35%, not much different from the KP treatment group (10% Bioplacenton) with an average percentage of 97.56%, while the lowest average percentage of burn wound healing was in the KN (ointment base) group of 89.32%. The high percentage of wound healing in the P3 treatment group was influenced by the content of snail mucus, namely Acharan sulfate, which moisturizes the skin. The wound moist is the most important thing and can significantly increase epithelialization in the wound-healing process [20]. The Bioplacenton gel treatment group had an average similar to the 5% snail slime gel treatment group because Bioplacenton gel is a medicine for external wounds, especially burns, specifically designed to treat and accelerate wound healing.

The percentage results show that bioplacenton gel has a percentage that is not much different from the 5% snail mucus gel treatment; this is because Bioplacenton contains neomycin sulfate and Placenta Extract, which functions to treat burns, wounds with infection, chronic wounds, and other types of wounds. Placenta extract worked to trigger the formation of new tissue, and neomycin sulfate prevented infection in the wound area [21]. Placenta extract has anti-inflammatory, antioxidant, and anti-melanogenic effects, provides a moisturizing effect, and is rich in collagen-forming material [22]. Neomycin sulfate is an aminoglycoside class of antibiotics used topically on the skin, working by preventing gram-negative bacterial infections in the wound area [23].

CONCUSSION

Based on the results of the study, it can be concluded that the most effective snail mucus gel (*Achatina fulica*) accelerates the healing of burns in mice (Mus musculus L.) in the 5% gel concentration compared to the 3% and 4% gel concentrations, with an average length of time for healing burns is 14 days from the healing time of 16 days, the mean final diameter of the burn is 0.33 mm from the initial diameter of 20 mm, and the average percentage of burn healing is 98.35%.

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