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Distribution of Snail Mucous Extract (Achatina Fulica) on the Number of Wound's Basal Epithelial Cells in Rats of Wistar Strain

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Abstract

Background: Acharan sulfate, a new glycosaminoglycan isolated from Achatina fulica, obtained from Kediri, East Java, Indonesia, plays a big role in wound healing process, because it can promote re-epithelialization. Purpose: This study aims to prove that extract of Achatina fulica mucous will increase the number of basal epithelial cells of the wound incision in the back of white male rats of wistar strain. **Method:** This research was done by an in-vivo experimental laboratory, using 14 white male rats of wistar strain that given an incision injury in the back with a length of ± 2 cm and depth of ± 2 mm. They were divided into two treatment groups, that are control group which is the incision wounds were given saline solutions; and treatment group which is the incision wounds were given an extract of Achatina fulica mucous with crude acharan sulfate level is 86,74%. This treatment is given 3 ml once a day for five days. On the sixth day, biopsy was carried out to make a histological specimen, then evaluate under a microscope to account the number of basal epithelial cells of the wound incision. Then statistical analysis was conducted by using independent samples t-test with α=0,05. **Results:** There were significant differences between two treatment group, that are the number of basal epithelial cells in control group and treatment group ($p<0,05$). **Conclusion:** Extract of Achatina fulica mucous with crude acharan sulfate level is 86,74% increase the number of basal epithelial *cells of the wound incision in the back of white male rats of wistar strain.*

Key words: *wound healing, re-epithelialization, achatina fulica, acharan sulfate, snail mucous*

Introduction

Wound is a type of damage to body tissues due to an injury or trauma that disrupts the regular continuity of tissue structure[1]. In dentistry, dental treatment procedures are often related to wounds, such as tooth extraction, scaling, thrush.

Achatina fulica, is an animal that can be found anywhere and still consumed by Indonesian[2], and it's a identity fauna for the city of Kediri[3]. Their mucous contains a glycosaminoglycan secreted from the grains in the snail's body located on the outer surface, as a result of exposure to stress on the snail[4]. This content is called the acharan sulfate that contains most of the repeated disaccharide units from →4)-2-acetamido-2-deoxy-α-D glucopyranose(1→4)-2-sulfo-α-L-idopyranosyluronic acid(1→ ($GicNAc\text{-}IdoA2SO_3^-$)[5].

Based on previous study by Martins, Maria de Fatima et.al, even though snail mucous applied in different forms, the results showed that both treatment group have faster wound healing, and better healing process than control group[6]. Therefore, Snail mucous is widely developed abroad for cosmetics.

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Tissue re-epithelialization is one of the stages in the wound healing process[9] after the inflammatory stage. In this stage, there is an interaction between cells and extracellular matrix[10,11], fibroblast proliferation, angiogenesis, and wound contraction[12]. In the proliferative phase, fibroblasts synthesize collagen and glycosaminoglycans, which will interact with each other and play a role in wound contraction. While glycosaminoglycans themselves can stimulate the re-epithelialization process by increasing the growth and differentiation of epithelial cells and stimulate cell migration[13,14,15]. This study is expected to prove that glycosaminoglycans in Achatina fulica mucous namely acharan sulfate can help the process of re-epithelialization in wound healing marked by an increase in the number of basal epithelial cells of the wound, so that later can be used as an alternative drug to accelerate wound healing[16].

Material and Method

This study is an experimental[17] laboratory conducted on 14 white wistar strain male rats, obtained from the Laboratory of Biochemistry, Faculty of Medicine, Airlangga University, around 2.5-3 months old, bodyweight ranges from 150-200 grams, physically healthy and possible to be sampled, after being maintained for 3-4 days to adapt, in the same places, conditions, and feed at the study site. The experimental animals were divided into two treatment groups, 7 male rats in control group (treated with saline water), and 7 male rats in treatment groups (treated with snail mucous extract).

Achatina fulica Mucous Isolation

This study used around 50 snails (Achatina fulica) obtained from Kediri, East Java. Snail mucous of Achatina fulica is collected by stimulating the body surface of the snails for 30-60 seconds using an electric shock from 6 pieces 1.5-volt battery that configured in parallel set up. Snail mucous obtained is around 550-600 ml and collected in a bottle, then stored in a freezer and will be defrosted when used[18,19].

Water-soluble snail mucous is obtained by mixing it with water (aquadest) twice the sample volume. Then add 1% potassium acetate to ethyl alcohol five times the volume of the sample, and the mixture is put on magnetic stirrer in refrigerator at 40°C overnight. After that, the water-soluble extract was centrifuged at 8000 X g for 30 minutes[20].

The sediment obtained was then measured in volume and dissolved in distilled water 2.5 times the sample volume and 5% cetylpyridinium chloride as much as 5/8 times the sample volume, then stored for 1 hour at room temperature. After 1 hour, the mixture was centrifuged at 8000 X g for 30 minutes, then the precipitate was dissolved in 2.5M NaCl by ¼ sample volume at 450°C for 30 minutes. After that, the sample is purified by adding ethanol 3 times the volume of the sample, then let stand for 1 hour and then the mixture is centrifuged at 2900 X g (4000 rpm) for 30 minutes.

Precipitation obtained was diluted again with tris-HCL pH 8.0 to obtain snail mucous extract of ethanol precipitation as a result of re-suspension. The results of the re-suspension were collected and then stored in -80°C, then freeze dry was carried out to obtain concentrated snail mucous extract which is easy to store and contains crude acharan sulfate[18–20]. The results obtained are in the form of brownish solids (granules) containing crude acharan sulfate, which is calculated using the Biuret method, amounting to 86.74% of the weight. The weight of snail mucous extract is 1260 mg using digital weighing instrument with an accuracy of up to 0.01 gr.

Treatment Procedure

Before doing the incission, shave the hair that covers the skin of rats's back that will be incised with a razor blade and then cleaned with antiseptic, and then giving inhalation anesthesia to experimental animals.

Each group was given the following treatment:

The Control Group (CG) and the Treatment Group (TG) made incisions with length ± 2 cm and depth of ± 2 mm, full thickness, until the muscular layer (fascia) is seen, using a scalpel.

The incision wound on CG was irrigated with saline water while in the TG was dripped with snail mucous extract diluted with distilled water (10 mg/ml) each of 3 ml, for 5 days by using sterile syringe.

On the 6th day, a biopsy was performed, including

the normal tissue, then the tissue was fixed with 10% formalin solution and microscopic preparations were made. For all specimens, cutting with a microtome was done with a thickness of 5 µ and stained with Hematoxylin Eosin (HE).

The wound on the back of rats that were exposed as a result of biopsy were sutured and given antiseptics.

After histological preparations were obtained, then photographed with a camera (min. 3 megapixels and 5x optical zoom) in one field of view with the aid of a 400x magnification light microscope. The number of basal epithelial cells wound was calculated by counting the number of cylindrical epithelial cells sitting on the basal stratum within the wound margins[23] then entered in a computer and calculated manually with the help of the Image Tool program from the computer.

Results

Data from calculation on the number of wound basal epithelial cells in each treatment group were then analyzed using a statistical test with a significance level (α) = 0.05.

Table 1: The number of basal epithelial cells along with their statistical test values.

Sample	Basal epithelial cells count	
	Control group	Treatment group
1	44	78
2	40	69
3	37	67
4	46	66
5	51	77
6	32	74
7	47	94
Mean	42.4286	75.0000
Standard Deviation	6.50275	9.62635
Sig. Levene's Test	0.546	
Sig. Kolmogorov-smirnov Test	0.990	0.835
Sig. Independent samples t-test	0.000	

Based on the table above, the results of the Levene's test, to find out the data variance, are the p value=0.546> α=0.05, which means that the average population of the two variants is the same or homogeneous data. Kolmogorov-smirnov test is to determine the distribution of data, the results obtained in CG that the p value=0.990> α=0.05, which means that in the CG, the distribution data is normal. And for TG, the results obtained are p value=0.835> α=0.05, which means that in the TG, the distribution data is normal. The results of the independent samples t-test, to find out whether there were significant differences between each treatment group, p value=0,000< α=0.05 which means that there are significant differences in the number of basal epithelial cells between the CG and the TG.

Discussion

Microscopically, the wound area looks different from the surrounding healthy tissue, namely the proliferation of fibroblasts, capillary proliferation, and collagen fibers appear irregular. In addition to the visible difference in the number of basal epithelial cells between the CG and the TG, there was also a difference in the wound closure process, namely in the CG there were three

preparations whose wounds had not closed or had not formed an intercellular bridge from the new epithelial cells, whereas in the TG there is only one preparation. This can be caused by several factors, intrinsic or extrinsic. Intrinsic factors include age, health status, nutrition, oxygenation and tissue perfusion. Extrinsic factors include poor surgical and suturing techniques, drugs, wound management, psychosocial/psychological stress, and risk of infection due to unclean wounds[26,27].

From the various factors, the probable cause in this study is poor wound management, because on the second day of evaluation it turned out that the gauze dressing detached in one of the TG so that the wound was not protected, making the wound area able to move and can be disturbed by other rat, causing the healing process to be interrupted. In addition, in the TG, the wound closure process was faster and an increase in the number of basal epithelial cells showed a thicker epidermal layer after measured using specific computer software.

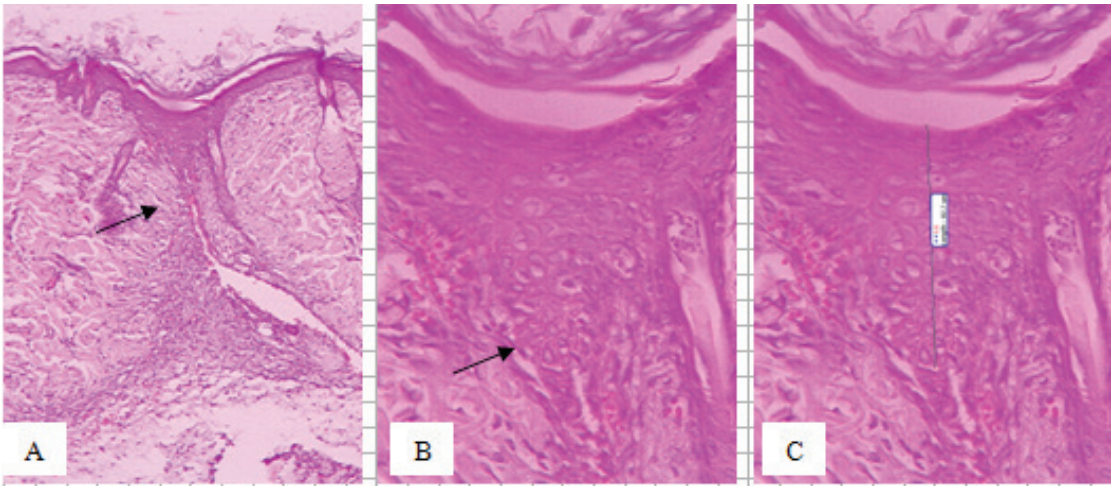

Figure 1. Microscopic features in the CG, location of the wound (A), basal epithelial cells in the basal stratum (B), epidermal thickness=82.7 um (C).

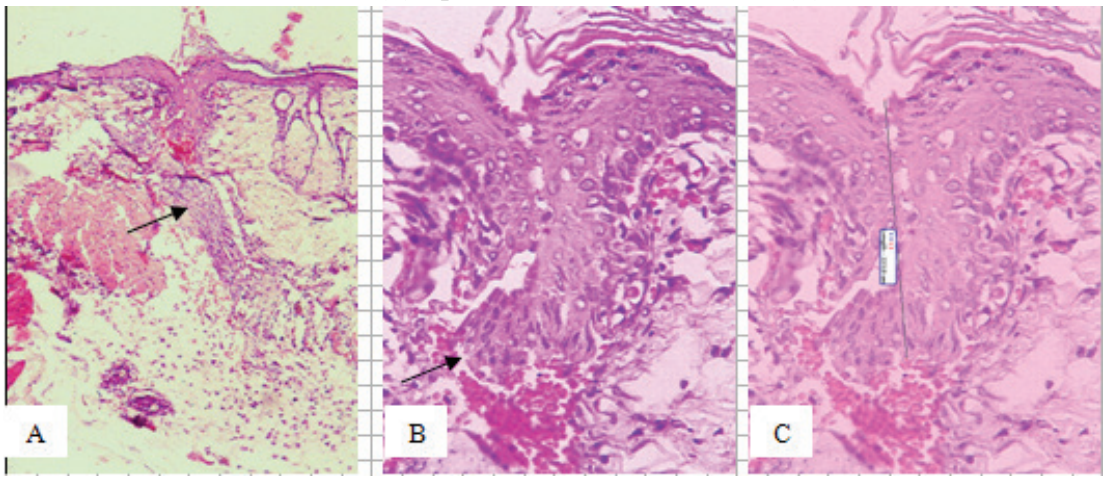

Figure 2. Microscopic features in the TG, location of the wound (A), basal epithelial cells in the basal stratum (B), epidermal thickness=223.8 um (C).

This is because the TG was administered with Achatina fulica mucous extract which contained crude acharan sulfate. Acharan sulfate plays a role in the integrity of the basal membrane by interacting with basal membrane components such as fibronectin, laminin, and type IV collagen. The level of expression and quality of it will have an effect on re-epithelialization, epidermal growth, and basal keratinocyte (epithelial cell) growth

reactions during re-epithelialization[28].

The basal epithelial cells plays a role in cell mitosis and renewal of epidermal cells[16]. In other words, the epithelium growth center is located in this layer. Therefore, if the number of basal epithelial cells increases, the growth of epithelial cells in the upper layers will also increase. This is what causes the thickness of the epidermis in the TG thicker than the CG.

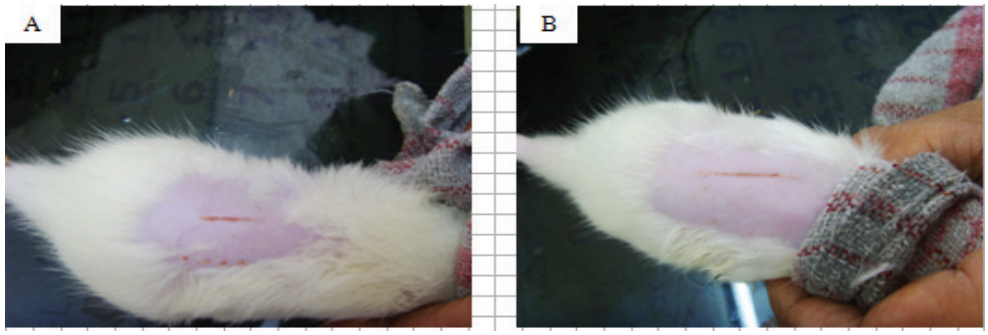

Figure 3. Macroscopic feautre of the incision wound on the 3rd day after injury, the CG (A), the TG (B).

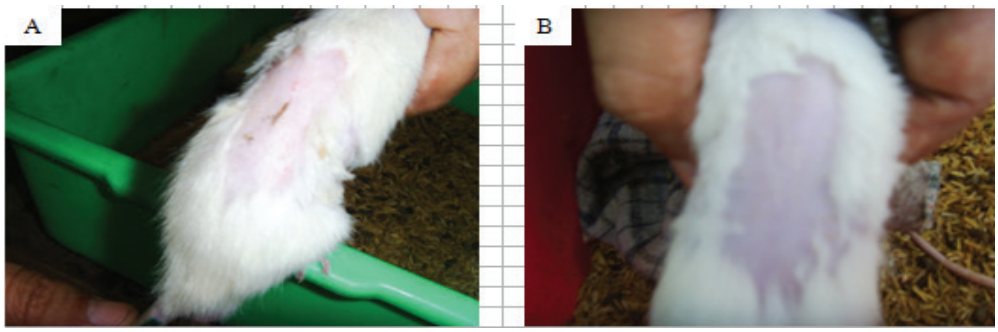

Figure 4. Macroscopic picture of the incision wound on the 5th day after injury, the CG (A), the TG (B).

Macroscopically, there was not too much difference between the CG and the TG. In the TG, it can be seen that the closure of the wound looks better, less crust, and the wound looks unmarked compared to the CG. This is because glycosaminoglycans when interacting with collagen will play a role in wound contraction, provide sufficient strength during the remodeling phase, inhibit excessive collagenesis by blocking the cleavage site of collagen so that it will affect collagen fibrous deposition in vivo[15]. Therefore, the formation of scar tissue is fewer in TG than the CG. This proves that the administration of Achatina fulica mucous extract plays a better role in wound healing than natural healing without outside help.

Conclusion

There was a significant difference between the number of basal epithelial cells in the CG and the TG with 86.74% crude archaran sulfate. This proves that the administration of Achatina fulica mucous extract with 86.74% crude acharan sulfate can increase the number of basal epithelial cells in the skin incision wounds of the white wistar strain male rats.

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Ethical Clearance : Approved

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