The Effectiveness of Snail Slime and Chitosan in Wound Healing

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***Abstract*—Snail slime (*Achatina fulica*) has many functions, including wound or scratch and gingivitis healings, and skin care. The essential substances contained in the snail slime involve glycosaminoglycans and proteins. The proteins have important biological functions, including as a bacterial protein (enzyme) binding receptor. Chitosan is a natural polymer containing a hydroxyl group (OH) and amine groups (NH2) which is positively charged in acidic solution. It is antimicrobial and polycationic, so that it can be used as a coagulant agent. The research aims at finding out the effectiveness of snail slime and chitosan in wound healing. The methods employed in the research included isolation of snail slime, the synthesis of chitosan (2%), and *in vivo* treatment stages using 5 groups of mice as negative controls, snail slime and chitosan (2%). The ratios of snail slime and chitosan examined were 1: 1, 1: 2, and 2: 1. The result shows that snail slime and chitosan (2%) with the ratio of 1: 2 is effective in wound healing. The content of the anti-inflammatory factor in snail slime and antimicrobial.**

***Index Terms*—chitosan, snail slime, wound healing**

I. Introduction

Wound healing is crucial to bring back skin integrity and it is also a complex and dynamic process with a predictable pattern. Proliferation phase is one of significant phases in wound healing, and it occurs after the inflammatory phase [1]. The proliferation or fibroblastic phase will occur quickly if there is no infection and contamination in the inflammatory phase [2].

The use of chemical compounds for wound healing or chemotherapy including povidone iodine sometimes gives a toxic effect in in vitro studies. Therefore, other alternative treatments using natural materials which are useful as antimicrobial factors, such as snail slime and chitosan, are highly required.

Wound healing with snail slime can be one of the alternatives because it is not only easy to use, but it also can spread well in the skin. In addition, it does not clog skin pores, and it has an antibacterial effect. Snail slime gives a positive reaction to test for protein contents, comprising amino acids and proteins which play role in cell regeneration and growth. The animal protein content of snail slime has a high biological value in wound healing and in the inhibition of inflammatory process. Slime of *Achatina fulica* can heal wound twice faster than the normal saline solution can. The essential content of snail slime includes *glycosaminoglycan* that can bind copper peptide. The snail's proteins have essential biological functions, comprising as bacterial protein (enzyme) binding receptor [3].

Chitosan is three-high-molecular weight natural polymer. It is nonpoisonous; it can accelerate wound healing, reduce blood cholesterol levels, stimulate the immune response and can be biologically decomposed. It has a stronger antimicrobial property compared to chitin in avoiding fungi because it has an active group that will bind to microbes, so it can inhibit microbial growth. Chitosan has a good chemical reactivity because it has a number of hydroxyl (OH) and amine groups (NH2) attaching to its chain. One of its important characteristics is that it has a positive charge in acidic solution. The substance is a stronger antifungal factor compared to chitin. In addition, chitosan is polycationic, so it can be used as a clotting agent [4].

The research aims at finding out the effectiveness of snail slime and chitosan in wound healing. The content of the antiinflammatory factor in snail slime and antimicrobial factor in chitosan is supposed to be potential and effective combination in wound healing.

II. Material and Methods

A. Material and Samples

The research was conducted at the Microbiology and Parasitology Laboratory of Medical Faculty of Sebelas Maret University and the Science Laboratory of Kusuma Husada Surakarta School of Health for 6 months.

The materials used included crude chitosan synthesized from crab shell waste and chitosan manufactured by Biotechsurindo in Cirebon.

B. Isolation of Snail Slime

The snail slime isolation as in Fig. 1 was obtained from 10-50 local snails (*Achatina fulica*) using an electric shock from 5-10 volt power supply for 30-60 seconds.

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The slime was macerated in water for 24 hours in 40 ℃. Fraction containing water-soluble slime was obtained from the procedure of mixing the water twice of the number of samples added to the slime. The supernatant was received as WSF (Water Soluble Fraction). The fraction of slime (mucin fraction) of the WSF was gained by using ethanol precipitation by mixing supernatant resulted from the water maceration with absolute ethanol ratio of 1: 3, and then it was centrifuged at 2900 r.p.m. for 30 minutes. The precipitation was re-dissolved with Tris -Cl and finally mucin fraction was obtained [5].

Figure 1. Isolation of snail slime.

C. Synthesis of Chitosan

Synthesis of chitosan from samples of shrimp shells or crab shells was made through deacetylation, demineralization, deproteination of chitin [6]. Meanwhile, industrial chitosan was obtained from PT. Biotech Surindo Cirebon Indonesia. Solution of 1.5% chitosan was then made in a solution of 10% acetic acid. Chitosan can be synthesized from deacetylation of chitin as in Fig. 2 through the process and have a degree of deacetylation 80%-90%. Chitosan is insoluble in water but soluble in acidic solvents with a pH below 6.0. Common solvent used to dissolve chitosan is 1 % acetic acid at a pH of about 4.0

Figure 2. Crab shells and chitosan product.

Chitin is a carbohydrate polymer, the N-acetylated polymer of (1- 4) linked N-acetylglucosamine. Chitosan, the deacetylated derivative of chitin is more valuable and having wide application than chitin. The main source of chitin is crustacean especially shrimp shell. The procedure are isolating chitin from shrimp shell by deproteination and demineralization step, followed by deacetylation using chitin deacetylase. At pH above 7.0 the stability is very limited solubility of chitosan polyelectrolyte complexes formed with anionic hydrocolloid gel so it can be used as an emulsifier, chelating and coagulant.

Chitosan, a polycationic polymer and waste product from the sea food processing industry, is an abundant natural resource that has, as yet, not been fully utilized. Advantages of this polymer include availability, low cost, high biocompatibility, biodegradability and easy of chemical modification. The physicochemical properties of chitosan, as well as its numerous applications, are reviewed with particular emphasis on its use in water treatment, pharmaceutics, agriculture and membrane formation. The multifunction of chitosan as antibacterial activity, antimetastatic , antisteoporotic, immuno adjuvant and in vitro biocompatibility of wound.

D. Treatment Stage

The experimented animals (mice) were wounded by incising them with the measure of 0.5 cm × 0.5 cm and depth of 0.3 cm on the back using sterile scalpel. The wound was made parallel to the spine. Before implementing the treatment to the mice, anesthesia using ether and cetamine, and epilation in the back were conducted. The ketamine dose applied was 0.02 ml per 20 gram body weight. The incision was conducted after the target area was applied with antiseptic solution. The research was conducted for 5 days, and on the 6^{th} day, the mice were determined on a microbiological level of wound healing by applying inoculation on Vogel-Johnson Agar medium to determine whether or not the *Staphylococcus aureus* bacterium exists. The mice meeting the inclusion criteria were divided into 7 treatment groups; each group consists of 5 mice. The treatment of research involved:

K1: negative control group (physiological saline of NaCl)
K2: the treatment group of snail slime
K3: the treatment group of 1.5% chitosan
K4: the treatment group with the ratio of snail slime and 1.5% chitosan = 1: 1
K5: the treatment group with the ratio of snail slime and 1.5% chitosan = 2: 1
K6: the treatment group with the ratio of snail slime and 1.5% chitosan = 1: 2
K7: positive control group (drug patent)

III. Results and Discusion

A. The Effectiveness Snail Slime and Chitosan in Wound Healing

The research results on the effectiveness test of snail slime and chitosan in wound healing are listed in Table I. The negative control group was applied using sterile physiological saline of NaCl, while the positive control treatment used patent pharmaceutical product which is so-called hydrocortisone. To determine the wound healing rate, the microbiological test was conducted to analyze the absence of *Staphylococcus aureus* as the indicator bacteria of pus producer in the wound.

Research related to the use of snail slime and chitosan to measure the speed of healing of cuts in mice. The treatment group with the ratio of snail slime and 1.5%

chitosan = 2:1 shows the optimum effectiveness of wound healing rate ± 1.2 days. Snail slime effect as antibacterial and anti-inflammatory will further accelerate the inflammatory phase that will sooner on the proliferative phase of wound healing. Scientifically never conducted research on the ability of fraction separation snail slime and the results indicate that the substance agglutinin, acharan sulfates, achasin protein and glikoconjugat an antimicrobial to *Escherihcia coli*, *Streptococcus haemoliticus*, *Salmonella typhi*, *Pseudomonas aeruginosa*, and *Candida albicans* and the effects of mucus [7].

TABLE I. THE EFFECTIVENESS OF SNAIL SLIME AND CHITOSAN IN WOUND HEALING RATE

No	Code	Treatment	The Grade of wound Healing	Specification
1	Negative control	NaCl	3.8	Red wound; 0.5 cm
2	Positive control	Drug patent	3.2	Red wound, dry; 0.3 cm
3	A 1	100 % Snail slime	2.5	Pink wound, dry; 0.2 cm
4	A 2	1.5% Chitosan	2.3	Pink wound, dry; 0.2 cm
5	A 3	Snail slime : chitosan = 1 : 1	2.1	Pink wound, dry; 0.1 cm
6	A 4	Snail slime : chitosan = 1 : 2	1.2	Wound is healed and dry
7	A 5	Snail slime : chitosan = 2 : 1	2.2	Pink wound, dry; 0.1 cm

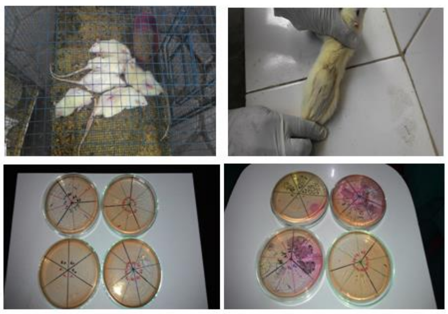

Figure 3. The effectiveness of snail slime and chitosan in wound healing rate.

Snail slime effectiveness as in Fig. 3 is influenced by the content of a substance snail mucus as beta agglutinins in the serum, achasin protein, acharan sulfate and glikoconjugate. Substance of beta agglutinin role in hemostasis process, where this process occurs blood clotting is physiological. Hemostasis process depends on coagulation factors, platelets and blood vessels. Substance beta agglutinin role in coagulation factors. There are three stages in the process of coagulation is the first stage is the stage of formation of thromboplastin and thromboplastin because it contains agglutinin. The second stage is the activation of prothrombin into thrombin and the third stage is the formation of fibrinogen to fibrin clot formation. Other ingredients in snail mucus that achasin protein which will inhibit the formation of the parts of the bacterial strains such as peptidoglycan layer and the cytoplasmic membrane. The absence of bacterial cell wall will cause the cells to die. The content of snail slime is a complex glikoconjugate which is actively controlling cell function and plays a role in cell matrix interactions, proliferation of fibroblasts, specialization, and migration, as well as effectively control the cellular phenotype. Giving snail mucus can increase the average number of fibroblast cells that can proliferate and binding elements of the extracellular matrix to form granulation tissue so that the number of fibroblasts at the injured area will be more and faster closing. Snail slime contains such active substances as isolates, heparan sulfate, and calcium. The isolate content is useful as antibacterials and analgesics, while calcium plays a role in hemostasis [8], [9].

Snail slime has antibacterial and antiinflammatory effects and therefore the proliferation phase will heal wounds immediately. The content of the snail slime supposed to be the most influential fibroblast proliferation is heparan sulfate which assists in blood clotting process and fibroblast proliferation. Heparan sulfate is also used for angiogenesis and inhibition of vascular endothelial growth factor and it decreases mitogenic activity from fibroblast growth factor. Heparan sulfate is included as one of proteoglycans that serves as a binder and a reservoir (storage) of basic fibroblast growth factor (bFGF), which is secreted into the ECM (Extra Cellular Muscular). ECM can release bFGF which will stimulate the recruitment of inflammatory cells, the activation of fibroblasts and the formation of new blood vessels when injury occurs. The research results demonstrate that the snail slime is potential and effective for in vivo wound healing [10], [11].

Multipurpose uses of chitosan cannot be both chemically and biologically separated from their natural characteristics. Chitosan is a linear-shaped polyamine polymer, having amino and active hydroxyl groups, and it also has the ability to chelate several metals [12]. Chitosan fibers are used as threads in surgery and are easily absorbed by the human body so that they can be used as a bandage covering the wound and medication carrier [13], [14]. Chitosan has influential role in the blood clotting and therefore it can be used as hemostatics; it can be biologically degradable, is non-toxic, non immunogenic and biocompatible with the body tissue of mammals [15], [16]. The research results successfully prove the potential use of chitosan biomembranes as wound dressing [17].

Wound healing is a process of normalization of skin integrity and its underlying tissues through the various stages of acute inflammation. Healing is closely related to inflammation. It is an early phase in the wound-healing process. Before the healing happens, the products of inflammation such as exudates and dead cells move out of the site as the dead tissues dissolve. This occurs due to both autolytic enzymes of the dead tissues (autolysis) and enzymes sent from inflammatory leukocytes (heterolysis). Liquid materials are absorbed into the lymphatic vessels and therefore this initiates the wound healing. Tissue repair involves two real processes including regeneration and fibrosis. The former replaces damaged cells or tissues with the new ones. Meanwhile, the latter comprises four phases including fibroblast migration and proliferation, extracellular matrix decomposition, formation of new blood vessels (angiogenesis), and scar tissue maturation. Wound healing consists of inflammatory phase, fibroblastic phase, maturation phase and retraction phase. It is very important to immediately restore its integrity and it is both complex and dynamic processes with a predictable pattern. One of the crucial phases of wound healing is proliferation phase and this occurs after inflammatory phase. The proliferation or fibroblastic phase will immediately occur in case that there exists no infection and contamination in the inflammatory phase. Fibroblasts play roles in the production of protein structures used for tissue reconstruction. Fibroblasts are particularly ground substances of collagen fibers that will form connections at wound edges. The cell proliferation in the injured tissues starts with the fibroblast growth factor (FGF), serving as a stimulation signal for fibroblast cell proliferation. Fibroblasts will proliferate to bind extracellular matrix elements in order to form scar tissues and accelerate wound healing. Activated fibroblasts will secrete extracellular matrix, and bind the extracellular matrix elements to form granulation tissue. The granulation tissue formation ends up the proliferation phase of wound healing process and therefore maturation in the remodeling phase begins [18], [19]. Drugs can be used to help accelerate the inflammatory and proliferation phases. Factors influencing the wound healing process include immunological status, glucose level (resulting in impaired white cell function), hydration (slowing down metabolism), nutrition, plasma albumin level (building blocks for repair, colloid osmotic pressure, edema), oxygen supply and vascularization, pain (causing vasoconstriction), and corticosteroid (depressing immune function) [20], [21].

IV. Conclusion

Snail slime and 1.5% chitosan have effective potentials to accelerate the wound healing process. The 1:2 ratio of 100% snail slime and 1.5% chitosan mixture indicates the effectiveness of the optimum wound healing process.

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