ORIGINAL RESEARCH

The Potential of Snail (*Achatina Fulica*) Mucus Gel as a Phythopharmaca to Accelerate the Inflammation Process during Wound Healing

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Abstract

Aim: To determine the potential of snail (*Achatina fulica*) mucus gel as a phytopharmaca material to accelerate the inflammatory process during wound healing

Materials and methods: Snail mucus gel was mixed with 3% CMC-Na to concentrations of 0% (control), 24%, 48%, and 96% snail mucus. Four 5-mm diameter punch biopsy excision wounds were made on the back skin of Wistar rats ($n = 6$). The snail mucus gel was applied to the back wounds of the mice. The mice were euthanized on days 2, 4, and 7 and hematoxylin and eosin-stained sections of the skin wound area were prepared. The number of polymorphonuclear leukocytes in the dermis was observed during wound healing using a binocular microscope (400x).

Results: A significant difference in the number of polymorphonuclear leukocytes ($p = 0.000$) was observed. The increase in the number of polymorphonuclear leukocytes peaked on day 4 in response to the 48% and 96% snail mucus gel concentrations. The 96% snail mucus gel had significantly more polymorphonuclear leukocytes than those in the other concentrations according to Tukey's test.

Conclusion: Snail mucus accelerated the inflammatory process during wound healing.

Clinical significance: Snail mucus is a potential material to be developed into a drug to accelerate wound healing. The toxicity, biocompatibility, and stability of the 96% snail mucus gel must be tested to produce a useful phytopharmaca product.

Keywords: Excision wound, Glycosaminoglycans, Heparan sulfate, Heparin sulfate, Hyaluronic acid.

World Journal of Dentistry (2022): 10.5005/jp-journals-10015-2056

Introduction

Polymorphonuclear leukocytes are a type of granulocyte and comprise 60–70% of the total number of leukocytes in the blood. Polymorphonuclear leukocytes are not needed in healthy tissue, but if there is injury, the tissue will release inflammatory mediators and chemotaxic factors that attract polymorphonuclear leukocytes to leave the blood vessels and enter the injured area in large numbers where they phagocytose antigens. The arrival of polymorphonuclear leukocytes is the first stage in a series of reactions called the inflammatory response.[1,2]

The snail (*Achatina fulica*) has been widely reported to produce glycoprotein secretions that play a beneficial role during wound healing. Snail mucus contains four natural and essential elements for the skin, such as allantoin, antimicrobial peptides, enzymes, and glycoproteins. Allantoin is believed to help regenerate skin cells, reduce scarring, and help heal burns. Antimicrobial peptides, such as mytimacin-AF, protect the skin from some microbes. Enzymes help eliminate dead skin. Glycoproteins, particularly the specific glycosaminoglycans found in snails, such as acharan sulfate, help the skin regenerate.[3,4]

Glycosaminoglycans stabilize membranes, increase the synthesis of hyaluronic acid, act as an anti-inflammatory agent, and accelerate angiogenesis.[5] Some studies have shown that systemically administered glycosaminoglycans accelerate wound healing and tissue regeneration, and reduce scar tissue formation.[6,7]

Various natural ingredients have the potential to accelerate the wound healing process. Indonesia has various natural materials that can be developed into phytopharmaca. Self-reliance technology is the focus of Indonesian research for medicinal raw materials in the field of health and medicine. Phyto-pharmacy products are widely used as alternatives to reduce the use of synthetic drugs. This study determined the potential of snail (*A. fulica*) mucus gel as a phytopharmaca material to accelerate the inflammatory process during wound healing.

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How to cite this article: Rosanto YB, Hasan CY, Rahardjo R, *et al.* The Potential of Snail (*Achatina Fulica*) Mucus Gel as a Phythopharmaca to Accelerate the Inflammation Process during Wound Healing. World J Dent 2022;13(3):224–227.

Source of support: Nil

Conflict of interest: None

Materials and Methods

This study was conducted in the pharmaceutical and microanatomy laboratories of the Universitas Gadjah Mada. The snails were obtained from the natural slime in rice fields and the species was identified in the laboratory. Snail (*A. fulica*) mucus was extracted by stimulating the snail's body with 6 volts of electricity for 60 seconds. Thirty snails were used in this study and produced 40 ml of mucus The snail mucus was filtered with a batis cloth. The snail mucus was sterilized by filtering it through Whatman No. filter paper. 4.

The medical ingredients given topically do not require sterilization with light or heat. The snail mucus was diluted with 3% CMC-Na to obtain concentrations of 24%, 48%, and 96% snail mucus. The gel was stored in a closed glass container at 8–15°C.

The subjects in this study were male Wistar rats. The number of samples was calculated using the equation method for 12 rats.[8] Ethical clearance was obtained from the Medical Research Ethics Commission of the Faculty of Dentistry, Universitas Gadjah Mada. The backs of the rats were shaved, and a 5-mm biopsy excision wound was made using a punch. The rats were separated into four groups, of three rats each. All rats were treated with 1 ml of 24%, 48%, or 96% snail mucous, or the control snail mucus using a micropipette. One rat from each group was sacrificed on days 2, 4, and 7. Skin specimens were taken for hematoxylin-eosin staining.

The numbers of polymorphonuclear leukocytes in the wound were counted using a binocular microscope (400x magnification) in five fields of view. Three observers counted the polymorphonuclear leukocytes. Fields of view were captured in photographs with Optilab. The number of polymorphonuclear leukocytes in each slide was added and then divided by five to obtain the average number of polymorphonuclear leukocytes.

A parametric test was used for the number of polymorphonuclear leukocytes. The mean polymorphonuclear leukocyte data were tested by two-way analysis of variance (ANOVA) to determine the differences in polymorphonuclear leukocytes among the treated groups. A *post hoc* test was carried out with Tukey's test to determine which groups were different. Statistical calculations were performed using IBM SPSS Statistic 23 software (IBM Corp., Armonk, NY, USA). A p-value <0.05 was considered significant.

Results

The results of the polymorphonuclear leukocyte calculations are recorded in Table 1 and are depicted in Figure 1. This graph shows an increase in the number of polymorphonuclear leukocytes from days 2–4 in all groups. The highest number of polymorphonuclear leukocytes was found in the 96% snail mucus treatment group on day 4 (mean 69). The number of polymorphonuclear leukocytes in the 96% and 48% snail mucus groups decreased on days 4–7. The number of polymorphonuclear leukocytes in the 24% snail mucus group and the control increased from day 4–7 (Fig. 2).

The two-way ANOVA results for the polymorphonuclear leukocytes revealed $p = 0.000$ (<0.05), indicating that all independent variables (day, concentration, and the day and concentration interaction) together had a significant effect on the difference in the number of polymorphonuclear leukocytes. The significance values for day, concentration, and the interaction between day and concentration were also 0.000 (<0.05), indicating that each of these independent variables had a significant effect on the difference in the average number of polymorphonuclear leukocytes. The R^2 value was 0.998, indicating that the independent variable had a strong effect on the dependent variable. These results show that snail mucus had a significant effect on polymorphonuclear leukocytes during the healing of skin wounds on the backs of rats.

Discussion

This study was conducted to determine the potential of snail mucus gel as a phytopharmaca material to accelerate the inflammatory process during wound healing in a male Wistar rat animal model with lesions on the back skin. An excision wound was selected because it represents a secondary healing condition, has a greater risk of impaired healing, and is a large area of injury for topical application. The excision wound model has been used in research on the healing of skin wounds with topical agents.[9]

The excision wound in this study was made using a round biopsy with a diameter of 5 mm. The 5-mm wound size was chosen because it was sufficient to represent a secondary wound that could be easily treated topically and was within the limits of the wound size that rats could tolerate. Physical and metabolic stress arises due to the large size of a wound compared to the body size of the mouse. Physical and metabolic stress can arise due to large wounds relative to the total body surface area, which could affect the results of wound healing studies. To prevent the effects of physical and metabolic stress, it is advisable to use a wound size <15 x 15 mm in rats weighing 200–350 g.[10]

Advances in wound drug technology require the latest research to achieve optimal rapid wound healing. Various natural ingredients with the potential to accelerate the wound healing process are becoming a trend. Indonesia has various natural ingredients that can be developed into phytopharmaca. This study contributes to advances in domestic medicines by maximizing the excellence of typical Indonesian cultivation, which often uses natural ingredients for treatment.

The number of neutrophils at a skin injury site increases within 12 hours and reaches the maximum on day 2 then decreases to 50% on day 5.[11] Many neutrophils were detected in the dermis on day 2 after the rat skin injury, but only a few neutrophils and monocytes or macrophages were found in the tissue on day 7, indicating that the inflammatory phase was almost over.

The stimulus given to the tissue in this study was an excision wound made with a punch biopsy. When tissue is exposed to a stimulus, cellular phospholipase A2 stimulates the phospholipid membrane to release arachidonate from the glycerol middle chain.[12,13] Enzymes in the smooth endoplasmic reticulum convert arachidonic acid into prostaglandins, starting with the formation of prostaglandin H2 (PGH2) which is catalyzed by the enzyme cyclooxygenase (COX).[14] COX-2 plays a role in the inflammatory process and can increase 20-fold in macrophages, monocytes, fibroblasts, and endothelial cells when stimulated under an inflammatory condition.[15]

The mean number of polymorphonuclear leukocytes on day 7 of observation in the 48% and 96% snail mucus gel groups was lower than those in the control and 24% snail mucus groups. The decrease in the number of polymorphonuclear cells in these groups means that these concentrations of snail mucus gel have

Table 1: The number of polymorphonuclear leukocytes between groups and between treatment days

	Control	*24%*	*48%*	*96%*
Day-2	3	4	6	8
Day-4	41	44	57	69
Day-7	44	55	35	34

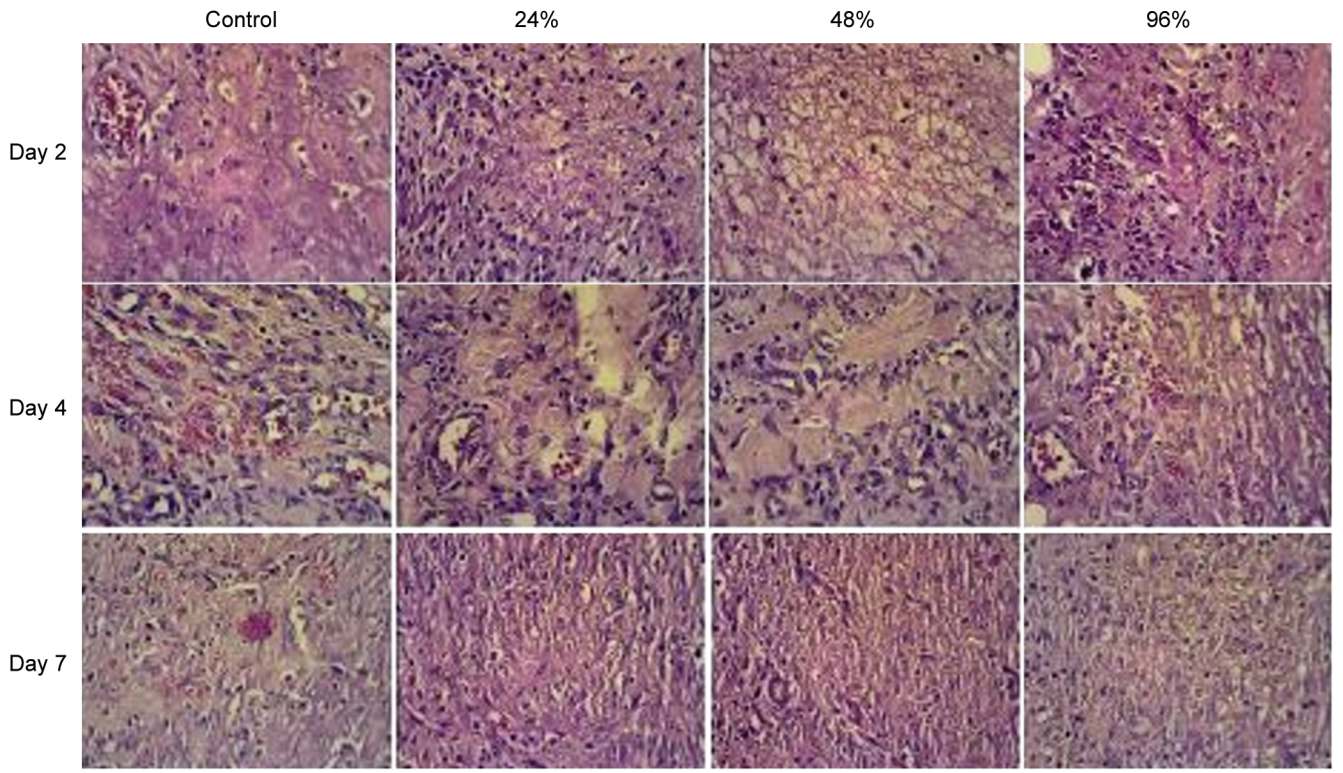

Fig. 1: Polymorphonuclear Leukocytes In the wound area on Days 2, 4, and 7 In hematoxylin and eosin-stained Histological preparations (Magnification, 400x). The 96% snail mucus gel had the Significantly Highest number of polymorphonuclear leukocytes on day 4 (p = 0.000)

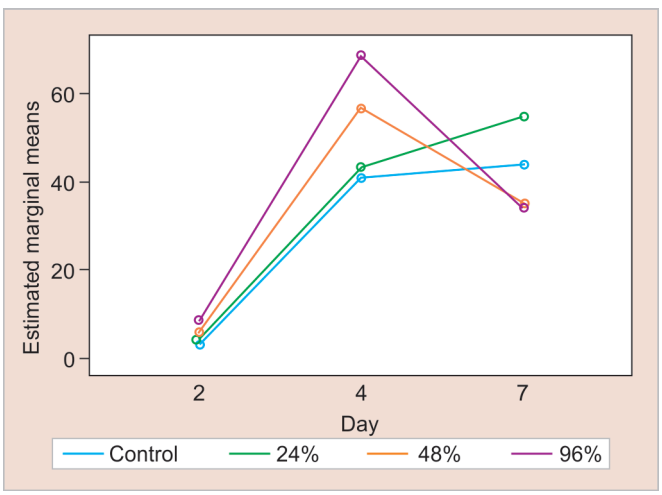

Fig. 2: Differences In the number of polymorphonuclear leukocytes between groups and between treatment days

anti-inflammatory properties. The potential of the snail mucus to stimulate wound healing has promising prospects due to its active ingredients.[16] Snail mucus contains achatin isolate, heparan sulfate, and calcium. Achatin isolate acts as antibacterial and analgesic,[4] which accelerates the inflammatory phase so that the proliferation phase of wound healing can occur immediately.[17]

The results of this study are in line with previous research conducted by Harti et al.[16] That study used wound color and size as parameters to state that snail mucus accelerates wound healing macroscopically. The current study demonstrated that the acceleration of wound healing by snail mucus occurred as a result of its effect on polymorphonuclear leukocytes. Research conducted by Gubitosa et al. showed that snail mucus has anti-inflammatory activity. Another study investigated the activity of macrophages and demonstrated a reduction in lipopolysaccharide-induced interleukin (IL)1-B and IL-6 levels.[18]

The decrease in the number of polymorphonuclear leukocytes by the snail mucus gel was due to the release of biological compounds that play a role in chemotaxis of polymorphonuclear leukocytes by bacteria in the wound area.[19] Thus, it is possible that the quicker inflammatory response was a result of the antimicrobial activity of the mucus, as observed in a previous study on antibacterial activity of snail mucus.[20] In addition, the release of factors, such as vascular-endothelial growth factor and platelet-derived growth factor, which stimulate angiogenesis, also accelerate the inflammatory process and wound healing.[21]

The limitation of this study is that the snail slime gel used was not tested for toxicity, biocompatibility, or stability. Research on the toxicity, biocompatibility, and stability of the 96% snail slime gel is currently being carried out by the author.

Conclusion

Snail mucus accelerated the inflammatory process during wound healing. Snail mucus is a potential promising material to be developed into a drug to accelerate wound healing. However, it is necessary to test the toxicity, biocompatibility, and stability of the 96% snail slime gel to produce a useful phytopharmaca product.

Acknowledgments

We thank our colleagues who provided insight and expertise that greatly assisted this research. This research was funded by Universitas Gadjah Mada.

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