Formulation Of Eel (Monopterus albus) Extract Gels For Accelerated The Wound Healing

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ABSTRACT

Eel contains a lot of amino acids and fatty acids that play an important role in wound healing processes. This study aims to formulate the eel extract gel to accelerate burn wounds healing. Polyvinyl alcohol (PVA) 146,000 and hydroxypropylmethyl cellulose (HPMC) 60SH-50 were used as gelling agents. Each gelling agent was used in three different concentrations i.e. PVA with concentration 8% (FA1), 10% (FA2) and 12% (FA3); HPMC with konsentrasi 4% (FB1), 5% (FB2) and 6% (FB3). Propylene glycol was used as a humectant. Methylparaben and propylparaben were used as preservatives. Vitamin E was used as an antioxidant. Evaluations included general appearance, homogeneity, pH, washed test, and viscosity. The best formula is then tested for its effect on the healing of burn wounds. The results showed that formula that used PVA as a gelling agent produced transparent gel, while the formula that used HPMC produced opaque gel. Increasing the gelling agent concentration, increasing the viscosity of the gel produced. According to the physical appearance of the gel produced a formula that used PVA 10% (FA2) as the gelling agent was the best formula among the other formulas. From the results of tests on burns healing, gel formulas containing eel extract heal wounds faster than base gel and positive control.

Keywords : Eel (Monopterus albus) extract, gel, wound healing, burn wound
INTRODUCTION

Wounds are tissue damage to the anatomy of the skin caused by various factors. Wounds can be grouped into two, i.e., closed wounds and open wounds. Examples of closed wounds are bruises and hematomas, while examples of open wounds are incisions wounds, gunshot wounds, and burns (Sezer, et al., 2007).

The ideal characteristics of wound treatments are retaining the moisture (like gel) and absorbent (like hydrocolloid) (Boateng, et al., 2008). Gel has several advantages, including non-sticky, easy to dry and form a thin layer of a membrane, which can also be a wound dressing (Akita, et al., 2006; Bowen, 2006). The active ingredient can be added to the gel to accelerate wound healing.

Some of the active ingredients that often used are silver sulfadiazine, metronidazole, and povidone-iodine. Amino acids, fatty acids, collagen, and amylase, can also be given to provide nutrients to the injured skin (Calder, 2005; Corsetti, et al., 2010). Some essential amino acids in wound healing include glutamine, arginine, cysteine, glycine, and proline. These amino acids are crucial for skin formation, constituents of collagen, stimulation of surface antigen expression, and as precursors of antioxidants (Williams, 2003; Witte, 2002). Fatty acids play a role in repairing damaged tissue, wound healing agents, and antithrombotic. In wound healing, fatty acids and proteins work together in the formation of keratinocytes and fibroblasts (Calder, 2005; Storey, 2005). These compounds widely found in fish species such as eels (Monopterus albus) (Razak, et al., 2001). A previous study also reported the amino acids and fatty acids contents on eels (Febriyenti(b), et al., 2019). From the description above, in this study, gels containing eel extract to accelerate the wound healing were formulated.

MATERIALS AND METHODS

Materials

Eels were purchased from Bukittinggi, West Sumatera, Indonesia. Eel extract was prepared according to Febriyenti et al. (Febriyenti(b), et al., 2019). PVA 146,000 was bought from VWR International, Belgium. HPMC 60SH-50 and vitamin E were supplied by Sigma Chemical Co., USA. Propylene glycol, methylparaben, and propylparaben were acquired from R&M Chemicals, UK. Pentobarbitone sodium was purchased from CEVA (Barcelona, Spain). Normal saline 0.9% was purchased from Thai Otsuka Pharmaceutical Co, Ltd. Distilled water was bought from Bratachem, Indonesia. Commercial burn gel was used as the positive control. All chemicals were used without further purification.

Preparation of Gel

Gelling agents were dissolved in distilled water. PVA was prepared by the hot mechanical method. HPMC was prepared by cold mechanical method (Febriyenti, et al., 2014; Shivhare, et al., 2009; USP, 2007). Eel extract, methylparaben, propylparaben, and vitamin E were dispersed in propylene glycol and then mixed with the gelling agent using Ultra turrax at 10,000 rpm for 15 min (Febriyenti, et al, 2014; Febriyenti, et al., 2011; Febriyenti, et al., 2008). The compositions could be seen in Table 1.

Table 1. Formulas of Eel (Monopterus albus) Extract Gel

| Ingredients             | Formula (%) |
|-------------------------|-------------|
|                         | FA 1 | FA 2 | FA 3 | FB 1 | FB 2 | FB 3 |
| Eel extract             | 10   | 10   | 10   | 10   | 10   | 10   |
| PVA                     | 8     | 10   | 12   | -    | -    | -    |
| HPMC                    | -     | -    | -    | 4     | 5    | 6    |
| Propylene glycol        | 10   | 10   | 10   | 10   | 10   | 10   |
| Methylparaben           | 0.1   | 0.1  | 0.1  | 0.1  | 0.1  | 0.1  |
| Propylparaben           | 0.02  | 0.02 | 0.02 | 0.02 | 0.02 | 0.02 |
| Vitamin E               | 0.01  | 0.01 | 0.01 | 0.01 | 0.01 | 0.01 |
| Distilled water up to   | 100   | 100  | 100  | 100  | 100  | 100  |

Gel Evaluations

The general appearance of the prepared gels, consistency, and transparency was observed visually (USP, 2007; Misal, et al., 2012). Homogeneity of all gel formulas was tested by visual inspection for their appearance and presence of any aggregates.
The pH of all gel formulas was determined by using a digital pH-meter (Shivhare, et al., 2009; Martin, et al., 2001). A washed test was conducted using one gram of gel that applied to the hand and then washed with a certain amount of water. Accounted for much of the water volume was used (Febriyenti, et al., 2014; Jellinek, 1970). Viscosities of all formulas were determined using Brookfield LV viscometer (Martin, et al., 2001).

Animal

The animal studies were conducted using the facility of Pharmacology laboratory, Akademi Farmasi Imam Bonjol, Bukittinggi. Adult, healthy, male Spraque-Dawley (SD) rats, weight ranging between 200 and 300 g were selected in this study. The rats were housed in standard environmental conditions under a 12-h light–dark cycle lighting in solid bottom cage with top-ventilated stainless steel cover. All animals were allowed to move freely and were provided access to food and water. Animal’s welfare guidelines were adopted from Guide to the Care and Use of Experimental Animals (Olfert, et al., 1993). The handling of rats followed the protocol described by Deacon (2006). The method’s procedures included for the animals have been approved by Animal Ethic Committee Universitas Andalas with Ref. No. 012/KEP/FK/2015.

Burn wound model

The gels were assessed for burn wound model using the test method of Laila, et al. (2011) and Febriyenti(a) et al. (2019). The rats were anaesthetized with pentobarbitone sodium (50 mg/kg body weight) that was administered intraperitoneally. Dorsal part of the animal was clipped with electric clippers followed by scrubbing the skin with 70% ethanol and normal saline. Burn wounds were created using a metal rod (1.5 cm diameter) heated to 95 ± 2°C in water and exposed for 10 seconds. Positive control rats were treated using commercial burn gel, while experimental rats were treated with formula FA (without active ingredient), and formula FA2. The gels were placed in such a way that the wounds could be completely covered. Animals were randomly divided into three groups with six rats for each group. Experiment was conducted for 24 days to see the wound contraction. The wound size was determined using the tracing method (Goldman, 2002; Nayak, et al., 2008). Wound closure was calculated as the percentage of wound area contraction using the formula:

\[
\text{Percentage of wound closure} = \frac{\text{Area (0 day)} - \text{Area (x day)}}{\text{Area (0 day)}} \times 100\%.
\]

Statistical analysis

Results of the wound contraction were represented as mean ± SD. One-way ANOVA followed by Duncan post hoc test was used to identify differences between groups. It showed a statistical difference if \( P < 0.05 \) was obtained.

RESULTS AND DISCUSSION

Gelling agent polymer for wound dressing should produce transparent gel (Heng, et al., 1996; Febriyenti(a), et al., 2019), so then it also could be a transparent membrane. Wounds easily observed through transparent membranes (Balakrishnan, et al., 2005). The results of the gel evaluations showed in Table 2. Formulas that used PVA as a gelling agent produced transparent gel while HPMC produced opaque gel. All gel formulas have good homogeneity. It means that the method used to prepare the gel was appropriate to mix all the ingredients. Gels that contained HPMC as a gelling agent had pH value slightly higher than gels that contained PVA. Nevertheless, the results are still within the normal skin pH range 4.2-6.5 (Febriyenti, et al., 2014; Gennaro, A. R., 1990) or 5-6.5 (Balsam, 1992). Washed tests carried out to determine the easiness of cleaning up the gel from the surface of the skin. The washed test is conducted by determining the amount of water needed to wash up the gel smeared on the skin. Polymer type and viscosity of the gel affect the amount of water needed. Usually, the dilute gels required less water to wash it up. Viscosities of the gels were determined using Brookfield LV viscometer. Gel viscosity increases with increasing the concentration of the gelling
agent used. Ideal gel viscosity value is 2000 – 4000 cPs (Garg, et al., 2002). Based on the results in Table 2, FA2 and FB3 have the ideal viscosity value. But if general apparent of FA2 is compared with FB3, FA2 is better than FB3 because it is transparent.

Table 2. Results of Gel Evaluations

| Formula | General appearance | Homogeneity | pH  | Washed test | Viscosity test |
|---------|--------------------|-------------|-----|-------------|----------------|
| FA1     | Transparent, dilute gel | Good | 6.16 | 15 ml | 110 cPs |
| FA2     | Transparent, thick gel | Good | 6.18 | 20 ml | 2850 cPs |
| FA3     | Transparent, thick gel | Good | 6.15 | 27 ml | 4235 cPs |
| FB1     | Opaque, dilute gel | Good | 6.82 | 15 ml | 108 cPs |
| FB2     | Opaque, thick gel | Good | 6.81 | 18 ml | 990 cPs |
| FB3     | Opaque, thick gel | Good | 6.82 | 24 ml | 3512 cPs |

Furthermore, the best formula (FA2) and gel base (FA) were used for the burns healing test (Table 3). For the comparison, the commercial burn gel was used as positive control. Results of the burns healing test presented in Table 4. The test was conducted 24 days. Group of animal that treated with FA2 have the wound closure more than 80% at day-12 while the other two groups were more than 18 days. It means that FA2 that containing eel extract could accelerated the burn wound healing. The result is similar with previous study using Haruan extract (Laila, 2011). Eel extract and Haruan extract were rich with essential amino acids and fatty acids that play important rule on healing process (Febriyenti(b), 2019; Febriyenti, 2012).

Table 3. Formulas of Gel Base (FA) and Eel (*Monopterus albus*) Extract Gel (FA2)

| Ingredients          | Formula (%) | FA | FA2 |
|----------------------|-------------|----|-----|
| Eel extract          | 10          |    |     |
| PVA                  | 10          |    |     |
| Propylene glycol     | 10          |    |     |
| Methylparaben        | 0.1         |    |     |
| Propylparaben        | 0.02        |    |     |
| Vitamin E            | 0.01        |    |     |
| Distilled water up to| 100         |    |     |

Table 4. Percentage of Wound Closure for Each Groups in Different Days. Positive Control, PC; gel base, FA; Formula Gel With Eel Extract, FA2; Data Presented as Mean ± SD, N = 6.

| Day | Percentage of wound closure (%) |
|-----|---------------------------------|
|     | P     | FA   | FA2   |
| 1   | 0.99^a±0.03 | 0.18^b±0.02 | 7.90^b±1.19 |
| 2   | 9.81^b±0.22 | 6.27^a±0.48 | 15.68^a±0.61 |
| 3   | 13.10^a±0.18 | 8.93^a±0.16 | 24.13^a±0.64 |
| 4   | 21.42^b±0.91 | 11.66^a±0.66 | 33.82^c±1.06 |
| 5   | 29.70^a±0.92 | 15.65^a±0.86 | 42.07^a±1.12 |
| 6   | 31.07^a±0.80 | 18.55^a±0.60 | 56.38^a±0.69 |
| 7   | 33.16^a±0.25 | 22.67^a±1.10 | 59.23^a±0.22 |
| 8   | 42.39^a±1.39 | 30.81^a±0.99 | 63.58^a±1.45 |
| 9   | 49.02^a±1.07 | 34.84^a±0.94 | 72.35^c±1.06 |
| 10  | 52.90^a±0.12 | 39.44^a±0.88 | 75.04^a±0.40 |
| 11  | 55.25^a±1.75 | 49.39^a±0.72 | 78.12^a±0.28 |
| 12  | 59.54^a±0.67 | 54.14^a±0.99 | 81.11^b±0.68 |
| 13  | 62.16^a±1.27 | 57.23^a±0.49 | 82.75^a±0.53 |
| 14  | 68.34^a±0.62 | 59.02^a±0.11 | 85.00^b±0.17 |
| 15  | 73.82^a±0.32 | 61.28^a±0.55 | 92.79^a±0.91 |
| 16  | 75.52^b±0.53 | 63.78^a±0.48 | 99.13^c±1.14 |
| 17  | 77.47^a±0.71 | 67.18^a±0.29 | 100.00^a±0.01 |
| 18  | 81.93^a±0.16 | 70.11^a±0.28 | 100.00^a±0.00 |
| 19  | 85.23^a±0.60 | 74.33^a±0.59 | 100.00^a±0.00 |
| 20  | 87.39^a±0.61 | 78.96^a±0.08 | 100.00^a±0.01 |
Means within a row with a different letter are significantly different (P<0.05)

CONCLUSION

Formula FA2 that contained PVA 10% as a gelling agent had the best physical appearance among the other formulas. The gel was transparent and had proper viscosity as gel preparation. Formula FA2 that containing eel extract could accelerated the burn wound healing compared with base gel and commercial burn gel.

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