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The Effectiveness of Catfish Oil in Accelerating the Healing of Incised Wounds in Mice

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ABSTRACT

The wound healing process naturally progresses through several phases, including hemostasis, inflammation, fibroplasia, and maturation. The present study aimed to evaluate the efficacy of gel and cream formulations of catfish oil in promoting wound healing in mice. The present study consisted of eight groups, each with five mice. The groups included a cream base of catfish oil (P1), a gel base of catfish oil (P2), fish gel at 1% (P3), fish gel at 2% (P4), fish cream at 1% (P5), fish cream at 2% (P6), povidone iodine (P7), and a commercial ointment (P8). The preparations were applied in an adequate amount to the injured skin surface. The mice were subjected to a 2-centimeter incision wound and treated once daily for 14 days. The parameters observed included signs of inflammation, such as redness, swelling, and discharge. Formulations containing 1% and 2% catfish oil facilitated faster wound healing compared to plain cream and gel, proving to be as effective as povidone iodine and commercial ointments. Specifically, the gel preparation containing 1% catfish oil, the cream formulation with 1% catfish oil, and the 2% catfish oil formulation yielded the most promising results in accelerating the healing time of incision wounds in mice.

Keywords: Catfish oil, Cream, Gel, Mice, Wound

INTRODUCTION

Wounds are injuries to the body tissues, often stemming from disruptions in the epidermis, which lead to skin injuries (Alpayet et al., 2023). These injuries can result from both medical conditions and intentional or accidental causes. Intentional causes include therapeutic actions such as surgeries or blood sampling, whereas unintentional causes generally arise from accidents involving blunt or sharp forces (Canpolat and Başa, 2017). Wounds, regardless of their cause, require a repair process to restore the affected areas to their original state (Alpayet et al., 2023).

Wound healing is a natural restorative process that unfolds through a series of intricate stages, including hemostasis, inflammation, fibroplasia, and tissue remodeling (Moenadjat, 2023). The method of wound healing generally requires 8 to 12 weeks to complete fully, varying based on the extent, depth, and severity of injury to the epidermal and dermal skin layers (Dhivya et al., 2015). Wound healing can be accelerated through specialized care and interventions that target healing factors, such as topical treatments. Specialized wound care generally involves debridement, which cleans the wound of necrotic tissue and bacteria, including *Staphylococcus aureus* or *Streptococcus pyogenes*, to facilitate healing (Mohanty and Kahoo, 2017). Necrotic tissue can become a focus for infection, prolong the inflammatory phase, mechanically hinder the healing process, and inhibit epithelialization. While debridement can happen naturally, assisted debridement facilitates a faster wound-healing process (Alpayet et al., 2023).

Wound care through dressing helps to prevent bacterial growth and reduces the risks associated with necrotic tissue. Dressings create a moist wound environment, which can speed up healing, reduce pain, and decrease inflammation compared to wounds exposed to air (Clark et al., 2023). Healing factors include local factors such as oxygenation, infection, foreign bodies, and the number of venous innervations, as well as systemic factors such as sex, age, stress levels, diseases, ischemia, obesity, alcohol, smoking, immunosuppressive conditions, medications, and nutrition (Dhivya et al., 2015). Proper nutrition is essential for optimal wound healing. Inadequate macronutrients, including protein, amino acids, carbohydrates, fats, and fatty acids, as well as micronutrients such as vitamins and minerals, can impair the healing process. Oral supplementation with essential nutrients, including arginine, glutamine, collagen, and omega-3 fatty acids, has been shown to support wound healing (Clark et al., 2023).

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Topical preparations can also support wound healing. Applying herbal remedies, such as honey, coconut oil, eucalyptus oil, and *Aloe vera*, has been shown to accelerate healing and improve the quality of wound healing. However, the use of herbal preparations as topical wound treatments remains limited (Abdelsattar et al., 2022; Gwarzo et al., 2022). Fish oil is a natural source of omega-3 fatty acids. The topical use of fish oil in the form of creams or gels is expected to provide localized effects on wounded tissues. Preliminary studies have shown the potential of fish oil cream to accelerate burn wound healing in mice (Fauziah et al., 2019). The catfish oil (*Clarias gariepinus*) is empirically used as an herbal remedy to accelerate wound healing (Khonsa and Setiawati, 2023). The high Omega-3 content in catfish oil aids in the healing of skin wounds (Clark et al., 2023). The present study aimed to evaluate the effectiveness of catfish oil gel and cream preparations on the healing of incisional wounds in mice.

MATERIALS AND METHODS

Ethical approval

The present study received ethical approval from the Animal Ethics Committee of the School of Veterinary Medicine, IPB University, Bogor, West Java, Indonesia, with approval number 139/KEH/SKE/XI/2023.

Study area

The study was conducted from November 2022 to January 2023 at the Pharmacy Laboratory and the Laboratory Animal Management Unit (UPHL) of the School of Veterinary Medicine and Biomedical Sciences, Bogor Agricultural Institute (IPB), Indonesia.

Materials

The equipment used in the current study included mouse cages (JICA, Japan), a digital scale with 0.01 gr accuracy (Taffware Digipounds, China), a ruler (Kenko, Japan), minor surgical instruments (Renz, Germany), a blender (Mitochiba, China), stirring rods (Pyrex, USA), measuring glasses (Pyrex, USA), volumetric flasks (Pyrex, USA), infusion pans (Oxone, Indonesia), and a thermometer (Hanna, USA). Fish oil (Pharmacy Laboratory, SKHB IPB, Indonesia), ketamine (Teaching Animal Hospital, IPB, Indonesia), xylazine (Teaching Animal Hospital, IPB, Indonesia), bedding (Laboratory Animal Management Unit, SKHB IPB, Indonesia), standard mouse feed (Table 1; PT. Indofeed, Indonesia), commercial ointment (Bioplasenton ointment, PT KalbeMed, Indonesia), and water.

Table 1. Composition of mice feed

Composition	Nutritional content (From dry matter)
Protein	18%
Fat	4%
Crude fiber	4-6%
Ash	4-7%
Metabolized energy	2850 kcal/kg

Animals

The experimental animals were 40 healthy Deutschland Denken Yoken (DDY) mice, weighing 25-30 grams, male, aged 2-3 months (Laboratory Animal Management Unit, SKHB IPB, Indonesia). The mice were acclimatized in cages (Five mice in each cage) for one week with established feed. Food was given by sprinkling it on the husks every 12 hours (Alpayet et al., 2023). The mouse cages were plastic boxes measuring approximately 40 cm x 30 cm x 18 cm, equipped with oven-dried rice husks as bedding, a water bottle, feed, and a wire cover. The mice were maintained in an environment with a temperature of 20-25°C, and they had access to drinking water *ad libitum*.

Fish oil extraction

The fish oil extraction method was adapted from Lestari et al. (2020). Cleaned catfish fillets (Skinless and boneless) were blended with water. The minced fish (500 g) was placed in a tray lined with transparent cloth and heated in an oven at 100°C for 20 minutes. After being removed from the oven, the sample was pressed using a hydraulic press. The extracted oil was stored in dark-colored beakers covered with aluminum foil and then refrigerated at a temperature of 1.7-3.3°C with a typical humidity of 60-80%.

Preparation of fish gel at 1% and 2% concentrations

Catfish oil was added to the manually prepared gel base at concentrations of 1% (0.5 mL) and 2% (1 mL) out of a total weight of 20 mL. The mixture was then adjusted to 10 grams (Gum arabic, glycerin, water, methylparaben, and triethanolamine). The preparation was applied sufficiently to the entire surface of the wound and stirred thoroughly until the catfish oil was evenly distributed throughout the gel. Finally, the gel was allowed to stabilize.

Preparation of fish cream at 1% and 2% concentrations

Catfish oil at 1% (0.5 mL) and 2% (1 mL) of the 20 mL total weight of the mixture was added to the manually prepared cream base until it became 10 grams (Tearic acid, acetyl alcohol, glycerin, water, methylparaben, triethanolamine), respectively. The preparation was applied sufficiently to the entire surface of the wound, and the mixture was stirred thoroughly until the catfish oil was evenly distributed throughout the cream. To stabilize the cream's viscosity, fish oil cream was added.

Experimental design

Forty DDY male mice weighing around 250 to 300 grams and aged 2-3 months were randomly divided into eight groups, each consisting of five mice. The groups were cream base of cat fish oil (P1), gel base of cat fish oil (P2), fish gel 1% (P3), fish gel 2% (P4), fish cream 1% (P5), fish cream 2% (P6), povidone iodine (P7; Betadine salep, PT.Mahakam Beta Farm, Indonesia), and commercial ointment (P8; Placenta extract, Bioplasenton, Kalbe Farm, Indonesia; Table 2). The administration of gel-based preparations (P2) and cream (P1) served as the negative control group, while the povidone iodine preparation group and commercial ointment served as the positive control groups.

Before being given the incision treatment, the mice were first given anesthesia, namely ketamine 80-100 mg/kg and xylazine 5-10 mg/kg (Alpayet et al., 2023). One part of the mice's dorsal edge was shaved, then incised two centimeters long using a scalpel. The incised wound was treated according to the conditions listed in Table 2, using 0.1g of the treatment, which was then spread evenly to cover the entire wound area and repeated once daily until the wound healed. Treatment was given for 21 days. Reapplication was done after cleaning the wound with cotton and distilled water. The wound length was measured, and wound scoring was assessed daily using a ruler and a wound healing scoring system. Measurements and assessments were discontinued once the wound had healed.

Table 2. Experimental design of catfish oil cream and gel test for wound healing in mice aged 2-3 months

Group	Treatment
Cream base (P1)	The mice's wounds were treated with a cream base without a fish oil mixture.
Gel base (P2)	The mice's wounds were treated with a gel base without a fish oil mixture.
Fish gel 1% (P3)	The mice's wounds were treated with fish oil cream with a concentration of 1%.
Fish gel 2% (P4)	The mice's wounds were treated with fish oil cream with a concentration of 2%.
Fish cream 1% (P5)	The mice's wounds were treated with fish oil gel with a concentration of 1%.
Fish cream 2% (P6)	The mice's wounds were treated with fish oil gel with a concentration of 2%.
Povidone iodine (P7)	The mice's wounds were treated with povidone-iodine.
Commercial ointment (P8)	The mice's wounds were treated with a cream base without a fish oil mixture.

Statistical analysis

The data on the number of days until healing were analyzed quantitatively using Minitab 18 with one-way Analysis of Variance (ANOVA) and Tukey's test methods with a P value less than 0.05. The data on wound length and wound healing scores were explained descriptively. Significant differences are evident from the superscript letters in the table.

RESULTS AND DISCUSSION

The wound-healing process consisted of hemostasis, inflammation, proliferation, and remodeling (Fauziah and Soniya, 2020). Catfish oil contains polyunsaturated fatty acids, specifically Omega-3 and Omega-6. Catfish fat has been proven to contain Eicosapentaenoic acid (EPA), Docosahexaenoic acid (DHA), and Arachidonic acid in proportions of 0.21-2.48%, 0.95-9.96%, and 0.349-1.105%, respectively (Khonsa and Setiawati, 2023).

The observation of incision wounds in mice was conducted over 21 days. The average healing time for incision wounds treated with catfish oil gel and cream on mice is presented in Table 3. The current results indicated that the gel and cream preparations of catfish oil were effective in accelerating the healing of incision wounds. Administration of 1% and 2% fish gel, specifically in groups P3 and P4, showed a significant difference ($p < 0.05$) in healing time compared to Group P2. The administration of 1% and 2% fish cream in groups P5 and P6 resulted in a faster healing duration (Approximately 16 to 17 days) than that of Group P2 ($p < 0.05$). The povidone-iodine and commercial ointment preparations served as positive controls and demonstrated similar average healing times, 16 to 17 days, as the catfish oil gel and cream. The current results indicated that the efficacy of catfish oil cream and gel was comparable to that of povidone-iodine and commercial ointments.

The clinical observation illustrated in Figure 1 depicts the healing phase of an open wound on days 1, 7, and 14. By day seven, the diameter of the incision wounds in mice started to decrease in all groups ($p < 0.05$), although the healing rates varied. Observations on day 14 revealed that groups P1, P2, P3, P6, P7, and P8 had completely closed wounds, while groups P4 and P5 still exhibited open wounds with small diameters. In addition to the diameter measurements, signs of inflammation, such as redness and swelling, were observed in groups P4 and P5. On the first day, the incision wounds in the mice were visible, showing redness and swelling. By day seven, Group P3 displayed no swelling and only slight redness. On day 14, groups P1, P2, P3, P6, P7, and P8 showed no swelling; however, slight redness on the skin persisted, accompanied by scabs in groups P4 and P5. The application of catfish oil cream and gel preparations at concentrations of 1% and 2% effectively reduced skin inflammation in mice. Groups P3, P5, and P6 demonstrated the best results in expediting the healing process of cut wounds in mice.

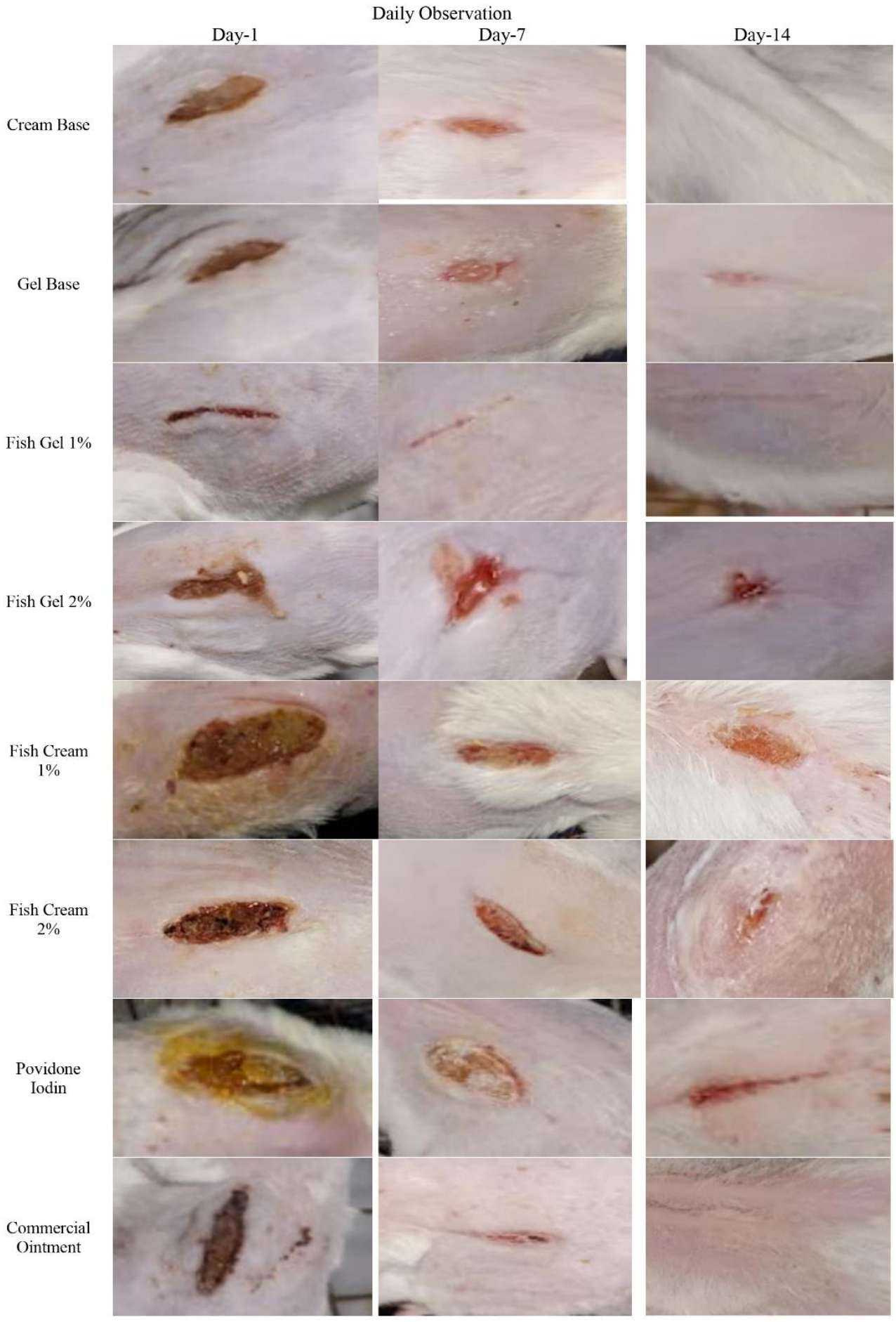

Figure 1. Daily observation of wound treatment in male mice aged 2-3 months. Source: Authors of the present study

Table 3. The average healing time of incision wounds in male mice aged 2-3 months

Group	Healing day
Cream base (P1)	22.400 ± 1.140^{ab}
Gel base (P2)	23.20 ± 2.680^{a}
Fish gel 1% (P3)	16.600 ± 2.074^{c}
Fish gel 2% (P4)	18.80 ± 2.280^{bc}
Fish cream 1% (P5)	17.00 ± 2.830^{c}
Fish cream 2% (P6)	16.600 ± 0.548^{c}
Povidone iodine (P7)	17.80 ± 3.56^{bc}
Commercial ointment (P8)	16.4 ± 1.949^{c}

[abc] Different superscript letters in the same column indicate significantly different values ($p < 0.05$)

The high protein content in fish oil can help to enhance the regeneration of dead skin cells (Khonsa and Setiawati, 2023). Catfish oil can stimulate platelet-derived growth factor and transforming growth factor through fibroblast interactions, as well as fibroblast growth factor, which is necessary for fibroblast proliferation, thus accelerating wound healing (Khonsa and Setiawati, 2023). One of the significant components of catfish oil is Omega-3, an unsaturated fatty acid rich in EPA and DHA, functioning as an anti-inflammatory agent (Khonsa and Setiawati, 2023).

The effectiveness of fish oil cream and gel preparations in the present study was influenced by different contents, one of which was omega-3. According to Peng et al. (2018), the administration of Omega-3 can enhance re-epithelialization in the skin, thereby accelerating the healing process. Omega-3 content can increase levels of Interleukin-6 (IL-6) and IL-10 (Ardha et al., 2020). Moreover, Omega-3 can improve collagen deposition, collagen-1 expression, hydroxyproline expression, and vascular endothelial growth factor (VEGF) expression (Peng et al., 2018). The VEGF plays a major role in angiogenesis to expedite the healing of incisional wounds (Peng et al., 2018; Ishak et al., 2018). Omega-3 also aids in increasing collagen synthesis (Senadheera et al., 2020), and appropriate anti-inflammatory administration can enhance procollagen during the wound healing process in mice. Collagen is the most abundant protein in the body. During wound healing, collagen is synthesized by cells, including fibroblasts. The type, amount, and arrangement of collagen undergo continuous changes throughout the wound-healing process (Senadheera et al., 2020; Alpayet et al., 2023).

CONCLUSION

The current results indicated that the gel and cream preparations of catfish oil, at concentrations of 1% and 2%, had efficacy in accelerating the wound-healing process. The 1% catfish oil gel preparation, as well as the 1% and 2% catfish cream preparations, demonstrated the best results in speeding up the healing period of incision wounds in mice, which was around 16 to 17 days. Based on the current results, it is recommended to further develop gel and cream formulations containing catfish oil, particularly at concentrations of 1% and 2%, as potential topical agents to promote wound healing. Additionally, further evaluations on formulation stability, potential skin irritation, and *in vitro* biological activity are essential to support progression toward clinical application.

DECLARATIONS

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Authors' contributions

Aulia Andi Mustika, Lina Noviyanti Sutardi, Andriyanto, and Cahyono did the conceptualization and methodology. Adwisto Saktika Purohita did the methodology. Nadiya Safitri wrote the original draft and handled the resources. All authors checked and approved the analyzed data and the last edition of the manuscript.

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Competing interests

The authors have not declared any conflict of interest.

Ethical considerations

This paper was originally written by the authors and has not been published elsewhere. The authors checked the text of the article for plagiarism index, and confirmed that the text of the article is written based on their original scientific results.

Availability of data and materials

The data to support the present study's findings is available upon reasonable request from the corresponding author.

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