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Original Research Article

Effectiveness of Black Soldier Fly (*Hermetia illucens*) Prepupa Oil Emulgel for Burn Wound Recovery

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ABSTRACT

High levels of fatty acids found in black soldier fly (BSF) prepupa oil have the potential to act as antimicrobials and anti-inflammatory agents. This study aimed to evaluate the efficacy of BSF prepupa oil emulgel as a topical treatment to hasten the morphological and histological healing of burn wounds. This study used 30 male BALB/c mice divided into six groups, namely the group without treatment (UT group), the group given emulgel base (EB group), the group given bioplacenton® (Bp group), the group given 5%, 10%, and 15% BSF prepupa oil emulgel (EBSFPO 5%, 10%, and 15% groups). Burns were made by exposing the skin of the back to 25% phenol for 30 seconds; the treatment was carried out for 14 days with application twice a day for all groups. The morphological results showed that the 15% emulgel treatment accelerated the decrease in scab score on day 5 and decreased the wound area faster on day 7 by 0.40 cm^2 compared to the other groups. Histological analysis also confirmed that the untreated group had a significant effect in reducing the thickness of the epidermis. In contrast, emulgel 15% significantly reduced the thickness of the dermis and the number of macrophage cells. Meanwhile, epithelialization and fibroblast formation were complete in all treatment groups after 14 days. The findings suggested that BSF 15% prepupa oil emulgel can be produced as a topical solution that works well for treating wounds.

***Keywords*:** Emulgel, fatty acid, macrophages, prepupa, wound healing

Introduction

Burns are one of the skin injuries responsible for many pathophysiological changes in the skin and other organs. If burns are left untreated, the clinical situation can develop into systemic conditions such as multi-organ failure, sepsis caused by damage to cells and blood vessels, and impaired blood supply to the wound.[1] Delay in burn wound healing is a serious concern for patients and healthcare providers worldwide. Delays in the healing process of severe burns can lead to serious infections. In addition, if the injury is not treated properly, chronic non-healing wounds or proliferative scar tissue may form. [2,3]
Until now, numerous antibiotics have been created from a wide range of biological resources and synthetic compounds to treat pathogenic microorganisms. Nevertheless, the excessive utilization of antibiotics has led to a rapid increase in bacteria resistant to multiple drugs.[4] Consequently, there is an urgent need to develop novel drugs with antimicrobial and anti-inflammatory properties and drugs that exhibit greater efficacy than the currently available antibiotics. There is also a need for more research aimed at developing new drugs or compounds derived from insects or arthropods.[5]
Hermetia illucens (Diptera: Calliphoridae) or black soldier fly (BSF) larva have been used extensively in the medical field for treating skin damage as a medical/insect medicine in Europe and America.[6,7,8] BSF larva are regarded as the emerging champions in the realm of sustainable feed products due to their ability to yield protein of exceptional quality.

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The protein component has already been subjected to industrialization as a superior food ingredient, while the residual oil extract remains significantly untapped. Notably, the oil derived from BSF larva exhibits a distinct composition comprising 40-50% lauric acid, which is classified as a saturated medium-chain fatty acid (MCFA).[9] MCFA has immunomodulatory properties and is reported to suppress inflammations.[10]
So far, there has been no investigation on the effectiveness of BSF prepupa oil in emulgel dosage form for burn wound healing. The selection of emulgel-based formulations is essential in drug delivery systems. A previously reported study showed that cork fish oil emulgel preparations at 5%, 10%, and 15% were able to reduce the diameter of burns compared to treatments with bases and natural healing.[11] Therefore, this study aimed to analyze the effectiveness of the BSF prepupa oil emulgel formulation as a topical dosage form to accelerate the healing process of burn wounds.

Materials and Methods

BSF Prepupa Collection
Black Soldier fly (BSF) prepupa was collected from BSF cultivators in Gunung Pangilun, Padang, West Sumatra Indonesia (-0.94924000, 100.35427000), and authenticated by Dr. Resti Rahayu from the Department of Biology, Faculty of Mathematics and Natural Sciences, Universitas Andalas in April 2023.

Extraction of BSF prepupa oil
Wet prepupa (6 kg) were dried in the sun, after which the prepupa were pulverized using a grinder. The powdered black soldier fly prepupa was extracted with hexane at room temperature for 24 hours. After that, the extract was filtered using Whatman no.1 filter paper, and the filtrate was then evaporated under low pressure using a rotary evaporator (BUCHI Rotavapor BL-710D, Switzerland) at 40°C. The crude extract was stored in a refrigerator (LG GN-B200SQBB, Indonesia) at 4°C until ready for use.

Preparation of emulgel

Three concentrations of emulgel formulations were prepared from BSF prepupa oil. Weighed amounts equivalent to 5%, 10%, and 15% BSF prepupa oil were incorporated into 50 g of gel base. The emulsion was made with BSF prepupa oil as the oil phase and the water phase which consist of the mixture of sodium lauryl sulfate with propylene glycol. The oil phase was added to the water phase at 70°C while continuously stirring using a 300 rpm magnetic stirrer (IKA C-MAG HS7, Malaysia) until an emulsion was formed. The gel base was prepared by dissolving Hydroxypropyl methylcellulose (HPMC) in hot water as much as 20 times the weight of HPMC, then allowing it to stand for 20-30 minutes, after which it was crushed to form a gel base. The emulsion was added to the gel base and crushed until homogeneous to form a good emulgel.[12]

Ethical approval

The procedure for handling and treating test animals was approved by the Research and Ethics Committee of Andalas University (Approval number: 195/UN.16.2/KEP-FK/2023).

Bioactivity testing of BSF prepupa oil emulgel on the burn healing process

Experimental animal preparation

Thirty healthy adult male BALB/c mice (body weight 25-30 grams, age 2-3 months) were obtained from Pondok Tikus, Lubuk Begalung, Padang, West Sumatera, Indonesia (-0.94924000, 100.35427000) for this experiment. Before the experiment, the mice were acclimatized to a laboratory setting for seven days. Fed with Rat Bio and water *ad libitum* was given to all of the mice.

Burn wound model

The mice were divided into six major groups, each with five mice: Untreated group (UT group), emulgel base-treated group (EB group), Bioplacenton®-treated group (Bp group), emulgel BSF prepupa oil 5%-treated group (EBSFPO 5% group), emulgel BSF prepupa oil 10%-treated group (EBSFPO 10% group) and emulgel BSF prepupa oil 15%-treated group (EBSFPO 15% group). Before the application of 25% phenol solution (induction of injury), the hair around the back of mice was shaved with a hair thresher (veet) and then cleaned with 70% alcohol. Mice were anesthetized by inhalation of diethyl ether for 10 seconds. Burns were conditioned by attaching 1 cm diameter filter paper that had been soaked in 25% phenol solution to the skin of the mice's back for 30 seconds.[13] The initial application was carried out the day after the wound was induced. The area of the wound was measured periodically (1, 4, 7, 10, and 14 days) and photographed with a digital camera, and the area was calculated with Image-j Launcher.

Burn healing activity assessments.

Burn wound healing was observed morphologically by calculating the scab score and wound area. Wound scab scoring was divided into five: scab has not yet formed (4), thin scab (3), thick scab (2), open scab (1), new skin was forming, and hair was growing (0).[14] Calculation of wound area was carried out using Image-J Launcher 1.4.3.67 software. After opening the software, the wound image was imported, the composition shape that fits the wound, and the average value.[15] On the 15th day, mice were euthanized. Skin tissues were fixated in 10% formalin solution, processed, and embedded in paraffin. Tissues were cut into 5 mm thick sections and stained with hematoxylin and eosin (HE). All parameters were recorded by microscope (Olympus Microscope BX51, Japan) and then using a computer program (Image-Launcher 1.4.3.67 version) to measure the thickness of epidermis, dermis, epithelialization score, macrophage count, and fibroblast score. Epithelization scoring: absent (comprehensive damage to the epidermis, scored 0), starting (initial formation of the epidermal layer, scored 1), incomplete (epidermal layer was formed but with thickening, scored 2), complete (epidermal layer is fully formed with no thickening, scored 3). Fibroblast scoring: none (score 0), 5-10 cells (1), 10-50 cells (2), >50 cells (3).[16]

Statistical analysis

Quantitative data were analyzed using SPSS 23 software (International Business Machines Corporation). One-way analysis of variance (ANOVA) was used to assess significant differences between groups in various treatments, followed by Duncan's new multiple range test (DNMRT). Differences were considered statistically significant at (p<0.05).

Results and Discussion

The wound-healing effect of BSF prepupa oil emulgel on the experimental animals after treatment with the different emulgel concentrations was examined in this study. The wound area was observed for 14 days, and the morphology was observed visually and calculated using the *Image J* program. Morphologically (Figure 1A), the results showed that the wounds in each treatment group gradually healed until day 14. In a healed wound, there was new tissue covering the wound, or there was no erythema and swelling.[17] Wounds in the EBSFPO 15% group heal faster, marked by the release of scabs on the wound area. The scoring of scabs on the wound (Figure 1B) shows that the scoring observations from day 1 to day 3 did not show any scabs (score 4). On days 3 to 6, there was a decrease in scab scores for all treatments. Based on statistical analysis, the wound scab score did not significantly affect all treatment groups on the same day. However, the 15% EBSFPO group decreased faster than other treatment groups. This finding was supported by data on the decrease in burn area that, for all treatments on the same day, based on statistical analysis, did not have a significant effect, but on the 7th day, the 15% EBSFPO group experienced a faster decrease in wound area than other treatments (0.40 cm^2). In contrast, the decrease in wound area in the UT, EB, Bp, 5% EBSFPO, and 10% groups was 0.76 cm^2, 0.77 cm^2, 0.47 cm^2, 0.73 cm^2, and 0.61 cm^2, respectively (Figure 2).

The wound healing activity was enhanced because BSF prepupa contains various types of fatty acids such as lauric acid, myristic acid, palmitate acid, stearate acid, capric acid, palmitoleic acid, oleic acid, and linoleic acid, which have been reported to promote wound healing.[18,19] An earlier investigation revealed that fatty acids can modify the skin's structure and immunological aspects. They can also modify skin permeability and suppress the synthesis of pro-inflammatory eicosanoids, reactive oxygen species (ROS), and cytokines, thereby influencing the inflammatory reaction and facilitating the process of wound healing.

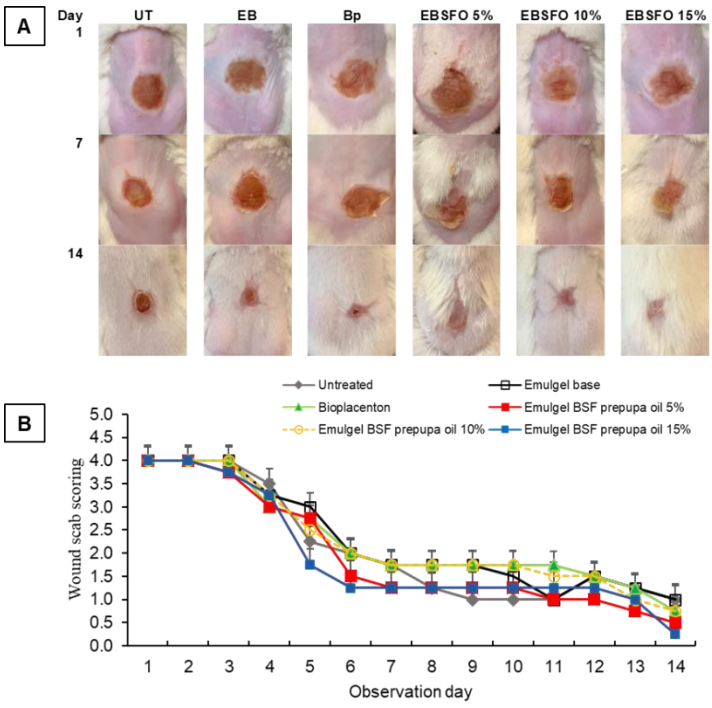

Figure 1: (A) Effect of BSF prepupa oil emulgel on wound healing in mice skin as indicated by the morphological condition of the wound on days 1, 7, and 14 (upper part). (B) Graph of wound scab scoring on days 1 until 14 (lower part).

The study was also supported by research by Richter *et al.*, which showed that BSF larvae oil modulates the decrease of TNFα, IL-6, and IL-1β secretion in macrophages.[20] Lauric acid suppressed the release of the pro-inflammatory cytokines TNFα, IL-6, and IL-1β. Reduced expression of TNF-α has the effect of reducing cellular oxidative stress, thereby aiding wound healing. Other ingredients, such as stearic acid, play a role in reducing inflammation by suppressing inflammatory cell accumulation or nuclear factor kappa B (NF-kB) activity. The inhibition of NF-kB activation is accompanied by the inhibition of IL-1 and TNFα.[21]

Previous studies also reported that *H. illucens* larva extract inhibited activity against gram-negative bacteria.[22] In addition, as shown in studies by Muller *et al*. Caligiani *et al*. Jabee *et al*. the content of saturated fatty acids (SFA), which includes palmitic acid, lauric acid, oleic acid, linolenic acid, and linoleic acid, showed antimicrobial activity against Gram-positive bacteria. Gram-positive bacteria such as *Staphylococcus aureus* are often the cause of infection in burn wounds.[23-25]

Based on histological observations of mice skin, which includes quantitative observations (epidermal thickness, dermis thickness, number of inflammatory cells) and semiquantitative observations (scoring the number of fibroblasts and re-epithelialization), the results of the histological picture of mice skin on day 14 shows that the group given EBSFPO 5% and 10% showed an increase in epidermal thickness except at a dose of 15% (Figure 3). This result indicates that in the EBSFPO 10% treatment group, the epithelial proliferation phase was ongoing, resulting in a thicker epidermis. This epithelial cell proliferation causes the epidermal layer to thicken. The epidermis then grows to the needed thickness and is finally projected into the dermis.[26,27]

In addition to histological observation of skin tissue, the thickness of the epidermis and dermis of mice skin tissue was also calculated. Based on statistical analysis the untreated group differed significantly from the other treatments, while the 15% EBSFPO group was not significantly different from BP and EB (Table 1). In addition, based on a statistical analysis of dermis thickness, EBSFPO 15% was significantly different from other treatments but not significantly different from EB. The EBSFPO 15% group showed changes in skin tissue towards improvement, as indicated by thinning the epidermis and dermis layers close to normal conditions. However, the UT group had a much thinner epidermis thickness compared to the EBSFPO 15% group, in which the dermis remained thickened. Fatty acids play a role in influencing the maturation and differentiation of the outermost skin layer, the stratum corneum. Additionally, they hinder the generation of pro-inflammatory eicosanoids, reactive oxygen species (ROS), reactive nitrogen species (RNS), and cytokines, impacting the inflammatory response and influencing the probability of wound healing.[28]

The calculation of the re-epithelialization score for all treatments showed a score of 3 (complete), which was characterized by the complete formation of the epidermal layer. Fatty acids aid the process of collagen and epithelial tissue formation in wounds. The faster the re-epithelialization, the faster the wound closes, resulting in faster wound healing.[29] The progress of wound healing was also observed in the average number of macrophages and fibroblasts (Figure 4 and Table 2).

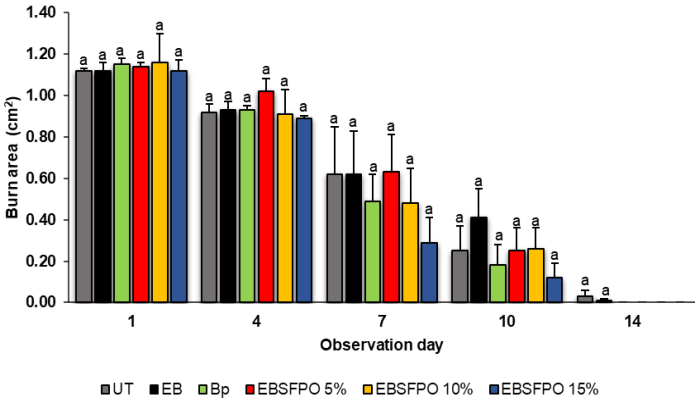

Figure 2: Effect of BSF prepupa oil emulgel on wound healing in mice skin as indicated by the wound area reduction on days 1, 4, 7, 10 and 14. Statistically, the same lowercase characters at the top of the bar graph indicate that all treatment groups are not significantly different (p>0.05).

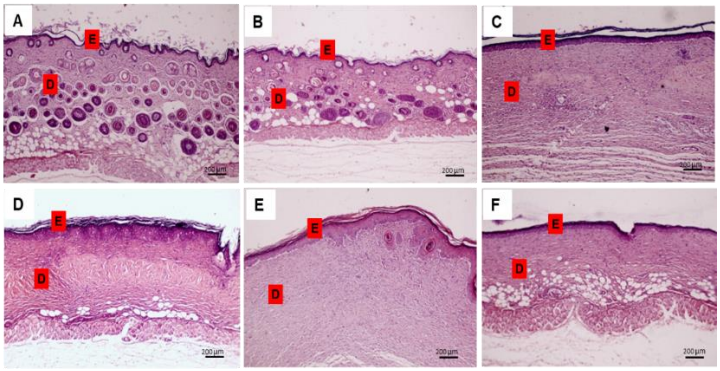

Figure 3: Histopathological analysis of epidermis and dermis thickness in mice skin tissue. (HE staining, 10x magnification). Epidermis (E), dermis (D). (A) Untreated group, (B) emulgel base-treated group, (C) bioplacenton-treated group (D) emulgel BSF prepupa oil 5% (E) emulgel BSF prepupa oil 10% and (F) emulgel BSF prepupa oil 15%.

Table 1: Effects of BSF prepupa oil emulgel on epidermis, dermis thickness, and re-epithelialization score in mice skin tissue

Groups	Mean thickness of epidermis and dermis (µm) ± SD		Re-epithelialization score
	Epidermis thickness	Dermis thickness	
Untreated	27.617 ± 8.15^{a}	479.847 ± 55.79^{b}	3 (complete)
Emulgel base	45.370 ± 7.73^{b}	289.048 ± 55.03^{a}	3 (complete)
Bioplacenton	44.533 ± 8.84^{b}	642.171 ± 45.11^{c}	3 (complete)
Emulgel BSF prepupa oil 5%	68.609 ± 16.58^{c}	438.629 ± 25.39^{b}	3 (complete)
Emulgel BSF prepupa oil 10%	71.713 ± 27.05^{c}	700.755 ± 57.82^{d}	3 (complete)
Emulgel BSF prepupa oil 15%	45.140 ± 7.49^{b}	295.745 ± 39.02^{a}	3 (complete)

Statistically different lowercase letters in the same column indicate that the group is significantly different (p<0.05) from the other groups.

Table 2: Effect of BSF prepupa oil emulgel on macrophage and fibroblast counts

Groups	macrophage cell count ±SD	Fibroblast scores ±SD
Untreated	104.2 ± 7.40^{c}	2.4 ± 0.00^{a} (10-50 cells)
Emulgel base	94.2 ± 6.57^{b}	2.0 ± 0.00^{a} (10-50 cells)
Bioplacenton	123.2 ± 5.76^{d}	2.6 ± 0.55^{a} (> 50 cells)
Emulgel BSF prepupa oil 5%	101.4 ± 6.31^{bc}	2.4 ± 0.55^{a} (10-50 cells)
Emulgel BSF prepupa oil 10%	167.8 ± 5.97^{e}	2.8 ± 0.55^{a} (> 50 cells)
Emulgel BSF prepupa oil 15%	82.8 ± 7.19^{a}	2.4 ± 0.55^{a} (10-50 cells)

Statistically different lowercase letters in the same column indicate that the group is significantly different ($p<0.05$) from the other groups.

Based on statistical analysis, the 15% EBSFPO group showed a significant decrease in the macrophage cell count compared to the other treatments. This data showed that the EBSFPO 15% group can effectively reduce inflammation in mice. The fatty acid content in prepupa oil, especially oleic acid, linoleic acid, and palmitic acid, acts as a chemotactic factor in the early stages of wound healing, which attracts inflammatory cells from the bloodstream to migrate to the tissue. This finding was related to de Oliveira *et al*.'s research, which demonstrated the wound-healing activity of avocado oil containing monounsaturated and polyunsaturated fatty acids, such as oleic acid, linoleic acid, and palmitic acid. Specifically, this activity results in a reduction in the number of inflammatory cells and an increase in collagen synthesis.[30]

Another aspect of wound healing can be identified with granulation tissue formation. The appearance of granulation tissue was characterized by the growth of fresh capillaries (angiogenesis) and the infiltration of inflammatory cells, mainly macrophages and fibroblasts (connective tissue), in the wound area; the proliferation of fibroblasts determines the outcome of wound healing.[31] Based on statistical analysis (Table 2), calculating the average fibroblast score for all treatment groups had no significant effect. The EBSFPO group was already in the healing phase, so it did not cause an increase in the number of fibroblasts needed for tissue regeneration. Bardia *et al*. reported that linoleic acid reduced the number of inflammatory cells in wound tissue, shortened bleeding time, and demonstrated fibrin stabilization and fibroblast migration.[32]

The emulgel formulation utilized in this investigation can facilitate the healing of wounds due to its cold properties, which alleviate pain and generate a moist setting. This moisturizing effect can enhance the migration efficiency of epithelial cells, thus expediting the wound re-epithelialization process. Furthermore, a controlled, humid environment can promote the assimilation of topical therapeutics, including antibiotics, analgesics, and other bioactive compounds, into the wound area.[33] The presence of fatty acids in prepupa oil, such as palmitic acid, oleic acid, linoleic acid, and stearic acid, worked as free radical scavengers and moisture guards that play a role in the wound healing process.[34,35]

Conclusion

The application of 15% BSF prepupa oil emulgel was morphologically practical in accelerating the healing of burn wounds, and histologically, it can reduce the thickness of the epidermis and dermis under normal conditions and reduce inflammatory cell count. Therefore, prepupa oil emulgel has excellent potential to be developed as a topical preparation for effective and affordable treatment of burns in the future.

Conflict of Interest

Authors declare no conflict of interest

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

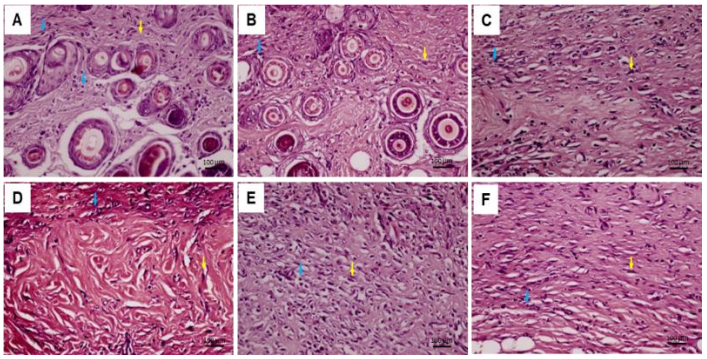

Figure 4: Histopathological analysis of the number of macrophage cells and fibroblast cells in mice skin tissue. (HE staining, 40x magnification). Macrophage cells (blue arrows), fibroblasts (yellow arrows). (A) Untreated group, (B) emulgel base-treated group, (C) bioplacenton-treated group, (D) emulgel BSF prepupa oil 5%, (E) emulgel BSF prepupa oil 10% and (F) emulgel BSF prepupa oil and 15%.

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