




The potential of black soldier fly prepupa oil (*Hermetia illucens* L.) on wound healing in mice (*Mus musculus* L.)

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ABSTRACT: The wound is a local response of a tissue caused by contact with a heat source, sharp object, or microbes. Some pathogens have developed resistance to several topical antibiotics and cause adverse side effects. Therefore, the search for natural anti-inflammatory drugs that are effective but have few side effects is urgently needed. One of the natural ingredients that have the potential as anti-inflammatory is Black Soldier Fly (BSF) prepupa oil. This study aims to reveal the potential of BSF prepupa oil in accelerating burn wound healing and explain the content of chemical compounds in BSF prepupa oil as an anti-inflammatory using gas chromatography-mass spectrophotometry (GCMS) analysis. This study used 48 male BALB/c mice with four treatment groups, namely control group (without any treatment), 0,1 g Bioplacenton® treated group three times a day, 20 µL BSF prepupa oil-treated groups with once a day or two times a day applications. Burn wound conditioning was done by administering 25% phenol for 30 seconds, after which it was left for one day and then treated. Furthermore, changes in wound morphology, a decrease in the wound area, and leukocyte count were observed. The results showed that administering 20 µL BSF prepupa oil twice a day had a considerable anti-inflammatory effect on burn wound healing, as indicated by morphological changes in scab detachment and a significant decrease in wound area on day 7. However, it did not affect the number of leukocyte components in mice. In addition, GC-MS analysis revealed several anti-inflammatory solid compounds, namely acetic acid, ethenyl benzene, lauric acid, propionic acid, stearic acid, palmitic acid, and linoleic acid.

KEYWORDS: Anti-inflammatory; black soldier fly prepupa; burns; hematology; linoleic acid.

1. INTRODUCTION

The wound is the loss or destruction of a portion of body tissue [1,2] due to mechanical, chemical, thermal, microbial, or immunologic interference [3]. Non-healing wounds significantly impact health, cause pain, loss of function and mobility, and increase morbidity and mortality [4]. The wound healing process includes three phases, the inflammatory phase that occurs after wounding (0-3 days), the proliferation phase characterized by increased tissue formation (3-14 days) [5], and the tissue remodeling phase, which is the phase of wound healing that continues for an extended period (3-6 months or even up to years) [6]. The wound recovery rate and quality depend on many factors, including nutrition [7]. The effects of selected nutrients on wound healing have been studied, for example, arginine [8], glutamine [9], vitamin C [10], and fatty acids [11].

So far, the use of topical antibiotics such as Bioplacenton, Cloramphenicol, Tetracycline HCL, Silver Sulvadiazine 1%, and Basitracin is not effective enough to speed up the wound healing process. Some pathogens have been reported to have multiresistance to several of these topical antibiotics. This is due to excessive administration of antibiotics and/or their inappropriate use [12]. In the last decade, traditional medicine has grown in popularity. Traditional medicines ingredients may originate in animals, plants or other microorganisms which are widely used as alternative medicines. A number of studies show that traditional medicines have potential as wound healing agents besides chemical treatments [13-15]. The use of traditional medicine for wound healing is based on its antiseptic, astringent, anti-inflammatory, antioxidant and antibacterial potential. One of the traditional medicinal ingredients that has the potential to be used is oil of BSF prepupa.

There are researches on the amino acids, fatty acids, and vitamins and minerals contents of BSF prepupa [16]. BSF contains a high amount of fatty acids (up to about 40%) and is rich in saturated fatty acids

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(S.F.A.), especially palmitic acid, lauric acid, oleic acid, linolenic acid, and linoleic acid, besides having antimicrobial activity against Gram-positive bacteria [17-19]. In another study, Rodrigues et al. [20] reported that oral administration of fatty acids such as linoleic acid (L.A.) in peanut oil improved the wound healing process in non-diabetic animals. The study of Collins and Sulewski [21] shows that the rich fatty acid content in cork fish extract oil is a nutrient that can accelerate the wound healing process [22].

Based on this, scientific information on the efficacy of BSF prepupa oil is still minimal. Therefore, further research is needed to test the effectiveness of prepupa oil in accelerating the healing process of burn wounds. This study aimed to investigate the potential of BSF prepupa oil in accelerating burn wound healing and the components of leukocyte values in mice and identify the compounds of prepupa oil that may be associated with anti-inflammatory properties.

2. RESULTS

2.1 Burn Morphology in Mice

After giving 25% phenol for 30 seconds on the back of the mice and left for one day, type two burns were obtained, characterized by brownish skin and uneven skin surface. Figure 2 illustrates the description of burns after 1, 4, 7, 10, and 14 days. The results showed that the wounds in each treatment group gradually healed over time. Injuries in the BSF prepupa oil treatment group healed faster on the 7th day of observation compared to the control (without any treatment) and Bioplacenton® treatments, which were characterized by the detachment of the scab on the wound area. The speed of scab removal indicates the rate of wound healing. At the end of the observation on day 14, it was seen that the wound was closed and healed for all treatment groups, but hair growth was faster and denser in the treatment group with BSF prepupa oil compared to the control (without any treatment) and Bioplacenton® groups.

2.2 Effect of BSF Prepupa Oil on Burn Area in Mice

Wound area measurements were conducted on day 1, day 4, day 7, day 10, and day 14 (Figure 3). Wounds in the test animals were declared healed, marked by changes in wound area that were getting smaller. The results showed that the administration of 20 µL BSF prepupa oil twice a day had a significant effect ($p < 0.05$) on reducing wound area on day seven compared to other treatment groups. Where in the treatment of giving 20 µL BSF prepupa oil twice a day, the decrease in wound area was faster, namely in the second three days of observation (range of day 4 to day 7), while the treatment of giving Bioplacenton® decreased the wound area faster, namely in the first three days of observation (range of day 1 to day 3) for the subsequent decrease in wound area to be slower. Although on day 14, observations showed the test animals for all groups healed.

2.3 Effects of BSF Prepupa Oil on the Leukocytes

To establish the modulating effect of black soldier flies prepupa oil on leukocyte counts, monocytes, granulocytes, lymphocytes, and total leukocytes were counted at three different time points (days 1, 8, and 15). The results presented in Figure 4 show that the number of monocytes, granulocytes, lymphocytes, and total leukocytes for all treatment groups at three different time points (days 1, 8, and 15) did not have a statistically significant effect.

2.4 Identification of Chemical Compound Components in BSF Prepupa Oil by Gas Chromatography-Mass Spectrophotometry (GC-MS)

The GC-MS analysis revealed 22 compounds identified in the black soldier fly prepupa oil as depicted in the chromatogram Figure 1, namely acetic acid, oxolane, artisan, difluoromethyl pyridine, 2-ethenyl- 2-deuterioethenylbenzene, ethenyl benzene, boric acid, lauric acid, propionic acid, adipic acid, odoantipyrine, valeric acid, myristic acid, stearic acid, docosanoic acid, palmitic acid, heptadecanoic acid, arachidic acid, tetracosanoic acid, octadecadienoic acid, and linoleic acid were determined according to NIST library. Among all compounds detected, 7 of them, namely acetic acid, ethenyl benzene, lauric acid, stearic acid, propionic acid, palmitic acid, and linoleic acid are suggested to contribute in the suppression of inflammatory response (Table 1).

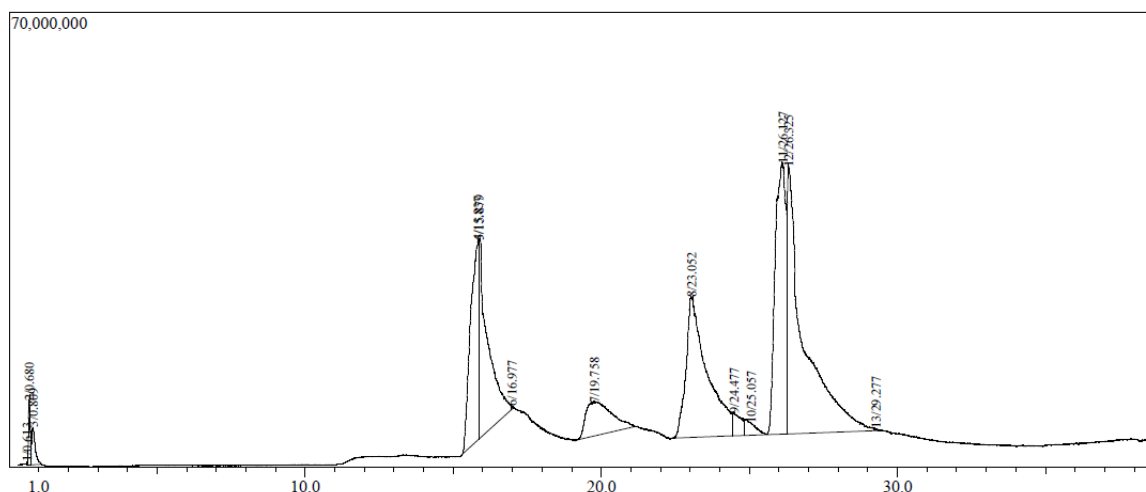


Figure 1. GC-MS Chromatographic of BSF prepupa oil

Table 1. Bioactive compounds detected in BSF prepupa oil based on GC-MS analysis

No	No Peaks	Compound Name	Retention times	Area (%)	Bioactivity
1	1	Acetic acid	0.613	0.01	anti-inflammatory [33]
2		Oksolana			Unknown
3		Arsan			Unknown
4	2	Difluoromethyl Pyridine, 2-ethenyl-	0.680	0.79	Unknown
5		2-deuterioethenylbenzene			Unknown
6	3	Ethenyl benzene	0.809	0.86	cyclooxygenase-2 inhibitors [38]
7		Boric acid			Unknown
8	4	Lauric acid	15.837	10.74	anti-inflammatory and antibacterial [28]
9	5	Propionic acid	15.879	11.71	anti-inflammatory [33]
10	6	Adipic acid	16.977	0.03	Unknown
11		odoantipyrine			Unknown
12		Valeric acid			Unknown
13	7	Myristic acid	19.758	5.82	Unknown
14		Stearic acid			anti-inflammatory [33]
15		Docosanoic acid			Unknown
16		Tetracosanoic acid			Unknown
17	8	Palmitic acid	23.052	19.54	antioxidant, anti- inflammatory, and regenerative agent [36]
18		Heptadecanoic acid			Unknown
19		Arachidic acid			Unknown
20		Tetracosanoic acid			Unknown
21	11	Octadecadienoic acid	26.127		Unknown
22	12	Linoleic acid	26.325	29.55	cyclooxygenase-2 inhibitor [37]

3. DISCUSSION

This study showed the potential of black soldier flies prepupa oil in accelerating wound healing. On visual observation of burn wounds, BSF prepupa oil treatment showed better wound healing, characterized by removing scabs on day seven, compared to other treatment groups. On the 14th day of observation, it was seen that the wounds on the test animals for all treatments had healed, and the skin surface was smooth

with standard skin color. In addition, BSF prepupa oil treatment administration showed faster and denser hair growth in mice. This is thought to be due to the content of prepupa oil, such as linoleic acid, palmitic acid, and stearic acid, which play a role in accelerating the process of hair growth in mice. Sajakala [23] reported that the fatty acid content in hazelnut oil, namely palmitic acid, linoleic acid, and stearic acid, plays a role in stimulating hair growth. While the control (without any treatment) and Bioplacenton® treatments start hair growth faster but not tightly, the range of days to achieve perfect hair growth is longer than the BSF prepupa oil treatment.

Based on morphology observation, the burn area was measured. The results revealed that administering BSF prepupa oil twice a day has the potential to accelerate burn wound healing in mice, as evidenced by a faster decrease in burn area on day 7. The beneficial effect of BSF prepupa oil twice a day on day 7 was significantly different, with a very noticeable reduction in wound area of 0.03 cm², while the wound area in the control (without any treatment) was 0.38 cm², and the Bioplacenton® was 0.34 cm²; this shows that BSF prepupa oil twice a day reduces the wound area 12 times faster than the control (without any treatment) and 11 times faster than the Bioplacenton® treatment. Giving BSF prepupa oil twice a day shows a shorter time for the re-epithelialization process. In connection with the decrease in the wound area, the percentage of burn wound healing has increased on day 7, with the administration of BSF prepupa oil twice a day showing a higher rate of recovery which is 98%, compared to control treatment of 61% and Bioplacenton® of 68%. However, on day 14, the test animals for all treatments were all healed, only differentiated by the length of healing time, where the four treatments with BSF prepupa oil twice a day administration provided faster wound healing than other treatments. This is in line with previous research,

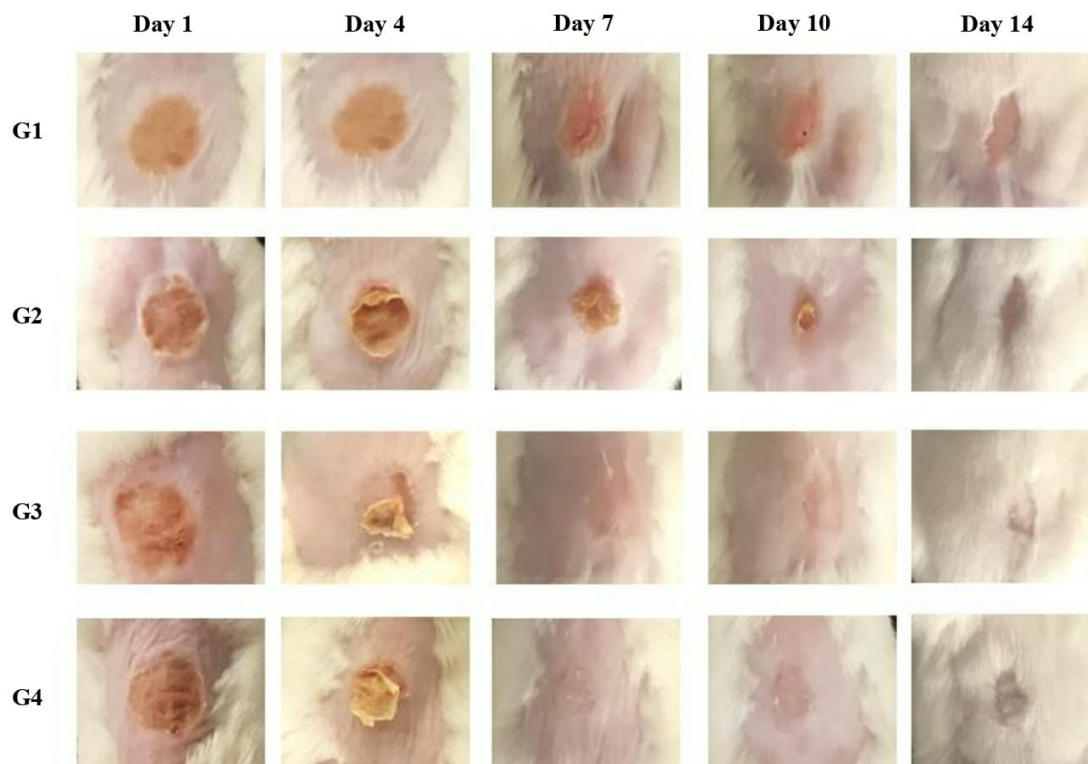


Figure 2. Effect of BSF prepupa oil on morphological feature of burn wounds. G1= burn+control (without any treatment), G2= burn +topical application of 0,1 g Bioplacenton® three times a day, G3= burns+topical application of 20 µL BSF prepupa oil once a day, G4= burns +topical application of 20 µL BSF prepupa oil twice a day

the effect of BSF maggot methanol extract at a concentration of 20% was able to provide a faster healing effect on open wounds compared to untreated wounds in the control group [24].

In this study, using GC-MS for evaluating bioactive compounds is a well-accepted approach [25]. GC-MS analysis of black soldier flies prepupa oil showed the presence of 22 compounds. 7 of the 22 identified compounds are known to have potential antimicrobial, anti-inflammatory, and prostaglandin inhibitors. Some of these compounds are acetic acid, ethenyl benzene, lauric acid, propionic acid, stearic acid, palmitic acid, and linoleic acid. Previous studies have shown that fatty acids play a significant role in the wound-healing process [26, 27, 12]

The content of bioactive compounds in BSF prepupa oil influences the events in burn wound healing. Based on the GC-MS analysis that has been carried out, BSF prepupa oil contains lauric acid. Dubo et al. [28] reported lauric acid has anti-inflammatory and antibacterial properties. Another study Nakatsuji et al. [29] said that lauric acid showed activity as an antimicrobial and anti-inflammatory agent. Lauric acid has a marked suppressive effect on the production of IL-8 and IL-6 and attenuates the expression of IL-8 and TNF- α (Tumor necrosis factor- α) [30]. TNF- α is a homotrimeric protein comprising 157 amino acids, mainly produced by activated macrophages, T lymphocytes, and natural killer cells [31]. It functionally triggers a series of various inflammatory molecules, including cytokines and other chemokines. TNF- α binds to its receptors, mainly TNFR1 and TNFR2, and then transmits molecular signals for biological functions such as inflammation and cell death. So if TNF- α is attenuated, inflammation can be alleviated. In addition, further testing on signaling pathways showed that lauric acid suppresses MAPK (mitogen-activated protein kinase) and NF- κ B (nuclear factor kappa B) activation. NF- κ B is a key factor for the proinflammatory cytokine release, If NF- κ B is inhibited it will prevent the inflammatory response [30, 32].

The following compound content in BSF prepupa oil, namely acetic acid, propionic acid, and stearic acid, has bioactivity as an anti-inflammatory [33]. Stearic acid attenuates induced inflammation by suppressing inflammatory cell recruitment/accumulation and NF- κ B activity [34]. In addition, acetic acid also plays a role in reducing cytokine expression and attenuating NF- κ B activity [35]. Palmitic acid is a fatty acid class compound with bioactivity as an anti-inflammatory. The role of other fatty acids has been studied in other studies, namely in sea buckthorn pericarp oil fatty acids, namely palmitic acid, can increase epithelialization of skin and mucosal tissue, besides being proven to play a role as an antioxidant, anti-inflammatory, and regenerative agent [36]. Epithelialization is one of the main phases in the wound healing process, in addition to forming granulation tissue with collagen and deposition of wound connective tissue proteins. As the most abundant compound, linoleic acid is a polyunsaturated essential fatty acid with antibacterial bioactivity and is a COX 2 (cyclooxygenase-2) inhibitor [37]. Hayun et al. [38] reported that ethylbenzene compounds also have a role as COX2 inhibitors. COX plays a role in prostaglandins; if prostaglandin production is inhibited, the wound-healing process will accelerate [39].

Upon the release of proinflammatory cytokines caused by burns, leukocytes will be recruited into the affected tissue. As a result, the number of leukocytes will increase markedly along with the inflammatory response [40]. Based on statistical analysis, BSF prepupae oil, negative control and Bioplacenton[®] were not significantly different, but the number of total leukocytes, granulocytes, and agranulocytes (lymphocytes and monocytes) in the BSF prepupae oil treatment increased in the circulatory system on the first day, indicating leukocyte recruitment. When inflammation occurs, the value of total leukocytes, granulocytes, lymphocytes and monocytes will increase, where platelets will secrete growth factors that signal leukocytes to enter the wound area. Neutrophils are the first to enter the injured area, followed by monocytes and lymphocytes [41]. Leukocytes have a major role in the body's defense system. An increase in leukocyte count supports immunosuppression and wound healing. This shows that the administration of BSF prepupae oil twice a day modulates the body's defense or immunity components in wound healing as indicated by a faster decrease in wound area on day 7 and supported by the values of total leukocytes, granulocytes, lymphocytes and monocytes that have decreased on day 8, indicating that the inflammatory phase is over.

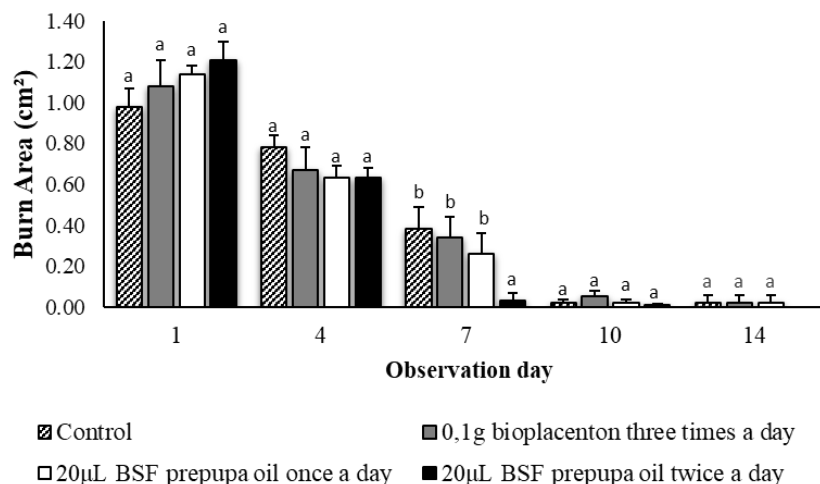


Figure 3. Effect of BSF prepupa oil on the burn wound in mice. G1= burn+control (without any treatment), G2= burn +topical application of 0,1 g Bioplacenton® three times a day, G3= burns+topical application of 20 µL BSF prepupa oil once a day, G4= burns +topical application of 20 µL BSF prepupa oil twice a day. Statistically different lowercase characters at the top of the bar graph indicate that the group given 20 µL BSF prepupa oil twice a day was significantly different ($p < 0.05$) from the other treatment groups.

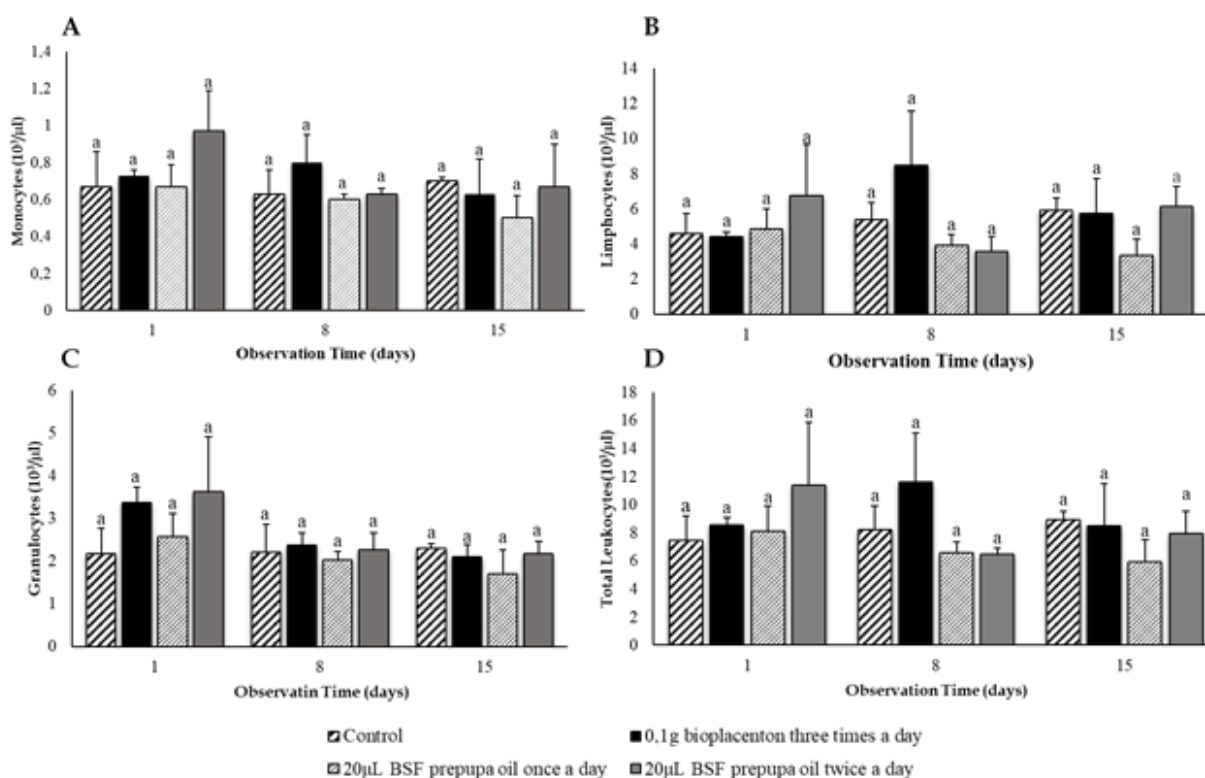


Figure 4. Mean number of monocytes, granulocytes, lymphocytes and total leukocytes on observation days 1, 8, and 15 (n=3). A). Monocytes, B). Granulocytes, c). Lymphocytes, d). Total leukocytes. G1= burn+control (without any treatment), G2= burn +topical application of 0,1 g Bioplacenton® three times a day, G3= burns+topical application of 20 µL BSF prepupa oil once a day, G4= burns +topical application of 20 µL BSF prepupa oil twice a day. Statistically the same lowercase characters at the top of the bar graph indicate that all treatment groups are not significantly different ($p < 0.05$).

4. CONCLUSION

Based on the results obtained, it is concluded that BSF prepupae oil 20 twice a day provides a faster burn wound healing effect and modulates leukocyte components to accelerate burn healing. The content of BSF prepupae oil consists of several strong anti-inflammatory compounds, namely acetic acid, ethenyl benzene, lauric acid, propionic acid, stearic acid, palmitic acid, and linoleic acid. therefore BSF prepupae oil has the potential to be developed as a natural burn medicine.

5. MATERIALS AND METHODS

5.1. BSF Prepupa Oil Extraction

BSF prepupa samples 6 kg were obtained from black soldier fly breeders in Gunung Pangilun, West Sumatra, Indonesia, fed fermented tofu pulp. Wet prepupa were dried under the sun. The resulting dried prepupa was 3 kg, then the prepupa was ground using a grinder. 3 BSF prepupa was extracted with hexane at room temperature for 24 hours. After filtering using Whatmann no.1 filter paper, the extract was evaporated under low pressure using a rotary evaporator at 40°C and stored in a refrigerator at 4°C until used.

5.2. Preparation of Test Animals

The adult male BALB/c mice (48 individuals, body weight 25-30 grams, 2-3 months old) were purchased from Pondok Tikus, Lubuk Begalung, Padang, West Sumatra, Indonesia. The mice were firstly acclimatized individually in a single cage and fed with BP-2 feed (Padang, West Sumatra, Indonesia) and tap water ad libitum for one week. The rearing room's temperature, humidity, and light-dark cycle were regularly controlled. The procedures for handling and treating test animals have been approved by the Research and Ethics Committee of Andalas University (Approval number: 195/UN.16.2/KEP-FK/2023).

5.3. Burn to Condition of Test Animals

Before wounding, the hair around the mice's back was shaved with a hair thresher and then cleaned with 70% alcohol. Mice were anesthetized by inhalation of diethyl ether for 10 seconds. Burn conditioning in mice using phenol solution with a concentration of 25% is characterized by the formation of type two burns. Burns were conditioned by attaching 1 cm diameter filter paper moistened with 25% phenol solution to the skin of the mice's back for 30 seconds. The wound was left for one day, then the initial wound area was measured, and then the test animals were treated according to the treatment group [42].

5.4. Anti-Inflammatory Testing Procedures

This study used an experimental method with a completely randomized design (C.R.D.) consisting of 4 treatments with 14 days of observation. The treatment was as follows:

G1: burns + control (without any treatment)

G2: burns + topical application of 0,1 g Bioplacenton® three times a day

G3: burns + topical application of 20 µL BSF prepupa oil once a day

G4: burns + topical application of 20 µL BSF prepupa oil twice a day

5.4.1. Burn Morphology

Visual observation of burns on mice was carried out daily for 14 days by looking at the condition of the burns and hair growth in the application area daily.

5.4.2. Calculation of Burn Area and Percentage of Wound Healing

Wound healing was measured by looking at the narrowing of the wound size. Wound area measurements were taken on days 1, 4, 7, 10, and 14. Image acquisition and wound area calculation were performed using Image-J software. The healing rate was calculated using the following equation [43]:

$$Ht = \frac{(S_0 - S_t)}{S_0} \times 100$$

where Ht represents the wound healing rate at time t after surgery, S₀ represents the initial wound area, and S_t represents the wound healing area at time t.

5.4.3. Analysis of the Quantity of Mice Leukocyte Components

Three blood samples from mice were taken for each treatment on days 1, 8, and 15; the spine of the mice was dislocated. Then, the mice were dissected, and blood was taken from the heart. A blood hematology examination was performed using an automatic hematology analyzer machine to determine the number of leukocytes (white blood cells), including lymphocytes, monocytes, and granulocytes. A complete blood sample of 500 μ L was inserted into the analyzer column, and then the quantity of blood values was presented automatically on the monitor screen [44].

5.5. Gas Chromatography-Mass Spectrometric (GC-MS) Analysis

BSF prepupae oil derivatization method: 350 mg BSF prepupae oil sample was weighed in a boiling flask, 6 ml NaOH-methanol 0.5 M was added and stirred, then 7 ml BF₃-methanol 12.5% was added. The mixture was then heated at 70 °C with a water bath equipped with a condenser for 2-3 minutes, then 5 ml of hexane was added and heated again for 1 minute. After finishing the mixture is cooled.

GC-MS analysis: GC-MS analysis of BSF prepupa oil was performed using GCMS-QP2010 Plus. The oven temperature was accomplished using a temperature-programmed route: accordingly, 60°C (hold for 1 min) was set as the initial temperature, then raised to 280°C (hold for 3 min). With a pressure of 31.5 kPa, with helium as the carrier gas used, helium flowed at a flow rate of 0.70 mL/min and a linear velocity of 30.5 cm/sec. Injector and detector temperatures were set at 220°C and 240°C. The oil is injected with a volume of 1 μ L and the ratio was 10.0. The eluted components will be detected on the mass detector. Spectra of known compounds will be stored in the NIST library.

5.6. Statistical analysis

The data were analyzed with SPSS 23 software (International Business Machines Corporation). The one-way analysis of variance (ANOVA) was deployed to elucidate the difference among treatment groups, followed by a least significant difference (L.S.D.) post-test.

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