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The Effect of Black Soldier Fly (*Hermetia illucens*) Maggot Oil Extract on Acute Incision Wound Healing in Mice (*Mus musculus*)

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Abstract
Wound treatment is usually carried out using antiseptics or antibiotics; however, if the dosage is not appropriate, it will cause bacterial resistance and infection. Black soldier fly (Hermetia illucens) maggot oil extract has antibacterial and anti-inflammatory properties, which can speed up the wound healing process. The aim of this research was to determine the effect of administering black soldier fly maggot oil extract ointment to accelerate wound healing. Black soldier fly maggot oil extract waas made into an ointment at different concentrations using vaseline solvent. This study used 24 male mice, each induced with incision wounds, which were then divided into six treatment groups: ointment treatment groups (25%, 50%, and 75%), natural control, negative, and positive, with four repetitions. The data was analyzed using ANOVA tests ($p<0.05$). The results of the study showed that the administration of black soldier fly maggot oil extract on wound closure, the distribution of collagen fibres, and the number of fibroblast cells had a significant effect ($p<0.05$). Black soldier fly maggot oil extract at a dose of 75% was the most optimal dose for healing cut wounds, as shown by the healing percentage (45%), collagen fibres scoring value (2.8), and number of fibroblast cells (2.8).

Keywords: BSF maggot oil; cut; wound healing; colagen fibres; fibroblast cells

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INTRODUCTION

A wound is a condition where the tissue, structure and normal anatomical function of skin is damaged due to an event that causes bleeding. Cuts from sharp objects, blunt trauma, exposure to chemicals, contact with hot objects, explosions, or animal bites can result in wounds. Wounds can cause pain, discomfort, and difficulty in performing daily activities. Without treatment, wound healing can takes up to four to six weeks (Wallace *et al*., 2022). Therefore, treatment is necessary to speed up the healing process and prevent the condition from worsening (Alpayet *et al*., 2023).

The wound healing process is the response of body to various injuries which involves a series of complex and dynamic stages. At each stage, healing cells and factors interact with one another, allowing the damaged skin tissue to gradually recover (Haryono and Utami, 2019). When a wound occurs, the body starts to rebuild the damaged tissue by restoring its form and function to that of the previous skin (Purnama *et al*., 2017). Physiologically, the wound healing process goes through several stages, starting from hemostasis, then continued to inflammation, proliferation, and finally remodelling (Rodrigues, 2019).

Currently, a lot of individuals prefer to treat a variety of illnesses with natural materials. Both plants and animals can provide these therapeutic ingredients (Paju *et al*., 2013; Čičková *et al*., 2015). The kind of treatment selected has a significant impact on how quickly wounds heal. The Black Soldier Fly (BSF) maggot is one of the components derived from animals that can be utilized to repair wounds. Research on the properties and oil content of black soldier flies (Hermetia illucens, Diptera: Stratiomyidae) is gaining popularity. BSF has expanded to other parts of the world since it began in the Americas (Čičková et al., 2015).

The high concentration of alkaline chemicals found in BSF maggots is essential for promoting wound healing by thwarting microbial infections. By increasing the density of collagen fibers and capillaries in the injured area of the skin, as well as the production of new epithelium and granulation

tissue, it accelerates wound healing (Madihah, 2019). With 40-50% protein and 29-32% fat, BSF maggots (Hermetia illucens) have a high content of protein and fat that helps in the formation of new tissue in wounds (Naveh et al., 2011). High lauric acid in BSF maggot oil extract. Lauric acid is a natural fatty acid that has anti-inflammatory and antibacterial qualities (Kim and Rhee, 2016; Widianingrum et al., 2019). Lauric acid is important in the healing process after a wound occurs (Widianingrum et al., 2019). By supporting the growth of T and NK cells, triggering surface antigens, producing pro-inflammatory cytokines by maximizing lymphocyte function, fibroblast production, and improving macrophage performance, the lauric acid in BSF maggot oil serves as an anti-inflammatory agent (Widianingrum and Salasia, 2021). Higher concentrations enhanced wound healing, according to a previous study by Marsela and Mangunsong (2021) that used the methanol extract of BSF maggots for open wound healing in male white rats at doses of 10%, 15%, and 20%. Similar to this, Surbakti *et al*. (2021) showed that rats' open wounds may be successfully cured by virgin coconut oil, which has a high lauric acid content, outperforming the control group. Based on the above, the lauric acid content is known to enhance the wound healing process. Therefore, this study aims to further investigate the benefits of using BSF maggot oil extract (*Hermetia illucens*), which has a high lauric acid content, as an effective topical agent for healing acute incisional wounds in mice (*Mus musculus*).

MATERIALS AND METHODS

This study was experimental research with a completely randomized design divided into six treatment groups: the positive control group (bioplacenton), the negative control group (pure vaseline), the natural control group (no treatment), Group A (25% formulation), Group B (50% formulation), and Group C (75% formulation), each with four replications.

The preparation of the Black Soldier Fly maggot oil extract began with drying through roasting or oven-drying, followed by processing with an expeller press machine, where the temperature during the oil extraction process reached 120 °C. The sterility of the oil was thus guaranteed (Lee *et al*., 2021). After the organic phase (containing oil) was separated from the solid sample and non-oil impurities were eliminated, the BSF maggot oil extract was produced. An internet marketplace was used to acquire the oil extract used in this investigation. The sterility of the oil was thus guaranteed (Lee *et al*., 2021). After the organic phase (containing oil) was separated from the solid sample and the impurities that were non-oily are removed, BSF maggot oil extract was produced. The BSF maggot in this study was obtained by buying at an online buying and selling place.

To make ointments with different concentrations of 25%, 50%, and 75%, BSF maggot oil extract formulations were prepared by combining oil extracts with pure vaseline. 2.5 milliliters of maggot oil extract and 7.5 milliliters of vaseline were mixed to make an ointment concentration of 25%. Five milliliters of maggot oil extract and five milliliters of vaseline were combined to obtain a 50% ointment concentration. Concentration of 75% was made by mixing 2.5 ml of vaseline and 7.5 ml of maggot oil extract.

Male mice weighing ±30 g age 2-4 months were used as animal models. To reduce stress and help the mice adapt to their new environment, they were acclimated for seven days. Wound incisions in mice were made after the acclimation period. Before making an incision, the dorsal fur was shaved. Mice were then anesthetized using inhalation. The shaved back was then cleaned with 70% alcohol. Incisions were made using a sterile razor blade, creating a cut 1 cm long and 0.2 cm deep on the back of the mice by lifting the skin with tweezers and then making the incision.

Macroscopic observations were carried out by measuring the initial and final wound lengths. Observations were conducted up to the sixth day. Wound length was measured by determining the distance between the farthest edges with scabs that had not yet fully closed. The wound length was measured using a digital caliper, and the percentage of wound healing was calculated using the following formula:

$$Wound\ percentage = \frac{Initial\ wound\ length - Final\ wound\ length}{Initial\ wound\ length} x100\%$$

Histopathological observations of skin tissue preparations were conducted microscopically, focusing on collagen fibres distribution and fibroblast cell count using a binocular microscope. Using scoring techniques, the distribution of collagen fibres and the number of fibroblast cells were

evaluated. A semi-quantitative method was used to analyse the tissue recovery characteristics as part of the evaluation (Paramita, 2016).

SPSS 25 was used to statistically analyse the study's data. First, normality tests were conducted using the Kolmogorov-Smirnov test and homogeneity testing for data of wound length and wound healing percentage. This was followed by ANOVA and Duncan's post hoc test. For scoring collagen fibres distribution and fibroblast cell count, statistical analysis was done using Kruskal-Wallis test, followed by the Mann-Whitney test. All statistical test were conducted at 0.05.

RESULTS

Based on macroscopic observations, including final wound length and percentage of wound healing, as well as microscopic observations, including collagen fibres distribution and fibroblast cell count, to understand the wound healing process, all three factors are related. Observations were conducted by measuring the incision wound length in mice using a caliper on the initial wound (day 1) and the final wound (day 6). Measurement results showed that the average shortest wound length was in the KP (positive control) group, with an average value of 0.5 cm, while the longest average wound length was in the KNo (negative control) group, with an average value of 0.78 cm. The treatment group with the shortest average length was KC (75% dosage), with an average length of 0.55 cm, which is almost the same as the KP (Positive Control) group. The final wound length data obtained was used to calculate the percentage of wound healing in each treatment group. Based on the calculations, the group with the highest percentage of wound healing after the KP (bioplacenton) group was the KC (75% dose) group, while the group with the lowest percentage of wound healing was the KNo (no treatment) group. The wound measurements were taken by measuring the farthest point of the scab on the mice in each group using calipers. The results of these measurements and percentage calculations indicate that different doses of BSF maggot oil extract have an effect on the rate of wound healing. The results of the final wound length measurements are presented, Percentage of Healing of Cut Wounds, The collagen fibres density score for each treatment, and Number of fibroblast cells in Table 1.

Table 1. Wound length after treatments, percentage of healing of cut wounds, the collagen fibres density score for each treatment, and number of fibroblast cells

	Final Wound Length (cm)	Wound Healing Percentage (%)	Collagen fibres density	Fibroblast score
KN group	0.65 ± 0.035^{b}	35 ± 3.54^{b}	2.2 ± 0.17^{a}	2.2 ± 0^{a}
KP group	0.5 ± 0.035^{a}	50 ± 3.54^{c}	3 ± 0^{b}	2.9 ± 0.13^{b}
KNo group	0.77 ± 0.043^{c}	22.5 ± 4.33^{a}	2.1 ± 0.1^{a}	2 ± 0.08^{a}
Oinment 25%	0.63 ± 0.041^{b}	37 ± 4.12^{b}	2.4 ± 0.17^{a}	2.3 ± 0.2^{a}
Oinment 50%	0.61 ± 0.021^{b}	38.75 ± 2.25^{b}	2.5 ± 0.1^{a}	2.6 ± 0.2^{b}
Oinment 75%	0.55 ± 0.035^{a}	45 ± 3.54^{c}	2.8 ± 0.13^{b}	2.8 ± 0.2^{b}

Description: KN: Negative Control; KP: Positive Control; KNo: Natural Control; KA: 25% Dose; KB: 50% Dose; KC: 75% Dose. Different notations indicate significant differences based on Duncan's test results ($p<0.05$). Collagen fibres and fibroblat cells : low (1), medium (2), high (3). *Notations indicate significant differences based on the Kruskal-Wallis test ($p<0.05$).

The next step is to score the histopathological preparations of the incision wounds microscopically by examining the distribution of collagen fibres and the number of fibroblast cells using a binocular microscope with 400x magnification on skin tissue preparations. Microscopic observations of collagen fibres were conducted in four fields of view for each sample, and the averages were taken for each treatment group (Herdiani, 2022). The results of the observations on collagen fibres density are presented in Figure 1 below.

The next step in scoring the histological preparations is to observe the number of fibroblast cells. Observations of fibroblast cells were also conducted in four fields of view for each sample, and the averages were taken for each treatment group (Herdiani, 2022). The scoring of fibroblast cell observations is shown in Table 4, and the observations of the number of fibroblast cells are presented in Figure 2.

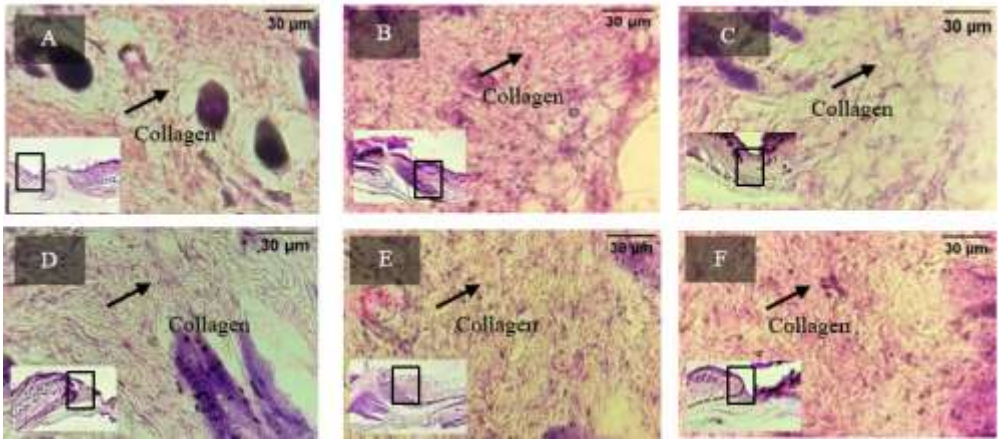

Figure 1. Histological observation of collagen fibres in wound tissue. Arrows indicate collagen fibres. A: Negative Control; B: Positive Control; C: Natural Control; D: 25% concentration; E: 50% concentration; F: 75% concentration.

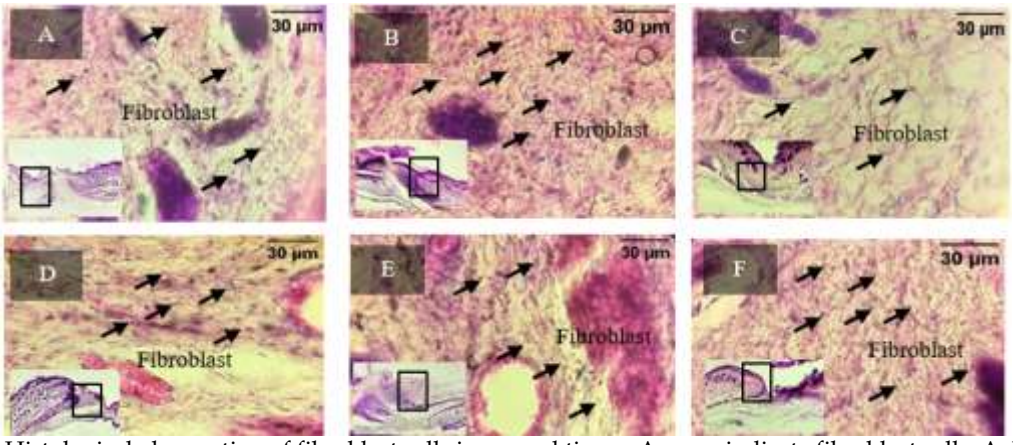

Figure 2. Histological observation of fibroblast cells in wound tissue. Arrows indicate fibroblast cells. A: Negative Control; B: Positive Control; C: Natural Control; D: 25% concentration; E: 50% concentration; F: 75% concentration.

DISCUSSION

Based on observations, scabs started to form on the third day in some treatment groups, namely the positive control group, the 75% dose group, and the 50% dose group. This scab formation is due to the adhesion of platelets that exit the blood vessels, leading to scab formation. Scabs can form when fibroblast cells develop and produce collagen, which functions to unite the wound edges (Perdanakusuma, 2017). The scab size decreased on the fourth and fifth days as the wound passed the haemostasis phase. Therefore, macroscopic observations were conducted until the sixth day because, in some groups, the scab size kept decreasing, necessitating immediate sample collection. If the tissue beneath the scab starts drying, the scab can detach from the skin as the wound edges begin to contract toward the center (Aponno *et al*., 2014). Skin scab samples were collected for microscopic observation to examine the presence of fibroblast cells and the density of collagen fibres in the histopathological preparations of each treatment group.

The percentage of wound healing was observed by measuring the initial wound length and the final wound length on the sixth day. A higher percentage indicates better wound healing, with the wound size decreasing day by day. Macroscopically, the proliferative phase is characterized by the presence of granulation tissue rich in new blood vessels, fibroblast cells, macrophages, granulocytes, endothelial cells, and collagen. These tissues form the extracellular and neovascular matrix, which fills wound gaps and creates scaffolds for cell adhesion, migration, growth, and differentiation processes (Landén et al., 2016).

Among the treatment groups of BSF maggot oil extract with different doses, the 75 percent dose group showed the best wound healing results when compared to the other treatment groups. The concentration of BSF maggot oil extract is different, which is 75 percent, where the concentration

has a much higher percentage value than that of the bioplacenta. This is due to the different concentrations of BSF maggot oil extract, which can affect how quickly the wound heals.

Lauric acid, is the highest content in BSF maggot oil extract with a content of 32.25%. Lauric acid has the ability to increase metabolism during the wound healing process and is easily absorbed by cells. To help wounds heal faster, cells work better to make new cells that replace damaged cells (Silalahi and Nurbaya, 2011). Lauric acid is converted into monolaurin, an antiviral, antibacterial, and antifungal substance, after entering the body (Pulung et al., 2016). In addition, by promoting angiogenesis and inhibiting inflammatory indicators, lauric acid promotes wound healing by increasing the delivery of oxygen and nutrients to the wound area. Lauric acid has previously been tested in vitro to promote collagen growth. An important structural element of connective tissue, collagen promotes the growth of new tissue and accelerates the wound healing process (Dewi, 2019). Linoleic acid is another substance that promotes wound healing. By strengthening fibrin tissue in the early phases of wound healing, linoleic acid, also known as omega-6, reduces the time it takes for bleeding to occur. By encouraging the production of fibroblast cells in the wound area, it also promotes collagen synthesis and fibroblast proliferation (Barda et al., 2016).

Studies have been conducted and proven that using virgin coconut oil with a high lauric acid content helps mice heal open wounds better than the control group (Surbakti et al., 2021). Based on the analysis of BSF maggot oil extract and supported by microscopic observations of collagen fiber distribution and fibroblast cell count, the 75% treatment group was found to be very successful in increasing collagen fiber density and fibroblast cell count, with an average score of 2.8. Given that the score was close to the average score of the positive control group (KP), the dense collagen fibers showed that the 75% treatment was very successful in increasing collagen fiber density.

The mean score for each treatment group was comparable to collagen fibers, according to subsequent observations of fibroblast cells. According to the data, the 75% treatment group (KC) had an average score of 2.8, which was comparable to the average score of the positive control group (KP). This shows that 75% treatment is very successful in increasing the production of fibroblast cells, as shown by a large number of fibroblasts.

The inflammatory phase, proliferative phase, and remodeling phase are the stages of wound healing (Amita, 2017). Each of these phases is intertwined, affects the other, and is crucial for wound healing. Increased blood flow to the wound site during the inflammatory phase helps in the development of fibrin threads, which protect the wound from bacterial infections. Fibroblasts produce collagen and connective tissue during the proliferative phase, and the dermal layer regenerates to aid in the healing process. Thick collagen fibres are formed to reconstruct the wound structure during the final stage, remodelling, while epithelialization makes sure the skin can heal correctly (Marwa, 2015)

Fibroblasts produce collagen and the extracellular matrix that is frequently referred to as granulation tissue. By the second day following a new wound, collagen begins to form. The skin tissue is made stronger and more elastic by collagen fibers. Collagen is released once fibrin threads develop, which starts the process of joining and combining the margins of the wound. As collagen ages, tension increases (Dealey, 2012).

Lauric acid is an active biomolecule that functions to alter the activity of growth factors, codes between cell signals, and cell proliferation. Fibronectin is then stimulated by growth factors to produce fibrin threads. According to Nevin (2010), this fibrin thread will serve as a temporary protector for fibroblast proliferation and re-epithelialization. By promoting mitochondrial biogenesis, which is important for IGF-1 signaling during tissue repair, lauric acid can also increase IGF-1 during the wound healing phase. The PI3K/AKT pathway, which contributes to re-epithelialization, angiogenesis, and inflammation reduction, is involved in this process (Ong et al., 2020; Bobiński et al., 2019). In addition, lauric acid can increase and initiate collagen synthesis in wounds. According to Sastrawan (2016), the density of collagen fibers seen in the treatment group shows that there has been a healing process in the wound.

Myofibroblasts are a type of fibroblast that has the ability to contract the initial phase of wound healing, which pulls the wound boundary closer until the wound merges and closes into one. Fibroblasts multiply as the wound heals as the wound heals. As granulated tissue builds up the connective tissue matrix, the production of collagen fibroblasts causes dense fibrosis to grow gradually in the wound tissue (Napanggala et al., 2014). Utilizing BSF maggot oil extract can maximize the wound healing process during the proliferation phase, as evidenced by the high average number of fibroblasts in the treated group. Fatty acids, which are bioactive compounds that promote the formation and division of new cells, are the cause of the higher number of fibroblasts in the BSF

maggot oil extract treatment groups. An increased fibroblast count speeds up epithelialization throughout the wound healing process (Sastrawan, 2016).

CONCLUSION

For acute incision wounds, applying a 75% concentration of Black Soldier Fly maggot oil extract yields the best results, with a 45% wound healing rate, dense collagen distribution, and a high fibroblast cell count.

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CONFLICT OF INTEREST

There is no conflict of interest

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