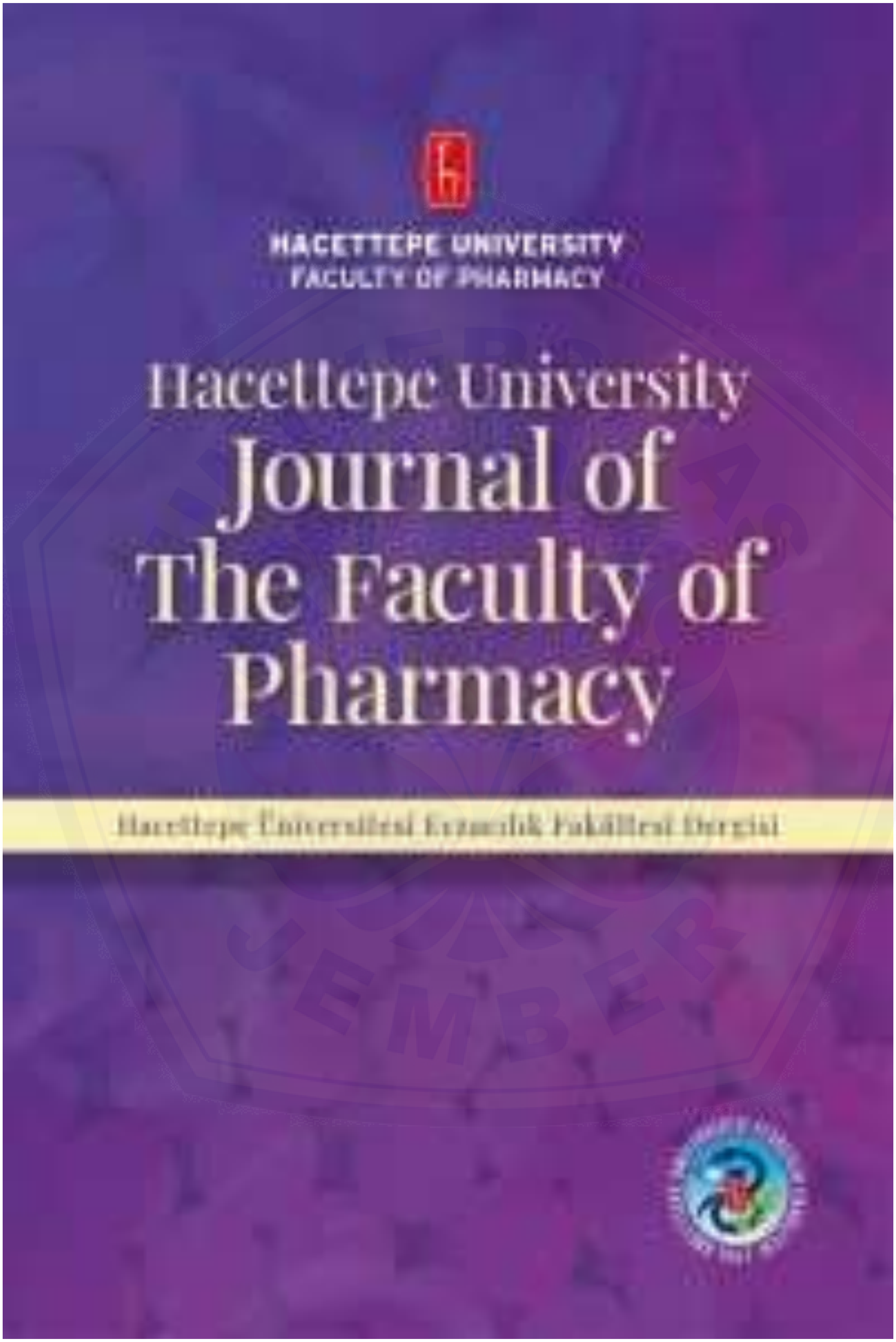
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# Effectiveness of Edamame (*Glycine max* L. Merril) Membrane in Accelerating The Wound Healing Process of Deep-Partial Thickness Burn

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**ABSTRACT**

The gold standard for deep-partial thickness burns is early excision and skin grafting. However, many hospitals in Indonesia still resort to conventional treatment due to the high cost of surgery and the need for qualified medical professionals. This research aimed to investigate the effectiveness of edamame (*Glycine max* L. Merill) membrane as an innovative therapy for deep-partial thickness burns. Forty-eight male Wistar rats with deep-partial thickness burns were randomly assigned into four groups: control, silver sulfadiazine treatment, the membrane with 40% edamame extract, and the membrane with 60% edamame extract. Multiple serial measurements were taken at 4, 10, and 16 days to evaluate wound healing parameters, including macroscopic evaluation, histopathological examination, and hydroxyproline assessment. The results revealed that the treatment groups using edamame membrane showed significantly improved wound healing compared to the control group. Macroscopic evaluation, histopathological findings, and hydroxyproline assay confirmed the efficacy of the edamame membrane with a 60% extract concentration, which yielded the best healing outcomes. This study concluded that edamame membrane is effective as deep-partial thickness burns wound dressing.

**Keywords:** burns, dressing, edamame, hydroxyproline, wound healing.

## 1. Introduction

Burns pose a significant global public health concern, resulting in an estimated 180,000 deaths annually [1]. The American Burn Association classifies burns into four categories based on depth: epidermal, superficial-partial thickness, deep-partial thickness, and full-thickness burns [2]. A study conducted by Wardhana in 2017 revealed that the highest prevalence of burns was observed in the full-thickness category, followed by deep-partial thickness burns [3]. Providing adequate treatment for deep-partial thickness burns is crucial to prevent their progression into full-thickness burns. Full-thickness burns are particularly severe compared to other burns, as they can lead to life-threatening complications such as respiratory distress and sepsis, potentially resulting in a critical state or death [4].

The primary objective of burns treatment is to prevent infection, which can hinder the natural healing process [5]. Early excision and skin grafting are the gold standard treatments for deep-partial thickness burns [6]. However, due to the high cost of surgery and the need for qualified medical professionals, many hospitals in Indonesia still rely on conventional treatment methods utilizing silver sulfadiazine (SSD) cream [7]. SSD is a topical antibacterial agent derived from sulfa that effectively controls the infection and facilitates healing in deep-partial thickness burns. Nevertheless, studies have been conducted on the long-term use of SSD cream, which has reported the formation of a dry, rough, and leather-like eschar on the wound surface. This characteristic can impede proper skin penetration and delay wound healing [5].

Wound healing can be divided into three interconnected phases: the inflammatory reaction, cell proliferation and extracellular matrix synthesis, and collagen maturation. During the proliferation phase, fibroblasts migrate to the site of the wound and initiate the production of collagen, which gradually contributes to the formation of granulation tissue covering the wound [6]. Collagens are rich in hydroxyproline, an amino acid that is a biochemical marker for assessing wound healing progress [7].

Edamame (*G. max*) is a soybean harvested when immature and still tender green, about 60 days after planting [8]. Missouri Botanical Garden Herbarium lists this plant at 04796514 [9]. It contains potassium, ascorbic acid, iron, vitamins A, B1, C, E, and isoflavones [8]. Genistein is the primary isoflavone in soybean, and evidence suggests that genistein has anti-inflammatory, antioxidant, and antibacterial effects that promote wound healing [10]. Edamame-based gauze dressings had created and maintained a moist environment for 3-4 days. Therefore it supported the healing of the wound area and prevented new injuries after the removal of rough scars [11]. This study aimed to determine the role of topical treatment with edamame membrane on deep- partial thickness burn wound healing in rats.

## 2. Material and Methods

### *2.1. Plant Collection and Edamame Membrane Preparation*

The researchers collected edamame (*G. max*) seeds from PT. Mitra Tani Dua Tujuh in Jember, January 2020. We macerated the powder of edamame with 96% ethanol and evaporated it on a rotary evaporator.We added 40% and 60% extract with 2 g of hydroxypropyl methylcellulose (HPMC) until it rose viscous within 24 hr. Moreover, we added 0,18 g of methylparaben, 15 mL of propylene glycol, and 0,15 g of propylparaben, then mixed it with 100 mL of water that contains 5% of DMSO. We poured the mixtures into a glass plate in which there was sterile gauze with a size of 2.5 cm in length, 2.5 cm in width, and ±1 mm thick. We made the sterile gauze covered by the mixtures and stored it in the refrigerator till use(4 ˚C) [11,12].

### *2.2. Animal and Experimental Control*

The male Wistar rats, weighing 200±50 g, were acclimated in the animal unit for a week. They were exposed to a 12-hour light/dark cycle at approximately 32 ˚C. The rats were individually housed in cages and provided unrestricted water and food pellets access. The forty-eight rats were randomly divided into four groups, each consisting of 12 rats. After inducing burn wounds, each group was assigned a different treatment: the positive control group received SSD cream, the negative control group received a membrane without extract, the group treated with a membrane containing 40% edamame extract, and the group treated with a membrane containing 60% edamame extract. Four animals from each group were sacrificed on days 4th, 10th, and 16th. This research was approved by the ethics team of the Faculty of Medicine, University of Jember number 1352/H25.1.11/KE/2019 on 27th December 2019.



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### *2.3. Skin Burn Injury Induction*

The rats were anesthetized using an intraperitoneal injection of ketamine (75 mg/kg) and xylazine (15 mg/kg). The backs of the animals were shaved, and a burn was induced by applying a hot aluminum plaque (2x2 cm). The aluminum plaque was heated to 70 ˚C in a dry oven and then pressed onto the skin of the rats for 10 seconds to create a deep-partial thickness burn wound. To ensure consistency, we allowed the temperature of the aluminum plaque to cool down within 5 seconds before each subsequent application. The same individual performed all procedures to minimize any bias resulting from differences in the application force [13].

### *2.4. Treatment*

The researchers categorized the animals into four groups for the experiment. Group one (SSD) received a topical application of SSD cream twice daily. Group two (Control) was treated with a membrane without any extract. Group three (40% MEE) and group four (60% MEE) were provided membranes containing 40% and 60% edamame extract, respectively. The membranes were renewed every three days to ensure the effectiveness of the treatment.

### *2.5. Wound Area and Closure Assessment*

The researchers captured photographs of the burn wounds immediately after their creation (on the first day) and on days 4, 10, and 16 using a Samsung Galaxy S10 Mobile Phone. The same camera settings, fixed camera distance from the wound, and rat positioning were maintained throughout the photography process to ensure consistency. We analyzed the photos by MATLAB R2009 software. We got data from MATLAB software: pixels of wound image per pixel of one $cm^2$. We compared the differences in wound size between the groups on the first day and the 4th, 10th, and 16th days.

### *2.6. Hydroxyproline Analysis*

A 100 mg of skin tissue sample was obtained from the burned area. It was placed on a petri dish and dried at 60 ˚C for 12 h. Subsequently, 3-5 mL of HCl 6 N was added to the dried sample and hydrolyzed at 130 ˚C for four h. Next, 2 mL of the hydrolyzed solution was transferred to an Eppendorf tube and centrifuged at 10,000 rpm for 5 minutes. The supernatant was carefully collected and moved to a test tube, which evaporated for 30-45 minutes at 60-80 ˚C. 500 µL of the vaporized solution was added to the evaporated solution, along with 30 µL of Chloramine T and 470 µL of pH six citrate buffer. The mixture was mixed and incubated for 20 minutes at room temperature. Following this, 250 µL of HClO4 0.4 M and 250 µL of Ehrlich solution were added to the mixture, then mixed and set aside for 90 minutes at 60 ˚C. The fluid was centrifuged at 3000-4000 rpm for 5 minutes, and the supernatant was transferred to a cuvette. The absorbance value of the solution was measured using a spectrophotometer at 557 nm. The absorbance value was calculated with the standard hydroxyproline curve to determine the levels of hydroxyproline [14,15].

### *2.7. Histopathologic Evaluation*

The researchers took the treated skin after the sacrificed animals and stored it in a 10% formalin-containing solution. We stained the sections with hematoxylin-eosin (HE) and observed under a light microscope at a 400x lens magnification in five fieldsof view, and the result was average. We carried out the observations by the blinding method. The histopathological examination includes an examination of fibroblast and epithelialization.

### *2.8. Statistical Analysis*

The researchers presented all data as the mean±standard deviation (mean±SD). Statistical analysis was performed using the SPSS software package. We tested the data for normality and homogeneity of variance, then analyzed it with analysis of variance (ANOVA), followed by Post hoc multiple comparisons. Statistical significance was determined at $p<0.05$.

## 3. Results and Discussion

Figure 1 shows a deep-partial thickness burn on the first day. This burn was under the characteristic of deep-partial thickness burns, based on the American Burn Association, in which the skin typically is white in color, splotchy red, dry, and blisters may occur. We evaluated the wound healing process on multiple serial days of 4, 10, and 16 after burning induction. We measured the wound area, hydroxyproline, number of fibroblasts, and epithelial thickness as wound healing parameters. Figure 2 shows every group's macroscopic evaluation of the wound healing process.

**Figure 1.** A Deep-partial thickness burn wound after induction. The wound appears white, splotchy red, dry, and it has blisters.

Table 1 shows the wound area of every group. On day 4, the 60% MEE group showed a significant reduction ($p>0.05$) in the wound size compared with other groups. In contrast, the average wound area between SSD, control, and 40% MEE groups was almost similar, and there was no significant difference between the groups ($p<0.05$). On the 10th-day induction, the wound area was significantly narrower in all groups compared with the 4th day. The difference was significant between all groups except SSD and 40% MEE groups ($p>0.05$). Furthermore, on the 16$^{th}$ day of the experiment, the group threatened with SSD, 40% MEE, and 60% MEE showed a significant reduction ($p<0.05$) in the wound size compared with the control group. The 60% MEE group gave the highest percentage of wound closure (Table 2).

Edamame (*G. max*) extract contains various active components, including isoflavones, which are phytoestrogens [16]. The primary isoflavones found in edamame are genistein, daidzein, and glycitein. Genistein, in particular, is present in the highest concentration [17]. These isoflavones exhibit similar efficacy to natural estrogens. Estrogens are known to enhance the expression of the TGF-β growth factor, which stimulates the proliferation of fibroblasts and accelerates the rate of epithelization [18,19]. Furthermore, genistein directly stimulates the proliferation of fibroblasts by modulating estrogen

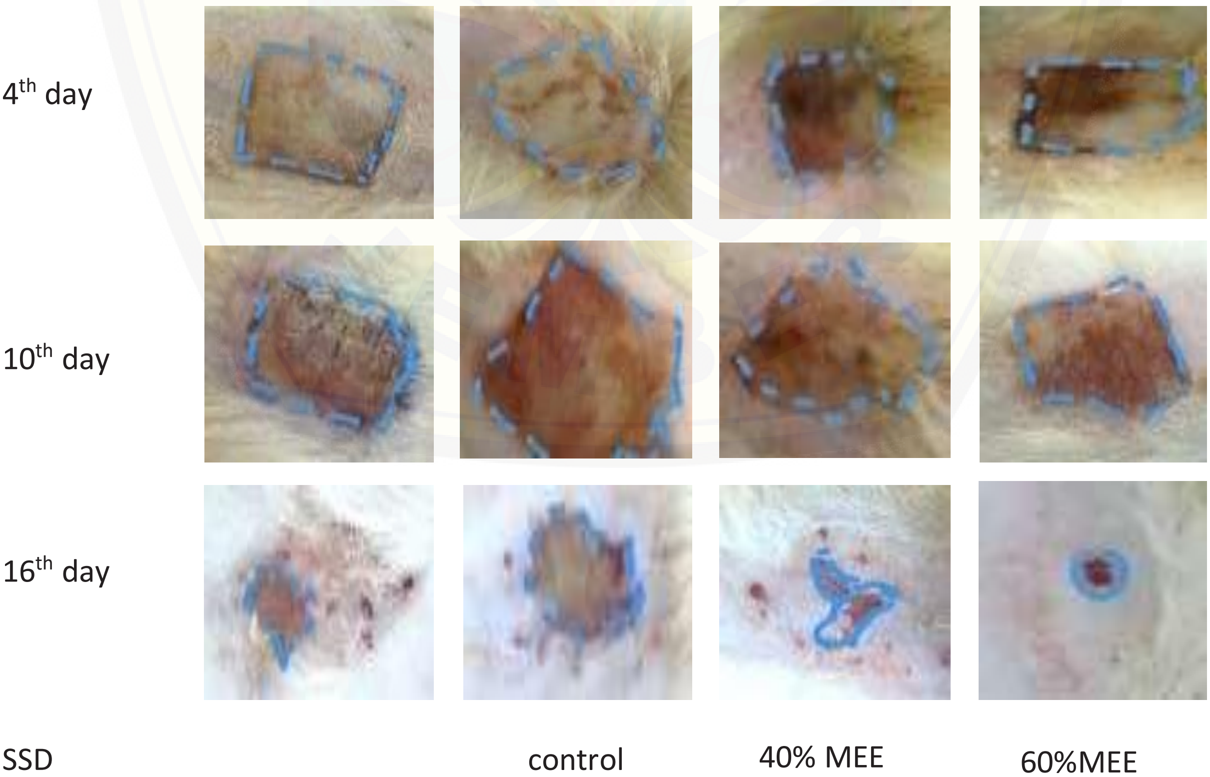


**Figure 2.** Macroscopic observations of the healing site of skin burn in different groups on days 4, 10, dan 16



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**Table 1.** The Average of Wound Area on Different Days

| Day | SSD ($mm^2$) | Control ($mm^2$) | 40% MEE ($mm^2$) | 60% MEE ($mm^2$) |
|---|---|---|---|---|
| 4 | 389.25±5.56 | 389±4.76 | 385.75±11.67 | 369.25±6.02 |
| 10 | 219.75±12.606 | 267±10.424 | 200.75±6.396 | 128.25±16.111 |
| 16 | 103.5±5.802 | 201.25±10.21 | 99±6.055 | 83.25±14.384 |

Each value represents the mean ± SD (n=4)

receptor alpha (ERα) and estrogen receptor beta (ERβ) [20]. Phytoestrogen also controls tissue inhibitor of metalloproteinase (TIMP) expression to inhibit excess collagen degradation [19]. As a result, the formation of keloids and hypertrophic scars is prevented [21].

Genistein, a primary active MEE compound, is also an antioxidant, antimicrobial, and anti-inflammatory in accelerating burn healing [22]. Normal oxygen metabolism stimulates the production of reactive oxygenspecies (ROS) or free radicals. Excessive free radicals are dangerous because they release various inflammatory mediators such as NF-κB and TNF to recruit neutrophils and other immune cells to the wound area, leading to tissue damage [23]. Genistein prevents cell death caused by ROS with the donor hydrogen ion mechanism and increases glutathione as a natural antioxidant [24,25]. As an anti-inflammatory agent, genistein suppresses the stimulation COX-2, so the secretion of pro-inflammatory molecules such as IL-1β, IL-6, IL-12, and TNF-α are inhibited [26]. Genistein was reported to have an antibacterial effect. Genistein represses topoisomerase's function and inhibits bacteria's DNA metabolism [27]. Edamame (*G. max*) is also rich in vitamins A, C, and E, which contribute to accelerating the wound healing process. Vitamin A plays a crucial role in reducing inflammation by promoting collagen synthesis. Vitamin C aids in strengthening and stabilizing the structure of collagen. Additionally, vitamin E is a vital antioxidant supporting wound healing [28].

Our study revealed a significant difference ($p<0.05$) in the mean percentage of wound closure among the experimental groups on days 4, 10, and 16. Notably, the group treated with the membrane containing 60% edamame extract demonstrated higher effectiveness. These findings are further substantiated by the hydroxyproline levels presented in Table 2 and the histological findings outlined in Table 3.

Table 2 shows the hydroxyproline levels of control and treated rats on different days of analysis. Hydroxyproline assessment on the 4th day after injury showed that 60% of MEE groups had the highest hydroxyproline level, while control groups had the lowest level. The difference was significant between all groups ($p<0.05$). An increased level of hydroxyproline is required for a faster wound healing rate.On the 10th day, membrane group hydroxyproline levels increased along with fibroblasts' proliferation process and collagen deposition. Sixteen days after induction, the hydroxyproline levels were going down as a sign that the proliferation phase would be complete, except for the control group, which maintained increasing hydroxyproline due to the deceleration of the proliferation phase. On the 16th day, the 60% MEE group had the lowest level of hydroxyproline among other groups. Hence, the 60% MEE group gave the best result for wound healing biochemically and macroscopically.

Histological assessment includes the counting of fibroblasts and the measurement of epithelial thickness. Table 3 presents that the control group had the smallest average number of fibroblasts compared to other groups on days 4, 10, and 16. MEE significantly increased fibroblast proliferation compared to the control group ($p>0.05$). However, statistics showed no difference between the MEE and SSD groups. Meanwhile, the 60% MEE groups gave the highest number of fibroblasts among other groups on days 4, 10, and 16.

Genistein accelerates burn wound healing by enhancing the proliferation of fibroblasts and keratocytes [24]. Data from days 4th, 10th, and 16th proved it when comparing the MEE and control groups. The Epithelial thickness and fibroblasts count each day always showed MEE significantly higher than the control group.

**Table 2.** The average of hydroxyproline levels on different days based on µg per 100 mg skin tissue

| Day | SSD | Control | 40% MEE | 60% MEE |
|---|---|---|---|---|
| 4 | 5198±272.58 | 2625±305.54 | 6368±312.24 | 7708±577.39 |
| 10 | 9681±711.93 | 7230±1092.68 | 9858±54.46 | 12213±632.61 |
| 16 | 6575±1461.19 | 9125±938.45 | 3660±702.06 | 3288±1006.26 |

Each value represents the mean ± SD (n=4)

**Table 3.** The Average of Fibroblasts on Days 4, 10, and 16

| Day | SSD | Control | 40% MEE | 60% MEE |
|---|---|---|---|---|
| 4 | 20.73±5.53 | 11.23±0.81 | 22.58±5.27 | 24.38±3.30 |
| 10 | 26.2±4.64 | 16.75±1.42 | 25.8±3.93 | 29.83±2.87 |
| 16 | 41±4.97 | 28.88±6.32 | 42.63±7.46 | 50.2±3.79 |

Each value represents the mean ± SD (n=4)

Furthermore, the increased fibroblast proliferation was accompanied by a large amount of collagen build-up due to the subcutaneous VEGF and TGF-β release by genistein [24]. Collagen contains the amino acid hydroxyproline, which plays a role in twisting the collagen triple-helix structure [23]. Therefore, hydroxyproline is used to measure the production of collagen. Hydroxyproline level decreased on day 16 in the MEE group, with 60% MEE showing the lowest level. This indicated that the MEE group had reached the remodeling phase and reduced collagen synthesis. So hydroxyproline production also decreased [29]. Although the hydroxyproline level showed decreased collagen, fibroblast as collagen-forming cells still increased on the last day. This phenomenon is expected due to the lack of negative feedback from collagen production [30].

Table 4 presents the result of epithelial thickness measurement from each group. The control group has the thinnest epithelial thickness compared to the other group. Meanwhile, the 60% MEE group has the thickest epithelial thickness. Microscopic observation on day 4 in the SSD group and 60% MEE group showed that the structure of epithelial tissue of the wound was more visible when compared to the 0% MEE and 60% MEE group. However, on days 10 and 16, the SSD group had thinner epithelial thickness than the 40% MEE and 60% MEE groups.

Statistics showed that on the 4$^{th}$ day, the average epithelial thickness of all groups had no difference ($p>0.05$). On the 10$^{th}$ and 16$^{th}$ days, there were significant differences between the MEE group with the SSD and control groups. 40% MEE and 60% MEE groups have thicker epithelial than the SSD and control groups. This indicates that edamame extract can accelerate the process of re-epithelization. On the 16$^{th}$ day, the SSD group showed no significant difference from the control group. This may occur because the SSD group formed a scar that was very hard and difficult to remove from the skin. The macroscopic observations show this rough scar.

In this study, 60% MEE and 40% MEE are more effective than the SSD group. It is due to the rough eschar formed in the SSD group from the 4$^{th}$ until the 10th-day afterburn induction. The rough eschar` was very difficult to remove from the skin. Meanwhile, 40% and 60% of MEE groups formed soft eschar, which can be removed before day 10. Hence, the40% and 60% groups result in a higher percentage of wound closure and a faster proliferation phase than the SSD group. The other study by Sutejo et al. investigated the healing effects of crude edamame extract and silver sulfadiazine on burn wounds in rats. Histopathological examination and hydroxyproline analysis were used to evaluate healing effects. They used crude edamame extract and SSD cream for 15


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**Table 4.** The Average Epithelial Thickness on Days 4, 10, and 16

| Day | SSD (μm) | Control (μm) | 40% MEE (μm) | 60% MEE (μm) |
|---|---|---|---|---|
| 4 | 11.13±3.29 | 8.9±2.47 | 10.25±2.58 | 14.73±2.07 |
| 10 | 23.52±1.62 | 20.78±1.58 | 26.92±0.43 | 31.17±2.17 |
| 16 | 36.65±1.54 | 34.33±2.17 | 41.70±3.40 | 45.20±2.29 |

days to improve the burned skin wounds in rat models [15]. According to this improved research, the edamame membrane is more beneficial since it has a more extended usage than crude extract. This dressing only needs to be applied once for three days, whereas crude extract needs to be smeared twice daily. Edamame membrane contains humectants, so it moisturizes the wound and does not cause another injury when removed. This research also performs serial measurements multiple times to describe the phases of burn wound healing.

## 4. Conclusions

We concluded that the edamame membrane causes the acceleration of wound healing in deep-partial thickness burn-proven macroscopically, histopathological and biochemical assays. The results suggest that this edamame membrane can be utilized as an effective treatment option for burns, serving as an alternative therapy for wound healing. Notably, the edamame membrane offers advantages over cream preparations, including an extended duration of use, moisturizing properties, and the prevention of rough scar formation.

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## Conflict of Interest

The author/editor has no conflicts of interest, financial or otherwise, to declare.

## Statement of Contribution of Researchers

Concept – I.R.S., D.D.W.; Design – I.R.S., D.D.W.; Supervision – I.R.S., D.D.W.; Resources – I.R.S., D.D.W.; Materials – B.S.E., A.N.A., S.F.Y.; Data Collection and/or Processing – B.S.E., A.N.A., S.F.Y.; Analysis and/or Interpretation – I.R.S., D.D.W.; Literature Search – B.S.E., A.N.A., S.F.Y.; Writing – I.R.S., D.D.W., B.S.E., A.N.A., S.F.Y.; Critical Reviews – I.R.S., D.D.W.

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