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# Comparison of Burn Wound Histopathology Imaging between Epidermal Growth Factor Spray and Silver Sulfadiazine Application: An in Vivo Study

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### ABSTRACT

**Background.** Burns are injuries to the skin or other organic tissue that can be caused by heat, radiation, electricity, and contact with chemicals. This study aims to determine the difference in the histopathological appearance of second-degree burns with the administration of epidermal growth factor (EGF) spray with silver sulfadiazine (SSD) in experimental animals. **Methods:** The research design is an experimental study with a post-test-only approach with a control group design. A total of 27 healthy male Wistar rats were used in this study (weighing 200-300 grams; 8 weeks of age). Experimental animals were grouped into 3 groups, namely the control group (K), the EGF spray (EGF) treatment group, and the 1% silver sulfadiazine (SSD) treatment group. Histopathological examination of the wound was performed with hematoxylin-eosin staining. histopathological assessment with Burkitt's criteria. **Results:** The average thickness measurement of the burn surface epidermis in both the SSD and EGF treatment groups showed a thicker epidermis than the control group. The leukocyte density in the granulation tissue of the SSD and EGF treatment groups was lower than the control group, namely 107.0 and 85. **Conclusion:** The administration of EGF spray showed a better histopathological picture than the control group and the SSD group in second-degree burns, especially in cell regenerative processes, suppression of inflammation, and epithelial repair.

### 1. Introduction

Burns is a health problem that often occurs in everyday life, both at home and at work.<sup>1</sup> Burn are injuries to the skin or other organic tissue that can be caused by heat, radiation, electricity, and contact with chemicals.<sup>1,2</sup> The high morbidity and mortality rates make the management of burns a special concern. Fatal injuries can lead to death, while non-fatal injuries can lead to increased morbidity such as disability. The prevalence of burns in the world according to the World Health Organization (WHO) is as many as 265,000 deaths per year.<sup>1,3</sup> Meanwhile in Indonesia, the prevalence of burns is 1.3% with the highest proportion

occurring in Papua with a prevalence of 2.1%. The high mortality and morbidity rates in burns require appropriate treatment so that the results of wound healing are optimal.<sup>2-6</sup>

Silver sulfadiazine (SSD) is used as the gold standard for the treatment of superficial or deep burns because it is considered to have the ability to not easily become resistant and is a broad-spectrum antibiotic. Silver absorbs exudate from burned skin. Silver is also known to be effective in increasing the effectiveness of sulfadiazine in inhibiting the growth or colonization of broad-spectrum bacteria, fungi, and viruses.<sup>2,5</sup>

Sulfadiazine itself has a mechanism of action by inhibiting the synthesis of folic acid. In addition, sulfadiazine can inhibit enzymes for cellular and denaturation of bacterial DNA molecules.<sup>2,7</sup> Therefore, the combination of silver sulfadiazine is an excellent combination to inhibit bacterial growth.<sup>8,9</sup> However, several studies have shown that SSD has a cytotoxic effect on fibroblasts and keratinocytes in vitro and can inhibit wound healing in vivo.<sup>10-13</sup>

Epidermal growth factor (EGF) is a polypeptide M-605 that can stimulate or inhibit the proliferation and differentiation of various cells. EGF is part of the growth factor complex and with its receptors it together helps to modulate cell growth.<sup>12,14</sup> EGF is released by cells and then stimulates growth alone, or with neighboring cells, and stimulates their ability to divide. Receptors on the cell surface bind to EGF and relay signals.<sup>12</sup> EGF stimulates the proliferation and keratinization of various epidermal tissues in vivo and in vitro.<sup>15</sup> In particular, EGF interacts with its receptors throughout the epidermis, especially in the basal layer, and promotes epithelial growth through the activation of several pathways. Binding of EGF to its receptor results in dimerization and autophosphorylation.<sup>16</sup> This process activates the active mitogens of the protein kinase pathway, ultimately influencing the phosphorylation of many transcription factors and calcium release by the activation of protein kinase C.<sup>12,15</sup> EGF also promotes epidermal regeneration and corneal epithelialization by several actions. These actions include increasing epithelial cell proliferation and migration to the wound, stimulating the production of proteins such as fibronectin, and increasing the number of fibroblasts in the wound. When fibroblasts there are large numbers of collagen is formed in  $\alpha$ -helical structure.<sup>16</sup> Crossing between strands of collagen fibers produces fibers that are quite resistant to damage.<sup>12</sup> Therefore collagen has an important role in mammalian structure in addition to its importance in wound healing.<sup>14</sup> EGF plays an important role in wound healing and applications using EGF can be in the form of an easy-to-use spray. EGF acts on epithelial cells and fibroblasts, promoting recovery from epithelial damage.<sup>12</sup> The use of SSD for

burns has a good antimicrobial effect but has less effect on the formation of post-burn epithelialization. This study aims to determine the difference in the histopathological features of second-degree burns with EGF Spray administration with SSD in experimental animals.

## 2. Methods

The research design is an experimental study with a post-test-only approach with control group design in vivo to determine differences in the histopathological features of second-degree burns with EGF Spray administration with SSD. A total of 27 Wistar rats were used in this study with inclusion criteria being male, weighing 200-300 grams, 8 weeks old, a health condition characterized by active movement, not being alone in the corner of the cage, clean fur, no defects, and clear eyes. This study has been approved by the Research Ethics Commission of the Faculty of Medicine, Andalas University (No.589/UN.16.2/KEP-FK/2021). Experimental animals were acclimatized for 7 days before induction of burns. Before burn induction, experimental animals were anesthetized with ketamine at a dose of 80 mg/kg BW intraperitoneally. Then, the burn was induced with an iron plate with a diameter of 2 cm which had been heated at a temperature of 100°C, for 3 minutes. The iron plate was then affixed to the back of the experimental animal for 3 seconds by cleaning the fur of the experimental animal and aseptic procedures. Experimental animals were grouped into 3 groups, namely the control group (K), the EGF spray (EGF) treatment group, and the 1% silver sulfadiazine (SSD) treatment group. The treatment was carried out for 1 week, then the wound was evacuated with the experimental animal under anesthesia. The wound was put into 10% NBF (normal buffered formalin) solution, then dehydrated with graded ethanol and xylene. After the dehydration process, paraffinization was carried out, then 2mm slides were made with a microtome. The microtome slide was placed on a glass object and the rehydration process was carried out. Furthermore, staining with hematoxylin-eosin (HE). Furthermore, the histopathological assessment was carried out with

Burkitt's criteria.

Data analysis was carried out with the help of SPSS version 25 software. Univariate analysis was carried out to present the frequency distribution of variables test Bivariate analysis was performed to assess the mean difference between the test groups, with  $p < 0.05$ .

### 3. Results

Based on table 1, it is known that the average thickness measurement of the burn surface epidermis in both the SSD and EFG treatment groups showed a thicker epidermis than the control group.

Table 1. Comparison of epidermal thickness between test groups

No	Group	Mean±SD ( $\mu\text{m}$ )	p-value*
1	K	63.7±8.32	0.001
2	SSD	72.25±6.76	
3	EGF	68.8±5.87	

\*Kruskall Wallis,  $p < 0.05$

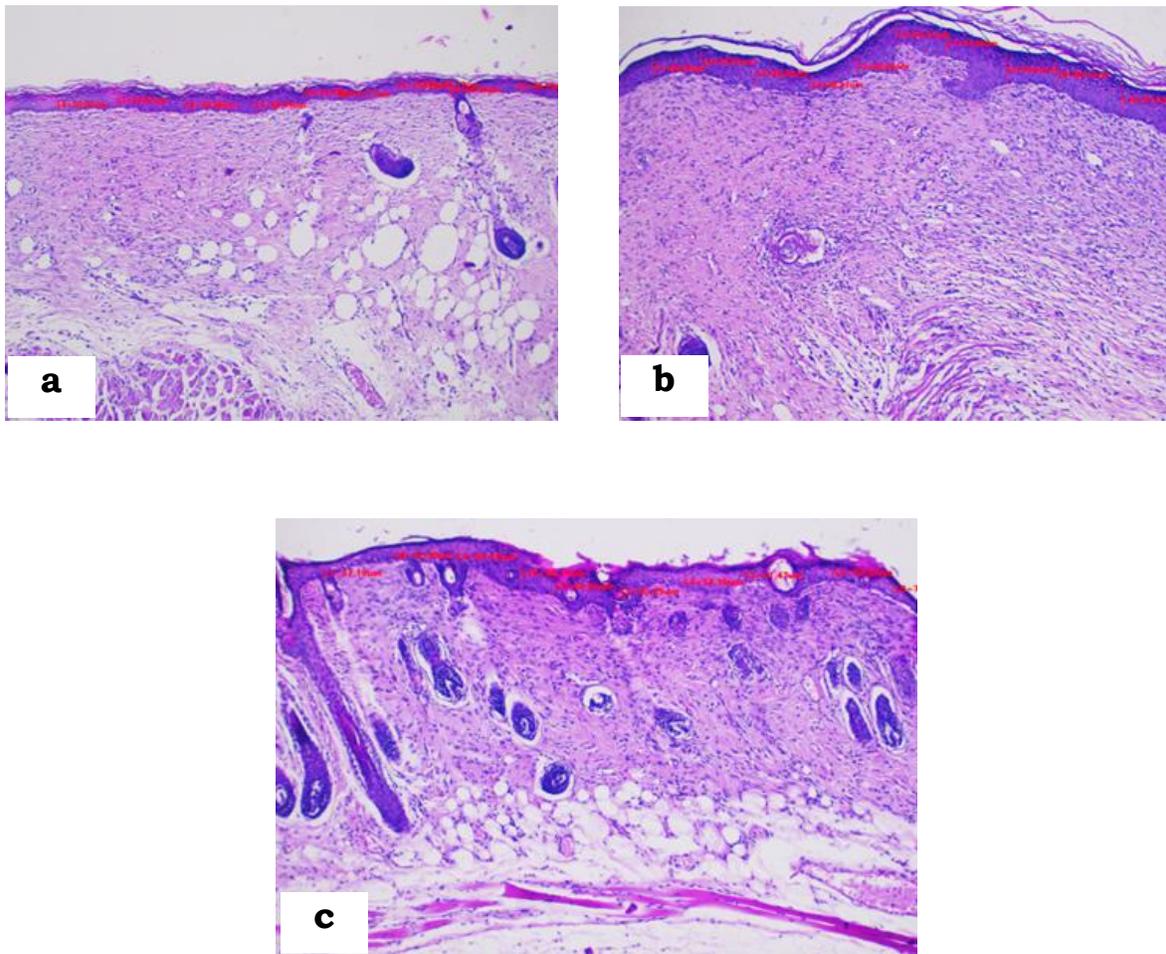


Figure 1. Overview of epidermal thickness measurement control group (a), SSD treatment group (b), EGF Spray treatment group (c)

Based on table 2 it is known that the average measurement of leukocyte density in the granulation network of SSD and EGF treatment groups is lower than the control group, which is 107.0 and 85. The

average density of leukocytes in the EGF group granulation network is lower than that of the SSD group.

Table 2. Comparison of leukocyte density in granulation tissue

No	Group	Mean±SD (cells)	p-value*
1	K	121.3±9.87	0.001
2	SSD	107.0±9.56	
3	EGF	85.0±7.76	

\*Kruskall-Wallis, p<0.05

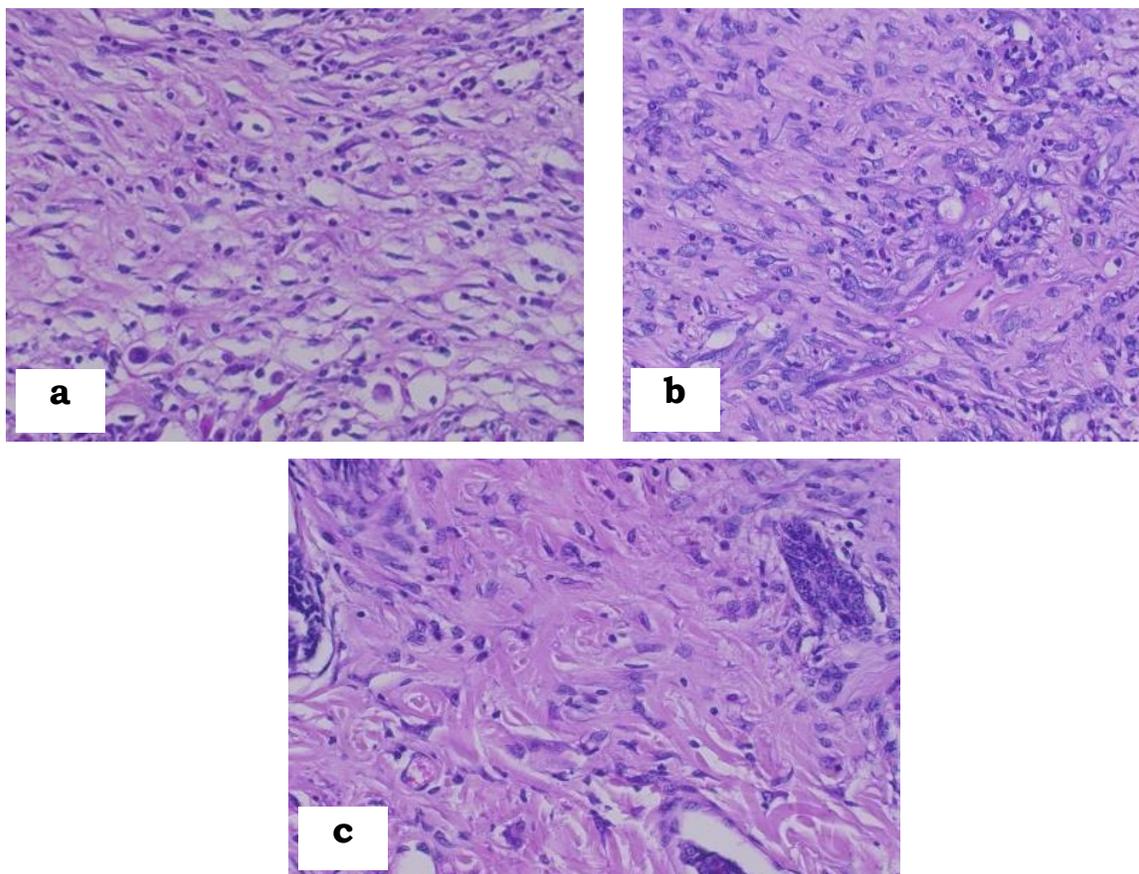


Figure 2. Overview of the calculation of leukocyte density in the control group (a), the SSD treatment group (b), the EGF Spray treatment group (c)

Table 3 shows that the average calculation of the fibroblast density in the granulation tissue of the two treatment groups is higher than the control group,

namely 183.6 and 117.8. The average density of fibroblasts in the SSD treatment group was higher than in the EGF treatment group.

Table 3. Comparison of fibroblast density in granulation tissue

No	Group	Mean±SD (cells)	p-value*
1	K	97.7±5.54	0.001
2	SSD	183.6±9.89	
3	EGF	117.8±7.87	

\*Kruskall-Wallis, p<0.05

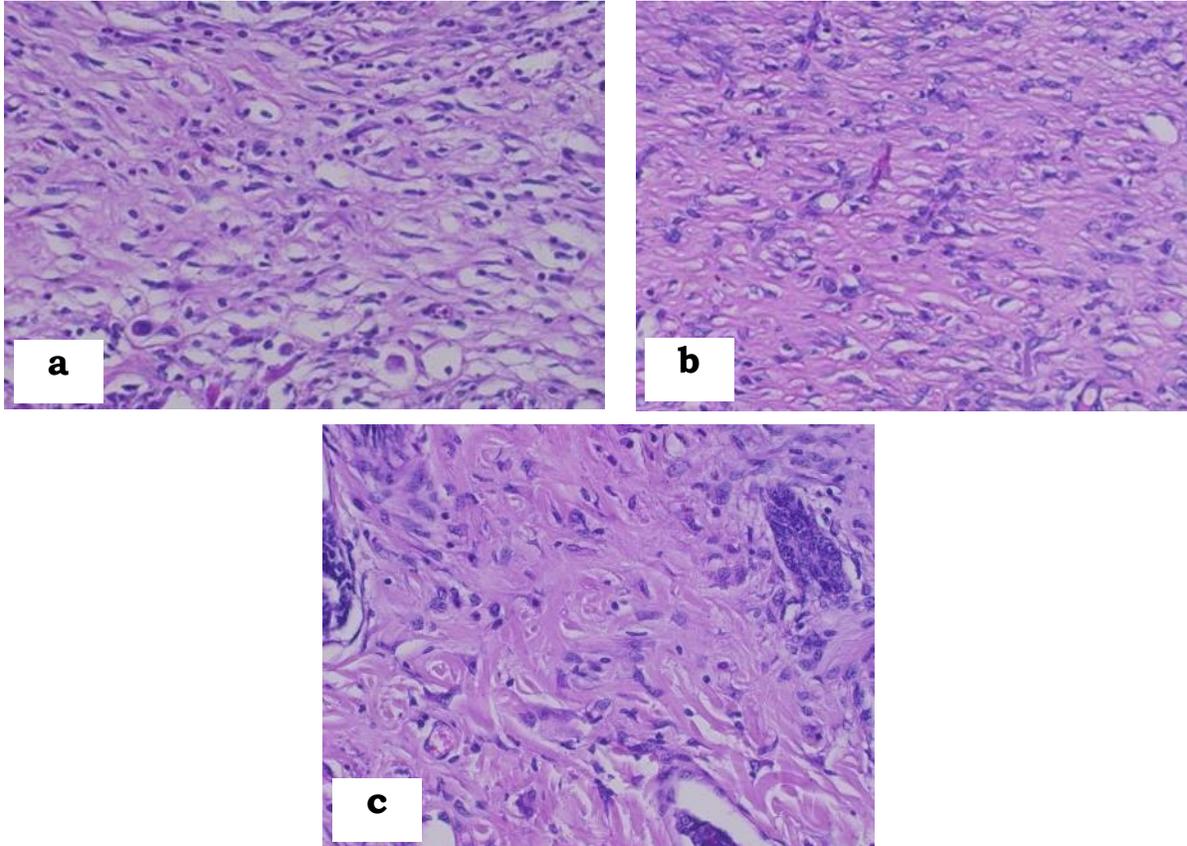


Figure 3. Overview of the calculation of fibroblast density in the control group (a), the SSD treatment group (b), the EGF Spray treatment group (c)

Table 4 shows that the average vascular density in the granulation tissue of the SSD treatment group shows a higher vessel density than the control group.

ie 15.6. The average vessel density of the EGF treatment group was lower than that of the SSD and control groups, which was 12.

Table 4. Comparison of neovascular density in granulation tissue

No	Group	Mean±SD (Gray)	p-value*
1	K	15.2±1.12	0.001
2	SSD	15.6±1.65	
3	EGF	12.0±1.21	

\*Kruskall-Wallis, p<0,05

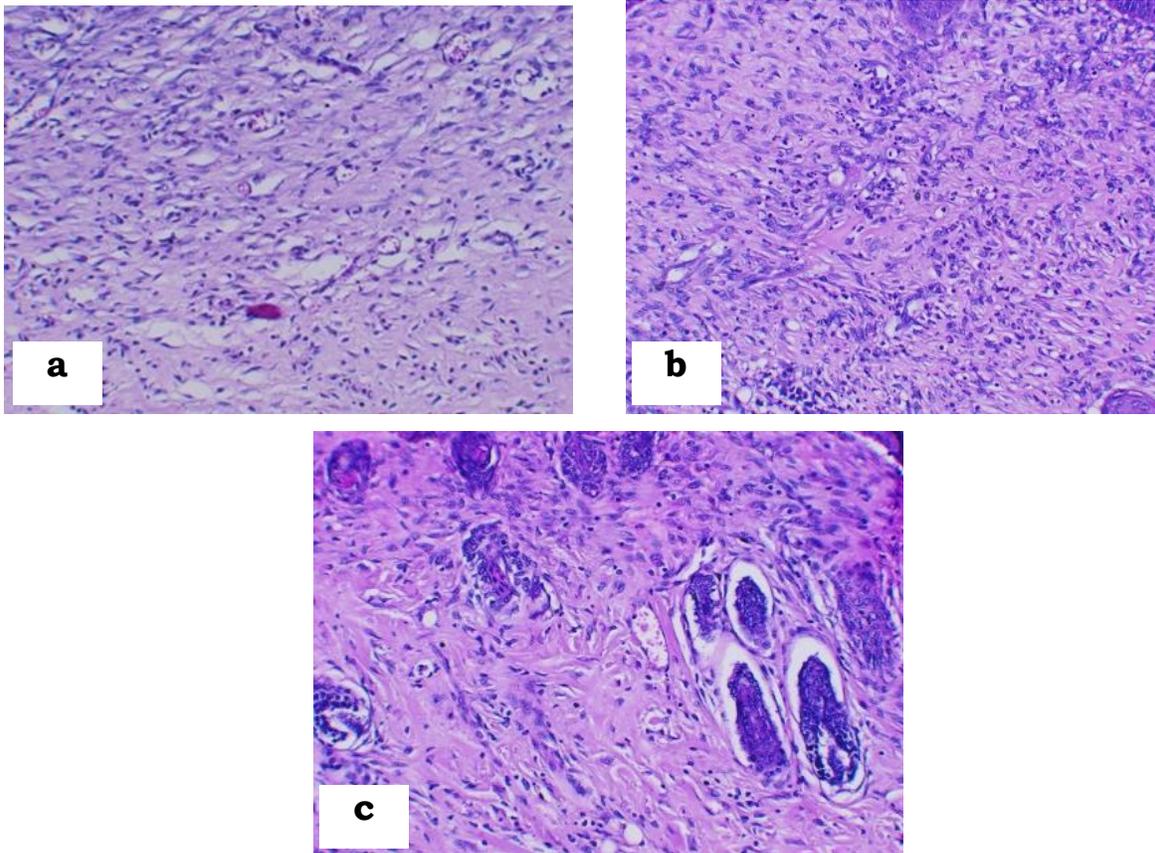


Figure 4. Overview of the calculation of neovascularization density in the control group (a), SSD treatment group (b), EGF Spray treatment group (c)

#### 4. Discussion

Based on the results of this study, the histological assessment of the effect of EGF spray on the skin of experimental animals after burns showed histological differences between the control and treatment groups and the standard drug, namely SSD. In the control group, the surface of the post-burn scars was covered with complete epithelialization, in all samples except one sample with non-complete epithelialization. Epidermal epithelium appears to be slightly thicker than the periphery of normal tissue, but the structure of the epidermis is not perfect, it appears beneath the granulation without adnexa or a few hair follicles. The dermis contains granulation tissue with some loose collagen, many inflammatory cells, and a few fibroblasts with partially dilated hyperemic vasculature.

In the treatment group with SSD, the post-burn wound surface was covered with complete epithelium in all samples. The dermis contains granulation tissue with mostly denser collagen than control and a higher

population of fibroblast cells, lower inflammatory cells than control. Vascularization appeared to be slightly higher than the control but with a lower impression of hyperemia. Adnexal regeneration was seen less in the dermis layer than in the treatment with EGF spray.

The treatment group with EGF spray showed complete epithelialization in all samples, epithelialization and thicker than control. The dermis contains granulation tissue with mostly moderate density collagen and minimal signs of inflammation. The granulation tissue area seemed less with lower fibrosis than the control and Sulfadiazine treatment. The dermis under the wound contains hair follicles and adnexa which exhibit a regenerating effect. There was an effect of wound repair compared to the control and standard drugs which were closer to the normal structure of the skin with lower fibrosis. In the treatment group with EGF spray, the post-burn wound surface was covered with complete epithelium, with lower granulation than sulfadiazine and lower inflammation. This suggests a faster wound healing

process with immediate epithelialization so that inflammation in the dermis produces less granulation than sulfadiazine. In the treatment with EGF spray, it appears that there is an epidermal tissue that is more like normal epidermis with dermal papillae and there is a regeneration of adnexa/hair follicles underneath; Follicular/adnexal regeneration may occur as a direct result of EGF on the adnexal dermis, but may also occur due to faster wound closure with minimal granulation.

Epidermal growth factor (EGF) was first discovered in 1962 which is a growth agent. EGF is a growth factor that has a low molecular weight polypeptide with 53 amino acids. The function and role of EGF is to stimulate cell growth, proliferation, and differentiation by attaching it to the EGF receptor (EGFR) on the cell surface. EGF binding to its receptor will modulate cell growth and differentiation. The action of EGF that is bound to its receptor begins by stimulating ligand-induced dimerization and then activating the intrinsic protein tyrosine kinase activity of the receptor. This tyrosine kinase activation process will activate a signal transduction cascade that will result in various biochemical changes in the cell, such as increased intracellular calcium levels, increased glycolysis, and protein synthesis, and increased gene expression including genes for EGFR. The role of EGF will stimulate the proliferation of various cells, such as glial cells, fibroblasts, and cells originating from the epithelium such as keratocytes.<sup>17-21</sup> EGF stimulates the proliferation and keratinization of various epidermal tissues in vivo and in vitro. In particular, EGF interacts with its receptors throughout the epidermis especially in the basal layer by promoting epithelial growth through the activation of several pathways. Binding of EGF to its receptor results in dimerization and autophosphorylation. This process activates the active mitogens of the protein kinase pathway, ultimately influencing the phosphorylation of many transcription factors and calcium release by protein kinase C activation. EGF also promotes epidermal regeneration and corneal epithelialization by several actions. These actions include increasing epithelial cell proliferation and migration to the wound,

stimulating the production of proteins such as fibronectin, and increasing the number of fibroblasts in the wound.<sup>22-25</sup>

## 5. Conclusion

The administration of EGF spray showed better histopathological features than the control group and the SSD group in second-degree burns, especially in cell regenerative processes, suppression of inflammation, and epithelial repair.

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