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Wound Healing Activity of ethanolic extract of *Scutellaria barbata* D. Don (Lamiaceae) leaves extract in excision and burn wound models

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ABSTRACT

***Objective:** To evaluate the wound healing effect of ethanolic extract of Scutellaria barbata D(Lamiaceae) in rats.*

***Methods:** Male Sprague Dawley rats weighing 250–300 g were randomly divided into incision and burn wound groups. Each group was stratified into three subgroups (1) untreated; (2) standard-; (3) test (extract) treated groups. The test substances were applied topically once daily. The general appearance and degree of wound healing were assessed on Days 4, 8, 12, 16 and 20 after burns and excision wound. The parameters studied were percentage of wound contraction and period of epithliazation in excision and burn wound model. Histopathological evaluation was carried out.*

Results: In excision and burn wound model, a significant decrease in period of epithelialisation was observed in all the treatment groups compared to the control group. Comparative analysis revealed that the plant extract Scutellaria barbata D. Don (10%) shows wound healing activity. There was significant reduction of wound contraction in the Standard Povidone Iodine Treatment group and standard Silver Sulphadiazine group. Histopathological findings on Day 21 revealed prominent fibrinoid necrosis and incomplete epithelialization in the control groups, whereas fully developed epithelialization and keratinization were observed in all extract-treated groups.

***Conclusions:** Ethanolic extract of Scutellaria barbata D(Lamiaceae) was found to possess significant wound healing property in both excision and burn wound models.*

Keywords: Scutellaria barbata D, wound healing, excision wound, burn wound

INTRODUCTION

Wound healing is a complex process that is dependent on several factors that may hasten or affect the process of wound healing activity. When wound occurs, the tissues are able to heal by the mechanism of regeneration or repair. Regeneration is the process of replacing the damage tissues by identical cells and more limited to repair. Regeneration of human tissues appears to have their own limit, epithelial tissue; liver and nerve cell have their own limit in regeneration. Wound healing activity involves hemostasis, vascular response, inflammatory phase, proliferation and maturation [1].

Wound is defined as an injury that is caused when a knife, bullets etc that cuts or breaks the skin. An injury to the body as from violence, accident or surgery that typically involves laceration or breaking of a membrane and usually damage to underlying tissues [2]. Wound healing is an important process because further delay of healing may leads to other complications such as septicemia and bacterial infection.

Several medicinal plants have been used since the ancient time for the treatment of cuts, wound and burn that they show significance in wound healing. Common plants like *Aloe vera, Carica papaya, Cinnamomum zeylanicium, Terminalia arjuna and Terminlia chebula* have been extensively reported for their wound healing potential [3].

Since ancient times, people have used plants and preparations thereof to accelerate the wound-healing process [4]. Recently, the interest of using alternative therapies and natural remedies in wound management has rapidly increased. There are hundreds of medicinal plants that have long histories of curative properties against various diseases and ailments. However, their use is merely based on tradition, without any scientific evidence of their efficacy or knowledge about putative active compounds or their mode of actions [5].

Scutellaria barbata D(Lamiaceae) is a well known traditional Chinese herbal plant also known as *ban zhi lian* in Chinese which is a perennial herb which is natively distributed throughout southern China. It common name is Barbate skullcap. Skullcaps genus named as Scutellaria which derived from the Latin word scutella which means a small dish or tray that resembles the appearance of sepal during the fruiting period. For centuries, the plant has been used to treat various disorders such as the bacterial infections, inflammation, hepatitis and cancer. It was believed that the plant *Scutellaria barbata* D. Don are having the potential activity of antineoplastic activity because it contains the component of antioxidant of flavone scutellarin and it may induce apoptosis of ovarian and breast tumor cells in in vitro studies. Many studies investigated that *Scutellaria barbata* D. Don possesses component such as antioxidant such as flavanones, alkaloids, steroid and polysaccharide has been shown to be effective as an antitumor agent and anti-inflammatory [6]. In this context, the present study was therefore, undertaken to evaluate the wound healing potential of ethanolic extract of *Scutellaria barbata* D. Don in burn and excision wound models in rats.

MATERIALS & METHODS

Plants Collection & Authentication

The plant *Scutellaria barbata* D. Don is also known as Barbate Skullcap was collected from the local market in Alam Damai, Kuala Lumpur Malaysia and it was taken to University Putra Malaysia for authentication. The plant *Scutellaria barbata* D. Don was successfully identified. The authentication voucher no. **SK 2308/13.**

Plant Extraction

The fresh leaves of the plant were washed under running tap water and again washed with distilled water. The cleaned plant was allowed to air dried in room temperature. After they plant was completely air dried, the leaves of the plant were grinded into fine powder by using a grinder. The powdered was measure about 50g and was soaked into 500mL of ethanol 70% according to the ratio of 1:10 [7]. The grinded fine powder was soaked in ethanol 70% for maceration for 7 days accompanied by continuous shaking in the room temperature. The suspension was extracted by using the cold extraction method [8]. The suspension was filtered by using filter paper through filter funnel filtration method [9].

Animal Ethics

University animal ethics committee approved the experimental protocol & animals were maintained well in an animal house approved by AMU animal ethics committee **(AMU/AEC/HSFOP/2014/07).**

Study design

The crude extracts that were obtained was then incorporated into the simple ointment to make 10%w/w. 10g of the plant crude extracts were incorporated into 90g of the simple ointment to make up to 100g of the crude extract ointment. The animals were grouped as such: 36 Albino Wistar male rats were used in this research. The male rats weighed about 150-250g each was acclimatized about 14 days prior to the experiment. The rats were housed at a temperature of 23 ± 2 ℃ and relative humidity about 30-70%. A 12:12 light:day cycle was followed. The rats were kept under normal routine laboratory condition where the temperature will be between 22-24.0° C. The rats were fed with standard pellets and allow to access to water. Cleaning and sanitisation were done every alternate day. Paddy husks were provided for the rats as their bedding.

The animals were randomly divided into six groups with six animals each for the two experimental models. Group 1 and 2 were control, group 3 and 4 were standard group and group 5 and 6 were test groups.

Experimental wound models

All wounding procedures were carried out under ketamine anaesthesia(10mg/kg)

Excision Wound

The back of each rat was shaved by using a electric shaver one day prior to the experiment. A total of 18 rats will be anaesthetized. An area of 500mm^2 circular was anticipated on the shaved area by marking using methylene blue. An impression was made on the dorsal thoracic region 1cm away from the vertebral column and 5cm away from ear on the anaesthetized rats. A full thickness of area of 500mm^2 and 2mm depth was created by using sterilised surgical set. Hemostasis was achieved by blotting cotton soaked with normal saline. The procedure was repeated on the rest of the rats in the excision wound model. [10]

Burn Wound

The dorsal part of each rat was shaved 1 day prior to the experiment. A total of 18 rats will be anaesthetized. An area of 500mm^2 circular was anticipated on the shaved area by marking using methylene blue [10]. The burn wound was created by using a device

with an iron piece and a wooden handle placed on the back of the rat. The iron was heated to red hot over flamed and was place on the shaved back of the skin up to 10 seconds without applying any pressure [11].

Application of Ointment on Wound

In excision model, Group 1 was the control group, group 2 was the standard group, treated with Povidone Iodine ointment, group 3 was the test experimental animal, the rats were treated with 10% *Scutellaria barbata* D extract ointment. The ointment was applied on the rats' for 20 days.

In burn model, Group 1 was the control group, group 2 was the standard group, treated with Silver sulphadiazine ointment and group 3 was the test experimental group, which the rats were treated with with 10% *Scutellaria barbata* D extract ointment. The ointment was applied on the rats' for 20 days.

Wound Contractility

Progressive changes of the wound area were observed on days 2, 4, 6, 8, 10, 12,14,16,18 and 20 and were measured by tracing the wound boundaries using millimeter-scale transparent graph paper with a permanent marker. The percentage of wound healing was calculated by using the formula:

$$Percentage\ of\ Wound\ Closure = \frac{Initial\ Wound\ Size - Final\ Wound\ Size}{Initial\ Wound\ Size} \times 100\%$$

The number of days for complete epithelialisation was noted [11]. The interpretation data of day 4, 8, 12, 16 and 20 was taken for data analysis. The results for the wound contractility were recorded.

Histopathological Evaluation

A specimen sample of tissue was taken from the skin of each group of rat the end of the experiment to evaluate for the histopathological alterations. Samples were fixed in 10% buffered formalin, processed and blocked with paraffin and then sectioned into 5 µm and stained with hematoxylin & eosin (HE) and Masson's Trichrome. Photomicrographs were captured at a magnification of 100 X. Re-epithelization, fibroblast proliferation, mononuclear and/or polymorphonuclear cells, neovascularization and collagen depositions in dermis were analyzed to score the epidermal or dermal re-modeling.

Statistical Analysis

All values are reported as mean ±S.E.M. the means of wound area measurements between groups at different time intervals were compared using one way ANOVA, followed by dunnett test used to examine the mean differences in wound healing between the groups. Data analysis was performed using statistical package programme SPSS version 20.0. A p value <0.05 was considered as statistically significant [12].

RESULTS

In the excision wound model, the wound contraction was 389.67±67.97, 354.67±71.84, 202.50±72.93, 160.50±73.31 and 79.83±32.08 on 4th, 8th, 12th, 16th and 20th day respectively in the control group. The wound contraction was significantly decreased on 12th, 16th and 20th day in the *Scutellaria barbata D. Don* (10%) extract ointment compared to control group. There was significant reduction of wound contraction in the Standard Povidone Iodine Treatment group which was 24.17±26.54 on 20th day. *Scutellaria barbata D. Don* (10%) extract ointment exhibited statistically significant as compared to control and standard group respectively. P<0.005) (Table 1)

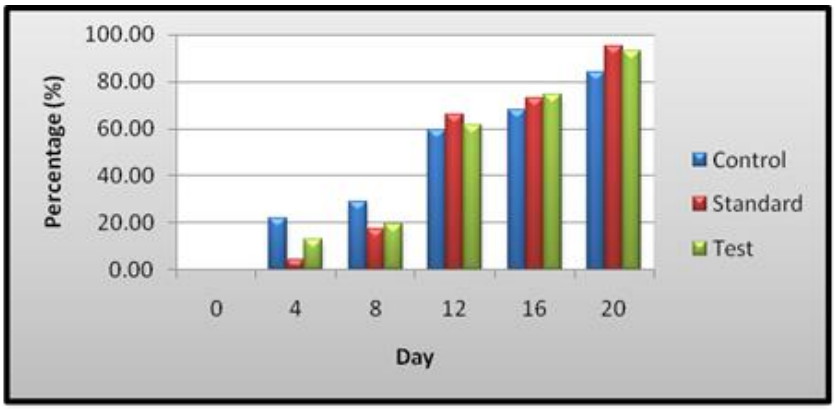

Figure 1: Percentage of Wound Contractility in Excision Wound Model

Group	Day 0	Day 4	Day 8	Day 12	Day 16	Day 20
Control	500 ± 0.00	389.67 ± 67.97	354.67 ± 71.84	202.50 ± 72.93	160.50 ± 73.31	79.83 ± 32.08
Standard	500 ± 0.00	478 .00 ± 35.93	412.67 ± 70.57	169.67 ± 39.06	134.00 ± 28.73	24.17± 26.54
Test	500 ± 0.00	435.00 ± 78.36	403.67 ± 81.74	192.33 ± 112.87	126.67 ±102.62	35.83 ± 36.66

Table 1: Effect on period of epithelialisation in Excision Wound Model.

In the burn wound model, the wound contraction was 424.67±54.43, 387.60±82.26, 237.67±117.97, 164.17±78.76 and 132.67±62.12 on 4th, 8th, 12th, 16th and 20th day respectively in the control group. The wound contraction was significantly decreased on 12th, 16th and 20th day in the *Scutellaria barbata D. Don* (10%) extract ointment compared to control group. There was significant reduction of wound contraction in the Standard Silver Sulphadizine Treatment group which was 64.00±29.24 on 20th day. *Scutellaria barbata D. Don* (10%) extract ointment exhibited statistically significant as compared to control and standard group respectively. P<0.005) (Table 2).

Group	Day 0	Day 4	Day 8	Day 12	Day 16	Day 20

Control	500.00 ± 0.00	424.00 ± 54.43	387.00 ± 82.26	237.67 ± 117.97	164.17 ± 78.76	132.67 ± 62.12
Standard	500.00 ± 0.00	373.33 ± 81.90	371.17 ± 83.15	277.33 ± 47.93	139.67 ± 49.87	64 ± 29.24
Test	500.00 ± 0.00	421.33 ± 87.71	364.83 ± 82.29	234.00 ± 85.53	205.50 ± 104.44	76.50 ± 37.01

Table 2: Effect on Period of Epithelialisation in Burn Wound Model.

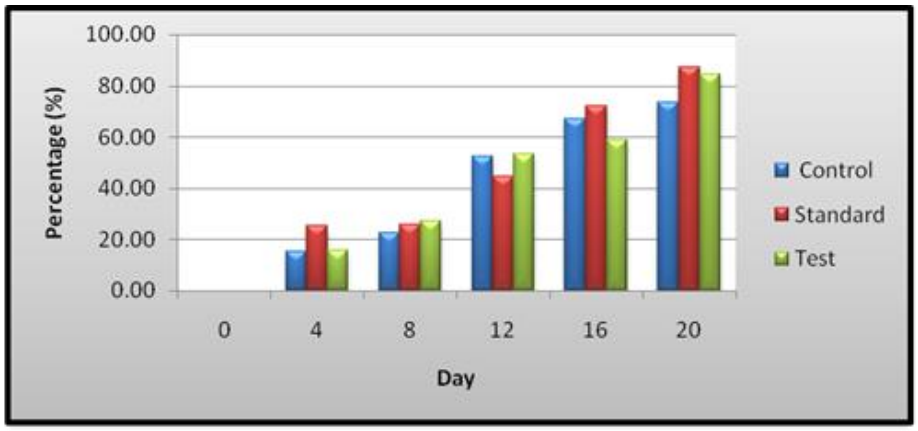

Figure 2: Percentage of Wound Contractility in Burn Wound Model.

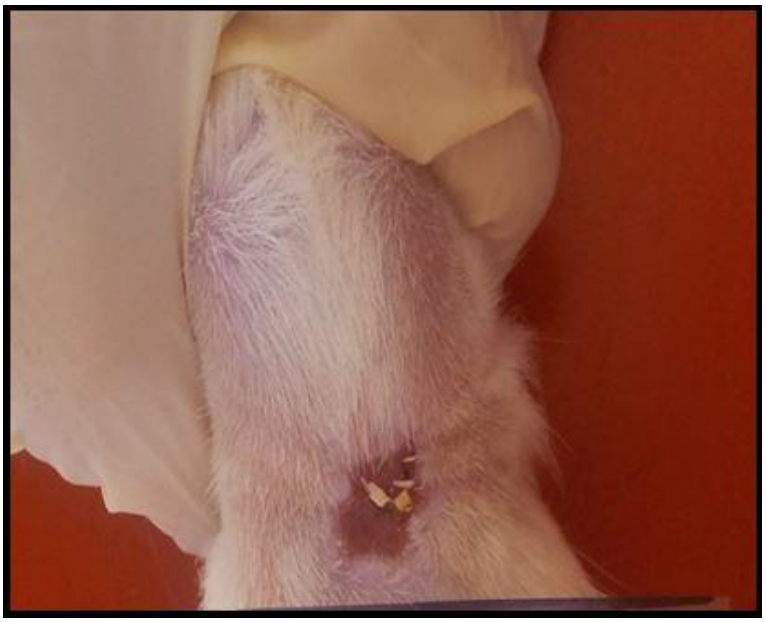

Figure 3: Excision Wound- Control(Day 20)

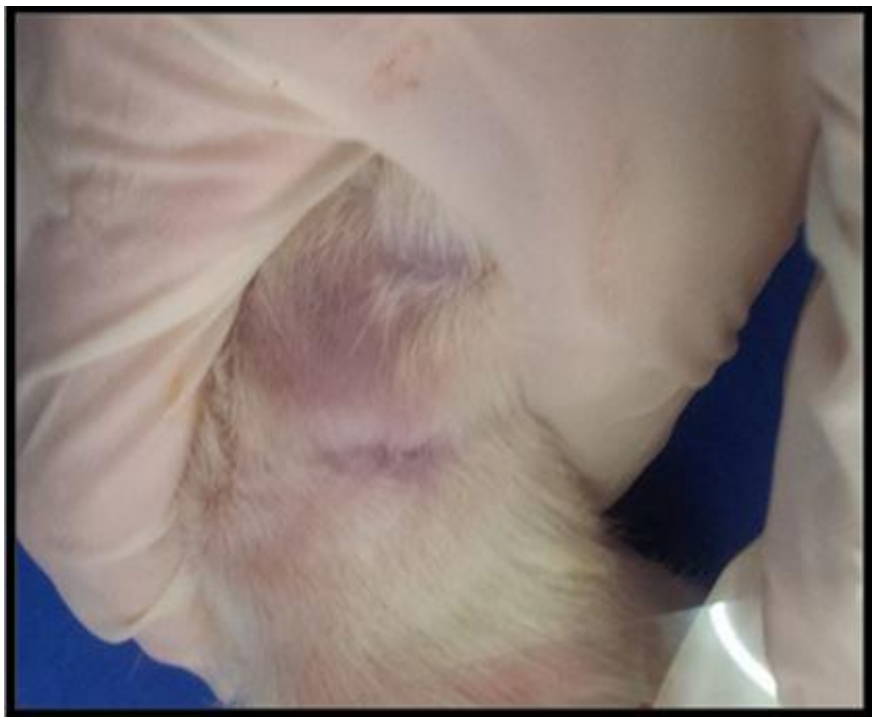

Figure 4: Excision Wound Model- Standard(Day 20)

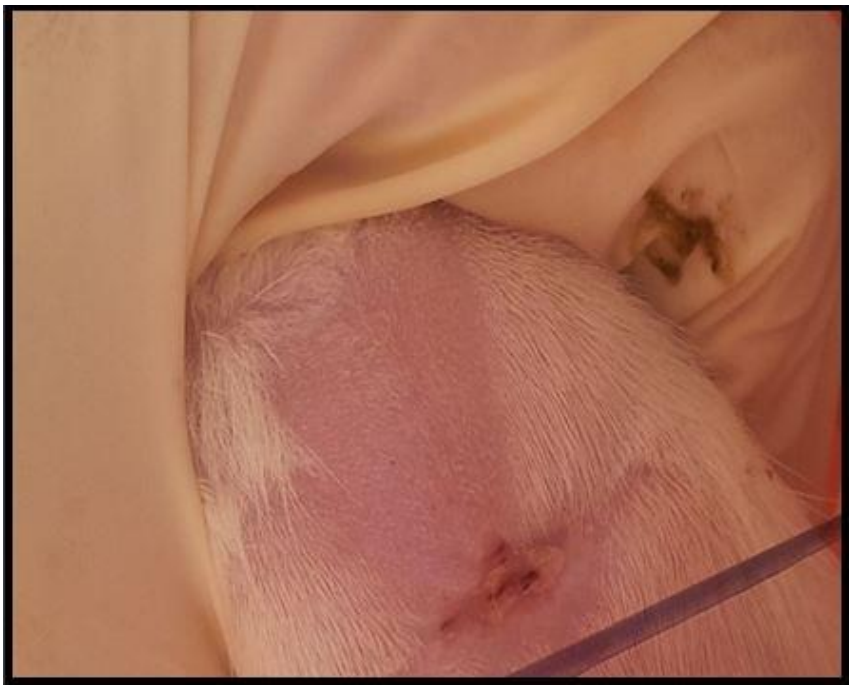

Figure 5: Excision Wound- SBDD Extract Ointment (Day 20)

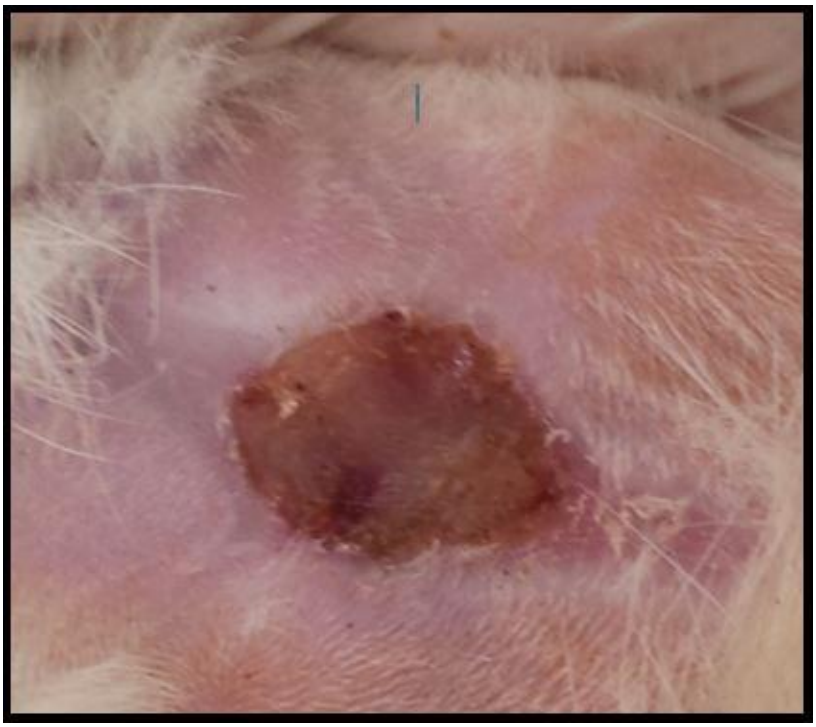

Figure 6: Burn Wound Model- Control(Day 20)

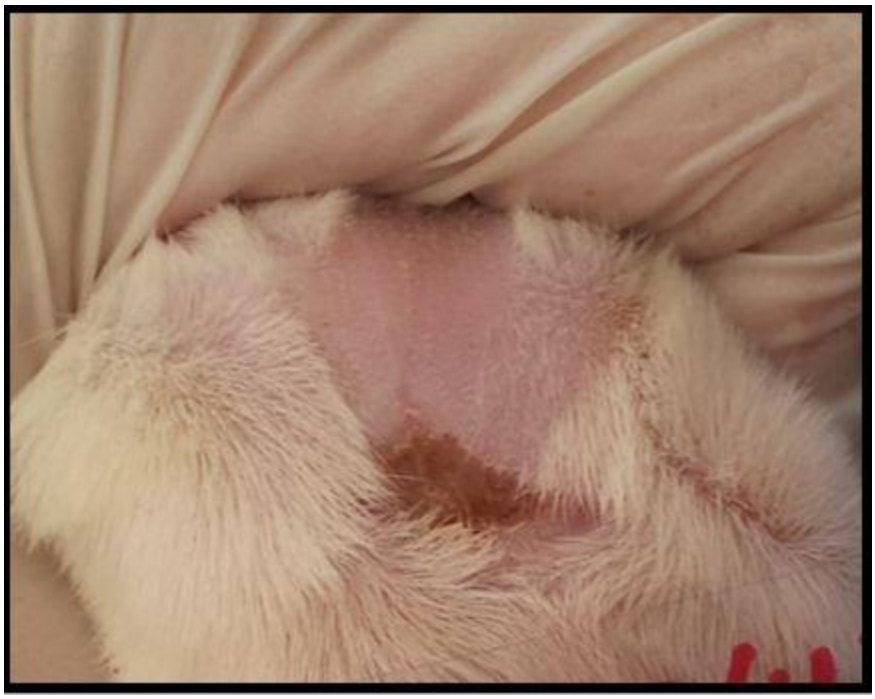

Figure 7: Burn Wound Model- Standard(Day 20)

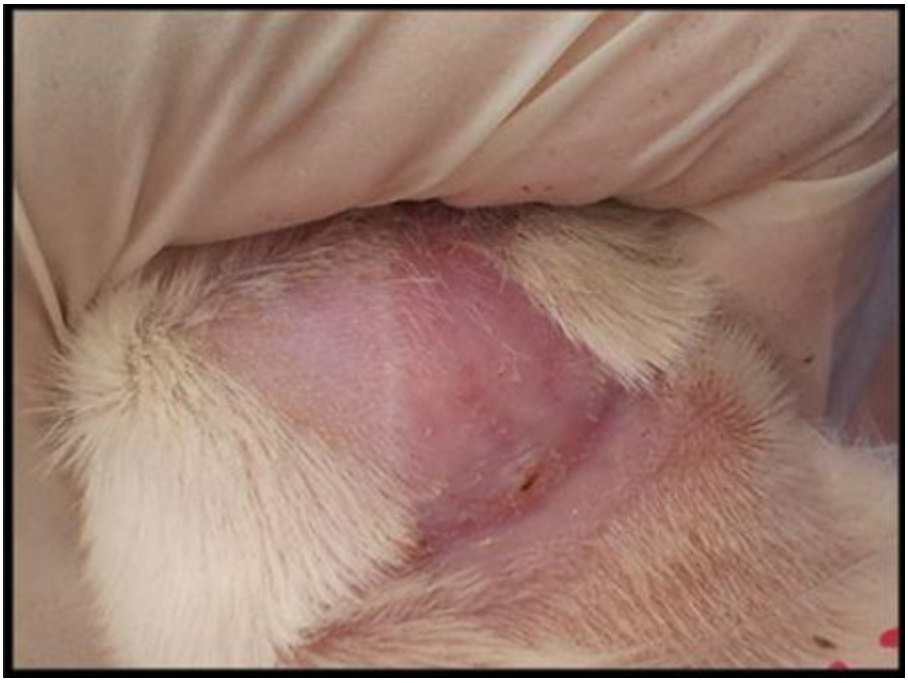

Figure 8: Burn Wound Model- 10%SBDD Extract Ointment (Day 20)

Histopathological Study

In excision wound model, the control group in H&E stains as in Figure 7 showed less fibroblast and fibrosis formation when compared to the tissue from the test group. In the test group, the tissue Figure 8 showed massive fibrosis formation. Besides that, there was also a bundle of lymphocyte presence in the tissue in the test group.

In the control group tissue, the Masson Trichrome stains show lesser collagen presence compare to the test group, Figure 9. In the test group, there are significant bundles of collagen presence as shown in Figure 10. There were also present of fibroblast when compared to the control group.

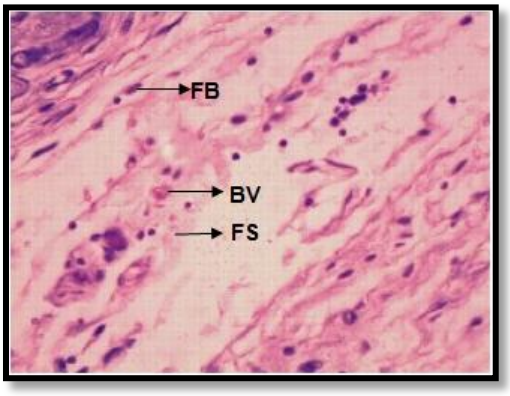

Figure 9: H&E Stain in Excision Model- Control

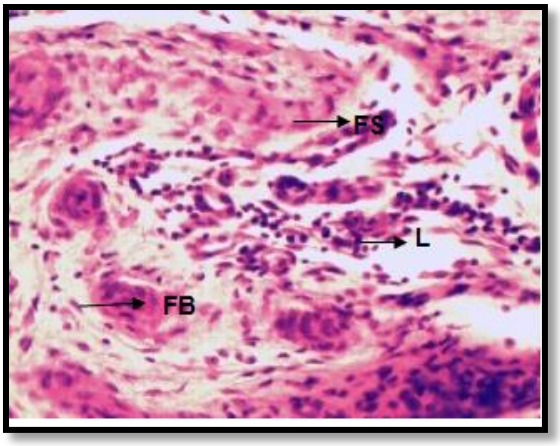

Figure 10: H&E Stain In Excision Model- SBDD Extract Ointment

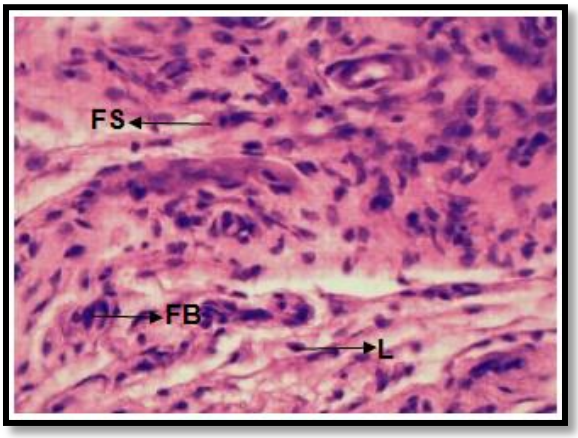

Figure 11: H&E Stain in Excision Model- Standard

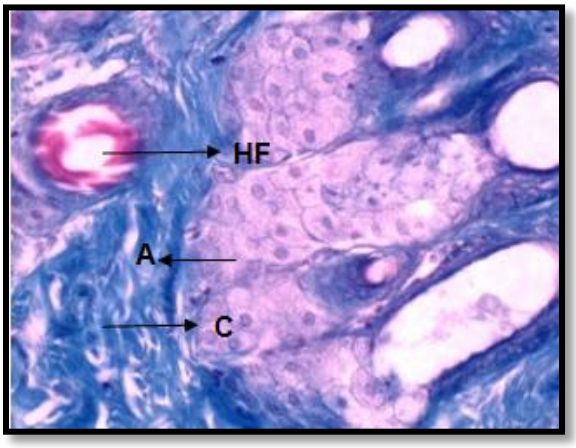

Figure 12: MC Stain in Excision Model- Control

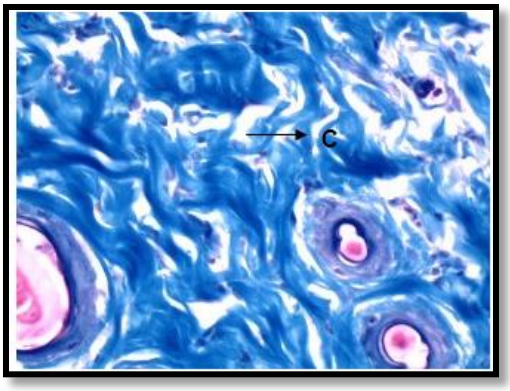

Figure13: MC stain in Excision Model- Standard

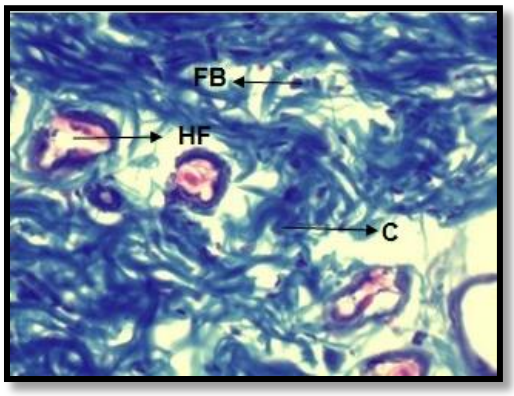

Figure 14: MC Stain in Excision Model- SBDD Extract Ointment

In burn wound model, H&E stains showed less fibroblast and fibrosis, as shown in Figure 11. However, the tissue in the extract treatment group showed more fibroblast and fibrosis present. There were also much lymphocytes that can be seen as shown in Figure 12. In Masson's Trichrome stains, the control group, Figure 13 shows lesser collagen. Unlike the extract treatment group, the tissue showed large amount of collagen present, beside that, there were also present of melanin and melanocyte and also fibroblast that can be seen as shown in (Figure 14).

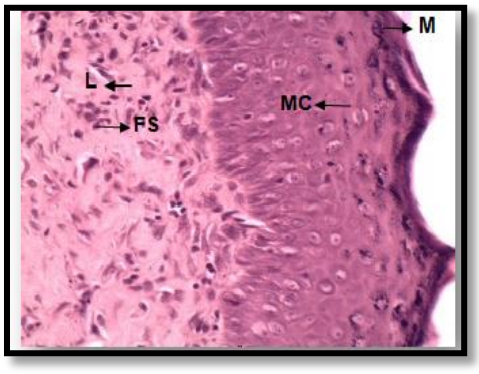

Figure 15: H&E Stains in Burn Model- Control

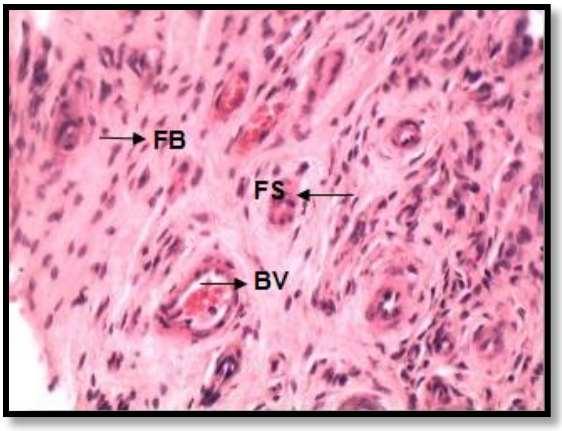

Figure 16: H&E Stain in Burn Model- SBDD Extract Ointment

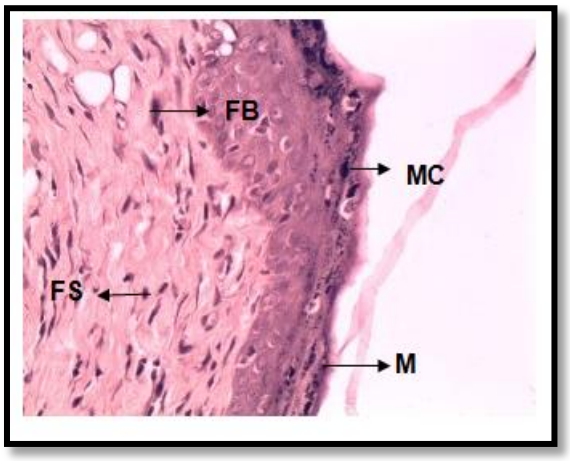

Figure 17: H&E Stain in Burn Model- Standard

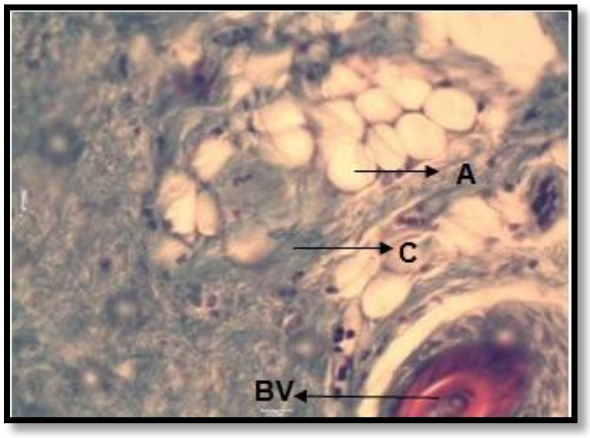

Figure 19: MC Stains in Burn Model- Control

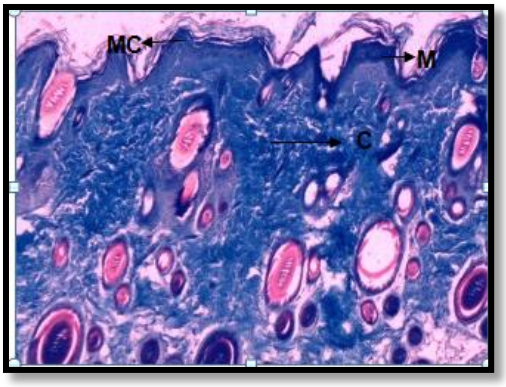

Figure 19: MC Stains in Burn Model- Standard

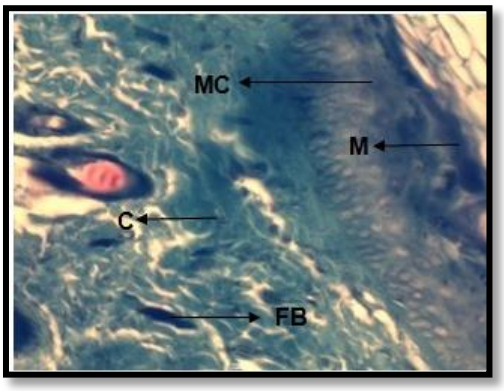

Figure 20: MC Stain in Excision Model- SBDD Extract Ointment

DISCUSSION

Wound healing is an orderly progression of events that establish the integrity of the tissues. Many studies have shown that plant products are preferred in wound healing since they are devoid of side effects and are more effective [13]. wound healing process begins with restoration of a damaged tissue as closely as possible to its natural state and wound contraction is the course of shrinkage in wounded area. The healing primarily depends on the repairing ability of the tissue in addition to type and degree of damage and general health status of the tissue. Wound contraction is the process of mobilizing healthy skin surrounding the wound to cover the denuded area and involves complex and superbly orchestrated interactions of cells, extracellular matrix and cytokines. The centripetal movement of wound margin is believed to be due to the activity of myofibroblasts [14]. since *Scutellaria barbata D. Don* enhanced wound contraction, it would have either enhanced contractile property of myofibroblasts or increased the number of myofibroblasts recruited into the wound area. Granulation, collagen maturation and scar formation are some of the many phases of wound healing which run concurrently, but independent of each other [15]. A significant increase in wound contraction was seen as compared to control. Hence *Scutellaria barbata D* has prohealing effect as evidenced by the above findings.

In the excision wound model *Scutellaria barbata D* accelerated the period of epithelialization significantly in later stages of wound contraction. It shows that *Scutellaria barbata D* has prohealing effect as evidenced by the above findings and was able to promote epithelialization either by facilitating the proliferation of epithelial cells or by increasing the viability of epithelial cells.

In burn wound model *Scutellaria barbata D* increased the rate of wound healing.in burn wounds, there is an extensive loss of cells and tissue and makes the repair process more complicated [16]. Wound healing activity of *Scutellaria barbata D* the phases of inflammation and proliferation gradually increased as comared to control group.

The study demonstrated that ethanolic extract of *Scutellaria barbata D. Don* were able to increase the rate of wound healing for both excision and burn wounds. Wound healing is a complex dynamic process that involves various phase, that initially involves hemostasis, vascular response, inflammatory phase by synthesis of collagen and extra cellular macromolecules which later removed to form a scar, proliferative phase and maturation [17]. Many plant extracts and medicinal herbs possess wound healing activity. The most main component that present in these herbal plants is Tannin that acts as free radical scavenger [18]. Tannin that possesses astringency property causes mild coagulations of skin protein, dry and hardens and protects the skin. Many researchers have been done on the role of antioxidant properties from plant extracts in wound healing has been published widely. Decrease in the period of epithelialisation indicates that there was rapid epithelialisation of the wound. In the histopathology study, the presence of fibroblast, fibrosis, melanin, melanocyte and collagen indicate the presence of wound healing. Moreover, the presence of lymphocyte shows significance in wound healing activity. Hence, the study that was conducted on the *Albino wistar* showed significant decrease in period of epithelialisation when compared to the control group. The extract ointment of *Scutellaria barbata D. Don* possesses wound healing activity on both excision and burn wound model.

CONCLUSION

In conclusion, the present study demonstrated that the ethanolic extract of *Scutellaria barbata D. Don* leaves promote wound healing activity in albino rats. In addition, *Scutellaria barbata D. Don* extract have shown to affect cellular growth and profileration in injured tissues. The wound healing activity of *Scutellaria barbata D. Don* extract may be related to the growth factors and vascular endothelial growth factor, fibroblast growth factor and vascular endothelial growth factor. Further studies with purified constituents compared to the crude extact might be needed to ratify the complete mechanism of wound healing activity of *Scutellaria barbata D. Don.*

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