Effect of Different Concentrations of Topically Applied *Boswellia Serrata* Extract on Excisional Wound Healing in Rats

Bushra Hassan Marouf,[*1] Dana Omer Ismaeel[2] and Goran Mohammad Raouf Abdulqader[3]

[1]Department of Pharmacology and Toxicology, College of Pharmacy, University of Sulaimani, Sulaimani, Kurdistan Region, Iraq.
[2]Department of Surgery & Theriogenology, College of Veterinary Medicine, University of Sulaimani, Sulaimani, Kurdistan Region, Iraq.
[3]Department of Pathology and Forensic Pathology, College of Medicine, University of Sulaimani, Sulaimani, Kurdistan Region, Iraq.
[*]**Corresponding author**

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Wound is known as one of the serious health issues. Boswellia serrata (BS) contains a wide range of phytochemicals with different pharmacological properties. The study aimed to assess the wound healing potential of Boswellia Serrata Extract (BSE) against an experimental model of excision wounds. A total of 45 rats in five equal groups were used in this investigation. The wound was created in the cervical dorsal region of each rat as a two-symmetrical circle pattern. All groups were being treated as follows: control group with ointment base, β-Sitosterol group with 0.25% w/w β-Sitosterol-containing ointment, three BSE ointment groups; treated with BSE ointment containing 5%, 10%, and 20% boswellic acid (BA) respectively. The wounds were followed up for 15 days. On days 5, 10, and 15, three rats from each group were sacrificed, and the wounds and scars were sent for histopathological study. The ointments containing 5% and 10% BA exerted a significantly increased wound contraction rate, lesser surface area and shorter re-epithelialization period in the macroscopical analysis in compare to the control group and the β-Sitosterol group. However, the microscopical results exhibited significant healing progress in a concentration-dependent manner. This was reflected by the formation of clear dermal granulation tissue, collagen deposition and angiogenesis in quantitative histological evaluation. In conclusion, this study provided a macroscopical and microscopical evidence for a potential wound healing effect of the topical application of BSE in concentration-dependent manner. Although the highest BA concentration in macroscopical analysis exhibited lesser efficacy.

Keywords: boswellic acid, epithelialization, excision wound, wound healing.

Introduction

A wound can be defined as dysregulation of the morphological and molecular compartments of the skin. It develops as a result of tissue damage by chemical, physical, thermal, microbial, or immunological factors [(1)]. Wound healing is the reestablishment of the structural integrity of the injured region. Once the injury occurs, it starts with a hemostasis state and coagulation to an inflammation phase followed by proliferation, tissue granulation, neovascularization, re-epithelialization, and restoration. After the injury, the hemostatic and inflammatory phase begins, however, the inflammatory phase can extend for up to six days. Proliferation is regarded as the start of both angiogenesis and the formation of extracellular matrix. An extended inflammatory and/or proliferative phase will hinder healing and promote the formation of excessive scar tissue. The remodelling stage typically initiates three weeks after damage [(2)]. Many therapeutic approaches exist for the treatment of wounds such as antibiotics and nonsteroidal anti-inflammatory drugs but the majority of these drugs produce numerous unwanted side effects. External application of herbal medicine has been shown in numerous studies to activate local macrophages, have a chemotactic effect on leukocytes, improve local immunity, regulate matrix metabolism, promote local microcirculation, and have anti-inflammatory and antibacterial effects, among other effects [(3,4)]. Several studies on herbal pharmaceuticals have recently been conducted to evaluate their potential in wound care. These natural therapies have proven to be effective as an alternative to the available synthetic drugs for wound treatment [(5)]. Many natural herbs have been shown to have strong wound-healing properties in pharmacological studies [(6)]. *Boswellia serrata* (BS) also known as olibanum Salai guggul, contains a wide range of phytochemicals, such as a mixture of terpenes in the resinous part and a mixture of polysaccharides in the gum part with numerous oxidizing agents and digestive enzymes. Furthermore, it contains a low percentage of essential oils such as monoterpenes, diterpenes, and

sesquiterpenes [7]. The resinous part contains pentacyclic triterpenes, in which boswellic acid is the major biologically active phytoconstituent of this portion [8]. There are four major boswellic acids (pentacyclic triterpenic acids) found in BSE: β-boswellic acid (BA), acetyl-β-boswellic acid, 11-keto-β-boswellic acid , and 3-*O*-acetyl-11-keto-β-boswellic acid (AKBA) , which are responsible for most of the pharmacological activities of the extract [9]. The AKBA has shown to be effective against a large number of diseases such as arthritis, ulcerative colitis, crohn's disease, osteoarthritis, cardiotoxicity, wound healing, diabetes, oxidative stress, bronchial asthma, inflammation, and cancer [10]. Despite the aforementioned pharmacological properties of BS, few studies have been conducted on its role in wound healing. According to the previous studies, BS oleo-gum dramatically increased wound contraction in diabetic foot ulcers and thermal burn wounds, as a result, there is a possibility that BS can help diabetic wounds recover faster [11,12]. In light of the encouraging results of BS for wound healing management, the present study is designed to assess the potential effect of topical application of BSE using different concentrations to ameliorate the complications that accompany the injured skin in an animal model of excision wound.

Materials and Methods

Animals and ethical consideration

A total of 45 healthy adult male Wistar albino rats weighing 240-260g were divided into five groups, each of nine rats (n =9) and they were housed individually in polypropylene cages for one week before the experiment to acclimatize to standard laboratory conditions [temperature (25±2ºC), relative humidity (44–56%), light and dark cycles (12:12 h)] of a well-ventilated animal house. They were freely accessible to the standard pellet diet and water *ad libitum* throughout the experimental study. The experimental protocol was registered and approved by the Ethical Committee of the College of Pharmacy, University of Sulaimani (ID: PH22-21 on 31.08.2021). All the procedures in the study were performed under the guidelines for the care and use of laboratory animals approved by the Research Registration and Ethics Committee of the College of Pharmacy, University of Sulaimani.

Preparation of BSE ointment

A standardized BSE powder containing 65% boswellic acid was used for the preparation of the ointment. The BSE powder was purchased from BULK SUPPLEMENTS-Henderson NV, the USA supplier. The BSE ointments containing 5%, 10%, and 20% boswellic acid were prepared by measuring an equivalent amount of BSE powder to give 5, 10, 20gram of BA then added and mixed to the ointment base "white petroleum jelly" to prepare 100g ointment utilizing a homogenizer for uniform formulation and smooth texture [13].

Generation of excision wound model and the treatment

The animals were anesthetized by intraperitoneal injection of ketamine (75 mg/kg) and Xylazine (10 mg/kg). A square of 2 x 2 cm skin area on the animal's cervical dorsal region was thoroughly shaved with an electric clipper at the dorsal back of the animal and cleaned with a disinfectant (70% ethanol). The wound model and surgical procedure were was performed as described previously [14,15]. Briefly, the wound was created in the cervical dorsal region of each rat as a two-symmetrical circle pattern. This technique involves the creation of two circular full-thickness wounds extending through the panniculus carnosus on the rat dorsum using a sterile dermal metal punch (8 mm in diameter) with a thickness comprising all the skin layers (epidermis, dermis, and subcutaneous fat). The excised skins of each animal were saved for subsequent histopathological assays, representing samples from the initial day (day 0) without treatment. The duration of the study was 15 days, and the follow-up monitoring was done by digital camera starting from day 0 and on days 4, 7, 10, 13, and 15 [15]. Additionally, periodically on the 5th, 10th, and 15th days of wound creation, three rats from each group were euthanized to follow up the wound healing and re-epithelialization period, and then the wound and the surrounding area were sent for histopathological evaluation [14] . During the whole experimental period, wounds were kept open without any dressing, and various wound-related parameters such as wound surface area, rate of wound closure, and epithelialization period were considered to be monitored in the progression of the healing.

Animal groups and treatment protocols

The animals were randomly divided into five groups (G) and treated as follows:

G1 (Control); with petroleum jelly (ointment base).

G2 (β-Sitosterol); with a moist exposed burn ointment (MEBO) (from Julphar, United Arab Emirates) was applied. The MEBO contains 0.25% w/w β-Sitosterol as a main active ingredients, sesame oil, and berberine combined with baicalin in a base of beeswax and sesame oil [16], it was used as a standard ointment in this study.

G3; with topical application of 5% BSE ointment

G4; with topical application of 10% BSE ointment.

G5; with topical application of 20% BSE ointment.

Wounds were treated according to the designed groups and the ointment were topically applied in the adjusted amount to coat the surface of the wound once daily for 15 days as described previously [17]. The treatment started in each group just 2 hours after the wound was created.

Macroscopic evaluation

Wound surface area, percent of wound contraction, and epithelialization time measurements

The wound area of each rat was measured six times: at the beginning (Day 0), the fourth, seventh, tenth, thirteenth, and fifteenth days. The progressive changes in the wound area were monitored by a professional camera. The wounds were photographed by a digital camera at these intervals and a precise measurement of the wound area was performed by Image J software [18]. The photographic measurement was done by a person who was blinded to the identity of the samples.The percentage of wound contraction was calculated using the following formula:

Wound contraction (%) = $[(A_0 – A_t)/A0] * 100$

Where A_0 represents the initial wound surface area and A_t represents the wound surface area at time t [3]. The epithelialization period was also recorded. The period was calculated as the number of days required for the dead tissue remnants (eschar) to fall without any residual raw wound [19].

Microscopic analysis

On the 5th, 10th, and 15th days, 3 rats (each possessing 2 wounds) from each group were sacrificed sequentially, and then the skins of the wound areas were dissected and isolated for histological examination.

Histotechnique protocol for hematoxylin and eosin (H&E) stained section

Following animal euthanization and postmortem findings, skin wound samples were collected and processed for histological preparation. First, samples were added to tissue cassettes and fixed in a 10% formaldehyde solution at least for 72 hours. Then, tissue sections were dehydrated by passing through ascending concentrations of ethanol (50%, 60%, 70%, 90%, and 100%), followed by two-step cleaning with xylene. After that, processed tissues were embedded in melted paraffin blocks at (60 -70°C) using an automated tissue embedder. Next, tissue samples within a paraffin block were sectioned to 5 µm using a semi-automated rotary microtome. Later on, sections will be mounted on glass slides from the warm water bath and dried using a hot plate tissue dryer. Next, the mounted skin sections were deparaffinized, and again cleaned with xylene solution for 20 minutes then oven-dried at 50°C for 10 minutes. At the end of the tissue processing technique, slide sections were stained with Harris's hematoxylin and eosin solutions then cleaned as a final step in xylene and covered with glass or plastic coverslip using DPX.

Quantitative histological evaluation

In general, tissue samples were analyzed under the light microscope (NOVEL XSZ-N107, China) by the mean of image-snap analyzer software (AmScope Ver. 3.7) using a microscope digital camera (MU300, 2019). Briefly, histological quantitative measures of skin wound sections from each animal were evaluated and measured in µm and statistically analyzed as mean percentage. On the other hand, inflammatory cells were counted in a randomly chosen 10 fields from different skin tissue sections under high power magnification (1000X). The mean average was calculated and measured statistically in percentage. Moreover, the area of granulation tissue distribution, newly formed blood vessels (angiogenesis), inflammatory exudates, proliferated collagen fibers and epidermal re-epithelization thickness were estimated in µm, then quantitatively measured in mean percentage. The mean percentage of calculated values were assessed as following lesion scores (score >75% as mild lesion; score 50-75% as moderate lesion; score <50% as severe lesion), while normal skin patch that sent to histopathology score <10% as no lesion.

Masson's Trichrome (MT) technique

Following the necropsy, skin samples were taken for histological preparation. Skin wound patches were deparaffinized and rehydrated throughout descending alcohol concentration starting from (100%, 90%, 80% and 70%). Then washed with distilled water and rinsed in running tap water for 5-10 minutes. Next, the tissue sections were stained with Weigert's iron hematoxylin working solution for 10 minutes. After that rinse in running tap water for 10 minutes and then wash in distilled water. Later on, the sections were stained again with Biebrich scarlet-acid fuchsin solution for 10 minutes, then as usual, washed in couple steps of distilled water. After that the samples were incubated with phosphomolybdic-phosphotungstic acid solution for 10 minutes as a differential satin. Finally, stain with aniline blue solution and for 5-10 minutes, then rinse in distilled water and as a final step differentiate in 1% acetic acid solution for 2-5 minutes. Again, wash in distilled water, then dehydrate through two-step of ethanol alcohol (95% and, absolute ethyl alcohol), then clear with xylene and cover with coverslip.

Quantitative histological evaluation for Masson's Trichrome stained sections

Briefly, morphometric quantitative measures of skin wound sections from each animal stained with the MT technique were evaluated and measured in µm and statistically analyzed as mean percentage. To be mentioned, the area of bluish-colored collagen fibers distribution and granulation tissue were estimated in µm from at least five different sections from each sample. The same technique was applied for calculating the inflammatory cells as in the histopathology protocol. The mean average was calculated and measured statistically in percentage. Moreover, area of granulation tissue distribution, newly formed blood vessels (angiogenesis), inflammatory exudates, proliferated collagen fibers and epidermal re-epithelization thickness were

estimated in µm, then quantitatively measured in mean percentage. Last but not least, the mean percentage of calculated values were assessed as following lesion scores (score >75% as mild lesion; score 50-75% as moderate lesion; score <50% as severe lesion), whereas normal skin patches score <10% as no lesion. All tissue samples were analyzed under the light microscope (NOVEL XSZ-N107, China) by the mean of image-snap analyzer software (AmScope Ver. 3.7) using a microscope digital camera (MU300, 2019).

Statistical analysis

The statistical analysis was performed using GraphPad Prism software, LCC version 9.4.1. The data was checked for normality using the Shapiro-Wilk test before performing other statistical analysis. Data was expressed as mean±SEM. Two-way ANOVA was used to analyze significant differences between groups, followed by Tukey's post hoc test. *P-value* <0.05 is considered as a statistically significant.

Results

Evaluation of Wound Surface Area

The macroscopical assessment of the excision wounds in the present study revealed that the topical application of BSE ointment in different concentrations decreased the wound surface area and epithelialization period in the treated groups compared with the control group and the standard group that used β-Sitosterol ointment. The appearance of the wound in the studied groups at different time points was described by the histopathologist during the follow up; at the baseline the wounds of all groups were identical, it showed a clean normal wound.Following wound creation, on day 4 and in BSE treated group, the reduction in wound surface area was significant (P<0.05) in comparison with the control group and standard group. Treatment with different concentrations of BSE ointment (5%, 10%, and 20%) decreased wound area from approximately initial 59-60 mm^2 to relatively complete healing on day 10±1 in both low and moderate doses and on day 12±1 in higher doses. On day 15, all the treated wounds were closed as shown in Table 1 and Figure 1.

Table 1. Changes in the excisional wound surface area after treatment with different concentrations of *Boswellia Serrata* extract ointment in rats

Groups	Wound surface area (mm^2)						*P*-value (ANOVA)
	Day 0	Day 4	Day 7	Day 10	Day 13	Day 15	
Control	59.8±4.4^{a}	57.8±3.9^{a}	50.8±5.6*^{a}	18.2±10.5*^{a}	12.7±9.9*^{a}	0.4±0.6*^{a}	0.0001
β-Sitosterol	59.3±4.3^{a}	50.7±4.4*^{b}	47.5±5.3*^{a}	23.7±12.5*^{a}	6.6±3.7*^{a}	1.6±0.5*^{b}	0.0001
5% BSE	59.8±5.8^{a}	42.7±5.5*^{c}	24.9±3.8*^{b}	0.98±1.3*^{b}	0.05±0.08*^{b,a}	0.00±0.0*^{a}	0.0001
10% BSE	59.9±1.9^{a}	37.4±4.0*^{c}	29.5±2.3*^{b}	1.2±1.9*^{b}	0.00±0.0*^{b,a}	0.00±0.0*^{a}	0.0001
20% BSE	60.03±1.4^{a}	45.4±7.3*^{d}	33.7±5.1*^{b}	6.6±1.7*^{b}	0.00±0.0*^{b,a}	0.00±0.0*^{a}	0.0001
P-value	0.097	0.0001	0.001	0.0001	0.0002	0.0001	

Values are presented as mean±SEM; BES: *Boswellia serrata* extract; * significantly different compared with baseline within the same group (*P*<0.05); values with non-identical superscripts (a,b,c,d) are significantly different among different groups within the same period (*P*<0.05).

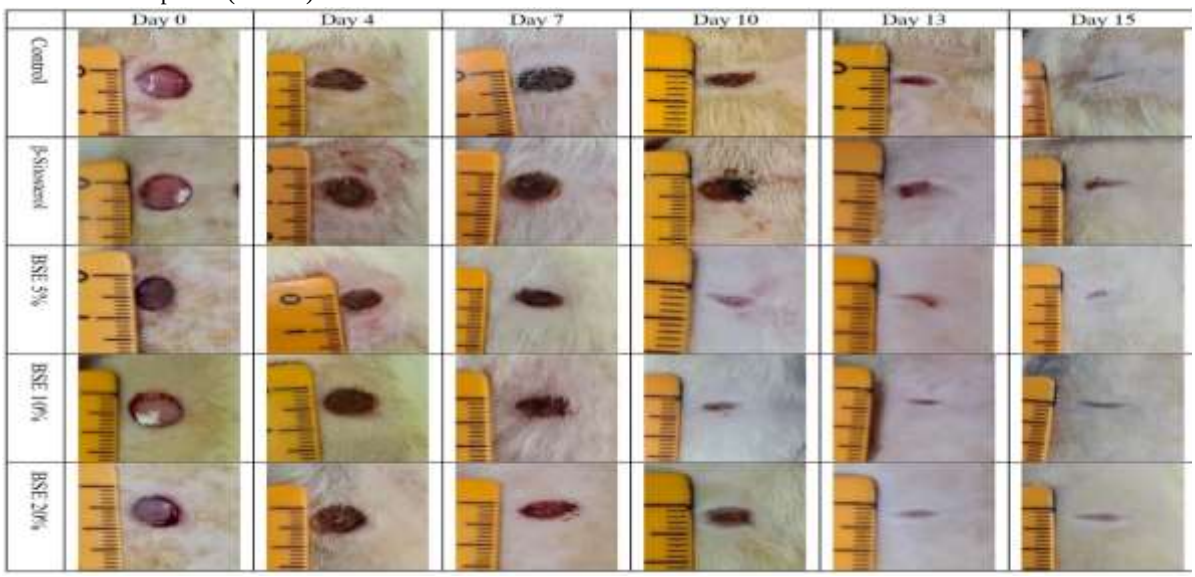

Figure 1.Morphological representation of the wounds created on the panniculus carnosus on the rat dorsal region after treatment on day 0, 4, 7, 10, 13 and 15 post-wounding. The progress of healing achieved by day 10 post-wounding in 5%, 10% and 20% BSE groups and progressed to full wound closure after 15 days in all groups.

Evaluation of Wound Contraction and Epithelialization Period

Re-epithelialization was determined through the percentage of wound contraction, which was obtained from analyzing the wound surface area calculated by the ImageJ software. When comparing epithelialization on the 4th day, the rate of wound contraction was significantly higher in all groups using BSE ointments. On the 7th day, all the BSE treated wounds showed a higher rate of wound contraction as compared to the control and standard groups ($p<0.05$). On the 10th day, wounds from both 5% and 10% BSE treated groups were relatively healed (Figure 2). The epithelialization period recorded in the 5% and 10% BSE treated groups was 10±1 days (this period was measured as the number of days required for the dead tissue remnants to fall without leaving any residual raw wound). While for the 20% BSE treated group, it was 12±1. On day 15th, the wounds of the control and standard groups showed relatively complete healing, although the quality of scars in both groups was not ideal (Table 2).

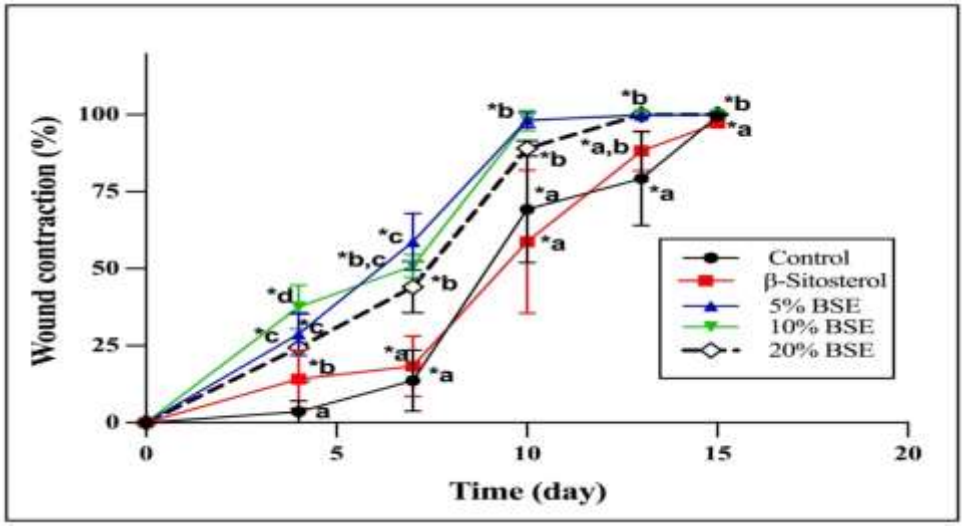

Figure 2. Effect of BSE on wound contraction rate

Data are expressed as mean±SEM; and analyzed by two-way ANOVA followed by Tukey's test. * significantly different compared with baseline within the same group ($P<0.05$); values with non-identical superscripts (a,b,c,d) are significantly different among different groups within the same period ($P<0.05$). BES: *Boswellia serrata* extract.

Table 2. Changes in the percentage of excisional wound contraction after treatment with different concentrations of Boswellia serrata extract ointment in rats

Groups	Wound contraction (%)						*P*-value (ANOVA)
	Day 0	Day 4	Day 7	Day 10	Day 13	Day 15	
Control	0.00±0.0	3.5±3.6^{a}	13.7±9.9*^{a}	69.2±17.4*^{a}	79.3±15.2*^{a}	99.4±1.0*^{a}	0.0001
β-Sitosterol	0.00±0.0	14.1±9.4*^{b}	18.4±9.8*^{a}	58.8±23.3*^{a}	88.2±6.4*^{a,b}	97.2±1.1*^{b}	0.0001
5% BSE	0.00±0.0	28.7±6.9*^{c}	58.7±9.2*^{b}	98.2±2.4*^{b}	99.8±0.13*^{b}	100±0.0*^{a}	0.0001
10% BSE	0.00±0.0	37.5±7.1*^{d}	50.9±3.8*^{b,c}	98.0±3.2*^{b}	100±0.0*^{b}	100±0.0*^{a}	0.0001
20% BSE	0.00±0.0	24.4±11*^{c}	44.0±8.4*^{c}	89.1±2.6*^{b}	100±0.0*^{b}	100±0.0*^{a}	0.0001
P-value (ANOVA)		0.0001	0.001	0.0001	0.025	0.0001	

Values are presented as mean±SEM; BES: *Boswellia serrata* extract; * significantly different compared with baseline within the same group ($P<0.05$); values with non-identical superscripts (a,b,c,d) are significantly different among different groups within the same period ($P<0.05$).

Histopathological morphometric quantitative assessment

Table 3 reveals histopathological morphometric quantitative assessment and lesion scoring from different treatment groups at different experimental intervals. In general, skin samples (skin patches) in groups (for simplicity G1-G5 was used in histopathological figures): control (G1), β-Sitosterol (G2), 5% BSE (G3), 10% BSE (G4) and 20% BSE (G5) show no significant morphological changes in all treatment groups at day 0 in comparison with the samples collected at days 5, 10 and 15. Likewise, at day 5 as shown in Figure 3 all groups demonstrate the hemato-inflammatory phase

which is evident by profound deposition of acidophilic inflammatory exudate together with diffuse infiltration of inflammatory cells mainly polymorphonuclear cells. Moreover, histopathology examination shows a small area of granulation tissue formation with nearly thin layers of regenerating epithelial cells, and the lesion scoring expresses severe score in term of healing intensity particularly in G1 in comparison to other treatment groups (Table 3). On the other hand, skin lesions at day 10 show a significant reduction in the lesion scoring intensity from severe to moderate and then to mild especially in G2 and G5 (Table 3), in which animals treated with 0.25% β-Sitosterol ointment (G2) shows a significant reduction in the inflammatory phase and wound cellularity apparent by clear collagen deposition, granulation tissue together with angiogenesis, in addition to epidermal re-epithelization, which is comparable with the same statistically significant lesion scoring in G5 (treatment with 20% BSE) in term of, reducing wound cellularity and augmentation of wound fibrous content. However, lesions in G1, G3 and G4 still show much cellular content in compare to G2 and G5. In contrast, lesion scoring as shown in table 3 displays a significant reduction $P<0.05$ in all treatment groups from moderate to mild at day 15 in comparison with the experimental period day 10, yet, it is much significant in G2 and G5 (β-Sitosterol and 20% BSE) respectively. Furthermore, lesion scoring also shows visible reduction in G3 and G4 (5% and 10% BSE) in a dose-dependent manner in comparison to skin lesions in G1 (natural healing) which also shows mild intensity, evidenced by a drop in the amount of inflammatory exudate and proliferation of more collagen fibers with clear re-epithelization of the epidermal layers. Therefore, accordingly topical treatment with 20% BSE ointment at days 10 and 15 shows genuine therapeutic effect against experimentally induced clean-cut skin wound, however topical application of 0.25% w/w β-Sitosterol ointment at same experimental intervals demonstrates similar significant effects with respect to wound healing improvement.

Table 3. Histological quantitative evaluation of skin wound with different treatment values

Experimental Groups N=12	Granulation Tissue Formation* (Mean%)**	Area of Angiogenesis* (Mean%)**	Inflammatory Cells Infiltration (Mean %)**	Collagen Fibers Proliferation * (Mean %)**	Skin Epidermal regeneration* (Mean %)**	Lesion Scoring (0 -100%)	Lesion Grading
Day 0							
(G1) CG†	3.56 % ^{A}	2.35 % ^{A}	4.61 % ^{A}	2.97 % ^{A}	2.76 % ^{A}	0-10 %	No lesion
(G2) 0.25% MEBO	4.57 % ^{A}	3.47 % ^{A}	4.73 % ^{A}	3.85 % ^{A}	1.88 % ^{A}	0-10 %	No lesion
(G3) 5% BSE	4.22 % ^{A}	2.79 % ^{A}	5.78 % ^{A}	4.11 % ^{A}	2.82 % ^{A}	0-10 %	No lesion
(G4) 10% BSE	4.68 % ^{A}	3.12 % ^{A}	5.32 % ^{A}	2.67 % ^{A}	3.17 % ^{A}	0-10 %	No lesion
(G5) 20% BSE	3.78 % ^{A}	3.26 % ^{A}	6.13 % ^{A}	2.86 % ^{A}	2.96 % ^{A}	0-10 %	No lesion
Day 5							
(G1) CG†	22.45 % ^{B}	23.67 % ^{B}	94.61 % ^{E}	19.78 % ^{B}	18.43 % ^{B}	10-50 %	Severe
(G2) 0.25% MEBO	34.75 % ^{C}	46.32 % ^{C}	89.22 % ^{E}	48.23 % ^{C}	26.84 % ^{B}	10-50 %	Severe
(G3) 5% BSE	27.56 % ^{B}	26.91 % ^{B}	90.18 % ^{E}	42.51 % ^{C}	20.61 % ^{B}	10-50 %	Severe
(G4) 10% BSE	28.72 % ^{B}	36.81 % ^{C}	87.45 % ^{E}	44.91 % ^{C}	21.84 % ^{B}	10-50 %	Severe
(G5) 20% BSE	38.24 % ^{C}	43.37 % ^{C}	86.92 % ^{E}	48.32 % ^{C}	22.17 % ^{B}	10-50 %	Severe
Day 10							
(G1) CG†	54.23 % ^{D}	55.49 % ^{D}	78.82 % ^{E}	60.32 % ^{D}	53.28 % ^{D}	50-75 %	Moderate
(G2) 0.25% MEBO	77.92 % ^{E}	75.89 % ^{E}	52.49 % ^{D}	77.35 % ^{E}	75.61 % ^{E}	75-100 %	Mild
(G3) 5% BSE	56.32 % ^{D}	64.55 % ^{D}	59.44 % ^{D}	73.88 % ^{D}	56.92 % ^{D}	50-75 %	Moderate
(G4) 10% BSE	70.54 % ^{D}	68.91 % ^{D}	57.16 % ^{D}	72.61 % ^{D}	58.69 % ^{D}	50-75 %	Moderate
(G5) 20% BSE	76.81 % ^{E}	75.48 % ^{E}	52.21 % ^{D}	77.13 % ^{E}	65.41 % ^{D}	75-100 %	Mild
Day 15							
(G1) CG†	76.68 % ^{D}	75.41 % ^{D}	55.71 % ^{D}	76.49 % ^{D}	75.34% ^{D}	75-100 %	Mild
(G2) 0.25% MEBO	84.43 % ^{E}	80.33 % ^{E}	32.61 % ^{C}	88.03 % ^{E}	93.76 % ^{E}	75-100 %	Mild
(G3) 5% BSE	77.65 % ^{E}	74.51 % ^{D}	56.46 % ^{D}	78.26 % ^{E}	76.48 % ^{E}	75-100 %	Mild
(G4) 10% BSE	79.21 % ^{E}	77.23 % ^{E}	54.47 % ^{D}	82.53 % ^{E}	78.64 % ^{E}	75-100 %	Mild
(G5) 20% BSE	81.72 % ^{E}	79.68 % ^{E}	43.60 % ^{C}	84.76 % ^{E}	86.52 % ^{E}	75-100 %	Mild

Notes: *Area of granulation tissue formation, angiogenesis, collagen fibers proliferation and skin epidermal regeneration were estimated in (µm). **Each value represents the mean percentage of (n=12). Statistical comparison among groups: Mean values with different capital letters have significant differences at ($P < 0.05$). (for simplicity G1-G5 was used in histopathological figures). †: **G1:** control group (CG) topical application with vehicle (ointment base as control) . **G2:** Standard ointment group, 0.25% w/w β-Sitosterol Ointment (MEBO). **G3:** 5% w/w BSE ointment group. **G4:** 10% w/w BSE ointment group. **G5:** 20% w/w BSE ointment group.

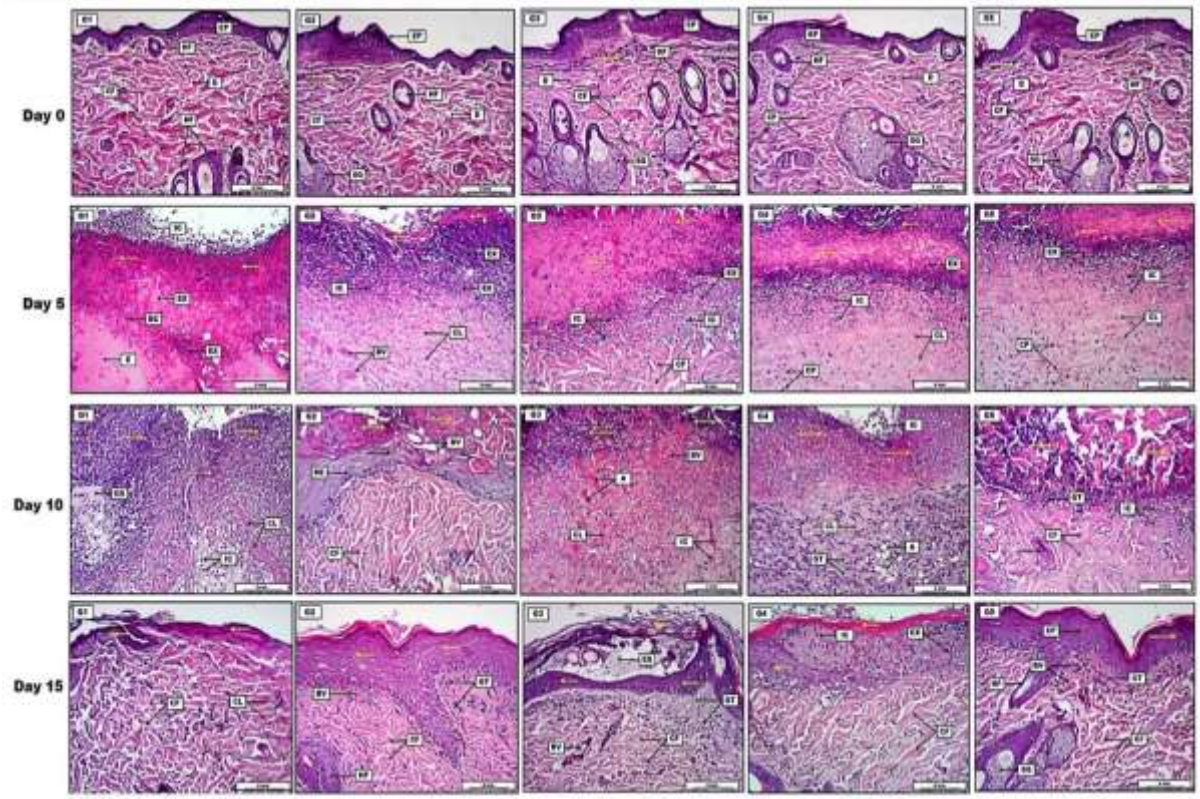

Figure 3. Photomicrograph of the skin from all groups

G1: control group topical application with vehicle (ointment base as control). **G2:** Standard ointment group, 0.25% w/w β-Sitosterol Ointment (MEBO). **G3:** 5% w/w BSE ointment group. **G4:** 10% w/w BSE ointment group. **G5:** 20% w/w BSE ointment group. In different time intervals; **(Day 0, 5, 10 and 15)** all stained with hematoxylin and eosin (H&E). **Day 0:** Skin samples in all groups display normal morphological organization of the epidermis (EP), presence of many sebaceous glands (SG) together with typically distributed hair follicles (HF) within the distinctive dermal layers (D) which consist of randomly oriented collagen fibers (CF). The sections also reveal some areas of non-significant infiltration of inflammatory cells (yellow arrows). **Day 5:** Samples in G1 show significant infiltration of mononuclear inflammatory cells (IC) above a severely crusted mixed purple to pinkish debris materials (yellow arrows), together with the presence of eosinophilic inflammatory exudate (EX) interlaced with deep-pinkish edematous fluid (E) which contain some bacterial colonies (BC). In G2, samples show reduction in the area of hematogenic crusty materials (yellow arrows), with still existing purplish-eosinophilic inflammatory exudates mixed with inflammatory cells within the dermis, together with deposition of light pinkish collagen materials (CL) and newly formed blood vessels (BV). Skin samples in G3, 4 and 5 show diffuse dissemination of lose crusty eosinophilic inflammatory edematous materials within the wound surface area (yellow arrows) mixed with a significant quantity of inflammatory exudates (EX), all sections show clear deposition of collagenous materials (CF, CL) with many newly formed capillaries (CP) particularly in G4 and G5 samples. **Day 10:** At 10^{th} day skin samples in G1 still show significant deposition of highly cellular inflammatory exudates (yellow arrows) which are interlaced with obvious numbers of extensively distributed inflammatory cells (CL) with many areas of edematous fluid, the section shows light eosinophilic deposition of the extracellular collagenous matrix (CL). Samples in G2 demonstrate significant and constant regeneration of the epidermal layers (RE) with the presence of newly formed blood vessel (BV) and many small capillaries (black arrow), the section reveals a proliferation of large bundles of light pinkish collagen fibers (CF) within the dermis, in addition to the presence of deep eosinophilic crusty inflammatory exudates over the re-epithelized area (yellow arrows). Skin sections in G3, 4 and 5 display clear dermal granulation tissue (GT) with distributed areas of collagen deposition (CL) interwoven with zones of angiogenesis (A) and many newly formed blood vessels (BV, black arrow), in which the above area of the healed wound still covered with crusty pretentious inflammatory exudates mixed with widely distributed mononuclear inflammatory cells (IC). **Day 15:** Skin sections in G1 reveals noticeable morphological regeneration in the epidermal layer (yellow arrows), together with the deposition of pinkish collagenous materials (CL) and proliferation of collagen fibers within the dermis (CF). G2 samples at 15^{th} day of treatment show significant regeneration of epithelial tissue (yellow arrows) leading to the formation of many epidermal papillae. In addition, the section shows a distinct proliferation of collagen fibers (CF) interlacing with granulation tissue formation (GT), and many newly formed blood vessels (BV), together with the presence of newly grown sebaceous glands and hair follicles (HF). Skin tissue samples in G3, 4 and 5 demonstrate significant re-epithelization with many layers of keratin deposition (yellow arrows), together with clear area of granulation tissue (GT) and collagenous connective tissue distribution (CF) intermixed with newly formed blood vessels (BV). Sections in G3 and G4 still show some areas of inflammatory exudates (EX) and many inflammatory cells (IC). Tissue samples in G5 display full epidermal growth (EP) and dermal hair follicles (HF) together with many sebaceous glands (SG). H&E. Scale bars: 4 mm.

Histological quantitative assessment using Masson's Trichrome-stained sections

Initially, complete histological quantitative assessment and lesion scoring from skin samples stained with Masson's Trichrome (MT) special stain from different treatment groups at different experimental intervals were illustrated in Table 4 and Figure 4. Following animal scarification, skin patches were collected for special stain preparation at days 0, 5, 10 and 15. To be mentioned, at day 0, skin samples in groups G1, G2, G3, G4 and G5 (for simplicity G1-G5 was used in histopathological figures) show no significant morphological changes in comparison to the samples collected at days 5, 10 and 15. In contrast, at day 5 samples stained with the MT technique show a severe bright-red hemato-inflammatory phase which is apparent by the heavy deposition of acidophilic inflammatory exudate mixed with diffuse inflammatory cells. Furthermore, histological evaluation shows slight granulation tissue formation with nearly thin layers of epithelial regeneration, also the lesion scoring express severe score in term of healing intensity predominantly in G1 in compare to other treatment groups (Table 4). Instead, at day 10 dermato-morphometric assessment showed significant reduction in lesion scoring intensity from severe to moderate even to mild particularly in G2 and G5 (Table 4), in which animals treated with 0.25% β-Sitosterol ointment and 20% BSE in G2 and G5 respectively, illustrate significant reduction in the inflammatory phase evident by strong collagen deposition, granulation tissue proliferation and clear angiogenesis, as well as prominent epidermal re-epithelization. On the other hand, amazingly at day 15, skin patches treated with the MT technique, show significant reduction in lesion scoring from all treatment groups (G1 to G5) expressed as mild scale, though it is much significant in G2 and G5 (0.25% β-Sitosterol and 20% w/w BSE) ointment respectively, evident with more bluish collagen deposition and granulation tissue formation, with complete epidermal reepithelization. Thus, according to our suggested data topical application of 20% w/w BSE ointment at days 10 and 15 shows significant therapeutic efficacy in wound healing improvement in comparison to the similar effect of 0.25% w/w β-Sitosterol ointment at the same experimental intervals.

Table 4. Quantitative evaluation of skin wound stained with Masson's Trichrome technique

Experimental Groups N=12	Granulation Tissue Formation* (Mean%)**	Area of Angiogenesis* (Mean%)**	Inflammatory Exudates+Cells (Mean %)**	Collagen Fibers Proliferation* (Mean %)**	Skin Epidermal regeneration* (Mean %)**	Lesion Scoring (0 - 100%)	Lesion Grading
Day 0							
(G1) CG†	4.76 % ^{A}	3.87 % ^{A}	5.23 % ^{A}	3.24 % ^{A}	1.82 % ^{A}	0-10 %	No lesion
(G2) 0.25% MEBO	5.19 % ^{A}	4.39 % ^{A}	3.49 % ^{A}	5.63 % ^{A}	1.34 % ^{A}	0-10 %	No lesion
(G3) 5% BSE	5.81 % ^{A}	4.47 % ^{A}	5.31 % ^{A}	5.41 % ^{A}	1.58 % ^{A}	0-10 %	No lesion
(G4) 10% BSE	5.48 % ^{A}	5.23 % ^{A}	5.73 % ^{A}	5.87 % ^{A}	2.45 % ^{A}	0-10 %	No lesion
(G5) 20% BSE	6.34 % ^{A}	5.41 % ^{A}	4.89 % ^{A}	6.12 % ^{A}	1.72 % ^{A}	0-10 %	No lesion
Day 5							
(G1) CG†	18.52 % ^{B}	21.37 % ^{B}	98.42 % ^{E}	17.84 % ^{B}	14.32 % ^{B}	10-50 %	Severe
(G2) 0.25% MEBO	32.65 % ^{C}	45.39 % ^{C}	87.46 % ^{E}	49.55 % ^{C}	24.78 % ^{B}	10-50 %	Severe
(G3) 5% BSE	25.39 % ^{B}	25.17 % ^{B}	89.62 % ^{E}	44.91 % ^{C}	19.32 % ^{B}	10-50 %	Severe
(G4) 10% BSE	30.25 % ^{B}	34.62 % ^{C}	86.41 % ^{E}	46.16 % ^{C}	20.47 % ^{B}	10-50 %	Severe
(G5) 20% BSE	37.44 % ^{C}	39.84 % ^{C}	84.73 % ^{E}	49.62 % ^{C}	20.34 % ^{B}	10-50 %	Severe
Day 10							
(G1) CG†	56.32 % ^{D}	57.46 % ^{D}	77.61 % ^{E}	60.49 % ^{D}	51.84 % ^{D}	50-75 %	Moderate
(G2) 0.25% MEBO	76.43 % ^{E}	75.62 % ^{E}	53.29 % ^{E}	76.35 % ^{E}	75.48 % ^{E}	75-100 %	Mild
(G3) 5% BSE	58.22 % ^{D}	65.43 % ^{D}	58.72 % ^{D}	71.34 % ^{D}	54.11 % ^{D}	50-75 %	Moderate
(G4) 10% BSE	66.49 % ^{D}	69.36 % ^{D}	57.41 % ^{D}	72.49 % ^{D}	58.25 % ^{D}	50-75 %	Moderate
(G5) 20% BSE	77.66 % ^{E}	75.52 % ^{E}	55.49 % ^{E}	75.12 % ^{E}	77.85 % ^{E}	75-100 %	Mild
Day 15							
(G1) CG†	76.45 % ^{E}	75.34 % ^{E}	54.78 % ^{E}	77.34 % ^{E}	75.53 % ^{E}	75-100 %	Mild
(G2) 0.25% MEBO	86.34 % ^{E}	81.76 % ^{E}	30.12 % ^{C}	90.21 % ^{E}	94.32 % ^{E}	75-100 %	Mild
(G3) 5% BSE	78.51 % ^{E}	76.23 % ^{E}	44.58 % ^{C}	80.51 % ^{E}	80.72 % ^{E}	75-100 %	Mild
(G4) 10% BSE	81.94 % ^{E}	79.88 % ^{E}	44.71 % ^{C}	83.13 % ^{E}	81.44 % ^{E}	75-100 %	Mild
(G5) 20% BSE	83.22 % ^{E}	81.29 % ^{E}	40.16 % ^{C}	86.55 % ^{E}	87.62 % ^{E}	75-100 %	Mild

Notes: *Area of granulation tissue formation, angiogenesis, collagen fibers proliferation and skin epidermal regeneration were estimated in (µm). **Each value represents mean percentage of (n=12). Statistical comparison among groups: Mean values with different capital letters have significant differences at ($P < 0.05$). †: **G1:** control group (CG) topical application with vehicle (ointment base as control). **G2:** Standard ointment group, 0.25% w/w β-Sitosterol Ointment (MEBO). **G3:** 5% w/w BSE ointment group. **G4:** 10% w/w BSE ointment group. **G5:** 20% w/w BSE ointment group.

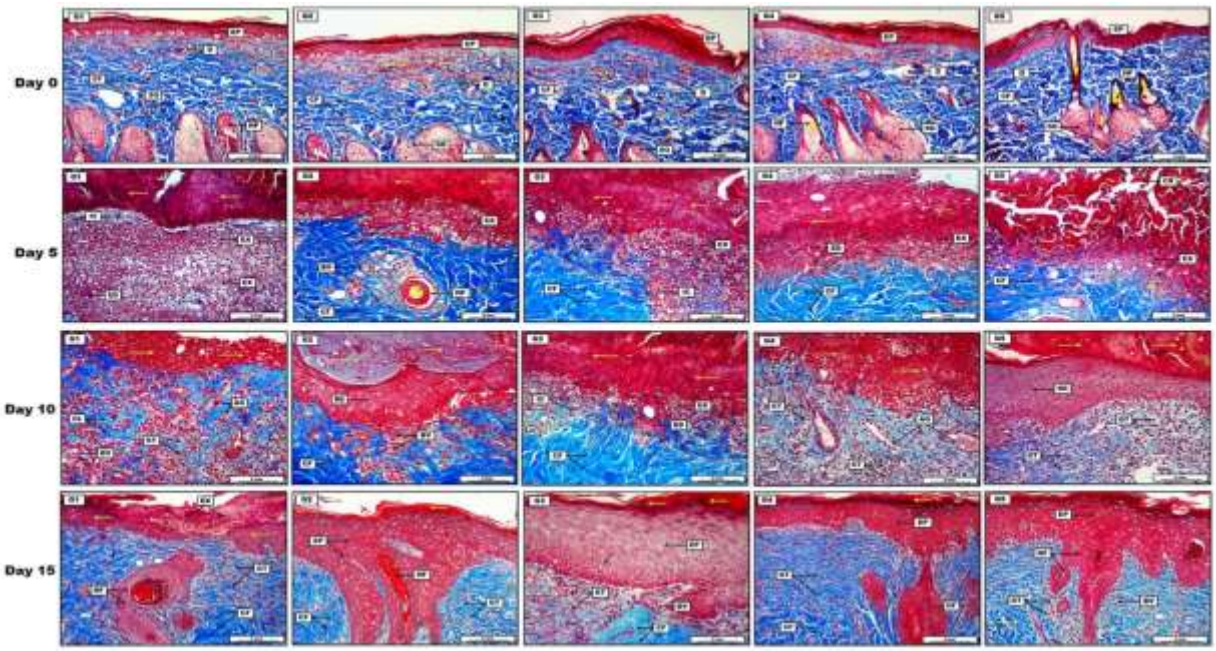

Figure 4. Photomicrograph of skin from all groups

G1: control group (CG) topical application with vehicle (ointment base as control). **G2:** Standard ointment group, 0.25% w/w β-Sitosterol Ointment (MEBO). **G3:** 5% w/w BSE ointment group. **G4:** 10% w/w BSE ointment group. **G5:** 20% w/w BSE ointment group. In different time intervals; **(Day 0, 5, 10 and 15) all stained with Masson's Trichrome stain**. **Day 0:** Skin samples in all groups show a normal arranged deep pinkish-colored epidermis (EP) with the typically distributed hair follicles (HF) within the distinctive blue-colored dermal layers (D) which consist of randomly oriented collagen fibers (CF) and of many sebaceous glands (SG) together with some congested blood vessels (yellow arrows). **Day 5:** Samples in **G1** show significant infiltration of mononuclear inflammatory cells (IC) just beneath a severely crusted mixed deep pinkish debris materials (yellow arrows), together with the presence of significant eosinophilic inflammatory exudate (EX) intermixed with deep-pinkish edematous fluid (E). In **G2**, samples show a reduction in the area of hematogenic bright pinkish crusty materials (yellow arrows), with still existing clear eosinophilic inflammatory exudates mixed with dark-pinkish inflammatory cells within the dermis, together with deposition of deep blue collagen fibers (CF) and newly formed blood vessels (BV), the section shows some clear hair follicle (HF). Skin samples in **G3, 4 and 5** display profound dissemination of loose crusty bright eosinophilic inflammatory edematous materials within the wound surface area (yellow arrows) with some areas of crusty slits mixed with a significant quantity of inflammatory exudates (EX) and inflammatory cells (CL), all sections show clear deposition of obvious blue collagenous materials (CF, CL). **Day 10:** At 10^{th} day skin samples in **G1** show significant deposition of eosinophilic cellular inflammatory exudates (yellow arrows) which interlaced with significant numbers of broadly distributed inflammatory cells (CL) with many areas of edematous fluid, the section also reveals significant distribution of granulation tissue (GT) with significant numbers of newly formed blood vessels (BV) and many areas of angiogenesis (AG), the section also shows deposition of bluish extracellular collagenous matrix (CL). Samples in **G2** demonstrate significant regeneration of bright pinkish epidermal layers (RE) with the presence of newly formed blood vessel (BV) within the dermis, the section reveals the proliferation of deep bluish collagen fibers (CF) in the dermal layer, in addition to the presence of light purplish to eosinophilic crusty inflammatory exudates over the re-epithelized area (yellow arrows). Skin sections in **G3, 4 and 5** display clear dermal granulation tissue (GT) particularly in G4 and G5 interlaced with significant numbers of newly formed blood vessels, with distributed areas of blue collagen deposition (CF) interwoven with zones of angiogenesis (AG), in which the above area of healed wound still covered with crusty pretentious inflammatory exudates mixed with widely distributed mononuclear inflammatory cells (IC). However, samples in **G5** displays clear regeneration layers of the epidermis (RE). **Day 15:** Skin sections in **G1** reveals visible structural regeneration in the epidermal layer (yellow arrows), together with the deposition of clear eosinophilic crusty inflammatory exudates (EX), with significant proliferation of collagen fibers within the dermis (CF), the section also shows apparent proliferation of granulation tissue together with newly formed hair follicles. **G2** samples at 15^{th} day of treatment show significant and profound regeneration of the epidermal layer (EP) with the deposition of bright pinkish keratin layers (yellow arrows) which results in the formation of many epidermal papillae toward the dermis. In addition, the section shows distinct proliferation of blue collagen fibers (CF) interlacing with granulation tissue formation (GT), and many newly formed hair follicles (HF). Skin tissue samples in **G3, 4 and 5** demonstrate significant re-epithelization of the epidermal layer (EP) with many layers of keratin deposition (yellow arrows), together with clear areas of granulation tissue (GT) and blue collagenous tissue distribution (CF) mingled with newly formed blood vessels (BV). Tissue samples in G5 display full epidermal growth (EP) and dermal hair follicles (HF) together with obvious bluish collagen fibers proliferation (yellow arrow). Masson's Trichrome. Scale bars: 4 mm.

Discussion

The principle outcome of this study was that the BSE-treated groups exhibited a higher wound healing rate in the macroscopical analysis in comparison with the control group (natural healing) and the group that used the commercial product MEBO containing 0.25% w/w β-Sitosterol. This finding was evident by the significant decrease in the wound surface area, higher wound rate contraction, and better quality of the wound scars of BSE-treated groups. The mean percentage of wound closure was significantly higher in the BSE groups than control and β-Sitosterol groups from 7th day, and this difference continued to the 15th day. Complete healing was achieved for 5% and 10% on 10th day of post-operation, while for the 20% BSE ointment and the other groups complete healing relatively occurred on day 15. The delay in full healing of the wound in the group that used 20% BSE ointment might be explained by a weak penetration capacity and more viscosity of the BSE 20% ointment into the surface layer of the excised skin. The beneficial effect of BSE is often attributed to its phytochemical contents, including major boswellic acids such as α-boswellic acid, β-boswellic acid, 3-acetyl-β-boswellic acid, 11-keto-β-boswellic acid, and AKBA[8]. The BAs; particularly AKBA is the most potent antibacterial component of boswellic acids obtained from the oleo gum resin of BS, they possess various antimicrobial[20], anti-inflammatory [9] and antioxidant [21], properties. During the previous years, some researches have been conducted to investigate the mechanisms of action of BSE in the context to the inflammatory process [9]. The *in-vivo* studies showed that the ingestion of the BSE decreased polymorphonuclear leukocyte infiltration and migration as well as primary antibody synthesis, [22] and led to almost total inhibition of the classical complement pathway [23]. Anti-inflammatory effect of BA has been documented in various acute and chronic rodents models of inflammation via the inhibition of cyclooxygenase and 5-lipoxygenase (LO), the key enzyme of leukotriene synthesis, [24]. In addition, Cuaz-Pérolin *et al*. reported AKBA as a natural inhibitor of the transcription factor nuclear factor kappa B (NF-kB), whose existence is required for the synthesis and function of pro-inflammatory cytokines and chemokines [25].A recent study also provided evidence for AKBA as an allosteric modulator of human 5-LO this led to the inhibition of the formation of classical 5-LO products and shifting a regiospecificity of 5-LO toward a 12-lipoxygenating enzyme. This innovative effect of AKBA has added a new and favorable pharmacological action for its profile which may be capable of converting pro-inflammatory products to anti-inflammatory ones consequently providing a new approach for clinical intervention in inflammation [26].Numerous factors contribute in the therapeutic effects of BSE and BAs. In recent years, BSE has been shown to target both the humoral and adaptive immune responses [21] eventually interfering with the inflammatory cascade [9]. The extract also ameliorates the production of different pro-inflammatory cytokines such as interleukins; IL-1, IL-2, IL-6, interferon-gamma (IFN-γ) and tumor necrosis factor- α (TNF-α). Furthermore, nuclear factor kappa B (NF-κB) has been known as a target molecule for AKBA, in Lipopolysaccharide (LPS) -stimulated human monocytes acetyl-α-BA and AKBA have inhibited the generation of TNF-α via indirect suppression of NF-κB and subsequent downregulation of TNF-α expression [27]. Thus, the rapid rate of wound closure observed in this study might be attributed to the aforementioned mechanisms. Additionally, various growth factors such as transforming growth factor beta (TGF-β), platelet activation factor, epidermal growth factor, and platelet-derived growth factors seem to be necessary for the initiation and promotion of wound healing [28]. According to the present investigation angiogenesis and collagen fibers have been promoted in the BSE-treated groups which is evident by the histopathological evaluation. Interestedly, the effect of BSE was verified in the Masson's Trichrome-stained histological sections which demonstrated a significant re-epithelialization of the epidermal layer with many collagen fiber proliferation, through a significant reduction in lesion scoring intensity from severe to moderate even to mild especially in the groups using standard ointment and 20% BSE ointment. Recently, the findings of Pengzong *et al* on the effect of the standardized extract of BS on the amelioration of diabetic foot ulcer were clarified by various organized mechanisms such as inhibition of oxidative stress and pro-inflammatory markers including TNF-α, ILs, and NF-κB, increased collagen synthesis such as hydroxyproline and collagen-1 and angiogenesis, promoting growth factors such as vascular endothelial growth factor (VEGF) and TGF-β, and inhibition of apoptosis to accelerate wound healing [11].Additionally, Bertocchi *et al*. reported the role of boswellic acid in promoting angiogenesis, this action of BA was bserved in our histopathological analysis therefore it is in line with the findings of the mentioned investigation [29]. Cellular recruitment to the wound tissue during the inflammatory phase is necessary to remove debris, dead cells, and necrotic tissue. The histopathological finding of the present study demonstrated an extreme inflammatory infiltration, many newly formed capillaries (angiogenesis) and deposition of collagenous materials in the beginning of the healing process (day 5) in the BSE treated group in concentration-dependent manner. According to the studies of Ammon [30] the presence

of α-boswellic acid in BSE has been shown to exhibit anti-inflammatory potential via inhibition of TNF-α, IL-1β, and IL-6 which might account as a contributing effect of BSE in the wound healing in the present investigation. The macroscopical finding of the present study was comparable with the histopathological analysis of the studied wounds, where 5% and 10% BSE ointment-treated groups in macroscopical presentation showed higher wound healing rate evidenced by higher contraction rate and lesser surface area and shorter re-epithelialization period in compare to the control group, this was reflected by formation of clear dermal granulation tissue, collagen deposition and angiogenesis in quantitative histological evaluation in concentration-dependent manner. As far as is known, the present study is the first of its kind to highlight the use of different concentrations of boswellic acid as a topical application in accelerating the wound healing.

Conclusion

This study provided macroscopical and microscopical evidence for the topical application of BSE ointment in the acceleration of the wound repair in a concentration dependent manner. Although the highest concentration in macroscopical analysis exhibited lesser efficacy. We suggest that BSE in the studied concentration can be topically used as an alternative therapeutic option for the treatment of cutaneous excision wounds.

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Conflict of Interest

The authors declared no conflicts of interest.

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Ethics Statements

The protocol of this experiment was registered and approved by the Ethical Committee of the College of Pharmacy, University of Sulaimani (Registration number: PH22-21 on 31 August, 2021).

Author Contribution

B.H.M. participated in conceptualization, study design, interpretation of the data, data analysis, writing the first draft, and revised the manuscript. D.O.I. participated in the practical work, statistical analysis, interpretation of the data and review the manuscript. Go..M.R; contributed in histopathological procedure and interpretation, review the manuscript, and revised the manuscript. All the authors have read and approved the submitted version of the manuscript.

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تأثير التركيزات المختلفة المستخدم موضعياً لمستخلص Boswellia Serrata بسويليا سيراتا في التئام الجروح الاستئصالية في الجرذان

بشرى حسن معروف *[1]، دانا عمر اسماعيل[2] و گوران محمد رؤوف عبدالقادر[3]

[1] فرع الادوية والسموم، كلية الصيدلة، الجامعة السليمانية، السليمانية، كردستان، العراق.

[2] فرع الجراحة والتوليد، كلية الطب البيطري، الجامعة السليمانية، السليمانية، كردستان، العراق.

[3] فرع علم الامراض والطب الشرعي، كلية الطب، الجامعة السليمانية، السليمانية، كردستان، العراق.

الخلاصة

يُعرف الجرح بأنه أحد المشكلات الصحية الجادة. نبات البسويليا سيراتا Boswellia Serrata يحتوي على مجموعة كبيرة من المواد الكيميائية النباتية ذات الخصائص الدوائية المختلفة. تهدف الدراسة إلى تقييم إمكانية مستخلص بسويليا سيراتا Boswellia Serrata Extract (BSE) في التئام نموذج تجريبي لجرح استئصالي في الجرذان. تم استخدام ما مجموعه ٤٥ جرذا في خمس مجموعات متساوية في هذا البحث. تم إنشاء جرحين لكل جرذ كنمط دائري متماثل في المنطقة الظهرية العنقية. تمت معالجة جميع المجموعات على النحو التالي: في مجموعة التحكم تم استخدام الفازلين (قاعدة المرهم)،و في مجموعة بيتا سيتوستيرول تم استخدام مرهم يحتوي على بيتا سيتوستيرول ٠,٢٥٪ ، و ثلاث مجموعات سميت بمجاميع BSE؛ التي عولجت بتركيزات مختلفة من مرهم BSE منها يحتوي على ٥٪، ١٠٪، و٢٠٪ حمض البوسويليك (BA) على التوالي. وتم متابعة الجروح لمدة ١٥ يوما. وفي الأيام ٥، ١٠، ١٥، تم قتل ثلاثة جرذان من كل مجموعة، وبعدها تم ارسال الجروح والندوب لدراسة الانسجة الطبية. أدت المراهم التي تحتوي على ٥٪ و ١٠٪ BA إلى زيادة ملحوظة في معدل تقلص الجرح وتقليل مساحة سطح الجرح وتقصير فترة إعادة تكوين الجلد في التحليل بالعين المجردة مقارنة بمجموعة التحكم ومجموعة بيتا سيتوستيرول. بينما، أظهرت النتائج المجهرية تقدمًا كبيرًا في الالتئام بشكل تدريجي معتمدا على تركيز المرهم. وقد انعكس ذلك من خلال تكوين أنسجة حبيبية جلدية واضحة، وترسيب الكولاجين وتكوين الأوعية الدموية في التقييم النسيجي الكمي. في الختام، قدمت هذه الدراسة الأدلة العيانية والمجهرية على فعالية استخدام الموضعي لمرهم BSE وتاثيره في التئام الجروح بشكل تدريجي معتمدا على تركيز المرهم. في حين أن أعلى تركيز لحمض البوسويليك في التحليل العياني أظهر أقل فعالية.

الكلمات المفتاحية: حمض البوسويليك، إعادة تكوين الجلد، الجرح الاستئصالي، التئام الجروح.