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The role of aloe vera gel, propolis ointment, and aloe vera with propolis gel to improve the burn healing in rabbits: A Comparative study

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Abstract

Propolis and Aloe vera gel have seen widespread clinical application since the approval for wound coverage by the FDA. They were because of properties that promote wound healing and their efficient cost in manufacturing. Therefore, the present study aimed to assess the efficacy of the Propolis and Aloe vera gel on burn wound healing in rabbit models. It was carried out on 20 adult male rabbits divided into 4 groups. Four burn wounds were applied to the deep third-degree burns on the right side of each animal. The first animal group was control (COL group); the second was treated with Aloe vera gel (AVG group); the third was therapy via Propolis ointment (PO group), and the last was lubricated with 1/1 mixture of Propolis with Aloe vera Gel (AVPG) as (AVPG group). The treatments were repeated once daily until complete healing. For four days of burns, the percentage of wound contraction was assessed. The results showed that the AVPG significantly accelerated wound healing activity compared to wounds dressed with AVG and the control burn wounds. However, the level of wound contraction was significantly higher, and the healing time was faster in the AVPG group than in the other groups. The mean epithelialization percentage of burn obtained in days were respectively 76.46±3.2 (AVPG group), 76.25±3.4 (AVG group), and 70.42±3.9 (COL group) with the inferiority of the PO group 68.75±5.3. However, micropathological examination revealed that the pathological signs showed wound healing in the AVPG, AVG, and PO was better than in the COL group. A complete healing process was observed in the treated burns group, where complete re-epithelialization was coated in all epidermis layers. We conclude that the AVPG can be used as an adjunctive or alternative agent to existing wound healing therapies in the future.

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Introduction

The skin is the layer of usually soft, flexible outer tissue covering the body of a vertebrate animal, with three major jobs; protection, regulation, and sensation (1). It protects the several layers of epidermal tissue and protects the soft tissues, muscles, ligaments, and internal organs, and hard tissue, bones, which underlie it (2). When it is defective, several microorganisms invade; the wound will easily infect, stimulating the tissue's immune response. Repair healing includes activating immune cells, cytokines, and growth factors. Therefore, the first aim of cutaneous wound healing is to return the damaged morphological structure and its function, which is mainly connected with therapy and management that regulates wound healing or returning tissue healthy (3). Wounds remain a clinically big challenge, with initial and terminal problems donating a recurrent effect of diseases and deaths (4). As a method to diminish the wound load, many attempts have been made to determine the healing physiology and injury care to ensure new therapeutic styles and the remaining development of technologies for severe and wound management in the long term (5). Therefore, the

main aim of cutaneous damage healing is to restore normal structure and function it is mainly supplemented by therapy and management, which binds wound healing rather than returning the tissue to health (3). Medicinal plants' advantage in treating injuries at various phases is common and nearly all classic medical systems worldwide. Significant potential for qualitatively improving and enhancing wound healing has appeared in numerous herbal-based medicines (6,7). Alternative therapeutics are based on natural elements. They have hypothesized that the heterogeneous structure of Aloe vera gel may provide them with treatment and pharmacological properties (8). There are several substances bioactive with medical and nutritious properties that were used as biomaterials which may reduce pain and accelerate/improve the healing in the juice of Aloe vera leaves (9), mentioned possibly using Aloe vera such as antimicrobial, anti-virus, and anti-inflammatory substances. Also, several studies mentioned that Propolis was used to treat skin injuries because of a higher percentage of wound healing than classic interventions. The propolis applications were developed by several studies that combined the Propolis with other materials to create synergistic effects in the regenerative skin process (10,11).

Therefore, our study suggested a new mixture of these classic compounds to study the medical characteristics of this combination.

Materials and Methods

Ethical approval

The work was approved by Kufa University's Faculty of Veterinary Medicine's Scientific Ethic Committee (no: 2043/ Aug 29, 2024).

Animals

Twenty adult male healthy New Zealand rabbits were used in this study. The average age was between 4 and 6 months old, and the body weight was 1.5 - 2.25 kg. They were kept in standard cages at 20-25°C in the Faculty of Veterinary Medicine laboratory house of Kufa University. The feed was mixed with pellets, vegetables, and ad libitum water. These rabbits were randomly divided into 4 groups.

Preparation of aloe vera gel (AVG)

The fresh Aloe vera gel was made (12). The Aloe vera leaves were collected from the local garden. These leaves were cleaned with fresh water, and the thick external greenish layer was carefully removed. The internal gel was isolated by a sterile spatula and retained at room temperature in a sterile ceramic dish until used.

Preparation of propolis ointment (PO)

The crude Propolis was isolated from honeybees in the Kufa region of Iraq. The extracted Propolis was prepared by " The raw Propolis collected from different apiaries of Najaf city in Iraq. Propolis was macerated with 70% ethanol alcohol to extract pure Propolis. This mixture was filtrated by filter paper and concentrated in a water bath at 50' (13). However, the Propolis ointment was made by mixing 50% of the extracted Propolis with 50% paraffin ointment (Vaseline) and retained at room conditions in a sterile ceramic dish until used.

Preparation of aloe vera with propolis gel (AVPG)

The extracted Propolis was prepared (13), and it was combined with 50% of Aloe vera Gel and retained at room conditions in a sterile ceramic dish until used.

Experimental design

All animals were prepared by clipping and shaving their hair of the right side with applied 70% Alcohol antiseptic solution and anesthetized generally via ketamine (30 mg/kg) (Fabriue par: KEPRO B.V.-Maagdenburgstraat 38-7421 ZE Deventer-Holland/Pays-Bas) and Xylazine (3 mg/kg) (XYLAZINE 20%; alfasan, WOERDEN-HOLLAND) intramuscularly injection (14,15). Also induced the four burns with a stainless-steel metal rod (2cm×2cm square the transverse surface) was placed in boiling water to heat to 100°C. This degree is enough for the third degree of burn (16). Rabbits are divided randomly into four groups as follows; Group I (AVG group) Five rabbits were treated with Aloe vera gel for one week, the first time daily. Group II (PO group) Five rabbits were treated with Propolis ointment for one week, the first time daily. Group III (AVPG group) Five rabbits were treated with Aloe vera and Propolis gel for one week, the first time daily. Group IV (COL group) Five rabbits were left without treatment as the control group. The therapy was placed topically with a sterilized spatula immediately following the burning protocol. It was returned daily for the first time for one week until epithelialization occurred. The size of the burn lesion was calculated by the digital caliper every seven days. The epithelial lesion period was measured daily until it reached the epithelialization point where the scab formed (17,18). The epithelialization of burn lesion was calculated by the contracted percentage of it, as described via the formula: Percent is [(initial lesion size - daily lesion size) / initial lesion size] ×100 (Figure 1).

Histopathological examination

The cutaneous biopsy was taken weekly after the scarification formation of the histopathological section for three weeks post-burning. Biopsies were fixed in 10% neutral buffered formalin for 72 hours, dehydrated, cleared in xylene, and embedded in paraffin wax using routine methods. The sections were re-hydrated and stained using Masson's trichrome stain to examine under a light microscope. The descriptive means of wound healing assessment was used depending on (19). Also, the Kruskal-Walli's test (is a non-parametric statistical test for testing whether samples originate from the same distribution and is

used for comparing two or more independent samples of equal or different sample sizes. The parametric equivalent of the Kruskal-Walli's test is the one-way analysis of variance ANOVA) to wound healing assessment as described (20).

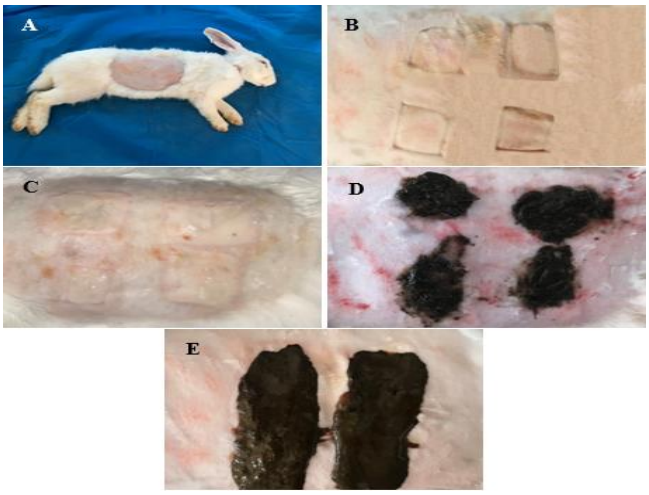

Figure 1: Groups of study.

Statistical analysis

The statistical analysis system SAS (2004) was used to affect study factors in traits. All data were expressed as Mean ± Standard Error (M±SE), and differences among the groups of animals were compared using one-way Analysis of Variance (ANOVA). The least significant difference (LSD) was used to compare means significantly. The level P<0.05 was considered to be significant.

Results

Table 1 shows no significant differences between first and second weeks in the COL and AVPG groups. However, they recorded the difference significance in the third week, and both AVG and PO groups documented significant differences in the second week. While the total means demonstrated superiority with closely the grades of AVG and AVPG groups 76.25±3.4 and 76.46±3.2 respectively compared to other groups, then came the COL group with 70.42±3.9 grade, and the last level was the PO group with 68.75±5.3 grade. Burn wounds in the COL group exposed high inflammatory signs. Three findings of the inflammatory signs (swelling, local fever, and redness, which showed the mildness of treated burn wounds with AVPG, AVG, and PO) represented this. Micropathological examination enhanced wound healing in the AVPG, AVG, and PO groups compared with the COL group (Table 2 and 3). The previous table showed an improvement in the assessment of wound healing in all treated groups in the COL group. It also demonstrated the superiority of the AVPG group over other groups, with the score grades of both AVG and PO groups closed.

Table 1: The epithelialization percentage of burn lesions was three weeks later

Group	COL	PO	AVG	AVPG
1st W.	59.38±5.14 A	50.625±7.7 A	63.75±4.3 A	67.5±4.9 A
2nd W.	68.125±5.5 A	69.375±5.1 B	77.5±1.8 B	76.25±3.6 A
3rd W.	83.75±2.9 B	86.25±3.8 B	87.5±3.5 B	85.625±4.8 B
Mean ± SD	70.42±3.9	68.75±5.3	76.25±3.4	76.46±3.2

The capital letter means the difference is significant among different groups P≤0.05.

Table 2: Descriptive means of wound healing assessment

Group	COL	PO	AVG	AVPG
Mean	5.33	6.67	7.00	9.67[a]
± SD	0.58	1.15	1.00	0.58

Table 3: Ranks of wound healing assessment

Group	COL	PO	AVG	AVPG
Mean Ranks	2.50	5.83	6.67	11.00

Likewise, the Kruskal-Walli's test demonstrated a greater mean rank of the AVPG group 11.00 in all groups, with closely both AVG and PO groups 6.67 and 5.83, respectively, superior to the COL group significantly 2.5 mean rank. In figure 2, the histopathological examination of the COL group in the first week exposed the inflammatory layer consisting of dead inflammatory cells (neutrophils), epithelial cells, and clots covering the wound area and attached to the dermis layer. Also, inflammatory cells were observed between the inflammatory and dermis layers and within the dermis layers. In 2nd week, the inflammatory layer was observed in the wound area with severe infiltration of inflammatory cells in the dermis layer of the wound area (Figures 3). Additionally, the absence of an epidermis layer is due to the failure of the re-epithelialization process to restore the epidermis layer and the severe infiltration of inflammatory cells, which occupy spaces in the collagen fibers of the dermis layer. In the 3rd week, the new epidermis layer was observed in the wound area. However, the new epidermis layer didn't cover wound area (Figures 4).

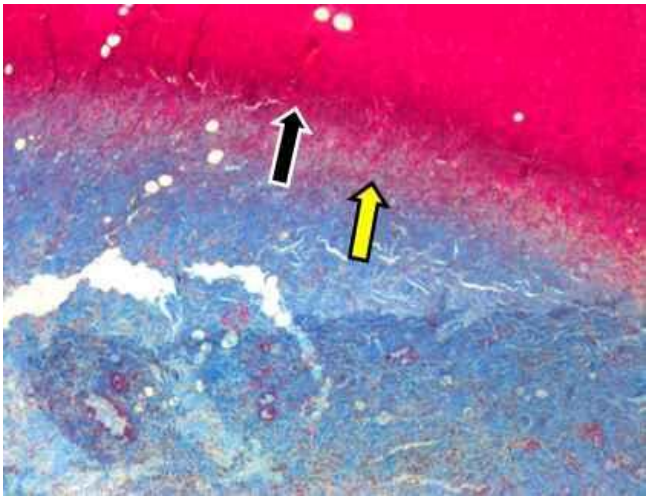

Figure 2: Photomicrograph of rabbit skin 1st week-post injury of COL group. The inflammatory layer (black arrow) consists of dead inflammatory cells, epithelial cells, and clots, covering the wound area and attached to the dermis layer. Also, inflammatory cells (yellow arrow) were observed between the dermis and dermis layers. MTS. 40X.

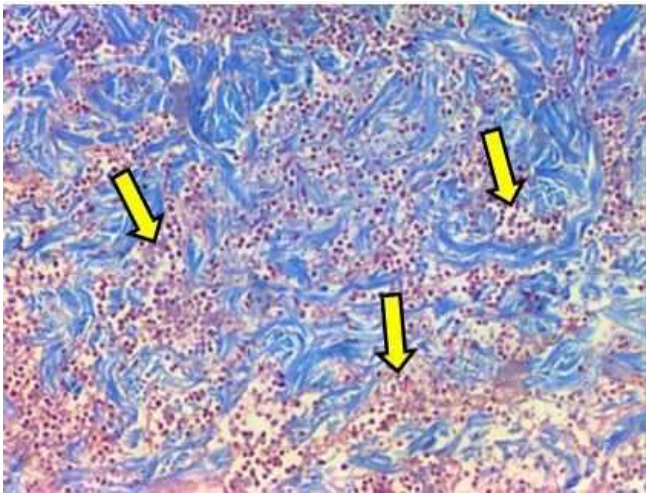

Figure 3: Photomicrograph of rabbit skin 2nd week-post injury of COL group. Severe infiltration of inflammatory cells (yellow arrow) that occupied spaces in collagen fibers of the dermis layer. MTS. 100X.

In the first week of the PO group, the inflammatory layer involved dead inflammatory cells, epithelial cells, and clots covering the wound area, which were attached to dermis layer (Figures 5). In the second week, a new epidermis layer was observed in the wound area, initiated from the wound margins due to the early re-epithelialization process and extended to the wound center. As well as inflammatory layer residue above the new epidermis layer (Figures 6). The third-week period revealed that the new epidermis layer covered all wound areas. In contrast, the new epidermis layer involved a complete growth pattern due to the success of the re-epithelialization process to form all layers of the epidermis. Also, inflammatory cell numbers were limited in the dermis layer below the epidermis layer (Figures 7).

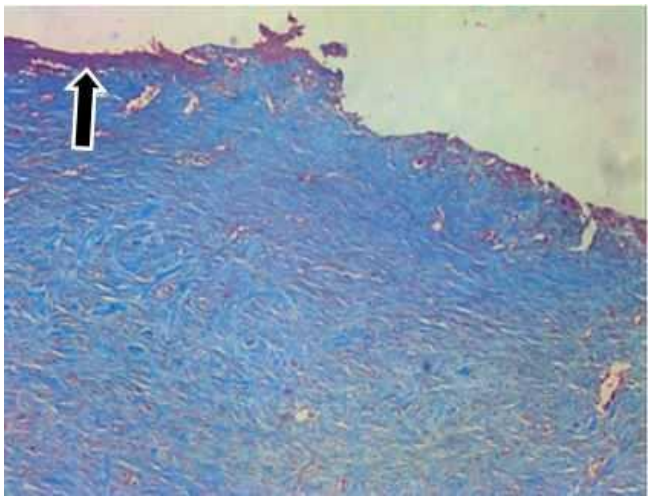

Figure 4: Photomicrograph of rabbit skin 3rd week-post injury of COL group. A new epidermis layer (black arrow) was observed in the wound area; however, the new epidermis layer did not cover the wound area. MTS. 40X.

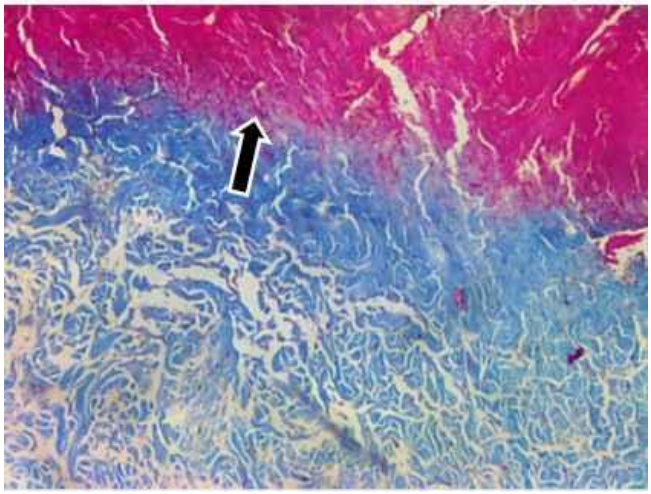

Figure 5: Photomicrograph of rabbit skin 1st week-post injury of PO treated group. The inflammatory layer (black arrow) consists of dead inflammatory cells, epithelial cells, and clots, covering the wound area and attached to the dermis layer. MTS. 40X.

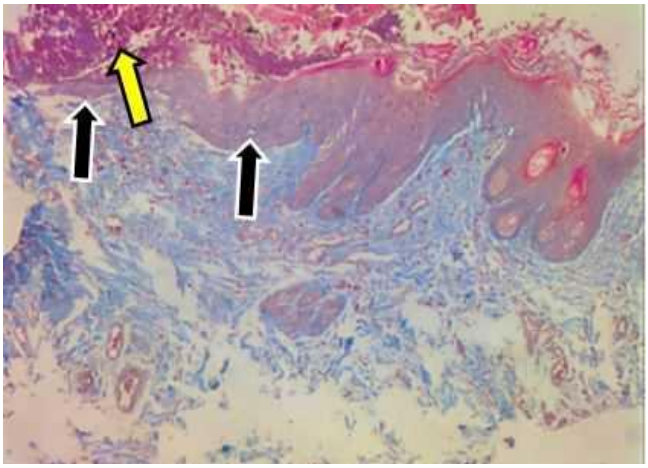

Figure 6: Photomicrograph of rabbit skin 2nd week-post injury of PO treated group. A new epidermis layer (black arrow) was observed in the wound area, initiated from the wound margins due to the early re-epithelization process, and extended to the wound center. Note the inflammatory layer residue (yellow arrow) above the new epidermis layer. MTS. 40X.

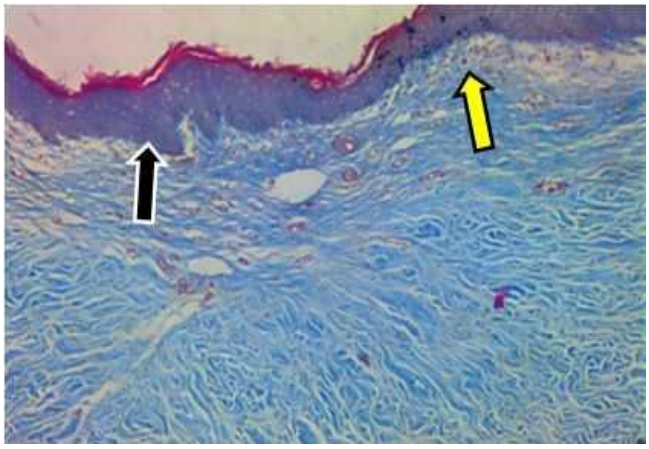

Figure 7: Photomicrograph of rabbit skin third week-post injury of PO treated group. The new epidermis layer (black arrow) covered all wound areas. In contrast, the new epidermis layer showed a complete growth pattern due to the success of the re-opertization process in forming all layers of the epidermis. Note the limited number of inflammatory cells (yellow arrow) observed in the dermis layer below the epidermis layer. MTS. 40X.

The inflammatory layer of the AVG group in 1st period consisted of dead inflammatory cells (neutrophils), epithelial cells, and clots covering the wound area and attached with dermis layer (Figures 8). Also, inflammatory cells in the dermis layer were observed. However, in 2nd period, a limited re-epithelialization process was observed in the margin of the wound area; however, the new epidermis layer did not extend to the center of the wound area (Figures 9). As well as an inflammatory layer attached to the wound area's dermis layer. At the same time, the third period showed a thin new epidermis layer that covered the wound area. However, this epidermis layer did not show a complete growth pattern due to the failure of the re-epithelialization process to form all layers of epidermis (Figures 10).

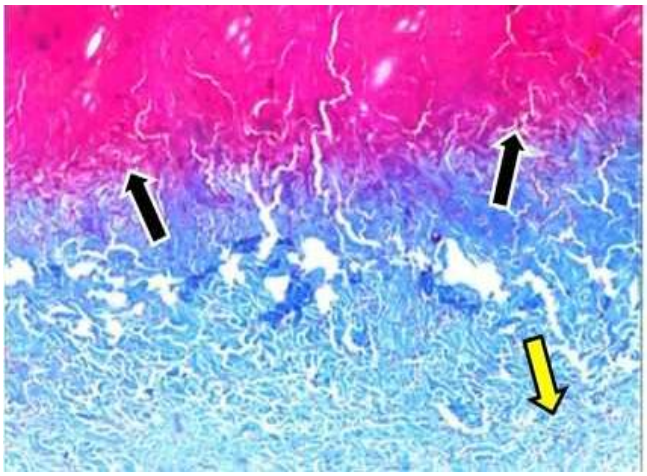

Figure 8: Photomicrograph of rabbit skin 1st week-post injury of AVG treated group. The inflammatory layer (black arrow) consists of dead inflammatory cells, epithelial cells, and clots, covering the wound area and attached to the dermis layer. Also, the dermis layer's inflammatory cells (yellow arrow) were observed. MTS. 40X.

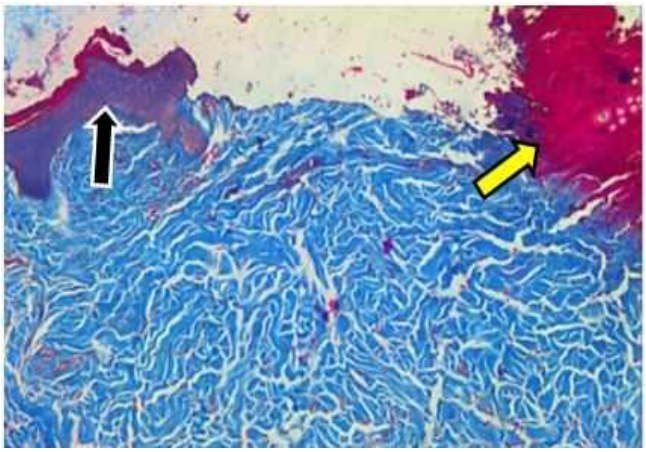

Figure 9: Photomicrograph of rabbit skin 2nd week-post injury of AVG treated group. A limited reepithelization process was observed in the margin of the wound area; however, the new epidermis layer (black arrow) did not extend to the center of the wound area. Note the presence of an inflammatory layer (yellow arrow) attached to the wound area's dermis layer. MTS. 40X.

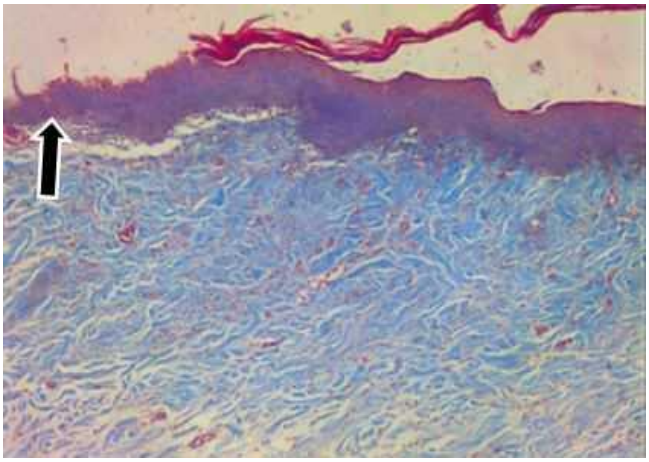

Figure 10: Photomicrograph of rabbit skin third week-post injury of AVG treated group. A thin new epidermis layer covered the wound area; however, this epidermis layer did not show a complete growth pattern due to the failure of the epithelialization process to form all layers of the epidermis. MTS. 40X.

The first period of the AVPG group also suffers from inflammatory processes within the dermis layer (Figures 11). Furthermore, 2nd, week recorded moderate epithelialization at the wound area and formed the early epidermis layer in all the pictures of the pathological section equally, with the inflammatory form (Figures 12). In the third period, the pathological sections emerged as a complete epidermis layer that covered the wound area and formed the primary hair follicles in dermis layers (Figures 13).

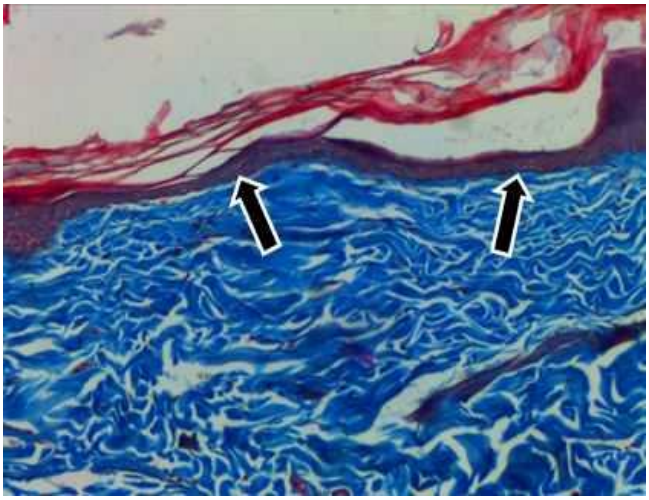

Figure 11: Photomicrograph of rabbit skin 1st week-post injury of AVPG treated group. The inflammatory layer (black arrow) consists of dead inflammatory cells, epithelial cells, and clots, covering the wound area and attached to the dermis layer. MTS. 40X.

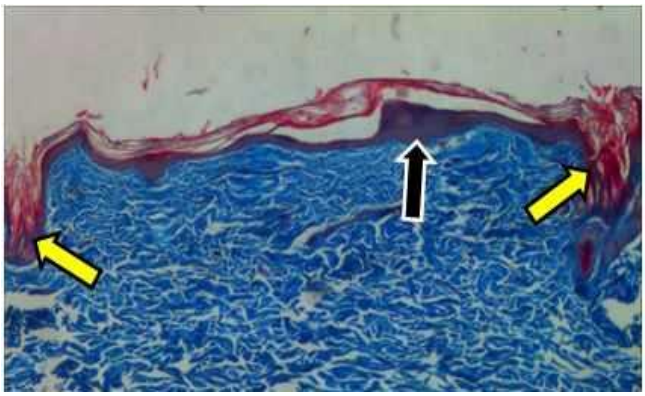

Figure 12: Photomicrograph of rabbit skin 2nd week-post injury of AVPG treated group. The new epidermis layer (black arrow) covered all wound areas. In contrast, the new epidermis layer showed a complete growth pattern due to the success of the re-opertization process in forming all layers of the epidermis. Note the limited number of inflammatory cells (yellow arrow) observed in the dermis layer below the epidermis layer. MTS. 40X.

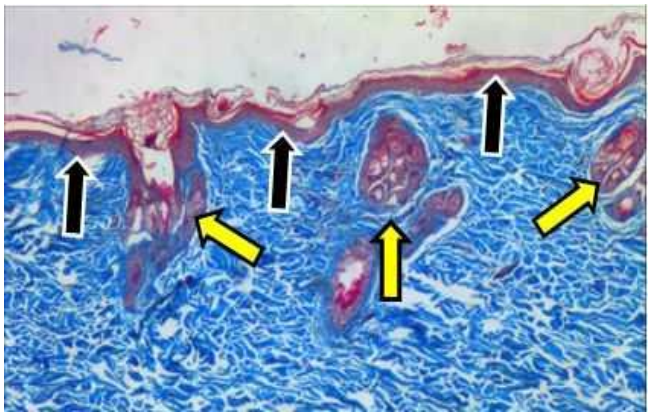

Figure 13: Photomicrograph of rabbit skin 3rd week-post injury of AVPG treated group. The new epidermis layer (black arrow) covered all areas. In contrast, the new epidermis layer showed a complete growth pattern due to the success of the re-opertization process in forming all layers of the epidermis. Note the presence of hair follicles (yellow is observed in the dermis layer below the epidermis layer). MTS. 40X.

Discussion

In the present study, burn healing via secondary wound healing is chosen as it looks like a clinical disorder that recurrently occurs in cases of skin burns in animals. The study period is limited to three weeks because the burned skin of rabbits is healing completely or closely via the terminal of this study period. The epithelialization percentage of burn lesions in total mean registered more

superiority at the grade of contraction in both AVG and AVPG groups compared with the COL group. This could result from Aloe Vera's ability to stimulate growth factors, also improving cell proliferation (9). This was described by (21), who said the Aloe vera gel contains substances that act as antiseptics and accelerate the growth of fibroblasts, which can help wound healing through the interaction of the growth receptors on fibroblasts and stem cells, which stimulates their proliferation and synthesis of collagen.

Furthermore, the present study results showed that the AVG and AVPG groups showed a significant difference in contrast with the PO group, which developed burn zone contraction and decreased epithelialization time. The treatment of burned wounds via Aloe vera and Propolis in mice and rabbits, respectively, contrast with the control group since 2nd week period while this group needed to another week for recorded the significant difference ($P<0.05$) at healing period due to the Aloe vera capability to stimulate production of collagen from the proliferative fibroblasts. At the same time, the Propolis has antiseptic and bioactive materials that accelerate releasing the growth factors, such as FGF and PDGF, by the migrated cells into the injury, mainly by fibroblast cells, which were dependable for the remodeling of the matrix in the injured area (22-24).

The pathological section recorded the proliferation of inflammatory cells in most layers (inflammatory, dermis, and epidermis layer) because the burn wounds distracted the physical skin barrier, allowing the infected microorganism to invade the affected tissue. Therefore, the healing was delayed in the COL group in the first- and second-week periods, and a thin new epidermis layer just appeared in the last period, which conformed to demonstrate (18) when they induced burn wounds in a rabbit model.

While the PO group sections appeared as the inflammatory layer in the first week, they also contained dead neutrophils, epithelial cells, and clots covering the wound area and attached to the dermis layer as a result of the Propolis had anti-inflammatory (25), antimicrobial and biologically active elements which accelerated burned wound healing, microbial blocking and avoidance of inflammation (26,27). Therefore, the 2nd and 3rd periods of PO sections recorded the new epidermis layer covering all wound areas with a complete growth pattern due to the success of the re-epithelialization process to form all layers of the epidermis as well as the presence of limited numbers of inflammatory cells (lymphocytes) that observed in dermis layer below the epidermis layer (28).

However, the sections of the AVG group revealed the new epidermis layer initiated since 2nd week with limited re-epithelialization process in the margin of the wound area, as well as the absence of an inflammatory layer that attached to the dermis layer of the wound area, which continued until third week as a thin new epidermis layer that covered the wound area without completed growth pattern due to failure of re-epithelialization process to form all layers of the epidermis. Also, proved the correction of this data when treating 2nd-degree burned wounds in male rabbits via fresh Aloe vera gel (29,30).

When the AVPG sections were examined, the percentage of re-epithelialization was more than the other groups with acceleration of the burn healing at three sectional periods until the terminated experimental time, which rebounded its structure to the semi-natural design of normal skin by creating the blood vessels, hair follicles, and waved epidermal layers that may be occurred due to accumulation of the compounded elements for both Propolis and Aloe vera gel which made synergistic effects.

Conclusion

Our study demonstrates the efficiency of fresh Aloe vera gel in treating burn wounds with enhancement and acceleration of wound healing by repairing damaged tissue without the capacity of pathogenic microorganisms to invade the affected tissue. Also, the Propolis ointment demonstrates its classical ability to heal burned wounds. However, their combination (Propolis /Aloe vera gel) generated the synergistic effect that created regenerative burn healing.

Acknowledgments

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Conflicts of interest

According to the authors, there is no conflict of interest.

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دور هلام الصبار ومرهم العكبر وهلام الصبار مع العكبر لتحسين التأم الحروق في الأرانب: دراسة مقارنة

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الخلاصة

لقد شهد العكبر وهلام صبار الالوفيرا تطبيقًا سريريًا واسع النطاق منذ الموافقة على تغطية الجروح من قبل إدارة الغذاء والدواء. كان ذلك بسبب خصائصه في تعزيز التئام الجروح وتكلفته الفعالة في التصنيع. ولذلك تهدف الدراسة الحالية إلى تقييم مدى فعالية هلام العكبر مع الصبار في التئام جروح الحروق المستحدثة في نموذج الأرنب. نفذت التجربة على ٢٠ أرنباً ذكراً بالغاً قسمت عشوائياً إلى ٤ مجموعات. حيث استحدثت أربع جروح (حرق عميق من الدرجة الثالثة) على الجانب الأيمن لكل حيوان. كانت مجموعة الحيوانات الأولى هي مجموعة السيطرة؛ والثانية عولجت بهلام الألوفيرا والثالثة عولجت بمرهم العكبر والأخيرة دهنت بـ ١/١ خليط من العكبر مع جل صبار الالوفيرا. كررت العلاجات مرة واحدة يومياً حتى الشفاء التام لمدة أربعة أيام بعد استحداث الحروق، حيث قيمت نسبة تقلص الجرح. أظهرت النتائج أن مجموعة الالوفيرا والكعبر سرع بشكل ملحوظ نشاط التئام الجروح مقارنة بالجروح المعالجة بالكعبر وحروق السيطرة. ومع ذلك، كان مستوى تقلص الجرح أعلى بكثير كما ان وقت الشفاء كان أسرع في مجموعة الالوفيرا والكعبر مقارنة بالمجموعات الأخرى. وقد كان متوسط نسبة نمو الظهارة للحروق بعد الشفاء خلال التجربة على التوالي: ٣,٢±٧٦,٤٦ الى مجموعة الالوفيرا والكعبر، و ٣,٤±٧٦,٢٥ الى مجموعة الالوفيرا، و٣,٩±٧٠,٤٢ لمجموعة السيطرة مع هبوط لمجموعة الكعبر الى مستوى ٥,٣±٦٨,٧٥. ومع ذلك كشف الفحص المرضي الدقيق أن العلامات المرضية أظهرت أن شفاء الجروح المجاميع الثلاثة المعاملة كان أفضل مقارنة مع مجموعة السيطرة. في حين لوحظت عملية الشفاء الكاملة في مجموعة الالوفيرا والكعبر حيث تمت إعادة التشكل الظهارة الكامل لتغليف جميع طبقات البشرة. نستنتج أنه يمكن استخدام الالوفيرا والكعبر كعامل مساعد أو بديل لعلاجات التئام الجروح الموجودة في المستقبل.