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Enhancing Surgical Wound Healing: A Synergistic Approach Using Coated Vicryl and Quercus Infectoria Galls in a Rat Model

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Abstract

OBJECTIVE: taking care of wound healing, and warning of surgical line infection are important concern after surgical operation. Quercus infectoria Olivier Galls (Q.infectoria) stimulates healing of cutaneous wound through involvement in granulation tissue formation, collagen deposition and tissue remodeling by different way of application. Material and Method: eighteen adult healthy male rats with 200-300 gm of weight were used. Longitudinal skin incisional wounds were formed on the back region of each rat, they were divided into two groups; control group (n=9 with 3 replication for each duration) were subjected to suturing through the subcuticular closure with Vicryl, while treatment group (n=9 with 3 replication for each duration) were sutured with Vicryl suture that impregnated within the *Q.infectoria extract* solution. Result: The rate of wound contraction was faster in the treatment than the control group. Histopathological changes on 0, 7th and 14th days showed significant difference between control and treatment group depending on the rate of angiogenesis, intensity of granulation tissue and epidermis re-epithelization, and the amount of collagen fibers which were higher in the treatment group than control group. Conclusion: Coating Vicryl suture with *Q.infectoria* extract solution enhances the cutaneous wound healing process in the animal model and reducing post-operative complication and can be used as effective medical agent to inhibit the development of inflammations.

Keywords: Olivier, Gall, Wound, Vicryl, Rat

Introduction

Post-operative incision care determines effective results for patients after surgery. Focusing on lowering the risk of infection and related problems should be the highest priority for all clinicians taking care of surgical incision wounds [1]. Surgical incision healing is a systemic process which starts with an injury and continues with a numerous of physiologic responses that will play important roles in the ability of the wound to heal. Different tissues have their own normal rates of growth during the process of healing [2]. Inflammation, destruction, proliferation and maturation are four distinct stages of wound healing [3]. In order for a wound to heal all four steps must occur in the correct order and time span. Many factors play a role in interruption one or more phases of this process, resulting in inadequate or delayed wound healing include oxygenation, infection, age, stress, diabetes, obesity, medications, alcoholism, smoking and diet [4]. *Quercus infectoria* is one of the most important medicinal plants, traditionally used in treatment of different ailments. This plant is a short tree or shrub its height is two meters and is found mainly in Asia, Greece and Iran. When stinging gall wasp (*Adleria gallae-tinctori)* attacks the oak tree lead to development of round abnormal growth on the young braches that are the galls (*Adleria gallae-tinctori) [5, 6]*. The ancient women believed that Q. infectoria galls, have been

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important to exhibit the most anti-inflammatory, antibacterial, and antifungal activities [7], and it has a magic fruit especially for gynecology [7, 8]. It has been mixed with different herbs as medication given after child delivery to recover the uterine *wall* [9]. Antimicrobial agents, which are widely used today, and oral bacteria have significant impact on increasing resistance to common antibiotics. So the galls can be considered as anti-infective herbal compounds for the inhibition of oral pathogens [10]. The plant is also a play important role in the treatment of tropical parasitic diseases such as blastocystis and amebiasis when methanol extract of *Q. infectoria* which inhabit 76% and kill 67% of B. hominis that cause blastocystis [11].

Subcuticular skin closure with suture compare to staple closure after incisional wound has been shown to result in lower rates of wound complications than with staple closure [12]. Vicryl are common suture materials used for skin suture during surgery. It has advantages such as avoidance of pain which caused by suture removal and tissue damage [13]. The aim of this study was to evaluate the potential use of vicryl plus *Quercus infectoria* Olivier and compare rate of wound complications with vicryl alone in subcutaneous skin suturing in a rate model.

Material and Methods

Animals Model and Experimental Design

Eighteen adult healthy male rats (200-300 g) were housing in a cage of identical environmental condition at Veterinary Teaching Hospital belonging to College of Veterinary Medicine at university of Sulaimani. They were kept in a 12 hrs light/ dark cycle at 22 ± 2EC, with unlimited access to water and food.

Ethical Approval

All the procedures and approaches of this study were conducted and approved according to the principles of the ethics by the college of the veterinary medicine research committee, University of Sulaimani, Kurdistan Regional Government, Kurdistan/ Iraq.

Extraction and Fractionation of Quercus infectoria (Olivier) Galls

The galls had been grinded into small portions with a sterile pestle and electric powered grinder. 100 mg of yellow powder had been soaked for seventy-two hours in 500 ml of sterile distilled water. Then, the aqueous result was boiled for 30 minutes' period at boiling point. By a Buchner funnel using Whatman No.1 filter paper the extract was filtered, then under decreased pressure and managed temperature (40-60 °C) by rotary evaporator it was concentrated to the level of semi dryness, the result was a mild brown colored residue. Then incubated in a clean Petri dish throughout the entire night at (37°C) to attain approximately 25% yield of crude extract. Then stored at (-4°C) in air tight glass bottles and used for the duration of 1-5 days. *Quercus infectoria* Olivier solution (treatment solution) was prepared by adding 5 mg of crude extract with 5 ml of distilled water then Vicryl suture material with size (0-3) immersed into treatment solution for 2 hours (figure 1: B), so that used to suture incisional wound by subcuticular closure to each rats in treatment group.

Anaesthesia Procedure

Rats were anesthetized with intraperitoneal injection of xylazine (5mg /kg) and ketamine (50 mg/kg). The back region was clipped of excess hair and routinely prepared aseptically for surgical wound. Longitudinal incision (2-3 cm) was created of the skin on the back region in each rat with scalpel as presented in (Figure 2). Rats were randomly allocated into control group and treatment group with n=9 and 3 replications for each duration. In the control group, the longitudinal incisions were sutured with subcuticular closure using vicryl suture (0-3) alone, while in the treatment group, the incision wounds were sutured with subcuticular closure using vircyle (0/3) impregnated within the *Q. infectoria* extract solution. Then the incision wounds were evaluated grossly and microscopically in both groups (Treatment & Control group) at 0, 7 and 14th and 28th days after the operation.

Morphological Evaluation

Digital photographs of rat's longitudinal incisions wound were taken on 0, 7, and 14th and 28th days after operation. The time at which the wound bed was filled with new tissue and completely healed, the wound closure grossly was distinct.

Histopathological analysis

For histopathological study sample were collected on 7th and 14th day of operation from rat's longitudinal incisions wound of treated and control groups. Samples were fixed in 10% PBS buffered formalin for 48hrs. After routine histological processing methods, samples embedded in paraffin blocks followed serial paraffin sections (5µm) were made by using a rotary microtome. The paraffin section samples were stained with harries hematoxylin and Eosin stain. Histopathological state of wound healing was analyzed by depending on a wide range of wound healing parameters of skin cells, including vascularity, collagen content, or number of capillaries, granulation tissue, abscess formation, necrotic epithelium. Incision sections were examined and photographed under a light microscope (AmScope, Japan).

Results

Morphological study

Morphological study of longitudinal incision wound healing of both groups were started on 0 day after operation. They were undergoing through the

principle's steps of wound healing, without any post-operative complications such as inflammation and abscess formation in both control and treated rat (Figure 3 A and B). Grossly, on 7th day post operation there was significant difference in wound scar size between the treated and control groups as present in (Figure 3 C and D), also on 14th day of experiment rat's longitudinal incisions wound was completely closed in treatment group but at the site of the longitudinal skin wound in control rats there was small amount of scar tissue as present in (Figure: 3 E and F). At the end of operation this study implies that *Q. infectoria* play an important role in inducing of healing of surgical incision when the suture material immersed into *Quercus infectoria* Olivier prepare solution. Table 1 showing all the parameters that were taken before and after the operations in both groups, such as wound size, and weight of the animals that were increases concomitantly with the healing process of the wound.

Statistically it was found that there is significant increase in the rate of wound healing in the treatment group when compared to the control group, particularly at day 7th and 14th after the operation. While at the day 28th, the process of wound healing and contraction they became equal and no statistically significant were found (Figure 4).

Histopathological findings

Histopathological evaluation was performed on 7^{th} and 14^{th} day of operation because grossly longitudinal incision wound healing of both group were started on 0 day after operation and there was not significant difference in the size of wound scar between the treated and control groups until 7th day of post operation.

a. On 7^{th} day of post operation

Histopathological evaluation of treatment group showed that a thin layer of epidermis was formed which closed the incisional site, in the sub-epidermal layer; there was a thick layer of fibrovascular granulation tissue which was constituted from newly formed blood vessels, proliferated fibroblasts and strands of collagen fibers. Granulomatous reaction also detected in the hypodermal layer which characterized by having more fibrous tissue and few giants' cells despite of mononuclear cells infiltrations (Figure 5: a and b). While the control group was represented a gap was formed from the epidermis which made a disruption in the continuity of epidermal layers and filled with necrotic debris (Figure 5: c). Marked inflammatory reaction was noted in the papillary layer characterized by infiltration of polymorphonuclear cells such as neutrophils and eosinophils (Figure 5: d). Minimum lesions were detected in the dermal reticular layer as detachment in the cells of sebaceous gland (Figure 5: e). Moreover, more lesions were seen in the hypodermis, especially in the area of suture material which formed the granulomatous mass in the surrounding of stitches. The granulomatous mass formed from aggregation of mononuclear cells and some foreign body giant cells (Fig. 5: f).

b. 14^{th} day of post operation

Histopathological evaluation of treatment group was represented Completer re-epithelialization with detectable amount of keratin was found within the epidermal layer. Below this region a thick fibrous granulation tissue was observed as it made from horizontally oriented collagen fibers with complete absence of angiogenesis, inflammatory reaction and skin appendages (Fig. 6: a). Regarding the hypodermal examination, still granulomatous masses were present but their walls were thinner than the granuloma in the 7th days of treatment, although they had mononuclear cells with few foreign body giant cells. Additionally, in the surrounding of granulomas complete loss of adipose tissue was detected and the whole areas replaced by fibrous tissue (Figure 6: b). However, control group was alterations in the papillary layer were demonstrated declining in the inflammatory cells and replaced by angiogenesis with many proliferated fibroblasts (Figure 6: c). Additionally displayed complete re-epithelialization, but an epidermal layer is thin layer from the stratum basal to the stratum corneum with observing acantholysis changes among the cells (Figure 6: d). In the hypodermis, multiple granulomatous masses were seen surrounded by foreign body giant cells, macrophages, lymphocytes, fibroblasts with strands of fibrous tissue (Fig. 6: e).

Discussion

The galls of *Q. infectoria* has been proved to have the activity of antibacterial [14], antifungals [15], antiviral [16], antioxidant agents [17]. *Q.infectoria* through involvement in remodeling of tissue, formation of granulation tissue, and dispositioning of collagen through different way of application it has been found to enhance cutaneous restoration [18]. The current study analyzed the effects of *Q.infectoria* coated Vicryl suture by subcuticular suturing incisional wound healing. The results from this study confirmed that the immersing of the Vicryl inside *Q.infectoria* solution on cutaneous wound in rats has significant effects on time of healing. This method of suture coating producing significant morphological and histological changes in treatment groups in compare with control groups. Various studies have shown that *Q.infectoria* on wound healing improves epithelial regeneration and enhances the increases of fibroblast proliferation and vascular density [19]. Morphologically, in this study there was significant improvement in skin wound healing present after 7 days in treatment group and tissue scar closely related solution while in control group huge amount of scar was obviously seen. Interestingly, at the end of experiments on the 14^{th} day of treatment, there

was complete closure of the skin wound in treatment group when compared to control group. As study on wound healing properties of *Quercus infectoria* was proven; The *Quercus infectoria* showed a clear positive effect on wound healing, with a significant increase in the levels of the antioxidant enzymes superoxide dismutase and catalase in granuloma tissue [20]**.** On our study, histological evaluation showed that the regeneration of epithelial density and collagen amount were significantly higher in treatment group than control group on 7^{th} day of operation. [21-23] Also in treated group the wound area showed late phase of granulation tissue (fibro granulation), in which the wound completely closed and there was only few scab necrotic tissue that covered the surface, in dermal layer collagen fibers were arranged in disorganized manner placed as small randomly distributed fibrils with few angiogenesis were distributed throughout the granulation tissue and the extreme inflammatory reaction, in compare to the control group on 14^{th} day of operation, in addition the researchers on the study of potential wound healing effects of *Quercus infectoria* formulation in diabetic rats investigated that untreated diabetic wound tissue compare with diabetic wounds treated with *Quercus infectoria* had incomplete epithelialization and minimal cellular infiltration ,*demonstrated nearly complete re-epithelialization, large numbers of cell migrate with deposition of collagen and blood vessel was demonstrated at the wound area* [22]. For this study choice Vicryl for suturing of longitudinal incision on the back region of the rats model because some researchers were exposed that patients in the Vicryl Plus suture group experienced fewer wound-related complications compared to the conventional braided silk suture group [23]**.**

Q. infectoria was showing that orally had high potential resist growth of oral bacteria. It led to the usage of the Q. *infectoria* extract in the traditional treatment of oral diseases associated with bacterial infections. In addition, it can be used effectively as a complementary agent in the clinical treatment of periodontitis [10]**,** it had same idea with our research that demonstrated that the *Q. infectoria* has anti-inflammatory activity and improve healing of longitudinal incision on the back region of rat model.

Finally, there are many studies show the positive effects of *Q. infectoria* on wound healing in different body regions with topical *Q. infectoria* application but there is no study investigating the effect of *Q. infectoria* extract coated the suture materials Vicryl on the cutaneous wound healing by subcuticular closure. According to the results of our study, Vicryl coating with *Q. infectoria* on cutaneous wound decreases the time of wound healing and it is a safe and potential medical agent that can play important role in the success of surgery when used during surgical operation.

Conclusion

Q. infectoria has greater effective on the regeneration and faster resolution of the surgical wound, particularly when coated with absorbable multifilament surgical suture, it aids in reducing post-operative complications and can be used as effective medical agent to inhibit the development of inflammations.

Conflict of Interest

The authors declare that there is no conflict of interest.

Ethical approval

All the procedures and approaches of this study were conducted and approved according to the principles of the ethics by the college of the veterinary medicine research committee, University of Sulaimani, Kurdistan Regional Government, Kurdistan/ Iraq.

TABLE 1. Show all the parameters that were taken before and after the operation in control and the treatment groups.

Control 7 days	**Weight before surgery**	**Zero day wound size**	**Weight after 7 days**	**Wound size after 7 days**
Rat 1	225 g	2.4 cm	231 g	2.2 cm
Rat 2	228 g	2.8 cm	220 g	2.4 cm
Rat 3	149 g	2.1 cm	155 g	1.8 cm
Treatment 7 days	Weight before surgery	Zero day wound size	Weight after 7 days	Wound size after 7 days
Rat 1	225 g	2.5 cm	231 g	1 cm
Rat 2	228 g	2.3 cm	220 g	1.2 cm
Rat 3	149 g	2 cm	155 g	1.2 cm
Control 14 days	Weight before surgery	Zero day wound size	Weight after 14 days	Wound size after 14 days
Rat 1	149 g	2 cm	158 g	1.4 cm
Rat 2	160 g	2.5 cm	175 g	1.2 cm
Rat 3	147 g	2 cm	138 g	0.8 cm
Rat 4	153 g	2.5 cm	178 g	1.5 cm
Treatment 14 days	Weight before Surgery	Zero day wound size	Weight after surgery	Wound size after 14 days
Rat 1	159 g	3 cm	168 g	0 cm
Rat 2	160 g	2.4 cm	175 g	0.3 cm
Rat 3	147 g	2.7 cm	138 g	0 cm
Rat 4	153 g	2.2 cm	178 g	0.2 cm
Control 28 days	Weight before surgery	Zero day wound size	Weight after surgery	Wound size after 28 days
Rat 1	300 g	3.2 cm	342 g	0 cm
Rat 2	303 g	3 cm	343 g	0 cm
Rat 3	295 g	3 cm	332 g	0 cm
Rat 4	341 g	3.3 cm	369 g	0 cm
Treatment 28 days	Weight before surgery	Zero day wound size	Weight after surgery	Wound size after 28 days
Rat 1	330 g	3 cm	362 g	0 cm
Rat 2	300 g	3.1 cm	340 g	0 cm
Rat 3	295 g	3.2 cm	328 g	0 cm
Rat 4	251 g	3 cm	379 g	0cm

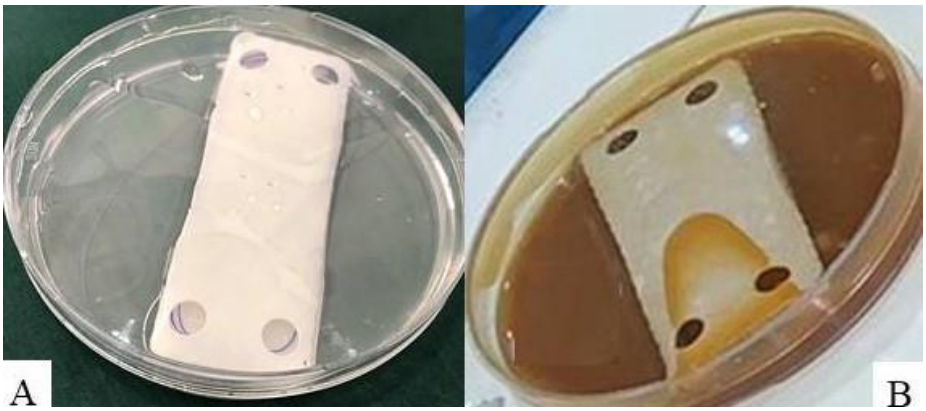

Fig. 1. A. Representing the vicryl suture (3-0) alone. B. Immersing vicryl suture (3-0) within *Quercus infectoria* oliver solution.

Fig. 2. Photograph showing the longitudinal incision on back region of rat.

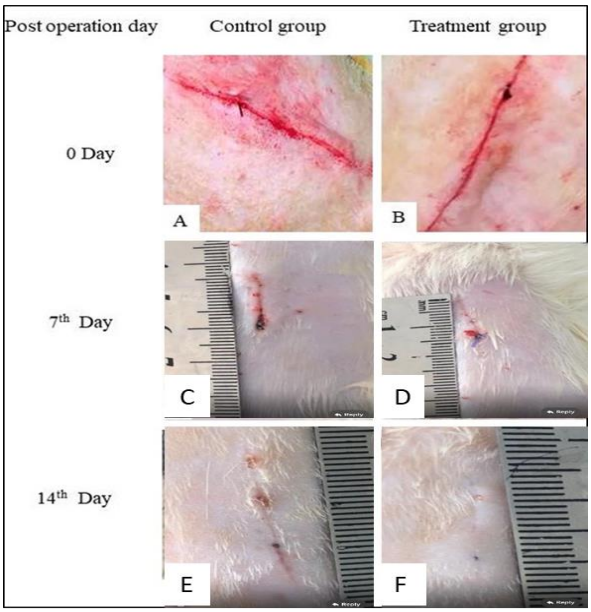

Fig. 2. Morphological post-operative longitudinal incision healing. A, it represents the longitudinal incision suture with vicryl only. B, it represents 0 day of longitudinal incision suture with vicryl that immersed in *Quercus infectoria* oliver solution. C, on 7th day of post-operative take care that the healing in control group is fair while D. it was showed the scar tissue is present clearly and the longitudinal incision started to healing completely. E. morphological figure showed that the scar tissue still remains in control group on 14th day of operation but F. treatment figure showed that the longitudinal incision healing completely not present any scar tissue.

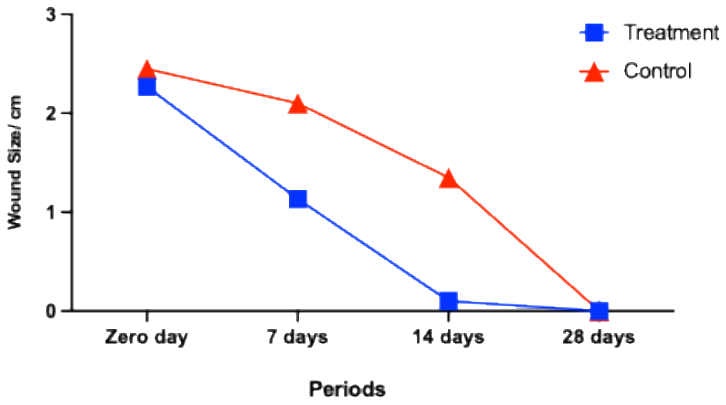

Fig. 4. Line graph showing the wound size in mm between the control and the treatment groups at days 0, 7, 14 and 28, there is a significant decrease in the size of the wounds in the treatment groups when compared to the control group, using two-way ANOVA test ($p<0.05$).

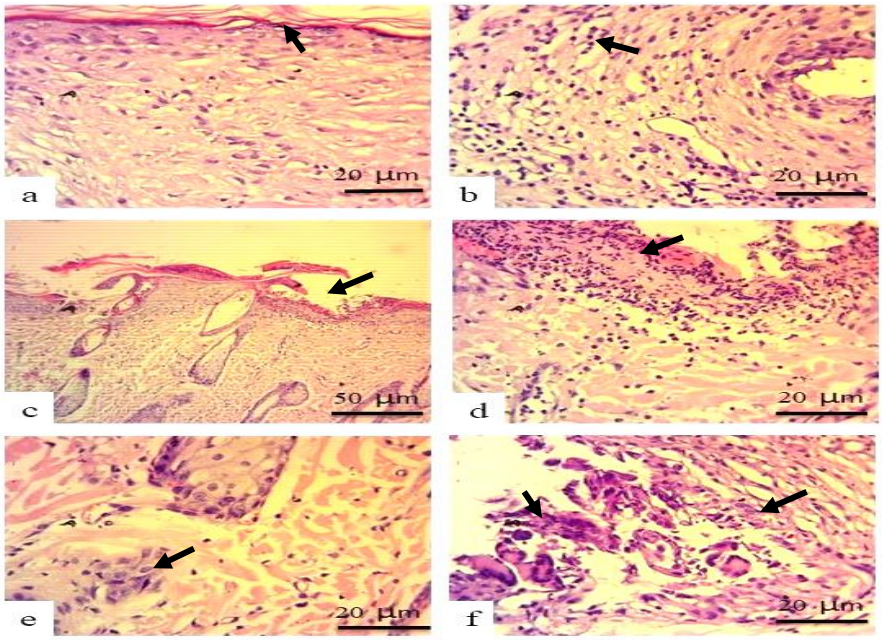

Fig. 5. light microscopical structure of longitudinal incision healing on 7th day of post-operation. a and b treatment group display re-epithelialization with fibrovascular granulation tissue and Granuloma mass consists of large number of mononuclear cells with fibrous tissue. c-f control group. c. Demonstrates the incision area with accumulation of necrotic debris and inflammatory cells. d. Accumulation of polymorphonuclear cells such as neutrophils and eosinophils beneath the damage area. e. Detachment in the cells of sebaceous gland is seen in the reticular layer. f. Granulomatous mass consists of aggregation of mononuclear cells (macrophages and lymphocytes) with foreign boy giant cells (H&E stain).

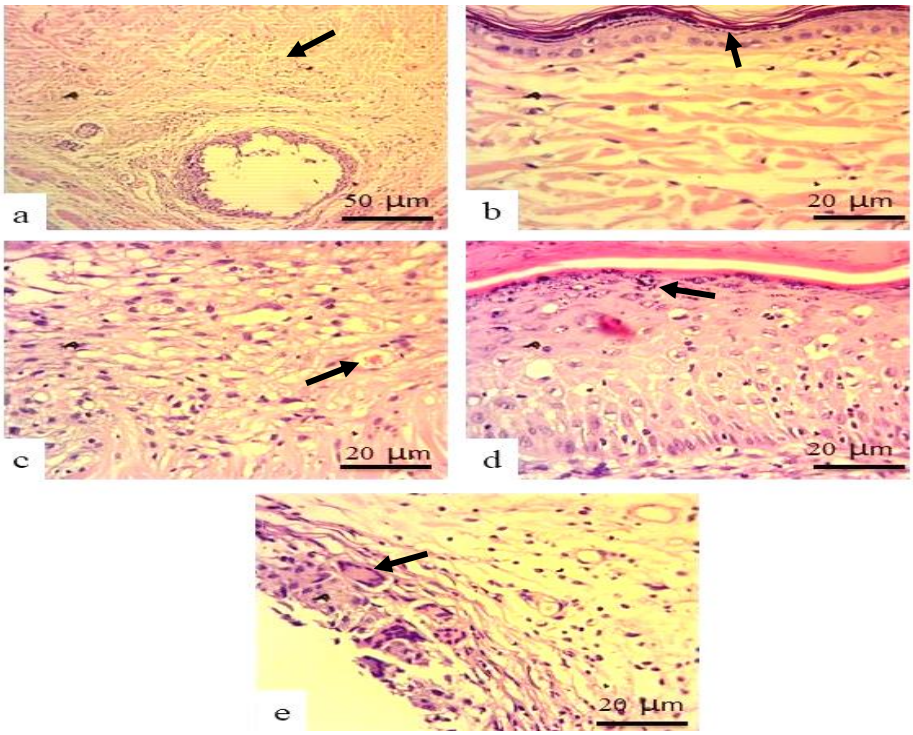

Fig. 6. Histopathological study of longitudinal incision healing on 14th day after operation. a and b treatment group. a. Granulomatous mass with few layers of fibrous tissue and complete replacement of hypodermal adipose tissue by fibrosis. b. complete re-epithelialization with thick layer of fibrous granulation tissue with loss of skin appendages in the incisional site. c-e. Control group. c. The damage area filled by newly formed blood vessels with the large numbers of fibroblasts. d. Demonstrates re-epithelialization with acantholytic changes among the keratinocytes. e. Granulomatous reaction in surrounding of stitches within the hypodermis (H&E stain).

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تعزيز التئام الجروح الجراحية: نهج تآزري باستخدام فيكريل المغلف وعفصات نبات السنديان في نموذج الفئران

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الملخص

إن العناية بشفاء الجروح والتحذير من عدوى الخطوط الجراحية؛ هي مصدر قلق مهم بعد العملية الجراحية، لذلك، يعزز (*Q. infectoria*) *Quercus infectoria* Olivier Galls التئام الجروح الجلدية من خلال المشاركة في إعادة تشكيل الأنسجة وتكوين أنسجة التحبيب وترسيب الكولاجين من خلال طرق تطبيق مختلفة. في هذه الدراسة، تم استخدام ثمانية عشر فأرًا بالغًا سليمًا، وتم إنشاء جروح جلدية طولية في منطقة ظهر كل فأر، ثم تم تقسيمهم إلى مجموعتين؛ المجموعة الأولى تعتبر مجموعة تحكم (ن = ٣ مع ٣ تكرارات لكل مدة) تم خياطة من خلال الإغلاق تحت البشرة بخيوط فيكريل فقط، بينما المجموعة الثانية تعتبر مجموعة علاجية (ن = ٣ مع ٣ تكرارات لكل مدة) تم خياطة من خلال الإغلاق تحت البشرة بخيوط فيكريل مدمجة داخل محلول مستخلص *Quercus infectoria*. تم التحقيق في القياسات المورفولوجية لشفاء الجروح في كلتا المجموعتين في اليوم ٠ و ٧ و ١٤. تم إجراء التقييمات النسيجية المرضية لمبدأ الشفاء في اليوم السابع والرابع عشر. كان معدل تكوين الأوعية الدموية وكثافة نسيج التحبيب وإعادة تكوين الظهارة في البشرة وكمية ألياف الكولاجين أعلى في مجموعة العلاج مقارنة بمجموعة التحكم. خلصت هذه الدراسة إلى أن طلاء خيوط فيكريل بمحلول مستخلص *Q. infectoria* يعزز عملية التئام الجروح الجلدية في النموذج الحيواني ويقلل من المضاعفات بعد الجراحة ويمكن استخدامه كعامل طبي فعال لمنع تطور الالتهابات.