OIL EXTRACT OF LORANTHUS EUROPEUS SEEDS PROMOTES WOUND HEALING

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ABSTRACT

The efficacy of oil extract of the seeds of Loranthus europaeus on wound healing was investigated. After the preparation of the oil extract, the oil ointment of L. europaeus was prepared. A preliminary biochemical analysis were carried out to find out the chemical contents of L. europaeus oil extract. The study involved 18 male rabbits, they were divided equally and randomly into 3 groups depending on post wounding biopsy: 3rd, 7th and 14th day post wounding. Two full thickness cut wounds were done on the both sides of shoulder regions (the left wound as control and the left as treated wound) of each group of animals. The treated and control wounds were treated continuously with (0.5mg) oil ointment and vaseline base respectively, for 14th day twice a day. All wounds were evaluate macroscopically which included “measurement of contraction rate, daily wound contraction, hyperemia, exudation and scab formation,” and microscopically for “neutrophil, macrophage infiltration, re-epithelization, fibroblast proliferation with collagen production and new blood capillary formation.” Both macroscopic and microscopic results showed the efficacy of L. europaeus seeds in promoting the healing process significantly as compared with control wounds (P<0.05). The oil extract treated wounds showed significant increase in hyperemia, exudation and scab formation, neutrophils and macrophages infiltration, fibroblast proliferation with collagen production and formation of new epithelium (re-epithelization), contraction rate and daily wound contraction at 3rd day post wounding, as compared with control wounds, but these categories showed reduction at 7th day except in macrophages, re-epithelization and fibroblast with collagen production which all showed significant increase at 7th and 14th days post wounding as compared with control wounds. The preliminary chemical analysis for oil extract showed the presence of Glycosides, Carbohydrates, Aldehydes & Ketones, Trifluorphenoides groups, protein and Polysaccharides, while Alkaloids, Flavonoids and Saponins are absent.

INTRODUCTION

Wounds can be defined as any processes which lead to disruption of normal architecture of a tissue (1). They may be: closed (e.g.: bruises, ruptures and sprains), or open (e.g.: abrasions, laceration, avulsions, hernias and excised or surgical wounds) (2). Wounds can be classified according to the number of skin layers affected to: Superficial wounds, Partial thickness wounds and Full thickness wounds (3). Generally, healing starts immediately after damage has occurred, but the mechanism, speed of healing and the eventual nature of the regenerated tissue depend on the type of wound. There are three main phases of wound healing: Inflammatory phase, repair phase (which may be further subdivided into proliferation and organization) and maturation / regeneration phase (including contraction and remodeling) (2,4-11).
Several field and laboratory studies explained that there are several plants which is used medically for treatment of skin wounds. *Loranthus europaeus* characterize by their medical and economical importance which is used in several countries of world (12).

In Iraqi folk medicine *L. europaeus* were used for treating abscesses, it is claimed that the poultice causes maturation and acceleration the drain of the pus from it. However, the mechanism of action of these seed is unknown till now. Consequently, it is thought to be interesting to investigate the wound healing efficacy of oil extract of *L. europaeus* seeds.

**MATERIALS AND METHODS**

**Preparation of oil extract ointment:** The seeds of *Loranthus europaeus* had been bought from local market in Baarha Province/Iraq. Kept in polyethylene bags at room temperature until the use time. After cleaning, the seeds were chopped were transferred to the thimble of soxhlet apparatus, extracted with (400 ml) petroleum ether (B.P 40-60 °C, BDH, England) for 24 hrs. Then the solution was concentrated by rotary evaporator, (Puchi Rotavapor, RE) at 50°C, the final dryness was done by the evaporation of remnant solvent by leaving the residue in room temperature, the resultant was (31gm) viscous oil. The ointment was prepared with vaseline base. The ratio of oil extract to vaseline was 3:1 (13).

**Preliminary chemical analysis for the extract:** To determine the chemical groups in the oil extract, the following Chemical tests had been done: *carbohydrates test:* by use Molish reagent (14), *Flavonoides test:* by use magnesium turnings and Alcoholic potassium hydroxide solution (Alcoholic KOH(5n)) (15), *Saponin test:* by use aqueous mercuric chloride (5%),(Hgcl2) (16), *Glycosides test:* by use Benedict’s reagent (17), *Alddehydes and Ketons test:* by use 2, 4 Dinitro phenyl hydrazine reagent (17), *Alkaloides test:* by use Dragendorf reagent (18), *Tri terpenoides test:* by use sulfuric acid and chloroform (19), *Protein test:* by use Biuret reagent (20) and *Polyuronidates test:* by use iodine reagent (21).

**Animals and housing:** The europaeus seeds were used for treating abscesses were housed in metal cages (0.5-1.5 meters) with a height of 0.5 meters. The rabbits were fed on alfalfa and water ab libitum.

**Experimental Design:** Eighteen male rabbits divided randomly into three groups of six animals per each group according to the date of post wounding biopsy; those groups were:

- **Group 1:** (3rd -day post wounding), **Group 2** (7th -day post wounding) and **Group 3** (14th day post wounding). All rabbits were clipped and prepared for aseptic surgery. They were anesthetized with intramuscular administration (I.M) of 10mg / kg body weight xylazin hydrochloride (Rompun, Haverlock Hart, Shawne, KS) and 50mg /kg body weight Ketamin hydrochloride (Ketanes, Areco. Fort Dodge, IA). Then at the dorsal aspect (shoulders of the animal near the neck), a circular open wound (cut) was done including a full thickness of skin layers after determination the area with a marker with a known diameter 2cm. The treated wounds were on the right side, and the control were on the left side of the animal.

- The treated wounds were on the right side, and the control were on the left side of the animal. The treated wounds were on the right side, and the control were on the left side of the animal.

**A- Macroscopic evaluation:** All wounds were examined at the determined intervals.

1.- wound contraction: At the day of wounding (day 0), each wound site from individual rabbit was digitally photographed at the indicated time intervals. The degree of wound contraction (expressed as a percentage) was calculated using the following equation (22):

\[
\left(\frac{A_{day0} - A_{day}}{A_{day0}} \right) \times 100
\]
Where: \( x = 3, 7, 14 \) day post wounding, \( A \) = wound surface area.

2. **Wound contraction per a day**: The rate of wound contraction per a day is calculated by dividing the difference between mean diameter of wounds at operative day (0 day; 2 cm) and the day of complete closure (or last experimental day, 14th day), by the number of days required for complete wound closure (or the number of experimental days, "14 days", if complete occlusion required longer time).

3. **Hypertension, exudation and scab formation**: using the following scores: 0 represent non or absence, 1 represent mild, 2 represent moderate and 3 represent severe.

**B-Microscopic evaluation**: Under general anesthesia, both control and treated wounds were excised at the determined date. Then formalin fixed, paraffin impeded, haematoxyline-eosin stained and histological sections were prepared. The prepared sections were evaluated for inflammatory cells, fibroblasts, capillary proliferation and re-epithelization, which is depending on the progression of new epithelium to cover the defect; using the following score: 0 represent no epithelium; 1 represent new epithelium covering up to \( \frac{1}{5} \) of the defect; 2 represent new epithelium covering up to \( \frac{2}{5} \) of the defect; 3 represent complete re-epithelization.

**Statistical analysis**: The results were analyzed by one-way ANOVA test using SPSS (version 9.0). All data are expressed as Mean ± SD, the difference between groups were considered significant at \( P<0.05 \).

**RESULTS**

**Preliminary chemical analysis for oil extract**: The preliminary chemical analysis for oil extract showed the presence of Glycosides, Carbohydrates, Aldehydes & Ketones, Trifurcenes, quinones and Polysaccharides, while Alkaloids, Flavonoids and Saponins are absent.

**Macroscopic Evaluation**: The effect of oil ointment of *L. europaeus* on the wound contraction rate is presented in Table (1). The wound contraction rate was significantly higher in treated group than in control group at all intervals (\( P<0.05 \)). A complete wound closure was achieved at 11th-12th day post wounding in all treated wounds. While the control wounds failed to achieve a complete closure till the end of experiment at 14th day post wounding.

Table (2) showed the result of decreasing in wound diameter for both treated and control wounds. At 3rd day post wounding, both treated and control wounds, showed non significant difference in decreasing the wound diameter. On 7th day, there is a significant decrease in diameter of *L. europaeus* oil extract treated wounds, and at 11th day the diameter for treated wounds a significantly decrease (\( P < 0.05 \)) and become (0 cm). The daily wound contraction in wounds treated *L. europaeus* oil extract was (1.8 mm / day), while in control wounds, was (1 mm / day) as shown in Table (3).

The results of the effect of *L. europaeus* oil extract on different wound healing categories (hypertension, exudation and scab formation) were explained in Table (4). On 3rd day post wounding, the treated wounds showed a significantly increased in all wound healing categories (i.e. hypertension, exudation and scab formation) than in control wounds (\( P<0.05 \)). On 7th day post wounding, there was more reduction in severity of hypertension and exudation in treated wounds than in control wounds (\( P<0.05 \)), while the scab still thicker in treated wounds. Both hypertension and exudation had disappear completely in treated wounds by the day 14th and the scab became thinner with persistence of very mild exudation in control wounds.

**Microscopic evaluation**: The results of the effect of oil extract on histological elements of wound healing were showed in Table (5) and Figures (1-10).
On 3rd day post wounding, the infiltration of neutrophils was significantly higher in treated than in control wounds (P < 0.05), figure (1), and (3). On 7th day, the neutrophil infiltration is significant higher in control than in treated wounds (P < 0.05), Figures (5,7). The infiltration became mild on 14th day in control wounds and disappeared completely in treated wounds, Figures (9,10).

The infiltration of macrophages was significantly higher in treated wound than in control wounds at 3rd and 7th day (P < 0.05), and their infiltration became more sever in both control and treated wounds at 14th-day. The fibro-vascular granulation tissue (new blood capillaries and proliferative fibroblast with collagen) started to appear on the 7th - day and became more obvious on 14th-day, the new capillaries and fibroblast proliferation was significantly higher in treated wounds (P < 0.05), Figures (1, 3, 7, 9, 10).

Throughout the period of experiment, the progression of new epithelium to cover the wound area is significantly higher in treated wound than control wounds (P < 0.05), Figures (2, 4, 6, 8, 10).

Table (1): Effect of *L. europaeus* oil extract on wound contraction rate (mean±SD) (n=6)

<table>
<thead>
<tr>
<th>Groups</th>
<th>Wound contraction rate %</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>3rd day</td>
</tr>
<tr>
<td>Controlled(C)</td>
<td>12.7±7.34</td>
</tr>
<tr>
<td>Treated(T)</td>
<td>*42.1±9.96</td>
</tr>
</tbody>
</table>

(*) Differences between (T&C) are significant at level (P< 0.05).

Table (2): The effect of *L. europaeus* oil extract on mean diameter of wounds at different intervals, Mean±SD (n=6).

<table>
<thead>
<tr>
<th>Groups</th>
<th>Mean diameter at different intervals (cm)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0 day</td>
</tr>
<tr>
<td>Control (C)</td>
<td>2.0±0.00</td>
</tr>
<tr>
<td>Treated(T)</td>
<td>2.0±0.00</td>
</tr>
</tbody>
</table>

Number of animals = 18 rabbits, Mean ± SD

(*) Differences between (T&C) are significant at level (P< 0.05).

Table (3): The rate of wound contraction per a day in the *L. europaeus* oil extract group "treated and control wounds", (n=6).

<table>
<thead>
<tr>
<th>Groups</th>
<th>Wound diameter (cm) at 0 day</th>
<th>Wound diameter (cm) at 11 day</th>
<th>Wound diameter (cm) at 14 day</th>
<th>Contraction rate per a day</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control(C)</td>
<td>2.0</td>
<td>0.9</td>
<td>0.5</td>
<td>0.1</td>
</tr>
<tr>
<td>Treated(T)</td>
<td>2.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.18</td>
</tr>
</tbody>
</table>
Table (4): Effect of *L. europeus* oil extract on macroscopic wound healing categories (Means±SD), (n=6).

<table>
<thead>
<tr>
<th>Wound duration</th>
<th>groups</th>
<th>Wound healing categories</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Hyperemia</td>
</tr>
<tr>
<td>3(^{rd}) day</td>
<td>Control(C)</td>
<td>1.66±0.51</td>
</tr>
<tr>
<td></td>
<td>Treated(T)</td>
<td><em>2.83±0.40</em></td>
</tr>
<tr>
<td>7(^{th}) day</td>
<td>Control(C)</td>
<td>0.86±0.51</td>
</tr>
<tr>
<td></td>
<td>Treated(T)</td>
<td><em>0.33±0.51</em></td>
</tr>
<tr>
<td>14(^{th}) day</td>
<td>Control(C)</td>
<td>0.00±0.00</td>
</tr>
<tr>
<td></td>
<td>Treated(T)</td>
<td>0.00±0.00</td>
</tr>
</tbody>
</table>

(*) Differences between (T&C) are significant at level (P<0.05).

Table (5): Effect of *L. europeus* oil extract on microscopic wound healing categories

<table>
<thead>
<tr>
<th>Wound duration</th>
<th>Groups</th>
<th>Wound Healing categories</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Neutrophils</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3(^{rd}) day</td>
<td>Control(C)</td>
<td>1.50±0.54</td>
</tr>
<tr>
<td></td>
<td>Treated(T)</td>
<td><em>2.83±0.40</em></td>
</tr>
<tr>
<td>7(^{th}) day</td>
<td>Control(C)</td>
<td>2.50±0.54</td>
</tr>
<tr>
<td></td>
<td>Treated(T)</td>
<td><em>1.66±0.51</em></td>
</tr>
<tr>
<td>14(^{th}) day</td>
<td>Control(C)</td>
<td>0.66±0.51</td>
</tr>
<tr>
<td></td>
<td>Treated(T)</td>
<td><em>0.00±0.00</em></td>
</tr>
</tbody>
</table>

(Mean±SD), (n=6).

(*) Differences between (T&C) are significant at level (P<0.05).
Figure 1- 3rd day post wounding (T).
Wound, there is high infiltration of neutrophils with presence of

Figure 2- 3rd day post wounding (T). clear re-epithelization. 40X.

Figure 3- 3rd day post wounding (C).
Infiltration of neutrophils and macrophages less than in treated wounds. 40X.

Figure 4- 3rd day post wounding (C). Re-epithelization less obvious than in treated wounds. 40X.

Figure 5- 7th day post wounding (T).
Decrease in neutrophils infiltration, and increase macrophages. (arrow) 40X.

Figure 7- 7th day post wounding (C). More infiltration of neutrophils and macrophages. 40X.
DISCUSSION

Wound contraction: In the present study, the topical application of L. caragana oil extract on full thickness skin ulcers result in more accelerated contraction of treated wounds with 100% contraction rate at 11th-day post wounding, while in control wounds the contraction rate was 87% at the end of the experiment (14th day).

The macrophages activated by polysaccharides which stimulate the fibroblast proliferation (23) with subsequent proliferation of myofibroblast at the periphery of the wound, the contraction of the contractile protein of myofibroblast play important role in wound.
contraction \(23,25\). Heggers et al. \(26\) found in their study, that the excisional wounds were treated \(26\) with Aloe vera ointment for 14th day showed significant increase in contraction rate as compared with control wounds, the authors attributed this result, to polysaccharides in Aloe which increase collagen activity and promote wound contraction. Romo \(23\), reported a maximal rate of the wound contraction as 0.7mm/day, depending on tissue laxity and shape of the wound, loose tissue contract more, and the square wound tend to contract more than circular wound. In the present study, the daily contraction rate of wounds treated \(24\) by \(L. euopeus\) oil extract was 1.8mm/day, in spite of the tissue laxity and circular wounds, and this may reflect the efficacy of \(L. euopeus\) oil extract in promoting wound contraction.

Wound healing categories: Both macroscopic and histopathologic results, indicate that topical application of \(L. euopeus\) seeds in oil extract for full thickness cut wounds could effectively induce healing process. The presence of polysaccharides in \(L. euopeus\) oil extract, polysaccharides promote macrophage activity \(27-29\). The activated macrophages secrete cytokines such as [platelet derived growth factor (PDGF), transforming growth beta factor (TGF), Interleukins (IL), Fibroblast growth factor (FGF), Insulin growth factor (IGF-1), epidermal growth factor (EGF) and others \(30-32\).] These cytokines are essential for fibroblast proliferation, angiogenesis and chemotaxis. Among these cytokines, PDGF and Interleukins (particularly IL-1) are chemotactic and activators for neutrophils \(33-38\), and this may explain the high infiltration of neutrophils in wound treated by \(L. euopeus\) oil extract and poultice particularly in the early inflammatory phase.

The obvious appearance of fibrovascular granulation tissue after 7th day post wounding, may be explained by the macrophages activation with subsequent fibroblasts proliferation and their migration to wound site with collagen production \(32,36\). The macrophage derived angiogenic factor released in response to low oxygen tension (hypoxic condition) can explain the proliferation of new capillaries \(39-42\).

Since oxygen is required for the synthesis of collagen by fibroblast \(43\), \(L. euopeus\) oil extract and poultice may improve angiogenesis or vascular supply and make more oxygen available to improve collagen formation for wound healing. During the wound healing process, epithelial cells, proliferate and migrate from the edges of the wound and eventually cover the wound with newly skin \(44\). By lysing collagen enzymes, the epithelial cells move across the wound and attach to a viable tissue, the proliferation and migration of the epithelial cells is dependant on adequate supply of oxygen \(45\), therefore; the increased presence of oxygen caused by \(L. euopeus\) oil extract and poultice, improving microcirculation, should greatly
improve re-epithelization and wound healing process, this may explain the increase fibrovascular granulation tissue and re-epithelization during the present experimental study.

There was a relationship between healing process and scab that covered both treated and control wounds in the two groups, but highly appearance of scab layer in treated wound of L. europaeus oil extract and less in poultice treated wound, may be due to nature of scab with can behave like semi-occlusive dressing, the role of scab has been reported by Bigbie et al. (46). he showed that a wound scab protect the wound, promote the migration of epithelium, and provides more cosmetically pleasing results. Apparently the scab formation in the present study bound with healing process which increased at the first period of experiment, later on later on decreased and disappeared in wounds treated with oil extract. This may due to the treated wounds in oil extract show a complete healing at 11th day of scab for treated wounds, while poultice treated wounds showed non complete healing till 14th day and this reflected by the presence of scabs in poultice and control wounds which also showed no complete healing.

Aldehydes also reported to induce healing properties by increased cellular proliferation and collagen synthesis at the wounded site, Suga et al. (47) found in their study on an alcoholic extract of Centella asiatica on rat dermal wound, the treated wound showed significant increase in the collagen content of granulation tissue, epithelialise and contract faster than control wounds, they regarded these healing properties due to aldehyde content in the extract of this plant, from this one can suggest that aldehyde in L. europaeus seed may considered another growth substance induce healing properties, in addition to polysaccharides.

المستخلص

لتدوير نبات حب الديق بأشجار النبات الجروج

علاء الدين حسن جوان** و ميثاق عبد الهاشم الريبي** و جاسم محمد الشاذلي

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الخلاصة

تم اختيار نبات حب الديق لتقوم كعامل في إصلاح الجروح. لهذا الفرضية تمضخت الدراسة تخضع لخلاصة الزيتية لتدوير نبات حب الديق. وقد أجريت تحليلات كيميائية لهذه الخلاصة تمزج الأحماض الدهنية في الزيتية.

كما تم تحصين المرهم الزيتي.

استخدمت المرهم الزيتي لخلاصة الزيتية ضمن هذه الفرضية (18) من نبات البارناب، فрактиك بشكل

عشوائي إلى 3 مجموعات فرعية (مجموعة اليوم ثلاثي و مجموعة اليوم السبع و مجموعة اليوم الرابع عشر). خضع كل

حيوان من كل مجموعة إلى جرحين، جرح دركي (فتق) مصنف كل طبقات الدهك في منطقة الظهر. عولجت

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الجرح (المعالجة والسيطرة) بمستحضر بـ(أعمال) من المرهم الزيتي والفانيتين على التوالي لمدة 14 يومًا مع مساعدة مرتين في اليوم.

فيما كم الجرح في كل مجموعة تقييمًا عمياءً ومجهوليًا. فالقيمة القياسية لمعدل تحسن الجروح، حسب تقارير الجروح في اليوم الواحد، وذات أيضًا قيادة درجة احترام الجروح، النموذج، وتكوين القروح. أما مجهوليًا فقد كم درجة (اريخ البالا، الابن، فنبرة الخداد، كلا، الإفرازات الدورية، مع إنتاج البلازما والبيتا واثنين ولوميني البلازما) المكروهات القروح في اليوم الواحد.

لقد أظهرت نتائج كل من هذه المعايير عدة نتائج حيًا لجرح سمني فعال بالجرح بجرح علاجية أمنة محسوبة (P=0.5). أظهر نتائج من القيم العمياء، للجرح، والقروح الممبردة في سلمة جروح درجة علاجية عالية في اليوم. للجرح المعالجة بالخلاصة الطبية زائدة على القيمة في اليوم الثالث في كل من درجة الإحترام، النموذج، فنبرة القروح، إحصاء الفعاليات والقيمات، والأورام، والقيمة، الأورام، والقيمة، الأورام، والقيمة، الأورام، والقيمة، الأورام، والقيمة، الأورام. فنبرة القروح في اليوم الواحد، لكل حيًا حيًا عاديًا وذات أيضًا قيادة درجة احترام الجروح، النموذج، وتكوين القروح. أما مجهوليًا فقد كم درجة (اريخ البالا، الابن، فنبرة الخداد، كلا، الإفرازات الدورية، مع إنتاج البلازما والبيتا واثنين ولوميني البلازما) المكروهات القروح في اليوم الواحد.

REFERENCES