Effects of *Loranthus europeus* seeds on pyogenic inflammation in rabbits

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Abstract : Within the interest in the study of plants and herbs that have anti-inflammatory effect in animals, seeds extract of *Loranthus europeus* (L.) were investigated. Twenty four female rabbits were divided randomly into four equal groups depending on the date of post wounding biopsy :(1^{st}, 3^{rd}, 7^{th}, and 14^{th} – day post wounding).Two full thickness incesional wounds (treated and control) were made on both sides of the shoulder region, polyvinyl sponge granuloma contaminated with *Staphylococcus aureus* bacteria was inserted into incesional wounds. The treatment continued for 14 days twice a day. Both (treated and control) wounds were submitted for macroscopic evaluation (for hyperemia and exudation) and microscopic evaluation (for inflammatory cells infiltration, fibroblast proliferation with collagen formation) in each of these intervals.

Both Macroscopic and microscopic results revealed highly increase in hyperemia, exudation, netrophils and macrophages infiltration at 1st and 3rd post wounding days, but these categories showed reduction at 7th day post wounding except macrophages infiltration and fibroblast with collagen production showed significant increase in treated wounds when compared with control wounds ($P<0.05$).

Introduction:

Several field and laboratory studies explained that there are several plants which are used medically for treatment of skin inflammation and wounds as *Urtica dioca* (1), *Cucurbita pipo* (2), *Aloe vera* (3), and *Achillea talagonica* (4).

The genus Loranthus, is now known as Dendrophathae in English (5). In Arabic the plant has different names such as Hib el-debag, Fulful Hawa and Habet pukour. Plants of this genus are common angiosperim parasites of some trees. The most common host is Mango, Fiscus sp., Albizzia sp. and Dalbergia sp., the species are semi-parasitic on tree trunks and branches and can be easily spotted on the branches of trees as a dense cluster of small twigs, bearing smooth broad leaves and long tubular, orange coloured flowers with red barriers (5).

Loranthus europeus is widely distribution in Tropic and semi-Tropic areas, also widely growth in India and Northern of America as recorded by (6). In Iraq, *L europeus* distributed in Northern areas of Irac especially in Amadia, Roundoze and Sulymania (7).

The Loranthus genus plants, including *L europeus* characterize by their medical and economical importance

which is used in several countries of world (7). The whole plant used as powerful stimulant and as accelerator for fetal delivary, also as anti hypertension resulting from arteriosclerosis (8). Chakrivarty (9) explained the benefit of aqueous extract of plant leaves in their useful as anti-tubercular. Also the aqueous extract used as drug against rat-bite poisoning (10).

In (1991) El-Saadany *et al.* (11) explained the role of *L europeus* seeds in treatment of hypercholesterolemia in rat. Also Gupta *et al.* (12) had been studied the effect of aqueous extract of *L europeus* seeds on the growth and development of tumor epithelial membranes, also on the life span of tumor bearing rats.

On other hand, the toxicity study of light ether extract of *L europeus* seeds had been done by AL-fartosy, (7), on experimental mice, he found that, the ether extract had no toxic effect on mice.

Loranthus europeus seed had a known importance in Iraqi folk medicine. In Iraq these seed were used in the form of poultice after mastication and moisture in mouth 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, and because of several medical benefits of the seeds extract, and the presence of these seeds in local market in cheap prices. Consequently, it is thought to be interesting to investigate the effect of *L. europeus* oil extract on pyogenic inflammation and determination the elements of pyogenic inflammation that may be affected by *L europeus* oil extract.

Materials and methods:

Preparation of oil extract: Seed of *Loranthus europeus* had been bought from local market in Basrha Province / Iraq. After cleaning, the seed were chopped using hummer and mortar, the result was viscous material. One hundred gram from chopped seeds were transferred to the thumble of soxhlet apparatus, extracted with (400 ml) petroleum ether (BDH, England) for 24 hrs. Then the solution was concentrated by rotary evaporator, (Puchi Rotavapor, RE) at 50C°, the resultant was (31gm) viscous oil, then kept in dark glass container at 4Co'Jawad, 1982).

Preparation of bacterial culture: The stock of *Staphylococcus aureus* was brought in a nutrient broth (Himedia Limited India), from the Biology Department, Collage of Science/ Basrah University. The stock was re-cultured on manitol salt agar (Oxoid LTD, England) by streak plate technique and incubated for 48 hrs.

One colony of *Staph aureus* was transferred into nutrient broth and incubated for 24 hrs, this bacterial culture was used to contaminate the sponge poly vinyl granuloma (13).

Bacteriological Count: the number of bacteria (*Staph aureus*) which contaminated the sponge poly vinyl granuloma ,the bacterial count has been cone using pouring method (14).

Animals and housing: The animals used in the present study were domestic rabbits *(Lepus domestica)* of (3-4) months age, body weight (1000- 1250) grams. The rabbits were housed in metallic cages (100 ×40 ×193 cm) and were fed on alfa alfa and water *ad libitum*, at room temperature.

Preparation of Poly vinyl sponge granuloma: The sponge poly vinyl granuloma was used in this stucy as a model for inducing and studyirg the inflammation process in the linear skin incisions. A sponge of size 2×1×1 cm was sandwiched between 2 discs cut from silicon rubber stoppers. (Arthur H. Thomas and CO.) Each disc was (0.2 cm) thick and was trimmed to fit the dimensions of the sponge. The 3 discs were then secured:ogether by a centrally located silk stich (Ethicon, INC.). The 3 discs technicue was used in order to eliminate cellular infiltration from top to bottom surfaces of the discs (15). The poly vinyl sponges were sterilized in autoclave for 15 min in 120 C°.

Experimental Design: Twenty four female rabbits were divided randomly into four equal groups depending on the date of post wouncing biopsy as follow: ***Group A:*** (1 -day post wounding). ***Group B:*** (3^{rd}-day post wounding), ***Group C:*** (7^{th} -day post wounding).& ***Group D:*** (14^{th} 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, Shawnee, Ks.) and 50mg /kg body weight Ke.amin hydrochloride (Ketanes, Areco. Fort Dodge, IA.).

In each animal, two standard linear skin incisions were made on both sides of back (on the shoulder, near the neck region) using a sterile blade. The incisions were made by a scalpel with aseptic technique through the epidermis , dermis anc subcutaneous fat, the length of each incision was 2cm . The right sided incision was used as treated wound, the le.t one used as control. Both treated and control wounds were widened by sterile

artery forceps, a poly vinyl granuloma then inserted in each wound. (15). Both wounds were contaminated by 0.1 ml *Staph. aureus* suspension (containing (1.95×10^{3} bacteria /ml) to ensure a poygenic inflammation.

This is followed by the addition of (0.5g) oil ointment to the treated wound. The control wounds were treated by (0.5g) vasaline. The wounds then sutured with 3-0 silk stitch (Ethicon. INC.). All wounds were covered with non-adherent occlusive gauzes to maintain the ointment, to keep the wounds clean and to prevent the animal from licking or scratching the wounds. Finally, a bandage was wrapped around the trunk of animals to fix the gauze dressing; the bandage in turn was externally strengthened with cotton vest to prevent detachment and self-infliction.

The ointment and Vaseline were applied to the tested and control wounds respectively, twice daily for 14 days.

Macroscopic evaluation: All wounds in this experiment were examined at the determined intervals (1st, 3rd, 7th, and 14th-day post wounding). Prior to wound examination, the cotton cloth vest and bandage were removed; the wound surfaces were cleaned gently with gauze soaked with normal saline (16).

The wounds (treated and control) were evaluated macroscopically regarding the severity of hyperemia (redness) and exudation (serous, seropurulant and purulent) using the following score: 0 **represented none or (absent), 1 represented mild, 2 represented moderate and 3 represented sever.**

Wound biopsy: Under general anesthesia and aseptic conditions, both control and treated wounds were excised at the determinec date by an elliptical incision

around the wound. The residual defected area was sutured and dressed carefully.

The excised wound biopsies were immediately put in10% formalin (BDH-England) for at least (48 hrs). After proper fixation, a perpendicular incision was made across the wound including the sponge .Then a slice (3-5 mm thickness)of the sponge was taken and submitted for histopathological examination.

The biopsies were dehydrated by several dilutions of ethanol alcohol (LIF.Germany). Dealcoholization with xylol (Switzerland, FLUKA), then embedec in paraffin wax (Pool, Ltd, England), blocked and (3-5 μ) thickness sections were obtained by the microtome. The sections were put on glass slides, deparrafinised with xylol, and rehydarated by alcohol and stained by Hematoxylin and Eosin. The sections (sponge slices) were examined microscopically to evaluate the pyogenic inflammation.

Histological evaluation: The histological sections prepared from the sponge were examined by light microscope to evaluate the degree of neutrophils and macrophages infiltration, fibroblast proliferation with collagen deposition and new capillaries formation (Granulation tissue).

Each of these categories was scored from (0-3) for statistical analysis as following: **0 represent none or absence, 1 represents mild, 2 represent moderate & 3 represent sever.**

Statistical Analysis: The results were analyzed by 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:

Macroscopic Evaluation: At the1st-day post wounding, topical application of (0.5) mg of oil extract ointment showed significant increase ($p<0.05$) in hyperemia and exudation as compared with control wounds (treated with 0.5mg Vaseline). At the 3rd-day post wounding, the hyperemia and exudation were significantly more sever in treated wounds than control wounds ($P<0.05$). At the 7th-day post wounding the severity of these categories was less in treated wound than in control wounds ($P< 0.05$). At the 14th -day, the hyperemia disappeared with very mild exudation in both treated and control wounds (Table-1).

Microscopic Evaluation: The effects of L europeus oil extract on pyogenic categories present in sponge implant are explained in Table (2) and Figure (1-8). At 1st day post wounding, the neutrophils and macrophages infiltration were significantly higher in treated wounds than in control wounds ($P<0.05$). No fibroblastic proliferation and no granulation tissue formation were seen in both treated and control wounds Figures (1&2).

At the 3rd-day post wounding, neutrophils and macrophages infiltration were still significantly higher in treated wounds than in control wounds ($P< 0.05$), new blood capillaries and fibroblast started to appear in both groups with significantly more capillary and fibroblastic proliferation in treated wounds ($P < 0.05$) Figures (3&4).

From 7th-day till 14th-day post wounding, there is a steady reduction in neutrophils and macrophages infiltration, the macrophages infiltration reach it's peak on 7th-day, the fibrovascular granulation tissue (blood vessels and proliferative fibroblast with collagen) became

obvious from 7th-14th day post wounding particularly in treated wounds Figures (5, 6, 7& 8).

Table (1): Effect of *L. europeus* oil extract on pyogenic inflammation categories

Wound Duration	Groups	Pyogenic inflammation categories	
		Hyperemia	Exudate
1st – day	Control (C)	0.83± 0.75	0.33 ± 0.51
	Treated (T)	* 1.50 ± 0.54	* 1.16 ± 0.40
3rd – day	Control(C)	1.50 ± 0.54	1.50 ± 0.54
	Treated(T)	*3.00 ± 0.00	*2.83 ± 0.40
7th – day	Control(C)	2.16 ± 0.40	1.50 ± 1.54
	Treated(T)	* 0.83 ± 0.75	1.16 ± 1.51
14th – day	Control(C)	0.66 ± 0.51	0.66 ± 0.51
	Treated(T)	*0.00 ± 0.00	0.33 ± 0.51

Number of animals = 24 rabbits, mean ± SD

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

Table (2): The effect of *L europeus* oil extract on pyogenic inflammation categories in implant sponge.

Wound duration	Groups	pyogenic inflammation categories			
		Neutrophil	Macrophage	Granulation Tissue	
				New blood capillaries	fibroblast + collagen
1st day	Control (C)	1.66 ± 0.51	0.16 ± 0.40	0.00 ± 0.00	0.00 ± 0.00
	Treated (T)	*2.50 ± 1.54	*0.83 ± 0.40	0.33 ± 0.51	0.00 ± 0.00
3rd day	Control (C)	2.00 ± 0.63	1.50±0.54	0.16 ± 0.40	1.00 ± 0.00
	Treated (T)	*3.00 ± 0.00	*2.33 ± 0.51	*1.16 ± 0.40	*1.83 ± 0.40
7th day	Control (C)	2.33 ± 0.75	1.16 ± 0.40	1.66 ± 05	2.00 ± 0.00
	Treated (T)	*3.00±0.00	*3.00 ± 0.00	*2.83 ± 0.40	*3.00 ± 0.00
14th day	Control (C)	1.33 ± 0.75	1.16 ± 0.40	2.50 ± 0.54	2.83 ± 0.40
	Treated (T)	*0.50 ± 0.54	*0.50 ± 0.54	*3.00 ± 0.00	*3.00 ± 0.00

Number of animals = 24 rabbits, mean ± SD.

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

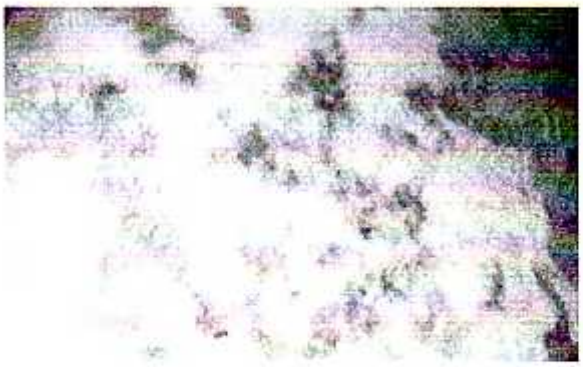

Figure (1) A sponge implant from 1st-day (1) incision. High infiltration of neutrophils. 40 X.

Figure (2) A sponge implant from 1st day (C) incision. Less neutrophil infiltration. 40X.

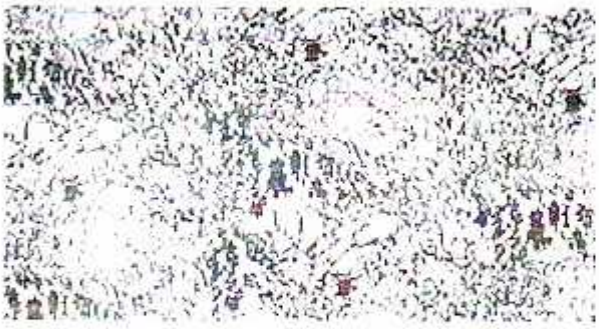

Figure (3) A sponge implant from 3rd-day (I) incision. Higher infiltration of neutrophils with more macrophages.40X

Figure (4) A sponge implant from 3rd-day (C) incision. Less infiltration of inflammatory cells 40X.

Figure (5) A sponge implant from 7th-day (T) incision .there is reductions in neutrophils infiltration but fibroblast appear in a huge number. with more macrophages 40X

Figure (6) A sponge implant from 7th day(C) incision. More neutrophi s and macrophages infiltration. and less fibroblasts 40X.

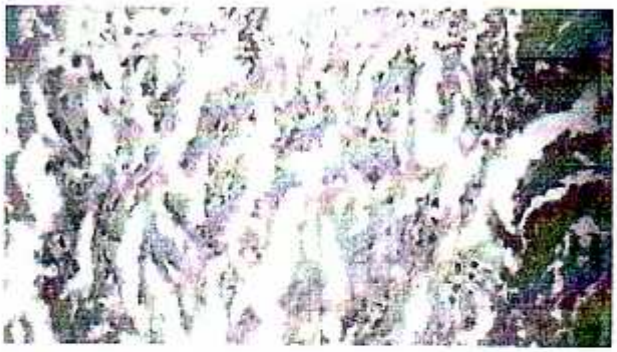

Figure (7) A sponge implant from 1-th-day (T) incision. Few inflammatory cells with abundant collagen. 40X.

Figure (8) A sponge implant from 14th -day (C) incision. Increase proliferation of fibroblast with less collagen fibers and infiltration of inflammatory cells still appear within. 40X.

Discussion:

In the present study the pyogenic inflammation was significantly more sever in wound treated by oil extract of *L europeus* particularly during the 1st and 3rd -days post wounding.

During bacterial infection, there is a massive production of pro-inflammation cytokines including Interlukin-1 (IL-1) and Interlukin-6 (IL- 6) (17), these cytokines mediators act as chemoattractants and activators for neutrophils (18;19;20;21;22;23;24) which are responsible for eradicating the invasive bacteria and necrotic materials from the wound site (25;26). This may explain the prominent hyperemia and exudation with obvious infiltration of phagocytic cells (neutrophils and macrophages) during 1st and 3rd -day in treated wounds. This is in agreement with Roseler *et al.*(27) who concluded that polysaccharides of *Echinacea purpura* increase the number of polymorph nuclear leukocyte (PMNs) released from the bone marrow, raising the white blood cells count and mobilizing PMNs into action, these effects increase the resistance of mice to lethal infection with Staphylococci, *Candida albican* and *Listreia monocytogenes*.

Following elicitation of pro-inflammatory cytokines, the levels of anti-inflammatory mediators such as IL-10 and MCP-1 (monocyte chemo attractant protein-1) are increased and suppress the activity of pro-inflammatory cytokines for resolution of inflammation by inhibiting neutrophil function (17;28) and this may explain the decrease in hyperemia, exudation and neutrophil infiltration in treated wound with *L europeus* oil extract after the 3rd day. Many authors emphasized that

polysaccharides promote macrophage activity through binding to glycoprotein surface receptor (29; 30).

After overcome of bacterial inflammation, activated macrophage by polysaccharides play a role in phagocytosis of killed bacteria and damaged tissue and stimulate the chemotaxis and proliferation of fibroblast with collagen production and secret substances that attract endothelial cells to the wound and stimulate their proliferation to promote angiogenesis (31) and this may explain the infiltration of macrophages increment and new blood vessels synthesis during 7th-day post wounding.

On the basis of the present results, one can concluded that the oil extract of *L. europeus* seeds may act as immuno-modulator during bacterial infection and may contain substances act as chemo-tactic agent for neutrophils and promote macrophages activity.

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تأثير بذور نبات حب الدبق على الالتهاب القيحي في الأرانب

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الخلاصة : ضمن الاهتمام بدراسة النباتات والأعشاب التي تمتلك تأثير في عمليات الالتهاب، اختيرت الخلاصة الزيتية لبذور نبات حب الدبق لتقييم قابليتها ضد الالتهاب القيحي في الحيوانات. وقد استخدمت لهذا الغرض ٢٤ من أناث الارانب قسمت وبشكل عشوائي إلى أربعة مجاميع متساوية اعتمادا على تاريخ اخذ العينة من الجرح: (اليوم الأول، اليوم الثالث، اليوم السابع و اليوم الرابع عشر) بعد العملية. كل مجموعة من هذه المجاميع مكونة من ٦ حيوانات. خضع كل حيوان إلى جرحين خطيين في جهتي منطقة الكتف متضمن كل طبقات الجلد قسمت إلى (جرح معالجة و جرح سيطرة) وقد خضع كل جرح إلى التهاب قيحي تجريبي بواسطة نظام لإحداث الالتهاب (الأسفنجة)حيث لوثت هذه الأسفنجة بـ(١مل) من النمو البرثومي لبكتيريا المكورات العنقودية الذهبية للتأكد من إحداث التهاب قيحي في هذه الجروح.بعدها عولجت الجروح بـ(٠,٥ملغم) من كل من المرهم الزيتي وقاعدة الفازلين لجروح المعالجة والسيطرة على التوالي. قيمت كل من جروح المعالجة والسيطرة عيانيا ومجهريا في كل من الفترات الأربعة

المذكورة. فقد قيمت الجروح عيانيا لتقدير كمية (الاحمرار، والتضوح) ومجهريا لتقدير درجة ارتشاح الخلايا الالتهابية (العدلات، البلعمات)، الارومات الليفية مع إنتاج ألياف الكولاجين وتكوين النسيج الدموية الجديدة الموجودة جميعا في الأنسجة.

اظهر كلا من التقييم العياني للجروح والفحص النسيجي زيادة عالية في درجة الاحمرار،التضوح،ارتشاح العدلات والبلعمات في اليوم الاول والثالث بعد العملية. لكن حدث انخفاض في تلك العوامل في اليوم السابع ما عدا البلعمات اضافة إلى ظهور الارومات الليفية مع الالياف الغروية حيث شوهدت جميعا زيادة عالية لجروح المعالجة عند مقارنتها مع جروح السيطرة.