

THE EFFECT OF OIL EXTRACT OF *THYMUS VULGARIS* ON FULL THICKNESS INCISION OF WOUND HEALING IN RABBITS

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(Received 30 March 2015 ,Accepted 3May 2015)

Key words: thymus vulgaris , rabbit , wound healing .

ABSTRACT

The present study was to determine wound healing properties of the oil extract of *thymus vulgaris*. The influence of the oil extract on rate of wound closure was investigated using the excisional wound model and histopathological investigation of treated and untreated wound tissue performed in 18 rabbit, divided into three groups, six animal in each group, the oil ointment were prepared and applied topically three times a day to excision wound for 14th day post operative and compared with control.

It is concluded the treated wound showed a faster rate of wound reduction compared with control, this was further supported with histopathological studies.

INTRODUCTION

Medicinal plants are used in many countries as an alternative to synthetic drugs. medicinal herbs are high natural source of medicinal products used in traditional medicine and chemical entities for modern drugs (1).

Thymus vulgaris L.(thyme) is an aromatic plant belonging to the Lamiaceae family, used for medicinal and spice purposes almost everywhere in the world (2). thyme has long history of been used in traditional medicine for treatment of various diseases for instance to treat respiratory diseases, it also used to prevent hardening of the arteries, treatment of toothache, urinary tract infection and dyspepsia (3), it also expels fungus from stomach and intestine and it has ability to increase appetite, antioxidant, carminative, antimicrobial, anthelmintic (4),anti-inflammatory and analgesic effect (5) and has late been recommended as substitute as cancer prevention agent (6). the remedial potential of *thymus vulgaris* is due to the presence of thymol, carvacrol and flavonoids. the known primary constituents of thyme include essential oil (borneol, carvacrol, linalool, thymol), bitter principle oleanic acid, rosmarinic acid, caffeic acid and triterpenic acids (7).

Wound healing is a dynamic , interactive process among several cell types, involving a cascade of events including inflammation, new tissue formation and tissue remodeling which finally lead to the reconstruction of the wounded area, which is eventually partial (8,9).

in this research we attempt to show the effect of oil extract of *thymus vulgaris* on improvement the wound contraction and re-epithelization in full thickness excisional wounds model in rabbits.

MATERIAL AND METHODS

- plant material:

The leaves of *thymus vulgaris* which was purchased from local market in Basra – Iraq.

- Preparation of oil extract:

The leaves were grind in mortar to powder and subjected to extraction with soxhlet with acetone solvent 250 ml (BDH England) for 24 hours. after complete extraction, the extract was concentrated by rotary evaporator (Punchi Rota vapor, RE) at 40°C to obtained oil extract, the final dryness was done by the evaporation of remnant, the solvent by leaving the residue in room temperature, the result was (20 gm) oil extract kept in dark glass container at (4°C).

- Oil ointment preparation:

The ointment was prepared with Vaseline base by triturating method using 2 spatulas to admix the oil extract of the plant with gradual addition of Vaseline to obtain a homogenous ointment with ratio 3:1 oil Vaseline (10). Then ointment had been kept at (4°C) until use time.

- Animal housing:

18 rabbit, 2-2.5 kg body weight were used for the study, the animals were housed in metallic cages. they were fed on *alfa alfa* and bread and water adlibitum at room temperature. the animals were divided in to 3 groups of 6 animals for each group as the following:

A: 3rd day post operative.

B : 7th day post operative.

C: 14th day post operative.

Excisional wounds:

wound healing study was done by an excisional model. the back of each animal was shaved and prepared after washing with alcoholic spirit. An area marked was defined

with a marker on the shaver back of the animals. The circular marked area was excised with its full thickness using a surgical sterile blade and scissors under anesthesia with xylazine hydrochloride (10mg/kg body weight) and ketamine hydrochloride (50 mg/kg body weight) intramuscularly .

The right sided excision was used as treated wound, the left one used as control. All wounds were covered with non adherent occlusive gauze. finally a bandage was wrapped around the trunk of animals in turn was externally strengthened with cotton vest(11) .

Determination of wound contraction:

This evaluation include determination the range of wound contraction of each wound after measuring the area of wound in indicated interval, and calculated by using the following equation to explain the wound contraction as percent (12).

$$\left[\frac{A \text{ day}_0 - A \text{ day}_x}{A \text{ day}_0} \right] * 100$$

Where, $X = 3, 7, 14$ day postoperative.

A = wound surface area.

Histopathological studies:

On each period of experimental day (3rd, 7th and 14th postoperative day). The regenerated tissue from the healing wounds collected and placed in 10% formalin for histopathological studies and dehydrated by several dilutions of ethanol alcohol dealcoholization with xylol, then embedded with paraffin wax blocked (3-5)mm thickness sections were obtained by microtome. The sections were put on glass slides deparaffinised with xylol, rehydrated by alcohol and stained by hematoxyline and eosin, the sections were examined for re-epithelization, inflammation, collagen and a fibroblast presence (13).

RESULTS

- wound contraction:

The effect of ointment of oil extract of *thymus vulgaris* on the wound contraction rate is presented in table (1). The wound contraction rate is greater in treated wounds than the control and complete wound closure was achieved at 14th day post wounding in treated wounds while the control wounds failed to achieved a complete closure till the end of experiment at 14th day post wounding.

Table 1: Effect of thymus oil extract on wound contraction rate.

Time Days	Contraction Rate	
	Treated Wound	Control Wound
3 rd	60.7%	23.1%
7 th	80.8%	43.5%
14 th	100%	74.1%

- Histopathological studies:

The result of the effect of oil extract on microscopic examination of sections prepared from the wounds of treated and control groups exhibited the following characteristics:

On 3rd day post wounding, neutrophils highly infiltration than control fig (1), on 7th day the neutropil infiltration highly in control than in treated wounds fig(2) . the infiltration become mild on 14th day in control wounds and disappeared completely in treated wounds fig(3). The infiltration of macrophage was more in treated wound than in control wounds at 3rd and 7th day. and their infiltration become 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 become obvious on 14th day. The new capillaries and fibroblast proliferation was more in treated wound.

Throughout the period of experiment, the progression of new epithelium to cover the wound area in treated wound more than the control wounds.



Fig. (1): 3rd day post operative day (Treated), exudates(E) with highly infiltration of neutrophils (N)and few macrophages(M).10x.H&E stain.

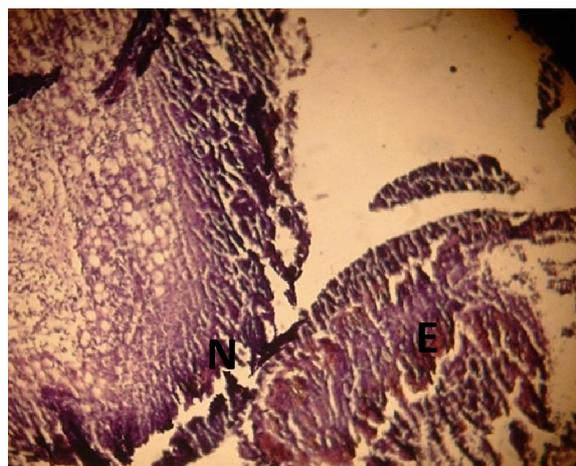


Fig. (1): 3rd day post operative day (Control), exudates(E) with highly infiltration of neutrophils (N)and no macrophages .10x.H&E stain.

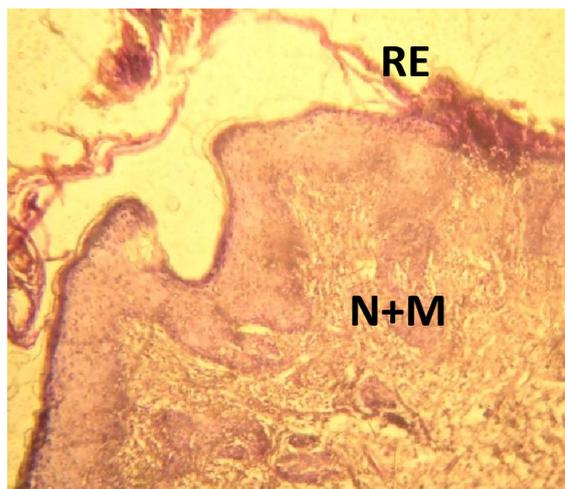


Fig. (2): 7th day post operative day (Treated), re-epithelization(RE) was more obvious, more macrophages and less neutrophils, 10x. H&E.



Fig. (2): 7th day post operative day (Control), re-epithelization(RE) was obvious, less macrophages and more neutrophils, 10x.H&E

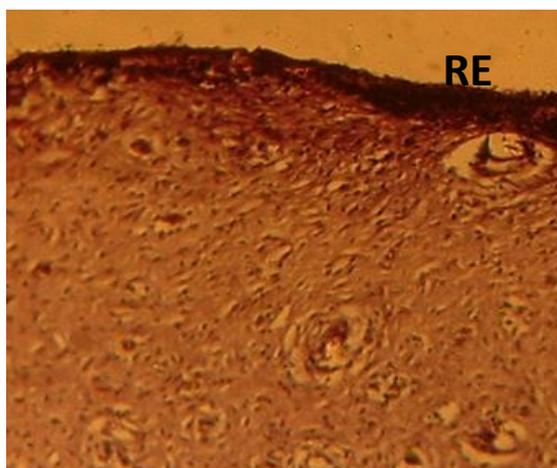


Fig. (3): 14th day post-operative day (Treated), complete healing. with complete re-epithelization, 10x. H&E stain.

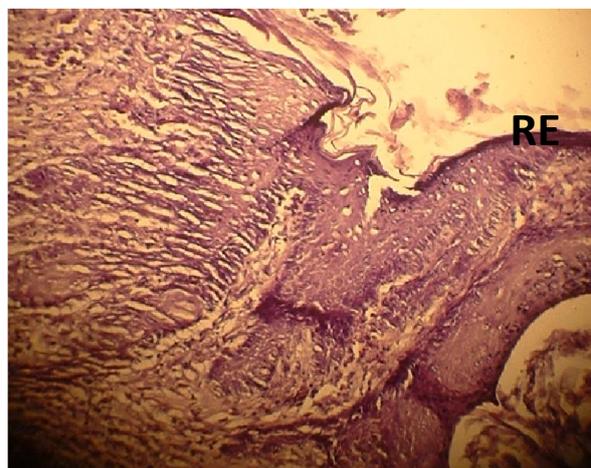


Fig. (3): 14th day post-operative day (Control), non -complete healing. 10x. H&E stain.

DISCUSSION

The present study clearly demonstrated that thymus vulgaris of full thickness excisional wounds have possessed a definite pro healing action in treated wound as compared with control wounds, and this observed in the rate of wound reduction with 100% contraction rate at 14th day post operative day, while in control wound was 74.1% at the end of experiment.

Thyme oil of *thymus vulgaris* contain thymol, phenol and triterpene (7), thymol can have helpful effects in controlling the inflammatory process that present in many

infections , therefore necessary for proper wound healing (14). Triterpene, the important constituent for wound healing (15).

(16) showed in their study that free triterpene in cumin extract show pro healing activity further more the complete Re-epithelization on 12th day post operative day in treated wounds and the increase in tensile strength of these wound as compared with control wounds ,since the above compound activate the infiltration of lymphocyte which in turn activate the formation of new epithelization (Re-epithelization) .from obtained result in the study, it can be stated that presence of thymol, triterpene in the thymus oil extract were responsible for wound healing.

تأثير المستخلص الزيتي للزعتر على التئام الجروح القطعية الكاملة الطبقات في الأرناب

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الخلاصة

وكانت هذه الدراسة لتحديد خصائص المستخلص الزيتي للزعتر للتئام الجروح . تم معرفة تأثير المستخلص على معدل إغلاق الجرح باستخدام نموذج الجروح القطعية الكاملة الطبقات ودراسة الامراضية النسيجية للأنسجة المعالجة والغير المعالجة والتي أجريت في ١٨ أرناب، حيث قسمت إلى ثلاثة مجاميع ، ستة حيوانات في كل مجموعة، تم تحضير المرهم الزيتي والذي استخدم موضعيا ثلاث مرات يوميا على الجرح ولمدة ١٤ يوم من إحداث الجرح وتم مقارنته مع مجموعة السيطرة . ويستنتج من ذلك أن معدل التقلص لوحظ في الجرح المعالج أسرع مقارنة مع مجموعة السيطرة وكان هذا مدعوما بالدراسة النسيجية المرضية .

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