

# Effect of Locally Applied Black-seed Oil and Honey Mixture on Wound Healing

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**Abstract:** Background: Wound healing is a complex process, which is influenced by multiple factors. Wound management still remains an important focus of researches. The use of natural products as an alternative treatment has been on the rise in the last few decades. Blackseed and honey are of the oldest alternative medicine. Objective: The aim of this study is to evaluate the effect of locally applied blackseed oil and honey mixture on experimental wound healing in rabbit. Material and methods: Twenty four young male rabbits from animal house, College of Medicine, University of Mosul were used in this study at 1st of June to 30 of November 2012. The animals divided into two groups after induction of circular wound by 4 mm sharp circular punch in both ears of rabbit under anesthesia. The first group is experimental, the wounds treated daily by local application of black seed oil and honey mixture, the second group is control, no local treatment applied. In the end of 1st, 2nd, 3rd, 4th weeks three animals from both groups were anesthetized and the wound area and excised. The wound area estimated and the specimens prepared for histological examination for the wounds healing. Results: The mean wound areas in experimental group at the end of 1st, 2nd, 3rd, 4th were 22, 11.7, 7.5, and 2.3 respectively, while in control group were 22.3, 13.8, 10.5, and 7 respectively, the difference between two means was significant only at end of 4th week (p value = 0.003). The histological examination of wound site in experimental group at 1st, 2nd, 3rd, and 4th weeks shows histological scoring 6.12, 8.33, 10.66, and 11.5 respectively, while in control group 5.66, 7.56, 8.2, and 10.16 respectively. The difference between the two means was significant at the end of 3rd and 4th week. Conclusion: The present study demonstrates that a locally applied blackseed oil and honey mixture enhances healing in experimental wound in rabbit ear.

Keywords: local, honey, blackseed, rabbit ear, Wound healing.

## INTRODUCTION

Wound healing is a complex biological cascade of cellular and biochemical events comprised of three phases: inflammation, proliferation, and maturation[1],[2]. Wound management still remains an important focus of researches[1],[2]. The use of natural products as an alternative treatment in wound healing and treatment has been on the rise in the last few decades[1],[2],[3]. Blackseed and honey are the most important natural remedies of Arabic Islamic medicine and used for various diseases for over 1500 years[3],[4],[5],[6],[7]. In Islam blackseed and honey are regarded as one of the greatest forms of healing medicine [3],[4],[5],[6],[7]. Topical application of honey to wounds has been found to enhance wound healing[5],[8],[9],[10]. Local application of blackseed oil experimental wounds has been found to enhance wound healing [11]. By reviewing the available literatures, there were no registered studies on effects of locally applied of the blackseed oil and honey mixture on wound healing. The aim of present study is to evaluate the effect blackseed oil and honey mixture as a topical agent in healing of identical experimental wound in rabbit ear.

## Material and methods:

This study was approved by the research ethics committee at the College of Medicine, University of Mosul and follows the council for international organization of medical sciences ethical code for animal experimentation. Twenty four young male aged 4 months locally bred New Zealand rabbits from animal house, College of Medicine, University of Mosul were used in this study at first of June to thirty of November 2012. Their average weight 1240 grams ranged between 1050 grams and 1500 grams. The animals were kept in separate metallic cages for one week for adaptation in animal's house. In each cage one animal feed with standard ration and water. The black seed oil and honey collected from local market from local production. A mixture of 100 ml from both of them prepared and preserved in refrigerator.

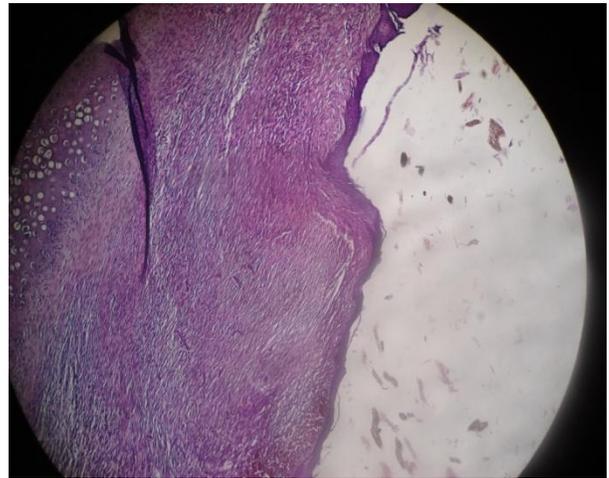
## Experimental technique

Food was suspended eight to ten hours prior to administration of anesthesia. Animals were anesthetized by intramuscular injection of ketamine (50 mg/kg of body weight) and intramuscular injection of diazepam (5.0 mg/kg of body weight). No preoperative antimicrobial prophylaxes given. The ear tip were cleaned by povidone iodine solution. Under an aseptic conditions technique, a circular wound created by compression of sharp terminal end 4mm diameter punch on the distal end of both ears, local bleeding controlled by local compression with povidone iodine soaked gauze for 5 minutes, no local dressing applied (figure – 1).

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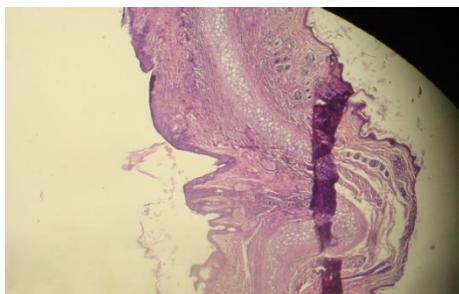


**Fig-1:** The circular wound induced in rabbit ear one week after induction of wound.



**Fig-3:** histological examination show thick, vascular mature fibrous tissue, and epithelium completely lining the wound, scored histologically 11.

The animals were assigned to two groups; the first group (12 animals) as experimental group treated by a daily local application of a mixture of black seed oil and honey to wound edges. The second group was control group (12 animals), no local application used. After 1st week, 2nd weeks, 3rd weeks, 4th weeks 3 animals from both groups were anesthetized again as described previously and the terminal end of ear were excised including the wound and area around it, and prepared for histological examination. The area of wounds were estimated after removal of marginal crust with aid of magnifying lens and tape measure; by taking the mean of mean of vertical and horizontal diameters, the means and standard deviations of wound area calculated, and samples prepared for histological examination. The excised samples prepared for histological examination by fixation with 10% formaldehyde solution, Then sections embedded in paraffin wax were cut and stained with haematoxylin and eosin. The site of wound examined histologically depending on technique described by Goncalves et. al, the wound healing histological scoring was based on the degree of cellular invasion (cellularity), granulation tissue formation, vascularity and re-epithelization 12. The histological scoring extend from 1 to 12 according to criteria wound healing, ( Fig - 2, 3). The code describing each animal treatment was broken after the histological scoring completed.



**Fig-2:** histological examination show immature granulation tissue dominated by inflammatory cells, small hematoma, and partial epithelialization overlying, scored histologically 5.

**Statistical analysis:**

Results are reported as mean ± standard deviation. The unpaired student (t) test used to calculate the differences between two means. The p value was considered a significant if it is less than 0.05.

**Results**

All animals survived to the end the study. No wound infection observed in the animals of either group, one animal develop transient ear paralysis resolve spontaneously in two weeks. The mean wound areas in experimental group at the end of 1st, 2nd, 3rd, 4th were 22 ± 4.6, 11.7 ± 3.9, 7.5 ± 2.9 ± 1.6, and 2.3 ± 2 respectively, while in control group were 22.3 ± 6.7, 13.8 ± 4.2, 10.5, and 7 ± 2.2 respectively, the difference between two means was significant only at end of 4th week (p value = 0.003), (Table - 1).

time of histological wound healing assessment t	Wound healing scoring in experimental group N= 6	Wound healing scoring in control group N= 6	P - value
	Mean ± SD	Mean ± SD	
end of 1 <sup>st</sup> week	6.12 ± 1.7	5.66 ± 1.52	0.632
end of 2 <sup>nd</sup> week	8.33 ± 1.42	7.56 ± 1.37	0.362
end of 3 <sup>rd</sup> week	10.66 ± 0.81	8.2 ± 1.32	0.003
end of 4 <sup>th</sup> week	11.5 ± 0.77	10.16 ± 0.72	0.011

**Table 1:** The wound areas in experimental and control group at different weeks of wound healing.

Wound healing Time	Wound areas mean in experimental group N= 6	Wound size in control group N= 6	P – value
	Mean $\pm$ SD	Mean $\pm$ SD	
1 week	22 $\pm$ 4.6	22.3 $\pm$ 6.7	0.930
2 weeks	11.7 $\pm$ 3.9	13.8 $\pm$ 4.2	0.391
3 weeks	7.5 $\pm$ 2.9	10.5 $\pm$ 1.6	0.05
4 weeks	2.3 $\pm$ 2	7 $\pm$ 2.2	0.003

The histological examination of wound site in experimental group at 1st, 2nd, 3rd, and 4th weeks shows histological scoring  $6.12 \pm 1.7$ ,  $8.33 \pm 1.42$ ,  $10.66 \pm 0.81$ , and  $11.5 \pm 0.77$  respectively, while in control group  $5.66 \pm 1.52$ ,  $7.56 \pm 1.37$ ,  $8.2 \pm 1.32$ , and  $10.16 \pm 0.72$  respectively. The difference between the two means was significant at the end of 3rd and 4th week only, (Table - 1 ).

**Table -2:** The histological wound healing scoring in experimental and control group.

## Discussion

In this study the macroscopic observations and wound areas evaluation shows that , the locally applied blackseed oil and honey mixture on experimental wound in rabbit ear shows late enhancement of wound healing by reducing the wound area significantly by the end of 4th week. This finding confirms the idea that the locally applied blackseed oil and honey mixture enhances wound healing. At the end of first week the wound was larger than expected in experimental and control group because of wound retraction and marginal slough. In this study also, the locally applied blackseed oil and honey mixture enhances wound healing in rabbits with experimental wound in ears by improving the wound healing scoring in histological examination of wound significantly at the end of 3rd and 4th weeks. The topical application is widely used as a therapeutic approach in the treatment of wounds[3]. Honey is the most commonly used complementary and alternative medicine topical preparation alone or in combination with black seeds[14]. Topical application of honey to wounds has been found to hastens wound healing[8],[9],[15],[16]. Microscopic evaluation demonstrated that there was a significant acceleration of dermal repair in wound treated with honey, and had have favorable influences on the various phases of wound healing[8],[15].Honey dressings make the wounds sterile in less time and have a better outcome in terms of hypertrophic scars[5],[8] . Studies show that topical honey application on wound healing include stimulation of tissue growth, enhanced epithelialization, improved angiogenesis granulation, improve fibrous connective tissue tensile strength and minimized scar formation[15],[17],[18],[19].Honey's antibacterial properties have been proved by many investigation[1],[6],[18],[19] . Many studies demonstrate that blackseed oil enhance wound healing in topical application, and it gained popularity as ingredients in topical skin care[11],[19],[20]. Histological studies confirm the improvement in the wounds healing of rabbit skin by blackseed oil[11],[19]. Blackseed (Nigella Sativa) extract, oil and ground seeds have a clear

and undeniable antibacterial effect[20],[21]. In this study, the blackseed oil and honey mixture shows clear evidence that it stimulate wound healing in histological examination and in macroscopical assessment of wound areas. The rabbit ear wound-healing model was used in this study, since this model allows to precisely measure the wound areas and new granulation tissue formation and re-epithelialization in a matched control manner, because the ear cartilage splints the wound with minimal contraction[22]. In conclusion, the present study demonstrates that a locally applied blackseed oil and honey mixture enhances healing in experimental wound in rabbit ear.

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#### تأثير خليط زيت الحبة السوداء و العسل الموضعي على التئام الجروح في الأرانب

##### المختصر

الخلفية : ان معالجة الجروح تبقى محور هام للباحثين. ان استخدام المواد الطبيعية كمعالجة بديلة للجروح اتسع في العقود القليلة الاخيرة. ان العسل والحبة السوداء مستخدمة كطب بديل منذ القدم.

الأهداف : تهدف الدراسة لتقييم تأثير خليط زيت الحبة السوداء والعسل كعلاج موضعي يومي على التئام الجروح المستحدثة في اذان الارانب .

المواد وطريقة العمل : تم استخدام اربع وعشرون من الأرانب النيوزلندية الذكور المكثرة محليا في هذه الدراسة و تمت هذه الدراسة في بيت الحيوانات بقسم الجراحة التجريبية في كلية الطب في الفترة من اول شهر حزيران الى اخر تشرين ثاني من عام 2012. تم إحداث جرح دائري بقطع جراحيا تحت التخدير العمومي وبواسطة مثقب دائري حاد قطره 4 في اذني الارانب. قسمت الحيوانات إلى مجموعتين كل مجموعة تتكون من اثنا عشر حيوانا. أعطيت المجموعة الأولى علاج موضعي يومي بخليط زيت الحبة السوداء والعسل فيما كانت المجموعة الثانية عينة ضابطة بدون علاج موضعي . وفي نهاية الاسبوع الاول والثاني والثالث والرابع تم قطع منطقة الجرح و ما حولها تحت التخدير العمومي لثلاثة افراد من كل مجموعة وتم تقدير مساحة الجرح لكل جرح وتم اجراء فحص نسيجي شكلي للعينات لتقدير درجة التئام الجرح.

النتائج : كان معدل مساحة الجرح في مجموعة التجربة خلال نهاية الاسبوع الاول والثاني والثالث والرابع 22 ، 11.7 ، 7.5 ، 2.3 على التوالي ، بينما كان في المجموعة الضابطة 22.3 ، 13.8 ، 10.5 ، 7 على التوالي ، وكان الفرق معنوي في نهاية الاسبوع الرابع فقط . اظهر الفحص النسيجي لمكان الجرح في مجموعة التجربة خلال نهاية الاسبوع الاول والثاني والثالث والرابع تقديرا نسيجيا ب 6.12 ، 8.33 ، 10.66 ، 11.5. على التوالي ، بينما كان في المجموعة الضابطة 5.66 ، 7.56 ، 8.2 ، 10.16 على التوالي. , وكان الفرق معنوي في نهاية الاسبوع الثالث والرابع .

الخلاصة : ان هذه الدراسة تبين بان خليط زيت الحبة السوداء والعسل كعلاج موضعي يومي يساعد في التئام الجروح في الحيوانات المختبرية ( الأرانب) المحدث لديها جرح في الأذن .