

Evaluation of the effect of Local exogenous application of extracted *Malva parviflora* and Honey on Wound healing in Rats

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Abstract

Wound healing is a complex dynamical interaction between various cell types, the extracellular matrix, cytokines, and growth factors. The present study was carried out for the purpose of verifying the speed of wound healing by herbs and honey, a surgical study for the rapid treatment of wounds in rats. Where he took 40 randomly and was divided into four groups of ten rats per group and as follows. The control group is composed of ten rats, a group of herb extract of the *Malva parviflora* consisting of ten rats, a group of honey consisting of ten rats and finally a group of herb *Malva parviflora* with honey consisting of ten rats. Where a wound was developed in the face of the rat and treated each according to his group for 5 days and 10 days to identify the changes that will occur in the wound area. The results of the present study showed that the best cure was in the fourth group, which is a mixture between the herb extract of the hibiscus and honey as explained in the results.

تقييم تأثير المستخلص الموضعي لعشبة الخبيزة والعسل في شفاء الجروح للجرذان

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الخلاصة

اجريت الدراسة الحالية لغرض التحقق من سرعة شفاء الجروح بواسطة الاعشاب والعسل وهي دراسة نسيجية جراحية لغرض سرعة علاج الجروح في الجرذان حيث اخذ اربعين جرذا عشوائيا وقسمت الى اربعة مجاميع بواقع عشرة جرذان للمجموعة الواحدة وكما يلي مجموعة السيطرة وهي مكونة من عشرة جرذان ، مجموعة مستخلص عشبة الخبيزة وهي مكونة من عشرة جرذان ، مجموعة العسل وهي مكونة من عشرة جرذان واخيرا مجموعة عشبة الخبيزة مع العسل وهي مكونة من عشرة جرذان. حيث تم استحداث جرح في وجه الجرذ وعولج كل حسب مجموعته ولمدة ٥ يوم و ١٠ يوم لغرض معرفة التغيرات التي سوف تحدث في منطقة الجرح. وقد بينت نتائج الدراسة الحالية ان افضل شفاء حدث في المجموعة الرابعة التي هي عبارة عن خليط بين مستخلص عشبة الخبيزة والعسل وكما موضحا في النتائج.

Introduction

Skin is the biggest external defense system(organ) ,it covers the outside of the body but has other functions beside the defense mechanism. It serves as a mechanical barrier between the inner part of the body and the external world (1). It consists of three layers, the outer layer is called epidermis, the middle layer is dermis

and the inner most layer is hypodermis. A wound is defined as a break or damage in the skin, resulting from physical or thermal damage or as a result of the presence of an underlying medical or physical condition, Wound healing is a complex process consisting of four steps, hemostasis, inflammatory reaction, proliferation and remodeling, all of which are regulated by

cytokines and growth factors released by cells in the wounded area. The phases are overlapping and linear for acute wounds, whereas the chronic wounds can be found at different stages of the healing process and do not heal in orderly manner[2]. *Malva* is a widespread tropical and temperate genus of the family Malvaceae [3].

Many species of this genus are efficient in cough, ulcers in the bladders, intestinal infections, colitis, tonsillitis, gastroenteritis, cholesterol and lipid-lowering, antihypertensive, antioxidant, analgesics, emollient, pectoral girdle and arteriosclerosis treatment. In addition, the plants are also used externally as antidandruff, demulcent softening of tumors and abscess, *Malva parviflora* (known as cheese weed) is growing in waste ground, roadsides and desert plains in Egypt [3]. Honey has been in use as a wound dressing for thousands of years[4,5]. In the past few decades, there has been a large amount of clinical evidence that has been accumulated that demonstrates the effectiveness of honey in this application[6,7]. However, it is only in more recent times that the science behind the efficacy has become available. It is now understood that honey is not just sugar syrup with certain physical properties that make it suitable as a wound dressing material, but that it is a biologic wound dressing with multiple bioactive components that can expedite the healing process.

Material and method

Study design

The animals were divided into following groups according to the applicable of materials.

1-(Control group): the skin defect treated with 1µl of normal saline and its number represented the all number of the following experimental groups as the left side of each animal considered to be the control.

2. Experimental group includes:

A. Group I contains (10) rats, the skin defect topical with 1µl of honey.

B. Group II contains (10) rats, the skin defect topical with 1µl of *Malva*.

C. Group III contains (10) rats, the skin defect topical with 1µl of 1µl combination of (Honey & *Malva*). Every single group will be composed of 15 rats that will be studied in three periods 5,10 days (5 rats for each period), we sacrifice the rats at the each periods.

Microscopic examination

Tissues were quickly excised and fixed in 10% formalin neutral buffer solution. The trimmed tissues were first washed with tap water followed by dehydration through a graded alcohol series and then passed through xylol and paraffin series before being embedded in paraffin. The paraffin blocks were cut into 5-6 µm sections stained with Hematoxylin and Eosin and examined under a light microscope

The Results

Control groups

Five days:

Histological findings at wound site of control group for 5 days duration shows complete epithelialization and there is decreased number of inflammatory cells together with formation of loose collagen fibers, The other view shows granulation tissue with irregular arrangement of collagen fibers and characteristic by proliferation of endothelial cell, new blood vessels.

Ten days:

Histological section at wound site of 10 days duration of control group shows reepithelialization with completely structure basal present, besides remodeling of collagen fibers can be detected, View shows formation new blood vessels and active fibroblasts with remodeling of collagen fibers can be detected.

Honey groups

Five days:

Histological findings of experimental group (Honey) of 5days duration showed reduction in inflammatory cells and replacement of granulation tissue by fibrous with scattered fibroblasts and complete epithelization is seen too The other microphotograph shows condensed collagen fibers with signs of remodeling accompanied by active fibroblasts and formation new blood vessel

Ten days:

Histological section at wound site of (Honey)10days duration view of skin section at wound site for experimental group revealing complete epithelization ,the new epithelium is thin with no rete-ridges, healed wound site with fibrous and reduced cellular component can be detected, Magnified of other view shows epithelial cell layers of the new epidermis and few fibroblasts are seen scattered throughout the fibrous of the underlying derim and formation of a new blood vessels.

Malva groups

Five days:

Histological findings of experimental group (*Malva*) of 5days duration showed reduction in inflammatory cells and replacement of granulation tissue by fibrous with scattered fibroblasts and complete epithelization is seen too The other microphotograph shows condensed collagen fibers with signs of remodeling accompanied by active fibroblasts

Ten days:

Histological section at wound site of (*Malva*)10days duration view of skin section at wound site is revealing complete

epithelization , narrow incision which characteristic by perfused fibrosis formation of new blood vessels, and thick keratized layer with normal epidermal layer, Magnified of other view shows epithelial cell layers of the new epidermis and few fibroblasts are seen scattered throughout the fibrous of the underlying derim

Combination groups

Five days:

Histological findings of experimental group combination(Honey & *Malva*) of 5days duration showed extension of epidermal layer in both edge of incision and reduction in inflammatory cells and replacement of granulation tissue by fibrous with scattered fibroblasts and complete epithelization is seen too The other microphotograph shows condensed collagen fibers with signs of remodeling accompanied by active fibroblasts and formation new blood vessel and proliferation of epidermal cells.

Ten days:

Microphotograph view at wound site of Honey and *Malva* group view of skin section at wound site is completed healing of incision which characteristic by fused of both edge together and revealing complete epithelization ,the new epithelium is thin with no rete-ridges, healed wound site with fibrous connective tissue with reduced cellular component can be detected, Magnified of other view shows epithelial cell layers of the new epidermis and few fibroblasts are seen scattered throughout the fibrous connective tissue of the underlying derim

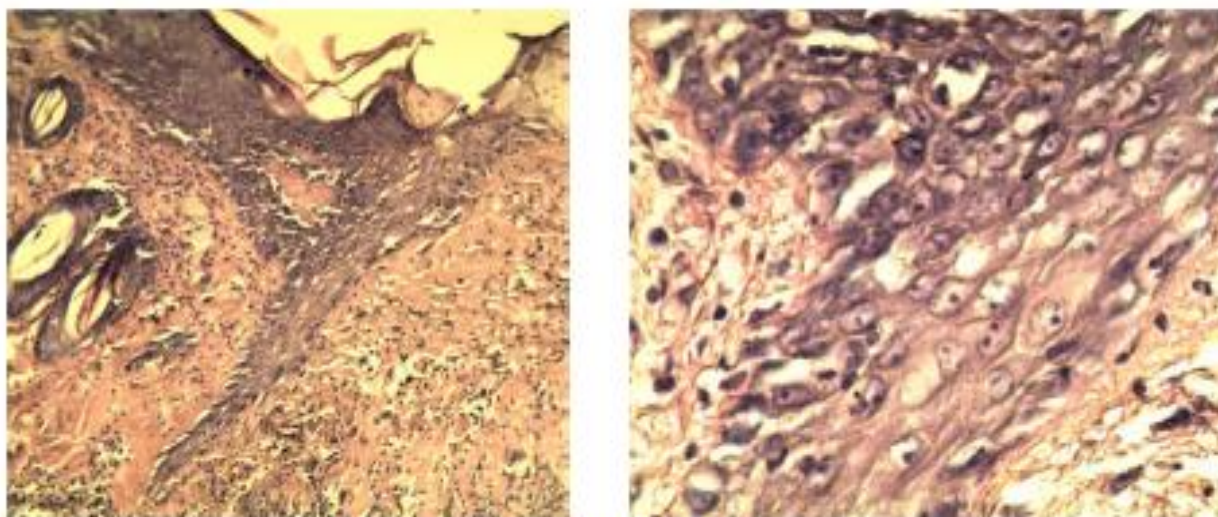


Fig1: 5 days control treated with normal saline

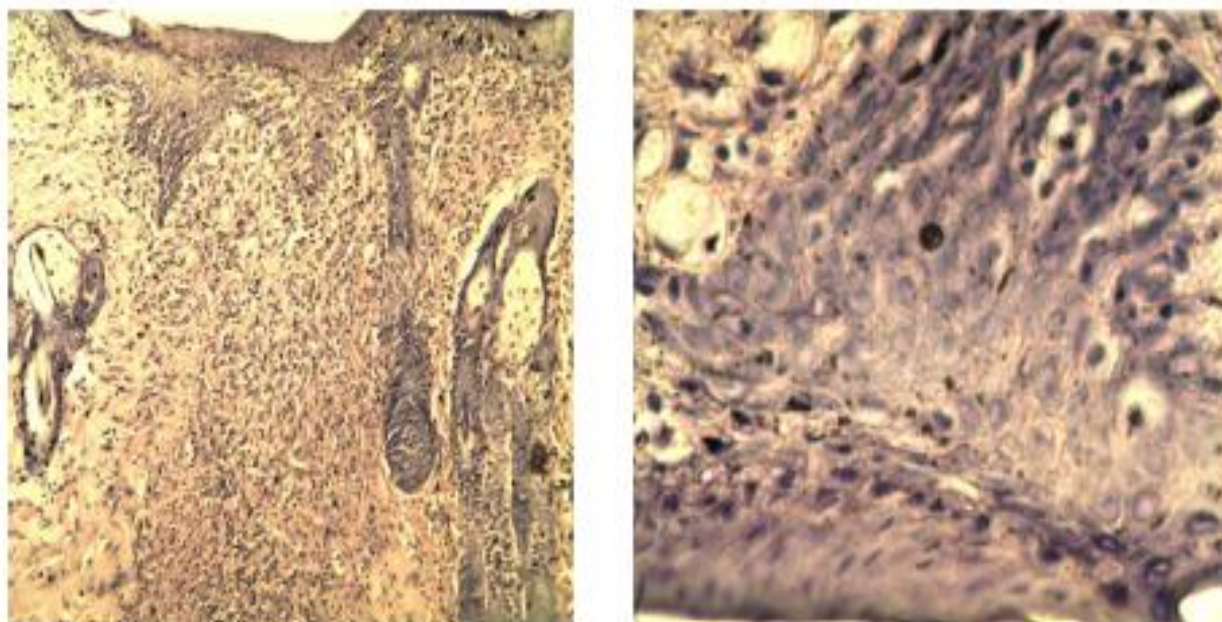


Fig2: 10 days control treated with normal saline

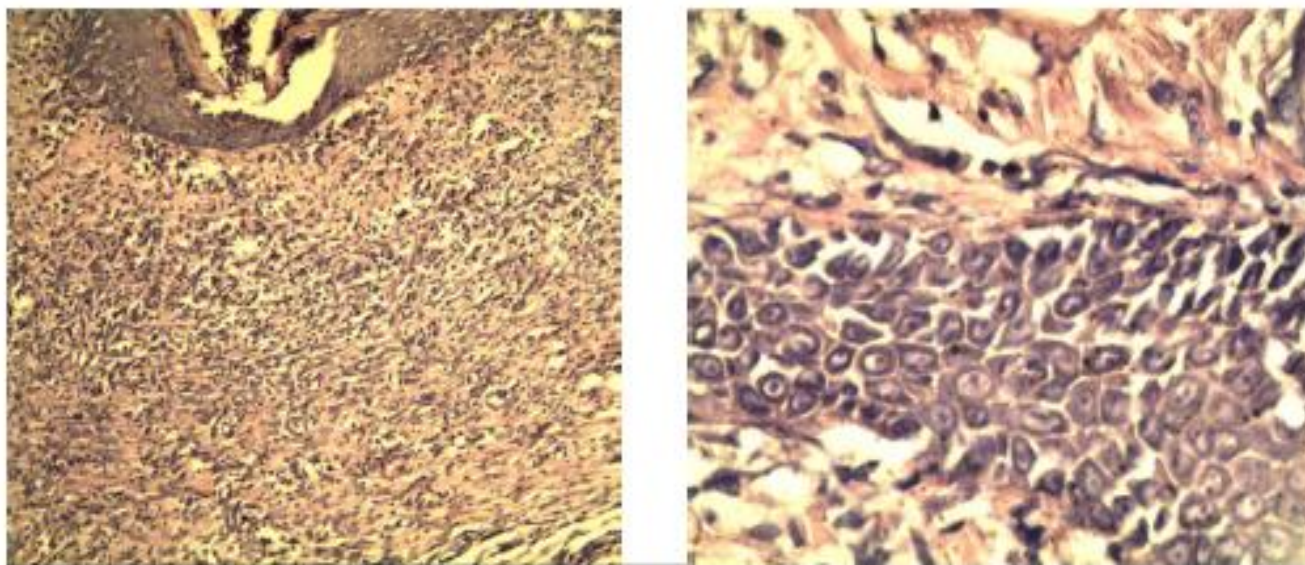


Fig3: 5 days Honey treated with Honey

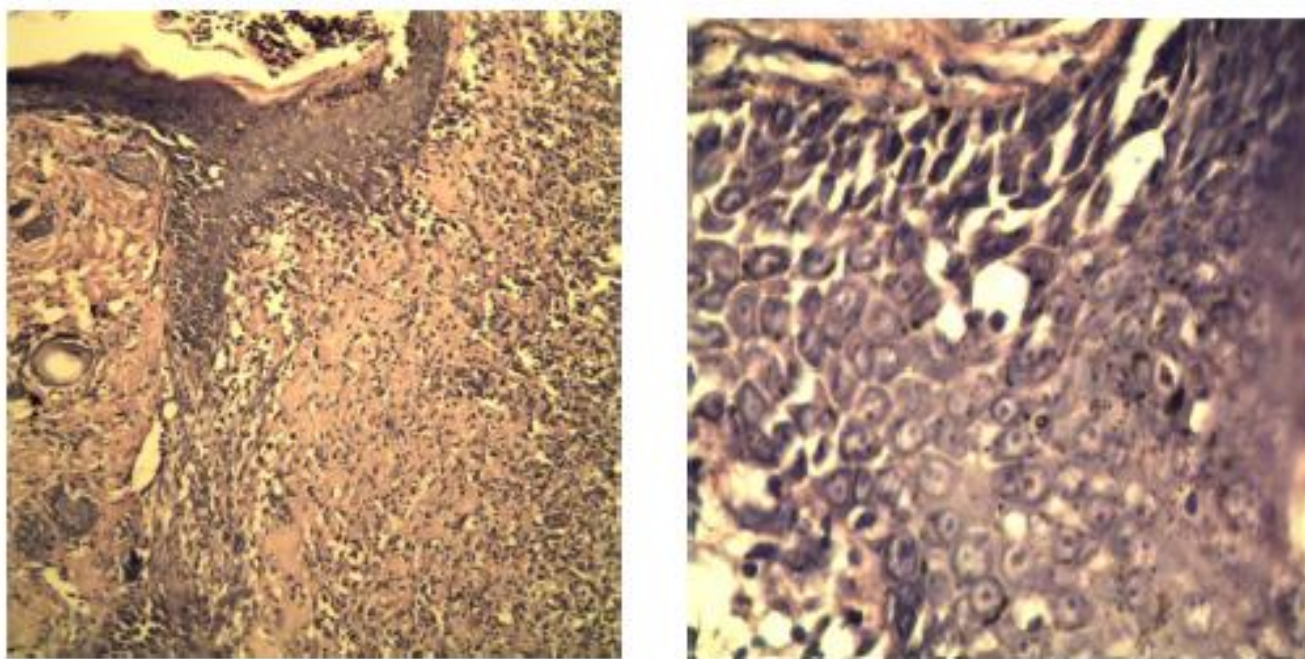


Fig4: 10 days Honey treated with Honey

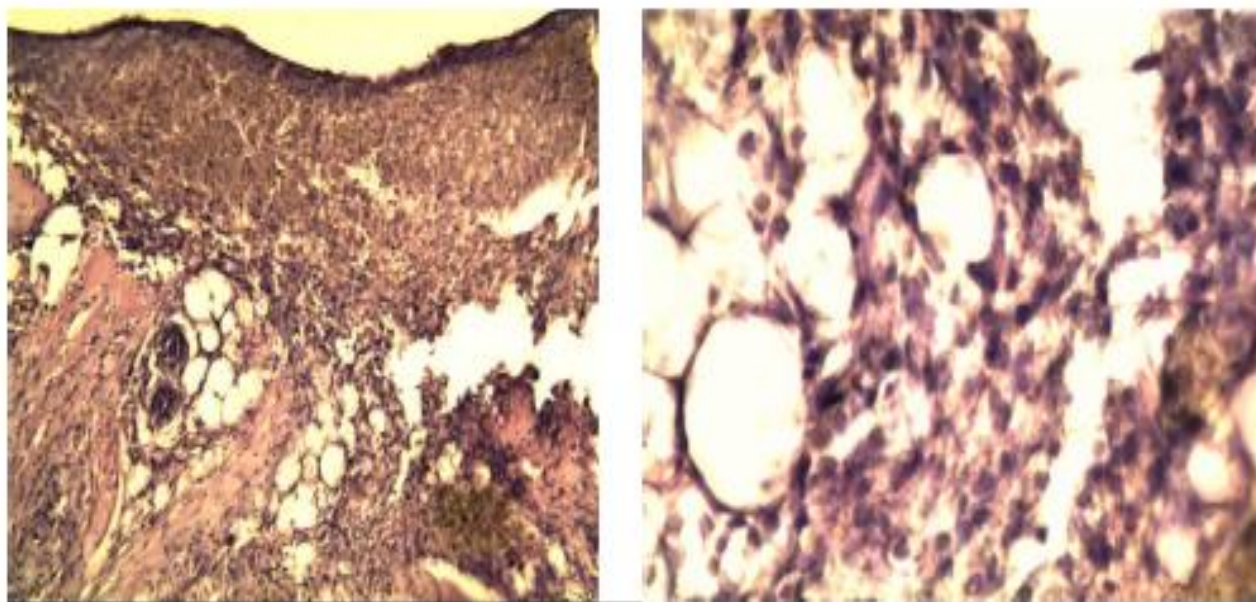


Fig5: 5 days *Malva* treated with extracted *Malva*

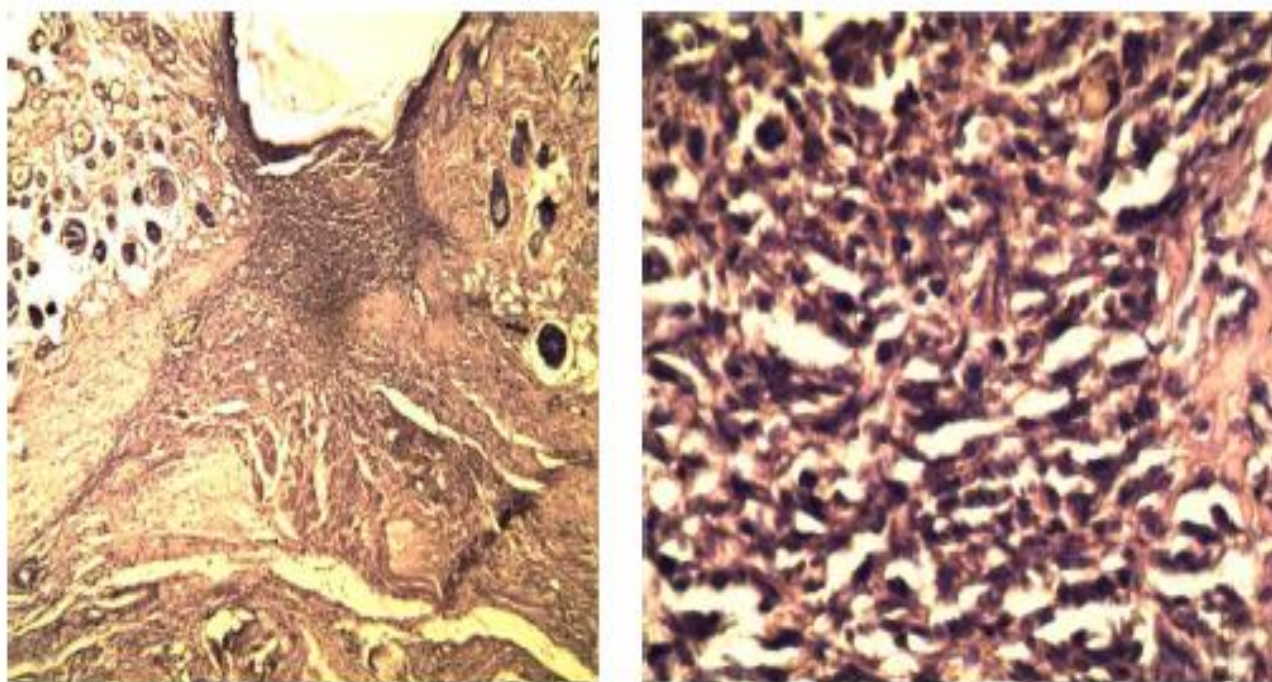


Fig6: 10 days *Malva* treated with extracted *Malva*

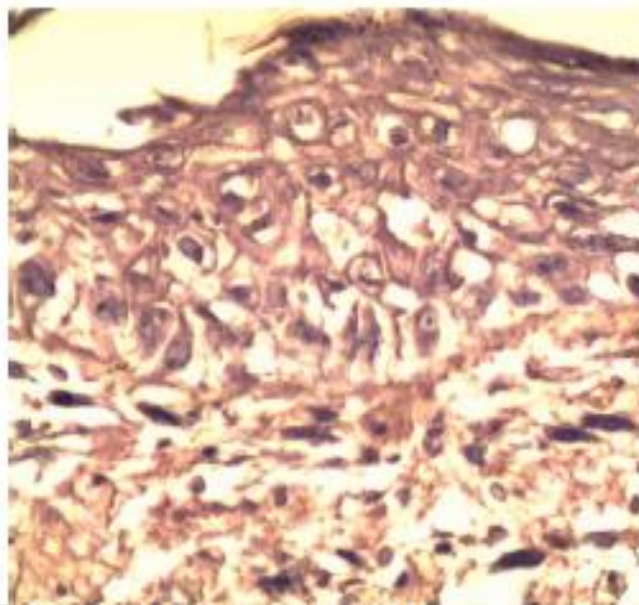
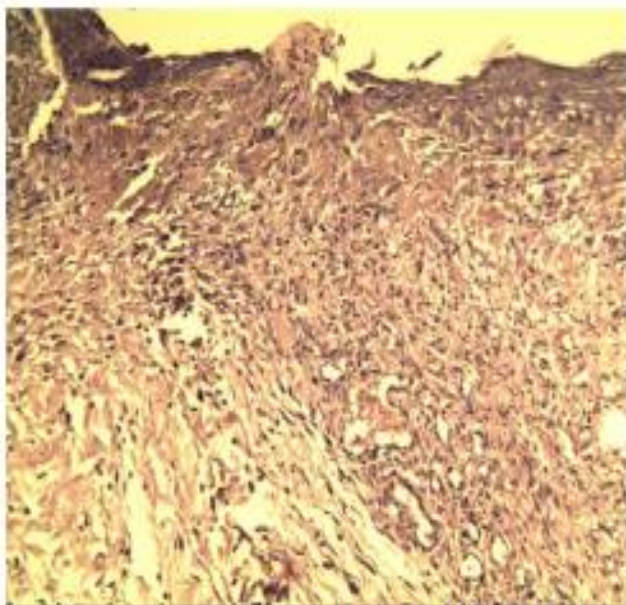


Fig5: 5 days combination treated with extracted *Malva* and Honey.

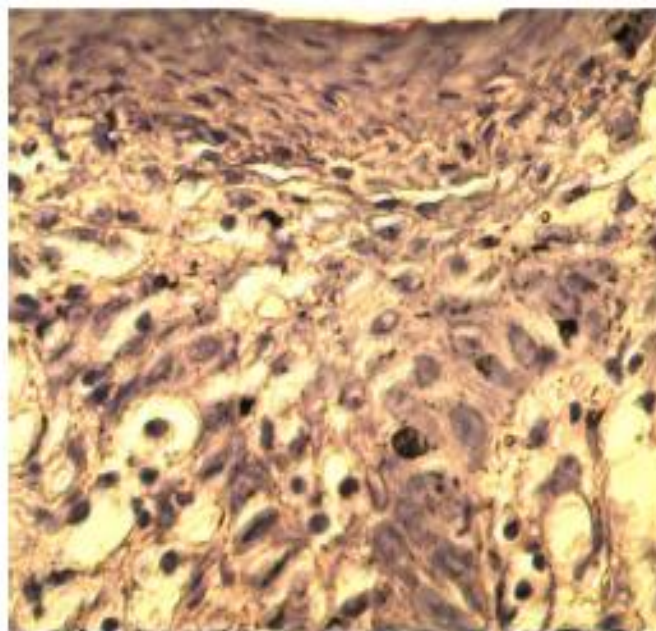
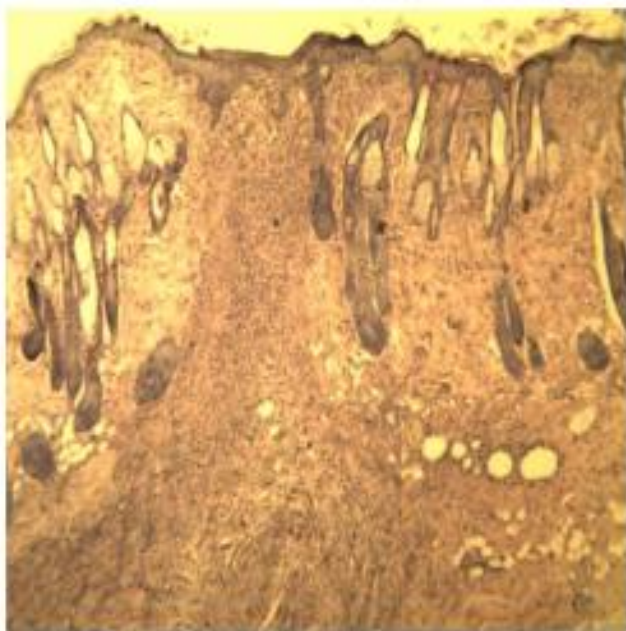


Fig10: 10 days combination treated with extracted *Malva* and Honey.

Discussion

Wound can be described as loss of tissue integrity which is a result of pathological change or physical trauma. Wound healing is a natural process to return the skin or mucosa back to its normal state [8]. Skin wound healing follow the same pattern of the four phases of haemostasis, inflammation, proliferation and maturation/matrix remodeling. Recently, there has been an increasing interest in alternative medicines and natural medicinal products for the local treatment of wounds because of the fast of wound healing of traditional drug treatments. The use of animal models has allowed for analysis of some aspects of the oral cavity and other associated structures

that would not be easily studied in humans. In the studies involving the skin tissue in face, rats are commonly used because they present some advantages in relation to other animals. Among these advantages are: the low cost, the easy manipulation, maintenance in controlled environmental and sanitary conditions including special diets. Another advantage is the similarity between the skin tissue in face of rats and humans, which is basically by made up of epithelial tissues on the surface and subjacent connective tissue, called basal lamina which offers support and nutrition to the epithelium [9]. Wound infection is an important factor that delays or inhibits wound healing. Wound healing needs a good healthy environment so that the normal physiological process will result in a normal healing process with minimal scar formation. One of the most important strategies to keep the process of healing ongoing is to sterilize damaged tissue from any microbial infection. Honey has many effects, such as antibacterial, antioxidant, antitumor, anti-inflammatory, and various metabolic effects. Regarding antibacterial activity, inhibition of bacterial growth has

been shown using impregnated honey disks or incorporating honey into agar plates[10]. How much of this inhibition is due to honey's antimicrobial properties or to its acidity and hyperosmolar nature is not well established[10]. In this regard, the hyperosmolar sugar paste also has antibacterial activity and is superior to antiseptics[11]. Various studies explore that honey has beneficial effects on wound healing apart from its antibacterial properties. The accelerative effect of honey in the wound healing process is related to its physical properties of hygroscopicity, hypertonicity, lower pH, and complex chemical composition. However, the stimulatory effects obtained when honey was administered orally or parenterally suggest that a tissue growth factor may be involved, rather than stimulation of growth being a consequence of wound acidification or improved nutrition of the tissue. Honey provides a moist healing environment yet prevents bacterial growth even when wounds are heavily infected. It is a very effective means of quickly rendering heavily infected wounds sterile, without the side effects of antibiotics, and it is effective against antibiotic-resistant strains of bacteria[12]. Its antibacterial properties and its viscosity also provide a barrier to cross-infection of wounds. It also provides a supply of glucose for leucocytes, essential for the 'respiratory burst' that produces hydrogen peroxide, the dominant component of the antibacterial activity of macrophages [13]. Furthermore it provides substrates for glycolysis, which is the major mechanism for energy production in the macrophages, and thus allows them to function in damaged tissues and exudates where the oxygen supply is often poor [13]. The acidity of honey (typically below pH4[14]) may also assist in the antibacterial action of macrophages, as an acid pH inside the vacuole is involved in killing ingested

bacteria[13]. Whether it is through this action, or through preventing the toxic unionized form of ammonia from existing that is involved[15], topical acidification of wounds promotes healing [16]. The high glucose levels that the honey provides would be used by the infecting bacteria in preference to amino acids [17] from the serum and dead cells, and thus would give rise to lactic acid instead of ammonia and the amines and sulphur compounds that are the cause of malodour in wounds. Honey gives a fast rate of tissue regeneration and suppression of inflammation, oedema, exudation and malodour in wounds, as evidenced in clinical observations and the results of animal studies and clinical trials. The antibacterial properties clearing infection could alone account for these effects by preventing the production of the products of bacterial metabolism which are responsible for the contrary conditions. But honey has a direct trophic and anti-inflammatory effect on wound tissues, as evidenced by the results of animal studies in which there was no bacterial infection involved, particularly in those where the honey was administered systemically. Honey can be expected to have a direct nutrient effect on regenerating tissue because it contains a wide range of amino acids, vitamins and trace elements in addition to large quantities of readily assimilable sugars [14]. (The vitamin C content of honey, which is typically more than three times higher than that in serum, and may be many times higher, could be of particular importance as because of the essential role of this vitamin in collagen synthesis.) In addition, the high osmolarity of honey causes an outflow of lymph which serves to provide nutrition for regenerating tissue which otherwise can only grow around points of angiogenesis (seen as granulation): healing is delayed if the circulation to an area is poor, or if a patient

is poorly nourished. Also it has been suggested that the decreased turgor resulting from the application of honey may increase oxygenation of tissues [14]. As well as providing an immediate anti-inflammatory treatment the honey would provide an antibacterial action and a barrier to further infection of the wound. *Malva* is a widespread tropical and temperate genus of the family Malvaceae [3]. Many species of this genus are efficient in cough, ulcers in the bladders, intestinal infections, colitis, tonsillitis, gastroenteritis, cholesterol and lipid-lowering, antihypertensive, antioxidant, analgesics, emollient, pectoralgia and arteriosclerosis treatment. In addition, the plants are also used externally as antidandruff, demulcent, softening of tumors and abscess, *Malva parviflora* (known as cheese weed) is growing in waste ground, roadsides and desert plains in Egypt [3]. *Malva parviflora* have higher antibacterial as well as antifungal activities against *Bacillus subtilis*, *Staphylococcus aureus*, *Escherichia coli*, *Proteus vulgaris*, *Aspergillus niger* and *Aspergillus oryzae* than hexane and ethanol extract showing values of growth inhibition zones in the range of 11.67 to 15.58, 10.96 to 14.32, 13.03 to 16.00, 10.38 to 15.37, 10.19 to 14.25 and 10.97 to 16.58mm, respectively. These findings agree with those found in the previous studies [18; 19].

Conclusion:

Through the results of the present study we recommend more use of plant extracts with honey in accelerating the healing process of wounds as well as the expansion of scientific research in this type of operation

References

- 1- Emmerson EP, The role of estrogen and MIF in cutaneous wound healing, phd thesis University of Manchester. 2010 ; ch1 :31,77.
- 2- Boateng JS, Matthews KH Stevens HNE and Eccleston GM. "Wound healing dressings and drug delivery systems: a

review." *Journal of Pharmaceutical Sciences*. 2008 ; 97: 2892-2923.

3- Zohary,M.(1987).Flora Palaestina.The Israel Academy of Sciences and Humanities part II. pp 315–319.

4- Forrest RD. Early history of wound treatment. *J R Soc Med*. 1982;75(3):198-205.

5- Majno G. *The Healing Hand. Man and Wound in the Ancient World*. Cambridge, Massachusetts: Harvard University Press; 1975.

6- Molan PC. The evidence supporting the use of honey as a wound dressing. *Int J Low Extrem Wounds*. 2006;5(1):40-54.

7- Molan PC. The evidence and the rationale for the use of honey as a wound dressing. *Wound Pract Res*. 2011;19(4):204-221.

8- Yuslianti,E.R , I.P. Rahmayani Sabirin and Afifah B. Sutjiatmo Effectiveness of Noni (*Morinda citrifolia* L.) Leaves Extract Gel as Standardized Traditional

Medicine to Accelerate Oral Mucosa Wound Healing on Wistar Rats, *Rsearch Journal of Medicinal Plants*. Year: 2014 | Volume: 8 | Issue: 4 | Page No.: 156-166.

9- Cavalcante CavGalyléia Meneses ; Renata Jana ına Sousa de PaulaI; Leonardo Peres de SouzaII; Fabr ıcio Bitu SousaIII; M ırio Rog rio LimaMotaIII; Ana Paula Negreiros Nunes Alves Experimental model of traumatic ulcer in the cheek mucosa of rats .*Acta Cir. Bras*. 26 (3) : 227-34 . 2011.

10-Karayil, S., Deshpande, S.D., and Koppikar, G.V. (1998) Effect of honey on multidrug resistant organisms and its synergistic action with three common antibiotics. *J. Postgrad. Med*. **44**, 93–96. 153.

11-Archer, H., Barnett, S., Irving, S., Middleton, K., and Seal, D. (1990) A controlled model of moist wound healing comparison between semipermeable film, antiseptics and sugar paste. *J. Exp. Pathol*. **71**, 155–170.

12- Molan P, Brett M. Honey has potential as a dressing for wounds infected with MRSA, The Second Australian Wound Management Association Conference, Brisbane, Australia, 18-21 March, 1998, 1998.

13- Ryan GB, Majno G. Inflammation. Kalamazoo, Michigan: Upjohn, 1977:80.

14-White JW. Composition of honey. In: Crane E, ed. *Honey: a Comprehensive Survey*. London: Heinemann, 1975:157206.

15- Leveen HH, Falk G, Borek B, et al. Chemical acidification of wounds. An adjuvant to healing and the unfavourable action of alkalinity and ammonia. *Ann Surg* 1973; 178(6):745-753.

16-Kaufman T, Eichenlaub EH, Angel MF, Levin M, Futrell JW. Topical acidification promotes healing of experimental deep partial thickness skin burns: a randomised double-blind preliminary study. *Burns* 1985; 12:84-90.

17-Nychas GJ, Dillon VM, Board RG. Glucose, the key substrate in the microbiological changes in meat and certain meat products. *Biotechnol Appl Biochem* 1988; 10:203-231.