Enhanced wound contraction in fresh wounds dressed with honey in wistar rats (*Rattus Novergicus*)

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Summary

Background: **Due to reports that honey accelerates wound healing, an investigation on its role in wound contraction in fresh wounds inflicted on wistar rats was carried out.**

Method: **Twenty adult male wistar rats had 2cm by 2cm square wound inflicted on their right dorsolateral trunk. They were divided into two groups. The experimental group had their wounds dressed with honey while the control group had normal saline dressing. Wound dressing was done every five days and measurements taken at each dressing. Wound morphology was also assessed.**

Results: **Dressing with honey significantly enhanced percentage wound contraction on day 10 with value of 79.20 ± 2.94 compared to control value of 53.50 ±4.32. p=0.0. The mean wound measurement on day 10 reduced significantly in honey group, 1.15±0.18 compared to control group 2.38 ± 0.28. p=0.002. However, there was no significant difference in fibroblast count per high power field in honey group 68.0 ±2.59 compared to control 90.2 ± 17.40, p=0.242.**

Honey dressing increased mean blood vessel count per high power field, 18.8±3.77 albeit non significantly when compared to control value of 13.4±2.44, p=0.264. Also honey dressing caused increased granulation tissue formation in wounds dressed with honey compared to control group.

Conclusion: **Our study suggests that honey dressing enhances wound contraction in fresh wounds which is one of the key features of wound healing.**

Keywords: ***Wound contraction, Honey, Healing.***

Résumé

Introduction: D'après des rapports que la consommation du miel accélere la cicatrisation de blessure, une enquête sur son effet en matiére du reserrement en ce qui concerne une plaie récente fait aux wister rats a été effectuée.

Méthode: On a fait une plaie de 2cm par 2cm carré dans le côté droit du trompe des vingt wistar rats du sexe masculin adultes. On a divisé ces rats en groupe de deux, et on a soigné le groupe d'experimentation avec le miel tandis que le groupe du contrôle était traité normalement avec le goutte-à-goutte de solution saline . On fait un pansement tous les cinq jours et on prend les mesures pendant chaque pansement. On a également évalué la morphologie de la blessure.

Resultats: Le pansement avec le miel a remarquablement amélioré le pourcentage du resserrement de la plaie pendant le dixième jour avec la valeur 79,20 ±2.94 par rapport à la valeur du groupe du contrôle de 53,50 ± 4.32 P =0,0 Le moyen de la mesure de la plaie dans le dixième jour était remarquablement en basse dans le groupe du miel, 1,15±0.18 par rapport au groupe du contrôle 2,38 ± 0.28, P=0,002. Toutefois, il y a aucune difference importante en matière du compte fibroblast par high power field dans le groupe du miel 68,0 ± 2,59 par rapport au groupe du contrôle 90,2 ± 17,40, P= 0.242.

Le pansement avec du miel a augmenté le moyen du compte du vaisseau sanguin par power field élevé dans le groupe du miel 18,8±3,77 albeit non important par rapport à la valeur du contrôle de 13,4 ± 2.44 P = 0,264. En plus, pansement avec du miel a provoque une augmentation dans la formation du tissu de la granulation en matière des blessures traitées avec du miel par rapport au groupe du contrôle.

Conclusion: Cette étude, évoque que traitement avec du miel provoque un resserrement d'une plaie en matiére des plaies récentes qui est l'un des trait clés de la cicatrisation des blessures.

Introduction

Healing of wounds caused by accidents, assault, warfare and surgical operations has always been of central consideration in surgical practice because any breach in the surfaces of the body-the skin and mucus membrane exposes the deeper tissues to the danger of infection[1].

Prompt attention is highly desirable in all cases of soft tissue injury[2]. One important aspect of wound management is dressing with topical healing agents. Honey has been used as a medicine since ancient times in many cultures[3]. With regards to wounds, honey has a well established usage as a wound dressing in ancient and traditional medicine[4]. In recent times this has been rediscovered, and honey is in fairly widespread use as a topical antibacterial agent for the treatment of wounds, burns and skin Ulcers.[5] Much of the effectiveness of honey as a wound dressing appears to be due to its Antimicrobial properties; since infection is one of the common impedi-

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ments to wound healing[6]. The healing process will not occur unless infection is cleared from a lesion: swabbing of wounds dressed with honey has shown that the infecting bacteria are rapidly cleared[7,8,9].

Wound healing in mammals is a complex interplay of connective tissue deposition, epithelialisation, neovascularisation and wound contraction. Despite the numerous documentation of wound healing properties of honey, its antimicrobial effect on wounds; surprisingly most of the works were on burns, infected wounds and chronic ulcers[10,11,12]. Literature is sparse on its role on wound contraction in freshly incised wounds, thus the reason for this study.

Materials and methods

Twenty adult male wistar rats weighing between 170 – 220gm were utilised for the experiment. The animals were gotten from the animal house of the Department of Anatomy, College of Medicine, University of Ibadan, Only animals without pre-existing skin lesion were used for the study and they were fed with standard rat pellet cubes from Ladokun feeds limited. Water was provided *ad libitum*. The animals were divided into two groups of 10 animals each. Group A animals served as the control group and had their wounds dressed with normal saline. Group B animals served as the experimental group and had their wounds dressed with honey.

Wounds were created by anaesthetizing the animals with chloroform inhalation, shaving the hair on the dorsolateral aspect of the trunk with an electric clipper and the skin cleaned with methylated spirit. A $2cm^2$ by 2^2cm square was traced on the skin with an already cut sterile template and wound incised along those margins as closely as possible till the panniculus carnosus layer was removed to prevent its presumed active role in wound contraction.[2] Sterile transparent cellophane was placed on the wound, the outlined traced on it to give the wound dimension on day 0 and the area determined using graph paper as described by Billingham[13].

Haemostasis was secured by direct application of pressure and the wounds dressed with gauze after the appropriate dressing agent has been applied to the wound surface. This was further covered with another layer of gauze and the dressing secured in place by plaster that was taped circumferentially round the trunk.

Dressings were changed every five days till complete re-epithelialisation was completed. During each dressing wound dimensions were measured and morphology assessed. An animal was selected randomly from each group on day 10 to assess granulation tissue and when re-epithelialisation was completed. The healed scar and the specimen harvested on day 10 were fixed in 10% buffered formalin for 72 hours and processed for histology using paraffin wax technique. This was subsequently stained with haematoxylin and eosin. Using light microscope, fibroblast and blood vessel count were done per high power field.

Granulation tissue pattern was reviewed.

Statistical analysis

Data were expressed in mean ± standard error of mean (S.E.M) and analysed using the students t-test where appropriate. Values of $p<0.05$ were considered significant.

Results

All the animals survived the period of experimentation and they tolerated the dressing materials well. The wounds in both experimental and control groups remained clean throughout the perior of the experiment. The granulation tissue profile in both groups appeared similar during the first 10 days of the experiment, however the granulation tissue in the group with honey dressing increased after day 10.

Both groups had adequate inflammatory infiltrate and new blood vessel formation. There was marked reduction in the wound dimension on day 10 in the honey group-1.15± 0.18 compared with a value of 2.38 ± 0.28

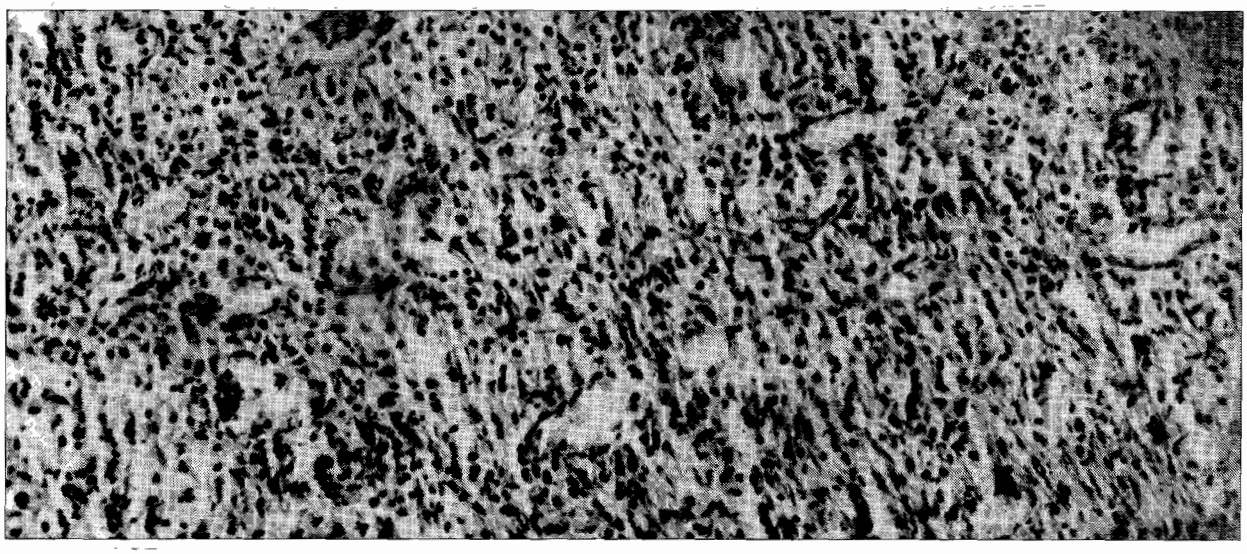

Fig. 1. ***Photomicrograph showing an H & E section of the granulation tissue from experimental animal. X160.***

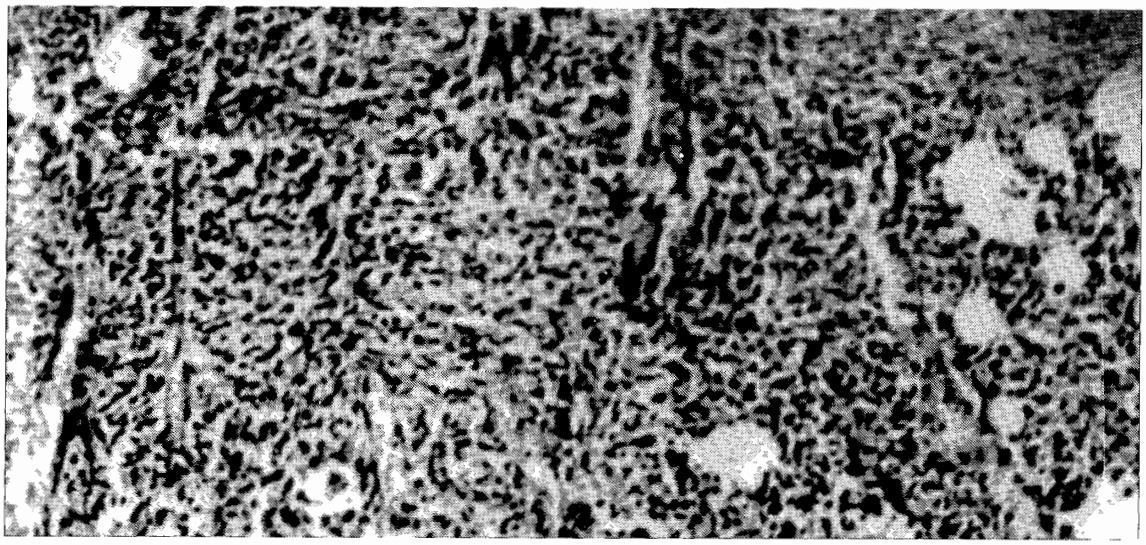

Fig. 2. *Photomicrograph showing an H & E section of the granulation tissue from control animal. X160.*

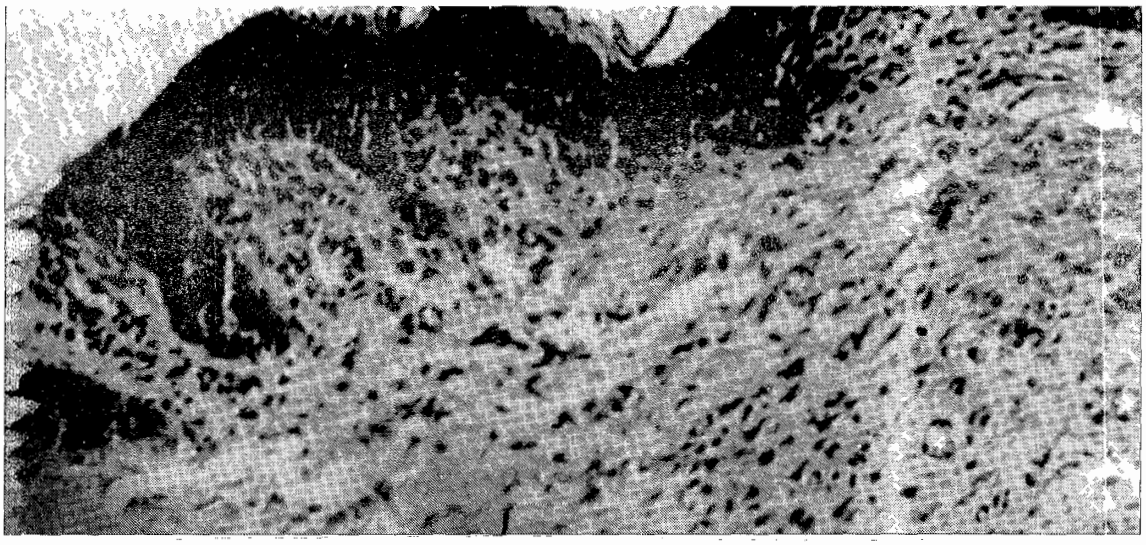

Fig. 3. *Photomicrograph showing an H & E section of the healed scar tissue from control animal. X160.*

observed in the control group. This is significant with a p value of 0.002 ($p<0.05$).

The percentage wound contraction was remarkably enhanced in the honey group with a mean value of 79.20 ± 2.92 compared with control group value of 53.50 ± 4.32. This is significant with a p value of 0.00 ($p<0.05$).

The values of wound measurements on day 0, 10 percentage wound contraction of day 10, mean fibroblast

Table 1 Wound and Dimensions on day 0.10 percentage wound contraction on day 10 mean fibroblast and mean blood vessel counts

	Wound dimension on day 0(cm^2)	Wound dimension on day 10 (cm^2)	Percentage wound contraction on day 10	Mean fibroblast count per high power field	Mean blood vessel count per high power field
Group A	5.38 ± 0.23	2.38 ± 0.28	53.50 ± 4.32	90.20 ± 17.40	13.40 ± 2.44
Group B	5.37 ± 0.41	1.15 ± 0.18	79.20 ± 2.92	68.00 ± 2.59	18.80 ±3.77
p-value	0.990 (p>0.05)	0.002 (p<0.05)	0.00 (p<0.05)	0.242 (p>0.05)	0.264 (p>0.05)

Values = mean ± standard error of mean; $p < 0.05$ is significant

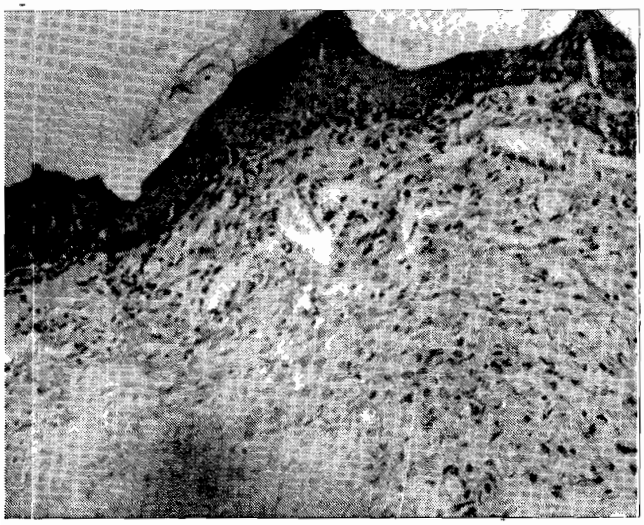

Fig. 4. ***Photomicrograph showing an H & E section of the healed scar tissue from experimental animal. X160.***

count and mean blood vessel count per high power field are outlined in table 1.

Discussion

Wound healing in its broadest sense implies the replacement of damaged tissues by living tissues which in mammals is essentially fibrous tissue. When there is significant tissue destruction the compound structures heal through synthesis of fibrous tissue by organisation of stimulated granulation tissue, wound contraction which ensures the inward movement of viable tissue margins into closer approximation and epithelialisation.

Wound contraction involves a great deal of passive or mechanical stresses responsible for elastic recoil of granulation tissue[14]. Honey have been demonstrated in previous studies to enhance wound healing when compared to conventional treatment[15]. Thus we did not look out for the exact day of complete re-epithelialisation in this study.

Honey has a rather low PH, high osmolality, peroxidases that combat microorganisms via free radicals thus conferring an antimicrobial role to it, honey also has vitamins, trace elements and an enzyme call inhibine. All these enable honey to keep the wound surface moist, well nourished thus allowing granulation tissue to proliferate[16]. Two opposing views have dominated thinking on the mechanism of wound contraction: first that the contractile force is located in the wound granulation tissue and secondly that the wound margins themselves are capable of active advance on account of centripetally disposed fibroblasts located beneath the edge, the picture frame area. The two hypotheses are not mutually exclusive however weight of evidence seems to favour the former view[2]. The enhanced wound contraction observed in the honey group during this study seems to lend credence to this view since honey dressing caused exuberant granulation tissue formation in the honey treated group compared to the control group. The exact mechanism via which this enhanced contraction was achieved in the granulation tissue would require further investigation since it cannot be ascribed to neovascularisation or fibroblast proliferation because there was no significant difference in the experimental versus control group in this study.

However, the reduced fibroblast count observed in the honey group may be ascribed to the fact that the wound in the honey group are at an advanced stage of healing compared to the control group since mitosis in fibroblasts are observed only when the organism requires additional fibroblasts for example when connective tissue is damaged and this mitosis ceases or reduces once the process of repair has been completed[17].

Finally, the effect of honey on the following growth factors like transforming growth factor beta (TGF-b) which is involved in wound contraction, vascular endothelial growth factor (VEGF) which is involved in angiogenesis, epidermal growth factor (EGF) which is involved in epidermal growth, cellularity, epithelialisation and thickness of granulation tissue would be pertinent in addition to its effect on specific fibroblast cell lines like myofibroblast.

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