AFRICAN JOURNAL OF MEDICINE and medical sciences

Volume 32, No 2 June 2003

EDITOR
B. O. OSOTIMEHIN

ASSISTANT EDITOR
A. O. UWAIFO

ISSN 1116—4077

Afr. J. Med. med. Sci (2003) 32, 193-196

A comparative study of the wound healing properties of honey and *ageratum conyzoides*

OW Oladejo, IO Imosemi, FC Osuagwu, OO Oyedele, OO Oluwadara, OE Ekpo, A Aiku, O Adewoyin and EEU Akang

Departments of Anatomy, Physiology, Pharmacognosy and Pathology, College of Medicine, University of Ibadan, Nigeria

Summary

The present study investigates the wound healing properties of methanolic extracts of *Ageratum conyzoides* leaves compared with those of honey. Thirty Wistar rats were randomized into 3 groups of 10 animals each. They were fed with standard rat cubes and Tap water weighed and acclimatized to laboratory conditions for one week. Under anesthesia, each animal had the skin of its dorsolateral flank shaved after which an area of the skin was excised. On achieving haemostasis, the wounds were packed with gauze soaked in the appropriate dressing for each group. Measurement of wound size, and wound biopsies were taken on the 10th day post-wound creation. Together with healed wound samples, these were processed for histology. Fibroblast and blood vessel densities per unit area of wound were determined for the healed wound samples. Histologically, the day 10 *Ageratum* sections showed fewer inflammatory cells compared with similar honey and Control sections. Also, healed scar sections of wounds dressed with the herb extract showed more fibrosis. Honey and *Ageratum* caused significant greater wound contraction than controls (p=0.001 and 0.005 respectively). Healed wounds from the *Ageratum* group had significantly fewer fibroblasts than honey and controls (p=0.012 and 0.036 respectively).

Keywords: *Wound-healing, ageratum, honey, herbal traditional medicine.*

Résumé

L'étude présente enquête les propriétés curatives d'extraits du methanolic des feuilles d'Ageratum conyzoides comparé avec ce de miel. Trente rats Wistar ont été randomisés dans 3 groupes de 10 animaux chacun. Ils ont été nourris avec les cubes normaux du rat, l'eau était pesé et acclimaté aux conditions du laboratoire pour une semaine. Sous anesthésie, chaque animal s'est rasée dans le peau de épine dorsale et après, une région de peau était excisé. En accomplissant l'haemostasis, les blessures étaient emballées avec gaze trempée dans l'assaisonnement approprié pour chaque groupe. Le dimension de la blessure était mesure et les Biopsies de la blessure ont été prises dix jour après, une échantillon des blessures guéries ont été développés pour l' histologie. Le Fibroblaste et les densités du vaisseau sanguin ont été déterminées par région de l'unité de la blessure guéris. Histologiquement, les sections Ageratum de 10 jours ont montré à moins cellules provocatrices comparées avec des sections du miel et du contrôle. Aussi, les sections de la cicatrice guérie avec l'extrait de l'herbe a montré plus de fibrose. Le miel et Ageratum ont causé la plus grande contraction de la blessure considérable que contrôles. (p=0.001 et 0.005 respectivement). Les blessures guéris du groupe Ageratum avait moins fibroblastes que miel et contrôles. (p=0.012 et 0.036 respective).

Correspondence: Dr. OW Oladejo, Department of Anatomy, College of Medicine, University of Ibadan, Nigeria.

Introduction

Attempts at finding a perfect wound healing agent have a long history. The emphasis has been on finding agents that are affordable, effective and with minimal side effects. One agent, which has received wide usage as an adjunct to wound healing, is honey [1].

Specifically, it is in widespread usage as a topical antibacterial agent for the treatment of burns wounds and skin ulcers, with varying degrees of effectiveness [2].

Ageratum conyzoides is an annual herb with a long history of traditional medicinal uses. In many countries of the world and possessing a wide range of isolates, including flavonoids, chromenes, benzoflurans and terpenoids [3] Its extracts have long been used in India as an antilithic, antidysentery and a bacteriocide [4]. In Africa, Asia and South America, its aqueous extracts have been used as a bacteriocide [5,6] and in the treatment of fevers and cold, [7,8] arthroses and rheumatism [9] as well as an analgesic [10] in humans.

In 1977, Durodola demonstrated the effectiveness of crude extracts of *Ageratum* in the treatment of burns wounds in experimental animals. Like honey, its value in this regard is thought to result from bactericidal action against staphylococcus aureus, which is a major wound pathogen [5,11].

The present study is aimed at comparing the effects of these two agents on the healing of freshly excised wounds. It is hoped that results obtained will be of use in the fields of general and plastic surgery, and allied disciplines.

Materials and methods

Thirty male Wistar rats were selected based on the non-presence of any pre-existing skin lesion. They were subsequently split into three groups (Normal saline controls, CG; Honey, HG; and *Ageratum*, AG) with 10 animals in each group.

All the animals were acclimatized in the lab for one week and fed with standard rat cubes and water ad libitum. The animals were weighed and anaesthetized using inhalational chloroform. Each animal had the skin of its right dorsolateral flank shaved, then swiped with 70% alcohol. Thereafter, a 2cm by 2cm area of skin was measured and excised care being taken to remove full-thickness skin (below the panniculus carnosus layer) in each case. Homeostasis was secured by direct application of pressure. The wounds were then packed with gauze soaked in the appropriate dressing agent for each group. A further layer of gauze was placed on this and the dressing secured in place with a zinc oxide plaster taped circumferentially round the animals' trunk along the area of skin previously shaved. Change of wound dressing was done at five daily intervals until complete wound Re-epithelization. Subsequent measurement of wound size was done on the 10th day after the creation of the wound, using a transparent plastic, which was previously cleaned with Sodium hypochlorite solution. The plastic was placed on the wound, whose outline was then traced on to it. This outline was then traced on a graph paper. A random biopsy of the wound in each group was taken on day 10 for histology. Similarly, another sample of the completely healed wound (end scar) was taken in each group. Fibroblast counts and blood vessel density per unit area of wound were determined in the end scar samples.

For methanol extraction of *Ageratum conyzoides*, 250grams of the dried leaves were crushed and immersed in methanol for 72 hours at room temperature after which the extract was concentrated by exposure to air. After further drying, 8 grams of the powder were made into a 1.5% suspension in distilled water. This suspension was used for the wound dressing.

Results

The results of the study are shown in figures1a-c, 2a-c and 3; and tables 1&2. Histological sections of the wounds dressed with normal saline, honey and *Ageratum* obtained

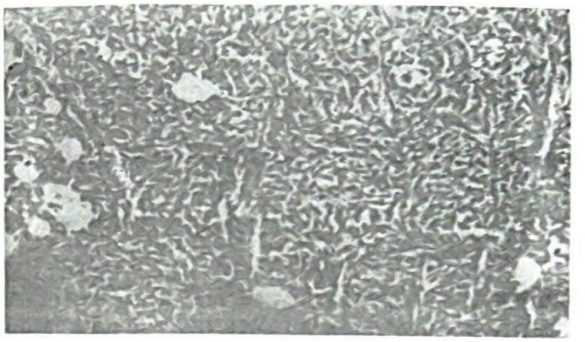

Fig. 1a: Micrograph showing 10th day wound dressed with normal saline. There is abundant granulation tissue formation ∞ 320.

Fig. 1b: Micrograph showing 10th day wound dressed with honey. There are numerous inflammatory cells ∞ 320.

Fig. 1c: Micrograph showing 10th day wound dressed with *Ageratum* extract. The inflammatory cells density appears less than in figures 1a&1b ∞ 320.

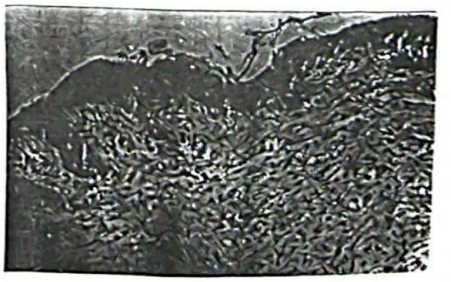

Fig. 2a: Micrograph showing the healed scar from wound dressed with normal Saline. Complete re-epithelization has occurred and there is dermal fibrosis ∞ 320.

Fig. 2b: Micrograph showing healed scar of wound dressed with honey. The features are here are similar to those in Figure 2a ∞ 320.

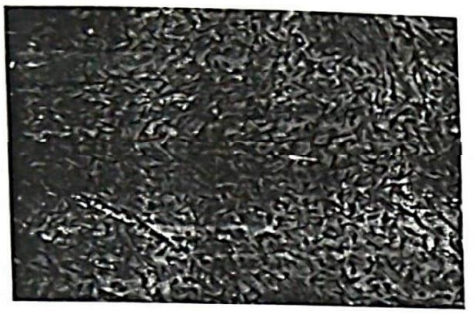

Fig. 2c: Micrograph of healed scar from wound dressed with *Ageratum* extract. Epithelial surface not visualized. While cellular content is slightly less, dermal Fibrosis is slightly more pronounced than earlier figures ∞ 320.

Table 1.Comparison of the relative wound-contracting effects of Honey and *Ageratum conyzoides.*

Agent	Mean contraction(cm^2)	Mean % wound contraction	P values in comparison with control	P values honey compared *with Ageratum*
Saline (control) (n=10)	3.06	55		
Honey(n=10)	4.42	80	0.001	
Ageratum (n=10)	4.06	82	0.005	0.334

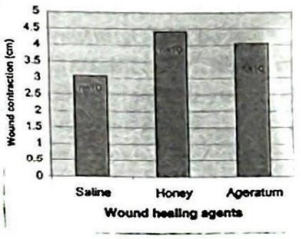

Fig. 3: Mean wound contraction achieved by various agents.

on the 10th day of wound healing all showed similar features (figures1a-c) These include robust granulation tissue formation and inflammatory cell infilteration. The *Ageratum* wound sample however shows fewer inflammatory cells than either the saline or honey sections. Similarly, the healed wounds from these three sources were also very similar, with the *Ageratum* section again showing features suggesting slightly more advanced dermal fibrosis than the other specimens (figures2a-c).They all showed complete re-epithelization with collagen fibers arranged in irregular whorls in the sub-dermal connective tissue.

Actual measurements show that both honey and *Ageratum* had significantly greater wound contracting effect than the control (p=0.001 and 0.005 respectively); Table 1and figure 3), but no significant difference in this regard when compared with each other. (p=0.33). Concerning fibroblast proliferation, only *Ageratum* significantly reduced the number of fibroblasts per high power field (p=0.04).Honey also reduced the fibroblast count in the healed scars but non-significantly (p=0.24;

Table 2: Comparison of the relative effects of Honey and *Ageratum conyzoides* on wound fibroblast and blood vessel proliferation in the healed wound samples.

Agent	Mean no of fibroblasts(f)	Mean no of blood vessels(BV)	P Values: compared with *control* F	BV	P Values: compared with *Ageratum* F	BV
Saline(control)	90.2	13.4				
Honey	68	18.8	0.24	0.26		
Ageratum	44.2	25.8	0.04	0.07	0.01	0.32

Table2). As regards the effect of these agents on blood proliferation in the wounds, both honey and *Ageratum* increased the number of blood vessels observed. These outcomes were however not significantly different from observed events in the control wounds (p =0.26 and 0.07 respectively, Table2).Wounds treated with *Ageratum* had significantly less fibroblasts (p=0.01), but non-significantly more blood vessels (p=0.32; Table2) than those in the honey group.

Discussion

Probably for the first time, this study has demonstrated significant wound-contracting effects of honey and *Ageratum conyzoides* in freshly excised wounds. That this property did not differ significantly between these two agents suggests that other considerations, such as cost or availability may determine which one of them practitioners will prefer.

Rural dwellers, who are widely acknowledged to form majority of African populations, and who already use herbs for medicinal purposes, may find *Ageratum* particularly useful.

The significantly reduced end-scar fibroblast count produced by *Ageratum* compared to both saline controls and honey is also reflected in the fewer cellular content of the *Ageratum* sections (figures1c&2c). In the light of the above it is curious that no significant difference from controls exist in the blood vessel count observed in all the samples (Table2).This may mean that honey and *Ageratum* enhance wound healing in ways other than by promoting angiogenesis. Considering, the effect of *Ageratum* on fibroblasts referred to above, a study in which the local concentrations of the numerous mediators involved in physiological wound healing, such as transforming growth factor beta (TGF-β) and cytokines[12] are measured, should prove rewarding.

This study has demonstrated that both honey and extracts of *Ageratum conyzoides* herb are potent accelerators of wound healing process in freshly excised wounds and should receive more attention in this regard in future investigations.

References

1. Juma A, Lulat A. Honey-a remedy rediscovered.J.Roy.Soc.Med.1989; 82:384-385.
2. Green, A.E.Wound Healing Properties of honey.Br.J.Surg.1988; 75, 1278.
3. Okunade, AL Ageratum *conyzoides L.* (Austeraceae).Fitoterapia 2002; 73(1):1-16
4. Borthakur N, Baruah AKS.Search for Precocenes in *Ageratum conyzoides linn.* Of North-East India.J.India Chem.Soc 1987; 64:580-581.
5. Almagboul AZ, Farroq AA, Tyagi BR.Antimicrobial activity of certain Sudanese plants used in folkloric medicine; screening for antibacterial activity, part 11.Fitoterapia 1985; 56:103-109.
6. Ekundayo OS, Sharma S, Rao EV.Essential oil of *Ageratum conyzoides.*Planta Med.1988;54:55-57.
7. Penna. A.Notas Sobre Plantas Brasilieras. Araujo Penna Filhos, Rio de Janeiro, 1921.
8. Abena AA, Kintangoula-Mboya GS, Diantama J, Bioka D.Analgesic effects of a raw extract of *Ageratum conyzoides* in the rat. Encephale 1993; 19(4):329-332.
9. Marques-Neto JF,Lapa A, Kubota M.Efeitos do *Ageratum conyzoides* linee no tratamento da artrose.Rev.Bras.Reumat 1988;28(4):34-37.
10. Bioka D,Banyikwa FF,Choudhuri MA.Analgesic Effect of crude extracts of *Ageratum conyzoides* in the rat.Acta Hort.1993;332:171-176.
11. Durodola J.J.Antibacterial property of crude extracts from a herbal wound healing remedy-*Ageratum conyzoides*.Planta Med.1977; 32(4):388-390
12. Komarcevic A, Pejakov L, Komarcevic M.New views on the physiology of wound healing.Med.Pregl.2000; 53(9-10):479-483