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VASCULAR PERMEABILITY- INCREASING EFFECT OF THE LEAF ESSENTIAL OIL OF *OCIMUM GRATISSIMUM* LINN AS A MECHANISM FOR ITS WOUND HEALING PROPERTY.

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Abstract

Persistent microvascular hyperpermeability to plasma proteins is a characteristic feature of normal wound healing. Does the leaf essential oil of *Ocimum gratissimum* heal wounds by promoting this feature? Evan's blue dye (20mg/kg body weight) in normal saline was administered intravenously through marginal ear vein of experimental rabbits (n=5). Each animal served as its own control. One hour after Evan's blue dye administration, 0.1ml each of Ocimum oil, histamine dihydrochloride (30µg/ml) and normal saline were randomly administered by intra-dermal injection at the prepared sites on each of the animals. Increase in vascular permeability was assessed by dye effusion test. Analysis of the differences in vascular permeability between treatment groups showed that, Ocimum oil, in intensity and duration, was significantly ($p<0.05$) more effective in increasing cutaneous capillary permeability over a 24h period after treatment. The ability of Ocimum oil in increasing vascular permeability may be one of the factors that contribute to its wound healing property.
Key words: *Ocimum gratissimum*, vascular permeability, mechanism of action.

Introduction

Wounds occurs as a result of physical injuries that break and expose the skin epidermal and dermal layers, causing a damage to the blood vessels and leading eventually to bleeding. Wound healing is a complex sequence of events intiated by the stimulus of injury to tissues. These events involve four main processes: (a) coagulation, (b)inflammation and debridement of wound, (c)epithelia repairs and (d)tissue remodeling and colleagen deposition (Rashed et al 2003). It is known that

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any agent which accelerates one or two of the above events is a promoter of wound healing (Abu-Al-Basal 2001). Medicinal plant extracts, decoctions and concotions have been in use traditionally to treat various skin lesions(burns and wounds), and these plants have demonstrated wound healing properties in various animal models (Suguna et al., 1996., Chithra et al 1998., Bale and Sheikh 2000a, b., Abu-Al-Basal 2001, Rashed et al 2003).

Formulations of the leaf essential oil of *Ocimum gratissimum* Linn., Family Lamiaceae, (Ocimum oil) have been made in a variety of bases as topical antiseptics for use in the treatment of minor wounds, boils and pimples (Orafidiya *et al*, 2001) basically for its established antimicrobial properties (El-Said et al, 1969; Begum *et al*, 1993). Ocimum oil contains mainly thymol, (48.1%), p-cymene, (12.5%), and 40 other constituents in trace amounts (Martins *et al*, 1999). The oil significantly resolves the inflammatory lesions of *Acne vulgaris* (Orafidiya *et al*, 2002, 2004a), promotes the inflammatory and proliferative phases of wound healing and possesses proven efficacy against a variety of clinical isolates and type strains of micro-organisms including wound contaminants (Orafidiya *et al*, 2003). Being a potential wound healing agent, we sought to investigate possible mechanisms for its wound healing property.

Material and methods

Plant material and preparation of Ocimum oil.

The leaves of *O. gratissimum* were collected and identified as earlier reported (Orafidiya *et al*, 2003, 2004a, b, c) Ocimum oil was extracted from the fresh leaves by hydro-distillation using the British Pharmacopoeia method. The oil was then stored in glass containers in the refrigerator until needed.

Experimental animals

The "principles of laboratory animal care" [NIH Publication No. 85-23] were followed in this study. Five adult albino rabbits (average weight 4.4kg) were used for the experiment. They were procured from the laboratory animal resource section of the College of Health Sciences, Obafemi Awolowo University Ile-Ife. They were acclimatized for 10 days before commencement of the experiment and maintained on rabbit pellets (Ladokun Feeds, Ibadan, Nigeria) and water *ad libitum.*

Preparation of animal skin sites

Skin sites were prepared 24h prior to administration of the test samples by clipping the hairs on the flanks, extending toward the dorsal region of the animals. All the sites were depilated with Royal Crown® Depilatory Shaving powder (J. Strickland & Co. Memphis. TN 38106 USA) which was applied for 5 min and rinsed off subsequently with fresh water. Three skin sites of approximately 4cm^2 each (on either side) were demarcated on each rabbit for the application of Ocimum oil, control (normal saline) and reference agent (histamine dihydrochloride, 30µg/ml).

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Animal treatment and evaluation

Evan's blue dye (20mg/kg body weight) in normal saline was administered intravenously through the marginal ear vein of the rabbit. Each animal served as its own control. One hour after Evan's blue dye administration, 0.1ml each of Ocimum oil, histamine dihydrochloride (30µg/ml) and normal saline were randomly administered by intra-dermal (i.d.) injection at the prepared sites in each of the 5 rabbits. Increased vascular permeability was assessed by dye effusion and observations were recorded at 0.5, 1, 2, 3, 4, 6, 8, 10, 12, 18 and 24hr after i.d. injections of the test and control agents. The intensity of the reaction was graded grossly as 1=negligible, 2=mild, 3=moderate and 4=marked; according to the method of Miles and Whihelm (1955) and expressed as average increase in vascular permeability.

Statistical analysis

Group data difference was evaluated by a modified t-test [n<20] (Gardiner, et al, 1998). Statistical analysis was done using the SPSS version 11.0 software program package (SPSS Inc., Chicago, IL). Test for statistical significance was by paired sample student t-test and one- way analysis of variance at 95% confidence interval.

Results

The vascular permeability-increasing (VPI) effect of Ocimum oil was grossly significant ($p<0.01$) relative to reference and control agents from 0.5h onwards after i.d. injection (Figure 1). Ocimum oil VPI activity reached moderate levels (3.2±0.84) within the first 1h, became marked (3.82±0.22) first at about 3h, and was maintained over a 6h period (3h→9h) after i.d. injection of Ocimum oil (Figure 1). VPI activity of the oil declined very slowly; mild values (2.07±0.64) were recorded grossly between 10h and 18h. Activity was negligible and comparable to histamine ($p=0.07$) 22h after oil administration (Figure 1). Normal saline showed no VPI activity after administration; sites appeared as when untreated throughout the 24h test period.

VPI activity of histamine was mild (3h→4h) and negligible (0.5h→2h, 6h→24h) during the test period; maximum activity (1.6±0.41) was recorded at 3h after i.d. injection (Figure 1). One-way analysis (ANOVA) of the differences in vascular permeability between treatment groups showed that, Ocimum oil, in intensity and duration, was significantly ($p<0.05$, Std. Error= 0.14-0.15) most effective in increasing cutaneous capillary permeability over a 24h period after administration.

Discussion

Vascular hyperpermeability is a characteristic feature of wound healing that has been found to continue at wound sites for a considerable time after the cessation of overt bleeding (Brown *et al*, 1988; Breuing *et al*, 1992). Thus, blood vessels continue to leak plasma proteins for several days after the initiating trauma has ceased and even for a short time after a complete epidermal covering has been restored. The result is persistent extravasations of fibrinogen and ongoing deposition of provisional fibrin gel matrix, events that favor the continued induction of granulation tissue. (Clark *et al*, 1988; Van De Water *et al* 1992).

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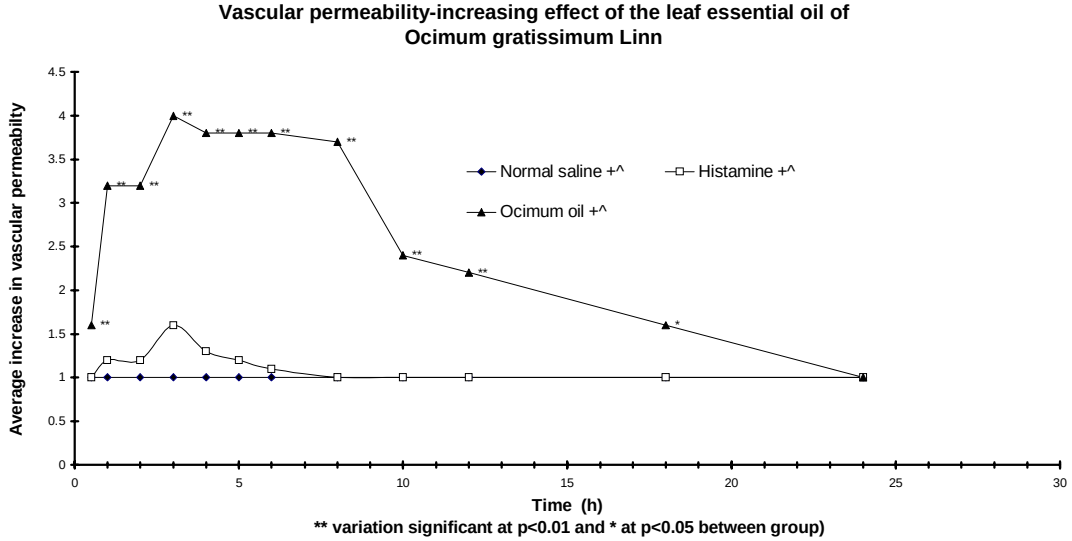
Figure 1



+^ (n=5; 1=negligible, 2=mild, 3=moderate, 4=marked).

This ascertion is buttressed when Evan's blue dye, injected intravenously , forms coloured complex with serum albumin, which leaks out into the extracellular space only when vascular capillary permeability is sufficiently increased to allow the passage of albumin molecules leading to blue discoloration of the skin (Carpenter and Lynn, 1991). The area and intensity of the blue coloration is proportional to increase in vascular permeability within limits (Spector, 1958). The results of this study showed that, Ocimum oil, in intensity and duration, was significantly ($p<0.05$) more effective in increasing cutaneous capillary permeability over a 24h period after administration (Figure 1).

Mechanism for the initiation and maintenance of persistent vascular hyperpermeability in healing wounds has been reported to be due to a vascular permeability factor (VPF) (Clauss *et al*, 1990). VPF is a selective mitogen for endothelial cells and a reported chemotactic for monocytes (Clauss *et al*, 1990) that enhances the permeability of local venules and small veins with a potency some 50,000 times that of histamine (Senger *et al*, 1990; Keck *et al*, 1989). Brown *et al*, (1992) reported that VPF and its mRNA are expressed in normal keratinocytes, both in situ and in tissue culture, and that VPF expression is markedly increased in the activated, migrating keratinocytes involved in wound healing. Hints that Ocimum oil invokes inflammatory responses and promotes the proliferation of matrix keratinocytes (which are physiological events that occur in wound healing) were

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given in recent studies investigating the toxicity and the effects of the oil in cyclophosphamide induced hair loss (Orafidiya *et al*, 2004b; 2004c).
In conclusion, this study has demonstrated the ability of Ocimum oil in increasing vascular permeability, a possible additional feature to its wound healing property.

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