

COMUNICACIONES

**PRELIMINARY STUDY OF THE CICATRIZANT ACTIVITY OF
AQUEOUS EXTRACT OF *Acalypha langiana***

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Introduction

Wound healing is a highly complex, but orchestrated cascade of events which can roughly be divided into three overlapping phases-inflammation, granulation tissue formation and remodelling of the extracellular matrix. These events involve several cellular phenomena such as migration, proliferation, adhesion, phenotypic differentiation, etc. Immediately after injury, there is clot formation and the earlier phase of wound repair involves inflammation and synthesis of ground substance. The ground substance mainly consists of proteoglycans (PGs), which are the heterogenous, non-fibrillar components of the extracellular matrix (1).

Wound healing is a complex process involving a highly regulated series of biological events. These include a set of coordinated interactions between cells in the dermis and the epidermis, and important relationships have been found to exist between fibroblasts, keratinocytes and resident dermal cells. Healing of wounds, a fundamental response to tissue injury, occurs by a process of connective tissue repair. A fibrous scar is the end product of this process, the predominant constituent of which is collagen. Collagen and other components of the ground substance are synthesized by the highly vascular granulation tissue that is formed within the wound space. Since collagen provides strength and integrity to the dermis and all other supporting tissues, the synthesis, secretion and subsequent organization of collagen plays an integral role in wound healing (2).

Several indigenous drugs have been described in folkloric Mexican medicine for the management of cuts, bruises, burns and wounds. One of them is *Acalypha langiana* Muell. (Euphorbiaceae) is commonly known as "arloromo". It is a common herb that grows wild and abundantly in the fields of Mexico. A water extract of the leaves has long been used for Guerrero indigenous for the treatment of wounds and the use in Aguascalientes of powdered leaves to treat ulcers was reported a long time ago. In Guerrero a water extract of the leaves has long been used for indigenous to clean wounds and when abundant hemorrhages are present during postpartum, it is applied as a vaginal wash (3). Based on our primary ethnobotanical field research with healers in, various uses were attributed to the plant related to anti-inflammatory effects, like hepato-protective and wound-healing. Decoction of the roots is drunk for the treatment of hepatitis. Decoction of the root or the leaves is also used to wash

wounds and a powder of the same plant parts is applied topically as a wound healer. The leaves are also used as wound dressings to prevent suppuration.

The genus *Acalypha* with about 450 species is the fourth largest genus of Euphorbiaceae are used in Central America as folk medicine (4). There have been no scientific studies chemistry, and no studies of the possible cicatrizant activity of *A. langiana*.

Here we report the positive influence of topical administration of *Acalypha langiana* on wound healing in rats.

Material and Methods

Experimental animals

Young adult, Wistar rats weighing 170-200 g were used. The animals were maintained with pellet food (Purina), while tap water was available *ad libitum* in the Bioterio of Universidad Autónoma Metropolitana-Xochimilco (México, D.F.). The animals were housed in a cage under conditions of standard light (light on from 7.0 a.m. to 7.0 p.m.), temperature ($22\pm 1^{\circ}\text{C}$) and room humidity ($60\pm 10\%$) conditions for at 1 week before the experimental sessions. The procedures involving animals and their care conformed to the international guidelines Principles of Laboratory Animals Care.

Sixty white Wistar rats were divided into six groups of ten rats per group. The control group received 0.5 ml saline (topical), others group received 0.05, 0.1, 0.2, 0.4 and 0.5 % of aqueous extract of *A. langiana*.

Plant material

The leaves were collected in near of Puerto Vallarta state of Nayarit, Mexico, in July 2004 and were taxonomically authenticated in the Department of Botany of ENEP-Iztacala UNAM and a voucher specimen (7916) of the plant is stored in the herbarium of this Department for reference.

Preparation of the aqueous extract

The extract was prepared as follows: 100 g of the dried powdered leaves, were boiled in 500 ml of distilled water for 25 min. The aqueous extract was filtered, the filtrates obtained were lyophilized.

Wound creation

Excision wound: On the stretched skin of a rat, five scratches were made with a sharp needle, deep enough without cutting, to draw blood. The assay products were applied immediately. The aspect and evolution of the scratch was quantified again by a blind assay. The surface area of healing (7 day) wound was measured by tracing the boundary of still open wound on semi-transparent paper and calculation of area was done by using a graph paper (5,6). An untreated group served as control.

Incision wound: The test was carried out on the rat's shaved back where, after anesthesia, two transversal cuts of 2.5 cm were made (paravertebral incisions) cutting through the full thickness of the skin. Interrupted sutures, 1 cm apart, were placed to approximate the cut edges. The sutures were removed on the 7th post-wounding day. Throughout the period, the extract were given topical daily, at the same doses as for excision wounds. Aqueous extracts solution (200 ml/wound) was applied topically in concentrations of 0.05, 0.1, 0.2, 0.4 and 0.5% twice daily for 7 days. The control group received an equal amount of vehicle. The surface area of healing (7 day) wound was measured every alternate day by tracing the boundary of still open wound on

semi-transparent paper and calculation of area was done by using a graph paper (7,8). Joint of both sides of the wound and its general aspect were taken into account.

Epithelisation time was noted as the number of days after wounding required for the scar to fall off leaving no raw wound behind (9).

All surgical procedures were carried out under thiopentone sodium (25 mg/kg, i.p.) anesthesia. Animals were allowed to recover and were housed individually in metallic cages containing autoclaved paper cuttings. They received food and water *ad libitum*.

Statistical Analysis

The data were statistically analysed by the Student's t-test, using the program "Microcal Origen". All group treated with aqueous extract of *Acalypha langiana* were compared with group control. Student's t-test was used to identify differences between groups, and data were considered significant at P-values of 0.05 or less.

Results

Topical applications of the leaves extract of *A. langiana* showed effect on the healing process on rats. The test results are showing in Table 1. The most remarkable effect of cicatrizant activity was observed in the group treated with aqueous extract of leaves of *A. langiana* with 85% in excision wound test and 74% in incision wound test at doses of 0.5%. While the group treated with aqueous extract at doses of 0.05% showed 20 and 15% in excision wound test and incision wound test respectively.

Table 1. Wound healing properties of *Acalypha langiana* on normal rats

Extract %	% Cicatrizant activity (M ± DE) Excision wound test	% Cicatrizant activity (M ± DE) Incision wound test
Vehicle control	19 ± 1.1	13 ± 2.5
0.05	20 ± 1.4*	15 ± 2.9**
0.1	45 ± 2.5**	39 ± 3.9*
0.2	65 ± 3.7**	61 ± 4.1**
0.4	71 ± 4.7*	67 ± 4.2*
0.5	85 ± 5.1**	74 ± 6.1*

Values are mean ± D.E. (n = 10 animals). * P<0.05 and ** P<0.01 as compared to vehicle control.

Table 2 showed the aspect and evolution after topical application on rats of the extract of *Acalypha langiana*. The congestion and oedema wounds decreased significantly in the groups treated with aqueous extract of leaves of *A. langiana* after 7 days of treatment at doses of 0.2 at 0.5 %. The wounds after 7 days treatment with plant extract exhibited marked dryness of wound edges with regeneration of healing tissue and the wound area was also considerably reduced compared to controls indicating the healing potential of *A. langiana*.

Table 2. Aspect and evolution finding of 7 days of normal rats after topical application of extract of *Acalypha langiana*

Treatment Extract (%)	Congestion	Oedema
Control	+++	++++
0.05	+	++
0.1	+++	++++
0.2	-	-
0.4	-	-
0.5	-	-

Slight +, moderate ++, marked +++, extensive++++, absent -

Discussion y Conclusions

Healing is a physiological process and does not normally require much help but still wounds cause discomfort and are prone to infection and other complications. Therefore, use of agents expediting healing is indicated. Further, some diseases like diabetes, immunocompromised conditions, ischaemia and conditions like malnourishment, ageing, local infection, local tissue damage due to burn or gun shot wounds lead to delay in healing. Such conditions specially require the use of agents, which can facilitate healing (10).

For the cicatrization test, wound creation, there is a better response in animals treated with *A. langiana*, while the control samples present the worst profile. Wound contraction in specimens from the groups treated, respectively, with 0.5 and 0.4% of extract was significantly enhanced when compared with controls, in which the wound healing is not complete yet at the end of the

experiment.

A. langiana, topically applied, enhanced the anti-inflammatory response. It can be hypothesized that *A. langiana*, leaves extract, contains active components capable of exerting their action mainly during the first hours after topical application

The present investigation clearly showed that the topical application of aqueous extract of the leaves of *A. langiana* produced significant wound healing activity and showed an activity dose-dependently.

The wound healing properties of the plant validate their uses in traditional medicine for treating injured. The results obtained encourage us to carry out a wider and more profound study of this plant to obtain better knowledge of its therapeutic possibilities.

Abstract

Aqueous extract of leaves of *Acalypha langiana* were studied for their wound healing properties on excision wound and incision wound tests. The most remarkable effect of cicatrizant activity was observed in the group treated with aqueous extract of leaves of *A. langiana* with 85% in excision wound test and 74% in incision wound test at doses of 0.5%.

Key words: Acalypha langiana, leaves, cicatrizant activity

Resumen

Se determinó el efecto del extracto acuoso de las hojas de *Acalypha langiana* en la cicatrización de heridas producidas quirúrgicamente (incisión) y por un rasguño profundo (excisión) en animales de experimentación. El efecto más importante producido por el extracto acuoso de las hojas de *A. langiana* fue de 85% en la prueba de rasguño profundo (incision wound) y 74% en el ensayo de heridas producidas quirúrgicamente a dosis de 0.5%.

Palabras clave: *Acalypha langiana*, hojas, actividad cicatrizante

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