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Double incision wound healing bioassay using *Hamelia patens* from El Salvador

Alfredo Gomez-Beloz[a,*], James C. Rucinski[b], Michael J. Balick[c], Camille Tipton[d]

[a] Biology Department, Saint Martin's College, 5300 Pacific Avenue SE, Lacey, WA 98503, USA
[b] Department of Surgery, New York Methodist Hospital and Weill Medical College of Cornell University, Brooklyn, NY 11215, USA
[c] Institute of Economic Botany, The New York Botanical Garden, Bronx, NY 10458, USA
[d] Program in Biology, The Graduate School and University Center of The City University of New York, 365 Fifth Avenue, New York, NY 10016, USA

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Abstract

Hamelia patens Jacq. (Rubiaceae) has received little attention in the laboratory for its wound healing ability even though it is commonly used as a treatment for wounds throughout Central America. A double incision wound healing bioassay was carried out with a crude extract of *Hamelia patens* collected from El Salvador. Animals were divided into three groups. Group I ($n = 14$) had the left incision treated with 5% (w/w) *Hamelia patens* and the contralateral side with petroleum jelly (PJ). Group II ($n = 14$) had the left incision treated with 10% (w/w) ointment and the contralateral side with petroleum jelly. Group III ($n = 10$) had the left incision treated with petroleum jelly and the contralateral side left untreated. Breaking strength of the incisions was measured on day 7 and day 12. For Groups I and II, there was no significant difference between treatment and control incisions at day 7. On day 12, there was a significant difference between the treated and control incisions for Groups I and II. There was no significant difference between petroleum jelly and untreated incisions for Group III on day 7 and day 12. *Hamelia patens* does increase breaking strength of wounds significantly more than the control group. Further wound healing studies of this plant are warranted.

Keywords: Wound healing; *Hamelia patens* Jacq.; Double incision wound healing bioassay; Ethnobotany

1. Introduction

Hamelia patens Jacq. (Rubiaceae) (Fig. 1) has received little attention for its wound healing ability. This species is a bush or small tree, 1.4–3.0 m tall with deeply veined leaves and red to bright orange-red, fine hairy flowers. *Hamelia patens* is commonly used as a treatment for wounds in Central America (Ayala, 1994). In El Salvador, where it is known as *chichipince*, a crude extract of *Hamelia patens* is blended into soap and sold as a cleansing agent for wounds. Antibacterial activity attributed to *Hamelia patens* (Misas et al., 1979) may be responsible for the purported effectiveness of this soap. There are many other uses for this species. Notes on ethnobotanical collections made in Belize in deposit at the New York Botanical Garden have shown that the plant is used for herbal baths to treat skin sores and swellings [herbarium specimen, *Arvigo 71*], to treat fevers [*Balick 2533*], to treat skin fungus [*Balick 1777, 2055*], as an analgesic for headache [*Balick 2293*], to treat cradle cap on children [*Balick 2409*], to treat skin rash as part of a mixture of other herbs [*Balick 2099*], to treat itching skin from scabes [*Arvigo 316*], and to treat open sores [*Arvigo 931*]. The leaves are dried over a fire, mashed and applied to blistered hands, and this procedure is said to "harden the blisters" (Steggerda, 1943). The fruit is said to be edible, "but of poor flavor" (Standley and Record, 1936).

The plethora of common names which identify this species attests to its popularity and wide neotropical distribution. These names include: chactoc, red-fowl, ix-canan, klaush-pam, neanan, readhead, sac-te-much, scarlet bush, xcanal (Belize); leoncito (Colombia); coral, coralillo, coloradillo, achiotillo, achiotillo colorado (Honduras); zorillo real, pala camarón (Costa Rica); panasi (Cuba); busunuco (Dominican Republic); sisipince, chichipinte, shishipince, coralillo, zambumbia, sancocho, doncella, canilla de venado, and bálsamo cimarrón (El Salvador); hierba del cáncer, chichipin, cuetillo, sisipince, clavito, flor de cangrejo, canuto, hierba de erisipela (Guatemala); koray (Haiti); xcanan, ncanan, pie de pajaro, kanan, chacoco (México)

* Corresponding author. Tel.: +1-360-438-4327.
E-mail address: agomezbeloz@stmartin.edu (A. Gomez-Beloz).

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Fig. 1. *Hamelia patens* Jacq. (Photo by M.J. Balick.)

(Balick et al., 2000; Robineau, 1989; Arvigo and Balick, 1993; De Lezama, 1994, and various herbarium specimens cited above). Although its ethnobotanical use as a wound healing plant is popular, there have been no published experimental reports on its wound healing ability.

Two types of measurements can be taken from in vivo linear incision wound healing models—tensile strength and breaking strength. Tensile strength is "measured in terms of load applied per unit of cross section area ... [and] is given as pounds per square inch or kilograms per square centimeter" (Peacock, 1984). On the other hand, breaking strength is the "measurement of force required to break a wound without regard to its dimensions" (Peacock, 1984). Wound healing is of the utmost importance to conventional medicine, especially surgery. Because surgeons are interested in the disruption of skin, breaking strength is the measurement of most interest to them (Peacock, 1984; Mustoe et al., 1987). Therefore, breaking strength was measured for *Hamelia patens* collected from El Salvador using a double incision wound healing bioassay.

2. Methodology

2.1. Plant material

The entire plant *Hamelia patens* was collected from the vicinity of Volcan de San Salvador, El Salvador, and was identified by Raúl Francisco Villacosta Munzón of the Escuela de Biología, Departamento de Química, Universidad de El Salvador. The plant sample was dried at room temperature and frozen at −30 °C in San Salvador prior to shipment. A voucher specimen (ITIC-152) was deposited in the Herbarium of the University of El Salvador. Additional voucher specimens cited in this publication have been deposited at The New York Botanical Garden Herbarium as well as the Herbarium of the Forestry Department of Belize.

2.2. Experimental animals

Thirty-eight male Sprague–Dawley rats weighing 300–324 g each were used for this study. The rats were purchased from a commercial supplier. They were fed commercial rat feed and provided water ad libitum.

2.3. Extract and ointment preparation

The above ground portion of the dried plant (394 g), including leaves, stems, and flowers, was exhaustively extracted in 2 l ethanol. The mash was mixed every day for 4 days. The mixture was concentrated and dried under reduced pressure. The resultant ethanol-free crude extract was ready for use in the ointment preparation.

A 5 and 10% (w/w) *Hamelia patens* ointment was prepared from the plant extract using a petroleum jelly (PJ) base. The 5% (w/w) ointment was prepared by mixing 15 g of crude *Hamelia patens* extract with 285 g of petroleum jelly for a total of 300 g. The 10% (w/w) ointment was prepared by mixing 30 g of *Hamelia patens* extract with 270 g of petroleum jelly for a total of 300 g.

2.4. Double incision wound healing bioassay

The protocol for the double incision wound healing bioassay was approved by the Institutional Animal Care Use Committee of Lehman College (Protocol No. 98-9).

The rats were acclimated for 10 days in cages at the CUNY-Lehman College Animal Care Facility and fed water ad libitum. Eighteen hours prior to the incisions, the rats were fasted but given water ad libitum. Rats were anesthetized with pentobarbital (35 mg/kg, intraperitoneal) and monitored for respiration, color, and temperature. After successful anesthesia, the back of each rat was depilated using a commercial barber's hair cutter. Two paravertebral incisions, 6 cm long, located 1.5 cm from the midline on either side of the rat, were made through the skin and panniculus carnosus muscle to the deep fascia of each rat. The parted skin was joined together with three surgical clips placed 1.5 cm apart and the wound left undressed. The rats were given butorphanol (4 mg/kg) post-operatively twice a day for 2 days to alleviate discomfort.

2.5. Administering the treatments

The rats were divided into three groups. For Group I ($n = 14$), the left wound was treated with 5% (w/w) *Hamelia patens* and the contralateral side, which served as a control, received only petroleum jelly. Group II ($n = 14$) had the left wound treated with 10% (w/w) ointment and the contralateral side with PJ. Group III ($n = 10$) had the left wound treated only with petroleum jelly ointment base and the contralateral side was left untreated. All treatments were applied once every 24 h. Petroleum jelly served as a positive control and the no-treatment wounds (NT) served as negative controls.

2.6. Wound breaking strength

Because wound healing breaking strength is not significant until approximately 5 days post-wounding (Dunphy and Udupa, 1955), half the rats of each of the groups were retrieved on day 7. The remaining half were retrieved on day 12. This is when collagen levels approximately reach normal skin collagen levels. For each group, rats were euthanised individually using carbon monoxide narcosis followed by cervical dislocation to insure death. The staples were removed and wound breaking strength measured for each of the incisions using a tensometer built from the guidelines described in Saha et al. (1997) (Fig. 2). Breaking strength of the wound was measured as the minimum force required to break the incision apart. The force, presented in Newtons (N), is calculated as mass (kg) times acceleration (9.8 m/s^2). Water and dry weight of the container applied the force. Upon completion of each set of measurements, the animals were properly disposed.

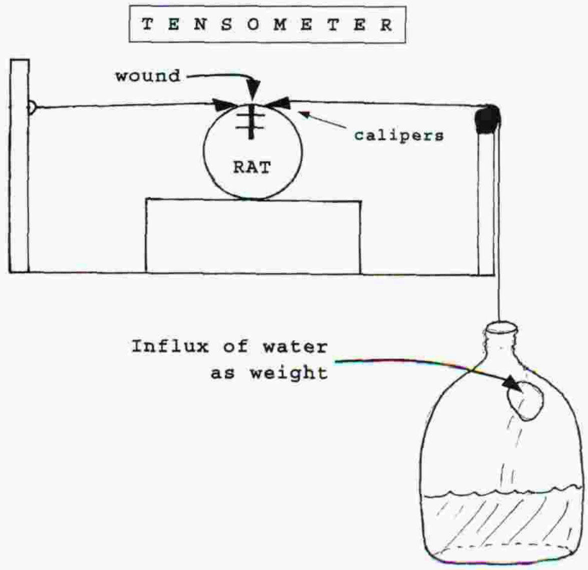

Fig. 2. Tensometer used for the double incision wound healing bioassay.

3. Results

Data analysis was carried out using the SPSS statistical software package. Comparisons of groups of means were carried out using paired sample *t*-tests and considered significant at $P < 0.05$ and highly significant at $P < 0.01$. Results are presented in Table 1 and Fig. 3. For the 5% (w/w) treatment group (Group I), the difference between treatment and control incisions at day 7 was not significant. For the day 12 group, the difference was significant. In Group II, the 10% (w/w) treatment group, again, the difference at day 7 between treatment and control incisions was not significant. At day 12, however, a significant difference was observed. Between petroleum jelly and no-treatment incisions (Group III), for either day 7 or day 12, no significant difference was found.

Table 1
Effects of *Hamelia patens* extract on incision wounds using the double incision wound healing model bioassay

Days post-wounding	Treatment	Tensile strength, mean ± S.D.	Control	Tensile strength, mean ± S.D.
7	5% (w/w)	8.90 ± 0.99	PJ	8.71 ± 1.04
12	5% (w/w)*	17.13 ± 1.42	PJ	15.23 ± 2.10
7	10% (w/w)	10.55 ± 1.62	PJ	9.85 ± 0.62
12	10% (w/w)*	17.22 ± 2.04	PJ	15.00 ± 1.69
7	PJ	8.52 ± 1.14	NT	8.26 ± 1.18
12	PJ	13.45 ± 1.72	NT	14.46 ± 1.59

Results are compared with control (PJ = petroleum jelly and NT = no treatment) and *P*-values were calculated by paired sample *t*-test.
* $P < 0.05$.

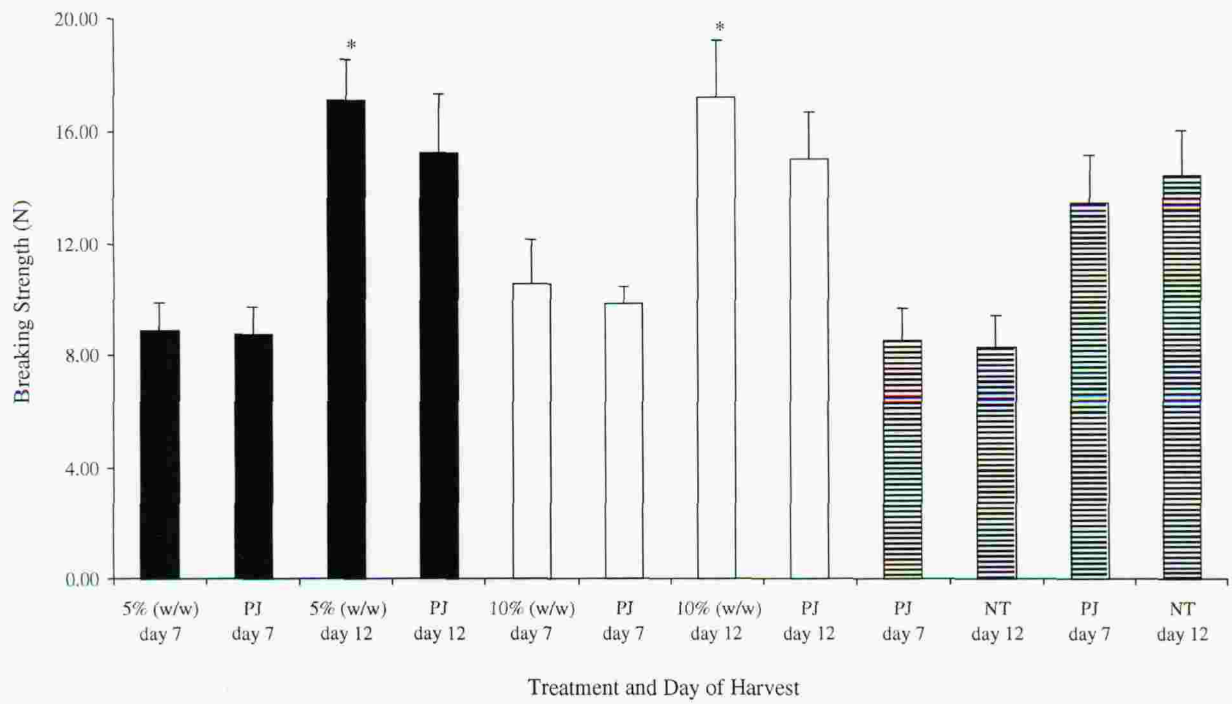

Fig. 3. Breaking strength of wounds treated with 5 and 10% (w/w) *Hamelia patens* Jacq. and petroleum jelly (PJ) and no treatment (NT) control groups. Results are presented as means ± S.D. Statistically significant results are given as $^{*}P < 0.05$.

4. Discussion and conclusions

Linear incision wound healing models are used for wound healing bioassays because they "represent a true surgical wound that could be reproducibly analyzed in a nonsubjective, highly controlled manner" (Mustoe et al., 1987). Normal wound healing occurred in all groups. However, a significant difference was found at day 12 for 5 and 10% (w/w) treatments when compared with their controls. No difference in breaking strength means was found at day 12 when comparing 5 and 10% (w/w) treated incisions. Group III (PJ versus NT) was used as an overall control group to measure any significant difference between PJ and NT because PJ was used as the suspension for the extract. No significant difference was found between the two on breaking strength, indicating that petroleum jelly had no significant effect on normal wound healing.

This study demonstrates that *Hamelia patens* increased breaking strength of wounds significantly, more so than the control group. In order to better understand the specific wound healing mechanisms of *Hamelia patens*, more extensive studies need to be performed, as has been done with other well known wound-healing plants, most significantly *aloe* and *sangre de grado* (Vaisberg et al., 1989; Pieters et al., 1992; Heggers et al., 1993; Pieters et al., 1995; Heggers et al., 1996; Heggers et al., 1997). Since popular *Hamelia patens*-derived products are sold or used throughout Central America as a natural remedy for treating wounds and other skin conditions, further studies of *Hamelia patens* for its wound healing properties would be useful in ascertaining its efficacy and range of uses.

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