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Therapeutic effectiveness of a *Mimosa tenuiflora* cortex extract in venous leg ulceration treatment

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Abstract

The cortex of *Mimosa tenuiflora* is a popular remedy utilized in Mexico for the treatment of skin lesions. Modern studies support the existence in this cortex of compounds with cicatrizing properties. In the present study the therapeutic effectiveness of an extract elaborated with this bark in the treatment of venous leg ulceration disease was explored. A randomized, double-blind, placebo-controlled clinical trial was conducted with ambulatory patients distributed into two groups, one receiving a hydrogel containing 5% of a crude extract standardized in its tannin concentration (1.8%), while the control group, was administered the same hydrogel but without addition of the extract. In both aseptic washings were performed initially followed by topical application of the corresponding hydrogel and dressing. Follow-up lasted 13 weeks and ulcer healing was determined through measurement of the lesion area by digital-photographic parameters. Therapeutic effectiveness occurred in all patients of the extract group; after the 8th treatment week, ulcer size was reduced by 92% as mean value in this group, whereas therapeutic effectiveness was observed only in one patient of the control group (χ^2, $p = 0.0001$). No side effects were observed in any patient in either group.

Keywords: *Mimosa tenuiflora*; Venous leg ulceration disease; Phytomedicine; Complementary and alternative medicine

1. Introduction

Venous leg ulceration (VLU) disease is a common and disabling condition that often recurs as a consequence of chronic vascular insufficiency. The condition affects up to 1% of adults during their lifetime, and the earliest symptoms (vessel wall deterioration, vein valve modifications, and varicose veins) are related with venous hypertension that results in a local metabolic-events cascade, originating skin-ulcer formation in distal leg regions (Jones and Nelson, 2005; Katsenis, 2005). A significant number of VLU treatments that comprise wound-healing fundamentals have been reported: first, the importance of daily washings with sterile water and neutral soap, and second, the role of oxygen in wound healing, the effects of pH, and use of antimicrobial medicines, and including debridement, simple dressing and compression bandaging (Cullum, 1994). Review studies have indicated that no single treatment method takes precedence as possessing unsurpassed effectiveness. Unfortunately, in many cases these treatments are ineffective, with ulcers remaining open for months or years, producing chronic pain and disability (Bandolier, 1994).

There are reports for VLU treatment costs in certain countries. In Sweden, for example, the cost of one treatment varies between $1,300 and $2,500 US dollars during the average 52-week treatment period. Best estimate of the annual cost of this disease according to epidemiologic data is 100–120 million

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UK pounds (Ragnarson and Hjelmgren, 2005). Evidently, new procedures to reduce VLU healing time are very important to decrease treatment costs; consequently, new products are especially welcomed as coadjutants of wound healing that may improve the skin's cicatrizing process.

Among the design and production alternatives of novel remedies, we highlight the use of medicinal plants from Traditional Herbal Medicine with long-time demonstration of a rich history of therapeutic utility and safety. This is the case of *Mimosa tenuiflora* (Willd.) Poiret (Leguminosae), a tree from Mexico known by the popular name of *tepescohuite*. According to Mexican Medical Ethnobotany, dried and powdered *tepescohuite* bark applied directly on the lesion is an effective remedy to treat skin burns and wounds (Sánchez-León and Yashté, 1991; Camargo-Ricalde et al., 1994; Camargo-Ricalde, 2000). In 1984 in Mexico, this material was used empirically to alleviate the suffering of hundreds of burn victims of a large natural gas-depot explosion; on this occasion, direct application of *Mimosa* bark powder by emergency-services medical personnel on the lesions of many burn patients resulted in facilitating skin regeneration and prevented scarring (Lozoya, 1988). Later, pharmacologic and phytochemical studies performed in Mexico and France supported the existence in this cortex of certain natural compounds with cicatrizing properties. Basic pre-clinical studies report that water and alcoholic extracts from dried *Mimosa* bark are particularly rich in tannins that possess *in vitro* antimicrobial properties against a broad group of Gram-positive and -negative microorganisms, yeasts, and dermatophytes (Lozoya et al., 1989); these same extracts induced the growth of different human cells under cultivation conditions (Villarreal et al., 1991). Other studies allowed identification of the bark of a group of triterpenoidal saponins, designated as Mimonosides A–C (Jiang et al., 1991a,b), which according to *in vitro* observations induced cultured human-cell proliferation, possess immunomodulation capacity, and therefore were attributed to at least part of the potential cicatrizing properties of the plant's bark (Jiang et al., 1992; Anton et al., 1993).

Taking into consideration experimental and anecdotal information in the literature regarding this bark's medicinal properties in cicatrizing processes, we decided to perform a double-blind, randomized, placebo- controlled clinical trial to evaluate the therapeutic effectiveness of a phytodrug developed in our laboratory from an *Mimosa tenuiflora* bark extract in VLU treatment.

2. Materials and methods

2.1. Plant material and extraction

Mimosa tenuiflora (Willd.) Poiret (Leguminosae) bark was collected from a controlled crop from Chiapas State in Mexico and authenticated at the IMSS-M Ethnobotanical Herbarium under code #14841. Once dried and milled, the plant material was extracted by maceration with ethanol during 72 h at room temperature. The extract obtained was then filtered and concentrated under reduced pressure at 60 °C and stored until its use for pharmaceutical presentation. The obtained bark extract was mainly constituted of polyphenols (36% by Folin-Denis method) also containing triterpenoidal and steroidal saponins (<1%) as also reported by Jiang et al. (1991a,b) and a low quantity of indolic alkaloids (<0.05%), as also reported by Meckes et al. (1990). The bark extract did not contain flavonoids.

For phytodrug preparation, a portion of the extract was mixed with polyethylene glycol (PEG-200) and incorporated into Carbopol® 940 with a mixture of sterile water and triethanol amine (pH 7). The phytomedicine obtained was standardized in its polyphenolic content (1.8 g tannins/100 g hydrogel) as determined by the method described for this plant in the Mexican Herbal Pharmacopoeia (2001). Tannin content was utilized as chemical fingerprinting, considering that this group of compounds is reported as useful in the cicatrizing process (Brown and Dattner, 1998). For the control group, a hydrogel was also prepared that was composed of Carbopol® 940, PEG 200, triethanol amine (pH 7), and sterile water, with the addition of Red #3 and Green #3 to the mixture to confer the brown appearance of the phytomedicine. According to clinical protocol requirements, treatments were blinded and products were bottled in similar plastic containers accomodating 100 g of hydrogel for topical application.

2.2. Subjects

The study was carried out on ambulatory patients from the Mexican Institute of Social Security's (IMSS) Regional Hospital No. 1 (HGR No. 1) in the city of Cuernavaca, Morelos, Mexico. With the institution's scientific committee approval, a total of 40 adult ambulatory patients diagnosed with VLU were invited to participate in the study according to requirements established by the IMSS Clinical Studies Ethics Commission. Control or experimental treatments were assigned at random.

2.3. Inclusion criteria

Adult subjects of both sexes between 30 and 70 years of age complied with the following criteria: (a) resident of Cuernavaca City; (b) diagnosed with VLU by the project's medical team; (c) no treatment for at least 1 month prior to study initiation; (d) VLU without clinical infection; (e) provision of signed consent for participation in this clinical trial.

Non-inclusion criteria included pregnant patients and women who were breast-feeding.

2.4. Assay procedures

The protocol corresponded to that of a double-blind, randomized, placebo-controlled clinical trial. Patients were initially informed concerning the possible risks and benefits of this study and were free to leave the protocol at any time or due to the occurrence of adverse reactions to the treatments. Written informed consent was obtained from each participant, and the study obtained the approval of the Ethics Committee of the medical institution where it was performed. The experimental procedure was conducted over 13 weeks. Each patient received his/her assigned product and instructions on how to use it daily for treatment. This consisted of once-daily washings of the

ulcerated area with clean boiled water and neutral soap followed by application of the hydrogel; afterward, the lesion was covered with a simple dressing and compression bandaging. Patients were referred under a weekly schema for continuous medical consultation. At first and final consultations, blood samples were obtained from patients for hepatorenal-function monitoring total gross output [TGO] and total gross product [TGP], creatinine, and urea to determine therapeutic treatment safety.

Healing of ulcers was assessed for all patients by measuring the lesion area once weekly throughout the treatment duration with the aid of a digital-photographic camera and data processing in an imaging analyzer (LSM5 Browser, Carl Zeiss). Therapeutic effectiveness was established by weekly comparison of the lesion-area value in cm^2 with the initial pre-treatment value and expression of this as a cicatrizing percentage ≥80%.

2.5. Statistical analysis

Data obtained from measuring instruments were analyzed with STATA software. Inferential analysis consisted of comparing the different variables evaluated in the experimental group with those of the control group. χ^2-test was used to determine differences among proportions, ANOVA-test for mean differences, Mann–Whitney U-test for comparing independent samples, and $p \leq 0.05$ values were considered for establishing significance.

3. Results

Statistical analysis of the selected parameters with respect to general study-population characteristics did not display important differences between treatment groups. Forty patients clinically diagnosed with VLU were included in the trial: 20 were assigned at random to the experimental group and 20 to the control group. Mean age in the study population was 61 years, this showing that the ailment is characteristic of the second half of a human lifetime. Body mass index (BMI) showed a mean value of 30.6 for the collective, indicating that the majority of patients (82.5%) had overweight and obesity. Only 22.5% of patients manifested the existence of VLU antecedents in their families. All patients presented varicose veins in the same leg where ulceration was found: evolution time of this symptom showed a mean value of 16.5 years, while the mean value in evolution time of ulcers (VLU) was 8.5 years. In 72.5% of cases, patients reported having previously used other medication without success; during the disease-evolution time, 51% of patients were orally administered with different products, while 20% of patients received local applications of diverse compounds. The majority of patients in both groups showed edema, ocher dermatitis, local pain in the ulcer area, and were infection-free in the majority of cases.

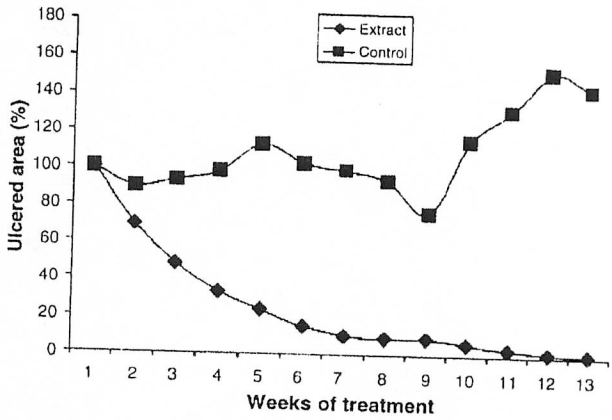

Fig. 1. Comparative effects of treatments on percentage of residual ulcer area during 13 weeks of clinical trials. Data correspond to mean values. ANOVA, $p < 0.01$ in all weeks of treatment.

Table 1 and Fig. 1 show the different evolutions between the groups receiving the *Mimosa tenuiflora* extract-containing hydrogel and the control product. On measuring the lesions in the group receiving the extract, we found that ulceration areas were reduced dramatically after the 3th week of treatment; at the 8th treatment week, ulcer-size reduction was 93% as the group's mean value. At the end of the study, all patients were cured regardless of ulcer size and previous disease-evolution time, the latter in many cases counted in years. The extract's cicatrizing healing effect was observed from the first weeks of

Table 1
Effect of treatments on the accumulated therapeutic effectiveness (cured ≥80% ulcer lesion)

Week	*Mimosa tenuiflora* extract			Control			χ^2 p
	n	f	%	n	f	%	
1	20	0	0	20	0	0	NS
2	20	1	5.00	19	0	0	0.32
3	19	5	26.32	19	0	0	0.01
4	19	11	57.89	19	0	0	0.0001
5	19	14	73.68	19	0	0	0.0001
6	19	17	89.47	18	1	5.56	0.0001
7	19	18	94.74	18	1	5.56	0.0001
8	19	19	100.00	17	1	5.88	0.0001
9	19	19	100.00	15	1	6.67	0.0001
10	19	19	100.00	15	1	6.67	0.0001
11	19	19	100.00	11	2	18.18	0.0001
12	19	19	100.00	11	2	18.18	0.0001

Values correspond to population (n), absolute frequencies (f), and relative frequencies (%). NS = not significant.

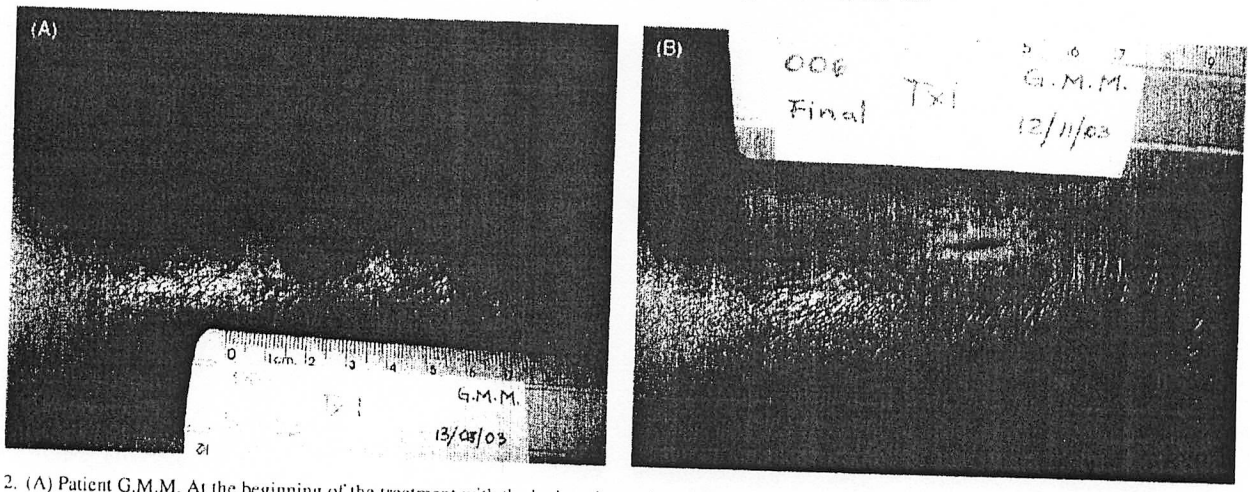

Fig. 2. (A) Patient G.M.M. At the beginning of the treatment with the hydrogel containing *Mimosa tenuiflora* extract. (B) The same patient at the end of treatment.

treatment, and the complete cicatrizing process required different time periods depending the ulcer-area size. Nonetheless, at the end of the 12th week all cases were cicatrized. Contrariwise, in the control group administered the placebo hydrogel no reduction of lesion areas was observed in any case during the first 9 weeks. Moreover, after the 10th and 11th treatment weeks, a lesion-surface increment was observed in some patients in comparison with original study conditions (Fig. 1).

Table 1 compares therapeutic effectiveness between groups. Under experimental treatment, 57.89% of patients exhibited

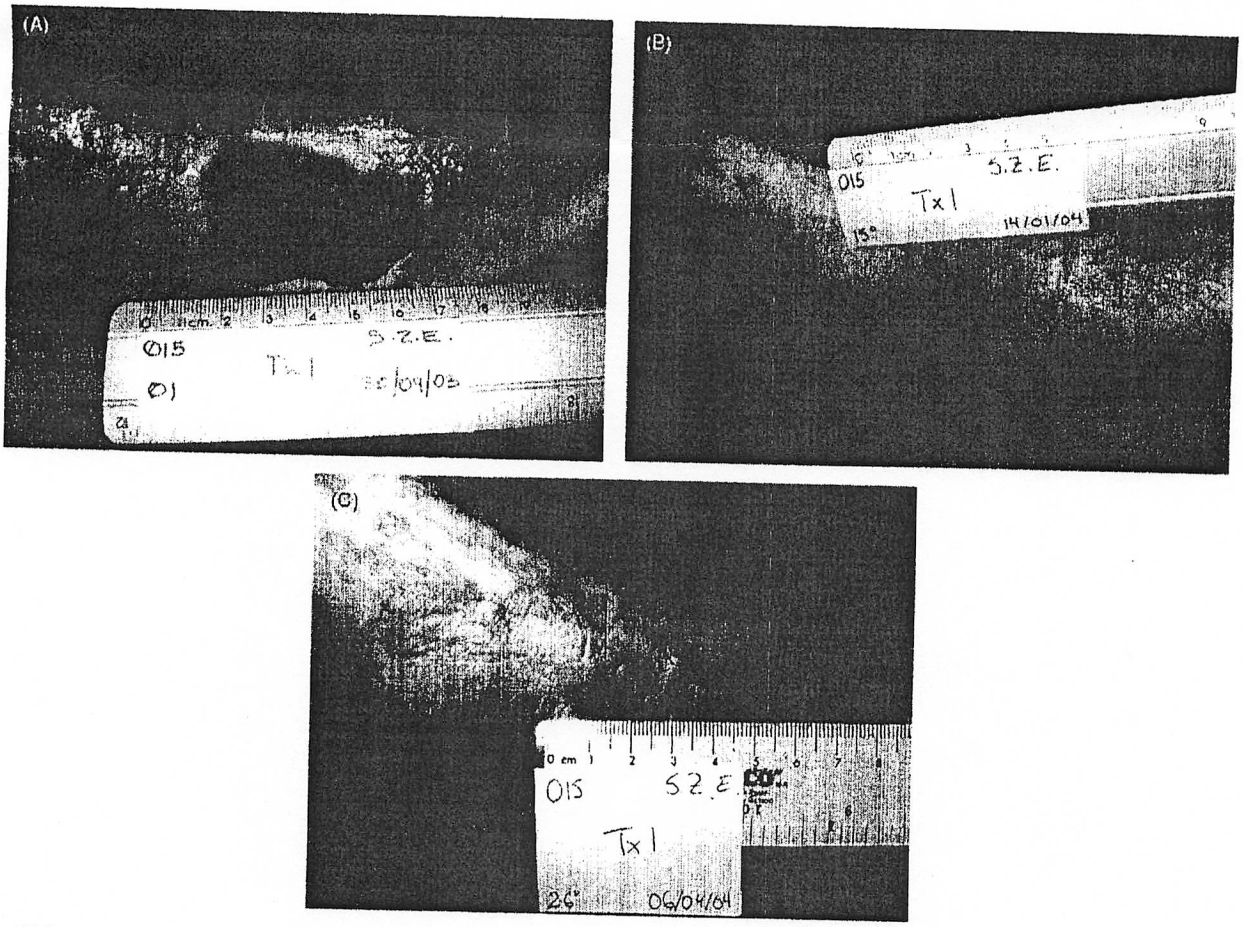

Fig. 3. (A) Patient S.Z.E. At the beginning of the treatment with the hydrogel containing *Mimosa tenuiflora* extract. (B) Same patient in the middle of treatment. (C) Same patient after total recovery.

therapeutic effectiveness at the end of the 4th treatment week and nearly 100% demonstrated this at the end of 8th week, whereas in placebo treatment only one patient showed therapeutic effectiveness from the 6th week ($p = 0.0001$). On the other hand, one patient in the experimental group (5%) and nine in the control group (45%) abandoned treatment due to lack of a good therapeutic response ($p = 0.003$). With respect to the treatments' therapeutic safety, hepatic and renal parameters showed no differences in the groups' blood samples either prior to or after treatment (Figs. 1A and B, 2A–C).

4. Discussion

Results showed that the extract obtained from *Mimosa tenuiflora* bark facilitated skin-ulcer cicatrization when used as a coadjuvant in conventional VLU treatment in ambulatory patients; in the majority of cases and depending on lesion-area size, total ulcer cicatrization was obtained at the end of 8–13 weeks of treatment with the *Mimosa tenuiflora* bark standardized extract-containing hydrogel. The efficacy of this phytomedicine was demonstrated in patients with ulcerations who had suffered for many years (mean, 8–9 years) from this ailment and who had attempted to cure this unsuccessfully with different topical remedies and procedures. No side effects or adverse reactions were detected during 13 weeks of daily application of the extract-containing hydrogel, which supported the safety and good tolerabilty of this phytomedicine.

In the control group, a small increment of the ulcered area in some patients was observed. Although it is teorically considered that leg ulcers may show improvement even with hydrogel use alone, in our study some patients of the control group presented a secondary infectious process at the end of treatment perhaps precisely due to the absence in the hydrogel of the extract, for which antimicrobial properties are well known (Lozoya et al., 1989). Some of these patients abandoned treatment due to absence of clinical improvement. With regard to the possible healing mechanisms of the *Mimosa tenuiflora* bark extract used, these mechanisms are difficult to determine at present. According to the literature, healing mechanisms could be related with either the tannins and/or saponins present in the extract; in the case of saponins, although these demonstrate very low concentrations in the bark, mimonosides are described as possessing cicatrizant properties *in vitro* and as inducing certain immunostimulant effects (Anton et al., 1993). On the other hand, high tannin content in the same extract must be considered as the predominant composition involved in the healing effect. It is well known that natural products containing condensed tannins are used in medicine to aid wound and burn healing (Brown and Dattner, 1998); in addition, according to the modern herbal medicine vademecum plant extracts from *Centella asiatica, Tabebuia impetiginosa*, or *Quercus robur*, among many others, containing up to 2.0% of condensed tannins (the *Mimosa* extract here used contained 1.8%) are used commercially for skin-ulcer treatment (Vanaclocha and Cañigueral, 2003). Notwithstanding this, the role of this group of compounds in the cicatrizing proccess is not completely clear. Tannins are reported to possess broad antimicrobial properties by means of different mechanisms that include enzyme inhibition, oxydative phosphorylation reduction, and iron deprivation, among others (Scalbert, 1991). On the other hand, the well known astringency of tannins has not been explored in the light of modern molecular biology; nevertheless, it is clear that effects produced by tannins on live skin are different from those of the leather tanning process. Finally, a combined saponin/tannin effect cannot be discarded.

The medicinal properties of other groups of compounds obtained from plants are being studied through the molecular scope of their local effects on skin. Protection of microcirculation by inhibiting endothelial activation has been reported as the mechanism of action of different flavonoidal extracts with therapeutic efficacy in chronic venous insufficiency management (Katsenis, 2005). The medical use of *Hamamelis virginiana* cortex extract in vascular and mucosa inflammation treatment is based on the fact that its procyanidins – ET1 inhibitors – restore endothelian function (Corder et al., 2004). The anti-inflammatory and cicatrizing activity of *Echinacea pallida* root extracts is reported to be related with the antihyaluronidase activity of its echinosides, a group of compounds derived from caffeic acid (Speroni et al., 2002).

Studies to determine the precise identity and properties of the tannin group present in *Mimosa tenuiflora* bark extract are in progress in our laboratory to dilucidate the possible mechanism of action involved in its cicatrizing effect, which has been traditionally observed and now clinically confirmed.

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