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Wound healing activity and systemic effects of *Vernonia scorpioides* extract in guinea pig

S.N. Leite*, G. Palhano, S. Almeida, M.W. Biavatti

Núcleo de Investigações Químico-Farmacêuticas (Niqfar), Curso de Farmácia, CCS, Universidade do Vale do Itajaí (UNIVALI). Rua: Uruguai, 458 Itajaí, Santa Catarina (SC), 88302-202, Brazil

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Abstract

The ethanolic leaves extract of *Vernonia scorpioides* was investigated for its wound healing activity in guinea pigs. Administered once a day for 30 days, 200 mg of a hydrogel containing 50% of extract did not accelerate the closure time, but improved regeneration and organization of the new tissue.

Keywords: *Vernonia scorpioides*; Wound healing; Hematological and biochemical activity

1. Introduction

The fresh leaves ethanolic extract of the *V. scorpioides*, vernacular names: Piracá, Erva-de-São-Simão, has been utilized for the treatment of chronic skin ulcers, by elderly people living at the municipal institution ('Centro de Convivência para Idosos—CCI') in Itajaí (Santa Catarina, Brazil).

The *Piracá* extract is reported to promote the cure of this chronic skin condition.

This tropical lianous herb is easily grown in poor and deforested soils all over the country, and native people recommend the external use of *Piracá* aqueous extract to treat various skin conditions: allergies, skin parasites, irritations, skin

*Corresponding author. Tel.: +55-47-341-7600; fax: +55-47-341-7600.
E-mail address: snleite@ccs.univali.br (S.N. Leite).

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injuries, itching, among others [1,2]. Many *Vernonia* species are used medicinally [3]. In Brazil there are an estimated 200 species, some traditionally used, such as the fresh leaves macerate of *V. condensata,* known as fel-de-bugre, fel-de-índio (*Indian-bitterness*) or alumã—swallowed to treat gastrointestinal disturbance [4].

Searching through the scientific literature, only three studies describing the isolation and characterization of sesquiterpene lactones such as scorpioidin [5], scorpiolide [6] and others [7,8] were found. On the contrary, beside a preliminary evaluation of the antifungal activity of the aerial parts extract [9] no papers were found that corroborates *V. scorpioides* popular use. Considering the wide traditional use of this species and the lack of information about its safety and efficacy, the wound healing effect of the freshly ethanol extracted leaves of Piracá in the guinea pig was investigated.

2. Experimental

2.1. Plant material

Leaves of *V. scorpioides* (Lam.) Pers., Asteraceae were collected in March (2001) from cultivated specimens obtained in the municipal Elderly Living Center ('Centro de Convivência para Idosos—CCI') in Itajaí (SC), Brazil, and were identified by Dr Ana Claudia Araújo (Universidade do Vale do Itajaí, Santa Catarina, Brazil). Voucher specimens [M. Biavatti 11 (15/03/01)] were deposited at the Herbário Barbosa Rodrigues (HBR), Itajaí, Santa Catarina, Brazil.

2.2. Tested material

The extract was prepared according to the traditional method in use at the Elderly Living Center (CCI): fresh leaves were cleaned and extracted (15%, w/v) with 96% commercial ethanol for 7 days in the absence of light.

The obtained extract was reduced up to 50% of it initial volume. A hydrogel containing Carbopol 2% was prepared incorporating 50 g of the concentrated extract in 50 g of the gel. For the control group, 30 g of 96% commercial ethanol was incorporated in 50 g of the gel.

2.3. Animals

Guinea pigs (H–D) of both sex weighing 650 ± 50 g were used. They were housed in standard environmental conditions and fed with rodent diet with water ad libitum.

2.4. Wound healing activity

Animals were divided into two groups of six animals each (3 males and 3 females): *V. scorpioides* hydrogel group (treated) and ethanol gel group (control).

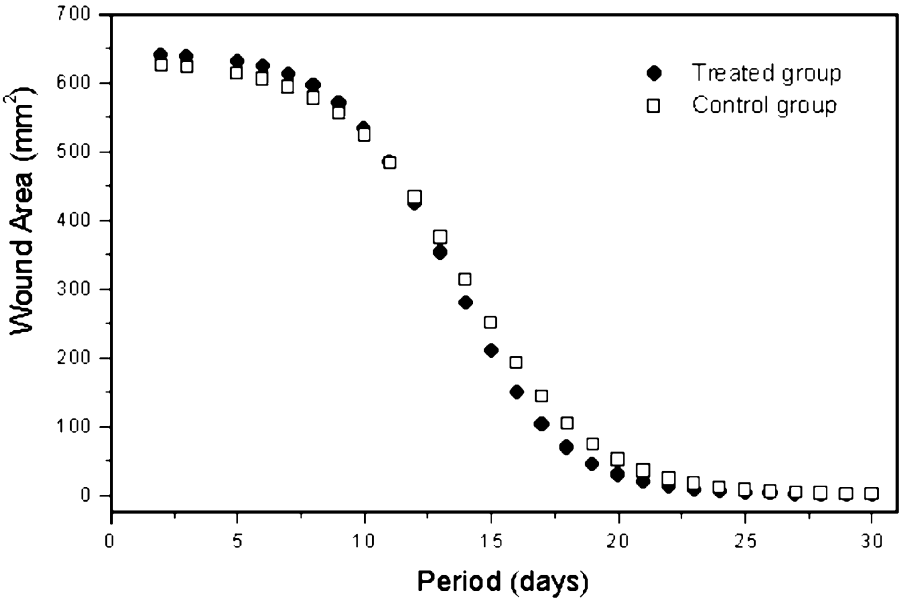

Fig. 1. Evaluation of *V. scorpioides* ethanolic leaves extract applied topically on wound healing in guinea pigs.

All animals were anesthetized and the excision procedure was performed according to Cross et al. [10]. Then, the chirurgical wounds were treated daily topically with 200 mg of the *V. scorpioides* hydrogel while the control group were treated with ethanol gel during 30 days. The measurement of the wounds area (mm^2) was carried out every 7 days and wound healing was expressed as percentage of reduction of original wound. The crust formation, granulation tissue and inflammation presence were analyzed.

Samples of the skin tissues from the healed wounds of each animal were taken for histopathological examinations. The thin sections of tissues were stained with Hematoxilin/Eosin solution and observed under microscope.

At the end of the experiment, blood samples were collected by cardiac puncture for the evaluation of hematocrit, erythrocyte, leukocyte counting and blood cell differentiation.

3. Results

Fig. 1 shows the wound healing for the two groups. Cicatrisation time curves of both groups (treated and control) were not significantly different (t-test, $P < 0.05$). The treated group presented a rigid, dark and thick crust. It is probably due to proteins and wound exudates interconnected with the extract constituents favoring the local homeostasis and protecting the new tissue by forming an external cover

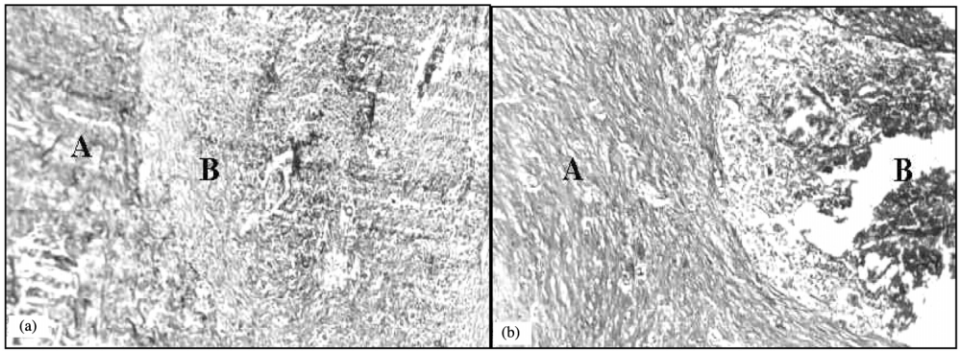

Fig. 2. (a) Histopathological characteristics of healed tissue after treatment with *V. scorpioides* hydrogel. A: connective tissue (collagen), B: granulation tissue. (b) Histopathological characteristics of the control (ethanol gel) healed tissue. A: granulation tissue, B: tissue necrosis. Plates stained with H/E, pictures taken with Olympus PM 20 Photomicroscope 20× magnification.

that furnished mechanic protection. In the control group a thin and transparent crust that detached and bled until the 10th day was observed.

The study of the histological structure (Fig. 2) showed that the tissue regeneration was greater in the skin wound treated with the gel containing the extract. The control group presented edema, monocyte cells and areas with cellular necrosis that were not observed in the treated group.

The analysis of the hematological data (Table 1) indicated great intra-group data variability; statistical significant difference was only found for the monocyte cells ($P > 0.05$). High values in the control group revealed inflammation presence due to the higher mobilization of these cells to the healing area in the control group [11].

Table 1
Effect of *V. scorpioides* ethanolic leaves extract applied topically on guinea pigs in some hematological parameters

Variable	Control Mean ± S.E.	*V. scorpioides* hydrogel Mean ± S.E.
Erythrocyte (M/μl)	4.88 ± 0.1751	4.81 ± 0.1381
Hemoglobin (g/dl)	13.0 ± 0.3997	13.1 ± 0.30443
Hematocrit (%)	40.16 ± 1.0619	39.78 ± 0.3549
Platelets (K/μl)	335.16 ± 45.8427	290.66 ± 32.2786
Leucocytes (10^3/μl)	3.93 ± 0.6370	5.35 ± 0.5789
Basophils (10^3/μl)	0.1 ± 0.010	0.04 ± 0.0489
Neutrophils (10^3/μl)	1.48 ± 0.0945	1.76 ± 0.2123
Lymphocytes (10^3/μl)	1.36 ± 0.2985	1.91 ± 0.4671
Eosinophils (10^3/μl)	0.45 ± 0.2924	1.25 ± 0.7068
Monocytes (10^3/μl)	0.61 ± 0.1046	0.3 ± 0.0258

$n = 6$; $P < 0.05$.

4. Conclusions

Even if the wound healing total time was not different for the two groups, the histological evaluation of healed tissue revealed that the connective tissue of the treated group was well organized and rounded by granulation tissue. On the contrary, inflammation and necrosis focus associated with higher monocyte values was observed in the control group (Fig. 2).

Further studies are requested to evaluate the healing effect of *V. scorpioides* extracts accordingly with the popular use, especially in venous stasis ulcers, diabetic foot ulcers and pressure ulcers models. Wounds differ pathophysiologically, making it difficult to spread results obtained with one type of wound study [12].

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