

## Tissue injuries of wistar rats treated with hydroalcoholic extract of *Sonchus oleraceus* L.

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The use of plant species is emerging as an important alternative in the treatment of injuries. Therefore, the extract of *Sonchus oleraceus* 10% was employed in the repair of skin lesions. A total of 36 male Wistar rats were subjected to a punch injury and divided into three groups: a negative control, receiving no treatment, a positive control, treated with Dersani, and the experimental group treated with the extract. The injury was assessed macroscopically and microscopically. Morphometric data was collected at the 3<sup>rd</sup>, 5<sup>th</sup> and 7<sup>th</sup> postoperative day, and the experimental group showed greater changes in shrinkage of the lesion compared to control groups. On the 3<sup>rd</sup> postoperative day, the injury in the experimental group showed less necrotic tissue, lower slough and more granulation tissue in relation to the positive control group. On the 7<sup>th</sup> and 10<sup>th</sup> postoperative day, the injury in the experimental group showed lower slough compared to the positive control group. Microscopic analysis of lesions on the 5<sup>th</sup> postoperative day revealed increased fibroplasia in the experimental group compared to control groups, while on the 14<sup>th</sup> postoperative day less neovascularization was evident in the experimental group and increased formation of hair follicles in the negative control group. The extract of *S. oleraceus* provided tissue repair in accordance with normal physiological patterns thus confirming empirical evidence for its use.

**Uniterms:** *Sonchus oleraceus*/pharmacognosy. Skin lesions/treatment/experimental study. Wound healing/experimental study. Medicinal plants.

O emprego de espécies vegetais vem surgindo como alternativa no tratamento de lesões. Dessa forma, foi utilizado o extrato hidroalcoólico de *Sonchus oleraceus* a 10% na reparação de lesões cutâneas. Trinta e seis ratos machos Wistar, foram submetidos a uma lesão com “punch” e distribuídos em três grupos: controle negativo, não recebeu tratamento; controle positivo, tratado com Dersani; e o experimental, tratado com extrato. A lesão foi avaliada macroscopicamente e microscopicamente. Os dados morfométricos mostraram que no 3<sup>o</sup>, 5<sup>o</sup> e 7<sup>o</sup> dia pós- operação (DPO), o grupo experimental apresentou maior evolução na retração da lesão em relação aos grupos controles. No 3<sup>o</sup> DPO a lesão do grupo experimental apresentou menor tecido necrótico, menor esfacelo e maior tecido de granulação em relação ao grupo controle positivo. No 7<sup>o</sup> e 10<sup>o</sup> DPO, a lesão do grupo experimental apresentou menor esfacelo em relação ao grupo controle positivo. A análise microscópica das lesões revelou no 5<sup>o</sup> DPO maior fibroplasia no grupo experimental em relação aos grupos controles; no 14<sup>o</sup> DPO ocorreu menor neovascularização no grupo experimental e maior formação de folículos pilosos no grupo controle negativo. O extrato hidroalcoólico de *S. oleraceus* propiciou reparo tecidual obedecendo a padrões fisiológicos normais e confirmando os dados empíricos de sua utilização.

**Unitermos:** *Sonchus oleraceus*/farmacognosia. Lesões cutâneas/tratamento/estudo experimental. Cicatrização/estudo experimental. Plantas medicinais.

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## INTRODUCTION

In recent years, a return to medical treatment, considered by many health professionals as folk or nonscientific medicine, has intensified, along with the gradual reintroduction of many plant species, including weeds as an alternative or complementary therapy (Garros *et al.*, 2006). The use of these plant species is an emerging alternative to some ineffective synthetic products that is linked to socioeconomic factors and a search by the population for treatments which are less aggressive to the body and produce fewer side effects (Coelho, 1998). *Sonchus oleraceus* L. belongs to the Asteraceae family and is a grass-weed affecting several crops in Brazil. Originally from North Africa, Asia Minor and Europe and naturalized in Brazil, it is spreading to all regions with balmy weather, where it grows from September to March. It is an annual plant that is herbal, milky and popularly known by the name of milkweed, wild chicory, smooth, jealousy or locksmith (Jorge, Ferro 1987; Vieira, Barreto, 2006). The leaves of *S. oleraceus* are eaten as salad in some regions. The whole plant is used in folk medicine in several regions of the country for stomach disorders, liver disease, a rheumatoid against wounds, sores, rashes, eczema, varicose ulcers and applied in the form of compresses or infusions. It has anti-inflammatory and diuretic properties, as well as a healing action when used externally (Lorenzi, Matos, 2008).

The plant's chemical composition includes essential oils, steroids, resins, carbohydrates, phytosterine, alkaloids, tannins, coumarins, flavonoids and saponins (Lorenzi, Matos, 2008; Miyase, Fukushima, 1987; Guarrera *et al.*, 2008). Therapeutic studies with the plant species *S. oleraceus* are still scarce, although recent studies report its contraceptive action, and anxiolytic, anti-inflammatory and antioxidant activity (Vilela *et al.*, 2010; Nehir El, Karakaya, 2004; Schaffer *et al.*, 2005). *Sonchus oleraceus* L. Milkweed is a weed that has been shown empirically to treat wounds and ulcers. Thus, the purpose of this study was to evaluate the effect of the extract of milkweed in the process of healing of skin in rats.

## METHODS

The experiment was carried out at the Laboratories of Vegetable Biotechnology, Animal Experimentation, Microtécnica and Histology of the Regional Integrated University of High Uruguay and Missions - URI - Campus Erechim/RS. All the ethical principles in animal experimentation advocated by the Brazilian Society of Laboratory Animal Science (SBCAL) were adopted. The research project was submitted to and approved by the Re-

search Ethics Committee (CEP) of the Regional Integrated University of Alto Uruguai and Mission, URI-Campus Erechim, under Protocol No. 32/TCA/09.

### Collection and preparation of extract of *S. oleraceus*

Leaves, flowers and stems of milkweed (*S. oleraceus*) were collected in January 2009 at a private estate in Upper Uruguay, Rio Grande do Sul, Brazil (herbarium specimens of the species were deposited at the Herbarium Father Baldwin Rambo, Regional Integrated University of High Uruguay and Missions - Campus Erechim, under No. 11 506).

The plant material was dried in an oven with forced air flow at a temperature of  $35 \pm 1$  °C to stabilize the active principles and achieve constant weight. The dried material was then pulverized in a blade grinder to obtain fine particles which were not sieved. Extraction was by cold maceration with vehicle extractor of 70% ethanol. A total of 50 grams of dried and ground plant material was added to 1000 mL of 70% ethanol solution for 24 hours for extraction, with maceration performed after 48 and 72 hours. The solution was kept closed and protected from light. The liquid from filtration was evaporated in an oven with forced air flow at a temperature of  $35 \pm 1$  °C to a constant weight for a period exceeding 24 hours. Residue from evaporation was considered the crude extract, which was then eluted with distilled water to obtain the concentration treatment of 10%. The yield of extract was calculated using the equation  $TEA (\%) = (Mf / Mi) \times 100$  where, TEA refers to the total extract amount (%), Mi the initial mass of the sample (grams), and Mf the final mass of extract (g) according to Pansera *et al.* (2003). A yield of 11.6 grams was obtained corresponding to 23.22% (w / v) of crude extract.

### Experimental animals

A total of 36 male Wistar rats aged 60-90 days, supplied by the vivarium of the URI - Campus Erechim were used. During the study period, the animals were kept individually in polyethylene boxes under controlled temperature ( $22 \pm 2$  °C), humidity ( $50 \pm 5\%$ ) and lighting, with access to water and food “*ad libitum*” and a 12-hour light/dark cycle.

### Experimental model and treatment of injuries

Mechanically induced tissue damage was inflicted to the dorsal region of the animals for subsequent analysis of healing. For induction of tissue injury, the animals were immobilized using intraperitoneal anesthesia with sodium

thiopental 2.5% at doses of 40 mg/kg according to Carregaro *et al.* (2005). After immobilization the animals were placed in the prone position to perform shaving and antiseptics of the dorsal region and posterior injury induction. A small fragment of skin (0.8 cm diameter) was removed on the dorsal cranial area extending to the dorsal muscle fascia using a dermal metal punch. The lesion was measured and then considered day 0 (zero) postoperatively (POD).

The animals were randomly divided into three groups where each group was subdivided according to the analysis period on the 5<sup>th</sup> and 14<sup>th</sup> postoperative days (POD). This gave a total of 18 animals for the 5<sup>th</sup> postoperative day (POD) and 18 for the 14<sup>th</sup> postoperative day (POD). The treatment for all groups was given after the induction of injury and was in the form of an open curative applied twice a day (following the same schedule) in an amount sufficient to fully cover the lesion (0.1 mL).

The negative control group (C-) received no treatment, but control animals were handled equally. The positive control group (C+) was treated with application of Dersani<sup>®</sup>, a curative medicine obtained commercially. This consists of an oily lotion based on essential fatty acids, with vitamins A and E, which revitalizes and maintains the skin's moisture balance, improving its elasticity. The medicine contains caprylic acid, capric acid, lauric acid, soy lecithin, vitamin A and E and sunflower oil (linoleic acid) that helps prevent the formation of scabs and contributes to the restoration of skin integrity. The experimental group was treated with the extract of milkweed (*S. oleraceus*) at a concentration of 10% (100 mg/mL).

### Macroscopic analysis

At the 0, 3<sup>rd</sup>, 5<sup>th</sup>, 7<sup>th</sup>, 10<sup>th</sup> and 14<sup>th</sup> postoperative day (respectively), animals of all groups were weighed and lesions analyzed for size of their fields and macroscopic aspects (quantity of exudate and tissue type). All tests carried out before applying any topical agent, and for these tests as well as for all other painful procedures and/or stress, the animals were subjected to inhalation of anesthesia with diethyl ether 98%. Morphometric analysis was performed using calipers, where the transverse and longitudinal area of the lesion was measured. From these elements the area of the ellipse was calculated using the formula:  $\text{Area} = \pi \times a \div 2 \times b \div 2$ , where  $\pi = 3.1416$ , **a** corresponds to cross-sectional area and **b** corresponds to the area of longitudinal lesion (Callegari-Jacques, 2008).

For morphological analysis, a hand-held magnifying glass with a two-times magnification was used, and the

parameters evaluated included the amount of exudate, and type of injury, classified according to the scores: absent (0), little (1), moderate (2) and intense (3). The appearance of the wound was defined as per Santos *et al.* (2005) into: Necrotic Tissue, yellow or white; Slough, tissue stained black, brown or chestnut; Granulation Tissue, pink or red, moist and grainy; Epithelial Tissue, pink or bright tissue and Wound Closed, completely covered with epithelium. The data were evaluated according to the scores 0 (closed wound), 1 (epithelial tissue), 2 (granulation tissue), 3 (slough) and 4 (necrotic tissue).

### Microscopic analysis

Histological analysis was performed with six animals in each group. The animals were killed sequentially on the 5<sup>th</sup> postoperative day (POD) or 14<sup>th</sup> postoperative day (POD). The fragment of skin surrounding the lesion was collected for later histological processing in paraffin and routine analysis by light microscopy. Histology slides with 4 mm-thick sections were stained with hematoxylin-eosin (HE) for general histological observation and with Mallory Trichrome (TM) for the study of collagen fibers, in accordance with the directions of Carvalho *et al.* (1991) and Santos *et al.* (2002). In the epidermis, the formation of layers, degree of reepithelialization such as loose and dense dermis was evaluated while vasodilation, neovascularization, leakage of erythrocytes, vascular congestion, fibroplasia, the presence of sebaceous glands, sweat glands and hair follicles was also analyzed. These parameters were quantified by scores defined as: 0: absent (0%), 1: minimal (0% to 25%), 2: moderate (25% to 50%) and 3: severe (50% to 75%) and then tabulated for statistical analysis.

### Statistical analysis

The statistical data related to the animals' body weight and morphometry were assessed using ANOVA followed by factor of Duncan. For the analysis of qualitative assessment of morphology (amount of exudate, wound type and total scores) and histological examination, the non-parametric Kruskal-Wallis and Mann-Whitney test were employed. The significance level was set at less than 5% ( $p < 0.05$ ) using Statistica software for Windows 5.1 (1997).

## RESULTS

All animals recovered well from anesthesia and no deaths occurred during the experimental process. The

daily clinical evaluations showed adequate recovery, with all groups maintaining general condition, presence of physical activity and willingness to forage. During the observation period, no significant differences in body weight between groups and days after surgery were recorded, revealing that even the animals submitted to inhalation anesthesia and surgery during the analysis showed no significant changes in body weight or toxic action mediated by the application of topical agents.

## Macroscopic evaluation

### Morphometric

The morphometric results of the area of lesions in control and experimental animals are shown in Table I. It was observed that the lesion area decreased gradually with time in all groups. This reduction was found in a linear fashion over the POD.

On the 3<sup>rd</sup> postoperative day the negative control group and the experimental group had onset of tissue repair, with significant reduction of injury when compared to the positive control group. On the 5<sup>th</sup> postoperative day, the lesion area remained with significant reduction in negative controls and experimental animals versus the control group. On the 7<sup>th</sup> postoperative day, a significant reduction in lesion area (cm<sup>2</sup>) was observed among experimental rats compared to the control group. The lesion area of the negative control group showed no significant difference in relation to areas of injury compared to the positive control and experimental groups. On the 10<sup>th</sup> postoperative day, reduction in lesion area in animals of the experimental group was higher. However, no significant difference was seen compared to control groups. On the 14<sup>th</sup> postoperative day, areas of the lesions showed no significant differences between the experimental and

control groups because the lesions of experimental rats had self-repaired (Table I).

### Morphology

On the 3<sup>rd</sup> postoperative day, lesions of the animals showed significant differences in necrotic tissue, slough and granulation. The experimental group showed a reduction of necrotic tissue and slough and increased granulation compared to the control group. In contrast, the negative control group showed significant reduction only in the granulation tissue when compared to the experimental group.

On the 5<sup>th</sup> postoperative day, significant differences in the slough and granulation tissue in the lesions of the groups was evident. The experimental group showed significant difference with less slough and increased granulation tissue in relation to the positive control group. Comparing the experimental group with the negative control group, a significant decrease was observed only for slough. No significant differences were observed in lesions in epithelial tissue necrosis.

On the 7<sup>th</sup> postoperative day, a significant increase in the slough in the injury of the animals was evident in the positive control group compared to the experimental group. The control groups also showed greater intensity and thickening of crust formation. For the necrotic tissue, granulation and epithelial no significant differences were identified between the lesions of experimental and control groups. On the 10<sup>th</sup> postoperative day, the lesions of animals in the positive control group showed significant increase in slough when compared to the experimental group. For the granulation and epithelial tissues, no significant differences were found between the experimental and control groups.

On the 14<sup>th</sup> postoperative day, there were no significant differences in tissue and epithelial sloughing of the

**TABLE I** - Morphometric analysis of tissue repair of injuries (cm<sup>2</sup>) among positive control, negative control and experimental groups at 0, 3<sup>rd</sup>, 5<sup>th</sup>, 7<sup>th</sup>, 10<sup>th</sup> and 14<sup>th</sup> postoperative days (POD)

Postoperative Days (POD)	Groups			Value p
	Positive Dersani®	Negative	Experimental Extract 10%	
0	0.501 ± 0.116	0.543 ± 0.071	0.500 ± 0.109	0.5079
3 <sup>rd</sup>	0.612 ± 0.160	0.458 ± 0.082	0.496 ± .126	0.0037 <sup>ab</sup>
5 <sup>th</sup>	0.527 ± 0.142	0.369 ± 0.095	0.346 ± 0.073	0.0007 <sup>ab</sup>
7 <sup>th</sup>	0.423 ± 0.104	0.359 ± 0.097	0.234 ± 0.115	0.0219 <sup>b</sup>
10 <sup>th</sup>	0.136 ± 0.040	0.127 ± 0.069	0.088 ± 0.009	0.6052
14 <sup>th</sup>	0.006 ± 0.016	0.013 ± 0.018	-	0.2941

Values expressed as mean ± standard deviation, **a** significant difference between positive control and negative control groups, **b** significant difference between positive control and experimental groups, for p < 0.05 by Duncan's parametric test.

experimental and control groups. However, all animals in the experimental group had complete reepithelization of the lesion compared to control groups.

## Microscopic evaluation

### Epidermis

Judging from the histological parameters of healing of the epidermis on the 5<sup>th</sup> and 14<sup>th</sup> postoperative day, no significant differences were evident. The lesions in the animals from all groups showed complete formation of layers by the 5<sup>th</sup> and 14<sup>th</sup> postoperative days. The experimental group showed greater intensity of reepithelialization, without significant difference compared with the control groups.

### Dermal

#### • Loose connective tissue

On the 5<sup>th</sup> postoperative day, no significant differences for the parameters of loose connective tissue were evident in experimental and control groups. The control group showed negative scores with greater intensity for fibroplasia, whereas the experimental group showed a lower intensity scores for vasodilation, with neither reaching significant differences.

On the 14<sup>th</sup> postoperative day, the positive control group showed a significant increase in neovascularization compared to the experimental group. In contrast, the negative control group, showed a significant increase in hair follicles compared to the positive control and experi-

mental groups. No changes between groups for the other parameters was shown (Table II).

#### • Dense connective tissue

On the 5<sup>th</sup> postoperative day, a significant increase in fibroplasia was observed in the experimental group compared to control groups, indicating intense tissue repair. The negative control group showed a lower intensity of vasodilatation with no significant difference in relation to positive control and experimental groups. The other parameters showed no significant differences between groups (Table III).

On the 14<sup>th</sup> postoperative day, no significant differences were identified for the parameters analyzed in dense connective tissue between the experimental and control groups.

## DISCUSSION

Conventional wisdom drawn from the culture of people is considered the starting point for scientific knowledge. Based on such knowledge, it is assumed more than 70% of drugs derived from plants were developed. Although there are counterpoints between scientific knowledge and popular knowledge in relation to the demands of building an accepted scientific knowledge of medicinal plants, the steady integration of this knowledge contributes greatly to the acquisition of new knowledge, helping researchers in pursuit of cure for several diseases (Bittencourt *et al.*, 2002).

**TABLE II** - Number of animals according to intensity of loose dermal tissue repair (0 = absent, 1 = minimal, 2 = moderate, 3 = intense) in groups on 14<sup>th</sup> postoperative day (POD)

Loose Connective Tissue	Groups												Value H	Value p
	Positive Dersani®				Negative				Experimental Extract 10%					
	0	1	2	3	0	1	2	3	0	1	2	3		
Neovascularization	-	3	2	1	1	3	2	-	4	2	-	-	7.29	0.0261 <sup>b</sup>
Vasodilation	4	2	-	-	5	-	1	-	4	2	-	-	0.14	0.9315
Vascular congestion	1	3	2	-	5	-	1	-	4	2	-	-	4.19	0.1228
Leak of erythrocytes	5	1	-	-	5	1	-	-	4	1	1	-	0.43	0.8059
Hair follicles	5	1	-	-	-	2	3	1	4	2	-	-	9.94	0.0069 <sup>ac</sup>
Sebaceous glands	5	1	-	-	3	3	-	-	5	1	-	-	1.28	0.5247
Sweat glands	3	3	-	-	2	3	1	-	2	4	-	-	0.70	0.7042
Fibroplasias	-	-	2	4	-	-	-	6	-	-	1	5	2.26	0.3220

<sup>a</sup> significant difference between positive control and negative control groups, <sup>b</sup> significant difference between positive control and experimental groups, <sup>c</sup> significant difference between negative control and experimental groups, for  $p < 0.05$  by Kruskal-Wallis non-parametric test.

**TABLE III** - Number of animals according to intensity of dense dermal tissue repair (0 = absent, 1 = minimal, 2 = moderate, 3 = intense) in groups on 5<sup>th</sup> postoperative day (POD)

Dense Connective Tissue	Groups												Value H	Value p
	Positive Dersani®				Negative				Experimental Extract 10%					
	0	1	2	3	0	1	2	3	0	1	2	3		
Neovascularization	-	2	4	-	-	2	4	-	-	2	4	-	-	1
Vasodilation	-	2	3	1	-	1	5	-	1	3	2	-	1.04	0.5931
Vascular congestion	-	1	3	2	-	1	4	1	1	3	2	-	0.27	0.8727
Leak. of erythrocytes	1	4	1	-	2	2	2	-	1	4	1	-	3.70	0.1565
Hair follicles	6	-	-	-	5	1	-	-	6	-	-	-	0.31	0.8539
Sebaceous glands	5	1	-	-	6	-	-	-	6	-	-	-	0.31	0.8539
Sweat glands	5	1	-	-	6	-	-	-	5	1	-	-	0.31	0.8538
Fibroplasias	-	2	4	-	-	2	3	1	-	-	-	6	11.09	0.0039 <sup>bc</sup>

<sup>b</sup> significant difference between positive control and experimental groups, <sup>c</sup> significant difference between negative control and experimental groups, for  $p < 0.05$  by Kruskal-Wallis non-parametric test.

Analyzing the results of morphometric and morphological evolution was observed in tissue repair in animals treated with the extract of *S. oleraceus*. The more pronounced tissue repair in the experimental group may have been due to less formation of crusts on the wound and the lower amount of exudate, particularly attributed to flavonoids in the extract conferring these anti-inflammatory, antioxidants and antibacterial properties (Schaffer *et al.*, 2005; Yin *et al.*, 2007; Vilela *et al.*, 2010). According to Eurides *et al.* (1998), lower crust formation explains the performance of the product, facilitating reepithelialization since the presence of liquid in injury can affect the process of bacterial growth. The crust also impairs collagen deposition and epithelialization of the lesions (Stadelmann *et al.*, 1988) and also prevents the products applied topically from coming into close contact with the injured area.

At the 3<sup>rd</sup> and 5<sup>th</sup> postoperative days, the area of injury of experimental rats showed significant differences in the lesion area versus the positive control group, as well as showing lesions with less exudates, reduced formation of necrotic tissue and slough and increased formation of granulation tissue compared to the positive control group. This factor was related to improvements in the process of shrinkage of lesions and may be linked to the activity of the extract of *S. oleraceus* 10%. This effectiveness in wound healing can be attributed to the flavonoids in extract of *S. oleraceus*, corroborating their use as anti-inflammatory, antibacterial, antipyretic and antioxidant agents (Nehir El, Karakaya, 2004; Schaffer *et al.*, 2005; Yin *et al.*, 2007; Vilela *et al.*, 2010) essential in the healing of wounds. However, the acceleration of the healing process displayed

on the 3<sup>rd</sup> and 5<sup>th</sup> postoperative day, although beneficial through avoiding infection, can result in final healing of low quality (Campos *et al.*, 2000).

The occurrence of the granulation tissue and reduction of exudate in the experimental group at the 3<sup>rd</sup> and 5<sup>th</sup> postoperative days, indicate an improvement in retraction of the lesions as a result of shifting centripetal and contractile activity of myofibroblasts in granulation tissue from the margins to the center of the lesion. After the initial reduction of the area, there is the resetting of the basement membrane, optimizing reepithelialization (Mott *et al.*, 2003). This process of wound contraction is totally independent of the epithelialization process, occurring below the new epithelium formed and gradually disappearing, to the extent that the margins of the wound move to the center until joining (Swain, 1980). In the phase of fibroplasia that occurs after 48 hours of injury, the fibroblasts multiply and produce collagen. There is also intense endothelial proliferation at this stage, resulting in the formation of granulation tissue (Sanchez-Neto *et al.*, 1993).

From the 7<sup>th</sup> postoperative day, the lesion area in the experimental group was found to be well-differentiated compared with the control groups, especially versus the positive control group. The morphological analysis also showed lower formation of slough for the experimental group compared to the positive control group, indicating again a breakthrough in the repair process by the extract. From the 10<sup>th</sup> postoperative day, lesions in the experimental group had already self-repaired compared to control groups. The fact that there was no significant difference in lesion area among groups at both the 10<sup>th</sup> and the 14<sup>th</sup>

postoperative days was due to morphological repair of lesions in the experimental rats.

In the epidermis, based on the microscopic ratings of lesions on the 5<sup>th</sup> and 14<sup>th</sup> postoperative days showing no significant differences, better reepithelialization of experimental and positive control groups was seen compared to the negative control group where the greater intensity of reepithelialization in these groups indicated efficient healing. In contrast, for layering, all groups independent of treatment, showed gradual and complete layering over the postoperative days. On the 14<sup>th</sup> postoperative day, the loose connective tissue lesions in experimental rats showed less neovascularization compared with the control groups, indicating that lesions in the experimental group had been in the process of remodeling. According to Solórzano *et al.* (2001), the process of remodeling of the wound involves the balance between synthesis and degradation of collagen, reducing the vascularization and infiltration of inflammatory cells until the lesion reaches maturity. Thus, a tissue injury is completely repaired when it presents formation of its skin appendages, increased fibroplasia and decreased neovascularization (Kierzenbaum, 2004). According to Stashak (1994), neovascularization promotes healing by increasing the supply of nutrients and cells in the affected area, reducing the retraction time of the lesion. In contrast, the oxygen tension decreased in the lesion site due to insufficient blood supply causes the inhibition of migration of fibroblasts and collagen synthesis resulting in risks and complications in wound healing (Gogia, 2003).

On the 14<sup>th</sup> postoperative day, it was also possible to verify in loose connective tissue lesions of the animals in the negative control group, a significant increase in the number of hair follicles versus the positive control and experimental groups, evidencing that the negative control group showed adequate compensation for the injured tissue. The negative control group also showed no significant difference and greater formation of glands. An injury is considered fully repaired when presenting skin appendages, as these are requirements of the skin so that it can fully perform its function (Kierzenbaum, 2004).

In the analysis of dense connective tissue, on the 5<sup>th</sup> postoperative day, the experimental group showed a high degree of fibroplasia with thicker collagen fiber compared to control groups. The formation of collagen is crucial when it comes to tissue repair, however, if production is exaggerated in pathological situations featuring an excessive inflammatory phase, the limited production of collagen results in a scar with susceptibility to dehiscence with formation of broad, inelastic, slim and fragile scar tissue (Weinzweig, 2001). Collagens are major extracellular matrix proteins comprising approximately 25% of total body

protein mass with a fundamental role in tissue architecture, resistance of the tissues and in a wide variety of cell-cell and cell-matrix interactions (Baynes, Dominickzac, 2000).

According to Tiago (1997), the evolution and maturation of the scar occurs by the deposition of collagen at the wound site. Moreover, to bring this about, maturation is required for neovascularization of tissue oxygenation (Greca *et al.*, 2000), as anoxia leads to the synthesis of unstable collagen and formation of fibers with less mechanical strength. Microscopic analysis of lesions in the experimental group showed intense fibroplasia on the 5<sup>th</sup> postoperative day. However, the large increase in fibroplasia did not occur simultaneously with increased neovascularization, which implies an inadequate process of tissue repair. Adzick (1999) suggested that the formation of new vessels simultaneously with the fibroplasia is essential for healing since the growth of new blood vessels have to follow the progress of fibroblasts into the wound to provide their metabolic needs. If failures occur in angiogenesis, migration of fibroblasts and the healing of the lesion are impaired.

The results of this study, considering the various parameters, showed gradual and continuous evolution of the phases of tissue repair in animals treated with the extract of *S. oleraceus* and the Dersani commercial product (positive control). It was found that the extract acted more promisingly in the repair of injuries since, provided adequate conditions for the restoration of the lesions, it accelerated external repair, followed by gradual repair procedure with the formation of thick collagen fibers. In contrast, the Dersani provided slower external repair concomitantly with internal repair and gradual formation of thinner collagen fibers. The healing effect of hydroalcoholic *S. oleraceus* may be strongly attributed to the strong presence of tannins and flavonoids, since the participation of these compounds have proven action in the healing process, as well as possessing anti-inflammatory, analgesic, wound healing, antimicrobial and antioxidant properties (Cowan, 1999; Carvalho *et al.*, 2003).

## CONCLUSIONS

The similarity of the extract of *S. oleraceus* at 10% in terms of healing, with the commercial product Dersani, which has a proven effect, confirms the importance of studies related to the effects of medicinal plants. The progression of lesions is critical in the process of repair by secondary intention. Thus, we suggest carrying out further studies to examine the therapeutic effect of *S. oleraceus* with other concentrations and formulations such as ointments, gels and creams, as well as toxicity tests

detrimental to the organism, because there is a strong relationship between the concentration of an extract and its activity coefficient in the vehicle (Lachman *et al.*, 2001). Thus, the use of *S. oleraceus* for skin lesions in humans remains an open field of study and its therapeutic actions are still being explored in our laboratories.

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