

Effect of *Opuntia ficus-indica* (L.) Mill. Cladodes in the Wound-Healing Process[♦]

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ABSTRACT

Opuntia ficus-indica (L.) Mill. cladodes contain a polysaccharide fraction that can retain water. The cladodes are used in Sicilian folk medicine as cicatrizant. We studied the healing activity of a base cream containing 15% lyophilized cladodes and of a commercial ointment on the wound produced on the backs of rats. The treatment was carried out for 3 and 5 days.

After three days of treatment, the scar tissue is evident, both fibers and fibroblasts in the derma are properly arranged. The dermal vessels are reduced in lumen and the keratinocytes show proliferation areas. In the 5 days' treatment, the wound healing process is almost completed and the piliferous bulbs are recovering. Complete epithelization occurs. Evidently, the *O. ficus-indica* treatment accelerates wound healing, probably by involving the proliferation and migration of the keratinocytes in the healing process.

Keywords: *Opuntia ficus-indica*, cicatrizant activity, soluble fibres, polysaccharides, traditional use, waste matter.

1. INTRODUCTION

Cladodes of *Opuntia ficus-indica* (Cactaceae), a plant widespread in dry regions of the world, show interesting biological properties and are widely used in traditional medicine (Barbera and Inglese, 1993; Cruse, 1973; Meyer and Mc Laughlin, 1981; Camacho-Ibanez et al., 1983; Frati et al., 1990; Hegwood, 1990; Pimenta, 1990; Fernandez et al., 1992; Fernandez et al., 1994).

Cladodes are particularly rich in soluble fibres (Kurasawa et al., 1992; Sanchez-Castillo et al., 1995; Burkitt et al., 1985) and, moreover, contain mineral substances: vitamins and flavonoids (Rosado and Diaz, 1995; Abramovitch et al., 1968; Burrett et al., 1982; Endo et al., 1987; El-Moghazy et al., 1984; Pinto and Avecedo, 1983; Salt et al., 1987; Sawaya et al., 1983; Teles et al., 1994).

In Sicilian folk medicine, they are used for healing wounds (Cacioppo, 1991) In order to give this a scientific basis, we have studied in the rat the healing effect of an ointment containing 15% lyophilized cladodes originating from the waste of *O. ficus-indica* cultivation.

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2. MATERIALS AND METHODS

2.1. Plant Material

Opuntia ficus-indica (L.) Mill. cladodes were obtained from a cultivation located in a commercial orchard at S. Cono (CT- Sicily). The cladodes, deprived from epidermis and glochids, were homogenized in Ultra-Turrax for 5 min and lyophilized at once. The yield was 1%.

The lyophilized cladodes (15%) were added in a base cream (Egeria natural s.a.s.).

2.2 Animal

Male Wistar rats, weighing 180 g to 200 g, kept in standardized conditions (temperature $22 \pm 2^\circ\text{C}$; humidity $60 \pm 4\%$; natural light), maintained on a standard diet (S. Morini Mill rat GLP), and water *ad libitum*, were used.

The experimental procedures were carried out in accordance with the internationally accepted principles and the national laws concerning care and use of laboratory animals.

2.3. Treatment

The rats were depilated on the back and were divided into five groups of five animals each. The skin was washed with sterile physiological solution and, by a sterile lancet, a standard wound (14 x 3 x 2 mm) was produced on the back of the rats.

The wounds of the Group I animals (controls) did not undergo any treatment. The wounds of Group II rats were treated with 0.60 g of base cream. The wounds of Group III rats, were dressed with a commercial preparation (0.60 g) containing sodic salt of hyaluronic acid (Connettivina[®] crema 0.2%, Fidia Farmaceutici S.p.A., Abano Terme, Padova). The wounds of Group IV animals were dressed with a cream containing 15% *Opuntia ficus-indica* lyophilized cladodes (0.60 g/rat).

All the treatments were carried out twice a day (9 a.m. and 5 p.m.) for 3 and 5 days. During the treatment, the wounds were observed daily and measured. At the third and the fifth day of treatment the animals were sacrificed under ether anaesthesia and biopsies were performed using a biopsy punch (Stiefel \varnothing 8 mm).

2.4. Histology

The skin samples were fixed in a solution of paraformaldehyde 4% (Immunofix[®], Bioptica, Milano) in phosphate buffer 0.06 M for 5 h at 4°C . The samples were washed, twice, for one hour with phosphate buffer 0.2 M and dehydrated in graded ethanol (30° to 100°) and, finally, embedded in paraplant.

Sections (5μ), obtained by a rotative microtome, were observed and photographed using an optical microscope (Axiophot, Zeiss, Germany).

Polymorphonucleate cells (PMN_s) in dermal aggregates were not considered lesions.

3. RESULTS

3.1. Macroscopic Observation

On the 3rd day of treatment the wounds of the nontreated rats appear reddened and infected, with thickened and irregular margins.

The lesions treated with a commercial preparation are clear, the length reduced and the width remained unaltered. Cream containing 15% lyophilized cladodes made the wounds dry, clean, with distinct margins and with reduced length.

On the 5th day of treatment the nontreated rat lesions presented clear sign of suppuration.

With the commercial preparation, the wounds still present open margins all along, whereas the cream containing 15% lyophilized cladodes resulted in complete healing.

3.2. Microscopic Observation

Three days after the incision, the skin of the animals of Group I (nontreated wounds) and Group III (treated with base cream) presents a marked disorganization of the derma. Indeed, in the deep layers a vascular network can be perceived characterized by dilated veins while no piliferous formations are detectable in the overlying layers (Figure 1). At the same time, the skin of the animals of Group III (treated with a commercial preparation) present a disorganized fibrotic mass, widely dilated veins in the vascular network, no epidermal or piliferous production, and a considerable number of inflammatory cells (Figure 2).

After treatment with lyophilized *Opuntia ficus-indica* cladodes, the lesion is clearly healed. In this area, the cells responsible for the inflammatory response in an amorphous matrix can still be found. Under the scar, a few layers of keratinocytes, migrating from the surrounding regions, are evident (Figure 3).

Apart from the infiltrating lymphocytes in the derma, there are a large number of fibroblasts. The vascular network shows moderately dilated veins in the deep layers together with a vascular neoformation (Figure 4).

After 5 days' treatment the skin of the animals of Group I (nontreated wound) and Group II (treated with base cream) presents a fibrous mass with a large amount of macrophages in correspondence to the scar (Figure 5).

Treatment with a commercial preparation (Group III) resulted in an evident proliferative organization in the dermic region. The cicatrization shows margins that scale off from the superficial skin layers and are full of numerous infiltrating elements, responsible for the inflammatory response. In the deep layers of the derma, in proximity to the cicatrizing area, a rapid reorganization of the piliferous bulb can be observed. Numerous dilated veins, and others that are forming, can be detected in the deep layers (Figure 6). Almost uniformly distributed lymphocytes can be found all through the derma. The healing processes are mostly dermal, while re-epitheliazation is ascribable to a thin layer of flattened keratinocytes (Figure 7).

On the 5th day of treatment with lyophilized cladodes, healing is at an advanced stage. The skin of healed rats has a normal organization of all layers. In the deep strata of the derma, piliferous bulbs are present together with a normal disposition of the veins and connective bands (Figure 8). The derma thus shows a normal vascular as well as fibrous expression. All the typical layers from the basal to the corneal can be observed in the epidermis. The scar appears as a crust mass that comes off in the healed area. Around the borders of the lesion, there is an area of epidermal proliferation made up of a few layers with clear signs of the cytomorphous process: in this area, all the keratinocytes tend to form a compact corneal stratum with cells having a highly-flattened nucleus organized in several layers. There is a considerable reduction in the number of infiltrating elements and neo-formation of veins in the vascular network (Figure 9).

4. DISCUSSION AND CONCLUSION

As the results of our experiments show, it is evident that in animals with nontreated wounds, the cutaneous layers show typical characteristics of the healed scars. Indeed, veins with a dilated lumen and the presence of inflammatory cells are very evident; in the superficial area underneath the scar no keratinocytes can be detected.

In samples treated with the commercial preparation, the veins are wide with a good organization of the collagen fibres. A thin layer of migrated keratinocytes can be seen underneath the scar. In the more advanced stages of healing (5 days' treatment) a reorganization of piliferous bulbs occurs.

Treatment with the preparation containing the lyophilized *Opuntia ficus-indica* cladodes produces a better and more organized tissue reconstruction due to an improved organization of the dermal constituents. Indeed, from the first stage of the healing process, an ordered disposition of the collagen fibres can be seen, as well as the vascular neo-formations, which are characterized by the presence of smaller, and more numerous dermal veins than those in the control animals (Brown et al., 1992; Norris et al., 1982).

In the more advanced stages of the healing process, there is a decrease in the number of inflammatory cells with respect to the control animals. The role of these cells is fundamental in the first stages of tissue reparation because they stimulate the synthesis of cytokines such as TGF- α , β (Rappolee et al., 1988; Sporn et al., 1987) and EGF (Igarashi et al., 1991) that are the more important molecules among those involved in this process. Immediately after macrophage invasion, a significative migration of fibroblasts takes place, they synthesise the molecules responsible for the formation of the extracellular matrix (fibronectin, collagen and glycosaminoglicans) (Pierce et al., 1994). The skin preparations examined show an organization of the collagen fibres analogous to that in the normal skin. Treatment with *Opuntia ficus-indica* cladodes influences the remodelling phase of the macrophages that reabsorb the excess of connective tissue with a scavenging action on the cellular debris.

Moreover, the epidermal layers are more numerous and tend toward cytomorphosis, so that the process of re-epitheliazation is surely in a more advanced stage with respect to wounds treated with the commercial preparation. This is supported by a good reorganization of the derma; in fact, it is well known in literature that arrangement of epidermal layers is backed up by the component of the extracellular matrix.

Furthermore, in the skin of animals treated with *Opuntia ficus-indica* cladodes, it is evident that the normalization of the derma favours the reorganization of the piliferous bulbs that are known to accelerate the process of skin re-epitheliazation in the rat. Finally, *Opuntia ficus-indica* cladodes treatment decreases the inflammatory cells, stimulates the migration of fibroblast with the consequent enhancement of collagen formation, stimulates angiogenesis and skin and piliferous bulb organization.

Opuntia ficus-indica cladodes contain a polysaccharide fraction (Karawya et al., 1980), and various reports show that polysaccharides from different plants can be responsible for the effects associated with the healing of wounds. Some polysaccharides have an influence on the immune system and the polysaccharides, all coming from plants used for wound healing in folk medicine, can influence the complement system (Tomoda et al., 1981; Yamada et al., 1991 a,b,c). In a previous work, we reported the antiinflammatory activity of *Opuntia ficus-indica* cladodes (Galati et al., 2000), and a correlation between the two effects could be possible. Besides the angiogenesis is an essential process in wound healing (Brown, et al., 1992; Norris, et al., 1982), and it is known that some angiogenic activators have been shown to promote wound healing. These activators could be a low-molecular-weight component of cladodes (Lee et al., 1998) as monosaccharide residues, polyphenols or β -sitosterol, that is the predominant sterol in Cactaceae (Salt et al., 1987).

The present study demonstrates that the treatment accelerates wound healing. Probably, *Opuntia ficus-indica* cladode components are capable of maintaining high humidity of the wound-dressing interface, but perhaps they also could influence inflammation, fibroplasias, and collagen synthesis.

5. REFERENCES

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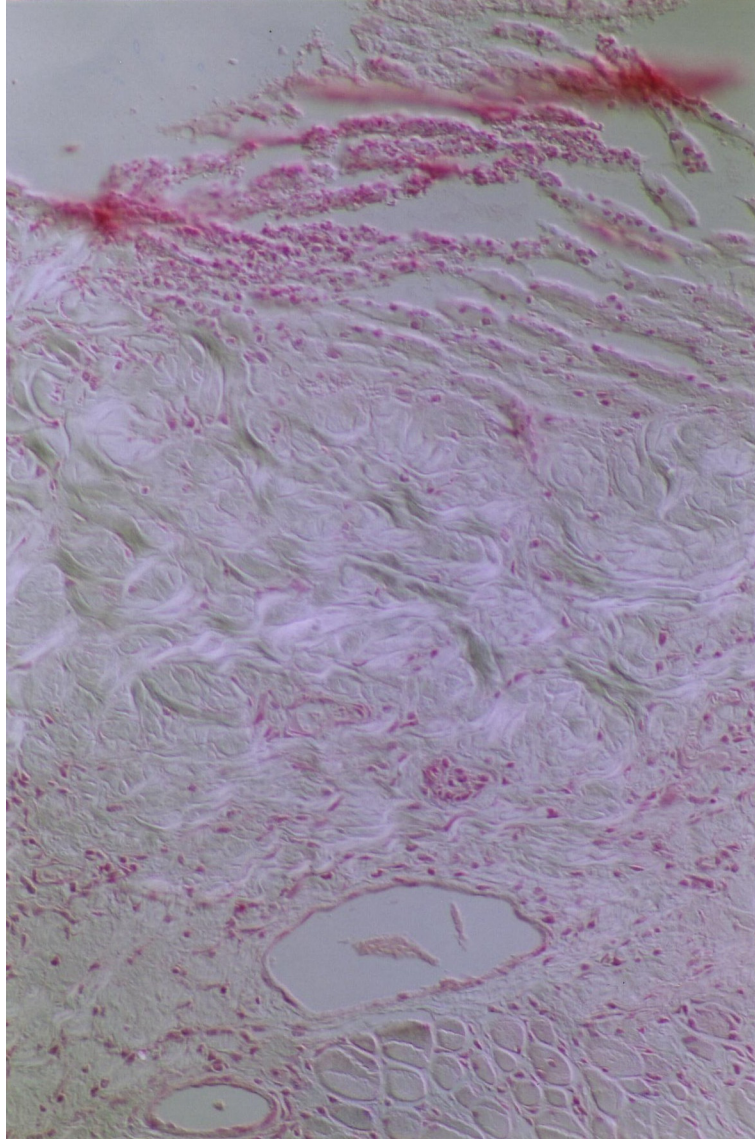


Figure 1. Skin of the back of the nontreated rats and treated with base cream (3rd day). The wound appears, with a marked disorganization of the derma. (Magnification 20X)

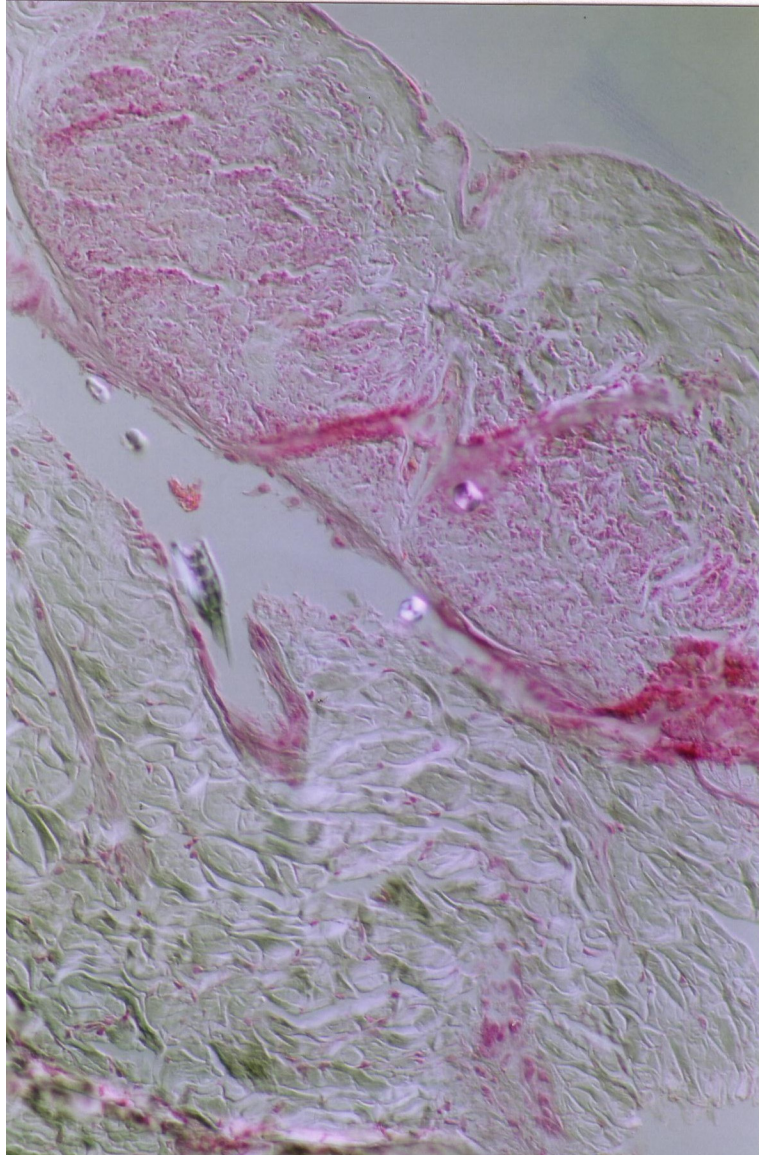


Figure 2. Skin of the back of the rats treated with a commercial preparation (Connettivina®) (3rd day). In the lesioned area, the formation of the scar can be detected. (Magnification 20X)

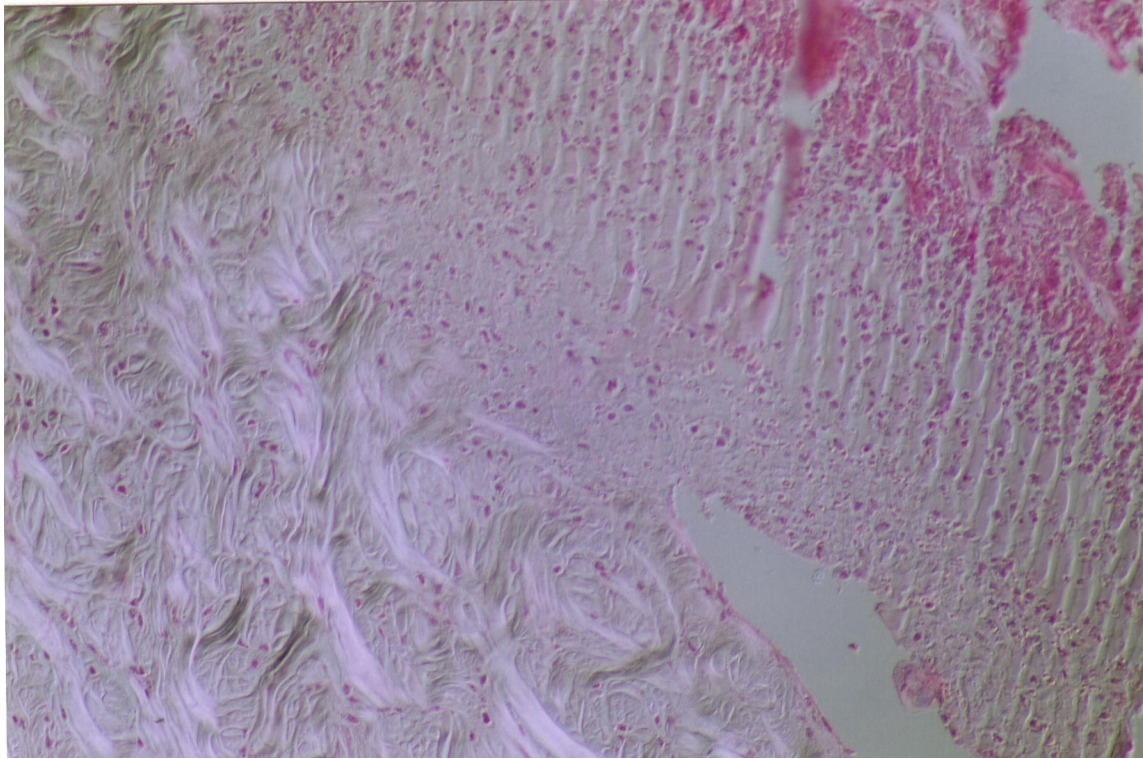


Figure 3. Skin of the back of the rats treated with *O. ficus-indica* lyophilized cladodes (3rd day). Under the scar, there is a layer of flattened keratinocytes. (Magnification 20X)

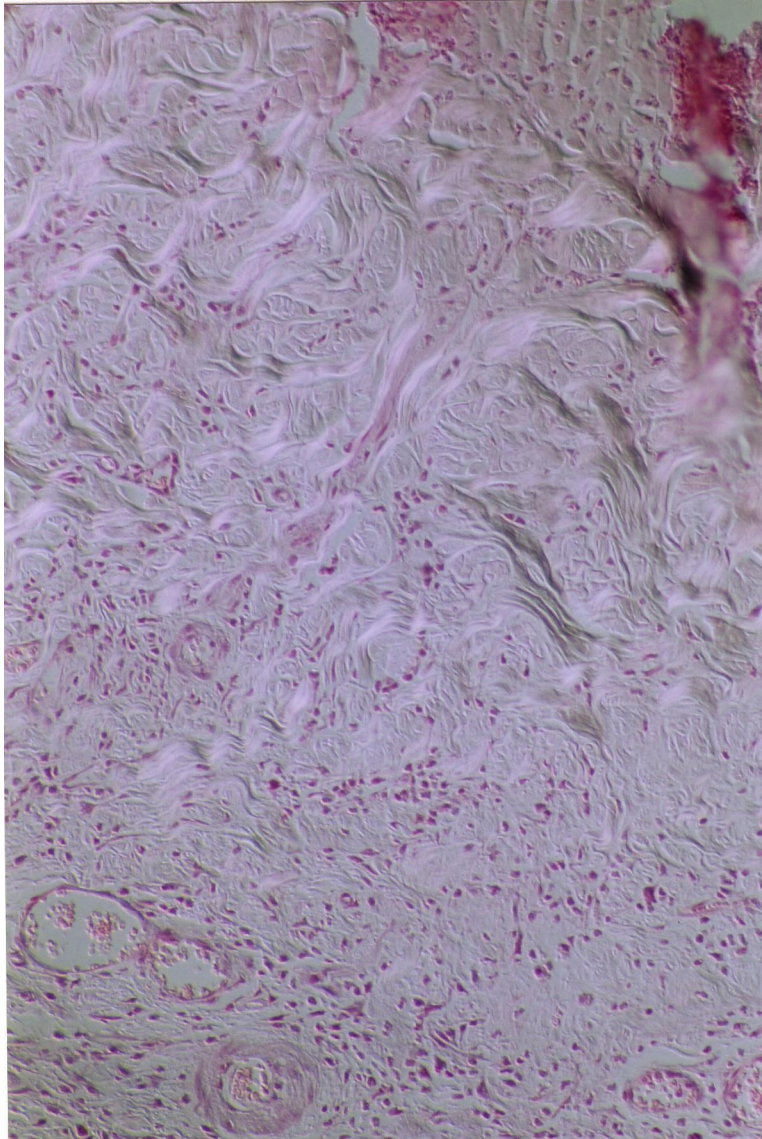


Figure 4. Skin of the back of the rats treated with *O. ficus-indica* lyophilized cladodes (3rd day). In the dermic region the veins appear moderately dilated versus control animals, and there are vascular neoformations and infiltrating elements responsible for the inflammatory response. (Magnification 20X)

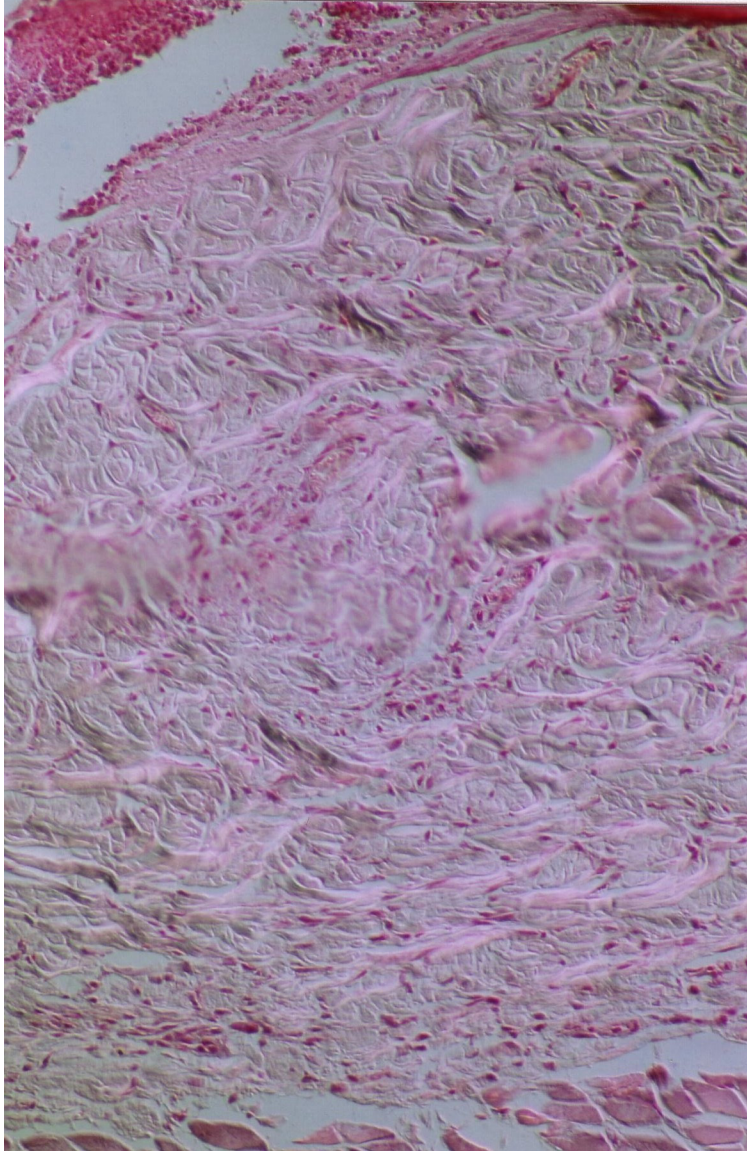


Figure 5. Skin of the back of the nontreated and treated with base cream (5th day). The wound presents a marked disorganization of the derma. In the dermic region there are inflammatory cells. (Magnification 20X)

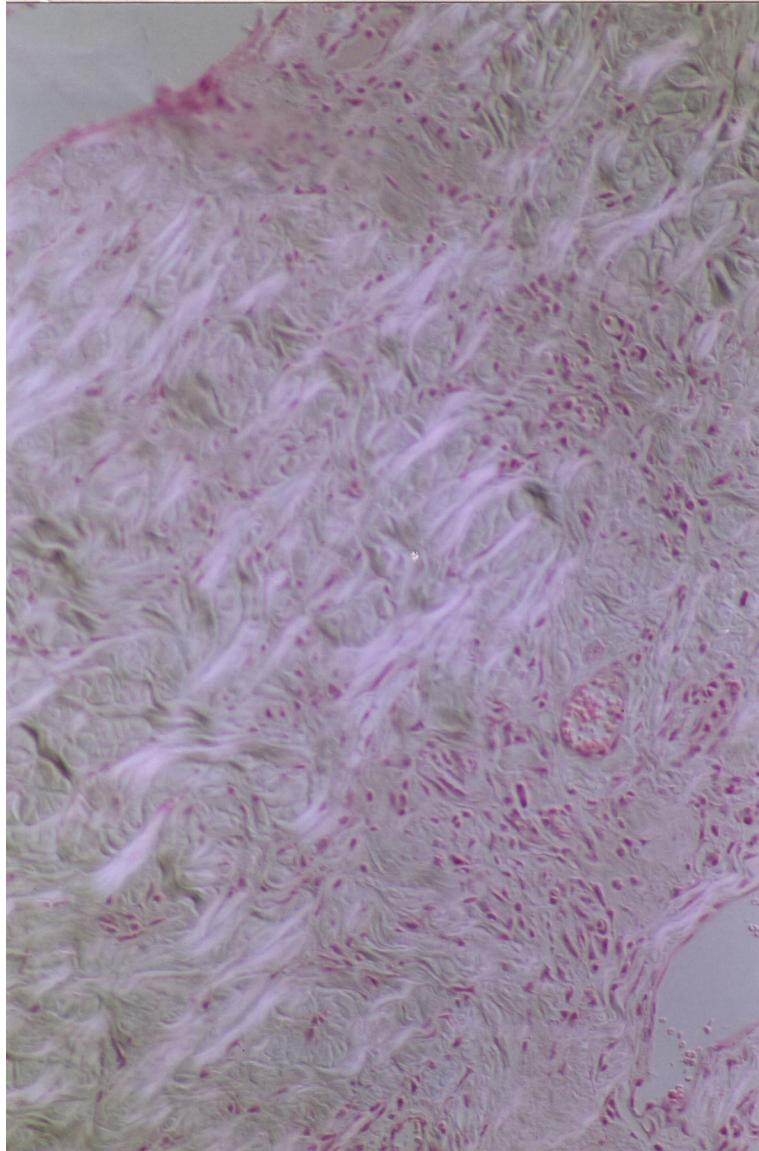


Figure 6. Skin of the back of the rats treated with a commercial preparation (5th day).
The veins show a widely dilated lumen. It is possible to see
some vascular neoforations. (Magnification 20X)

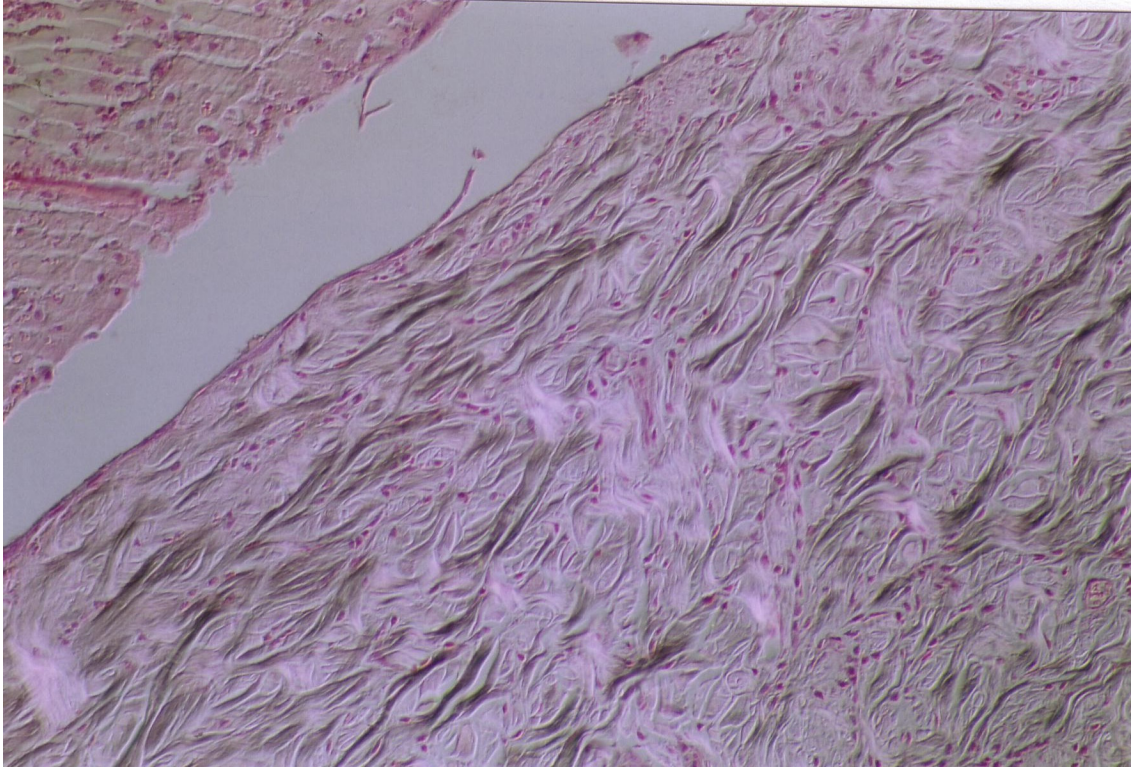


Figure 7. Skin of the back of the rats treated with a commercial preparation (Connettivina®) (5th day). Under the scar there is a continuous layer of keratinocytes. (Magnification 20X)

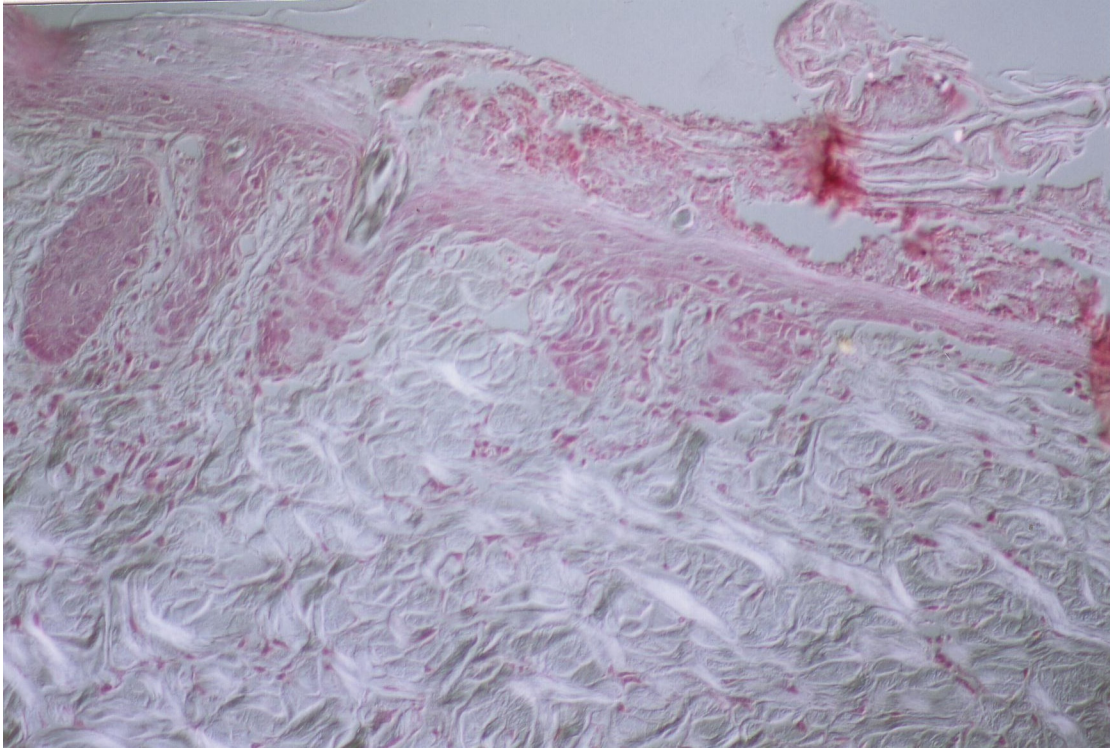


Figure 8. Skin of the back of the rats treated with *O. ficus-indica* lyophilized cladodes (5th day). Under the scar, the reorganization of the piliferous bulbs is detectable. (Magnification 20X)

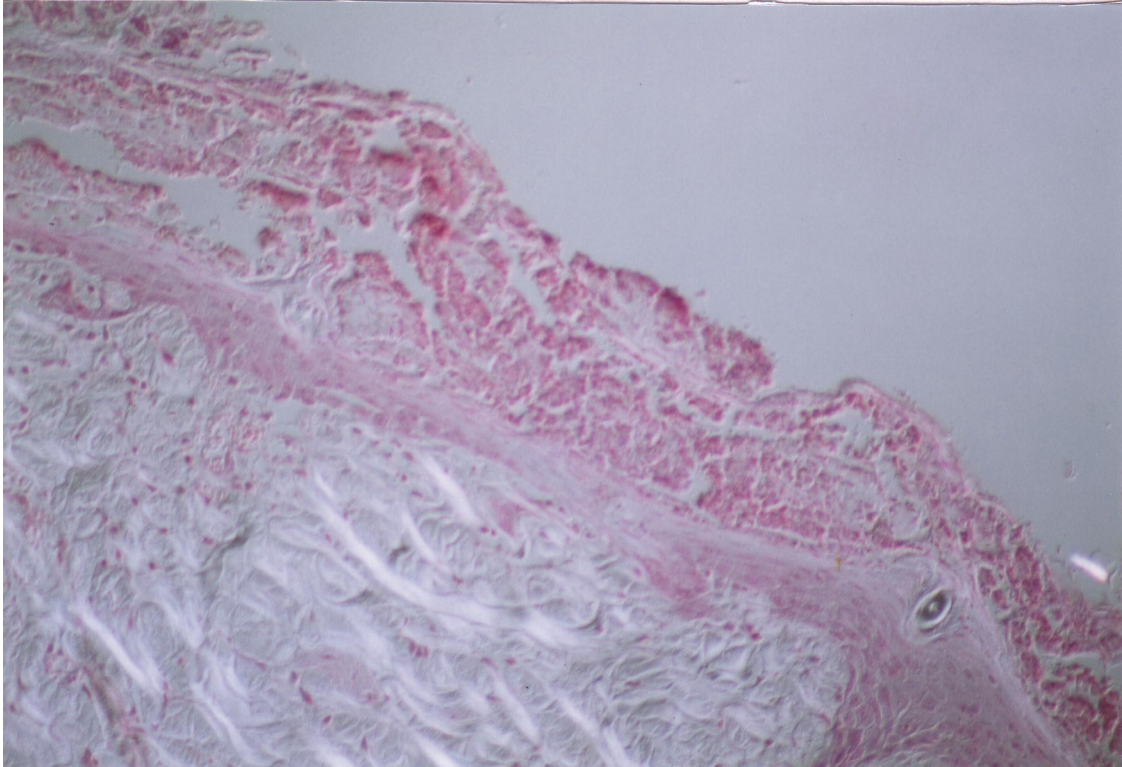


Figure 9. Skin of the back of the rats treated with *O. ficus-indica* lyophilized cladodes (5th day). The veins appear with a reduced lumen. There are vascular neoformations. (Magnification 20X)