Histological evaluation of the efficacy of Centella asiatica and bisabolol ointment in thermal injuries

Evaluarea histologică a eficacității unguentului cu Centella asiatica și bisabolol în leziunile termice

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Abstract

Centella asiatica is widely used for its antimicrobial, antioxidant, anti-inflammatory, neuroprotective and wound-healing properties. Bisabolol is an unsaturated sesquiterpene found in increased amounts in chamomile and clary sage oil known for its multiple skin-repairing and soothing virtues. The aim of the present research was to histologically evaluate the efficacy of ointment with Centella asiatica extract and bisabolol. 14 mice from the balb/c line were included in the study, which were divided into 2 groups (experimental, control) and each animal included in the study was clipped before applying the mechanical burn. The duration of the experiment was 14 days with collections of biological material at 3.7 and 14 days. Following the study, we found that, in thermal burns, the use of ointment in which Centella asiatica 2% and bisabolol 1% were incorporated speeds up the skin healing process, compared to the untreated injury, and prevents bacterial superinfections from setting in.

1. Introduction

The structure of the epidermis creates a formidable barrier against harmful substances. Its complex architecture is a challenge that must be overcome in order to allow percutaneous drug penetration. A new approach is the incorporation into topical dermatological formulations of compounds that improve the transdermal permeability of drugs.

Centella asiatica, also known as Gotu Kola, Bua-bok, Tiger grass, is a perennial herbaceous plant member of the Apiaceae family, with an important traditional value, especially in Southeast Asia, due to its nutritional and therapeutic properties [1, 2, 4].

Centella asiatica is widely used due to its antimicrobial, antioxidant, anti-inflammatory, neuroprotective and wound healing properties [3, 6, 9].

Triterpenic compounds: asiatic acid, madecassic acid, asiaticoside and madecassoside are the main components of Centella asiatica plant responsible for wound healing. This action has been demonstrated for both extracts and triterpene compounds in a large number of scientific research involving in vitro studies [10, 11].
Studies in rats have shown that topically applied *Centella asiatica* extract leads to increased levels of enzymatic and non-enzymatic antioxidants in newly created tissues [20].

There are also authors who have confirmed the influence of the triterpenes in *Centella asiatica* on increasing the metabolism of lysine and proline, the amino acids that build the collagen molecule. In addition, these compounds increase the synthesis of tropocollagen and mucopolysaccharide in connective tissues [26].

Experimental studies in various animal wound healing models, including phthalic anhydride-induced atopic dermatitis, burn, excision, and incision, have shown that topical and oral administration of alcoholic extract of *Centella asiatica* reduces massive neutrophil recruitment accompanied by reduced the release of TNF-α, IL-1β, IL-6 and IgE, and the inhibition of the expression of COX-2, NF-κB and activated lipoxygenase (LOX) activity at the level of the skin lesion [7, 13].

*Bisabolol* is an unsaturated sesquiterpene found in increased amounts in chamomile and clary sage oil. In addition to the dermatological benefits, it also has numerous pharmacological properties such as analgesic, antioxidant and antitumor. It is a compound for which, in studies carried out on laboratory animals, dermal toxicity was not observed. Used together with propylene glycol, they synergistically greatly improve skin penetration [5].

## 2. Materials and methods

The ointment with *Centella asiatica* extract 2% and *bisabolol* 1% was prepared within the Pharmacology and Pharmacy discipline of the Faculty of Veterinary Medicine Timișoara. The ointment was prepared according to the ointment preparation technique from FR 10th Edition. The base used by Ultrabasic Creme Ratiopharm GmbH.

### 2.1. The experimental protocol

In total, 14 mice from the balb/c line were included in the experiment, which were divided into 2 groups (experimental, control).

Mice were housed individually, in standard 40X20X30 cm cages in the Toxicology and Pharmacology laboratory, with constant temperature conditions.

Each animal included in the study was clipped before the mechanical burn was applied. The burn was induced by applying a hot round-headed iron rod of known diameter to an open flame for 30 seconds to keep the temperature constant.

Under the effect of narcosis (isoflurane) the hot iron rod was applied for 5 seconds on the skin of the animals, in the lumbar area, according to the data described in the specialized literature.

Immediately after induction of the mechanical burn, the tested ointment was applied.

The duration of the experiment was 14 days, with collections of biological material (skin) on day 3, 7 and 14 of the experiment.

For the euthanasia of the animals, the association between Ketamine (50-100mg/kgc) and Xylazine (2-8mg/kgc im) was chosen. Daily measurements were taken with an electronic caliper for each individual mouse. The ointment was applied of 2xd, in a uniform amount for each mouse, using a standard double-ended spatula.

### 2.2. The histological study

The skin fragments were fixed in ethyl alcohol 80° for 7 days, after which they were washed, dehydrated and embedded in paraffin. The sections thus prepared were processed for the histological study by the usual Hematoxylin – Eosin method. Histological images were captured using Olympus CX41 microscope software.

## 3. Results and discussion

According to data from the specialized literature, burns produced by thermal agents (hot liquids, hot solid objects, flames, etc.) on the skin can also be classified from a histological point of view, depending on the depth of the affected component tissues.
Thus, first degree or superficial burns affect only the epidermis, the affected area being congested, dry and painful, but which can heal on its own within five days without the formation of scars.

If the alterative process extends to the level of the dermis, we are dealing with second degree or partially deep burns. They can present a mild form, when the epidermis and half of the structure of the dermis are involved. The area is painful, blisters appear, but the corneous productions (hairs) are intact. Often, this type of injury heals in one to three weeks without surgery. The severe form involves the destruction of the dermis in depth, the area is discolored, devoid of hair and is accompanied by the formation of scar connective tissue with partial loss of functions.

Third-degree or deep burns involve the dermis entirely and often require surgical intervention using skin grafts.

In the present study, the action of the ointment whose base included *Centella asiatica* extract (2%) and *bisabolol* 1% was monitored. The microscopic examination of the skin of the control group was taken as a comparative benchmark for our investigations, highlighting the classic structure and appearance, with the component tissue types, which assembled, form the epidermis, dermis and hypodermis (Fig. 1).

![Figure 2](image_url)

**Figure 2.** Histological section performed through the skin harvested from the control group with thermal injury absence of epidermis (→), thermal coagulation of collagen fibers in the dermis (→), glandular structural alterations (→) col. HE, 100X

After three days of treatment using the test ointment that had *Centella asiatica* and *bisabolol* incorporated, the proliferation of keratinocytes can be seen in the marginal area, expressed by the increase in the thickness of the epidermis. Epithelial cells of the epidermis have migrated to the affected area, being present in a newly formed layer, under the crust (Fig. 3).
Figure 3. Histological section performed through the skin harvested from the treated group with ointment, after 3 days: proliferation of keratinocytes (→), col. HE, 40X

Figure 4. Histological section performed through the skin harvested from the group treated with ointment, after 3 days: inflammatory infiltrate (→), col. HE, 100X

In the area destroyed by the thermal agent, following the application of the test ointment, the microscopic examination revealed the presence of a rich inflammatory infiltrate, forming a real cell layer, arranged in the dermis, partially replacing the collagen fibers (Fig. 4).

By measuring the contraction of the wound, after three days of treatment and without, we found that, in the case of applying the ointment with *Centella asiatica* and *bisabolol*, the diameter was reduced from 14.75 mm to 11.14 mm, and for the untreated skin, the diameter was reduced by at 14.75 by 10.21 mm. Microscopic examination after seven days of treatment showed that the epithelial cells in the epidermis of the area adjacent to the lesion had migrated to the center. The tissue remodeled area revealed the proliferation of epithelial cells, expressed by the thickening of the newly formed epidermis and the formation of new hair roots (Fig. 5).

Wound diameter was reduced to 9.69 mm for treated skin and 9.32 mm for untreated skin, with no significant differences between the two measurements. The microscopic examination performed at 14 days of treatment revealed the extension, through migration and proliferation of epithelial cells, of the epidermis towards the center of the affected area, its diameter reaching 7.64 mm (Fig. 6). Inflammatory cells retreated subcutaneously, under the hypodermis (Fig. 7).

Figure 5. Histological section performed through the skin harvested from the group treated with ointment, after 7 days: proliferation of epithelial cells (→), col. HE, 40X

Figure 6. Histological section performed through the skin harvested from the group treated with ointment, after 7 days: expansion, by migration and proliferation of epithelial cells (→), col. HE, 40X

Figure 7. Histological section performed through the skin harvested from the group treated with ointment, after 7 days: withdrawal of the inflammatory infiltrate (→), col. HE, 200X
The wound healing process is a biological, tissue-regenerating process that involves a complex cascade of events that are divided into three unique main phases that include inflammation, proliferation, and maturation. In addition, the wound healing mechanism involves complex interactions between different cell types, extracellular matrix components, and cytokine mediators [1].

Aqueous, ethanolic and methanolic extracts of Centella asiatica not only increase the viability of skin cells, but significantly affect their migratory properties, which gives this plant powerful regenerative properties that are used in wound healing processes.

Conclusions

In thermal burns, the use of ointment in which Centella asiatica 2% and bisabolol 1% have been incorporated accelerates the healing process of the skin, compared to the untreated lesion. Although the reduction in the diameter of the wounds resulting from the application of the thermal agent was faster in the case of the untreated lesion, due to the massive presence of inflammatory cells, the tissue remodeling, with the recovery of the architectonics, was more evident in the case of the use of the test ointment.

The use of ointment with Centella asiatica and bisabolol prevented the establishment of bacterial superinfections, which, once established, can complicate and prolong the healing process of this type of acute injury.

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References


