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The action of ointment with extracts of *Centella asiatica* and *Croton lechleri* on the integument in severe thermal burns

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Abstract: The health of the skin, both in humans and in animals, has been and continues to be an ongoing concern for the medical and pharmaceutical fields. Accidental or inflicted, wounds are represented by cuts, lacerations, burns, tears or other injuries, and for them to heal without complications, certain trauma management protocols must be followed. In the present study, the action of the ointment in which were incorporated two extracts obtained from *Centella asiatica* and from *Croton lechleri* was followed on the integument of laboratory mice. The thermal burn was treated for 7 days by twice daily application of the ointment and to assess its action samples were collected from the integument on day 0, day three and day seven for histological investigations. Because the use of the respective ointment in burns requires additional care, future investigations will be conducted to elucidate the action on the skin in the case of this type of trauma.

• Introduction

Skin health, both in humans and animals, has been and continues to be a continuing concern for the medical and pharmaceutical fields. In addition to the knowledge and understanding of the mechanisms regarding the homeostasis of the best represented organ of the body, with multiple functions (of which the sensory and barrier functions are the most well-known), over time, solutions and remedies have been sought regarding the prevention the installation of conditions at this level, which are as numerous as the causes that can produce them. The reasons why skin care is vital are plentiful, starting with the fact that, first and foremost, it is a barrier against external threats such as pathogenic bacteria, allergens and trauma. At the same time, the skin can be an indicator of internal threats regarding the proper functioning of the body, such as allergies or nutritional and/or drug intolerances, various diseases.

• Material and method

The present study was performed on a total of 12 Balb/c mice, which were separated into two groups: the experimental and the control. The duration of the experiment was 7 days, with collections skin samples on days 0, 3, and 7 of the experiment. Mice were housed singly in standard plastic cages in the Toxicology/Pharmacology/Biobase laboratory from Faculty of Veterinary Medicine Timișoara, where the temperature conditions (20°C) were constant. Each animal included in the study was clipped before the mechanical burn was applied. The burn was induced by holding a hot round iron over an open flame for 30 seconds to keep the temperature constant. Under the effect of narcosis (isoflurane), the hot iron was applied for 5 seconds to the skin of the animals, in the lumbar area of the back. Immediately after the induction of the mechanical burn, the phytotherapeutic ointment made at the Department of Pharmacology of Faculty of Veterinary Medicine Timișoara was applied, for each individual of the experimental group. The ointment was applied 2 × 1/day, in an equal amount (1g/application), for each mouse. The collection of skin samples was carried out on days 0, 3, and 7, and they were fixed in ethyl alcohol 800 for 7 days, after which they were washed, dehydrated and embedded in paraffin. The prepared sections were processed for the histological study by the standard Hematoxylin – Eosin method. For the euthanasia of the animals, the combination of Ketamine (50-100 mg/kg) and Xylazine (2-8mg/kg IM) was used.

• Results and discussions

From a structural point of view, mammalian skin has a similar architecture, even if there are differences (especially in thickness) between species, within the same species or between different regions of the body (Fig 1). Microscopically, after the application of the thermal aggressor, at the level of the impact zone, the installation of alternative changes was observed, including the epidermis and the dermis (Fig. 2). Thus, the epidermis is absent almost entirely, the area being covered by a thin, detached crust due to reduced adhesion. Following coagulation, the collagen fibers in the dermis formed a homogeneous mass. Sebaceous glands and hairs have undergone necrosis phenomena. In the deep area, vascular phenomena can also be noted, expressed by congestion. In the adjacent area, where the thermal aggressor propagates, the spaces that separate the epidermis from the dermis appeared, a sign that indicates the destruction of the junctional complex between the two structures. The burn, as an acute pathological entity, entails a dynamic healing process, which, like any other reparative process, involves the passage of four phases, which can evolve distinctly, gradually or overlapping, respectively: the hemostasis phase, the inflammatory phase, the of proliferation and tissue remodeling phase. In the impact area, three days after the application of the thermal aggressor, without being followed by the institution of any treatment, microscopically, the absence of the epidermis was found, which signifies the absence of the initiation of epithelization. The accentuation of the process of coagulation and protein denaturation was revealed by the appearance of a compact mass of collagen fibers, which are not individualized and not anastomosed. In the dermis, localized glandular productions and hairs were completely necrotic. In the deep dermis, adipocytes were the predominant cells, among which inflammatory cell infiltration was observed (Fig.3). At the periphery of the affected area, the inflammatory infiltrate was very obvious. All these identified elements represent moment 0 of the initiation of the healing process. After three days of treating the wound caused by a thermal aggressor with the ointment obtained at the Discipline of Pharmacology, which contained extracts of *Centella asiatica* and *Croton lechleri*, by applying it twice a day, the microscopic examination revealed the reduction of necrotic tissue in the area of impact, under which an extensive layer of adipocytes is found. At the base of this layer, the appearance of immature hair follicles was noted (Fig. 4). The massive presence of adipocytes is a specific element of the third day post-burn, their involvement in the healing process being still unknown. After three days of treatment, the epidermis is absent in the affected area, and a thin, adherent crust is found in its place. The compact mass of collagen fibers is reduced, with inflammatory cellular infiltrates. Structurally, the area peripheral to the aggression showed much more advanced changes in the healing process, with a thickened epidermis, as a result of the proliferation of keratinocytes, which form dermal papillae. Inflammatory cells invaded the entire structure of the skin, the cells being present under the epidermis, among the collagen fibers, among adipocytes, above and below the muscle layer (Fig. 5). 7 days after the action of the aggressor agent on the skin, at the structural level the numerical reduction of adipocytes and the significant increase of the inflammatory infiltrate were evident. In the impact area, a thick crust was present, with relative adhesion, arranged over the inflammatory infiltrate under which rare collagen fibers and sebaceous glands are found, the two structures being newly formed. In the region adjacent to the impact zone, an exaggerated proliferative phenomenon of keratinocytes was found, with thickened epidermis and the appearance of dermal papillae. The dermis shows newly synthesized collagen fibers and keratinocyte proliferations that will form hair follicles and sebaceous glands. The ratio of adipocytes / inflammatory cells is in favor of the second category, and vascular phenomena were observed under the muscle layer, expressed by hyperemia/vasodilatation. After 7 days of application of the ointment with extract of *Centella asiatica* and *Croton lechleri*, the microscopic examination revealed denudation of the impact area, of direct action of the thermal aggressor (Fig. 6). Thus, after this time interval, the lysis of the healing components was found, although the peripheral zoo thermal trauma is in the phases of tissue proliferation and remodeling, revealed by the thickened epidermis, with the formation of dermal papillae, a sign of complete epithelization and by the presence of inflammatory infiltrate. The number of adipocytes is relatively increased compared to that of the untreated group, in this case, the layer formed by them being sensitive to lytic processes. In both cases, the maintenance of environmental hygiene conditions and appropriate food prevented the installation of secondary infections, which would have hindered the healing processes.

Conclusion: Denudation of the impact area will generate the establishment of additional protection methods.

