



## HISTOLOGICAL AND CLINICAL ASSESSMENT OF A BIOACTIVE OINTMENT CONTAINING HONEY, PROPOLIS, POLLEN, AND SEA BUCKTHORN OIL FOR SKIN INJURY REPAIR

Diana Brezovan, Jelena Savici, Maria Patricia Pâinișoară, Mălina Oprescu, Larisa Rusu Ardelean, Iacob Răzvan, Eugenia Dumitrescu

University of Life Sciences, Faculty of Veterinary Medicine, Iacob Vet- Cabinet Veterinar

**Abstract:** The aim of this research was to evaluate histologically and clinically the action of an ointment containing bioactive substances (honey, pollen, propolis, sea buckthorn oil) on different skin lesions, represented by burns and open wounds. Researchers are concerned and continue to invent new ointments, because skin lesions are still a major medical problem, and current treatments do not completely solve infections, slow healing, scars or chronic wounds. For the histological evaluation, an experiment was set up, lasting 14 days, in which thermal burns were induced in balb/c mice and treated with the ointment under investigation. Skin samples were collected on the 3rd, 7th and 14th day and processed for evaluation using the Hematoxylin - Eosin method. For the clinical evaluation, the ointment was used in the clinic on three patients: 2 cats and a dog. For the experimental part, the use of the ointment in the case of severe skin burns proved effective, with the installation of reparative phenomena appearing much earlier compared to the absence of any treatment, and in the case of traumatic wounds, by simply applying the ointment, the results regarding their healing were more than satisfactory.

### • Introduction

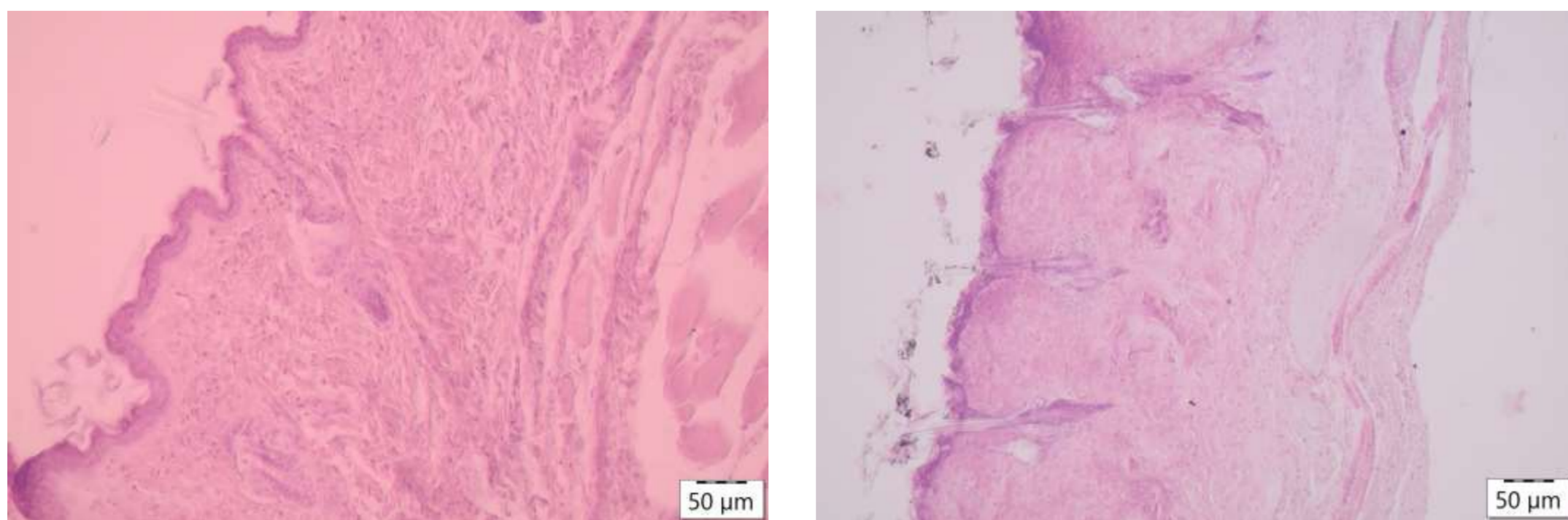
Ointments have long been used in the treatment of skin burns and lesions because they provide a protective barrier over damaged tissue, helping to maintain moisture and support the natural healing process. Their semi-solid consistency allows active ingredients to remain in contact with the affected area for a longer period, enhancing therapeutic effectiveness. In burn and wound management, ointments can reduce water loss, prevent infection, relieve pain, and promote tissue regeneration. Additionally, many formulations contain antimicrobial, anti-inflammatory, or soothing agents that help minimize complications and accelerate recovery. For these reasons, ointments remain an essential component in dermatological and wound-care therapy.

### • Material and method

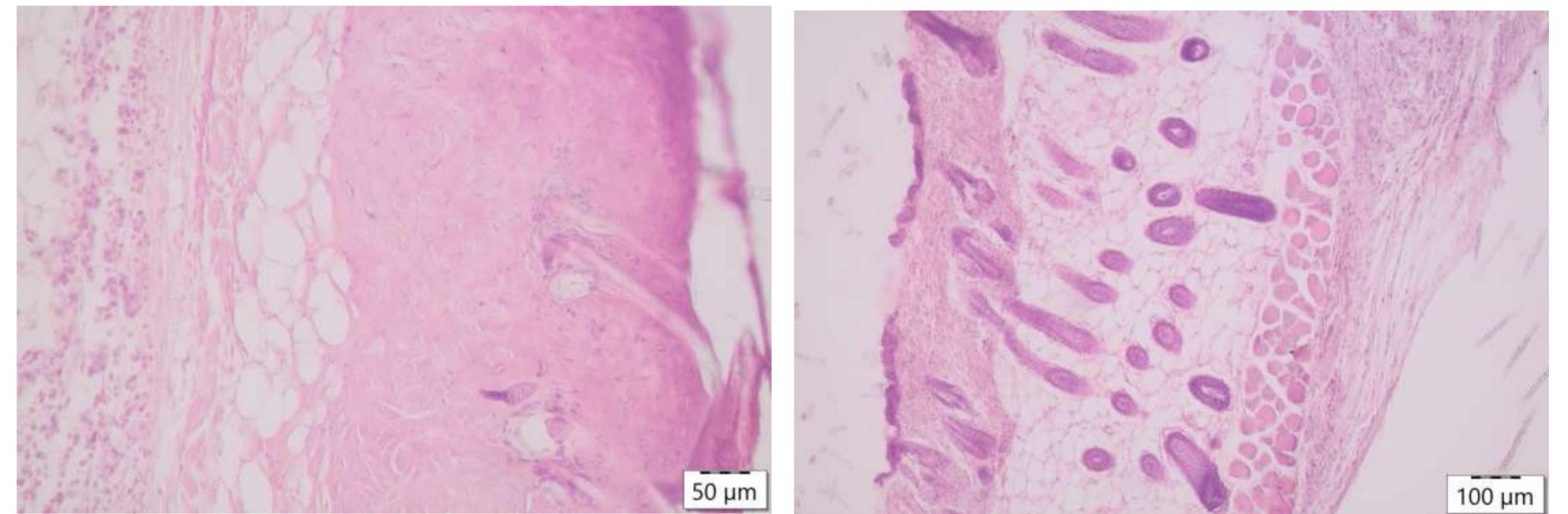
The present study was performed on a total of 12 Balb/c mice, which were separated into two groups: the experimental and the control. The duration of the experiment was 14 days, with collections skin samples on days 0, 3, and 7 and 14 of the experiment. Each animal included in the study was clipped before 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. The skin sections were processed for the histological study by 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. For the clinical evaluation, the ointment was used in the clinic on three patients: 2 cats and a dog.

### • Results and discussions

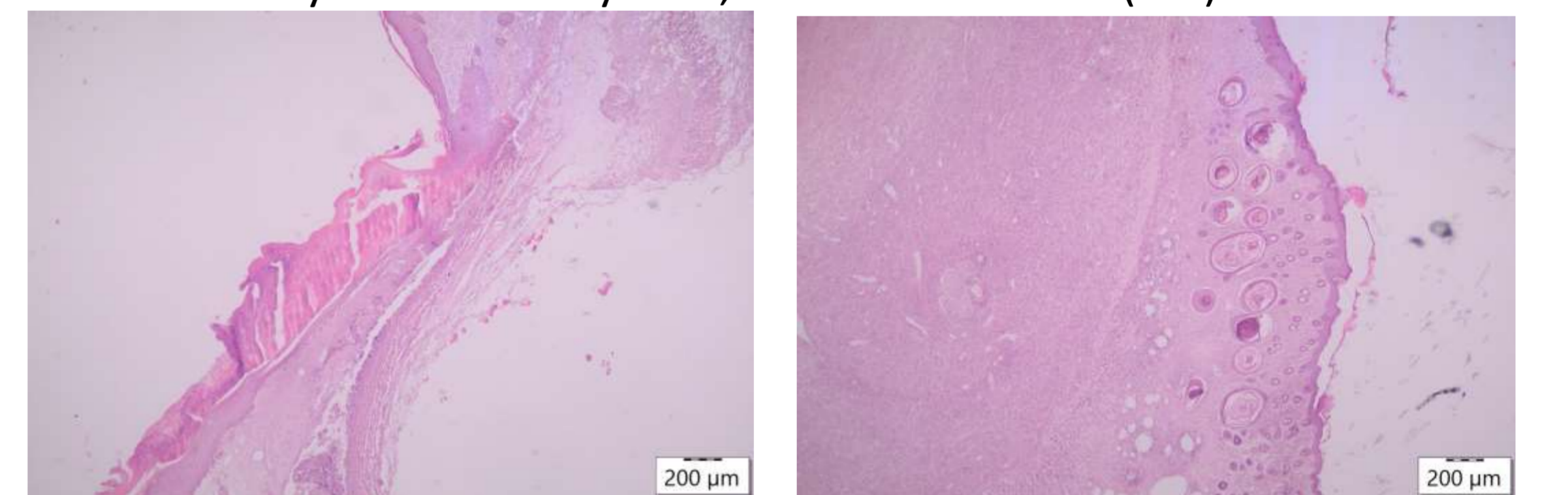
Microscopic examination of the skin in the control group mice revealed the normal appearance of this organ, with the three well-structured layers, namely the thin continuous epidermis, the dermis, in whose connective tissue are found the glandular and corneous structures, and the hypodermis rich in adipocytes (1). Microscopic examination of the skin in mice that immediately suffered a thermal traumatic phenomenon revealed the presence of numerous alterative phenomena specific to this type of condition. Thus, it was possible to observe the loss of the epidermis on extensive surfaces and the weakening of the dermo-epidermal junction, with elongated basal cells, in areas where the epidermis did not definitively lose contact with the dermis. Also, the denaturation by coagulation of collagen molecules is evident, the fibers formed by them being structurally uniform and here too alterative changes are observed at the level of glandular and corneous structures. Regarding the *panniculus carnosus* muscular layer, the reduction of muscle fascicles was observed, with the increase of the spaces between them (2).



After 3 days of daily administration of the ointment, microscopically, it was found, compared to the untreated control, the appearance of new and numerous roots of hair follicles, with the extension of the hypodermis (3-4). After seven days, the untreated skin showed massive infiltrations with inflammatory cells, as a reaction to tissue destruction and as a healing reaction. In the infiltrative area, the destroyed epidermis lost contact with the dermis, with numerous spaces and cracks visible). Numerous inflammatory cells surrounded the corneous and glandular structures, also located between the collagen fibers of the dermis.



After the same period, and after daily application of the ointment, the skin showed detachment of the destroyed epidermis, under which a thin, newly formed epidermis was located. In the dermis, the appearance of spaces of varying sizes, surrounded by an inflammatory infiltrate and new blood vessels. After 14 days, in untreated skin, left to follow its natural healing mechanisms, retraction of the lesional area was observed, where a thin, new epidermis is visible under the crust. The inflammatory infiltrate is still present. In the adjacent areas, the skin has regained its normal structural appearance. After two weeks of application of the ointment, the lesional skin has overcome the alteration process, presenting an apparently normal-looking structure, with numerous hair follicles and neovascularization. In the dermis, the presence of spaces, although smaller in size and reduced in number, surrounded by inflammatory cells, was still observed (5-6).



Case 1. The cat presented with a wound on the right pelvic limb, one week old. After debridement of the open wound, measuring 7 cm x 3 cm, the ointment was applied 4 times a day. At discharge on request, after 10 days of local treatment with the ointment, the wound size was 3 cm x 1 cm.

Case 2. European breed cat with an open wound on the ventral neck (bite trauma). The owners report that 5 days ago there was an altercation between their cat and the neighbor's cat, and as a result of the interaction, their cat was bitten on the neck. After consultation, the diagnosis of an open wound was made. After 5 days of treatment (with local application of the ointment 4 times a day), the wound had reduced considerably.

Case 3. Bichon male. The dog presented with a wound on the side of the neck resulting from a trauma caused by a fence wire. As a therapeutic protocol, it was decided to use the ointment with local application 5 times a day. The initial size of the wound was 3.5 cm x 1.5 cm. On the 6th day of treatment, the wound closed.



### • Conclusions

For the experimental part, the use of the ointment in the case of severe skin burns proved effective, with the installation of reparative phenomena appearing much earlier compared to the absence of any treatment, and in the case of traumatic wounds, by simply applying the ointment, the results regarding their healing were more than satisfactory.