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Evaluation of the efficacy of *Centella asiatica* 5% alcoholic extract ointment in thermal trauma in mice

Evaluarea eficacității extractului alcoolic de Centella asiatica 5%. unguent în traumatisme termice la șoareci

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Rezumat

Centella asiatica **sau Brahmi~Gotu Kola este o plantă folosită din cele mai vechi timpuri ca plantă medicinală în țări precum India, Nepal, China, Sri Lanka sau Madagascar.**
Planta este considerată ca fiind una dintre cele mai utile plante pentru revitalizarea celulelor nervoase, motiv pentru care în India este cunoscuta drept „planta creierului" sau utilizată pentru tratarea afecțiunilor pielii și vindecării rănilor. Planta este încă des folosită în două ramuri tradiționale ale medicinei indiene Ayurveda și Unani. Scopul acestui studiu a fost de a evalua eficacitatea unguentului cu extract de *Centella asiatica* la șoareci cu traumă termică. Acest studiu a fost efectuat pe un număr total de 12 de șoareci din linia Balb/c, în vârstă de 24-26 săptămâni, care au fost lotizați în două loturi: unul experimental și unul martor. Durata experimentului a fost de 14 zile, cu recoltări de material biologic în ziua 3, 7 și 14 a experimentului. În cazul unguentului testat vindecarea clinică a avut loc după ziua a 9 de tratament prin debridarea crustei formate și punerea în evidență a unui epiteliu de regenerare.

Abstract

Centella asiatica **or Brahmi~Gotu Kola is a plant used since ancient times as a medicinal plant in countries such as India, Nepal, China, Sri Lanka, and Madagascar. The plant is considered to be one of the most useful plants for revitalizing nerve cells, which is why in India it is known as the "brain plant" or used to treat skin conditions and heal wounds. The plant is still often used in two traditional branches of Indian medicine, Ayurveda and Unani. The aim of this study was to evaluate the efficacy of *Centella asiatica* extract ointment in thermally injured mice. This study was performed on a total of 12 Balb/c mice aged 24-26 weeks, which were divided into two groups: one experimental and one control. The duration of the experiment was 14 days, with collections of biological material on days 3, 7, and 14 of the experiment. In the case of the tested ointment, clinical healing occurred after the 9th day of treatment by debridement of the formed crust and highlighting a regeneration epithelium**

1. Introduction

Centella asiatica is an ethnomedicinal plant used on different continents in various ancient cultures and tribal groups. It is mentioned in ancient traditional Chinese texts from 2000 years ago and in traditional Indian medicine Ayurveda from 3000 years ago.

Centella asiatica (Gotu Kola) is a perennial plant belonging to the *Umbelliferae family (Apiaceae)* that is found throughout India, growing in humid places at an altitude of up to 1800 m. It is found in most tropical and subtropical countries, growing in marshy areas, including parts of India, Pakistan, Sri Lanka, Madagascar, South Africa, the South Pacific, and Eastern Europe [13].

The whole plant is used for medicinal purposes. It is widely used as a blood purifier, as well as for treating high blood pressure, improving memory, and promoting longevity. In Ayurveda, it is one of the main herbs used to revitalize the nervous system [12].

Numerous scientific studies claim that *Centella asiatica* extracts have many excellent features:

- analgesic,
- anticonvulsant,
- antidiabetic,
- antidepressant,
- antifilarial,
- antipsoriatic,
- antiinflammatory,
- antioxidant,
- antileprotic,
- antimicrobial,
- antispastic,
- antituberculosis,
- antitumor,
- antiulcer,
- anxiolytic,
- immunomodulatory,
- sedative,
- stimulant and
- cicatrizing role [1,3,8,9,10].

The chemical composition of the plant is considered to be well studied, its pharmaceutical activities being associated with triterpenoid constituents.

The dry plant contains volatile oil 0.1%, germacrene, caryophyllene, p-cymol, pinene; flavone derivatives – quercetin glycoside, kaempferol; sesquiterpenes – caryophyllene, elemen and bicycloelemene, trans-farnesene, germacrene D; triterpenic steroids - sitosterol, stigmasterol; triterpenic acids – asiatic acid, 6-hydroxy asiatic acid, madecassic acid, madasiatic acid, betulinic acid, thankunic acid, isothankunic acid; saponins or pseudosaponins (1-8%) – asiaticoside, asiaticoside A, asiaticoside B, braminoside, brahmoside, brahminoside, thankunizide, isothankunizide [7].

These triterpene saponins and their sapogenins are mainly responsible for wound healing and vascular effects by inhibiting collagen production at the wound site.

2. Materials and methods

2.1. The technique of obtaining the extract of Centella asiatica

10 g of *Centella asiatica* were weighed and macerated according to the indications of the Romanian Pharmacopoeia Ed. X, for 10 days, shaking three times a day, in 100 ml of alcohol of 70 (v/v) in brown bottles.

Fig.1. Obtaining *Centella asiatica* extract

After extracting and pressing the residue, the extractive liquid was left to settle at a temperature of 5-10 °C, for six days after which it was filtered.

2.2. Preparation of ointment with *Centella asiatica* extract

The preparation of the ointment was carried out in the Pharmacy Laboratory of FMV Timișoara.

A professional Mayam ointment base (procured from the company Elemental SRL) was chosen as a base, which is a complex biomimetic base, composed of ingredients identical to the skin.

It is suitable for the protection and regeneration of the skin barrier, moisturizing, soothing, and maintaining skin health.

The appearance of product is a white cream, very fine, soft, and easy to apply on the skin. It absorbs quickly and does not leave a sticky or greasy layer on the skin. The smell is neutral.

Ingredients: water, triglycerides, pentylene glycol, hydrogenated lecithin, glycerin, cocoa butter, palmitic acid, squalane, and ceramides.

To make the ointment with *Centella asiatica* extract, 100 ml of Mayam ointment base was used, which was melted in a water bath. After melting, 5 ml of *Centella asiatica* extract was gradually incorporated (fig. 2.)

After homogenization, the obtained ointment was transferred into sterile containers with a capacity of 50 ml until use.

Fig.2. The ointment base used in the preparation

2.3. Animals

This study was performed on a total of 12 Balb/c mice aged 24-26 weeks, which were divided into two groups: one experimental and one control.

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

Mice were housed singly in standard 40x20x30 cm plastic cages in the Toxicology/Pharmacology/Biobase laboratory.

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, exactly as described in the specialized literature. 12 h after the induction of the mechanical burn, the application of the ointment to the experimental group began.

The collection of the biological material (skin) was carried out on days 3, 7, and 14.

For the euthanasia of the animals, the combination of Ketamine (50-100 mg/kg) and Xylazine (2-8mg/kg IM) was used.

The ointment was applied 2 × 1/day, in an equal amount (1g/application), for each mouse, using a standard two-headed spatula.

3. Results and discussions

In the case of the tested ointment, clinical healing occurred after the 9th day of treatment by debridement of the formed crust and highlighting a regeneration epithelium.

The appearance of the thermal injury in the treated group and the control group for days 3, 7, and 14 are shown in fig. 3.

Fig.3. Appearance of lesions in treated and control group

Collections of biological material were made on days 3, 7, and 14 of the experiment and the histological aspects for the control group and the treated group are presented in fig. 4 -7. The sections were stained by the usual Hematoxylin-Eosin method.

Histological images shown were captured using Olympus C×41 microscope software.

Image 4 shows the normal appearance of mouse skin.

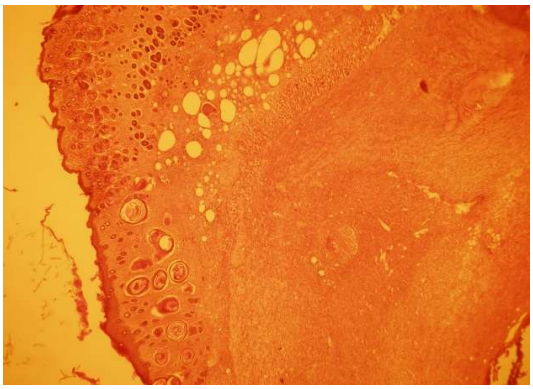

Fig. 4. Normal histological appearance of mouse skin, H.E. stain, 100X

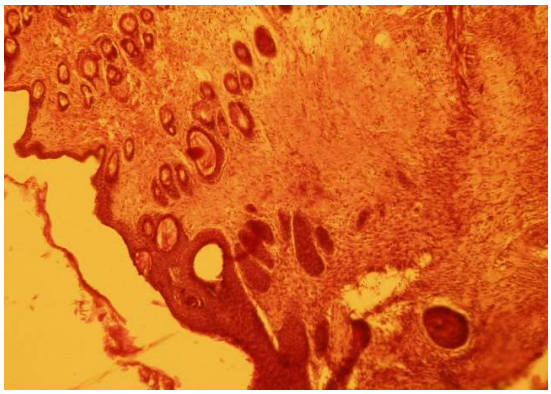

Fig. 5. Histological appearance of the mouse skin in the treated group, 3 days after the production of the thermal injury and the application of the ointment, H.E. stain, 100X

In figure 5, the histological image of thermal injuries highlighted the massive presence of fibroblasts in the dermis and a thickened epidermis.

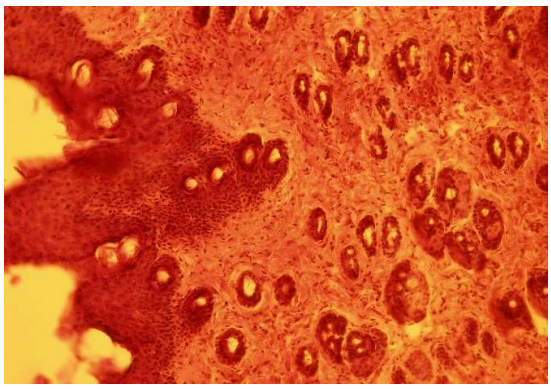

Fig. 6. Histological appearance of the mouse skin in the treated group, 7 days after the production of the thermal injury and the application of the ointment, H.E. stain, 200X

The histological appearance of the skin, 7 days after the injury, in the treated group reveals a thickened epidermis, the presence of fibroblasts and newly formed collagen fibers but also hair follicles and glandular structures.

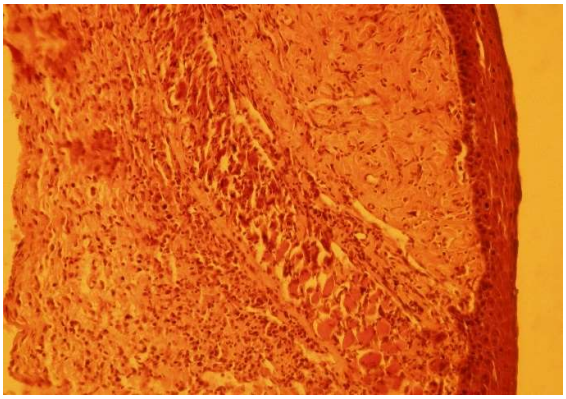

Fig. 7. Histological appearance of the mouse skin in the treated group 14 days after the production of the thermal injury and the application of the ointment,. H.E stain., 200X

14 days after applying the ointment, the skin structure is normal with well-defined collagen fibers and fibroblasts present.

Our results are consistent with those of other researchers who claim that *Centella asiatica* stimulates the production of fibroblasts, and collagen fibers and thus helps the formation of the extracellular matrix.

In scientific journals, there are numerous studies that show that *Centella asiatica* extracts have been traditionally used to heal wounds, and recent research has contributed more and more to support these claims [6].

A preclinical study showed that different formulations (ointment, cream, and gel) containing an aqueous extract of *Centella asiatica*, applied to open wounds in rats (3 times a day for 24 days) led to increased cell proliferation and collagen synthesis at the wound site [2].

The authors found that wounds treated with an aqueous extract of *Centella asiatica* epithelialized faster and the rate of wound contraction was higher compared to untreated control wounds.

Healing was more prominent after the application of the aqueous extract in gel form. The authors claim an effect on keratinization,

which helps thicken the skin in areas of infection [11].

Authors such as Poizot et al., [11] and Rosen et al., [12] argue that asiaticoside, a constituent of the *Centella asiatica* plant, exhibits wound healing activity by increasing collagen formation and angiogenesis.

Apart from stimulation of collagen synthesis in various cell types, asiaticoside increases the tensile strength of newly formed skin, continuing their healing.

It has also been shown to inhibit the inflammatory process that can cause hypertrophy in scars and improve capillary permeability.

4. Conclusions and recommendations

Following the study, we can conclude that the topical application of ointment with 5% *Centella asiatica* extract to mice with thermal trauma led to the massive appearance of fibroblasts in the dermis and well-defined collagen fibers, and stimulation of the extracellular matrix.

The obtained results recommend the use of *Centella asiatica* extract ointment in thermal injuries in animals.

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