THE WOUND HEALING EFFECT OF A DERMAL PREPARATION WITH *HYPERICUM PERFORATUM* IN SKIN INCISIONS

EFECTUL CICATRIZANT AL UNUI PREPARAT DERMIC PE BAZĂ DE *HYPERICUM PERFORATUM* ÎN LEZIUNILE DE TIP INCIZIE

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Abstract. *St. John's wort (Hypericum perforatum) is a plant used in traditional and modern medicine for treating skin disorders, peptic ulcer disease, infections, inflammatory affections and depresive disorders. The present experiment aims to prepare a new ointment formulation, based on the total extract of Hypericum perforatum included into a natural and biocompatible ointment base, determination of the total flavone content, and the evaluation of the wound healing potential using an in vivo experimental model of skin incision. The spectrophotometric results emphasize the presence of a high concentration of polyphenols that act sinergically with the ointment base in healing the lesions with lack of continuity. The histopathological results demonstrate the rehabilitation of the normal dermal tissue after only 6 days of treatment, showing the formation of a mature granulation tissue and the reconstruction of the epithelial matrix.*

Key words: *Hypericum perforatum, dermal formulation, wound-healing*

Rezumat. *Sunătoarea (Hypericum perforatum) este o plantă utilizată în medicina tradiţională şi modernă în tratamentul afecţiunilor dermice, a ulcerului gastric, a infecţiilor, bolilor inflamatorii şi tulburărilor depresive. Prezentul experiment îşi propune ca obiective prepararea unei noi formule de unguent, având la bază extractul total de sunătoare înglobat într-o bază de unguent naturală şi biocompatibilă, determinarea conţinutului în flavone şi evaluarea efectului cicatrizant la nivel dermic, utilizând un model experimental in vivo de tip incizie. Rezultatele analizei spectrofotometrice evidenţiază un conţinut ridicat în flavone, ce acţionează sinergic cu baza de unguent în cicatrizarea leziunilor cu pierderea soluţiei de continuitate. Rezultatele histopatologice demonstrează reabilitarea ţesutului dermic normal după 6 zile de tratament, cu formarea ţesutului de granulaţie matur şi refacerea matricei epiteliale.*

Cuvinte cheie: *Hypericum perforatum, formulare dermică, cicatrizare*

INTRODUCTION

Natural biocompatible products are gaining more attention in medical research and industry to the detriment of synthetic compounds that lack

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biocompatibility and their production is polluting the environment (Pavlenkov J., 2010). *Hypericum perforatum* L. (St. John's Wort) is a member of the genus *Hypericum* that includes about 400 species worldwide (Saddiqe et al., 2010). It is native to Europe, West Asia, North Africa, Madeira and the Azores and is naturalized in many parts of the world. The plant spreads rapidly by means of runners or from the prodigious seed production and can invade pastures, disturbed sites, dirt roads, the sides of roads and highways, and sparse woods. The plant has a long history of more than 2400 years and has enjoyed a reputation as a wound healer since the fifth century B.C. (Gerard, 1597; Withering., 1796).

The present study aims to develop a new wound-healing ointment based on the total extract of the aerial parts of *Hypericum perforatum*, advantaged by the simple, low-cost and environmentally friendly preparation.

MATERIAL AND METHOD

II.1. Chemical and materials

Ethyl alcohol, petrolatum, lanolin, methanol (R), aluminium chloride, sodium acetate and olive oil *(Olivae oleum virginale)* were purchased from Sigma-Aldrich. The aerial parts of *Hypericum perforatum* (St. John's wort) were collected from the Botanic Garden, Iaşi, Romania and a voucher specimen was identified by the staff of the same institution.

II.2. Hypericum perforatum histological analysis

The plant material has been fixed and preserved in 70% ethylic alcohol. The sections were cut by microtome, coloured with iodine green and rutheniu red, then analyzed in a Optika light microscope. The light micrographs were performed by means of the same light microscope, using Canon A540 camera.

II.3. Preparation of the Hyperici herba ointment

After collection, the plant material was dried in a dark room with controlled temperature and relative humidity. Samples with 10% moisture content were mechanically ground to obtain a homogenous drug powder. The extracts were prepared by macerating 50 g powder in 500 mL virgin olive oil/500 mL 70% ethanol, at room temperature, for 2 weeks. In the end, the extracts were filtered and placed in dark brown jars with stoppers. The ointment base was prepared by mixing white petrolatum and lanolin in equal amounts on water bath (40ºC) until a homogenization. Fifteen mL of each extract were gradually included into the ointment base.

II.4. Determination of total flavonoids

The hydroalcoholic extracts prepared from three different samples of *Hypericum perforatum* as described above were analyzed for the total flavonoid content, by the spectrophotometric method (FRX). The extracts were diluted in the proportion of 1:10, mixed with 5 mL of sodium acetate 100 g/l (R), 3 mL of aluminum chloride 25 g/l (R), and completed with methanol (R) to 25 mL. The samples were kept at room temperature for 40 min and the absorbance read at 430 nm. Rutine was used as the standard to produce the calibration curve. The mean of three readings was used and the total flavonoid content was expressed in rutoside (g%).

II.5. Experimental model of dermal injury

All the experimental proceedings achieved on laboratory animals (Wistar rats) in this study were in agreement with the European Council Directive of 24 November 1986 (86/609/EEC). The experiment included 3 groups of Wistar rats (7 animals per group): negative control group (incision, not treated), *Hyperici herba* ointment group (treated with

Hyperici herba ointment), and ointment base group (treated with the ointment base). The incision wound model was based on a previously described model (Süntar et al., 2010), with some modifications. Two linear paravertebral incisions (1 cm long) were made with a sterile surgical blade through the full thickness of the skin at 1.5 cm distance from the midline of each side of the vertebral column. The *Hyperici herba* ointment and the ointment base were applied topically once a day for 6 days.

RESULTS AND DISCUSSIONS

III.1. Hypericum perforatum histological analysis

In cross-section, the stem has a circular contour, modified by two opposing ridges. The epidermis contains large cells, slightly tangentially elongated, with a very thick external wall and covered by a thin striated cuticle. Conductive tissues are largely of secondary origin, cambium making a thin ring of phloem towards the outside and a very thick ring of xylem towards the inside. The phloem consists of sieve-tube cells, companion cells and few parenchymatous cells. The xylem has vessels irregularly disspersed in the libriform bulk. The pith is parenchymatous-cellulose, meatic, the size of the cells increasing towards the inside. The center section is occupied by a large lacuna (fig. 1). The front view of the leaf epidermis (fig. 2) shows cells of irregular shape, with wavy side walls. The lower epidermis shows anisocytic stomata, sometimes in large numbers, even intertwined, other times very rare or absent, so the lamina is hypostomatic. In cross section (fig. 3) the midvein protrudes from the underside of the lamina. The two epidermis contain isodiametric or slightly tangentially elongated cells, bigger on the upper side and with some stomata amongst them on the underside. The midvein has several layers of hypodermic collenchyma and a vascular bundle with primary structure.

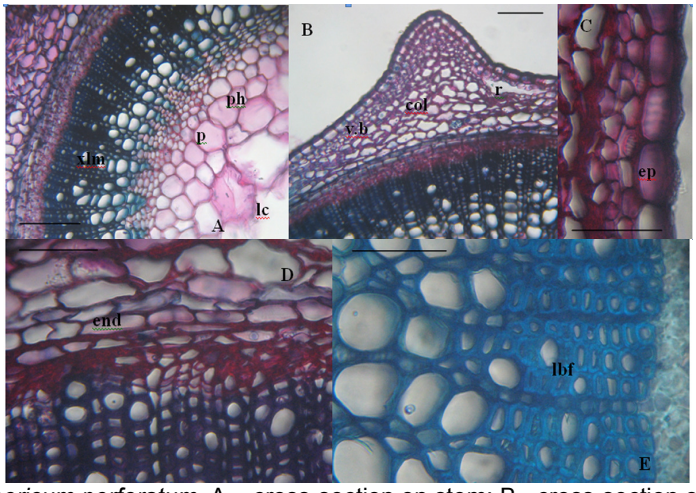

Fig. 1 - *Hypericum perforatum*. A – cross-section on stem; B - cross-section on stem with detail of ridge; C – cross-section on stem with detail of epidermis; D – cross-section on stem with detail of cork and central cilinder; E – cross-section on stem with detail of libriform

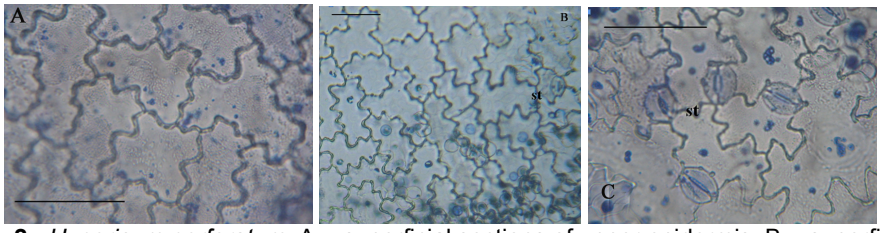

Fig. 2 - *Hypericum perforatum*. A. - superficial sections of upper epidermis. B. - superficial sections of lower epidermis; C. – superficial sections of lower epidermis

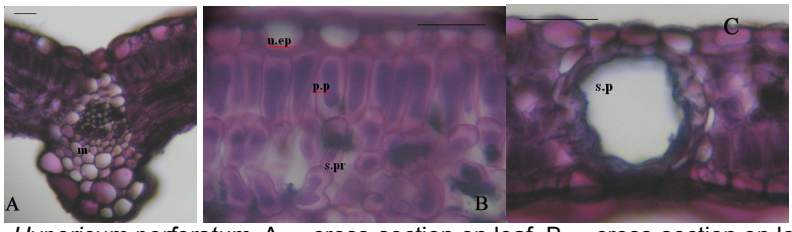

Fig. 3 - *Hypericum perforatum*. A. – cross-section on leaf. B. – cross-section on leaf with detail of mesophyll; C. – cross-section on leaf with detail of secretory pocket; Abbreviations: c – cork; col – colenchyma; end – endodermis; ep – epidermis (u – upper, l – lower); lbf – libriform; lc – lacuna; m – midvein; mes – mesophyll; mxlm – metaxylem; p – pith; ph – phloem; p.p – palisade parenchyma; r – ridge; rz – rhizodermis; s.p – secretory pocket; s.pr – spongy parenchyma; st – stomata; v.b – vascular bundle; xlm – xylem.

III.2. Determination of the total flavonoids

The content of flavonoids in 3 different samples of *Hypericum perforatum* is presented in Table 1. The results are expressed as g% rutoside.

Table 1

Concentrations of total flavonoids in *Hypericum perforatum* samples

***Hypericum perforatum* samples**	**Total flavonoids (g% rutoside)**
Sample 1	3.900
Sample 2	3.825
Sample 3	3.705

III.3. Histopathological results

The histopathological results after 6 days of topical treatment with Hypericum perforatum ointment are presented in Fig.4. If in the first day the edema is severe, the epidermis is atrophic and focal fibrosis is present, the third day of treatment reveals an improvement in the clinical and histopathological signs (mild inflammation in the hypodermis, normal aspect of the muscle tissue, small intracellular abcess). In the 6th day of treatment, the ulceration is replaced by granulation tissue, that spreads from the epidermis towards the hypodermis, where a mild inflammatory infiltrate with nodular perivascular disposition can be found. The muscle fibers have normal appearance, with interstitial edema.

Normal wound healing follows a predictable pattern that can be divided into overlapping phases: (a) hemostasis and inflammation, (b) proliferation, and (c) maturation and remodeling (Barbul , 2010). The total extract of the aerial parts of St. John's wort interferes in all the stages of the wound-repair process, improving

the healing of the skin injuries due its antibacterial, antioxidant, and anti-inflammatory effects.

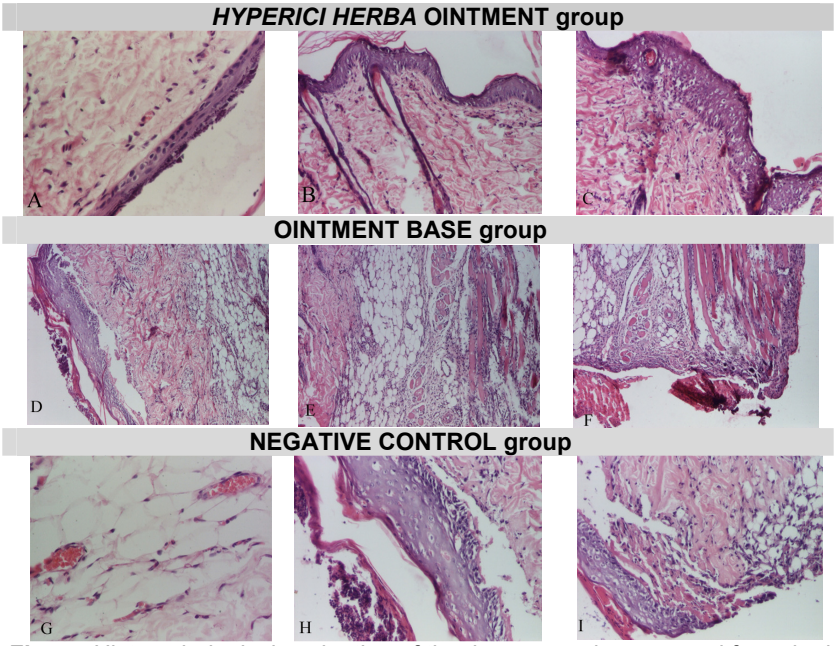

Fig. 4 - Histopathological evaluation of the tissue samples removed from the healed skin of the Wistar rats at the end of the treatment

***HYPERICI HERBA* OINTMENT group**: **A** - Epidermis with regenerated aspect (HEx100); **B** - Epidermis with ortokeratosis and rare perivascular lymphocytes (HEx100); **C** - Epidermis with mild spongiosis and vacuolar degeneration of keratinocytes. Transversal section of the hair follicle (HEx100); **OINTMENT BASE group**: **D** - Vascular congestion at the level of hypodermis (HEx40); **E** - Abscess in the keratin crust, parakeratosis and the absence of the granular tissue (HEx40); **F** - Microabscess in the keratin crust, spongiosis and vacuolar degeneration in the epidermis. (HEx40); **NEGATIVE CONTROL group G** - Abscess in the keratine crust, vacuolar degeneration of the keratinocytes; mild inflammatory infiltrate with perivascular disposure (HEx200); **H** - Inflammatory infiltrate in hypodermis and muscle tissue with aspects of necrosis and muscle regeneration (HE x100); **I** - Inflammatory infiltrate at the level of hypodermis and muscle tissue (HEx100)

Wounds represent an environment that facilitates the development of micro-organisms that hinder the wound healing process. Hyperforin from the *Hyperici herba* extracts shows antibacterial properties against Gram-positive bacteria, along with tannins, hypericin and volatile oil. Although the anti-inflammatory effect of the *Hypericum* extracts was attributed mainly to the inhibitory action of quercetin upon the signal transduction pathway, recent experiments reveal the inhibitory effect of hyperforin upon the lymphocyte reaction at the level of the epidermal cells and upon the T-lymphocyte proliferation. On the other hand, hyperforin is one of the natural compounds with a strong inhibitory effect upon cyclooxygenase-1 and lipoxygenase-5 (Albert et al., 2002). Recent studies demonstrate that hyperforin may interfere with other inflammatory responses of the leukocytes, including the marked inhibition of the reactive oxygen species and release of elastase (Feißt and Werz, 2004).

The ointment base itself contributes to wound healing, the ingredients being biocompatible and efficient in the re-epithelialization process. The oils found in lanolin are similar to those oils we secrete from within our own skin, which is why lanolin is so effective. Lanolin is a natural moisturizer with powerful emollient and protective properties. Vaseline (white petroleum) is a mixture of saturated petroleum hydrocarbons that was used in World War I to treat cuts and bruises and prevent sunburn, while during World War II, it was used to prepare sterile antiseptic wound dressing (Wiles et al., 2010).

CONCLUSIONS

1. The dermal formulation based on the total extract of *Hypericum perforatum* is efficient in wound-healing.

2. The ingredients used in the preparation of the ointment are natural and biocompatible.

3. The method of preparation is friendly to the environment, leaving no toxic or polluting compounds.

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