

# THE WOUND-HEALING EFFECT OF SOME NATURAL VEGETAL AND APITHERAPY EXTRACTS IN SKIN LESIONS

## EFFECTUL CICATRIZANT AL UNOR EXTRACTE VEGETALE SI APITERAPICE ASUPRA LEZIUNILOR DERMICE

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**Abstract.** *The present experiment evaluates the wound-healing effect of oil extracts of onion, St. John's wort and propolis tested intraoperatively and postoperatively on a cutaneous excision wound model in Wistar rats. The experimental model included five groups of Wistar rats: negative control group (non-treated control group), EUC group (treated with onion oil extract), EUP group (treated with propolis oil extract), EUS group (treated with St John's wort oil extract), and EUSCP group (treated with the mixture of all three extracts). In the end, a specimen sample of tissue removed from the healed skin of all rats was taken in order to be analyzed by histopathological examination. Clinical and histopathological results demonstrate the efficiency of oil extracts of onion, St. John's wort and propolis in the treatment of lesions with loss of cutaneous substance.*

**Key words:** *skin lesion, onion, St. John's wort, propolis.*

**Rezumat.** *Prezentul experiment evaluează efectul cicatrizant al extractelor uleioase de ceapă, sunătoare și propolis testate intraoperator și postoperator la șobolani Wistar, utilizând un model de afectare dermică de tip excizie. Modelul experimental include cinci loturi de șobolani: lot control (lot martor netratat), lot EUC (lot tratat cu extract uleios de ceapă), lot EUP (lot tratat cu extract uleios de propolis), lot EUS (lot tratat cu extract uleios de sunătoare), lot EUSCP (lot tratat cu amestec de extracte uleioase de ceapă, sunătoare și propolis). În final, s-au prelevat probe de țesut dermic din zona reepitelizată pentru efectuarea examenului histopatologic. Rezultatele clinice și histopatologice demonstrează eficiența extractelor uleioase de ceapă, sunătoare și propolis în tratamentul leziunilor dermice cu pierdere de substanță.*

**Cuvinte cheie:** *leziune dermică, ceapă, sunătoare, propolis.*

### INTRODUCTION

Wounds represent the result of an injury at the level of skin integrity, and if the process of tissue repair following an inadequate treatment fails, they become chronic wounds. Besides the fact that these chronic dermal injuries affect negatively the quality of patients' life, their management and care need high

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economical resources, a rather important problem especially for the developing countries (Schreml et al., 2010).

The last decades bring the complementary medicine into the medical forefront, having as major role the use of plants in the treatment of different disorders. The concept of *phytotherapy treatment* is reconsidered by achieving *in vivo* and *in vitro* studies regarding the confirmation of the healing effects of plants, the determination of the active principles responsible for these effects, and the elucidation of their mechanism of action (Adetutu et al., 2011).

The present experiment aims to confirm the traditional use of onion, St. John's wort and propolis in the treatment of wounds, by demonstrating the efficacy of their oil extracts in the processes of reepithelization and cicatrization on an experimental model of excision closed with one surgical suture exerted on Wistar rats.

## MATERIAL AND METHOD

### **Preparation of extracts**

Bulbs of *Allium cepa* and aerial parts of *Hypericum perforatum* were collected from the Botanical Garden, Iași, Romania and a voucher specimen was identified by the staff of the same institution. Propolis was purchased from *Stupina LLC*, Bălănești, Gorj, Romania. The fresh vegetal products (the bulbs of *Allium cepa* and aerial parts of *Hypericum perforatum*) were grounded, weighted (50.00 g) and macerated in 500 mL of virgin olive oil in dark brown jars, at room temperature, for 2 weeks.

The propolis was grounded and 20 mL virgin olive oil was added. The extract was placed on a magnetic stirrer for 72 hours (700 rpm).

In the end, the extracts are filtered through gauze and placed in dark brown jars with stoppers. Furthermore, 150 mL of each filtered extract are mixed in a separate jar on a magnetic stirrer to obtain the mixture of extracts (EUSCP extract).

### **Experimental model**

All the experimental proceedings achieved on laboratory animals (Wistar rats) in this study were in agreement with the guidelines of animal bioethics from the Act on Animal Experimentation and Animal Health and Welfare Act from Romania and were in compliance with the European Council Directive of 24 November 1986 (86/609/EEC).

The experiment included 5 groups of Wistar rats (6 animals per group): negative control group (control group with excisions, not treated), EUC group (treated with onion oil extract), EUP group (treated with propolis oil extract), EUS group (treated with St John's wort oil extract), and EUSCP group (treated with the mixture of all the three oil extracts).

The experimental model was achieved by making two paravertebral excisions (1x1 cm) with a sterile surgical blade through the full thickness of the skin at a distance of 1.5 cm from midline of each side of the vertebral column (Süntar et al., 2010). The wounds were closed with one surgical suture.

During the surgical operation, the natural oil extracts were applied. The postoperative treatment lasted 7 days (1 application per day), until the complete healing of the wound. In the end, a specimen sample of tissue removed from the healed skin of all rats was taken with a 3 mm biopsy punch in order to be analyzed by histopathological examination.

## RESULTS AND DISCUSSIONS

### *Clinical results*

The macroscopic evaluation of epidermal lesions for all the 4 experimental treated groups demonstrated the efficacy of the treatment with oil extracts of onion, propolis and St. John's wort. The complete healing occurred after 7 days of treatment, the time needed for reepithelization of the wounded skin being much shorter than in other studies (Akkol et al, 2011) (Table 1).

### *Histopathological results*

In all the 4 treated groups, the epidermis is intact, rectilinium, represented by 3-4 layers (EUSCP group) or 4-5 layers (EUP group). Isolated, there can be noticed areas completely denuded of epithelium, with small foci of regeneration (EUS group) and subepithelial microhemorrhage in the regenerating foci. Especially in the regenerating foci, hyperchrome nuclei are to be seen in the basal layer, slightly increased in volume, and also vacuolar degeneration (Table 1).

Discrete or no vascular congestion can be seen in the superficial papillary dermis and the deeper reticular one. Edema is present with different intensities, from discrete (EUS group), to moderate (EUP group) and significant edema (control group) (Fig. 1, Table 1). No edema could be observed in the group treated with onion oil extract and the mixture of all three oil extracts, thus resulting that onion extract exerted the most important anti-inflammatory effect (Fig. 1, Table 1). Lymphocytes and fibroblasts are seen mainly perivascular, but also with diffuse disposition in the superficial and deeper dermis (fig. 2, table 1). As in the case of edema, the most decreased number of lymphocytes and fibroblasts appears in the groups treated with onion oil extract (EUC and EUSCP groups), conducting us to the idea that the wound healing effect has been exerted much faster in these groups (Fig. 2, Table 1). Discrete aspects of collagenisation or fibrosis are seen in some cases (control group) (Table 1). The hair follicles present in biopsies do not show any modification.

The wound healing effects of oil extracts of onion, St. John's wort and propolis are mainly based on the anti-inflammatory, antioxidant and antibacterial effects of their components, as the regulation of inflammation, immunomodulation and oxidation plays an important role in re-establishing the normal function and anatomy of the affected skin (Koltuksuz et al., 2011).

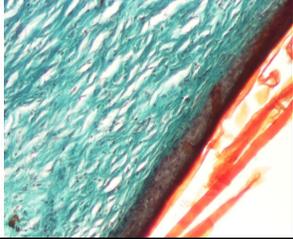
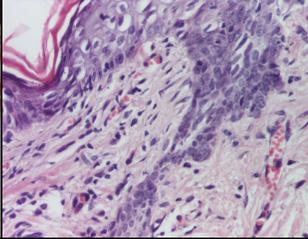
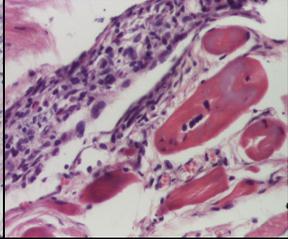
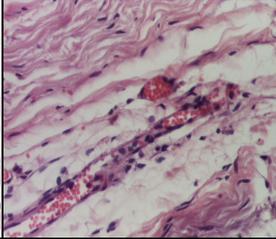
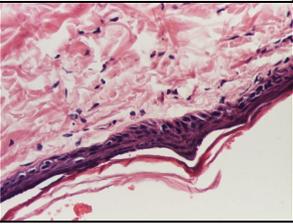
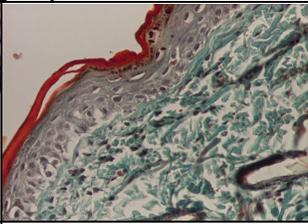
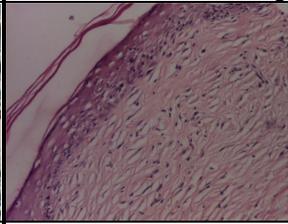
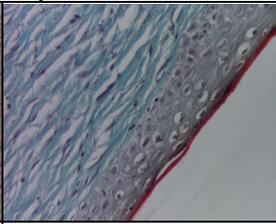
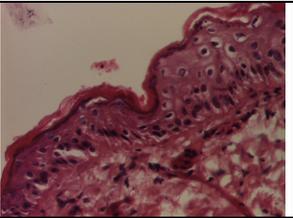
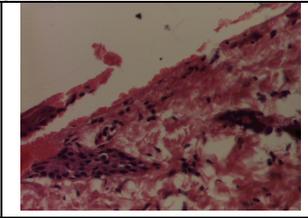
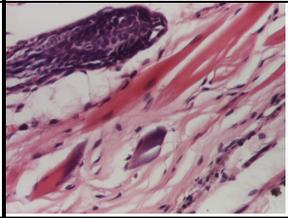
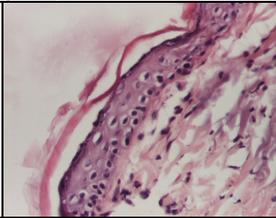
First of all, the tested oil extracts interfere in the first phase of tissue repair process – the inflammatory phase. The anti-inflammatory mechanism is exerted by: 1) quercetin, the major flavonol in onion, that acts by inhibiting cyclooxygenase (COX) and lipoxygenase (LOX) (Singh et al., 2009); 2) quercetin and hyperforin from the oil extract of St. John's wort (Istudor, 1998; Süntar et al., 2010); 3) flavones and caffeic acid phenethyl ester (CAPE), a specific inhibitor of the kappa B transcription nuclear factor, that inhibits LOX-5 by a non-competitive mechanism (Serarslan et al., 2007).

Recent experiments demonstrate the inhibitory effect of hyperforin upon the lymphocyte reaction at the level of epidermal cell and also upon the lymphocyte T proliferation (Schempp et al., 2000). We have also revealed in the present

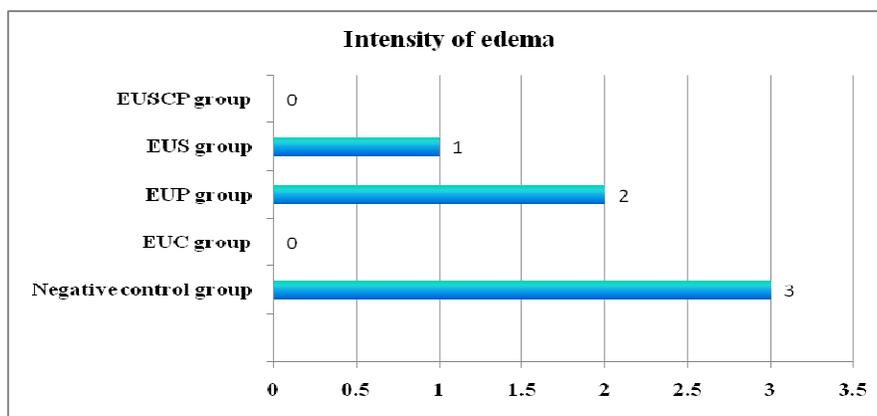
experiment good results in this regard for the St. John's Wort oil extract, but even better results for the onion oil extract.

Table 1

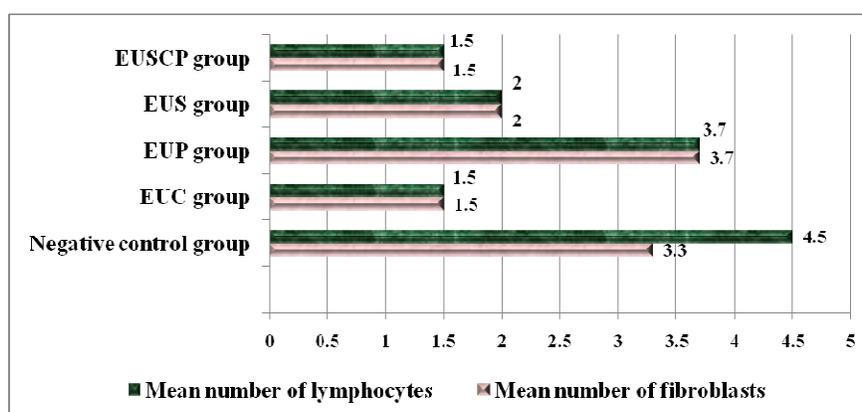
Histopathological evaluation

MICROGRAPHIES and DESCRIPTION			
<b>Control group</b>			
			
Collagenized epidermis and dermis, edema (Sz x100)	Epidermis and dermis with leukocytes (HEx200)	Muscle inflammation (HEx 200)	Stasis and edema in deep dermis (HEx200)
<b>EUC group</b>		<b>EUP group</b>	
			
Regenerating epidermis and dermis (HEx200)	Epidermis and dermis, slight accentuation of basement membrane (Sz x200)	Epidermis and dermis – collagenization and slight edema (HEx100)	Epidermis and dermis – collagenization (Sz x200)
<b>EUS group</b>		<b>EUSCP group</b>	
			
Epidermis (HEx200)	Without epidermis (HEx200)	Dermis with appendages and striated muscle (HEx200)	Epithelium (HEx200)

The oil extracts tested in this experiment exert their antibacterial effect by the presence of the following compounds: 1) alliin and compounds of volatile oil from onion (Grigorescu et al., 2001); 2) hyperforin from St. John's wort extract, that has activity against a number of Gram-positive bacteria (*Staphylococcus aureus*, *Corynebacterium diphtheriae*), its effect being sustained by tannins, hypericine, and volatile oil (Istodor, 1998); 3) flavones from propolis (mainly galangin, pinocembrin and pinostrobin), along with acids and aromatic esters (Dimov et al, 1992).



**Fig. 1** - Intensity of edema at the end of the treatment (evaluation scale of edema: 0 – absent; 1 – discrete; 1-2 – moderate edema; >2 – severe edema)



**Fig. 2** - Mean number of fibroblasts and lymphocytes

The antioxidant effect of propolis is due to the inhibition of leukocyte myeloperoxidase by the means of its rich content in flavonoids and polyphenols, mainly kaempferol and CAPE (Kujumgiev et al., 1993).

Among the minerals present in onion and St. John's wort, calcium interferes in the wound healing process mainly by regulating the differentiation of keratinocytes, acting in a more discrete manner upon their proliferation (Tu et al., 2001).

It is worth mentioning that the clinical and histological results obtained for onion oil extract (EUC and EUSPC groups) are superior to all the other extracts tested in this experiment, showing regeneration of the epidermis, with minimal, hardly visible scars (Tables 1), no presence of edema (Fig. 1), and reduced number of lymphocytes and fibroblasts (Fig. 2).

Another thing that is worth mentioning is that the number of lymphocytes and fibroblasts has similar values for all the experimental groups, excepting for the negative control group (Fig. 2).

## CONCLUSIONS

1. Clinical and histopathological results demonstrate the efficacy of the treatment with oil extracts of onion, propolis and St. John's wort in wound-healing of excision lesions.
2. The complete healing occurred after 7 days of treatment, the results being clearly superior to those cited in other studies
3. Clinical and histological results obtained for onion oil extract are superior to all the other extracts tested in this experiment.

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## REFERENCES

1. **Adetutu A., Morgan W.A., Corcoran O., 2011** - *Ethnopharmacological survey and in vitro evaluation of wound-healing plants used in South-western Nigeria.* J Ethnopharmacol., 137(1), p. 50-56;
2. **Akkol E.K., Suntar I., Keles H., Yesilada E., 2011** - *The potential role of female flowers inflorescence of Typha domingensis Pers. in wound management.* J Ethnopharmacol., 133, p. 1027–1032;
3. **Dimov V., Ivanovska N., Bankova V., Popov S., 1992** - *Immunomodulatory action of propolis: Prophylactic activity against Gram-negative infections and adjuvant effect of water-soluble derivative.* Vaccine, 10, p. 817-823;
4. **Grigorescu E., Lazăr M. I., Stănescu U., Ciulei I., 2001** - *Index fitoterapic,* Editura Cantes, Iași, p. 31;
5. **Kujumgiev A., Bankova V., Ignatova A., Popov S., 1993** - *Antibacterial activity of propolis, some of its components and their analogs.* Pharmazie, 48, p. 785-786;
6. **Schempp C.M., Winghofer B., Ludtke R., Simon-Haarhaus B., Schopf E., Simon J.C., 2000** - *Topical application of St John's wort (Hypericum perforatum L.) and of its metabolite hyperforin inhibits the allostimulatory capacity of epidermal cells.* British Journal of Dermatology, 142(5), p. 979–984;
7. **Schreml S., Szeimies R. M., Prantl L., Landthaler M., Babilas P., 2010** - *Wound healing in the 21st century,* J Am Acad Dermatol, 63(5), p. 866-881;
8. **Serarslan G., Altuğ E., Kontas T., Atik E., Avci G., 2007** - *Caffeic acid phenetyl ester accelerates cutaneous wound healing in a rat model and decreases oxidative stress.* Clinical and Experimental Dermatology, 32, p. 709–715;
9. **Singh B.N., Singh B.R., Singh R.L., Prakash D., Singh D.P., Sarma B.K., Upadhyay G., Singh H.B., 2009** - *Polyphenolics from various extracts/fractions of red onion (Allium cepa) peel with potent antioxidant and antimutagenic activities.* Food and Chemical Toxicology, 47, p. 1161–1167;
10. **Süntar I. P., Akkol E. K., Yilmazer D., Baykal T., Kirmızıbekmez H., Alper M., Yeşilada E., 2010** - *Investigations on the in vivo wound healing potential of Hypericum perforatum L.* J Ethnopharmacol., 127, p. 468–477;
11. **Tu C.L., Chang W., Bickle D.D., 2001** - *The extracellular calcium-sensing receptor is required for calcium induced differentiation in human keratinocytes,* J. Biol. Chem., 276(44), p. 41079-41085;