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Hypericum perforatum for experimental skin burns treatment in rats in comparison to nitrofurazone

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Abstract
Overexposure to sun, radiation, chemical or electrical contact resulting tissues damages.
The treatment of burns depends on the location and severity of the damage. Burns are characterized by degree, based on the severity of the tissue damage. Damage also may extend to the underlying fat, muscle, or bone. *Hypericum perforatum L* (HP - St. Johns wort) has been used widely for the treatment of burn injuries for many years in traditional medicine.
The aim of study was to investigate HP treatment in comparison to nitrofurazone in experimental thermal burns in rats.
Administration of HP four times a day within the first 24 hours is clearly effective in wound healing in the experimental thermal second degree burn modality as a plant with various biological activities such as antidepressant, wound-healing, anti-inflammatory, and antimicrobial activity against bacterial and fungal strains is significantly superior to the antimicrobial nitrofurazone treatment.

Keywords: Skin burn, *Hypericum perforatum*, nitrofurazon, metalloproteinases

Introduction
Overexposure to sun, radiation, chemical or electrical contact resulting tissues damages. Burns are characterized by degree, based on the severity of the tissue injuries and several systemic derangements as well.
Burn injury triggers a systemic inflammatory response, with a rapid increase in cytokine levels. In burns the inflammatory response may be generalized, leading to increased capillary permeability, fluid shifts, increased risk of sepsis, with multiple organ dysfunctions. Burns are characterized by different degree, based on the tissue injuries severity and several systemic derangements as well. Damages also may extend to the underlying fat, muscle, or bone. Burn injury triggers a systemic inflammatory response, with a rapid increase in cytokine levels. In burns the inflammatory response may be generalized, leading to increased capillary permeability, fluid shifts, increased risk of sepsis, with multiple organ dysfunction. The treatment of burns depends on the location and severity of the damage.
Acute or chronic inflammation are characterized by several fundamental processes including exudation of plasma proteins, recruitment of leukocytes, and activation of cell and plasma derived inflammatory mediators. Increased expression of matrix metalloproteinases (MMPs) has been observed in almost every human disease with and recent insights from *in vitro* and mouse models of human disease processes suggest that MMPs have evolved to serve broad functions in defense, injury, inflammation and restore. Matrix metalloproteinases (MMPs) may contribute to the permeability changes associated with thermal injury, since they have a role as inflammation modulators [1, 2].
Burn wound healing is a complicated process including inflammation due to disruption of blood vessels and extravasation of blood constituents, re-epithelialization that begins several hours after injury, formation of granulation tissue, neovascularization, migration and mitogenic stimulation of endothelial cells and finally wound contraction as a result of interaction between cells, extracellular matrix and cytokines. Severe burn wounds need to be treated as soon as possible since any delay can postpone the healing process or result in infection. The lipid protein complex (LPC) released from burned skin lead to profound immune suppression. Thermal injury represents a pathophysiological condition in which hyperactive macrophages are primed to stimulate the down regulation or up regulation of certain inflammatory cytokines.

Abnormal levels of proinflammatory mediators, such as tumor necrosis factor alpha (TNF-α), interleukin-1b (IL-1b), interleukin-6 (IL-6), interleukin-8 (IL-8), and interleukin-10 (IL-10), have been reported both systemically and locally in burn experimental animals and patients (MMP) metalloproteinase expression can be induced in response to a range of signals, including cytokines, hormones.

MMPs are a group of endopeptidases that share the ability to degrade almost all components of the extracellular matrix and basement membranes [3-6].

Medicinal plants have recently received much attention for their various medicinal properties. *Hypericum perforatum* (HP-St.John wort), a member of the genus *Hypericum* family, is found in Europe, West Asia, North Africa, Madeira, and the Azores. The extract of HP contains naphthodianthrones, phloroglucinols, flavonoids, bioflavonoids, and phenylpropanoids which promote the wound healing process and have antifungal, anti-inflammatory, antimycobacterial, and antiviral activities. The plant has a wide range of medicinal applications such as skin wounds, eczema, burns, diseases of the alimentary tract, and psychological disorders [7-9].

Nitrofurazone (INN, trade name Furacin) is an antimicrobial organic compound belonging to the nitrofuran class. It is most commonly used as a topical antibiotic ointment. It is effective against gram-positive bacteria, gram-negative bacteria, Nitrofurazone was previously available as a prescription, and was indicated as a topical solution, topical cream, or topical ointment for the treatment of bacterial skin infections, wounds, burns, and ulcers. It was also used as a prophylactic measure to prevent infection that could potentially result in skin graft rejection. Nitrofurazone is still very popular as a topical solution. or cream (Alfred L. Weiner, M.D.; Z. Charles Fixier, M.D. JAMA. 1959;169(4):346-347). Its mechanism of action is not fully understood, but nitrofurazone's antimicrobial properties are suspected to be due to the interference of DNA synthesis in the microorganism by inhibiting certain enzymes that are involved with glycolysis.

Material and methods

The aim of study was to investigate local treatment by *Hypericum perforatum* (HP) oil [Liquid extract (DER 1:4-20), extraction solvent vegetable oil (e.g. olive oil, sunflower oil, linseed oil)]* compared to nitrofurazone cream (as antimicrobial agent) in experimental thermal burns in rats.

The animals were housed and cared according to the "Guide for the Care and Use of Experimental animals" and have permission of the Greek Ethical EL.25BIO 009. Committee on Care and Use of Laboratory animals. Guides for the care and use of laboratory animals. Washington, DC: Institute of Laboratory Animals and Resources. National Research Council.1985:p 83.

* Preparation: According to the German "Ergänzungsbuch" to the German Pharmacopoeia 6 (Erg.-B6. 1941): The crushed fresh flowers of H. perforatum (25 parts) are doused with olive oil (100 parts).

Twenty four male rats of 250g weight were randomized into four groups, 6 rats in each. A second-degree thermal burn was perfomed on the rats shaven dorsal areas of 3cm^2 with 100 °C boiled water for 10 seconds.

Group A was served as control.

Group B (Thermal Burn Control Group) was administered only irrigation with saline solution.

Group C (topical nitrofurazone cream 2%) was administered twice, daily since nitrofurazone cream is included among the agents tested for effectiveness against various bacteria [16].

Hypericum genus (St John's wort), is a common perennial herb of bright yellow flowers originating in Europe, West Asia and North Africa and one of the oldest herbal remedies in various cultures. Different species of the genus *Hypericum* (Hypericaceae) have been used in traditional medicine to treat a variety of internal and external ailments. The use of this species as an herbal remedy dates back to the time of the ancient Greeks since Hippocrates era [10, 11].

St. John's wort name derives from the herb's tendency to flower around a 24 June feast of St. John's birthday (wort means plant in Old English). The species name *perforatum* derives from the watermarking of translucent dots that can be seen when the leaf is held up to the sun. One of the earliest known mentions of HP as a medical plant is found in the Naturalis Historiae by Pliny the Elder (23–79 A. D.) as a treatment for burns, but also internally as an astringent.

The most important ingredients of *Hypericum perforatum* are hyperforin, a potent antimicrobial and anti-inflammatory agent and hypericin. Both are particularly active against gram-positive and gram-negative bacteria. Hypericin negatively regulated IL-12 production at the transcription level [12- 14].

The treatment principles of burns insist to preventing microvascular deterioration and edema reducing: positioning, early escharotomies and, infections preventions (debridement, necrectomies, local and systemic antibiotic therapy [15].

Group D (topical application of HP Hypericum oil (hypericin 110 microg/mL) and Hypericum ointment (hypericin 30 microg/mL) was administered four times a day (every six hours according Ute Wölfle 2013. Wound site healing on the skin was histopathologically evaluated [17].

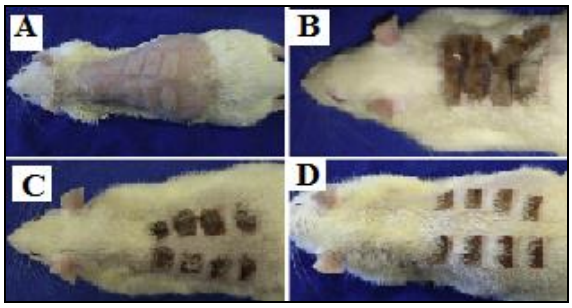

Fig 1.

After 24h the animals were euthanized by decapitation and blood was collected, where Metalloproteinases M-8 and M-9 were estimated in serum. The levels of MMP-8 were determined by immunofluorometric analysis (IFMA) (Medix Biochemica, Kauniainen, Finland). The levels of MMP-9 and TIMP-1 were analyzed by enzyme-linked immunosorbent assay (ELISA) using commercial kits (Biotrak ELISA System; Amersham Biosciences). Statistical analysis was performed by t-test.

Results

Table 1.

Metalloprot. (pg/mL)	Control	Burn-control	Nitrofurazone	Hypericum perforatum
MM-8(M±SD) (p<0,05)	6,64 (±4)	72.1 (± 44.9)	47,97 (±13,2)	22 (±7)
MM-9(p<0,05)	9,4.8 (±5,2)	90.9 (±21.3)	56,36 (±17,3)	27 (±9,1)
Collagen discoloration (p<0.5)	0	intense	mild	<+
Degenerated follicles (p<0.5)	0	significant	moderate	+
Epidermal thickness (p<0,005)	0	intense	mild	+++
Oedema (p<0.5)	0	significant	mild	+
Vascular injury (0,001)	0	intense	moderate	0

Histological evaluation was performed by stain eosin-haematoxylin. It was found that collagen discoloration of the HP treatment group was localized in the lower part of the epidermal layer and did not go up to the depth of dermis compared to the other groups, and epidermis, hair follicles and sebaceous glands remained protected compared to the burn, groups under administration of *Nitrofurazone Ointment* USP (0.2%) every 12 hour. Epidermal thickness and vessels number of the HP Group were significantly higher compared to the other groups (p<0.05). The number of degenerated hair follicles in the HP Group was significantly less than the other groups (p <*0*.05), and it was determined that the total number of hair follicles significantly increased in the 8th (p<0.05) and this number did not differ by the control group (p>0.05).

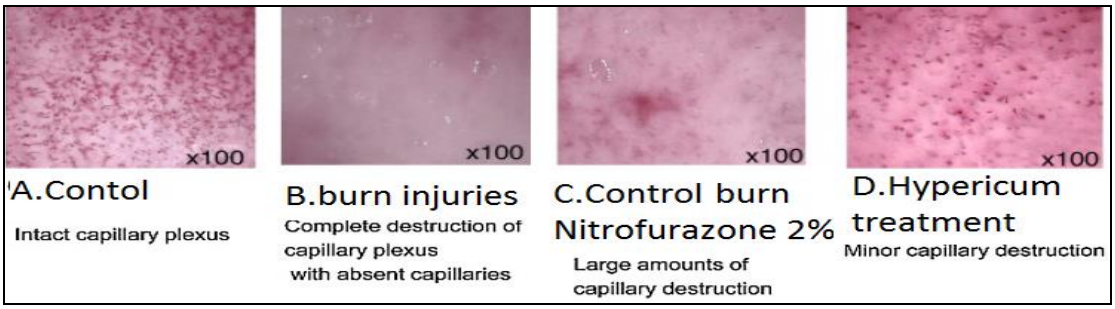

Fig 2.

Plasma MMP-8 and -9 were higher in burned animals than in healthy controls (p P<0.001 and P = 0.016).

The observed levels increase in the MMP-8 and MMP-9 likely was caused by their rapid release from the pre-formed granulae in neutrophil granulocytes, which is known to occur within minutes to a few hours after inflammatory stimuli such as interleukin-8 (IL-8), tumor necrosis factor alpha (TNF-α), and granulocyte colony-stimulating factor (G-CSF)(Owen CA, 2004). TNF-α can also stimulate the synthesis of MMP-8 and MMP-9 [18, 19].

The increase in metalloproteinases leads to breakdown of proteins thus providing more nutrients for microbes and making microbial penetration into the tissue easier. The particular pathophysiology of burns does predispose them to infection with a variety of pathogens. Topical treatment produces better outcomes than traditional systemic antibiotics.

The development of these topical antimicrobial approaches has been a fertile field for both small and large biotechnology companies. It is referred that topical antimicrobial agents will only continue to produce effective effects as are observed by traditional preparations for the third world [19-22].

Pharmacological research supports the use of *Hypericum* preparations, due to its constituents, naphthodianthrones (e.g. hypericin) and phloroglucinols (e.g. hyperforin) have interesting pharmacological profiles, including antioxidant, anti-inflammatory, anticancer, and antimicrobial activities.

Furthermore in an evaluation study of *Hypericum* patulum leaf extract skin wound healing activity in rats compared with nitrofurazone ointment it was documented that leaf extract ointments lead to more effective tissue regeneration than the antibacterial agent [16].

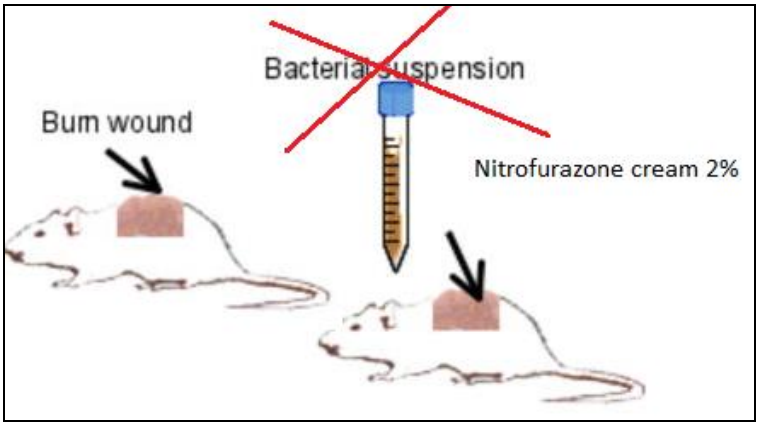

Fig 3.

Discussion-Conclusion

Administration of HP four times a day within the first 24 hours is clearly effective in wound healing in the experimental thermal second degree burn modality as a plant with various biological activities such as antidepressant, wound-healing, anti-inflammatory, and antimicrobial activity against bacterial and fungal strains is significantly superior to nitrofurazone treatment.

Moreover the healing properties of hypericum preparations are demonstrated in previous reports [24, 25].

Finally the amelioration of skin burn healing was more progressed by the Hypericum preparetions treatment as by nitrofurazone application possibly due to its effectiveness against the local microbial flora.

The aim of wound repair/healing consists of minimizing tissue damage proper nutrition and wound healing environment in order to restore the anatomical continuity and function of the affected areas [2]. Wound healing involves continuous cell–cell and cell–matrix interactions leading to proceed in three phases: protection against inflammation cellular proliferation and remodeling.

These phases under the plant-based remedy are implicated according its traditional use [3, 12]. The aerial parts of *Hypericum perforatum* improve the healing of the skin injuries by its antibacterial, antioxidant, and anti-inflammatory properties. Burn wound is an environment for microorganisms growth [13]. The aim of the study was to prove theplant preparation ability to prevent infection leading to rapid and effective wound restoration. The infected wounds attract high levels of phagocytic cells can damage the host cells and delay the healing process. Hyperforin from the Hyperici herbal extracts,and the presence of tanninand hypericin shows antibacterial properties against Gram-positive bacteria. In parallel hyperforin exerts inhibitory effect upon the lymphocyte reaction at the level of the epidermal cells. On the other hand, hyperforin is one of the natural compounds with a strong inhibitory effect upon cyclooxygenase-1 (COX-1) and lipoxygenase-5 (LOX-5) [18]. This dual mechanism offers the rational basis for the traditional use of St. John's wort in inflammatory dermal disorders. Recent studies demonstrate that hyperforin may interfere with other inflammatory responses of the leukocytes, including the marked inhibition of the reactive oxygen [26].

On the other hand nitrofurazone has a wide spectrum against Gram-positive and Gram-negative bacteria. However, the compound is not particularly active against most strains of P. aeruginosa and does not inhibit fungi or viruses S. aureus and E. coli can develop resistance to nitrofurazone.

Nitrofurazone is topically used as adjunctive therapy in patients with second- and third-degree burns. It has good eschar penetration, and can be used in the management of invasive burn wound infections with sensitive microorganisms. The drug presents some advantages for the ambulatory patient as tissue granulation begins sooner and separately crusts more rapidly.

The treated patients may be affected by allergic contact dermatitis, occurring in approximately 1% of the treated patients. Moreover the polyethylene glycols of nitrofurazone ointment (but not in cream) can be absorbed through denuded skin and may cause renal impairment [27].

At the end in comparison of the effectiveness and the clinical progress of the two used remedies in rat skin burn it is demonstrated that *Hypericum perforatum* treatment may be superior to nitrofurazone. The metalloproteinases levels are suppressed under hypericum and the skin damage indexes as well [28].

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