Introduction

Skin, the largest organ of the body, functions as the necessary interface between the internal and the external environment. Thus, it continuously protects the body from noxious stimuli, e.g., microorganisms, ultraviolet (UV) irradiation, allergens, and irritants. Its unique role and function is a direct result of its structure and makeup, particularly of the most superficial part, the epidermis. The main cellular component of the epidermis includes keratinocytes, but there are also melanocytes, Merkel cells, gamma delta T-lymphocytes, and Langerhans cells. Keratinocytes in the basal layer of the epidermis preserve their ability to proliferate upward to form the spinous layer and the granular layer. Beyond the granular layer, the keratinocytes terminally differentiate into corneocytes in the horny layer. In the outmost part of epidermis, corneocytes (compact keratinocytes without nuclei), together with the intercellular lamellar compartment (lipids), contribute to the structure and function of the stratum corneum (SC).

A PubMed literature search was performed using the following terms: plant oils and atopic dermatitis (AD), skin aging, skin barrier function, skin cancer, and wound healing (WH). Focusing on potential benefits of topically applied plant oils, we chose those that have been previously investigated in human skin, animal skin (mainly murine models of skin disease), or in vitro studies with keratinocytes. The search included clinical use of topical oil plants, but excluded more specific studies related to the biochemical extraction, purification, and modification of these plant oils and their byproducts.

Stratum Corneum Structure and Function

The structure of SC is like a brick wall, in which the corneocytes or “bricks” are surrounded by the intercellular lipid lamellae that act like the “mortar” to maintain both SC integrity and skin permeability barrier [1]. The skin’s barrier function depends mainly on the integrity of the SC. During differentiation, the plasma membrane of outer keratinocytes is replaced by the specialized cornified envelope (CE) of corneocytes. The CE gives corneocytes their rigidity. The development of the CE is attributed to the crosslinking of insoluble proteins (involucrin and loricrin) by transglutaminases. Some of the lipids (precursors of ceramides, free fatty acids (FFAs), and part of cholesterol) are synthesized in the keratinocytes at the stratum granulosum (SG) and then released from the lamellar bodies (LBs) into the SG-SC interface, whereas the remaining lipids are secreted onto the skin surface from the sebaceous glands (sebum). The permeability barrier is provided by the intercellular lipid-enriched matrix, which is composed of ceramides, FFAs, and cholesterol. Following the secretion of LBs, intercellular lipids are enzymatically modified to become the highly hydrophobic and organized lamellar structure. SC lamellar membranes are mostly composed of saturated FFAs of significantly longer chain length, which varies between C16 and C26. The main FFAs in the lamellar membranes are palmitic acid (C16:0) by 10% (mass/mass), stearic acid (C18:0) by 10% (mass/mass), behenic acid (C22:0) by 15% (mass/mass), lignoceric acid (C24:0) by 25% (mass/mass), and hexacosanoic acid (C26:0) by 10% (mass/mass) constitution of the total FFAs in SC. Other FFAs that present less in the SC include oleic acid (C18:1, n-9), eicosapentaenoic acid (C20:5, n-3), arachidonic acid (C20:4, n-6), docosahexaenoic acid (C22:6, n-3), linoleic acid (C18:2, n-6) as well as its derivatives that are linolenic acids [α-linolenic acid (C18:3, n-3), γ-linolenic acid (C18:3, n-6) and dihomo-γ-linolenic acid (C20:3, n-6)]. The C22 and C24 saturated FFAs are

present in relatively large amounts among the saturated FFAs, whereas the C18 unsaturated FFAs are the major constituents in unsaturated FFAs. In fact, linoleic acid is the most abundant polyunsaturated fatty acid. Aside from linoleic acid and arachidonic acid, the remaining FFAs can be synthesized in the keratinocytes.
The SC acts as a permeability barrier and an antimicrobial barrier. This antimicrobial barrier is attributed to the weak acidity of skin surface pH, free sphingoid bases generated from epidermal ceramides, and antimicrobial peptides within the intercellular compartment. Hydration of the SC is also crucial for the SC integrity and the maintenance of the skin barrier homeostasis. Natural moisturizing factor (NMF) components within the corneocytes contribute to the hydration of the SC. The composition of NMF includes free amino acids, pyrrolidone carboxylic acid, lactic acid, urocanic acid, organic acids, peptides, sugars, urea, citrate, glycerol, etc. Filaggrin, one of the terminal differentiation markers of the epidermis, also aids in SC hydration. Filaggrin is degraded into free amino acids in the SC. These amino acids are further metabolized into hygroscopic derivatives such as pyrrolidone carboxylic acid from glutamine and urocanic acid from histidine. This makes filaggrin one of the major factors influencing the hydration status of the SC.

Plant oils have been utilized for a variety of purposes throughout history, with their integration into foods, cosmetics, and pharmaceutical products. They are now being increasingly recognized for their effects on both skin diseases and the restoration of cutaneous homeostasis. This article briefly reviews the available data on biological influences of topical skin applications of some plant oils (olive oil, olive pomace oil, sunflower seed oil, coconut oil, safflower seed oil, argan oil, soybean oil, peanut oil, sesame oil, avocado oil, borage oil, jojoba oil, oat oil, pomegranate seed oil, almond oil, bitter apricot oil, rose hip oil, German chamomile oil, and shea butter). Thus, it focuses on the therapeutic benefits of these plant oils according to their anti-inflammatory and antioxidant effects on the skin, promotion of wound healing and repair of skin barrier.

Wound Healing

Wound healing (WH) is a dynamic and tightly regulated process of cellular, humoral, and molecular mechanisms. The process is depicted in four phases: hemostasis, inflammation, proliferation, and tissue remodeling. In the hemostasis phase, the clotting cascade is instantly activated following an injury, creating a temporary wound matrix. The inflammation phase consists of an innate immune response crucial in the breakdown and cleanup of tissue and pathogen debris at the site of injury. Polymorphonuclear neutrophils (PMNs) release reactive oxygen species (ROS) and nitric oxide, facilitate degradation of foreign organisms, and initiate phagocytosis of pathogens. Additionally, PMNs secrete high levels of PMN collagenase, elastase, and matrix metalloproteinases (MMPs), which break down damaged cells and extracellular matrix. Macrophages work through phagocytosis of pathogens and cell debris. Increased numbers of macrophages along with persistent inflammation are observed in chronic wounds. In contrast to acute wounds, where inflammation is crucial in the initial phases of wound repair, chronic non-healing wounds could result from the aberrant inflammatory response in proportion to its intensity and duration. Therefore, inflammation can positively or negatively affect the WH process. Excessive inflammation and/or duration is correlated with increased number of macrophages, resulting in compromised WH outcomes. Additionally, excessive levels of MMPs that are released from PMNs and macrophages, lead to extensive damage of extracellular matrix. This interferes with the normal formation of the scaffold for

new cells to migrate and proliferate in wounded areas. Studies of impaired WH models of obese (ob/ob) and diabetic (db/db) mice have shown that the number of macrophages is elevated in those models. Wound closure in obese mice (ob/ob) can be improved by systemic anti-tumor necrosis factor-alpha (TNF-α) treatment through inactivation of macrophages. Similarly, ROS and their oxidative reaction products present in the wound may also play a major role in tissue damage. Although ROS are part of normal regulatory circuits of skin barrier function, inflammation, and WH under physiological conditions, an excess in ROS is detrimental to the WH process.

Skin Inflammation and Proliferation

The skin encounters daily onslaught by exogenous stimuli. Noxious stimuli sometimes result in injuries and/or infections, leading to wound, inflammatory dermatoses, skin aging, or skin carcinogenesis. Inflammation takes place in response to these damages to the normal skin barrier. At the molecular level, the inflammatory response participates in a series of complex repair pathways related to the innate immune response, cutaneous differentiation, and skin barrier repair. Initially, upon inflammatory response, the keratinocytes and the innate immune cells such as leukocytes (PMNs, macrophages, and lymphocytes), mast cells, and dendritic cells are activated. Secreted cytokines such as IL-1α, TNF-α and IL-6 induce the chemokines of chemotaxis that attract the immune cells to the site of injury and infection. ROS are produced by activated keratinocytes and immune cells. Immune cells also secrete elastases and proteinases. The inflammatory microenvironment contributes to tissue repair and infection prevention/control. However, the chemokines produced by activated keratinocytes and immune cells are also able to damage the skin tissue in proximity to the target of the inflammatory response. Therefore, the intensity of inflammation and the time to resolution are critical in avoiding or at least limiting damage to normal skin tissue. Thus, modulation of inflammation is important in maintaining skin homeostasis.

If the initial acute response fails to resolve the causative factor, then the inflammatory response will continue and the subsequent inflammatory microenvironment will disrupt skin homeostasis. If the dysregulation of inflammatory skin response persists, chronic inflammatory dermatoses such as AD or psoriasis will arise.

In the epidermis, the metabolism of polyunsaturated fatty acids (PUFAs) is highly active. Linoleic acid, the major 18-carbon n-6 PUFA in normal epidermis, in the epidermis is metabolized via the 15-lipoxygenase pathway mainly into 13hydroxyoctadecadienoic acid, which possesses anti-proliferative properties. Dietary deficiency of linoleic acid results in a scaly and pruritic skin disorder similar to AD in hairless mice. Arachidonic acid, the second major PUFA in the skin, is another substrate of 15-lipoxygenase, by which it is transformed to 15hydroxyeicosatetraenoic acid (15-HETE). 15-HETE specifically inhibits leukotriene B4-induced chemotaxis of human PMNs. However, arachidonic acid is mainly metabolized via the cyclooxygenase (COX) pathway into the prostaglandins E(2), F(2α), and D(2). At low concentrations, the prostaglandins function to modulate skin homeostasis, whereas, at high concentrations, they induce skin inflammation and hyperproliferation of keratinocytes. Moreover, squamous cell carcinoma of skin is the neoplasm that consistently overexpresses COX-2 in the parenchyma and the mesenchyma of premalignant and malignant lesions. Increased levels of prostaglandins E(2) and F(2α) in premalignant and/or malignant epithelial skin cancers are due to the constitutive upregulation of enzymes such as COX-2, causing

increased prostaglandin biosynthesis and the downregulation of 15hydroxy-prostaglandin dehydrogenase (15-PGDH), which is involved in the inactivation of prostaglandins. Thus, topical supplementation with plant oils that provide local cutaneous anti-inflammatory and anti-proliferative metabolites could serve as the monotherapy or as adjuncts to standard therapeutic regimens for the management and prevention of both inflammatory skin disorders and actinic keratoses.

Reactive Oxidative Stress, Skin Aging and Skin Cancer

The aging of our skin can be discussed as two entities: chronological and environmentally-influenced aging. Clinically, chronological and environmentallyinfluenced aging show skin changes including thinning, loss of elasticity, roughness, wrinkling, increased dryness, and impairment of the skin barrier. Chronological aging depends on a decrease in cellular replacement (senescence) of the epidermis, dermis, and hypodermis, but also from impairment in the remodeling of the extracellular matrix (e.g., collagen bundles and elastic fibers). The second type of skin aging is mediated by extrinsic factors such as UV radiation, air pollution, smoking, changes in external temperature, and other agents of skin aging exposome. Photoaging by chronic exposure to UV radiation is the best characterized. Clinical signs of photoaging include dyspigmentation (mostly lentigo and freckling), solar elastosis, actinic keratosis, and seborrheic keratosis. Photoaging is attributed to photo-oxidative damage to skin, mainly by high levels of ROS induced by UV radiation. ROS result in collagen degradation and its accumulation in the dermis, also known as solar elastosis. ROS levels are regulated by anti-oxidant enzymes in skin such as superoxide dismutase (SOD), catalase (CAT), and glutathione (GSH). If anti-oxidant defenses are overwhelmed after extensive UV light exposure, ROS production exceeds the capacity of antioxidant defenses in the skin. This causes oxidative stress, which damages skin cells and alters their gene expression, leading to photoaging, but also promoting cutaneous carcinogenesis (non-melanoma and melanoma skin cancers).

The Constituents of Plant Oils

Plant oils have long been used on the skin for cosmetic and medical purposes because they have been found to have many positive physiological benefits. For example, plant oil application may act as a protective barrier to the skin by an occlusive effect, allowing the skin to retain moisture, resulting in decreased TEWL values. Additionally, topical products have the benefit of higher bioavailability in the skin and having a localized effect rather than systemic effects. Previous research on plant oils have demonstrated that almond, jojoba, soybean, and avocado oils, when applied topically, mostly remain at the surface of skin, without deep penetration into the first upper layers of the SC. Although triglycerides do not penetrate deeper in SC, glycerol contributes to the SC hydration. Free fatty acids (FFAs), specifically monounsaturated FFAs such as oleic acid, may disrupt skin barrier and act as permeability enhancers for other compounds present in plant oils. Other components such as phenolic compounds and tocopherols exhibit an antioxidant effect and may modulate physiological processes such as skin barrier homeostasis, inflammation, and WH. When topically applied to hairless mice, sodium dl-α-tocopheryl-6-O-phosphate, a water-soluble derivative of vitamin E (dl-α-tocopherol), enhances ceramide synthesis and gene expression of differentiation markers (transglutaminase 1, cytokeratin 10, involucrin, and loricrin). Phospholipids, another component of plant oils, mainly fuse with the outer lipid layer of the SC, potentially acting as chemical permeability enhancers. In a study of the murine AD model with given dietary phospholipid

supplementation, phospholipids have been shown to enhance skin barrier and display the anti-inflammatory effect by regulating the covalently bound ωhydroxy ceramides in the epidermis and decreasing the gene expression of both thymus activation-regulated chemokine (TARC) and thymic stromal lymphopoietin (TSLP). Even without penetrating deeper into the epidermis, the occlusive effect of the plant oil topical application decreases the loss of water from the SC and regulates keratinocyte proliferation.

Plant oils can be classified into **essential oils** and **fixed oils**. Fixed plant oil components include triglycerides, FFAs, tocopherols, sterols, stanols, phospholipids, waxes, squalene, phenolic compounds, etc. These different compounds, when topically applied, influence skin physiology (skin barrier, inflammatory status, antioxidant response, and proliferation) differently. Plant oils also vary by the type and the amount of triglycerides and FFAs, e.g., straight-chain saturated fatty acids (SFAs) and unsaturated fatty acids (UFAs). Topical applications of SFAs and UFAs in healthy volunteers showed differences in TEWL and irritant skin response. Since composition and concentration of SFAs and UFAs are important in topical products, it is important to characterize them in each type of plant oil. Particularly, UFAs show different physiological responses when topically applied compared to TEWL. Linoleic acid, for example, has a direct role in maintaining the integrity of the water permeability barrier of the skin. The major metabolite of linoleic acid in the skin is 13-hydroxyoctadecadienoic acid (13-HODE), which possesses anti-proliferative properties. In contrast, oleic acid is detrimental to skin barrier function. Oleic acid causes barrier disruption and eventually induces dermatitis under continuous topical application. In addition to their role in skin barrier restoration/disruption, enriched FFA plant oils have also been studied as penetration enhancers (e.g., transepidermal drug delivery). Research has suggested that oils composed mostly of monounsaturated oleic acid increased skin permeability more than oils containing an almost even mixture of both monounsaturated and polyunsaturated fatty acids. Viljoen et al. has suggested that the lipid penetration within the epidermis follows the order: olive oil > coconut oil > grape seed oil > avocado oil.
Moreover, the concentration of FFAs such as oleic acid with respect to triglycerides correlates with clinical measures of skin barrier function (TEWL). This ratio determines molecular interactions with SC lipids and the extent of their penetration within the epidermis.

Poly- and monounsaturated fatty acids may influence the inflammatory responses either as soluble lipoic mediators or in the form of phospholipids anchored in the cell membrane. Topical applications of linolenic (n-3), linoleic (n-6), and oleic (n9) FFAs can modulate the closure of surgically induced skin wounds. n-9 FFAs induced faster wound closure when compared to n-3, n-6, and control. In fact, n9 FFAs strongly inhibited the production of nitric oxide at the wound site. A mild improvement on wound closure was observed in the n-6 FFA-treated animals, correlating with a peak in nitric oxide production at 48 hours post-operatively. n3 FFAs treatment significantly delayed wound closure, which correlates to a peak in nitric oxide at three hours post-operatively. According to a previous study about the administration of pequi (Caryocar brasielense) almond oil in an acute hepatic injury model in rat, topical applications of poly- and monounsaturated FFAs may have a relevant role and potential therapeutic implication on WH through their modulatory effects on the inflammation rather than effects on cellular proliferation.

Unsaponifiables are also essential in the biological function of plant oils. They have a high potential for antioxidant activity. Antioxidant activity is derived from tocopherols, carotenoids, triterpenes, flavonoids, and phenolic acids that protect from ROS.

Phenolic Compounds

Phenolic compounds are present in all vegetable oils in different concentrations. Phenolic compounds are the main antioxidants found in virgin olive oil, a well characterized oil known for its health benefits. These compounds are very important for the oxidative stability of the PUFAs within the oil. The main phenolic subclasses present in olive oil are phenolic alcohols, phenolic acids, flavonoids, lignans, and secoiridoids. Another plant oil, grape seed oil, contains a large amount of similar phenolic compounds, including flavonoids, phenolic acids, tannins, and stilbenes. The main polyphenols in grape seed oil are catechins, epicatechins, trans-resveratrol, and procyanidin B1.

Figure The potential benefits of plant oil topical application are diverse. Physiological responses are a result of the interaction between the bioactive compounds and the pathophysiological context of the skin.

Conclusions

Topical applications of plant oils may have different effect on the skin according to their composition and the pathophysiological context of the skin. The composition varies by different extraction methods. When applied topically, constituents of plant oils (triglycerides, phospholipids, FFAs, phenolic compounds and antioxidants) may act synergistically by several mechanisms: (i) promoting skin barrier homeostasis; (ii) antioxidative activities; (iii) anti-inflammatory properties; (iv) direct and indirect (upregulation of antimicrobial peptides) antimicrobial properties; (v) promoting wound healing; and (vi) anti-carcinogenic properties. Future studies can add to current findings to allow for better understanding of these oils, with the potential to develop dermatological treatments and skin care products using these oils.

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Organic extra virgin olive oil

1. A Donato–Trancoso; A Monte–Alto–Costa; B Romana–Souza Olive oilinduced reduction of oxidative damage and inflammation promotes wound healing of pressure ulcers in mice *Journal of Dermatological Science* 83(1), July **2016**, 60-69 https://doi.org/10.1016/j.jdermsci.2016.03.012Get rights and content

2. Olive oil accelerated ROS and NO synthesis in mice pressure ulcers.
3. Olive oil decreased inflammatory response and oxidative damage in pressure ulcers.
4. Olive oil improved re-epithelialization loss in pressure ulcers.

5. Olive oil accelerated dermal reconstruction and wound closure in pressure ulcers.
6. Olive oil improved the wound healing process pressure ulcers, in mice.

Very recently in this study investigated the effect of olive oil administration on wound healing of pressure ulcers in mice.

In this Male Swiss mice were daily treated with olive oil or water until euthanasia. One day after the beginning of treatment, two cycles of ischemia-reperfusion by external application of two magnetic plates were performed in skin to induced pressure ulcer formation.

Results

The olive oil administration accelerated ROS and nitric oxide (NO) synthesis and reduced oxidative damage in proteins and lipids when compared to water group. The inflammatory cell infiltration, gene tumor necrosis factor-α (TNF-α) expression and protein neutrophil elastase expression were reduced by olive oil administration when compared to water group. The re-epithelialization and blood vessel number were higher in the olive oil group than in the water group. The olive oil administration accelerated protein expression of TNF-α, active transforming growth factor-β_1 and vascular endothelial growth factor-A when compared to water group. The collagen deposition, myofibroblastic differentiation and wound contraction were accelerated by olive oil administration when compared to water group.

Conclusion

Olive oil administration improves cutaneous wound healing of pressure ulcers in mice through the acceleration of the ROS and NO synthesis, which reduces oxidative damage and inflammation and promotes dermal reconstruction and wound closure.

Graphical abstract

Introduction

Pressure ulcers may be defined as injuries in skin, which are compressed between the bony prominences of the patients and an external surface leading skin necrosis. Pressure ulcer is an important health problem due to their impact on the morbidity and suffering of patients, especially senior citizens and individuals with spinal cord injury. Without appropriate treatment, pressure ulcer may lead to cellulitis, osteomyelitis, sepsis and death. In Brazil, the incidence of pressure ulcer is 40% in surgical units, but higher in intensive care units. Although the etiology of pressure ulcers remains unclear, the cycles of ischemia-reperfusion (IR)-induced injury may be a factor to formation these type of chronic wounds. Previous studies in animal models have showed that repeated cycles of IR in skin causes exacerbated synthesis of reactive oxygen species (ROS) that do not scavenge by the local oxygen radical scavengers inducing elevated inflammatory response and skin necrosis.

The ROS are produced by all cells during the course of aerobic metabolism and for cells in wounded and inflammated tissues. The ROS have a positive role in the wound healing for participating pathogen destruction and angiogenesis process. However, high levels of ROS cause oxidative damage in lipids, proteins and nucleic acids leading to tissue damage. An increase in the concentration of 8isoprostanes, a marker of lipid peroxidation, has been shown

in fluid from chronic venous ulcers. Pressure ulcers present a massive invasion of numerous neutrophils, and their destructive enzymes, leading to tissue destruction. Thus, in chronic lesions, the oxidative damage and persistent inflammatory infiltrate may promote the impairment in healing of these lesions. Therefore, the administration of edible oil with anti-inflammatory and antioxidant proprieties, as olive oil, may be a good therapeutic strategy for promoting healing of pressure ulcers.

The olive oil is rich in oleic acid (C18:1 cis 9) (a n-9 MUFA) and phenolic compounds (hydroxytyrosol and oleuropein), which have antioxidant properties. Topical or oral administration of oleic acid in rats accelerates inflammatory cell migration, pro-inflammatory cytokines and ROS production in acute cutaneous lesions improving cutaneous lesions closure.

The dietary supplementation with olive oil in chronically stressed mice reduces the inflammatory response, lipid peroxidation and protein carbonylation improving the wound closure and collagen deposition. In *in vitro* study, olive oil administration promotes cell migration and collagen deposition and reduces lipid peroxidation in murine skin fibroblast cultures exposed to high epinephrine levels. In clinical trials, the application of a cream with *Aloe vera* and olive oil in pressure ulcers decreases wound size, necrotic area and pain. Another study demonstrates that the topical administration of extra-virgin olive oil prevents the formation of pressure ulcers in immobilized-patients. In burned patients, a diet supplemented with 20% of olive oil reduces the period of the permanence at hospital and promotes healing of second degree burns. However, neither study showed if olive oil administration may promote wound healing of pressure ulcers in mice.
Thus, the aim of this study was to investigate the effect of olive oil administration on wound healing of pressure ulcers using a murine model of IR-induced skin injury.

Fatty acid composition of olive oil.

Components
Myristic acid (C14:0)
Palmitic acid (C16:0)
Palmitoleic acid (C16:1)
Heptadecanoic acid: *cis* (C17:1)
Stearic acid (C18:0)
Vaccenic acid: cis 11 (C18:1)
Oleic acid *cis 9* (C18:1) (n-9)

Linoleic acid: *cis 9,12* (C18:2) (n-6)	
Linolenic acid: *cis 9,12,15* (C18:3) (n-3)	
Octadecatetraenoic acid (C18:4) (n-3)	
Arachidic acid (C20:0)	

Eicosapentaenoic acid: *cis 11* (C20:1) (n-9) 0,4 Arachidonic acid (C20:4) (n-6) ND Behenic acid (C22:0) 0,2 Lignoceric acid (C24:0) 0,2

ND, not detected. All values are expressed in% (w/w) of fatty acid methyl esters.

Results

Olive oil administration modulates the ROS and NO synthesis and reduces oxidative damage

In pressure ulcers, the reperfusion causes a tissue damage and necrosis due to mainly exacerbated synthesis of ROS and NO. Recently, it was demonstrated that olive oil administration is capable to reduce psychological stress-induced oxidative damage in lipids and proteins in cutaneous wound healing of mice. We evaluated if the olive oil administration could reduce the synthesis of ROS and NO and oxidative damage in mice pressure ulcers. The levels of ROS were increased 3 days after ulceration, but decreased 7 days later, in the olive oil group when compared to water group (Fig A). In addition, the nitrite levels were increased 3 days after ulceration, but reduced 7 days later, in the olive oil group when compared to water group (Fig. 1B). To evaluate the oxidative damage, the protein levels of nitrotyrosine, lipid peroxidation and carbonylated protein levels were measured. The olive oil administration reduced the protein levels of nitrotyrosine when compared to water group 7 days after ulceration only . The olive oil administration reduced the carbonylated protein levels when compared to water group 7 and 14 days after ulceration, but the lipid hydroperoxide levels was reduced only 7 days later.

Fig. Effects of olive oil on oxidative damage in pressure ulcers of mice. Mice were orally treated with olive oil or water daily until euthanasia. Two pressure ulcers were created in each mice using ischemia-reperfusion-induced skin injury model. Mice were killed 3, 7 or 14 days after ulceration and lesions were collected and lysated. (A) The levels of reactive oxygen species (ROS) in wound area of olive and water groups 3 and 7 days after ulceration. (B)

The levels of nitrite in wound area of olive and water groups 3 and 7 days after ulceration. (C) Densitometry expressed as arbitrary units (a.u.) for immunoblotting of nitrotyrosine (85 kDa) in wound area of olive and water groups 3 and 7 days after ulceration. The β-actin (42 kDa) was used as loading control protein. (D) The levels of carbonylated proteins in wound area of olive and water groups 3, 7 and 14 days after ulceration. (E) The levels of lipid hydroperoxides in wound area of olive and water groups 3, 7 and 14 days after ulceration. Data (n = 10 lesions per group) are expressed as mean ± SEM. *p < 0.05 vs. water group.

Dermal reconstruction and wound closure is promoted by olive oil administration in mice pressure ulcers

Olive oil increases collagen deposition and wound closure of chronically stressed mice. To evaluate the effects of olive oil administration on dermal reconstruction of pressure ulcers, the myofibroblastic differentiation, active TGF-β_1 expression and collagen deposition were measured. The gene expression of TGF-β_1 was 4X (QR = 4.82) greater in the olive oil group than in the water group 3 days after ulceration. In addition, the protein levels of active TGF-β_1 were greater in the olive oil group than in the water group 3 days after ulceration, but smaller 7 days later . The olive oil administration increased the protein levels of collagen type III when compared to water group 7 days after ulceration, but reduced 14 days later. The protein levels of collagen type I were increased in the olive oil group when compared to water group 14 days after ulceration. The myofibroblasts density was increased in the olive oil group when compared to water group 3 and 7 days after ulceration, but reduced 14 days later .
Effect of olive oil administration in experimental pressure ulcers.

Acute phase (day 3)	Chronic phase (
↑ ROS	↓ ROS
↑ NO (nitrite)	↓ NO (nitrite)
=peroxynitrite	↓ peroxynitrite
=carbonylated protein	↓ carbonylated p
=lipid hidroxyperoxides	↓ lipid hidroxype
=neutrophils	↓ neutrophils
=neutrophil elastase	↓ neutrophil elas
↓ MCP-1	–
=macrophages	↓ macrophages
↓ gene TNF-α expression	–
↑ protein TNF-α levels	↓ protein TNF-α
↑ protein MMP-1 levels	↓ protein MMP-1

–	↑ re-epithelializa
–	↓ necrotic area
↑ blood vessels	↑ blood vessels
–	↑↓ protein VEGF-
↑ gene TGF-β1 expression	–

↑ protein TGF-β1 expression ↓ protein TGF-β1 expression

– ↑↓ collagen type III

Acute phase (day 3)	**Chronic phase**
–	↑ collagen type I
↑ myofibroblasts	↓ myofibroblasts
=wound contraction	↑ wound contrac

ROS, reactive oxygen species; NO, nitric oxide; MCP-1, monocyte chemoattractant protein-1; TNF-α, tumor necrosis factor-α; MMP-1,

matrixmetalloproteinase-1; VEGF-A, vascular endothelial growth factor-A; TGF-β_1, transforming growth factor-β_1.

Discussion

The pressure ulcer are chronic nonhealing lesions that develop mainly in elderly and immobilized patients and enhance the period of hospitalization and the costs of treatment. These chronic lesions are a serious health problem, since there are not effective treatment to promote the wound healing of these chronic lesions. Recently, it was demonstrated that the usual application of olive oil-based formulas could be helpful in preventing the appearance of pressure ulcers in immobilized patients. In addition, the topical administration of *aloe vera* and olive oil cream may reduce the wound size and necrotic area of chronic lesions (mainly pressure ulcers) in patients. However, the mechanism by which olive oil may improve the cutaneous wound healing of pressure ulcers it is still undetermined. Recently, it was also demonstrated that the olive oil administration reduces the oxidative damage and inflammation improving the wound healing of cutaneous lesions in chronically stressed mice. Thus, we hypothesized that the olive oil administration could be a good alternative to improve the wound healing of pressure ulcers due to its anti-inflammatory and antioxidant properties. For this, mice were submitted to experimental model of pressure ulcer based on IRinduced skin injury

and orally treated with olive oil. This experimental model reproduces the IR-induced injury and the role of ROS and cell death prior to necrosis in the development of pressure ulcers. The re-epithelialization is essential event to the closure of chronic lesions, since the impairment of the neo-epidermis formation contributes to nonhealing of these lesions. In experimental pressure ulcers, the delay in the neo-epidermis formation compromises the loss of necrotic area, which delays the wound closure.

The Potential Beneficial Effects of Topical Application of Plant Oils on Skin

Olive Oil

Olive oil comes from the fruits of *Olea europaea* trees. It consists mainly of oleic acid, with smaller quantities of other fatty acids such as linoleic acid and palmitic acid. More than 200 different chemical compounds have been detected in olive oil, including sterols, carotenoids, triterpenic alcohols, and phenolic compounds. Hydrophilic phenols are the most abundant antioxidants of olive oil. The phenolic contents have antioxidant properties higher than those of vitamin E. In fact, these phenolic compounds and their antioxidant activity exhibit anti-inflammatory properties when olive oil is included in regular diet. Unsurprisingly, olive oil has been used as a skin product and hair cosmetic for a long time in several cultures. Studies on mice have shown that topical application of olive oil on pressure ulcers improves WH through the effects of anti-inflammation, reducing oxidative damage, and promoting dermal reconstruction. In rat experiments, wound contraction of full-thickness burns occurred faster with olive oil treatment when compared to the silver sulfadiazine and normal saline (control) group. Studies have also shown that concomitant use of other oils such as buckthorn oil with olive oil have positive effects on the skin. In a randomized controlled trial by Zahmatkesh et al., a mixture of olive oil, sesame oil, and honey was demonstrated to be a useful treatment for burns, by preventing infections, accelerating tissue repair, and facilitating debridement. Moreover, in a murine study with UVB radiation, Ichihashi et al. found that extra virgin olive oil applied to the skin delayed the onset and reduced the incidence of skin cancer development, likely secondary to reduced number of 8-hydroxy-2′-deoxyguanosine (8-OHdG) positive cell formation (a biomarker of oxidative stress and carcinogenesis). It has also been demonstrated that daily consumption of olive oil phenolics protect from DNA oxidation in postmenopausal women and interfere with G1 cell cycle in human colon adenocarcinoma cells and promyelocytic leukemia cells.

In contrast to its positive role in WH promotion and reducing skin cancer development, topically applied olive oil has a detrimental effect on SC integrity and skin barrier function. There is evidence of increased TEWL after topical application to the skin of the forearms of adult volunteers with and without AD. Experiments on mice also elicited similar results. Although skin barrier restoration is a key event in WH, olive oil may promote WH by modulating early phases such as inflammation, and stimulating dermal reconstruction, both of which are not related to subsequent re-epithelialization and the consequent permeability barrier restoration. At the present, it is widely accepted that minor components of olive oil also exert potent anti-inflammatory activities.

Olive pomace oil, a natural by-product of olive oil production, has also been found to contain minor constituents with antioxidant, antithrombotic, and antiatherogenic activities when it is included in the regular diet. However, the effects of olive pomace oil in the **skin have not been characterized yet**.

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Lavandula

Mercedes Pérez–Recalde; Ignacio E.Ruiz Arias; Élida B.Hermida Could essential oils enhance biopolymers performance for wound healing? A systematic review *Phytomedicine*, 38, 2018, 57-65

Millions of people in the world suffer from chronic wounds of different etiologies such as diabetic foot and leg ulcers, without solutions nowadays. Molecules obtained from plants offer an alternative to aid wound healing. Strong evidence about essential oils (EO) anti-inflammatory and antimicrobial properties is thoroughly described in literature and their chemical compositions are well characterized. More recently, EO effects in experimental wounds have begun to be analyzed. We aim to summarize the evidence of EO in experimental wounds, and the possibility of combining them with biopolymers commonly used in skin regeneration.

Treatments with Ess Oils from species of genders Lavandula, Croton, Blumea, Eucalyptus, Pinus, Cymbopogon, Eucalyptus, Cedrus, Abies, Rosmarinus, Origanum, *Salvia* and *Plectranthus,* have shown positive results in rodent wounds. Experimental wounds in rodents have shown faster closure rate, better collagen deposition and/or enhanced fibroblasts proliferation. In blends with biopolymers, several EO combined with chitosan, alginate, gelatin or collagen, were processed to give active films or nanofibers, with antioxidant, anti-inflammatory or antimicrobial activities. Curiously, all of these works were carried out since 2010.

There is significant evidence about the effectivity of EO as wound healers. The incorporation of EO into a polymer matrix that contributes to wound healing is still incipient. However, scientific based evidence of the EO incorporation in resorbable polymeric scaffolds was found and analyzed herein. In summary, EObiopolymer dressings or scaffolds have become promising artifacts regarding wound treatments, especially in chronic wounds, where treating infection and inflammation are still important issues

Challenges in wound healing

Wound healing, triggered by a skin damage, consists of a cascade of biochemical processes carried out to restore the structure and function of the injured or diseased tissue. Four overlapped phases must be accomplished for the restitution of normal skin: blood clotting, inflammation, new tissue formation and tissue remodeling. The last two phases comprise complex mechanisms like cellular proliferation, collagen synthesis and granulation tissue formation as well as matrix degradation and new collagen deposition, concurrently with wound contraction and scar formation. When an extensive dermo-epidermal skin loss— such as a deep burn—occurs, dressings are quite suitable to enhance the first phases of the healing process but not enough to achieve the last two. The challenge to build up an artifact that allows wound healing in both chronic and acute wounds has promoted research and development of dressings and scaffolds during the last decades. It is worthwhile to notice that chronic wounds are considered a major burden worldwide, by the number of affected

people and by the different stages and complexities involved under the label “chronic wound”. In fact, several million people in the world that suffer from chronic wounds, remain stalled typically in the inflammatory phase, affecting their normal activities and diminishing their quality of life. These lesions proceed from different etiologies like diabetic, venous or pressure ulcers and, in particular, chronic lower extremity ulcers: all of them with enormous social and economic implications. For example, the annual cost of caring for chronic wounds in the United States approaches US $25 billion.

Plant molecules effects on skin regeneration

A large amount of surveys and experimental evidence sustain plant beneficial properties on wound healing as well as on a wide range of skin diseases. The effectiveness of those active principles used for wound treatments has been demonstrated rather recently by many biochemical, molecular and pharmacological studies.

Ess Oils is the largest group of secondary metabolites produced by plants. Chemically, EO consist of a complex mix of monoterpenes—10 carbons—and sesquiterpenes—15 carbons—in minor proportion; they can be extracted by water or steam distillation, or by cold pressing in the case of citric fruits. Due to their uses in cosmetics, fragrances and food the chemical composition and effects on skin of EO have been thoroughly studied since decades. In addition, strong evidence of EO anti-inflammatory, antioxidant and antimicrobial effects—three crucial issues in chronic wounds treatment—has been reported In fact, face an infection, the normal healing is disrupted by the inflammatory phase, the wound becomes chronic and promotes a delay in the proliferation phase. Regarding diabetic wounds, *Candida* is the most common yeast that infects them and leads to the delay in wound healing process.
However, despite this background, EO and their main components are scarcely used for wound healing. Therefore, the research work on their effects in experimental wounds is meaning enough to deserve a summary according to their main properties, as stated in Table 1. Before this summary, considerations of the current knowledge of EO cytotoxicity are exposed.
Data were collected and examined using standard procedures. Chemical structures of the EO, details of the animal model, doses and time of administration, histological assessment and biochemical mechanisms highlighted from the chosen articles were analyzed and summarized. Similarly, data about chemical blends between polymers and EO were compiled.

Methodological quality and assessment

Correct positive and negative controls were assessed to guarantee the experiment quality. Linear incision and circular full-thickness excision were the typical wound models; wound contraction rate and complete histological analysis were the experimental methods usually considered for the wound assessment. For the histological analysis, hydroxyproline content and Masson trichrome stain were preferred to evaluate collagen content and maturation within the dermis at different times.
Results: terpenes and terpenoids from essential oils in wound healing
Experimental enhancement of healing process in animal models

Alginate

Regarding both the 1,8-cineole anti-inflammatory and antiseptic properties of 1,8-cineole and alginate fibers ability for caring moderate to highly exuding chronic and acute wounds.

In a recent experimental work, alginate films were separately mixed with ten different EO in order to assess antibacterial and antifungal properties of the films. Sodium alginate was dissolved in hot water (3% w/v) and, after cooling, glycerol was added (1% v/v) as plasticizer. EO from *Helicrysum italicum* (immortelle), chamomile blue, cinnamon, lavender, tea tree, peppermint, eucalyptus, lemongrass or lemon were separately added. Each EO was slowly blended to alginate/glycerol solutions and Igepal 1% was added as surfactant; final solutions were casted and left to dry under ambient conditions. 16%, 50% and 66% of the film dry weight were the used concentrations. Films proved to be stable under different humidity environments and inhibited bacterial and fungal growth, according to the EO type and concentration. The authors suggest that these results might be useful to design novel antimicrobial wound dressings, as well as biodegradable coatings for other medical applications.
H-M Mori, H Kawanami, H Kawahata and M Aok Wound healing potential of lavender oil by acceleration of granulation and wound contraction through induction of TGF-β in a rat model *BMC Complementary and Alternative Medicine BMC series* – 2016 **16**:144 https://doi.org/10.1186/s12906–016–1128–7
Although previous studies have suggested that lavender oil promote wound healing, no study has examined the molecular mechanisms of its effect. In this study, we investigated the effect of lavender oil on various steps of wound healing and its molecular mechanism, focusing on transforming growth factor-β (GF-β).

Methods Circular full-thickness skin wounds were produced on rats. Control solution or lavender oil was topically applied to the wounds on alternating days for 14 days.

Results

The area of wounds topically treated with lavender oil was significantly decreased as compared to that of wounds of control rats at 4, 6, 8, and 10 days after wounding. Topical application of lavender oil induced expression of type I and III collagen at 4 days after wounding, accompanied by an increased number of fibroblasts, which synthesize collagen. Induced expression of type III collagen by topical application of lavender oil was reduced to control level at 7 days after wounding although increased expression of type I collagen still continued even at 7 days, suggesting rapid collagen replacement from type III to type I in wounds treated with lavender oil. Importantly, expression of TGF-β in wounds treated with lavender oil was significantly increased as compared to control. Moreover, an increased number of myofibroblasts was observed in wounds treated with lavender oil at 4 days after wounding, suggesting promotion of differentiation of fibroblasts through induction of TGF-β, which is needed for wound contraction.

Conclusion

This study demonstrated that topical application of lavender oil promoted collagen synthesis and differentiation of fibroblasts, accompanied by up-regulation of TGF-

β. These data suggest that lavender oil has the potential to promote wound healing in the early phase by acceleration of formation of granulation tissue, tissue remodeling by collagen replacement and wound contraction through upregulation of TGF-β. The beneficial effect of lavender oil on wound healing may raise the possibility of new approaches as complementary treatment besides conventional therapy.

The use of complementary and alternative medicines (CAMs) to treat a variety of conditions is increasing, and interest in their potential has been growing all over the world. Aromatherapy, which employs essential oils extracted from various plants and herbs, is widely used and is becoming a major CAM. Among various CAMs, inhalation aromatherapy has especially received attention mainly for its effects of relaxation and improvement of emotional or psychological conditions, and some clinical trials have suggested the potential of aromatherapy for anxiety, insomnia, stress, and pain. Regarding anxiety, efficacy on the clinical outcome is partially supported by a few studies demonstrating an anti-conflict effect of essential oils and a metabolic response to inhalation of essential oils in anxiety model rats. However, despite the reported beneficial effect in such clinical trials, these effects are still controversial because of lack of adequate basic experiments, the small number of recruited subjects, and lack of rigorous analytical methods. Further studies, especially basic studies to elucidate the detail mechanisms of the effects of essential oils, are needed.

In such situations, the researchers' interest in the biological and physiological activities of essential oils has been increased. In in vitro and in vivo experiments, some essential oils were suggested to act as anti-inflammatory, anti-viral, antitumor, anti-hyperglycemic, and anti-carcinogenic agents. In response to these results, targets of aromatherapy and its therapeutic potentials have expanded from emotional and psychological symptoms to various physical diseases. Moreover, these reports unexpectedly suggest that aromatherapy could be effective through not only inhalation, but also topical application of essential oils, and the medical indications of aromatherapy have expanded. Wound healing is one of the expected targets of topical application of essential oils. This has arisen because a more efficient strategy is still required in cases of unsuccessful or deficient repair. Useful CAMs that offer an easy approach, less toxicity and fewer side-effects in combination with conventional therapy are anticipated for severe ischemic ulcers or bedsores that are intractable due to insufficient growth of granulation tissue and lack of blood supply. It is already reported that some essential oils promote wound healing.

Lavender essential oil is expected to have a beneficial effect on wound healing because a few evidences for its effect were already reported. A previous randomized control trial conducted on 120 women demonstrated that treatment with lavender oil significantly reduced pain after episiotomy and redness of incision sites as compared to control. More recently, another randomized clinical trial for episiotomy demonstrated the similar results; significant reduction of REEDA (redness, edema, ecchymosis, discharge and approximation) score and visual analogue scale score for pain, as compared to control. Both clinical trials suggest beneficial effect of lavender oil on wound healing. Also, it was reported that topical treatment with lavender oil on aphthous ulceration showed a significant ulcer size reduction as compared to control in both an animal experiment and a clinical study. Moreover, there is a report evaluating the mechanism of effect of lavender oil on cutaneous wound healing in an animal experiment. This paper demonstrated that wound closure progressed more rapidly with topical application of lavender oil as compared to the control, accompanied by increased expression of PDGF-A and EGF, which are growth factors playing important roles in wound healing process such as tissue remodeling and re-epithelialization.

These clinical trials and animal experiments strongly suggest wound healing potential of lavender oil. However, elucidation of the mechanisms, especially the molecular mechanism, is not enough, and it is still unclear how essential oils act on various parts of the wound healing

process. Thus, in this study we investigated effect of lavender oil on wound healing and its molecular mechanism, using a cutaneous wound animal model. As wound healing process consists of sequential events such as formation of granulation tissue, collagen replacement from type III to type I and wound contraction (wound shrinking), we evaluated the influence of lavender oil on each part of wound healing in this study. Moreover, expression of transforming growth factor-β (TGF-β) was evaluated as a key molecule playing a role in healing of wounds topically treated with lavender oil, because TGF-β is known to regulate proliferation of fibroblasts, collagen synthesis in fibroblasts, production of wound granulation tissue, and differentiation of fibroblasts to myofibroblasts in granulation tissue. Here, we demonstrated the wound healing potential of lavender oil through induction of TGF-β in an animal model.

Methods

Procedure of animal experiment

Male Sprague-Dawley rats obtained from Japan SLC (Shizuoka, Japan), weighing about 250–270 g, were used in this study. The rats were maintained under constant room temperature (20–25 °C) with free access to water and a standard diet throughout the study.

The rats were anesthetized with 1.5 % halothane using an induction chamber and intraperitoneal administration of pentobarbital (0.5 ml/kg). After shaving the hair on their back and cleaning with 70 % ethanol, a circular full-thickness skin wound

(10 mm in diameter) was made in the midline of the back of each animal. Lavender oil (*Lavandula angustifolia* 0.8896 g/ml density) was dissolved up to 1 % in solution containing 0.1 % DMSO and Tween 20 because of its lipophilicity. Rats were randomly divided into three groups: (1) Untreated group; wound surgery only, (2) Control group; wound topically treated with control solution containing 0.1 % DMSO and Tween 20, and (3) Lavender group; wound topically treated with 1 % lavender oil dissolved in control solution. Then, 50 μl of each solution was applied to the wound area just after wound surgery, and each treatment was continued on alternating days till 14 days after surgery. As application of diluted essential oils to the skin or a wound is a popular approach in humans, diluted lavender essential oil (1 % solution) was applied to wounds without any ointment base or oleaginous base, in order to avoid the additional effect of these bases on the wound healing process. Each rat was separated to prevent licking the solution and to avoid serious infection of the wound. The wound area was digitally photographed at 0, 2, 4, 6, 8, 10, 12 and 14 days after wound surgery using a digital camera (Canon Power Shot S200, Tokyo, Japan), then the area was quantified using an image analysis system, Image J (NIH). Measurements were performed in a blind manner.
Each investigator was blinded to group assignment and other data concerning the animals, as well as to the results of the other investigator. Rats were sacrificed by intraperitoneal administration of an overdose of pentobarbitonein, to isolate tissue samples from skin for investigations.

Chemicals and reagents

Lavender oil was purchased from Pranarom, Int. (Ghislenghien, Belgium). Details about the chemical composition of lavender oil are shown in Table 1. The lavender oil we used was extracted by the hydrodistillation method from Lavandula angstiforia ssp. angstiforia. It was a pure essential oil, and no other substances including ointment base were added to the distilled

extract, in order to exclude the effect of other components. Tween 20 and DMSO were purchased from Wako Pure Chemical Industries (Osaka, Japan) and Sigma-Aldrich Co. (St Louis, MO, USA), respectively. All other chemicals were analytical grade. Table 1
Details about the chemical composition of lavender oil

Constituent	%	Constituent
Monoterpene alcohols	47.52 %	Monoterpene hydroc
linalool	43.00 %	trans-β-ocimene
borneol	1.80 %	cis-β-ocimene
α- terpineol	1.02 %	camphene
terpinen-4-ol	0.91 %	limonene
geraniol	0.59 %	others
others		
Esters	34.81 %	Sesquiterpene hydro
linalyl acetate	32.09 %	β-caryophyllene
lavandulyl acetate	1.29 %	β-farnesene
1-octen-3-yl acetate	0.59 %	germacrene D
Hexyl acetate	0.40 %	others
others		Ketones
		3-octanone
		Camphor
		others

Effect of lavender oil on wound healing

Representative photographs of the process of wound healing in each group are shown. The photographs suggest that topical application of lavender oil promotes wound closure, with a reduction in wound area. To perform accurate and quantitative analysis, the area of wound lesions in each group was measured using Image J at 0, 2, 4, 6, 8, 10, 12, and 14 days after wounding (Fig. 2)

There was no significant difference in the wound area between untreated and control rats at each time point. However, wound closure was observed to progress more rapidly with topical application of lavender oil. The wound area of rats treated with lavender oil was significantly decreased as compared to that of untreated rats and control rats at 4, 6, 8, and 10 days after wounding (at day 4, 6, 8: p <0.01 vs untreated and control; at day 10: p <0.05 vs untreated and control. There was no significant difference in wound size at 12 and 14 days. These data suggest wound healing potential of lavender oil in the early phase. Serious infection was not observed in each animal of each group, and it was considered that there was little effect of infection on wound healing in our experiment.

Fig. 1

Representative photographs of transition of wound closure in rat model. Untreated; wound surgery only, Control; wound topically treated with control solution containing 0.1 % DMSO and Tween 20, Lavender; wound topically treated with 1 % lavender oil dissolved in control solution

Fig. 2

Transition of wound area measured by Image J. Untreated (▲); rats with surgery only, Control (■); rats treated with control solution containing 0.1 % DMSO and Tween 20, Lavender (◆); rats treated with 1 % lavender oil dissolved in control solution. Values are mean ± SEM. n = 6 in each group. **: P <0.01 vs untreated, *: P <0.05 vs untreated, ##: P <0.01 vs Sham, #: P <0.05 vs Sham

Proliferation of fibroblasts and synthesis of collagen

Immunohistochemical studies demonstrated an increased number of P4H-positive cells, indicating fibroblasts that synthesize collagen, in wound lesions topically treated with lavender oil as compared to that in wound lesions treated with control solution.

Fig.

Representative photomicrographs of immunohistochemical studies. **a**, **b** Immunohistochemical staining for P4H at 4 days after wounding. **c**, **d** Immunohistochemical staining for type III collagen at 4 days after wounding. Magnification; × 100. Control; wound topically treated with control solution containing 0.1 % DMSO and Tween 20, Lavender; wound topically treated with 1 % lavender oil dissolved in control solution

Also, expression of each collagen was confirmed by RT-PCR. At 4 days after wounding, expression of mRNA of both type III collagen (Col IIIa1) and type I collagen (Col Ia2) in skin tissues of wound lesions topically treated with lavender oil was significantly increased as compared to that of those treated with control solution (p <0.01 vs control), suggesting acceleration of formation of granulation tissue in the early phase of wound healing. Moreover, expression of Col IIIa1 in wound lesions topically treated with lavender oil rapidly decreased to the control level by 7 days after wounding, while significantly higher expression of Col Ia2 by treatment with lavender oil as compared to control was still observed even at 7 days after wounding. This suggests that treatment with lavender oil results in promotion of tissue remodeling by rapid replacement of type III collagen with type I collagen. There was no

significant difference in expression of Col Ia2 and Col IIIa1 between the untreated group and control group (4 days; Col Ia2/GAPDH of untreated: 0.93 ± 0.19, Col IIIa1/GAPDH of untreated: 1.04 ± 0.16, ns vs that of Control, respectively) (7 days; Col Ia2/GAPDH of untreated: 0.93 ± 0.19, Col IIIa1/GAPDH of untreated: 1.08 ± 0.08, ns vs that of Control, respectively)
Fig. 4

Expression of mRNA of type I collagen and type III collagen. **a** Relative mRNA expression of type III collagen (Col IIIa1) at 4 days after wounding. **b** Relative mRNA expression of type I collagen (Col Ia2) at 4 days after wounding. **c** Relative mRNA expression of type III collagen (Col IIIa1) at 7 days after wounding. **d** Relative mRNA expression of type I collagen (Col Ia2) at 7 days after wounding. Control; wound topically treated with control solution containing 0.1 % DMSO and Tween 20, Lavender; wound topically treated with 1 % lavender oil dissolved in control solution. Values are mean ± SEM. n = 6 in each group. *: p <0.05 vs Control, **: p <0.01 vs Control

Expression of TGF-β and differentiation of fibroblasts to myofibroblasts

To investigate the detailed molecular mechanism of the effect of lavender oil on wound healing, we focused on expression of TGF-β, as it is already known to induce proliferation of fibroblasts and synthesis of both type I and type III collagen. Interestingly, as shown in Fig. 5, our ELISA study demonstrated that the protein level of TGF-β in wound lesions topically treated with lavender oil was significantly increased as compared to that in those treated with control solution, at both 4 and 7 days after wounding (at day 4, p <0.05 vs control, at day 7: p <0.01 vs control). There was no significant difference in expression of TGF-β between the untreated group and control group (4 days; untreated: 134.73 ± 3.94 pg/ml, Control: 136.44 ± 12.91 pg/ml, ns. 7 days; untreated:
134.17 ± 1.9 pg/ml, Control: 130.97 ± 4.89 pg/ml, ns).

Fig. 5

Expression of TGF-β protein determined by ELISA. **a** Expression of TGF-β protein at 4 days after wounding. **b** Expression of TGF-β protein at 7 days after wounding. Control; wound topically treated with control solution containing 0.1 % DMSO and Tween 20, Lavender; wound topically treated with 1 % lavender oil dissolved in control solution. Values are mean ± SEM. *: p <0.05 vs Control, **: p <0.01 vs Control. n = 6 in each group
In addition, TGF-β was reported to promote differentiation of fibroblasts into myofibroblasts in wound granulation tissue. Differentiation to myofibroblasts in wound lesions is essential for wound contraction. Consistent with the previous report, an increased number of myofibroblasts positively stained for α-SMA (open arrow heads) was observed in wound lesions topically treated with lavender oil at 4 days after wounding, in the early phase of the wound healing process (Fig. 6). This suggests that treatment with lavender oil accelerates wound contraction by myofibroblasts.
Fig. 6
Representative photomicrographs of immunohistochemical studies. **a**, **b** Wound lesion stained by hematoxylin-eosin staining at 4 days after wounding. Magnification; ×
40. **c**, **d** Immunohistochemical staining for α-SMA at 4 days after wounding. Magnification; ×
200. Control; wound topically treated with control solution containing 0.1 % DMSO and Tween

20, Lavender; wound topically treated with 1 % lavender oil dissolved in control solution. *Gr* granulation, *Nt* normal tissue. Closed arrow heads indicate representative myofibroblasts and open arrow heads indicate representative vascular smooth muscle cells around vasculature

Wound healing is a natural physiological process that develops in response to tissue damage, to restore the function and integrity of damaged skin tissues. The wound healing process is divided into four overlapping phases; blood clotting, inflammation, new tissue formation, and tissue remodeling. These processes, especially new tissue formation and tissue remodeling, consist of sequential and coordinated events including angiogenesis, cellular proliferation, collagen synthesis followed by formation of granulation tissue, matrix degradation followed by replacement of collagen, wound contraction, and scar formation. These healing processes are regulated by a large number of growth factors, cytokines, mitogens and chemotactic factors. Among them, epidermal growth factors (EGFs), insulinlike growth factors (IGFs), platelet-derived growth factors (PDGFs), and fibroblast growth factors (FGFs) are considered to play an important role in wound healing because these growth factors regulate cell migration and proliferation and the synthesis of extracellular matrix proteins, which are essential for formation of granulation tissue. Indeed, there have been several studies of the transfer into wounds of some of these genes to promote wound healing and accelerate wound repair. Besides these growth factors, there has been a focus on TGF-β in the wound healing process as it is considered to have the broadest spectrum of effects.

Lavender oil is extracted from *Lavandula angstiforia ssp. angstiforia* and is popularly used as a CAM in various fields of health promotion. There are many reports suggesting beneficial effects of inhalation of lavender oil on pain, allergic airway inflammation of asthma, anxiety disorder, quality of sleep, and dementia. Besides these expected effects, the influence of topical application of lavender oil on wound healing has already been evaluated. Although previous studies suggested a beneficial effect of lavender oil on wound healing, the detailed mechanisms of the effect have not been fully elucidated. However, there is an interesting paper demonstrating that wound closure progressed more rapidly with topical application of lavender oil as compared to the control, and that expression of PDGF-A and EGF tended to increase, although there was no significant difference as compared to the control. As PDGF-A is known to induce the secretion of matrix metalloproteinases (MMPs) from fibroblasts, this study suggests that lavender oil may accelerate wound closure through a rapid decrease in granulation tissue induced by PDGF and progression of reepithelialization induced by EGF. To our knowledge, this was the only study to refer and suggest molecular mechanisms of the effect of lavender oil on wound healing.

In contrast, in the present study, we demonstrated that acceleration of the formation of granulation tissue in the early phase, rather than a rapid decrease in granulation as previously reported, leads to rapid remodeling by collagen replacement and promotes wound closure. The current study showed that topical treatment with lavender oil increased the number of fibroblasts positively stained for P4H, which catalyzes proline hydroxylation of procollagen and is essential for collagen maturation and synthesis in fibroblasts, and induced expression of both type I and III collagen in wound lesions. The obtained data suggest that topical treatment with lavender oil accelerates formation of granulation tissue in the early phase of wound healing. Moreover, topical application of lavender oil to wounds is suggested to advance collagen replacement from type III to type I, based on our findings that increased expression of type I collagen was observed even at 7 days, although expression of type III collagen rapidly decreased to the control level by 7 days. As formation of granulation tissue consisting of

collagen and replacement of type III collagen with type I collagen are essential for tissue remodeling in the wound healing process, acceleration of formation of granulation and collagen replacement in the early phase is considered to promote wound healing. In fact, the wound area of rats treated with lavender oil was significantly decreased as compared to that of untreated rats and control rats at 4, 6, 8, and 10 days after wounding.

A novel finding of the current study is the induction of expression of TGF-β by treatment with lavender oil. There has been no report of a complication of TGF-β induction in the wound healing activity of essential oils. It has been reported that TGF-β stimulates angiogenesis, proliferation of fibroblasts, and matrix production by fibroblasts, and that it could be one of key molecules in the wound healing process. From these previous reports, TGF-β is considered to play a prominent role in cutaneous wound healing by acceleration of formation of granulation tissue, accompanied by increased production of collagen by fibroblasts. Thus, the upregulation of TGF-β by treatment with lavender oil observed in the present study can rationally explain the proliferation of fibroblasts which synthesize collagen and increased mRNA expression of collagen. TGF-β and collagen are considered to be expressed in a coordinated manner to form granulation in the wound. Also, TGF-β was reported to induce secretion of MMP-13, so-called collagenase-3, by fibroblasts. MMP-13 is essential for degradation of type III collagen, followed by replacement by type I collagen and tissue remodeling in the process of wound healing. In the present study, despite a significant increase in type III collagen at 4 days after the start of topical treatment with lavender oil, it rapidly decreased to the control level by 7 days. Rapid degradation of type III collagen is strongly suggested to be mediated by MMP-13, which is induced by up-regulation of TGF-β. Taken together, these findings indicate that induction of TGF-β by lavender oil functions to accelerate not only the formation of granulation tissue but also the replacement of collagen.

Another important finding of the present study is that topical treatment with lavender oil promoted differentiation of fibroblasts to myofibroblasts in wound granulation in the early phase of wound healing. This can also be explained by up-regulation of TGF-β, because TGF-β has been reported to stimulate differentiation of fibroblasts to myofibroblasts, which play a major role in tissue shrinking/contraction in the wound healing process. Also, it was reported that stimulation of the TGF-β signaling pathway by angiotensin II induces granulation tissue contraction via the angiotensin type 1 receptor.

Thus, the present data also showed that induction of TGF-β by topical application of lavender oil promotes not only formation of granulation tissue, but also wound shrinking/contraction. Moreover, a previous study demonstrated that suppression of type III collagen in the wound area enhanced myofibroblast expression, and that diminished type III collagen promotes myofibroblast differentiation. From this point of view, lavender oil may promote wound shrinking/contraction, because our data demonstrated that rapid degradation of type III collagen was observed in wounds topically treated with lavender oil.

Conclusions

The present study demonstrated that topical treatment with lavender oil significantly increased collagen synthesis by fibroblasts, accompanied by enhanced expression of TGF-β in wound lesions. Also, rapid replacement of type III collagen with type I collagen in wounds treated with lavender oil was suggested by the finding that increased expression of type III collagen

decreased to the control level by 7 days, while type I collagen was not reduced even at 7 days. Moreover, an increased number of myofibroblasts, probably due to upregulation of TGF-β, was observed in the early phase of the wound healing process in wound lesions topically treated with lavender oil, suggesting that treatment with lavender oil also accelerates wound contraction by myofibroblasts. Overall, the present data demonstrated that topical application of lavender oil to wounds accelerates wound healing through 1) formation of granulation tissue by collagen synthesis, 2) tissue remodeling by collagen replacement from type III to type I, and 3) wound contraction (wound shrinking). Of importance, this paper firstly suggests that TGF-β is involved in the mechanism of the effect of lavender oil on wound healing. The beneficial effect of lavender oil on wound healing may raise the possibility of new approaches as complementary treatment besides conventional therapy.

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Organic Calendula Oil

European Union herbal monograph on *Calendula officinalis* L., flos EMA/437450/2017
European Union herbal monograph on Hyperici herba EMEA/HMPC/745582/2009

S Jarić; O Kostić; Z Mataruga;D Pavlović; M Pavlović; M Mitrović ; P Pavlović Traditional wound-healing plants used in the Balkan region (Southeast Europe) J Ethnopharmacol 211, 2018, 311-328

Result

An ethnobotanical analysis showed that 128 plant species (105 wild, 22 cultivated and 1 wild/cultivated) are used in the treatment of wounds. Their application is external, in the form of infusions, decoctions, tinctures, syrups, oils, ointments, and balms, or direct to the skin. Among those plants recorded, the most commonly used are Plantago major, *Hypericum perforatum*, *Plantago lanceolata*, *Achillea millefolium*, *Calendula officinalis*, *Sambucus nigra*, Tussilago farfara and *Prunus domestica.*
The study showed that the traditional use of plants in wound healing is confirmed by *in vitro* and/or *in vivo* studies for *P. major* and *P. lanceolata* (3 laboratory studies for *P. major* and 2 for *P. lanceolata*), *H. perforatum* (5 laboratory studies and 3 clinical trials), *A. millefolium* (3 laboratory studies and one clinical trial), *C. officinalis* (6 laboratory studies and 1 clinical trial), *S. nigra* (3 laboratory studies) and *T. farfara* (one laboratory study) Arora, D.; Rani, A.; Sharma, A. A review on phytochemistry and ethnopharmacological aspects of genus *Calendula* (Review) Pharmacognosy Reviews 7 (13), 2013, 179-187
This review includes 84 references on The genus Calendula (Asteraceae) and comprises ethnopharmacology, morphology and microscopy, phytoconstituents, pharmacological reports,

clinical studies and Toxicology of The prominent species of Calendula. Triterpene alcohols, Triterpene saponins, flavonoids, carotenoids and polysaccharides constitute major classes of phytoconstituents of The genus. A few species of This genus have medicinal value, among These Calendula officinalis Linn., has been Traditionally used in The Treatment of various skin Tumors, dermatological lesions, ulcers, swellings and nervous disorders as well as almost 200 cosmetic formulations, i.e., creams, lotions, shampoos. Despite a long Tradition of use of some species, The genus has not been explored properly. In The concluding part, The future scope of Calendula species has been emphasized with a view To establish Their multifarious biological activities and mode of action.

The Genus Calendula

1. *officinalis* Linn. (Pot marigold) has been traditionally used in the treatment of inflammations of internal organs, gastrointestinal ulcers and dysmenorrhea and as a diuretic and diaphoretic in convulsions. It is also used for inflammations of the oral and pharyngeal mucosa, wounds and burns. *Calendula* is a cleansing and detoxifying herb and the infusion treat chronic infections. The dried flower heads have been used for their antipyretic, anti-tumor and cicatrizing effects. Topical application of infusion of flowers is used as antifungal and antiseptic in wounds, marks, freckles, sprain and conjunctivitis. *Calendula* tea is used as eyewashes, gargles, diaper rashes and other inflammatory conditions of the skin and mucous membranes. Mother tincture of *C. officinalis* is used in homoeopathy for the treatment of mental tension and insomnia.
2. Medicinal properties of *C. officinalis* have been mentioned in Ayurvedic and Unani system of medicine indicating that leaves and flowers are antipyretic, antiinflammatory, antiepileptic and antimicrobial. In traditional and homoeopathic medicine, *C. officinalis* has been used for poor eyesight, menstrual irregularities, varicose veins, hemorrhoids and duodenal ulcers. In the middle ages, *Calendula* flowers were used for liver obstructions, snake bites and to strengthen the heart. It was used in the 18 th century as a remedy for headache, jaundice and red eyes. The plant was employed in the civil war to treat wounds and as a remedy for measles, smallpox and jaundice.
3. Decoction and infusion of *Calendula persica* C.A. Mey aerial parts are employed for the treatment of kidney stones.

Alternative and *complementary* medicinal uses

Among the various species of the genus *Calendula*, *C. officinalis* is the only one, which is extensively used clinically throughout the world. The plant is listed in German Commission E, European Scientific Co-operative on Phytotherapy, British Herbal Pharmacopoeia, World Health Organization monographs for wound healing and anti-inflammatory actions. *C. officinalis* preparations are used in various complementary and alternative medicine systems mainly for burns, cuts, rashes, dermatitis and varicosis. It is also included as part of treatment for dry skin, bee
stings and foot ulcers. The essential oil of the plant is used for soothing central nervous system and as a wound healer.

1. *officinalis* preparations currently in use include carophyllenic ointment (containing carotenoids extracted from the flowers) and pot marigold tincture. It is one of the constituents of proprietary homoeopathic medicine Traumeel ®, used for treating the symptoms associated with acute musculoskeletal injuries including pain and swelling. Otikon otic solution and naturopathic herbal extract ear drops solution, ear drop formulations of naturopathic origin containing *Calendula* flowers, have been reported to be effective for the management of otalgia associated with acute otitis media in children.

Pharmacological Reports

Preparations of *C. officinalis* are mainly applied in the form of infusions, tinctures and ointments as a wound healing remedy for inflammations of the skin, mucous membranes, for poorly healing wounds, bruises, boils and rashes, e.g., pharyngitis and leg ulcers. In the mixed lymphocyte reaction, 70% ethanol extract showed stimulatory effects at 0.1-10 μg/ml, followed by inhibition at higher concentrations. Phagocytosis of human granulocytes was stimulated by polysaccharides isolated from aqueous extract of *Calendula* flowers. Extracts of *Calendula* flowers of differing polarities exhibited anti-oxidative effects on liposomal lipid peroxidation induced by Fe^{2+} and ascorbic acid. Isorhamnetin 3glycosides from *Calendula* flowers inhibited lipoxygenase from rat lung cytosol at a concentration of 1.5×10^{-5} M. In a test system based on porcine buccal membranes, strong concentration dependent adhesive processes were observed with a low viscosity polysaccharide enriched extract (98% carbohydrates) of *Calendula* flowers. These findings suggested that the polysaccharides may contribute to therapeutic effects in the treatment of irritated mucosa. A triterpene enriched fraction given orally to mice inoculated with Ehrlich mouse carcinoma prevented the development of ascites and increased survival time compared to control. Triterpenes such as faradiol and taraxasterol inhibit experimental tumor promotion and are therefore considered as inhibitors of tumor growth. A saponin rich fraction administered orally at 50 mg/kg body weight to hyperlipemic rats reduced the serum lipid level. The aqueous alcohol extract of *C. officinalis* showed central nervous system inhibitory effect with marked overall sedative activity as well as hypotensive effect. The alcohol extract of flowers of *C. officinalis* possesses anti-HIV properties. A cream containing *calendula* extract has been reported to be effective in dextran and burn edemas as well as in acute lymphedema in rats. Activity against lymphedema was primarily attributed to enhancement of macrophage proteolytic activity.
The essential oil of the flowers inhibited the growth *in vitro* of *Bacillus subtilis*, *Escherichia coli*, *S. aureus*, *Pseudomonas aeruginosa* and *Candida albicans.* Acetone, ethanol or water extracts inhibited the growth *in vitro* of the fungus *Neurospora* crass. A flavonoid fraction isolated from the flowers inhibited the *in vitro* growth of *S. aureus*, *Sarcina lutea*, *E. coli*, *Klebsiella pneumonia* and *Candida monosa*. The 50% ethanol extract of the plant showed spermicidal activity in rats at 2% concentration.

Clinical studies

In a randomized, open controlled study, the effects of three ointments were compared after topical treatment of patients with 2 nd or 3 rd degree burns for 17 days: *Calendula* flower

ointment (prepared by digestion in vaseline) (n = 53) or vaseline only (n = 50) or a proteolytic ointment (n = 53). The success rates were considered to be 37/53 for *Calendula* flower ointment, 27/50 for vaseline and 35/53 for the proteolytic ointment. [78] In an open uncontrolled pilot study, 30 patients with burns or scalds were treated 3 times/day for up to 14 days with a hydrogel containing 10% of a hydro-ethanol extract. The symptoms reddening, swelling, blistering, pain, soreness and heat sensitivity were scored before, during and at the end of treatment. Total score and individual scores for each symptom improved. In women with surgical wounds, local application of a mixture containing 70% oily extract of *Hypericum perforatum* and 30% oily extract of Phase III randomized single blinded trial of *C. officinalis* compared with trolamine for the prevention of acute dermatitis during irradiation for breast cancer was conducted. Patients who had been operated on for breast cancer and who were to receive post-operative radiation therapy were randomly allocated to application of either *Calendula* ointment containing 20% of fresh *Calendula* aerial parts in petroleum jelly (126 patients) or trolamine (128 patients) on the irradiated fields after each session. The primary end point was the occurrence of acute dermatitis of grade 2 or higher. Secondary end points were the occurrence of pain, the quantity of the topical agent used and the patient satisfaction. The occurrence of acute dermatitis of grade 2 or higher was significantly lower (41% vs. 63%; P < 0.001) with the use of *Calendula* than with trolamine. Moreover, patients receiving *Calendula* had less frequent interruption of radiotherapy and significantly reduced radiation-induced pain. Clinical examination of an ointment with *C. officinalis* extract was carried out in 34 patients with venous leg ulcer. A total of 21 patients with 33 venous ulcers were treated with ointment, applied twice a day for 3 weeks. Control group that consisted of 13 patients with 22 venous ulcers were treated with saline solution dressings, applied to ulcers for 3 weeks. In the experimental group, the total surface of all the ulcers at the beginning of the therapy was 67,544 mm ^{2} . After the 3 rd week, the total surface of all the ulcers was 39,373 mm ^{2} (a decrease of 41.71%). In seven patients, complete epithelialization was achieved. In the control group, the total surface of all ulcers at the beginning of the therapy was 69,722 mm ^{2} . After the 3 rd week, the total surface of all ulcers was 58,743 mm ^{2} (a decrease of 14.52%). In four patients, complete epithelialization was achieved. There was a statistically significant acceleration of wound healing in the experimental group (P < 0.05), suggesting the positive effects of the ointment with marigold extract on venous ulcerepithelialization.

Toxicology

Although rare, skin contact with *Calendula* preparations may result in an allergic reaction to the herb. Sensitization to *Calendula* and allergic contact reactions have been reported. There have also been incidents of anaphylactic shock after gargling with an infusion of *Calendula*.

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perfoliatum) Comparative Clinical Pathology May 2017, 26(3), 611–615 |

St. John's Wort (SJW) is an herbaceous medical plant. Since ancient times, SJW has been used to treat different kinds of mental and physical diseases and, for its antiseptic, anti-inflammatory and antibacterial properties. The aim of this study was to evaluate the wound healing activity of a phytotherapeutic product from St. John's Wort (SJW), herbaceous medical perennial plant, on skin lesions of different origins, in horses housed in Sicily (Italy). Six horses of different breeds, age and attitude were enrolled in the study. All the lesions were treated with an oil prepared from the aerial parts of *Hypericum perfoliatum* (*H. perfoliatum)*, macerated in oil under the sun. Topical application of the oil determined a significant improvement of skin lesions in all the horses involved in the study. Wound healing begun (Time1) in a period ranging from 2 days to 2 weeks (mean ± SD, 4 ± 1.89 days). The treatment resulted in complete resolution of wounds (Time2) in a period between 1 and 5 weeks (mean ± SD, 14.5 ± 8.2 days), while hair re-growth (Time3) was completed in period between 25 days and 2 months (mean ± SD, 33.25 ± 12.58 days). This study has certainly given a scientific point of view to a century of empiricism about the use of SJW in veterinary practice. The findings of this study seem to suggest that SJW may be considered an "all-in-one" remedy for wound healing, in order to make the management of skin injuries easier even for severe and complicated lesions.

St. John's Wort (SJW) is an herbaceous medical perennial plant belonging to the family of Hypericaceae, genus *Hypericum* (APG III classification) this common denomination is used both for *Hypericum perforatum* (*H. perforatum*) and *Hypericum perfoliatum* (*H. perfoliatum*). SJW grows on dry lands, as high as 1200–1600 m above sea level, in almost all of Europe, in North Africa, in West Asia and in many other parts of the world. The flowering season is between May and August. SJW shows a reddish and branching stem, yellow leaves with black spots and height between 20 and 80 cm. *H. perfoliatum* is higher than *H. perforatum* which, instead, presents a more branching stem. Beyond this, the sepals are smooth in *H. perforatum* and fringed in the *perfoliatum.*

Since ancient times, SJW has been used to treat mild to severe forms of depression, anxiety and in psychiatric illness in general, and as a topical remedy for skin wounds, abrasions and burns, and for its antiseptic and healing properties. Leaves and flowering tops are commonly used for their antiviral, antibacterial (against Gram +), antitumor, antiangiogenic and anti-inflammatory properties. The most common side effects reported after using SJW are photosensitivity and chemical interaction with some drugs, like indinavir, cyclosporine, digoxin, wafarin and others. This can be due to the formation of microparticles, nanoparticles and precipitates or to the induction of the intestinal P-glycoprotein drug transporter and drug-metabolizing enzyme (cytochrome P-3A4, isoenzyme of cytochrome P-450). The main constituents of the aerial part of these plants are the flavonids (luteolin, myricetin, quercitrin, hyperoside, rutin), followed by naphthodianthrones (hypericin, pseudohypericin), essential oil (terpenoids), acylphloroglucinols (hyperforin), phenilpropanes, proanthocyanidins (tannins) and others (carotenoids, amino acids, nicotinamide, vitamin C). However, the greatest medical and therapeutic activity is mainly comprised in two substances: hyperforin and hypericin. Hyperforin is the major lipophilic constituent, even if it is unstable and susceptible to oxidative degradation. It seems to play the greatest role in the antidepressant effect of SJW. However, its anti-inflammatory and anticarginocenic effects are probably due to the inhibition of 5-lipoxygenase and the production of prostaglandin E_2 (PGE_2). It showed the ability to relieve neuropathic pain in a rat model by the inhibition of the protein kinase C (PKC) activity.

The use of topical formulations of SJW (*H. perforatum*) for wound healing, alone or in combination with other herbaceous medical remedy, has been widely described in rats, golden hamsters, humans, in vitro, on cultured NIH3T3 fibroblasts. To the authors knowledge, few scientific works have been carried out on the use of *H. perfoliatum* in any species;, and no scientific evidences exist on the use of SJW as an antibacterial remedy and for wound healing in horses.
In view of such considerations, the aim of the present study was to evaluate the wound healing activity of a phytotherapeutic product from *H. perfoliatum* plants on skin lesions of different origins in horses of different breeds housed in Sicily, Italy.
The ointment was prepared according to an ancient Sicilian recipe. Three hundred grams of the aerial flowering parts of *H. perfoliatum* was macerated in 1 l of biological extra virgin olive oil (Biancolilla-Nocellara, Sicily) under the sun, for 40 days, in transparent hermetically sealed jars. Afterwards, the moisture was filtered through sterile gauzes and placed in dark brown jars with hermetic plastic stoppers, kept in a dark place.

At first, all the skin lesions were cleaned with gauzes soaked in sterile saline or with Marseille soap, in order to remove all traces of dried exudates. In inveterate lesions, a surgical or with hypertonic saline solution debridement was performed, in order to remove any eschar or shreds of necrotic tissues. This pre-treatment was carried out for 3–5 days. After that, the oil was applied with a brush, drawing a thick layer over the entire affected skin surface.

The treatment was carried out once a day, in the afternoon, until complete healing of the wounds. In the next half hour after the medication, licking of lesions and exposure to direct sunlight were avoided. In chronic cases and for very extended wounds, the oil was applied twice a day.

Topical application of the oil was used as the sole therapy. Animals were checked daily for 1 week, then every 2 days, and then weekly.

Results

Topical application of the oil determined a significant improvement of skin lesions in all the horses involved in the study. In all animals, wound healing begun in a period ranging from 2 days to 2 weeks (mean ± SD 4 ± 1.89 days). The treatment resulted in complete resolution of wounds (Time2) in a period between 1 and 5 weeks (mean ± SD 14.5 ± 8.2 days), while hair re-growth (Time3) was completed in period between 25 days and 2 months (mean ± SD

33.25 ± 12.58 days). Only in one horse (horse 9), it was necessary to stop the application of the oil because of the onset of an allergic reaction after 4 days of treatment. In horse 4, in skin lesions not treated with the oil, the healing times were significantly prolonged, with a complete healing after 3 weeks and hair regrowth after 7 weeks, instead of 1 week and 1 month respectively in the ones under treatment. Only in one case (horse 7), after the suspension of the treatment, an alopecic area of 4 × 4 cm was still present. In any cases, a concomitant systemic therapy with antibiotics and/or anti-inflammatory was considered to be necessary.

The optimal wound healing consists in reducing the tissue damage and in providing adequate tissue perfusion and oxygenation in order to restore the anatomical continuity and function of the affected areas. Wound healing proceeds in three phases: inflammation, cellular

proliferation and remodelling. The total extract of the aerial parts of St. John's Wort improves the healing of skin injuries by its antibacterial, antioxidant and anti-inflammatory effects.

The *H. perfoliatum* extract used for the clinical trial of this study has shown the necessary therapeutic effect to control the multifactorial process of wound healing, by synchronizing the physiological processes and eliminating or preventing complications. The antibacterial activity of SJW not only prevented the onset of bacterial complications but also determined the healing of wounds complicated by bacterial infection. Moreover, in the same horse (horse 4), healing times were longer in untreated skin lesions compared with the ones treated with the oil. Treatment of wounds with the oil presented high repeatability of the results, even when used in lesions different for origin, extension and severity.
In addition, animals did not show any discomfort, pain or adverse reaction due to the application of the product. Only in horse 9, the treatment was stopped because of the occurrence of an allergic reaction. However, this horse had previously shown hypersensitivity events to many drugs already used to treat the lesions. Anyway, in the first days of treatment, there was a significant improvement of the lesions. In all the cases of the study, the disappearance of the inflammatory phase, which precedes the step of granulation, took place within 2 ± 1 days of treatment; the sequence of events that characterise the scarring process and the step of re-epithelialisation were completed according to the physiological timing of the equine species. In particular, it was not observed the typical complication of the granulation phase in horses, the keloid. Indeed, the product was effective in the treatment of this injury (horse 6), with relatively short recovery time, although different therapeutic protocols had already been used unsuccessfully. The granulation step was completed for all the wounds within 7 ± 3 days after the beginning of the treatment, even in cases of serious lesions. Only in one case (horse 7), after the suspension of the treatment, an alopecic area of 4 × 4 cm was still present. However, at the first clinical examination, the horse showed a very severe lacerated and contused wound with irregular margins, which went from the hock to the lower third of the right shin of the right hind limb, on the cranio-medial side. The lesion presented a great lose of substance of the skin and subcutaneous layers, with considerable swelling of the surrounding tissues. The area appeared warm and extremely painful.

Pain and itching components were quickly and completely solved, too. This greatly helps the management of wounds in animal deeply sensitive to pain, like the horses. SJW also showed its effectiveness during summer seasonal recurrent dermatitis (*sweet itch*). This is a hypersensitivity reaction (type 1), linked to histamine release consequent to insect bites, in particular those belonging to the genus *Culicoides* that primarily affects horses living outdoors during the warmer months of the year.
Although the literature reports that the stability of SJW oil should last for an indefinite period, our product showed a reduction in effectiveness after 1 year, compared to fresh oil. It is advisable, therefore, to renew it annually to increase the effectiveness of the medication.

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