# The Benefits of Olive Oil Compounds in Healing Burned Skin Lesions

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*Olive oil was used for decades as a skin protective agent against sunburns and for healing skin lesions. The healing properties of chemical compounds contained in olive oil are an interesting research topic. We study herein the healing properties of olive oil on skin burn lesions in two groups of Wistar rats: Group 1 without olive oil treatment (control, n=10) and Group 2, treated with olive oil (n=10). The healing process was monitored by collagen fibers occurrence from the granulation tissue and re-epithelialization of the epidermis. Our results showed that the rats treated with olive oil had a shorter healing time compared to untreated animals (16±2.10 days vs. 22±2.90 days, p = 0.006). Our previous CG-MS studies analysed the presence of fatty acids in olive oil. In this context, we suppose that these compounds are responsible for the healing properties of olive oil on skin lesions. Since skin burns are encountered in surgery clinics and our results showed that the chemical compounds contained in olive oil speed up the healing process of skin burn lesions, we create herein the premises for meaningful studies on healing thermally damaged skin as part of the wound-healing process.*

*Keywords: olive oil components, burned skin lesion, treatment, healing*

The human skin is a complex organ that regulates heat and water loss, at the same time protecting the organism from different toxic substances. When exfoliation or a lesion occurs, the epidermis increases the penetration of active ingredients in the case of topical application [1]. Furthermore, topical application or oral administration of antioxidants has been recently suggested as preventive therapy for skin photoaging and UV-induced cancer [2].

Some authors showed that antioxidant supplements which could prevent the reactive oxygen species signals interfere with the health-promoting and life-span-extending capabilities of calorie restriction and physical exercise in diverse model organisms including mice and rats [3].

Essential fatty acids (EFAs) are long-chain polyunsaturated fatty acids derived from linolenic, linoleic and oleic acids. They cannot be produced in the human body and they have to be consumed through our daily dietary intake. EFAs have also been known as vitamin F. Arachidonic acid is a semi-EFA, as it can be synthesized in the body from linoleic acid. They are present in multiple food sources such as fish and oils, being essential for the synthesis of tissue lipids, playing an important role in the regulation of cholesterol levels and are precursors of prostaglandins [4].

Since olive oil has been used for decades for skin protection against sunburns, based on our previous studies on olive oil we carried out a prospective study to investigate its healing properties on skin burned lesions of Wistar rats. The aim of our multidisciplinary team was to asses the impact of olive oil components on improving the skin healing process, as an important part of shortening the hospitalization and, by consequence, of decreasing the hospitalization costs.

## Experimental part

### *Olive oil sample characterization*

Refined olive oil was used. The oil was analyzed by GC-MS using an Agilent GC 6890N model coupled with a quadrupolar MS 5973. Olive oil samples were pre-prepared for testing using two methods of derivatization: by methylation with methanol and by using bis-(trimethylsilyl)-trifluoroacetamide (BSFTA) as derivatization agent [5, 6].

### *Animals and experimental design*

Our study was carried out on male Wistar rats, weighing 250 ± 10 g. All the animals were individually housed in thermostated (22 ± 2°C) windowless plexiglass cages having constant humidity and controlled lighting conditions (i.e. 12 h of light and 12 h of darkness per day). The animals had free access to tap water. Feeding was done according to laboratory conditions.

Each animal was firstly anesthetized with an intraperitoneal (ip) injection of 5% ketamine (Narkamon inj., Leciva, Czech Republic) in a dose of 90 mg/kg. The hair of the dorsum was deeply shaved with an electric razor and the wound was subsequently induced as described elsewhere with hot water (90°C, 6 s) on the shaved area, resulting in a clearly distinguished burn [7].

Twenty male Wistar rats were randomly divided into two groups, according to the treatment, as follows: group

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1 (control, n=10): rats with an induced skin burn without any treatment and group 2 (n=10): rats with an induced skin burn treated with olive oil (0.2 mg/kg, and twice a day, topically applied).

Experiments were terminated at the time when comparable healed wounds where achieved in the two groups of rats: controls and olive oil treated. The wounds were evaluated histologically according to the method described by Allred and contributors (i.e. - = negative; + = weak; ++= moderate; +++ = strong) [8].

*Skin Sample preparation*

The Wistar rats were anesthetized using i.p. injections of 90 mg/Kg ketamine and 15 mg/Kg xylazine (Rometar inj., Leciva, Czech Republic). Tissue samples were taken when macroscopic healing was achieved and then treated with 10% formaldehyde solution and embedded in paraffin [6]. Tissue sections (5μm) were Haematoxylin-Eosin stained [9]. The tissues were then mounted in Canada balsam. A Leica DM L 32 microscope was used in the study (20 ×10 magnification).

*Statistical analysis*

Values were expressed in median ± standard deviation. Mann-Whitney U test was applied to compare the groups. We used Instat Software (GraphPad Corporation) for analysis. Probability values $< 0.05$ were considered to indicate significant difference.

*Ethical issues*

The experiment was carried out in accordance with the Helsinki Declaration and guidelines of the Ethics Committee of the International Association for The Study of Pain. The publication of results was approved by the institutional board of the Faculty of Medicine from the Ovidius University of Constanta, Romania.

## Results and discussions

*Gas chromatography-mass spectrometry (GC/MS) analysis*

Chemical compounds contained in olive oil were identified by qualitative and quantitative GC/MS analysis. The relative proportions of the compounds were obtained for fatty acids after derivatization by methylation using methanol and derivatization with BSFTA, as a derivatizing agent. Various saturated, mono-, di- and polyunsaturated FAs were found in olive oil. The differences between the FA compositions obtained by the two derivatization methods are showed in table 1.

Palmitic acid was found in similar quantities for both methods applied (i.e. 9.84% vs. 7.93%, 16:0, table 1, entry 1). Palmitoleic acid (16:1n-7, table 1, entry 2) and stearic acid (18:0, table 1, entry 3) were found in smaller quantities (i.e. 0.88% and 1.93%) using the derivatization by methylation in comparison with the second method when derivatization with BSFTA as a derivatizing agent was used (i.e. 4.14% and 10.18%).

Interestingly, the linoleic acid (18:2n-6, table 1, entry 5) and cholesterol (table 1, entry 8) were found only by using the derivatization by methylation method. Moreover, the myristic acid (14:0, table 1, entry 7) was found only by using the derivatization with BSFTA as a derivatizing agent.

Nevertheless, the oleic (18:1n-9, table 1, entry 4) and arachidic acid (20:0, table 1, entry 6) showed to be contained in higher quantities in olive oil (i.e. 1.93 and 18.81% by using derivatization by methylation method and 30.08 and 3.98% by using the derivatization with BSFTA as a derivatizing agent). The 15-isopropenyl-3-oxa-ciclo-pentadecan-2-ona was only 7.96%, and the difference to 100 corresponds to other compounds such as sterols.

Although the beneficial role of olive oil in the Mediterranean diet is mainly due to oleic acid, the presence of other FAs which depend on the cultivar among other factors (such as temperature) needs to be further

**Table 1**

GC-MS PRODUCT DISTRIBUTION AND RELATIVE PROPORTIONS (% OF THE VOLUME FROM TOTAL FATTY ACIDS) OF OLIVE OIL USING DERIVATIZATION BY METHYLATION WITH METHANOL AND DERIVATIZATION WITH BSFTA AS A DERIVATIZING AGENT.

| | | Olive oil (% of the volume) | |
|---|---|---|---|
| Entry | Fatty acid | Derivatization by methylation method | Derivatization using BSFTA method |
| 1. | 16:0 (palmitic acid) | 9.84 | 7.93 |
| 2. | 16:1n-7 (palmitoleic acid) | 0.88 | 4.14 |
| 3. | 18:0 (stearic acid) | 1.93 | 10.18 |
| 4. | 18:1n-9 (oleic acid) | 60.12 | 30.08 |
| 5. | 18:2n-6 (linoleic acid) | 8.10 | - |
| 6. | 20:0 (arachidic acid) | 18.81 | 3.98 |
| 7. | 14:0 (myristic acid) | - | 3.94 |
| 8. | Cholesterol | 0.29 | - |
| 9. | 15-isopropenyl-3-oxa-ciclo-pentadecan-2-ona | - | 7.96 |
| 10. | Sterols | - | 31.79 |

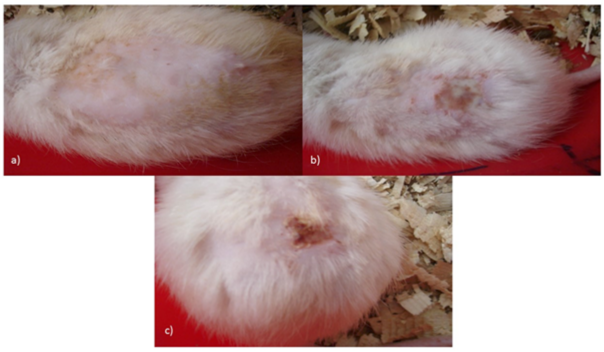

Fig. 1. a) Healthy Wistar rats before the burn was induced; b) untreated Wistar rats 22 days after injury (Group 1) and c) Wistar rats after 16 days of topical olive oil application (Group 2)

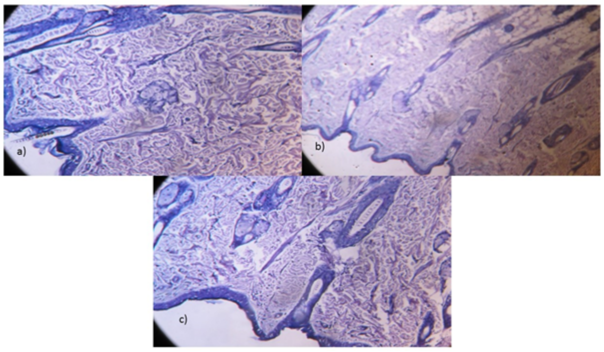

Fig. 2. High-power photomicrographs (20x10, HE) of the tissue sections of a) Healthy rats before the skin burn was induced; b) Untreated rats with skin burns after 22 days (Group 1, control); c) Rats with skin burns treated with olive oil after 16 days (Group 2)

**Table 2**
THE HISTOLOGICAL SCORE OF THE REGENERATED TISSUE WOUND INTO THE TWO GROUPS OF WISTAR RATS

| Group | Histological score |
|---|---|
| Group 1- Control | + |
| Group 2 – Olive oil treated | +++ |

considered. In the same context, it was found that 18:1/ 18:2 ratio of FAs was consistently correlated with the intracellular total triacylglycerol accumulation [10].

Another study showed similar results to ours as far as the content of olive oil is concerned by using ultra-performance liquid chromatography coupled to MS for the identification and quantitation of a few saturated and unsaturated FAs in olive oils from various countries. The total content of FAs in each individual oil was found within the range of 472.63 and 7751.20 µg/mL of olive oil, while oleic acid was found to be the major FA among all analyzed oils with the amount of 3785.94 µg/mL in Syrian olive oil [11].

*Studies of regenerative properties of olive oil*

The potential healing activity of olive oil was investigated in induced skin burns in rats treated with topical application versus controls (fig. 1).

Macroscopic evaluation showed that the tissue of treated rats revealed moderate to complete re-epithelialization with a subepithelial edema. Interestingly, granulation tissue was associated with edema including inflammatory cells which limit the wound margins. Furthermore, there was no evidence of hemorrhage or fibrin accumulation (fig. 1c). In contrast, untreated animals presented only partially recovered skin with a visible scar tissue, edematous and inflammatory granulation tissue without regenerated epithelium (fig. 1b).

Histological examination of the tissues of treated Wistar rats disclosed moderate to complete re epithelialization, minimal intercellular or subepithelial edema, without crusting, while some scar tissue was still visible for control group. Table 2 summarizes the main scoring employed for the histological features of the wound tissue samples.

Compared with untreated animals, those treated with olive oil exhibited an almost completely regenerated skin including epidermis, dermis and hypodermis (table 2, Group 2, +++ scoring). New epithelium was being laid down and newly formed vessels, collagen fibers, and basal membrane could be visualized in provisional fibrin matrix. The inner epithelial layer was almost completely remodeled by day 16 and granulation tissue thickened close to the base of the wound. Skeletal muscle cells looked more like a border between normal and affected tissue compared with untreated rats (fig. 2c).

In contrast, untreated animals presented few newly-formed and immature vessels along with fibrin deposition, hemorrhage, interstitial edema and evidence of generalized vascular congestion. Undeveloped collagenous connective tissue stroma and moderate granulation tissue accumulation with minimal adipose tissue substitution could also be seen (fig. 2b).

The results pointed out that the untreated wounds required a time to heal of approximately 3 weeks (22±2.90 days) to achieve a + score (fig. 2b) whereas the use of olive oil speeded the healing to 16±2.10 days ($p=0.006$) and to achieve a +++ score (fig. 2c, table 2).

Essential FAs are long-chain poly-unsaturated FAs consisting in linolenic, linoleic and oleic acids. Although there is no direct experimental evidence, they cannot be produced by the organism in normal conditions. The important polyunsaturated FAs in conventional beef are linoleic acid (C18:2), alpha-linolenic acid (C18:3), described as the essential FAs, and the long-chain fatty acids including arachidonic acid (C20:4), eicosapentaenoic acid (C20:5), docosanpetaenoic acid (C22:5) and docosahexaenoic acid (C22:6). The significance of nutrition on fatty acid composition is clearly demonstrated when profiles are examined by omega 6 (n-6) and omega 3 (n-3) families [12].

The composition of biological membranes is influenced by the type of fat present in the diet. Thus, diets rich in oleic acid (OA) (such as the Mediterranean diet) are associated with increased levels of this FA in various plasma membranes of rats and humans [13].

Unless indicated otherwise, essential FAs together with ω-9 fatty acids are important for the synthesis of lipids, being a representative of antioxidants. Animal experiments and clinical intervention studies indicate that omega-3 fatty acids have anti-inflammatory properties and, therefore, might be useful in the management of inflammatory and autoimmune diseases [14].

Dietary consumption of certain plants or fish oil is known to modulate the balance of lipid inflammatory mediators and, therefore, is valuable in the treatment of inflammatory skin disorders. It was concluded that nutritional factors exert promising actions on the skin regarding basal skin properties, including hydration, sebum production, and elasticity [15].

Cho and contributors examined the effect of oral administration of the antioxidant mixture of vitamin C, vitamin E, pycnogenol, and oils on ultraviolet radiation-induced wrinkle formation in mouse dorsal skin. The results of the study showed that antioxidant mixture significantly reduced radiation-induced wrinkle formation, accompanied by significant reduction of epidermal thickness, and UVB-induced hyperplasia, acanthosis, and hyperkeratosis [16].

In our previous CG/MS studies we detected only few monounsaturated acids (16:0, 16:1n-7, 18:0, 18:1n-9, 18:2n-6, and 20:0) in olive oil [17]. Since the biosynthesis of FAs within the plant is variable depending on the species considered, the explanation of FAs normally absent in olive oil is indicative of adulteration of the product with oils obtained from different seeds. In particular, some relative percentages of myristic, linolenic, arachidic, eicosenoic, behenic, and lignoceric acids were found in other studies and it was showed that the chromatographic separation on an appropriate capillary column allows separating and quantifying not only the normal (cis) FA, but also their trans-isomers [18].

Our results reveal a link between skin lesions healing and the properties of these compounds found in olive oil. Even if these findings do not yet fully explain the mechanism, the effects of olive oil appeared to be relevant. The Mediterranean diet shows an implication in the chronic inflammatory diseases due to its antioxidant and anti-inflammatory properties, which may not only act on classical risk factors, but also on inflammatory biomarkers such as adhesion molecules and cytokines [19]. In his work, Perona and contributors [20] noted an improvement in endothelial function in 22 hypercholesterolemic subjects fed with a Mediterranean diet.

Our study is relevant from a clinical point of view, too. Skin burns are encountered mainly in burn surgery units. However, they can occur in other surgical specialties as general or plastic surgery, urology, gynecology etc. as well, mainly due to the extensive use of electrocautery. In these situations, the wound healing process can be significantly prolonged. For this reason, the aim of our multidisciplinary team was to asses the impact of olive oil components in improving the skin healing process, as an important part of shortening the hospitalization and, by consequence, decreasing the hospitalization costs.

## Conclusions

In conclusion we present a promising method to support skin lesion healing. Further studies carried on with purified substances are necessary to clarify exactly which of these compounds are responsible in the healing process and which is the exact mechanism involved. The promising results obtained in our prospective, experimental study create the premises for introducing the method in humans, in order to improve the healing of the thermally damaged skin as an important part of the wound healing process.

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Manuscript received: 28.01.2016