MEDICAL APPLICATION OF LIPOPEROXIDE AND OZONIDE DERIVED FROM OZONIZED OILS
The Physiology

*Antibody-catalyzed ozone generation by human neutrophils*

2003. O₃ is present in human physiology !!!!!!

Authors demonstrate the physiological presence of an ozone-similar mediator during inflammation, indicating ozone as a new bio-molecule with striking effects which must be considered and studied following new strategies with newly constructed randomized-standardized clinical studies.

Bernard M. Babior et al. PNAS  March 18, 2003  100(6):  3031–3034

The Mechanism

**DIRECT OXIDATION** (germicide): slowly release of O₃, formaldehyde, trioxolane and lipoperoxides can destroy by oxidation the infective germs. Involve 1,2,4-trioxolane present in the ozonized oil, when added to the warm exudates film of the ulcer, slowly decomposes generating local oxygen, H₂O₂ as reactive oxygen species (ROS), and a trace of lipid oxidation products (4-HNE). Such a cascade can explain the **prolonged disinfectant action** and stimulation of proliferative activity of fibroblasts and keratinoblasts.


**CYTOTOXICITY:** Trioxolane, lipoperoxides and aldehydes are cytotoxic to microorganism; they can inactivate enzymatic pathways by mechanism involved disruption of nuclear mediators.

Inactivation of proteins, unsaturated lipid, peptidoglycans, DNA, RNA, respiratory enzymes

**Lysis / death**


**GROW FACTORS RELEASE:** O₃ and other oxidized oil components can release grow factor from platelets or from the local tissues (increased expression of PDGF, TGF-β, and VEGF) that act as tissue remodeling factors and stimulating proliferative activity of fibroblasts and keratinoblasts.


**OXIDATIVE PRE-CONDITIONING:** local oxidation of tissue by oxidized oil components can stimulate the expression of endogenous antioxidant mechanism (SOD, CAT, GPx) and promote tissue reparation.


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1. Introduction

Early evidence on the clinical use of ozonized oils first appear in scientific literature in 1859. This paper reviews the general and main clinical applications of ozonized oils that have appeared in scientific literature between 1859 - 2014. The oxidation products generated after the reaction of ozone with fatty acids and other substrates can acts as a germicide, immune stimulant and tissue restoration agent. The biological activities and stability of the ozonized oils allows the development of standard formulations that deliver the benefits of ozone, supported by pre-clinical and clinical studies. The main clinical studies that support the use of ozonized oils apply ozonized sunflower oil or ozonized olive oil in different clinical conditions. The applications are essentially for external use, however there is evidence of immune-stimulating and repair effects when used orally (Martínez-Sánchez et al. 2012).

In the world, there are millions of people affected by dirty traumatic lesions, infected wounds, chronic torpid ulcers, bed sores, burns, herpetic lesions, fungal infections and insect stings, who suffer for a long time because the conventional topical treatments based on antibiotics and anti-inflammatory drugs are not sufficiently effective.

Antibiotic-resistant strains of pathogenic bacteria are increasingly prevalent in hospitals and the community. New antibiotics are needed to combat these bacterial pathogens, but progress in developing them has been slow. Historically, most antibiotics have come from a small set of molecular scaffolds whose functional lifetimes have been extended by generations of synthetic tailoring. The emergence of multidrug resistance among the latest generation of pathogens suggests that the discovery of new scaffolds should be a priority (Fischbach and Walsh, 2009). Unfortunately, most physicians and nurses are not aware of the potency and efficacy of ozonated oil (Bocci, 2005).

I would like to predict that the application of ozonated oil, a simple and inexpensive remedy, will become far more useful than expensive pharmaceutical creams and will herald a medical evolution for the topical treatment of topic ulcers and wounds. Under
Interestingly, in spite of its instability, the O$_3$ molecule can be stabilized as an ozonide between the double bonds of a monounsaturated fatty acid such as oleic acid (Bocci, 2002). Ozonation of edible oil is performed by bubbling the gas mixture (O$_2$/O$_3$) for either five min or up to two days, respectively. One gram of oil can bind up to 160 mg of ozone. As a consequence, ozonized olive oil remains stable for 2 years at 4 ºC. This preparation is proving to be ideal for the topical use of O$_3$ in the treatment of chronically infected cutaneous and mucosal areas of the body (Valacchi et al., 2005). O$_3$ is widely recognized as one of the best bactericidal, antiviral and antifungal agents and therefore it is profitably and practically employed as ozonized olive oil with well defined peroxide contents.

The ozonized oil is now used topically for the treatment of war wounds, anaerobic infections, herpetic infections (HHV I and II), trophic ulcers and burns, cellulitis, abscesses, anal fissures, decubitus ulcers (bed sores), fistulae, fungal diseases, furunculosis, gingivitis and vulvovaginitis (Bocci, 2005). Even radiodermatitis lesions in patients with cancer have been found to be beneficially influenced by exposure to a simple application of ozonated oil (Matsumoto et al., 2001).

2. Chemistry of the ozonized vegetable oils

To obtain ozonized oil, edible oil is bubbling with the gas mixture (O$_2$/O$_3$). During the reaction of O$_3$ with the fatty acid present in vegetable oils, lipoperoxides and ozonides (derived in: aldehydes, ketones, peroxides) are formed. In chemistry, especially biochemistry, a fatty acid is a carboxylic acid often with a long unbranched aliphatic tail (chain), which is either saturated or unsaturated. For example, oleic acid is a mono-unsaturated omega-9 fatty acid found in various animal and vegetable sources. It has the formula CH$_3$(CH$_2$)$_7$CH=CH(CH$_2$)$_7$COOH). The term Oleic means related to, or derived from, oil or olive (Fig. 1).
The reaction of $O_3$ essentially with the unsaturated double bond of the fatty acid form different derivates as organic ozonides and lipoperoxides.

**Organic ozonides** are formed by addition reactions of ozone and unsaturated compounds. They are intermediates in ozonolysis and have a *trioxolane* ring structure with a five-membered C-O-O-C-O ring (Criegee, 1975; Diaz *et al.*, 1997) (Fig. 2). They usually appear in the form of foul-smelling oily liquids, and rapidly decompose in the presence of water to carbonyl compounds: aldehydes, ketones, peroxides.

![Fig. 2. Representation of the mechanism of Criegee reaction.](image)

In the generally accepted mechanism proposed by Rudolf Criegee in 1953, the alkene and ozone form an intermediate molozonide in a 1,3-dipolar cycloaddition. Next, the molozonide reverts to its corresponding *carbonyl oxide* (also called the *Criegee intermediate*) and aldehyde or ketone in a retro-1,3-dipolar cyclo-addition. The oxide and aldehyde or ketone react again in a 1,3-dipolar cyclo-addition or produce a stable ozonide intermediate (a trioxolane) (Fig. 3). Evidence for this mechanism is found in isotopic labeling. When $^{17}$O-labelled benzaldehyde reacts with carbonyl oxides, the label ends up exclusively in the ether linkage of the ozonide. There is still dispute over whether the
molozonide collapses via a concerted or radical process; this may also exhibit a substrate dependence.

**Fig. 3.** Representation of the steps of Criegee reaction to form trioxolane.

**Lipid peroxidation** refers to the oxidative degradation of lipids. This process proceeds it most often affects polyunsaturated fatty acids, because they contain multiple double bonds in between which lie methylene -CH2- groups that possess especially reactive hydrogen (Fig. 4).

**Fig. 4.** Formation of lipid peroxide during reaction of unsaturated lipid with ozone.

The reaction of ozone with vegetable oils occurs almost exclusively with the carbon-carbon double bonds present in un-saturated fatty acids producing, in addition to lipid peroxides and ozonides, several oxygenated compounds: aldehydes, diperoxides and polyperoxides;
and these compounds could be also responsible for the wide antimicrobial activity of ozonized oils (Ledeá, 2003).

Unsaturated lipid substrates react with insufflated gaseous O₂/O₃ mixture leading to therapeutically active ozonized derivatives (Figure 5).

![Chemical structures of ozonized derivatives](image)

**Fig. 5.** Representative chemical structures of ozonized derivatives which are formed by chemical reaction of ozone with unsaturated triglycerides. The primary ozonides are transient, unstable species which rearrange in the normal, secondary ozonides also known as Criegee ozonides (Travagli *et al.*, 2010).

In summary, main oxygenated compounds that could possibly be obtained in the reaction of a fatty acid with ozone are: peroxides and aldehydes (Fig.6), (Almeida *et al.*, 2012). The peroxides are the most important products formed. This group includes ozonides, hydroperoxides, polymeric peroxides and other organic peroxides and, probably, is responsible for the wide biological activity of described ozonized vegetable oils (Díaz *et al.*, 2006).
3. Quality of ozonized vegetable oils

From an industrial applicative viewpoint, the overall quality of ozonized derivatives depends upon several parameters, such as: 1) the type and the quality of ozone generators; 2) the ozonation conditions, in terms of reactors and time, material type and amount, presence of water and/or catalyzers; 3) the efficacy of the ozonizer, in terms of O$_3$ concentration output, gas flow, gas carrier. As for the latter, the use of medical grade O$_2$ instead of air is an important point to be considered; in fact, air feedstock (containing about 78% of nitrogen) used for the ozonation of unsaturated substrates could lead to the production of potentially toxic nitrated by-products, and to a significant decrease of the ozonation efficiency. Another important feature is that ozonized oil has to be unequivocally characterized in terms of the species contents as well as the reaction kinetics. For these purposes, the knowledge of the physicochemical properties of ozonized vegetable oils during production has a great importance for their characterization and identification. (Travagli et al. 2010).

The study of the physico-chemical properties of ozonized vegetable oils has great importance for their characterization and identification. For determining the quality of ozonized products, analytical methods such as peroxide, acidity and iodine values, relative density, viscosity are usually carried out.
The peroxide value (PV) represents the quantity of peroxide in the sample; acid value (AV) represents the present free acids; and iodine value (IV) is a measure of total number of double bonds in the sample. All values are well described according to the European pharmacopoeia (Eur. Pharm., 2010) and Official Methods of Analysis of the Association of Official Analytical Chemists (AOAC, 1984 and OACS, 1995).

The peroxide value represents the quantity of peroxide expressed in milliequivalents of active oxygen contained in a 1000 g sample (mEq/kg). In the case of materials characterized by a high peroxide content, some authors determined the PV introducing changes into the method described in the official monograph due the slow iodide reactivity with diallylperoxides (Richaud et al., 2006; Zanardi et al., 2008). In accordance with the official methods of analysis, after addition of potassium iodide, the sample is allowed to stand for 1 min so that the peroxide oxidizes iodide to iodine. During the ozonolysis of sunflower oil, polymeric peroxides and other organic peroxides have been formed, and due the high concentration of peroxides a long reaction time is required for these compounds the oxidize iodide to iodine (Tellez et al., 2006). Some methods include increased reaction time and reflux until from 30 °C to 60 °C. Peroxide content of ozonized sunflower oil using iodometric assay achieved the maximum values at 24 h of reaction time. Other difficult found in the iodometric assay is susceptible to interference by molecular oxygen as well as the reaction of liberated iodine with other components in the systems (Nourooz-Zadeh et al., 1995).

The assay of peroxides concentration is essential in order to establish the therapeutic dose of ozonized oil. The lack of a standardized method adapted to high levels of peroxide is one of the challengers in the quality control of ozonized oil. Table 1 show an example of a quality control report of the sunflower ozonized oil Ozonia 3000®.

Table 1. Chemical and physical characteristics of Ozonia 3000®

<table>
<thead>
<tr>
<th>Analysis</th>
<th>Method</th>
<th>Limits</th>
</tr>
</thead>
<tbody>
<tr>
<td>Density</td>
<td>European Pharmacopoeia</td>
<td>0.98 ± 0.02 g/mL</td>
</tr>
<tr>
<td>Acid Index</td>
<td>European Pharmacopoeia</td>
<td>26.50±5.50 mg KOH / 1 g</td>
</tr>
<tr>
<td>Index of peroxides</td>
<td>European Pharmacopoeia Modified</td>
<td>3000±300 mEq O₂ / kg</td>
</tr>
<tr>
<td>Iodine value</td>
<td>European Pharmacopoeia</td>
<td>160.00 ± 30.00 g / 100 g</td>
</tr>
<tr>
<td>Viscosity</td>
<td>European Pharmacopoeia</td>
<td>625.00±40.00 mPa·s</td>
</tr>
</tbody>
</table>
4. How ozonated oil acts?

How ozonated oil acts? remains an open question. Probably, when the stable triozonide comes into contact with the warm exudates of the wound, it slowly decomposes to reactive ozone, which readily dissolves in water, generating hydrogen peroxide and lipoperoxides that can explain the prolonged disinfectant and stimulatory activity. If it is correct, this reasoning implies that we should have titrated preparations with high, medium or low triozonide concentrations to be used during the inflammatory septic phase I, regenerating phase II or remodeling phase III, respectively. These phases have been related to the rapidly changing cell types and to the release of cytokines and growth factors that modulate the complex healing process (Bocci, 2005).

On the other hand, it has recently been observed that olive oil, which during ozonation traps O_3 in the form of a stable ozonide, when applied to all sorts of acute and chronic cutaneous infections, slowly release O_3 which, in comparison with conventional creams, displays effective disinfectant and stimulatory activities that lead to rapid healing (Valacchi et al., 2005).

It has been demonstrated that antimicrobial effect is not only attributable to the ozonides present in the ozonized oil, but to the all complex mixture of compound derived from the ozonization process (Menendez et al., 2008) like formaldehyde (Guinesi, 2011). After the contact ozonized oil - microorganism it was observed severe alteration of the cytoplasm (Sechi et al., 2001). In addition, application of ozonized oil leads to a significant reduction in amylase, lipase, keratinase and urease enzyme activities in the microorganism in line with a reduction in nucleic acid content (Neveen, 2006).

A recent study (Kim et al., 2008) was undertaken to evaluate the therapeutic effects of topical ozonized olive oil on acute cutaneous wound healing in a guinea pig model and also to elucidate its therapeutic mechanism. After creating full-thickness skin wounds on the backs of guinea pigs by using a 6 mm punch biopsy, authors examined the wound healing effect of topically applied ozonized olive oil (ozone group), as compared to the pure
olive oil (oil group) and non-treatment (control group). The ozone group of guinea pig had a significantly smaller wound size and a residual wound area than the oil group, on days 5 ($p<0.05$) and 7 ($p<0.01$ and $p<0.05$) after wound surgery, respectively. Both hematoxylin-eosin staining and Masson-trichrome staining revealed an increased intensity of collagen fibers and a greater number of fibroblasts in the ozone group than that in the oil group on day 7. Immunohistochemical staining demonstrated upregulation of platelet derived growth factor (PDGF), transforming growth factor-$\beta$ (TGF-$\beta$) and vascular endothelial growth factor (VEGF) expressions, but not fibroblast growth factor expression in the ozone group on day 7, as compared with the oil group. In conclusion, these results demonstrate that topical application of ozonated olive oil can accelerate acute cutaneous wound repair in a guinea pig in association with the increased expression of PDGF, TGF-$\beta$, and VEGF.

Even when the exact action mechanism of the ozonized oil was not described there are much pre-clinical and clinical evidence of it antimicrobial and as wound healing beneficial efficacy. As antimicrobial the most sensible bacteria was *Staphylococcus aureus* and the main resistant *Pseudomonas aeruginosa* (Menendez et al., 2008). A recent *in vitro* study confirms the microorganism sensibility to ozonized oil in that way (from more to less sensibility): *Staphylococcus aureus > Candida albicans > Escherichia coli > Pseudomonas aeruginosa > Enterococcus faecalis* (Zanardi et al., 2013).

In general a lethal effect of ozonized oil is evident when it was applied to multi-resistant strain of *Staphylococcus epidermis*, *Stafilococcus aureus*, also when was applied to fungi from the genus *Trichophyton*, *Epidermophyton* and *Microsporum*, yeast as *Candida albicans* and protozoan as *Giardia lamblia* (Menéndez et al., 2002; Neveen, 2006; Hernandez et al., 2009).

A comparison regarded to the antimicrobial effectiveness of ozonized extra virgin olive oil (peroxide value of 560/590 mEq/kg) with 0.2% chlorhexidine digluconate and 10% povidone-iodine through a disk diffusion test was done recently (Montevecchi et al., 2013). Ozonized oil shown a significant better behavior that the references. This effects on one of the main periodontal pathogens, suggest its potential applicability for periodontal treatment (Montevecchi et al., 2013).
The wound healing action mechanism of ozonized oil may be connected in part to its antimicrobial effect, but also with its ability to promote the liberation of gown factors (Schulz, 1981), activate local antioxidant mechanism (Zamora et al., 2007, 2008) and promote tissue reparation (Silveira et al., 2007).

The theoretical sequence of wound healing has been schematically represented to happen in three successive stages. The scheme presented in Figure 7 shows three phases: Phase I indicates the inflammation stage, normally lasting 2-3 days. The bacterial infection successive to a trauma, diabetes, local ischaemia and possibly antibiotic resistance, can become chronic unless and can be intervene with ozonized oils. Phase II corresponds to the intermediate stage and normally lasts two weeks. The synthesis of extracellular matrix (fibronectin, collagen III/I, hyaluronic acid and chondroitin sulphate) is accompanied by an active proliferation of fibroblasts and keratinocytes. The use of ozonized oil, not only prevents a superinfection, but stimulates the initial tissue reconstruction. Phase III, includes the final healing and scar tissue remodeling and may take a long time in elderly and/or diabetic patients. In some cases, excessive release of Transforming Growth Factor (TGF beta 1) may stimulate excessive fibrogenesis with cheloid formation (Bocci, 2005).

Sunflowers ozonized oil (Peroxide value 75 mEq/kg – 100 mEq/kg, Campanati et al., 2014), topically applied for 12 weeks in a total of 30 patients suffering from second-degree skin burns in the phase of reepithelisation were study recently (Campanati et al., 2013). Skin burn was subdivided in two symmetrical parts. One part was treated with occlusive application of ozonized oil; the contralateral part of the lesion was treated with topical application of hyaluronic acid gel, once a day for 12 weeks. Ozonized oil was more effective than hyaluronic acid in reducing symptoms related to skin burns, but it could be more effective in preventing the post-lesional hyperpigmentation (Campanati et al., 2013).
Fig. 7. Probably action mechanism of ozonized oil during wound healing. The entire wound healing process is a complex series of events that begins at the moment of injury and can continue for months to years. Ozonized oil component may act in different step of the wound healing proceed by different action mechanism. However, the word *ozonated* is itself without scientific meaning if it is not associated with *how much* peroxides are present in the oil. In fact, from a therapeutic point of view, the ozonide compositions have the capacity to deliver active O₂ and/or other useful species deep within the lesion without causing primary skin irritation. The few studies concerned with the therapeutic effects of ozonated oils on acute cutaneous wound healing in animal models do not investigate the dose/effect response, expressed as the amount of peroxides existing in the ozonated derivative used (Kim *et al.*, 2009). Recently, a quantitative evaluation of the therapeutic effect of topically applied ozonized sesame oil on acute cutaneous wound healing in mice as animal model has been developed (Valacchi *et al.*, 2010). The results indicate that both low (<1000) and high doses (>3000), as expressed in terms of peroxide value, delay cutaneous wound healing. Such evidence is reinforced by a
number of results between groups where the «middle» concentration (about 1500) has the most beneficial effect in accelerating the wound closure ratio.

In summary, the probably action mechanism of ozonized oil should be due to: 1) **Direct oxidation (germicide)**: slowly release of O₃, formaldehyde, trioxolane and lipoperoxides can destroy by oxidation the infective germs (Sechi et al., 2000; Valacchi et al., 2005; Guinesi et al., 2011). Is hypothesis that the antimicrobial mechanism of ozonized oil involve 1,2,4-trioxolane present in the ozonized oil, when added to the warm exudates film of the ulcer, slowly decomposes generating local oxygen, H₂O₂ as reactive oxygen species (ROS), and a trace of lipid oxidation products (4-HNE) (Zanardi et al., 2013). Such a cascade can explain the prolonged disinfectant action and stimulation of proliferative activity of fibroblasts and keratinoblasts (Valachhi et al., 2011).

2) **Cytotoxicity**: Trioxolane, lipoperoxides and aldehydes are cytotoxic to microorganism; they can inactivate enzymatic pathways by mechanism involved disruption of nuclear mediators (Neveen, 2006). 3) **Grow factors Release**: O₃ and other oxidized oil components can release grow factor from platelets (Bocci, 2005) or from the local tissues (increased expression of PDGF, TGF-β, and VEGF) that act as tissue remodeling factors (Kim et al., 2008). 4) **Oxidative pre-conditioning**, local oxidation of tissue by oxidized oil components can stimulate the expression of endogenous antioxidant mechanism (Zamora et al., 2007, 2008) and promote tissue reparation (Silveira et al., 2007).

5. **Uses of ozonized oils in dermatology**

As soon as the medical community will appreciate their efficacy, ozonized oil will become indispensable tools in chronic wound healing units (Bocci, 2005).

The germicidal properties of the ozonized vegetable oil It has been already established. The ozonized vegetable oils have been used in the treatment of microbial infections of the skin (dermatitis, sores, infected wounds, fistulas, acne, infected burns and ulcers), in the treatment of nasal, ear and vaginal infections (U.S. Pat. No. 984,722, U.S. Pat 5,270,344, U.S. Pat 5,364,879, U.S. Pat 2,356,062; U.S. Pat 3,504,038) and in post-operative disorders. They have been also used in the treatment of gastroduodenal ulcers (Pat. WO
01/37829 A1), against intestinal infections (U.S. Pat 5,364,879) or erysipelas (Pat RU 2040235 A) they have been recently used in the treatment of the *Giardia lamblia* (Pat. WO 01/37829 A1) Tynea Pedis, recidivating genital *Herpes simplex*, *Helicobacter pylori*, infection and in external hemorrhoids and bedsores (Menendez et al., 2005). Treatment of asthma, gastroduodenal ulcers (US 925590 A), treatment of infections caused by pinworms, genital herpes simplex, human papilloma virus (HPV), and fungi, such as microorganisms of the genus *Candida* (WO 03/085072 A1).

The ozonized vegetable oils and fats have been also used in cosmetics. Since the 1950s, in France, the ozonized solutions have been used as cosmetics, directly on the skin or in baths, as stimulants, purifiers, as decongestant, tranquilizers and regenerating substances of the epidermal tissue. The properties for stimulating the tissue regeneration, the oxygenation of the cells and tissues and the moderated whitening properties are added to the acknowledged germicidal activity of the products from the ozonation of unsaturated compounds, such as terpenes, fatty acids, triglycerides and vegetable oils in the cosmetic applications. The highly oxygenated compounds, such as the ozonized vegetable oils, favor the flexibility and the softening of the skin and is used also to prepare creams for repairing the epithelial tissue (Pat. WO 01/37829 A1).

How and when ozonized oils are used? Chronic wounds range from diabetic foot to putrid and deep ulcers due to limb atherosclerosis, or trauma and burns. Moreover, both immunosuppressive chemotherapy and/or malnutrition cause abscesses, anal fissures and fistulae, bed sores, furunculosis, and osteomyelitis which are difficult to treat and often fail after prolonged treatments. About 7 million patients in the United States are affected with a cost over US$ 25 billion annually. Various types of disinfectants, antibiotics, antifungal, antiprotozoal, and growth factors are scarcely effective because the deranged metabolism and local hypoxia are not modified. Several other approaches such as vacuum therapy, maggot therapy and devices for providing topical oxygen therapy in a clinical setting have been proposed and variably used. This last approach has a rationale in the sense that enhanced oxygenation is useful for activating the metabolism and cell proliferation of ischemic tissues. However, it has also considerable limitations because it is a cumbersome
therapy, with minimal disinfectant activity and modifications of the fundamental pathogenetic mechanisms (Travagli et al. 2010).

Ozonized oil preparation is proving to be ideal for the topical use in the treatment of chronically infected cutaneous and mucosal areas of the body. In addition, it was used to reduce the muscular fatigue (Paoli et al. 2011). Ozonized oil has been applied, in human pathologies involve germs, some example of clinical trial are the following:

5.1 Bacterial infections

Application of the Ozonized Sunflower Oil in Periodontitis: In this study, we used ozonized sunflower oil in order to evaluate their effect on the treatment of moderate simple periodontitis and for preventing its recidivation. A random, controlled and single-blind phase III clinical trial was performed in 84 patients, older than 35 years, from both sexes. Ozonized sunflower oil was topically applied to 42 patients on the operated area and on the 7.sup.th, 14.sup.th and 21.sup.st days after operation on the adjacent periodontal tissues. The control group was formed by 42 patients that received the conventional treatment with chlorhexidine (aqueous solution 0.2%). An analytic index of hygiene, clinical and radiographical tests and microbiological controls was applied to the patients, at the beginning, on the 21.sup.st, 90.sup.th and 180.sup.th days and with intervals of 1 month until 9 months after operation.

The effectiveness of the treatment after 180 d was considered as: good (satisfactory clinical and microbiological assessments) in 98 % of the patients of the group treated with ozonized sunflower oil and in 78 % of the control group; fairly good (some of the assessments were not satisfactory) for 2 and 17 %, respectively, and in the category of bad (both assessments were not satisfactory) only 5 % appears in the control group. Recidivation was more frequent in the control group (15 %) than in the group treated with ozonized sunflower oil (5 %). In general, the best clinical results (best evolution and healing during the study) and microbiological results were obtained in the group treated with ozonized sunflower oil, and also a lower percent of recidivation was found in that group. No side effects were observed (CU patent 22749; Menendez et al., 2008).
Application of the Ozonized Sunflower Oil in the Treatment of Alveolitis: The ozonized sunflower oil was used as the only drug in the treatment of alveolitis. The results were compared to those corresponding to a control group, where Alvogil® (iodine) was used as local treatment, besides applying an oral antibiotic. The sample was formed by 100 adult patients, randomly distributed into two groups, with 50 patients each. Healings were performed every 72 h and visits to the doctor were carried out as required. The healing criterion considered was the formation of a healing tissue and the decrease or elimination of the pain. The healing was reached in 43% of the patients treated with ozonized sunflower oil and in 41% the patients treated with Alvogil®, without any significant differences between both groups. However, patients treated with ozonized sunflower oil healed most rapidly and they only required two or three visits to the doctor, regarding the patients healed with Alvogil® that required four to six visits to the doctor (CU patent 22749; Menendez et al., 2008).

Treatment of the Acute Ulcer-Necrotizing Gingivitis with Ozonized Sunflower Oil: A random phase III clinical assay was performed in a group of 48 patients suffering from acute ulcer-necrotizing gingivitis. From those patients, 24 formed the group with ozonized sunflower oil, by topical applications on the lesions, three times a day, for 7 d. The control group (24 patients) was treated with local applications of aqueous solution of sodium perborate, as risings, with similar periodicity to that of the group treated with ozonized sunflower oil. The tests were performed 3 d or 7 d after the beginning of the treatments. In the group treated with ozonized sunflower oil, 75% of the patients were healed compared to the control group that reached 29.2%, with a significant difference (p <0.01). Regarding the signs and symptoms assessed: gingival bleeding, signs of local acute swelling and gingival pain, they disappeared more rapidly in the group treated with ozonized sunflower oil (CU patent 22749; Menendez et al., 2008).

Application of the Ozonized Sunflower Oil in the Treatment of Infected Radicular Ducts: The sample was constituted by 200 adult patients presenting radiolucent rarefaction areas,
with or without fistulas in monoradicular teeth. The patients for the study were allocated at random. The sample was distributed into two groups of 100 patients each. The test group received healings with ozonized sunflower oil, by sterile cotton balls impregnated with the oil and put it in the cavity, at the entrance of the ducts.

The change of the cure was performed every 48 h. In the control group, the healing was made at the same place and similarly, using a liquid bactericide (Cresophen®). In this group, a similar application was performed seven days later. Radiological and clinical tests were carried out to the patients at the start and at the end of the treatment. In the group treated with ozonized sunflower oil, the results were better, with 91 % of improvement compared to the control group (55 %) with significant differences (p<0.01). 88 % and 5 % of the patients healed with ozonized sunflower oil and Cresophen®, respectively, showing significant differences between both groups. Patients treated with ozonized sunflower oil needed two or three visits to the doctor's office, while most of the healed patients of the control group required four to six visits to the doctor's office (CU patent 22749, Menendez et al., 2008).

In addition I was recently demonstrated, in animal models, that topically-applied ozonized oil, may has a positive influence in bone density and in the quality of osseointegration around dental implants (El Hadary et al., 2010).

Comparative Study of the Effect of the Ozonized Sunflower Oil in Gingivostomatitis in Relation with Conventional Treatments: One hundred sixty children suffering from aphthous gingivostomatitis, between 0 and 15 years old were treated. The clinical symptoms of the children were fever, marked anorexia, salivation, gingival pain, asthenia and uneasiness of several days of evolution. The experimental group (60 children) were daily treated with touches of ozonized sunflower oil and the control groups with three different products (by following a similar procedure that the used for the experimental group): iodoxuridine (60 children), hibitane (20 children), boroglycerine (20 children). Between the third and the seventh days of treatment, the complete healing of the lesions was reached in 75 % of the patients treated with ozonized sunflower oil and in 6 % of the patients from the control group, with statistically significant differences (p<0.001), regarding
the other control treatments applied in a similar period of time (CU patent 22749; Menendez et al., 2008).

**Application of Ozonized Sunflower Oil in Acute Tonsilitis:** Fifteen patients suffering from acute tonsilitis were studied and daily treated with ozonized sunflower oil in the oropharyngeal area for a week. Microbiological controls (pharyngeal exudate) and physical tests of the oropharyngeal area were performed to those patients at the beginning and at the end of the treatment. Among the microorganisms in the first exudate we found *Streptococcus pyogenes*, *Haemophylus influenzae*, *Bordetella pertussis*, and others. At the end of the treatment, all patients were cured, taking into account the microbiological and clinical tests performed (CU patent 22749; Menendez et al., 2008).

### 5.2. Viral infections

**Application of the Ozonized Sunflower Oil in the Treatment of the Acute Herpetic Gingivostomatitis:** This study covered the treatment of 113 patients with antecedents of acute herpetic gingivostomatitis, and they were daily treated with ozonized sunflower oil. In 76.9 % of those patients, the symptoms disappeared after a three-day treatment; in 20.4 %, they disappeared on the seventh day of treatment and in 2.7%, the symptoms disappeared on the tenth day. The microorganism most frequently isolated in the lesions was the *Staphylococcus aureus* (CU patent 22749; Menendez et al., 2008a). An additional study involved 2596 patients, the efficacy was 92.7 % (2007 patients cured) with 0.3 % of adverse reactions (Menendez et al., 2008b).

**Ozonized Sunflower Oil in the Treatment of the Infection Caused by the Human Papilloma Virus:** Sixteen women with the human papilloma virus (HPV) in the vagina or in the cervix were studied and treated with embrocations of ozonized sunflower oil on the affected areas, using the speculum for the curing. The treatment was daily performed for 15 d. The results, by colposcopy and cytology, showed an effectiveness of 94 %. Application of Ozonized Sunflower Oil in the Treatment of Lower Limb Ulcers Caused by Chronic Venous Insufficiency (CU patent 22749; Balkanyi, 2006; Menendez et al., 2008).
Ozonized Sunflower Oil in the Treatment of Epidemic Hemorrhagic Conjunctivitis (EHC).

EHC is a self-limited, conjunctiva inflammation of viral etiology which affects all ages and takes place in epidemic form. Its main symptoms are sensation of foreign bodies, lacrimation, photosensitivity, general discomfort and pain. Its critical signs are subconjunctival hemorrhages, follicular reaction and pre-auricular adenopathy. Also, serous secretion, chemosis, superficial punctate keratitis and palpebral ptosis are observed. Taking into account the broad spectrum germicide of ozonized sunflower oil, as well as its degree of anti-inflammatory character, a study evaluate the effectiveness of this medication in its collyrium form for the treatment of EHC. In “Dr. Salvador Allende” Clinical Hospital, 20 patients were treated with EHC in October, 2009. Twelve of them received treatment with ozonized sunflower oil collyrium (one drop twice per day) and 8, was used as control group, and received conventional treatment (cold compresses, non steroidal antiinflammatory drugs, yodoxuridine in collyrium or recombinant alfa-2b interferon). All patients treated with ozonized sunflower oil underwent a fast evolution toward recovery. In 72 h, they showed signs of great improvement and in 1 week they were totally cured. No patients presented any complications. In the control group the evolution was more prolonged, mainly in patients showing complications (3 with keratitis). Treatment of EHC with ozonized sunflower oil collyrium provides very positive results in this disease (Copello et al. 2012).

5.3. Fungal infections

Application of Ozonized theobroma Oil in the Treatment of Tynea Pedis: Fifty patients with a diagnosis of tynea pedis, randomly distributed into two groups of study, 25 patients in each group, were studied. The experimental group was treated with an ointment containing 20 % ozonized theobroma oil, for 6 weeks, twice a day and the control group was treated with Whitfield ointment with no sulfur with a similar plan of treatment. The healing criterion was the presence of negative microbiological exudate.

A healing of 85 % and 20 % in the experimental and control groups, respectively, with significant differences between both groups was obtained (CU patent 22749; Menendez et al., 2008).
Sun Flower Oil and Olive ozonized oil are fungicide, active against fungi, produces of superficial mycosis in human, such as *Candida albicans*, *Trichophyton mentagrophytes*, *Microsporum canis*, *Thichophyton rubrum* (CU patent 22749; Balkanyi, 2006; Menendez et al., 2008a). Topical Sun Flower ozonized oil was evaluated in a controlled randomized phase III assay, using ketoconazole (Nizoral®) as the comparing group. The results demonstrated no significant differences between the two medications, nor side-effect or bacterial superinfection were observed in the study (Menendez et al., 2008a).

5.4. Mix infections

Application of Ozonized Sunflower Oil in the Treatment of Lower Limb Ulcers Caused by Chronic Venous Insufficiency: A study was performed with 20 patients with lower limb ulcers caused by chronic venous insufficiency with less than five years of evolution. Both groups were treated with venous rest, hyposodic diet and analgesic drugs. Besides, a mechanical disinfection with benzalconium chloride 1/5000 was performed twice a day. After disinfection, ozonized sunflower oil was applied to the experimental group and antibiotic ointments, according to the isolated germ, were applied to the control group. An amelioration of the inflammatory signs after 72 h and the appearance of granulation tissue after the fifth day were observed in the experimental group, whereas in the control group, both the evolution and the disappearance of signs and symptoms lasted more (CU patent 22749; Menendez et al., 2008).

Application of the Ozonized Sunflower Oil in the Treatment of Bedsores

Twenty patients suffering from bedsores in the sacral region were studied and randomly distributed into two groups of 10 patients each. The experimental group was treated with ozonized sunflower oil, twice a day, and the control group was treated with ointments, according to the germ present, with a similar plan of treatment. All the patients succeeded in the healing of their wounds. In the group treated with ozonized sunflower oil, the time of healing was shorter and it was not necessary to perform any anti-biogram, because of its wide germicidal power (CU patent 22749; Menendez et al., 2008).
Application of the ozonized oil in the treatment of fistulae and chronic surgical wounds: In an study involved 28 patients suffering from fistulae and chronic surgical wounds a fully effective in 27 cases without side effect was found (Matsumoto et al., 2001). Ozonized oil was also used the treatment of osteonecrosis of the jaw in patients with bone metastases (Ripamonti et al., 2011). Ozonized oil has also proved to be very effective in burns (Bocci, 2005). In addition Ozonized oils are used for the long-term treatment of injuries, burns and local infections such as skin and nail mycosis, as well as in the follow-up treatment of ulcus cruris and decubitus ulcers (Beck et al., 1995).

5.5. Protozoa

Ozonized sunflower oil showing inhibition and lethal activity in case of Giardia lamblia infections (Mirabal et al., 2002; CU 22749 A1).

6. Toxicology

The diverse tests performed with ozonized sunflower oil showed the safety of this kind of products: toxicological tests, histological tests, mutagenic tests, genotoxic tests and teratogenic tests (Menendez et al., 2008). In clinical assays using ozonized oil in the treatment of infective lesion, side-effect was not reported (Matsumoto et al., 2001; Valacchi et al., 2005).

7. Uses

Wide range of antimicrobial effect. Useful to treat topical fungi, bacterial and virus infections. Useful in the treatment of bedsores and in prophylaxis of diabetic food.

8. Contraindications

Allergy to formula components.
9. Warnings

Keep away from eyes.

10. Interactions, incompatibilities

Interactions: None well documented.
Incompatibilities: Do not mix ozonized oil with any other drugs or cosmetic.

11. Adverse reactions

Dermatologic: Skin rashes (rarely), skin-burning sensation, pruritus and erythema (0.3%).

12. Intoxication / first aid

Wash affected area and suppress treatment immediately.

13. Route / Doses

Topical apply lightly to affected area twice daily or as prescribed by the physician. Before the application, the damaged skin surface must be cleaned by removing necrotic tissue, pus, loose fibrin deposition, and excess of fluid exudates. Use smallest amount possible. Continue treatment to maintain remission.

14. Quality

A quality ozonized oil to be used with medical purpose should be prepared follow the good manufacture practice. That mean a strictly quality control during it production in a high quality reactor by fixing the quality of the raw materials and important reaction variables as: time of reaction, ozone concentration, ozone sources, burbling size, reaction temperature and others.

A quality control of the active component (ozonized oil) should involve chemical-physical analysis, microbiological analysis and biological analysis. Biological analysis should be demonstrated the pharmacological effect attributed to the oil and the absence of toxicity. Microbiology should demonstrate the microbiological quality of the preparation. Chemical /
physical analysis will be done to guaranty the homogeneous chemical content of active component and the stability.

Chemical analysis will involve the measurement of the content of lipoperoxides and aldehydes, iodine and saponification indices. Physical analysis will take into consideration the acid values, density and viscosity of the active component. Test will be do according to the pharmacopeia methods and should be also used to demonstrate the stability of the preparation (Sega et al., 2010).
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