Websites:
http://www.sciencepub.net
http://www.sciencepub.net/stem

Emails:
editor@sciencepub.net
sciencepub@gmail.com

Stem Cell

MARSLAND PRESS
Multidisciplinary Academic Journal Publisher

Formulation and clinical evaluation of ozonated olive oil for the treatment of acne vulgaris lesions

Xio Khaoshi*, Choan Zhang

Department of Pharmacy, Nankai University, Tianjin, China
khaoshixio@gmail.com

Abstract: The ozonides produced by ozonation procedure have significant killing effects on bacteria, fungi and yeasts. They have effects on activation of local microcirculation, tissue growth, and on other processes involved in the treatment of skin disorders. The purpose of present study is to formulate and evaluate the healing effects of ozonated extra virgin olive oil on acne vulgaris as a single-blind, non-randomized clinical trial. Each patient received a placebo on his/her lesions for one month and after 10 days of washouts, they were treated with ozonated olive oil applied on their whole lesions for one month. The results were expressed as the percentages of reduced inflammatory versus non-inflammatory lesions. After one month of ozonated olive oil treatment, a seventy-five percent healing was observed in 50% of patients while under the same condition the remaining twenty five percent of the patients had 50% healing. After one month of applying ozonated olive oil, both inflammatory and non-inflammatory acne lesions were decreased by 65%. In conclusion, topical application of ozonated olive oil has shown significant healing effects on patients suffering from acne vulgaris. Biocompatibility behavior of ozonated olive oil makes it an excellent candidate to be prescribed as a highly potential anti-acne medicine.
[Xio Khaoshi, Choan Zhang. **Formulation and clinical evaluation of ozonated olive oil for the treatment of acne vulgaris lesions.** *Stem Cell* 2020;11(2):54-60]. ISSN: 1945-4570 (print); ISSN: 1945-4732 (online). http://www.sciencepub.net/stem. 8. doi:10.7537/marsscj110220.08.

Keywords: Ozonated Olive oil, Acne vulgaris, Treatment

1. Introduction

Some purified herbal medicine extracts play critical roles in providing high potential biocompatible drugs to treat skin diseases 1. Employing vegetable oils in the preparation of drugs for topical application on the skin, goes back to many years ago 2. Plant oils are often composed of polyunsaturated fatty acids rich in triglycerides, antioxidant agents, liposoluble vitamins, and a number of interesting compounds which are used for dermatological and cosmetic purposes3. Among different plant oils, some including olive oil, coconut oil, wheat oil, and borage oil are the most common used in cosmetics 2. The advantage of vegetable oils in comparison to other non-vegetable oily substances is in their particular lipid composition which has a very similar structure and function as with physiologic sebum of the epidermis 4. Having a high affinity to the skin sebum makes such oils to be excellent skin healing agents. Olive oil is mainly composed of triacylglycerols (triglycerides such as oleic acid, arachidic acid, linoleic acid, stearic acid, and palmitic acid), small amount of FFA (free fatty acid), glycerol, phosphatides, pigments, flavoring agents, and sterols. Olive oil has anti-inflammatory and antimicrobial effects and is considered as one of the most superlative herbal oils used for the treatment of the skin diseases5. Epidemiological investigations have revealed that high consumption of olive oil in the mediterranean diet has decreased the incidences e of inflammatory, cardiovascular, and cancer disorders due to the presence of vitamin E, essential fatty acids, and high potential antioxidants especially tocopherols in olive oil 6. The topical application of olive oil has displayed healing effects on eczema, surface wounds, and burns 7, 8. Phytosterols and triterpenoid ingredients in olive oil have shown to have regenerating and relaxing effects on the skin 2. Furthermore, vitamins E and A present in olive oil have shown to inhibit skin irritation and aging and also to maintain the skin elasticity, and smoothness 2. Olive oil is the major ingredient in topical semi-solids used in the healing of inflammatory and mycotic skin disorders 9. An attractive and useful feature of virgin olive oil is when it is combined with ozone gas (O3). In this process, ozone gas is combined with olive oil producing a great healing topical substance which is used for a wide variety of skin problems 10, 11. Ozone has been utilized in numerous ways in medicine for a long time. 12, 13. Ozone gas is highly unstable and cannot actively penetrate the skin cells, but can be stabilized by being trapped inside vegetable oils and which can then interact majorly with polyunsaturated fatty acids (PUFAs) present in olive oil to form reactive oxygen species (ROS) 14 like

hydrogen peroxide causing the production of growth factors and the activation of redox transcription factors such as nuclear factor-kappa B (NF-kB) and subsequently accelerating the cell cycle 15, 16. Reaction steps of classical ozonolysis of alkenes adapted to oleic acid were shown in Fig. 1.

acne vulgaris is a pilosebaceous inflammatory disorder that affects more than 80% of teenagers and youth 18. Acne, also known as acne vulgaris, is a long-term skin disorder that happens when skin dead cells and skin oil clog hair follicles 19. General signs of acne are oily skins, blackheads or whiteheads, pimples, and possibly scars. Acne is the most prevalent skin disease among teenagers, though it also affects all age ranges 20. The most widespread topical prescription medications for acne are retinoid, antibiotics, retinoid-like drugs, salicylic acid, azelaic acid, and dapsone 21. Using such topical medicines have several side effects like skin discoloration, minor skin irritation, redness, dryness and some other skin disorders. For moderate to severe acne, patients are recommended to start oral consumption of antibiotics like tetracycline (minocycline or doxycycline), or a macrolide to kill bacteria and trigger inflammation process 22.

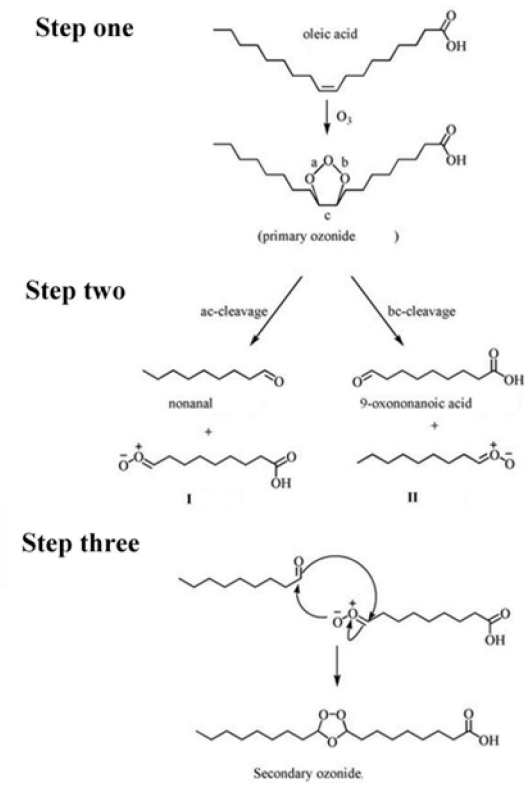

Figure 1. Three-step reaction sequence for classical ozonolysis of alkenes adapted to oleic acid 17.

Unfortunately, using antibiotics has some undesired side effects including upset stomach and dizziness. Various side effects of the available drugs (topical and systemic) have attracted the attention of many researchers to find biocompatible alternatives, such as herbal medicines, as compared to chemical drugs. The aim of the present study is to investigate the therapeutic effects of topical application of ozonated olive oil on patients affected by skin acne.

2. Material and Methods

Determination of acid and peroxide values, and iodine index

Extra virgin olive oil was purchased from Armaghan Tooba Company in Iran. First, the acid value of olive oil was determined according to the following method: 10 g of olive oil was added to 50 ml of a mixture of ethanol/ ether (1:1). After adding 1 ml phenolphthalein as an indicator, the solution was titrated by 0.1 N KOH until a stable pink color appears for 30 seconds indicating the titration end point. To determine the iodine index, 1 g of olive oil was transferred to a 250 ml Erlenmeyer flask and dissolved in 10 ml chloroform. Then, 25 ml Iodobromide (0.2N) was added to the olive oil-chloroform mixture and incubated in the dark for 30 min while shaking every 10 min. Next, 30 ml potassium iodide (15% w/v) and 100 ml ddH2O were added to the flask and was titrated using 0.1 N sodium thiosulfate. After a pale pink appears, 3 ml starch glue solution was added and the titration was followed until a blue color appears. These steps were carried out in a blank sample simultaneously. The peroxide value of the ozonated olive oil was determined according to the standard method of AOCS (American Oil Chemists Society) for both extra virgin and ozonated olive oil. First, 5 g potassium iodide was dissolved in ddH2O and then it was added to 1 g olive oil. In the presence of acetic acid, peroxides were reacted with 0.1 N KOH and the released iodide was titrated by 0.1 N sodium thiosulfate solution. The chemical reactions take place in order to determine the peroxide index, are illustrated as below:

$$2KI + 2CH3COOH \rightarrow 2HI + 2CH3COO^- + K^+$$

$$R{\bullet}OO{\bullet}H + 2HI \rightarrow ROH + H2O + I2$$

$$I2 + 2S2O3^- \rightarrow 2I^- + S4O6^{2-}$$

Ozonation process

Extra virgin olive oil was ozonated using an ozone generator (Model CD-0013.5). This unit produces 13.5 g of ozone per hour embedded in a current of medical oxygen gas at a rate of 3 L.min-1 at 25 °C for 240 hours. During ozonation process, olive oil is converted to an ozonated viscous gel with a defined peroxide value. This product will be stable in a refrigerator at (2-8°C) for two years.

Study design

This study provides futuristic, single-blind, well-defined, and controlled clinical trials. Our purpose was to investigate the therapeutic effects of using the ozonated olive oil on acne vulgaris lesions as compared to control. The inclusion criteria were subjects aging 10 to 30, not taking antibiotics, cortone, and anti-inflammatory drugs for one month before the beginning of the study. Exclusion criteria were hypersensitivity, pregnancy, and lactation. The test group consisted of twenty-five patients aging 10 to 30 years suffering from acne vulgaris. Clinical trials were carried out according to the method described by Campanati et al., 2013 16.

Sample volume was calculated using the following equations:

$$n = \frac{(s_1^2 + s_2^2)(\mathcal{E}_1 - \alpha/2 + \mathcal{E}_1 - \beta)^2}{(\overline{x_1} - \overline{x_2})^2}$$

Where, α= 0.05 and β= 0.1, the number subjects in each sample was calculated to near 19. However, to increase the accuracy of the data, the number subjects in each sample was increased to 25 patients. Patients were selected among males or females who have mild to moderate symptoms of acne vulgaris. Using some drugs was forbidden before and during the treatment including topical anti-acne medicines (two weeks), oral antibiotics (four weeks), hormone therapy (twelve weeks), and retinoids (six weeks). During the treatment, patients should not receive systemic treatments, should not have any other skin disorders, not being pregnant, no lactating, no sensitivity to ingredients used in the formulation of drugs.

To eliminate the possible effects of personal differences to the therapeutic response, treatments with both placebo and the drug were carried out in each patient independently. Furthermore, to illustrate the positive influence of the ozonation process on the therapeutic effects of the olive oil, acnes were treated with unprocessed olive oil (placebo) once every night for one month each time covering one cm2 of each acne lesion with 0.1 milliliter of olive oil. After a 10-day washout period, a thin layer of ozonated olive oil was rubbed on acne lesions once a night. Clinical responses were evaluated by photographs taken from acne lesions before and after the end of treatment period. Clinical responses were expressed as the percentage of the reduced number of acne lesions (total inflammatory and non-inflammatory lesions). Global evaluation was carried out according to the scoring method described as following and meantime. Possible side effects were investigated by a specialist. In the scoring method, scores of zero to six were considered according to the therapeutic response observed as (S0) full recovery, (S1) 90% recovery, (S2) 75% recovery, (S3) 50% recovery, (S4) 25% recovery, (S5) no change, and (S6) acne lesions progression. All data were recorded in Excel and analyzed using SPSS version 16. Kolmogorov-Smirnov test was used to evaluate the normality of data. Statistical analysis t-test and Mann-Whitney test were applied for independent samples and data analysis.

3. Results

Ozonation process and olive oil characteristics

Acid and peroxide values and iodine index for olive oil before and after ozonation process are reported in Table 1. Based on the results, the acid value of olive oil was increased after the ozonation process which can be due to the degradation of by-products produced by the ozonation reaction. Conversely, the ozonation process led to the decrease in iodine index originating from the reduction in ozone reaction with double bonds in olive oil. Iodine index represents the total number of double bonds in the sample and can be used to investigate the reduction of double bonds during the ozonation process and the formation of ozonides. Acid and peroxide values of the ozonated olive oil in this study was compared with those reported by other literature (Fig. 2 A and B). It has been reported that ozonides can easily penetrate the cell membrane and show several biological activities including skin cells stimulation and accelerating the repairing process of skin disorders by increasing fibroblasts and also the production of collagen, elastic and reticular fibers 2. Some literatures have reported the beneficial effects of ozone on the healing of sores by decreasing bacterial infection and enhancing oxygen pressure in the wounds2. Furthermore, the production of various species of peroxide resulting from ozone reaction with double bonds and their oxidation have caused a remarkable increase in peroxide value (Table 1) which confirms the report cited in literature 2. The final product is a viscous oil which is colorless and smells like ozone.

Table 1. Comparison of some physicochemical properties between extra virgin olive oil and ozonated olive oil

	Acid value (mg KOH g^{-1})	Iodine index (g Iodine per 100 g)	Peroxide value (mmol-equv.Kg^{-1})
Extra virgin olive oil	0.28 (±0.02)	81.8 (±1.28)	10 (±0.12)
Ozonated olive oil	17.3 (±0.06)	0	2439 (±13.3)

Clinical evaluations

Olive oil has several beneficial effects on human skin such as elasticity, moisturizing, protection against ultraviolet radiation and reactive oxygen species and more 23. Olive oil is one of the non-comedogenic oils. In topical application, it is anti-inflammatory and has healing effects on acne lesions, dermatitis atopic, psoriasis, and eczema. The presence of β-sorbitol in olive oil inhibits the conversion of testosterone to dihydrotestosterone (DHT) and regulates sebum secretion which prevents acne formation 24. Accordingly, olive oil is one of the potent ingredients of anti-acne drugs.

Design of study

This study was carried out as a futuristic, non-random, single-blind, and controlled clinical trial for the first time in Iran dealing with twenty-five patients suffering from acne vulgaris in the age range of 10 to 30 years. Considering the effects of personal differences on the healing rate of acne lesions and possible deviations resulting from such individual differences, we studied intervention effects and adopted negative control for each patient independently. The study was designed by applying a thin layer of olive oil as a placebo on part of acne lesions every night for one month. After a 10-day washout period, a thin layer of ozonated olive oil was rubbed on all acne lesions once every night, and the clinical responses were evaluated after one month. The objective was to investigate the healing effect of ozonated olive oil on acne vulgaris. Non-ozonated extra virgin olive oil was used as a placebo for comparison.

Clinical responses of patients suffering from acne vulgaris treated with ozonated olive oil were recorded according to the method described by Layegh et al. 2013 25 who investigated the effects of isotretinoin on the healing of acne lesions and recorded the number of inflammatory and non-inflammatory lesions at the beginning and at the end of the treatments and expressed as the percentage of the acne reduction. Moreover, they carried out the global evaluation of patients using the scoring method based on the percentage of the acne healing. According to clinical studies and the results obtained in the present study, using ozonated olive oil for a month can significantly reduce the number of acne lesions compared to placebo (Fig. S1 A to C). After one month of using ozonated olive oil, nearly 65% of acne lesion numbers (inflammatory and non-inflammatory) were decreased while in the placebo group, a maximum of 14.5% reduction in the number of acne lesions was observed (Fig. 3). After using ozonated olive oil for one month, 50% of patients revealed 75% healing while, 24% of others showed 50% healing.

Based on t-test statistical analysis, using placebo before starting treatment with ozonated olive oil has not shown a significant effect on the healing rate (p value~0.71).

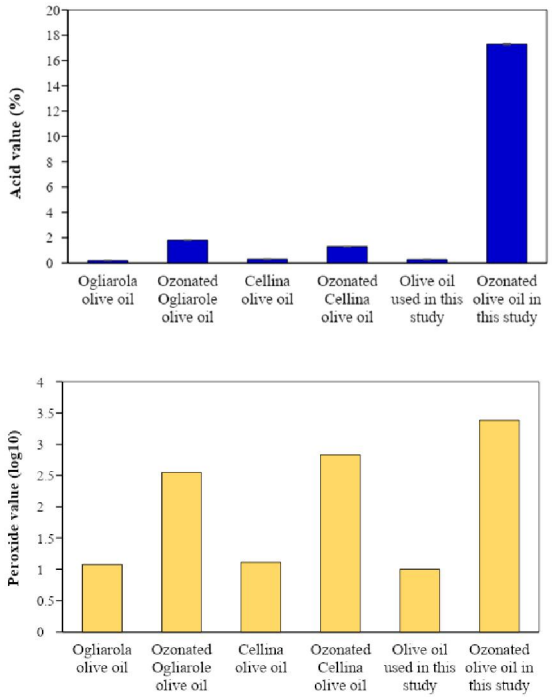

Figure 2a. Comparison of acidity and peroxide indexes of the ozonated olive oil in this study with those reported by other literature.

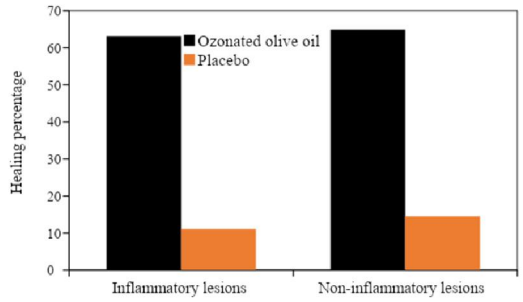

Figure 2b. Haling average of inflammatory and non-inflammatory lesions using t-test statistical analysis (p-value <0.01)

It has been reported that four factors play a role in acne lesions pathology including epidermal follicles proliferation rate, sebum hypersecretion, inflammation, and hyper-activity of propionium acne bacterium 26, 27. Among these factors, inflammation, as a key factor in acne pathogenesis, is known as the most important factor at all stages of acne lesions even before the formation of comedonal acne 28. Accordingly, in the current study we investigated the

influence of the use of ozonated olive oil on both inflammatory and non-inflammatory acne lesions. Several evidences indicated that inflammation started at the beginning of early acne (microcomedones) and also before the increase in keratinocyte proliferation. It has been shown that there is a relationship between inflammation and the development of microcomedon acne lesions 28. Several clinical trials have been carried out to investigate the role of inflammation in the early stages of acne formation 29, 30. These studies were based on the direct and indirect effects of anti-inflammatory drugs like dapsone, retinoid, antibiotics, and benzoyl peroxide on comedonal acne formation. Regarding the possible mechanism involved in the relationship between propionium acne bacterium and the immune response, it has been reported that these bacteria can trigger the innate immune response in the early and last stages of the disease by activating TLR2 (toll-like receptor2) factor 31. TLR is part of the innate immune system against the invasion of microorganisms and their activation will lead to the expression of immune response genes including those genes encoding cytokines and chemokines which stimulate host immune cells 32. A relationship between propionium acne and the release of inflammatory mediators has been detected in the biopsy samples of patients suffering from acne vulgaris 28. Propionium acne releases various proteases and enhances the transcription of pro-inflammatory cytokines such as Interleukin 1 alpha (IL-1α), IL-8, tumor necrosis factor-alpha (TNFα), matrix metalloproteinase, and IL-37. Hence, in both pathways, TLR2, and PAR2 (proteinase-activated receptor 2) are activated. Some studies have reported that the presence of propionium acne bacterium is not required for comedogenesis and hence some kinds of comedones can be formed in the absence of propionium acne bacterium 29. The occurrence of inflammatory responses in the absence of propionium, indicates that inflammatory process is derived from other immunochemical pathways independent of propionium. Many investigators have insisted on the role of sebaceous glands in inflammation development 33. Pre-inflammatory cytokines can begin the remodeling process in pilosebaceous units and accelerate comedone formation. The increased production of sebum does not have a direct relationship with the development of lesions. However it can lead to inflammatory changes in acne lesions. Production of fatty acids in the absence of bacteria along with the increased expression of IL-1 in cultured human sebocytes, has led researchers to suggest the hypothesis that cultured sebocytes can create acne lesions by unique mechanisms. The lipid components of sebum have pre-inflammatory effects while oleic acid and palmitic acid have shown to have antimicrobial effects 29.

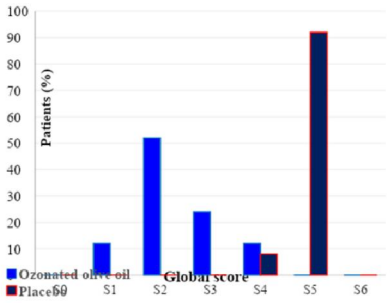

Figure 3. Evaluation of global response in patients suffering acne vulgaris using Mann-Whitney test (p value <0.01)

The main reason which encouraged us to apply the ozonation process on extra virgin olive oil is the presence of antimicrobial and antiseptic potentials in formulations containing ozonides which can kill bacteria in the skin and mucous membranes by disrupting the bacterial membrane via phospholipids peroxidation 34. Furthermore, the inhibitory effects of ozone on NF (nuclear factor)-κB makes it a powerful anti-inflammatory agent. In aqueous media, ozone can chemically react in two ways: (1) directly through molecular reactions; (2) indirectly via the formation of free radicals. Both ways can be involved in the antimicrobial potential of ozone. Moreover, the degradation of ozone derivatives by increasing the availability of oxygen in ischemic and inflamed tissues can cause an improvement in both topical metabolism and tissue proliferation which are needed to accelerate skin injuries healing 34. Topical application of ozonated oils on skin injuries has shown to have rapid antiseptic and recovery effects 35. Moreover, such oils have a wide range of inhibitory effects against gram-positive and negative bacteria (ref?). We speculate that the healing effects of the formulated anti-acne oil is derived from its antiseptic and antimicrobial potential possibly by oxidizing the microorganisms through releasing peroxides which is consistent with other reports 36.

The results obtained in this study indicate that ozonated olive oil can effectively heal acne vulgaris both inflammatory and non-inflammatory ones. Based on the high potential of formulated ozonated oil and its biocompatibility, it can be prescribed as an anti-acne drug.

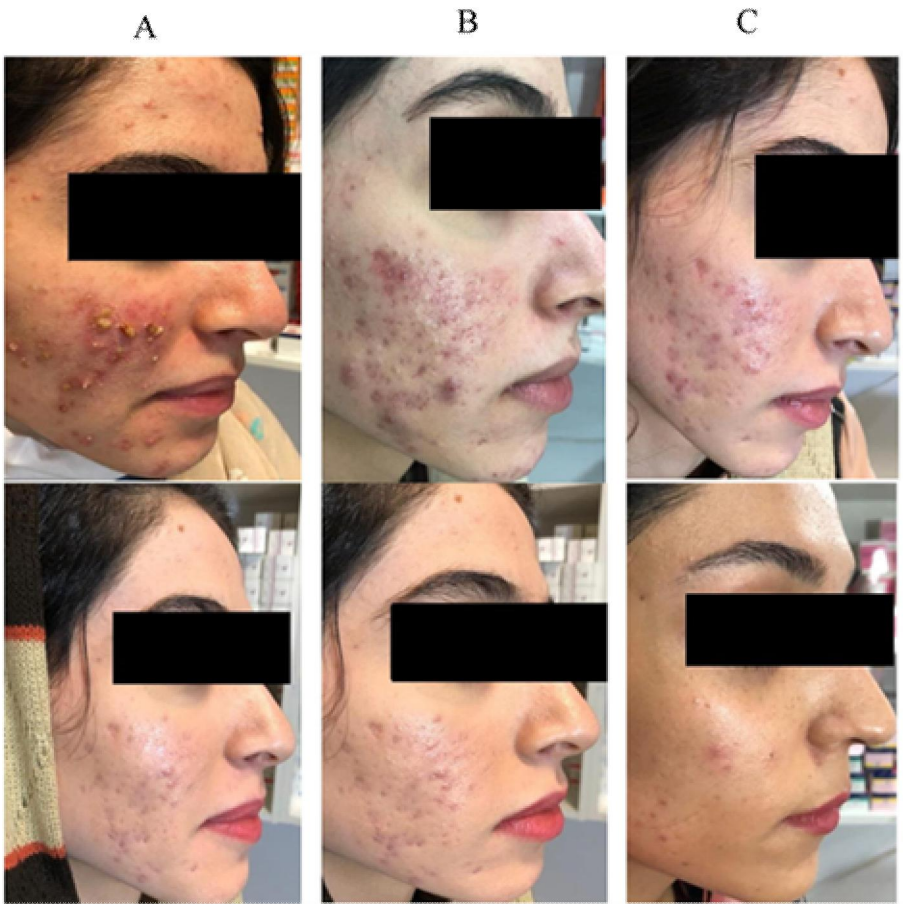

Figure S1. Healing effect of using ozonated olive oil on patients suffering from papulopustular lesions before treatment (A), after two weeks (B), and one month (C).

Acknowledgements:

This work was supported by a grant from Department of Pharmacy, Nankai University, Tianjin, China (Grant number 92104).

Corresponding Author:
Dr. Xio Khaoshi
Department of Pharmacy, Nankai University, Tianjin, China
khaoshixio@gmail.com

References

1. Yuan H, Ma Q, Ye L, et al. The traditional medicine and modern medicine from natural products. Molecules. 2016; 21:559.
2. Carata E, Tenuzzo BA, Dini L. Powerful Properties of Ozonated Extra Virgin Olive Oil. Herbal Medicine: Intech Open. 2018.
3. Rabasco Álvarez AM, González Rodríguez ML. Lipids in pharmaceutical and cosmetic preparations. Grasas y Aceites, 51 (1-2), 74-96. 2000.
4. Kamboj VP. Herbal medicine. Current science. 2000; 78:35-39.
5. Cicerale S, Lucas L, Keast R. Antimicrobial, antioxidant, and anti-inflammatory phenolic activities in extra virgin olive oil. Current opinion in biotechnology. 2012; 23:129-135.
6. Trichopoulou A, Katsouyanni K, Gnardellis C. The traditional Greek diet. European journal of clinical nutrition. 1993; 47:S76-81.
7. Tabassum N, Hamdani M. Plants used to treat skin diseases. Pharmacognosy reviews. 2014; 8:52.
8. Al-Waili NS. Topical application of natural honey, beeswax and olive oil mixture for atopic dermatitis or psoriasis: partially controlled, single-blinded study. Complementary therapies in medicine. 2003; 11:226-234.
9. Baumann LS. Less-known botanical cosmeceuticals. Dermatologic therapy. 2007; 20:330-342.
10. Valacchi G, Fortino V, Bocci V. The dual action of ozone on the skin. British Journal of Dermatology. 2005; 153:1096-1100.

11. Travagli V, Zanardi I, Valacchi G, et al. Ozone and ozonated oils in skin diseases: a review. Mediators of inflammation. 2010; 2010.
12. Bocci VA. Scientific and medical aspects of ozone therapy. State of the art. Archives of medical research. 2006; 37:425-435.
13. Bocci V, Borrelli E, Travagli V, et al. The ozone paradox: ozone is a strong oxidant as well as a medical drug. Medicinal research reviews. 2009; 29:646-682.
14. Sharma H. Innovative approaches in cosmeceuticals_ developing anti_aging topical application forms. 2008.
15. Werner S, Grose R. Regulation of wound healing by growth factors and cytokines. Physiological reviews. 2003; 83:835-870.
16. Campanati A, De Blasio S, Giuliano A, et al. Topical ozonated oil versus hyaluronic gel for the treatment of partial-to full-thickness second-degree burns: A prospective, comparative, single-blind, non-randomised, controlled clinical trial. Burns. 2013; 39:1178-1183.
17. Criegee R. Mechanism of ozonolysis. Angewandte Chemie International Edition in English. 1975; 14:745-752.
18. Purdy S, Langston J, Tait L. Presentation and management of acne in primary care: a retrospective cohort study. Br J Gen Pract. 2003; 53:525-529.
19. Draelos ZD. The effect of a daily facial cleanser for normal to oily skin on the skin barrier of subjects with acne. Cutis. 2006; 78:34-40.
20. Collier CN, Harper JC, Cantrell WC, et al. The prevalence of acne in adults 20 years and older. Journal of the American Academy of Dermatology. 2008; 58:56-59.
21. Zaenglein AL, Pathy AL, Schlosser BJ, et al. Guidelines of care for the management of acne vulgaris. Journal of the American Academy of Dermatology. 2016; 74:945-973. e933.
22. Das S, Reynolds RV. Recent advances in acne pathogenesis: implications for therapy. American journal of clinical dermatology. 2014; 15:479-488.
23. 23) Lin T-K, Zhong L, Santiago JL. Anti-inflammatory and skin barrier repair effects of topical application of some plant oils. International journal of molecular sciences. 2018; 19:70.
24. Viola P, Viola M. Virgin olive oil as a fundamental nutritional component and skin protector. Clinics in dermatology. 2009; 27:159-165.
25. Layegh P, Mosallaei N, Bagheri D, et al. The efficacy of isotretinoin-loaded solid lipid nanoparticles in comparison to Isotrex® on acne treatment. Nanomedicine Journal. 2013; 1:38-47.
26. Morohashi M, Toyoda M. Pathogenesis of acne: medical electron microscopy. Med Electron Microsc. 2001; 34:29-40.
27. Whitney KM, Ditre CM. Management strategies for acne vulgaris. Clinical, cosmetic and investigational dermatology. 2011; 4:41.
28. Makrantonaki E, Ganceviciene R, Zouboulis CC. An update on the role of the sebaceous gland in the pathogenesis of acne. Dermato-endocrinology. 2011; 3:41-49.
29. Tanghetti EA. The role of inflammation in the pathology of acne. The Journal of clinical and aesthetic dermatology. 2013; 6:27.
30. Gollnick H, Cunliffe W, Berson D, et al. Management of acne: a report from a Global Alliance to Improve Outcomes in Acne. Journal of the American Academy of Dermatology. 2003; 49:S1-S37.
31. Kim J. Review of the innate immune response in acne vulgaris: activation of Toll-like receptor 2 in acne triggers inflammatory cytokine responses. Dermatology. 2005; 211:193-198.
32. Man SM, Kanneganti T-D. Converging roles of caspases in inflammasome activation, cell death and innate immunity. Nature Reviews Immunology. 2016; 16:7.
33. Zouboulis CC. Acne and sebaceous gland function. Clinics in dermatology. 2004; 22:360-366.
34. Sega A, Zanardi I, Chiasserini L, et al. Properties of sesame oil by detailed 1H and 13C NMR assignments before and after ozonation and their correlation with iodine value, peroxide value, and viscosity measurements. Chemistry and Physics of Lipids. 2010; 163:148-156.
35. El Hadary AA, Yassin HH, Mekhemer ST, et al. Evaluation of the effect of ozonated plant oils on the quality of osseointegration of dental implants under the influence of cyclosporin a: an in vivo study. Journal of Oral Implantology. 2011; 37:247-257.
36. Montevecchi M, Dorigo A, Cricca M, et al. Comparison of the antibacterial activity of an ozonated oil with chlorhexidine digluconate and povidone-iodine. A disk diffusion test. New Microbiologica. 2013; 36:289-302.