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Letter to the Editor

OZONIZED ORAL GEL AS AN ADJUVANT IN THE TREATMENT OF PERIODONTAL DISEASE: A PRELIMINARY REPORT

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ABSTRACT

Ozonized oils have been demonstrated to induce the reduction of many oral microorganisms. The aim of this study was to evaluate the efficacy of a new ozonized oil formulation for the treatment of periodontal disease. A total of 10 patients with a diagnosis of chronic periodontitis were randomly selected, and a split-mouth scheme was used. All patients underwent to support periodontal therapy at the baseline measurement. Microbial sampling and analysis were performed in each selected site before supporting periodontal therapy. The selected site corresponded to the deepest periodontal pocket of the oral cavity. *Aggregatibacter actinomycetemcomitans, Porphyromonas gingivalis, Tannerella forsythia, Treponema denticola, Fusobacterium nucleatum, Campylobacter rectus*, and Total Bacterial Loading were evaluated through the quantification of total bacterial genome copies by PCR. Then, support periodontal therapy was done using an ultrasonic scaler. After support periodontal therapy, each patient was given ozonized sunflower seed oil [Ozoral gel, Innovares SRL, Sant'Ilario d'Enza (RE), Italy]. The patients were instructed to apply the gel daily after evening oral hygiene at home. After 2 weeks, microbiological samples were collected again in each patient and analyzed. A statistically significant difference was detected between Total Bacterial Loading ($p<0.014$) and *Tannerella Forsythia* ($p<0.012$) pre and post-ozonized sunflower seed oil treatment. Ozoral has demonstrated antiseptic properties. Additional studies with larger sample sizes are needed to confirm this preliminary result.

KEYWORDS: *ozonized oil, ozone therapy, periodontal disease, periodontal pathogens*

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INTRODUCTION

Ozonized oils, like ozonized sunflower oil, have been demonstrated to induce the reduction of many oral microorganisms (1). Ozonation of edible oil is performed by bubbling the gas mixture (O2/O3) into the oil under a controller reaction environment. This preparation is ideal for topical use in treating chronically infected cutaneous and mucosal areas of the body (2). Ozonized oils are widely recognized as one of the best bactericidal, antiviral and antifungal agents, and, therefore, it is profitably and practically employed in medicine and odontology. In this sense, studies have been carried out in peri-implant mucositis (3), caries prevention (4), periodontal diseases (5), regeneration and wound healing of the extraction socket and surgical site (6, 7).

Plaque biofilm is the main cause of both caries and periodontal disease. Ozonized oils have been proven useful in controlling oral infectious microorganisms in dental plaque (3). The antimicrobial property of ozonized oils effectively reduces the number of various periodontal bacteria (8). Ozonized oil seems to exert its antimicrobial action through different mechanisms, including: 1) Direct oxidation (germicide) (2, 9, 10); 2) Cytotoxicity (11); 3) Grow factors Release (12) and 4) Oxidative pre-conditioning (13).

Various etiological factors cause oral lesions, and microorganisms play a major role (14). Elimination of these microbial pathogens is the aim of most dental treatments. It has been demonstrated that ozonated sunflower oil effectively kills the biofilms formed by Candida species and the bacterium *Streptococcus mutans* (15).

The efficacy and safety of ozonized oil is closely linked to its quality control. The peroxide value is one of the basic parameters to define the dosage and its clinical application (16). This indicator is critical to define the proper indication. Lack of standardization and quality control of ozonized oils may cause variability when the germicide capacity is assayed. A new ozonized sunflower seed oil [Ozoral gel, Innovares SRL, Sant'Ilario d'Enza (RE), Italy] has recently been introduced in the market for periodontal treatments (3, 5). Ozoral® is a mucoadhesive hydrogel containing 15% of Ozonia3000® Sunflower, an ozonized sunflower seed oil registered to ECHA (European Chemical Agency https://echa.europa.eu) in compliance with the Reach Regulation https://echa.europa.eu/it/regulations/reach/legislation and classified as non-toxic, non-irritating and non-hazardous by ingestion. The muco-adhesiveness of Ozoral® is due to a polysaccharide of vegetable origin, which favours adhesion and permanence of the product on the oral mucosa despite the humidity.

In the present study, the antimicrobial properties of this new ozonized sunflower seed oil against oral and periodontal pathogens have been evaluated using the quantification method of total bacterial genome copies by PCR. The study's null hypothesis was that the ozonized sunflower seed oil did not demonstrate antibacterial effects; it does not affect antibacterial capabilities in addition to supporting periodontal therapy (SPT).

MATERIALS AND METHODS

The study was a single-centre clinical trial. A total of 10 patients with a diagnosis of chronic periodontitis were randomly selected. Patients enrolled in this study were 35-55 years old. Subjects had not previously received any surgical or non-surgical periodontal therapy. The patients were excluded from the study if they met the following criteria: pregnancy; a history of taking antibiotics or using antibacterial mouth rinses for the past 6 months; teeth with furcation involvement; smoking, and drug or alcohol abuse. Subjects participating in the study volunteered to follow a detailed verbal description of the procedure and signed consent forms. This trial was approved by the Albanian University Ethical Committee n 232.

A total of 10 patients were selected, and a split-mouth scheme was used. The patients were treated with SPT. Before SPT, microbial sampling and analysis were performed in each selected site. The selected site corresponded to the deepest periodontal pocket of the oral cavity. Microbiological samples were collected from each patient. For bacteria analysis, sites were isolated using cotton rolls. Sterile absorbable paper points (size 60) were used to collect subgingival samples and were immediately transferred to the microbiological laboratory for processing. *Aggregatibacter actinomycetemcomitans, Porphyromonas gingivalis, Tannerella forsythia, Treponema denticola, Fusobacterium nucleatum, Campylobacter rectus*, and Total Bacterial Loading were evaluated. Then SPT was done using an ultrasonic scaler. After SPT, ozonized oil (Ozoral®) was given to each patient for use on the left side of the oral cavity. The patient was instructed to apply the gel once a day after evening oral hygiene. After 2 weeks, microbiological samples were collected again in each patient.

Ozoral gel was supplied by Innovares SRL, Sant'Ilario d'Enza (RE), Italy. The manufacturer did a quality control report of the batch. The method to assay the peroxide values was ISCO3 (2016) (17). Peroxide Values in Ozonized Oils - www.isco3.org.

Probes oligonucleotides were designed based on 16S rRNA gene sequences of the Human Oral Microbiome Database (HOMD 16S rRNA RefSeq Version 10.1), counting 845 entries. All the sequences were aligned to find either a consensus sequence or less conservated spots. Two real-time polymerase chain reaction (PCR) runs were performed for each sample. The first reaction quantified the total amount of bacteria using two degenerate primers and a single probe matching a highly conservated 16S ribosomal RNA gene sequence. The second reaction detected and quantified the following bacteria: *Aggregatibacter actinomycetemcomitans, Porphyromonas gingivalis, Tannerella forsythia, Treponema denticola, Fusobacterium nucleatum, Campylobacter rectus*. Oligonucleotide concentrations and PCR conditions were optimized to ensure sensitivity, specificity, and no inhibitions in case of unbalanced target amounts. Absolute quantification assays were performed using the Applied Biosystems 7500 Sequence Detection System. The amplification profile was initiated by a 10 min incubation period at 95°C to activate polymerase, followed by a two-step amplification of 15 s at 95°C and 60 s at 57°C for 40 cycles. All these experiments, including non-template controls, were performed to exclude reagent contamination.

Plasmids containing synthetic DNA target sequences (Eurofin MWG Operon, Ebersberg Germany) were standard for the quantitative analysis. Standard curves for each target were constructed in a triplex reaction using a mix of the same plasmids in serial dilutions ranging from 101 to 107 copies. There was a linear relationship between the threshold cycle values plotted against the copy number log over the entire range of dilutions. The copy numbers for individual plasmid preparations were estimated using the Thermo NanoDrop spectrophotometer; the absolute quantification of total bacterial genome copies in samples allowed for calculating a relative number of bacterial species. Plasmid purification and handling was performed in a separate laboratory with dedicated pipettes to prevent samples and polymerase chain reaction contamination.

Descriptive statistics (mean, standard deviation, minimum, median, and maximum) were calculated for each group and variable. The data normality of the distributions was calculated with the Kolmogorov–Smirnov test. The Friedman non-parametric test was then performed, followed by Dunn's post hoc test. Significance was predetermined as $p < 0.05$ for all the tests performed. SPSS program and paired simple statistic T-test were used to detect significant differences.

RESULTS

A statistically significant difference was detected between Total Bacterial Loading ($p<0.014$) and *Tannerella forsythia* ($p<0.012$) pre and post-ozonized oil treatment (Table I). When the p-value is less than 0.05, the difference between the two compared bacterial loadings is statistically significant.

Table I. *paired sample test.*

	Pairwise differences					t	Degree of freedom	*p value*
Couple	Media	Standard deviation	Standard error	Confidence interval for the 95% difference				
				inferior	superior			
AA1 - AA2	130450	334141	105664	-108579	369481	1.235	9	.248
PG1 - PG2	-136	2700	854	-2068	1795	-.160	9	.877
TF1 - TF2	18552	18768	5934	5126	31978	3.126	9	.012
TD1 - TD2	19213	32478	10270	-4020	42446	1.871	9	.094
FN1 - FN2	1107097	1682268	531979	-96324	2310519	2.081	9	.067
CR1 - CR2	68025	187066	59155	-65793	201844	1.150	9	.280
TBL1 - TBL2	1365269	1429368	452005	342761	2387778	3.020	9	.014

AA: *Aggregatibacter actinomycetemcomitans;* ***PG:*** *Porphyromonas gingivalis;* ***TF:*** *Tannerella forsythia;* ***TD:*** *Treponema denticola;* ***FN:*** *Fusobacterium nucleatum;* ***CR:*** *Campylobacter rectus;* ***TBL:*** *Total Bacterial Loading 1 pre-treatment, 2 post-treatment. The table reports data on the treated side only, pre-and post-treatment.*

DISCUSSION

Ozonized oil seems to strongly inhibit the formation of dental plaque and reduce the number of pathogens, both Gram-positive and Gram-negative organisms, including: *Staphylococci*, *Streptococci*, *Enterococci*, *Pseudomonas*, *Escherichia coli* and, above all, against Mycobacteria (18, 19) This effect of oxidation gives to its bactericidal, virucidal, and fungicidal activity. After contact with ozonized oil - microorganism, severe alteration of the cytoplasm was observed (20). In addition, applying ozonized oil reduces amylase, lipase, keratinase and urease enzyme activities in the microorganism significantly, in line with a reduction in nucleic acid content (11). This action seems not to damage human body cells; the reason attributed to this is the antioxidant ability of mammalian cells (21).

Even when the exact action mechanism of the ozonized oil is not described, there is much pre-clinical and clinical evidence of its antimicrobial and wound healing beneficial efficacy. As an antimicrobial, the most sensible bacterium is *Staphylococcus aureus,* and the primary resistant is *Pseudomonas aeruginosa* (22). 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* (23).

In general, a lethal effect of ozonized oil is evident when it is applied to a multi-resistant strain of *Staphylococcus epidermis*, *Staphylococcus aureus*, also when it is applied to fungi from the genus *Trichophyton*, *Epidermophyton* and *Microsporum*, yeast as *Candida albicans* and protozoan as *Giardia lamblia* (24, 25).

A comparison regarded 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 (8). Ozonized oil showed a significantly better behaviour than the references. This effect on one of the main pathogens suggests its potential applicability for periodontal treatment (8).

However, the word *ozonized* is without scientific meaning if it is not associated with *how much* peroxides are present in the oil. Probably the leading cause of variability regarding the microbiological efficacy of these active components is closely connected with the lack of standardization. The few studies on the therapeutic effects of ozonized oils on acute cutaneous wound healing in animal models did not investigate the dose/effect response, expressed as the number of peroxides in the ozonized derivative used (26).

Our study evaluated the antibacterial properties of a standardized ozonized sunflower seed oil in a group of patients who used it as a home-care praesidium. Ozoral gel has been statistically significant ($p<0.05$) in reducing Total Bacterial Loading and *Tannerella forsythia* bacterial loading. This last bacterium belongs to Socransky's red complex, a periodontal pathogen (27-32). Thus, Ozoral gel demonstrated antibacterial effects. In contrast with previous studies, based on the only analysis of traditional microbiology, these results demonstrate for the first time the reduction of the total bacterial load by ozonized oils, using quantification of total bacterial genome copies by PCR; this should explore the significance of the results of the work, not repeat them. A combined Results and Discussion section is often appropriate. Avoid extensive citations and discussion of published literature.

CONCLUSIONS

It is our knowledge, however, that additional studies with a larger sample size and a higher number of home-care applications are needed to firmly demonstrate the effectiveness of ozonized oil as a viable antimicrobial agent in routine dental therapies.

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Informed Consent Statement: Informed consent was obtained from all subjects involved in the study.

Conflicts of Interest: The authors declare no conflict of interest.

REFERENCES

1. Saini R. Ozone therapy in dentistry: A strategic review. *J Nat Sci Biol Med.* Jul 2011;2(2):151-153.
2. Valacchi G, Fortino V, Bocci V. The dual action of ozone on the skin. *Br J Dermatol.* Dec 2005;153(6):1096-1100.
3. Butera A, Pascadopoli M, Gallo S, et al. Ozonized Hydrogels vs. 1% Chlorhexidine Gel for the Clinical and Domiciliary Management of Peri-Implant Mucositis: A Randomized Clinical Trial. *J Clin Med.* Feb 12 2023;12(4).
4. Nardi GM, Fais S, Casu C, et al. Mouthwash Based on Ozonated Olive Oil in Caries Prevention: A Preliminary In-Vitro Study. *Int J Environ Res Public Health.* Dec 6 2020;17(23).
5. Tete G, D'Amicantonio T, Polizzi E. Efficacy Ozone Therapy in Reducing Periodontal Disease. *Materials (Basel).* Mar 16 2023;16(6).
6. Anzolin AP, da Silveira-Kaross NL, Bertol CD. Ozonated oil in wound healing: what has already been proven? *Med Gas Res.* Jan-Mar 2020;10(1):54-59.
7. Lim Y, Lee H, Woodby B, Valacchi G. Ozonated Oils and Cutaneous Wound Healing. *Curr Pharm Des.* 2019;25(20):2264-2278.
8. Montevecchi M, Dorigo A, Cricca M, Checchi L. Comparison of the antibacterial activity of an ozonated oil with chlorhexidine digluconate and povidone-iodine. A disk diffusion test. *New Microbiol.* Jul 2013;36(3):289-302.
9. Guinesi AS, Andolfatto C, Bonetti Filho I, Cardoso AA, Passaretti Filho J, Farac RV. Ozonized oils: a qualitative and quantitative analysis. *Braz Dent J.* 2011;22(1):37-40.
10. Lezcano I, Nuñez N, Espino M, Gómez M. Antibacterial Activity of Ozonized Sunflower Oil, Oleozón, Against Staphylococcus aureus and Staphylococcus epidermidis. *Ozone: Science & Engineering: The Journal of the International Ozone Association.* 2000 2000;22(2):207.
11. Neveen SI. Antifungal activity of ozonized Olive Oils (Oleozone). *International J of Agriculture and Biology* 2006;8(5):670-675.
12. Bocci V. *Ozone A New Medical Drug*. Dordrecht, The Nederlands: Springer; 2005.
13. Zamora Z, Gonzalez R, Guanche D, et al. Ozonized sunflower oil reduces oxidative damage induced by indomethacin in rat gastric mucosa. *Inflamm Res.* Jan 2008;57(1):39-43.
14. González-Munoz L, Flichy-Fernández AJ, Ata-Ali J, Pascual-Moscardó A, Penarrocha-Diago MA. Effect of ozone therapy upon clinical and bacteriological parameters of the oral cavity: an update. *Journal of Clinical and Experimental Dentistry.* 2011;3(4):e325-e327.
15. Higa B, Cintra BS, Alvarez CM, et al. Ozonated oil is effective at killing Candida species and Streptococcus mutans biofilm-derived cells under aerobic and microaerobic conditions. *Med Mycol.* Aug 8 2022;60(8).
16. ISCO3, Martínez-Sánchez G, Lozano ÓL. Physico-chemical characterization of ozonized oil. Peroxide Value. http://isco3.org/officialdocs/#4. 2016;ISCO3/LAB/00/04
17. Zanardi I, Travagli V, Gabbrielli A, Chiasserini L, Bocci V. Physico-Chemical Characterization of Sesame Oil Derivatives. *Lipids*. 2008;43(9):877-886. doi:https://doi.org/10.1007/s11745-008-3218-x
18. Sechi LA, Lezcano I, Nunez N, et al. Antibacterial activity of ozonized sunflower oil (Oleozon). *J Appl Microbiol.* Feb 2001;90(2):279-284.
19. Rodrigues KL, Cardoso CC, Caputo LR, Carvalho JCT, Fiorini JE, Schneedorf JM. Cicatrizing and antimicrobial properties of an ozonized oil from sunflower seeds. *Inflammopharmacology.* 2004;12(3):261-270.
20. Sechi LA. Antibacterial activity of ozonized sunflower oil (Oleozon). *Journal of Applied Microbiology.* 2001;90:279–284.
21. Izzotti A, Fracchia E, Rosano C, et al. Efficacy of High-Ozonide Oil in Prevention of Cancer Relapses Mechanisms and Clinical Evidence. *Cancers (Basel).* Feb 24 2022;14(5).
22. Menéndez s, González R, Ledea O. *Ozono, aspectos básicos y aplicaciones clínicas*. La Habana: CENIC; 2008.
23. Zanardi I, Burgassi S, Paccagnini E, Gentile M, Bocci V, Travagli V. What is the best strategy for enhancing the effects of topically applied ozonated oils in cutaneous infections? *Biomed Res Int.* 2013;2013:702949.
24. Menendez S, Falcon L, Simon DR, Landa N. Efficacy of ozonized sunflower oil in the treatment of tinea pedis. *Mycoses.* Oct 2002;45(8):329-332.

25. Hernandez F, Hernandez D, Zamora Z, et al. Giardia duodenalis: effects of an ozonized sunflower oil product (Oleozon) on in vitro trophozoites. *Exp Parasitol.* Mar 2009;121(3):208-212.
26. Kim HS, Noh SU, Han YW, et al. Therapeutic effects of topical application of ozone on acute cutaneous wound healing. *J Korean Med Sci.* Jun 2009;24(3):368-374.
27. Haffajee AD, Socransky SS. Microbial etiological agents of destructive periodontal diseases. *Periodontol 2000.* Jun 1994;5:78-111.
28. Lauritano D, Carinci F, Palmieri A, Cura F, Caruso S, Candotto V. Reuterinos((R)) as adjuvant for peri-implant treatment: A pilot study. *Int J Immunopathol Pharmacol.* Jan-Dec 2019;33:2058738419827745.
29. Roncati M, Lauritano D, Cura F, Carinci F. Evaluation of light-emitting diode (LED-835 NM) application over human gingival fibroblast: an in vitro study. *J Biol Regul Homeost Agents.* Apr-Jun 2016;30(2 Suppl 1):161-167.
30. Lopez MA, Andreasi Bassi M, Confalone L, Carinci F, Ormianer Z, Lauritano D. The use of resorbable cortical lamina and micronized collagenated bone in the regeneration of atrophic crestal ridges: a surgical technique. Case series. *J Biol Regul Homeost Agents.* Apr-Jun 2016;30(2 Suppl 1):81-85.
31. Scarano A, Murmura G, Carinci F, D. Lauritano D. Immediately loaded small-diameter dental implants: Evaluation of retention s, and comfort for the edentulous patient (2012) European Journal of Inflammation, 10 (1), pp. 19-23. . Immediately loaded small-diameter dental implants: Evaluation of retention, stability, and comfort for the edentulous patient. *Eur J Inflamm.* 2012;10(1):19-23.
32. Carinci F, Girardi A, Palmieri A, et al. LAB®-Test 1: Peri-Implantitis and bacteriological analysis *Eur J Inflamm.* 2012;10(1):91-93.