Regenerative Effects of Ozonized Oils REGEN(PJ)FACEZONE® and REGEN(PJ)BODYOZONE® on Skin Lesions

Peeter Jaramillo-Cazco [a]

a. Bachelor's Degree in Health. Centro de Especialidades Médicas Doctor Ozono. Ecuador. ORCID: https://orcid.org/0009-0009-4619-6006.

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Abstract

Introduction: The aim of this study was to analyze the effects of ozonized oils REGEN(PJ)FACEZONE® and REGEN(PJ)BODYOZONE® on skin regeneration in patients with cutaneous lesions, through microbiological testing and dermal analysis.

Materials and Methods: An experimental, quantitative, cross-sectional study was conducted. Inductive–deductive methods, data triangulation, and comparative analysis were employed. The applied techniques included direct observation, data tabulation, medical history, dermal analysis, and microbiological evaluation. The sample was divided into two groups: Group A (control), treated with conventional management (n=5), and Group B (experimental), treated with REGEN(PJ)FACEZONE® and REGEN(PJ)BODYOZONE® (n=5).

Results: Group A showed high levels of bacterial resistance to tetracycline, minocycline, and clindamycin, which limited their effectiveness against Cutibacterium acnes and Staphylococcus aureus. In contrast, Group B demonstrated that REGEN(PJ)FACEZONE® achieved significant inhibition of C. acnes and S. aureus bacterial growth. Furthermore, REGEN(PJ)BODYOZONE® showed notable improvements in skin hydration and regeneration.

Conclusions: Ozonized oils REGEN(PJ)FACEZONE® and REGEN(PJ)BODYOZONE® represent an effective alternative for the treatment of resistant bacterial infections, particularly in acne, pressure ulcers, and dermatitis. They are also effective in improving facial elasticity and reducing solar lentigines.

Keywords: Ozone; acne; dermatitis; lentigo; pressure ulcers

Introduction

The skin, as the largest organ of the human body, fulfills fundamental functions in protecting against both external and internal aggressions. Its main roles include the regulation of water balance, immune function, protection against ultraviolet (UV) radiation, thermal regulation, absorption and excretion of fluids, sensory perception, as well as cosmetic and emotional functions related to the hydrolipidic mantle and the expression of emotional state (1).

Alterations in skin integrity can be caused by multiple factors, including inflammatory diseases, infections, pH imbalances, aging, trauma, and chronic exposure to solar radiation (2–4). Among the most frequent conditions are acne, eczema, psoriasis, solar lentigo, diaper dermatitis, and pressure ulcers, which affect not only health but also patients' quality of life (2–4).

Ultraviolet radiation is one of the main etiological agents of skin aging. This phenomenon, known as photoaging, is characterized by a progressive loss of skin regenerative capacity, alterations in its morphophysiological structure, thinning of the epidermis and dermis, and the appearance of hyperpigmentation, deep wrinkles, and loss of elasticity (5–8). It is estimated that up to 80% of facial aging is a direct consequence of prolonged sun exposure (9), and approximately 90% of visible aging is attributed to cumulative ultraviolet radiation damage (10).

A key mechanism in this process is oxidative stress, generated by the accumulation of reactive oxygen species (ROS), which exceed the antioxidant capacity of cells. This imbalance induces damage to lipids, proteins, and DNA, accelerating extracellular matrix deterioration and cellular dysfunction (6–8). UVA radiation in particular decreases the activity of essential antioxidant enzymes such as superoxide dismutase (SOD), catalase, and glutathione peroxidase, thereby favoring premature aging, solar lentigo formation, and loss of elasticity (9,10).

On the other hand, dermal lesions of infectious or pressure-related origin, such as pressure ulcers and diaper dermatitis, pose a significant clinical challenge due to their slow healing, high bacterial load, and risk of complications. The proper management of these lesions requires therapeutic strategies that not only control infection but also promote tissue regeneration (11,12).

In this context, ozone therapy has proven to be an effective alternative in the treatment of skin lesions. Several studies highlight that ozonized oils

possess antimicrobial, anti-inflammatory, and regenerative properties by releasing active oxygen and stable peroxides at the application site, thereby promoting tissue oxygenation, stimulation of cellular repair, and control of bacterial growth (3,13,14). Furthermore, these compounds act as modulators of oxidative stress, restoring cellular redox balance and favoring tissue homeostasis (15–22).

Ozonized oil is a 100% organic product that does not require preservatives. It is activated by body temperature, releasing oxygen for several hours, has high durability, and its efficacy is comparable to or even superior to some conventional antimicrobials, with a broad spectrum of action and no reported toxic effects (23). Its topical use has demonstrated efficacy in wound healing, treatment of burns, pressure ulcers, vascular ulcers, diabetic foot, dermatitis, acne, and solar lentigo, thanks to its ability to stimulate cellular regeneration, re-epithelialization, and reduction of microbial load (24).

Within this framework, the development of the ozonized oils REGEN(PJ) FACEZONE® and REGEN(PJ)BODYOZONE® emerges. These formulations are intended for application in dermatological, cosmetic, and clinical contexts. Designed under quality and standardization criteria, they aim to provide an effective alternative for skin repair and the management of resistant infections (25).

Therefore, the objective of this study was to analyze the effects of the ozonized oils REGEN(PJ)FACEZONE® and REGEN(PJ)BODYOZONE® on skin regeneration in cutaneous lesions through microbiological testing and dermal analysis.

Materials and Methods

Study design

An experimental, quantitative, and cross-sectional study was conducted.

Methods

Inductive–deductive methods, data triangulation, and comparative analysis were applied.

Techniques

The techniques employed included direct observation, data tabulation, medical history review, dermal analysis, and microbiological evaluation.

Inclusion criteria

- Patients with facial lesions, specifically solar lentigo, loss of elasticity, and comedogenic acne.
- Patients with pressure ulcers.
- Patients with diaper dermatitis.

Samples

- **Comedogenic acne:** Group A (control) with 9 samples, Group B (experimental) with 5 samples.
- **Pressure ulcers and diaper dermatitis:** Group A (control) with 99 samples, Group B (experimental) with 6 samples.

Variables and indicators

- **Variable 1:** Comedogenic acne
  Indicator: Presence of *Cutibacterium acnes*.
- **Variable 2:** Pressure ulcers
  Indicator: Presence of *Staphylococcus aureus*.
- **Variable 3:** Diaper dermatitis
  Indicator: Presence of *Staphylococcus aureus*.
- **Variable 4:** Facial photoaging
  Indicator: Presence of solar lentigo.

Procedure

Phase 1: Literature search

A search was conducted in databases such as PubMed, ScienceDirect, Scielo, Dialnet, and Cochrane on skin aging, prevention, treatment, and the use of ozonized oils in dermatology.

Phase 2: Sample selection

Patients who met the inclusion criteria were selected, and informed consent was obtained for their participation in the study.

Phase 3: Group A (control) – conventional treatment

- **Culture media:**
  - Mueller-Hinton agar for *Staphylococcus aureus*.
  - Brucella or Mueller-Hinton agar supplemented with blood and vitamin K for *Cutibacterium acnes*.
- **Antibiotics used:**
  - **For C.** ***acnes:*** Tetracycline, minocycline, and clindamycin, in serial concentrations (µg/mL), diluted in distilled water.
  - **For S.** ***aureus:*** Clindamycin (2 µg).
- **Equipment and materials:** Flasks, sterile Petri dishes, pipettes, test tubes, and incubator.
- **Incubation conditions:**
  - ***C. acnes:*** Anaerobic, at 35–37 °C, for 48–72 hours.
  - ***S. aureus***: Aerobic, at 35–37 °C, for 18–24 hours.

- **Preparation of media with antibiotics:**
  Antibiotics were incorporated into agar before solidification (at 45–50 °C) in serial concentrations:
  - **Tetracycline and minocycline:** 0.125, 0.25, 0.5, 1, 2, 4, 8, 16, and 32 µg/mL.
  - **Clindamycin:** 0.125, 0.25, 0.5, 1, 2, 4, and 8 µg/mL.
    The mixtures were poured into sterile Petri dishes and allowed to solidify.
- **Preparation of bacterial suspension:**
  Adjusted to a density of 0.5 on the McFarland scale (~1.5 × 10^8 CFU/mL) using sterile saline solution.
- **Inoculation:**
  Drops of 2–5 µL of bacterial suspension were placed on the surface of each plate, at separate points.
- **Controls:**
  Plates without antibiotics were used as bacterial growth controls.

Phase 4: Group B (experimental) – application of REGEN(PJ) FACEZONE® and REGEN(PJ)BODYOZONE®

- **Comedogenic acne (Variable 1):**
  - *Medium with ozonized oil:* Mueller-Hinton agar supplemented with blood and vitamin K, incorporating serial concentrations of REGEN(PJ)FACEZONE® (0.25%, 0.5%, 1%, 2%, and 4%) before solidification (45–50 °C).
  - *Bacterial suspension:* C. acnes cultured under anaerobic conditions (48–72 h) at 0.5 McFarland density (~1.5 × 10^8 CFU/mL).
  - *Inoculation:* 2 µL of bacterial suspension was placed on plates with different concentrations of ozonized oil.
  - *Control:* Plate without ozonized oil to verify normal bacterial growth, incubated under anaerobic conditions (5% CO_2, 10% H_2, 85% N_2) at 35–37 °C for 48–72 hours.
- **Pressure ulcers and diaper dermatitis (Variables 2 and 3):**
  - *Medium with ozonized oil:* Mueller-Hinton agar with REGEN(PJ) BODYOZONE® in concentrations of 0.125%, 0.25%, 0.5%, 1%, 2%, and 4% before solidification.
  - *Bacterial suspension:* S. aureus (ATCC 25923 as control and one MRSA strain), adjusted to 0.5 McFarland (~1.5 × 10^8 CFU/mL).

- ***Inoculation:*** 2 µL of bacterial suspension was applied to plates with different concentrations of ozonized oil.
- ***Control:*** Plate without ozonized oil, incubated under aerobic conditions at 35–37 °C for 18–24 hours.

- **Facial photoaging (Variable 4):**

REGEN(PJ)FACEZONE® was applied for six months in patients with solar lentigines and facial loss of elasticity

Evaluation: Dermal analysis using the Multi Skin Test Center® equipment, models MC750 and MC900, with Skin Check Up software (Courage + Khazaka Electronic GmbH).

Parameters evaluated: Hydration, elasticity, size and intensity of lentigo pigmentation, with macro- and microscopic skin analysis before and after treatment.

Results

The findings of this study show that conventional antibiotic treatment exhibited high levels of bacterial resistance in both Cutibacterium acnes and Staphylococcus aureus, which significantly limited its clinical effectiveness. In contrast, the ozonized oils REGEN(PJ)FACEZONE® and REGEN(PJ) BODYOZONE® demonstrated high antimicrobial effectiveness in the tests performed. For C. acnes, complete bacterial inhibition was achieved at concentrations of 4% of REGEN(PJ)FACEZONE®, while for S. aureus, including resistant strains (MRSA), REGEN(PJ)BODYOZONE® showed total inhibition from the same concentration.

Additionally, patients with solar lentigines and facial photoaging treated with REGEN(PJ)FACEZONE® exhibited significant clinical improvements in hydration, elasticity, and pigmentation reduction, according to dermal analysis performed before and after treatment.

Detailed results by groups are presented below:

Group A – Conventional treatment (antibiotics)

- Variable 1: Comedogenic acne (Cutibacterium acnes)

  The results obtained revealed high levels of bacterial resistance to tetracycline, minocycline, and clindamycin. Table 1 presents the minimum inhibitory concentrations (MICs) and the outcomes of antibiotic susceptibility testing.

Table 1. Results for *Cutibacterium acnes* – Group A

Patient.	Antibiotic	MIC (µG/ML)	Results
1	Tetracycline	>16	Resistant
2	Minocycline	>16	Resistant
3	Clindamycin	>2	Resistant

Variables 2 and 3: Pressure ulcers and diaper dermatitis *(Staphylococcus aureus)*

The bacterial isolates showed resistance to clindamycin, with MIC values greater than 2 µg/mL, indicating a reduced effectiveness of conventional treatment.

Table 2. Results for *Staphylococcus aureus* – Group A

Patient	Antibiotic	MIC (µG/ML)	Results
1.V3	Clindamycin	>4	Resistant
2.V3	Clindamycin	>2	Resistant

Group B – Treatment with ozonized oils

Variable 1: Comedogenic acne *(Cutibacterium acnes)*

The ozonized oil REGEN(PJ)FACEZONE® demonstrated significant efficacy against C. acnes, with inhibition rates above 85% starting at a concentration of 2% and complete inhibition at 4%. The Chi-square test showed statistically significant differences between low and high concentrations ($p < 0.01$), with a 95% confidence interval.

Table 3. Results for Cutibacterium acnes – Group B

Concentration of REGEN(PJ)FACEZONE®	Inhibition rate (%)
0,25 %	20 %
0,5 %	35 %
1 %	60 %
2 %	85-95 %
4 %	100 %

Variables 2 and 3: Pressure ulcers and diaper dermatitis *(Staphylococcus aureus)*

The ozonized oil REGEN(PJ)BODYOZONE® showed an overall inhibition rate of 61.67% against S. aureus. Low concentrations (0.125% and 0.25%) were minimally effective (<30% inhibition), whereas from 1% upwards the inhibition was significant, ranging between 80% and 100%. Complete inhibition of bacterial growth was observed at 4% concentration.

Table 4. Results for Staphylococcus aureus – Group B

Concentration of REGEN(PJ)FACEZONE®	Inhibition rate (%)
0,125 %	25 %
0,25 %	30 %
0,5 %	60 %
1 %	80 %
2 %	95 %
4 %	100 %

Variable 4: Facial photoaging (solar lentigo)

Dermal analysis results showed significant improvements after six months of treatment with REGEN(PJ)FACEZONE®. A reduction in solar lentigo size, decreased pigmentation intensity, improved skin hydration, and increased elasticity were observed.

Table 5. Results for solar lentigo – Group B

Parameter	Before treatment	six months later	Change (%)
Lentigo size(mm)	10 mm	6 mm	-40 %
Intensity pigmentation	4 (moderate-severe)	2 (mild-moderate)	Obvious reduction
Edges of lentigo	Well defined	More diffuse	Aesthetic improvement
Skin hydration (%)	40 %	65 %	+25 %
Skin elasticity (%)	30 %	50 %	+20 %

Discussion

The results obtained in this study reveal that the bacteria Cutibacterium acnes and Staphylococcus aureus exhibited high levels of resistance to conventional antibiotic treatments such as tetracycline, minocycline, and clindamycin, representing a significant therapeutic limitation in managing skin infections. This finding is consistent with recent reports indicating a concerning increase in bacterial resistance associated with the indiscriminate and prolonged use of topical and systemic antibiotics in the treatment of dermatological diseases (2–4).

In contrast, the ozonized oils REGEN(PJ)FACEZONE® and REGEN(PJ) BODYOZONE® demonstrated remarkable antimicrobial efficacy. In the tests performed with C. acnes, REGEN(PJ)FACEZONE® achieved complete bacterial inhibition at concentrations of 4% or higher, while for S. aureus, including resistant strains (MRSA), REGEN(PJ)BODYOZONE® also reached total inhibition at the same concentration. These results suggest a dose-dependent effect, with increasing inhibition rates as the oil concentration rises.

The observed efficacy can be explained by the mechanism of action of ozonized oils, which release stable peroxides and reactive oxygen species, creating a hostile environment for pathogenic microorganisms without affecting healthy cells (15–22). This mechanism is supported by scientific literature, which documents their antimicrobial, anti-inflammatory, regenerative, and oxidative stress-modulating activity (3,13,14).

Additionally, patients treated with REGEN(PJ)FACEZONE® for facial photoaging showed significant improvements in hydration, elasticity, reduction in size and intensity of solar lentigines, and overall skin texture, consistent with the reparative effects and stimulation of cellular metabolism attributed to ozonized oils (23,24).

The findings from the reviewed literature support these results, as summarized in Table 6.

Table 6. Research Background

Author	Main contribution
Stable-García (3)	Demonstrates the effectiveness of ozonated oils in healing skin wounds.
Darias-Domínguez (8)	It reports a high prevalence of grade II photoaging and melasma in 61.54% of patients.
Shanbhag (9)	It relates oxidative stress to loss of skin elasticity and aging.
Guillamón (11)	Identify factors that favor the appearance of pressure injuries.
GNEAUPP (12)	Establishes the categorization of pressure ulcers and the associated shear mechanisms.
González (13)	It recommends that topical photoprotection be accompanied by systemic strategies.
Sifontes (22)	Documents the clinical use of ozonated oils and their broad therapeutic spectrum.
Instituto IVO (23)	Evaluates the activity and clinical efficacy of ozonated healing oil.
Jaramillo (25)	Presents the development of REGEN(PJ) ozonated oil for human and veterinary use.
Bello-Expósito (24)	Evidence of the effectiveness of topical ozone in the healing of chronic wounds.
Díaz (26)	Analyzes the ozonation of genetically modified and unmodified sunflower oils.
Martínez (27)	Evaluates the effectiveness of ozone therapy in the treatment of diabetic foot ulcers.
Ozonoterapia Hoy (28)	Reports successful cases of atopic dermatitis treated with ozonated oil.
Contreras (29)	Systematizes the use of ozone therapy in three human dermatological conditions.

Overall, this background supports the hypothesis that ozonized oils represent an effective therapeutic alternative for the treatment of infectious and degenerative skin lesions, offering advantages over conventional treatments. Furthermore, their safety profile, prolonged action without toxic ef-

fects, and ability to modulate inflammatory and oxidative processes position them as a valuable tool in clinical and aesthetic dermatology.

Therefore, the results of this study not only align with previous evidence but also provide concrete data on the specific efficacy of REGEN(PJ)FACEZONE® and REGEN(PJ)BODYOZONE® in managing conditions such as acne, pressure ulcers, dermatitis, and solar lentigines, highlighting their potential as a safe and effective alternative in the face of the increasing bacterial resistance observed in clinical practice.

Conclusions

The results of this study allow us to conclude that conventional antibiotic treatments, specifically tetracycline, minocycline, and clindamycin, exhibited high levels of bacterial resistance in both Cutibacterium acnes and Staphylococcus aureus, significantly limiting their clinical efficacy in managing acne, pressure ulcers, and diaper dermatitis. In contrast, the ozonized oils REGEN(PJ)FACEZONE® and REGEN(PJ)BODYOZONE® demonstrated high antimicrobial efficacy, achieving complete bacterial inhibition at concentrations of 4 % for both C. acnes and S. aureus, including resistant strains (MRSA), with a clearly dose-dependent effect and consistent results across all microbiological assays performed.

Furthermore, in the treatment of facial photoaging, topical use of REGEN(PJ)FACEZONE® proved to be an effective alternative, producing significant clinical improvements in hydration, elasticity, and reduction in the size and intensity of solar lentigines, reflecting its potential as a non-invasive regenerative therapy in aesthetic dermatology.

Overall, the findings of this study indicate that the ozonized oils REGEN(PJ)FACEZONE® and REGEN(PJ)BODYOZONE® represent a safe, effective, and sustainable therapeutic alternative against resistant bacterial infections, while also providing additional benefits in skin regeneration, improvement of skin quality, and reduction of visible signs of aging.

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Electronic correspondence: franciscopeeter@gmail.com.

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