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LITERATURE REVIEW:

Is the Use of Ozonated Oil Effective in the Treatment of Oral Lesions? Systematic Review of Clinical Studies

¿Es el uso del aceite ozonizado efectivo en el tratamiento de las lesiones orales? Una revisión sistemática de estudios clínicos

Jusef Yabar Condori[1] https://orcid.org/0000-0003-2932-9849; Jonathan Meza-Mauricio[2] https://orcid.org/0000-0002-4878-9835
Gerardo Mendoza-Azpur[3] https://orcid.org/0000-0002-6548-1285; Yuri Castro-Rodríguez[4] https://orcid.org/0000-0002-9587-520X

1. Dental Surgeon, Periodontics specialist. Department of Periodontology. School of Dentistry. Universidad Científica del Sur. Lima. Peru.
2. Periodontics master. Department of Periodontology and Oral Implantology, Dental Research Division, University of Guarulhos, SP, Brazil.
3. Periodontics master. Department of Periodontology. School of Dentistry. Universidad Científica del Sur. Lima. Peru.
4. Education Sciences PhD. Department of Periodontology. School of Dentistry. Universidad Científica del Sur. Lima. Peru.

Correspondence to: Yuri Castro-Rodríguez - yuricastro_16@hotmail.com
Jusef Yabar Condori - yabarjusef@gmail.com

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ABSTRACT: The aim of this study was to perform a systematic review (SR) of the therapeutic effect of ozonated oil for oral lesions treatment. A SR was conducted according to the PRISMA guidelines. The Medline (PubMed), Embase, Cochrane Library, Scielo and LILACS were investigated, together with manual searches, to extract all publications until December 2020, including randomized and non-randomized clinical trials reporting the effects of ozonated oils on the management of oral lesions when compared with other methods. The risk of bias (RoB) of the studies included were assessed by using the RoB 2 tool and ROBINS-I. After analyzing the titles and reading the abstracts, 1932 articles were excluded; the remaining 25 passed a full-text evaluation. Ultimately, 13 articles were included in this SR. There was heterogeneity of the results regarding healing times and intervals of ozonated oil application for the treatment of each type of oral lesion, but in general, there was a shorter healing time when ozonated oil was used as therapy, and no adverse effects were reported. Despite the limited information found and the lack of rigorous methodological standards for the use of ozonated oil on oral lesions, a positive effect was suggested. The findings indicated an advantage in terms of shorter healing times when compared with other conventional treatments. No adverse effects were reported, showing safety and reliability for patient's treatment.

KEYWORDS: Mouth; Ozone; Ozoile; Therapeutics; Wounds and injuries.

RESUMEN: El objetivo de este estudio fue realizar una revisión sistemática (RS) del efecto terapéutico del aceite ozonizado sobre las lesiones orales. Se realizó una RS siguiendo las directrices PRISMA. Se realizaron búsquedas en Medline (PubMed), Embase, Cochrane Library, Scielo y LILACS, y búsquedas manuales, que abarcaron hasta diciembre de 2020, de ensayos clínicos aleatorizados y no aleatorizados que informaran sobre el efecto de los aceites ozonizados en el tratamiento de las lesiones orales en comparación con cualquier otro método. El riesgo de sesgo (RoB) de los estudios incluidos se evaluó mediante la herramienta RoB 2 y ROBINS-I. Tras analizar los títulos y leer los resúmenes, se excluyeron 1932 artículos; los 25 restantes pasaron una evaluación exhaustiva del texto completo. Finalmente, se incluyeron 13 artículos en esta RS. Hubo heterogeneidad de resultados en cuanto a los tiempos de cicatrización y los intervalos de aplicación del aceite ozonizado para el tratamiento de cada tipo de lesión oral, pero en general, hubo un menor tiempo de cicatrización cuando se utilizó el aceite ozonizado como terapia, y no se comunicaron efectos adversos. A pesar de la limitada información encontrada y de la falta de normas metodológicas rigurosas sobre el uso de aceite ozonizado en lesiones orales, se sugirió un efecto positivo del uso de aceite ozonizado para el tratamiento de lesiones orales. Los resultados indicaron una ventaja en términos de menor tiempo de curación en comparación con otros tratamientos convencionales; además, no se notificaron efectos adversos, por lo que se demostró una opción de tratamiento segura y fiable para los pacientes.

PALABRAS CLAVE: Boca; Ozono; Terapéutica; Heridas y lesiones.

INTRODUCTION

Oral mucosa lesions could be presented as changes in color, appearance, swelling or loss of the integrity of the tissue surface. Some types of these lesions may be benign and do not require any treatment, others may present acute symptoms, affecting patient's quality of life, causing difficulties with swallowing and speech, with symptoms such as burning, irritation and pain (1). The prevalence of oral lesions in the general population worldwide ranges from 4.9% to 64.7% (2, 3).

The oral mucosa could be an easily accessible point of entry for infectious agents, such as bacteria or viruses. Therefore, an early diagnosis and treatment is essential to avoid future complications or the development of serious, even malignant lesions (4). There are several alternatives for conventional treatment according to the type of mucosal lesion the patient has, however, some of these have been shown to be inefficient, by generating discomfort to the patients and requiring relatively extended treatment times. In view of the foregoing, there are treatment alternatives in

natural and traditional medicine that are used as therapy for oral diseases, such as ozone therapy that has shown some positive findings (5).

Ozone is a natural gaseous molecule formed by 3 oxygen atoms. Ozone has been recognized for being immunostimulant, analgesic, anti-hypnotic, detoxifying, antimicrobial, and being capable of performing bioenergetic and biosynthetic actions. Due to its atraumatic, painless, non-invasive characteristics, it can be considered highly reliable and has shown a high acceptance rate among patients (6). There are 3 ways to apply ozone: ozone gas, ozonated water and ozonated oil (7).

Ozonated oil results from the reaction of ozone with fatty acids and other substances, by means of bubbling ozone through of a generator. During this reaction, substances such as peroxides, ozone, aldehydes and ketones are formed (8, 9). Ozonated oil that comes into contact with exudates or injuries is divided and generates ozone, releasing hydrogen peroxide and lipoperoxides, promoting regenerative and disinfectant effects. This slow release of ozone further promotes the healing process by acting as a local antimicrobial agent and promoting cytokine release with an effect on tissue repair (10).

There are many studies where ozonated oil has been used as a therapeutic agent for different types of oral lesions, showing better results than control conventional treatments. Some benefits described in the literature include quicker healing, less discomfort and absence adverse effects (5, 11-22). However, there is no standardized methods to apply it, benefits or the ideal treatment time. Therefore, a systematic review was conducted to answer the following focused question based on a PICO strategy: Is the use of ozonated oil effective in the treatment of ulcerated lesions of the oral mucosa? And: is the use of ozonated oil effective in the treatment of alveolar bone lesions?

MATERIALS AND METHODS

PROTOCOL AND REGISTRATION

This SR was conducted in accordance to the Transparent Reporting of Systematic Reviews and Meta-Analysis-PRISMA Statement (23) and was registered at PROSPERO with number CRD42021237068.

FOCUSED QUESTION

The focused questions to be addressed were:

1. Is the use of ozonated oil effective in the treatment of ulcerated lesions of the oral mucosa?
2. Is the use of ozonated oil effective in the treatment of alveolar bone lesions?

ELIGIBILITY CRITERIA

The inclusion criteria for this SR were the following: Clinical studies that used ozonated oil as a treatment for different oral lesions (subprosthetic stomatitis, recurrent aphthous stomatitis, herpetic gingivoestomatitis, pericoronaritis, alveolitis and fibroedematous gingivitis). Literature reviews, letters to the editor, short communications, case reports, pilot studies and trials that analyze other applications of ozonated acceptance not related to oral injuries were excluded.

SEARCH STRATEGY

The MEDLINE (PubMed), Embase, Cochrane Library, Scielo and LILACS databases were searched up to March 2021 by two independent reviewers (J.Y and J.A). The search was performed without restrictions on dates or language. The search strategy was applied as follows: PubMed: ("Aphthous stomatitis" OR "Dry Socket" OR "Pericoronitis" OR "Herpetic Stomatitis" OR "subprosthetic stomatitis" OR

"fibro edematous" OR “alveolitis” OR osteomyelitis OR "Aphthous Ulcer" OR "Aphthous Stomatitides" OR "Alveolar Osteitis" OR "Herpetic Gingivostomatitis" OR "Oral Herpes Simplex" OR "oral injury" OR "oral lesion") AND ("ozonated oil" OR "Ozonated" OR "ozonation" OR "ozone" OR "Ozonized" OR "ozone therapy" OR "ozonotherapy" OR "ozonating" OR "oleozon" OR "vegetable oils" OR "plant oils" OR "ozonation"). In addition, the grey literature in the System for Information on Grey Literature in Europe (http://www.opengrey.eu) and Google Scholar databases were screened electronically, as recommended by the high standards for systematic reviews (AMSTAR guideline) (24). Finally, the list of references of studies included were also hand searched to capture any potential additional records, as suggested by Greenhalgh and Peacock (25).

DATA COLLECTION, EXTRACTION AND MANAGEMENT

SCREENING AND SELECTION OF ARTICLES?

Titles and abstracts were screened by two independent reviewers (JY. and JA). Full-texts were obtained and reviewed for studies that appeared to meet the inclusion criteria. Kappa scores (Cohen’s Kappa coefficient) were used during full-text assessment to ensure eligibility and level of agreement between the reviewers. Disagreements were solved by discussion and consulting a third reviewer (GM).

DATA EXTRACTION

The studies that fulfilled the eligibility criteria were processed for data extraction, conducted by two independent researchers, using predefined spreadsheets. Disagreements were resolved by discussion with a third reviewer. In the event of missing data, a request was sent to the authors by e-mail. For each study selected, the following variables were collected: 1) characteristics of the study: author, year of publication, country, design of the study (randomized clinical trial and non-randomized); 2) demographic data: sample size, mean age (years) and gender (male/female); 3) outcome measures: healing time, application interval and adverse effects (presence or absence).

RISK OF BIAS IN INDIVIDUAL STUDIES

Two reviewers (J.M.M and J.Y) evaluated the risk of bias in studies selected, using the Cochrane tool for risk of bias assessment, RoB 2 (version 2, available at: https://www.riskofbias.info/welcome/rob-2-0-tool/current-version-of-rob-2).

The authors of this SR decided to evaluate the result related to "assignment to the intervention (the effect for the purpose of treating)" and five domains were examined: randomization bias and treatments concealment, bias due to deviations from the planned interventions that affected the involvement of the participants and a team of researchers, although there were missing result data, the result measurement and the result selection were selected. Based on the responses to the questions and the algorithms of the tool, it was considered that each domain had “low risk of bias”, "some concerns related to risk of bias" or "high risk of bias". The studies were categorized as low risk of bias (all domains had low risk of bias), high risk of bias (one or more domains had high risk of bias) and concerning (when one or more domains had some concerns) (26). The decisions were resolved by means of discussion, consulting a third advisor (G.M).

The non-randomized controlled trials were evaluated by means of the ROBINS-I tool. (27) Each controlled trial was classified as low information, or as low, moderate, severe and critical risk of bias. The discrepancies between the reviewers were resolved by means of open discussion. In the event that an agreement could not be achieved, the final decision was made by another co-author (G.M).

The following domains have evolved to not randomized controlled trials (27):

Preintervention
- Confusion bias
- Selection bias

Intervention
- Bias in the classification of interventions

Post-intervention
- Bias due to deviations from planned interventions
- Bias due to missing data
- Bias in the results measurement
- Bias in the selection of the informed result

To demonstrate the general risk of bias, each study included was classified as low, moderate, severe and critical risk of bias or the absence of information according to the following criteria:

- Low risk of bias: the study is considered to have a low risk of bias for all domains.
- Moderate risk of bias: the study is considered to have a low to moderate risk of bias for all domains.
- Severe risk of bias: the study is considered to have a severe risk of bias for at least one domain, but it does not have a critical risk of bias for any domain.
- Critical risk of bias: the study is considered to have a critical risk of bias in at least one domain.
- No information: there is no clear indication that the study has a serious or critical risk of bias and there is a lack of information on the most key domains for bias (a judgment is required for this).

RESULTS

STUDY SELECTION

The electronic search strategy identified 1957 articles. After analyzing the titles and abstracts, 1932 studies were excluded, the remaining 25 were subject of a full text exhaustive evaluation, from which 12 were excluded. As a result, 13 papers were included in this SR (Figure 1).

STUDY CHARACTERISTICS

The thirteen randomized (5, 11, 12, 14, 16, 18-20) and non-randomized clinical trials (13, 15, 17, 21, 22) included were conducted between 2010 and 2020, and their main methodological characteristics are presented in Table 1. All clinical studies showed the effective outcome of the ozonated oil on different types of oral lesions: subprosthetic stomatitis, recurrent aphthous stomatitis, herpetic gingivostomatitis, pericoronitis, alveolitis and fibro edematous gingivitis; and used as a control and conventional treatment for each type of oral lesion. A total of 1644 patients from different age groups were included in the present SR. Participants in 3 studies suffering from pericoronitis (11, 12, 22), participants in 3 studies developing alveolitis (5, 18, 22), 5 studies conducted in patients with recurrent aphthous stomatitis (14, 16, 19, 21, 22), 3 studies based on subprosthetic stomatitis (15, 17, 22), 2 studies were based on patients with fibroedematous gingivitis (20, 22), lastly a single study contemplating the use of ozonated oil for patients with herpetic gingivostomatitis (13). In all studies the local application of ozonated oil was used as therapy. The follow-up periods varied from 72 hours to 6 weeks, depending on the type of wound.

OZONATED OIL IN PERICORONITIS

In 2 randomized clinical trials (RCT) (11, 12) and 1 non-RCT(22) authors reported the use of the ozonated oil as therapy for pericoronitis and compared it with conventional therapy consisting of 50% trichloroacetic acid and mouthwashes with 0.2% chlorhexidine for a period of time between 3 to 7 days, an interval of application was 3 to 4 times a day, with a healing time between 72 hours and 7 days, a larger number of patients recovered in less time in the study group (ozonated oil), while the control groups showed lower satisfaction rates in

the same period of time, showing statistically significant differences. No adverse effects were reported.

OZONATED OIL IN ALVEOLITIS

In 2 RCT (5, 18) and 1 non RCT (22) Ozonated oil treatment was used as a treatment for alveolitis and compared it with alvogyl. All the studies had a 7-day follow-up period, the time interval in which the healing took place. The lesions healing times were heterogeneous, varying between 4 days (18), 5 days (5) or up to 7 days (22) for study group (ozonated oil), the interval of application varied from 4 times a day (22) up to every 72 hours (5), no adverse effects were reported. In all cases, a shorter recovery time was observed when Ozonated oil was used as therapy. However, only one study (5) reported statistically significant differences.

OZONATED OIL IN FRIBROEDEMATOUS GINGIVITIS

Only 2 studies: 1 RCT (20) and 1 non-RCT (22), used the Ozonated oil therapy for fibroedematous gingivitis and it was compared with the use of chlorhexidine mouthwashes at 0.2%, the healing time varied between 7 days (22) showing only favorable results in study group (ozonated oil) and 6 weeks (20) (in which 96.6% of patients healed in the study group), the interval of application varied from 4 times a day (22) to 2 times a week (20), no adverse effects were reported. In both studies there was a shorter healing time when ozonated oil was introduced.

OZONATED OIL IN SUBPROSTHETIC STOMATITIS

Three (15, 17, 22) of the studies included in this SR (non-RCT) used ozonated oil as treatment for subprosthetic stomatitis and compared it with conventional treatment consisting of nystatin. The follow-up time ranged from 7 days (17, 22) to 15 days (15), on the other hand, the interval of application of ozonated oil for this type of injury varied from once a day (15, 17) to 2 times a day (22). The healing time found in the studies varied from 7 days (17, 22) up to 12 days (15). In all the studies, there was a shorter recovery time when Ozonated oil was used as therapy compared with treatment with nystatin. No adverse effects were reported.

OZONATED OIL IN RECURRENT APHTHOUS STOMATITIS

In 3 RCTs (14, 16, 19) and 2 non-RCT (21, 22) studies the Ozonated oil was used to treat recurrent aphthous stomatitis and compared it with other therapies such as: multivitamins and 0.2% chlorhexidine mouthwashes (14, 19), sodium perborate alkaline mouthwashes and 0.2% chlorhexidine mouthwashes (16, 22), and finally a comparative study with metronidazole gel (21), with a follow-up time ranging from 7 (14, 16, 19, 22) to 14 days (21), an interval of application of between 2 (14, 19, 22) to 4 (16, 21) times a day, as a healing time between 5 (14) and 7 days (16, 19, 21, 22). In all cases, a shorter recovery time was observed when Ozonated oil was implemented as therapy. Only 2 studies (19, 21) reported statistically significant differences. No adverse effects were reported.

OZONATED OIL IN HERPETIC GINGIVOESTOMATITIS

Only 1 non RCT study (13) included in the present SR used the Ozonated oil as therapy for herpetic gingivoestomatitis and compared it with the use of idoxuridine at 0.1% with a follow-up period of 10 days, the healing time was ≤8 days for the control group, the interval of application was once a day, without specifying the presence of the adverse effects. The patients treated with the Ozonated oil had a shorter healing time of the lesion.

RISK OF BIAS IN THE STUDIES SELECTED

In RCT studies, inadequate methods of generating sequences and assigning participants were found in all of the publications (90%) included and only one study showed a correct assignment process between groups (Figure 2). Among the deviations from the planned interventions, 60% of the studies were classified as "high" due to problems with the masking process (Figure 3). However, it was not possible to mask the entire staff due to the experimental context. Finally, all studies reported results without a pre-specified plan. In general, it was considered that only one study had a clear risk of bias. Figures 2 and 3 show a summary of the results.

Three of the non-RCT studies were classified as having a severe risk of bias (15-17), one study with a moderate risk of bias (21) and one study was classified as having a critical risk of bias (13) as shown in Figure 4.

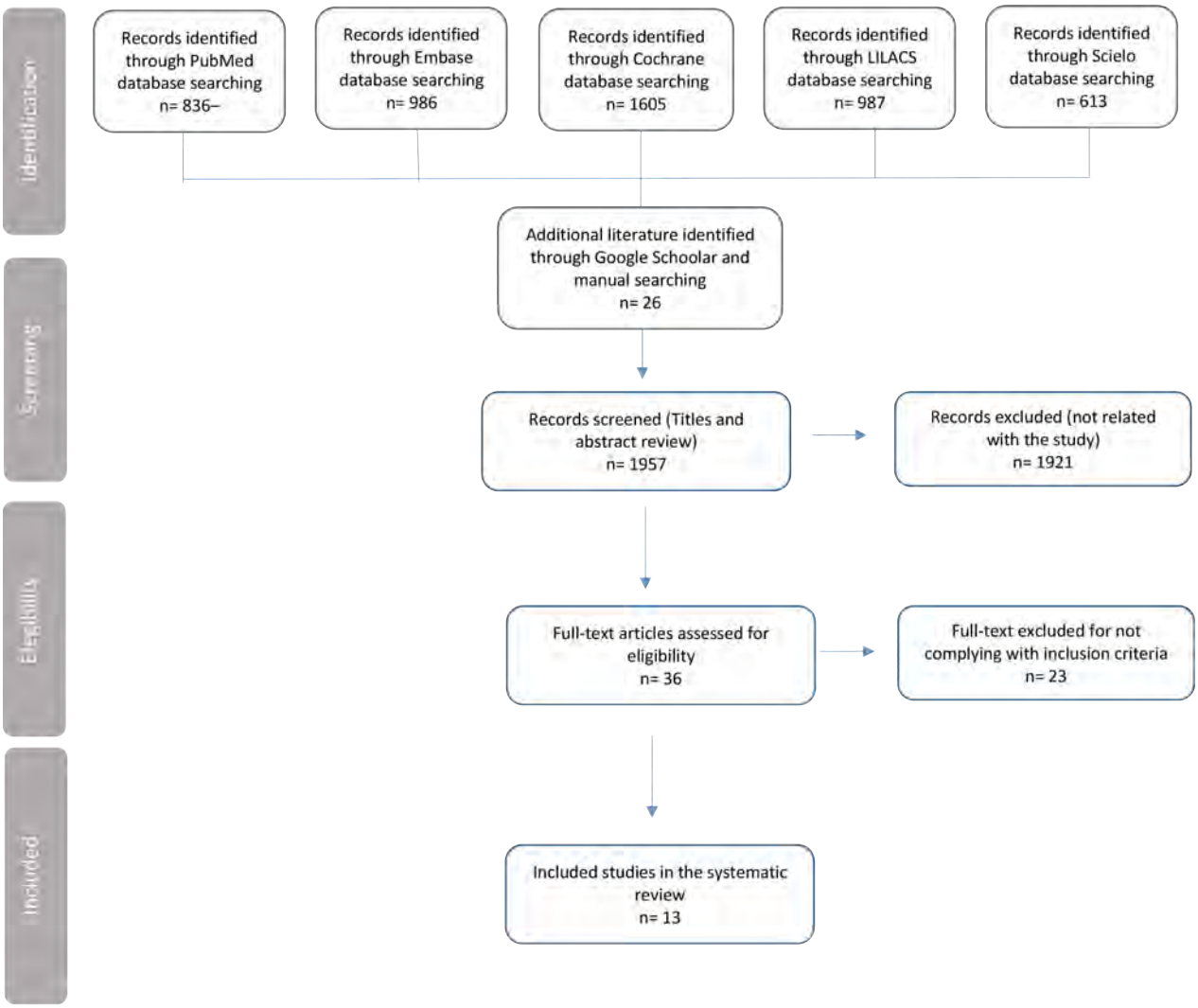

Figure 1. Flow chart of manuscripts selected through the review process.

Table 1. Main characteristics of the included studies in the systematic review.

Author and year	Design	N	Age	Gender (M/F)	Type of oral lesion	Test group	Control group (CG)	Assessed parameters	TF	AI	HT SG/CG	AE	Results
Mayor & col. (11) 2010	RCT	90	> 15	25/65	pericoronitis	Ozonated oil	50% trichloroacetic acid	*Clinical evaluation*: satisfactory (improvement or cure at 72h), unsatisfactory (equal to or worse at 72h). *Efficacy of the study drug*: good (satisfactory clinical evolution), poor (unsatisfactory clinical evolution)	72h	3 times a day	72h/ >72h	no	100% good efficacy for SG (OO), 62% good efficacy and 38% poor efficacy for CG (trichloroacetic acid).
Mayor & col. (12) 2011	RCT	90	> 15	Not specified	pericoronitis	Ozonated oil	50% trichloroacetic acid	*Microbiological evaluation*: satisfactory (field absent or scarce, Gram- not predominant), unsatisfactory (abundant field or covered field, Gram- predominate). Degree of patient satisfaction (satisfied vs not satisfied, according to the patient's appreciation) and adverse effects (yes or no)	72h	3 times a day	72h/ >72h	no	Microbiological evaluation, satisfactory SG in 43 patients (96%), satisfactory CG in 18 patients (40%). Patient's satisfaction: Satisfactory SG 43 patients (96%), CG 30 patients (66%).
Rodriguez & col. (13) 2012	No RCT	250	<9	82/168	Herpetic gingivo-estomatitis	Ozonated oil	0.1% idoxuridine	*Evolution: Cured*: When there are no clinical signs of Acute Herpetic Gingivostomatitis. Not cured: When there are clinical signs of Acute Herpetic Gingivostomatitis in the oral cavity	10d	1 time a day	≤8d/ 8a 10d	no	79 patients treated with OO (63.2%) cured in < 6 days, 78 patients treated with iodoxuridine (62.4%) were cured in 9 to 10 days; the treatment with OO minimized the healing time.
Martinez-Abreu & col. (5) 2015	RCT	100	20-59	56/44	Alveolitis	Ozonated oil	Alvogyl	*Clinical*: recovered (pain and inflammation disappear), improved (pain and inflammation decrease), the same (pain maintains intensity and inflammation persists), worse (pain and inflammation persists or worsens). *Microbiological*: infected (presence of mopyogens), free of infection (absence of mopyogens Efficacy evaluation: good (clinical criterion of recovered and microbiological free of infection), acceptable (clinical criterion of improved and microbiological free of infection) and poor (clinical criterion of equal or worse and microbiological infected).	7d	Every 72h	3 to 4 days/ >3,4 days	Not specified	100 patients in 2 groups: 50 SG (OO) and 50 CG (alvogyl) The patients were evaluated at 72h (2nd visit), 96h (3rd visit) and at one week of treatment (4th visit). 41 SG patients only needed 2 visits to recover, CG only 29 patients. For the microbiological evaluation: From 20 patients in the SG 13 patients showed signs of recovery from infection, while in the CG from the 20 evaluated patients only 6 showed the same result.

Author and year	Design	N	Age	Gender (M/F)	Type of oral lesion	Test group	Control group (CG)	Assessed parameters	TF	AI	HT SG/CG	AE	Results
Fontaine & col. (14) 2016	RCT	80	> 12	26/54	recurrent aphthous stomatitis	Ozonated oil	vitamins and mouthwash with 0.2% chlorhexidine.	*Clinical evaluation*: satisfactory (the pain decreases or disappears; the size of the lesion decreases or disappears) not satisfactory (the pain persists and there is no change in the lesion). *Healing*: cured (there are no clinical signs of stomatitis) and not cured (there are clinical signs of stomatitis in the mucosa). Degree of satisfaction: satisfied and not satisfied (for both criteria the perception of the patients was taken into account).	7d	2 times a day	4 to5 days/ >7 days	no	SG and CG 40 patients each. OS: 34 patients needed only two to three applications to consider a satisfactory clinical evaluation, 38 patients only needed a maximum of 4 to 5 days to consider themselves cured. CG: 18 patients needed more than 7 days. Degree of satisfaction: 97.5% of the GE satisfied.
Castillo & col. (15) 2018	No RCT	68	>19	17/51	subprosthetic stomatitis	Ozonated oil	nystatin	*Clinical evaluation*: healing was determined as the lesion disappeared and the physiological mucosa became.	15d	1 time a day	4 to 12d/ 7 to 15d	no	CG the mucosa recovered normality between 10 to 12 days (52.9% of the sample). Only 14.7% achieved it between 7 and 9 days; no patient was healed during the first week of treatment. SG 44.1% of patients reached normality of the mucosa between 7 to 9 days, 32.4% reached normality between 4 to 6 days. 76.6% of the SG patients healed before 10 days of application.
Diaz-Couso & col. (16) 2018	RCT	32	> 12	12/20.	recurrent aphthous stomatitis	Ozonated oil	alkaline mouthwashes sodium perborate and mouthwashes 0.2% chlorhexidine	*Cured*, there is a good general condition of the affected area (remission of symptoms) and disappearance of the ulcer. *Not cured*, permanence of the ulcer, the symptoms do not subside with the application of the medicine. For the criterion of adverse reactions, the presence in the affected area of burning, redness, heat, tumor and pain was taken into account.	7d	4 times a day	3 to 7 days on both groups	no	On the third day, the eight SG patients evolved favorably (50%) and in the CG it was 12.5%. In general the evolution of the treatment, cured patients: SG 93.7% and CG 68.7%.

Author and year	Design	N	Age	Gender (M/F)	Type of oral lesion	Test group	Control group (CG)	Assessed parameters	TF	AI	HT SG/CG	AE	Results
Jimenez & col. (17) 2018	No RCT	40	> 20	17/23	subprosthetic stomatitis	Ozonated oil	nystatin	*Treatment effectiveness*: Very effective, effective and not very effective.	7d	1 time a day	4 to days/ >7 days	no	Evaluation of the effectiveness of treatment in sub-prosthesis stomatitis: SG, effective treatment in 14 patients (35.0%), CG, ineffective treatment with 18 patients (45.0%). The daily application of OO was effective for the treatment of this disease between four and seven visits to consultation, healing time was shorter compared to nystatin cream in the CG.
Souto & col. (18) 2018	RCT	100	20-59	Not specified	alveolitis	Ozonated oil	alvogyl	*Pain* (referred by the patient at the beginning and after the application of the treatments at 5, 15, and 30 minutes, visual analog scale). *Inflammation* (state of the mucosa surrounding the alveolus, by direct observation: inflamed (hyper-colored, increased in volume, smooth); slightly inflamed (slightly hyper-colored and slight increase in volume) and non-inflamed (normally colored, resilient and normal size). *Scarring*: non-healing (delayed clot formation, stench, hypersensitivity in the walls of the alveolus), partial scarring (despite the new clot formation, presents hypersensitivity, slight stench) and total scarring (alveolus occupied by a new blood clot) , with formation of granulation tissue and proliferation of the epithelium on the external surface). *Response to treatment*: no response to treatment (same patient , pain does not disappear, no clot formation, hypersensitive alveolus walls and stench), improved (not intense pain, slight stench and new clot formation) and healed (the clot disappears). pain, clot formed and granulation tissue formation).	7d	1 application	4 to 5d/ >7 d	Not specified	*Pain*: SG, 40 patients (80%) did not report pain 5 minutes after applying the OO. CG, 13 patients did not report pain 5 minutes after applying the drug, there were statistically significant differences. *Grade and inflammation*: SG, 40 patients with inflammation at 96 hours after applying the treatment, however, there were no statistically significant differences. *Healing*: SG, 46 patients (92%) healed at 96h. CG, 44 healed patients (88%). *Response to treatment*: 86% of SG was cured at 96h and 80% of CG in the same time period.

Author and year	Design	N	Age	Gender (M/F)	Type of oral lesion	Test group	Control group (CG)	Assessed parameters	TF	AI	HT SG/CG	AE	Results
Leal-Rodríguez & col. (19) 2019	RCT	120	15-35	40/80	recurrent aphthous stomatitis	Ozonated oil	vitamins, diphenhydramine and mouthwash with 0.2% chlorhexidine.	They were evaluated from the clinical point of view the status of the lesions and all the data referring to the time of evolution, to establish the diagnosis and establish treatment	7d	2 times a day	7d / >7d	no	Evaluation on the seventh day: SG, 93.33% cured. CG only 56.67% evolved satisfactorily. Therefore, the time required for the clinical signs of the disease to disappear was shorter when OO was applied than when conventional treatment was applied.
Peña & col. (20) 2019	RCT	60	> 15	Not specified	chronic fibroedematous gingivitis	Ozonated oil	mouthwashes with 0.2% chlorhexidine	The status of the lesions was evaluated from the clinical point of view: healed, improved, equal or worse	6 weeks	2 times a week	6 weeks	no	In both groups, most of the patients evolved satisfactorily during the first 2 weeks of treatment. GE after the fourth visit or week of treatment, it was clinically observed the resolution of all inflammation symptoms in 70% of patients, while in CG 56.6%. In the sixth week in the EG, 96.6% of the cases were given a cure criterion, while in the CG it was 90%.
Alexandrina & col. (21) 2020	No RCT	30	Not specified	Not specified	recurrent aphthous stomatitis	Ozonated oil	Metrºogil denta gel (metronidazole 10mg)	*Clinical examination*, including a survey, clarification of complaints, and a visual examination. For more detail, they used a bacterioscopic study.	14d	4 times a day	3 to 7 days for both groups	Not specified	Compared with traditional dental treatment, the advantage of using OO was noted, on the second day after treatment, patients reported a decrease in pain. The oxygen-ozone irrigation method was less effective. Subjects in the third group, who were treated with Metrogil Denta applications, showed the worst results and clinical improvement was observed between 6 and 7 days after treatment.

Author and year	Design	N	Age	Gender (M/F)	Type of oral lesion	Test group	Control group (CG)	Assessed parameters	TF	AI	HT SG/CG	AE	Results
Díaz-Couso (22) 2020	No RCT	464	> 12	215/249	Alveolitis, subprosthetic stomatitis, chronic fibromatous gingivitis, recurrent aphthous stomatitis, pericoronitis	Ozonated oil	Subprosthetic stomatitis (Nystatin), recurrent aphthous stomatitis (alkaline sodium perborate mouthwashes and 0.2% chlorhexidine antiseptic rinses), alveolitis (alvogyl), Pericoronitis (0.2% chlorhexidine). Chronic edematous gingivitis (0.2% chlorhexidine mouthwash)	The effectiveness of the treatment was taken into account through its favorable or unfavorable evolution to treatment during seven days, remission of symptoms and / or disappearance of the disease. For the criterion of adverse reactions, the presence of burning, redness, heat, tumor and pain in the affected area was taken into account.	7d	2 times a day	7d / >7d	no	On the third day, SG had a favorable evolution of 97 patients (41.8%); Already on the seventh day there were 209 patients (90.1%). CG on the third day, only 26.2% had evolved favorably, growing to 53.3% on the fifth day. It was observed that on the seventh day there was a total absence of pain and remission of all the symptoms of oral diseases in a greater number of patients, belonging to the SG.

RCT: Randomized clinical trial, No RCT: Non-randomized clinical trial, N: number of participants, M: male, F: female, TF: Time of follow-up, d: Time in days, h: time in hours, AI: Application interval, SG: Study group, CG: Control group, HT: healing time, AE: Adverse effects

Table 2. Studies excluded from and reasons for their exclusion.

First author (Year)	Title of the paper	Reason for the exclusion
Soler y col (2020)	Application of ozonated sunflower oil in patients affected with chronic fibroedematous gingivitis	Does not present a control group
Frías y col (2020)	Effectiveness of ozone therapy in the treatment of sub-prosthesis stomatitis in Manzanillo 2018-2019.	Does not present a control group
Céspedes y col (2019)	Usefulness of topical Oleozón® in oral ulcers in patients with mechanical artificial ventilation	There is no comparison with conventional therapy
Télles y col (2018)	Use of oleozón® in sub-prosthetic stomatitis	Does not present a control group
Jorge y col (2017)	Effectiveness of oleozón in the treatment of sub-prosthetic stomatitis in patients over 60 years of age.	Does not present a control group
Casado y col (2017)	Effectiveness of oleozon treatment versus aloe vera cream in subprosthetic stomatitis	There is no comparison with conventional therapy
Al-Omiri y col (2016)	Ozone treatment of recurrent aphthous stomatitis; a double blinded study	Does not present a control group
Kumar y col (2016)	Efficacy of Ozonated olive oil in the management of oral lesions and conditions: a clinical trial	Does not present a control group
Milanés y col (2016)	Ozone therapy in sub-prosthesis stomatitis, 2016	Does not present a control group
Armas y col (2015)	Efficacy of two natural therapies in the remission of pain in recurrent aphthous stomatitis	There is no comparison with conventional therapy
Báez y col (2015)	Ozone therapy in a patient with bucal sores	Case Report
Colás-Costa y col	The effectiveness of oleozon in the treatment of recurrent aphthous stomatitis	Does not present a control group

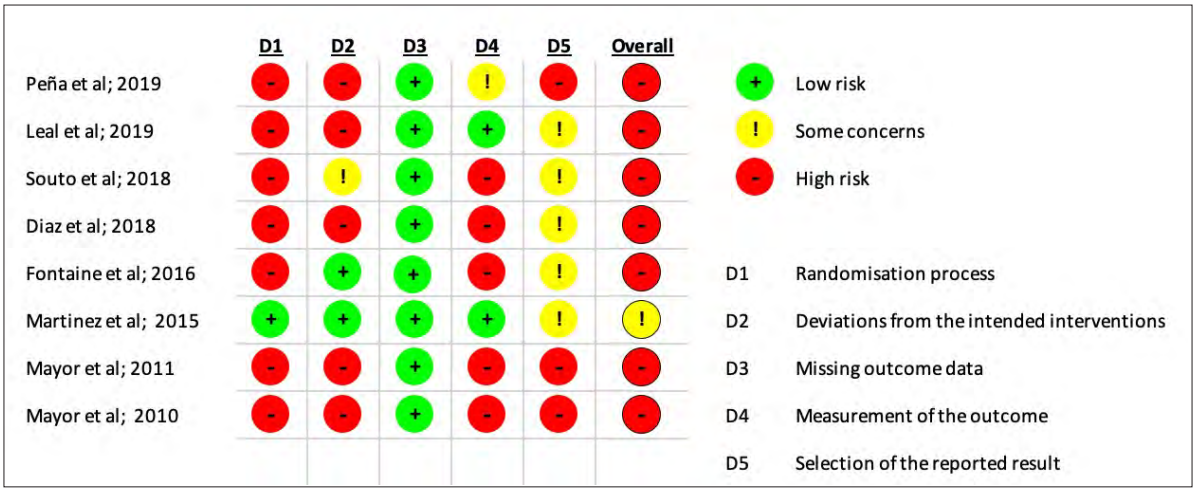

Figure 2. Risk of bias summary of the clinical trials, included in the systematic review, based on the Cochrane risk of bias tool, RoB 2.

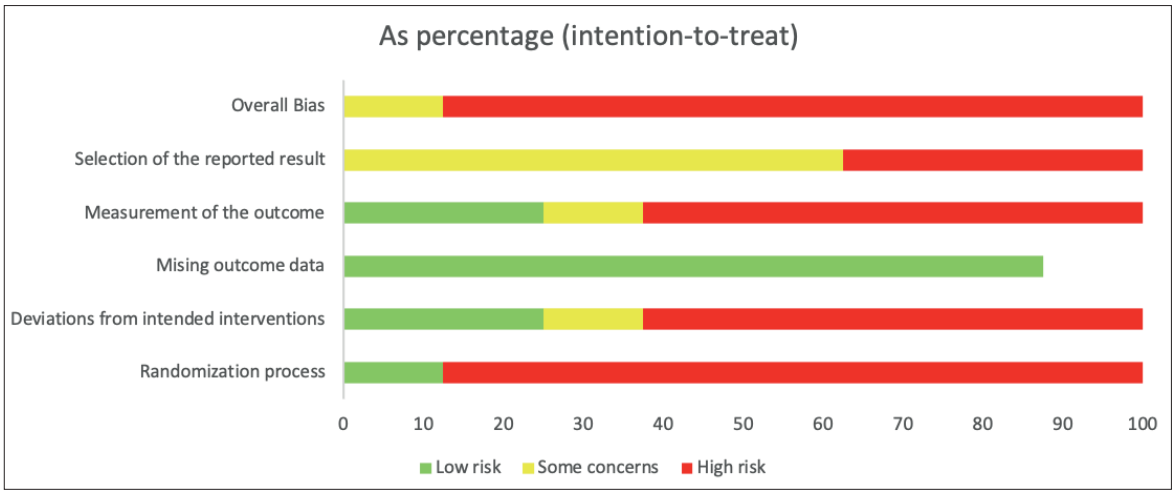

Figure 3. Risk of bias summary as a percentage of the clinical trials included in the systematic review.

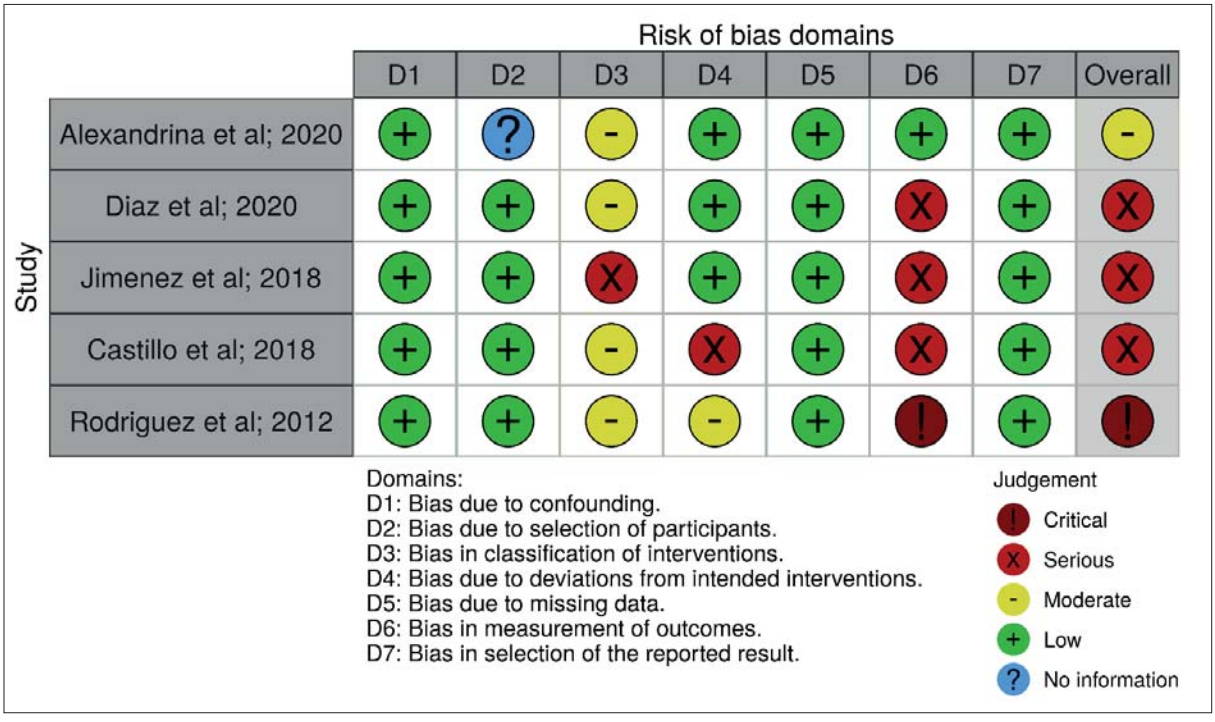

Figure 4. Risk of bias summary of the non-randomized clinical trials included in the systematic review, based on the tool ROBINS-I.

DISCUSSION

In this SR, the effect of Ozonated oil was studied in oral lesions such as subprosthetic stomatitis, recurrent aphthous stomatitis, herpetic gingivostomatitis, pericoronitis, alveolitis and fibroedematous gingivitis. Despite the differences of the oral lesions described, there was always a positive therapeutic effect when using ozonated oil when compared with the corresponding conventional treatment. In many cases this effect was reflected as a shorter healing time or less discomfort for the patient. In the majority of the studies included, the lack of adverse effects was another point favoring Ozonated oil, showing to be a safe therapy for patients.

Relative to pericoronitis, in a SR about the standard of care for pericoronitis based on evidence, Wehr *et al*. (28). informed that receiving antibiotics and follow-up appointment was the most common treatment option for these cases. In spite of this, in the 3 studies included in the present SR, which were related to this event, trichloroacetic acid and 0.2% chlorhexidine mouthwashes were used and compared with the local use of the Ozonated oil, showing the latter reduced healing times with statistically significant differences (11, 12).

Alvogyl is widely used for the treatment of alveolitis. However, Alexander (29) and Bloomer (30) do not recommend it for this type of condition. Furthermore, in this SR the superiority of Ozonated oil was shown when compared with the Alvogyl with positive results in a shorter time (5). It is worth mentioning that other studies have shown some other products that produced better results than those of alvogyl. For example, Taberner *et al*. (31) in a SR concerning the methods used for the management of dry alveolitis, recommending laser therapy, eugenol zinc oxide and growth factor-rich plasma; whereas, Ozonated oil appears to be a simpler alternative than the growth factors-rich plasma since the Ozonated oil costs less and even allows patients to apply the product on themselves (28).

Regarding fibro edematous gingivitis, Gawron *et al*. (32), in their review, mentioned that the routine treatment of this type of lesion - when it is minimal and local- is based on maintaining proper oral hygiene and performing a scaling and root planing. For diffuse and advanced gingival enlargement, however, surgical intervention is necessary, with the indication of chlorhexidine mouthwashes in all cases, and the recommendation is to investigate less invasive therapeutic methods. In the present SR, there are 2 studies (20, 22) in which the Ozonated oil, combined with a good oral hygiene, obtained favorable results and a shorter healing time when compared with the use of chlorhexidine. This could be debated considering the great antibacterial effect that has been reported for Ozonated oil in some microbiological studies (33, 34). In the cited studies, the potential of Ozonated oil to reduce the presence of periodontal pathogens and other opportunistic bacteria was shown to be equally effective than chlorhexidine (33, 34).

Subprosthetic stomatitis is regularly treated with nystatin, as its efficacy for treatment of this disease has been shown in several studies (35). Nevertheless, despite the afore-mentioned favorable results, 3 studies included in this SR compared the use of nystatin with ozonated oil (15, 17, 22), in which patients achieved recovery in a shorter time interval in study group (ozone oil). It is worth to mentioned that ozone oil not only has antimicrobial and anti-fungal properties, but also stimulates the immune system and promotes a site-specific wound healing (36).

Recurrent aphthous stomatitis (RAS) is the most common lesion in the oral mucosa, and although its natural history has shown that it is cured without any damage, the most severe forms need a treatment focused on reducing the pain and promoting the healing of ulcers (8,12). Corti-

costeroids are normally used, as well as topical application of chlorhexidine, tetracycline and diluted hydrogen peroxide (37, 38). Although there is no evidence of the bacterial origin of recurrent aphthous stomatitis, in this SR, in the 5 studies that tested treatments for this type of lesion (14, 16, 19, 21, 22), the majority compared the use of ozonated oil with local application of chlorhexidine and vitaminic supplements taken orally. The findings showed that compared with Ozonated oil, the treatments tested needed longer recovery times. These results could be associated with the antimicrobial and healing properties of the Ozonated oil (36), conferring a therapeutical advantage on it.

Herpetic gingivostomatitis is an oral lesion common in children and it is usually treated by controlling the symptoms and complemented by oral administration of Acyclovir (39, 40). In one study included in this SR, the authors used an anti-viral agent (idoxuridine) and showed a longer recovery time when compared with the Ozonated oil. In spite of the fact that there is no evidence of Ozonated oil having any antiviral properties, its known properties of promoting and improving healing (36) allowed a quick recovery of patients, once the viral cycle of the disease had passed.

In this SR there was diversity of information and methodology of the studies included that did not allow a metanalysis to be performed. The poor methodology was reflected in the high risk of bias. Therefore, the authors suggest that more clinical studies with higher standards of methodological rigor, need to be conducted. However, in spite of the diverse presentations in the studies selected, it was possible to observe advantages for the use of Ozonated oil in the treatment of oral lesions, achieving faster healing and the absence of adverse effects. As a result, the authors suggest that the use of ozone oil should be widely divulged throughout the dental community and scientific world, so that it may have a promising future in different types of treatment in our specialty.

CONCLUSION

In summary, in spite of the limited information found and the lack of methodological meticulousness of the included studies included in this SR, it was suggested that positive effects were obtained with the use of ozonated oil for the treatment of oral lesions, when compared with conventional alternatives. This effect on various types of oral lesions was found to be beneficial in terms of shorter healing times and no adverse effects, presenting a safe and reliable alternative treatment for patients suffering from soft tissue lesions.

CONFLICT OF INTEREST

The authors show no conflict of interest with respect to the article.

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AUTHOR CONTRIBUTION STATEMENT

Conceptualization and design: J.Y.C. and J.M.M.
Literature review: J.Y.C. and G.M.A.
Methodology and validation: J.Y.C., Y.C.R. and G.M.A.
Formal analysis: J.Y.C., Y.C.R. and J.M.M.
Investigation and data collection: J.Y.C.
Resources: J.Y.C. and G.M.A.
Data analysis and interpretation: J.Y.C., Y.C.R. and J.M.M.
Writing-original draft preparation: J.Y.C., and Y.C.R.
Writing-review & editing: J.Y.C., Y.C.R. and J.M.M.
Supervision: G.M.A and J.M.M.
Project administration: G.M.A.
Funding acquisition: J.Y.C.

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