International Journal Dental and Medical Sciences Research
Volume 6, Issue 5, Sep - Oct 2024 pp 587-592 www.ijdmsrjournal.com
ISSN: 2582-6018

Assessing the genotoxic safety of *Rosmarinusofficinalis* essential oils in the treatment of periodontitis

Ortíz Ortíz E[1], Pérez-Flores GA[2], Valencia-Quintana R[2], Sánchez-Alarcón J[2], García-Torres E[1*]

[1]*Facultad de Odontología, Universidad Autónoma de Tlaxcala. Mexico. Rivereña S/N, Centro, 90000 Tlaxcala de Xicohténcatl, Tlaxcala, Mexico.*

[2]*Facultad de Agrobiología, Universidad Autónoma de Tlaxcala. Mexico. CA Ambiente y Genética UATLX-CA-223*

Corresponding Author: García-Torres E; qcegt2015@gmail.com

Submitted: 25-10-2024 Accepted: 05-11-2024

ABSTRACT: Periodontitis is a multifactorial chronic inflammatory disease that causes the loss of dental organs and also contributes to the activation of factors that can induce genetic damage in relation to the periodontal state. Among the most used chemical treatments for periodontal disease is the application of chlorhexidine as the antiseptic agent of choice due to its antimicrobial effects and extracts from medicinal plants have also been used. Recently, the use of rosemary essential oils as anti-inflammatory and antibacterial agents has been considered, but their possible genotoxic effects are unknown. The objective of this study was to determine the genotoxic effect of an oral solution based on *Rosmarinus officinalis* essential oils in patients with periodontitis. For this, 10 subjects with a diagnosis of periodontitis in a range of 18 - 60 years old were included. At the beginning and end of treatment with 0.14% rosemary solution (for 14 days), a sample of epithelial cells was taken from the oral cavity and a smear was performed to evaluate the frequency of cellular abnormalities. The results indicate that there are no significant changes in the frequency of cellular abnormalities after treatment with rosemary solution for 14 days of use. This study provides evidence for the safe use of *Rosmarinus officinalis* essential oils as an alternative herbal therapeutic for periodontitis.

KEYWORDS: Rosmary, periodontitis, genotoxicity.

I. INTRODUCTION

Periodontitis is a multifactorial inflammatory disease affects individuals of all ages, is associated with accumulation of dental plaque resulting in the progressive destruction of dental tissues [1,2]. Some distinctive features of periodontitis include gingival inflammation, alveolar bone loss, mobility and bleeding [2]. The advanced stage of this disease causes loss of dental organs and can have a negative impact on systemic health [3]. Also, periodontitis contributes to the formation of reactive oxygen species (ROS) and with this, the activation of nuclear transcription factors is generated that can induce genetic damage in relation to the periodontal state. Some studies report genotoxic damage in patients with periodontitis [4]. For example, there is an increase in the frequency of micronuclei in patients with periodontitis compared to healthy subjects [5-7].

The most commonly used treatments for periodontitis are focused on limiting plaque accumulation and soft tissue inflammation and generally use mechanical and chemical techniques. In mechanical techniques, scaling and root planing are used, while in chemical techniques, chlorhexidine is generally applied as the antiseptic agent of choice due to its antimicrobial effects [8]. But, chemical treatments can cause side effects on oral tissues such as allergies, erosion of the oral mucosa and discoloration of teeth when these products are used for a long period of time [9]. Given this situation, the effect of medicinal plant extracts on different oral diseases and infections has been evaluated, obtaining benefits in terms of reducing dental plaque and inhibiting oral pathogens [10]. For example, to treat periodontal disease, herbal alternatives have been explored that include Curcuma longa [11,12], *Camellia sinensis, Cinnamomum zeylanicum, Aloe barbadensis, Allium sativum*, among others, whose antimicrobial properties are effective and safe [12].

Rosmarinus officinalis (rosemary) is an aromatic plant of the Lamiaceae Family whose chemical components have multiple biological

International Journal Dental and Medical Sciences Research
Volume 6, Issue 5, Sep - Oct 2024 pp 587-592 www.ijdmsrjournal.com
ISSN: 2582-6018

properties such as antioxidant, antifungal, anticancer, analgesic, antimicrobial and anti-inflammatory [13,14]. Its therapeutic efficacy has been shown to eliminate mono- and polymicrobial oral biofilms [15,16]. The anti-inflammatory properties of rosemary essential oil have been demonstrated in strains of Gram-positive and Gram-negative pathogenic bacteria, suggesting that it could be used as an antimicrobial and anti-inflammatory agent in topical creams, gels, disinfectants and mouthwashes aimed at oral pathologies [17]. Regarding toxicity, it has only been shown in mice and rabbits that the essential oil of *Rosmarinus officinalis* applied cutaneously and orally is not toxic for a period of 14 days nor does it produce adverse effects [18] and its bioactive components they do not generate mutagenicity [19].

These studies provide relevant information to consider the potential use of *Rosmarinus officinalis* as a safe and effective herbal therapeutic alternative to alleviate the inflammatory process of periodontitis in humans [12,17). Therefore, the objective of this study was to determine the genotoxic effect of *Rosmarinus officinalis* in patients with periodontal disease.

II. MATERIAL AND METHOD

Subjects of study

The present study was carried out at the Facultad de Odontología of the Universidad Autónoma de Tlaxcala, Mexico, in which 13 subjects with a diagnosis of periodontitis in an age range of 18 - 60 years old were included. The participants signed an informed consent. The procedure of this research adhered to the ethical standards of the General Health Law on Research and the standards of the Declaration of Helsinki. This study was approved by the bioethics and research committee of the Facultad de Odontología of the Universidad Autónoma de Tlaxcala, Mexico (Of. No.:FO-027-2003).

Study criteria for sample selection

Inclusion criteria: Adult between 18 and 60 years old, have a diagnosis of periodontitis, voluntary participation, indistinct sex, without systemic involvement, without consumption of medications or oral antiseptics. Exclusion criteria: Patients with orthodontic treatment. Elimination criteria: do not attend the post-treatment sample collection.

Procedure

At the first appointment, the clinical history of the study subjects was taken and a gingival diagnosis was performed. In addition, the study subject was instructed on the modified Bass technique and the rosemary-based oral solution was given with the following instructions: after brushing teeth, rinse with 10 ml of the solution for 1 minute, twice a day (after breakfast and dinner) for 14 continuous days without stopping [20].

Preparation of oral solution with Rosemary (0.14%)

To prepare 500 ml of herbal solution, 500 ml of distilled water was used and 700 µl of rosemary essential oil was added. The rosemary essential oil was obtained in Rancho San Martin Shop, Puebla, Mexico.

Sampling of bucal epithelial cells, fixation and staining

Before (Day 1) and after (Day 15) treatment, a cell sample was obtained from the oral mucosa to evaluate the frequency of cellular abnormalities. To do this, the patients were asked to rinse their mouth with water and saline solution in order to remove some bacteria, after which a gentle scraping of the inside of each cheek was performed with a microbrush. Subsequently, a smear was made, fixed with methanol acetic acid 3:1 and stored at room temperature until processing for staining. Fixed slides were treated for 1 min each in 50 and 20% ethanol and washed with deionized water for 2 min prior to staining. The slides were then treated with 5.0 M hydrochloric acid for 30 min followed by washing for 3 min in running tap water. Moist slides were treated with Schiff's reagent at room temperature in the dark for 60 min, washed in running tap water for 5 min and rinsed in deionized water. Slides were stained in 0.2% Light green for 30 s, rinsed indeionized water and allowed to air-dry at room temperature prior to being mounted with Entellan resin.

Microscopic analysis of cellular abnormalities

For each slide (two per patient), 1500 cells were examined and cellular abnormalities (MN, binucleate cells, karyorrhexis, karyolysis, condensed chromatin, lobed cells, pyknotic nuclei) were recorded at 400x magnification using a bright-field microscope (VELAB VE-B15 ®).

Statistical analysis of the data

Comparison of different cell types before and after using rosemary herbal solution was performed using Wilcoxon tests for dependent data.

International Journal Dental and Medical Sciences Research
Volume 6, Issue 5, Sep - Oct 2024 pp 587-592 www.ijdmsrjournal.com
ISSN: 2582-6018

The statistical analyzes were carried out in GraphPad version 6.0 considering a significance level of $p \leq 0.05$.

III. RESULTS

The initial oral examination indicated that all patients showed generalized gingival diagnosis. Table 1 shows the data on the characteristics of the patients who underwent treatment with rosemary herbal solution for 14 days.

The comparison of the frequency of the different cellular abnormalities analyzed did not show significant changes after treatment with the rosemary-based solution for 14 days of use (table 2).

Table 1. Description of factors in patients with periodontal disease exposed to treatment with rosemary oral solution.

Characteristic	
n	10
Sex M/F	3/7
Age (years; mean, SD)	39.6 ± 17.7
Cigaretteconsumption	
Smoker n (%)	1 (10)
Non smoker n (%)	9 (90)
Alcohol consumption	
Yes n (%)	3 (30)
No n (%)	7 (70)
Consumption of non-bottled water	
Yes n (%)	3 (30)
No n (%)	7 (70)
Fishconsumption	
Yes n (%)	6 (60)
No n (%)	4 (40)
Orthodontics	
Yes n (%)	0 (0)
No n (%)	10 (100)
Gingival Dx (mean, SD)	2 ± 0

Table 2. Comparison of the frequency of normal and abnormal cells in patients with periodontal disease before and after treatment with rosemary oral solution.

Cell type	**Before**	**After**	**P value**
Normal cells	95.8±1.4	93.0±5.2	0.37
Binucleated	2.3±1.5	3.7±2.5	0.27
Kariorrhexis	0.9±0.7	1.3±1.5	0.76
Chromatins	0.5±0.4	1.3±1.9	0.35
Karyolysis	0.3±0.3	0.3±0.5	0.76
Lobulated	0.03±0.07	0.1±0.4	0.87
Pyknosis	0.11±0.1	0.09±0.2	0.62
Micronucleus	0.1±0.2	0.06±0.09	0.90

Mean ± SD/1500 cells

International Journal Dental and Medical Sciences Research
Volume 6, Issue 5, Sep - Oct 2024 pp 587-592 www.ijdmsrjournal.com
ISSN: 2582-6018

IV.

V. DISCUSION

The findings of this research show that in patients with periodontitis the use of rosemary essential oil for a short period of exposure does not generate an increase in the cellular abnormalities evaluated after its application.

In periodontitis, it has been reported that non-beneficial microorganisms in the oral cavity cause cellular inflammation, thus producing ROS that, on the one hand, damage chromosomes and cause the loss of genetic material, generating cellular abnormalities such as micronuclei and nuclear budding.

On the other hand, ROS also damages the cell membrane and causes lipid peroxidation and multipolar mitosis, resulting in binucleation and multinucleation [4]. Thus, to evaluate the genotoxic effects, biomarkers such as micronuclei and binucleation of epithelial cells of the oral mucosa are used. This technique, in addition to monitoring genetic damage in humans, provides a clinically useful tool in the prognosis of patients with periodontitis [4].

Other studies on genotoxicity have included lifestyle factors such as smoking, alcohol intake, radiotherapy and chemotherapy treatments, occupational exposure to radiation, potentially carcinogenic chemicals, the consumption of xenobiotics and chemicals derived from medicinal plants [4,5,7,21].

Rosemary is an aromatic plant that contains various bioactive molecules such as phenolic acids, flavonoids, saponins, tannins and essential oils, among others, whose antibacterial, cytoprotective and anti-inflammatory properties have been demonstrated in previous research [13-17,22,24]. But in some cases, these chemicals can also cause genotoxicity and mutagenicity. For example, rosemary essential oil has been reported to have effects on the increase of micronucleated cells and chromosomal aberrations at a high dose in mice [24] and its chronic use can cause adverse effects on the liver, kidneys and reproductive system [23], while cutaneous and oral cavity application in rabbits and mice does not indicate toxicity or mutagenicity [18,19].

Another study mentions that rosemary essential oil has cytoprotective effects at the cell membrane level, which protects them from oxidative damage and the generation of ROS [22]. This information is relevant in periodontitis because it would prevent the increase in cellular abnormalities caused by inflammation and the intracellular process that it causes. This could explain why in our results there was no increase in cellular abnormalities after using the rosemary solution for 14 days at a low concentration (0.14%). However, it is important to consider the possible side effects of rosemary essential oil for therapeutic applications, particularly in people sensitive to the different active components of the plant and to take into account the dosage to be used.

With the information obtained, further studies could be carried out to evaluate, in the medium and long term, the effect of rosemary essential oil on periodontal disease. Likewise, it could be considered as an active substance to generate products such as toothpastes, gels and mouthwashes.

VI. CONCLUSION

This research indicates that *Rosmarinus officinalis* is an herbal alternative that can be safely used as an adjuvant treatment plan in patients with periodontitis without generating genotoxicity.

Acknowledgements

This research was made in Facultad de Odontología, Universidad Autónoma de Tlaxcala, Mexico. We thank Rosario Lechuga and Yoali Pérez Zempoalteca for technical assistance.

Conflict of interests

The authors declare that they have no competing interests.

REFERENCES

[1]. Arigbede AO, Babatope BO, Bamidele MK. Periodontitis and systemic diseases: A literature review. Journal of Indian Society of Periodontology. 2012;16(4):487-91. Doi:10.4103/0972-124X.106878

[2]. Kwon T, Lamster IB, Levin L. Current concepts in the management of periodontitis. International dental journal. 2021;71(6):462-76. Doi:10.1111/idj.12630

[3]. Sojod B, Périer JM, Zalcberg A, Bouzegza S, El Halabi B, Anagnostou F. Enfermedad periodontal y salud general. EMC-Tratado de Medicina. 2022;26(1):1-8. Doi:10.1016/S1636-5410(22)46043-0

[4]. Sawhney T, Manaktala N, Pralhad S, Natarajan S. Assessment and comparison of nuclear changes seen in gingivitis and periodontitis using fluorescent microscopy. Revista Española de Patología. 2019;52(4):208-13. Doi:10.1016/j.patol.2019.03.002
[5]. Bastos-Aires D, Azevedo Á, de Lurdes Pereira M, Pérez-Mongiovi D, Teixeira A. Preliminary study of micronuclei levels in oral exfoliated cells from patients with periodontitis. Journal of Dental Sciences. 2013;8(2):200-4. Doi:10.1016/j.jds.2012.12.007
[6]. Tadin A, Gavic L, Roguljic M, Jerkovic D, Zeljezic D. Nuclear morphological changes in gingival epithelial cells of patients with periodontitis. Clinical Oral Investigations. 2019;23:3749-3757. Doi:10.1007/s00784-019-02803-5
[7]. Serikova OV, Shumilovich BR, Filippova ZA, Kalaev VN, Kalaeva EA, Larina AV. Nuclear aberrations in the gingival epithelium of patients with chronic periodontitis. Journal of Indian Society of Periodontology. 2023;27(4):374-80. Doi:10.4103/jisp.jisp_18_22
[8]. Stoyell KA, Mappus JL, Gandhi MA. Clinical efficacy of turmeric use in gingivitis: A comprehensive review. Complementary Therapies in Clinical Practice. 2016;25:13-7. Doi:10.1016/j.ctcp.2016.08.004
[9]. Farjana HN, Chandrasekaran SC, Gita B. Effect of oral curcuma gel in gingivitis management-a pilot study. Journal of Clinical and Diagnostic Research 2014;8(12):ZC08-ZC10. Doi:10.7860/JCDR/2014/8784.5235
[10]. Carrol DH, Chassagne F, Dettweiler M, Quave CL. Antibacterial activity of plant species used for oral health against Porphyromonasgingivalis. PLoS ONE 2020;15(10):e0239316. Doi:10.1371/journal.pone.0239316
[11]. Ángeles CD, Hernández EL, Hernández AL, Martínez IO. Curcumina, unaalternativaterapéutica para la clínica dental (Parte I): antiinflamatorioy analgésico. Revista ADM. 2016;73(5):245-9.
[12]. Mosaddad SA, Hussain A, Tebyaniyan H. Green alternatives as antimicrobial agents in mitigating periodontal diseases: a narrative review. Microorganisms. 2023;11(5):1269. Doi:10.3390/microorganisms11051269
[13]. Santoyo S, Cavero S, Jaime L, Ibañez E, Señoráns FJ, Reglero G. Chemical composition and antimicrobial activity of Rosmarinusofficinalis L. essential oil obtained via supercritical fluid extraction. Journal of Food Protection2005; 68(4): 790–795.
[14]. Flores-Villa E, Sáenz-Galindo A, Castañeda-Facio AO, Narro-Céspedes RI. Romero (Rosmarinusofficinalis L.): su origen, importancia y generalidades de sus metabolitos secundarios. TIP. Revista Especializada en Ciencias Químico-biológicas. 2020;23:1-17. Doi:10.22201/fesz.23958723e.2020.0.266
[15]. De Oliveira JR, Camargo SE, De Oliveira LD. Rosmarinusofficinalis L.(rosemary) as therapeutic and prophylactic agent. Journal of Biomedical Science. 2019;26(1):1-22. Doi:10.1186/s12929-019-0499-8
[16]. Günther M, Karygianni L, Argyropoulou A, Anderson AC, Hellwig E, Skaltsounis AL, Wittmer A, Vach K, Al-Ahmad A. The antimicrobial effect of Rosmarinusofficinalis extracts on oral initial adhesion ex vivo. Clinical Oral Investigations. 2022;26(6):4369-80. Doi:10.1007/s00784-022-04400-5
[17]. Lorenzo-Leal AC, Palou E, López-Malo A, Bach H. Antimicrobial, cytotoxic, and anti-inflammatory activities of Pimentadioica and Rosmarinusofficinalis essential oils. BioMed Research International. 2019;2019(1):1639726. Doi:10.1155/2019/1639726
[18]. Mengiste B, Dires K, Lulekal E, Arayaselassie M, Zenebe T, Feleke G, Makonnen E, Mekonnen A. Acute skin irritation, acute and sub-acute oral toxicity studies of Rosmarinusofficinalis essential oils in mice and rabbit. African Journal of Pharmacy and Pharmacology. 2018;12(26):389-96. Doi:10.5897/AJPP2018.4957
[19]. Kalaimathi RV, Jeevalatha A, Basha AN, Kandeepan C, Ramya S, Loganathan T, Jayakumararaj R. In-silico absorption, distribution, metabolism, elimination and toxicity profile of isopulegol from Rosmarinusofficinalis. Journal of Drug Delivery and

Therapeutics. 2022;12(1):102-8. Doi:10.22270/jddt.v12i1.5188

[20]. Mahyari S, Mahyari B, Emami SA, Malaekeh-Nikouei B, Jahanbakhsh SP, Sahebkar A, Mohammadpour AH. Evaluation of the efficacy of a polyherbal mouthwash containing Zingiberofficinale, Rosmarinusofficinalis and Calendula officinalis extracts in patients with gingivitis: a randomized double-blind placebo-controlled trial. Complementary Therapies in Clinical Practice 2016;22:93-98. Doi:10.1016/j.ctcp.2015.12.001

[21]. LópezNigro MM, Portmann E, Angeleri G, Gurni A, Carballo MA. Biomarcadores para evaluación de genotoxicidadpotencial. BoletínLatinoamericano y del Caribe de PlantasMedicinales y Aromáticas. 2009;8(2):154-159.

[22]. Karimi G, Hassanzadeh M, Mehri S. Protective effect of Rosmarinusofficinalis L. essential oil against free radical-induced erythrocyte lysis: cytoprotective effect of Rosmarinusofficinalis. Iranian Journal of Pharmaceutical Sciences. 2005;1(4):231-236.

[23]. GhasemzadehRahbardar M and Hosseinzadeh H. Toxicity and safety of rosemary (Rosmarinusofficinalis): a comprehensive review. Naunyn-Schmiedeberg's Archives of Pharmacology. 2024.Doi:10.1007/s00210-024-03336-9

[24]. Maistro EL, Mota SF, Lima EB, Bernardes BM, Goulart FC. Genotoxicity and mutagenicity of Rosmarinusofficinalis (Labiatae) essential oil in mammalian cells in vivo. Genet Mol Res. 2010;9(4):2113-22. Doi: 10.4238/vol9-4gmr857. PMID: 21038297