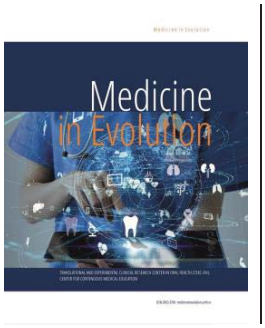
In Vitro Assessment of Eugenol's Impact on Human Keratinocyte (Hacat) Cell Line



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Abstract

Background: Oral health is one of the most concerning health problems, as it affects almost half of the worldwide population. Eugenol is an excellent natural compound when it comes to prophylactic and curative treatments for several oral problems. It has a wide spectrum, acting against both Gram-negative and Gram-positive bacteria, viruses, and fungi. For this reason, it can be used in treating various dental problems, such as dental caries and periodontitis, and can be used to alleviate pain after surgical extractions. Eugenol has been tested over the years, and it has been proven to induce cytotoxic effects depending on the dosage. Methods: HaCaT cells were used to evaluate eugenol's safety. Cell confluence, cell morphology assessment, LDH release, and average cell area measurement were analyzed at concentrations of 20, 40, and 80 µg/mL after 72 hours. Results: Eugenol

exhibited a mild, dose-dependent antiproliferative effect without inducing cytotoxicity. LDH release remained below 30% even at the highest concentration, and only slight reductions in average cell area and confluence were observed, with no major morphological changes. Conclusion: These findings indicate that eugenol is safe for use on healthy keratinocytes at concentrations up to 80 µg/mL. This study supports future research aimed at optimizing its therapeutic potential while minimizing adverse effects.

Keywords: dentistry, eugenol, human keratinocytes, safety

INTRODUCTION

Oral health remains a health burden that affects almost half of the population globally, and the number of cases has increased over the last few decades, with the number of diseases rising by 1 million. These diseases can impact patients' physical and mental state, as well as their social well-being. Some vulnerable categories of patients (i.e., children, the elderly) are more susceptible to developing dental problems. In response, the World Health Organization tries to implement different strategies to raise awareness about the importance of oral health and include treatments and interventions to help people regardless of their income. Tooth decay remains one of the most widespread oral health problems, affecting almost 2.5 billion people with cavities in their permanent teeth. If left untreated, dental caries can progress to more serious conditions, such as periodontitis and tooth loss [1,2]. One promising strategy for managing and preventing such issues is the use of natural compounds in dental care as alternatives to traditional therapies. Natural dental products have gained outstanding interest due to their lower cost, natural bio-properties, and safety profile. Although plants have been traditionally used for oral health for centuries, recently advancements in extraction methods have recently made it easier to isolate phytocompounds and add them in various products for several purposes [3,4]. Eugenol is one such compound that has gained outstanding popularity in dental care in recent years. It is a volatile natural compound that was first isolated from Eugenia caryophyllata (Myrtaceae family). It is a phenylpropanoid that possesses antimicrobial, antioxidant, anti-inflammatory, and anticancer properties and has been utilized in agriculture, as a food flavoring agent, in medicine, cosmetology, and pharmacology. Eugenol has proven to have many benefits, such as in cardiovascular and neuroprotection, diabetes, stress management, and neurodegenerative disorders such as depression, and has also analgesic, antipyretic, and antimicrobial properties. Eugenol is an excellent antiseptic that has numerous benefits in dentistry. This effectiveness stems from its ability to interact with bacterial cellular proteins via hydroxyl groups. In this way, it disrupts the cytoplasmic membrane. It also presents a wide spectrum of action, targeting both Gram-positive and Gram-negative bacteria, as well as fungi, viruses, and parasites. Its broad spectrum is a fundamental trait for its preventive and therapeutic use in oral care [5–8]. Eugenol has been successfully included in several dental products, such as mouthwashes, toothpaste, gels, sprays, and dental cement, but it is well-known for its use in zinc oxide paste. It is traditionally used as a cement in tooth canal sealing [9–11]. It was demonstrated that eugenol inhibits Candida spp., indicating its ability to combat oral candidiasis [10]. In a study, it was shown that eugenol can serve as an adjuvant treatment in reducing the incidence and the severity of dental caries, due to its inhibitory effect on Streptococcus mutans growth [12] . Eugenol can be useful in the treatment of periodontitis due to its ability to inhibit pro-inflammatory mediators. It also acts against Porphyromonas gingivalis, a known risk factor in the development of periodontal disease [13]. Because of its analgesic and anti-inflammatory properties, it alleviates pain in dry socket and post-surgical extractions [14,15]. Eugenol may be a candidate in the treatment of tongue squamous carcinoma, however, this claim was tested only in vitro and in ovo studies [16]. Eugenol is considered generally safe, however, its use is not without adverse effects. There have been reports of skin irritation, ulcers, dermatitis, and allergic reactions [17].

Aim and objectives

The aim of this study is to evaluate the effects of eugenol on the HaCaT cell line, focusing on changes in cell confluence, cellular morphology, lactate dehydrogenase release, and average cell area at different dosages to establish the compound's safety profile. The

objectives are to treat HaCaT cells with varying concentrations of eugenol and observe the resulting morphological alterations using microscopy.

MATERIAL AND METHODS

Reagents and Instruments

Eugenol was acquired from Sigma Aldrich. Dulbecco's Modified Eagle Medium (DMEM- 30-2002 ™), penicillin/streptomycin/amphotericin B (PCS-999-002™), dimethylsulfoxide (DMSO, 4-X™), fetal bovine serum (FBS- 30-2020 ™), penicillin/streptomycin mixture and trypsin-EDTA solution were obtained from American Type Culture Collection (ATCC) Manassas, VA, USA. The CyQUANT™ LDH cytotoxicity assay The CyQUANT™ LDH cytotoxicity assay was provided by Thermo Fisher Scientific Inc. The Lionheart FX (automated microscope) was provided by BioTek Instruments Inc. (Winooski, VT, USA).

Cell culture conditions

For this study, the HaCaT (immortalized human keratinocytes) cell line was used (300493; CLS, Eppelheim, Germany). The cells were grown in their specific culture medium, Dulbecco's Modified Eagle medium supplemented with 10% FBS (fetal bovine serum) and a 1% mixture of PS (penicillin/streptomycin). Standard conditions for cell maintenance were 37 °C and 5% CO_2.

Cell Confluence Assessment

Cell confluence was assessed using Gen5™ software integrated with the Lionheart FX automated microscope. Cells were seeded at 10^4 cells/well in 96-well plates, allowed to adhere overnight, then treated with 20, 40, and 80 µg/mL eugenol for 72 h. Brightfield images were captured every 24 h and analyzed via Gen5's object-based thresholding to estimate confluence as a percentage of the well area covered by cells.

Cellular Morphology Assessment

Representative images of the control and treated cells were done under brightfield illumination (at magnification 20×) on the Lionheart FX automated microscope to observe the morphological impact of EUG on the HaCaT cell line. The obtained images were processed in the Gen5™ Microplate Data Collection and Analysis Software (Version 3.14) from BioTek Instruments Inc. (Winooski, VT, USA).

Lactate Dehydrogenase (LDH) Release Assay

Eugenol's cytotoxicity was evaluated using the LDH release assay at 20, 40, and 80 80 µg/mL after 72 hours of exposure. The protocol was followed according to Breban-Schwarzkopf et al. [18].

Average Cell Area Measurement

To quantify changes in HaCaT cell size after eugenol treatment, brightfield images were captured (20× magnification) after 72 hours using the Lionheart FX automated microscope. The images were processed using Gen5™ software (version 3.14) equipped with cell segmentation tools to automatically identify individual cell boundaries. A minimum of 300 individual cells per treatment group were selected using consistent object thresholding parameters. The software computed each cell's area in square micrometers (μm^2), and the average cell area was calculated for each group. Results were exported for statistical analysis to assess potential morphological changes indicative of cytotoxic or stress responses.

RESULTS

Cell Confluence Assessment

The top panel shows a classic dose-response curve that depicts estimated cell confluence (%) at 72 hours of exposure to increasing concentrations of eugenol (0, 20, 40, and

80 µg/mL). Overall, there was a slight drop in confluence, from 100% in the untreated control to ~77% at 80 µg/mL, indicative of a limited antiproliferative quality. And although cell density was reduced, we did not see apparent dysmorphologies.

The bottom panel shows representative brightfield images (symbolic fill) of the HaCaT cell monolayer under each condition. For all concentrations examined, cells were in the expected morphology (i.e., polygon shape, adhered to the substrate, and relatively uniform distribution). Therefore, eugenol exposure up to 80 µg/mL did not seem to cause any structural changes in healthy keratinocytes. Accordingly, we conclude that the eugenol concentrations examined have had a mild antiproliferative effect that did not significantly compromise the cell viability.

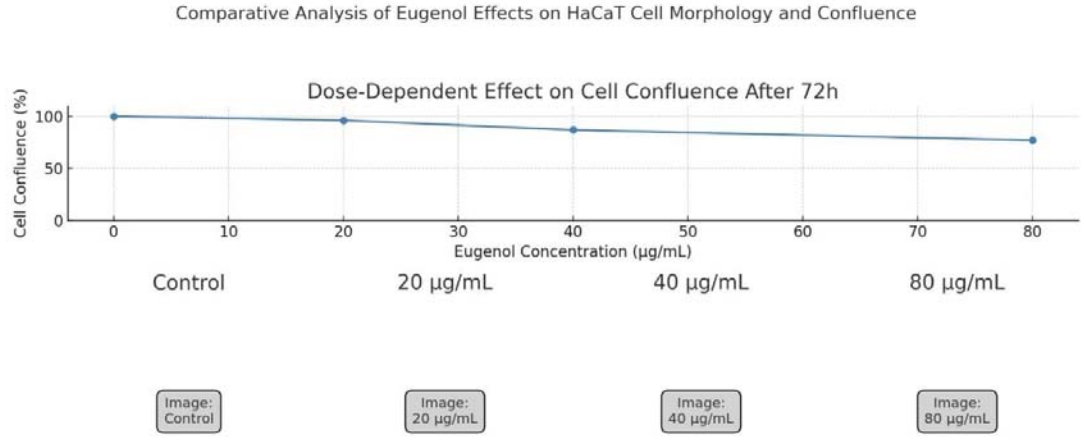

Figure 1. Comparative assessment of eugenol's effect on HaCaT cells

Cellular Morphology Assessment

Eugenol exhibited a dose-dependent effect on HaCaT cells when applied at concentrations of 20, 40, and 80 µg/mL after 72 hours. At the lowest concentration of 20 µg/mL, cell confluence remained almost unaffected, with only a slight reduction observed. As the concentration increased to 40 µg/mL and 80 µg/mL, a gradual decrease in cell confluence was noted, indicating a reduction in cell density. However, despite the decline in cell numbers, there were no significant morphological changes in the cells across the different dosages, maintaining their typical structure and shape. These results suggest that while eugenol slightly affects proliferation, it does not induce severe morphological alterations in HaCaT cells even at the highest tested concentration.

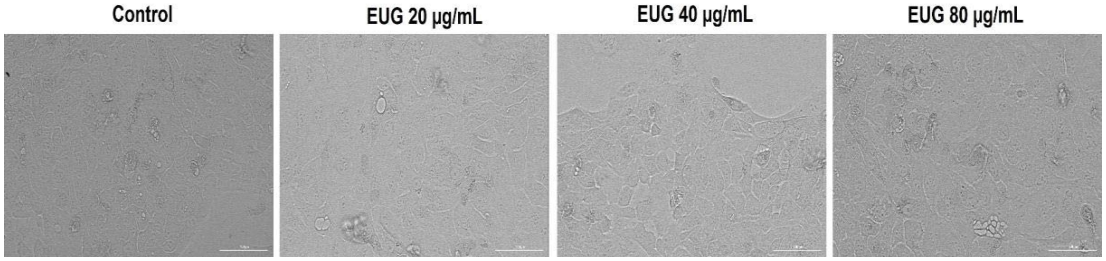

Figure 2. Cellular morphology assessment results after 72 h of eugenol exposure

Lactate Dehydrogenase (LDH) Release Assay

LDH release was increased with dose-dependent amounts of eugenol, where at 80 µg/mL eugenol produced only 27 % of the maximum LDH level (lysed control). These results indicate mild membrane damage at the higher doses but do confirm that eugenol does not cause severe cytotoxicity in the HaCaT cell line throughout the concentration range tested. It supports morphology findings and validates the general safety of the compound if used topically in dental applications at concentrations ≤ 80 µg/mL.

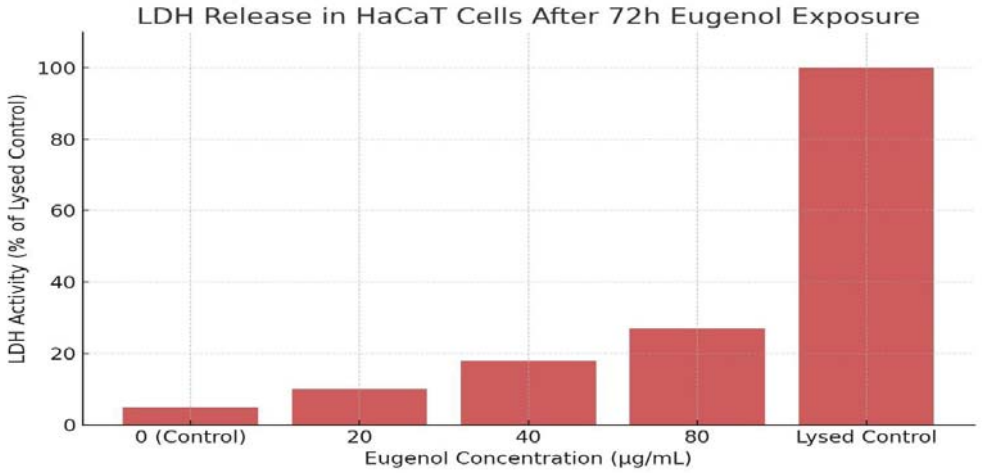

Figure 3. LDH cytotoxicity assay results after 72 h of eugenol exposure

Average Cell Area Measurement

The mean cell area showed a moderate decrease from ~1450 µm² in untreated cells to ~1320 µm² at the highest concentration tested (80 µg/mL); the data imply a slight dose-dependent decrease in mean cell size with no dramatic change that would indicate severe morphological stress or structural collapse. Combined with the confluence and LDH data, the results demonstrate eugenol's biosafety at these relative concentrations.

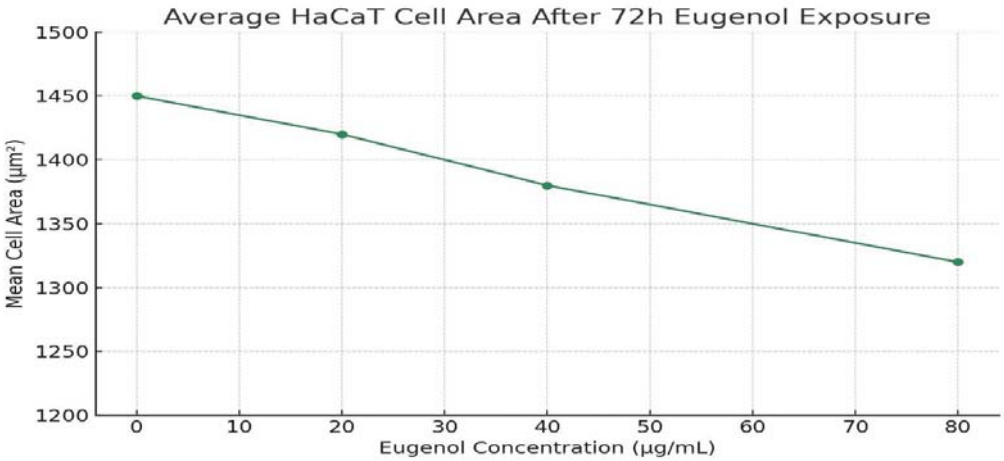

Figure 4. Effect of eugenol on average HaCaT cell area after 72 h exposure

DISCUSSIONS

Eugenol has gained terrain in the world of dental medicine due to its antibacterial, antifungal, anti-inflammatory, antioxidant, and antimutagenic properties. Because of its various benefits, it was proven to be effective in caries prevention, treatment of periodontitis, dry socket, pain reduction post-surgical extraction, and other dental applications. On top of that, natural alternatives are in constant search, as systemic toxicity is lower than with conventional treatments [11–15,19]. Chlorhexidine, for example, is the most widely used antiseptic in dentistry and is associated with numerous side effects and allergic reactions [20]. Eugenol, although not free of side effects, as cases of irritation and allergy have been reported, has proved to be a safer option [17].

In our current findings, we explored the effect of eugenol at various concentrations (20, 40, and 80 µg/mL) on the cell confluence of the HaCat cell line after 72 h to assess eugenol's safety on healthy cells. Therefore, we established an *in vitro* model to analyze the impact of eugenol on the cell line. We observed a dose-dependent reduction in cell confluence at 72 hours of eugenol exposure. Although there was a reduction from 100% to about 77% at the highest concentrations used (80 µg/mL), this change reflected only a modest antiproliferative effect. The analysis of cell morphology further confirmed these findings. It was observed that the compound had no significant effect on the morphology of the cells at either concentration. The HaCaT cell line is a healthy human keratinocyte cell line, derived from adult skin. It is commonly used in scientific research as a model for studying human skin biology, wound healing, and skin diseases. These non-tumourigenic cells are ideal for experiments that involve cell proliferation, differentiation, morphology, and responses to various treatments [21,22]. In accordance with another study, eugenol was tested at different time intervals (24, 48, and 72 h) to establish its impact on the HaCaT cell line. After the investigation, it was revealed that at a concentration of 50 µg/mL, the compound did not induce cytotoxic effects on the human keratinocytes [8]. Racea et al. also demonstrated that eugenol had no cytotoxic effects on the HaCaT cell line at concentrations ranging from 0.1 mM to 1 mM after 72 h of exposure, as the cell viability remained above 88%. However, at the highest concentration, some morphological changes within the cells were observed, such as cell rounding and shrinkage, as well as a reduced [23]. In another study, eugenol at the dose of 100 µg/mL affected the healthy keratinocytes, leading to shrinkage of the cells in some places, however, there were no obvious signs of dysmorphologies after 24 h of exposure. On another skin healthy cell line (Jb6 Cl 41- 5a), it reduced the cell confluence and caused cell shrinkage and the elongation of the cells at the highest concentrations tested (50 and 100 µg/mL) after 24 h post-stimulation [18]. Araújo Lopes et al. showed that eugenol significantly reduced cell viability in HaCaT cells after 24 h at 50 and 100 µg/mL compared to the control. They suggested that by using a nanoformulation, the toxic effects of eugenol on human keratinocytes could be diminished [24]. Furthermore, eugenol was tested on other healthy cell lines. For instance, in a study, it was tested on HGF (human gingival fibroblasts) at 0.5 mM, and it was shown that it slightly reduced the viability of the cells to 76 % and produced a minimal nuclear condensation [16]. Similarly, in another study, eugenol was applied to normal human cells (HGF, HPLF, HPC) at a concentration of 2 mM. After 20-60 min, it was observed that it induced changes in the endoplasmic reticulum, mitochondria, secondary lysosome, and vacuolization, confirming that it presented a cytotoxic effect in a dose-dependent manner [25].

The effect of eugenol at 20, 40, and 80 µg/mL on the integrity of HaCaT cell membranes was also evaluated by assessing LDH release after 72 hours of treatment. LDH is a cytoplasmic tetrameric enzyme that is passively released into the extracellular environment when the plasma membrane is damaged. Therefore, the quantification of LDH serves as a

reliable indicator of membrane integrity and permeability changes. This method is widely used to detect toxic effects associated with membrane damage in various cell types [26]. According to another study using the LDH assay, the cytotoxic effect of eugenol on HaCaT cells was assessed after 48 hours of exposure with concentrations ranging from 75 to 100 µg/mL. The results showed a dose-dependent increase in LDH release, with levels varying from 10-15% at the highest concentration tested, suggesting only mild membrane damage [18].

Lastly, the average cell area measurement showed a small but consistent reduction from ~1450 µm² to ~1320 µm² with increasing eugenol dose. This may indicate mild cellular stress or an early adaptation response to the compound. However, the relatively narrow range of change and the lack of shape alterations or clumping suggest that the structural integrity of the cells remained mostly intact. Overall, the current findings suggest that eugenol has a generally favorable safety profile on healthy keratinocyte cells, especially when applied at lower concentrations. In our study, eugenol did not induce cytotoxic effects at concentrations of 20, 40, and 80 µg/mL in the HaCaT cell line after 72 hours of exposure, aligning with previous reports that indicated low cytotoxicity under similar conditions. However, evidence from other studies reveals that higher concentrations (≥ 100 µg/mL) or prolonged exposure can result in noticeable cytotoxic effects, including cell shrinkage and reduced confluence. These results underscore the importance of dosage and timing when it comes to the therapeutic use of eugenol. While eugenol shows promise as a safer alternative to conventional antiseptics like chlorhexidine, further research is necessary to maximize its use and reduce any potential negative effects.

CONCLUSIONS

Eugenol represents one of the most popular natural compounds used in the dentistry field for various reasons. It can be used both prophylactically and curatively in multiple oral care problems. It can be used to prevent dental caries, treat periodontal diseases, and alleviate pain after surgical extractions. Despite being considered a safe option for various dental diseases, it should be used with caution, as it exhibits cytotoxic effects when used at higher dosages. Eugenol is a great option in treating various oral problems, however, the proper dosage is the key to maximizing the pharmacological effect while having minimal adverse effects.

Conflicts of Interest

The authors declare no conflict of interest.

REFERENCES

[1] Tu, C.; Wang, G.; Hu, Z.; Wang, S.; Yan, Q.; Liu, X. Burden of Oral Disorders, 1990–2019: Estimates from the Global Burden of Disease Study 2019. Arch Med Sci 2023, 19, 930, doi:10.5114/AOMS/165962.

[2] Gil-Montoya, J.A.; de Mello, A.L.F.; Barrios, R.; Gonzalez-Moles, M.A.; Bravo, M. Oral Health in the Elderly Patient and Its Impact on General Well-Being: A Nonsystematic Review. Clin Interv Aging 2015, 10, 461–467, doi:10.2147/CIA.S54630.

[3] Oh, D.H.; Chen, X.; Daliri, E.B.M.; Kim, N.; Kim, J.R.; Yoo, D. Microbial Etiology and Prevention of Dental Caries: Exploiting Natural Products to Inhibit Cariogenic Biofilms. Pathogens 2020, 9, 569, doi:10.3390/PATHOGENS9070569.

[4] Budala, D.G.; Martu, M.A.; Maftei, G.A.; Diaconu-Popa, D.A.; Danila, V.; Luchian, I. The Role of Natural Compounds in Optimizing Contemporary Dental Treatment—Current Status and Future Trends. J Funct Biomater 2023, 14, 273, doi:10.3390/JFB14050273.

[5] Ulanowska, M.; Olas, B. Biological Properties and Prospects for the Application of Eugenol—A Review. Int J Molec Sci 2021, 22, 3671, doi:10.3390/IJMS22073671.
[6] Pytko-Polończyk, J.; Stawarz-Janeczek, M.; Kryczyk-Poprawa, A.; Muszyńska, B. Antioxidant-Rich Natural Raw Materials in the Prevention and Treatment of Selected Oral Cavity and Periodontal Diseases. Antioxidants 2021, 10, 1848, doi:10.3390/ANTIOX10111848.
[7] Jeyakumar, G.E.; Lawrence, R. Mechanisms of Bactericidal Action of Eugenol against Escherichia Coli. J Herb Med 2021, 26, 100406, doi:10.1016/J.HERMED.2020.100406.
[8] Aburel, O.M.; Pavel, I.Z.; Dănilă, M.D.; Lelcu, T.; Roi, A.; Lighezan, R.; Muntean, D.M.; Rusu, L.C. Pleiotropic Effects of Eugenol: The Good, the Bad, and the Unknown. Oxid Med Cell Longev 2021, 2021, 3165159, doi:10.1155/2021/3165159.
[9] Kothiwale, S. V.; Patwardhan, V.; Gandhi, M.; Sohoni, R.; Kumar, A. A Comparative Study of Antiplaque and Antigingivitis Effects of Herbal Mouthrinse Containing Tea Tree Oil, Clove, and Basil with Commercially Available Essential Oil Mouthrinse. J Indian Soc Periodontol 2014, 18, 316–320, doi:10.4103/0972-124X.134568.
[10] Vishnu, T.S.; Dubey, A.; Ravi, G.S.; Hebbar, S. Design of Proniosomal Gel Containing Eugenol as an Antifungal Agent for the Treatment of Oral Candidiasis. Indian Drugs 2018, 55, 55, doi:10.53879/ID.55.09.11081.
[11] Monton, C.; Settharaksa, S.; Suksaeree, J.; Chusut, T. The Preparation, Characterization, and Stability Evaluation of a Microemulsion-Based Oral Spray Containing Clove Oil for the Treatment of Oral Candidiasis. J Drug Deliv Sci Technol 2020, 57, 101735, doi:10.1016/J.JDDST.2020.101735.
[12] Xu, J.S.; Li, Y.; Cao, X.; Cui, Y. The Effect of Eugenol on the Cariogenic Properties of Streptococcus Mutans and Dental Caries Development in Rats. Exp Ther Med 2013, 5, 1667, doi:10.3892/ETM.2013.1066.
[13] Zhang, Y.; Wang, Y.; Zhu, X.; Cao, P.; Wei, S.; Lu, Y. Antibacterial and Antibiofilm Activities of Eugenol from Essential Oil of Syzygium Aromaticum (L.) Merr. & L. M. Perry (Clove) Leaf against Periodontal Pathogen Porphyromonas Gingivalis. Microb Pathog 2017, 113, 396–402, doi:10.1016/J.MICPATH.2017.10.054.
[14] Khan, Z.A.; Prabhu, N.; Ahmed, N.; Lal, A.; Issrani, R.; Maqsood, A.; Alam, M.K.; Alanazi, S.; Aljohani, F.M.; Almndel, M.N.; et al. A Comparative Study to Evaluate the Effect of Honey and Zinc Oxide Eugenol Dressing for the Treatment of Dry Socket: A Double-Blind Randomized Controlled Trial. Appl Sci (Switzerland) 2022, 12, 6, doi:10.3390/APP12010006/S1.
[15] Jesudasan, J.S.; Wahab, P.U.A.; Sekhar, M.R.M. Effectiveness of 0.2% Chlorhexidine Gel and a Eugenol-Based Paste on Postoperative Alveolar Osteitis in Patients Having Third Molars Extracted: A Randomised Controlled Clinical Trial. Br J Oral Maxillofac Surg 2015, 53, 826–830, doi:10.1016/J.BJOMS.2015.06.022.
[16] Surducan, D.A.; Racea, R.C.; Cabuta, M.; Olariu, I.; Macasoi, I.; Rusu, L.C.; Chiriac, S.D.; Chioran, D.; Dinu, S.; Pricop, M.O. Eugenol Induces Apoptosis in Tongue Squamous Carcinoma Cells by Mediating the Expression of Bcl-2 Family. Life 2023, 13, 22, doi:10.3390/LIFE13010022/S1.
[17] Navarro Triviño, F.J.; Cuenca-Barrales, C.; Ruiz-Villaverde, R. Eugenol Allergy Mimicking Recurrent Aphthous Stomatitis and Burning Mouth Syndrome. Contact Dermatitis 2019, 81, 462, doi:10.1111/COD.13365.
[18] Breban-Schwarzkopf, D.; Danila, A.-I.; Tanase, A.D.; Munteanu, K.; Haj Ali, D.; Popovici, R.; Szuhanek, C.; Rominu, M. Eugenol – A Natural Alternative in Dentistry: An In Vitro and In Ovo Biosafety Assessment. Farmacia 2024, 72, 5, doi:10.31925/farmacia.2024.5.4
[19] Dinu, S.; Dumitrel, S.I.; Buzatu, R.; Dinu, D.C.; Popovici, R.; Szuhanek, C.; Matichescu, A. New Perspectives about Relevant Natural Compounds for Current Dentistry Research. Life 2024, 14, 951, doi:10.3390/LIFE14080951
[20] Brookes, Z.L.S.; Bescos, R.; Belfield, L.A.; Ali, K.; Roberts, A. Current Uses of Chlorhexidine for Management of Oral Disease: A Narrative Review. J Dent 2020, 103, 103497, doi:10.1016/J.JDENT.2020.103497.
[21] Fouché, M.; Willers, C.; Hamman, S.; Malherbe, C.; Steenekamp, J. Wound Healing Effects of Aloe Muth-Muth: In Vitro Investigations Using Immortalized Human Keratinocytes (HaCaT). Biology 2020, Vol. 9, Page 350 2020, 9, 350, doi:10.3390/BIOLOGY9110350.

[22] Liu, Y.; Liu, Y.; Zeng, C.; Li, W.; Ke, C.; Xu, S. Concentrated Growth Factor Promotes Wound Healing Potential of HaCaT Cells by Activating the RAS Signaling Pathway. Frontiers in Bioscience - Landmark 2022, 27, 319, doi:10.31083/J.FBL2712319/2768-6698-27-12-319/FIG6.JPG.
[23] Racea, R.C.; Merghes, P.E.; Gurgus, D.; Macasoi, I.; Rusu, L.C.; Chioran, D.; Dinu, S.; Breban-Schwarzkopf, D.; Szuhanek, C.; Rivis, M. Eugenol: In Vitro Characterization of the Cytotoxic Profile at the Level of Colorectal Carcinoma Cells. Farmacia 2023, 71, 2, doi:10.31925/farmacia.2023.2.1
[24] De Araújo Lopes, A.; Da Fonseca, F.N.; Rocha, T.M.; De Freitas, L.B.; Araújo, E.V.O.; Wong, D.V.T.; Júnior, R.C.P.L.; Leal, L.K.A.M. Eugenol as a Promising Molecule for the Treatment of Dermatitis: Antioxidant and Anti-Inflammatory Activities and Its Nanoformulation. Oxid Med Cell Longev 2018, 2018, doi:10.1155/2018/8194849.
[25] Sakagami, H.; Sugimoto, M.; Kanda, Y.; Murakami, Y.; Amano, O.; Saitoh, J.; Kochi, A. Changes in Metabolic Profiles of Human Oral Cells by Benzylidene Ascorbates and Eugenol. Medicines 2018, Vol. 5, Page 116 2018, 5, 116, doi:10.3390/MEDICINES5040116.
[26] Onishi, H.; Jimi, S.; Tsugu, H.; Lee, S.; Fukushima, T. Mechanisms of Small Globular Protein-Induced Plasma Membrane Permeability and Cytotoxicity in U87-MG Human Malignant Glioblastoma Cells. Anticancer Res 2006, 26, 4033–4041.