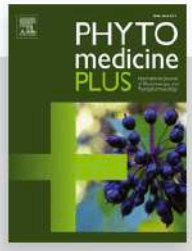
ISSN: 2667-0313

ELSEVIER

PHYTO medicine PLUS

International Journal of Phytotherapy and Phytopharmacology



Phytomedicine Plus
Open access
3.7
CiteScore

Volume 4, Issue 2

May 2024

Download full issue

Previous vol/issue | Next vol/issue

Actions for 45 selected articles

Select all / Deselect all

Download PDFs

Export citations

Show all article previews

Receive an update when the latest issues in this journal are published

Sign in to set up alerts

Short communication • *Open access*

The attitude of medical students concerning herbal medicinal products/phytopharmaceuticals/phytotherapy in different regions of Europe

Liselotte Krenn, Martin Burkart
Article 100525

View PDF | Article preview

Research article • *Open access*

Juniperus oxycedrus leaves and berries extracts: HPLC-PDA-ESI/MS^2 phenolic characterization and *in vitro* anti-inflammatory effects

Mohamed Reda Kachmar, Yassine Oulad El Majdoub, Andreia P. Oliveira, Aziz Bouymajane, ... Said Chakir
Article 100528

View PDF | Article preview

Research article • *Open access*

Evaluation of the neuropharmacologic potentials of methanol leaf extract of *Cnidoscolus aconitifolius* in mice

Wilson F. Iyare, Israel O. Bolanle, Abigail M. Akhigbemen, Dickson O. Uwaya, ... Raymond I. Ozolua
Article 100529

View PDF | Article preview

Research article • *Open access*

Actions for 45 selected articles
Select all / Deselect all

Download PDFs

Export citations

Show all article previews

Research article • *Open access*

Phytochemical characterization and assessment of antitumor activity of the aqueous extract of *Acmella caulirhiza* in female *Wistar* rats induced by 7,12 dimethylbenz (a) anthracene

Huiny Mirlone Tienoue Fotso, Mary-Ann Mbong Angie, Françoise Raïssa Ntentie, Felix Nwang Ngalla, ... Julius Oben Enyong
Article 100527

View PDF Article preview

Research article • *Open access*

In-vitro and *in-vivo* anti-inflammatory properties of extracts and isolates of Pangdahai

Mahmood B. Oppong, Shijie Cao, Shi-Ming Fang, Seth K. Amponsah, ... Qiu Feng
Article 100533

View PDF Article preview

Research article • *Open access*

Assessment of antioxidant potential, cytotoxicity, and anticancer activity of methanolic extracts from selected wild medicinal plants

Yaseen Al Qaisi, Ibrahim Alfarrayeh, Ahmad Alsarayreh, Khaled Khleifat, Noor Abu-Nwas
Article 100534

View PDF Article preview

Research article • *Open access*

Green Robusta Coffee Bean Extract (GRCBE) inhibits bone loss in wistar rat models of Lps *P. gingivalis* and NiTi wire-induced experimental periodontitis

Dessy Rachmawati, Tantin Ermawati, Nanda Innayatur Rahmatillah, Nurwandani Meylina, ... Ineke D.C. Jansen
Article 100535

View PDF Article preview

Research article • *Open access*

Evaluation of antioxidant-rich bioactive plant extracts with special reference to *Moringa oleifera* Lam. for their interference to bacterial quorum sensing and biofilm

Zarrin Haris, Iqbal Ahmad
Article 100511

View PDF Article preview

Review article • *Open access*

Nutraceutical-based telomerase inhibitors: Renewed hope for cancer therapy

Yasser Fakri Mustafa

Actions for 45 selected articles
Select all / Deselect all

Download PDFs

Export citations

Show all article previews

Research article • *Open access*

Enhanced photodynamic effect of a natural anthraquinone on *Candida tropicalis* biofilms using a stepwise irradiation strategy

Juliana Marioni, María L. Mugas, Florencia Martinez, Bianca C. Romero, ... Susana C. Núñez-Montoya
Article 100539

View PDF Article preview

Research article • *Open access*

Evaluation of antioxidant properties from purple tubers and their ability to improve glucose and lipid metabolism in streptozotocin-induced diabetic rats

Abdul Gofur, Siti Nur Arifah, Mochammad Fitri Atho'illah, Esha Ardiansyah, ... Mardiana Lelitawati
Article 100542

View PDF Article preview

Research article • *Open access*

Renoprotective effects of *Cucumeropsis mannii* seed oil on cisplatin-induced nephrotoxicity in Wistar rats

Boniface Anthony Ale, Patrick Maduabuchi Aja, Ikechukwu Jacob Okoro, Felix Emmanuel Nwite, ... Victor Nwadiogbu Ogugua
Article 100536

View PDF Article preview

Research article • *Open access*

Prophylactic effects of *Combretum molle* boughs on changes in the hematological, biochemical, and histological parameters in dexamethasone-induced insulin-resistant rats

David Miaffo, Exodus Ntumah Ankang, Barthelemy Maidadi, Absalom Beram Gode
Article 100544

View PDF Article preview

Research article • *Open access*

Radical scavenging dihydroxycinnamic natural compounds from *Trianthema pentandra*

Sadiq Abubakar, Ahmed A. Yakasai, Melati Khairuddean, Thomas J. Simpson, Habiba I. Rasheed
Article 100546

View PDF Article preview

Research article • *Open access*

The impact of combination therapy of *Croton membranaceus* and finasteride on benign prostatic hyperplasia (BHP) histo-morphology

Actions for 45 selected articles
Select all / Deselect all

Download PDFs

Export citations

Show all article previews

Research article • *Open access*

Ocimum gratissimum L. leaf flavonoid-rich extracts reduced the expression of p53 and VCAM in streptozotocin-induced cardiomyopathy rats

Basiru Olaitan Ajiboye, Courage Dele Famusiwa, John Adeolu Falode, Adetutu Omolola Ojelabi, ... Oluwafemi Adeleke Ojo
Article 100548

View PDF Article preview

Research article • *Open access*

Oral administration of quercetin and fisetin potentiates photocarcinogenesis in UVR-exposed hairless mice

Celina Pihl, Jonatan Riber Granborg, Fernanda Endringer Pinto, Peter Bjerring, ... Catharina Margrethe Lerche
Article 100547

View PDF Article preview

Research article • *Open access*

Effects of Chinese ginger *Alpinia officinarum*-based phytopharmaceutical compound on gastric motility

Laura Menne, Gemma Mazzuoli-Weber, Kristin Elfers
Article 100552

View PDF Article preview

Research article • *Open access*

Cepharanthine inhibits influenza A virus replication by impairing viral polymerase activity and regulating influenza-induced immune response

Yinyan Li, Huiting Sang, Qinxian Mo, Linrui Jiang, ... Jie Yang
Article 100553

View PDF Article preview

Research article • *Open access*

Extracts of *Cistus creticus* cultivated at different salinity levels exhibit promising therapeutic potential for pancreatic cancer cell lines

Burcin Ozbekle, Yagmur Arikan, Elif Damla Arisan, Bahar Yildiz Kutman
Article 100551

View PDF Article preview

Research article • *Open access*

Ameliorative influence of Baijin Pills on lithium-pilocarpine-induced epilepsy in mice: role of oxidative stress, neurotransmitters and multidrug resistant transporters

Jingbo Li, Xiaolan Cheng, Lian Xue, Hongquan Liu

Actions for 45 selected articles
Select all / Deselect all

Download PDFs

Export citations

Show all article previews

Research article • *Open access*

Liquid chromatography–mass spectrometry and xCELLigence real time cell analyzer revealed anticancer and antioxidant metabolites in *Trianthema portulacastrum* L. (Aizoaceae)

Muhali Olaide Jimoh, Mahboob Adekilekun Jimoh, Nasifu Kerebba, Olalekan Olanrewaju Bakare, ... Charles Petrus Laubscher
Article 100550

View PDF Article preview

Research article • *Open access*

Metabolite profiling of potential bioactive fractions from ethanol extract of *Boehmeria nivea* flowers by GC–MS/MS analysis

Asri Peni Wulandari, Zahra Khira Nafisa, Tati Herlina, Rani Maharani, ... Rahadian Zainul
Article 100557

View PDF Article preview

Research article • *Open access*

CGA mitigates HMGB1 mediated TLR4 activated hepatic cancer in urethane primed mice

Alok Tripathi, Kriti Shrinet, Arvind Kumar
Article 100554

View PDF Article preview

Review article • *Open access*

Terpenoids in essential oils: Chemistry, classification, and potential impact on human health and industry

Tohfa Siddiqui, Mohammad Umar Khan, Vikram Sharma, Komal Gupta
Article 100549

View PDF Article preview

Review article • *Open access*

Multi-therapeutic potential of flavonoids as an essential component in nutraceuticals for the treatment and management of human diseases

Stanley I.R. Okoduwa, Ibrahim Abdulwaliyu, Bernard E. Igiri, Shefiat O. Arekemase, ... Razaq A. Mustapha
Article 100558

View PDF Article preview

Research article • *Open access*

Ludwigia octovalvis (Jacq.) P.H. Raven extract improves memory performance in mice with chron kidney disease

Hao-Wei Wang, Yi-Hsuan Huang, Hsiao-Jou Tung, Wei-Sheng Lin, ... Pei-Yu Wang

Actions for 45 selected articles
Select all / Deselect all

Download PDFs

Export citations

Show all article previews

Qualitative and quantitative studies on the chemical changes during Ayurvedic detoxification process of *Acorus calamus* Linn. (Indian Vacha)

Debanjan Chatterjee, Ashutosh Kumar, Inder Pal Singh
Article 100574

View PDF Article preview

Research article • *Open access*

5-Methylcoumarin-4β-glucoside mitigated colon tumor progression in mice with AOM/DSS-induced colon carcinogenesis

Ibrahim Malami, Alhassan Muhammad Alhassan, Qamar Uddin Ahmed, Syed Adnan Ali Shah, ... Bilyaminu Abubakar
Article 100568

View PDF Article preview

Research article • *Open access*

Biochemical and Histoarchitectural evaluation of 4-Vinylcyclohexane induced ovarian cancer against *Alpinia purpurata* (Vieill). K. Schum

Arul Raj Cinthamony, Anusooriya Palanirajan, Manikandan Vani Raju, Meenakshi Kaniyur Chandrasekaran, ... Gopalakrishnan Velliyur Kanniappan
Article 100569

View PDF Article preview

Research article • *Open access*

Comprehensive characterization of the compositional differences in essential oils prepared from *Artemisiae argyi* during different picking seasons using gas chromatography Orbitrap high-resolution mass spectrometry

Huan Yao, Qianwen Cao, Mengge Liu, Yanbo Luo, ... Xia Xu
Article 100572

View PDF Article preview

Review article • *Open access*

Cardioprotective potential of botanical agents against anthracycline-induced cardiotoxicity

Parisa Firoozbakhsh, Zahra Ghaffarinejad, Mahta Arbabi, Negar Dokhani, Azin Alizadehasl
Article 100575

View PDF Article preview

Phytomedicine Plus 4 (2024) 100535

Contents lists available at ScienceDirect

Phytomedicine Plus

journal homepage: www.sciencedirect.com/journal/phytomedicine-plus

Green Robusta Coffee Bean Extract (GRCBE) inhibits bone loss in wistar rat models of Lps *P. gingivalis* and NiTi wire-induced experimental periodontitis

Dessy Rachmawati [a,b,*], Tantin Ermawati [a], Nanda Innayatur Rahmatillah [a], Nurwandani Meylina [a], Novia Yolanda Safitri [a], Rina Sutjiati [c], Ineke D.C. Jansen [d]

[a] *Department of Dental Biomedical Science, Faculty of Dentistry, University of Jember, Indonesia*
[b] *Center of Excellence for Agromedicine (CEAMED), University of Jember, Indonesia*
[c] *Department of Orthodontia, Faculty of Dentistry, University of Jember, Indonesia*
[d] *Department of Periodontology, Academic Centre for Dentistry (ACTA) Amsterdam, the Netherlands*

ARTICLE INFO

Keywords:
P. gingivalis
NiTi wire
Oral inflammation
Green Robusta coffee bean extract

ABSTRACT

Oral exposure to alloys frequently leads to negative reactions, both in the immediate area and across the body. The usage of coffee extracts is anticipated to enhance cellular function by counteracting the effects of pro-inflammatory signals that are involved in chronic inflammatory responses triggered by inflammatory agents like bacteria and metal ions. The objective is to develop a new approach using an extract obtained from Robusta coffee beans as an anti-inflammatory substance, which may have therapeutic benefits in preventing bone loss in Periodontitis Rat. The main cause is primarily attributed to the existence of metals and LPS *P. gingivalis* in the oral cavity. Methods. A randomized controlled trial was conducted, enroling 35 male Wistar rats that were divided into 7 groups and used as the experimental animals. Groups 1–4 consisted of rats that did not receive any therapy, while groups 5–7 comprised rats that were treated with GRCBE. The rats were subjected to decapitation after 14 days. The Green Robusta Coffee bean extract (GRCBE) was derived from the unprocessed green beans of Javanese Robusta coffee. We discovered in prior experimental experiments that GRCBE at a concentration of 500 mg/ml can inhibit the progression of periodontitis in rats. The substances used to cause inflammation and immune system impairment were NiTi wire and LPS *P. gingivalis*. Immunohistochemistry was used to assess the expressions of OCN, BMP2, and COX-2. Results. The simultaneous exposure of P. *gingivalis* lipopolysaccharide (LPS) and NiTi wire in rats led to an upregulation of OCN and BMP2 expression, while the expression of COX-2 reduced. The administration of GRCBE resulted in a steady drop in pro-inflammatory biomarkers, specifically OCN and BMP2, while the anti-inflammatory biomarker COX2 rose. In conclusion. In the field of dentistry, the coexistence of *P. gingivalis* LPS and metallic biomaterials can influence the occurrence of alveolar bone resorption in a rat model of periodontitis. GRCBE exhibits potential as an innovative approach for reducing periodontitis induced by the simultaneous activation of metal and LPS *P. gingivalis*. In this setting, it has the potential to be employed as an anti-inflammatory remedy.

1. Introduction

Inflammation of the oral cavity can be caused not only by lipopolysaccharides (LPS) but also by the presence of exposure to foreign substances in the oral cavity such as the presence of metals. Inflammatory reactions associated with contact with nickel ions (Ni^{2+}) are mediated by transcription factor NF-κB and through signalling TLR-4, which is a receptor that mediates the inflammatory process (Rachmawati et al., 2016, 2017). Nickel Titanium (NiTi) is a metal mixture (alloy) often used in biomedical applications, such as implants and orthodontic wires due to its good corrosion resistance properties and biocompatibility. However, its prolonged use is still controversial due to the release of ions in the oral cavity (Ševčíková et al., 2018). NiTi is an alloy consisting of 55% nickel and 45% titanium, aluminium, silicon, and other materials

Abbreviations: AB, Alveolar bone; B, Buccal; BE, Basal Epithelial cells; BMP2, Bone Morphogenetic Protein 2; COX-2, Cyclooxygenase 2; EG, Gingival Epithelium; JE, Junctional Epithelium; L, Lingual; M, Macrophages; M1, 1st Molar; NiTi, Nickel T itanium; OCN, Osteocalcin; IL, interleukin; LPS, lipopolysaccharide; PL, Periodontal ligament; RCBE, Robusta Coffee Bean Extract; TLR, toll like receptor.

* Corresponding author at: Dept. of Dental Biomedical Science, Faculty of Dentistry, University of Jember, Jl Kalimantan 37, Jember, East Java 68121, Indonesia.
E-mail address: d.rachmawati@unej.ac.id (D. Rachmawati).

https://doi.org/10.1016/j.phyplu.2024.100535

Available online 15 February 2024

(Briceño et al., 2013; Es-Souni et al., 2005). NiTi has a TiO_2 layer which functions to block the oxidation of nickel ions. However, several *in vitro/in vivo* studies have shown that NiTi can still corrode (Ševčíková et al., 2018). The corrosion processes may occur due to the wet environment of the oral cavity and are mediated by the interaction between the metal and saliva (Rachmawati et al., 2021). In addition, metal-based alloys such as nickel (Ni) are known for their reactive properties in triggering immunotoxicity and in causing sensitivity reactions (Rachmawati et al., 2013, 2015). Therefore, the use of NiTi *wire* is thought to worsen periodontitis due to the interaction between the Ni^{2+} ions release and *LPS P. gingivalis*. Our previous study shows that exposure to some types of dental *alloys* combined with the addition of LPS can increase the inflammatory response compared to that of without bacterial endotoxins. This indicates the presence of synergy between metal exposure and endotoxins characterized by the production of pro-inflammatory cytokines (Rachmawati et al., 2017).

Recently, research focusing on the use of natural materials has been developed (Gościniak et al., 2021). Research on alveolar bone regeneration using herbal plants as alternatives also continues to be developed. One of the herbal plants used is Robusta coffee (*Coffea canephora*). The Green Robusta coffee bean extract (GRCBE) has been used *in vitro* and *in vivo* inflammatory research (Paur et al., 2010; Vamanu et al., 2020; Weber et al., 2020). Coffee is one of the most popular beverages all over the world. Besides its function for consumption, coffee also has beneficial health properties. Robusta coffee contains phenolic compounds i.e. caffeic acid, chlorogenic acid, ferulic acid and other phytochemicals which have anti-inflammatory and antioxidant abilities (Cagliani et al., 2013; Fischer et al., 2001; Kim et al., 2018; Martín et al., 1998; Panusa et al., 2017). In previous experiments, we conducted a series of trials and determined that a concentration of 500 mg/ml of GRCBE is beneficial in treating periodontitis in rats. Based on this background, this study investigated the stimulating effect of GRCBE in periodontal tissue regeneration by measuring Osteocalcin (OCN), Bone Morphogenetic Protein 2 (BMP2) and Cyclooxygenase (COX-2) in Wistar rats exposed to a combination of LPS *P. gingivalis* and NiTi wire.

2. Materials and methods

2.1. Animals criteria

The study design was a randomized controlled trial (RCT). Thirty-five Wistar rats (*Rattus novergicus*) with the following inclusion criteria: white male Wistar rats, body weight of 180–200 gs, age ± 2–3 months, healthy and good physical condition (clean, white fur colour with normal red eyes) were used in this study. The PRIASE 2021 guidelines were developed in accordance with the recommendations given in the Guidance for Developers of Health Research Reporting Guidelines (Nagendrababu et al., 2021). The animals were acclimatized for 1 week before the overall experimentation. The Wistar rats were divided into 7 groups. Groups 1–4 were rats without therapy: 1) Control rats (placebo), 2) rat periodontitis models (LPS), 3) NiTi wire-fitted rats (NiTi), 4) rats periodontitis models with LPS and NiTi wire (LPS+NiTi); Group 5–7 were rats treated with GRCBE; 5) LPS + GRCBE, 6) rats-fitted NiTi wire + GRCBE, 7) LPS+ NiTi wire + GRCBE. The study obtained approval from the Ethical Committee of Medical Research of the Faculty of the Dentistry University of Jember, Indonesia for Experimental Animal Use Number 1337/UN.25.8/KEPK/DL/2021. All procedures were made to minimize surgery-induced suffering and reduce the overall number of animals used.

2.2. Exposure of LPS P.gingivalis

Male Wistar rats were induced with LPS *P. gingivalis* (LPS-PG Ultrapure, Invivo Gen-Sandiego USA) concentrations of 100 μg/mL in the gingival sulcus lower left molar 1st (M1) in samples 2, 4, 5 and 7. The injection was done in 0.05 ml using a needle with a size of 30 G. The injection was carried out once every 3 days for 14 days. The procedure was proven effective in inducing periodontal inflammation (Dumitrescu et al., 2004). After 14-day treatment, a rat model of periodontitis was obtained with characteristics of sulcular epithelial damage, pocket formation, and signs of inflammation: redness, swelling, and deep probing. In addition, the X-ray photo was taken to ensure that the inflammation was formed as characterized by the appearance of alveolar bone resorption (Fig. 3).

2.3. NiTi wire installation

Rats were treated using ketamine at a dose of 0.04 – 0.08 mL which was injected intramuscularly in the rat's hamstrings muscles. NiTi *wire* (Dentsply GAC Low Land 0.016″, GAC International, Bohemia, NY, USA) was attached gripping to the molar of the left lower jaw forming a C-like shape that circles the buccal up to the palatal parts. Of note that during the installation, NiTi wire needed to be adjusted to the shape of the cervical dental arch (Fig. 1). We ensured that the wire grips attached perfectly to prevent this from coming off easily when installed to the rat. Wires were installed on groups 3, 4, 6 and 7 of the rats. Induction of a rat model of periodontitis using LPS *P. gingivalis*, NiTi wire and combination of LPS *P. gingivalis* and NiTi wire ligation was carried out for 14 days.

2.4. Green Robusta coffee bean extract preparation

Prior to the start of the study, identification of the Robusta Coffee bean was obtained from the The Crop Laboratory, Department of Agricultural Production Jember State Polytechnic's. Indonesia (53/PL17.3.1.02/LL/2018). The Robusta coffee beans were acquired from the coffee and cocoa plantations managed by the Indonesian Coffee and Cocoa Research Institute (ICCRI) in Jember, East Java, Indonesia. The ingredients were dehydrated and subsequently pulverized using a combination of crushing and mixing using a blender machine, resulting in the formation of fine granules. Extraction of GRCBE was carried out using maceration techniques. 250 g. GRCBE granules were macerated in 1500 mL, 96% ethanol solution for 72 h. The maceration results were then filtered and subsequently evaporated using a rotary evaporator at a temperature of 30–40 °C with a pressure of 880 mBar for 6 h to obtain GRCBE concentration of 100%. The extract was stored in a sealed glass container. Prior to the experiment, the extract was diluted using a d-PBS solvent into a concentration needed (Dulbecco's Phosphate Buffer Saline, Gibco).

2.5. Green Robusta coffee bean extract application

GRCBE administration was started after treatment with LPS *P. gingivalis* and/or NiTi wire for 14 days until obtaining a rat model of periodontitis. GRCBE was applied with a final concentration of 500 mg/mL to the rats in groups 5, 6, and 7. Application were done once a day for 14 days in the gingival sulcus area of mesial Molar 1st (M1) using a tuberculin syringe size 16 gauge in total volume of 0.05 mL

2.6. Wire installation of the lower left jaw of rats

anaesthesia induction was performed with ketamine at a dosage of 1 ml until the rat became unconscious. Subsequently, the wire was positioned around the buccal to palatal region of the left mandibular molar (M) teeth, generating a C-like shape. This was done to modify the cervical arch on the tooth and ensure a secure grasp of the wire, minimizing the risk of detachment when attached to the rat. The wires were positioned on the groups 3, 4, 6 and 7, cohorts of rats.

2.7. Rat lower left jaw sampling

At day 15 after treatment with GRCBE, rats were euthanized using ketamine HCl at a dose of 0.2 mL/250 g. per body weight, intra-

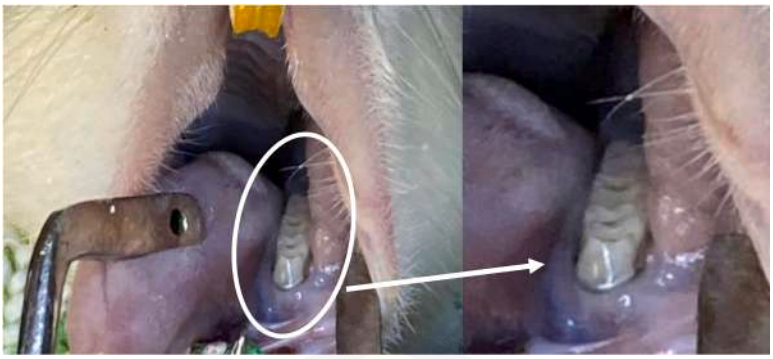


Fig. 1. NiTi wire position. Installation of NiTi wire on rat samples in the area M1 left lower jaw.

muscular in the hamstring muscles. The tissues taken included the three teeth of M1, M2, and M3 lower mandible left along with their periodontal tissue. Next, the tissues were immersed in a formalin solution for 24 h to avoid damage, and autolysis, to maintain cell morphology and prevent the growth of bacteria and fungi. Paraffin blocks were created particularly from the bone and tissue of the M1 left lower jaw.

2.8. Histological preparations

Tissues were immersed in 10% formaline solution for at least 24 h and continued with the decalcification process using a 10% solution of formic acid until the tissue was soft. The dehydration stage was done using alcohol with a low to high concentration. Xylol was used in the clearing stage. The tissue was wrapped in filter paper labelled with the identity of the sample and put into the embedding material, *i.e.* paraffin with a boiling point of 56–60°C for 2 h. Procedures were repeated 3 times. Next, the tissue was put into a block printing tool containing liquid paraffin and was left until frozen. Cutting was done in mesiodistal direction with a thickness of 6–7 μm using rotary microtome (Leica, Chicago, USA). Staining was done with Haematoxylin Eosin (HE) followed by Immunohistochemistry (IHC) staining. Sections were incubated for 1 h at 37 °C with the primary antibodies anti-OCN, (1:300), anti-BMP2 (1:300), and anti-Cox-2 (1:300) (Invitrogen, Thermo Fisher Scientific, Eugene, OR, USA), followed by the secondary antibody (Universal Excell Stain, Biogear Scientific). Observation was done using an optical microscope (Olympus, Tokyo, Japan) with a magnification of x40, x100 and x400.

2.9. Immunohistochemistry analysis of OCN, BMP2 and COX-2

The staining of OCN, BMP-2 and COX-2 were determined by calculating the number of osteoblasts/macrophages cells that developed brownish colour. Biomarker expressions were calculated based on three selected fields of view, particularly in the alveolar bone area of the buccal part from coronal to apical. Three different examiners were involved to observe the intensity of the colour expressed. Histological images are presented in Fig. 2.

2.10. Statistical analysis

The statistical significance was analysed by using the non-parametric test Kruskal-Wallis and Mann-Whitney, with the statistic program

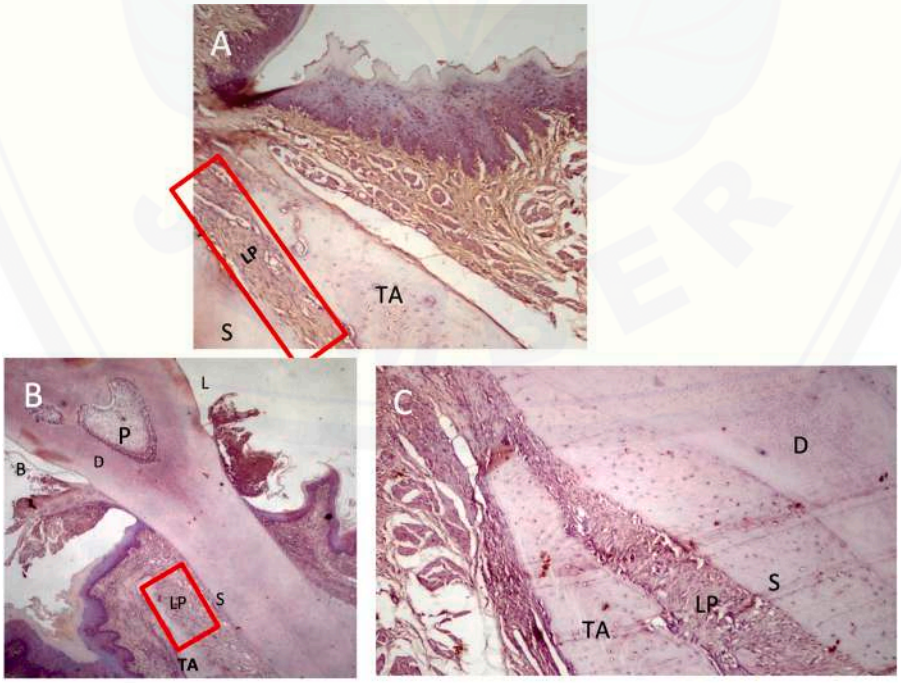


Fig. 2. Represent histological images of the buccal-lingual direction of the 1st molar of the rats with Immunohistochemistry staining A: Control (magnification x100); B: Rat Periodontitis (LPS) (magnification 40x); C: Rat Periodontitis (LPS) (magnification x100). (C: Cementum; M: Mucosa; P: Pulp; D: Dentin; AB: Alveolar bone; PL: Periodontal ligament; B: Buccal Section; L: Lingual section; Red Box: Observed segment).

GraphPad Prism Software version 9.0 (San Diego, CA, USA). $p \leq 0.05$ was considered statistically significant. All data were presented as mean ± SD.

3. Research results

3.1. Quantitative expression of OCN, BMP2 and COX-2

The immunohistochemistry staining (IHC) showed that most staining of OCN was found in the control group (10 ± 0.83), which gradually decreased in the groups without therapy (groups 2–4). An increase in OCN staining occurred in all GRCBE therapy groups (groups 6–7) and the highest increase was found in group 7 (LPS + NiTi wire + therapy) (10 ± 0.36). Similar trend was also present for BMP-2. The number of BMP-2 positive cells was higher in the GRCBE therapy groups (groups 5–7). The largest number of BMP-2 cells was found in the group given LPS + therapy (group 5: 10.5 ± 1.73), followed by the NiTi wire + therapy (group 6) and group 7: group with combination of LPS + NiTi wire + therapy.

The COX-2 staining exhibited contrasting outcomes in comparison to the staining of OCN and BMP2. The group with the highest COX-2 staining was group 4, which consisted of LPS Pg + wire NiTi (13.75 ± 0.833). This was followed by group 3, which consisted of wire NiTi, and group 2, which consisted of LPS *P gingivalis*. The administration of GRCBE treatment resulted in a notable reduction in the quantity of COX-2. The levels of OCN, BMP2, and COX-2 expression, as well as the impact of GRCBE therapy, may be observed in Table 1 and Fig. 5.

In addition, the Mann-Whitney test was conducted to ascertain the extent of the disparity in the number of OCN, BMP2, and COX-2 expressions between the groups. Fig. 5 demonstrates a notable disparity between the therapy and non-therapy groups, with a p-value of ≤ 0.05 indicating statistical significance. There was a notable difference between the control placebo group and the group exposed to LPS *P. gingivalis* (group 1 vs group 2 (NiTi wire) and 3 (LPS *P.gingivalis* + wire NiTi)).

3.2. Histological overview of OCN, BMP-2 and COX-2

Light microscope with a magnification of x400 was used. Histological images of the representative immunohistochemistry sections of OCN, BMP-2 and COX-2 in the gingival mucosa were observed from the epithelium of the gingival sulcus to the connective tissue under the junctional epithelium as shown in Fig. 6.

Histological images of OCN and BMP-2 (Figs. 6A and 6B) show similar trends. Control rat (1A, 1B) showed the presence of OCN and BMP-2 which were characterized by a brownish colour. This was confirmed by the colour intensity and was classified as normal (Table 2).

Table. 1
Average Expressions of OCN, BMP2 and COX-2 in Rat Periodontitis After Treatment For 14 Days ($n = 5$).

No.	Group	*n*	$\bar{X}$ ± SD		
			OCN	BMP2	COX-2
1	Control	5	10 ± 0.83	9 ± 0.82	7.75 ± 2,06
2	LPS *P. gingivalis*	5	6 ± 0.83	7 ± 1,15	10.67 ± 1,44
3	NiTi wire	5	5 ± 0.72	6 ± 1.15	12.00 ± 1,05
4	LPS *P. gingivalis* + NiTi wire	5	5 ± 0.88	4.5 ± 0.58	13.75 ± 0.83
5	LPS *P. ginivalis* + therapy	5	7 ± 0.37	10.5 ± 1.73	8.25 ± 0.87
6	NiTi wire + therapy	5	8 ± 0.62	10.25± 1.26	8.67 ± 0.82
7	LPS *P. gingivalis* + NiTi *wire*+ therapy	5	10 ± 0.36	10 ± 1.41	10.33 ± 0,72

Table 2
Colour Intensity of Expression of OCN, BMP-2 and COX-2 Based on the Average Number of cells expressing biomarker proteins.

No.	Group	OCN	BMP2	COX-2
1	Control	++	++	++
2	LPS *P.gingivalis*	+	+	+++
3	Wire NiTi.	+	+	+++
4	LPS *P.gingivalis*+wire NiTi	+	+	++++
5	LPS *P.gingivalis*+therapy	++	+++	++
6	NiTi *Wire*+therapy	++	+++	++
7	LPS *P.gingivalis*+NiTi *wire*+therapy	+++	+++	++

Description of the Intensity of OCN, BMP2, COX-2 Expressions in the cytoplasm of the cells that are brown.
[+]: Low; the average of the expressed cells OCN, BMP-2, COX-2 (3.1 – 6).
[++]: Normal; the average expressed cells OCN, BMP-2, COX-2 (6.1 – 10).
[+++]: Medium; the average of the expressed cells OCN, BMP-2, COX-2 (10.1 – 13).
[++++]: High; the average of the expressed cells OCN, BMP-2, COX-2 (13.1 – 16).

These indicated that in a healthy state, the periodontal tissue is already expressing OCN and BMP-2 in normal quantity and intensity. In contrast, rats that were not treated with GRCBE (Figs. 6A and 6B, 2, 3, 4) showed less staining for OCN and BMP-2 than those in the control group (1). Furthermore, rats treated with GRCBE (5, 6, 7) clearly showed an increase in the number of positive cells for OCN and BMP-2 as characterized by a brownish colour with normal (++) to moderate (+++) intensity (Table 2) compared to a normal histological image. The staining of OCN and BMP-2 was widely found along the alveolar bone region. This shows that in rats treated with GRCBE, there was a process of alveolar bone regeneration visible by an increase in OCN and BMP-2.

Whereas Fig. 6C shows that COX-2 staining had an inverse trend compared to the staining of OCN and BMP-2. The histological images show that there was a stronger staining of COX-2 in the untreated rats (Figs. 6C, 2, 3, 4) compared to the treated groups with GRCBE (5, 6, 7). COX-2 staining with normal intensity was present in the control group (Fig. 6C (1)). The intensity gradually increased (+++) in the state of periodontitis (LPS *P. gingivalis* (Fig. 6C (2)), NiTi wire (Fig. 6C (3)). Fig. 6C (4)). Robust COX-2 staining with the highest intensity (++++) was found when rats were exposure to the combination of LPS+NiTi wire. Overall, the colour intensity of COX-2 decreased in all groups after treated with GRCBE (Groups 5, 6 and 7) which showed COX-2 with normal intensity (++).

4. Discussion

Our study confirms that OCN, BMP-2 and COX-2 were present in the control rats (Table 1 and Fig. 5). Expressions with normal intensity shown in Table 2 strengthen these findings. This was due to the osteoblast activity that secretes OCN and BMP-2 as a natural bone remodelling process due to the absence of pathogen. Moreover, a combination of LPS *P. gingivalis* + NiTi wire a decrease in the number of osteocalcin and BMP-2 positive cells in group 2 and 3, and an increase in the number of COX-2 positive cells was found. This proves that exposure to LPS *P. gingivalis* and/or NiTi wire alone can trigger the inflammation as confirmed by radiographic images that there was resorption of alveolar bones up to 1/3 apical part (Fig. 2). Robust inflammation signs were even detected in the combination between LPS Pg and NiTi wire (group 4). This occurs due to an imbalance in bone remodelling between osteoclasts and osteoblasts, triggering inflammation of the periodontal tissue.

The use of a wire is at risk in combination with poor oral hygiene resulting in plaque accumulation in the oral cavity. This can lower the pH of the oral cavity (Ripamonti and Renton, 2006). In accordance with the case studies founded, patients undergoing fixed orthodontic treatment may find it more difficult to maintain the oral cavity hygiene since

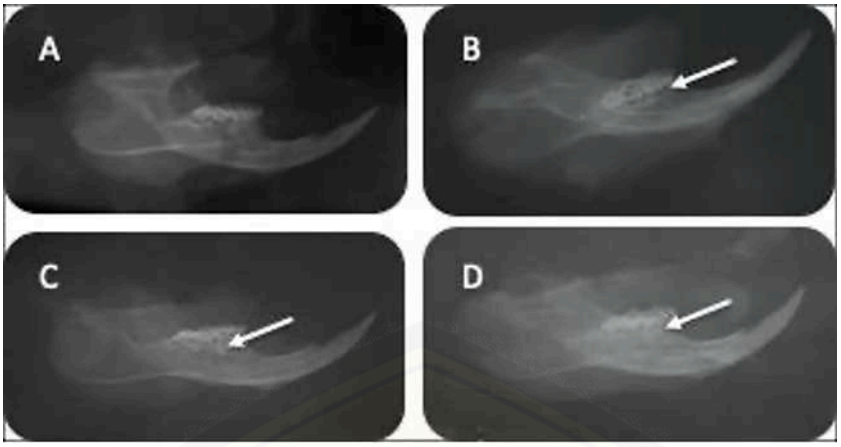


Fig. 3. Represent radiological images of molar 1st left mandibular rat (A: Control; B: LPS P. gingivalis; C: NiTi wire; D: LPS P. gingivalis + NiTi wire; white arrow: resorption of the alveolar bone).

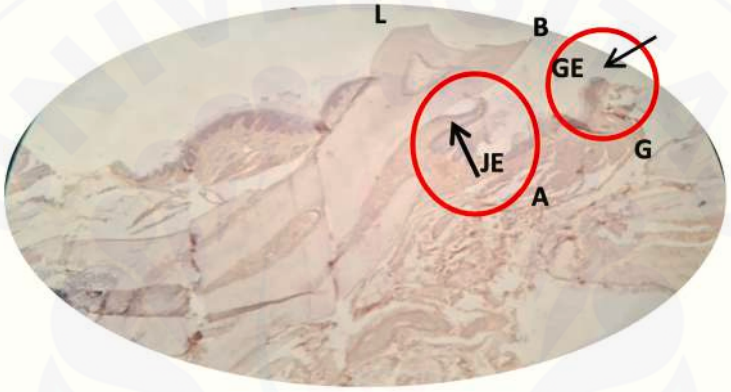


Fig. 4. Represents histological image of the buccal-lingual direction of the M1 teeth in periodontitis rat with exposure to LPS P. gingivalis 100 μg/ml (magnification of 40) with Immunohistochemistry staining (A: Picture showing loss of attachment to the junctional epithelium; Buccal (B); Lingual (L); Gingival (Red Circle G); Gingival Epithelium (GE); Junctional Epithelium (JE).

the food is easily trapped on the wire. They are more susceptible to periodontal disease which enhance gingival recession and plaque retention (Atassi and Awartani, 2010). Of note, the plaque index and orthodontic plaque index are high in patients undergoing orthodontic treatment. In addition, nickel ions (Ni^{2+}) composition in the NiTi wire can be released at acidic pH conditions caused by organic acids formed by the decomposition of food residues attached to the wire and the metabolic results of bacteria that multiply on plaques inside the oral cavity (Rachmawati et al., 2021).

The oral cavity is an ideal environment for metal biodegradation due to its temperature, the quality and pH of saliva which can affect the stability of metal ions (Azizi et al., 2016; Fors and Persson, 2006; Rachmawati et al., 2021). Fors and Persson (36) conclude that Ni^{2+}ions can be released due to plaque and saliva in orthodontic patients with NiTi wire. The biological effects of metals to cause increased inflammation in the oral cavity are caused by the influence of the body's response to the release of Ni^{2+}ions from corroded NiTi (Pataijindachote et al., 2018). A case report shows that a patient experiences an inflammatory reaction in the tissues of his oral cavity after 3 days of using nickel-containing wire. In addition, nickel is the most common metal that causes contact dermatitis. Reports indicate that a nickel concentration of about 30 mg/L can cause contact dermatitis (Noble et al., 2008). Additionally, the release of Ni^{2+}ions in the use of NiTi wire can cause a gingival recession, up to the resorption of the alveolar bone through increase the biosynthesis of prostaglandins E2 (PGE2) and IL-1 which is characterized by the increased expression of COX-2 (Branco-de-Almeida et al., 2020).

Our study confirms that the increased expression of OCN, BMP-2 and the decrease of COX-2 were significantly potent in the group of periodontitis rat model with a combination of LPS + *wire* NiTi induction (group 4) compared to the group of rats that were injected with LPS (group 2) or NiTi wire-installed only (group 3). This explains that there is an adequate increase of inflammation occurring in combination of periodontitis (LPS) with the installation of NiTi wires. Heavy metals cannot be metabolized by the body hence they will merge and bind to a single atomic molecule of ROS from the inflammatory response, which will cause the existing inflammation worsened. In a state of oxidative stress, an increase in lipopolysaccharides (LPS) are some of the factors that can decreasing OCN and BMP2 and an increase in anti-inflammatory cytokines such as COX-2 (Latvala et al., 2016). Such situation was proven *in vitro* in the research of Rachmawati et al. (2017) that exposure to several types of alloys after immersion with the addition of LPS *P. gingivalis* increased the innate immune response to the presence of pathogens compared to exposure without bacterial endotoxins. This indicates a synergy between metal exposure and endotoxins as characterized by the production of pro-inflammatory cytokines (Rachmawati et al., 2016).

This study aimed to determine the effect of administering GRCBE (*C. canephora*) on the amount of OCN, BMP-2 and COX-2 in a rat periodontitis model with and/or without therapy. The study shows that there was a significant decrease in the average number of positive cells stained for OCN and BMP-2 of the LPS exposure group, NiTi wire and the

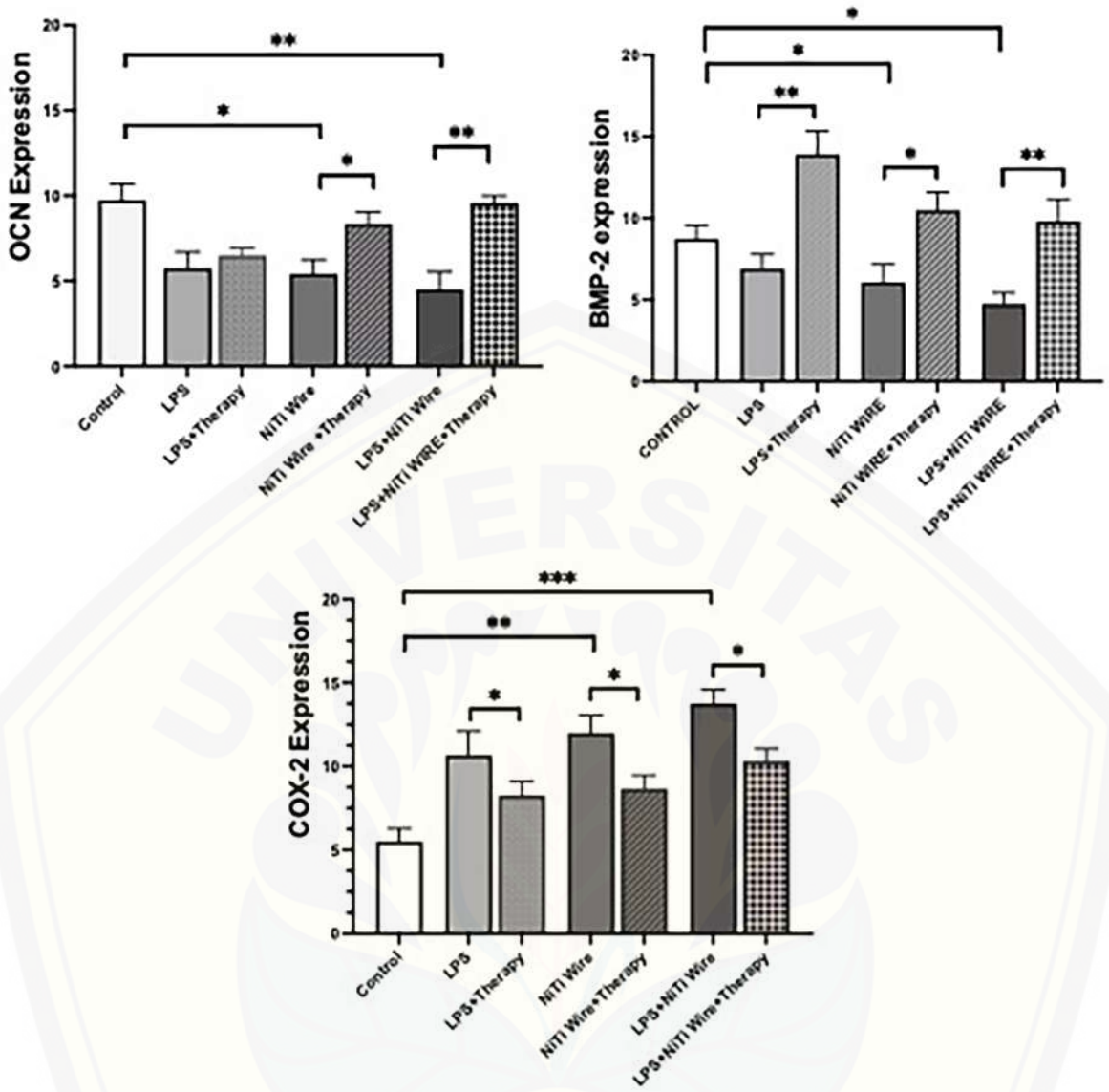


Fig. 5. Graph of Average Expression of OCN (A), BMP2 (B) and COX-2 (C) In the presence and absence of GRCBE Therapy. Graphical bars (Fig 5) and numerical expression count (Table 1), the data represent mean ± SD from five samples (*nn* = = 5). Asterisks specify statistically significant (Kruskal-Wallis and Mann-Whitney, (non-parametric) differences with and without therapy and amongst the groups as compared to the control group, $p<0.05$ (*), $p<0.01$ (**) and $p<0.001$ (***).

combination of LPS + wire NiTi (groups 2–4) compared to the group given GRCBE therapy (5–7). The positive cells stained of OCN and BMP-2 in the group with a combination of LPS *Pg* + NiTi wire were significantly smaller than those of the group receiving GRCBE therapy (Fig 5). This proves that GRCBE can increase the amount of OCN, BMP2 and therefore increase alveolar bone regeneration due to the anti-inflammatory and antioxidant properties of Robusta coffee components such as caffeine, ferulic acid, chlorogenic acid, and caffeic acid.

Deterioration of the cell in inflammation triggers the release of lysosome enzymes from leukocytes through their action on cell membranes and secreting substances endogenously known as inflammatory mediators. In addition, arachidonic acid is also released from the cell membrane by the enzyme phospholipase (Ezzo and Cutler, 2003). Arachidonic acid is one of the inflammatory mediators that play a crucial role in the biosynthesis of prostaglandins through the cyclooxygenase (COX) pathway. Cyclooxygenase first catalyzes 2 stages of prostaglandin biosynthesis and is present in 2 forms, namely COX-1 and COX-2. Cyclooxygenase 1 plays a role in homoeostasis, while cyclooxygenase 2 increases when inflammation occurs and plays a role in the synthesis of prostaglandins, especially PGE2 which causes increased infiltration of inflammatory cells (Cekici et al., 2014). COX-2 is an enzyme of which presence is influenced by the presence of inflammation in the tissues, one of which is periodontitis caused by the tissue response to the presence of LPS *P.gingivalis* (Ribeiro-Santos et al., 2019). This is supported by a study conducted by Lazăr (2015) which reports that the amount of COX-2 expression is higher in periodontitis patients compared to patients who have a healthy periodontal tissue condition (Morton and Dongari-Bagtzoglou, 2001).

Robusta coffee bean contains active compounds with pharmacological effects as anti-inflammatories with inhibition of cyclooxygenase pathways, such as flavonoids, polyphenols, and chlorogenic acid, thus play a role in reducing inflammatory symptoms (Song et al., 2022; Tsou et al., 2019). In the injured tissues, there will be a healing process which begins with the formation of blood vessels and subsequently followed by the inflammatory phase on the 1st to 3rd day. Likewise, followed by the proliferation phase on the 3rd to 7th days and remodelling on the 7th to 14th days after pathogenic exposures.

The results show that the average expression of OCN and BMP-2

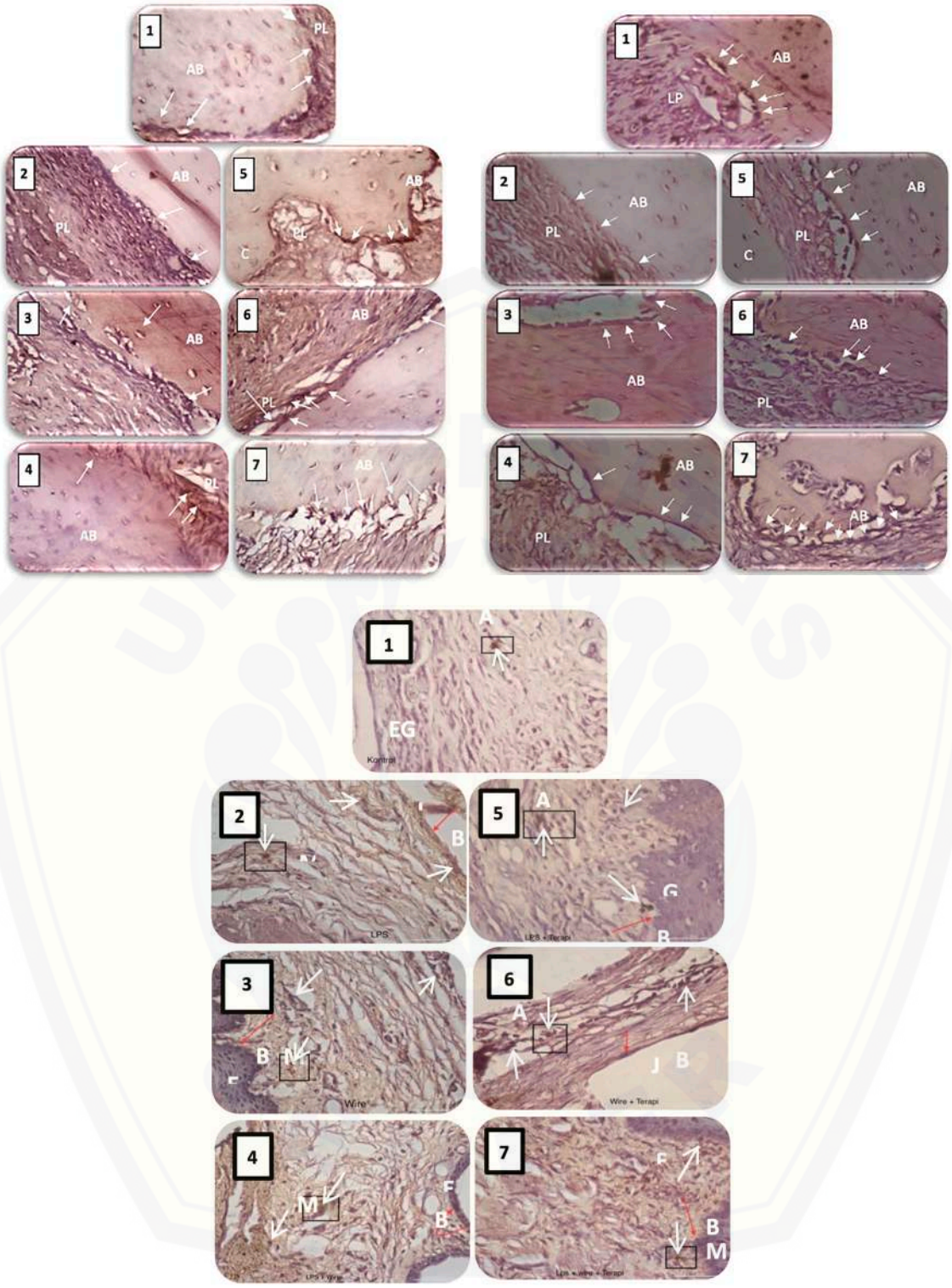


Fig. 6. Represent histological images with a magnification of x400 expression of OCN (A), BMP2 (B) and COX-2 (C) by immunohistochemistry staining of gingival mucosa region 1st molar showed a distribution of brown colour around the gingival sulcus extending to the junctional epithelium (JE) marked with a white arrow (1) Control; (2) LPS; (3) NiTi wire; (4) LPS+NiTi Wire; (5) LPS + therapy; (6) NiTi Wire + therapy; (7) LPS + NiTi wire + therapy. EG: Gingival Epithelium; JE: Junctional Epithelium; AB: Alveolar bone; PL: Periodontal ligament M: Macrophages; BE: Basal Epithelial cells; The White Arrow shows the brown-coloured cell area in the gingival epithelium and extracts the biomarker.

increased while COX-2 decreased in the inflammatory due to GRCBE therapy concentration of 500 mg/mL. Decreased expression of COX-2 is effective in all groups of inflammatory therapy due to LPS (group 5), NiTi wire (group 6) and combination of LPS + NiTi wire. Therefore, therapy by utilizing GRCBE containing anti-inflammatory compounds accelerates healing as characterized by a decrease in COX-2 expression.

GRCBE contains flavonoids that can inhibit the occurrence of inflammation in two ways, particularly by inhibiting capillary permeability, metabolism of arachidonic acid and the secretion of lysosome enzymes. Flavonoid compounds can inhibit the release of arachidonic

acid and the secretion of lysosome enzymes from cell membranes by blocking the cyclooxygenase pathway. The cyclooxygenase expressed at the time of inflammation is COX-2. Inhibition in the synthesis of COX-2 can decrease the metabolism of arachidonic acid in the cell membranes so that prostaglandin biosynthesis is reduced, and the inflammatory response decreases due to inhibition of NFκB activation, thus inhibiting the synthesis of IL-1 and TNF-α.

Anti-inflammatory and antioxidant compounds of chlorogenic acid compounds, according to research conducted by Park (2022) are described through the interaction of chlorogenic acid with amino acid residues that are divided into several proteins that form hydrogen bonds. The bond allows for a strong bond, with energy of −198.95 cal/mol. Hence, it has the potential to inhibit the performance of COX-2 in producing prostaglandins during inflammation. In addition, Robusta coffee also contains an anti-inflammatory agent which has an inhibitory power against the activity of the cyclooxygenase (COX) enzyme *in vitro* (Park and Yoon, 2022). The reduction of inflammatory reactions carried out by inhibiting the COX-2 pathway has been proven by Landete's *in vitro* research (2012) showing that polyphenol extract from the content of Robusta coffee beans can reduce the inflammatory response as characterized by a decrease in COX-2 expression. This is in line with the results of Chul Lee et al. (2019), that Robusta coffee bean is more effective for reducing the degree of inflammation which is characterized by decreased secretion of tumor Necrosis Factor-α (TNF-α). The decrease in TNF-α as a pro-inflammatory cytokine can decrease the amount of COX-2 expression (Lee et al., 2019).

GRCBE can increase the amount of expression of OCN and BMP-2 which are biomarkers for the regeneration of alveolar bones. Caffeine has anti-inflammatory properties that can inhibit the production of TNF-α stimulated by LPS *P. gingivalis*, thus it can reduce the inflammation (Hall et al., 2015). *Chlorogenic acids* are reported to have anti-inflammatory properties that function to anchor proinflammation cytokines, i.e. TNF- α, IL-1, PGE-2, and IL-6 in macrophage cells that can inhibit osteoclasts (Hall et al., 2015; Hienz et al., 2015; Xu et al., 2014). *α*Ferulic acid can reduce the production of TNF- so that it can repair osteoblasts and regenerate alveolar bones (Shen et al., 2012). Caffeic acid has the potential as a powerful antioxidant and anti-inflammatory. Caffeic acid can suppress the activation of NF-kβ, which is important in inflammatory processes (Hall et al., 2015). Decreased bone resorption activity by osteoclasts, providing an opportunity for the process of osteoblast differentiation through the activation of TGF-beta by regulating the synthesis of BMP-2 through core binding factor-1 (C-bfa1), is a transcription factor in progenitor cells into osteoblasts. These osteoblast precursors will explore and differentiate to form pre osteoblasts and then will become mature osteoblast (Hienz et al., 2015).

It is in line with the results of a study showing that inhibition of pro-inflammatory cytokine products will result in the decreased formation of osteoclasts, and osteoblasts increase (Farah and de Paula Lima, 2019). The increase in osteoblasts is also followed by osteocalcin expression. This research is supported by Shu-jem's (2013) study showing that Robusta coffee with 0.1 mM of caffeine can effectively improve OCN regulation (Su et al., 2013). Bone is a complex tissue that will change throughout the life span through the process of bone formation by osteoblasts and osteoclasts (Gruber, 2019). In a normal alveolar bone, the number of osteoblasts and osteoclasts are balanced, but the non-balanced bone formation will result in the alveolar bone resorption (Kapinas and Delany, 2011). Therefore, therapy with the administration of GRCBE in a rat periodontitis model induced by a combination of LPS *P. gingivalis* and installed NiTi wire is effective in increasing the regeneration of alveolar bones. The bioactive components in the GRCBE have shown to have a great potential as immunomodulatory substances in the treatment and prevention of periodontal disease. Further, *in vitro,* and *in vivo* studies are required to show the efficacy of GRCBE as the future alternative in the periodontitis therapy.

A limitation of our study is that we solely assessed tissue inflammation using immunohistochemical staining, without considering the overall antioxidant capacity. To gain more insights, the anti-inflammatory properties of GRCBE can be elucidated through future investigations utilising techniques such as Western blot, PCR, immunohistochemistry, or ELISA. Based on the constraints of this animal investigation, it may be inferred that the administration of 500 mg/mL GRCBE promoted bone growth and inhibited inflammation. Additional research focusing on the examination of other biomarkers, such as the TNF-α-dependant signalling system or the Wnt/β catenin pathway, can provide insight into the mechanism by which GRCBE affects periodontal disease. Our investigation demonstrated that administering GRCBE can effectively prevent bone destruction in periodontitis, especially when caused by the combination of LPS P. gingivalis and NiTi wire.

5. Conclusion

In conclusion, our study revealed a new possible therapeutic agent for periodontal inflammation using GRCBE. Our novel findings highlight the antiinflammation efficacy of GRCBE at 500 mg/mL which is effective in improving alveolar bone regeneration in rat periodontitis models induced by a combination of LPS *P. gingivalis* and NiTi wire.

Financial support

This research was funded by the Directorate of Higher Education of the Ministry of Education and Culture of the Republic of Indonesia with the UNEJ Higher Education Research Grant scheme 2022 (contract No 110/E5/PG.02.00.PT/2022) for funding support for some parts of this research.

CRediT authorship contribution statement

Dessy Rachmawati: Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, Project administration, Resources, Software, Supervision, Validation, Visualization, Writing – original draft, Writing – review & editing. **Tantin Ermawati:** Data curation, Investigation, Methodology, Supervision, Visualization. **Nanda Innayatur Rahmatillah:** Formal analysis, Investigation, Methodology, Validation, Visualization. **Nurwandani Meylina:** Data curation, Formal analysis, Investigation, Methodology, Resources. **Novia Yolanda Safitri:** Data curation, Formal analysis, Investigation, Methodology, Resources. **Rina Sutjiati:** Formal analysis, Methodology, Project administration, Software. **Ineke D.C. Jansen:** Investigation, Supervision, Validation, Visualization, Writing – review & editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

All data generated for this study are included in the article.

Acknowledgments

The authors thank the Directorate of Higher Education of the Ministry of Education, Culture, Research, and Technology of the Republic of Indonesia with the UNEJ Higher Education Research Grant scheme 2022 for funding support for some parts of this research.

References

Atassi, F., Awartani, F., 2010. Oral hygiene status among orthodontic patients. J. Contemp. Dent. Pract. 11, 25–32.

Azizi, A., Jamilian, A., Nucci, F., Kamali, Z., Hosseinikhoo, N., Perillo, L., 2016. Release of metal ions from round and rectangular NiTi wires. Prog. Orthod. 17, 10. https://doi.org/10.1186/s40510-016-0123-3.

Branco-de-Almeida, L.S., Franco, G.C.N., Castro, M.L., 2020. Protective effects of desipramine on alveolar bone in experimental periodontitis. J. Periodontol. 91, 1694–1703. https://doi.org/10.1002/jper.19-0569.

Briceño, J., Romeu, A., Espinar, E., Llamas, J.M., Gil, F.J., 2013. Influence of the microstructure on electrochemical corrosion and nickel release in NiTi orthodontic archwires. Mater. Sci. Eng. C Mater. Biol. Appl. 33, 4989–4993. https://doi.org/10.1016/j.msec.2013.08.024.

Cagliani, L.R., Pellegrino, G., Giugno, G., Consonni, R., 2013. Quantification of Coffea arabica and Coffea canephora var. robusta in roasted and ground coffee blends. Talanta 106, 169–173. https://doi.org/10.1016/j.talanta.2012.12.003.

Cekici, A., Kantarci, A., Hasturk, H., Van Dyke, T.E., 2014. Inflammatory and immune pathways in the pathogenesis of periodontal disease. Periodontology 64, 57–80. https://doi.org/10.1111/prd.12002, 2000.

Dumitrescu, A.L., Abd-El-Aleem, S., Morales-Aza, B., Donaldson, L.F., 2004. A model of periodontitis in the rat: effect of lipopolysaccharide on bone resorption, osteoclast activity, and local peptidergic innervation. J. Clin. Periodontol. 31, 596–603. https://doi.org/10.1111/j.1600-051X.2004.00528.x.

Es-Souni, M., Es-Souni, M., Fischer-Brandies, H., 2005. Assessing the biocompatibility of NiTi shape memory alloys used for medical applications. Anal. Bioanal. Chem. 381, 557–567. https://doi.org/10.1007/s00216-004-2888-3.

Ezzo, P.J., Cutler, C.W., 2003. Microorganisms as risk indicators for periodontal disease. Periodontology 32, 24–35. https://doi.org/10.1046/j.0906-6713.2003.03203.x, 2000.

Farah, A., de Paula Lima, J., 2019. Consumption of chlorogenic acids through coffee and health implications. Beverages 5, 11.

Fischer, M., Reimann, S., Trovato, V., Redgwell, R.J., 2001. Polysaccharides of green Arabica and Robusta coffee beans. Carbohydr. Res. 330, 93–101. https://doi.org/10.1016/s0008-6215(00)00272-x.

Fors, R., Persson, M., 2006. Nickel in dental plaque and saliva in patients with and without orthodontic appliances. Eur. J. Orthod. 28, 292–297. https://doi.org/10.1093/ejo/cji091.

Gościniak, A., Paczkowska-Walendowska, M., Skotnicka, A., Ruchała, M.A., Cielecka-Piontek, J., 2021. Can plant materials be valuable in the treatment of periodontal diseases? Practical review. Pharmaceutics 13. https://doi.org/10.3390/pharmaceutics13122185.

Gruber, R., 2019. Osteoimmunology: inflammatory osteolysis and regeneration of the alveolar bone. J. Clin. Periodontol. 46 (Suppl 21), 52–69. https://doi.org/10.1111/jcpe.13056.

Hall, S., Desbrow, B., Anoopkumar-Dukie, S., 2015. A review of the bioactivity of coffee, caffeine and key coffee constituents on inflammatory responses linked to depression. Food Res. Int. 76 (Pt 3), 626–636. https://doi.org/10.1016/j.foodres.2015.07.027.

Hienz, S.A., Paliwal, S., Ivanovski, S., 2015. Mechanisms of bone resorption in periodontitis. J. Immunol. Res. 2015, 615486 https://doi.org/10.1155/2015/615486.

Kapinas, K., Delany, A.M., 2011. MicroRNA biogenesis and regulation of bone remodeling. Arthritis Res. Ther. 13, 220. https://doi.org/10.1186/ar3325.

Kim, W., Kim, S.Y., Kim, D.O., Kim, B.Y., Baik, M.Y., 2018. Puffing, a novel coffee bean processing technique for the enhancement of extract yield and antioxidant capacity. Food Chem. 240, 594–600. https://doi.org/10.1016/j.foodchem.2017.07.161.

Latvala, S., Hedberg, J., Di Bucchianico, S., 2016. Nickel release, ROS generation and toxicity of Ni and NiO micro- and nanoparticles. PLoS One 11, e0159684. https://doi.org/10.1371/journal.pone.0159684.

Lee, I.C., Lee, J.S., Lee, J.H., Kim, Y., So, W.Y., 2019. Anti-Oxidative and anti-inflammatory activity of Kenya grade AA green coffee bean extracts. Iran J. Public Health 48, 2025–2034.

Martín, M.J., Pablos, F., González, A.G., 1998. Discrimination between Arabica and Robusta green coffee varieties according to their chemical composition. Talanta 46, 1259–1264. https://doi.org/10.1016/s0039-9140(97)00409-8.

Morton, R.S., Dongari-Bagtzoglou, A.I., 2001. Cyclooxygenase-2 is upregulated in inflamed gingival tissues. J. Periodontol. 72, 461–469. https://doi.org/10.1902/jop.2001.72.4.461.

Nagendrababu, V., Kishen, A., Murray, P.E., 2021. PRIASE 2021 guidelines for reporting animal studies in Endodontology: explanation and elaboration. Int. Endod. J. 54, 858–886. https://doi.org/10.1111/iej.13481.

Noble, J., Ahing, S.I., Karaiskos, N.E., Wiltshire, W.A., 2008. Nickel allergy and orthodontics, a review and report of two cases. Br. Dent. J. 204, 297–300. https://doi.org/10.1038/bdj.2008.198.

Panusa, A., Petrucci, R., Lavecchia, R., Zuorro, A., 2017. UHPLC-PDA-ESI-TOF/MS metabolic profiling and antioxidant capacity of arabica and robusta coffee silverskin: antioxidants vs phytotoxins. Food Res. Int. 1, 155–165. https://doi.org/10.1016/j.foodres.2017.05.017.

Park, C.M., Yoon, H.S., 2022. Chlorogenic Acid as a Positive Regulator in LPS-PG-Induced Inflammation via TLR4/MyD88-Mediated NF-κB and PI3K/MAPK Signaling Cascades in Human Gingival Fibroblasts. Mediators Inflamm. 2022, 2127642 https://doi.org/10.1155/2022/2127642.

Pataijindachote, J., Juntavee, N., Viwattanatipa, N., 2018. Corrosion analysis of orthodontic wires: an interaction study of wire type, pH and immersion time. Adv. Dentist. Oral Health 10, 1–7.

Paur, I., Balstad, T.R., Kolberg, M., 2010. Extract of oregano, coffee, thyme, clove, and walnuts inhibits NF-kappaB in monocytes and in transgenic reporter mice. Cancer Prev. Res. (Phila.) 3, 653–663. https://doi.org/10.1158/1940-6207.

Rachmawati, D., Bontkes, H.J., Verstege, M.I., 2013. Transition metal sensing by Toll-like receptor-4: next to nickel, cobalt and palladium are potent human dendritic cell stimulators. Contact Derm. 68, 331–338. https://doi.org/10.1111/cod.12042.

Rachmawati, D., Cahyasari, D.A., Febiantama, A.T., Hidayati, L., Kleverlaan, C.J., 2021. Brewed Robusta coffee increases nickel ion release from dental alloys: an in vitro study. Materials 14, 22. https://doi.org/10.3390/ma14227069.

Rachmawati, D., Muris, J., Scheper, R.J., 2015. Continuing the quest for autoimmunity due to oral metal exposure. Autoimmunity 48, 494–501. https://doi.org/10.3109/08916934.2015.1033688.

Rachmawati, D., Peferoen, L.A., Vogel, 2016. Metal ions potentiate microglia responsiveness to endotoxin. J. Neuroimmunol. 291, 89–95. https://doi.org/10.1016/j.jneuroim.2015.12.013.

Rachmawati, D., von Blomberg, B.M.E., Kleverlaan, C.J., Scheper, R.J., van Hoogstraten, I.M.W., 2017. Immunostimulatory capacity of dental casting alloys on endotoxin responsiveness. J. Prosthet. Dent. 117, 677–684. https://doi.org/10.1016/j.prosdent.2016.08.013.

Ribeiro-Santos, F.R., Silva, G.G.D., Petean, I.B.F., 2019. Periapical bone response to bacterial lipopolysaccharide is shifted upon cyclooxygenase blockage. J. Appl. Oral Sci. 27, e20180641 https://doi.org/10.1590/1678-7757-2018-0641.

Ripamonti, U., Renton, L., 2006. Bone morphogenetic proteins and the induction of periodontal tissue regeneration. Periodontology 41, 73–87. https://doi.org/10.1111/j.1600-0757.2006.00155.x, 2000.

Ševčíková, J., Bártková, D., Goldbergová, M., 2018. On the Ni-Ion release rate from surfaces of binary NiTi shape memory alloys. Appl. Surf. Sci. 427, 434–443.

Shen, W., Qi, R., Zhang, J., 2012. Chlorogenic acid inhibits LPS-induced microglial activation and improves survival of dopaminergic neurons. Brain Res. Bull. 88, 487–494. https://doi.org/10.1016/j.brainresbull.2012.04.010.

Song, J., Kim, B., Kim, O., 2022. Effect of coffee on lipopolysaccharide-induced immortalized human oral keratinocytes. Foods 11, 15. https://doi.org/10.3390/foods11152199.

Su, S.J., Chang, K.L., Su, S.H., Yeh, Y.T., Shyu, H.W., Chen, K.M., 2013. Caffeine regulates osteogenic differentiation and mineralization of primary adipose-derived stem cells and a bone marrow stromal cell line. Int. J. Food Sci. Nutr. 64, 429–436. https://doi.org/10.3109/09637486.2012.759184.

Tsou, S.H., Hu, S.W., Yang, J.J., Yan, M., Lin, Y.Y., 2019. Potential oral health care agent from coffee against virulence factor of periodontitis. Nutrients 11, 9. https://doi.org/10.3390/nu11092235.

Vamanu, E., Gatea, F., Pelinescu, D.R., 2020. Bioavailability and bioactivities of polyphenols eco extracts from coffee grounds after in vitro digestion. Foods 9, 9. https://doi.org/10.3390/foods9091281.

Weber, L., Kuck, K., Jürgenliemk, G., Heilmann, J., Lipowicz, B., Vissiennon, C., 2020. Anti-Inflammatory and barrier-stabilising effects of myrrh, coffee charcoal and chamomile flower extract in a co-culture cell model of the intestinal mucosa. Biomolecules 10, 7. https://doi.org/10.3390/biom10071033.

Xu, X.C., Chen, H., Zhang, X., 2014. Simvastatin prevents alveolar bone loss in an experimental rat model of periodontitis after ovariectomy. J. Transl. Med. 12, 284. https://doi.org/10.1186/s12967-014-0284-0.