ORIGINAL ARTICLE

Role of Caffeine and Ethanol in Modulating Expression of Receptor Activator of Nuclear Factor κβ (RANK) and Osteoprotegerin (OPG) during Orthodontic Tooth Movement: An In Vivo Study

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ABSTRACT

Background and Objective. Orthodontic tooth movement is driven by bone remodeling influenced by systemic factors, including caffeine and ethanol. This study aimed to investigate the effects of caffeine and ethanol on the expression of Receptor Activator of Nuclear Factor κβ (RANK) and Osteoprotegerin (OPG), key bone remodeling biomarkers, during orthodontic tooth movement.

Methods. A laboratory experimental study was conducted on 30 male Wistar rats divided into three groups: K1 (orthodontic force only), K2 (force + caffeine), and K3 (force + ethanol). Orthodontic force was applied using Ni-Ti coil springs. Caffeine and ethanol were administered orally daily. On days 7 and 14, maxillary tissues were collected and analyzed via immunohistochemistry for RANK and OPG expression. Data were analyzed using One-Way ANOVA and Independent Sample T-tests with significance at $p < 0.05$.

Results. Caffeine and ethanol administration increased RANK and OPG expression compared to controls; however, only the ethanol group showed a significant increase in RANK expression on day 14 ($p = 0.044$). OPG expression was significantly higher in treatment groups at both time points ($p < 0.05$). These changes suggest modulatory effects on bone remodeling biomarkers without direct measurement of tooth movement.

Conclusion. Caffeine and ethanol modulate bone remodeling marker expression during orthodontic force application, with ethanol significantly increasing RANK expression at later stages. Further studies are needed to clarify the clinical implications for orthodontic treatment.

Keywords: caffeine, ethanol, orthodontic tooth movement, bone remodeling, RANK, OPG, immunohistochemistry, rat model

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INTRODUCTION

Coffee and alcoholic beverages are among the most widely consumed drinks globally, including Indonesia. Knapik et al. reported that caffeine consumption among adults reaches approximately 89%.[1] According to the World Health Organization (WHO), alcohol consumption has shown an upward trend across Southeast Asia in 2018.[2] Both caffeine and ethanol are believed to potentially influence orthodontic treatment, particularly in the context of orthodontic tooth movement (OTM).

The two main types of coffee commonly consumed are Arabica and Robusta. Kristiyanto et al. reported that Robusta contains a higher caffeine content (2.4%) compared to Arabica (1.9%).[3] Arabica coffee also contains various health-promoting bioactive compounds, including polyphenols, flavonoids, proanthocyanidins, coumarins, chlorogenic acids, trigonelline, tocopherols, cafestol, and kahweol, and it is generally more preferred due to its superior aroma.[4]

Studies have shown that caffeine administration during orthodontic treatment can increase tooth mobility. For example, Herniyati et al. found that caffeine intake elevated the expression of Receptor Activator of Nuclear Factor-κB ligand (RANKL) and osteoclast numbers during OTM.[5] Supporting this, Golshah et al. also demonstrated that caffeine injections significantly enhanced tooth movement in rats undergoing orthodontic therapy.[6] Berman et al. noted that excessive caffeine intake may negatively impact bone density, which could subsequently influence tooth movement during treatment.[7]

In contrast, alcohol consumption is expected to rise in half of the Asian region. According to the WHO, Southeast Asia is projected to see the largest increase, with India expected to lead, followed by Indonesia and Thailand.[2] In Indonesia, 3.3% of individuals over the age of 10 consume alcohol, with rates exceeding 10% in five provinces: North Sulawesi, East Nusa Tenggara (NTT), Bali, Gorontalo, and Maluku.[8] Alcohol consumption in Indonesia is also deeply rooted in cultural traditions, with beverages such as *tuak*, *arak*, *sopi*, and *badeg* commonly consumed during customary and ceremonial events. These practices remain prevalent in areas such as North Toraja Regency in South Sulawesi.

de Araujo reported that ethanol disrupts the balance between bone formation and resorption, potentially impairing bone apposition.[2,8,9] Schröder added that ethanol may stimulate osteoclast activity while simultaneously inhibiting osteogenesis by suppressing preosteoblast markers.[10]

Orthodontic tooth movement involves coordinated biological responses in the alveolar bone, including interactions among periodontal ligament fibroblasts, mesenchymal stem cells, immune cells, osteoblasts, osteocytes, and osteoclasts.[11] These cells contribute to bone remodeling, where resorption by osteoclasts is followed by bone formation by osteoblasts.[12] On the pressure side, the mechanical stimulus induces an inflammatory response that promotes the release of proinflammatory cytokines such as interleukin-6 (IL-6), tumor necrosis factor (TNF), macrophage colony-stimulating factor (M-CSF), and especially interleukin-1 (IL-1). These mediators activate preosteoclasts, leading to osteoclast formation and subsequent alveolar bone resorption. Conversely, on the tension side, an anti-inflammatory response is triggered, resulting in the production of cytokines and growth factors including IL-6, IL-10, transforming growth factor-β (TGF-β), and osteoprotegerin (OPG), which promote osteoblast differentiation and bone apposition.[13]

Among these signaling molecules, IL-1 plays a central role by stimulating osteoclastogenesis through the activation of RANK. RANK, expressed by osteoclast precursors, binds to its ligand RANKL, facilitating bone resorption on the pressure side. However, RANKL can also bind to OPG, a decoy receptor that inhibits the RANK–RANKL interaction. The binding of OPG to RANKL suppresses osteoclast differentiation and activity, thereby limiting bone resorption.[13]

Based on this background, the present study aims to explore and compare the effects of caffeine and ethanol on the expression of RANK and OPG during orthodontic tooth movement, in order to clarify the potential impact of these commonly consumed substances on bone remodeling in the orthodontic context.

MATERIALS AND METHODS

This animal study was conducted following ethical approval obtained from the Ethics and Advocacy Unit, Faculty of Medicine, Hasanuddin University (Recommendation No. 420/UN4.6.4.5.31/PP36/2023). A total of 15 healthy male Wistar rats (aged 2–3 months; body weight 200–250 g) were acclimatized for one week under standardized laboratory conditions prior to the experiment.

The animals were randomly divided into three groups (n = 5 per group):

- **Group K1:** Application of orthodontic force only
- **Group K2:** Orthodontic force + caffeine administration
- **Group K3:** Orthodontic force + ethanol administration

Inclusion and Exclusion Criteria

Inclusion criteria included good general health, complete dentition (no missing incisors or molars), and absence of anatomical abnormalities. Animals displaying weight loss, lethargy, or appetite loss were excluded from the study.

Installation of Closed Coil Spring

The appliance installation procedure is illustrated in Figure 1 using this procedure:

- Before the procedure of installation and activation of the Nickel–Titanium (Ni–Ti) closed coil spring, intramuscular injection of ketamine anesthesia is administered. The Ni–Ti closed coil spring is then installed on one side with fixation using 0.1 stainless steel ligature wire.

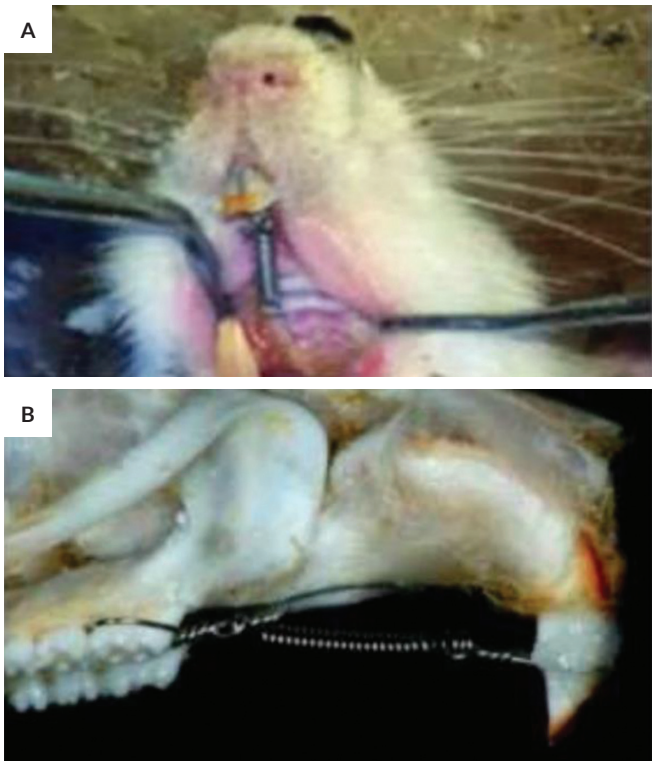

Figure 1. ***Orthodontic appliance placement.*** **(A)** Shows the rat after appliance placement with orthodontic force applied. **(B)** Provides a close-up of the coil spring delivering a force of 50 g/cm^2.

- The force magnitude is then measured using a tension gauge to achieve a force of 50 g/cm^2.
- Once the desired force magnitude is obtained, the closed coil spring is fixed on the opposite side using 0.1 stainless steel ligature wire.
- To enhance retention, flowable composite is applied.
- After installing the Ni–Ti closed coil spring on the same day, caffeine and ethanol, as well as a combination of caffeine and ethanol, are administered to the test animals.

Substance Administration

On the same day as appliance installation, caffeine and ethanol were administered to the respective groups. Caffeine (prepared from Arabica coffee extract at a concentration of 5 mg/mL) was delivered orally via gavage. Each rat received 0.25 mL of extract, equivalent to 1.25 mg caffeine per 250 g body weight. Ethanol was administered orally at a dose of 2.06 mL/kg BW, equating to 0.515 mL per rat per day.

Tissue Harvesting and Histological Processing

After the 3- and 7-day intervention period, all rats were euthanized via cervical dislocation following ketamine anesthesia. The maxillae, including incisors and surrounding alveolar bone, were dissected and fixed in 10% neutral buffered formalin (pH 7.0) for 24 hours. Standard histological procedures, including decalcification, embedding, sectioning, and mounting, were followed.

Immunohistochemical (IHC) Analysis

IHC staining was performed to evaluate the expression of Receptor Activator of Nuclear Factor κβ (RANK) and Osteoprotegerin (OPG) in the periodontal tissues. Tissue sections were deparaffinized, rehydrated, and subjected to antigen retrieval using heat-induced epitope retrieval (HIER) in citrate buffer (pH 6.0; Abcam, UK), maintained at 95°C for 20 minutes.

After cooling, sections were incubated overnight at 4°C with the following primary antibodies:

- Anti-RANK (1:100 dilution; Santa Cruz Biotechnology, USA; Cat. No. sc-52950)
- Anti-OPG (1:200 dilution; Santa Cruz Biotechnology, USA; Cat. No. sc-390518)

The next day, after washing with PBS, the sections were incubated with a biotinylated secondary antibody (Goat anti-rabbit IgG, 1:500; Vector Laboratories, USA) for 1 hour at room temperature. Detection was performed using the VECTASTAIN® Elite ABC-HRP Kit (Vector Laboratories, USA), followed by chromogenic visualization with DAB Substrate Kit (3,3′-diaminobenzidine; Abcam, UK).

Counterstaining was carried out using Hematoxylin Solution (Merck, Germany) for 1 minute to enhance nuclear contrast. The slides were then dehydrated, cleared, and mounted using DPX mounting medium (Sigma-Aldrich, USA).

Quantitative assessment of RANK and OPG expression was conducted using light microscopy (Leica ICC50W, Germany) at 400× and 1000× magnifications across three anatomical regions (cervical, middle, and apical thirds) within both tension and pressure areas. Expression levels were analyzed using ImmunoRatio (ImageJ plugin, University of Helsinki), and mean values were calculated from three representative fields per section.

Statistical Analysis

All data were analyzed using SPSS version (25.00). The normality of RANK and OPG expression data was first assessed using the Kolmogorov–Smirnov test. If the data met the normality assumption ($p > 0.05$), intergroup comparisons were conducted using the independent samples T-test. A p-value of less than 0.05 was considered statistically significant.

RESULTS

Expression of RANK and OPG under Orthodontic Force with Caffeine and Ethanol Treatment

IHC analysis quantified the expression of **Receptor Activator of Nuclear Factor Kappa-B (RANK)** and **Osteoprotegerin (OPG)** in periodontal tissues of rats subjected

to orthodontic force alone (K1) or combined with caffeine (K2) or ethanol (K3) administration on Days 7 and 14.

RANK Expression

The expression of RANK (Receptor Activator of Nuclear Factor Kappa-B), a key marker of osteoclastogenesis, showed an increasing trend from the control group to the caffeine and ethanol treatment groups at both time points (Table 1). However, statistical analysis revealed that these increases were not statistically significant at Day 7. On Day 7, the mean RANK expression in the control group (K1) was 9.00 ± 1.58, while the caffeine (K2) and ethanol (K3) groups showed slightly higher values of 10.60 ± 1.14 and 10.80 ± 1.64, respectively. One-Way ANOVA analysis (Table 2) indicated no significant differences between the groups (p = 0.148), suggesting that although the treatment groups exhibited higher RANK expression, the differences were not statistically meaningful at this time point.

At Day 14, RANK expression continued to increase across all groups. The control group (K1) showed a mean expression of 10.00 ± 1.58, while the caffeine and ethanol groups exhibited higher values (11.80 ± 2.59 and 12.00 ± 1.00, respectively). Although One-Way ANOVA again showed no significant differences overall (p = 0.210), pairwise comparison using the Independent Sample T-test (Table 3) revealed a statistically significant increase in RANK expression in the ethanol group (K3) compared to the control group (K1) (p = 0.044). This suggests that ethanol has a more pronounced effect on osteoclast activity, potentially accelerating bone resorption in orthodontic treatment.

OPG Expression

The expression of OPG (Osteoprotegerin), a critical inhibitor of osteoclastogenesis, was significantly elevated in the caffeine and ethanol treatment groups compared to the control group at both Day 7 and Day 14. On Day 7, the mean expression of OPG in the control group (K1) was 7.00 ± 1.58, while the caffeine (K2) and ethanol (K3) groups exhibited higher values of 9.40 ± 1.14 and 9.20 ± 1.30, respectively. One-Way ANOVA revealed a statistically significant difference among the groups (p = 0.029) (Table 4), indicating that both caffeine and ethanol administration led to a notable increase in OPG expression relative to the control group at this early time point.

By Day 14, OPG expression remained significantly elevated in both treatment groups. The control group (K1) showed a mean expression of 7.80 ± 1.30, whereas both the caffeine (K2) and ethanol (K3) groups displayed the highest expression values of 10.80 ± 1.30. One-Way ANOVA again confirmed a significant difference among the groups (p = 0.004) (Table 4), suggesting that the effects of both caffeine and ethanol on OPG expression persisted throughout the study. Independent Sample T-test pairwise comparisons revealed significant differences between the control and both the caffeine and ethanol groups at both time points (Table 5), reinforcing the treatment-dependent increase

Table 1. Expression of RANK and OPG in Treatment Groups on Days 7 and 14

Treatment	Biomarker	Day 7 (mean ± SD)	Day 14 (mean ± SD)
Force (K1)	RANK	9.00 ± 1.581	10.00 ± 1.581
	OPG	7.00 ± 1.581	7.80 ± 1.304
Force + Caffeine (K2)	RANK	10.60 ± 1.140	11.80 ± 2.588
	OPG	9.40 ± 1.140	10.80 ± 1.304
Force + Ethanol (K3)	RANK	10.80 ± 1.643	12.00 ± 1.000
	OPG	9.20 ± 1.304	10.80 ± 1.304

Table 2. One-Way ANOVA Results for RANK Expression

Day	*p*-value (One-Way ANOVA)
7	0.148
14	0.210

Table 3. Pairwise Comparisons of RANK Expression (Independent Sample T-test)

Treatment	Day 7 (*p*-value)	Day 14 (*p*-value)
K1 vs K2	0.148	0.221
K1 vs K3	0.116	**0.044***
K2 vs K3	0.829	0.876

Table 4. One-Way ANOVA Results for OPG Expression

Day	*p*-value (One-Way ANOVA)
7	0.029
14	0.004

Table 5. Pairwise Comparisons of OPG Expression (Independent Sample T-test)

Day	Day 7 (*p*-value)	Day 14 (*p*-value)
K1 vs K2	**0.025***	**0.007***
K1 vs K3	**0.043***	**0.007***
K2 vs K3	0.803	1.000

in OPG expression. However, no significant difference was observed between the caffeine and ethanol groups, indicating that both treatments may similarly influence OPG production.

These findings suggest that caffeine and ethanol stimulate the production of OPG, potentially as a compensatory mechanism in response to elevated osteoclast activity during orthodontic tooth movement. The increase in OPG expression may serve to inhibit excessive bone resorption, contributing to bone remodeling during the orthodontic process.

Immunohistochemical Visualization

IHC analysis of RANK and OPG expression further corroborated the quantitative findings. Representative images from Figures 2 to 5 showed stronger staining intensity for both RANK and OPG in the caffeine and ethanol groups compared to the control group. Staining was predominantly localized in the periodontal ligament and alveolar bone adjacent to the orthodontically moved teeth, indicating an active bone remodeling process influenced by caffeine and ethanol administration.

DISCUSSION

This study evaluated the modulatory effects of caffeine and ethanol on the expression of Receptor Activator of Nuclear Factor κβ (RANK) and Osteoprotegerin (OPG), which are critical regulators of bone remodeling during orthodontic tooth movement (OTM) in a rat model. Both substances significantly influenced these biomarkers, with ethanol showing a more prominent effect, particularly with the increased expression of RANK observed on Day 14.

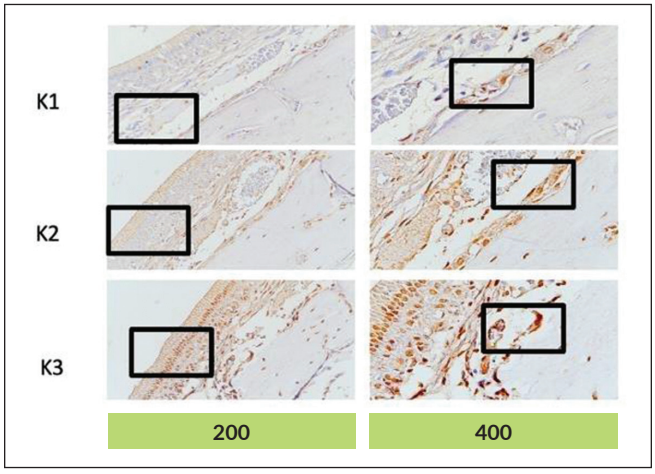

Figure 2. RANK expression on day 7 using immunohistochemistry (IHC) techniques.
K1 = Force, K2 = Force + Caffeine, K3 = Force + Ethanol

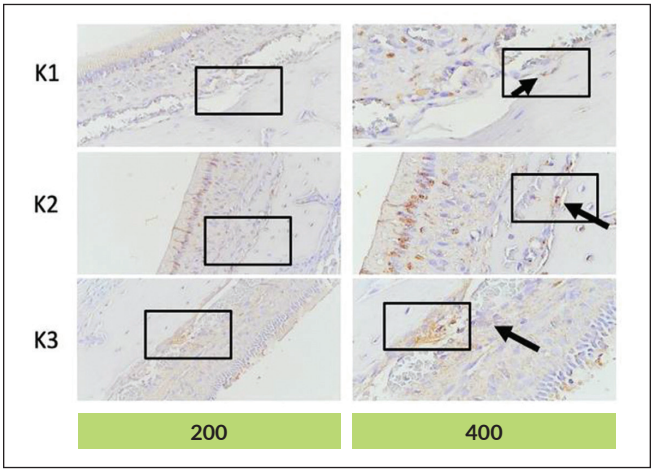

Figure 4. OPG expression on day 7 using IHC techniques.
K1 = Force, K2 = Force + Caffeine, K3 = Force + Ethanol

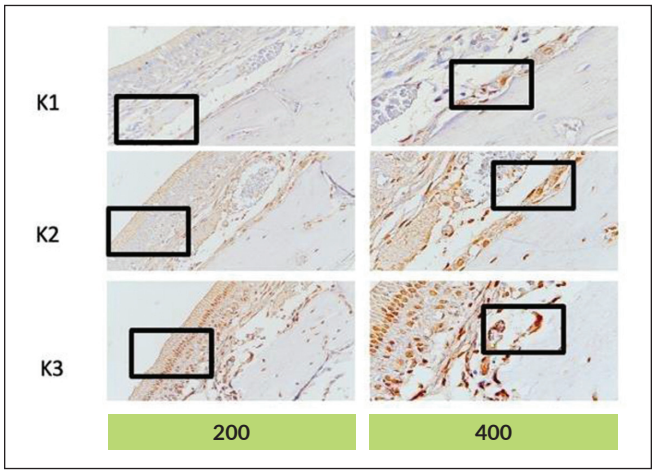

Figure 3. RANK expression on day 14 using IHC techniques.
K1 = Force, K2 = Force + Caffeine, K3 = Force + Ethanol

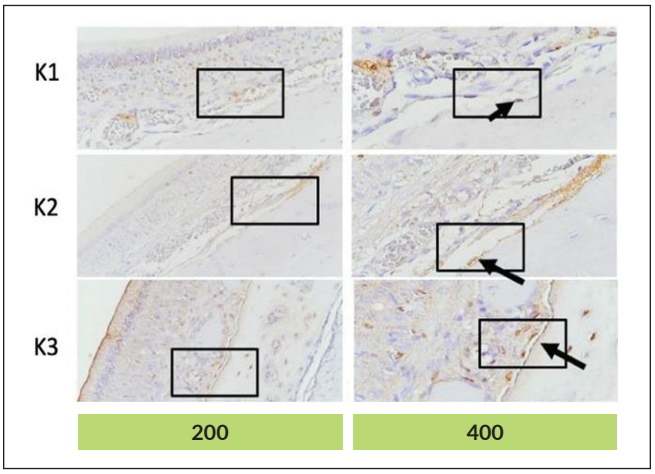

Figure 5. OPG expression on day 14 using IHC techniques.
K1 = Force, K2 = Force + Caffeine, K3 = Force + Ethanol

RANKL is a cytokine known to affect osteoblast formation. When RANKL binds to RANK, it can inhibit osteoblast differentiation.[14] The RANK/RANKL/OPG signaling axis plays a pivotal role in orchestrating osteoclastogenesis and maintaining bone homeostasis. OPG acts as a decoy receptor by binding to RANKL, thereby preventing its interaction with RANK and inhibiting osteoclast differentiation and activity.[15] This balance is essential for coupling bone resorption and formation during physiological bone remodeling, including that induced by orthodontic forces.

On Day 7, RANK expression was elevated in both the caffeine (K2) and ethanol (K3) groups compared to the control group (K1), although the differences were not statistically significant ($p > 0.05$). This trend persisted in the caffeine group on Day 14, with a modest, non-significant increase in RANK expression ($p = 0.221$).

In contrast, the ethanol group demonstrated a statistically significant increase in RANK expression on Day 14 ($p = 0.044$) compared to the control. This suggests that ethanol exerts a stimulatory effect on osteoclast differentiation over a prolonged period, especially during the later stages of bone remodeling. These results are consistent with previous studies reporting that ethanol enhances osteoclastogenesis by upregulating RANK expression, ultimately accelerating bone resorption.[16] Meanwhile, caffeine produced a non-significant increase, possibly due to its context-dependent effects on osteoclastogenesis under specific conditions.

This response may be attributed to caffeine's ability to promote osteoclastogenesis, as shown by a study conducted by Yi et al., which demonstrated that caffeine enhances osteoclastogenesis triggered by periodontal ligament (PDL) cells.[17] Osteoclasts are multinucleated cells formed through the fusion of mononuclear precursors derived from the hematopoietic lineage.[17] Osteoclast differentiation is primarily mediated through c-fms, the receptor for macrophage colony-stimulating factor (M-CSF), in precursor cells, which modulates RANK expression. RANKL, regulated by various hormones and cytokines, binds to RANK on osteoclast precursors, facilitating their rapid development into mature, bone-resorbing osteoclasts. The transcription factor, nuclear factor of activated T-cells, cytoplasmic 1 (NFATc1), serves as a key regulator of RANKL-induced osteoclastogenesis, promoting osteoclast fusion and activation by upregulating genes involved in osteoclast attachment, migration, acidification, and degradation of the bone matrix.[18]

Upon RANKL–RANK binding, multiple intracellular signaling cascades are activated, including c-Jun N-terminal kinase (JNK), p38, and extracellular signal-regulated kinase (ERK) MAPK pathways in preosteoclasts. Activation of the p38 MAPK pathway is essential for osteoclast development, as it controls the expression of osteoclast-specific genes. This includes stimulation of the microphthalmia-associated transcription factor (MITF), which regulates the expression of genes such as tartrate-resistant acid phosphatase (TRAP; encoded by Acp5) and cathepsin K (CtsK).[19] Both TRAP and CtsK contribute to osteoclast functionality by regulating bone matrix degradation and collagen turnover.

Although ethanol increased RANK expression, its impact on OTM may not differ significantly from the orthodontic force alone. This aligns with the findings of Iitsuka et al., who reported that ethanol enhances both osteoclast number and activity, contributing to elevated RANK expression.[16] Ethanol's enhancement of osteoclastogenesis appears closely tied to its regulatory effects on bone metabolism.[16,18]

OPG functions as a critical regulator against osteoclastogenesis by acting as a decoy receptor for RANKL.[20,21] In this study, OPG expression significantly increased in both the caffeine and ethanol groups at Days 7 and 14. As shown in Table 4, caffeine-treated rats (K2) exhibited a significant increase in OPG expression compared to the control (K1) on both days ($p = 0.030$ on Day 7), suggesting an osteoblastic feedback mechanism designed to counterbalance elevated osteoclast activity and preserve bone integrity.

Similarly, the ethanol group exhibited a marked increase in OPG expression, consistent with a compensatory response to elevated osteoclast activation. Increased OPG levels are part of the physiological regulation of bone remodeling, wherein osteoblasts upregulate OPG secretion in response to enhanced RANKL–RANK activity.

These results support the hypothesis that both caffeine and ethanol influence OPG expression as part of an adaptive regulatory mechanism to control osteoclastogenesis and bone resorption during OTM. The rise in OPG expression may help protect bone tissue from excessive resorption in response to increased RANK expression.[22]

While both caffeine and ethanol modulated RANK and OPG expression, their effects differed in magnitude and direction. Ethanol significantly elevated RANK expression on Day 14, indicating a more potent stimulatory effect on osteoclastogenesis and bone resorption. In contrast, caffeine had a more modest effect on RANK and a stronger stimulatory effect on OPG expression.[18]

Caffeine concentration has been reported to increase OPG levels in osteoblasts, thereby potentially promoting bone apposition during orthodontic treatment. Holland et al. found that orthodontic force in rats induced preosteoblast proliferation as early as Day 2.[23] However, this contrasts with findings by Liu et al., who emphasized that bone remodeling involves a synergistic balance between bone resorption and formation, regulated by multiple factors including prostaglandins (PGs), 1,25-dihydroxyvitamin D3, parathyroid hormone (PTH), nitric oxide, sex hormones, calcitonin, growth factors, and cytokines.[24]

Bone resorption, primarily regulated by the RANK/RANKL/OPG axis, involves the binding of RANKL (secreted by osteoblasts) to RANK on osteoclast progenitors in the presence of M-CSF, promoting differentiation into mature osteoclasts. OPG, also secreted by osteoblasts, acts as a decoy receptor that binds to RANKL and blocks its interaction

with RANK, thereby preventing osteoclastogenesis. Furthermore, COX-2 expression and arachidonic acid availability are required for osteoblast-mediated PG synthesis. PGs increase RANKL expression in osteoblasts, thus facilitating osteoclastogenesis.[24]

Okada et al. demonstrated that COX-2 protein expression and associated PG synthesis are essential for bone resorption responses to $1,25(OH)_2D_3$ and PTH. Additionally, PGE_2 production mediated by COX-2 plays a key role in LPS-induced bone resorption. Bradykinin enhances cytokine-stimulated prostaglandin production by increasing COX-2 expression, which in turn raises RANKL expression. Compressive force promotes osteoclastogenesis by upregulating RANKL and M-CSF synthesis, while simultaneously downregulating OPG via the COX-2/PGE_2 pathway.[25] Liu et al. also found that low concentrations of caffeine increased RANKL expression and decreased OPG levels in osteoblast cultures.[24]

A study by Naghsh et al. further emphasized the importance of the OPG–RANK–RANKL system in bone remodeling, particularly the role of OPG as a soluble TNF receptor that prevents osteoclastogenesis by sequestering RANKL.[26] This finding is supported by Capati et al., who reported an inverse relationship between OPG and RANKL levels: decreased OPG results in increased RANKL expression, thereby promoting osteoclast activity and bone resorption.[27] OPG binds to RANKL and inhibits its binding to RANK, reducing osteoclast activity. Osteoblasts thus regulate osteoclast development by controlling the RANKL/OPG interaction.[27]

Coffee-derived caffeine also contains caffeic acid, a flavonoid with antioxidant properties that may reduce oxidative stress in osteoblasts. Oxidative stress has been shown to impair osteoblast differentiation and function, thereby slowing bone formation.[28,29] In this study, the application of orthodontic force combined with either caffeine or ethanol similarly affected RANK expression and increased osteoclast numbers. RANKL, a regulator of bone remodeling during tooth movement, binds to RANK on osteoclast precursors, initiating their differentiation, activation, and ultimately bone resorption.[30] Roodman's research highlights the critical role of osteoclastogenesis in facilitating orthodontic tooth movement.[31]

Although this study provides novel insights into the molecular responses to caffeine and ethanol during orthodontic force application, several limitations should be acknowledged. First, only two time points (Days 7 and 14) were evaluated for RANK and OPG expression, which may not fully capture the dynamic process of bone remodeling during orthodontic therapy. Second, the study did not directly measure orthodontic tooth displacement, limiting the ability to draw functional correlations between molecular findings and clinical outcomes.

Moreover, the use of a rat model presents limitations in extrapolating the results to human physiology. Future studies should assess these effects in clinical settings. Longitudinal studies incorporating both orthodontic tooth movement measurements and biomarker profiling, including oxidative stress and hormone levels, are needed to validate these findings and translate them into evidence-based clinical practice.

CONCLUSION

This study highlights the modulatory effects of caffeine and ethanol on the expression of RANK and OPG, crucial regulators of osteoclastogenesis and bone remodeling during orthodontic tooth movement (OTM). The findings indicate that ethanol significantly increases RANK expression at Day 14, suggesting its potential to stimulate osteoclast differentiation and enhance bone resorption over time. Conversely, caffeine exhibited a more modest effect on RANK expression but significantly elevated OPG expression in both treatment groups, suggesting an adaptive response by osteoblasts to counterbalance heightened osteoclast activity.

While ethanol and caffeine both influence bone remodeling biomarkers, ethanol appears to have a more pronounced impact on osteoclast differentiation, whereas caffeine seems to contribute to maintaining bone integrity by increasing OPG levels. These findings underscore the importance of considering lifestyle factors such as caffeine and ethanol consumption in orthodontic treatment planning, as they may modulate the efficiency and outcome of tooth movement.

However, further studies are necessary to directly measure the impact of these substances on actual tooth displacement and to explore their long-term effects in human clinical settings. Additionally, future research should investigate other biomarkers and systemic factors such as oxidative stress, hormonal regulation, and cytokines, which could provide a more comprehensive understanding of the molecular mechanisms involved in orthodontic tooth movement.

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Statement of Authorship

All authors certified fulfillment of ICMJE authorship criteria.

Author Disclosure

All authors declared no conflicts of interest.

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