

## The Effect of Red Betel Leaf Extract (*Piper crocatum*) on Changes in the Number of Osteoclasts in the Pressure Area of Orthodontic Movement in Rat Teeth

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### Abstract

Orthodontic mechanical forces produce pressure and tension areas as a process of alveolar bone remodeling for successful orthodontic treatment. These pressure areas prepare space for tooth movement, which is formed as a result of osteoclast activity. This study aims to determine the effect of red betel leaf extract (*Piper crocatum*) on changes in osteoclast number in the pressure area of orthodontic tooth movement in rats.

This study is a laboratory experimental study with a post-test only control group design. The sample consisted of 36 male Wistar rats divided into 9 groups: negative control (no force, no extract), positive control (given mechanical force), and treatment (given mechanical force + red betel extract 20 mg/kgBW) with observation times of 1, 3, and 7 days. Mechanical force was applied to the maxillary first molar using a Nickel-Titanium Closed Coil Spring with a force of 2.116 oz. The results showed the highest average number of osteoclasts was in the treatment group for 1 day (36.25), 3 days (33.5), and 7 days (34.75), which was significantly increased compared to the control group. The antioxidant activity of the extract was very strong with an IC50 value of 40.2977 µg/ml. Based on the observations, there was an increase in the number of osteoclasts in the pressure area compared to the control group, particularly on day 7, which may potentially accelerate orthodontic tooth movement.

**Keywords:** red betel leaf, orthodontic mechanical force, osteoclasts, pressure area, bone remodeling

### Abstrak

Gaya mekanis ortodonti menghasilkan area tekanan dan tarikan sebagai proses remodeling tulang alveolar untuk keberhasilan dalam perawatan ortodonti. Area tekanan tersebut untuk menyiapkan ruang sebagai arah pergerakan gigi dan ruang tersebut akan terbentuk sebagai akibat aktifitas osteoklas. Penelitian ini bertujuan untuk mengetahui efek ekstrak daun sirih merah (*Piper crocatum*) terhadap perubahan jumlah osteoklas pada area tekanan pergerakan gigi ortodonti tikus. Desain penelitian adalah eksperimental laboratoris dengan rancangan *post-test only control group design*. Sampel berupa 36 ekor tikus Wistar jantan yang dibagi menjadi 9 kelompok: kontrol negatif (tanpa gaya, tanpa ekstrak), kontrol positif (diberi gaya mekanis), dan perlakuan (diberi gaya mekanis + ekstrak sirih merah 20 mg/kgBB) dengan waktu pengamatan 1, 3, dan 7 hari. Gaya mekanis diberikan pada gigi molar 1 rahang atas menggunakan *Nickel-Titanium Closed Coil Spring* dengan gaya sebesar 2,116 oz. Hasil penelitian menunjukkan jumlah osteoklas rata-rata tertinggi terdapat pada kelompok perlakuan 1 hari (36,25), 3 hari (33,5), dan 7 hari (34,75) yang meningkat secara signifikan dibandingkan kelompok kontrol. Aktivitas antioksidan ekstrak sangat kuat dengan nilai IC50 sebesar 40,2977 µg/ml. Berdasarkan pengamatan terjadi perubahan peningkatan jumlah osteoklas pada area tekanan dibandingkan kelompok kontrol, terutama pada pengamatan hari ke-7, yang berpotensi mempercepat pergerakan gigi ortodonti.

**Kata kunci:** daun sirih merah, gaya mekanis ortodonti, osteoklas, area tekanan, remodeling tulang.

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## INTRODUCTION

Malocclusion is a highly prevalent oral health problem in Indonesia, affecting approximately 80% of the population. This condition can interfere with chewing and speech function, and can even cause temporomandibular joint disorders. Therefore, it requires appropriate treatment through orthodontic treatment<sup>1,2</sup>. Orthodontic treatment uses appliances such as brackets or coil springs to apply continuous force to move teeth into their ideal position.

Tooth movement occurs through bone remodeling, specifically alveolar bone resorption by osteoclasts in areas of pressure that serve as spaces for tooth movement. Orthodontic forces trigger a mechanosensitive response in the periodontal ligament, which increases the production of inflammatory mediators such as RANKL, thereby stimulating osteoclast differentiation and activity<sup>3,4</sup>. However, excessive osteoclast activity can lead to undesirable bone resorption, necessitating control of osteoclastogenesis<sup>5</sup>.

One approach is to use natural ingredients such as red betel leaves (*Piper crocatum*), which contain flavonoids, alkaloids, saponins, and tannins<sup>6</sup>. Flavonoids have antioxidant and anti-inflammatory activities that can reduce oxidative stress and modulate the RANKL/OPG ratio, thus potentially controlling osteoclast activity. Red betel leaf extract is known to have strong antioxidant activity with an IC50 value of 16.15 mg/L<sup>7</sup>.

Based on this, this study aims to evaluate the effect of red betel leaf extract (*Piper crocatum*) on changes in the number of osteoclasts in the pressure area during orthodontic tooth movement and to determine its antioxidant content and activity.

## RESEARCH METHODS

This study obtained Ethical Clearance from the Faculty of Dentistry, University of Jember with number

3262/UN25.8/KEPK/DL/2025. The research design used a post-test only control group design. The study population used 36 male *Rattus norvegicus* Wistar strain rats. Sample selection used purposive sampling with the criteria of healthy rats, body weight 250-300 grams, and age 3-4 months. Rats were randomly divided into 9 groups, consisting of 3 negative control groups (without mechanical force, without extract), 3 positive control groups (induced by mechanical force, without extract), and 3 treatment groups (induced by mechanical force and given red betel leaf extract) with each observed for 1, 3, and 7 days.



**Figure 1.** Installation of Ni-Ti orthodontic closed coil spring

Orthodontic mechanical force was applied by attaching a 6 mm Ni-Ti closed coil spring to the left maxillary first molar and attaching it to both maxillary incisors. The applied force was a mesial tipping movement of 2.116 oz (60.01 grams force). Red betel leaf extract was administered orally using a gastric tube at a dose of 20 mg/kgBW dissolved in 2 ml of distilled water.

After the treatment period, the mice were euthanized using lethal doses of ketamine and decapitated to harvest maxillary alveolar bone tissue. Histological preparations were prepared through decalcification, dehydration, clearing, impregnation, embedding,

sectioning, and hematoxylin and eosin (HE) staining. Osteoclast cell counts were performed using a binocular light microscope at 400x magnification in the pressure area (mesial root of M1 tooth), counted by three different observers. Data were analyzed using the Shapiro-Wilk test, Levene test, One-Way ANOVA test, and followed by a Post-Hoc LSD test ( $\alpha = 0.05$ ).

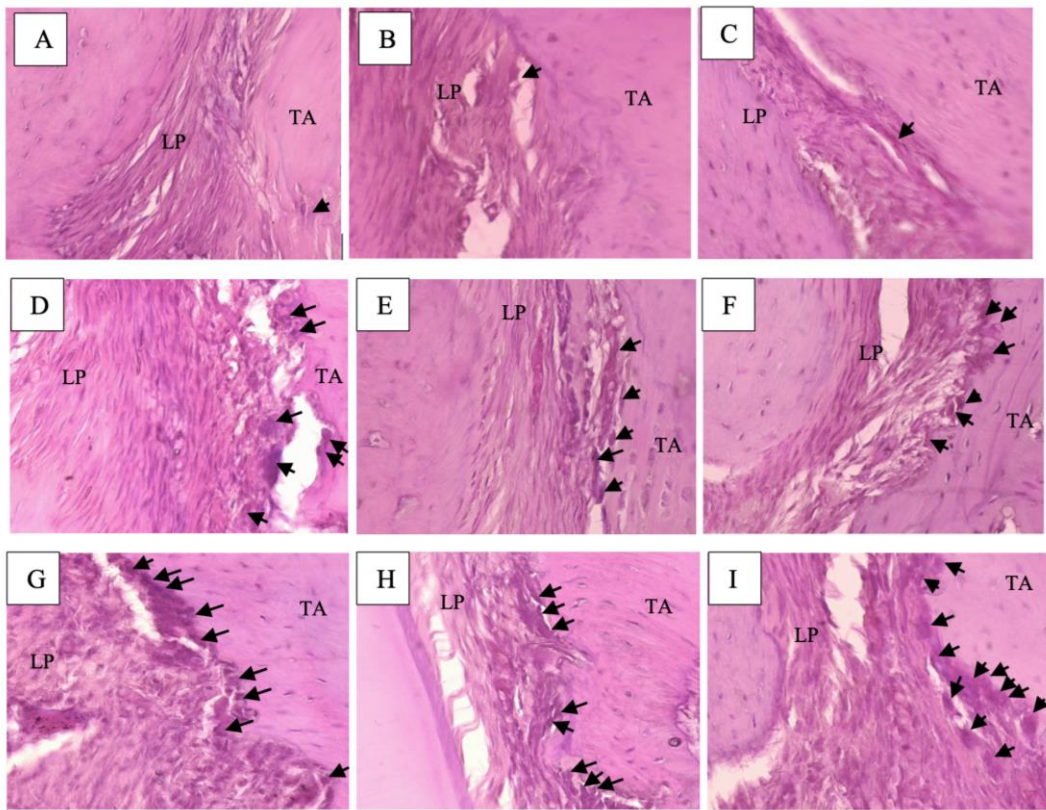
## RESULT

Based on phytochemical screening tests and antioxidant activity using the DPPH method and UV-Vis spectrophotometry, the results showed that red betel leaf extract has very strong antioxidant activity.

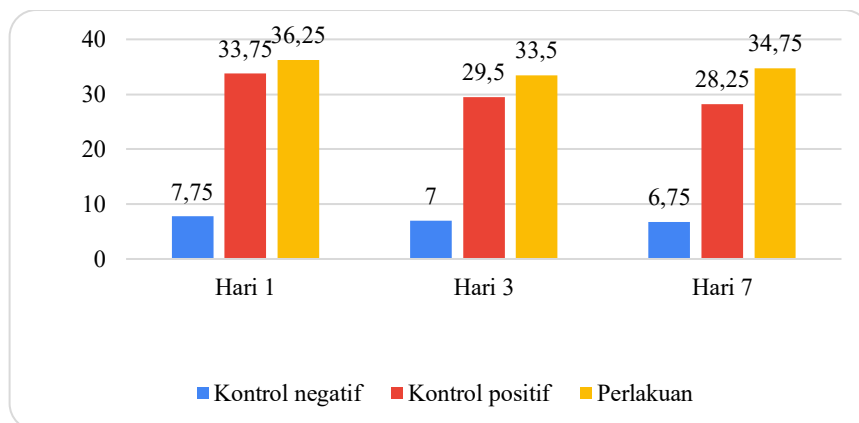
**Table 1.** Phytochemical and Antioxidant Test Results of Red Betel Leaf Extract

Parameter	Total Content (g/100g Extract)	IC50
Total Phenol	0.176g GAE/100g	-
Flavonoid	0.043g QE/100g	-
Antioxidants	-	40.2977 $\mu\text{g/ml}$

QE: Quercetin Equivalent, GAE: Gallic Acid Equivalent, IC50: Inhibition Concentration 50, g/100g: grams of extract concentration per 100 grams of sample (in every 100 grams of extract there are compounds equivalent to grams of standard solution),  $\mu\text{g/ml}$ : micrograms of extract concentration per milliliter of sample.



**Figure 2.** Histological image of osteoclasts with HE staining at 400x magnification of the pressure area in one of the fields of view. (A) Negative Control 1 Day; (B) Negative Control 3 Days; (C) Negative Control 7 Days; (D) Positive Control 1 Day; (E) Positive Control 3 Days; (F) Positive Control 7 Days; (G) Treatment 1 Day; (H) Treatment 3 Days; (I) Treatment 7 Days; TA: Alveolar Bone; LP: Periodontal Ligament; Black Arrow: Osteoclasts



**Figure 3.** Histogram of the average number of osteoclasts in the alveolar bone pressure area of the negative control group, positive control group, and treatment group.

Counting the number of osteoclast cells in the alveolar bone of the pressure area using a binocular light microscope with a 400x magnification objective lens by looking at 3 fields of view, namely in the alveolar bone of the pressure area starting from the peak of the alveolar bone towards the apical then observed by 3 different observers and counting the number of osteoclast cells. The average number of osteoclasts in the alveolar bone of the pressure zone is shown in Figure 3.

## DISCUSSION

Orthodontic forces induce tooth movement through a process of alveolar bone remodeling involving resorption in areas of compression by osteoclasts and apposition in areas of tension by osteoblasts. This process is also accompanied by an inflammatory response that increases the production of reactive oxygen species (ROS). ROS play a role in stimulating osteoclastogenesis, thereby influencing bone resorption activity<sup>8,9</sup>.

The results showed that the number of osteoclasts increased on day 1, then decreased on day 3 and tended to stabilize on day 7. However, the treatment group with red betel leaf extract (*Piper crocatum*) showed a higher number of osteoclasts than the positive control group. This is thought to be related to the content of bioactive compounds such as flavonoids, phenolics, tannins, and saponins, which have antioxidant activity.

Flavonoids in red betel leaf extract act as antioxidants through the hydrogen atom transfer (HAT) mechanism, which can neutralize free radicals and reduce oxidative stress<sup>10,11</sup>. Flavonoids also inhibit ROS-forming enzymes and modulate

The results of the One-Way ANOVA statistical analysis showed  $p < 0.001$  ( $p < 0.05$ ), indicating a significant difference between groups. Further LSD tests showed that all treatment groups had a significant increase in the number of osteoclasts compared to the negative control. Compared to the positive control, the largest and statistically significant difference in the number of osteoclasts was observed on day 7 ( $p < 0.05$ ).

osteoclast signaling pathways, thereby helping control bone resorption activity<sup>12,13</sup>. Phenolic compounds also contribute to antioxidant activity through their hydroxyl groups, which can stabilize free radicals<sup>14</sup>.

Tannins and saponins in red betel leaf extract also play a role in reducing ROS levels through free radical scavenging mechanisms, inhibiting pro-oxidative enzymes, and modulating pro-inflammatory cytokines. These ROS can control osteoclast differentiation and activity and prevent excessive bone resorption<sup>15-17</sup>.

The controlled increase in osteoclast numbers at the beginning of the treatment period is in line with the initial phase of bone remodeling that occurs 24–48 hours after the application of force from the orthodontic appliance, this is in line with the need for space that is expected to be immediately available to provide a place for the direction of tooth root movement when applying compressive force from the installed orthodontic appliance. Over time, the number of osteoclasts decreases slightly due to the tissue adaptation process, this occurred because the

active compressive force exerted by the orthodontic appliance diminished as the tooth root moved. The significant difference observed between the control and treatment groups—particularly on day 7—indicates that red betel leaf extract has the potential to modulate the osteoclast activity required for the controlled resorption of alveolar bone.

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## CONCLUSION

Red betel leaf extract increases the number of osteoclasts in the pressure area of the alveolar bone of rat teeth induced by orthodontic mechanical force and the antioxidant activity in red betel leaf extract measured using the IC50 parameter is included in the very strong category.

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