# EFFECTIVENESS OF KATUK LEAVES ETHANOL EXTRACT TO AMOUNT OF OSTEOBLAST AND OSTEOCLAST IN ORTHODONTIC TREATMENT (EFEKTIVITAS EKSTRAK ETANOL DAUN KATUK TERHADAP JUMLAH OSTEOBLAS DAN OSTEOKLAS PADA PERAWATAN ORTODONTI)

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## ABSTRACT

The optimal achievement of orthodontic treatment is determined by a bone remodelling process involving osteoblast, osteoclasts, and the estrogen hormone. Estrogen deficiency can increase osteoclast age and decrease osteoblast, resulting in an imbalance between osteoclasts and osteoblasts. One natural alternative that can replace the role of the hormone estrogen is phytoestrogens. *Sauropus androgynus* (L.) Merr (katuk) is a phytoestrogen that contains isoflavones with many similarities with estrogens. This research aims to determine the effectiveness of the various doses of ethanol extract of katuk leaves orally on the number of osteoblasts and osteoclasts. This research was conducted using experimental laboratory methods using 24 female Guinea pigs divided into a control group and three groups with various doses of 39.15 mg/BW, 78.3 mg/BW, and 156.5 mg/BW. The observations made in this test were the number of osteoblasts and osteoclasts on the alveolar guinea pig on day 14 and analysed using the one way ANOVA test ($p < 0.05$). All guinea pigs have applied a rubber separator to the left incisor and given a dose according to the group, and after 14$^{th}$ days, histological preparations were made. The results showed that the highest number of osteoblasts was at a dose of 78.3 mg/BW, and the lowest number of osteoclasts was at a dose of 39.15 mg/BW with values of 15.03 ± 2.27 and 1.73

± 0.56, respectively. Statistically, the number of osteoblasts between the treatment and control groups significantly differed (p = 0.04), while the number of osteoclasts between the treatment and control groups had no significant difference (p = 0.228). This study concludes that katuk leaves extract has effectiveness in increasing the number of osteoblasts in orthodontic treatment, while the decrease in osteoclasts is not statistically proven.
***Keywords**: : Isoflavones, katuk leaves, orthodontic treatment*

**ABSTRAK**

Keberhasilan perawatan ortodonti ditentukan dengan proses remodeling tulang yang melibatkan osteoblas dan osteoklas serta dikendalikan oleh faktor hormon. Defisiensi hormon estrogen dapat meningkatkan umur osteoklas dan menurunkan umur osteoblas sehingga terjadinya ketidakseimbangan antara osteoklas dan osteoblas. Salah satu alternatif alami yang dapat menggantikan peran hormon estrogen adalah fitoestrogen. Daun katuk (*Sauropus androgynus* (L.) Merr.) mempunyai kandungan isoflavon yang merupakan kelompok utama dari fitoestrogen yang memiliki banyak kesamaan dengan estrogen. Penelitian ini bertujuan untuk mengetahui efektivitas ekstrak etanol daun katuk per oral dengan berbagai dosis terhadap jumlah osteoblas dan osteoklas. Penelitian ini dilakukan dengan metode laboratorium eksperimental menggunakan 24 ekor marmut betina yang dibagi menjadi kelompok kontrol dan 3 kelompok variasi dosis 39,15 mg/kgBB, 78,3 mg/kgBB, dan 156,5 mg/kgBB. Pengamatan yang dilakukan pada pengujian ini yaitu jumlah osteoblas dan osteoklas pada tulang alveolar marmut pada hari ke-14 dan dianalisis uji *one way ANOVA* (p<0,05). Semua marmut diaplikasikan karet separator pada gigi insisif kiri dan diberikan dosis sesuai kelompok lalu setelah hari ke-14 dilakukan pembuatan preparat histologi. Hasil penelitian diperoleh bahwa jumlah osteoblas paling tinggi terdapat pada dosis 78,3 mg/kgBB dan jumlah osteoklas paling rendah terdapat pada dosis 39,15 mg/kg BB dengan nilai masing-masing 15,03 ± 2,27 dan 1,73± 0,56. Secara statistik jumlah osteoblas antara kelompok perlakuan dan kontrol memiliki perbedaan yang signifikan (p=0,04) sedangkan jumlah osteoklas antara kelompok perlakuan dan kontrol tidak memiliki perbedaan yang signifikan (p=0,228). Kesimpulan pada penelitian ini adalah ekstrak daun katuk memiliki efektivitas

terhadap peningkatan jumlah osteoblas pada perawatan ortodonti sedangkan penurunan osteoklas tidak terbukti secara statistik.
***Kata kunci:*** *Daun katuk, isoflavon, perawatan ortodonti*

## INTRODUCTION

Orthodontic treatment is a treatment carried out in dentistry to correct unsuitable tooth alignment and position to obtain a stable occlusion relationship to restore masticatory function, muscle balance, and facial aesthetic harmony. Based on the Riset Kesehatan Dasar (RISKESDAS) in 2018, the prevalence of dental andoral health problems in Indonesia is 57.6%. The majority of malocclusion in Indonesia is still very high, namely 80% of the population. Malocclusion ranks third as a dental and oral health problem after caries and periodontal disease. Dayataka et al. (2019) reported malocclusion of junior high school(adolescents aged 12-15 years) in Cimahi was 96.7%. Treatment of malocclusion with an orthodontic approach takes a long time to obtain efficient results.[1-3]

Tooth movement in orthodontic treatment occurs due to biological processes in the tooth-supporting tissue or periodontium caused by mechanical stress so that bone changes. When the teeth are moved orthodontically, there will be stress and strain activity. The pressure side of the periodontal ligament space will experience vascular constriction and constriction and cell replication, and decreased collagen fibre production resulting in bone resorption due to osteoclast activation. Osteoclasts absorb bone during the remodelling process. The stretch side periodontal ligament space has widened. This result caused vascularity, cell replication and increasing collagen fibres production. The work is resulting in bone apposition by osteoblasts. Osteoblasts function to secrete the organic matrix of bone to help mineralisation of the non-calcified matrix. The stress and strain activity causes the tooth's socket to move according to the movement of the tooth through the alveolar bone. The process of bone remodelling is controlled by several hormones, one of which is the hormone estrogen.[2,3]

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Estrogen affects bone density by modulating TGF-β1, which is the most significant factor in bone formation and maintaining the balance of dynamic processes of bone formation and bone resorption.

Estrogen deficiency increases bone loss with each age leading to increased intensity of bone remodelling. Still, estrogen deficiency also increases osteoclasts' age and decreases the period of osteoblasts so that there is no balance between osteoclasts and osteoblasts, which causes less bone to be formed. Hormone replacement therapy used when estrogen decreases phytoestrogens. Phytoestrogens are substrates from plants that have estrogen-like activity. Phytoestrogens are divided into three classes isoflavones, coumestans, and lignans. Isoflavones are frequently studied because phytoestrogen levels in isoflavones are higher than coumestans and lignans.[4-14]

The community easily obtains Katuk leaves because they have many benefits, such as facilitating breastfeeding and maintaining bone health due to containing flavonoids. Isoflavones are included in flavonoid phytochemical compounds and have the highest levels of phytoestrogens. The enzymatically complex metabolic conversion in the digestive tract is composed of isoflavones. It caused heterocyclic phenols with the same structure as estrogen.[11] Indriasari et al. in 2019 reported that giving soy genistein isoflavones increase TGF-β1 levels in old rabbits during orthodontic tooth movement. The same results were also obtained from research by Sholillah (2015) using water extract of katuk leaves (*Sauropus androgynus* (L.) Merr.) At a dose of 45 mg/BW, which significantly reduced the number of osteoclasts of the femur in menopausal white rats.[15-18]

## METHOD

This research is an experimental laboratory study Post Test. Control Group Design with guinea pigs subjects was separated into four groups to investigate the effectiveness of katuk leaf ethanol extract on the number of osteoblasts and osteoclasts in orthodontic treatment of research on guinea pig alveolar bone. This research was conducted at the Laboratory of Pharmacology and Therapy, Faculty of Medicine, Padjadjaran University and the Cytohistotechnology Laboratory of STIKES Jenderal Achmad Yani from October to December 2020. This study received ethical approval from the Medical Research Ethics Committee of the Faculty of Medicine, Padjadjaran University, with letter number 1022 / UN6.KEP / EC / 2020.

**Samples and object of research**

Guinea pigs (*Cavia cobaya*) obtained from PT Biofarma Bandung. The guinea pigs have met the inclusion criteria, namely female guinea pigs, aged 2-4 months, body weight between 250-400 grams, guinea pigs with teeth without diastema, healthy, and originating from the breeding site and fed the same. Before the research, guinea pigs were acclimatized for seven days. The exclusion criteria were guinea pigs that were sick during transformation, rubber separator released before the observation period, and a weight loss of> 10% during the adaptation period. The number of experimental animals used was 24 female guinea pigs that counted using the Federer formula, with six guinea pigs in each treatment group. The experimental animals were divided into 4 groups: control group, the ethanol extract of katuk leaves or

EEDK group 39.15 mg/BW, the EEDK group78.3 mg/BW, and the EEDK group 156.5 mg/BW, all groups were given rubber separator to the left incisor. Katuk leaves (*Sauropus androgynus* (L.) Merr.) Obtained from the Medicinal Garden, Faculty of Pharmacy, Jenderal Achmad Yani University.

**Research tools and materials**

The tools used in this study were divided into extract-making tools, maintenance tools, tissue preparation tools, and treatment tools. The extract making tool consists of a rotary evaporator, Erlenmeyer tube, oven, analytical balance, waterbath, and pH meter. The maintenance equipment consists of guinea pigs cage covered by a tub and made of braided wire, a digital scale for weighing the guinea pigs, and a feeding and drinking area for the guinea pigs. The tools for making tissue preparations consist of minor surgical instruments, microtomes, slide glasses, and deck glass. The treatment tools consist of separator pliers and scalpels. The materials used in this study were divided into ingredients for making katuk leaves extract, colouring preparations, and materials for treatment. The components for the extract consisted of wet katuk leaves, filter paper, and ethanol solvent. The staining material for the preparation consisted of a solution of hematoxylin and eosin. The treatment material consisted of orthodontic separator rubber under the brand *Forestadent*.

**Procedures**

Preparation of katuk leaves ethanol extract: As much as 1 kg of cotton pad were applied to wet katuk leaves and left for 24 hours to make a dilute extract. The powder was carried away when it is removed from the outlet under the macerator filter using filter paper. The ethanol solvent was added to the drugs in the macerator until the solvent was colourless, approximately by soaking it for 5-6 times. Furthermore, the dilute extract was concentrated using a rotary evaporator until it was focused or no more solvent is dripping in the rotary evaporator condenser.[19,20] Treatment of experimental animals: After an adaptation period of seven days following the applicable rules and procedures at the Laboratory of Pharmacology and Therapy, Faculty of Medicine, Padjadjaran University. All groups of guinea pigs were given general anaesthesia with 20 mg/BW of ketamine injection and applied to the separator rubber on the left incisor.

Furthermore, the guinea pigs were given ethanol extract of katuk leaves previously diluted with CMC liquid according to the dose group orally. All guinea pigs were given ethanol extract of katuk leaves every day and observed until the 14$^{th}$ day. On the8$^{th}$ day, the rubber separator was replaced. The elastomeric rubber was easy to lose its force, especially during the first 24-48 hours and slowly decreased during the tooth movement process. On the 14$^{th}$ day, all guinea pig groups were terminated using ketamine 75 mg/BW. After sacrificing the subject, the maxillary bone and both incisors were removed for histological preparations. The histological preparations were made by using the Hematoxylin-Eosin staining method. The maxilla and incisors were fixed for 24 hours and then decalcified with 8% formic acid for ten days and continued with

dehydration, clearing, impregnation, embedding, and cutting processes. After the preparation was completed, the number of osteoblasts and osteoclasts was observed in five visual fields in the stress and strain area. These were then added and divided by five to obtain the average value. Observations were made at 400x magnification using a light microscope which at least two people observed.

**Data analysis**

The histological observations of osteoclasts and osteoblasts in the alveolar marmot were statistically analysed using the ANOVA test because the data were normally distributed and homogeneous. Statistical analysis using SPSS version 26.0 software with a significant level of 0.05 ($p = 0.05$) and a confidence level of 95% ($p \leq 0.05$) was considered significant.

## RESULT

**Microscopic Overview of the Number of Osteoblasts and Osteoclasts in the Research Group**

The results of the research group were made into microscopic preparations. The results of the microscopic preparations were then observed at 400x magnification using an Olympus binocular microscope. The data of this study were obtained by calculating the number of osteoblasts and osteoclasts in five viewing areas.

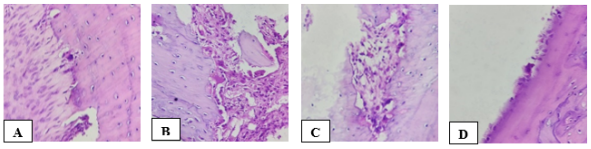


**Figure 1.** Histological results with 400x magnification with Hematoxylin Eosin staining.

In the control group (A) and the dose group 156.5 mg/BW (D), there were fewer osteoblasts and osteoclasts than the 39.15 mg/BW (B) dose group and 78.3 mg/BW (C).

**The Effectiveness of Katuk Leaf Ethanol Extract on the Number of Alveolar Osteoblasts and Bone Osteoclasts**

The results from microscopic observations were then performed quantitative measurements by counting the number of osteoblast cells. The number of osteoblasts was calculated from five fields of view, which were carried out randomly by following certain criteria. There were osteoclasts and osteoblasts. The results of the measurements can be seen in Table 1.

**Table 1.** The average number of osteoblasts

| No | Group | Average |
|---|---|---|
| 1 | Control | 13.56 |
| 2 | EEDK 39.15 mg/BW | 10.40 |
| 3 | EEDK 78.3 mg/BW | 15.03 |
| 4 | EEDK 156.5 mg/BW | 10.80 |

The measurement results from Table 1 show that the average number of osteoblast cells from the alveolar bone of guinea pigs in the control group, the EEDK group was 39.15 mg/BW, the EEDK group was 78.3 mg/BW. The EEDK group 156.5 mg/BW varied, with the highest number of osteoblasts, was in the EEDK group 78.3 mg/BW.

The normality test data for the average number of osteoblasts were using the Saphiro Wilk test showed that the data were normally distributed. The homogeneity test was carried out using the

Levene test. The homogeneous test results show that all data were homogeneous, so the statistical test used is the One Way ANOVA parametric test (Table 2).

**Table 2.** Effectiveness of katuk leaf ethanol extract on alveolar guinea pigs bone osteoblasts

| Group | P Value |
|---|---|
| Control<br>EEDK 39.15 mg/BW<br>EEDK 78.3 mg/BW<br>EEDK 156.5 mg/BW | 0.04* |

Descriptions: One Way ANOVA test, * p <0.05 (significant)

The One Way ANOVA calculation results with p value = 0.04, which means that there is a very significant difference in the average number ofosteoblasts between the four experimental animal groups. Then the Post Hoc Duncan test was carried out to determine which groups were different in Table 3.

**Table 3.** Duncan's post hoc test of the average number of osteoblasts

| Group | Average Number of Osteoblasts (α=0.05) | |
|---|---|---|
| | 1 | 2 |
| EEDK 39.15 mg/BW | 10.40 | |
| EEDK 156.5 mg/BW | 10.80 | |
| Control | 13.56 | 13.56 |
| EEDK 78.3 mg/BW | | 15.03 |

Description: Duncan's Post Hoc Test

**The Effectiveness of the Ethanol Extract of Katuk Leaves on the Amount of Alveolar Guinea Pigs Bone Osteoclasts**

The number of osteoclasts was calculated from five fields of view, which were carried out randomly by following certain criteria, namely, osteoclasts and osteoblasts. The results of the measurements can be seen in Table 4.

**Table 4.** The average number of osteoclasts

| No | Group | Average |
|---|---|---|
| 1 | Control | 2.56 |
| 2 | EEDK 39.15 mg/BW | 1.73 |
| 3 | EEDK 78.3 mg/BW | 2.27 |
| 4 | EEDK 156.5 mg/BW | 2.10 |

The measurement results from Table 4 shows the average number of osteoclasts of the alveolar bone of guinea pigs in the control group, the EEDK group 39.15 mg/BW, the EEDK group 78.3 mg/BW, and the EEDK group 156.5 mg/BW varied with the number of osteoclasts. The highest was in the control group, and the lowest was at EEDK 39.15 mg/BW.

The results of the data normality test for the average number of osteoclasts using the Saphiro Wilk test showed that the data were normally distributed, and the homogeneity test was carried out using the Levene test. The homogeneous test results show that all data are homogeneous, so the statistical test used is the One Way ANOVA parametric test (Table 5).

**Table 5.** Effectiveness of katuk leaves ethanol extract on alveolar guinea pigs bone osteoclasts

| Group | P Value |
|---|---|
| Control<br>EEDK 39.15 mg/BW<br>EEDK 78.3 mg/BW<br>EEDK 156.5 mg/BW | 0.228 |

Description: One Way ANOVA test

The results from Table 5 show that the value of p = 0.228 (p> 0.05), which means that there is no significant difference in the mean number of osteoclasts between the four experimental animal groups.

## DISCUSSION

Isoflavones could increase osteoblastogenesis by increasing the level of TGF-β1 during orthodontic tooth movement. Osteoblast differentiation from mesenchymal cells is stimulated by estrogen due to its positive effect on the production of TGF-β, IGF-I, BMP-6 and other factors that promote the process. Estrogen actively modulates TGF-β1 in osteoblasts and others. TGF-β1 is the most significant factor in bone formation and maintains a balance between the dynamic processes of bone formation and bone resorption.[20-22]

Katuk leaves (*Sauropus androgynus* (L.) Merr has a fairly high content of isoflavones, including genistein and daidzein. Isoflavones can work by binding to estrogen receptors and working to meet the lack of estrogen. The hormone estrogen can promote osteoclast apoptosis due to the stimulative effect of estrogen on apoptosis. Osteoclasts were mediated by upregulation of Fas ligand(FasL) in osteoblasts, and Fas expression in osteoclasts binding of two membrane proteins Fas and Fasl initiated the apoptosis process. Estrogen hormone is involved in regulating osteoclast autophagy. Depletion of estrogen increased the expression of osteoclast autophagy markers in the alveolar bone while estrogen replacement compensates.[23,24]

The results from Table 3 show that in subset one, there were data from the EEDK 39.15 mg/BW, EEDK 156.5 mg/BW, and the control group. Based on these results, the mean number of osteoblasts in the three groups did not significantly differ; in other words, the mean number of osteoblasts in the EEDK group was 39.15 mg/BW EEDK 156.5 mg/BW, and the control group was the same. The control group and the EEDK group 78.3 mg/BW were in subset two, meaning that there was a difference in the EEDK group 78.3 mg/BW compared to other groups but not different compared to the control group showed no significant difference.

Testing the effectiveness of katuk leaves extract on increasing osteoblasts is following previous theory and research. Indriasari et al. (2019) reported that the isoflavone content in soybeans could induce TGF-β1 levels during orthodontic tooth movement. Increased osteoblasts. The result follows Syarif et al. (2020), showing that moringa leaves extract containing flavonoids can increase the number of osteoblasts and reduce the number of osteoclasts in the stress area on the movement of the orthodontic teeth of guinea pigs (*Cavia cobaya*).[16,23,24]

An increase in osteoblasts can occur when katuk leaves contain flavonoid phytochemical compounds, antioxidants and anti-inflammatory. Antioxidants possessed by polyphenols can inhibit reactive oxygen species (ROS) and maintain the vitality of osteoblasts and osteocytes, which play a role in osteoblast activity and osteogenesis and reduce osteoclast differentiation and activity.

Flavonoid can manage cell function by stimulating TGF-β production, whereas TGF-β induces proliferation and migration of osteoblast. The increase of osteoblastic proliferation and activation may increase the amount of osteoblast, which further increase the expression of osteoprotegerin (OPG). An increase of OPG is very influential to the osteoclast activation, since OPG is the decoy receptor for Receptor Activator of Nuclear Factor Kappa-B Ligand (RANKL).[23]

The results follow Sholillah (2015) regarding the water extract of katuk leaves (*Sauropus androgynus* (L.) Merr.) which could significantly reduce the number of osteoclasts using several dosage levels on the femur of menopausal white rats. The number of osteoclasts in this study increased in number, and the ethanol extract concentration of katuk left up to a dose of 78.3 mg/BWand decreased again at a dose of 156.5 mg/BW. However, the difference in the number of osteoclasts in this study was not statistically significant, so it can be said that the ethanol extract of katuk leaves with various doses given did not affect the number of osteoclasts. Ideally, the number of osteoclasts in the remodelling process of orthodontic treatment should decrease. The increased dose can cause the result will cause a buildup in the treatment area resulting in a turnover. Turnover is what disrupts the remodelling process, especially in orthodontic treatment.[17,20]

Katuk leaves (*Sauropus androgynus* (L.) Merr) have many ingredients other than flavonoids or isoflavones (specifically vitamin C). Adnan (2019) showed vitamin C could increase osteoblasts and reduce the number of osteoclasts, statistically significant. Vitamin C does not affect the number of osteoclasts but can have a good effect on stabilising orthodontic treatment.[20,25]

## CONCLUSION

Based on the results of research, it can be concluded the ethanol extract of katuk leaves with 39.15 mg/BW, 78.3 mg/BW, and 156.5 mg/BW of body weight was effective in increasing the number of osteoblasts of the guinea pig alveolar bone. The EEDK dose of 78.3 mg/BW was the most effective dose to increase the number of guinea pigs osteoblasts. While for reducing guinea pigs alveolar bone osteoclasts, the doses of 39.15 mg/BW, 78.3 mg/BW, and 156.5 mg/BW of body weight were not proven to be effective statistically.

## CONFLICT OF INTEREST

As a result of this, we declare that there is no conflict of interest in the scientific articles.


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