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Research Article

Combination of *Channa striata* Extract Gel and HBOT on Trabeculae Area during Orthodontic Treatment

Bunga Fauzia[1*], Bambang Sucahyo[2], Pambudi Rahardjo[2], Arya Brahmanta[2]

[1]Department of Dental Materials, Faculty of Dentistry, Universitas Hang Tuah, Jl. Arief Rahman Hakim No. 150, Surabaya, 60111, Indonesia
[2]Department of Orthodontics, Faculty of Dentistry, Universitas Hang Tuah, Jl. Arief Rahman Hakim No. 150, Surabaya, 60111, Indonesia

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Abstract

Orthodontic pressure applied to the teeth causes stress and inflammation in the periodontal tissues. Channa striata extract gel and hyperbaric oxygen therapy (HBOT) accelerate the healing process of periodontal tissues under stress due to orthodontic pressure by stimulating osteoblasts to form bone trabeculae in tension areas on bone remodeling. To determine the effect of the combination of Channa striata extract gel and HBOT on the trabecular bone area during orthodontic tooth movement in the tension area. 30 male Cavia cobaya were randomly divided into 5 groups (n=6): negative control (K-) without orthodontic tooth movement (OTM), positive control (K+) with OTM, treatment (P1) with OTM and Channa striata extract gel, treatment (P2) with OTM and HBOT, and treatment (P3) with OTM and combination of Channa striata gel extract and HBOT. Rubber separators were attached to the maxillary central incisors. HBOT 2.4 ATA was administered for 7 days from day 17 to day 23, and Channa striata gel was administered for 14 days from day 10 to day 23. The trabecular bone area was examined using HE staining, and results were calculated using Image Raster. Data were evaluated using the One-Way ANOVA test and LSD ($p<0.05$). The mean trabecular bone area was the largest in the P3 group (1,126,649.83+90,817.17). Anova and LSD analysis showed that the most significant difference was found in group P2 ($p<0.05$). The combination of HBOT and Channa striata gel extract affects the trabecular bone area in the tension area during OTM, and the most effective therapy is HBOT.

Keywords: Orthodontic tooth movement, hyperbaric oxygen therapy, Channa striata, trabecular bon

INTRODUCTION

The application of orthodontic forces on the teeth causes tissue trauma, periodontal ligament compression, and bone deformation. It causes biochemical reactions at the cellular level that result in bone remodeling.[1] When orthodontic pressure is exerted, the trabecular bone in the stressed area undergoes resorption by osteoclasts. Conversely, in the tension area, osteoblasts will perform bone apposition, which is essential for new bone formation. This process creates the balance necessary for tooth movement.[2,3] The bone trabeculae are the parts located at the center of the alveolar bone and the cortical chip.[4] The main composition of bone trabeculae is type I collagen fibers and basic substance containing aggregates of proteoglycans and several specific multi-adhesive glycoproteins, including osteonectin.[5]

The high collagen and mineral content gives the tissue its hard properties. The trabeculae pattern has a variable shape, influenced by the amount of pressure. When pressure is applied to the tooth with too much force, it can damage the supporting tissue. This pressure can cause inflammation of the gingiva and periodontal tissues. In addition, many blood vessels in large areas of pressure will close so that there is no blood supply to the periodontal ligament.[5,6]

* Corresponding author, e-mail: bunga.fauzia@hangtuah.ac.id

Channa striata extract gel contains albumin and growth factors that can accelerate the healing process of periodontal tissues under stress due to orthodontic pressure.[7] Research conducted by Siswanto et al. (2016) showed that *Channa striata* extract with concentrations of 25%, 50%, and 100% was able to accelerate the wound healing process. Based on the results of this study, *Channa striata* extract with a concentration of 100% provides the best effect for the wound healing process.[8]

Vascularization disorders of periodontal ligaments and alveolar bone that occur due to orthodontic treatment can be treated by performing hyperbaric oxygen therapy (HBOT). HBOT is a therapy that involves inhaling 100% oxygen in a high-pressure chamber at 2.4 atmospheres absolute (ATA) for 90-120 minutes, 5-6 days, and 6-8 weeks.[9] HBOT can increase tissue oxygen, increase vascularization, and tissue perfusion to accelerate wound healing. HBOT stimulates specific growth factors involved in wound healing. One of the specific growth factors stimulated when HBOT is basic Fibroblast Growth Factor (bFGF), which is a specific growth factor that will stimulate collagen synthesis, cell proliferation, and increase angiogenesis during orthodontic tooth movement.[9,10]

Individual applications of hyperbaric oxygen treatment (HBOT) and Channa striata extract have been shown in recent research to improve bone growth and tissue repair. However, the synergy of using Channa striata gel extract in conjunction with HBOT for the trabecular bone area during orthodontic tooth movement has not been examined in any previous study. The purpose of this study is to determine the effect of the combination of Channa striata extract gel and HBOT on the trabecular bone area during orthodontic tooth movement in the tension area.

MATERIAL AND METHOD

Ethical Clearance and Research Design

This study is a true experiment with a randomized-only control group design using male guinea pigs (Cavia cobaya) in healthy conditions. A Certificate of Ethical Clearance was obtained with No. EC/051/KEPK-FKGUHT/XI/2019 issued by the Ethics Commission of the Faculty of Dentistry, Universitas Hang Tuah.

Gel Preparation of *Channa striata* Extract

Fresh *Channa striata* was purchased in the Bendul Merisi area of Surabaya City. Identification of *Channa striata* biota species was carried out at the Ecology Laboratory, Sepuluh November Institute of Technology, Surabaya. Fresh *Channa striata* was brought to the Chemistry Laboratory of Universitas Hang Tuah for extract preparation. *Channa striata* were gutted, washed under running water, and cut into 1 cm thick cross sections without removing the bones. The extract was obtained by steaming the fish and distilled water (1:1) at 70°C for 50 minutes. The resulting liquid was rotated for 5 hours to separate the distilled water, resulting in a thick, light yellow liquid, which was then filtered and stored.[11] *Channa striata* extract was gelled by mixing Na CMC 0.6%. To obtain *Channa striata* gel with 100% concentration, mix 25 ml of *Channa striata* extract into 100 ml of Na CMC.[11] The finished *Channa striata* extract gel was analyzed for protein, Fe, Zn, and Cu content in the Chemistry laboratory, ITS Surabaya.

Preparation and Group Distribution of Experimental Animals

This study used 30 male guinea pigs *(Cavia cobaya)* with an age of 3-4 months and a weight of 300-400 grams. Animals were acclimatized for 7 days, then given treatment for 14 days. All samples were sacrificed after 14 days of treatment by euthanasia with 10% ketamine at a dose of 0.1 ml/kg BW IM until overdose (Overdose of Chemical Anesthetics) and then decapitation to take the maxilla.

The experimental animal groups were divided into 5 (n=6) the Negative Control group (K-) without orthodontic tooth movement, the Positive Control group (K+)

with orthodontic tooth movement, treatment group 1 (P1) with orthodontic tooth movement and administration of *Channa striata* extract gel, treatment group 2 (P2) with orthodontic tooth movement and administration of Hyperbaric Oxygen Therapy (HBOT), and treatment group 3 (P3) with orthodontic tooth movement and administration of a combination of *Channa striata* extract the gel and Hyperbaric Oxygen Therapy (HBOT). Separators (orthodontic separator band S) were installed in groups K (+), P1, P2, and P3 on the left maxillary central incisor of Cavia cobaya on day 8 using a Zahnart plier separator, then replaced using a large separator on day 9 and glued using GIC GC Fuji I luting cement, so that the separator would not easily come off. The separator was in place for 14 days. The distance of incisive tooth movement was measured using a digital caliper (Krisbow) from the distal tip of the right incisor to the distal tip of the left incisor minus the mesiodistal width of both incisors.

Figure 1. Rubber separators were attached to the maxillary central incisors of Cavia cobaya

Hyperbaric Oxygen Therapy (HBOT) and *Channa striata* Extract Gel Administration

Channa striata extract gel was applied using an insulin syringe needle that was blunted at the end using scissors and sandpaper so as not to injure the guinea pig's gingival sulcus. The application of *Channa striata* extract gel in groups P1 and P3 on the gingival sulcus was 0.025 mL once a day. The treatment was carried out on day 10 to day 23. Group P3, *Channa striata* extract gel was administered once a day on day 10 to day 23 and then given hyperbaric oxygen therapy on day 17 to day 23.

In the P2 and P3 groups, hyperbaric oxygen was given (in the animal chamber) from day 17 until day 23 without removing the rubber separator on the experimental animals, then increasing the pressure in the chamber to 2.4 ATA and flowing pure oxygen (100%) for 90 minutes (3x30 minutes), after which it was stopped and lowered to the original condition (1 ATA). The guinea pigs were removed from the chamber and brought to the original cage.

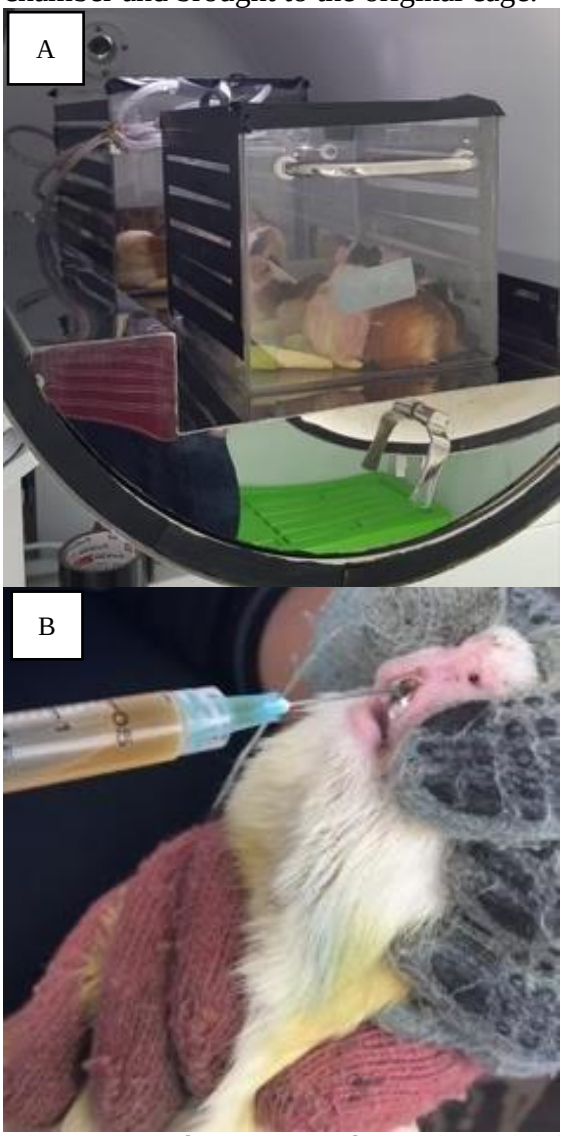

Figure 2. Hyperbaric Oxygen Therapy (HBOT) (A) and *Channa striata* Extract Gel (B) administration to Cavia cobaya

Preparation and HE Staining

All samples were sacrificed after 14 days of treatment. Anesthesia using 10% ketamine at a dose of 0.1 ml/kg BW IM was given until the animals overdosed (Overdose of Chemical Anesthetics) and then decapitated to take the maxilla. The extracted maxilla was cleaned of adherent tissue and fixed in a 10% buffered formalin solution for 4 days. The guinea pig maxilla was decalcified using a 6% formic acid solution for 21 days (until the bone became soft, checked using a needle). Tissue processing was performed by cleaning and impregnation. The trabecular bone area was examined by HPA with Hematoxylin Eosin (HE) staining and viewed using a microscope with 40x magnification in a 5x field of view. The results of the documentation of trabeculae bone were analyzed using Image Raster, and the area was calculated by giving points to all edges of the alveolar bone surface and bone marrow, which would later obtain a number with units of square micrometers (μm^2) (Primary Data, 2019). Trabeculae area is the result of reducing the total area of alveolar bone with bone marrow area (Primary Data, 2019).

Data Analysis

The data obtained was analyzed using SPSS 22.0. Data normality test using Shapiro-Wilk because the number of replications ≤ 50. The homogeneity test was conducted using Levene's Test. Numerical comparative hypothesis testing with One-way ANOVA was performed on all groups in the tension area and continued using Post Hoc LSD to see significant differences in the trabecular bone area in the tension area in each group ($p < 0.05$).

RESULTS

The largest mean area of bone trabeculae (Table 1) was found in group P3 (combination of *Channa striata* extract gel and HBOT) at 1,126,649.83 µm2 and the smallest mean area of bone trabeculae was found in group K(-) (negative control) at 533,295.83 µm2.

The results of the Shapiro-Wilk and homogeneity test showed a significance value of $p > 0.05$ in the tension area, so it can be said that the average area of trabeculae in the tension area of the alveolar bone during tooth movement of male guinea pigs is normally distributed and the data are homogeneous.

Table 1. Mean and standard deviation of the area of bone trabeculae in the tension region of alveolar bone (units in square micrometers (μm^2))

	Tension Areas	
Groups	**Mean of Area of Bone Trbaeculae**	**Standard Deviation**
K(-)	533.295,83	90.438,290
K(+)	780.411,00	68.144,036
P1	888.727,33	92.425,973
P2	1.083.744,00	80.726,332
P3	1.126.649,83	90.817,175

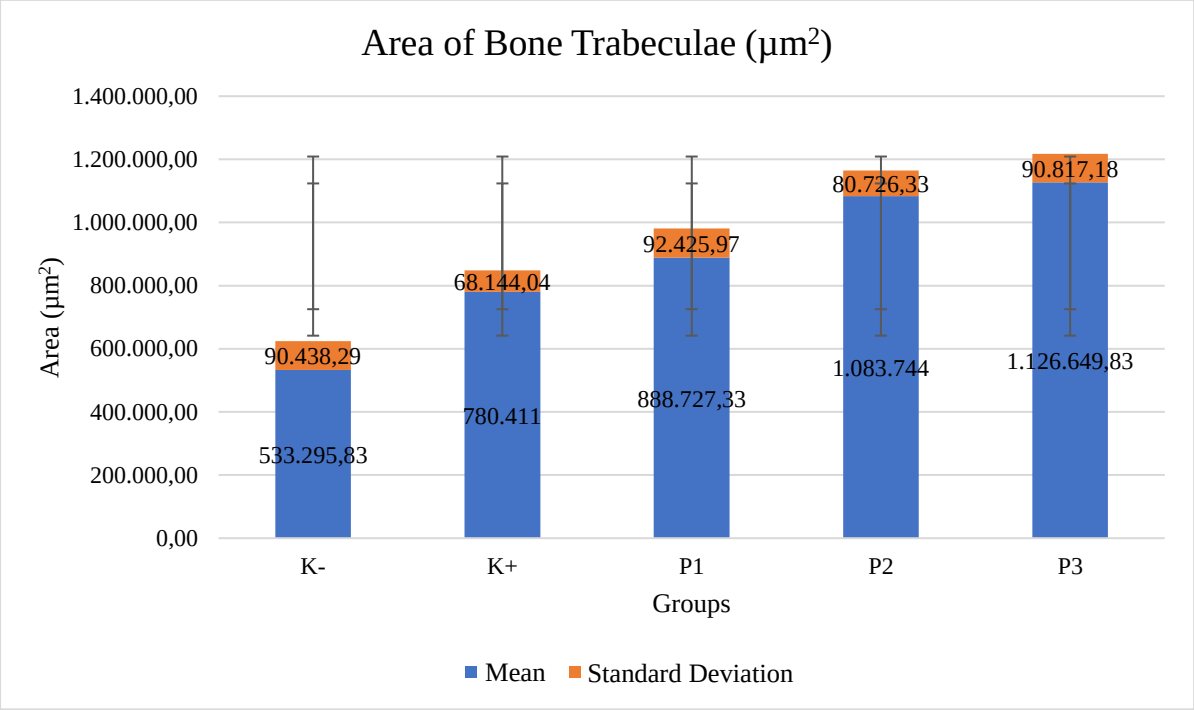

Figure 4. Diagrams of mean and standard deviation of trabecular bone area in all groups.

Table 2. Shapiro Wilk normality test result

Group	***Shapiro-Wilk* Sig.**
K(-)	,371*
K(+)	,859*
P1	,488*
P2	,713*
P3	,983*

Note *: p> 0.05 (data is normally distributed)

Table 3. Result of Levene's test of homogeneity

Homogeneity Test		
	Levene's Test	**Sig.**
Tension area	,208*	,931*

Note *: p>0.05 (homogeneous data)

Table 4. One-Way ANOVA test result

	Tension Area
Source of diversity	Sig.
Between Treatments	,000*
In treatments	
Total	

Table 5. LSD Post-Hoc test results

Kelompok/Rerata	K(+) (780.411,00)	P1 (888.727,33)	P2 (1.083.744,00)	P3 (1.126.649,83)
K(-) (533.295,83)	,000*	,000*	,000*	,000*
K(+) (780.411,00)		,037*	,000*	,000*
P1 (888.727,33)			,001*	,000*
P2 (1.083.744,00)				,390

The results of the LSD post-hoc test in Table 5 show that all groups have significant differences ($p < 0.05$), except for group P2 with P3. The group that has the most significant difference is group P2, which is a group of guinea pigs with orthodontic tooth movement with HBOT administration.

DISCUSSION

Channa striata is an aquatic biota that contains bioactive ingredients for protein synthesis, collagen synthesis, and cell proliferation that can help accelerate wound healing and bone trabecula formation and provide hard properties to bone tissue.[12] While HBOT is a therapy by inhaling 100% oxygen in a high-pressure room. Previous research stated that giving HBOT 2.4 ATA 90 minutes a day, for 7 days during tooth movement in rats increased trabecular bone volume, indicating osteoblastic activity.[13]

The results of the average area of bone trabeculae in the tension area of the alveolar bone in the negative control group (K-) amounted to 533,295.83 µm2, positive control (K +) amounted to 780,411 µm2, P1 amounted to 888,727.33 µm2, P2 amounted to 1,083,744 µm2, and P3 amounted to 1,126,649.83 μm^2. The lowest mean area of bone trabeculae in the tension area of the alveolar bone was obtained by group K (-), which is the group that was not given mechanical pressure on the rubber separator. In contrast, the highest average area of bone trabeculae was obtained by the P3 group, which was the group that was given a mechanical pressure rubber separator and combination therapy of HBOT and *Channa striata* extract gel. The results showed that group K (+), namely the group given a rubber separator, had a higher mean score than group K (-). Group K (-) was not given mechanical pressure on the rubber separator, so there was no bone remodeling process. Meanwhile, the K(+) group was given mechanical pressure from the rubber separator, which caused changes in the periodontal ligament and alveolar bone, causing apposition in the tension area.[14] The tension area will cause new alveolar bone formation carried out by osteoblasts. Osteoblasts are bone tissue cells that play a role in collagen synthesis to form osteoid as the basic material of bone. In the remodeling process, osteoblasts organize bone intercellular substances containing collagen and form osteoid, and then the complex will form bone trabeculae.[15]

The group that was given a mechanical pressure rubber separator and topically induced with 100% *Channa striata* extract gel (P1) had higher mean bone trabeculae area results than the K(-) and K(+) groups. There was a significant

difference in the P1 group with the K(-) and K(+) groups. Chana strata contain albumin, essential minerals such as zinc (Zn), copper (Cu), iron (Fe), and unsaturated fatty acids, which are also proven to facilitate collagen synthesis, angiogenesis, and connective tissue formation.[11,16] These bioactive components facilitate the differentiation and function of osteoblasts, which are important in osteoid deposition and cancellous bone formation. Zinc, especially, regulates inflammatory responses and encourages tissue regeneration. Copper and iron both aid in the enzymatic processes necessary for collagen.[22] Copper (Cu) and iron (Fe) minerals found in *Channa striata* extract are also compounds that help collagen synthesis and cell proliferation. This compound can increase growth factors that play a role in the maturation process of osteoblasts.[17] Osteoblast cells that have been differentiated will produce bone matrix (osteoid) deposited by osteoblasts containing type I collagen, then will be mineralized to form bone trabeculae.[4]

The group that was given mechanical pressure rubber separator and HBOT 2.4 ATA for 3x30 minutes a day (P2) had higher mean bone trabeculae area results than the K(-), K(+), and P1 groups. There was a significant difference in the P2 group with the K (-), K (+), and P1 groups. HBOT is administered systemically and can affect bone trabeculae formation in tooth movement and promote bone regeneration through OPG / RANK / RANKL expression. HBOT can indirectly stimulate osteoblast activity through its influence on the RANKL–OPG system that participates in the regulation of bone remodeling.[18]HBOT can stimulate osteoblasts directly through the bond between RANKL and OPG, which forms bone apposition so that bone trabeculae will also form quickly. HBOT increases the expression of Osteoprotegerin (OPG) and decreases the expression of RANKL (Receptor Activator of Nuclear Factor κB Ligand), thereby inhibiting osteoclast differentiation and activation.[20] By increasing the OPG/RANKL ratio, the balance of bone remodeling is shifted in the direction of new bone formation (apposition of bone) by osteoblasts. Therefore, HBOT systemically increases faster and more extensive bone trabecular development through increased osteoblastic and decreased osteoclastic activity during orthodontic tooth movement[9,18] HBOT stimulates specific growth factors involved in wound healing, one of which is basic Fibroblast Growth Factor (bFGF), which is a specific growth factor that will stimulate collagen synthesis, cell proliferation, and increase angiogenesis during orthodontic tooth movement.[19]

The administration of HBOT during tooth movement using orthodontic devices can affect vascularization and accelerate the ossification of new bone formation.[20] The group that was given a rubber orthodontic pressure separator with a combination therapy of HBOT and 100% *Channa striata* extract gel (P3) had higher mean bone trabeculae area results than groups K (-), K (+), P1, and P2. The P3 group has the highest mean compared to other groups due to the combination of the administration of *Channa striata* extract gel, which works locally, and HBOT, which works systemically so as to cause a synergistic effect on increasing bone trabeculae area. There was a significant difference in the P3 group with the K (-), K (+), and P1 groups. However, there was no significant difference between the P2 group and P3. Giving combination therapy works synergistically through *Channa striata* extract gel therapy with a concentration of 100% locally and HBOT 2.4 ATA systemically.

High gel concentration with high viscosity causes slow penetration of the drug into the body. In addition, the gelling agent used in this study was NaCMC, which provides a stable viscosity to the preparation but has a small spreading diameter.[21] Therefore, the group given HBOT 2.4 ATA was more effective than the group that was only induced with *Channa striata* extract gel (P1) and the combination group (P3). The results of this study indicate that the combination therapy of HBOT and *Channa*

striata extract gel affects the area of dental bone trabeculae in the tension area in orthodontic tooth movement and the most effective therapy that can be used to determine the area of bone trabeculae in the tension area in orthodontic tooth movement as an adjunct therapy to accelerate orthodontic treatment is HBOT 2.4 ATA 3x30 minutes a day for 7 days (starting from day 17 to day 23).

CONCLUSION

The combination of HBOT 2.4 ATA and *Channa striata* extract gel (Channa striata) 100% affects the area of bone trabeculae in the tension area in orthodontic tooth movement. The most effective therapy that can affect the area of bone trabeculae in the tension area on orthodontic tooth movement as an adjunctive therapy to accelerate orthodontic treatment is HBOT 2.4 ATA 3x30 minutes a day for 7 days (starting from day 17 to day 23).

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