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ORIGINAL ARTICLE

Channa Striata and Hyperbaric Oxygen Therapy Combination for Pressure Areas in Orthodontic Treatment

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ABSTRACT
Objective: To study the effect of using a combination of *Channa Striata* gel and hyperbaric oxygen therapy on pressure areas during orthodontic treatment. **Material and Methods:** The study was conducted using the ARRIVE Essential 10 guidelines. In this study, 35 3-4 months male guinea pigs (*Cavia Cobaya*) weighing 300-400 grams were used and divided into 5 groups (n=7). Decalcification was performed to dissolve the dental calcium and jawbone to cut the tissue properly. The decalcification was performed for 30 days. Then preparations were made with HE (Hematoxylin Eosin), observed using a microscope, and counted the number of osteoclasts and macrophages on a light microscope with 400 times magnification. The results of the preparations were analyzed using the SPSS program. **Results:** The Kruskal-Wallis test of macrophage cells and the ANOVA test of osteoclast cells showed significant results between all groups ($p<0.05$). **Conclusion:** The effect of hyperbaric oxygen therapy 2,4 ATA administered on days 8-14 and *Channa Striata* extract gel administered on days 3-14 can increase the number of macrophages in the periodontal ligament and osteoclasts in the alveolar bone in the pressure area during orthodontic tooth movement.

Keywords: Pressure; Orthodontics; Osteoclasts; Macrophages.

Introduction

Orthodontic treatment can be defined as treatment aimed at groups of people with malocclusion that was set as the treatment target. Orthodontic treatment aims to obtain optimum dentofacial function, health, stability, and esthetics [1]. Orthodontic forces will cause compression of the periodontal ligament and deformation of the alveolar bone. In the pressure area, this force causes the vascularity of the periodontal ligament and alveolar bone to narrow [2]. Impaired vascularization of the periodontal ligament and alveolar bone can be anticipated by performing HBOT (Hyperbaric Oxygen Therapy) and administration of *Channa Striata* extract, which contains albumin compounds to prevent the inflammatory process [3].

Tooth movement in orthodontics occurs due to mechanical forces that involve remodeling the alveolar bone and periodontal ligament. The bone remodeling process can be stimulated by mechanical forces obtained from activating orthodontic appliances that compress the teeth and transmit to the tissue surrounding the teeth, including the gingiva, periodontal ligament, and alveolar bone [4]. Mechanical forces cause a process of tension and pressure on the periodontal ligament. There is a process of resorption and apposition of the alveolar bone. In the tension area, the widening of blood circulation in the periodontal ligament results in a bone remodeling process that is activated by osteoblast cells. On the other hand, in the pressure area, osteoclast and macrophages cause the process of alveolar bone resorption so that the teeth will move toward the desired socket direction [2].

Periodontal ligament (PDL) is a connective tissue covering the root of the tooth that connects the cementum at the root of the tooth to the alveolar bone. During orthodontics, the load-displacement from the tooth to the surrounding bone is affected by PDL, in which fibrous connective tissue fills the space between the tooth root and the alveolar bone. PDL consists of elastic fibers (collagen) surrounded by matrix components such as blood vessels and lymph vessels. The cell types in PDL are fibroblasts, osteoblasts, osteoclasts, cementoblasts, cementoclasts, mast cells, and macrophages [5].

Alveolar bone is the part of the maxilla and mandible that forms and supports the tooth sockets (alveoli). Alveolar bone is formed when teeth erupt to provide bone attachment to the periodontal ligament. Alveolar bone can be divided into areas separated from the anatomical basis, but its function is unity with all the interconnected parts between the supporting tissues of the teeth. Typically, the crest of the alveolar bone is 1-2 mm apical to the cementoenamel junction. If there is bone loss, the alveolar crest is more than 2 mm apical to the cementoenamel junction.

Channa Striata are freshwater fish that have a reasonably high-altitude protein. Besides, this fish has a high albumin content. Albumin is very useful for postoperative wound healing. Hyperbaric oxygen therapy is commonly used effectively in wound healing, and the most crucial effect of adding oxygen to stimulate fibroblast proliferation and differentiation increasing collagen formation [6].

Osteoclasts come from differentiation monocytes/macrophages, are found in bone, and secrete various acids and enzymes that digest bone and facilitate its phagocytosis [7]. Under the influence of cytokine production, macrophages and monocytes can attract more IL-β, prostaglandin E2 and TNF-α, or hormones and dexamethasone that can be induced by the stimulation of PDL derived from RANKL. This study aimed to study the effect of using a combination of *Channa Striata* gel and hyperbaric oxygen therapy on pressure areas during orthodontic treatment.

Material and Methods

Study Design and Ethical Clearance

The study was experimental with a randomized control group post-test only design. ARRIVE Essential 10 guidelines were followed for this study. Ethical permission was obtained from the ethics and scientific research committee for experimental animal use in the Faculty of Dentistry Universitas Hang Tuah (S.Ket/006/KEPK-FKG UHT/VII/2019).

Sample and Experimental Procedures

The experimental group consisted of 35, 3-4 months male guinea pigs (*Cavia Cobaya*) weighing 300-400 grams and was divided into 5 groups. The sample size (n = 7) per group was determined depending on the ethics committee's decision. The materials used were *Channa Striata* extract, 100% pure oxygen in a hyperbaric animal chamber, 10% ketamine injection as an anesthetic drug, and a 0.1-0.2 ml/kg dose for *acepromazine* 0.5 ml, 10% buffered formalin, Betadine solution, and cotton. A single researcher performed all animal experiments according to the guidelines for the proper conduct of animal experiments.

The guinea pigs were monitored during the experiment, and all the groups were sacrificed for their jaws on the fourteenth day of the experiment. Afterwards, the maxillary teeth were dissected and placed in 10 % buffered formalin. Decalcification was performed to dissolve the dental calcium and jaw bone to cut the tissue properly. The decalcification was performed for 30 days with Ethylene Diamine Tetra Acid (EDTA). Histological analyses were performed at the Faculty of Dentistry Universitas Hang Tuah. Then preparations were made with HE (Haematoxylin Eosin) staining to determine the number of osteoclasts and macrophages in each group, then observed using a microscope and counted the number of osteoclasts and macrophages on a light microscope with 400 times magnification.

Data Analysis

The results of the preparations were analyzed using the Statistical Package for the Social Science (SPSS) Software, version 20.0 (IBM Corp., Armonk, NY, USA). The statistically significant differences among the group were determined and evaluated using one-way ANOVA and LSD tests ($p<0.05$).

Results

The data obtained from the study results were analyzed to obtain an overview of the distribution and a summary of the data to clarify the presentation of the results. The data obtained showed there were differences in the average number of macrophages between groups. The average number of macrophages was most abundant in the pressure area of group P3 (4,40), and the smallest average number of macrophages was found in group K(-) (2,90), as can see in Table 1 and Figure 1.

From the results of the Mann-Whitney test, groups with significant differences ($p<0.05$) are K(-) with K(+), K(-) with P2, K(-) with P3, K(+) with P1, K(+) with P3, P1 with P2, P1 with P3, and P2 with P3 (Table 2 and Figure 2).

Table 1. The number of macrophages observed among groups.

Group	Average Number of Macrophage Cells	Standard Deviation
K(-)	1,900	0,417
K(+)	2,900	0,417
P1	3,600	0,547
P2	4,300	0,904
P3	4,400	1,14

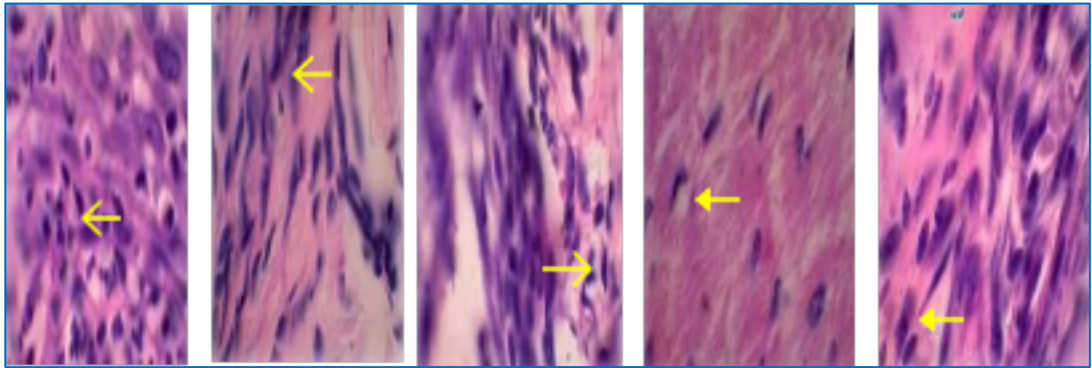

Figure 1. An overview of macrophage in the control group, orthodontic group, Chana group, HBO groups and combination group.

Table 2. The results of the Mann-Whitney test for the mean of macrophages in the pressure area.

Group/Mean	K(+) (2.90)	P1 (3.60)	P2 (4.30)	P3 (4.40)
K(-) (1.90)	0.007*	0.010*	0.439	0.008*
K(+) (2.90)	-	0.116	0.007*	0.034*
P1 (3.60)	-	-	0.006*	0.006*
P2 (4.30)	-	-	-	0.007*

*Statistically Significant.

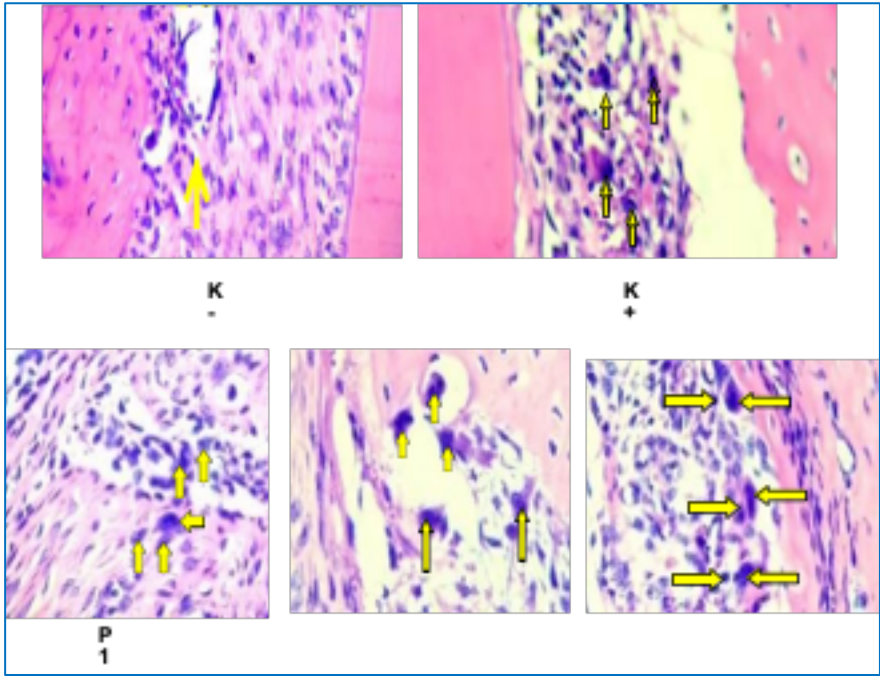

Figure 2. An overview of osteoclast in the control group, orthodontic group, Chana group, HBO group, and the Combination group.

Table 3 shows that the average number of macrophages was most abundant in the pressure area of group P3 (8,40), and the smallest average number of macrophages was found in group K(-) (4,80). The One-way ANOVA test was performed to determine an increase in the number of osteoclasts in the pressure area group.

In Table 4, it was obtained a significance value of $p<0.001$. This indicates a significant difference between the negative group and the positive group with the treatment group. Therefore, it was continued with the LSD test to find out which groups have differences.

Table 3. Average and standard deviation of osteoclasts in each treatment group.

Group	Mean	Standard Deviation
K(-)	4,800	0,837
K(+)	5,200	0,837
P1	6,800	0,837
P2	6,800	1,304
P3	8,400	1,517

Table 4. Results of the one-way ANOVA test for osteoclast cells in the pressure area.

Comparisons	Sum of Squares	df	Mean Square	F	p-value
Between Groups	41.600	4	10.400	8.525	<0.001
Within Groups	24.400	20	1.220		
Total	66.000	24			

The LSD test is a follow-up test used after finding a significant difference in the One-way ANOVA test. LSD test was performed to see the significant difference in the number of osteoclasts between each group (Table 5). Between two treatments were stated to be significantly different if the significant value of the results of the LSD test is less than the research error rate of 0.05 (5%), while if the significant value of the LSD test is greater than 0.05 (5%), then there is no significant difference between the two treatments. From the results of the LSD test above, it was obtained that there was a significant difference between the number of osteoclasts in all treatments because all the results of the standard deviation ($p<0.05$), except in the group between K- and K+ and P1 and P2, had an insignificant difference in the number of osteoclasts ($p>0.05$). The Kruskal-Wallis test of macrophage cells and the ANOVA test of osteoclast cells showed significant results between all groups (p<005).

Table 5. Results of post hoc LSD test for osteoclast cells in the pressure area.

Mean	K(+)	P1	P2	P3
Group Osteoclast	(5,200)	(6,800)	(6,800)	(8,400)
K- (4,800)	0.573	0.010*	0.010*	<0.001*
K+ (5,200)	-	0.033*	0.033*	<0.001*
P1 (6,800)	-	-	1.000	0.033*
P1 (6,800)	-	-	-	0.033*

*Statistically Significant.

Discussion

Channa Striata is one type of fish that has been known and trusted by the public as a food with the potential as medicine to accelerate the wound healing process. *Channa Striata* extract contains compounds that are important for the process of tissue synthesis, such as albumin, minerals Zinc (Zn), Cupprum (Cu), and Ferrum (Fe), as well as unsaturated fatty acids, which are known to accelerate wound healing and have anti-inflammatory effects [3]. Haruan fish extract contains important compounds for tissue synthesis such as albumin, zinc (Zn), Cupprum (Cu), and Ferrum (Fe) minerals, as well as unsaturated fatty acids. Albumin, Zn, Cu, Fe, and fatty acids are essential in accelerating the wound healing process to be anti-inflammatory and accelerate proliferation [3].

Hyperbaric oxygen therapy places the patient in a room containing 100% pure oxygen at higher-than-normal air pressure. Hyperbaric oxygen therapy can increase tissue oxygen, vascularity, and tissue perfusion, thereby accelerating wound healing [8]. This occurs because the administration of HBO therapy with a pressure of 2.4 ATA can increase Reactive Oxygen Species (ROS) and Reactive Nitrogen Species (RNS) and can cause tissue damage, including bone tissue [8]. ROS increases the expression of RANKL and RANK. The RANKL

will later bind to the RANK receptor (for Nuclear kB activator) on macrophages. This binding can also cause macrophage differentiation into osteoclast cells [9].

Channa Striata contains albumin, zinc (Zn), copper (Cu), iron (Fe), and unsaturated fatty acids that have an essential role in the wound healing process, function as anti-inflammatory, and accelerate proliferation [3]. The effect of HBOT 2.4 ATA and *Channa Striata* extract gel on macrophages and osteoclasts can accelerate tooth movement during orthodontic treatment.

This RANKL will later bind to its receptor RANK (for nuclear kB nuclear) found on macrophages. This binding triggers macrophages to differentiate into osteoclasts. However, in TNF-α, besides stimulating the expression of RANKL, it can also produce M-CSF (macrophage colony-stimulating factor), which will bind directly to its receptor c-Fms (macrophage colony-stimulating factor receptor) found in macrophages. This binding is also capable of causing the differentiation of macrophages into osteoclast cells [9].

Osteoclasts are the only cells capable of resorption of bone and are generally multinucleated. Osteoclasts resorb bone by sticking to the bone surface and lowering the pH of the surroundings to reach an acid level of about 4.5. The bone mineral then dissolves, and the collagen breaks down. Osteoclast differentiation and function are mainly regulated by macrophage colony-stimulating factor (M-CSF), a receptor for activation of nuclear factor-kappa 8 (RANK), and osteoprotegerin (OPG) [10].

Macrophages are inflammatory mediators in connective tissue infiltration. Macrophages produce several cytokines and provide antigens to T cells. They are an essential part of the innate immune response to intracellular infection and produce proinflammatory cytokines that enhance phagocytosis and differentiate osteoclast in response to TNF-α. It can be said that these cells form a link between the immune system and bone resorption [11]. One of the unique abilities of macrophages is to combine with other macrophages to form large multinucleated cells or macrophage giant cells (MGCs) with osteoclast type characteristics. Identification of colony macrophages stimulating factor (M-CSF) and receptor activator of nuclear factor kappa-B ligand (RANKL) as key molecules that induce monocyte and macrophage differentiation to become osteoclast [12].

This study used the combination of Hyperbaric Oxygen Therapy (HBOT) 2,4 ATA and *Channa Striata*, which were converted into a gel. HBOT was given systematically on male guinea pigs for 7 days from day 17 to day 23 and cork fish extract gel for 14 days from day 10 to day 23.

At the combination treatment group of *Channa Striata* extract gel and hyperbaric oxygen therapy, both tension and pressure areas had the highest collagen density mode values. It could happen because this combination group was given separator orthodontics rubber pressure with HBOT and Chana Striata. The HBOT application can release a high oxygen concentration in the injury area and increase the oxygen partial pressure that later blends into the blood. Therefore, it can affect the process of remodeling, angiogenesis, and blood supply [13]. The change in high oxygen rate can trigger the cellular alteration to take a role in orthodontic tooth movement [8].

TOHB is combined with a gel extracted from a Chana Striata, the main component that regulates the connective tissue found inside the Chana Striata. Albumin in *Channa Striata* can increase angiogenesis, which is a TOHB mechanism in promoting trauma wounds in the area so that a TOHB combination and a stylistic gel of cork significantly increase the effectiveness of treatment [14]. This is due to combined hyperbaric oxygen therapy 2.4 at systemically, and stylistic *Channa Striata* extract and synergistically increasing fibroblasts' proliferation in the pressure and pull areas. As hyperbaric oxygen therapy is administered, ROS (reactive oxygen species) and RNS (reactive nitrite of species) increase and serve as signal molecules in transduction or entry pathways for growth factors, cytokines, and hormones [15].

Two critical issues for a detailed understanding of orthodontic tooth movement are the periodontal ligament and the alveolar bone. There is extensive literature on descriptive studies involving multiple cells across the bone and the periodontal ligament. On application of orthodontic forces, there is remodeling of the periodontium [16]. On the compression side, there is an increase in the number of macrophages that leads to resorption of the hyalinized tissues, which eventually is followed by tooth movement [17]. Once alveolar bone resorption occurs, it leads to tooth movement. The tooth movement rate is determined by factors such as the magnitude and duration of applied force. These applied forces lead to vascular changes, which lead to the synthesis of cytokines, neurotransmitters, and colony-stimulating factors that lead to the release of macrophages and leucocytes [18]. An improved understanding of the tooth movement process with respect to tissue changes at the microscopic level and how these processes can be enhanced is essential for the advancement of Orthodontics.

Preclinical studies are essential in analyzing the feasibility or safety of any material used for clinical trials. The main objective of a preclinical study is to study and analyze a safe dose for human trials and we can locate any potential risks at this stage. The use of appropriate surrogates is essential for the study to deliver potentially predictive results. One of the limitations of such studies however has been the use of young healthy animals whereas a majority of human patients are affected by some disorders. As such adverse events reported in such studies remain subject to discussion and further exploration till these can be ratified by further experimentation and analysis.

The primary complications of using Hyperbaric oxygen in this study include Barotrauma due to barometric changes. Since the teeth and sinuses are intimately connected, pathologic phenomena may occur due to changes in pressure. However, in this experiment, the animals were provided food while delivering hyperbaric oxygen to ensure regular mouth opening. This would reduce pressure build-up, thereby reducing the chances of the subjects suffering from Barotrauma.

Conclusion

The *Channa Striata* extract gel administered on days 3-14 and hyperbaric oxygen therapy 2,4 ATA administered on days 8-14 can increase the number of macrophages in the periodontal ligament and osteoclasts in the alveolar bone in the pressure area during orthodontic tooth movement.

Authors' Contributions

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All authors declare that they contributed to critical review of intellectual content and approval of the final version to be published.

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Conflict of Interest

The authors declare no conflicts of interest.

Data Availability

The data used to support the findings of this study can be made available upon request to the corresponding author.

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