Indonesian Dental Association

Journal of Indonesian Dental Association

http://jurnal.pdgi.or.id/index.php/jida
ISSN: 2621-6183 (Print); ISSN: 2621-6175 (Online)

Research Article

Effectiveness of Flavonoid Isolate (*Glycine max* merill) on Distances of Orthodontic Teeth Movement

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KEYWORDS

estrogen;
malocclusion;
orthodontic treatment;
soy milk;
tooth movement

ABSTRACT

Introduction: The success of orthodontic treatment is influenced by a number of factors, including nutrition factor. Orthodontic force application is characterized by remodeling changes in the periodontal tissues leading to the phenomenon of tooth movement. Phytoestrogens are estrogen compounds that can maintain the balance of bone resorption formation and its impact on bone remodeling. Isoflavone, one of the phytoestrogen compounds, can be found in soybeans. **Objectives:** The aim of the study was to investigate the effectiveness of soy milk (*Glycine max* (L.) Merill) on tooth movement in orthodontic treatment. **Methods:** An experimental laboratory post-test with a control group design was used in this research. Twenty four female guinea pigs (*Cavia C*) were divided into 8 and divided into 3 groups used in this research. Each object was fed with soy-milk flavonoid isolate powder at doses of 3.12 g/kg BW and 6.25 g/kg BW. The data analysis used one-way ANOVA and Kruskal-Wallis, then continued with the Mann-Whitney test. **Results:** The result of the study showed, soy milk with 6.25 g/kg BW and 3.12 g/kg BW doses were effective on the distance guinea pigs' teeth movement who are undergoing orthodontic treatment. **Conclusion:** The most effective soy milk for the distance of teeth movement in guinea pigs in orthodontic treatment is a dose of 6.25g/kg WB. The soy milk was effective to the number of tooth distance movement with orthodontic treatment.

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DOI: 10.32793/jida.v7i1.1125

INTRODUCTION

Malocclusion is a condition of disharmony between maxillary and mandibular contacts and affects many aspects of function.[1,2] There are several factors influenced the occurrence of malocclusion including heredity, environment and nutritional factors.[3,4]

Orthodontic treatment will be done by moving the teeth in to the desired position by applying force to increase tooth movement is a complex biomechanical process occurred by involving forces delivered through orthodontic appliances to the cellular matrix, and interaction between component of metabolism including hormone and growth development factors.[3,5] Once the force is applied to the teeth, there will be delivered on the two sides of the apical tooth, namely the pressure side and the strain side.[6] The side that gets pressure will cause vasoconstriction and narrowing of the periodontal ligament, when vasoconstriction occurs, the flow of nutrients will decrease in areas that get pressure so that a hyalinization process and cell death are formed because osteoclasts are activated in the periodontal ligament, where osteoclasts are responsible for the resorption process and cause tooth decay. move.[7]

On the opposite side, which is the side that is stretched, a mechano-transduction process will occur, namely the ability of the osteoblasts lining the bone socket to react quickly and directly in the face of strain due to orthodontic tooth movement. Osteoblasts also have a secretory function and the formation of bone extracellular matrix also produces type 1 collagen fibers, so that they are able to carry out bone formation or bone apposition.[8] Tooth movement and bone remodeling process will occur with the formation of pressure and strain activities on the teeth that are given orthodontic devices through the alveolar bone.[7,9] Estrogen is one of the hormones that controls and plays a role in the process of bone remodeling. Estrogen has two receptors found in osteoblasts and osteoclasts namely alpha receptors (ERα) and beta receptors (ERβ) which can activate estrogen function. Estrogen can increase the function of osteoblasts in carrying out bone apoptosis and reduce resorption activation of osteoclasts.[9] Once there is a deficiency of estrogen, it will affect the balance of bone formation, namely that new bone formation does not occur following the process of bone resorption.[7,9] and nutrients that contain estrogen, can be obtained through natural sources called phytoestrogens, which will help prevent estrogen deficiency. Phytoestrogens are estrogenic compounds and are estrogenic and can help maintain the balance of bone resorption and formation.[10] One of the phytoestrogen compounds is isoflavones which can be found in nuts, one of which is soybeans.[11] The levels of phytoestrogens in isoflavones are higher than other types such as ligament and coumestan, so the consumption of isoflavones in soy is recommended.[12]

Consuming soy milk within three months for postmenopausal women who are at risk of osteoporosis has an effect on increasing bone density due to the flavone, prevents bone loss, and also the risk of cancer. [13] Similar research on the benefits of isoflavones obtained from other herbals assured can increase osteoblasts in tooth movement, which can help the process of bone remodeling in orthodontic treatment.[14]

Isoflavones are phytoprotein compounds that have the ability to help bone regeneration, including alveolar bone, and are one of the main ingredients found in soy milk. The process of bone reshaping is a biomechanical process that underlies tooth movement and assists tooth movement in orthodontic treatment. Based on this background, researchers are interested in conducting research on the effectiveness of soy milk on tooth movement distance in orthodontic treatment of guinea pigs.[15]

MATERIALS AND METHODS

This research was under ethical clearance approval #175/UN6.KEP/EC/2023 from the division of ethic Medical-Padjadjaran University. This study used an experimental laboratory posttest with control group design to compare and measure the effectiveness of soy milk changes occurring with distance of orthodontic force. The subject of this study was a healthy female guinea pig (*Cavia* c). The selected sample with the inclusion criteria weighing between 250 - 400 grams, no diastema, presence of anterior teeth, and obtained from the same farm breeding. The number of samples was taken using the Ferderer formula, and we obtained 24 guinea pigs as samples, with 8 guinea pigs in each group. There were three groups applied to this study, with 2 treatment groups and one control group. Each group was applied with a rubber separator on one of the lower incisors to get the movement of the anterior teeth and the treatment group was feed with soy milk flavonoid isolate with two different doses. Each group was subjected to a comparable environment, with identical nutritional parameters, including the width of the cage, the duration of feeding and drinking periods, and the administration of flavonoid isolates at the same time.

The orthodontic ring rubber applied on to lower incisors teeth with separator orthodontic device by (Medessy®). 2.5g and 5g of protein isolate flavonoids from of soy milk within 6.25g/kgBW and 3.12g/kgBW. Furthermore, the preparation of soy milk by mixing soy milk flavonoid isolate powder (Natlab®) with 50 ml of mineral water is then mixed thoroughly and placed in a measuring cup and given to group I as much as 6.25 g/kgBW, group II is given as much as 3.12 g/kgBW, group III as the control group was only given mineral water. The protein content given to guinea pigs is 400-500 grams containing around 2.5-5 grams of protein.

The orthodontic ring rubber applied on to lower incisors teeth with separator orthodontic device by (Medessy®). The dose of flavonoid-isolated soy milk administered to guinea pigs is divided into 2 doses, namely 3.12 g/kgBW containing 2.5 g protein and 6.25 g/kgBW containing 5 g protein so that each guinea pig receives the same amount of protein. Furthermore, the preparation of soy milk by mixing soy milk flavonoid isolate powder (Natlab®) with 50 ml of mineral water is then mixed thoroughly and placed in a measuring cup and given to group I as much as 6.25 g/kgBW, group II is given as much as 3.12 g/kgBW, group III as the control group was only given mineral water.

The observational action was to measure the distance of the movement of the guinea pig teeth within 21 days, where the measurements were carried out on days of 7, 14, and 21. The study was conducted for 21 days and measured every seven days. Its focus was on the timing of tooth movement in orthodontics, which is divided into phases. The initial phase occurs immediately after the application of force to the teeth, while the lag phase occurs after two to three weeks of applying force to the teeth. The distance formed in all the groups was observed by researchers and the greater distance of tooth movement performed in the treatment group indicates the effectiveness of soy milk in assisting the process of bone remodeling in orthodontic treatment. Every 7th, 14th, and 21st day the movement of the guinea pig's teeth will be measured using a 0.01 accuracy caliper (Vernier®) and data is recorded.

The first analysis was done for normality of the data using the Shapiro Wilk and homogeneity using the Levene test. Furthermore, since the data is normally distributed and homogeneous (p value > 0.05), the data continued to be analysed with the One-Way ANOVA statistical test and the Mann – Whitney test for comparisons between groups.

RESULTS

The results of this study were obtained from guinea pigs that had been adapted for 7 days and then treated for 21 days, the distance between the guinea pig teeth was measured on the 7th, 14th and 21st days. Measurements were carried out using the same method, with a caliper to measure the distance of the movement of the guinea pig mandibular incisor teeth from the mesial left incisor to the mesial right incisor (Figure 1). Data description of the mean value, and standard deviation (SD) of the distance of the guinea pig's tooth movement on day -7, day -14, and day -21 can be seen in Table 1. Table 1 is the average distance of guinea pig teeth after being treated. The results showed that on the 7th day, the 6.25 g/kgBW dose group had the highest average shift of 0.751 mm and the smallest shift in the control of 0.387 mm. Observations on the 14th day of the group dose of 6.25 g/kgBW has the highest average shift of 2.229 mm and the smallest shift in the control of 1.080 mm. Further observations on the 21st day of the group dose of 6.25 g/kgBW has the highest average shift of 3.226 mm and the smallest shift in the control of 1.444 mm. Based on table 4.1 shows that soy milk flavonoid isolate with dosage 6.25 g/kgBW has the highest average shift value for each day, while the control group has the lowest average shift value. On Day 7, Day 14 and Day 21 each group experienced an increase.

Figure 1. An Orthodontic rubber ring on the guinea pig's lower incisors.

Table 1. Mean and standard deviation of the distance between guinea pig teeth in each treatment group based on day 7, day 14, and day 21 in millimeters (mm)

Group	N	mean ± SD	Min	Max
Day 7				
Dosage 6.25g/kgBW	8	0.751±0.357	0.10	1.30
Dosage 3.12g/kgBW	8	0.612±0.298	0.30	1.10
Control	8	0.387±0.223	0.10	0.70
Day 14				
Dosage 6.25g/kgBW	8	2,229±0.732	1.01	3.03
Dosage 3.12g/kgBW	8	1.675±0.745	1.02	3.01
Control	8	1,080±0.390	0.70	2.00
Day 21				
Dosage 6.25g/kgBW	8	3,226±0.589	2.09	4.01
Dosage 3.12g/kgBW	8	2,221±0.643	1.03	3.04
Control	8	1,444±0.445	1.05	2.09

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the 6.25 g/kgBW dose group had the highest average shift of 0.751 mm and the smallest shift in the control of 0.387 mm. Observations on the 14th day of the group dose of 6.25 g/kg has the highest average shift of 2.229 mm and the smallest shift in the control of 1.080 mm. Further observations on the 21st day of the group dose of 6.25 g/kg has the highest average shift of 3.226 mm and the smallest shift in the control of 1.444 mm. Based on table 4.1 shows that soy milk flavonoid isolate with dosage 6.25 g/kgBW has the highest average shift value for each day, while the control group has the lowest average shift value. On Day 7, Day 14 and Day 21 each group experienced an increase.

The results of measuring the distance of the movement of the guinea pig's teeth were then carried out statistical analysis. Before statistical analysis is carried out, it is necessary to carry out a normality test using the Shapiro Will Test to find out whether the measurement results are normally distributed or not. Table 4.2 shows the 7th day for all groups, 14th day for the 6.25 g/kgBW and the 21st day for the 3.12 g/kgBW group and 6.25 g/kgBW obtained the results of normally distributed data (p value> 0.05). On Day 14 for the 3.12 dose g/kgBW and the control group is also the 21st day for the group control obtained data not normally distributed (p value <0.05).

Table 2. Test results of the Shapiro Wilk normality test and homogeneity test using the Levene test

Group	p-values (Normality)	p-values homogeneity	Interpretation
Day 7			
Dosage 6.25g/kgBW	0.862*	0.614**	Parametric
Dosage 3.12g/kgBW	0.387*		
Control	0.399*		
Day 14			
Dosage 6.25g/kgBW	0.174*	0.043	Non parametric
Dosage 3.12g/kgBW	0.046		
Control	0.001		
Day 21			
Dosage 6.25g/kgBW	0.790*	0.895**	Parametric
Dosage 3.12g/kgBW	0.240*		
Control	0.048		

*) data is normally distributed (p value> 0.05)
**) homogeneous data (p-values>0.05)

The results of measuring the distance of the movement of the guinea pig's teeth were then carried out statistical analysis. Before statistical analysis is carried out, it is necessary to carry out a normality test using the Shapiro Will Test to find out whether the measurement results are normally distributed or not. Table 2 shows the 7th day for all groups, 14th day for the 6.25 dose group g/kgBW and the 21st day for the 3.12 dose group g/kgBW and 6.25g/kgBW obtained the results of normally distributed data (p value> 0.05). On Day 14 for the 3.12 dose group g/kgBW and the control group is also the 21st day for the group control obtained data not normally distributed (p value <0.05).

After the normality test results are obtained, then a homogeneity test is needed to determine which statistical test to use. The homogeneity test uses the Levene test to find out whether the data is homogeneous or not homogeneous because the homogeneity test determines the statistical test that is used next. Homogeneity test results based on Table 2 data obtained on the 7th day and 14th day were obtained the data is homogeneous because the p-value >0.05, so that the interpretation becomes parametric. Therefore, the statistics used are the Kruskall-Wallis test. On the 14th day the data is not homogeneous because the p-value is <0.05, so that the interpretation becomes non parametric. Therefore, the statistics used are the One Way ANOVA test.

Table 3. The statistical test used the one way ANOVA test and the Kruskall Wallis test

Group	mean ± SD	p-values
Day 7		
Dosage 6.25g/kgBW	0.751±0.357	*0.070
Dosage 3.12g/kgBW	0.612±0.298	
Control	0.387±0.223	
Day 14		
Dosage 6.25g/kgBW	2,229±0.732	**0.006
Dosage 3.12g/kgBW	1.675±0.745	
Control	1,080±0.390	
Day 21		
Dosage 6.25g/kgBW	3,226±0.589	**0,000
Dosage 3.12g/kgBW	2,221±0.643	
Control	1,444±0.445	

*) One Way ANOVA, p≤0,05
**) Kruskall-Wallis, p≤0,05

Table 3 explains that this research was continued with the Oneway ANOVA and Kruskall Wallis tests to find out the differences between all groups. The hypothesis is Ho: a = 0 (no significant difference between groups on day 7, day 14 and day 21), or Ha: a ≠ 0 (there is a

significant difference each group on day 7, day 14 and day 21) with the test criteria, namely Ho is rejected if the significance of the P-value is <0.05.

The results of the three groups on day 7, day 14 and day 21 using the One Way ANOVA test and the Kruskall Wallis test showed that the effectiveness of soy milk flavonoid isolates on the distance of tooth movement in orthodontic treatment, namely on day 14 and day 21 21, which means that there is a significant and significant difference between the mean differences for each group on day 14 and day 21 so that it can be continued with the Mann – Whitney test. Whereas on the 7th day there was no significant and significant difference between the average differences for each group so that it could not be continued with Tukey's post hoc test.

In the paired comparison test, the results showed that on the 14th day there was a significant difference between the 3.12 dose groups g/kgBW with control group, there is a significant difference between dose 6.25g/kgBW with the control group, and There was no significant difference between groups dose 3.12 g/kgBW with a dose group of 6.25 g/kgBW.

In the paired comparison test, the results showed that on day 21 there was a significant difference between the 3.12 dose groups g/kgBW with the dose group of 6.25g/kgBW and there were significant differences between groups dose of 3.12 g/kgBW and a dose of 6.25 g/kgBW with the control group.

Table 4. Comparison differences in the distance of movement of guinea pig teeth in all groups

Day 14		
Group	Dosage 3.12g/kgBW	Dosage 6.25g/kgBW
Dosage 3.12g/kgBW		
Dosage 6.25g/kgBW	0.398	
Control	0.007*	0.007*
21st day		
Group	Dosage 3.12g/kgBW	Dosage 6.25g/kgBW
Dosage 3.12g/kgBW		
Dosage 6.25g/kgBW	0.005*	
Control	0.031	0.001*

* p<0.05

DISCUSSION

Orthodontic treatment is a treatment performed to obtain proper tooth and jaw alignment by correcting the function and aesthetics of the arrangement of the teeth such as aberrant occlusal contacts or crowding teeth.[1,4] Orthodontic treatment is carried out by moving the teeth in the desired direction by applying force or treatment to the teeth.[3] The process of tooth movement can be influenced by good nutrition, so that it can affect the speed of bone response in the bone remodeling process. Increased nutritional intake can affect the success of orthodontic treatment which can be seen by the number of osteoblast and osteoclast production which influence each other.[15] Intake of foods and nutrients that contain phytoestrogens, which will help prevent estrogen deficiency. Phytoestrogens in soybeans contain estrogen compounds and are estrogenic in nature which can help maintain the balance of bone resorption and formation.[10,11] Phytoestrogens are phytoprotein compounds that have the ability to help bone regeneration including alveolar bone, and are one of the main ingredients found in soy milk. The process of bone reshaping is a biomechanical process that underlies tooth movement and helps tooth movement in orthodontic treatment so that it can affect the distance of tooth movement.[12,14]

The effectiveness of soy milk flavonoid isolates on the distance of tooth movement can be seen in the following figure:

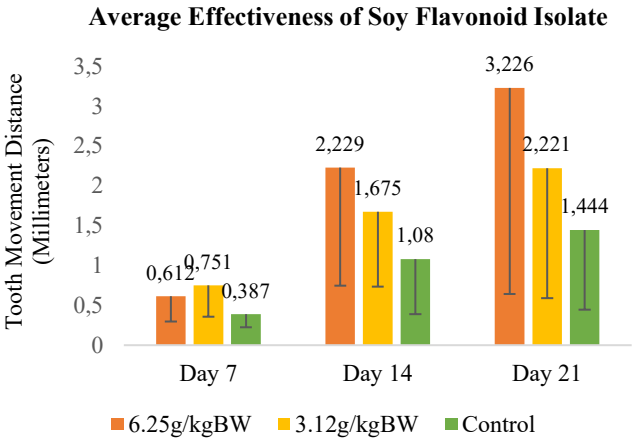

Figure 2. Increasing distance of guinea pig teeth movement on day 7, day 14, day 21 and the average.

Based on Figure 2, it can be seen that in the span of observation days there is an increase in the distance of tooth movement in guinea pigs which is getting bigger due to the application of orthodontic power which is carried out by placing rubber separators on the incisors of female guinea pigs and related to the process of bone remodeling. The results of measuring the distance of tooth movement during orthodontic treatment in guinea pigs showed that administration of soy milk isolate flavonoids at a dose of 6.25g/kgBW resulted in the greatest distance from the study group.

The greater the tooth movement distance in guinea pigs, the more proven that flavonoid isolates in soy milk at a dose of 6.25g/kgBW are effective in tooth movement in orthodontic treatment.[16] During orthodontic treatment, the estrogen hormone tends to decrease, so it requires intake of phytoestrogen nutrients which can bind to the estrogen hormone so that it can help the bone remodeling process so that the teeth can move faster, more constant and produce greater distances. Herawati et al in 2021 reported that the phytoestrogens in katuk leaves can affect the increase in osteoblasts during orthodontic treatment.[14] The results of measuring the distance of tooth movement at a dose of 3.12 g/kg BW also resulted in a larger distance than the control group because the intake of flavonoid isolates in soy milk phytoestrogens influences the process of bone remodeling through increasing the number of osteoblasts. The distance measurement results in the 3.12g/kgBW group were not as large as the 6.25g/kgBW group. This can be influenced by the lower levels of flavonoid isolates so that the binding of phytoestrogens with estrogen hormones is not as large as the 6.25g/kgBW group in assisting the process of bone remodeling and tooth movement in orthodontic treatment.[12,17,18]

The One Way ANOVA test were carried out to determine the effectiveness of soy milk in influencing the distance of guinea pig teeth during orthodontic treatment which is influenced by the bone remodeling process in increasing osteoblast production and decreasing osteoclasts and obtained significant results. This is in line with research conducted by Samantha (2021) and stated that consuming soy milk within three months of postmenopausal mothers who are at risk of osteoporosis has an effect on increasing bone density due to the isoflavone content in soy milk, and also research conducted by Chiuman et al. who stated that the phytoestrogens in soy milk can affect the production of osteoblasts and osteoclasts in the production of bone remodeling during orthodontic treatment and affect the distance of tooth movement.[13,19]

Flavonoid isolates of soy milk can accelerate the process of bone remodeling in orthodontic treatment because the phytoestrogens contained in soy milk can function as natural estrogen hormones derived from plants that can affect the production and activity of osteoblasts and osteoclasts, in accordance with the function of the hormone estrogen as an enhancer of bone cell regeneration. Recently, by increasing osteoblasts and decreasing osteoclasts during the bone remodeling process, soy consumption in orthodontic treatment can affect the biomechanical process of tooth movement.

CONCLUSION

Based on the results of research conducted by researchers, it can be concluded that soy milk flavonoid isolates (*Glycine max* (L.) Merill) doses of 6.25g/kgBW and doses of 3.12g/kgBW are effective on the distance of teeth movement in female guinea pigs in orthodontic treatment. The most effective soy milk (Glycine max (L.) Merill) on the distance of teeth movement of female guinea pigs in orthodontic treatment is a dose of 6.25g/kgBW. Male guinea pigs also possess estrogen, a sex hormone, albeit in smaller quantities than females. Consequently, administering soy milk flavonoid isolates (*Glycine max* (L.) Merill) to male guinea pigs can also influence the movement of guinea pig teeth, although to a lesser extent than in females.

CONFLICT OF INTEREST

No potential conflict of interest was reported by the authors.

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