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The Effect of Giving Wungu Leaves Extract (*Graptophyllum Pictum L. Griff*) on the Decrease in the Number of Osteoclasts in the Post Extraction Socket of Male Wistar Rats

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Abstract

In the process of wound healing after tooth extraction, there was resorption of damaged alveolar bone by osteoclasts so that new bone formation could occur. When the resorption process has ended, the activity of osteoclasts needs to be inhibited so that excessive bone resorption did not occur so that it could restore the function of the alveolar bone in supporting the teeth and protecting important structures in the bone.

Wungu (purple) leaves extract (Graptophyllum pictum (L). Griff) was known to have the main content of flavonoids, tannins, alkaloids, and saponins which have anti-inflammatory and antioxidant properties. These compounds could inhibit the formation of osteoclasts and improve the healing process of the socket. This type of research was experimental laboratory with a research group consisting of 6 groups, namely 3 control groups given aqua solution and 3 treatment groups given 10% wungu leaves extract. Each group was divided into observation groups on the 7th, 14th, and 21st days. The rats were then decapitated and the left lower jaw was taken for preparation then stained using Hematoxylin-Eosin followed by observation of the preparations in 3 fields of view.

The data for calculating the number of osteoclasts were tested by using one way ANOVA test and LSD test and there were significant differences between groups. The content in wungu leaves extract had a synergistic effect on reducing the number of post-tooth osteoclasts by blocking the RANK and RANKL bond so that new bone formation could occur immediately.

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Introduction

Tooth extraction was a process of removing the tooth from the alveolus. This action was one of the treatment actions that were often carried out by dentists that involve soft tissue and hard tissue.[1] Tooth extraction was performed when the tooth could no longer be retained in the oral cavity or to support orthodontic and prosthodontic treatment planning.[2]

After the tooth extraction procedure, resorption of damaged bone on the socket wall could occur.[3] Steiner *et al.,* (2008) reported that in the wound healing process there was resorption of damaged bone in the socket wall and alveolar *crest*. Osteoclasts have an important role in resorption of damaged bones so that new bone formation by osteoblasts could occur.[4,5,6]

When the resorption process has ended, differentiation and activity of osteoclasts needs to be inhibited so that excessive bone resorption did not occur. Excessive resorption could cause the alveolar bone to become brittle, the bone formation process to be slow, and the height and thickness of the alveolar bone could be reduced. This of course could be a problem, especially if it requires prosthodontic treatment. In addition, if bone healing was disturbed, the alveolar bone could not carry out its function to support other teeth around the extracted teeth, as well as protect important structures in the jawbone.[7] Therefore, we need a drug substance that could inhibit osteoclast activity so that excessive alveolar bone resorption did not occur.

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At this time, traditional medicine in the form of herbal medicines was in great demand by the public. Herbal medicine was thought to have relatively low side effects, and there are various useful ingredients in one plant so that it could provide more than one pharmacological effect.[8,9,10] One of the herbal plants that was easy to find and could grow in the lowlands and highlands so that its use could be developed was wungu leaves (*Graptophyllum pictum L. Griff)*[10].

Wungu leaves were known to have the main content of flavonoids, saponins, alkaloids, and tannins which have anti-inflammatory and antioxidant properties so that they could accelerate the wound healing process[9,10]. In addition, various studies related to the benefits of wungu leaves on the oral cavity have often been reported so that the use of this plant could continue to be developed.

The purpose of this study was to determine the effect of giving wungu leaves extract to decrease the number of osteoclasts in the post-extraction socket of male Wistar rats. The benefit of this research was to provide scientific information about the benefits of wungu leaves in the wound healing process after tooth extraction based on the number of osteoclasts so that the utilization of medicinal plants of wungu leaves could be optimized.

Materials and methods

This research was an experimental laboratory type. The wungu (purple) leaves used were *Graptophyllum pictum (L.) Griff.* The manufacture of wungu leaves extract was carried out by the maceration method to obtain a semisolid extract which was then diluted with a concentration of 10%.[11,12,13]

The number of samples used were 24 rats which were divided into 2 groups, namely the control group which was given aqua solution and the treatment group which was given 10% wungu leaves extract with each group being divided into 3 more groups, namely the observation group on the 7th day of observation, 14th, and 21st. The Wistar rats used had no anatomical abnormalities, and had the same weight and age range.[4,5,11,13]

Wistar rat tooth extraction was carried out on the left mandibular molar using a simple extraction method. After that, 10% wungu leaves extract was administered to the treatment group and aqua solution to the control group was administered orally using a gastric probe with a frequency of 1 time a day for 7, 14, and 21 days. Furthermore, histology preparations and Hematoxylin-Eosin (HE) staining were carried out. Observation and counting of osteoclasts were carried out under a microscope with a magnification of 400x and carried out by 3 observers. The number of osteoclasts were determined by calculating the average number of osteoclasts from 3 fields of view for each sample.[4,5,12,13]

The data obtained were analyzed using *Statistical Product and Service Solutions* (SPSS). Then the normality test was *carried* and the homogeneity test with the *Levene test*. The data obtained were normal and homogeneous so that it could be continued with the *one-way ANOVA* test and the LSD test. A significance value of less than 0.05 means that there were significant differences in each group being compared.[14]

Results

Group	7th day (mean±SD)	14th day (mean±SD)	21st day (mean±SD
Control	3,00±7,20	4,17±4,10	2,92±3,15
Treatment	4,25±3,99	2,92±2,08	1,78±6,07

Table 1. The results of the calculation of the average number of osteoclasts and the value of the standard deviation.

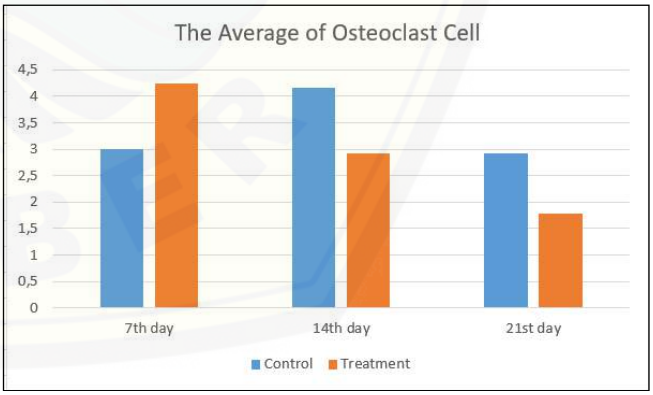

Figure 1. Graph of the average number of osteoclasts in each control group and the 7th, 14th and 21st day of treatment.

The highest osteoclast was in the 7th day treatment group. Based on the graph, it was also known that the control group experienced an increase from the 7th day to the 14th day and decreased on the 21st day. While in the

treatment group has increased on the 7th day and decreased on the 14th and 21st day.

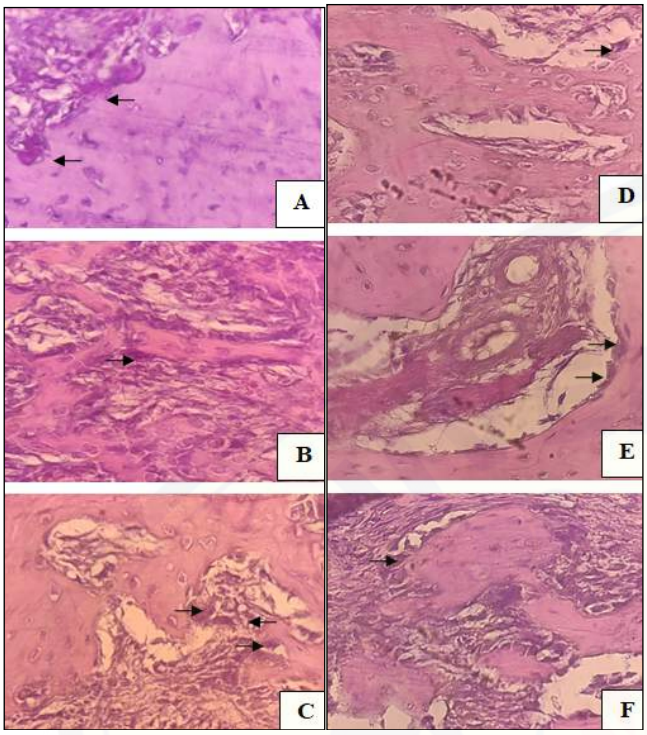

Figure 2. Microscopic view showing osteoclasts (black arrow) with HE staining and 400x magnification in the control group on the 7th day (A) the treatment group on the 7th day (B) the control group on the 14th day (C) the treatment group day 14th (D) control group day 21 (E) treatment group day 21st (F)

Discussion

Many studies have declared that wungu leaves (*Graptophyllum pictum (L.) Griff)* as medicine herbal that bioactive compound have anti-inflammatory, antioxidant, antibacterial and bone healing properties[10,11,13,14]. The 10% concentration was chosen based on previous research demonstrated that *Graptophyllum pictum L. Griff* leaves extract 10% could decrease osteoblasts and increase osteoblasts in gingival Wistar rats induced by *Porphyromonas* gingivalis effectively[13,14].

On the 7th day after tooth extraction, the treatment group had a higher average number of osteoclasts than the control group. The results of the LSD test showed that there was a significant difference between the treatment group and the control group. This indicates that the resorption of damaged bone in the treatment group occurred faster than the control group. Caused on the day 7 *woven bone* start performing from the base of the socket. In addition, osteoclasts were also seen around the socket wall to absorb damaged bones[4,5,15]

On the 14th day after tooth extraction, *woven bone* will be replaced with *lamellar bone* by osteoblasts. At this stage the regulation of the activity of osteoclasts and osteoblasts were important where osteoclasts could resorption bone and then osteoblasts could form new bone[4,14] The results of the LSD test on day 14 showed that there was a significant difference between the treatment group and the control group. The treatment group had a lower mean number of osteoclasts than the control group, indicating that bone formation occurred first in the treatment group. This was in accordance with the results of research by Vieira *et al* (2015) where normally the number of post-extraction osteoclasts could increase on day 14 and in the second week this is the peak of alveolar bone resorption[16].

On the 21st day after tooth extraction, it was seen that the bone would condense into bone trabeculae. At this stage, the osteoblasts have started to get trapped in the bone trabeculae and the osteoclast activity could decrease.[15,16] The results of the LSD test on day 21 showed that there was a significant difference between the treatment group and the control group. The treatment group had a lower mean number of osteoclasts than the control group. While the control group had a higher average number but still lower than the previous day, namely day 21. This shows that in the control group the osteoclasts have decreased while in the treatment group the osteoclasts have decreased from the previous day. This could occur because bone trabeculae have formed so that the activity of osteoclasts decreased. So it could be seen that in the control group without giving wungu leaves extract it takes longer to reduce the number of osteoclasts after tooth extraction.

Giving 10% wungu leaves extract to the treatment group on days 14 and 21 showed the number of osteoblasts was lower than the control group significantly. Quercetin was a flavonoid that has been identified from wungu leaves extract where quercetin could bind to TLR2 receptors on the surface of macrophages[11]. This binding was through the ERK 1/2 and JNK

signaling pathways which were members of the MAPK superfamily[4]. Macrophages through this signaling pathway could activate the transcription factor NFkB to decrease the secretion of pro-inflammatory cytokines such as TNF-α and IFN-ÿ[4,13,14]. This inhibition of pro-inflammatory cytokine secretion could also inhibit the differentiation of pre-osteoclasts into osteoclasts, so that the number of osteoclasts decreases in the treatment group[4,13].

Many studies said that the content in wungu leaves had several benefits. The main content of wungu leaves were flavonoids, alkaloids, saponins, polyphenols and tannins[10,11,13,14]. The synergistic effect as anti-inflammatory and antioxidant of these compounds could accelerate the healing process in soft and hard tissues[11,13,14], include of socket healing after tooth extraction[17].

An important mechanism for the wungu leaves extract in osteoclasts modulation was closely related to its role anti-inflammatory activity of these compounds is the inhibition of the enzyme phospholipase A2, cyclooxygenase, and lipoxygenase. Inhibition of these enzymes was able to prevent the migration of inflammatory cells. By preventing the migration of these inflammatory cells, it could inhibit the release of proinflammatory cytokines, namely IL-1, IL-6, TNF-α, and INF-ɣ[18] which had the potential to be stimulators of the formation of RANKL and OPG could be produced. OPG produced by osteoblasts could prevent the interaction of RANK and RANKL thereby inhibiting osteoclastogenesis. Therefore, the number of osteoclasts could decrease and new bone formation by osteoblasts could occur. Achieving a balance of resorption and bone formation was the key of the basic multicellular unit depend on the RANKL/OPG/RANK signaling system[4,19,20,21,22]

The antioxidant effect contained in the leaves works through 2 mechanisms, namely directly and indirectly. The direct mechanism was to donate hydrogen ions so that it could neutralize the toxic effects of free radicals. While the indirect mechanism was by increasing the expression of genes that play a role in the synthesis of endogenous antioxidant enzymes. Both of these antioxidant mechanisms could prevent cell death.[23,24] Antioxidants also had an important role in maintaining normal bone remodeling processes and protecting bone health by reducing inflammation and could induce osteogenesis.[22,25]

Conclusions

Based on the results of research that had been carried out, it could be concluded that wungu leaves extract had an effect in increasing the number of osteoclasts on the 7th day, then decreased on the 14th day, and decreased again on the 21st day in the socket area after tooth extraction male Wistar rat (*Rattus norvegicus)*.

Declaration of Interest

The authors report no conflict of interest.

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