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Who has more potential, Chlorella vulgaris 5% ointment or PRP preparation as a medicine for healing oral cavity wounds?

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ABSTRACT

Background: This study was conducted to analyze the effect of *platelet -rich plasma (PRP)* and chlorella vulgaris 5% ointment on the healing process and bone remodeling in the tooth sockets of experimental animals. We know that PRP is taken from the blood of experimental animals, then detrifused and re-inserted into the tooth sockets of experimental animals. The success is very high because it comes from oneself; the penning reaction is very small. Chlorella vulgaris comes from a single-celled aquatic plant also known as micro and, according to previous research, has properties as a wound healer. Use in the oral cavity is safe because it does not give toxic reactions and is widely used as a food supplement.

Methods: *Chlorella vulgaris* extract was prepared in 5% ointment preparation. *Platelet rich plasma* (PRP) was prepared from fresh blood taken from experimental animals and then centrifuged. A total of two *landrace* pigs were extracted so that 12 tooth sockets were available, then divided into two groups, namely the socket group with PRP and the socket group with Chlorella vulgaris ointment 5%. On days 0, 7, 14, and 21, IL-1 was examined, and on day 22, jaw bone tissue was sacrificed for scanning electron microscope (SEM) examination to see new bone formation in the tooth socket.

Results and discussion: IL-1 levels were examined from day 0 to day 21. Between days 0-7, it was seen that the decrease in IL-1 value was more in the PRP treatment sample by 0.35 than Chlorella vulgaris 5% ointment by 0.1. Likewise, on observation days 7-14, the decrease in IL-1 values was more in the PRP treatment sample of 0.39 compared to Chlorella vulgaris 5% ointment of 0.19. While on the observation of days 14-21, the sample given Chlorella vulgaris 5% ointment experienced a greater decrease with 0.62 while PRP with 0.41. SEM examination on day 22 showed the results of new bone formation in the tooth sockets treated with PRP and Chlorella vulgaris 5% ointment.

Conclusion: PRP and Chlorella vulgaris 5% ointment have an effect on the healing process and bone remodeling in tooth sockets because they have an effect on bone healing and remodeling, but the PRP material is proven to work faster.

Keywords: *Chlorella vulgaris* 5% *ointment*, PRP, bone remodeling, IL-1

INTRODUCTION

Platelet Rich Plasma (PRP)

Platelet-rich plasma (PRP) is a platelet preparation concentrated from the patient's own blood to accelerate tissue healing by releasing various growth factors that are essential for the regenerative process. PRP is produced through two main methods: centrifugation with two stages (soft spin and hard spin) and the Buffy Coat method, each offering a different approach to separate platelets from other blood components. These methods allow the production of PRP with a high concentration of platelets.The low plasma content of PRP makes it effective in medical applications such as wound healing, dental implant placement, and graft procedures. The advantages of PRP include its ability to reduce the risk of infection due to the use of autologous blood, its rapid preparation process, as well as its easy application in combination with bone graft materials and various surgical procedures.1-5

However, the use of PRP also has some disadvantages. The process of making PRP requires good clinical skills and efficient centrifugation equipment and may involve additional costs for procedures and equipment. In addition, the effectiveness of PRP may be affected by certain medical conditions, such as thrombocytopenia, use of certain medications, and active infections. Variability in the concentration of platelets and growth factors can also affect therapeutic outcomes, making it important to conduct more in- depth clinical evaluations to ensure the consistency and effectiveness of PRP in various medical applications.

Chlorella vulgaris

Chlorella vulgaris is a unicellular eukaryotic microalgae of the *Chlorellaceae* family known for its bright green color. This microbe has a diameter of 2-10 micrometers and functions as photosynthesis with the help of chlorophyll a and b pigments. Chlorella vulgaris also contains carotenoids such as lutein, which plays an important role in photosynthesis and provides various therapeutic benefits such as antioxidant protection and immune system support. This microalgae has a typical structure, can live in colonies in humid and aqueous environments, and reproduces asexually with a high growth rate. Its metabolic adaptations include autotrophic, heterotrophic, mixotropic, and photoheterotrophic, enabling effective growth under various conditions [7,8,9.10]

Chlorella vulgaris is rich in essential nutritional components, including proteins, fats, carbohydrates, and pigments such as chlorophyll and carotenoids. Protein is the main component in this microalgae, playing a role in various cellular functions and tissue maintenance. Fat content, especially glycolipids and phospholipids, as well as carbohydrates such as starch and cellulose, provide significant nutritional value. Chlorophyll and carotenoid pigments not only support photosynthesis but also have health benefits, including cell repair and protection against disease. In addition, Chlorella vulgaris produces Chlorella Growth Factor (CGF), which supports tissue growth and recovery.[11-15]

In medical applications, Chlorella vulgaris is used in various preparations, including ointments. Ointments are external preparations with a semi-solid consistency that are used on the skin and mucous membranes. Ointments from Chlorella vulgaris are designed to be stable, soft, easy to wear, and have even drug distribution. The quality of the ointment depends largely on the ointment base used, which must be physically and chemically compatible with the drug it contains. The ointment base should be stable, non- irritating, and able to release the drug effectively to the treated area. The ointment is used to treat various

skin conditions, such as hyperkeratosis and lichenification, and is not recommended for use on acute inflammations or hairy areas. The quality of the ointment ensures therapeutic effectiveness while facilitating application to the skin and mucous membranes. [11,12,13.]

RESEARCH METHODOLOGY

This research is experimental laboratory research with a quasi-experimental post- test group design. Conducted at the STIFA Pharmacy Laboratory (for the preparation of Chlorella vulgaris ointment). Anatomical Pathology Laboratory of UNHAS Animal Hospital (for centrifugation of pig blood for PRP). Veterinary Clinic "Doc Pet Clinic" for implant placement in test animals. UMI Faculty of Engineering microstructure laboratory for scanning electron microscope (SEM) examination. The research was conducted in July 2023. The samples used in this study were male landrace pigs aged more than 3 months, weighing approximately 30 kg, obtained from a pig breeding place in Moncongloe Maros. The number of samples used was 12 Landrace Pig teeth.

Preparation of *Chlorella vulgaris* Extract

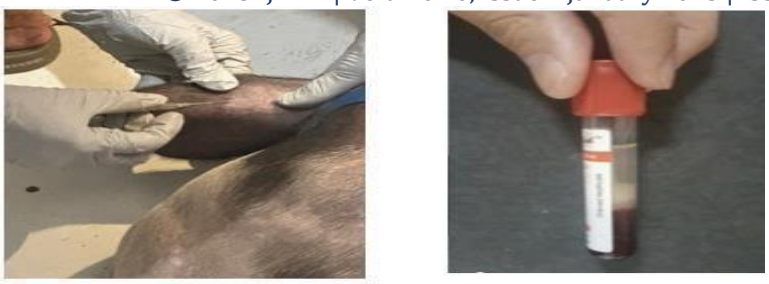
The research procedure includes making Chlorella vulgaris extract, then drying it with an herb dryer, and then using the maceration technique to obtain the filtrate. Furthermore, the finished extract is made into an ointment preparation.

Preparation of 5% concentration ointment:

1. Prepare tools and materials for the preparation of ointment base.
2. Weigh the ingredients, such as 5gr chlorella vulgaris extract, 80 gr white vaseline, and 20 gr lanolin.
3. Pour the ointment base ingredients, namely white vaseline and lanolin, which have been weighed into the mortar.
4. Stir the ointment base ingredients until homogeneous.
5. Then put the extract powder into the homogeneous base material and stir until homogeneous.

Preparation of Platelet Rich Plasma (PRP)

Blood was drawn from the ear of the experimental animal (landrace pig) and placed in a 6 ml sterile vacuum tube with anticoagulant.

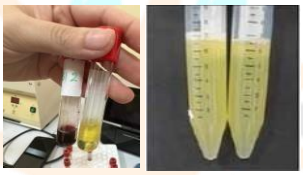


Pic.1 Blood collection of experimental animals (source of personal documents)

The first centrifugation was performed at a soft spin speed of 420 g for 16 minutes, which will produce three layers: platelets and white blood cells in the upper layer, white blood cell-rich plasma supernatant in the middle layer, and red blood cells in the lower layer.

Plasma supernatant with platelets and white blood cells transferred to another tube without anticoagulant

Image Plasma supernatant with platelets and white blood cells transferred to another tube



(Source: personal documentation)
for PRP screening

Second centrifugation at a higher speed (hard spin) of 800 g for 15 minutes, which will make the platelets concentrated in the bottom 1/3 of the tube, while 2/3 of the tube is Platelet Poor Plasma (PPP).

The PPP layer is separated from the PRP and removed from the tube, then the tube is shaken gently so that the platelets under the tube will mix and produce a PRP concentration.

Picture of PRP that is ready to use (Source: personal documentation)

Animal Test Treatment

All animal handling and surgical procedures were carried out in accordance with the 3R principles (replacement, reduction, refinement) and the animal center guidelines for the care and use of laboratory

animals and approved by the ethics committee with number 0112/PL.09/KEPK FKG-RSGM UNHAS/2023. Before entering the surgical stage of implanting implants in the jawbone of pigs, pig blood was first taken and then centrifuged to obtain platelet-rich plasma (PRP) and then disinfected with betadine in the area to be implanted.

Landrace pigs are sedated using zoletil 2-5 mg/kg BW via intramuscular and intravenous injection, followed by gas induction and maintenance using isoflurane (2-3%) and O2 3L/min) and fluids are given intravenously and monitored regularly by the veterinary team.

Sedation procedure and preparation for surgery on experimental animals (Source: personal documentation)

Figure 4. Sedation procedures and preparation for surgery on experimental animals (Source: personal documentation)

Disinfection of the surgical area using betadine. Local anesthesia with pehacaine, . Pig 1 extracted 2 left maxillary 2nd incisors, 2 2nd incisors and 2 left mandibular 1st premolars using dental forceps, filled the sockets with PRP and Chlorellaa vulagaris 5% ointment and sutured them. Do the same with the 2nd Pig
Research animals were put in portable cages, after which IL-1 observations were made on days 0, 7, 14 and 21 and SEM photographs were taken on day 22.

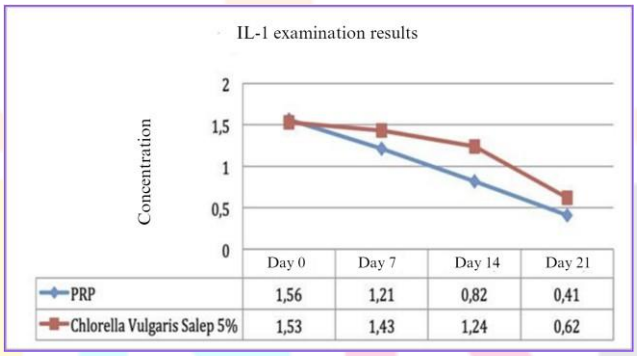
RESEARCH RESULTS

The study was conducted using 2 male landrace pigs, aged 3-6 months, weighing at least 30 kg. Each test animal consisted of 2 samples, namely a sample group of dental implants in pigs coated with Platelet Rich Plasma (PRP) and implants coated with 5% Chlorella vulgaris ointment extract as a treatment group. The manufacture of 5% Chlorella vulgaris ointment extract was carried out at the STIFA Pharmacy Laboratory, while the manufacture of PRP was carried out at the Anatomical Pathology Laboratory of the UNHAS Animal Hospital. Implant placement, blood collection, PRP coating, and 5% Chlorella vulgaris ointment extract were performed at the "Doc Pet Clinic" veterinary clinic. IL-1 cells were observed and counted gradually on days 0, 7, 14 and 21 after implant placement of both PRP-coated and 5% Chlorella vulgaris ointment-coated implants, and an electron microscope (SEM) examination was performed.

The research data were analyzed with the help of the IBM SPSS Statistics data analysis program. The data analysis used in this study used the Kolmogorov-Smirnov test. The statistical test results of the p value > 0.05 mean that the data is normally distributed or vice versa. For normally distributed data, parametric statistics can be used, namely the anova and T test, for data that is not normally distributed, using the mann- Whitney test to compare between groups and the Friedman test to test changes that occur in each repetition of observations.

Based on the Kolmogorov-Smirnov normality test, the Asymp. Sig. (2-tailed) of 0.200 indicates that the residual values are normally distributed (p > 0.05). The heteroscedasticity test using the Breusch- Pagan Test method produces a significance value of 0.297, which also indicates that no heteroscedasticity occurs (p > 0.05). With both assumptions met, the data can be analyzed using a two-way ANOVA with treatment (PRP and Chlorella vulgaris 5% Ointment) as the first factor and day (day 0, 7, 14, and 21) as the second factor.

Description : Day 0		
	Parameter	Result
Pig 1 (PRP)	IL 1	1,56
Pig 2 (Chlorella Vulgaris 5% ointment)	IL 1	1,53
Description : Day 7		
	Parameter	Result
Pig 1 (PRP)	IL 1	1,21
Pig 2 (Chlorella Vulgaris 5% ointment)	IL 1	1,43
Description : Day 14		
	Parameter	Result
Pig 1 (PRP)	IL 1	0,82
Pig 2 (Chlorella Vulgaris 5% ointment)	IL 1	1,24
Description : Day 21		
	Parameter	Result
Pig 1 (PRP)	IL 1	0,41
Pig 2 (Chlorella Vulgaris 5% ointment)	IL 1	0,62



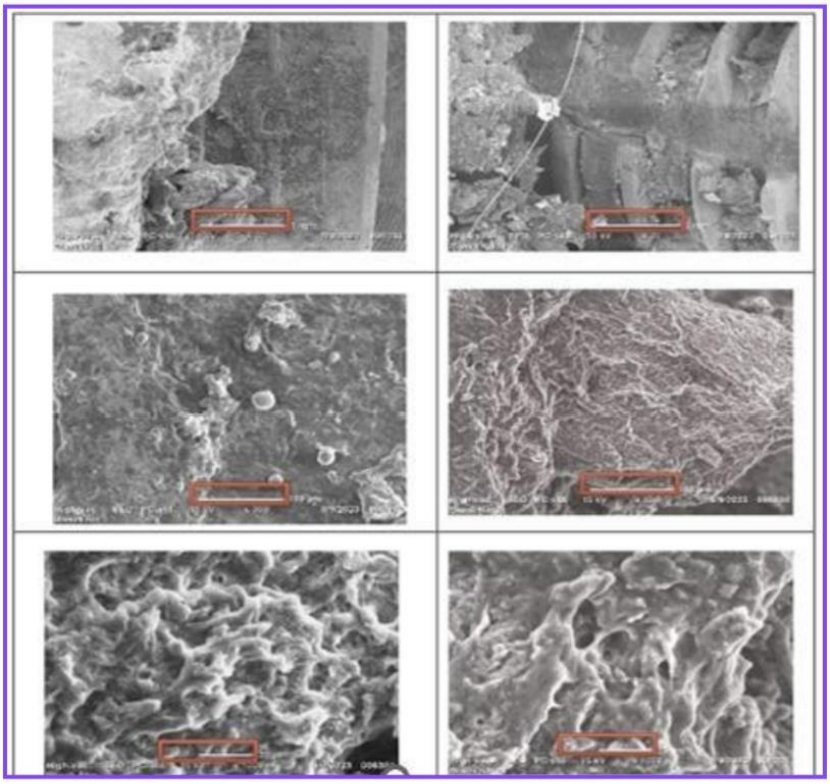
From the comparison diagram above, it can be seen that the largest decrease in the amount of IL-1 in observations from 0 to 21 days was seen in the treatment group given PRP. Between day 0 to day 7, it was seen that the decrease in IL-1 value was more in the PRP treatment sample of 0.35 compared to Ch;orella vulgaris 5% ointment of 0.1. Likewise, on observation days 7 to 14, the decrease in IL- 1 value was more in the PRP treatment sample of 0.39 than Chlorella vulgaris 5% ointment by 0.19.

intervention (CV 5% ointment) intervention (PRP)

30x magnification

300x magnification

1000x magnification



SEM on the group coated with 5% CV Ointment and coated with PRP on day 22 (Source: personal documentation)

Table The average Bone Implant Contact (BIC) value for implants coated with Platelet Rich Plasma (PRP)

Tooth	BIC Value
Maxillary Right Incisivus	6,42
Maxillary Right Incisivus	6,43
Mandibular Right Incisivus	4,02
Mandibular Right Incisivus	5,51
Mandibular Right First Premolar	6,22
Mandibular Right First Premolar	6,49

Table The Average Bone Implant Contact (BIC) value for implants coated with Chlorella Vulgaris Ointment 5%

Tooth	BIC Value
Maxillary Right Incisivus	3,89
Maxillary Right Incisivus	4,15
Mandibular Right Incisivus	3,11
Mandibular Right Incisivus	4,27
Mandibular Right First Premolar	5,06
Mandibular Right First Premolar	5,12

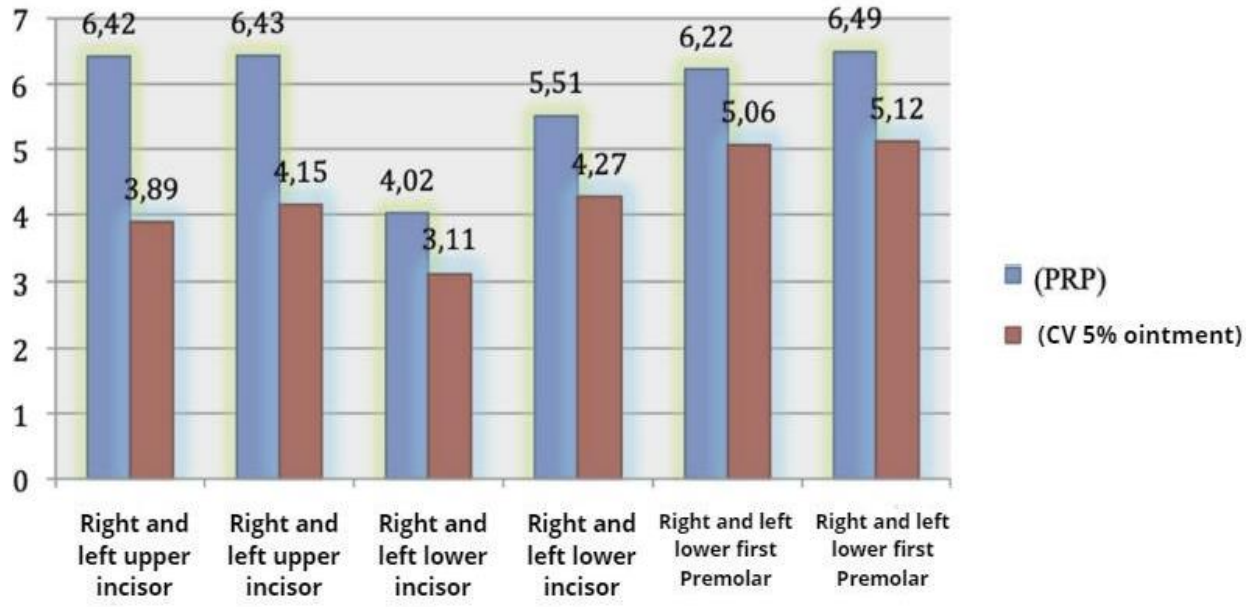


Table 5.7 Significant difference value between the weight of PRP Chlorella Vulgaris Ointment 5%

Intervention	PRP	Chlorella Vulgaris Ointment 5%
	(Mean±SD)	(Mean±SD)
Interleukin 1	5,848±0,967[a]	4,267±0,755[a]
P-Value	0,010*	

* Independent T Test **p<0,05

DISCUSSION

This study aimed to evaluate the effect of platelet rich plasma (PRP) coating and chlorella vulgaris 5% ointment on the bone remodeling process after dental implant placement. In this study, the test animals were divided into two treatment groups: the first group was given PRP coating, while the second group was given chlorella vulgaris 5% ointment. The study was designed to determine the effectiveness of these two types of coating materials in improving the quality and speed of bone formation around the implant. The use of these coatings is expected to improve implant osseointegration by accelerating the healing process and strengthening the overall stability of the implant, thus leading to better clinical outcomes.[13,14,16]

The application of PRP on the implant surface is a technique that aims to accelerate bone formation and improve implant stabilization. PRP contains various important growth factors, such as Platelet-Derived Growth Factor (PDGF), Transforming Growth Factor-beta (TGF-β), and Vascular Endothelial Growth Factor (VEGF). These factors play a crucial role in various stages of bone healing, including mitogenesis (cell proliferation), chemotaxis (cell movement), and cell differentiation. PRP can stimulate osteoblasts to form new bone and improve the quality of existing bone defects, thus accelerating the post-implant healing process. This study shows that the use of

PRP in implants can improve the quality and speed of osseointegration by stimulating more efficient bone healing mechanisms.[17,18,19,20]

In contrast, the use of chlorella vulgaris 5% ointment as a bioactive ingredient provides an alternative approach to enhance the healing process. The ointment contains active components such as beta-carotene and chlorellin, which have antiproteolytic and anti-inflammatory properties. These components improve tissue integrity and stimulate fibroblast formation, which is important in the wound healing process. In addition, chlorella vulgaris has antibacterial effects that can reduce inflammation and support the process of new tissue formation. By increasing the activity of leukocyte cells and accelerating the proliferation of fibroblasts and endothelial cells, chlorella vulgaris supports the formation of new bone tissue around the implant, having a positive effect on the wound healing process.[21,22,23,24]

The results showed that the use of PRP and chlorella vulgaris 5% ointment did not show a significant difference in interleukin-1 (IL-1) concentrations, indicating that PRP and chlorella vulgaris 5% ointment did not have a significant difference in interleukin-1 (IL-1) concentrations. that both coatings had no significant immunostimulating effects and were relatively safe for long- term use. However, analysis of new forceps bone formation showed significant differences between sockets treated with PRP and chlorella vulgaris 5% ointment, with PRP showing better results in terms of new bone formation around the socket. Limitations of this study include the

observation duration of only 22 days.[25,26,27,28]

CONCLUSIONS

The administration of Platelet Rich Plasma (PRP) and 5% Chlorella vulgaris ointment to the tooth socket showed decreased interleukin (IL-1) levels and increased bone formation on the 22nd day of observation, indicating that both materials accelerate the initial phase of bone remodeling. However, PRP was shown to work faster in the healing process, as it is derived from the patient's own blood, which reduces the risk of infectious disease transmission and immune reaction compared to chlorella vulgaris ointment.

Conflict of Interest

No conflict of interest

Acknowledgments

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