



## Comparison of the Effectiveness of Dermapen Using Plasma from Platelets with Dermapen on Pockmarked Skin Surface of Female Wistar Rats (*Rattus Norvegicus*)

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### Abstract

Various treatments can cure pockmarks. First, you have to know the cause of the pockmarks and the strength of the pockmarked skin, making it easier to choose the treatment, one of which is dermapen, which uses Plasma from platelets. Test and compare the effectiveness of the derma pen action using Plasma from platelets with the derma pen action on the pockmarked skin surface of female Wistar rats (*Rattus Copernicus*). This research is a laboratory experiment to compare the effectiveness of the dermapen action using Plasma from platelets with the dermapen action on the pockmarked skin surface of female Wistar rats (*Rattus norvegicus*) using a post-test with control group design or control samples based on treatment groups to analyze the dermapen action using Plasma from platelets with dermapenic action on the surface of rat skin pockmarks. The results showed that the dermapen treatment group with Plasma from platelets at a dose of 10% was more effective in healing cut wounds in white mice than the dermapen treatment group using Plasma from platelets at doses of 2.5% and 5%, respectively. This is because, at a concentration of 10%, blood plasma metabolite compounds from platelets that are applied to wounds already affect the wound. The effect only inhibits microorganisms at small doses, making it less effective in healing wounds. It is recommended that the minimum dose be 10% so that the metabolite compounds in the Plasma from platelets have a healing effect on wounds.

### Introduction

Various treatments can cure pockmarks. First, you have to know the cause of the pockmarks and the strength of the pockmarked skin, making it easier to choose the treatment, one of which is dermapen, which uses Plasma from platelets. Test and compare the effectiveness of the derma pen action using Plasma from platelets with the derma pen action on the pockmarked skin surface of female Wistar rats (*Rattus Copernicus*). This research is a laboratory experiment to compare the effectiveness of the dermapen action using Plasma from platelets with the dermapen action on the pockmarked skin surface of female Wistar rats (*Rattus norvegicus*) using a post-test with control group design or control samples based on treatment groups to analyze the dermapen action using Plasma from platelets with dermapenic action on the surface of rat skin pockmarks. The results showed that the dermapen treatment group with Plasma from platelets at a dose of 10% was more effective in healing cut wounds in white mice than the dermapen treatment group using Plasma from platelets at doses of 2.5% and 5%, respectively. This is because, at a concentration of 10%, blood plasma metabolite compounds from platelets that are applied to wounds already affect the wound. The effect only inhibits microorganisms

at small doses, making it less effective in healing wounds. It is recommended that the minimum dose be 10% so that the metabolite compounds in the Plasma from platelets have a healing effect on wounds.

## Methods

Laboratory experimental research compares the effectiveness of the derma pen action using Plasma from platelets with the derma pen action on the pockmarked skin surface of female Wistar rats (*Rattus norvegicus*) (Notoatmodjo, 2022). The research design uses a post-test with a control group design or control samples based on treatment groups to analyze the action of dermapen using Plasma from platelets with the movement of dermapen on the pockmarked surface of the mice's skin.

The samples in the study used female Wistar rats (*Rattus Norvegicus*). In determining the number of samples, researchers used the 3R Principle (Replacement, Reduction, and Refinement Reduction in determining the number of research samples (Kendall et al., 2018). The research sample, namely 20 female mice, will be divided into four groups: The first group consists of five female mice that received dermapen treatment using 2.5% platelet plasma. The second group consisted of 5 female mice who received dermapen treatment using 5% platelet plasma. The third group consisted of 5 female mice who received dermapen treatment using 10% platelet plasma. The fourth group consisted of 5 female mice who only received dermapen treatment.

Variables refer to characteristics or attributes that can be measured or observed and vary among the people or organizations studied (Creswell & Creswell, 2018). The variables in this study are the objects of research observation (Suwarno & Nugroho, 2023), in this case, Independent variables: dermapen action using platelet plasma and dermapen activity. The dependent variable is pockmarked wounds on the surface of the rat's skin. In this research, researchers used tools such as rat cages, gloves, cutting knives, digital scales, sondes, markers, blenders, pipettes, 3ml syringes, feed containers, rotary evaporators, mixers, freezers, freeze driers, masks. The materials used include healthy young and old female mice with no physical defects, distilled water, 96% ethanol, 1% HCL, label paper, rat food and drink, dermapen, and platelet plasma (PRP).

The research procedures that will be carried out are Acclimation of Test Animals, Production of Platelet Plasma, Treatment of Wistar strain female mice, and Analysis. Data on the skin surface of mice from each group was then analyzed with the help of SPSS. Because the number of samples was less than 50, the statistical test used was the normality test using Shapiro Wilk and continued with the paired T-test.

## Result and Discussion

The discussion begins with a general description of the research subject and then continues with the analysis and interpretation of research data. Then, a discussion of the research results is shown. The description of the research results will also illustrate how the research problem can be answered.

### Active Substance Content in PRP (Platelet Rich Plasma)

Ten minutes before the dermapen procedure, first take blood from the rat's vein using an eight cc syringe and put it in a sterile container containing sodium citrate (9:1), an anticoagulant used for all blood from the patient. The next stage is centrifugation, which is carried out on the whole blood citrate. The process is carried out by separating Plasma and platelets from red and white blood cells, requiring initial centrifugation (soft spin) at 2100 rpm for 3 minutes.



Figure 1.1 The Blood Process Is Centrifuged For 3 Minutes at 2100 Rpm



Figure 1.2 Red Blood Cells Separated from Plasma



Figure 1.3 Blood Centrifuged for 6 Minutes at A Speed of 4000 Rpm

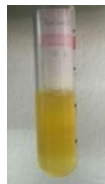


Figure 1.4 The Separation of Platelet Rich Plasma and Platelet Poor Plasma



Figure 1.5 Platelet-Rich Plasma Experiencing Precipitation and Forming a Gel

In Figure 1, the blood process is centrifuged for 3 minutes at 2100 Rpm. Separation of Plasma into PPP (platelet-poor Plasma) or upper two-thirds, and PRP (platelet-rich Plasma) or lower third, including suspended platelets. In Figure 2 are Red Blood Cells Separated from Plasma. Then, the blood plasma is taken using a syringe and transferred to a new container (tube) to be centrifuged again. The buffy coat and red blood cells are set aside. The serum was transferred to a sterile tube and spun again with a centrifuge at 4000 rpm for 6 minutes. This will produce the upper part of platelet-poor Plasma, and the lower part is called platelet-rich Plasma. Figure 3 shows blood centrifuged for 6 minutes at a speed of 4000 Rpm. Figure 4 shows the separation of Platelet Rich Plasma and Platelet Poor Plasma. After centrifugation, the resulting PRP concentrate is at the bottom of the 1-2 cc plasma tube. The resulting PRP is then taken using a syringe. Then, it is soaked using hot water (60–100° C) for one minute and a cold-water bath (8–0° C) to turn the Plasma into a thick gel, and it is ready for use. These results are shown in Figure 5, with platelet-rich Plasma experiencing precipitation and forming a gel.

Table 1. Phytochemical Test Results for PRP (Platelet-Rich Plasma)

Compound	Procedure	Result	Note
Glycogen	5 ml of sample plus ethanol plus iron (III) chloride	Blackish green colour	+
Lysosomes	0.05-gram sample + hot distilled water, shake vigorously	Foam forms	+
Serotonin	Sample + 5 ml distilled water + 2 ml HCL until acidic, filter	Orange precipitate	+
Calcium	Filtrate + 1 ml Dragendroff reagent, Sampel + Liebermann Bourchard	It forms a red-orange colour	+
Flavonoid	Sample + 5 ml distilled water + 0.1 mg powder + 1 ml alcohol solution + concentrated hydrochloric acid + amyl alcohol	A red-orange colour is formed on the amyl alcohol layer.	+

The results of the phytochemical test in Table 1 show that PRP (platelet-rich Plasma) contains positive tannin, saponin, alkaloid, triterpenoid, and flavonoid compounds. Meanwhile, several other studies state that platelets are formed from megakaryocytes and synthesized in the bone marrow (Casabona, 2018; El-Taieb et al., 2019; Everts et al., 2006; Kang & Lu, 2022; Long et al., 2020). Platelets have a ring of contractile microtubules around their exterior, containing actin and myosin. Inside the platelet, several intracellular structures contain glycogen, lysosomes, and two types of granules, namely dense granules and  $\alpha$  granules. Dense granules containing ADP, ATP, serotonin, and calcium.  $\alpha$  granules contain clotting factors, growth factors, and other proteins.

### Description of Research Results

Table 2. Mean Wound Length (cm)

Days to-	K	P1	P2	P3
2	0.96	0.88	0.79	0.73
4	0.84	0.73	0.63	0.56
6	0.75	0.62	0.51	0.43
8	0.63	0.43	0.36	0.18
10	0.49	0.22	0.16	0.09
12	0.28	0.07	0.06	0.01
14	0.07	0.04	0.02	0.00
<b>Mean</b>	<b>0.57</b>	<b>0.43</b>	<b>0.36</b>	<b>0.28</b>

The mean wound length (in cm) in the treatment group given PRP or platelet-rich Plasma at doses of 2.5%, 5%, and 10%, and the control group (only given dermapen) was measured on the first to the last day (14th day). Wound healing observations were carried out every two days for 14 days in 4 treatment groups, namely the group that received dermapen and those given PRP or platelet-rich Plasma at a dose of 2.5% (P1), PRP or platelet-rich plasma at a dose of 5% (P2), PRP or platelet-rich plasma with an amount of 10% (P3) and control treatment (K), which only involves dermapen. In the P3 treatment group, the wound healed completely and without scars on the 14th day. Meanwhile, in groups P1, P2, and K, complete wound healing had yet to occur on the 14th day, although in the P2 group, on the 14th day, the total healing rate had almost reached 0.02 cm.

Based on the average length of wounds in each group, it can also be concluded that wound healing in the P3 group occurred more quickly, followed by the P2 group. Wound healing was the slowest in the control group (K).

### Description of Normality Test

The normality test in this study used the Shapiro-Wilk SPSS normality test. This method was used because the number of data samples for each group was less than 50. So, using the Shapiro-Wilk Technique to detect data normality in this study was the most appropriate. This test was carried out to determine whether the research data was normally distributed. Data normality is essential because, with normally distributed data, the data is considered to represent the population (Ghozali, 2018). If the p-value is  $> 0.05$ , then the data is declared customarily distributed, and conversely, if the p-value  $< 0.05$ , then the data is reported as not normally distributed.

Table 3. Results of the Shapiro-Wilk Normality Test

Group	Statistics	Significance
Control (K)	0,961	0,814
Treatment 1 (P1)	0,833	0,146
Treatment 2 (P2)	0,908	0,453
Treatment 3 (P3)	0,953	0,758

Based on Table 3, the data obtained shows that the control and treatment groups for the wound length variable from day 1 to day 14 all show significant values. The significance value (p) in the Shapiro-Wilk Test is the value that exceeds the standard margin of  $p > 0.05$ , namely 0.814 for Group K, 0.146 for Group P1, 0.453 for Group P2 and 0.758 for Group P3. So, based on the Shapiro-Wilk normality test, the data is usually distributed.

### Description of Data Homogeneity Test Between Groups

The healing process of incision wounds in each group K, P1, P2, and P3, observed after 14 days of treatment in each group, was tested for homogeneity using the One Way ANOVA Test. The results show that the data variance from research variables for the control group (K), group P1, group P2, and group P3 is homogeneous or comes from a population that has the same variance, namely 0.215 ( $p > 0.05$ ).

Table 4. Results of the ANOVA Test of Homogeneity of Variances

Category	Statistic Levene	Significance
Mean	1,664	0,215
Median	0,975	0,429
Trimmed Mean	1,599	0,229

From Table 4, the results of the ANOVA test show differences in length between the four groups that underwent research or observation. Based on the data in the table, in the "Sig." The obtained p-value (p-value) is 0.000. Thus, at the actual level = 0.05, Ho is rejected, so the conclusion is that there is a significant difference in the average (mean) length of the wound based on the four groups.

### Advanced Test Description Post Hoc Test

From the previous Anova test results, data was obtained that the test results showed that Ho was rejected (there was a difference), so it was necessary to carry out further tests (Post Hoc Test). The further test (Post Hoc Test) that will be carried out will be carried out event. To determine which additional test to use, please look at/refer to the Test of Homogeneity of Variances table. The conclusion from this test is that the test results show the same variance, so the further test used is the Bonferroni Test.

Table 5. Post Hoc Bonferroni Test Results

Multiple Comparisons								
Dependent Variable: Length Wound								
(I) Group	(J) Treatment	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval			
					Lower Bound	Upper Bound		
Bonferroni	Control	1 <sup>st</sup>	.12800*	0.01432	0.000	0.0849	0.1711	
		2 <sup>nd</sup>	.18800*	0.01432	0.000	0.1449	0.2311	
		3 <sup>rd</sup>	.25400*	0.01432	0.000	0.2109	0.2971	
	Treatment 1	Control	-.12800*	0.01432	0.000	-	-	
		2 <sup>nd</sup>	.06000*	0.01432	0.004	0.0169	0.1031	
		3 <sup>rd</sup>	.12600*	0.01432	0.000	0.0829	0.1691	
	Treatment 2	Control	-.18800*	0.01432	0.000	-	-	
		1 <sup>st</sup>	-.06000*	0.01432	0.004	0.1031	0.0169	
		3 <sup>rd</sup>	.06600*	0.01432	0.002	0.0229	0.1091	
	Treatment 3	Control	-.25400*	0.01432	0.000	-	-	
		1 <sup>st</sup>	-.12600*	0.01432	0.000	0.1691	0.0829	
		2 <sup>nd</sup>	-.06600*	0.01432	0.002	0.1091	0.0229	
	*. The mean difference is significant at the 0.05 level.							

Table 5 shows the results of further tests using the Post Hoc Bonferroni Test. Comparison of group I and group J shows that the comparison between all groups indicates that there is a difference in the average length of healing of incision wounds in the Wistar strain of white rats (*Rattus norvegicus*), which is marked with an asterisk "\*." The group testing through the Bonferroni Post Hoc Test was carried out using the SPSS program.

## Discussion of Research Results

The wound healing phase consists of the inflammatory, proliferation, and maturation phases (Sorg et al., 2017). The average phase of wound healing on the first day to the 4th day in the group that was given dermapen treatment using Plasma from platelets in groups P2 (5%) and P3 (10%) occurred in the inflammatory phase, which showed a faster reduction in wound length compared to the other group. P1 (2.5%) and the control group (K) experienced the inflammatory phase until day 6. This occurs because the plasma content of platelets can regenerate cells and tissue, and the substance content can stop bleeding in wounds and act as an anti-inflammatory agent, which will influence the production of inflammatory cells in the inflammatory phase of wound healing. As an astringent, Tannin can reduce mucosal permeability, and the bonds between mucosa become more robust, thus preventing irritants. Also, Tannin affects the permeability of the mucosa and bacterial walls, so the bacteria shrink and die. The phenolic acid content in lemongrass plays a role in preventing cell damage caused by free radicals, thereby preventing inflammation and inflammatory processes.

Platelet-rich Plasma is enriched with platelets, which contain various compounds important for pharmacological effects. These platelets will release Platelet Angiogenesis GF and Growth Factors (GF), including Vascular Endothelial GF, Platelet Derived GF, Platelet Derived Endothelial GF, Fibroblast Growth Factors, Transforming GF - Beta, Insulin-like GF, Hepatocyte Growth Factor, Epidermal Growth Factor (Amable et al., 2013; Everts et al., 2006; Gentile et al., 2020; Nugroho et al., 2020; Peng, 2019; Sorg et al., 2017; Theoret & Schumacher, 2017). The primary way PRP works is through the platelets themselves by releasing various types of Growth Factors (GF) that have been activated and degranulated, which act as signal messengers to regulate multiple processes. Once activated, the growth factor  $\alpha$ -granules found in platelets will release various growth factors. After being formed from the coagulum platelets, activated thrombocytes between the fibrin strands form a matrix in the coagulum, which helps develop growth factors and eventually spreads into the surrounding tissue.

This study showed that the dermapen treatment group with Plasma from platelets at a dose of 10% was more effective in healing cut wounds in white mice than the dermapen treatment group using Plasma from platelets at doses of 2.5% and 5%, respectively. This is because, at a concentration of 10%, blood plasma metabolite compounds from platelets that are applied to wounds already affect the wound. Still, they only inhibit microorganisms at small concentrations, so they are less effective in healing wounds. This is the opinion of several studies, which state that if antibacterials are used at small concentrations, they are only inhibitory (bacteriostatic), but if used at high concentrations, they will kill microorganisms (Mohd Yusof et al., 2021; Naseem & Durrani, 2021; Shehabeldine et al., 2023; Vahdati & Tohidi Moghadam, 2020).

The results of this research are also in line with studies conducted by Peng (2019), Hesseler & Shyam (2019), Long et al. (2020), and Kelm & Ibrahim (2022), who used PRP in action research to overcome ageing on the skin (Hesseler & Shyam, 2019; Kelm & Ibrahim, 2022; Long et al., 2020; Peng, 2019). The investigation concluded that the treatment given to the patients (in the study) was combination therapy with fractional Er: YAG laser and platelet-rich plasma (PRP) 2 times. After treatment, subjective improvements were found by the doctor and the patient in the form of smoother facial skin texture and thinning of the brown spots on the cheeks. After two sessions of combination therapy observed on the 23rd day, the photoaging score, according to Glogau, was still type 3, Fitzpatrick was grade 2, and the wrinkle severity rating scale (WSRS) was also grade 2.

The research of Salem et al. (2018) and Gentile et al. (2020) also explained that transforming growth factor-beta 1 (TGF $\beta$ 1) carries out the proliferation and differentiation of mesenchymal

stem cells and encourages angiogenesis, the Epithelial Growth Factor (EGF) content in PRP increases regeneration and repair of cells experiencing inflammation or damage thereby accelerating restoration of function: the organ or tissue (Gentile et al., 2020; Salem et al., 2018). Meanwhile, Vascular Endothelial Growth Factor (VEGF) triggers chemotaxis and proliferation of endothelial cells and increases angiogenesis, vascular hyperpermeability, and cell differentiation. Basic Fibroblast Growth Factor (b-FGF), Insulin-Like Growth Factor (IGF), Adenosine Triphosphate (ATP), angiopoietin-2, fibronectin, osteocalcin, and Thrombospondin-1 (TSP-1) are growth factors released by PRP. EGF promotes cell growth in tissue experiencing necrosis. IGF is a hormone that relieves acute tubular necrosis, TGF-B1 increases anti-apoptosis, and Bcl-2 maintains epithelial homeostasis and prevents cell apoptosis. VEGF protects the peritubular endothelium, increases epithelial cell proliferation, induces angiogenesis, and promotes healing in tissues or cells.

Stress is a possible factor in the rat's body that can also influence the results, which cannot be ignored because it can affect the wound healing process. In the reference stated by Salem et al. (2018), Cusack & Buggy (2020), Kelm & Ibrahim (2022), and Decker (2021), stress can trigger an increase in cortisol, which has an impact on suppressing cellular immunity so that it can slow down wound healing (Cusack & Buggy, 2020; Decker et al., 2021; Kelm & Ibrahim, 2022; Salem et al., 2018). Furthermore, another factor that could also influence the results of this research is that the number of samples used was smaller, namely only 20 white mice. The large number of samples used will affect a study because the greater the number of samples used, the smaller the chance of generalization errors.

## Conclusion

The conclusion is based on research comparing the effectiveness of the dermapen action using Plasma from platelets with the dermapen action on pockmarked wounds on the skin surface of female Wistar rats (*Rattus norvegicus*) for 14 days. The obtained Platelet Rich Plasma is enriched with platelets, where the platelets contain various compounds that are important for pharmacological effects. These platelets will release Platelet Derived Angiogenesis GF and growth factors (GF), including Vascular Endothelial GF, Platelet Derived GF, Platelet-Derived Endothelial GF, Fibroblast Growth Factors, Transforming GF – Beta, Insulin-like GF, Hepatocyte Growth Factor, and Epidermal Growth Factor. The action of dermapen with blood plasma from platelets (PRP) was more effective than that of the control group, which only used dermapen.

The average wound healing phase on the first day to the 4th day in the group given the dermapen procedure with plasma application from platelets P2 (5%) and P3 (10%) saw a faster reduction in wound length compared to the P1 group (2.5 %) and control group (K). This happens because the content of bioactive substances in blood plasma from platelets (PRP) can speed up healing and remove scars. In this study, the results showed that the group given the dermapen treatment with the application of Plasma from platelets at a dose of 10% was more effective in healing pockmarked wounds in mice compared to the group given the dermapen treatment with the application of Plasma from platelets at a dose/level of 2.5% and 5%. This is because, at a dose of 10%, the metabolite compounds in Plasma from platelets already affect wounds. Still, they only inhibit microorganisms at small concentrations, so they are less effective in healing wounds.

As for what can be recommended as a suggestion from this research, it is necessary to carry out further research with higher plasma levels of platelets (PRP) and compare them using additional variables, for example, a comparison with the negative control group (those without treatment). It is also necessary to carry out further research on the action of dermapen with the application of Plasma from platelets in preventing/removing pockmarked wounds, which is safer when applied to humans. Likewise, further research needs to be done on other

forms/preparations of Plasma from platelets (PRP), which are more valuable and accessible to produce on a large scale, especially in North Sumatra or other areas that cultivate it.

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