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The Effect of Giving Mangrove Leaf Extract *(Rhizophora apiculata)* on the Healing of Burn Wounds in Male White Rats *(Rattus novergicus)* of the *Sprague Dawley* Strain

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Abstract

A burn wound is damage or loss of tissues caused by contact with a heat source and can be classified into four degrees: I, IIA, IIB, and III. Anti-inflammatory and antioxidant agents such as mangrove leaves can be applied to aid the healing process of burns. One alternative method is to use mangrove leaf extract. This study aims to understand the acceleration of burn healing by applying mangrove leaf extract *(Rhizophora apiculata)*. This study was a true experimental study with a post-test-only control group design, divided into five groups: Knormal (without treatment), K+ (bioplacenton), P1 (20% extract), P2 (30% extract), and P3 (40% extract). Burn wounds were observed for 21 days and assessed with average wound shortening, healing time, and the Bates-Jensen wound assessment tool. Based on the results of statistical tests, it was found that the application of Rhizophora apiculata leaf extract affected the healing of burns in white rats, resulting in a reduction in the burn area (P Value: 0.001). The application of mangrove leaf extract had an effect on the healing time of burns in white rats (P Value: 0.001). An effective dose of mangrove leaf extract was a concentration of 40% for healing burns, a higher effectiveness in healing burns compared to the standard drug, bioplacenton. The administration of mangrove leaf extract positively affects the healing of burns in male white rats *(Rattus novergicus)* of the Sprague Dawley strain, especially with an extract concentration of 40%.

Keywords: Burn wound, *Rhizophora apiculata* extract, rats

Full length article **Corresponding Author*, e-mail: syazili.mustofa@fk.unila.ac.id Doi # https://doi.org/10.62877/66-IJCBS-24-25-19-66

1. Introduction

Burns are injuries that occur on the skin due to direct or indirect contact with a heat source, resulting in skin tissue damage. The length of exposure to heat with skin tissue affects the degree of burns and the wound healing process. The high prevalence of burns is a serious global problem, as it is a cause of death and disability. Based on data from WHO in 2017, the WHO Global Burden Disease, it was found that the prevalence of burns cases was 180,000 victims who died with an age range of <20 years around 30% [1]. The prevalence of burn injuries in Indonesia is 2.2%. The provinces of Nanggro Aceh Darussalam and Riau Islands had the highest incidence of 3.8%, while Lampung province accounted for 1.7% of all injuries. Household burns are the most common [2]. Burns can be classified into four degrees: IIA, IIB, III, and I. Each degree of burn will have certain limitations and different clinical manifestations. In the case of burns of degree IIA, damage can be found in the epidermis and dermis, with some supporting organs such as hair follicles, sebaceous glands, and sweat glands still healthy. Second-degree burns can cause erythema, pain, watery surfaces, hyperesthesia, sensitivity to low temperatures, edema, and ulcers [3]. In general, the burn wound healing process includes four phases: the homeostasis phase, inflammation phase, proliferation phase, and maturation phase. If one of these processes is disrupted, such as in the inflammatory phase, wound healing cannot progress to the

next phase. For these processes to take place appropriately and avoid chronic inflammation, it is necessary to use substances that support the wound healing process, such as anti-inflammatory drugs [4]. The homeostatic phase occurs several hours after the burn event. Damaged blood vessels are the first thing that happens when an injury occurs, resulting in vasoconstriction in the arterioles. The inflammatory phase lasts 3 - 4 days post-injury. Signs of the inflammatory phase are bleeding and clotting due to smooth muscle retraction of the injured vascular wall. The proliferation phase starts from day 4-20 post-wounding. This phase consists of neoangiogenesis, granulation tissue formation, and re-epithelialization. The next phase is maturation, after entering the third week to 1 year. In this phase, the tissue will replace the injured area with a stronger structure [5]. Several things can affect the burn wound healing process, including oxygenation, infection, age, hormones, stress, diabetes, medication, obesity, alcohol, smoking, and nutritional intake. Burns can also develop complications if not treated properly, including infection of the burn wound, disruption of blood supply and circulation, and long-term complications [6]. Some important compounds in the mangrove plants' leaves *(Rhizopora apiculata)* include flavonoids, saponins, steroids, and tannins [7]. Flavonoids provide a protective effect against reperfusion in body tissues due to ischemia and become an antioxidant agent to reduce lipid peroxide profiles and increase the process of re-epithelialization in the proliferation phase [8]. Saponins can accelerate hemolytic activation because they can be antibacterial, antiviral, and antioxidant compounds [9]. Steroids can increase the speed of epithelialization formation in the body. As an antimicrobial that can increase epithelialization, tannins can increase cicatrix formation and wound contraction [10]. This study aims to determine the acceleration of burn wound healing by mangrove *(Rhizopora apiculata)* leaf extract.

2. Materials and Methods

2.1. Research Design, Time, and Place of Research

This study was a qualitative analytical research true experimental design with a post-test control group design method. Giving mangrove leaf extract *(Rhizophora apiculata)* to male white rats *(Rattus norvegicus) Sprague dawley* strain given burns to find out the effect on the length of wound healing. The research was conducted from September 2022 to November 2022 at the Faculty of Medicine, Universitas Lampung (FK Unila). Experimental animals *(Rattus novergicus* of *Sprague Dawley* strain*)* were kept in the animal house of the Faculty of Medicine, Universitas Lampung. Determination of mangrove leaves *(Rhizophora apiculata)* was carried out in the Botany Laboratory, Department of Biology, Faculty of Mathematics and Natural Sciences, Universitas Lampung, and the Preparation of mangrove leaf extracts *(Rhizophora apiculata)* was carried out in the organic chemistry laboratory (Faculty of Mathematics and Natural Sciences), Universitas Lampung. Observations were carried out in the animal house of the Faculty of Medicine, Universitas Lampung.

2.2. Population and Sample

This study's population (experimental animals) were male white rats *(Rattus norvegicus) Sprague Dawley* strain aged 2.5 to 3.5 months, weighing 150-200 grams, taken from the animal vet laboratory services Bogor. A total of 30 samples of male white rats were divided into five treatment groups. The sample was calculated using the Federer formula (Ridwan, 2013). The final sample size was 30 male white rats *(Rattus norvegicus) Sprague dawley* strain in 5 groups, and each group consisted of 6 rats.

2.3. Sample Criteria

The inclusion criteria in this study were adult white male rats *(Rattus norvegicus) Sprague Dawley* strain with an age of 2.5 - 3.5 months having a rat body weight ranging from 150 - 200 grams with second-degree burns. Criteria for healthy rats included the appearance of intact fur without falling out, no scars found on the body of rats, and no exudate found in all parts of the body. In addition, the rats were free from drug administration and treatment other than research. Meanwhile, the exclusion criteria were sick and dead rats during the study and burns not of degree II.

2.4. Tools and Materials

2.4.1. Tools and Materials for Creating Burn Wound

The tools and materials used for the Preparation of burns include an animal shaver, iron plate (2×2 cm in size), portable stove, portable stove gas, caliper, sterile handscoon, bent, sterile kom, clean cloth, lab coat, xylazine 2%, ketamine 10%, syringe, 70% alcohol, sterile gauze, razor blade, stopwatch, Aquadest, and cotton wool.

2.4.2. Tools and Materials for Burn Wound Care

Tools and materials used for burn wound treatment include instrument combs, sterile gauze, sterile handscoon, sterile anatomical tweezers, cirugis tweezers, rolled gauze, twisted, Cot Sheet, NaCl 0.9%, Bioplacenton® gel, 10 CC syringe, also mangrove leaf extract concentrations of 20%, 30%, and 40%.

2.4.3. Tools and Materials for Mangrove Leaf Extraction (Rhizophora apiculata)

Tools and materials used for raw leaf extraction include oven, scales, Erlenmeyer glass, glass funnel, filter paper, evaporator flask and alcohol container, evaporation machine, spiral condensator, water pump hose, water bath, vacuum pump, dried mangrove leaves *(Rhizophora apiculata)* and 95% ethanol.

2.5. Research Procedure

2.5.1. Animal Adaptation

A total of 30 rats were divided into 5 treatment groups with adaptation for seven days in the animal house of the Faculty of Medicine, Universitas Lampung, and weighed as a marker to determine the grouping of rats. Rats were fed

according to 10% of their weight, about 1.5-2 grams/day. Feed was given daily at 08.00, and drinks were given ad labitum. Cage cleaning was carried out by replacing wood shavings every 3 days [11].

2.5.2. Simplisia Examination

Prior to the research, mangrove leaves *(Rhizophora apiculata)* were first determined at the Botany Laboratory, Department of Biology, Faculty of Mathematics and Natural Sciences, Universitas Lampung.

2.5.3. Simplisia Preparation

Simplisia of mangrove leaves *(Rhizophora apiculata)* was obtained from Sawadaya Winawiyata Widiyakarya Forestry Business Training and Management Institute, Pasir Sakti District, East Lampung Regency. Then, wet sorting, washing, cutting, drying, dry sorting, and pollination of mangrove leaves were carried out. The pollinated simplisia was placed in a tightly closed place and protected from light.

2.5.4. Extraction of Mangrove Leaves (Rhizophora apiculata)

Mangrove leaves *(Rhizophora apiculata)* as much as 600 g. Mangrove leaves were cleaned with water, dried in the sun without direct light to dry to the minimum water content, and then heated in an 80℃ oven.

2.5.4.1. Maceration Process

Dried mangrove leaves *(Rhizophora apiculata)* were pulverized with a chopper. Then weighed 600 grams of mangrove leaf powder and put it into a glass Erlenmeyer size 1.5 L. Put 95% ethanol up to 1500 ml to soak for 6 hours until mangrove leaf powder completely dissolved while stirring occasionally, then let stand for 18 hours and let settle. The mixture with 95% ethanol solution was filtered with filter paper to get the filtrate and then evaporated with a rotary evaporator. To determine the specific gravity of 1 ml, the extract was allowed to dry for 24 hours at room temperature [12].

2.5.4.2. Determination of Mangrove Leaf Extract (Rhizophora apiculata) concentrations of 20%, 30% and 40%

The results of the study reported that the best extraction carried out on the best Folium eucommiae using 40% ethanol solvent produced the highest total flavonoids of 17.2% [13].

3 concentrations have been calculated consisting of:

1) 20% concentration consists of 6 ml of extract = 24 ml of distilled water
2) The 30% concentration consists of 9 ml of extract = 21 ml of distilled water
3) The 40% concentration consists of 12 ml of extract = 18 ml of distilled water

2.5.4.3. Animal Treatment

Before shaving, rats were given total anesthesia with a combination of xylazine and ketamine. Giving the combination of ketamine - xylazine produces better anesthesia and has a very strong analgesic effect, and produces sedation and hypnotics as well as a long duration of anesthesia in rats [14]. The best injection volume for rats is xylazine (5 mg/kg bw), and ketamine (2.5 mg/kg bw), given by subcutaneous injection in the gluteus. Sleep usually lasts 1-2 hours, with analgesics effective for 15-30 minutes [15]. Under anesthesia, cleanly shave the rat's back using scissors and a razor to minimize irritation to the rat's skin. Then, sterilize the shaved area with 70% alcohol. Induction of burns in rats was done using a circular iron plate with a diameter of 2 cm that was heated on a fire for 2 - 3 minutes with a temperature reaching 260-280 ℃. Position the rat on one side and place the metal plate slowly without any pressure by hand or other objects for 10 seconds. Then, the wound is compressed with distilled water for 1 minute [16].

2.5.4.4. Treatment and Calculation of Burn Wound Closure

Treatment and calculations were done by cleaning the burn area with normal saline solution, drying, and then measuring the burn wound using a caliper to calculate the surface area. Then, the extract would be reapplied based on the treatment given. Wound treatment was given once a day. Treatment was carried out for 21 days [17].

2.5.4.5 Wound Assessment

2.5.4.5.1 Burn Area Assessment

The experimental observation technique was applied with the division of 5 treatment groups for further observation and measurement once a day to see the shrinkage of the wound diameter macroscopically. This observation was carried out from the start of treatment for each group until the 21st day to determine changes in burn wounds. The continuation of wound healing on the rat's back was carried out by measuring the diameter with a 0.01 mm scale caliper and then calculating the area [18].

2.5.4.5.2 Scoring modified macroscopic Bates-Jensen wound assessment tool

This study also used the modified Bates-Jensen Wound Assessment Tool scale. The wound assessment instrument consisted of 13 question items, including wound induration, wound color in the surrounding area, edema, amount and type of exudate, granulation tissue, epithelialization tissue, type of size, depth, wound edges, undermining, type of necrotic tissue and amount of necrotic tissue [19].

2.6. Data Analysis

The data obtained would be analyzed statistically with the Shapiro Wilk Test data normality test because of the number of samples ≤ 50 (p=0.05). A variance test was used to determine the differences between 2 or more sample groups using Levene's test method. The results of this test

were decided to be homogeneous (p>0.05). If the data variance was normally distributed and homogeneous, it would be continued with the One-way ANOVA parametric test. The hypothesis was considered meaningful if the p-value was 0.05. However, if the data distribution was not normal, the Kruskal-Wallis nonparametric test would be used. The hypothesis was considered meaningful if the p-value was <0.05. If the One-Way ANOVA test results in p < 0.05, it would be followed by conducting Post Hoc LSD analysis, and if the Kruskal Wallis test results in a p-value < 0.05, it would be followed by Post Hoc Mann-Whitney analysis.

2.7. Research Ethics

The implementation of this study used an ethical clearance letter obtained from the Universitas Lampung, Health and Medical Research Ethics Commission, No. 4394/un26.18/pp.05.02.00/2022: This study used *Sprague dawley* white rat experimental animals by observing the principles of research ethics 3R and 5F.

3. Results and discussion

This study was conducted to assess whether there is a relationship between the administration of mangrove leaf extract *(Rhizohopra apiculata)* given topically with several concentrations, 20%, 30%, and 40% of the burn wound healing process in male white rats *(Rattus novergicus)* of *Sprague dawley* strain. The following are the results of the data obtained from the results of the study as shown in table 1. There were 5 observation groups that were treated and observed every day, and 4 days of observation were taken for data analysis, exactly day 4 as the final stage of inflammation, day 10 and 15 as the middle value in the proliferation phase, and day 21 as the beginning of the maturation phase. From the observations, it was found that the wound area in rats in treatment groups 2 and 3 was completely healed on day 21, and the other groups could not be said to be completely healed and the following is a description of the wound area observed per day. On the fourth day of treatment, all treatment groups showed the same results, with the burn area being 3.14 cm^2, the same as at the beginning of the burn treatment. This shows that it is still in the final stage of the inflammatory phase, so there is no significant change in burn surface area on day 4. This is in line with research conducted by Zahra in 2017 regarding the burn wound healing activity of 96% alcohol extract fraction of cocor bebek *(Kalanchoe pinnata)* leaves where on day 3-4 is the inflammatory phase where in this phase, bleeding occurs, then clotting or stopping blood through the permeability of smooth muscle vascular walls of the injured area [20]. Furthermore, day 10 was taken, which is the middle of the proliferation phase. On the 10^{th} day of the study, the researcher found that the burns had caused crusts (scabs), especially in the positive control group, and also for treatment groups 1, 2, 3, caused crusts but not as thick as the positive control group. In practice, bioplacenton is a topical drug containing placenta extract and neomycin sulfate, which can accelerate the formation of epithelial and granulation tissue, while boost the immune system [21]. In this proliferation phase, granulation tissue forms from fibroblast cells, inflammatory cell deposits, and new blood vessels resulting from angiogenesis [22]. From the results of the post hoc test, a significant difference if the Sig value <0.05 and no significant difference if the Sig value > 0.05, which means there is a significant difference between the normal treatment group with treatment group 1, normal control group with treatment group 2, normal treatment group with treatment group 3, positive treatment group with treatment group 1, positive treatment group with treatment group 2, positive treatment group with treatment group 3. On day 15, the proliferation phase begins. In this study, researchers found that on day 15, the crusts that existed on day 10 had begun to slough off and become new tissue. This made the burn surface area smaller, and found that treatment groups 3 and 2 were the groups with the smallest average burn area. This crustal sloughing has occurred from day 12. This is in line with research conducted by Sumiati in 2017, where changes occurred on day 11 to day 16 crusts (scabs) detached from the skin and began to show shrinkage of the wound diameter, while the wound can be said to heal on day 19 to day 21 where the diameter of the wound has closed even though there is still minimal granulation [23]. Another study conducted by Rezai et al. showed that burn patients who were given antioxidants in the form of Marigold flowers as much as 2 g / day orally within 15 days effectively healed second-degree burn patients [24]. After obtaining the wound area data on day 21, the Saphiro Wilk data normality test was carried out, and the results were Sig. > 0.05 in the KN, K+, and P1 groups, but for treatment groups 2 and 3, the data does not appear because the value is constant, so it can be concluded that the data is normally distributed, followed by Levene's test, the Sig value is obtained, 0.002, the data is not homogeneous. The next test uses the ANOVA test because the data is normally distributed, but the data variance is not homogeneous; the Sig value is obtained, 0.007, a significant difference between variables in the 21^{st}-day wound area data and can be continued for the Games-Howell post hoc test to see the differences in each variable, which obtained the following data as shown in table 3. The post hoc test data is said to have a significant difference in the Sig value. <0.05 and said to have no significant difference if the Sig value > 0.05 means that there are no significant differences between all groups. On day 21, the final phase of proliferation, at the beginning of week 3, is the initial phase of maturation. In this phase, researchers found that treatment groups 2 and 3 had fully recovered. In other groups, some rats still have not healed completely. This is because several factors affect wound healing. Putri (2021) said several things affect the healing process of burn wounds, including oxygenation, infection, age, hormones, stress, and nutrition [25]. During the study, which lasted ± 26 days, the average shrinkage of the burn area per day was assessed and then calculated for the difference per day and then divided by the healing time so that the burn shrinkage per day was obtained as follows in graph 1. From the graph 1, it can be observed that the P3 group tends to experience the most significant shrinkage of the burn area and is followed by P2, P1, and positive control, while the normal control can be seen to experience the longest process of shrinkage of the burn area compared to other groups. The shrinkage of the rat burn area shrinks every day. From the data taken, it is found that the average shrinkage of the burn area per day is as follows in table 4. Wound area shrinkage is one of the indicators for burn wound healing; in the table above, it is found that the normal treatment group has an average wound area shrinkage of 0.128 cm^2 per day.

Table 1. Average burn area in rats

Day of treatment	Wound area (cm^2)					P
	KN	K(+)	P1	P2	P3	
Day 4th	3.14	3.14	3.14	3.14	3.14	0. 406
Day 10th	2.75	2.86	2.79	2.74	2.73	0.669
Day 15th	2.24	2.25	1.28	1.11	1.09	0.000
Day 21st	0.16	0.11	0.01	0	0	0.007

Table 2. LSD post hoc test on wound area data on day 15

Groups	Control N	Control +	Treatment 1	Treatment 2	Treatment 3
Group N		.964	.001*	.001*	.001*
Group Control +	.964		.001*	.001*	.001*
Group treatment 1	.001*	.001*		.567	.516
Group treatment 2	.001*	.001*	.567		.938
Group treatment 3	.001*	.001*	.561	.938	

Note: * = Significantly different

Table 3. Games-Howell post hoc test of wound area data on day 21

Groups	Control N	Control +	Treatment 1	Treatment 2	Treatment 3	Control N
Group N	Group N		.945	.335	.264	.264
Group Control +	Group Control +	.945		.248	.161	.161
Group treatment 1	Group treatment 1	.335	.248		.424	.424
Group treatment 2	Group treatment 2	.264	.161	.424		-
Group treatment 3	Group treatment 3	.264	.161	.424	-	

Table 4. Average wound area shrinkage per day

Treatment Group	Average Wound Area Shrinkage	P
KN	0.128	0.001
K(+)	0.130	
P1	0.144	
P2	0.148	
P3	0.154	

Table 5. Length of burn wound healing

White rats	Length of burn wound healing (day)					P
	KN	K+	P1	P2	P3	
Average of burn wound healing	24.4	23	21.2	20.2	19.8	0.001

Table 6. Average Bates-Jensen score

Treatment Group	Average *Bates Jensen SCore*	P
K+	13.2	
KN	13.2	1.000
P1	13.2	
P2	13.2	
P3	13.2	

Note:
KN : Rat group without treatment.
K (+) : Treatment group of rats given bioplacenton.
P1 : Treatment group of rats given 20% mangrove leaf extract.
P2 : Treatment group of rats given 30% mangrove leaf extract.
P3 : Treatment group of rats given 40% mangrove leaf extract.

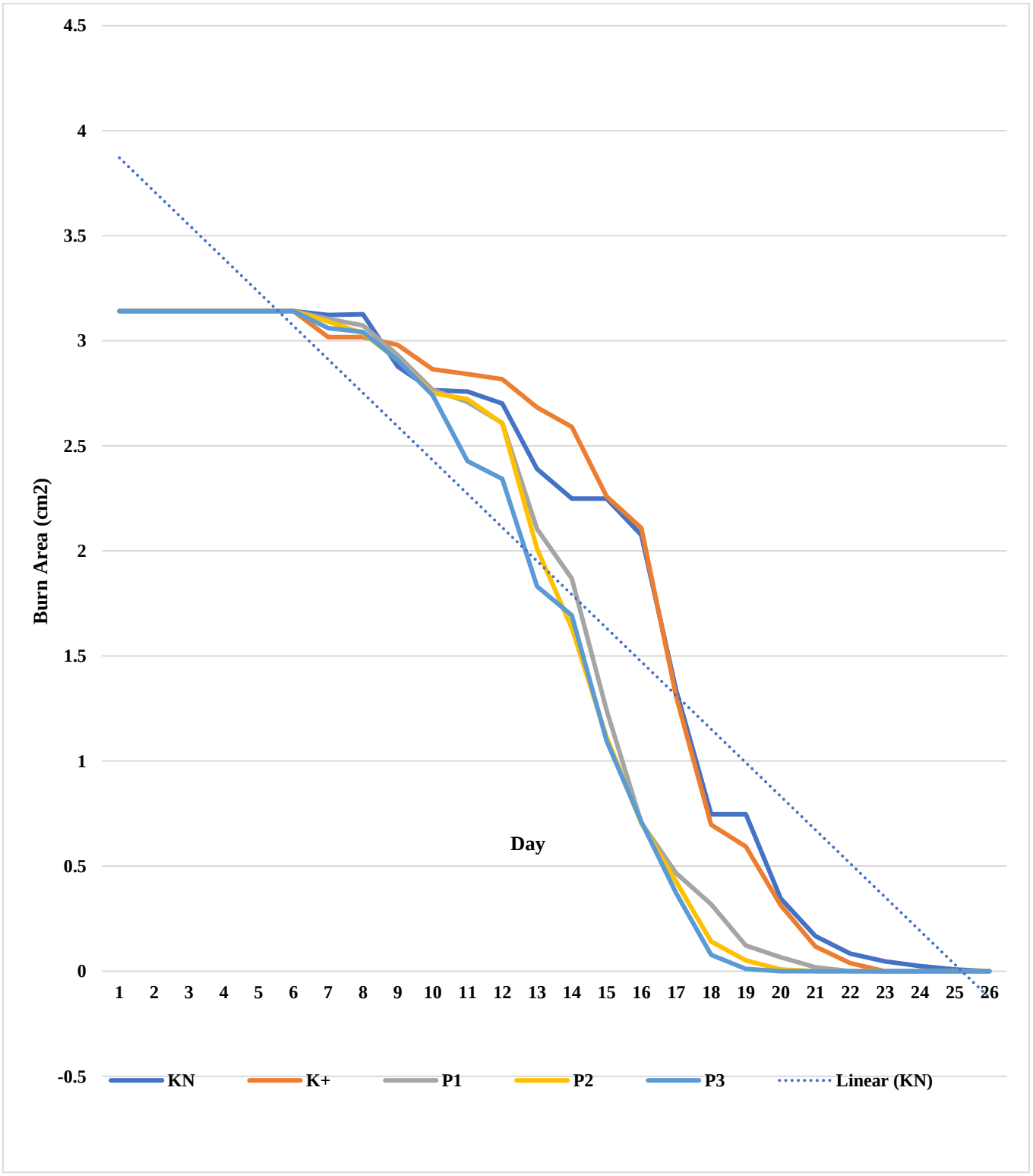

Figure 1. Graph of burn area shrinkage

KN : Rat group without treatment.
K (+) : Treatment group of rats given bioplacenton.
P1 : Treatment group of rats given 20% mangrove leaf extract.
P2 : Treatment group of rats given 30% mangrove leaf extract.
P3 : Treatment group of rats given 40% mangrove leaf extract.

The positive treatment group had an average burn area shrinkage of 0.130 cm^2 per day. Treatment group 1 had an average burn area shrinkage of 0.144 cm^2 per day. Treatment group 2 had an average burn area shrinkage of 0.148 cm^2 per day. Treatment group 3 had an average burn area shrinkage of 0.154 cm^2 per day. Based on the data above, it is found that there are differences in the length of healing of burn wounds in each rat in each group. In the normal group given aquadest, the fastest healing time was obtained on day 22 and the longest on day 26. In treatment group 1, with 20% mangrove leaf extract concentration, the fastest healing time was obtained on day 19, and the longest occurred on day 22. In treatment group 2 with 30% mangrove leaf extract concentration, the fastest healing time was obtained on day 19, and the longest occurred on day 21. In treatment group 3, with 40% mangrove leaf extract concentration, the fastest healing time was obtained on day 19, and the longest occurred on day 20. Based on the data obtained under burns, with treatment group 3, with 40% extract concentration, has the fastest average burn healing of 19.8 days, followed by treatment group 2, with 30% extract concentration with an average burn healing of 20.2 days, and for treatment group 1 with 20% concentration with an average burn healing of 21.2 days. Meanwhile, the normal control group had the longest average burn healing compared to the other groups, 24.4 days, and the positive control group had an average burn healing of 23 days. This is in accordance with previous research, which shows that high antioxidant levels play an important role in wound healing and contribute to reducing infection rates in burn patients [26,27]. The data obtained in the table 6 are the results of macroscopic observations based on the Bates-Jensen criteria after ± 26 days of observation. In the results of the Bates-Jensen score, it can be seen that all groups have the same value of 13.2 because in each group, there are still groups that are observed to have wound edges whose edges are visible, fused with the wound, while for other groups the wound edges are faint and not visible. So of the 5 rats observed, 4 of them scored 13, and 1 rat scored 14. The maximum score is 65, and the minimum score is 13. So, the smaller the value, it can be concluded that the wound-healing process is getting better. After obtaining data related to the Bates-Jensen score, the statistical analysis will be tested by testing the normality of the data first using Shapiro Wilk, and the Sig value will be obtained. 0.000 this is because the data is constant, and then Levene's test obtained a Sig value of 1.000, so the data is homogeneous and can be continued for the next test using the Kruskal Wallis test and obtained a Sig value. 1.000, then there is no significant difference; if there is no significant difference, there is no need to continue with the next post hoc test. In line with previous studies, antioxidant administration in second-degree burn patients can significantly affect the decrease in Bates-Jensen score in the treatment after day 7 [24].

4. Conclusions

An effect of mangrove leaf extract *(Rhizopora apiculata)* on burn wound healing in male white rats *(Rattus novergicus) Sprague Dawley* strain is detected. Mangrove leaf extract at 40% concentration is more effective in healing burns than the standard drug bioplacenton. It is expected that further researchers can conduct a microscopic comparison of the effects of mangrove leaf extract administration with various concentrations on burns.

Conflict of Interest

The authors state that there are no potential conflicts of interest associated with the research, authorship, or publication of this article.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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