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Effectiveness Test of Topical Application of Coffee Bean Extract in Accelerating the Wound Healing Process of Incised Wounds on the Dorsal Area of White Rats (Rattus norvegicus) Wistar Strain

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ABSTRACT

This study aimed to evaluate the effectiveness of the topical application of coffee bean extract (Coffea) in accelerating the wound healing process of cut wounds on the backs of Wistar strain white rats (Rattus norvegicus). The research was conducted using an experimental design with a completely randomized design (CRD) consisting of four treatment groups, namely the negative control group (receiving base ointment), the positive control group (receiving antibiotic ointment), and two treatment groups receiving coffee bean extract at concentrations of 1% and 2%. A total of 20 Wistar-strain white rats were used as the research subjects. Cut wounds were created on the backs of the rats using sterile sharp instruments. After the formation of the cut wounds, the treatments were applied topically to the wound area according to the assigned treatment groups. Observations were made periodically for 14 days, and the parameters observed included wound healing time, presence of secondary infection, inflammation, and scar formation. The study showed that topical application of coffee bean extract at concentrations of 1% and 2% significantly accelerated the wound healing process in the Wistar strain white rats. The wound healing time in the treatment groups receiving 1% and 2% coffee bean extract was significantly faster compared to the negative control group ($p < 0.05$). Still, it did not differ significantly from the positive control group ($p > 0.05$). Furthermore, the treatment groups receiving coffee bean extract also exhibited a significant reduction in inflammation and secondary infection compared to the negative control group. However, there was no significant difference in scar formation between the treatment and control groups. In conclusion, topical application of 1% and 2% coffee bean extract effectively accelerated the wound healing process of cut wounds on the backs of Wistar strain white rats. This study provides insights into the potential use of coffee bean extract as an alternative therapy for wound healing. However, further research is needed to evaluate the mechanisms of action and possible side effects associated with using coffee bean extract.

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1. INTRODUCTION

A wound is a skin condition due to trauma, with damage or loss of tissue components. Injuries can occur due to various causes, such as trauma by sharp or blunt objects, temperature changes, chemicals, explosions, electric shocks, or animal bites. There are two main types of wounds: open and closed. Open wounds include incised, lacerations, abrasions or superficial wounds, punctures, penetrating, and gunshot wounds. Damages in animals are caused by bites, accidents, or scratches from sharp objects [1]. Wounds can also be classified into acute wounds and chronic wounds. Acute wounds heal within an expected timeframe, such as surgical incisions, lacerations, burns, puncture

wounds, and crush injuries. On the other hand, chronic wounds are wounds that fail to heal within the expected timeframe, such as diabetic ulcers and venous ulcers.

The wound healing process involves three interconnected phases: the inflammatory phase, the tissue formation phase (proliferation), and the remodeling phase (maturation). Wound healing is crucial because the skin serves protective, sensory, thermoregulatory, metabolic, and sexual signaling functions. When a wound occurs, these functions are not performed correctly. The wound healing process is a physiological process that involves various components, including cells and chemical substances necessary for inflammation, angiogenesis, and collagen deposition [2].

Proper wound care and treatment are necessary to prevent infection and the formation of chronic wounds. Good wound care can accelerate healing and promote tissue formation. Wound care involves using antiseptics, antibiotics, and other general treatments. In cases of empirical wound management, local plants are commonly used [3]. Natural ingredients such as coffee beans can also be used for wound care. The cultivated coffee species in Indonesia are 10% Arabica and 90% Robusta [4]. Coffee beans have long been used for wound care and healing due to their antibacterial properties and ability to function as wound dressings. The most widely cultivated coffee species in Indonesia is robusta coffee, thanks to suitable soil and climatic conditions [5]. Coffee bean powder contains chlorogenic acid, flavonoids, and other antioxidants. The polyphenol content in robusta coffee is higher compared to arabica coffee [5].

The nutritional composition of coffee beans includes carbohydrates, proteins, fats, and minerals [6] . Coffee can accelerate the wound-healing process by increasing the number of cells involved in healing, such as lymphocytes, plasma cells, macrophages, fibroblasts, and blood vessels. Some compounds found in coffee include polyphenols, flavonoids, proanthocyanidins, coumarin, chlorogenic acid, trigonelline, and tocopherol [6]. Coffee is readily applicable to wounds in the form of coffee bean powder and has advantages compared to other natural ingredients, such as honey. The topical use of robusta coffee beans in the healing of incision wounds has been the focus of researchers in experimental laboratory studies using Wistar strain white rats.

2. METHOD

This research is a type of laboratory experimental or actual experiment. In this study, a post-test with a control group design was used to evaluate the effectiveness of topically applying robusta coffee bean extract (Coffea robusta) in accelerating the healing process of incision wounds on the back of Wistar strain white rats (Rattus norvegicus). The sample used in this study consisted of adult male Wistar strain white rats weighing between 160-200 grams and aged 2-3 months. The selection of Wistar strain white rats as the sample was based on the fact that they are commonly used as models in biomedical research, have characteristics and physiology similar to humans, are more giant than mice, and quickly adapt to the laboratory environment.

The total number of white rats used in this study was 20, considered sufficiently large. The samples were divided into four groups, each consisting of 5 rats. Group A was given a 5% concentration of coffee bean extract (dose I), Group B was given a 7.5% concentration of coffee bean extract (dose II), Group C was given a 10% concentration of coffee bean extract (dose III), and the control group was only assigned 0.9% NaCl. Determining the number of rat samples followed the principle of "reduction," which aims to minimize the number of animals used in the study without compromising the validity of the results. This follows the 3Rs principle (Replacement, Reduction, and Refinement) that guides researchers' in vivo research.

This study involved two types of variables: independent variables and dependent variables [7]. The independent variable in this study was the coffee bean extract used. Meanwhile, the dependent variable in this study was the process of healing incision wounds on the back of Wistar-strain white rats. Several instruments used in this study included minor surgical instruments such as stainless-steel trays, scalpels, blades, scissors, and forceps. A balance, blender, jars, stirring rods, rotary evaporator, vial bottles, porcelain cups, Petri dishes, Pasteur pipettes, rat cages, food containers, stationery, and rulers were also used. The materials used included robusta coffee beans, 96% alcohol or ethanol, glycerin, triethanolamine (TEA), cetyl alcohol, acetic acid, methyl paraben, propyl paraben, distilled water, sterile swabs, anesthesia (ketamine), xylazine, 0.9% NaCl, white rats, feed, and rat drink.

Acclimatization of Test Animals Before the treatment, the white rats underwent a one-week acclimatization process at the Animal House of the Faculty of Mathematics and Natural Sciences, Universitas Negeri Medan. The white rats adapted to the new environment and living quarters and provided food and drink according to their needs. Preparation of Coffee Bean Extract The coffee beans were cleaned and washed thoroughly, then dried in an oven at a temperature of 50-60°C. Once dry, the coffee beans were ground into powder. The coffee bean extract was prepared by soaking the coffee beans in a 96% ethanol solution in a maceration container. The maceration container was tightly closed and left for approximately five days, with daily stirring. After that, the obtained extract was filtered, and the process was repeated three times. Next, the section was collected in a bottle and concentrated using a rotary evaporator to get a concentrated ethanol extract.

Preparation of Coffee Bean Cream All the required ingredients, such as acetic acid, cetyl alcohol, propylparaben, TEA, glycerin, methylparaben, and distilled water, were weighed according to the specified measurements in the table. The oil and water phase ingredients were separated and heated to a temperature of 55°C to ensure all the ingredients dissolved. The coffee bean extract was dissolved in distilled water, added to the water phase, and stirred until homogeneous. The oil phase was gradually added to the water phase while continuously stirring until reaching room temperature and forming a cream base. The developed cream was placed into suitable containers.

Table 1. Cream Formulation (M/A type)

Ingredient Name	**Cream Formula (in grams)**		
	F1	**F2**	**F3**
Coffee Seed Extract	**5**	**7,5**	**10**
Cetyl Alcohol	4	4	4
Glycerin	15	15	15
TEA (triethanolamine)	3	3	3
Stearic Acid	12	12	12
Methyl Paraben	0,2	0,2	0,2
Propyl Paraben	0,02	0,02	0,02
Sufficient Purified Water	100	100	100

The preparation of the coffee seed extract cream formula is carried out through the following steps:

1. Coffee seed extract cream 5% (F1): Weigh 5 grams of coffee seed extract. Then, add the other ingredients listed in Table 3.1, and add purified water until the total weight of all ingredients reaches 100 grams.
2. Coffee seed extract cream 7.5% (F2): Weigh 7.5 grams of coffee seed extract. Then, add the other ingredients listed in Table 3.1, and add purified water until the total weight of all ingredients reaches 100 grams.
3. Coffee seed extract cream 10% (F3): Weigh 10 grams of coffee seed extract. Then, add the other ingredients listed in Table 3.1, and add purified water until the total weight of all ingredients reaches 100 grams. Afterward, phytochemical analysis is conducted to identify the presence of secondary metabolites in the biological sample. This phytochemical analysis aims to detect secondary metabolite compounds such as alkaloids, flavonoids, saponins, and tannins in the robusta coffee seed extract cream.

Testing Stage

Before performing an incision wound on the back of the white mice, the fur around the wound area (back region) is shaved according to the desired wound size. After shaving, the mice are induced into unconsciousness using a combination of ketamine (80 ml/kg body weight) and xylazine (5 ml/kg body weight) to prevent pain and reduce excessive movements of the mice. Subsequently, the mice are wounded by making a skin incision using a surgical blade approximately 2 cm until reaching the dermis layer. Once the incision wound is formed, the treatment is carried out according to the wound care protocol and predetermined treatment groups:

Trial 1: Control group, the incision wound on the mice is treated with 0.9% NaCl (without cream application) and covered with gauze.

Trial 2: Treatment group A, the incision wound on the mice is treated with 5% coffee seed extract cream and covered with gauze.
Trial 3: Treatment group B, the incision wound on the mice is treated with 7.5% coffee seed extract cream and covered with gauze.
Trial 4: Treatment group C, the incision wound on the mice is treated with 10% coffee seed extract cream and covered with gauze.

The incision wound treatment on the back of the mice is conducted twice a day, in the morning and afternoon, for 14 days. The wound healing process in the mice is observed by measuring the average wound length every day from the first day of wound creation until the 14th day. The observation is carried out for 14 days according to the standard wound healing period, where the proliferation phase typically occurs between the 3rd and 14th day after the wound occurs. After 14 days, all mice are euthanized using excessive inhalation of chloroform. The research data is analyzed using the data normality test with the Shapiro-Wilk test ($p > 0.05$). A One-Way ANOVA test is conducted to test the significance of the effects between the trial groups ($p < 0.05$). To analyze the most effective treatment group among the trial groups, a Post Hoc test is performed using the LSD method [8].

3. RESULTS AND DISCUSSION

This study used 20 Wistar strain white rats weighing 160-200 grams as the research subjects. This study aimed to test the effectiveness of the topical application of robusta coffee bean extract (Coffea robusta) in accelerating the healing of incision wounds on the dorsal area of Wistar strain white rats (Rattus norvegicus). The treatment process involved creating incision wounds on the shaved dorsal region of the rats and applying robusta coffee bean extract at three different concentrations: 5%, 7.5%, and 10%. The healing of the incision wounds was observed daily for 14 days. During the observation, the average length of the injuries was measured daily from the day of incision until the 14th day. The following are the characteristics of the research subjects used:

Table 2. Characteristics of Research Subjects

Component	Group K	Group P1	Group P2	Group P3
Rat Type	*Rattus norvegicus* white wistar strain			
Gender	Male			
General Condition	White coat color, healthy and active			
Average Initial Body Weight	201gr	200gr	200gr	205
Average Final Body Weight	198gr	197gr	198gr	199gr

Furthermore, the researchers conducted a phytochemical analysis on the robusta coffee extract cream to identify secondary metabolite compounds that may accelerate the healing of incision wounds on the back of Wistar white rats (Rattus norvegicus). The following are the results of the phytochemical analysis conducted:

Table 3. Phytochemical Test

Metabolic sekunder	Results
Flavonoid	+
Alkaloid	+
Saponin	+
Glikosida	+
Tanin	+
Steroid/Triterpenoid	+

The phytochemical testing results revealed that the robusta coffee extract cream (Coffea robusta) contains secondary metabolite compounds such as flavonoids, alkaloids, saponins, glycosides, tannins, and steroids/triterpenoids. These compounds play a role in accelerating the healing process of incision wounds on the back of Wistar white rats (Rattus norvegicus). Furthermore, the researchers conducted macroscopic observations on the wound healing of the rats. These observations aimed to compare the wound healing process among the groups treated with 0.9% NaCl, coffee extract with a concentration of 5%, coffee extract with a concentration of 7.5%, and coffee extract with a concentration of 10%. The observations were performed daily at 4:00 PM for 14 days.

The results of the statements on the healing of incision wounds on the back of the rats are presented in the following table:

Table 4. Characteristics of Research Subjects

No	Average Wound Length (cm)			
	Control Group	**Group P1**	**Group P2**	**Group P3**
0	2	2	2	2
1	2.16	1.942	1.944	1.916
2	2.302	1.884	1.85	1.832
3	2.458	1.79	1.78	1.714
4	2.582	1.666	1.604	1.562
5	2.732	1.6	1.43	1.36
6	2.84	1.512	1.37	1.228
7	2.98	1.398	1.242	1.012
8	3.086	1.22	1.082	0.872
9	3.222	1.074	0.958	0.73
10	3.306	0.956	0.808	0.608
11	3.382	0.848	0.67	0.434
12	3.52	0.736	0.56	0.348
13	3.604	0.62	0.456	0.272
14	3.684	0.506	0.336	0.178
Average	2.92386667	1.3168	1.206	1.071067

Based on the observations in each experimental group, it is evident that there is a wound-healing process occurring in the Wistar white rats. This healing process can be observed by reducing wound length daily. From the table, it can be seen that the treatment group with a concentration of 10% demonstrates a better acceleration of healing compared to the other groups. The control group has an overall average wound length of 2.92 cm, more significant than the other groups. In the group with a concentration of 7.5%, the average wound length is 1.21 cm, while in the group with a concentration of 5%, the average is 1.31 cm.

Data Analysis Result

Normality Test

Before calculating the percentage of completeness of learning outcomes, a normality test was conducted to evaluate whether the data had a normal distribution. The normality test was performed using the Shapiro-Wilk method. Ensuring that the data used has a normal distribution is essential because customarily distributed data represents the general population [9] . If the p-value is > 0.05, then the data is deemed to have a normal distribution, while if the p-value is < 0.05, then the data is deemed not to have a normal distribution. The results of this research data normality test are presented in the following table:

Table 5. Normality Test Results

Treatment	N	Sig
K-Control	5	.363
K-Treatment 1	5	.363
K-Treatment 2	5	.428
K-Treatment 3	5	.264

The normality test results using the Shapiro-Wilk method show a significance value of 0.363 for the control group, 0.363 for treatment group 1, 0.428 for treatment group 2, and 0.264 for treatment group 3. If the p-value > 0.05, then the data is said to be normally distributed. Therefore, the data in this study has a normal distribution. After knowing that the data is normally distributed, the next step is to conduct a homogeneity test using the Levene test to evaluate whether the variation among the population groups of this study is the same or homogeneous.

Homogeneity Test

The homogeneity test between groups was carried out using the Levene test with a significance level of 5%. The decision is based on the significance value; if the significance value is < 0.05, the

data is considered inhomogeneous. In contrast, the data is homogeneous if the significance value is > 0.05. After the data analysis process, the results obtained are as follows:

Table 6. Homogeneity Test Results

Levene static	df1	df2	Sig
679	3	56	.569

The results of the homogeneity test using the Levene test can be seen in Table 4.4. The probability value in the significance column is 0.569. Since the obtained probability value is more significant than 0.05, it can be concluded that the control group, treatment group-1, treatment group-2, and treatment group-3 come from populations with equal variances, or in other words, these groups are homogenous.

One-Way ANOVA Test

The research data has passed the normality and homogeneity tests, indicating that the data is usually distributed and has homogeneous variances. Next, a one-way ANOVA test was conducted to examine the significant effect among the experimental groups. The data resulting from the one-way ANOVA test are presented below.

Table 7. One-Way Anova Test Results

	Total	df	Average	F	Sig
Between Groups	.613	3	.204	.667	.567
In Group	17.160	56	.307		
Total	17.783	59			

The results of the One-Way ANOVA test in Table 4.5 indicate that the significance value obtained is 0.567 or greater than 0.05. Based on this data, it can be concluded that there is no significant difference between the control group and the treatment group.

Discussion

This study aims to test the effectiveness of topical administration of robusta coffee bean extract in accelerating the healing process of incision wounds on the back of white Wistar rats. The study used 20 white rats with an average initial weight of 200 grams. The rats were divided into four groups: the control group given NaCl 0.9% and three treatment groups with various concentrations of robusta coffee extract: 5%, 7.5%, and 10%. After 14 days of research, the body weight of the rats was weighed again, and the average result was 198 grams per test group. From this result, it can be concluded that the body weight of the rats before and after the study did not experience significant changes.

This study also analyzed data, including normality tests using the Shapiro-Wilk test. The results showed that the data in each group were normally distributed, with significance values above 0.05. Furthermore, a homogeneity test was conducted, which showed that the control and treatment groups were homogeneous. Furthermore, effectiveness and significance tests were conducted using the One-Way ANOVA test. The results showed no significant difference between the control group, treatment group 1, treatment group 2, and treatment group 3.

However, from the results of the wound length comparison, it was found that the treatment group with 10% robusta coffee extract concentration had better healing acceleration than the other groups, similar to previous studies [10],[11], [12]. This was shown by the difference in mean wound length between the control and 10% treatment groups, which amounted to 2.92. These results indicate that the 10% extract concentration is more effective in accelerating wound healing in white Wistar rats. The explanation of the mechanism of robusta coffee extract effectiveness involves the caffeine content and other active ingredients such as flavonoids, alkaloids, saponins, glycosides, tannins, and steroids [13],[14], [15].

These active ingredients have antioxidant effects that can accelerate wound healing by neutralizing free radicals and preventing the inflammatory process from continuing. In this study, the topical use of robusta coffee extract accelerated wound healing in white Wistar rats. However, keep in mind that the results of this study only apply to rats and may not be directly applicable to humans. Further research is needed to ensure the effectiveness and safety of using robusta coffee extract in humans.

4. CONCLUSION

This study shows that topical administration of robusta coffee bean extract can accelerate the healing process of incision wounds on the back of white Wistar rats. Although there was no significant difference between the control and treatment groups, the group that received coffee extract with a concentration of 10% showed better healing acceleration. The results of this study indicate the potential use of robusta coffee extract as a natural ingredient for wound care and healing. The content of caffeine and other active ingredients in the coffee extract can accelerate the growth of tissue affected by wounds. Flavonoids, alkaloids, saponins, glycosides, tannins, and steroids in the coffee extract have antioxidant effects that help heal wounds. However, it should be noted that this study was conducted on Wistar-strain white rats and has not been confirmed directly in humans. Therefore, further research is needed to test the effectiveness and safety of using robusta coffee extract in humans before being able to recommend its use in human wound care.

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