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ORIGINAL ARTICLE

The effectiveness of butterfly pea flower extract cream on collagen synthesis in post-dermapen wound healing

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Abstract

Skin wounds, whether acute or chronic, disrupt the integrity of the largest organ in the human body. The healing process involves intricate stages, with collagen playing a crucial role. Microneedling therapies, such as dermapen, stimulate collagen production and improve skin texture. This study investigated the effect of butterfly pea flower (Clitoria ternatea) extract cream on collagen production during the healing of dermapen-induced wounds in obese Wistar rats. Dermapen treatment, a minimally invasive cosmetic procedure, induces controlled skin punctures to stimulate collagen production. However, obesity can impair wound healing. This study aimed to determine if butterfly pea extract, rich in antioxidants, could enhance collagen synthesis and accelerate wound closure in obese rats. The results demonstrated that all treatment groups with butterfly pea extract cream exhibited significantly faster wound healing compared to the control group. Histopathological analysis revealed denser collagen deposition in the treatment groups, particularly those treated with 7% and 10% extract cream. These findings suggest that butterfly pea extract cream may have therapeutic potential in promoting efficient wound healing, particularly in obese individuals, by enhancing collagen production and accelerating the recovery process.

Keywords: dermapen, wound healing, collagen production, butterfly pea extract

Introduction

The skin is the largest organ in the human body, consisting of various cells, layers, and adnex, such as hair follicles, sebaceous glands, sweat, and nerve endings that feel pain, pressure, vibration, and temperature. All these entities act together to sense and protect us from the outside world, for example, physical, chemical, and biological influences (temperature, radiation, drying, trauma, chemicals, microbes, etc.). The skin is also significant for us to understand our environment and communicate through the receptors in the skin.[1] It is known that the skin plays an essential role in various processes such as hydration, protection from chemicals and pathogens, initiation of vitamin D synthesis, excretion, communication, and thermal regulation. Therefore, any form of skin damage is significant to pay attention to.[2]

Skin wounds are a typical damage caused by diseases, injuries, or physicochemical factors. Wounds can be classified as acute or chronic, depending on their cause and healing time. Acute wounds typically result from traumatic physical or chemical damage or surgical procedures, whereas chronic wounds are often linked to underlying health conditions such as infections, diabetes, vascular diseases, or cancer, which hinder the healing process over time. The healing of a wound depends on its size, depth, and the extent of damage to the epidermis and dermis. Chronic wounds, which are slow to heal, are often more complex due to factors like systemic nutritional deficiencies, immune system weaknesses, age, chronic stress, and other comorbid conditions that impair recovery.[3]

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Normal wound healing progresses through three phases: inflammation, proliferation, and remodeling, as the body's response to tissue injury. Collagen, a vital component of the extracellular matrix, plays a crucial role throughout the wound healing process, whether in its original form, as fibrillar collagen, or as a soluble component in the wound environment.[4] The final stage of skin wound healing results in scar formation. Scars consist primarily of collagen, similar to that found in the skin, covered by the epidermis. In the early stages of scarring, inflammation occurs to close the wound. The source of this inflammation is the blood vessels, which increase vascular permeability following injury. This allows inflammatory factors and immune cells to enter the wound site. In the initial phase of wound healing, resident cells, including fibroblasts that secrete collagen, accumulate in the damaged area. Over time, collagen, blood vessels, and nerve fibers are produced, forming an immature scar, which appears red, raised, hard, and painful.[5]

Scars can form on any part of the body, including the face, which is particularly important as it directly affects one's appearance and confidence. Facial scars often lead to a decline in self-esteem for both women and men.[6] However, this can be prevented by adopting proper skincare routines tailored to the skin's specific needs. Today, numerous skincare innovations offer various benefits, including microneedling technology. Microneedling is a relatively new, minimally invasive procedure involving controlled, superficial skin punctures using a roller with fine needles. This technique has quickly gained widespread popularity and acceptance due to its simplicity, affordability, safety, and effectiveness, requiring only minimal training.[7]

Microneedle therapy system is divided into 2 types, namely dermaroller and dermapen. Dermapen is a *mesotherapy* method using a device equipped with needles of varying sizes and amounts. One of the benefits of dermapen therapy is smoothing the skin and disguising acne scars or pockmarks. Dermapen is the latest development in *microneedling* therapy and has replaced *the dermaroller*. This ergonomic device is designed to be more comfortable in reaching tight areas (nose, around the eyes, and lips) and overcoming the problem of varying pressure applications.[7]

Dermapen is a type of *microneedle therapy system* tool shaped like a motorized pen, dermapen can be adjusted to skin problems. The dermapen can change the needle depth from 0.25 – 2 mm. This ergonomic device utilizes a disposable needle and guides the needle length adjustment. The tip has 9-12 needles arranged in a row. The dermapen should use sterile needles and be worn individually. That is, one demapen is only for one person. Dermapen is safer and more convenient for treating narrow areas such as the nose, around the eyes, and lips without damaging the adjacent skin.[8]

In response to injury, collagen induces platelet activation and aggregation, resulting in the deposition of fibrin clots at the injury site. In the inflammatory stage of wound healing, the activation of immune cells promotes the secretion of pro-inflammatory cytokines that affect the migration of fibroblasts, epithelial cells, and endothelial cells. Fibroblasts contribute to collagen deposition. Simultaneously, collagen degradation releases fragments that promote the proliferation of fibroblasts and the synthesis of growth factors, leading to angiogenesis and reepithelialization.[4]

The importance of skin to the human body requires us to pay close attention to various forms of skin damage. While natural healing processes exist, wound care management, such as skin dressing, can further enhance recovery. According to manuscripts found on clay tablets from ancient civilizations, wound dressings date back to 2000 BC. Archaeological evidence shows that the ancient Egyptians developed the first wound dressings. Their wound care system involved cleaning, applying a paste, and bandaging the wound. Today, these pastes are known as wound dressings, typically made from a mix of substances like oils, mud, clay, and medicinal plants.[3] One dressing mixture can be made using the butterfly pea extract (*Clitoria ternatea*). Traditionally, medicine has used butterfly peas to treat various health issues, including digestive problems, constipation, arthritis, skin conditions, and liver and intestinal disorders. It is widely used as an ornamental flower and natural food dye.[9] In Indonesia, Clitoria ternatea is a native plant with great potential for further development due to its numerous uses—such as a natural dye, cancer prevention due to its high antioxidant content, and as an ornamental plant.[10] The butterfly pea flower is rich in anthocyanidin/anthocyanin, potentially treating various conditions like allergies, rheumatism, neuroprotection, liver protection, diabetes, inflammation, cancer, and other life-threatening diseases. This prophylactic activity is attributed to its diverse polyphenolic compounds (such as polyphenols, flavanols,

flavonoids, anthocyanins, etc.). This study aims to investigate the effect of butterfly pea flower extract on collagen production during the healing process of dermapen-induced wounds in white rats, along with its histopathological features.

Method

Design

This study is an experimental study using a post-test-only control group design, a type of research that only observes the control group and treatment after specific actions are taken. The research was conducted from October to December 2023 at the Pharmacology Laboratory, Faculty of Medicine, Universitas Sumatera Utara, and the Laboratory of Anatomical Pathology, Universitas Sumatera Utara.

Sampling

This study used male white rats of the Wistar strain weighing 200-300 grams and aged 2-3 months as test samples. These animals have almost the same characteristics and physiology as humans and are one of the most widely used animals in biomedical science research. White rats also have an excellent ability to adapt to a laboratory environment. The sample in this study was divided into 4 groups, and Federer's formula calculated the magnitude: $(n-1)\times(t-1)\geq 15$. In this study, the researcher used 6 Wistar strain rat for each experimental group, so the total number of test animals in this study is 24. The grouping of test animals was carried out randomly into 4 test groups.

Tools and Material

The equipment used for the ethanol extraction of butterfly pea flower (*Clitoria ternatea* L.) includes a maceration set, filter, rotary evaporator, evaporating dish, water bath, 10 ml, 25 ml, and 100 ml volumetric flasks, test tubes, test tube rack, BioHit 1000µL micropipette, graduated pipette, spatula, vials, incubator, pH meter, cuvette, centrifuge, centrifuge tubes, UV-Vis spectrophotometer, beaker glass. The chemicals used include trichloroacetic acid (TCA), ethanol p.a. (*Brataco*), and distilled water (*aquades*)

Extract and cream creation

The raw material, butterfly pea flowers, obtained from farmers in Medan, undergoes a wet sorting process to separate rotten flowers. The flowers are then washed and dried using an oven at a temperature of 50°C. The dried flowers are ground using a blender and sieved with a 40-mesh sieve. The extraction of the dried flowers is carried out using the maceration method. 100 grams of butterfly pea flower powder (Clitoria ternatea) is weighed and mixed with 96% ethanol in a 1:10 ratio. The extract is then filtered to separate the solid residue from the filtrate. The filtrate is evaporated using a rotary evaporator to obtain a thick extract. The thick extract is dissolved in distilled water, added to the aqueous phase, and stirred until homogeneous. The oil phase is then gradually added to the aqueous phase, mixed, and stirred constantly until the mixture reaches room temperature and forms a cream base. The cream is then placed into containers.

Treatment procedures

In this study, male Wistar rats who had passed the acclimation period and consumed a high-fat feed diet were randomly divided into four groups. The mice were treated for 14 days, including Control Group (K-0), the mice were fed regular rat pellet feed and given aquades/day/tail for 14 days + base cream (0%); Treatment-1 (K-1) group, rats were fed regular pellet feed and aquades + smeared with telang flower extract cream with a concentration of 5% daily for 14 days; Treatment-2 (K-2) group, rats were given regular pellet feed and aquades + smeared with telang flower extract cream with a concentration of 7% every day for 14 days; and Treatment-3 (K-3) group, rats were fed regular pellet feed and aquades + smeared with telang flower extract cream with a concentration of 10% every day for 14 days.

Heart function examination

Cardiac biomarkers are substances released into the blood when the heart is damaged or under stress. Measuring these biomarkers is used to help diagnose acute coronary syndrome (ACS), cardiac

ischemia, and heart failure. Cardiac biomarker tests can also be used to assess a person's risk of developing these conditions or to assist in monitoring and management. These markers include enzymes, hormones, and proteins. Cardiac troponin, by far the most commonly used biomarker, has the highest known sensitivity. It enters the bloodstream immediately after a heart attack. A Troponin T (TnT) test can be performed by taking a sample of venous whole blood in a heparin tube, which is then analyzed using the Troponin-T Cardiac Reader from Roche Diagnostics. On day 15, the rats were anesthetized, and blood samples were collected from the orbital vein using a capillary pipette. 3 cc of blood was collected in EDTA (Ethylenediamine Tetraacetic Acid) tubes and placed in a cool box."

Skin histopathological preparations

Histopathological preparation is carried out by binding the skin organs using a 10% Neutral Formalin Buffer solution, then cutting and putting it into a plastic specimen place. Then, the dehydration process using alcohol with a tiered concentration, starting from 70%, 80%, 90%, and absolute I and II, every 2 hours. Then, it was clarified with xylol and printed using paraffin. The cut is floated in warm water at a temperature of 60 0C to stretch the tissue so it does not fold. The preparation is then lifted and placed in an object glass for Hematoxylin and Eosin (HE) staining. Next, it is examined under a microscope.

Histopathological observation process

Observation of skin tissue histopathology was carried out by comparing the control group and treatment. The changes observed will be the presence of fatty degeneration (vacuolization), necrosis and hydrophilic degeneration. To obtain quantitative data, scoring is carried out on each change found. The parameter observed in this study was the distribution of collagen tissue formed in wound healing. Histopathological scoring parameters for collagen tissue distribution density were performed based on the calculation of 1 field of view, on a magnification object of 400 x. The scale consisted of: 0= No collagen fibers were found in the wound area, +1= Collagen fiber density in the wound area was low (less than 10%), +2= Collagen fiber density in the wound area was moderate (10-50%), +3= Collagen fiber density in the wound area was tight (50-90%), and +4= Collagen fiber density in the wound area was very tight (90-100%).[11]

Data analysis

Histopathological data from microscopic examinations were collected and scored. The research data were tabulated, and observed changes were analyzed and presented descriptively. The data were then statistically analyzed using SPSS (Statistical Package for Social Sciences) version 25.0. Normality tests were conducted using the Kolmogorov-Smirnov test ($p > 0.05$). To test the significance between the experimental groups, a one-way analysis of variance (One-Way ANOVA) was performed at a 95% confidence level ($p < 0.05$). Further analysis was conducted using a Post Hoc Test with the Least Significant Difference (LSD) method.

Results

The researchers conducted macroscopic observations of wound healing in rats by measuring the length of the wounds using calipers. The healing process produced scars visible to the naked eye, allowing for macroscopic observations without a microscope. The aim of this observation was to compare wound healing between the group treated with a base cream and the groups treated with Clitoria ternatea (butterfly pea) extract cream at three different concentrations: 5%, 7%, and 10%. Observations were made daily for 14 days.

As shown in Table 1, the wounds in treatment groups 1, 2, and 3 were fully closed by day 14, with a wound length of 0 cm. On the other hand, the control group did not achieve complete wound closure, with a wound length of 0.55 cm on day 14. The wound length was measured daily to compare the percentage of wound healing after dermapen treatment across the different groups. The initial wound length was considered 0.00%, meaning the rate of wound healing before treatment was the same across all groups.

The average dermapen wound healing percentage on the control group's final day was 72.5%. Treatment group 1 achieved 100% wound healing by day 13, while treatment groups 2 and 3 fully recovered by day 12. The researchers concluded that the control group did not experience complete healing, and treatment groups 2 and 3 required the least time for full recovery compared to group 1.

Phytochemical tests revealed that butterfly pea flower extract contains secondary metabolites, such as flavonoids, saponins, tannins, and triterpenoids. These results are consistent with previous research by Cahyaningsih et al. (2019), which found that 80% ethanol extract of butterfly pea flowers contains flavonoids, saponins, triterpenoids, and tannins.

Histopathological examination was performed using a light microscope at 400x magnification. The purpose was to observe the structure and morphology of the cells, particularly collagen cells, in dermapen-induced wounds in the treatment groups using base cream (control) and butterfly pea extract cream at concentrations of 5%, 7.5%, and 10%. The cream was applied twice a day, in the morning and evening. Wound healing from dermapen treatment involves inflammation, proliferation, and maturation. Histopathological observations at 400x magnification revealed differences in collagen density. During the proliferative phase, fibroblasts play a crucial role in producing proteins, such as collagen, for wound healing. In the control group, which only received base cream, collagen growth was fragile, earning a score of 1 (less than 10%) due to the skin tissue being in an inflammatory state, where collagen formation was minimal.

Table 1. Average wound healing (cm (%))

Day	Control	P1	P2	P3
1	2 (0,0)	2 (0,0)	2 (0,0)	2 (0,0)
2	1.97 (1,5)	1.94 (3,0)	1.93 (3,5)	1.91 (4,5)
3	1.91 (4,5)	1.83 (8,5)	1.73 (13,5)	1.73 (63,5)
4	1.85 (7,5)	1.75 (12,5)	1.51 (24,5)	1.48 (26,0)
5	1.76 (12,0)	1.60 (20,0)	1.29 (35,5)	1.21 (39,5)
6	1.62 (19,0)	1.39 (30,5)	1.09 (45,5)	1 (50,0)
7	1.47 (26,5)	1.14 (43,0)	0.86 (57,0)	0.77 (61,5)
8	1.35 (32,5)	1.04 (48,0)	0.63 (68,5)	0.56 (72,0)
9	1.23 (38,5)	0.81 (59,5)	0.43 (78,5)	0.48 (76,0)
10	1.11 (44,5)	0.58 (71,0)	0.23 (88,5)	0.33 (83,5)
11	0.96 (52,0)	0.41 (79,5)	0.02 (99,0)	0.12 (94,0)
12	0.84 (58,0)	0.20 (90,0)	0 (100,0)	0 (100,0)
13	0.71 (64,5)	0 (100,0)	0 (100,0)	0 (100,0)
14	0.55 (72,5)	0 (100,0)	0 (100,0)	0 (100,0)

Table 2. *One-Way Anova* test results

	Sum	df	Mean square	F	Sig
Between Groups	1.222	3	.407	622.834	.000
Within Groups	.013	20	.001		
Total	1.235	23			

Table 3. *Post-Hoc* LSD Test Results on ALT Levels

Groups		Mean difference	Sig
Control	Treatment 1	.33500*	.000
	Treatment 2	.54833*	.000
	Treatment 3	.55500*	.000
P1	Control	-.33500*	.000
	Treatment 2	.21333*	.000
	Treatment 3	.22000*	.000
P2	Control	-.54833*	.000
	Treatment 1	-.21333*	.000
	Treatment 3	.00667	.657
P3	Control	-.55500*	.000
	Treatment 1	-.22000*	.000
	Treatment 2	-.00667	.657

In contrast, the groups treated with butterfly pea extract (*Clitoria ternatea*) showed dense and thick collagen growth. Treatment group 1 scored 3 (50-90%). Meanwhile, treatment groups 2 and 3 exhibited rapid and dense collagen growth, scoring 4 (90-100%). The histopathological images for groups 2 and 3, treated with 7% and 10% butterfly pea extract cream, showed the densest collagen arrangement compared to the control group and treatment group 1.

The results of the One-Way ANOVA test in Table 2 show that the resulting significance value is 0.000 or < 0.05. Based on these data, it can be concluded that there is a significant difference between the control group and the treatment group. The Post Hoc LSD test was used to determine whether the group had significant differences in wound healing compared to other groups. The results of the analysis showed that there was a significant difference between the control group and treatment groups: 1 (p= 0.000), 2 (p= 0.000), and 3 (p= 0.000). Meanwhile, there was no significant difference between treatment group 2 and treatment group 3 (p= 657).

Discussion

This research was conducted to test and analyze the effectiveness of butterfly pea flower (Clitoria ternatea) extract cream on collagen production during the wound healing process in dermapen-induced wounds on male Wistar obese rats (Rattus norvegicus). The sample for this study consisted of male Wistar rats weighing between 200-300 grams and aged 2-3 months. The sample size was determined using Federer's formula for four groups, resulting in 24 rats divided into four groups. The first group was the

control group, where rats were only given base cream. The treatment groups were given butter pea extract cream at 5%, 7%, and 10%.

The initial discussion explained that the skin is an essential organ involved in various processes such as hydration, protection from chemicals and pathogens, initiating vitamin D synthesis, excretion, communication, and thermal regulation. Therefore, any form of skin damage is critical and requires attention.[2] One type of skin damage is a wound. A skin wound is a pathological condition caused by disease, injury, or physicochemical damage. Wounds are classified as acute or chronic based on the cause and healing time. Traumatic physical or chemical damage or surgical procedures result in acute wounds. In contrast, diseases like infections, diabetes, vascular diseases, and cancer contribute to wounds that do not heal over time, referred to as chronic or difficult-to-heal wounds.[3]

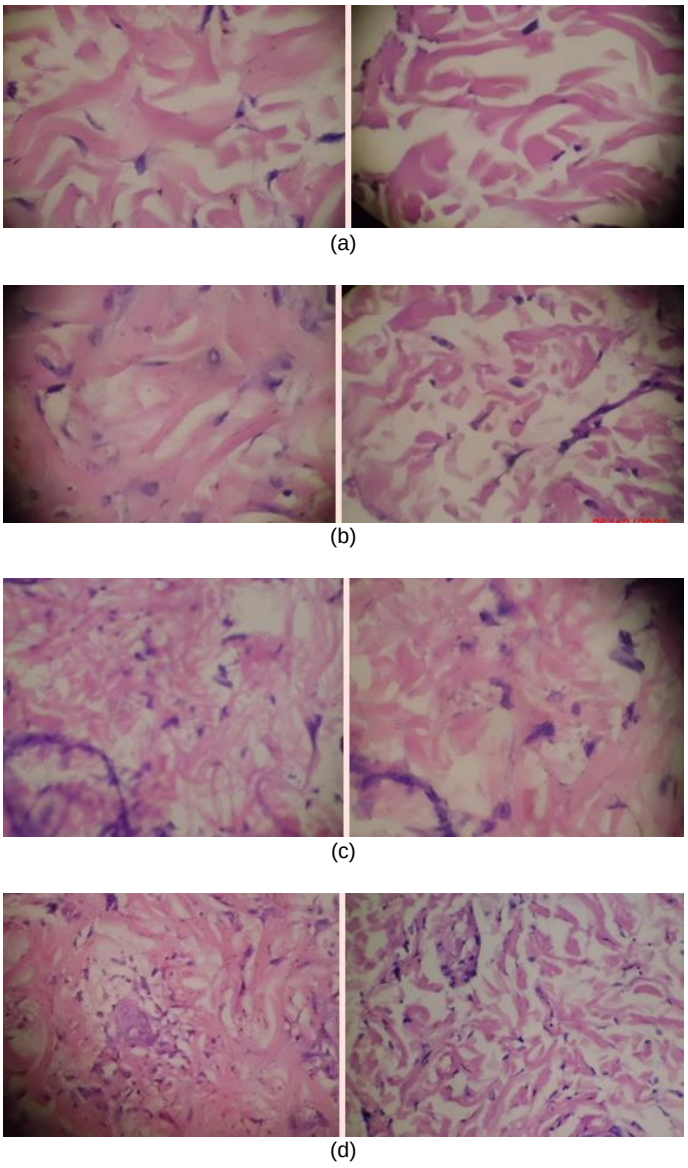

Figure 1. Overview of skin tissue histopathology
(a) Group (0%); (b) Treatment 1 (5%); (c) Treatment 2 (7%);
(d) Treatment 3 (10%)

After a wound is formed, the healing process begins. Routine wound healing occurs through three phases: inflammation, prolife-ration, and remo-deling, in response to tissue injury. Collagen, a vital extracellular matrix component, plays a critical role in regulating these phases, either in its native form, fibrillar conformation or as a soluble component in the wound environment.[4]

To accelerate wound healing, one approach is to apply a cream containing butter-fly pea flower extract. The phytochemical content of butterfly peas includes tannins, phlorotannins, carbohydrates, saponins, triter-penoids, phenols, flavonoids, flavanol glyco-sides, proteins, rutin, alkaloids, anthraquin-ones, anthocyanins, essential oils, and steroids. Its fatty acid composition includes palmitic, ste aric, linoleic, and oleic linoleic acids. The antioxidants in butterfly peas, such as phenols, flavonoids, anthocyanins, flavonol glycosides, kaempferol glycosides, quercetin glycosides, myricetin glycosides, terpenoids, tannins, steroids, are particularly beneficial for wound healing.[12]

The researchers hypothesized that applying butterfly pea flower extract cream would positively affect the healing of dermapen-induced wounds in obese male Wistar rats. To test this hypothesis, they experimented on male Wistar rats. The study involved collecting data related to the treatment procedure. First, the rats were subjected to a pre-treatment high-fat diet to induce obesity, with quail egg yolks as part of the high-fat diet. Obesity in the rats was confirmed using the Lee Index, a commonly used parameter. The results showed that after the high-fat diet, all test animals had a Lee Index value of 0.33 or greater (0.3 or more indicates obesity).

After confirming obesity, the dermapen-induced wounds were created. The procedure began by anesthetizing the test animals using a combination of ketamine (80 mg/kg body weight) and xylazine (5 mg/kg body weight) to prevent pain and reduce excessive movement caused by the dermapen treatment.

The dermapen was applied perpendicularly to the stretched skin with a needle depth of 2.5 mm. The pressure was applied point by point to all the wound areas, with the endpoint being the appearance of bleeding. The resulting wounds were treated with base cream in the control group and butterfly pea (Clitoria ternatea) extract cream in the treatment groups for 14 days. Data from the wound healing process required statistical analysis, including normality, homogeneity, and significance tests.

The normality test was conducted using SPSS with the Kolmogorov-Smirnov test. The results indicated that the wound healing data in each group were normally distributed, with a significance value of 0.200 in all groups. This means the data were normally distributed and representative of the population. Next, the homogeneity of the data was tested using Levene's test to determine if the data came from populations with equal variances. The homogeneity test results showed a significance value of 0.223, more significant than 0.05, indicating that the control group, Treatment 1, Treatment 2, and Treatment 3 groups were homogeneous or from the same population. The normally distributed and homogeneous data were subjected to effectiveness and significance testing using One-Way ANOVA. The results showed a significance value of 0.000, indicating a significant difference between the control group and Treatment groups 1 ($p = 0.000$), 2 ($p = 0.000$), and 3 ($p = 0.000$). However, there was no significant difference between treatment groups 2 and 3 ($p = 0.657$). The average percentage of wound healing in the control and treatment groups demonstrated apparent differences. On the final day, the control group achieved a 72.5% healing rate, while Treatment Group 1 reached 100% healing by day 13, and Treatment Groups 2 and 3 achieved 100% healing by day 12. Therefore, it can be concluded that the control group did not experience complete healing, and Treatment groups 2 and 3 required the least amount of time to achieve full recovery compared to Treatment group 1.

Observation continued on the histopathological picture of skin tissue to see the collagenization of wound healing. The results showed that the Control group that only received treatment in the form of a base cream produced fragile collagen growth, so it got a score of 1 (less than 10%). This is because the skin tissue is still inflammatory, so collagen is not seen much. The Treatment 1 group scored 3, which is 50-90%. Meanwhile, the Treatment 2 and 3 groups experienced rapid and dense collagen growth, so they were included in the score category 4 (90-100%). The histopathological picture in the Treatment 2 and 3 groups smeared with telling flower extract cream with concentrations of 7% and 10% showed the densest collagen composition compared to the Control and Treatment 1 group. The acceleration of wound healing occurs because of the content of secondary metabolites in telang flower extract in saponins, tannins, triterpenoids, and flavonoids, which are carriers of free radicals arising from dermapen scars and obesity. This study's results align with research conducted by Hotimah et al.[13]. The research tested the effect of ethanol extract ointment of telang flower on the healing of cut wounds on the back of white rabbits. The results showed that the ointment of ethanol extract from telang flower was effective in healing cut wounds on the back of rabbits.

Conclusion

The application of butterfly pea flower extract cream at concentrations of 5%, 7%, and 10% significantly affected the healing process of dermapen-induced wounds in obese Wistar rats. The treatment groups experienced complete healing, while the control group, which was given only base cream, did not. Histopathological observations showed that the control group exhibited fragile collagen growth, whereas the treatment groups that received butterfly pea extract at 5%, 7%, and 10% concentrations demonstrated full and dense collagen formation.

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