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ORIGINAL ARTICLE

Effectiveness of red betel leaf extract cream for healing burn wounds

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Abstract

Burns are a frequent type of wound that can cause complications, infection and bleeding. The use of plants and natural materials as wound-healing remedies has been known for a considerable period, although more scientific proof is required. The objective of this investigation is to examine the efficacy of red betel leaf extract ointment in curing burns on the backs of white wistar rats. The study used a pre-test post-test design with a control group. Twenty white rat samples were employed. The samples were split into four groups, consisting of five rats each. Betel leaf extract was prepared through maceration, whilst betel leaf extract cream was made using the oil-in-water emulsion method (M/A). A histopathology test was carried out to observe growth of fibroblast cells. Rat skin biopsy was taken from a 2 x 2 cm area, and data normality was tested with the Shapiro-Wilk test ($p > 0.05$). The One-way Anova test ($p < 0.05$) was used to assess the differences between the groups. To determine the most effective treatment group, a post hoc test using LSD technique was performed. The findings showed that the average percentage of burn wound healing in the control group (P0) compared to groups P1, P2, and P3 was very different. The analysis reveals that the application of 15% betel leaf extract cream proves to be superior in treating burns in white mice in contrast to 7.5% and 10% betel leaf extract cream. At a 15% concentration, secondary metabolite compounds found in betel leaf extract are already effective in treating wounds. However, at lower concentrations they only inhibit microorganisms, making them less effective in promoting wound healing.

Keywords: red betel leaf extract cream, burn, wistar rat

Introduction

Burn injuries are a common occurrence in daily life, resulting from various sources such as hot water, fire, chemicals, electricity, and radiation.[1] Burn injuries cause localized tissue damage with or without a systemic response to energy transfer from physical (mechanical, thermal, radiation, electrical) or chemical sources. Burn injuries extend beyond affecting the skin and subcutaneous tissues, also impacting surrounding bodily systems.[2] The detrimental effects of burn injuries on individuals are both psychological and physical, as they are severe forms of trauma dating back through history.[3] According to the World Health Organization (WHO), burns rank as the seventh most common injury worldwide, causing over 7.1 million injuries and 265,000 deaths annually. Burn-related deaths account for approximately 5% of all injuries, with nearly a quarter of these deaths attributed to traffic accidents.[4,5] In Indonesia, there were around 3,518 reported burn cases, showing an increasing trend from 2012 to 2014.[6] The 2018 Basic Health Research in Indonesia indicated a burn prevalence of 1.3%.[7]

While some burn wounds may heal naturally, unattended burns can lead to complications, infections, and bleeding. Therefore, burn wound management aims to prevent secondary infections, promote collagen formation, and facilitate the proliferation of epithelial cells for wound closure.[8] Therefore, the management

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of burn wound healing aims to prevent secondary infections and provide an opportunity for the proliferation of residual epithelial cells to close the burn wound surface. Actions that can be taken for burn wounds include providing local therapy to prevent infections, stimulate collagen formation, and ensure the development of residual epithelial cells to cover the wound surface.[9] The wound healing process is generally similar to the healing of other wounds and is commonly divided into three phases, including the inflammatory phase, the proliferation phase, and the maturation phase. The fundamental principle behind optimal wound healing is to minimize tissue damage and provide adequate tissue perfusion, oxygenation, and proper nutrition for the tissues.[10] The speed of wound healing can be influenced by substances present in the administered medication. If the medication can accelerate the healing process by stimulating the growth of new skin cells, compounds or substances that play a role in the wound healing process include alkaloids as antibacterials, flavonoids as anti-inflammatory and antibacterial agents, saponins as antiseptics, and tannins and triterpenoids as antioxidants.[11–13]

The use of plants and natural substances as medicine to reduce pain, heal, and prevent specific diseases has been known to society since ancient times. One plant or herb that is easily accessible and considered to have wound-healing properties is the red betel leaf (*Piper crocatum*).[14] Red betel leaves contain compounds such as flavonoids, alkaloids, polyphenols, tannins, and essential oils, where the main components of the essential oil in red betel leaves consist of phenols and their derivatives such as chavicol, eugenol, chavibetol, tannins, saponins, allylpyrocatechol containing antiseptic and antifungal substances.[15] Saponins, tannins, sterols, and polyphenols can heal burns. Saponins can act as cleansers, effectively healing open wounds, while tannins can be used to prevent infection in wounds due to their antiseptic and burn healing properties. Flavonoids, saponins, and tannins also act as antioxidants, pro-angiogenesis, and can enhance oxygen and nutrient supply to injured skin.[16] Flavonoids in the process of burn wound healing play a role as anti-inflammatory agents by inhibiting cyclooxygenase and lipoxygenase, resulting in a restriction of the number of inflammatory cells migrating to the wound tissue, consequently shortening the inflammatory phase. Flavonoids also act as powerful antioxidants in granuloma tissue, protecting the surrounding area of the wound from free radicals that can hinder wound healing by destroying fats, proteins, collagen, proteoglycan acid, and hyaluronic acid.[17,18] Saponins can act as cleansers and antiseptics, killing or preventing the growth of microorganisms that commonly occur in wounds, and preventing severe infections. In the skin that has suffered injuries such as wounds, saponins are useful for stimulating the formation of collagen, a structural protein that plays a role in the wound healing process. Meanwhile, tannins can be used as a preventive measure against wound infections due to their antiseptic and burn healing properties.[16]

The management of burns is typically done by providing topical medications. The topical application of herbal ingredients is known to yield more optimal results in wound healing, especially in accelerating wound contraction. This is because, with topical use, the drug compounds accumulate more on the wound side. Direct application of concentrated extracts on the skin is less practical and not optimal; therefore, formulations that can adhere to the skin surface for an extended period and have occlusive properties are needed to effectively heal wounds.[19] Topical treatment of burns is more effective because the drugs are easily absorbed by the skin, and their moisturizing function lasts longer. The use of topical therapy enhances efforts to transform open and dirty wounds into closed and clean wounds.[20,21] Cream is a common form of topical preparation with a local purpose. Cream formulations spread easily on the skin, are non-sticky, and are user-friendly. Oil-in-water type creams are suitable for topical use, such as in burn wounds, as they can absorb water.[22] This study aims to test the effectiveness of applying a cream with red betel leaf extract in the process of burn wound healing on the back of Wistar strain white rats.

Method

Study design

This research belongs to the category of laboratory experimental or true experiment. The research design utilizes a pre-test post-test with a control group design to examine the effectiveness of applying a cream with red betel leaf extract in the process of burn wound healing on the back of Wistar strain white rats.

Sampling

The research sample consists of adult male Wistar strain white rats weighing 160-200 grams and aged 2-3 months. The selection of Wistar strain white rats as the research sample is based on considerations that these rats have characteristics and physiology almost similar to humans, are larger in size compared to rats, and can adapt well to laboratory environments. A total of 20 white rats were used as samples, divided into 4 groups, with each group consisting of 5 rats. Group I is the control group (P0) receiving only 0.9% NaCl. Group II is treatment group 1 (P1) receiving 7.5% red betel leaf extract cream (dose 1). Group III is treatment group 2 (P2) receiving 10% red betel leaf extract cream (dose 2). Finally, Group IV is treatment group 3 (P3) receiving 15% red betel leaf extract cream (dose 3).

Materials

The tools used in this study include various minor surgical instruments such as stainless steel trays, scalpels, blades, scissors, and forceps. Additionally, a modified hot solder with stainless steel plates measuring 2 × 2 cm, a scale, blender, jars, stirring rods, rotary evaporator, vial bottles, porcelain cups, petri dishes, Pasteur pipettes, gauze, rat cages, feeding containers, markers, writing tools, and calipers with a precision of 0.1 mm was employed. The materials used consist of red betel leaves, 90% ethanol, glycerin, triethanolamine (TEA), ethyl alcohol, acetic acid, methyl paraben, propyl paraben, distilled water (aquades), sterile tampons, anesthesia (ketamine), xylazine, 0.9% NaCl, Wistar white rat, and rat feed and beverages.

Procedure

Preparation of red betel leaf extract

Previous literature indicates that the preparation of red betel leaf extract mostly involves the use of water or distilled water as a solvent; 70% ethanol[23]; and methanol as a solvent.[15,24] To differentiate from previous studies, the red betel leaf extract in this research was prepared using 90% ethanol as the solvent. The extraction process began with the selection of leaves, followed by cutting and drying them using an oven at a temperature of 50-60°C. The dried leaves were then blended into a powder. The extraction of red betel leaf was conducted through the maceration method, which involves soaking the leaves in a maceration container separately and then adding a 90% ethanol solution until the leaves are completely submerged. The maceration container was tightly closed and left for approximately 5 days, with stirring once a day. The obtained results were filtered and repeated three times, then collected in bottles for further concentration using a rotary evaporator until a concentrated ethanol extract was obtained. The extract obtained was evaporated using a rotary evaporator at a temperature of 70°C, aiming to remove the ethanol and obtain a concentrated extract from the red betel leaves.

Preparation of cream and dosing of red betel leaf extract

Cream is a commonly used form of topical preparation for local purposes. Creams spread evenly on the skin, are non-sticky, and easy to use. The formulation of red betel leaf extract cream was made as an oil-in-water (O/W) emulsion, as O/W creams are suitable for topical use, especially in burn wounds, as they can absorb water. The oil phase ingredients included stearic acid (emulsifier), cetyl alcohol (emulsifier and thickener), and propylparaben (preservative). The water phase ingredients included TEA (emulsifier), glycerin (humectant), methylparaben (preservative), and distilled water (solvent). Each phase was separated, and then the oil and water phases were heated to a temperature of 55°C until everything melted. The red betel leaf extract was dissolved in part of the distilled water, added to the water phase, stirred until homogeneous, and then the oil phase was added gradually to the water phase, mixed, and continuously stirred until room temperature was reached, forming the cream base. The cream was then placed in a container. All cream ingredients were weighed according to the specified doses (Table 1).

Table 1. Formula for red betel leaf extract cream preparation (O/W)

Ingredients	Cream formula (gram)		
	F1	F2	F3
Red betel leaf extract	7,5	10	15
Cetyl alcohol	4	4	4
Glycerin	15	15	15
TEA (triethanolamine)	3	3	3
Stearic acid	12	12	12
Methylparaben	0,2	0,2	0,2
Propylparaben	0,02	0,02	0,02
Aquades (distilled water)	100	100	100

The preparation of dose 1 (cream of red betel leaf extract 7.5%) is carried out by incorporating 7.5 grams of red betel leaf extract, dose 2 (cream of red betel leaf extract 10%) includes 10 grams of red betel leaf extract, while dose 3 (cream of red betel leaf extract 15%) requires 15 grams of red betel leaf extract. All these doses are supplemented with ingredients as listed in Table 1 and distilled water is added until the total weight of the ingredients reaches 100 grams.

Rat burn wound creation

All acclimatized white rats for 7 days (one week) were randomly divided into 4 groups, each consisting of 5 rats. Each rat was labeled on its tail according to its group using a waterproof marker. A modified hot solder with a stainless-steel plate measuring 2 × 2 cm was used. Fur or hair around the burn area (back part) was shaved according to the desired burn area. After shaving, the rats were anesthetized using a combination of ketamine (80 ml/kg BW) and xylazine (5 ml/kg BW) to ensure the rats did not feel pain and to avoid excessive movement caused by the rats. Subsequently, the rats were injured by applying hot solder to their backs for 2 seconds, causing blistering and peeling of the skin in certain areas, affecting the dermis and the tissue bound beneath.

Handling and observation of burn wounds

Handling followed wound care protocols and continued according to predetermined treatment groups. Burn wound care for rats was performed once a day in the morning for 14 days. Burn wounds were treated openly until healed, marked by the closing and covering of the wounds. Changes in burn wound healing in rats were observed by measuring the average surface area of the wounds each day, starting from the first day of burn injury until the 14th day, using calipers. The percentage of burn wound healing was calculated by subtracting the burn area before treatment from the burn area after treatment. The result was then multiplied by 100% and divided by the burn area on the first day. The burn wound healing process is generally similar to that of other wounds, and observation in this study continued for 14 days, aligning with the typical duration of the normal wound healing process, reaching the proliferation phase around days 3 and 14 after the wound occurred. After 14 days, all rats were euthanized with excess technical chloroform inhalation.

Histopathological observation

Histopathological testing was performed microscopically by observing the growth of fibroblast cells. The procedure began by conducting a skin biopsy of rats measuring 2 x 2 cm. The cross-section of the skin tissue was cut to observe the number of fibroblast cells. Wistar rat skin tissue was stored in urine pots, immersed in 10% formalin, and then stained with Hematoxylin and Eosin at the Microbiology Laboratory of the Universitas Sumatera Utara. The fibroblast cell count was done using a digital analysis method, with each specimen photographed with a camera and microscope at 100x magnification, capturing 4 images and storing them. The fibroblast cell count analysis photo was done using Image Raster 3 software. Fibroblast cell tissue visible from the histological examination was recorded as red-colored pixels by the software. Meanwhile, other tissues with different colors were selected and noted as pixels of other tissues. Fibroblast count was determined by the percentage of fibroblast cells visible in the field.

Data Analysis

The research data were analyzed using the SPSS program. The normality of the data was assessed using the Shapiro-Wilk test ($p > 0.05$). To examine differences between groups, One-way Anova test was conducted ($p < 0.05$). A post hoc test with LSD technique was used to determine which treatment group was most effective.

Results

The analysis of the content and phytochemical test of red betel leaf extract was conducted at the Faculty of Mathematics and Natural Sciences, Universitas Sumatera Utara. The sample used was red betel leaf extract originating from the Berastagi area, Samosir Island, North Sumatra, cultivated by farmers

Table 2. Phytochemical test results

Content	Reagent	Result	Remark
Flavonoid	Mg, Concentrated HCL	Yellow	Positive
Saponin	Aquades (Distilled Water)	Foaming observed	Positive
Tanin	$FeCl_3$	Dark green to black	Positive
Alkaloid	Reagen Warner	Brown-colored precipitate	Positive

around Lake Toba. The phytochemical content found in the extract included flavonoids, saponins, tannins, and alkaloids. Thus, it can be concluded that red betel leaf extract contains phytochemical compounds that can be utilized as medici nal ingredients due to its high antioxidant content.

Burn wound healing was observed in four treatment groups: 7.5% red betel leaf extract cream (P1), 10% red betel leaf extract (P2), 15% red betel leaf extract (P3), and a control group (P0). The average percentage of burn wound healing in the P3 group was higher, reaching 27.73% on the 14th day. In contrast, the P1, P2, and P0 groups had average burn wound healing percentages of 17.47%, 14.07%, and 6.47%, respectively, on the same day. The P3 group showed faster healing than the other groups, with a significant difference observed on the 14th day.

Table 3. Average burn wound healing (%)

Days	P0	P1	P2	P3
2	0	0	0	0
4	1.44	5.11	7.80	9.26
6	3.94	10.10	14.90	16.17
8	8.23	18.31	20.13	23.74
10	9.92	18.47	20.17	40.79
12	10.16	21.24	26.06	45.06
14	11.59	25.46	33.23	59.12
Average	6.47	14.07	17.47	27.73

The Shapiro-Wilk test results showed p-values greater than 0.05 for the control group (P0=0.114) and the treatment groups (P1=0.107; P2=0.871; P3=0.404), indicating that the data were normally distributed (p>0. 05). The test of homogeneity of variance results showed that the data variance in the control group (P0), Group P1, Group P2, and Group P3 was homogeneous (p>0.05). One-way ANOVA results indicated a significant difference in the percentage of burn wound healing among all observation groups (p<0.05). Post hoc tests were conducted to identify which groups differed, revealing differences in the average percentage of burn wound healing among all observation groups. Only the comparison between Groups P1 and P2 did not show any significant difference in the averages.

Table 4. Post Hoc test

(I) Group	(J) Group	p
Group P0	Group P1	0.000
	Group P2	0.000
	Group P3	0.000
Group P1	Group P0	0.000
	Group P2	0.163
	Group P3	0.000
Group P2	Group P0	0.000
	Group P1	0.163
	Group P3	0.000
Group P3	Group P0	0.000
	Group P1	0.000
	Group P2	0.000

Visual observations showed the average physiological changes that occur in burn wounds from day 1 to day 14 (see Figure 1). The control group (P0) and treatment group P1 experienced a longer healing process until day 14, as seen from the change in the color of the burn wounds, the time it took for scab formation, and the time it took for the scab to fall off. This indicates that the normal healing process occurred as the P0 group did not receive any treatment. Meanwhile, for treatment group P1 with the application of cream containing 7.5% extract, it did not have a significant effect on burn wound healing. For groups P2 and P3, it is evident on day 14 that there has been a change in color to reddish-brown, and the scab has already detached, indicating healing from the burn wound. Faster healing occurred in group P2.

The occurrence of necrosis in the specimen indicates that the burn injury has damaged the epithelial layer and some layers of the dermis, consistent with the characteristics of a second-degree burn. The parameter of infiltration of inflammatory cells and macrophages indicates that the wound has entered the inflammatory phase. Observation of neo capillarization indicates the presence of blood supply to the wound area, confirming the occurrence of the wound healing process.

Discussion

The decrease in the percentage of burn wound area in the control group (P0) resulted in the lowest reduction compared to other treatment groups. This is because the control group does not contain active substances that can assist in the acceleration of burn wound healing. The negative control acts only as a wound dressing that inhibits water evaporation on the skin layer.[25] Treatment with red betel leaf extract cream in the P3 group with a concentration of 15% resulted in a greater reduction in burn wound area compared to treatment groups P1 (7.5% extract concentration) and P2 (10% extract concentration). This is because the cream formulation containing 15% red betel leaf extract has more active ingredients that can

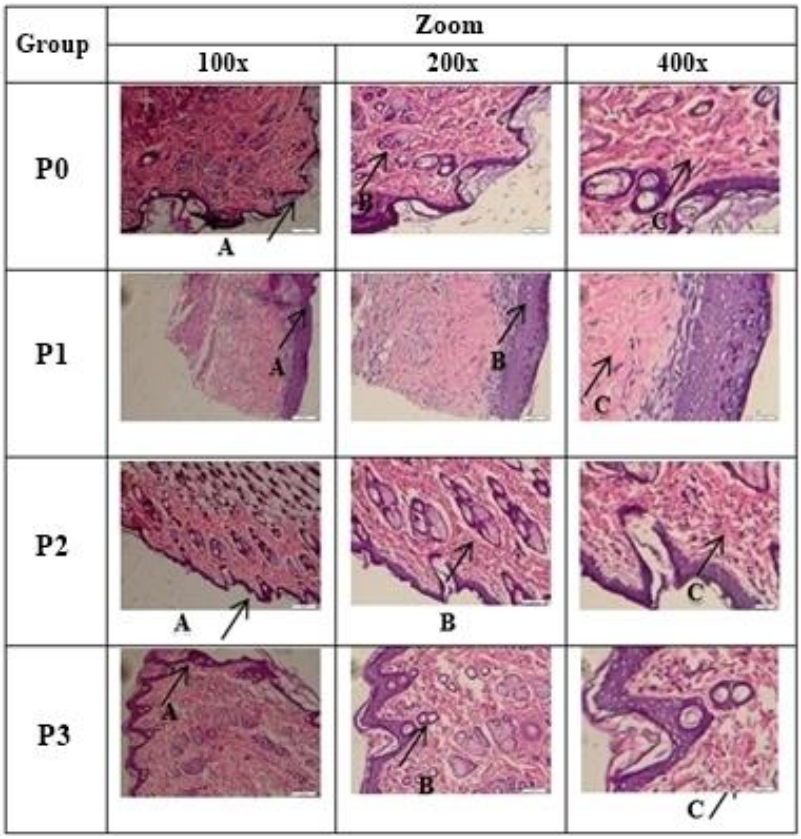

Figure 1. Results of histopathological specimen observations

(A) Necrosis in the epidermis; (B) Infiltration of inflammatory cells and macrophages; (C) Neovascularization

help reduce the burn wound area or percentage of healing. This is in line with the opinion of Purbowati et al.,.[26] stating that if antibiotics are used in small concentrations, they only inhibit (bacteriostatic), but if used in high concentrations, they will be bactericidal.

The reduction in wound area in the cream formulation is better than the gel formulation because the cream formulation contains both oil and water phases, so it will adhere to the skin longer than the gel formulation, which only contains water-soluble ingredients, resulting in a higher effect produced by the cream compared to the gel.[27] The activity of reducing burn wound area is influenced by the content of secondary metabolites in moringa leaves. Flavonoids function as antibacterial agents by forming complex compounds with extracellular proteins that disrupt the integrity of bacterial cell membranes. Flavonoids promote blood circulation throughout the body and prevent blood vessel blockage, acting as an anti-inflammatory, antioxidant, and helping to reduce pain in case of bleeding or swelling.[28] Flavonoids inhibit the enzyme lipoxigenase, which plays a role in the biosynthesis of leukotrienes. In addition, flavonoids inhibit the metabolism of arachidonic acid, reducing prostaglandin production. Flavonoids also inhibit the secretion of lysosomal enzymes, which are inflammatory mediators. Inhibition of these inflammatory mediators can inhibit the proliferation of inflammation processes. [29] Research by Sutrisno et al[30] reported that quercetin can accelerate the healing of second-degree burns by increasing the acceleration of wound diameter shrinkage, reducing the intensity of wound color, increasing collagen formation, and sebaceous gland formation. Antioxidants from quercetin compounds can trigger collagen production and increase Vascular Endothelial Growth Factor (VEGF). Collagen plays a crucial role in the wound healing process as it is an essential protein that makes up the body's connective tissue and enhances the strength of new tissue after the wound.

Tannins have anti-inflammatory and antioxidant activities. Antioxidants act as anti-inflammatory agents in various ways. The first way is by inhibiting the production of oxidants by neutrophils, monocytes, and macrophages. Inhibition of the production of hypochlorous acid (HOCl) and OH oxidants will be inhibited. In the second way, tannins directly inhibit reactive oxidants such as hydroxyl radicals (OH) and hypochlorous acid.[31] Shin et al.[32] found that when saponin was used to treat skin tissue, the synthesis of fibroblast collagen increased, and the expression of matrix metalloproteinases was inhibited. Saponins increase collagen synthesis in skin fibroblasts through protein phosphorylation. Saponin promotes matrix re-synthesis at the wound site. Saponins not only promote wound re-epithelization but also effectively inhibit inflammatory reactions during the initial phase.

The results of this study are in line with the research by Hair[33] which concluded that lemongrass extract at 100% and 50% concentrations could accelerate the healing of mouse labial mucosal wounds based on wound length. The higher the extract concentration, the faster the wound healing, possibly due to more active ingredients in the higher concentration extract. However, there was no significant difference between lemongrass extract concentrations of 100%, 50%, and 25%. Furthermore, another factor that may

also affect the results of this study is the smaller number of samples used, namely 20 white mice. The number of samples used will affect a study because the more samples used, the smaller the chance of generalization error. Another possible factor from within the mouse's body that can affect the results is stress as an unavoidable factor because it can affect the wound healing process. Previous studies have shown that stress can trigger an increase in cortisol, which impacts cellular immune suppression, thus slowing down wound healing.[34]

Conclusion

Red betel leaf extract contains phytochemicals that can be utilized as a medicinal ingredient due to its high antioxidant compound content. The average percentage of burn wound healing in the control group (P0) compared to groups P1, P2, and P3 is significantly distant. This is because the control group (P0) does not contain active substances that can assist in accelerating the burn wound healing process. In this study, it was found that the group receiving 15% red betel leaf extract cream was more effective in healing burn wounds in white mice compared to the groups receiving 7.5% and 10% red betel leaf extract cream. This is because, at a concentration of 15%, secondary metabolites in red betel leaf extract already have an impact on wounds, while at lower concentrations, they only inhibit microorganisms, making them less effective in wound healing.

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