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Original Article

In vivo study of *Centella asiatica* (L.) Urban as a drug gel for diabetes wounds

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A B S T R A C T

Background: Centella asiatica L. Urban (CA) is a tropical plant whose spread is quite broad as Indonesia. One of the ingredients of CA is asiaticoside which has excellent wound healing abilities. However, research on diabetic wound healing with CA extract formulation in the form of a gel has not been found. Therefore, it is necessary to look at the healing activity of diabetic wounds using Centella asiatica L. Urban extract in the form of a gel.

Objective: This experimental study aims to explore the effect of gel extract derived from the CA on the length of time for wound healing.

Methods: The subjects in this study were eight weeks old Balb-C mice conditioned to hyperglycemia and were divided into five groups. The CA extract is provided in three concentration levels, with 3%, 5%, and 7%. As a form of negative control, used gel without CA extract and positive control without gel, only hydrocolloid dressing.

Results: CA at concentrations of 3% (with the value of Sig. > 0.05), 5%, and 7% showed the ability to heal wounds.

Conclusions: CA gel extract with a concentration of 3% had a significant effect on wound healing compared to other preparations.

INTRODUCTION

Some Indonesian tormented by diabetes mellitus in 2000 reached 8.43 million human beings, and it'll growth to 333 million human beings in 2025. If not treated, people with diabetes can experience one problem of foot blood vessel disorders.[1] Approximately 15 to 25% of diabetic patients will develop a foot ulcer in their lifetime, which is the leading cause of non-traumatic amputations worldwide. The overall prevalence rate of complications is between 1.3 to 4.8%.[2] Diabetes is the most common cause of foot ulcers, infection, and ischemia, leading to hospitalization and the most frequent cause of non-traumatic lower-extremity amputations. In a study conducted on 64 respondents, the amputation rate was 39.1% with poor glycemic control, osteomyelitis, vasculopathy, and peripheral neuropathy.[3] Diabetic foot ulcer (DFU) is a severe complication of diabetes that results in morbidity and mortality. The mortality rate due to the development of diabetic foot ulcers is estimated at 5% in the first 12 months, with a 5-year mortality rate estimated at 42%.[4]

It is necessary to treat wounds in diabetes to prevent diabetic wounds from getting worse.[5] The latest wound care techniques have been using modern dressings. The dressings create a moist wound environment so that the host defense mechanism (neutrophils, macrophages) can clear devitalized tissue using the body's enzymes.[6] The principle of modern wound care products is to maintain and maintain a moist wound environment to facilitate the wound healing process. The variety of wound dressings available makes dressing selection difficult for practitioners. The selection of the proper dressing can reduce healing time, reduce the time to provide efficient care and improve the patient's quality of life. Research into wound healing mechanisms has improved our ability to heal chronic wounds faster through the use of dressings that have moisture.[7] Previous studies have shown that modern dressings are better at necrotic debridement, pain reduction during dressing changes, infection control, and wound closure.[8] One of the wound dressings used in modern

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wound care is a hydrogel, which reduces pain and accelerates wound healing, granulation growth, and epithelialization.[9] Hydrogel is one of the candidates with the most significant potential to mimic the microenvironment of genuine leather. It can be applied as a permanent or temporary dressing for different wounds to support the regeneration and healing of the injured epidermis, dermis, or both.[10] Gel is a topical dosage form often used for wound treatment. Gels were chosen. They can penetrate further than creams and have advantages over ointments because they provide a comfortable feeling and can be used in folds and hairy areas.[11]

Asiaticoside has excellent wound healing abilities. Many studies on asiaticoside reveal that it is a golden herbal compound used to manage wounds.[12] *Centella asiatica* (L.) Urban (CA) is one of several plants easily found around the home environment, and its presence has not been widely used as a potential herbal medicine. One of the active components of CA that is important in wound healing is asiaticoside which functions as an antioxidant and also supports angiogenesis in the wound healing process.[13] Asiaticoside promotes fibroblast proliferation and extracellular matrix synthesis in wound healing.[14] A study found that the ethanol extract of the CA plant contained asiaticoside, tannins, saponins, and steroids.[15]

Previous research on wound healing activities using CA extract has found that CA extract facilitates the wound healing process in both cuts and burns.[16] Another study found that CA extract capsules could shorten the course of diabetic sores.[17] Some have even found that the combined effect of asiaticoside and Nitric Oxide gel can accelerate the healing rate of diabetic skin ulcers.[18] Research on diabetic wound healing in experimental animals with ethanolic extract of CA has been carried out with the results of a definite healing action in routine healing and diabetes-induced wound healing.[19] However, no research has been found on the effect of CA extract gel in diabetic wound healing, so it is necessary to research its effect.

METHOD

Study Design

This study is a true experimental study using animals with Posttest Only Control Group Design.

Study Site

This research was conducted in three laboratories at two different universities; the CA was then determined at the Biology Laboratory of Ahmad Dahlan University. The extraction of CA carried out at the Pharmacy Laboratory of the University of Muhammadiyah Magelang, the making of a model of mice with diabetes mellitus wounds was carried out at the Laboratory of Basic Natural Sciences, Faculty of Health, University of Muhammadiyah Magelang, as well as giving extract gel, as well as wound measurements were carried out in the same laboratory. In contrast, the mice were obtained from the LPPT, Gadjah Mada University, Yogyakarta.

Materials

The surgical equipment used is a sterile scalpel, bisturi, hecting set, shaver, gloves, cotton bud, wound ruler, 1cc syringe, set mouse cage, scissors, glucometer, glucotest strip (Glucodoctor Korea), plastic, ruler, tissue. Tool for CA herb extraction process, including macerator, water bath, porcelain cup. The phytochemical screening equipment includes porcelain dishes, test tubes, stirring rods, filter paper, and a water bath.[15] The main ingredients used in this study were CA, Nicotinamide extract (70 mg/kg B.W. dissolved in physiological NaCl), Streptozotocyn with three dosage variations (120,150 and 180 mg/kg B.W. dissolved in 0.1M citrate buffer pH 4, 5), anesthetic (Ketamine HCl and Xylazine) lethal dose (200mg/Kg B.W).[20] Materials for phytochemical screening are 70% ethanol, 2 N HCL, reagent Meyer, Dragendorf reagent, powder Magnesium Sulfate, aquabides, chloroform, Concentrated H2SO4, FeCl3. Material for the material extraction process is 70% alcohol. Preparation of gel using materials CA leaf extract ratio 10:1, vascular, glycerin, propylene glycol, dimethylol dimethyl hydantoin (DMDM hydantoin), triethylamine (TEA), and distilled water.

Plant Extraction

CA herb extraction is carried out by maceration using 70% ethanol solvent. Simplicia herbs CA as much as 433 grams macerated in 10 liters of ethanol for three days and occasionally stirred. Extract the result of maceration later evaporated to produce a thick extract. The maceration process is then continued by making a thick extract where maceration results are collected later evaporated using a water bath. After obtaining a thick extract, we then calculated extract yield and tested extract phytochemical content.

CA Gel Preparations

The manufacture of gel preparations starts by dissolving the extract with distilled water, mixing viscometer and some water first, using a stirrer, adding glycerin and propylene glycol, and adding DMDM hydantoin. CA herbal extract (L) urban), and distilled water to volume desired with gentle stirring continuously to form a homogeneous gel. Then add TEA drop by drop until the pH is which are desired. The gel is stored in a closed container at room temperature.[21] Gel making with extract concentrations of 3%, 5%, and 7%.

In Vivo Procedure

Animal Preparation

Experimental animals used in this study were Balb-C mice aged eight weeks with a bodyweight of 25-35 grams as

many as 30. The first step is the adaptation of experimental animals to the new environment for three days. Experimental animals were placed in individual cages and received Ad libitum food and drink. The food given is in the form of Cutis brand pellets.

Making Mice with Diabetes Mellitus Model

The next step is to fast the rats overnight before being induced but still drink. After fasting, the rats were checked for blood glucose levels and given Nicotinamide injection (70 mg/kg B.W dissolved in physiological NaCl) 15 minutes before Streptozotocyn injection with three different doses (120,150 and 180 mg/Kg B.W dissolved in citrate buffer 0.1M pH 4.5). Blood glucose levels are checked on the day 8th of induction. Blood glucose levels were checked using a glucostrip test (Glucodoctor Korea)[6]. Animals that do not succeed in diabetes are sacrificed by giving lethal doses of anesthesia (Ketamine HCl and Xylazine) (200mg/Kg B.W).

Making Mice with Diabetes Wound

Experimental animals were male Bulb-C mice induced to become diabetic with a fasting blood sugar value of >120 mg/dl, and then an incision was made to produce an open wound of 10 mm. After undergoing a period of adaptation, the mice were shaved on the back and performed antiseptic action and topical anesthetic on the area to be treated slashed. Then a 10 mm long incision is made using a sterile scalpel; then, the wound is cleaned by flowing distilled water until it bleeds stop. After the wound-making procedure is complete, appropriate interventions are given differently for each group.

Experimental Procedure

Thirty mice which were declared diabetic wounds based on examination were divided into two groups, the treatment group of diabetes mice (three treatment groups used gel CA extract with concentrations of 3%, 5%, and 7%, and the control group of diabetes mice (the negative control group used gel without CA extract, and the positive control group used hydrogel) with each group used six mice. Intervention and observations were made every day. The length of the cut is measured by using a wound ruler. Each group recorded the changes on days 0, 2, 8, 14, and 21. Any changes were observed and recorded in each group.[22]

Determination of the Percentage of Wound Healing Effect

The percentage of wound healing was measured based on the wound area and was measured using a caliper to the nearest 0.1 mm. Measurements were carried out on test animals in all replications and groups, namely in the transverse, longitudinal, and diagonal directions from day 2 to day 21st. The treatment of giving the solution was carried out daily. The average diameter of the measurements is used as data.[23]

The Variable, Instrument, and Measurement

The dependent variable in this study was the change in wound size, which was measured using a wound ruler. Measurements were carried out every day to monitor glucose levels every day was checked using glucotest.

Statistical Analysis

Data that has been coded is analyzed using One Way ANOVA. Then the analysis was continued with the Post Hoc Test to find out the differences between groups.

Ethical Consideration

This research has received ethical approval from the Laboratorium Penelitian dan Pengujian Terpadu (LPPT) of Universitas Gadjah Mada with certificate number 00070/04/LPPT/V/2017.

RESULTS

From the second day of observation, there was a difference between the control and treatment groups. The differences began to be seen in the negative control group and the positive control group on the 8th day to the 21st day of observation. In contrast, in the negative control group with the CA extract treatment group, the concentration of 3%, 5%, and 7% differences began to be seen on the observation day second. The healing process starts from the second day to the fifth day but with different degrees of healing. There was rapid tissue formation from the sixth day to the twelfth day.

The result of the wound is that the wound closes entirely until the hair grows back, although not completely. This study indicates that topical application of CA gel extract in the form of a gel in mice with diabetic wounds can shorten the three phases of wound healing, which usually occurs until the maturation phase, which starts on day 24. In this case, the wound healing has occurred from the 21st day. The best wound healing occurred in the group of mice given 3% CA gel extract, which can be seen in Figure 1.

There was a similarity in the variance of the percentage of wound healing between the five treatment groups. There is a significant difference in the average percentage of wound healing between all treatment groups ($p<0.05$). Then a test was conducted to determine the differences between groups. The test results showed a significant difference in the negative control group compared to the positive control group and CA. Gel extract treatment group with concentrations of 3% and 5%. The existence of this significant difference indicates that the gel without extract added with hydrocolloid (positive control) and the CA. Gel extract at concentrations of 3% and 5% has a wound-healing effect because it significantly differs from the test results. In table 1, it can be seen using the percent wound-

Table 1. The Difference in The Mean Value of Wound Diameter Between Groups

Group (I)	Group (J)	Mean Difference (I-J)	Std. Error	Sig.	95% C.I	
					Lower	Upper
Positive Control	Negative Control	-3.66667*	.22361	.000	-4.3550	-2.9784
	Extract 3%	.33333	.22361	1.000	-.3550	1.0216
	Extract 5%	-.58333	.22361	.151	-1.2716	.1050
	Extract 7%	-1.58333*	.22361	.000	-2.2716	-.8950
Negative Control	Positive Control	3.66667*	.22361	.000	2.9784	4.3550
	Extract 3%	4.00000*	.22361	.000	3.3117	4.6883
	Extract 5%	3.08333*	.22361	.000	2.3950	3.7716
	Extract 7%	2.08333*	.22361	.000	1.3950	2.7716
Extract 3%	Positive Control	-.33333	.22361	1.000	-1.0216	.3550
	Negative Control	-4.00000*	.22361	.000	-4.6883	-3.3117
	Extract 5%	-.91667*	.22361	.004	-1.6050	-.2284
	Extract 7%	-1.91667*	.22361	.000	-2.6050	-1.2284
Extract 5%	Positive Control	.58333	.22361	.151	-.1050	1.2716
	Negative Control	-3.08333*	.22361	.000	-3.7716	-2.3950
	Extract 3%	.91667*	.22361	.004	.2284	1.6050
	Extract 7%	-1.00000*	.22361	.001	-1.6883	-.3117
Extract 7%	Positive Control	1.58333*	.22361	.000	.8950	2.2716
	Negative Control	-2.08333*	.22361	.000	-2.7716	-1.3950
	Extract 3%	1.91667*	.22361	.000	1.2284	2.6050
	Extract 5%	1.00000*	.22361	.001	.3117	1.6883

Bonferroni test

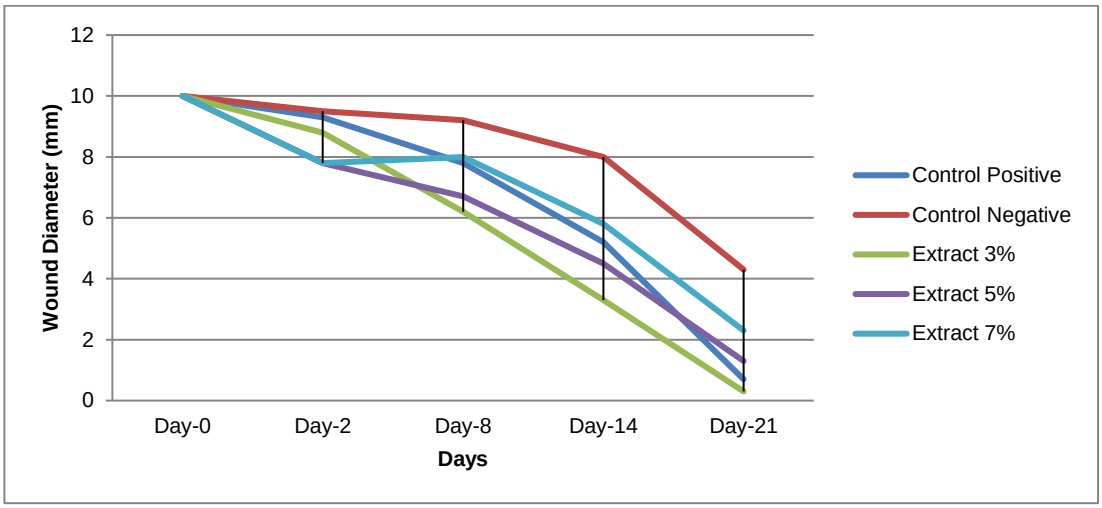

Figure 1. The Average Wound Diameter

healing parameter, the effectiveness of the CA gel extract at a concentration of 3% was better when compared to a concentration of 5%. Based on the test, it is also seen that the positive control group, when compared to the treatment group of CA gel extract with a concentration of 3%, has a p-value > 0.05, this indicates that there is no significant difference in the effectiveness of wound healing between the CA plant gel extract with a concentration of 3% and a gel without extract plus hydrocolloid which is the current standard for wound care, it can be concluded that the CA plant gel extract with a concentration of 3% has comparable effectiveness with hydrocolloid and gel without any extract.

DISCUSSION

This study showed that the concentration of CA extract 3%, 5%, and 7% had wound healing activity in diabetic wounds. The CA plant gel extract with a concentration of 3% has effectiveness comparable to hydrocolloid and gel without any extract. CA extract has a wound-healing effect because, based on the test results, it was found that the ethanol extract of the CA plant contained tannins, saponins, and steroids. The presence of chemicals such as saponins is thought to have a wound-healing effect. Saponins like soap form colloidal oceans in water and form foam.[24] Saponins are known to have antimicrobial effects.[25] Some saponins work as antimicrobials (source of anti-bacterial and anti-viral), increase the immune system,

increase vitality, blood sugar levels, reduce blood clotting, and saponins also affect collagen (the initial stage of tissue repair) by inhibiting the production of excessive wound tissue. Triterpenoid saponins are saponins that have a wound-healing effect. Functions improve the repair and strengthening of skin cells and stimulate the growth of nails, hair, and connective tissue. This content causes the CA plant to have the ability to reduce the inflammatory process and accelerate wound healing compared to the negative control group.[26] CA plant extracts facilitate the wound healing process in the incision and burn wounds; the active substance that plays the most role is the acid contained in the CA plant extract, showing itself as the most active component in wound healing.[27] Supported by the other results research with recent findings showing that the methanol fraction of CA exhibits polyvalent activity with outstanding results, potentially as an effective wound healer.[28] Saponins stimulate the formation of collagen, a protein structure that plays a role in wound healing.[29]

In the CA plant gel extract with a concentration of 7%, there was a decrease in effectiveness when compared to the CA plant gel extract at a concentration of 5%; this happened because the increase in concentration had an impact on the concentration of the solution, on the other hand, the absorption ability of the skin was limited, so not all compounds contained in the solution could be absorbed. Optimally even at higher concentrations. Increasing the concentration should increase the response in proportion to the increased concentration, but in this study, it decreased between concentrations of 5% to 7%; this could be due to the optimal concentration and response being achieved were increasing the dose was no longer able to increase the response in wound healing. Compounds in natural ingredients are not single but are still a collection of compounds; this can cause interactions between compounds to reduce activity.[23] Extracted from CA increases the percentage of collagen in the fibronectin cell layer and thus may assist in promoting wound healing.[14]

The phases of wound healing can be divided into three phases: the inflammatory phase, the reconstruction phase, and the maturation phase.[26] Damaged tissue will respond by way of mast cells releasing histamine and other mediators, causing vasodilation of intact surrounding blood vessels and increasing blood supply to the area, becoming red and warm. The wound on the eighth day shows that it is in the reconstruction phase starting from day 2 to 24 days (6 weeks). This phase is divided into a destructive phase and a proliferative or fibroblastic phase. This phase with high activity is a method of cleaning and replacing temporary tissue. PMN will kill pathogenic bacteria, and macrophages phagocytize dead bacteria and debris to clean the wound. In addition, macrophages are essential in the wound healing process because they can stimulate fibroblastic cells to make collagen. Angiogenesis, epithelial cell migration, and contraction will occur here to reduce the wound surface area.[25]

Here it is seen that the wound has begun to dry and is not wet. The wound does not look paler, and it is not too warm. In this phase, fibroblasts form collagen and connective tissue, forming new capillaries. There appears to be epithelialization starting from the wound margin. There is still a red color (velvety) and granulation tissue in this phase. This process indicates the occurrence of healing that starts from the growth of capillaries and the growth of granule tissue starting from the wound bed. The granulation process goes hand in hand with the re-epithelialization process. Until the final stage of this process, there will be an epithelialization process on the wound surface.[12] Is the remodeling phase, where the primary function is to increase the tensile strength of the wound. The remodeling phase can last from day 21 to more than two months or even years after injury. In this phase, collagen bonds occur, which preserve the scar tissue and the process of epithelialization that covers the skin. The original collagen will be produced during the reconstruction phase, organized with minimum tensile strength. During maturation, collagen is slowly replaced by a more organized form, increasing tensile strength. This coincides with a decrease in vascularity and scar size 10.

Hydrogel functions to keep the wound environment moist, soften, and destroy necrotic tissue without damaging healthy tissue, which is then absorbed into the gel structure and removed with dressings (natural autolytic debridement). Hydrogel is a treatment method that contains water in a gel composed of a polymer structure that contains water and helps lower temperatures up to 5ºC. Moisture is maintained in the wound area to facilitate the autolysis process and remove damaged tissue. Indications for using this hydrogel dressing are to maintain the water content of dry wounds, tenderness, and as a moisturizer and remove necrotic tissue.[27] Another advantage is that it can be used with a topical anti-bacterial. A moisture-balanced wound environment facilitates cell growth and collagen proliferation in a healthy noncellular matrix. In acute wounds, moisture balance facilitates the action of growth factors, cytokines, and chemokines that promote cell growth and stabilize the wound tissue matrix; therefore, wounds must be kept moist.[7] An environment that is too moist can cause maceration of the wound edges. In contrast, less humid conditions cause cell death, and there is no displacement of the epithelium and matrix tissue. The selection of wound care using gel-based materials in which there are active compounds from the CA plant will make the wound healing process faster supported by a moist environment.[28]

This study's results follow several previous studies with the content of saponins. Another study stated that all CA extracts facilitate the wound healing process in cuts and burns. The asiatic acid in the ethyl acetate extract appears to be the most active component for wound healing.[16] In addition, some studies are consistent with this study using the ethanolic extract of CA. There is definite healing activity in routine healing and wound healing induced diabetes.[19] This study used the extract in the form of a gel.

In contrast, there have been previous studies using CA extract capsules and have been shown to shorten the course of diabetic wounds and can be safely prescribed to diabetic patients.[17] Using a gel base. It has been proven that Nitric Oxide asiaticoside gel can enhance diabetic skin ulcer (DCU) wound healing by regulating the Wnt/β-Catenin signaling pathway.[18] The results of this study are in line with previous studies where CA extract gel affects diabetic wound healing, especially in 3% extract gel.

CONCLUSIONS AND RECOMMENDATION

Based on the results of observations of wound size in the 3% extract group, the most effective in showing wound repair activity was a decrease in wound size. Further research can be carried out on testing CA gel extract with a concentration of 3%. Researchers suggest that this study can conduct further research, such as nanotechnology for wound care drug preparations.

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