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Original Article

Comparison between the Effect of Precipitate and Supernatant *Aloe vera* Gel on Experimental Cutaneous Wound Healing using Optical Coherence Tomography

Kusmardi Kusmardi[1], Nurrashida Binti Mok Hallim[1], Aryo Tedjo[2], Anwar Ibrahim[3], Salinah[1,*]

ABSTRACT

Background: Wound healing is a fundamental response to injured tissue that results in the restoration of tissue integrity. One of the famous herbs that promote wound healing is *Aloe vera*. Despite well known for its therapeutic effect, several studies reported inconclusive evidence regarding this. Besides, lack of evidence to postulate the superior effect of two components of *Aloe vera* which are the precipitate and supernatant. **Objective:** Study compares the effects of precipitate and supernatant in promoting tissue repair. Evaluation takes place by using Optical Coherence Tomography (OCT) and is comparable with histopathology study. **Methods:** Twelve male mice were randomly divided into four groups (precipitate, supernatant, control 50% ethanol, and normal). 200 mg of *Aloe vera* was extracted. A standardized 2 cm longitudinal incision wound was created. All mice were given topical *Aloe vera*, 0.5 g each, once daily and assessment of wound surface was performed using OCT. The animals were sacrificed on day 10 to evaluate histopathologically. **Results:** R parameter from the OCT was utilized to analyze the data. There is no significant difference in the treatment effect between *Aloe vera* treated group and control on day 10 post-injury. Treated animals with precipitate did not differ significantly from supernatant treated group. Nevertheless, from histopathology analysis, precipitate showed better wound reepithelialisation, collagen formation and angiogenesis despite having numerous inflammatory cells. **Conclusion:** OCT using R parameter is not the best choice to detect wound healing. Nevertheless, from histopathological perspective, *Aloe vera* accelerates wound healing and precipitate *Aloe vera* gel does have a superior effect from supernatant in promoting wound healing.

Key words: Wound healing, *Aloe vera*, Precipitate, Supernatant, OCT.

Kusmardi Kusmardi[1], Nurrashida Binti Mok Hallim[1], Aryo Tedjo[2], Anwar Ibrahim[3], Salinah[1,*]

[1]Departement of Anatomical Pathology, Faculty of Medicine, Universitas, INDONESIA.
[2]Departement of Medicinal Chemistry, Faculty of Medicine, Universitas, INDONESIA.
[3]Departement of Medicinal Physic, Faculty of Medicine, Universitas, INDONESIA.

Correspondence

Dr. Salinah, MD, M.Biomed

Departement Patologi Anatomi, FKUI, Jalan Salemba Raya No 6, Jakarta, INDONESIA.

Phone no : (62) +81355001101

E-mail: salinahsyarif@gmail.com

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INTRODUCTION

Wound healing is a fundamental response to injured tissue that results in the restoration of tissue integrity. It is a complex process of connective tissue repair that involves a highly regulated series of biological events. This cascade of events involves several cellular phenomena such as migration, proliferation, adhesion, phenotypic differentiation, etc. It includes a set of coordinated interactions between cells in the dermis and the epidermis. There are important relationships exist between fibroblasts, keratinocytes and resident dermal cells.[1] Wound healing process can be roughly divided into three overlapping phases which are inflammation, proliferation and remodelling of the extracellular matrix.[2] The ability of an organism to activate these three processes effectively and promptly is essential for its survival since it is a necessary safeguard against long-term infection.

Previously, wound healing process often possessed problems in clinical practice. It was because of the delayed of natural investigative curiosity in order to promote healing. The aim was to shorten the time required for healing and to minimize the undesired consequences. Since the ancient times, Egyptian, Greek, Indian and European physicians had been attempting to treat wounds in a shorter time with fewer side effects.[3] Acceleration in wound healing with an extra stimulation of a factor can decrease the time of patient motionlessness and therapeutic costs. A lot of research has been envisaged to develop better healing agents and it has been a challenging task to generate them and keep pace with the problems encountered. As previously argued, wound healing agents should adhere to certain specifications. It has been suggested that these agents should facilitate granulation and collagen formation, promote normal immunity, debride wound slough and necrotic tissues, minimize microbial colonization, alleviate pain and facilitate angiogenesis and tissue perfusion.[4]

Nowadays the use of natural medicines, especially pharmaceutical herbs, has been the major cure for several years. They have their specific unique characteristics in wound healing properties. Either orally

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or topically, these herbs are helpful in aiding the healing process. They encourage blood clotting, fight infection and accelerate the rate of healing.[5] One of the famous herbs that promote wound healing is *Aloe vera*. It is widely known for its therapeutic effects and has been used against a variety of skin disorders including burns and wounds. This tropical cactus-like plant has been used for medicinal purposes in several cultures for a long period of time such as in Greece, Egypt, India, Mexico, Japan and China.[6]

Aloe vera is a complex plant that contains many biologically active substances. It contains 75 potentially active constituents: vitamins, enzymes, minerals, sugars, lignin, saponins, salicylic acids and amino acids.[7] These substances have various pharmacological properties that responsible for its therapeutic effects in wound healing. Evidence has shown that *Aloe vera* is effective in wound healing and inflammation reduction.[8-9] Several studies postulated that *Aloe vera* or one or more of its constituents, promote wound healing in various animal models.[9-10] In addition, *Aloe vera* when applied topically is useful in healing minor burns and that such application of the gel is harmless as hypersensitive reactions to it are rare. However, in the other three studies, they found that the clinical evidence of *Aloe vera* was inconclusive and it may actually slow down the healing process in severe burns.[6,10-11] Reviews of the evidence regarding the wound healing efficacy of this herb also showed that the results of various pieces of research are still contradictory.[12]

Besides, in spite of the wide use of *Aloe vera* as therapy to enhance wound healing, there is no strong evidence on the biochemical basis of its mechanism of action or its influence on the various phase of wound healing. The influence of *Aloe vera* in wound healing appears to be exerted by the synergistic action between all the component ingredients, giving a result which is greater than the sum of the individual action.[13] Nevertheless, lack of evidence showed whether precipitate or supernatant of *Aloe vera* exert the superior effect from each other in wound healing process.

In order to demonstrate the effect of precipitate and supernatant of *Aloe vera* gel on dermal wound healing in this study, Optical Coherence Tomography (OCT) was used as a diagnostic tool. OCT is a relatively new, noninvasive procedure that concentrates a beam of near-infrared light on tissue. It is an optical analog of ultrasound. The light penetrates a few millimeters (2-3mm) and is reflected back. This reflection generates cross sectional images of tissue structure.[14] It provides much higher resolution (10μm) because of shorter wave length of light. Although most of skin diseases can be diagnosed by clinical inspection, these characteristics make OCT particularly well suited for evaluation of the superficial structures of the skin. It has been used extensively in dermatology field as one of the diagnostic tools besides invasive tissue biopsies remains the gold standard. The usage of OCT in the assessment of the dermal wound healing was described in a study in China.[15] The study demonstrated that OCT accurately detects the presence or absence of wound reepithelialisation. Nevertheless, this field is still lacking in well-designed clinical trials. The outcome measures of wound healing are still not reliable and valid. Therefore, this study will discuss the utility of OCT in detecting and comparing the effect of precipitate and supernatant of *Aloe vera* on wound healing.

The widely used *Aloe vera* to accelerate dermal wound healing is still lacked in evidence. This is supported by anecdotal data and the safety and effectiveness have not always been proven. Moreover, the effect of *Aloe vera* components, precipitate and supernatant, on better and faster wound healing process is less described previously. Whether precipitate or supernatant accelerates the healing process is still uncertain. In order to observe this effect, OCT was used. Less number of studies described the utility of OCT for measuring wound healing process using red visible light. Histopathology studies as the gold standard were conducted to confirm the findings of OCT. The research questions are: does *Aloe vera* effectively accelerates the dermal wound healing in mice when compared with alcohol only with 1 time per day application within 10 days? Can OCT be used as a diagnostic tool to evaluate the treatment effect of *Aloe vera* in dermal wound healing process.

MATERIALS AND METHODS

Research design

The research design used in this study was experimental study. The study was conducted using two intervention groups and a control group (parallel). The *Aloe vera* extraction procedure was conducted in Department of Medical Chemistry Faculty of Medicine, Universitas Indonesia, while the procedure for making *Aloe vera* gel was held in Department of Pharmacy Faculty of Medicine, Universitas Indonesia. Experiment on mice to observe the effect of *Aloe vera* on wound healing was conducted in Department of Anatomical Pathology Faculty of Medicine, Universitas Indonesia. While collection of data using OCT was conducted in Department of Physics Faculty of Medicine, Universitas Indonesia.

Animal

The sample used in this study was male and relatively healthy mice (*Mus musculus*), strain *Swiss Webster*, age more than 4 months and weighs in the range of 30 to 40 grams. All animal have been obtained from Animal Laboratories, National Institute of Health, Research and Development, Indonesian Ministry of Health. All protocols and surgical procedures were approved by the Institute of Animal Care and Use Committees of Medical Faculty University of Indonesia. In this study, 28 mice were utilized and were allocated according to these groups (7 mice per group). Group 1: wounded mice treated with *Aloe vera* precipitate, Group 2: wounded mice treated with *Aloe vera* supernatant, Group 3: wounded mice treated with ethanol only and Group 4: normal mice without wound. All the mice were housed at Department of Anatomical Pathology laboratory in the cages with controlled light, temperature and humidity. The mice were fed commercial mice feed and water *ad libitum*.

Extraction of *Aloe vera* gel

Extraction of *Aloe vera* gel was conducted at Chemistry laboratory, Department of Medical Chemistry and these are the following steps. *Aloe vera* mature fresh leafs were collected and cleaned with distilled water. The rind was peeled off and removed leaving the colorless parenchyma gel. The gel was weighed and a total weight of 130 g was collected. The gel was ground in a blender until it became semi-liquid. Ethanol 50% was added until the volume of the conical flask reached 200 ml. The mixture was left for two days at room temperature, covered with aluminum foil. After two days, the mixture was filtered through filter papers to separate the precipitate and supernatant. The vaporization of supernatant *Aloe vera* gel was performed at Pharmacy laboratory by using Rotavapor Buchi with temperature of 40°C.

Preparation of *Aloe vera* gel

This procedure which was also conducted at Department of Pharmacy, Faculty of Medicine, Universitas Indonesia involved in the making of 20% *Aloe vera* gel. Two hundred mg *Aloe vera* gel was mixed with 50 mg Sodium benzoate in a test tube. Distilled water was added until the total volume became 10 ml. Fifty mg of 3% CMCNa (carboxymethyl cellulose Natrium) powder was added until the mixture expanded for at least 15 min. The mixture was stirred until well-mixed.

Wound model

All mice in *Aloe vera* groups (precipitate and supernatant) and control positive group were shaved on the back (2cmx3cm) with anesthesia. A standardized 1 cm longitudinal incision wound was created in the

shaved skin using a scissor. The wounds were of full-thickness type with approximately 0.2 cm in depth extending up to the subcutaneous tissue. The incision was not sutured. After wound creation, the mice were divided randomly into three groups as previously described. Animals were closely observed for any infection and those which showed signs of infection were separated and excluded from the study and replaced.

Administration of *Aloe vera*

The proposed treatment with precipitate and supernatant *Aloe vera* were started one hour after the surgical intervention and after the wound was observed under OCT. From day 0, wound surfaces were administered with precipitate of *Aloe vera* and supernatant once daily corresponding to group 1 and group 2 respectively. Each mouse received 0.5 g of the *Aloe vera* gel (0.1 % of wound surface area ≈ 0.05 g × 1 times × 10 days). Wound surfaces of mice in group 3 were covered with ethanol only. The *Aloe vera* and ethanol were applied at the same time for 10 days. The wounds remained uncovered in the three groups throughout the experiment.

Collection and preparation of wound samples

On day 11 after the treatments of mice in experimental groups, the mice were euthanized and sacrificed, then a sample from the wound matrix and healthy skin beside that was made in each mouse. The sample was fixed with formaldehyde 4%, stained with Hematoxylin Eosin staining and stored at room temperature until analysis. The tissue was observed under microscope with 10x and 40x magnification for histological changes.

After wound creation, all the mice in every intervention groups were observed under OCT. This procedure was performed at Physics Laboratory. Mice were sedated with ether before put under the OCT to capture the optical image of the wound. The OCT used red visible light with wavelength of 400-700 nm in the form of Light Emitting Diode (LED). With visible light, only image on the skin surface was visualized since visible light could not penetrate into the skin. The source of light was directed to the beam splitter before it deflected to the area of interest of each mouse (Figure 1).[16] The light reflection from the sample was captured by the Charge-Coupled Device (CCD) camera and was transferred to the computer for visualization. The image was converted to numerical values with Red (R), Green (G), Blue (B), Hue (H), Saturation (S), Intensity (I), Lumination (Y), Inphase (IN) and Quadrature (Q) as parameters. Results were compared between the treatment group and the control group as well as comparing the OCT results with histopathology findings.

Data analysis

The data had normal distribution by Kolmogorov-Smirnov test. Analysis was continued using R parameter after analysis discriminant test was conducted. Student's t test was used to evaluate the significant differences between *Aloe vera* treated group and ethanol treated group and with those of their normal contra-lateral skin samples. One-way analysis of variance (ANOVA) was used to evaluate the differences between the two treated groups, one control positive group and one negative control group. Differences were considered significant when $P<0.05$.

RESULTS

OCT findings

Table 1 shows the mean value of all the OCT parameters on day 10 post injury according to each group. However, from all the parameters, the wound closure analysis was preceded with R parameter only.

On day 10, there is no significant difference between the *Aloe vera* treated group (precipitate and supernatant) and the control group ($p>0.05$). The R mean value of *Aloe vera* treated group is higher (139.41 ± 10.94) compared to those treated with ethanol only (138.16 ± 11.53). However, it is far away from the intact skin where the mean value of R is only 128.04 ± 1.32. From Figure 2, we can see that the distribution of control group (group 2) is nearer to normal group (group 3) followed with the *Aloe vera* treated group.

In addition, neither precipitate nor supernatant showed significant different from the control group ($p>0.05$). However, these two groups did not significantly different from the normal group as well. Figure 3 shows the comparison of R values in all groups on day 10 of post injury. In precipitate treated group, the mean value of R is 132.86 + 7.94. The figure was increased in supernatant treated group with mean value of 145.96 + 10.37. Control and normal group reported mean value of 138. 16 + 11.53 and 128.04 + 1.32 respectively. From this, it is clearly shown that mice in precipitate group had mean value near the normal group.

The effect of each intervention was investigated on day 1, day 5 and day 10 (Figure 4). On that three particular days, the result shows no significant

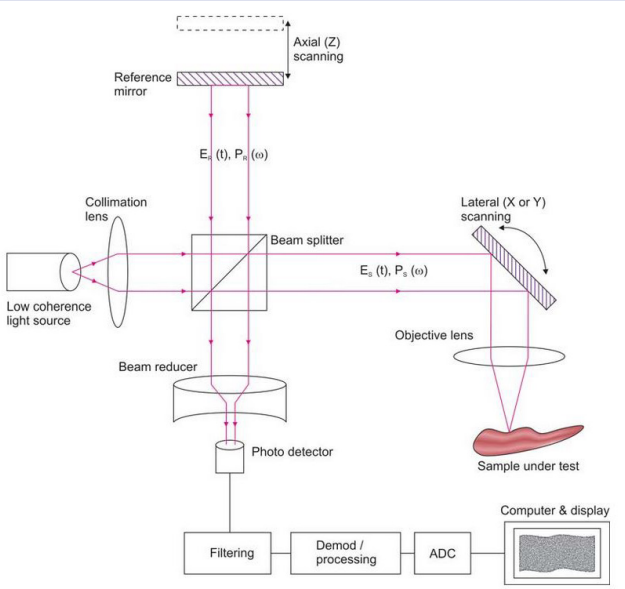

Figure 1: Optical coherence tomography uses light to generate high-resolution Images of tissues.

Table 1: The mean ± SD value of OCT parameters in precipitate, supernatant, control group, obtained on day 10 post injury.

Groups	R	G	B	H	S	I	Y	IN	Q
1	132.86 ± 7.94	110.05 ± 2.52	102.94 ± 1.29	34.00 ± 7.77	26.97 ± 4.71	115.29 ± 4.71	116.04 ± 4.00	140.80 ± 3.03	129.99 ± 0.71
2	145.96 ± 10.37	114.27 ± 3.16	105.70 ± 2.42	29.60 ± 3.84	32.87 ± 4.16	121.97 ± 5.29	122.76 ± 5.23	145.61 ± 3.84	131.33 ± 1.21
3	138.16 ± 11.53	112.35 ± 3.51	103.28 ± 2.10	28.82 ± 4.83	30.08 ± 5.57	117.92 ± 5.62	119.02 ± 5.72	142.81 ± 4.47	130.01 ± 1.12
4	128.04 ± 1.32	109.61 ± 0.72	103.26 ± 0.69	39.32 ± 3.25	23.48 ± 0.53	113.64 ± 0.89	114.38 ± 0.89	138.40 ± 0.36	129.33 ± 0.09

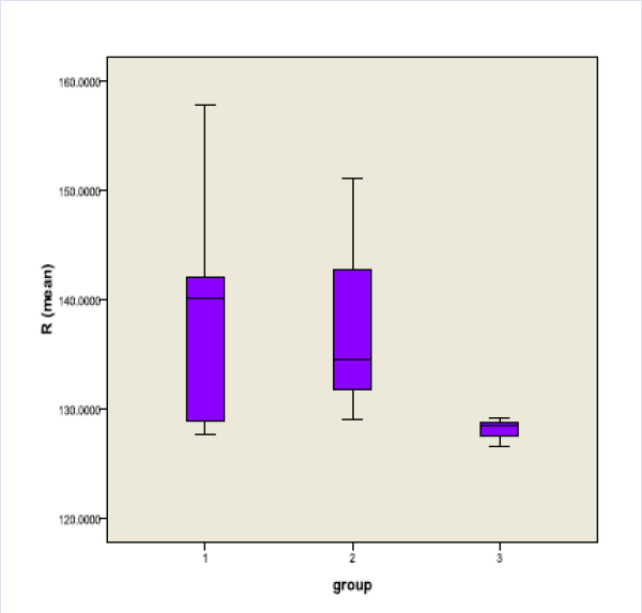

Figure 2: The comparison of R values between *Aloe vera* treated group and control wound tissue with normal tissue in mice on day 10.

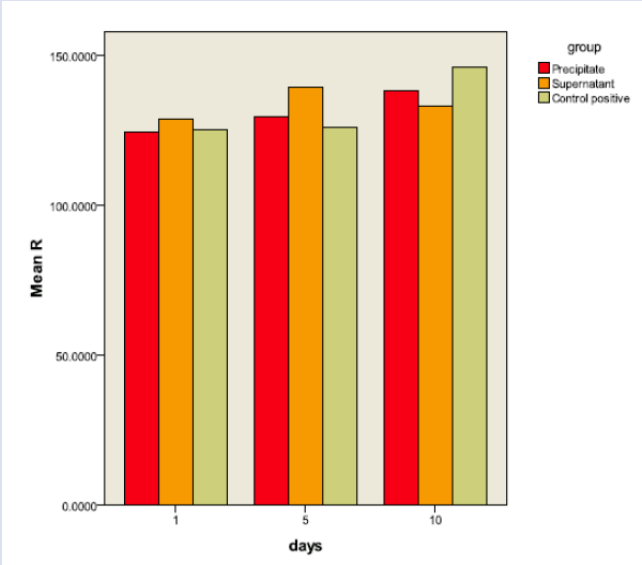

Figure 4: The mean value of R of *Aloe vera* treated groups and control wound tissue in mice on day 1, 5 and 10. Each bar represents the mean for 3 animals in each group.

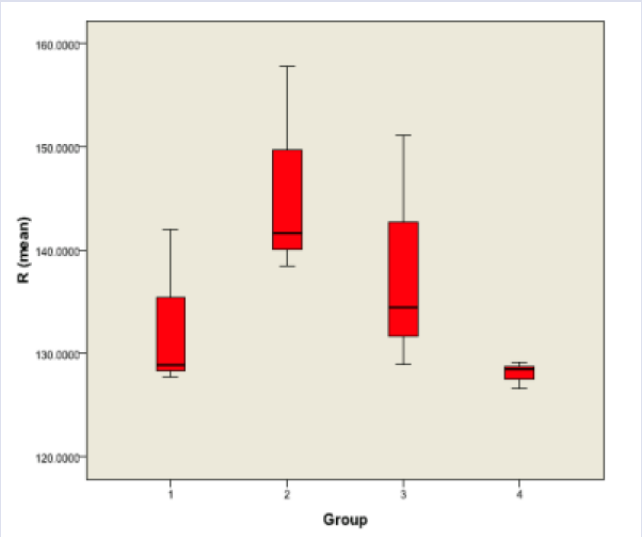

Figure 3: The comparison of R values between *Aloe vera* treated groups and control wound tissue with normal tissue in mice on day 10. Data are given as mean for 3 animals in each group.

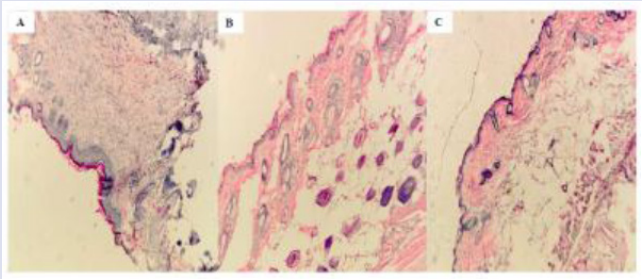

Figure 5: Cross-sections of a surgical wound obtained on day 10 post-injury from A) the treated group with *Aloe vera* precipitate B) the treated group with *Aloe vera* supernatant C) the control group. (H and E; x1 00).

difference between the experimental and control groups (p>0.05). Both precipitate and supernatant showed no significant difference from each other when evaluation was performed on day 1, day 5 and day 10 post injury (p>0.05). Nevertheless, there is an increment pattern of mean value of R in both precipitate and control positive group towards the end of the study. The mean value of R in the supernatant group fluctuated throughout the course and lower on day 10 compared to precipitate group even though the value was higher at the beginning.

Histopathological findings

Study included all the 12 samples but only 1 sample from each group was analyzed microscopically. Structural analysis was performed on day 10 and tissue repair was observed on that particular day (Figure 5). In comparison with group 2 and group 3, animals treated with precipitate of *Aloe vera* (Figure 5A) showed complete near-reepithelialisation with well-developed cornification. The growth of epidermis has almost completed with restoration of multilayered epidermis and it is thicker in contrast to samples from group treated with supernatant and ethanol only. The collagen fibers deposition showed well-distributed pattern and the tissue alignment is greater compared to the other two groups. The number of blood vessels is less. However, the infiltration of mononuclear inflammatory cells in the dermis is quite numerous.

On the other hand, those treated with supernatant of *Aloe vera* (Figure 5B), showed incomplete reepithelialisation and the stratum corneum was in continuous showing delayed cornification. The formation of complete multi-layered epidermis has not been reached, thus making it thinner.

The new collagen fibers are partially formed and unorganized with haphazardly distributed pattern. The number of blood vessels is higher and the interrupted connective tissue of the dermis is highly infiltrated with macrophages and lymphocytes especially in the deeper dermis.

In the control group (Figure 5C), the same pattern of histologic findings was observed similar to those treated with supernatant. However, control group showed better reepithelialisation as well as cornification and the dermis is more packed with the newly collagen fibers. The evaluation of wound healing from histopathological perspective also can be marked by observing fibroblast proliferation and mitotic body, which is not visible in these three samples where 100 x magnification was used.

DISCUSSION

This study investigates the effect of *Aloe vera* in promoting wound healing using OCT. The effect of the treatment was explored and analyzed using R parameter. This parameter was used because discriminant function analysis showed that R can discriminate all the four groups based on their treatment effect. Moreover, the OCT itself used red visible light as the light source. To evaluate this, the effect of the interventions was observed 10 days after dermal incision. Generally, at this stage, tissue repair has entered the proliferative phase by first intention wound healing. However, tissue repair may be delayed and stayed in inflammatory stage like in those closed by secondary or tertiary intention and will continue until epithelialization is completed.

This study demonstrates that there is no significant difference between *Aloe vera* and control group on day 10 post injury. From this, it can be inferred that the effect of *Aloe vera* and ethanol 50% in tissue repair is similar. Aloe treated group did not show a better wound healing effect and this finding is in accordance with study conducted by Zhang *et al*.[11] This study showed a significant delay in wound repair in those treated with *Aloe vera* gel and there is no benefit of therapy to the patients.

In addition, the present study showed the mean R value in the control group is nearer to normal skin tissue. In a standard wound healing process, the wounded area attempts to restore its normal state and eventually, becomes normal again after undergo all the tissue repair stages. This shows wounded tissue treated with ethanol is faster in restoring its normal condition thus, having a mean value near the intact skin tissue. However, both of these values were lower compared to *Aloe vera* treated group and in fact, normal group reported the lowest mean R value. An issue rises from this finding because from the OCT principles, the more light reflection takes place, the more light will be captured by the CCD camera and thus, the higher the R value will be. The condition of the skin surface influences this process where the better the surface reflects light, the better the image is visualized. Here, reflection light means light reflected from the wounded skin and backscattered light that took place at that particular area.

Initially, after the mice were incised, the skin surface was uneven and rough due to the broken skin. At this stage, less light was reflected back when it hit the surface since light could not be focused accurately to the area of interest. As it heals, the wounded surface was covered up with granulation tissue and later was sloughed off to become smooth again. A smooth skin surface produces a good light reflection. However, in this study, the mean R value in the normal group has become a matter of concern and all the findings were not accurate enough to prove whether *Aloe vera* has positive effect in speeding up the dermal wound healing process.

On day 10 post injury, the mean value of R could not illustrate the different in treatment outcome between precipitate and supernatant group. There is no significant difference between these two groups which indicates similarity in their effect on wound healing. As a matter of fact, both groups report no difference with either control or normal group. From these findings, it is likely to suggest that there is a possibility that precipitate and supernatant are effective to promote tissue repair since the mean R values for both groups were not significantly different from the normal group. Besides, precipitate demonstrated a mean R value near to normal group which implies this treatment accelerates wound healing better than supernatant and ethanol. Once again, this result is unreliable since the R mean value in the normal group is the lowest among all of the four groups.

The comparison of the treatment effects between precipitate and supernatant were evaluated on day 1, day 5 and day 10 post-injury (Figure 4). There is no significant difference in the treatment outcomes between precipitate, supernatant and control group on these three different days. Whether on day 1, day 5 or day 10, neither precipitate nor supernatant could demonstrate a superior significant effect in wound healing. They might be in the similar stage of wound healing process and were not different from each other. Longer duration of tissue repair is likely needed like one reported by Negahdari *et al*. to demonstrate the significant different between groups.[17] This study reported that application of *Aloe vera* has beneficial effects to the morphology of dermal wound healing and improved significantly on day 20 post injury.

Nevertheless, the mean R value for precipitate and ethanol treated group increases from day 1 to day 10 after dermal incision. These results point out that the wounded skin has progressively restored its original structure and its surface is becoming more suitable for light reflection to occur. From this, the OCT is able to detect the tissue repair progression that has taken place as time passed by. The surface has becoming smoother to reflect more light and thus, increased the mean R value at the end of the study. Therefore, it can be justified that precipitate promotes wound healing better than supernatant where the mean R value in the latter group fluctuated throughout the study. Yet, the increment pattern of mean R value in control group has not been justified.

The OCT findings in the present study were compared with the gold standard. From histopathological perspective, it was shown in Figure 5A that sample from *Aloe vera* precipitate group had better wound healing outcome 10 days post-injury in terms of wound reepithelialisation, formation of collagen and angiogenesis. However, the supernatant treated group did not show the same effect. In fact, when the wounded mice were given ethanol as in control group, the outcome was better.

Basically, wound healing process consists of three main continuous, overlapping and precisely programmed phases. It involves the inflammatory, proliferative and remodelling phase. *Aloe vera* has been shown to have several different effects in promoting various phases of wound healing like fibroplasia, collagen synthesis and contraction resulting in faster healing.[2,18-20] This plant acts as anti-inflammatory agent that enhances the proliferation phase as well as induces angiogenesis reaction. The active component inside the gel, mainly the mannose-6-phosphate, promotes wound healing and has potent anti-inflammatory activity in a dose response fashion.[2] Mannose-6-phosphate is the main constituent of the polysaccharide chain that attached to a protein in aloe. It is a growth substance capable of yielding the same response as insulin-like growth factor II and binds to fibroblast to activate fibroblast proliferation. Upon that, collagen synthesis will be initiated. Collagen deposition is a fundamental step in wound healing that provides the matrix for angiogenesis and tissue remodelling. Besides, the high percentage of glucose present in gel prevents bacterial growth due to the high osmotic virtue.[21]

In this study, the precipitate *Aloe* group showed an increased collagen and proteoglycan synthesis although the fibroblast cells could not been visualized. The other two groups, treated with supernatant aloe and ethanol 50%, demonstrated not well-developed collagen with haphazardly

pattern collagen fibers. This result shows that precipitate has the tendency to promote wound healing faster than supernatant.

The newly formed collagen promotes tissue repair since it provides strength and integrity to the wounded area. Wound strength is acquired from both remodelling of collagen and the formation of stable intra- and inter- molecular cross-links. Hashemi *et al*. demonstrated that incisional wounds that have been treated with the *Aloe vera* gel showed greater tensile strength.[9] From this study, the author concluded that *Aloe* not only increases collagen synthesis per cell, but it also aids in cross-linking of the protein enhancing the rapid maturation of collagen thus reducing the period of epithelialization.[9,22]

The main active component of *Aloe vera*, mannose-6-phosphate, contains enzymes, glycoproteins, growth factors, vitamins and minerals that have been shown to improve healing with enhanced reepithelialisation and rapid formation and maturation of granulation tissue in burn wounds.[23] On contrary, the multi-layered epidermis has not yet completed and even interrupted in supernatant Aloe group and group treated with ethanol 50% (Figure 5B and 5C). Again, this indicates that precipitate has the superior effect in promoting tissues repair.

In addition, *Aloe vera* gel has an inhibitory action on the arachidonic acid pathway via cyclooxygenase, which influences the inflammatory stage in wound healing progression.[18] It was found that the aqueous extract of aloe containing *Anthraglycosides,* reductor sugar, cardiotonic glycosides, mucilagus and pectins, has cyclooxygenase inhibition properties which lead to reduction in inflammation reaction. *Lupeol,* one of the sterol compounds found in *Aloe vera*, was the most active and reduced inflammation in a dose dependent manner.[24] Improved tissue repair is associated with reduce wound inflammation. The findings from histological view are not convincing enough to suggest whether precipitate has better anti-inflammatory activity compared to supernatant. This is because the number of inflammatory cells is numerous even though the other component of tissue repair was well-formed. The only possible reason for this outcome is this skin tissue has undergone wound healing by secondary intention which might due to infection along with the course of the study. Therefore, a lot of inflammatory cells were observed in this sample. According to Muller *et al*. infection is a common local wound factor that generates hindrance to wound healing.[25]

Another factor that plays a role in wound repair is the angiogenesis process. It is a critical process for the successful healing of wounds. The aim is to provide more blood and oxygen supply and therefore an enhanced wound healing outcome. Evidence shows that *Aloe vera* consists of angiogenic factor that accelerate tissue repair. It was found that β-sitosterol strongly induced angiogenesis.[19] It is a major active component of *Aloe vera* gel which has the angiogenic activity. β-sitosterol is the most common plant-derived steroids with a structure similar to that of cholesterol and it has the ability to induce angiogenesis directly and/or indirectly. However, it did not promote endothelial cell proliferation. From histopathological findings, it is obviously shown that precipitate enhances the presence of more blood vessels. Less number of blood vessels is visible in the other two groups. It shows that the active component of β-sitosterol might be higher in precipitate aloe compared to supernatant treated group. By increasing the rate of angiogenesis, adequate oxygen supply can be delivered and more proliferation and migration of the epithelial cells and fibroblast can happen. Hence, the rate of wound healing process will be much faster.

In another study conducted by Davis *et al*. they demonstrated that aloe precipitate has as much as 160% more wound healing activity than the original aloe by having a stimulatory system.[26] This system stimulates antibody production which is for wound healing and even more significantly, the precipitate wound healing activity was increased by an average of 47.5%. In addition, this study also found that supernatant part of *Aloe vera* inhibits the production of free oxygen radicals and involved in inhibitory system. This system on contrary, promotes anti-inflammatory activity. The findings from this study correlates with the present study where microscopically, precipitate enhances the tissue repair compared with supernatant where better reepithelialisation, collagen formation and angiogenesis were observed. The inflammation reaction, however, is greater in this group suggesting that precipitate has lower effect of anti-inflammatory. Comparing the effect of control and supernatant treated group histologically, the effect of supernatant is not overwhelming and is far away from the postulated hypothesis. This might be due to low concentration of active components in this form where 99.5% of supernatant consists of water mostly.[27]

As a whole, after comparing OCT findings and histopathological findings in this study, the therapeutic effect of *Aloe vera* was best demonstrated using the gold standard which is the histopathology study. The OCT findings were inconsistent with the gold standard results and were not convincing enough to draw the conclusion to detect wound healing process in mice. This is because several limitations have been identified when using this tool. Firstly, the OCT is a simple, newly designed tool which is an operator-dependant tool and skills are needed to operate it. Besides, the correct technique in order to run this OCT was lacked and researchers were not familiar enough with the tool itself before using it. Moreover, this OCT used red visible light as its light source and the wavelength is shorter where it just visualized the optical image on the skin surface and did not penetrate the epidermis. Previous study that used infrared as the light source demonstrated a significant effect in evaluating the dermal wound healing effect.[15] This is because infrared light can penetrate the epidermis until 2-3 mm and produces a better optical image.

Another limitation that was found in this study is the usage of R parameter to analyze the treatment effect. It is not reliable to use R since it produced the lowest mean value in the normal group where it is supposedly to be the highest value among all of the groups. Even so, the light reflection produced using this parameter was impeded by light scattering. This is due to the development of scar tissue during the tissue repair, the fur of the mice that blocked the reflection of the light, the *Aloe vera* gel that may cover the wounded surface and the back of the mice that were not flattened when measurement was taken. All of this affects the light reflection and thus, affect the production of the R values. As a result, the OCT findings were inconsistent with those presented with histopathology findings. To add, the type of wound also influences the light reflection since light could not be focused precisely in the incisional wound created on the mice. The uneven skin surface explains this where the light scattering is more than light reflection.

Furthermore, this study was lacked in sample size where only three mice were utilized in each group. Initially, seven mice were intended to be used but due to financial problem, the sample size was reduced. In fact, only 1 sample from each group was analyzed histologically. The smaller sample size causes the true differences between groups are less likely to be recognized. The treatment effects of *Aloe vera* were harder to detect in these smaller samples. Lastly, researchers were not blinded during the OCT measurement and the histopathological reading. Researchers knew which mice were receiving a particular treatment they received and consciously or subconsciously, the interpretation of the results could have been altered in a different way, thus creating the measurement bias.

CONCLUSION

The present study investigated the effect of precipitate and supernatant in promoting wound healing process using OCT. The OCT findings were not reliable to conclude OCT is useful and can be used to evaluate the dermal wound healing process. However, from histopathological

perspective, study concludes that *Aloe vera* accelerates wound healing and precipitate treatment does have a superior effect from supernatant in promoting wound healing.

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CONFLICT OF INTEREST

The authors declare no conflicts of interest.

ABBREVIATIONS

Anova: One Way Analysis of Variance; **CCD:** Charged-Coupled Device; **CMCNa:** Carboxymethyl Cellulose natrium; **g:** Gram; **LED:** Light Emitting Diode; **Mm:** Milimeter; **OCT:** Optical Coherence Tomography; **µm:** Micrometer..

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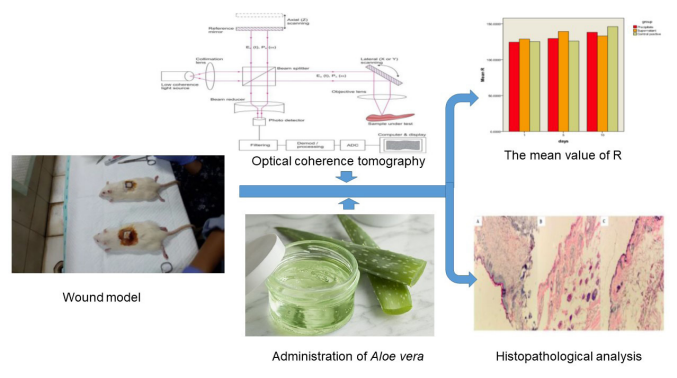
SUMMARY

- Wound healing is a fundamental response to injured tissue that results in the restoration of tissue integrity. It involves three overlapping phases which are inflammation, proliferation and remodeling. Acceleration in wound healing with an extra stimulation of a factor can decrease the time of patient motionless and therapeutic costs. One of the famous herbs that promote wound healing is *Aloe vera*. Despite well known for its therapeutic effect, several studies reported inconclusive evidence regarding this. Besides, lack of evidence to postulate the superior effect of two components of *Aloe vera* which are the precipitate and supernatant.
- This study evaluates the wound healing properties of *Aloe vera* on dermal wound healing in mice. Study compares the effects of precipitate and supernatant in promoting tissue repair. Evaluation takes place by using Optical Coherence Tomography (OCT) and is comparable with histopathology study.
- Twelve male mice were randomly divided into four groups (precipitate, supernatant, control 50% ethanol and normal). 200 mg of *Aloe vera* was extracted and filtered to yield precipitate and supernatant. A standardized 2 cm longitudinal incision wound was created with approximately 0.2 cm in depth. All mice were given topical *Aloe vera*, 0.5 g each, once daily and assessment of wound surface was performed using OCT. The animals were sacrificed on day 10 to evaluate histopathologically.
- R parameter from the OCT was utilized to analyze the data. There is no significant difference in the treatment effect between *Aloe vera* treated group and control on day 10 post-injury. Treated animals with precipitate did not differ significantly from supernatant treated group. Nevertheless, from histopathology analysis, precipitate showed better wound reepithelialization, collagen formation and angionesis despite having numerous inflammatory cells.
- The OCT using in this study was for analyzed effect of the treatment in promoting wound healing. The parameter used red visible light as the light source. During the study, it was found that the R mean value in the control group was nearer to normal skin tissue before intervention was given and still lower than *Aloe vera* group after the intervention. On day 10 post injury, the mean value of R could not illustrate the different in treatment outcome between precipitate and supernatant group. This become a matter of concern and all the findings were not accurate enough to prove whether *Aloe vera* has positive effect in speeding up the dermal wound healing process.
- From histopathological perspective, sample from *Aloe vera* precipitate group had better wound healing coutcome 10 days post-injury in terms of wound reepithelialisation, formation of collagen and angiogenesis. However the supernatant treated group did not show the same effect.
- OCT using R parameter is not the best choice to detect wound healing. Nevertheless, from histopathological perspective, *Aloe vera* accelerates wound healing and precipitate *Aloe vera* gel does have a superior effect from supernatant in promoting wound healing.

GRAPHICAL ABSTRACT



ABOUT AUTHORS

Kusmardi Kusmardi is working as a Senior Lecturer and Researcher at Anatomic Pathology, Faculty of Medicine, Universitas Indonesia. The major research focus on colorectal and breast cancer, include the potential inhibition of some Indonesian natural medicine on the both carcinogenesis, the indentification of normal tissue vs cancer development using some molecular marker and computational model. He wrote the mouse model for breast cancer book, the mouse model for colorectal adjuvant chemopreventive book and Lunasin: a soybean polypeptide as chemopreventive adjuvant for colon cancer. His Research Areas are Immunology, Pathobiology, Cancer Biology, Herbal Medicine, Biomedical Sciences, Oncology, Animal Model for Cancer Research, Biostatistics and Research Methodology, computational molecular medicine.

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