ORAL AND TOPICAL *Aloe vera* EXTRACT ADMINISTRATION IN DIABETIC MICE (*Mus musculus*) WOUND HEALING PROCESS

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ABSTRAK

*Tanaman Aloe vera atau lidah buaya telah digunakan untuk tujuan pengobatan di beberapa budaya selama ribuan tahun. Pada beberapa penelitian yang telah dilakukan pada hewan coba, pemberian Aloe vera telah terbukti efektif untuk mempercepat penyembuhan luka, baik pada diabetes maupun non-diabetes. Dalam percobaan ini, peneliti ingin membandingkan pengaruh pemberian ekstrak Aloe vera secara oral dan topikal terhadap luka diabetes. Metode penelitian yang digunakan ini berjenis eksperimental dengan menggunakan hewan coba mencit jantan yang telah diinduksi diabetes menggunakan STZ dan dibagi dalam 3 kelompok (kontrol, oral, topikal). Kemudian mencit dilukai dengan sayatan 1x1 cm pada punggung. Pemberian ekstrak Aloe vera dilakukan pada kelompok oral dan topikal. Pengamatan dilakukan pada hari ke-3 dan ke-6 dengan mengeuthanasia mencit dari tiap kelompok, kemudian diambil jaringan sekitar kulit yang dilukai untuk dibuat sediaan histopatologi. Masing-masing sediaan diamati di bawah mikroskop dengan mengamati jumlah makrofag dan fibroblas di area perlukaan sebanyak 6 lapang pandang dengan perbesaran 400x. Hasil pengamatan disajikan dalam bentuk deskriptif. Pada hari ke-3, jumlah makrofag dan fibroblas pada kelompok topikal lebih banyak dibandingkan kelompok oral. Namun pada hari ke-6, sediaan dari kelompok oral tidak memperlihatkan adanya infitrasi sel makrofag maupun fibroblas sehingga peneliti kesulitan untuk membandingkan efek ekstrak Aloe vera pada kelompok oral dan topikal pada hari ke-6. Sebagai kesimpulan, aplikasi ekstrak Aloe vera secara topikal memberi efek infiltrasi sel makrofag dan fibroblas pada luka diabetes lebih baik dibandingkan secara oral pada proses penyembuhan luka fase inflamasi.**(FMI 2012;48:150-155)***

Kata Kunci : *ekstrak Aloe vera, luka diabetes, wound healing, oral, topikal*

ABSTRACT

*Aloe vera has been used for medicinal purposes in some cultures for thousands years. In some of the studies that have been conducted in experimental animals, administration of Aloe vera has been proven effective to accelerate wound healing, both in diabetic and non-diabetic wound. In this experiment, the researcher wanted to compare the effect of Aloe vera extract orally and topically to diabetic wounds. The type method of this research is experimental research, which use male mice as experiment animal that had been induced diabetes using STZ. They were divided into 3 groups (control, oral, topical). Then the mice were wounded with a 1x1 cm incision on the back. Aloe vera extract is conducted in oral and topical group. Observations were made on days 3 and 6, mice were euthanized from each group and area around the wound were excised in order to make histopathology preparations. Each preparation was observed under microscope by observing the number of macrophages and fibroblasts in the area of injury as much as 6 field of view with 400x magnification. The results are presented in descriptive observation. On day 3 observation, the number of macrophages and fibroblasts in the topical group more than the oral group. But on the 6th day, the histopathology preparations of the oral group showed no macrophage and fibroblast cell infiltration, so it is difficult for researcher to compare the effects of Aloe vera extract on oral and topical group on day 6. In conclusions, application of Aloe vera extract topically have a better effects on the number of macrophages and fibroblasts infiltrate than oral application in the inflammatory phase of the diabetic wound healing process. **(FMI 2012;48:150-155)***

Keywords: *Aloe vera extract, diabetic wounds, wound healing, oral, topical*

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INTRODUCTION

According to Davis (Surjushe et al 2008), the plant *Aloe vera* or *Aloe vera* has been used for medicinal purposes in some cultures for thousands of years. Start of beauty recipes daughter up to treat soldiers wounded. In the early 1800s, *Aloe vera* has been used as a laxative in the US, but in mid-1930, a turning point occurs when it has been successfully used to treat chronic and acute dermatitis radiation. *Aloe vera* is rich in nutrients and biological benefits, such as wound healing, immune system modulation, antimicrobial, accelerating the

proliferation of cells of the skin and mucous membranes, especially in the wound, the effect on the skin to UV radiation and gamma rays, as well as anti-diabetic (Moghaddasi & Verma, 2011).

In the wound healing process, *Aloe vera* can give effect to epithelization, collagenation, increase vascularity in the wound, removing necrotic tissue, stimulates cytokine production of macrophages, influence inflammatory processes, as well as accelerate the maturation and wound contraction (Reddy et al, 2011). In some of the studies that have been conducted in experimental animals, administration of *Aloe vera* has been proven effective to accelerate wound healing, both in diabetic and non-diabetic.

In diabetes, the wound healing process is longer because of the neuropathy, making it easier to avoid infection and can be fatal even to lead to amputation. This is related to some process that decreases or disruption of growth factor production, angiogenic response, macrophage function, accumulation of collagen, the barrier function of the epidermis, the quantity of granulation tissue, proliferation and migration of keratinocytes and fibroblasts, the number of epidermal innervation, bone healing, and the balance between matrix components extracellular and remodeling components (Brem & Tomic-Canic 2007).

According Atiba et al (2011), oral administration of *Aloe vera* can accelerate the production of TGF-β1 (Transforming Growth Factor- β1) which then activates macrophages to bind mannose receptor binding. In addition, the effect of oral administration of *Aloe vera*, among others, can increase the production of extracellular matrix not only directly, but also through the stimulation of TGF- β1 on fibroblast wound, increasing the speed of wound contraction, accelerate myofibroblast proliferation through stimulation of TGF- β1. And in conclusion, they stated that the oral administration of *Aloe vera* will provide a systemic effect on wound healing through increased production of growth factors (growth factors) and angiogenesis.

According to research conducted by Chithra et al (1998), the provision of *Aloe vera* orally and topically proven to increase the amount of collagen type 3 are useful in the early wound healing process so that the later stages become better organized, which later will support the formation of collagen type 1 which is both useful in the final stages or the final scar. But until now, further research on the benefits of *Aloe vera* in the wound healing process continues to be deepened; given the necessary processes in wound healing is complex and various diseases. Including injuries that occur under certain circumstances, eg in case of diabetes. Therefore it is necessary to investigate the effect of *Aloe vera* orally and topically in these conditions and compare which one is more effective. This study aimed to determine the effect of *Aloe vera* extract in the process of wound healing in mice with diabetes.

MATERIALS AND METHODS

This study was designed using the method Controlled Randomized Post Test Design. The sample used in this study were mice (*Mus musculus*) adult male, with about 3 months of age, the weight range of 30-40 grams, and with a healthy physical condition. Then the mice will be induced with STZ to get the state of diabetes. Animal experiments performed in this study were mice (*Mus musculus*). Mice were obtained from the Experimental Animal Unit, Faculty of Medicine, Airlangga University. Selected adult mice, approximately 3 months old, 30-40 g body weight range, and in a healthy condition, namely morphology complete physical and psychological well. Used 12 mice were divided into 3 groups: control group (C), the treatment group M1 and M2. Each group contains ± 4 mice. Ingredients *Aloe vera* extracts obtained from a chemical industrial materials supplier store in Surabaya. Streptozotosin used as Induction of Diabetes. Streptozotosin dose given to make experimental animals with diabetes was 4.5 mg/tail dissolved in about 0.2 ml of distilled water. Process of care and treatment of experimental animals conducted at the Laboratory Animal Unit Try University Press. Process of assessing the injury and the histopathological treatment of wounds conducted at the Laboratory of Pathology, Airlangga University.

The whole process of investigation of the influence ratio of *Aloe vera* gel extract orally and topically in cutaneous wound healing in mice with diabetes will be implemented within a period of approximately 3 months. To make the experimental animals (mice) had diabetes, given Streptozotosin of 4.5 mg/tail on each mouse, and injected only once, namely on day 0 intraperitoneally. Then on day 2, blood sugar levels checked each mouse. Diabetic mice can be said if the blood sugar levels reach ≥ 200 mg/dL. All mice were anesthetized with ether anesthetics through the intraperitoneal injection. The hair on the back of each mouse was shaved and sterilized with 70% alcohol. Then excision wound on the skin with an area measuring 1 cm x 1 cm on the back of each mouse. Each wound periodically observed on days 3 and 6. Provision of *Aloe vera* extract in mice after the mice tested positive do suffer from diabetes and has been given the injury. In the group of mice M1, *Aloe vera* extract is given by the sonde. While the M2 group, *Aloe vera* extract topically applied directly to the wound area.

This treatment is done every day after the injury. Then in each group of mice (control group, M1, and M2) 2 experimental animals euthanized on days 3 and 6 post-treatment and then made preparations for histopathology. In this study, we carried out micro-scopic observation of lesions treated and untreated. Microscopic observations made by examining the injured skin tissue histopathologically and assess the number of macrophages and fibroblasts were viewed under a microscope at a magnification of 400 x which occurred in each treatment group.

RESULTS

Research data in the form of the number density of macrophages and fibroblasts which occurs in the three treatment groups on day 3 and 6 after injury and then made a descriptive analysis of the data. From the histopathological examination, the observation day 3, the number of macrophages is most prevalent in the control group. The number of macrophages in the topical group slightly below the control group, while the differences are quite large number of macrophages in the category sonde (oral) which is much less than the control group and topical. On day 6, the average number of macrophages in the group more topical than the control group, whereas in the group sonde histopathological examination did not reveal any lesion former injury, so there was no infiltration of macrophages were seen, and the only visible thickening of the epithelium.

From the histopathological examination, the observation of the 3rd day, the highest number of fibroblast cells existed in the control group, then topical groups, and the least in the category sonde. On the 6th day of observation, the number of fibroblasts in the control group and the group of topical almost the same, but the number of fibroblasts are more found in the control group. While the histopathological observations on the sonde group (oral) at day 6 was not found fibroblast cells throughout the field of view because there was no injury to the former network examined histopatho-logically.

DISCUSSION

This study was conducted to compare the effect of *Aloe vera* extract orally and topically to the skin wound healing in mice with diabetes. This study was a laboratory experimental research. The treatment was given to the experimental unit in the form of mice (*Mus musculus*) male with an average weight of 30 grams. There are six research groups are groups of mice with control wounds were observed on day 3, groups of mice with control wounds were observed on day 6, a group of mice with wounds treated with *Aloe vera* extract orally observed on day 3 , groups of mice with wounds treated with *Aloe vera* extract orally observed on day 6, a group of mice with wounds treated with topical *Aloe vera* extract was observed on day 3, and groups of mice with wounds treated with the extract *Aloe vera* orally observed on day 6. Minimum sample size calculation was based on the formula of the tail of each group. Each mice from each group were induced diabetes by using streptozocin number of 4.5 mg of each tail. On the second day after the induction, mice were taken little part blood samples to ensure that the mice had been conditioned in a state of diabetes. After the mice was confirmed in the state of diabetes, each mouse was given injury to his back for a 1x1 cm by first cutting the hair around the area to be harmed.

Once injured, each mouse was given appropriate treatment group. Mice in the control group were treated wounds, mice in the oral group were given extracts of *Aloe vera* in the sonde, and the mice in the group were given topical *Aloe vera* extract topically directly on the wound area. *Aloe vera* extract dose used to treat oral and topical wound was 1 ml/mouse. The treatment is done once each day. On day 3 and 6, with a minimum number of mice from each group on each day of observation, mice were euthanized and tissue taken wound and surrounding skin tissue else made preparations for histopathology.

Wounds were observed microscopically. Variables examined on microscopic examination are macrophages and fibroblasts were examined in two time, ie at day 3 and day 6 after injury. All specimens be making preparations histopathology with hematoxylin-eosin staining. Preparations are already finished and then observed under a microscope with a magnification of 400 times as much as 6 visual field examination, mainly carried out in the wound area with most cell infiltration. From the results of microscopic examination and counting macrophages and fibroblasts, the result is written in the form of descriptive analysis to compare the number of cells in all three experimental groups.

On the 3rd day of observation, the number of macrophages in the topical group was higher than that in the oral group. While the number of macrophages in the control group even more than the two treatment groups. From these results indicate that the application of topical *Aloe vera* extract gives a better effect than orally. However, when viewed from the control group, the group that received treatment (topical and oral) experienced a macrophage cell infiltration fewer.

Table 1. The number of macrophages from reading histopathologic preparations

No.	Day	Groups	Slide Codes	Macrophage Count						Total
				Visual Fields						
				1	2	3	4	5	6	
1.	Day 3	Control	K3	10	14	7	5	8	8	52
		Sonde	S3	3	2	2	3	3	2	15
		Topical	T3	7	5	6	9	4	10	41
2.	Day 6	Control	K6.1	6	5	9	3	5	3	31
			K6.2	3	-	-	-	-	-	3
			K6.3	4	14	20	12	11	10	71
		Sonde	S6	-	-	-	-	-	-	-
		Topical	T6.1	20	15	5	23	9	6	78
			T6.2	6	7	10	7	8	4	42

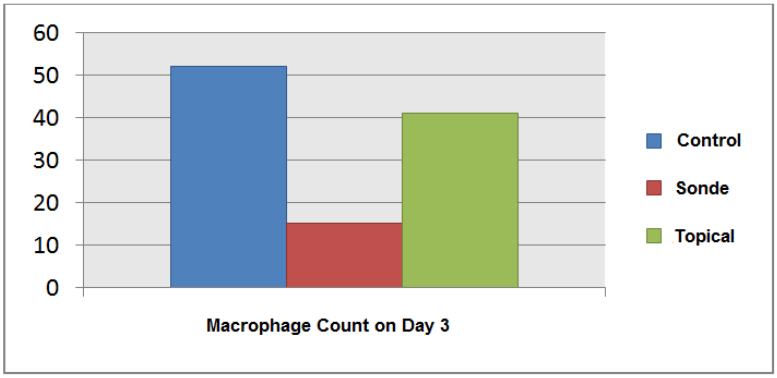

Figure 1. Diagram of the number of macrophages in the observation day 3

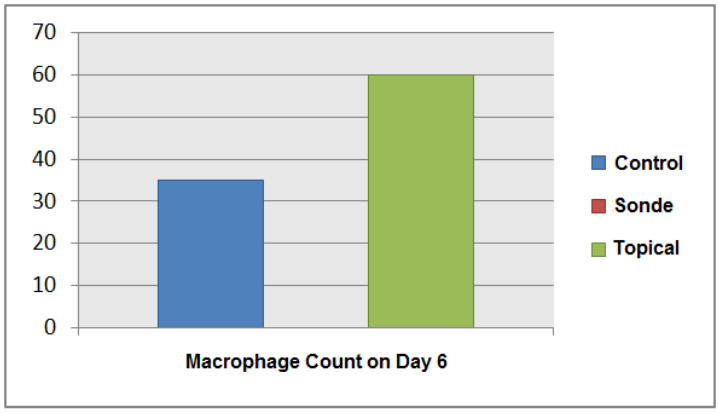

Figure 2. Diagram of the number of macrophages in the observation of the 6th day

Table 2. Number of fibroblasts from a reading of the histopathologic preparations

No.	Day	Groups	Slide Codes	Fibroblast Count						Total
				Visual Fields						
				1	2	3	4	5	6	
1.	Day 3	Control	K3	30	40	50	54	55	45	274
		Sonde	S3	10	15	14	17	20	12	88
		Topical	T3	35	22	15	35	20	22	149
2.	Day 6	Control	K6.1	15	35	32	27	25	20	154
			K6.2	16	-	-	-	-	-	16
			K6.3	13	12	15	17	19	17	93
		Sonde	S6	-	-	-	-	-	-	-
		Topical	T6.1	18	30	14	27	10	9	108
			T6.2	14	24	12	8	12	12	82

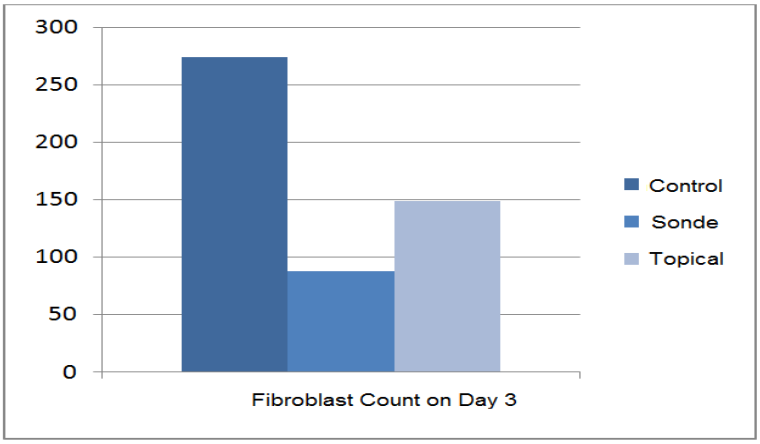

Figure 3. Diagram of the number of fibroblasts in the observation day 3

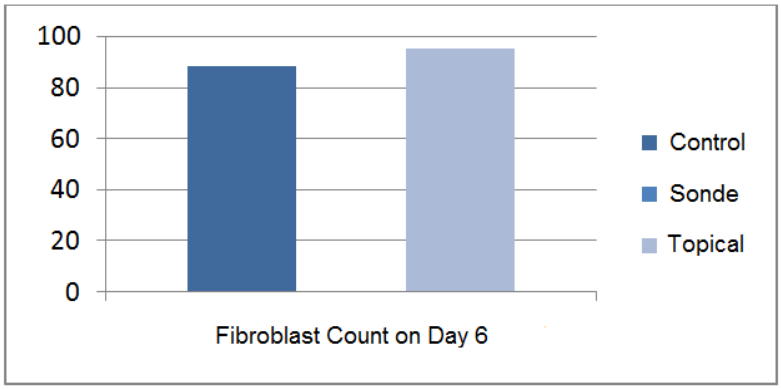

Figure 4. Diagram of the number of fibroblasts in the observation of the 6th day

This can be caused by anti-inflammatory effects of *Aloe vera* extracts. Antiinflammatory effect on *Aloe vera* is obtained from one of its components, namely acemannan which inhibit inflammatory mediator bradykinin (Cowsert 2010). On the 6th day of observation, the topical group had increased numbers of macrophages, whereas in the control group the average number of macrophages was lower than on the 3rd day of observation. The decrease in the control group is expected to occur due to the down-regulation

of specific mRNA for macrophage inflammatory protein receptor-2 and macrophage chemoattractant protein-1 (Wetzler et al 2000). While the observation of the oral group at day 6, microscopically not found infiltration of macrophages in the entire field of view, just look epithelial thickening in the field of view of the alleged former location of injury. Many factors can cause this, among others, the less the injury, resulting in wound healing process that occurs faster so overlooked in research observations.

Observations fibroblasts on day 3, the sequence number of the most widely fibroblasts to the least each obtained in the control group, the group topical and oral group. From these results, it appears that the effect of *Aloe vera* extracts against fibroblast cell infiltration in the topical group is better than the oral group. Fibroblasts on the 6th day of observation decreased when compared with the observation of the 3rd day, both in the control group and topical groups. Although both groups experienced a decrease in the number of fibroblasts, the average number of fibroblasts was more abundant in the control group. There is a difference greater decrease in the control group compared with the decline in the topical group, this happens because of a decrease in macrophages in the control group were more sharply between days 3 late day 6, whereas macrophages in the topical group increased from 6th day compared to when observations 3rd day. Macrophages themselves produce cytokines, one of which serves as a chemoattractant in the process of migration and proliferation of fibroblasts (Monaco & Lawrence 2003). Therefore, the number of fibroblasts in the control group which was originally on the 3rd day is much higher than the topical group, on the 6th day is lower than the topical group. While experiments in the oral group, the results of which showed the density of fibroblasts only on day 3, the day the number of fibroblasts was lower than the other two groups. While the 6th day, there were no scars from histopathological preparations throughout the field of view, which is prominent only visible epithelium proliferates. Allegedly many factors that led to the apparent absence of macrophages and fibroblasts in group oral dosage 6th day, among other injuries had healed faster, less the incision preparation, or other factors which might affect.

This study showed that application of *Aloe vera* extract on wound with diabetes topically give effect fibroblasts and macrophage infiltration were more than the application orally on day 3 which represents the inflammatory phase. While the comparison between the administration of *Aloe vera* extract orally and topically on the further phases, this research has not shown satisfactory results due to many factors. These factors, among others, *Aloe vera* extraction methods are different, the area where the source of *Aloe vera* plant derived, animal initial systemic conditions, until the severity of diabetes suspected of giving a significant influence on the wound healing process. In addition, from this experiment also proved that *Aloe vera* extracts provide anti-inflammatory effects influence.

CONCLUSION

Application of *Aloe vera* extract topically have a better effects on the number of macrophages and fibroblasts infiltrate than oral application in the inflammatory phase of the diabetic wound healing process.

REFERENCES

Atiba A, Ueno H, Uzuka Y (2011). The effect of *Aloe vera* oral administration on cutaneous wound healing in type 2 diabetic rats. J Vet Med Sci 73, 583-589

Brem H and Tomic-Canic M (2007). Cellular and molecular basis of wound healing in diabetes. J Clin Invest 117, 1219-1222

Chithra P, Sajithlal GB, Chandrakasan G (1998). Influence of *Aloe vera* on collagen characteristics in healing dermal wounds in rats. Mol Cell Biochem 181, 71-76

Cowsert LM (2010). Biological Activities of Acemannan. Available from http://www.symmetry direct.com/pdf/BioActivofAcemannan.pdf. Accessed April 9, 2011

Moghaddasi SM and Verma SK (2011). *Aloe vera* their chemicals composition and applications: a review. Int J Biol Med Res 2, 466-471

Monaco JL and Lawrence WT (2003). Acute wound healing an overview. Clin Plast Surg 30, 1-12

Reddy CHU, Reddy KS, Reddy JJ (2011). *Aloe vera*-a wound healer. Asian Journal of Oral Health & Allied Sciences 1, 91-92

Surjushe A, Vasani R, Saple DG (2008). *Aloe vera*: a short review. Indian J Dermatol 53, 163-166

Wetzler C, Kämpfer H, Stallmeyer B, Pfeilschifter J, Frank S (2000). Large and sustained induction of chemokines during impaired wound healing in the genetically diabetic mouse: prolonged persistence of neutrophils and macrophages during the late phase of repair. Journal of Investigative Dermatology 115, 245-253.