

Therapeutic Effects of *Aloe vera* on Cutaneous Microcirculation and Wound Healing in Second Degree Burn Model in Rats

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Abstract

Objective : To demonstrate the microcirculatory and wound healing effects of *Aloe vera* on induced second degree burn wounds in rats.

Method : A total of 48 male Wistar rats were equally divided into 4 groups as follows : sham controls, untreated burn-wound rats, those treated with once-daily application of normal saline (NSS) and those treated with once-daily application of lyophilized *Aloe vera* gel. The animals in each group were equally subdivided into 2 subgroups for the study of cutaneous microcirculation and wound healing on day 7 and 14 after burn. Dorsal skinfold chamber preparation and intravital fluorescence microscopic technique were performed to examine dermal microvascular changes, including arteriolar diameter, postcapillary venular permeability and leukocyte adhesion on postcapillary venules.

Results : On day 7, the vasodilation and increased postcapillary venular permeability as encountered in the untreated burn were found to be reduced significantly ($p < 0.05$) in both the NSS- and *Aloe vera* - treated groups, but to a greater extent in the latter. Leukocyte adhesion was not different among the untreated, NSS- and *Aloe vera* - treated groups. On day 14, vasoconstriction occurred after the wound had been left untreated. Only in the *Aloe vera* - treated groups, was arteriolar diameter increased up to normal condition and postcapillary venular permeability was not different from the sham controls. The amount of leukocyte adhesion was also less observed compared to the untreated and NSS- treated groups. Besides, the healing area of the *Aloe vera*-treated wound was better than that of the untreated and NSS-treated groups during 7 and 14 days after burn.

Conclusion : *Aloe vera* could exhibit the actions of both antiinflammation and wound healing promotion when applied on a second degree burn wound.

Key word : *Aloe Vera*, Cutaneous Microcirculation, Wound Healing, Second Degree Burn Model

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The family name of *Aloe vera* is *Liliaceae* and the scientific name is *Aloe vera* (Linn.) Burm. f.(1). Up to now, *Aloe vera* has been recognized as a good herbal medicine for analgesia, anticancer, antiviral, mutagen, antiulcer, cough suppressant, hyperglycemia, antifungal, antiarthritis, antiparasite, antifertility, cathartic, cosmetics, fat production, decongestion and antiinflammation(2).

Several pharmacological studies have been performed in an attempt to identify the active substances for antiinflammatory action of *Aloe vera*. Lectin aloctin A in *Aloe vera* was discovered to inhibit biosynthesis of PGE₂, an important inflammatory agent(3). Moreover, Heggers and Robson (4,5) suggested that *Aloe vera* gel products contained anthraquinone and related compounds such as barbaloin and aloe-emodin in sufficient quantities to act as competitive inhibition, called false substrate inhibitor blocking prostanoid synthesis, since they had a similar chemical structure to prostaglandin substrates.

Conversely, Capasso et al (1983) found that aloin and 1,8-dioxanthraquinone stimulated prostaglandin production in isolated rat colon(6). Besides, the presence of cyclooxygenase enzyme in *Aloe vera* extracts was demonstrated to increase prostanoids synthesis(7). Moreover, *Aloe vera* was reported to be an antiinflammatory agent and could dilate blood vessels through its action of inhibiting TXA₂ and maintaining the PGE₂ and PGF₂α ratio(8). However, the mechanism of antiinflammatory action of *Aloe vera* could not be confirmed at this point. The major purpose of this investigation was to demonstrate more scientific data for microcirculatory effects of *Aloe vera*, especially, on a burn wound model. The effects of *Aloe vera* as an anti-inflammatory agent for burn wound injury will be visible by the technique of intravital fluorescent microscopy. The parameters that will be determined are changes of arteriolar diameter, microvascular permeability, and incidence of leukocyte adhesion.

MATERIAL AND METHOD

Animal preparation

The experiment was conducted on 48 male Wistar rats weighing 200 to 250 grams. The animals were housed with free access to water and standard laboratory food. They were divided into four groups of 12 animals each as follows.

Control group. The animals were anesthetized with ether. As a sham group, the back of the

animals between the lower part of both scapulas was shaved and depilated without any treatment.

Burn wound group. The animals were anesthetized with ether before being shaved and depilated. Then a hot plate, 3.5 cm x 4.6 cm in size, with temperature maintained at 75°C was put on the prepared area for 10 seconds to make a partial thickness burn or second degree burn(9). The burned area accounted for 10 per cent of the total body surface. The animals in this group did not receive any treatment.

Burn wound with normal saline treatment group (NSS-treated). The same procedure of producing a secondary degree burn wound was performed as that of the burn wound group. Afterwards, 1 ml of normal saline was applied topically to the burned wound immediately after burn and then once daily until the day of experiment.

Burn wound with *Aloe vera* treatment group (*Aloe*-treated). After the burn wound was produced, the animals were immediately treated with topical *Aloe* gel powder (300 mg/kg body weight) in sterile water (10 ml/kg body weight)(10). *Aloe vera* used in this study was "lyophilized *Aloe vera* gel" prepared by the Lipo Chemical Co., USA. Lyophilization is a process for the preparation of dried *Aloe vera* gel with unchanged properties(11). The same dose of *Aloe* gel powder was applied topically to the burn wound once daily.

The animals in each group were equally subdivided into two subgroups for the experimental periods of seven and fourteen days. For further evaluation of wound healing effect, the burned areas were monitored immediately after burn and on the seventh and fourteenth days just before the examination of dermal microcirculation.

Experimental protocol for intravital fluorescence microscopic study of dermal microcirculatory changes.

In order to examine dermal microvascular changes, dorsal skinfold chamber preparation and intravital microscopic technique(12-14) were performed on the seventh and fourteenth days after burn.

On the day of experiment, the animal was anesthetized with intraperitoneal injection of sodium pentobarbital (50 mg/kg BW). A constant level of anesthesia was maintained throughout the experiment by intraperitoneal injection of supplement dose (20% of original dose) of the anesthetic agent every 30 to 95 minutes(15).

Under spontaneous respiration, the trachea was cannulated to facilitate respiration. A fine polyethylene catheter (PE 10, inner diameter of 0.28 mm) was inserted into the right common carotid artery until it reached the aortic arch. The common carotid arterial pressure was recorded via this catheter using pressure transducer (Nikon model RM 6000). The other catheter was placed into the jugular vein for injection of fluorescent-labelled macromolecule (fluorescein isothiocyanate labelled dextran, M.W. 150,000, FITC-dx-150) or fluorescent-labelled leukocyte (fluorescein marker-acridine orange) (Sigma Chemical Co.) into the blood stream. Both catheters were filled with heparinized saline. The dorsal skinfold chamber was implanted as described previously(12).

After completion of chamber implantation, the animal was placed on the microscopic stage of the fluorescent microscope equipped with transillumination and epiillumination optics (Nikon Optiphot-2). After intravenous application of FITC-dx-150, epiillumination was achieved with a 50 W, mercury lamp with a 488 nm attached to excitation filter and 515 emission barrier filter. An intravital microscope with a 20x long working distance objective (CF Achromat) was used to observe microvessels in the chamber. The video images of microvessels were stored on videotape (Sony, SLV-X311) using SIT-video camera (Dage MIT) for playback analysis. A videotape was connected to a video timer (UTG 33) for time recorder. During the experiment, microvessel images could be printed by video graphic printer (Sony, UP-890 CE).

Quantitation of diameter changes in arterioles

To measure diameter of arterioles a fluorescent plasma marker (0.2 ml of FITC-dx-150, 5 mg/100 μ l of physiological saline solution) was injected into the jugular vein to provide immediate contrast enhancement within the intravascular space(14). Then the areas of the second-and third-order arterioles were selected and recorded on videotape for further analysis of diameters using the software called "Global Lab Image". The diameter of each selected arteriole was determined as number of pixels which could be converted to micrometer by means of the software.

Determination of the permeability of postcapillary venules

Under pathologic condition, release of endogenous factors selectively enhanced the per-

meability of postcapillary venule (10-60 μ m) more than that of capillaries (4-9 μ m)(16-19) and the postcapillary venule displayed the primary site of neutrophil exudation and plasma leakage(20,21). Therefore, these vessel segments were chosen for investigation. In this experiment, postcapillary venules between 20-40 μ m in diameter were selected for the study.

After the arteriolar diameter recording, the field of postcapillary venule was chosen and recorded for further digital image analysis of intensity within inside (I_{in}) and outside (I_{out}) vessels. The ratio of I_{out}/I_{in} was calculated at zero time and thirty minutes after the intravenous injection of FITC-dx-150.

Studies of leukocyte-endothelium interaction in postcapillary venule

For visualization of the leukocyte, the fluorescent marker acridine orange was infused intravenously (0.5 mg/kg/min) for 5 minutes(14,19). During experiment, leukocytes were recorded on videotape for further observation of leukocyte adhesion.

Videotape of each experiment was played back and then adherent leukocytes were monitored. Adhesive leukocytes were defined as cells which did not move or detach from the endothelial lining within the entire observation for a period of 30 seconds or more, whereas, rolling leukocytes were defined as nonadherent leukocytes passing through the observed vessel segment during the observation period(22).

Statistical analysis

Data are shown as means \pm standard error. One-way analysis of variance (ANOVA) was performed to examine the difference of each parameter between control rats, burn wound rats, normal saline-treated burn wound rats and *Aloe vera*-treated burn wound rats.

RESULTS

From the intravital fluorescent microscopic monitoring, the video image of selected arterioles showed that the arteriolar diameter in burn wound rats increased largely with significant difference compared to control rats on the seventh day (Fig. 1). In *Aloe*- and NSS-treated burn wound rats, vasodilation was reduced significantly, but the reduction was more pronounced in *Aloe*-treated burn

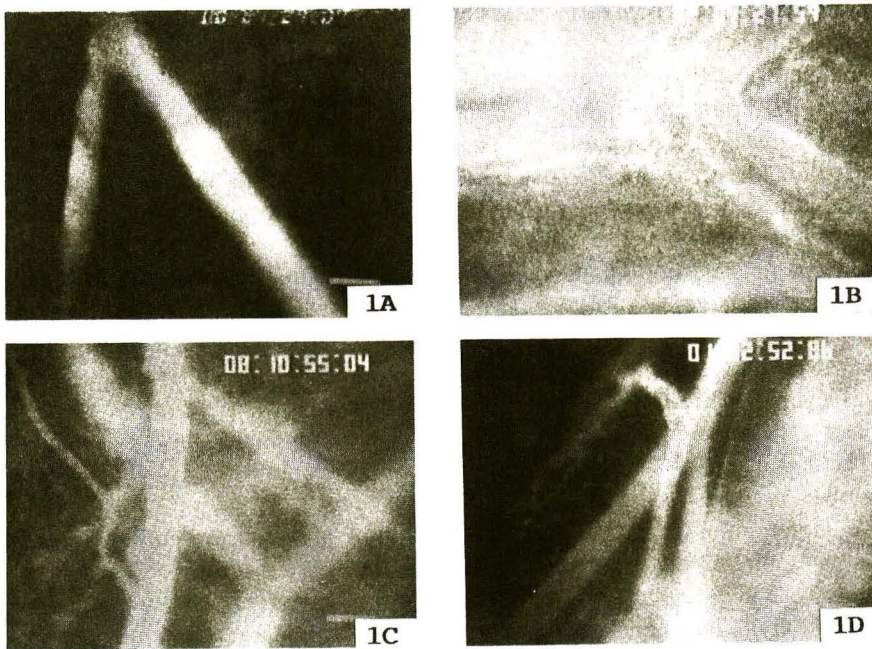


Fig. 1. Video microscopic image of subcutaneous vessels demonstrates the diameter changes of arterioles in the control rat (A), burn wound-rat (B), NSS-treated burn wound-rat (C), and *Aloe*-treated burn wound-rat (D) on day 7. Bar represents 100 micrometer.

Arteriolar Diameter

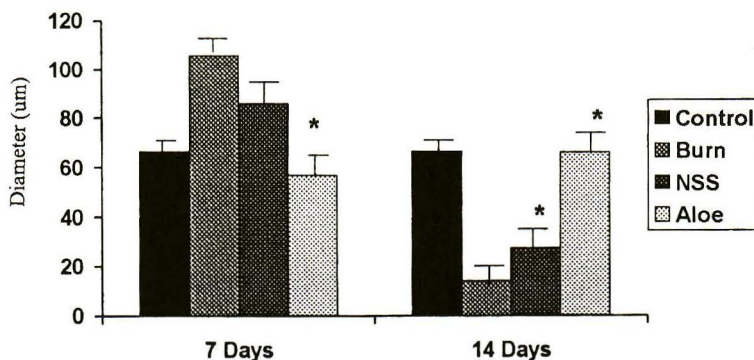


Fig. 2. Effects of *Aloe vera* on changes of arteriolar diameters.
* Significant difference compared to burn group. ($p < 0.05$)

wound rats than in NSS-treated burn wound rats as demonstrated graphically in Fig. 2. In aloe- and NSS-treated burn wound rats, there was no significant difference in arteriolar diameter compared to control rats.

However, on the fourteenth day, the arteriolar diameters of burn wound rats reduced largely with significant difference compared to control rats (Fig. 2). The arteriolar diameter in NSS-treated burn wound rats enhanced slightly but there was signi-

Healing Area

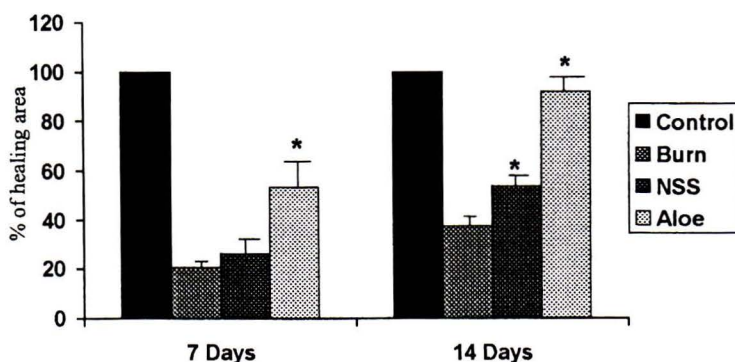


Fig. 3. Percentage of healing area of burn wound injury treated by normal saline and *Aloe vera*.

* Significant difference compared to burn group. ($p < 0.05$)

ficant difference from that of control rats. Only the arteriolar diameters of *Aloe*-treated burn wound rats was not significantly different from those of control rats.

These data represented that on the seventh day after burn, marked vasodilation occurred. *Aloe vera* reduced vasodilation better than NSS and both of these agents reduced vasodilation to normal condition. On the fourteenth day after burn, vasoconstriction was observed. Only *Aloe vera* increased the arteriolar diameter to normal condition.

Besides, it was demonstrated that the healing area of the *Aloe vera*-treated group was greater than that of NSS-treated and untreated groups, which was related to maintenance of normal arteriolar condition during one and two weeks after burn as shown in Fig. 3.

Studies of the permeability of postcapillary venules

There was leakage of fluorescein dye from the intravascular compartment of the postcapillary venule into the extravascular space. As described previously, this leakage of FITC-dx-150 was the indicator of permeability changes of postcapillary venule. These intensity ratios between outside and inside selected venules were determined at the eleventh and thirtieth minutes after fluorescence injection. Means and standard errors of means of these intensity ratios are summarized in Table 1. On

the seventh day, the intensity value in burn wound rats was increased significantly compared to the control rats. In *Aloe*- and NSS-treated burn wound rats, the intensity values were decreased significantly compared to the burn wound rats. However, the intensity value in the *Aloe*-treated burn wound rats was significantly different from those of the NSS-treated and untreated groups.

On the fourteenth day, the intensity values in the untreated and NSS-treated burn wound rats were reduced significantly compared to those values determined on the seventh day. Interestingly, there was no significant difference between those of the *Aloe*-treated burn wound-rats and control rats.

Table 1. Intensity value was assessed inside (I_{in}) and outside (I_{out}) portion of the postcapillary venules at 0 and 30 minutes. Then the intensity ratio was calculated using the equation: $(I_{out}/I_{in})_{30} - (I_{out}/I_{in})_0$. NS non-significant difference
* Significant difference compared to burn group ($p < 0.05$).

Group	Intensity ratio	
	Day 7	Day 14
Burn	0.63 ± 0.1	0.30 ± 0.11
NSS-treated	$0.24 \pm 0.001^*$	0.15 ± 0.0003 NS
<i>Aloe</i> -treated	$0.11 \pm 0.00022^*$	0.0^*

Studies of the leukocyte-endothelium interaction in postcapillary venule

Leukocytes sticking on postcapillary venules was observed in parallel with the increase of macromolecular permeability, especially, in the burn wound rats. Moreover, most tortuosity of capillaries on the seventh day was observed in the untreated groups. It was noted that on the seventh day, accumulation of leukocytes sticking and transmigration of leukocytes into the interstitium were not different among the groups of untreated, NSS-

and *Aloe*-treated burn wound rats. However, the amount of leukocyte adhesion were significantly less observed in the *Aloe*-treated burn wound rats compared to the others on the fourteenth day (Fig. 4).

DISCUSSION

This present study was designed in order to provide scientific evidence for the therapeutic effects of *Aloe vera*, especially, *in vivo* demonstration called the technique of intravital fluorescent microscopy. As demonstrated in this investigation, the model of partial thickness burn wound caused microvascular injury, endothelial injury, stimulating inflammatory response, and microvascular morphological changes. Even though this second degree burn seems to recover by itself within fourteen days, normal saline and *Aloe vera* have indicated further benefit for the healing process. Especially, *Aloe vera* seems to have great therapeutic effect. The hypothesis we would like to propose for the possible mechanisms of *Aloe vera* on the second degree burn model is summarized in Fig. 5. Such mechanisms could be both actions of antiinflammation and wound healing promotion. In this present study, the antiinflammatory effects of *Aloe vera* have been characterized by the inhibition of the abnormalities of vascular diameter changes, vascular permeability and leukocyte adhesion.

According to the study on prostaglandin and thromboxane production in the secondary degree burn model using immunoperoxidase technique, it was found that the burned tissue showed high levels of prostaglandin E_2 (PGE_2) and thromboxane A_2 (TXA_2)⁽²³⁾. Many investigations have revealed that *Aloe vera* has both antithromboxane and antiprostaglandin activities, implying its inhibition of arachidonic acid metabolism *via* cyclooxygenase pathway. It was found to reduce vasoconstriction and increase tissue survival or preserve tissue necrosis by actively inhibiting the localized production of thromboxanes^(8,24).

In the lipoxygenase pathway of arachidonic acid metabolism, the metabolites called leukotrienes cause intense vasoconstriction as well as increased permeability and leukocyte adhesion. It is evident in the present study that on the fourteenth day after the burn, postcapillary venule permeability and leukocyte adhesion were reduced

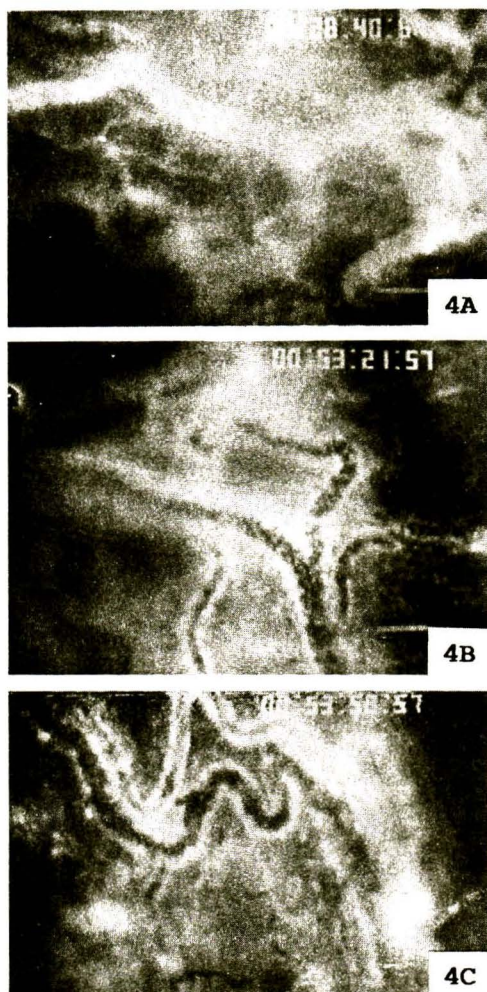


Fig. 4. On day 14, the leukocytes adhering on postcapillary venules are observed in burn wound-rat (A) much more than in NSS-and *Aloe*-treated group (B, C).

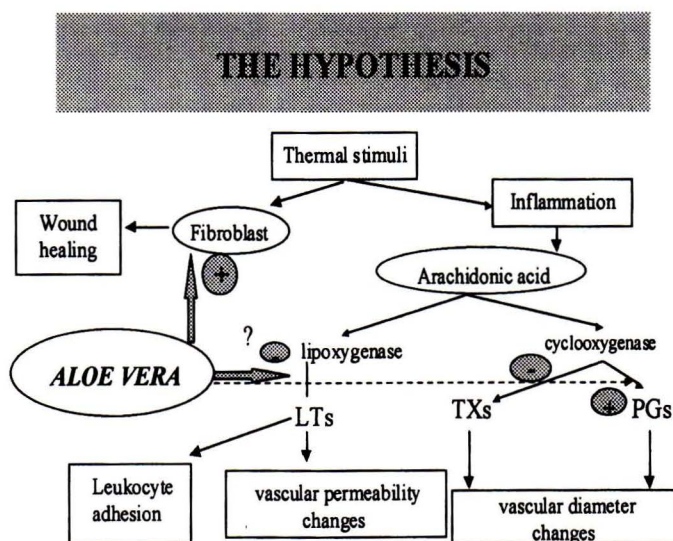


Fig. 5. The proposed mechanisms of *Aloe vera* on antiinflammation and wound healing.

significantly in *Aloe*-treated burn wounds compared to the untreated- and NSS-treated groups. Consequently, these findings possibly reflect the role of *Aloe vera* in the inhibition of lipoxygenase pathway.

The antiinflammatory action of *Aloe vera* was also shown to be attributable to some active components through the other activities in addition to antithromboxane and/or antiprostaglandin. Moreover, it was demonstrated that *Aloe vera* contained bradykininase(25), carboxypeptidase(26) and glycoprotein(27) which has antibradykinin activity with subsequent suppression of vasodilation and pain. Magnesium lactate in *Aloe vera* was found to inhibit the conversion of histidine to histamine while barbaloin and aloin could inhibit histamine release from mast cells(28). The antihistamine activity then results in decreasing vasodilation and inflammation. Furthermore, various types of sterols present in *Aloe vera* have been proposed as the antiinflammatory agents(10). Therefore, we believe that these various kinds of active ingredients can restore the normal endothelial functions of cutaneous microvasculature, including arteriolar diameter change, vascular permeability and leukocyte adhesion, after inflammatory responses to thermal injury.

Regarding the wound healing property of *Aloe vera*, the result of the present study is in agreement with that of previous studies(8,29). Lectin-like compounds(30), polysaccharides, amino acids, ascorbic acid, lignin(31,32) as well as the growth factors including gibberellin, auxins and mannose-6-phosphate(10) have been reported to be stimulators of fibroblast synthesis. By using the same partial thickness burn wound model, the *Aloe vera* gel-treated area had the average healing time of about 11.89 days while that of the vaseline gauze-treated area was 18.18 days(29).

In conclusion, we have made the hypothesis that since *Aloe vera* is composed of a combination of active components, then it could exhibit the actions of both antiinflammation and wound healing promotion as observed in our study. Therefore, in the future, *Aloe vera* might be a great therapeutic agent for burn wound patients.

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ผลการรักษาของว่านหางจระเข้ต่อการไหลเวียนเลือดในหลอดเลือดขนาดเล็กของผิวหนัง และการสมานแผลในแบบจำลองแผลไหม้ระดับที่สองในหนูแรท

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อัมพร จาริยะพงศ์สกุล, วท.ม.*, สุทธิลักษณ์ ปทุมราช, วท.ด.*

ได้ศึกษาผลของว่านหางจระเข้ต่อการไหลเวียนเลือดในหลอดเลือดขนาดเล็กของผิวหนังและการสมานแผล หลังจากทำให้เกิดแผลไหม้ระดับที่สองในหนูแรทเพศผู้จำนวน 48 ตัว โดยแบ่งเป็น 4 กลุ่ม ๆ ละเท่า ๆ กัน คือ กลุ่มควบคุมที่ไม่ได้ทำให้เกิดแผลไหม้ กลุ่มแผลไหม้ที่ไม่ได้รับการรักษา กลุ่มแผลไหม้ที่ได้รับการรักษาโดยการทาสารละลายน้ำเกลือวันละครั้ง และกลุ่มแผลไหม้ที่ได้รับการรักษาโดยการทาเจลว่านหางจระเข้วันละครั้ง หนูแต่ละกลุ่มถูกแบ่งออกเป็น 2 กลุ่มย่อยสำหรับใช้ศึกษาผลในวันที่ 7 และ 14 หลังจากเกิดแผลไหม้ ใช้เทคนิคดอร์ซัล สกินฟลัด แชมเบอร์ ร่วมกับกล้องจุลทรรศน์ฟลูออเรสเซนซ์เพื่อศึกษาการเปลี่ยนแปลงการไหลเวียนเลือดในหลอดเลือดขนาดเล็กของผิวหนัง อันได้แก่เส้นผ่าศูนย์กลางของหลอดเลือดแดงรอง ความซึมผ่านผ่านได้ของหลอดเลือดดำฝอยและการเกาะติดของเม็ดเลือดขาวบนหลอดเลือดดำฝอย

ผลปรากฏว่าในวันที่ 7 การขยายตัวของหลอดเลือดและการเพิ่มความซึมผ่านผ่านได้ของหลอดเลือดดำฝอยซึ่งเกิดในแผลไหม้ที่ไม่ได้รับการรักษานั้น พบว่ามีการลดลงอย่างมีนัยสำคัญทางสถิติทั้งในกลุ่มที่ได้รับการรักษาด้วยสารละลายน้ำเกลือและว่านหางจระเข้ แต่กลุ่มหลังมีการลดลงมากกว่า การเกาะติดของเม็ดเลือดขาวไม่มีความแตกต่างระหว่างกลุ่มที่ไม่ได้รับการรักษาและกลุ่มที่ได้รับการรักษาด้วยสารละลายน้ำเกลือและว่านหางจระเข้ ในวันที่ 14 เกิดการหดตัวของหลอดเลือดในแผลไหม้ที่ไม่ได้รับการรักษา มีเพียงกลุ่มที่ได้รับการรักษาด้วยว่านหางจระเข้เท่านั้นที่พบว่าเส้นผ่าศูนย์กลางของหลอดเลือดแดงรองเพิ่มขึ้นจนถึงภาวะปกติ และความซึมผ่านผ่านได้ของหลอดเลือดดำฝอยไม่แตกต่างจากกลุ่มควบคุมปริมาณการเกาะติดของเม็ดเลือดขาวก็น้อยกว่าเมื่อเทียบกับกลุ่มที่ไม่ได้รับการรักษาและกลุ่มที่ได้รับการรักษาด้วยสารละลายน้ำเกลือ นอกจากนี้พื้นที่การสมานแผลยังมากกว่าทั้งในระยะ 7 และ 14 วัน หลังเกิดแผลไหม้

สรุปได้ว่า ว่านหางจระเข้สามารถออกฤทธิ์ด้านการอักเสบและเร่งการสมานแผลได้ เมื่อใช้ทาบนแผลไหม้ระดับที่สอง

คำสำคัญ : ว่านหางจระเข้, การไหลเวียนเลือดในหลอดเลือดขนาดเล็กที่ผิวหนัง, การสมานแผล, แบบจำลองแผลไหม้ระดับที่สอง

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