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Effects of *Aloe vera* on leukocyte adhesion and TNF-α and IL-6 levels in burn wounded rats

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Abstract. The effects of *Aloe vera* on microcirculation and levels of TNF-α and IL-6 were investigated in rats after inducing burn. Seventy-two male Wistar Furth rats were equally divided into four groups as follow: controls (CON), untreated burn-wound rats (BURN), normal saline-treated burn-wound rats (BURN-NSS) and *Aloe vera*-treated burn-wound rats (BURN-ALOE). The animals in each group were equally subdivided into three subgroups for the study on day 3, 7 and 14 post-burn. Dorsal skinfold chamber preparation and intravital fluorescence microscopic technique were performed to examine leukocyte adhesion on postcapillary venules. ELISA techniques were performed to examine serum TNF-α and IL-6 levels. It was found that the amount of leukocyte adhesion was significantly reduced in the BURN-ALOE group compared to rats in the BURN group on day 14. Levels of TNF-α and IL-6 were also decreased significantly compared to BURN at all three monitored time points. Aloe vera could inhibit the inflammatory process following burn injury, as characterized by the reduction of leukocyte adhesion, as well as those pro-inflammatory cytokines.

Keywords: *Aloe vera*, leukocyte, TNF-α, IL-6 levels, burn wounded

1. Introduction

Local responses after burn injury include an acute inflammatory process, infection, activation of leukocyte–endothelium interaction and an alteration in circulating cytokines. These may all contribute, leading to systemic effects. Cytokines have been considered to be important factors in the post-burn pathophysiological process [1–4] and in the pathophysiology of sepsis and septic shock [5]. It has been recognized that at the site of tissue injury or infection, the local production of proinflammatory cytokines will activate host non-specific immunity. The first-wave cytokines such as interleukin-1 (IL-1) and tumor necrosis factor-α (TNF-α) were produced mainly by the tissue macrophages [6,7]. In addition to the induction of adhesive molecule expression on endothelial cells, IL-1 or TNF-α can potently stimulate cyclooxygenase activity. As well as the second-wave cytokines such as IL-6 and IL-8 which are generally chemotactic, neutrophils can be produced subsequently [8]. The cascade release of those secondary cytokines and hormonal factors may lead to local and systemic inflammation.

Aloe vera has been recognized as a good traditional medicine for its various pharmacological actions e.g., analgesia, antiviral, antifungal, antiparasite, and anticancer, etc. *Aloe vera* gel has also been used for the topical treatment of wounds, minor burns, and skin irritations. The scientific evidence for therapeutic properties of *Aloe vera* on burns wounds has demonstrated that the various constituents in *Aloe*

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vera may be responsible for these healing actions on burns, including anti-inflammation, antimicrobial, wound healing promotion and, possibly, immunomodulation. However, the antiinflammatory mechanism underlying the therapeutic effects of *Aloe vera* is largely unknown. In particular, there is no report indicating how *Aloe vera* works on the cytokines, the active inflammatory substances released following thermal injury. Therefore, in the present study, we aim to investigate the properties of *Aloe vera* by using the second-degree burn wound model.

2. Materials and methods

2.1. Animal preparation and induction of burn

Male Wistar Furth rats ($n = 72$; 200–250 g) were used for this study. All rat were received from the National Laboratory Animal Center of Mahidol University Salaya Campus, Bangkok. The animals were divided into four groups: control (CON), burn wounded (BURN), burn wounded with normal saline treatment (BURN-NSS), and burn wounded with *Aloe vera* treatment (BURN-ALOE). For the induction of burn wound injury, a partial thickness burn injury, or second-degree burn, was performed using the model of Zawacki [9]. Briefly, a hot plate measuring 3.5×4.6 cm^2 with temperature maintained at 75°C was placed on the prepared skin area for 10 seconds.

In the BURN-ALOE group, the animals were immediately treated with topical *Aloe vera* solution at dose of 300 mg/kg BW [10]. *Aloe vera* used in this study was lyophilized *Aloe vera* (Lipo Chemical Co., USA). All animals were given with free access to water and standard laboratory food until experiments were carried out, which were 3, 7 and 14 days post-burn.

2.2. Intravital fluorescent microscopic study

After dorsal skinfold chamber implantation, each animal was placed on the stage of a fluorescent microscope equipped with transillumination and epiilumination optics (Nikon Optiphot-2, Japan). After intravenous application of fluorescent marker (FITC-dextran-200, Sigma Co, USA), epiillumination was achieved with a 50-W mercury lamp with a 488 nm excitation filter and 515 nm emission barrier filter. Selected images were recorded in real time on videotape by video camera (Dage, SIT, USA).

For visualization of the circulating and adhering leukocytes, the fluorescent marker acridine orange was infused intravenously (0.5 mg/kg BW/min) for 5 minutes [12,13].

The leukocyte which was considered to be adherent had to remain stationary for a period of 30 seconds or more. The number of leukocyte adhesion was expressed as a percentage along a 100-μm length of the postcapillary venule.

2.3. Enzyme-linked immunosorbent assay (ELISA)

Blood samples were collected from each rat after the intravital fluorescent procedures. Then the blood sample were centrifuged at 1,500 g. Serum was stored at -70°C until it was analyzed.

2.4. IL-6 assay

Serum levels of IL-6 were determined by using the enzyme-linked immunosorbent assay (ELISA) kit by Endogen, Inc. (Woburn, MA, USA). The proportional concentration of IL-6 in each sample was determined using a spectrophotometer (DRGANON TEKNIKA micro-system, USA) with 450-nm wavelength.

2.5. TNF-α assay

Serum levels of TNF-α were determined by using the enzyme-linked immunosorbent assay (ELISA) kit by Endogen, Inc. (Woburn, MA, USA). The experimental protocol used for a TNF-α assay is similar to that of an IL-6 assay. However, the incubation time and reagent concentration were different.

2.6. Data analysis

Results were expressed as mean $\pm$ standard deviation (SD). Statistical analysis was carried out using ANOVA followed by a Student's 't'-test. A probability of less than 0.05 was considered to be significant.

3. Results

3.1. Leukocyte adhesion

The amount of leukocyte adhesion counted number of leukocytes was determined and expressed as a percentage per 100 μm length of the selected postcapillary venules. The percentages of leukocyte adhesion shown in Table 1 were not different in any groups of burn-wound rats, NSS-treated burn-wound rats, or the aloe-treated burn-wound rats on days 3 and 7 post-burn. However, on day 14 the amount of leukocyte adhesion was significantly decreased in the aloe-treated burn-wound rats as compared to the other burn-wound rats. Consequently, it was surmised that *Aloe vera* could reduce the amount of leukocyte adhesion to endothelium of postcapillary venules. Example videoimages of such evidence on 14 days post-burn are shown in Fig. 1.

3.2. TNF-α and IL-6 levels

Serum TNF-α and IL-6 levels were shown in Tables 2 and 3, respectively. The results indicated that TNF-α and IL-6 levels were increased after burn injury (Figs 2 and 3). Interestingly, *Aloe vera* helped to reduce TNF-α and IL-6 at all three time points monitered.

4. Discussion

In our experiment, after the burn injury, marked enhancement of leukocyte adhesion and transmigration was observed through an intravital fluorescence microscope using acridine orange labeled leukocytes as

Table 1

Means $\pm$ SD of leukocyte adhesion (percentage of cell numbers/100 μm) on postcapillary venules of control rats (CON), burn-wound rats (BURN), NSS-treated burn-wound rats (BURN-NSS) and aloe-treated burn-wound rats (BURN-ALOE)

Duration (days)	Leukocyte adhesion (percentage of cell numbers/100 μm)			
	CON	BURN	BURN-NSS	BURN-ALOE
3	3.84 ± 1.14	$26.33 \pm 4.80^{**}$	$22.98 \pm 2.97^{**,ns}$	$23.60 \pm 9.02^{**,ns,nss}$
7	2.31 ± 1.23	$24.77 \pm 10.31^{**}$	$19.95 \pm 0.55^{**,ns}$	$19.15 \pm 5.03^{**,ns,nss}$
14	4.74 ± 2.40	$22.12 \pm 1.75^{**}$	$18.77 \pm 3.81^{**,ns}$	$15.40 \pm 2.75^{**,\#,nss}$

**Significant difference compared to CON ($p < 0.01$); $^{\#}$Significant difference compared to BURN ($p < 0.05$); ^{ns}No significant difference compared to BURN; ^{nss}No significant difference compared to BURN-NSS.

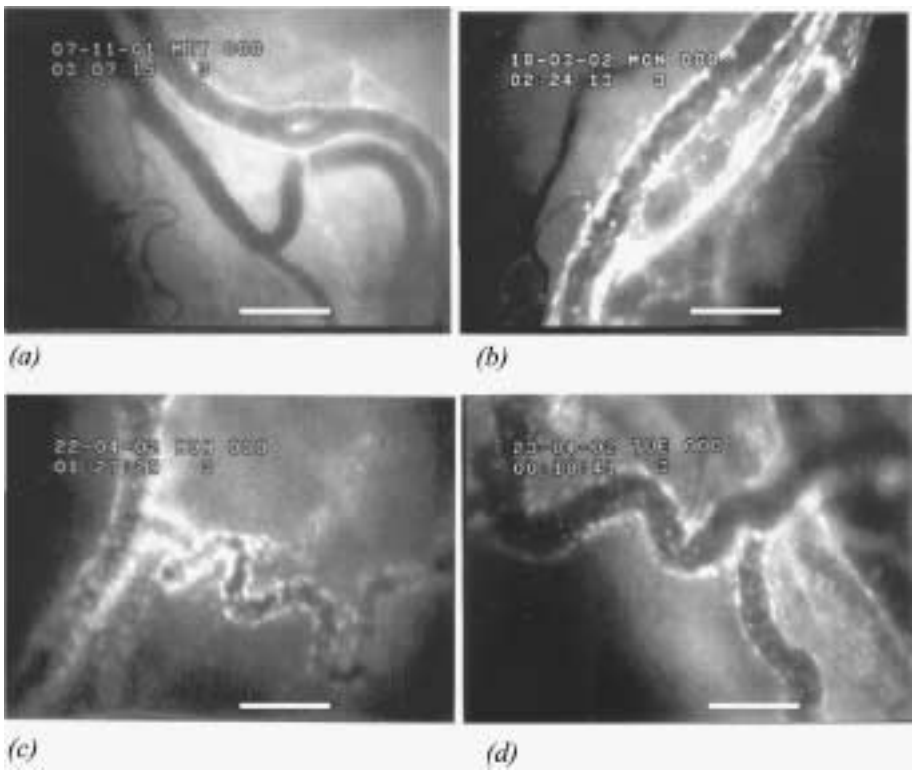

Fig. 1. Videomicroscopic images demonstrate leukocytes adhering to skin postcapillary venular endothelium observed at 14 days post-burn. (a) CON, (b) BURN, (c) BURN-NSS, (d) BURN-ALOE. White dots represent leukocytes stained by the intravenous injection of the fluorescein marker, acridine orange (0.5 mg/kgBW/min). (White bars represent 100 microns.)

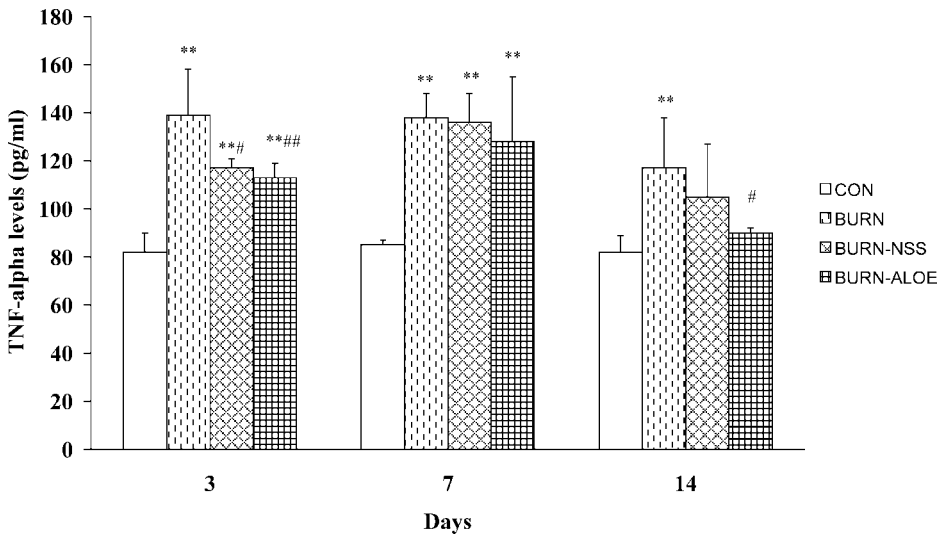

Fig. 2. Bar graphs represent means $\pm$ SD of TNF-α levels in all four groups: (a) CON, (b) BURN, (c) BURN-NSS, (d) BURN-ALOE. *Significant difference compared to CON ($p < 0.05$); **Significant difference compared to CON ($p < 0.01$); $^{\#}$Significant difference compared to BURN ($p < 0.05$); $^{\#\#}$Significant difference compared to BURN ($p < 0.01$).

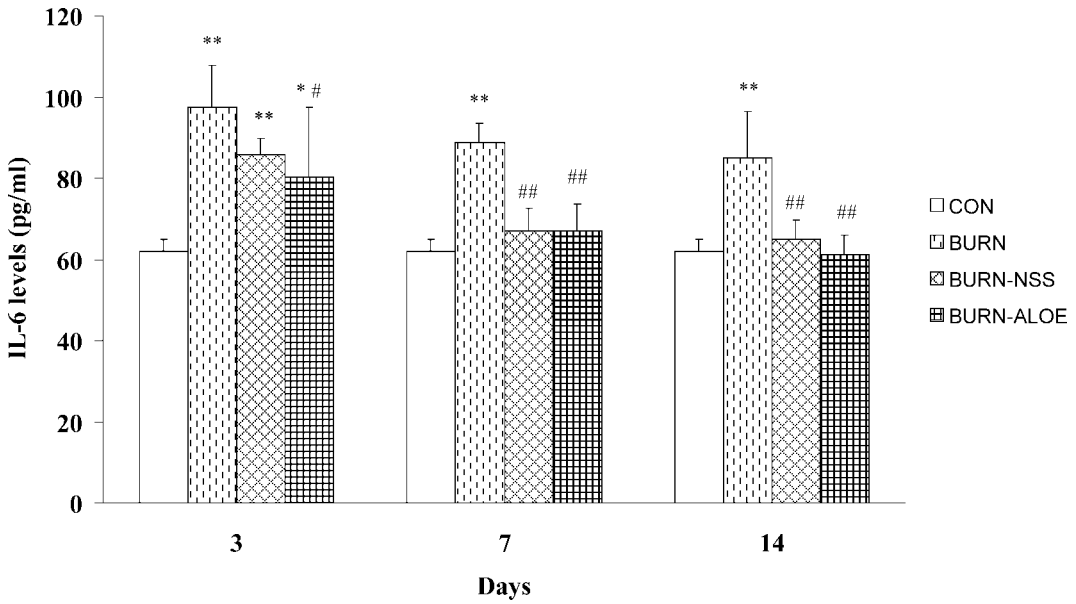

Fig. 3. Bar graphs represent means ± SD of IL-6 levels in all four groups: (a) CON, (b) BURN, (c) BURN-NSS, (d) BURN-ALOE. *Significant difference compared to CON (p < 0.05); **Significant difference compared to CON ($p < 0.01$); #Significant difference compared to BURN ($p < 0.05$); ##Significant difference compared to BURN ($p < 0.01$).

Table 2

Means ± SD of TNF-α levels of (CON), burn-wound rats (BURN), NSS treated burn-wound rats (BURN-NSS) and aloe treated burn-wound rats (BURN-ALOE)

Duration (days)	TNF-α levels (pg/ml)			
	CON	BURN	BURN-NSS	BURN-ALOE
3	82.0 ± 8.0	$139.0 \pm 19.0^{**}$	$117.0 \pm 4.0^{**,\#}$	$113.0 \pm 6.0^{**,\#\#,nss}$
7	85.0 ± 2.0	$138.0 \pm 10.0^{**}$	$136.0 \pm 12.0^{**,ns}$	$128.0 \pm 27.0^{**,ns,nss}$
14	82.0 ± 7.0	$117.0 \pm 21.0^{**}$	$105.0 \pm 22.0^{ns,ns}$	$90.0 \pm 2.0^{ns,\#,nss}$

*Significant difference compared to CON ($p < 0.05$); **Significant difference compared to CON ($p < 0.01$); #Significant difference compared to BURN ($p < 0.05$); ##Significant difference compared to BURN ($p < 0.01$).

Table 3

Means ± SD of IL-6 levels of (CON), burn-wound rats (BURN), NSS-treated burn-wound rats (BURN-NSS) and aloe treated burn-wound rats (BURN-ALOE)

Duration (days)	IL-6 levels (pg/ml)			
	CON	BURN	BURN-NSS	BURN-ALOE
3	62.1 ± 2.9	$97.4 \pm 10.5^{**}$	$85.9 \pm 3.8^{**,ns}$	$80.2 \pm 17.2^{*,\#,nss}$
7	62.1 ± 2.9	$88.8 \pm 4.8^{**}$	$66.9 \pm 5.7^{ns,\#\#}$	$66.9 \pm 6.7^{ns,\#\#,nss}$
14	62.1 ± 2.9	$85.0 \pm 11.5^{**}$	$64.9 \pm 4.8^{ns,\#\#}$	$61.1 \pm 4.8^{ns,\#\#,nss}$

*Significant difference compared to CON ($p < 0.05$); **Significant difference compared to CON ($p < 0.01$); #Significant difference compared to BURN ($p < 0.05$); ##Significant difference compared to BURN ($p < 0.01$).

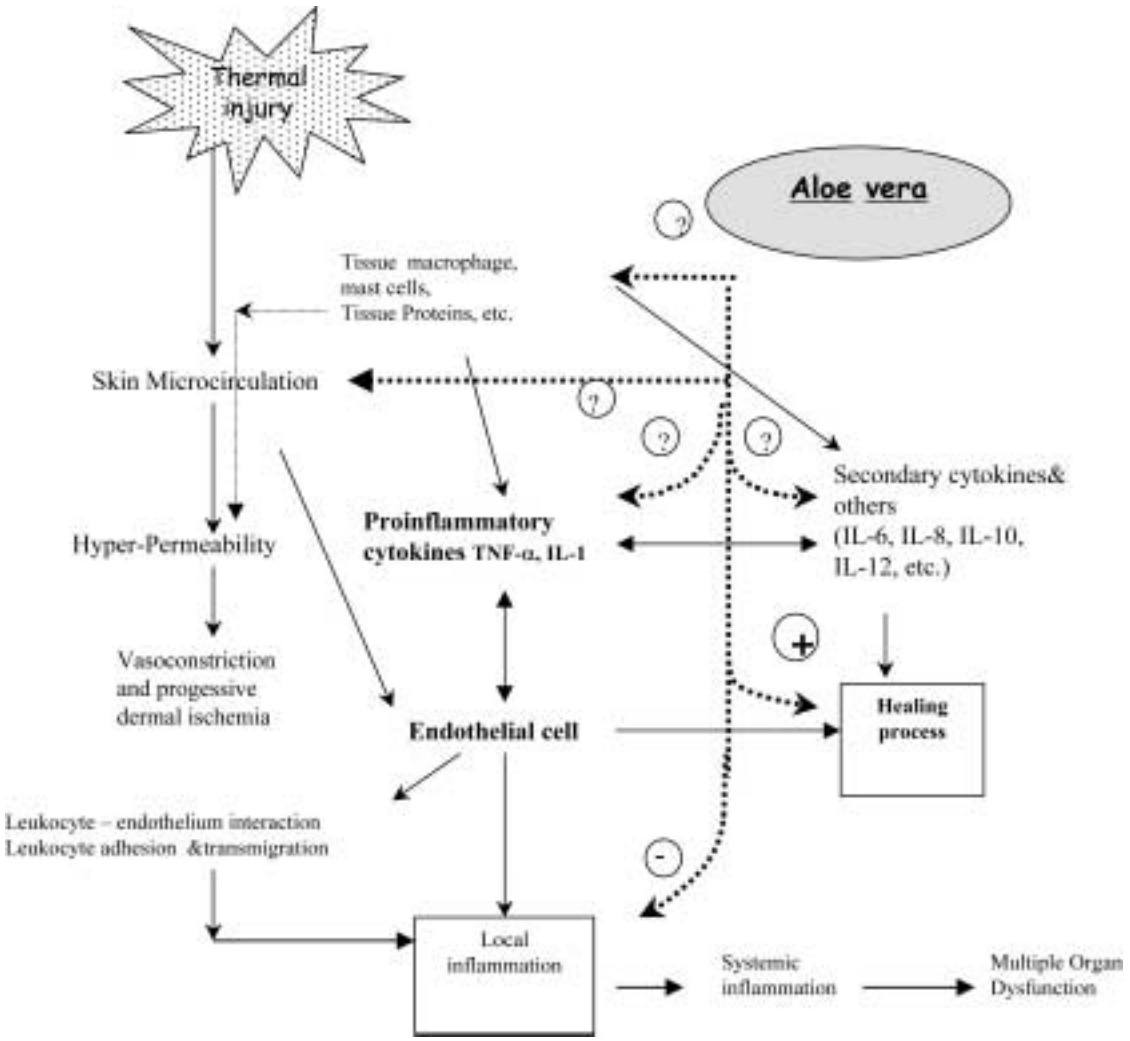

Fig. 4. The diagram demonstrates the proposed hypothesis summarized from our findings.

shown in Fig. 1. The results of digital-image analysis for leukocyte adherence demonstrated sustained adhesion in both groups of BURN and BURN-NSS for all three monitored time points. In the early burn phase (0–72 hours), the products of vasoactive amine and kinin systems modulate the inflammatory response. The release of proinflammatory mediators is initiated by tissue macrophages, mast cells, or other tissue cells such as damaged fibroblasts. Therefore, soluble mediators such as histamine, interleukin-1 (IL-1), and tumor necrosis factor-alpha (TNF-α) are obtained. These mediators induce the expression of endothelial–leukocyte interactions. TNF-α and IL-1 later induced the "secondary cytokines" (sometimes called "intermediate cytokines") such as IL-6, and IL-8. Deveci and co-workers [14] explained their experimental results on full-thickness burn wound rat models that IL-6 may inhibit the severity of the inflammatory response in the early period of thermal injury. They also reported that high levels of IL-6 decreased the levels of TNF-α. Moreover, it has been also suggested that a prolonged increase in levels of TNF-α may play an important role in the development of multiple organ failure after thermal injury [15,16]. Therefore, transiently increased circulating levels of TNF-α in burns indicate a poor prog-

nosis [17–19]. Interestingly, not only the inflammatory mediated action of TNF-α was documented, but in the primary phase, the role of TNF-α as a stimulators for fibroblast and angiogenesis production was also widely introduced [17,20]. It would be fair to say that transient changes in TNF-α and other cascade cytokines are important for both inflammatory response and the wound healing process.

Interestingly, our experimental data have demonstrated that daily treatment of *Aloe vera* (300 mg/kg BW) could reduce both TNF-α and IL-6 levels, and also the endothelial–leukocyte interaction significantly. Hart et al. [21] and Davis et al. [22] also demonstrated these effects of *Aloe vera* on leukocyte recruitment. As a result of this leukocyte recruitment evaluation, it is possible to say that *Aloe vera* may have some active ingredients which are able to inhibit or suppress the serial sequence of TNF-α on enhancement of endothelial–leukocyte interactions.

Results obtained in our study showed that there was a marked increase in serum TNF-α and IL-6 in BURN-group at 3 days post-burn. In the present study, we observed that treating burn-wounds with *Aloe vera* could prevent the elevation of TNF-α and IL-6 at all three monitored time points. Interestingly, the recent study by Blumenfeld et al. [20] demonstrated that the major carbohydrate substance of *Aloe vera*, 'acemannan', was responsible for the wound healing process in deep partial-thickness skin burn guinea-pigs. In addition, our previous investigation demonstrated that *Aloe vera* can stimulate the wound healing process as well [11]. The significant increase in healing in areas treated with *Aloe vera*, especially, epithelialization, was indicated on day 14 post-burn. As an overall conclusion, we summarize our findings as shown in Fig. 4. The effects of *Aloe vera* on TNF-α and IL-6, and on endothelium–leukocyte interaction indicated in our present study might be due to the active ingredients of *Aloe vera*, and such ingredients might play an important role in anti-inflammatory and growth promotion, especially epithelialization. With regards to the essential, but complex, issue of TNF-α and IL-6, we believe that *Aloe vera* may speed up the turnover rate of these transient substances. However, many more experiments need to be carried out before we have the information we need to clarify such mechanisms of *Aloe vera.*

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