

# Anti-inflammatory effects of *Aloe vera* on leukocyte–endothelium interaction in the gastric microcirculation of *Helicobacter pylori*-infected rats

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**Abstract.** This research was aimed to investigate anti-inflammatory effects of *Aloe vera* on leukocyte–endothelium in the gastric microcirculation of *Helicobacter pylori* (*H. pylori*)-infected rats. Thirty-six male Sprague-Dawley rats were divided into 3 groups: control, *H. pylori*-infected, and *A. vera*-treated group (200 mg/kg b.w., twice daily). *H. pylori*-inoculation was induced in the rats by the administration of *H. pylori* solution. Intravital fluorescence videomicroscopy was used to examine leukocyte adhesion in postcapillary venules on the posterior surface of stomach area on different periods after administration of *A. vera*. Serum tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ) level was measured in blood collected at the end of experiment by using ELISA technique. The results showed that in *H. pylori*-infected group on day 8, the leukocyte adhesion was  $13.40 \pm 1.00$  cells/100  $\mu\text{m}$  vessel length and the TNF- $\alpha$  was  $76.76 \pm 23.18$  pg/ml, which increased significantly ( $p < 0.05$ ), compared with the control group (leukocyte adhesion<sub>control</sub> =  $2.54 \pm 0.6$  cells/100  $\mu\text{m}$  vessel length and TNF- $\alpha$ <sub>control</sub> =  $9.92 \pm 2.62$  pg/ml). Treatment with *A. vera* reduced the leukocyte adhesion ( $5.5 \pm 0.5$  cells/100  $\mu\text{m}$  vessel length), and TNF- $\alpha$  ( $26.31 \pm 6.38$  pg/ml) significantly ( $p < 0.05$ ). In conclusion, *H. pylori* enhanced leukocyte–endothelium interaction in the posterior stomach area markedly. This enhancement in leukocyte–endothelium interaction could be improved by the treatment of *A. vera*, associated with reduction in TNF- $\alpha$  level.

Keywords: *Helicobacter pylori*, gastric microcirculation, leukocyte adhesion, tumor necrosis factor- $\alpha$ , *Aloe vera*

## 1. Introduction

In developing countries, there is a high incidence of *Helicobacter pylori* (*H. pylori*) infection in people [1]. Infection by this gastrointestinal bacterium induces gastric ulcer, duodenal ulcer, or chronic gastritis. Such *H. pylori*-associated chronic gastritis is generally characterized by infiltration of lamina

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propria with inflammatory cells, enhanced release of pro-inflammatory cytokines (for instance; IL-1 $\beta$  and IL-8, and TNF- $\alpha$  [2,3]) as well as the generation of reactive oxygen species [4].

*A. vera* is a kind of plant medicines commonly used for basic health care. This plant is separated into two portions: *Aloe vera* gel and latex portion. The gel portion of *A. vera* contains many biologically active substances [5]. In fact, glycoprotein extracted from *A. vera* has a strong anti-inflammatory effect [6–10], which may reduce leukocyte adherence, promote wound healing and reduce both tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ) and IL-6 levels [11–13]. Moreover, oral administration of the mucilaginous gel has prophylactic and curative effect against gastric lesion induced by hydrochloric acid and acetic acid. Mucopolysaccharide and glycoproteins content in *A. vera* gel show synergistic antagastric ulcer action [14].

Certainly, *A. vera* is of clinical benefit to patients with chronic gastritis, but few studies have been made on its effect on the gastric microcirculation *in vivo*. This study was aimed to examine the effect of *A. vera* on the gastric microcirculation using a newly developed *H. pylori*-infected rat model [15]. We made intravital observation of gastric microcirculatory changes in the posterior stomach area on different periods after the administration of *A. vera*. By measuring leukocyte adhesion in gastric venules, we evaluated the effect of *A. vera* on leukocyte–endothelium interaction in relation to changes in serum TNF- $\alpha$  levels.

## 2. Material and methods

### 2.1. Animal preparation

Male Sprague-Dawley rats (200–250 g b.w.) were used for the present experiment. The rats were divided into three groups: control ( $n = 12$ ), *H. pylori*-infected ( $n = 12$ ), and *A. vera*-treated group ( $n = 12$ ) as follows.

(a) *Control group*. The shammed operation for the inoculation of *H. pylori* was performed on the rats. The rats were housed with free access to water and standard chow until 14 days. Then, the rats were treated with distilled water at a volume of 1 ml/rat by gavage twice daily at an interval of 4–6 hours. They were treated with distilled water until the day of experiment.

(b) *H. pylori*-infected group. The rats were inoculated with *H. pylori*, according to the procedure developed by Thong-Ngam et al. [15]. Briefly, *H. pylori* suspension ( $10^{10}$  CFU/ml; 1 ml/rat) was given to the rats by gavage twice daily, with an interval of 4 hours during 3 days. Two weeks after the inoculation of *H. pylori*, the rats were treated with distilled water (*A. vera* vehicle) at a volume of 1 ml/rat by gavage twice daily at an interval of 4–6 hours until the day of experiment. The positive result of *H. pylori* infection was determined based on a rapid urease test and histological examination.

(c) *H. pylori* infection with *A. vera* treatment group (*A. vera*-treated group). Fourteen days after the *H. pylori* inoculation, the rats were gavaged with *Aloe vera* suspended in distilled water at a dose of 200 mg/kg b.w. twice daily with an interval of 4–6 hours during 3 or 8 days [12]. For the present treatment, lyophilized powder of *A. vera* (Lipo Chemical Co, USA) was used.

### 2.2. Intravital fluorescence videomicroscopy

The rat was anesthetized with intraperitoneal injection of sodium pentobarbital (45 mg/kg b.w.). A constant level of anesthesia was maintained throughout the experiment by supplement dose (20%

of original dose) every 30–45 minutes [16]. The tracheotomy was performed. The arterial blood pressure was recorded in the common carotid artery via a pressure transducer (Nihon-Kohden, Japan). The jugular vein was cannulated for injection of acridine orange (Sigma Chemical Co., USA; 5 mg/100 ml in normal saline) to label leukocytes. The abdominal cavity was opened via a midline laparotomy, and a loop of mesentery was exteriorized through the midline incision and placed on a Plexiglas chamber for microscopic observation. The mesentery was superfused continuously with Krebs Ringer solution (pH 7.4, 37°C).

The posterior surface of stomach microcirculation was observed under an epi-illumination fluorescence videomicroscope (Optiphot-2, Nikon, Japan) with a 50 W mercury lamp and a fluorescence filter. The video images were achieved through a silicon intensified target television camera (Dage-SIT 68, USA) [10,11], which was projected on a video monitor (GM-1411 QM, Sony, Japan) using  $\times 10$  (or  $\times 40$ ) objective lens (CF Plan Fluor, Nikon, Japan). The selected area was then recorded in real-time by a videotape recorder (SLV-X311, Sony, Japan) connected to a video timer (VTG-55, FOR-A, Japan) throughout the experimental period.

### 2.3. Leukocyte adhesion in postcapillary venules

Based on the recorded video images, we counted the number of leukocytes which adhered to the endothelial wall or remained stationary during 30 seconds or more in postcapillary venules (15–30  $\mu\text{m}$  in diameter). Using the mean total number ( $N$ ) of adherent leukocytes measured from three venules in one rat with the length  $L$  ( $\mu\text{m}$ ), we expressed the degree of leukocyte adhesion ( $Cn$ ) in terms of cells/100  $\mu\text{m}$  vessel length as follows [17]:

$$Cn = \frac{N}{L} \times 100.$$

### 2.4. Enzyme-linked immunosorbent assay (ELISA)

At the end of each experiment, blood sample was collected by means of cardiac puncture, and the serum was stored at  $-70^\circ\text{C}$  until the day of analysis. The enzyme-linked immunosorbent assay (ELISA) kit (R&D Systems, Minneapolis, USA) was used to measure the level of TNF- $\alpha$  in the collected serum.

### 2.5. Statistical analysis

Results were expressed as mean  $\pm$  SEM. One-way analysis of variance (ANOVA) was made to examine the difference of each parameter. The data were analyzed by using SPSS program (version 11.5) for Windows.

## 3. Results

*H. pylori* infection in the rat was judged based on a rapid urease test and histological examination. Figure 1 demonstrates histological changes between control and *H. pylori*-infected groups. The successful rate of infection was about 83%.

The TNF- $\alpha$  levels measured in three groups (control, *H. pylori*-infected and *A. vera*-treated) are listed with blood pressure, heart rate and body weight in Table 1. On both 3 and 8 days after treatment, the

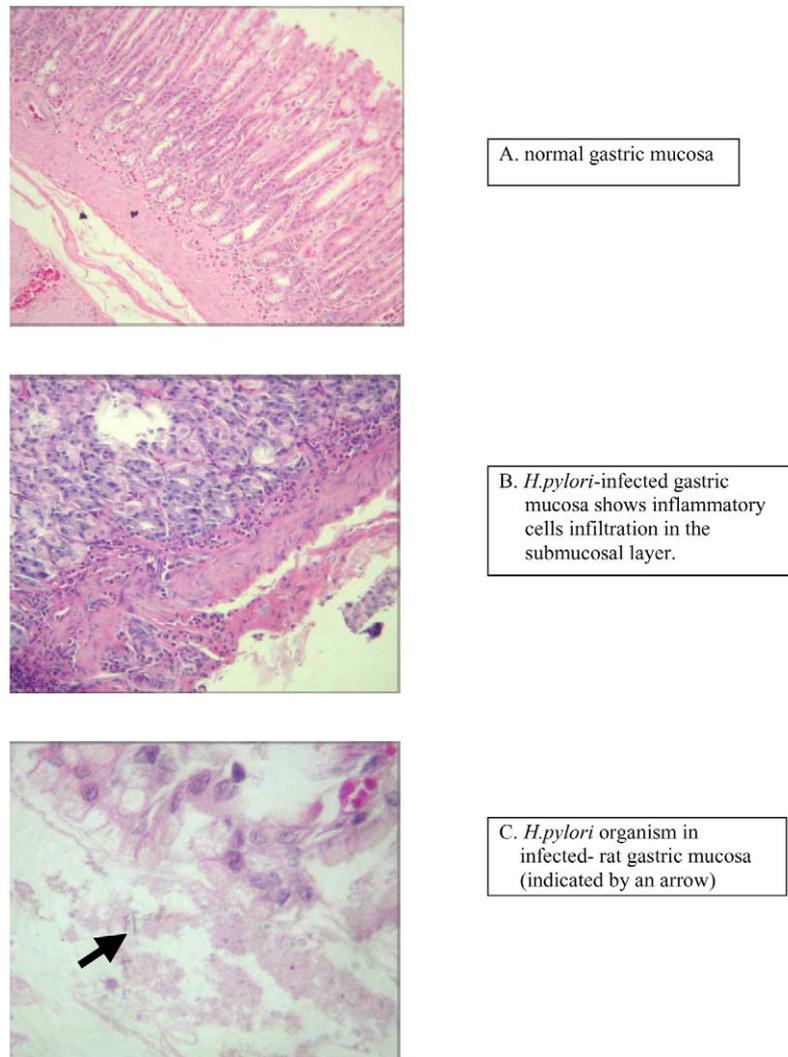


Fig. 1. An example of images to demonstrate histological changes between control (A) and *H. pylori*-infected group (B and C).

TNF- $\alpha$  levels were significantly higher in *H. pylori*-infected group than the control levels, but they were significantly reduced in *A. vera*-treated group, compared to *H. pylori*-infected group.

Figure 2 showed an example of fluorescence videoimage to demonstrate leukocytes adhesion to the venular wall in three groups. The degrees of leukocyte adhesion ( $C_n$ ) calculated for control, *H. pylori*-infected and *A. vera*-treated group are shown in Fig. 3. On day 8 after treatment, in *H. pylori*-infected group, the leukocyte adhesion was increased significantly ( $p < 0.01$ ), compared to the corresponding level of control group. However, in *A. vera*-treated group, the leukocyte adhesion was reduced significantly ( $p < 0.01$ ), compared to *H. pylori*-infected group.

Figure 4 shows the relation between the leukocyte adhesion and TNF- $\alpha$  level where all data measured on both day 3 and 8 in three groups were plotted. Interestingly, the degree of leukocyte adhesion is correlated fairly well with the level of TNF- $\alpha$ .

Table 1

TNF- $\alpha$  (pg/ml), body weight (g), systolic pressure (mmHg), diastolic pressure (mmHg) and heart rate (beats/min) of control group, *H. pylori*-infected group, and *A. vera*-treated group on 3 and 8 days after treatment (means  $\pm$  SEM)

Day of treatment	Control group (n = 6)	<i>H. pylori</i> -infected group (n = 6)	<i>A. vera</i> -treated group (n = 6)
TNF- $\alpha$ (pg/ml)			
3 Days	8.65 $\pm$ 1.79	61.98 $\pm$ 18.74*	14.52 $\pm$ 5.53 <sup>†</sup>
8 Days	9.92 $\pm$ 2.62	76.76 $\pm$ 23.18*	26.31 $\pm$ 6.38 <sup>†</sup>
Body weight (gm)			
3 Days	230.8 $\pm$ 5.6	227.7 $\pm$ 8.3	223.3 $\pm$ 11.5
8 Days	242.0 $\pm$ 4.8	239.2 $\pm$ 9.3	240.7 $\pm$ 10.3
Systolic pressure (mmHg)			
3 Days	111.8 $\pm$ 2.4	121.3 $\pm$ 2.6	113.1 $\pm$ 1.7
8 Days	108.2 $\pm$ 4.2	123.8 $\pm$ 4.4*	118.9 $\pm$ 3.7*
Diastolic pressure (mmHg)			
3 Days	94.1 $\pm$ 2.8	103.0 $\pm$ 5.3	100.6 $\pm$ 4.6
8 Days	93.2 $\pm$ 2.5	100.7 $\pm$ 1.6	96.7 $\pm$ 1.4
Heart rate (beats/min)			
3 Days	407 $\pm$ 9.9	373 $\pm$ 6.7	390 $\pm$ 13.4
8 Days	403 $\pm$ 6.2	339 $\pm$ 19.1*	370 $\pm$ 22.9

\*Significant difference, compared to control group ( $p < 0.05$ ).

<sup>†</sup>Significant difference, compared to *H. pylori* infection group ( $p < 0.05$ ).

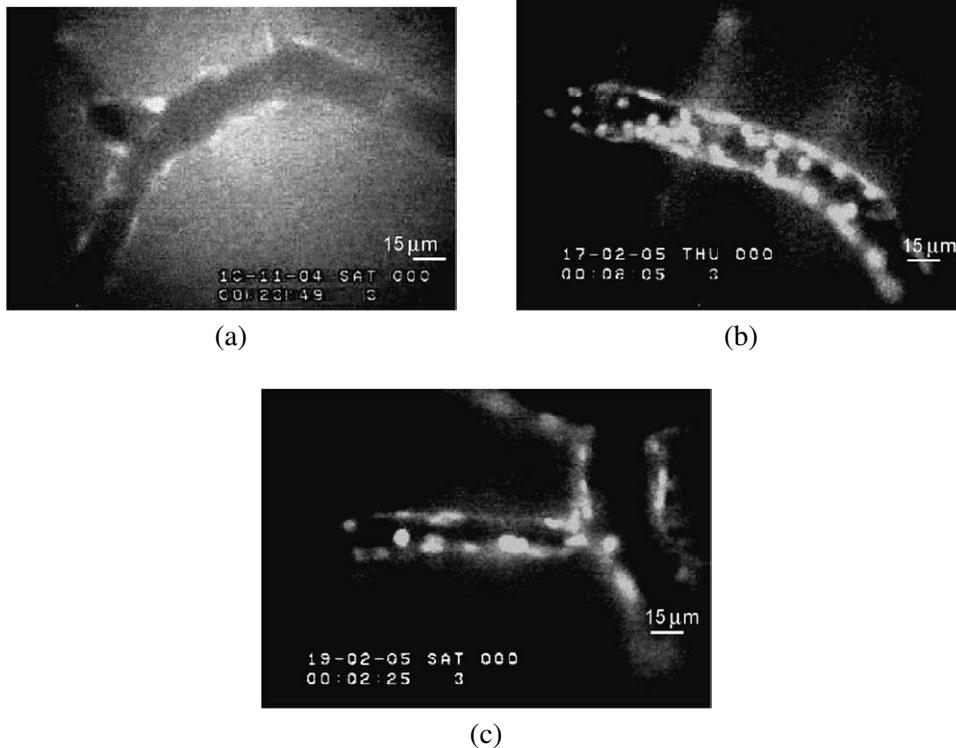


Fig. 2. Fluorescence videomicroscopic images to demonstrate leukocyte adhesion on 8 days after treatment in control (a), *H. pylori*-infected (b), and *A. vera*-treated group (c) ( $\times 40$ ).

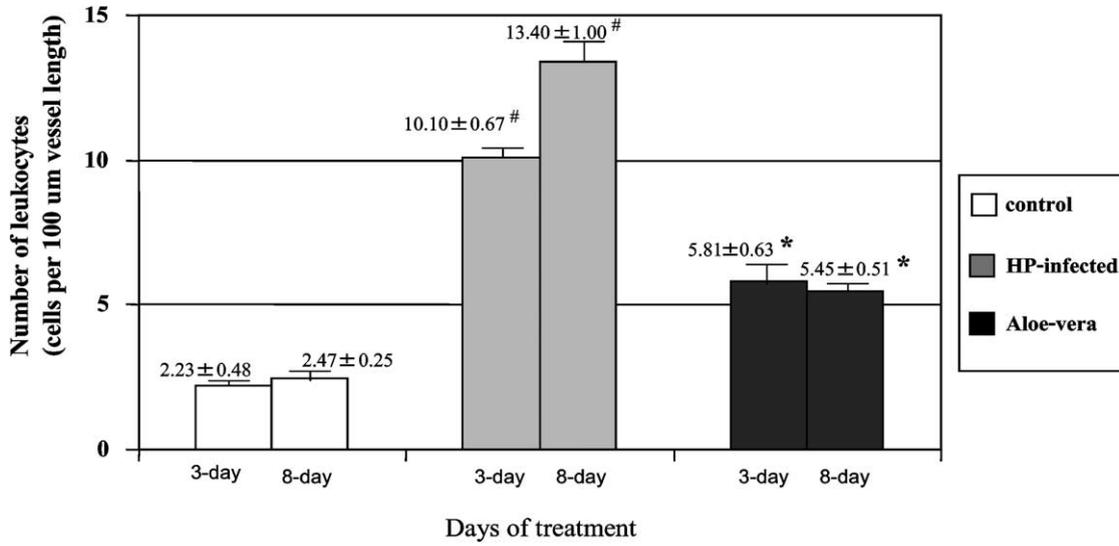


Fig. 3. The number of adherent leukocytes (cells/100 μm vessel length) measured in three groups (control, *H. pylori*-infected, and *A. vera*-treated) on 3 and 8 days after treatment (mean ± SEM). #Significant difference to control group ( $p > 0.05$ ). \*Significant difference compared to *H. pylori*-infected group ( $p < 0.05$ ).

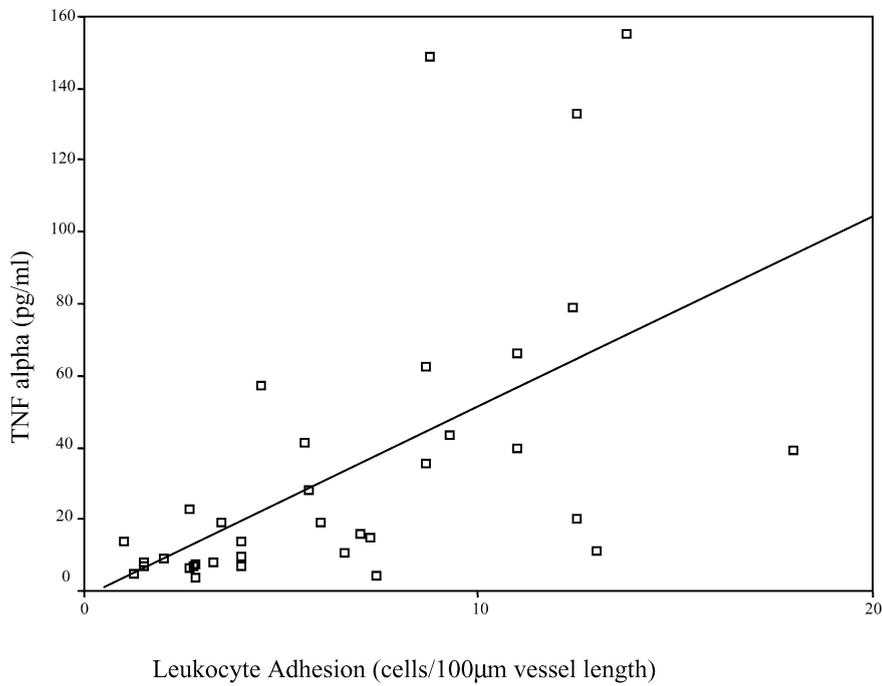


Fig. 4. Correlation between the leukocyte adhesion (cells/100 μm vessel length) and TNF-α (pg/ml). The solid line indicates the regression line, which is expressed in terms of the leukocyte adhesion ( $x$ ) and TNF-α ( $y$ ) as follows:  $y = 5.31x - 1.65$  ( $R = 0.575$ ,  $P < 0.0001$ ).

#### 4. Discussion

The present study has developed a rat model infected by *H. pylori* for intravital videomicroscopic examination of the anti-inflammatory effect of *A. vera* on the gastric microcirculation. Previously, Suzuki et al. reported about the procedure of microvascular evaluation of *H. pylori*-colonized gastric mucosa in Mongolian gerbils [17]. It is, in general, difficult to colonize *H. pylori* to rat stomach more than mouse or Mongolian gerbil ones. In our rat model, a modified procedure has been used for induction of *H. pylori* infection to rat stomach [13,15]. The histological examination has shown the colonization of *H. pylori* to the rat stomach (Fig. 1). It is to be mentioned that our modified procedure could earn a high rate of successful infection.

Two weeks after *H. pylori* inoculation, the video images of 8-days stomach microcirculation exhibited marked enhancement of leukocyte adhesion (Figs 2b and 3). The present water-extract *H. pylori* could exhibit chemotactic substances and further activate the expression of adhesive molecules on both leukocytes and postcapillary venular endothelium. Thus, our findings revealed that the inflammatory response occurred at the gastric mucosa caused by *H. pylori* infection. This alteration was in good agreement with previous reports using histopathological examination [18,19].

*A. vera* is composed of many biologically active substances [5]. In particular, glycoprotein extracted from *A. vera* has a strong anti-inflammatory response [6–9]. In *A. vera*, various types of sterols have been found, and believed to be responsible for their anti-inflammatory action [6–9]. The present results showed that twice daily treatments of *A. vera* (200 mg/kg b.w.) could reduce the leukocyte–endothelium interaction significantly (Figs 3c and 4). As shown in Table 1, the level of serum TNF- $\alpha$  was increased markedly in *H. pylori*-infected group, and such increment was inhibited by treatment of *A. vera*. Therefore, the present data have demonstrated positive histopathological results for *H. pylori*-infected and *A. vera*-treated groups.

It must be mentioned here that *A. vera* has no anti-biotic effect on *H. pylori*, and also no direct effect on the inhibiting growth of culture media with *H. pylori* in our other studies (data are not included). According to Duansak et al. study [11] on burn-wounded effects, *A. vera* active ingredients inhibit or suppress pro-inflammatory cytokines, TNF- $\alpha$  and IL-6 level, and accordingly leukocyte–endothelium interaction.

In conclusion, *A. vera* is effective for improving the interaction between leukocyte and endothelium interaction, associated with reduction in the TNF- $\alpha$  level. It might be a novel therapeutic strategy against *H. pylori* infection in the gastric microvasculature.

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#### References

- [1] R.E. Pounder and D. Ng, The prevalence of *Helicobacter pylori* in different countries, *Aliment. Pharmacol. Ther.* **9**(Suppl. 2) (1995), 33–39.
- [2] T. Brzozowski, P.C. Konturek, S.J. Konturek et al., Water extracts of *Helicobacter pylori* delay healing of chronic gastric ulcers in rats: role of cytokine and gastrin-somatostatin link, *Digestion* **60** (1999), 22–33.
- [3] J.E. Crabtree, Gastric mucosal inflammatory responses to *Helicobacter pylori*, *Aliment. Pharmacol. Ther.* **10** (1996), 29–37.

- [4] G.R. Davis, N.J. Simmonds, T.R. Stevens et al., Helicobacter pylori stimulates antral mucosal reactive oxygen metabolite production in vivo, *Gut* **35** (1994), 179–185.
- [5] A.D. Klein and N.S. Penneys, A. vera, *J. Am. Dermatol.* **18** (1988), 714–720.
- [6] R.H. Davis, A. vera: a natural approach for treatment wounds, edema, and pain in diabetes, *J. Am. Podiatr. Med. Assoc.* **78** (1988), 60–68.
- [7] R.H. Davis, W.R. Parker, R. Samson et al., The isolation of an active inhibitory system from an extract of A. vera, *J. Am. Podiatr. Med. Assoc.* **81** (1991), 258–261.
- [8] R.H. Davis, G.J. Stewart and P.J. Bregman, A. vera and the inflamed synovial pouch model, *J. Am. Podiatr. Med. Assoc.* **82** (1992), 140–148.
- [9] R.M. Shelton, A. vera: its chemical and therapeutic properties, *Int. J. Dermatol.* **30** (1991), 679–683.
- [10] J. Somboonwong, S. Thanamitramanee, A. Jariyapongskul et al. Therapeutic effects of A. vera on cutaneous microcirculation and wound healing in second degree burn model in rat, *J. Med. Assoc. Thai* **83** (2000), 417–425.
- [11] D. Duansak, J. Somboonwong and S. Patumraj, Effects of A. vera on leukocyte adhesion and TNF-alpha and IL-6 levels in burn wounded rats, *Clin. Hemorheol. Microcirc.* **29** (2003), 239–246.
- [12] S. Mahattanadul, Antigastric ulcer properties of A. vera, *Songklanakarinn J. Sci. Technol.* **18** (1995), 49–57.
- [13] D. Grindlay and T. Reynolds, The A. vera phenomenon: A review of the properties and modern uses of the leaf parenchyma gel, *J. Ethnopharmacol.* **16** (1986), 117–151.
- [14] J.J. Blitz, J.W. Smith and J.R. Gerard, A. vera gel in peptic ulcer therapy: preliminary report, *J. Am. Osteopath. Assoc.* **62** (1963), 731–735.
- [15] D. Thong-Ngam, R. Prabjone, N. Visedopas and T. Chatsuwana, A simple rat model of chronic Helicobacter pylori infection for research study, *Thai J. Gastroenterol.* **6** (2005), 2–6.
- [16] A. Jariyapongskul, S. Patumraj, Yamaguchi and H. Niimi, The effect of long-term supplementation of vitamin C on leukocyte adhesion to the cerebral endothelium in STZ-induced diabetic rats, *Clin. Hemorheol. Microcirc.* **27** (2002), 67–76.
- [17] H. Suzuki, M. Mori, K. Seto et al., Polaprezinc attenuates the Helicobacter pylori-induced gastric mucosal leucocyte activation in Mongolian gerbils – a study using intravital videomicroscopy, *Aliment. Pharmacol. Ther.* **15** (2001), 715–725.
- [18] R. Kozol, R. Domanowski, R. Czanko et al., Neutrophil chemotaxis in gastric mucosa: a signal-to-response comparison, *Dig. Dis. Sci.* **36** (1991), 1277–1280.
- [19] E. Bayerdorffer, N. Lehn, R. Hatz et al., Difference in expression of Helicobacter pylori gastritis in antrum and body, *Gastroentero.* **102** (1992), 1575–1582.

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