CHAPTER V

DISCUSSION

The imbalance between gastrotoxic agent such as acid, pepsin and protective mechanism such as mucus, gastric mucosal barrier and prostaglandin caused gastric mucosal injury, microcirculatory disturbances and ulceration.

The objective of this study was to study effects of *Aloe vera* on changes of gastric microcirculation, TNF-α, and IL-10 levels and gastric ulcer healing compared to sucralfate.

In this study, we found that the orogastric administration of 20% acetic acid resulted in gastric hemorrhage, congestion, edema of mucosa, inflammation, and gastric ulceration. Moreover, the 20% acetic acid also increased in leukocyte adherence, elevated TNF-α, and reduced IL-10 levels. Moreover, our findings also demonstrated that *Aloe vera* has similar effects to sucralfate. Both *Aloe vera* and sucralfate could reduce leukocyte adherence and TNF-α, but elevated IL-10 level, and promoted gastric ulcer healing.

I. The effect of 20% acetic acid induced gastric ulcer.

In this study, after the administration of 20% acetic acid the inflammation and gastric ulceration were produced. The 20% acetic acid caused the irritation on gastric mucosa resulted to erosive and ulcerative lesion. Accordingly, these effects of 20% acetic acid, therefore, could produce severe ulceration resemble to human chronic gastric ulcer

(Takagi et al., 1969). The pathogenesis mechanism of acetic acid induced gastric ulcer may be similar to NSAIDs induced gastric injury. The previous studies indicated that depletion of prostaglandin, neutrophil accumulation, impairment of mucosal blood flow and reduction of mucosal cell proliferation are all contributed to the pathogenic mechanisms of NSAIDs induced gastric injury (Scarpignato, 1995; Wallace, 1992).

However, in this study the control group was also found some of gastric erosions. Since, in our experimental protocol the control group was also gavaged by distilled water, therefore, it might cause some stress or trauma to the animal. Since the stress condition is able to produce the followings : *a) acute gastric mucosal lesion by complex psychological factors influencing individual vulnerability, b) stimulation of specific brain pathways regulating autonomic function, c) decreasing blood flow to the mucosa, increasing in muscular contractility, mast cell degranulation, leukocyte activation, d) and increase free radical generation resulting in increase lipid peroxidation* (Andrade et al., 2001; Overmier et al., 2000; Tuncel et al., 1998; Yelken et al., 1999)

In generally, the gastric ulcer can be spontaneous healing. The period of healing could be divided by 3 phases: the early lag phase which is initiated by replacement of necrotic tissue. Necrotic portion of the mucosa detach into the gastric lumen or it might be removed by scavenging macrophages. The early lag phase end when granulation tissue forms below the ulcer crater (0-3 days after ulceration). The rapid healing phase is a second phase of healing. This phase is characterized by formation of new microvessels (angiogenesis), migration of regenerated epithelial cells to re-epithelialize the ulcer cater and by intensive

epithelial cell proliferation in the ulcer margin (3-10 days after ulceration). Finally, the late lag phase is characterized by complete re-epithelialization of the ulcer crater, shrinkage of the granulation tissue in the ulcer bed, which is converted to fibrous tissue (10-20 days after ulceration) (Halter et al., 1995).

The results of our study on day 8 which is in the rapid healing phase, also demonstrated for epithelial cell proliferation as well (Figure 4.9).

II. Leukocyte-endothelial cell interaction in 20% acetic acid induced gastric ulcer.

In this study, after the administration of 20% acetic acid induced gastric ulcer and gastric inflammation resulted to increase leukocytes adherence on endothelial surface of postcapillary venules. Besides, it also produced gastric inflammation as characterized by the migration of macrophages and PMNs in the ulcer area. The migrated macrophages were then released proinflammatory cytokines such as TNF-α and interleukin-1β (IL-1β). As those proinflammatory cytokines will then promote the up-regulation of adhesion molecules expression on endothelial cells and leukocytes (Pober et al., 1986; Pohlman et al., 1986), therefore, caused leukocytes recruitment (Watanabe et al., 1997). Adhesion molecules on both endothelial cells and leukocytes involved rolling, adhesion, and transmigration of leukocytes in the gastric inflamed area. Huang et al (2001) suggested that the leukocytes adherence on gastric microcirculation and then transmigration into the gastric tissue were thought to be a major cause in the pathogenesis of gastric inflammation. Previous study suggested that the increment of PMNs may

play an important role in the pathogenesis of nonsteroidal anti-inflammatory drugs (NSAIDs) induced gastropathy (Morise et al., 1998). In the other hand, NSAIDs may enhance the expression of cell adhesion molecules on the surface of endothelial cells (Wallace et al., 1993). Adhesion molecules play an important role in the recruitment of leukocyte to sites of inflammation, leading to gastric mucosal injury (Wallace et al., 1991, 1993). Some studies also suggested that the leukocyte adhesion and/or aggregation occluded the microcirculation might result to ischemic mucosal injury (Andrews et al., 1994; Wallace et al., 1993). Recently, there was a report that the leukocytes infiltration in gastric mucosa caused tissue damage leading to the ulcerative lesion (Wada et al., 1996).

The leukotrienes (LTs) important in inflammation are a novel group of mediators derived from arachidonic acid following initial oxygenation by stimulated inflammatory cells such as PMNs and macrophages and hence may be constituent of inflammatory exudates. They have the potential to mediate inflammatory reaction through their ability to stimulate leukocyte accumulation, to increase vascular permeability, to cause changes in blood flow, and through their possible modulation of pain responses (Otterness et al., 1984). *In vivo* study found that the administration of Leukotriene B_4 (LTB_4) caused leukocyte adherence and then passage through vascular endothelium in hamster check pouch. Therefore, LTB_4 has the potential to be an important mediator or modulator of leukocyte recruitment within inflammatory exudates (Bray et al., 1981).

III. TNF-α and IL-10 levels in 20% acetic acid induced gastric ulcer.

In this study, we found that the administration of 20% acetic acid induced gastric ulcer caused the elevation of TNF-α levels at both experimental periods of day 1 and day 8. Besides, the findings also demonstrated for the cascade result of such elevated pro-inflammatory cytokines in inducing leukocyte-endothelial cell interaction. It was suggested that 20% acetic acid caused macrophages to be stimulated and released proinflammatory cytokines which was mentioned above for TNF-α. As then TNF-α was caused further result as to stimulate ICAM-1 expression on vascular endothelial cells. ICAM-1 is an adhesion molecule, which has a pivotal role in the development of the inflammatory reaction by increasing the leukocyte adhesion to endothelium and promoting the transendothelial migration of leukocytes to inflammatory sites (Konturek et al., 2000). Moreover, it was reported that TNF-α was also stimulated the expression of LFA-1 (CD11a/CD18) (Dustin et al., 1989), which it is adhesion molecule on leukocyte. And this might be the reason for our findings of increasing in both TNF-α and leukocytes-endothelium interaction in the inflammatory area.

However, we have found that after the administration of 20% acetic acid induced gastric ulcer resulted in reduced IL-10 level on day 1, but it could elevate spontaneously on day 8 as compared to the control groups (Table 4.7 and Figure 4.5). The explanation for this result of IL-10 might be resided on the fact that when the gastric mucosa was damaged by the application of acetic acid, therefore, T and B lymphoctyes in submucosal beneath the damaged area that typically produced basal level of IL-10 were damaged as well. From Figure 5.1 , we can see that the

location of macrophages was actually beyond the damage area, the application of acetic acid might not reach to, therefore, the macrophages were then survived. The survival macrophages were then able to be stimulated and released TNF-α in response to acetic acid injury. Therefore, our findings showed that TNF-α was synthesized more than IL-10, since on day 1.

When the inflammation was occurred as activated from TNF-α, the IL-10 was synthesized later on. The increment of IL-10 level caused to reduce gastric inflammation through its feedback inhibition of TNF-α production. Therefore, we concluded that the spontaneous elevation of IL-10 response in the chronic gastric inflammation. The elevation of IL-10 was then feedback to reduce gastric tissue inflamed simultaneously.

IV. The effect of *Aloe vera* on leukocyte adherence compare to sucralfate.

In this study, we found that *Aloe vera* and sucralfate treatments could reduce leukocyte adherence after the 20% acetic acid induced gastric ulcer. *Aloe vera* has been previously reported for its antiinflammatory activity which decreased carrageenan- induced edema and neutrophil migration in rats (Vazquez et al., 1996). In addition, *Aloe vera* has antiinflammatory effect by reducing leukocyte adhesion in burn wound rats (Somboonwong et al., 2000; Duansak et al., 2003). In the other hand, *Aloe vera* is able to inhibit prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) and thromboxane B_2 production in burn wound quinea pig (Robson et al., 1979). Thromboxanes and prostaglandins (PGs) have been shown to elicit platelet aggregation, leukocyte adherence and vasoconstriction result in

enhancing ischemia. Moreover, there was reported that *Aloe vera* possesses bradykininase activity and also decreases inflammation (Davis et al., 1989). Bradykinin causes the increase in vascular permeability and then stimulate inflammation (Qu et al., 1990). Therefore, we concluded that these effects of *Aloe vera* might reduce the causes of inflammatory process including the effects of *Aloe vera* on pro-inflammatory cytokines which will be discussed in the following, therefore, *Aloe vera* could reduce leukocyte adherence after the 20% acetic acid induced gastric ulcer.

V. The effects of *Aloe vera* on TNF-α and IL-10 levels compared to sucralfate.

In this study, we found that *Aloe vera* and sucralfate treatment could reduce TNF-α level both on day 1 and day 8 after gastric ulcer induction (Table 4.6 and Figure 4.4). *Aloe vera* has cytoprotective effect by stimulate endogenous prostaglandins production (Robert et al., 1979). Sucralfate is a cytoprotective drug which also stimulate PGs production. Prostaglandins (PGs), in particularly protaglandin E_2 (PGE_2) is protecting the gastric mucosal from various irritant, promote mucus production and increase mucosal blood flow (Hollander, 1994; Wallace et al., 1995; Linder et al., 2000). Previous study reported that, PGE_2 has a modulatory role on TNF-α production and is also a potent inhibitor of neutrophil adherence and chemotaxis (Watanabe et al., 1994). Ding et al. (1998) reported that PGE_2 inhibits TNF-α released in the gastric mucosa lead to a reduction in neutrophil activation and subsequently decreased ischemia and mucosal damage from the administration of indomethacin. And they also reported that inhibition of TNF-α by PGE_2 could result either directly

or indirectly in the reduction of neutrophil CD11b/CD18 and endothelial ICAM-1 expression, which subsequently reduce neutrophil adhesion on vascular wall.

Besides, after *Aloe vera* and sucralfate treatment could elevate IL-10 level both on day 1 and day 8 after gastric ulcer induction. IL-10 is antiinflammatory cytokine produced by various cell including monocytes/macrophages and T lymphocyte. IL-10 has inhibitory effect on cytokines synthesis by macrophages (David et al., 1991). In addition, the mild antiinflammation effects of IL-10 may be due to the suppression of TNF-α production (Karen et al., 1997). Bodger et al. (1997) has shown that the mucosal secretion of IL-10 and TNF-α were raised in severe chronic inflammation from *H. pylori* gastritis, which the release of IL-10 may be protective, limiting tissue damage caused by inflammation. Therefore, the elevation of IL-10 is down-regulated TNF-α production from macrophage. These reasons to confirm the effect of *Aloe vera* and sucralfate on the reduction of TNF-α level.

VI. The effect of *Aloe vera* on gastric ulcer healing compared to sucralfate.

In this study, *Aloe vera* and sucralfate treatment could reduce inflammation and promote gastric ulcer healing, which confirm by the histopathological examination. The histopathological examination found that *Aloe vera* and sucralfate treatment promoted epithelial cell proliferation, elongation and dilatation of oxyntic grand. *Aloe vera* and sucralfate have a cytoprotective effect by stimulate PGE_2 production. PGE_2 play an important role in the maintenance of mucosal integrity and

mucus production. The previous study has reported that, *Aloe vera* could promote burn wound healing in rats (Somboonwong et al., 2000; Duansak et al., 2003). *Aloe vera* can promote wound healing by fibroblast activation (Davis et al., 1994). In addition, the compound agent in *Aloe vera* is β-sitosterol could induce angiogenesis *in vivo* (Moon et al., 1999). The mechanism of angiogenesis play an important role in wound healing. The antithromboxaneB_2 effect of *Aloe vera,* resulted in reduced vasoconstriction and improved perfusion of gastric mucosal capillaries which promoted healing ulcer (Grindlay and Reynolds, 1986; Blitz et al., 1963; Barry, 1983).

Furthermore, gastric acid is considered as an important aggressive factor in the stomach and is known to produce gastric injury (Brzozowski et al., 2000). *Aloe vera* is able to decrease gastric acid secretion and increase in mucus secretion (Suvitayavat et al., 2004).

As the overall results of this study, we would like to conclude that:

1. The administration of 20% acetic acid induced gastric ulcer resulted in gastric ulceration and gastric inflammation by stimulated TNF-α production from macrophages and stimulated leukocyte adherence on vascular endothelial cells.

2. *Aloe vera* treatment could reduce gastric inflammation by reduced TNF-α production from macrophages and by reduced leukocyte adherence on vascular endothelial cells.

3. *Aloe vera* treatment could also reduce gastric inflammation by elevated IL-10 levels.

4. *Aloe vera* treatment could promote gastric ulcer healing by stimulating proliferation of epithelial cells and elongation and dilatation of oxyntic gland.

5. *Aloe vera* treatment has similar effect to sucralfate treatment on gastric ulcer therapy.

Hypothesis for the effect of *Aloe vera* on the gastric ulcer

As the overall results of this study, we would like to propose the possible mechanism of *Aloe vera* shown in Figure 5.1. The mechanism of *Aloe vera* could be explained by both action of antiinflammation and promotion ulcer healing. The results of this study have shown that *Aloe vera* could decrease leukocyte adherence at inflammatory tissue and reduced TNF-α levels. Our findings originally demonstrated that *Aloe vera* could act as an antiinflammatory agent for gastric ulcer by using intravital fluorescent microscopic evidence based.

Furthermore, the results of this *Aloe vera's* effect was also confirmed by the histological examination. As which *Aloe vera* could promote ulcer healing by promoting reepithelialization, elongation and dilatation of oxyntic gland. Our findings also defined that ulcer healing effect of *Aloe vera* actually mediated through its property on increasing IL-10, an important cytokines for wound healing process.

As an overall conclusion, we have made the hypothesis that since *Aloe vera* is composed of a combination of active components, therefore, the action of both antiinflammation and ulcer healing could be observed in this study.

In the future, *Aloe vera* might be a great therapeutic agent used for gastric ulcer patients in the future.

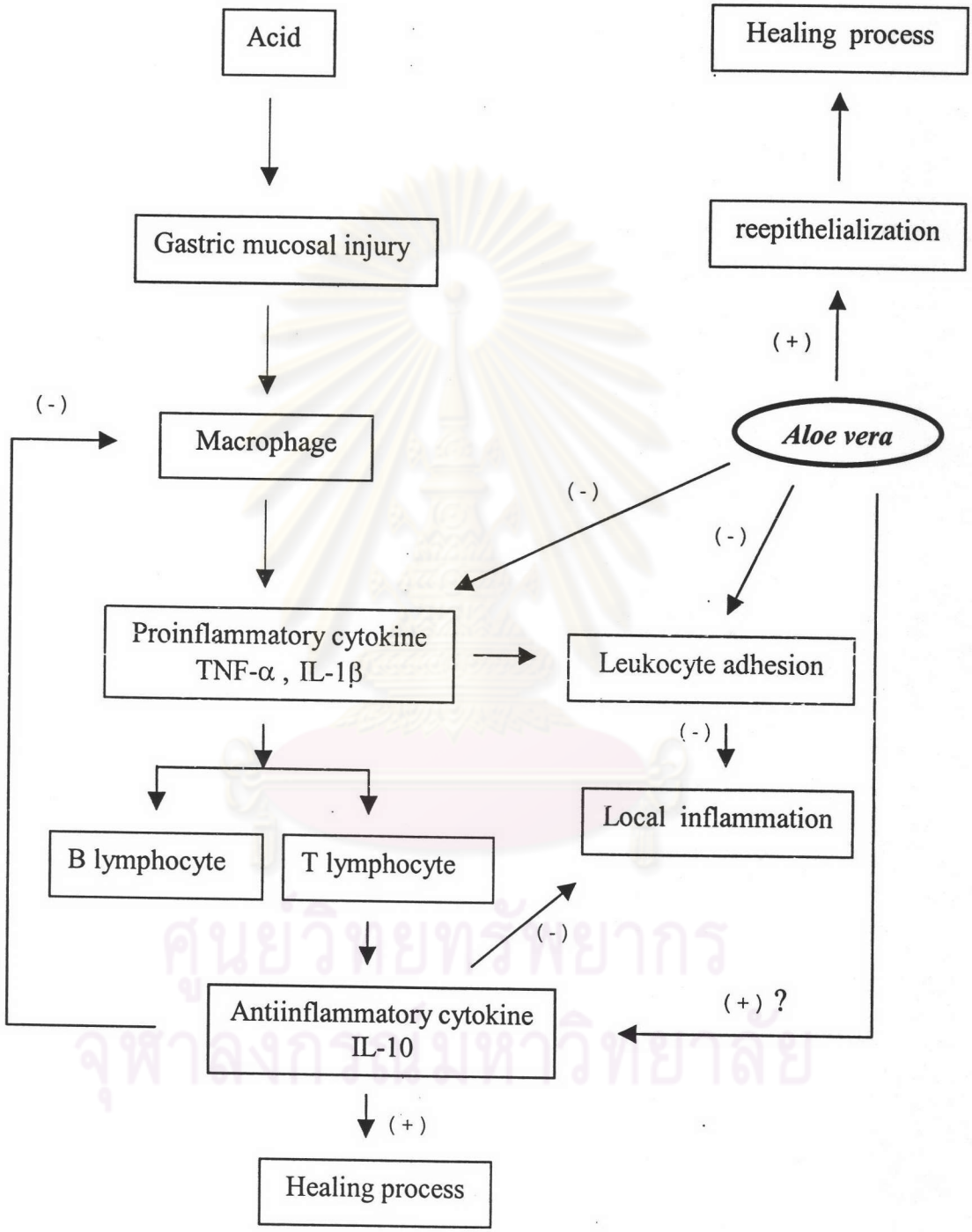

Figure 5.1 The purposed mechanism of *Aloe vera* as antiinflammation and gastric ulcer healing.