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Aloe vera Improved Gastric Injury on Nonsteroidal Anti-Inflammatory Drugs-Induced Gastropathy in Rats

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ABSTRACT

Background: Non-steroidal anti-inflammatory drugs (NSAIDs) are one of the most commonly prescribed worldwide and known to induce gastric injury from multiple mechanisms. *Aloe vera* is known to effectively decrease inflammation and promote ulcer healing but there are still limited data.

Objective: To investigate the effects of *Aloe vera* on NSAIDs-induced gastropathy and its effects on serum TNF-α, IL-1β, CINC-1, and tissue MDA in rats.

Materials and Methods: Male Wistar rats were randomly divided into three groups (n=6, each). Group 1 (control group): was fed with sterile water. Group 2 (NSAIDs group): was fed with 150 mg/kg indomethacin dissolved in 5% sodium bicarbonate at 0 h and 4 h. Group 3 (*Aloe vera*-treated group): was fed with 150 mg/kg indomethacin as previously described and 200 mg/kg *Aloe vera* at 0 h and 4 h. The stomach was removed to study gastric histopathology at 8 h after experiment. Serum and gastric tissue were collected to determine TNF-α and CINC-1 using ELISA technique and gastric MDA using TBARS assay kit.

Results: In NSAIDs group, serum TNF-α, CINC-1and gastric MDA were significantly increased when compared to control group (27.8±1.5 vs. 85.1±49.1 pg/mL, 104.5±45.8 vs. 1054.7 ± 20.4 pg/mL and 1.7±0.2 vs. 9.4±1.1 nmol/mg protein, $p<0.05$, respectively). The mean level of TNF-α, CINC-1and gastric MDA in *Aloe vera*-treated group were improved as compared with NSAIDs group (85.1±49.1 vs. 35.2±1.6 pg/mL, 1054.7 ± 20.4 vs. 813.6±239 pg/mL and 9.4±1.1 vs. 2.7±0.6 nmol/mg protein, $p < 0.05$, respectively). Five rats in NSAIDs group developed moderate to severe gastric inflammation and erosions. The gastric erosions and neutrophil infiltration scores were significantly reduced in Aloe vera treated group.

Conclusion: *Aloe vera* attenuated NSAIDs induced gastropathy in rats by the reduction of oxidative stress, inflammatory cytokines, and improvement of gastric injury.

Key words : NSAIDs, *Aloe vera,* gastropathy, Inflammatory cytokines

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INTRODUCTION

NSAIDs-induced gastropathy is a common medical problem in clinical practice. NSAIDs including aspirin are used by approximately 11% of the U.S. population on a regular basis[1]. NSAIDs are the second most important risk factor for peptic ulcer disease following *H.pylori* infection. The use of these medications are likely to increase with population ages, therefore it is more likely to develop the serious ulcer-related complications. In a study from Thailand, the rate of hospital admission due to peptic ulcer disease was 111-112/100,000[2].

The NSAIDs-induced gastropathy has various pathogenesis mechanisms, including directed inhibition of the prostaglandins, directed toxic effect of NSAIDs and indirect effects through stimulation of proinflammatory cytokines such as TNF-α, IL-1β, IL-6, IL-8, IFN-γ and inflammatory cells infiltration of lamina propria which lead to a reduction in mucosal blood flow, hypoxia, and a reduction in mucosal defense[3-6]. *Aloe vera* plant (*Aloe vera barbadensisis*) has long been widely used for the treatment of various diseases such as burn and skin ulcer[7]. Aloe leaf exudates also possess anti-diabetic and cardiac stimulatory activity[8]. It is known to promote wound healing and effectively decrease tissue inflammation.

There are several studies that demonstrate the effect of *Aloe vera* on gastric ulcer protection. It can protect gastric mucosa by its anti-inflammatory[9], cytoprotective, healing and mucus stimulatory effects[10].

A study of Eamlamnam K and *et al.*[10] found that *Aloe vera* treatment can reduce leukocyte adherence and TNF-α level, elevate IL-10 level and promote gastric ulcer healing in 20% acetic acid induced gastric ulcer.

A mechanism of action of *Aloe vera* is directed toward the pathogenesis of NSAIDs induced gastropathy. Therefore, *Aloe vera* is an interesting option for the treatment of this disease.

To date, there was no experimental data to prove its beneficial effects on the inflammatory cytokine change.

We conducted the randomized control experimental study to determine the effects of *Aloe vera* on NSAIDs-induced gastropathy in rats by evaluation of the histopathological improvement and the level of TNF-α, CINC-1 and tissue MDA.

MATERIAL AND METHODS

Material

Male Wistar rats, weighing 180-220 gm, were obtained from the National Laboratory Animal Center, Mahidol University (Bangkok, Thailand). Rats were housed in a controlled temperature room at 25±1°C under standard conditions (12-h dark-light cycle). The experimental protocol was approved by the Ethical Committee of Faculty of Medicine, Chulalongkorn University, Thailand.

Indomethacin and *Aloe vera* extract preparation

A single dose of 150 mg/kg of indomethacin was dissolved in 5% sodium bicarbonate that was freshly prepared for the experiment. A single dose of 200 mg/kg of *Aloe vera* extract (Namsiang International, Bangkok, Thailand) were dissolved in distilled water that was freshly prepared for the experiment.

Experimental protocol

All rats were fasted with free access to water ad libitum for 24 hours before the experiment. They were randomly assigned into 3 groups.

Group 1 (control group, n=6); rats were fed with distilled water 1 mL/rat via an intragastric tube.

Group 2 (NSAIDs group, n=6) served as gastropathy group without *Aloe vera* treatment. Rats were fed with indomethacin (150 mg/kg body weight dissolved in 5% sodium bicarbonate 1 mL) via an intragastric tube at 0 h and 4 h.

Group 3 (*Aloe vera*-treated group, n=6) served as gastropathy group with *Aloe vera* treatment. Rats were fed with indomethacin (150 mg/kg body weight dissolved in 5% sodium bicarbonate 1 mL) plus *Aloe vera* extract (200 mg/kg body weight dissolved in distilled water 1 mL) via an intragastric tube at 0 h and 4 h.

8 hours after the experimental process, all rats were anesthetized with intraperitoneal injection of thiopental (60 mg/kg, Jagsonpal Pharmaceuticals Ltd Haryana, India). The stomach was removed and dissected along the greater curvature and washed twice with ice-cold phosphate-buffered saline, frozen in liquid nitrogen, and stored at -80°C to examine tissue malondialdehyde (MDA) using thiobarbituric acid reactive substances (TBARS) assay kit (Cayman, USA) and was expressed as nanomole per milligram (nmol/mg) protein[11]. The remaining stomach was cut into multiple 5-micron-thicked sections, fixed in 10% formalin solution, which were later stained with hema-

toxylin and eosin (H&E) for histopathologic examination. Then, the blood sample was collected by cardiac puncture. The blood was allowed to clot at room temperature for 2 hours before centrifuged at 3000 rpm. for 20 minutes and serum was separated and stored at -80˚C to measure the TNF-α and CINC-1 levels using sandwich enzyme linked immunosorbent assay (ELISA) technique (ELISA kit R&D systems, USA) and was expressed as picogram per milliliter (pg/mL).

Histopathology

Tissue samples of stomach were excised and fixed with 10% formalin then were processed by routine technique before paraffin embedding. Sections were cut at 5 μm thickness and stained with Hematoxylin and Eosin (H&E). One experienced gastrointestinal pathologist examined all blinded samples by using light microscope with magnification × 20. All histopathological findings were recorded and graded by using gastric erosion and polymorphonuclear leukocyte infiltration score as follows, score 0: no erosion, score1: erosion 1/3 of epithelium depth, score2: erosion 2/3 of epithelium depth, or develop ulcer. For PMN infiltration score, score0: no infiltration, score1: PMN infiltrate 1/3 of epithelium, score2: PMN infiltrate 2/3 of epithelium, score3: PMN infiltrate all depth of epithelium which represent normal, mild, moderate and severe gastric mucosal injury respectively.

Statistical analysis

All continuous data were presented as mean and standard deviation (SD). One-way analysis of variance (one-way ANOVA) was used to compare outcome among three experimental groups and Tukey PostHoc comparison was employed for post hoc analysis. All statistical tests were performed by using SPSS for windows version 17 (SPSS Inc., Chicago, IL, United States). *P* value at less than 0.05 was considered statistically significant.

RESULTS

Histological changes

The stomach histology was normal in the control group. In the NSAIDs group, almost all of the stomach tissues show some degree of mucosal injury from mild to moderate severity (Figure 1). Five rats developed gastric erosion (score 2) and one rat developed 0.5 cm gastric ulcer. Polymorphonuclear infiltration score in the NSAIDs group were score 1 (number = 4) and score 0 (number = 2). In the *Aloe vera*-treated group, stomach histopathology was improved when compared to the NSAIDs group, especially in the reduction of polymorphonuclear leukocyte infiltration (score 1: n=2; score 0: n=4). The severity of gastric erosion was reduced from score 2 to score 1. Additionally, no gastric ulcer was found as shown in Table 1.

Changes of TNF-α level

The mean serum TNF-α level in the NSAIDs group was significantly higher than in the control group (27.8±1.5 vs. 85.1±49.1 pg/mL, p=0.01). In *Aloe vera*-treated group, the serum TNF-α was significantly reduced when compare to the NSAIDs group (35.2±1.6 vs. 85.1±49.1 pg/mL, p=0.025). The averages of serum TNF-α level of all groups were shown in Figure 2A.

Changes of CINC-1level

The average concentrations of serum CINC-1were 104.5±45.8 pg/mL, 1054.7±20.4 pg/mL, and 813.6±239 pg/mL in the control group, the NSAIDs group, and the *Aloe vera*-treated group respectively. In the Aloe vera-treated group, there was a significant decrease of serum CINC-1 level compared with NSAIDs group (p = 0.03). The averages of serum TNF-α level of all groups were shown in Figure 2B.

Changes of gastric MDA level

The level of gastric MDA was significantly higher in the NSAIDs group compared with the control group (9.4±1.1 vs. 1.7±0.2 nmol/mg protein, p<0.05). In the Aloe vera-treated group, there was a significant decrease in the elevated gastric MDA level compared with NSAIDs group (2.7±0.6 nmol/mg protein, p<0.001). The averages of serum TNF-α level of all groups were shown in Figure 2C.

DISCUSSION

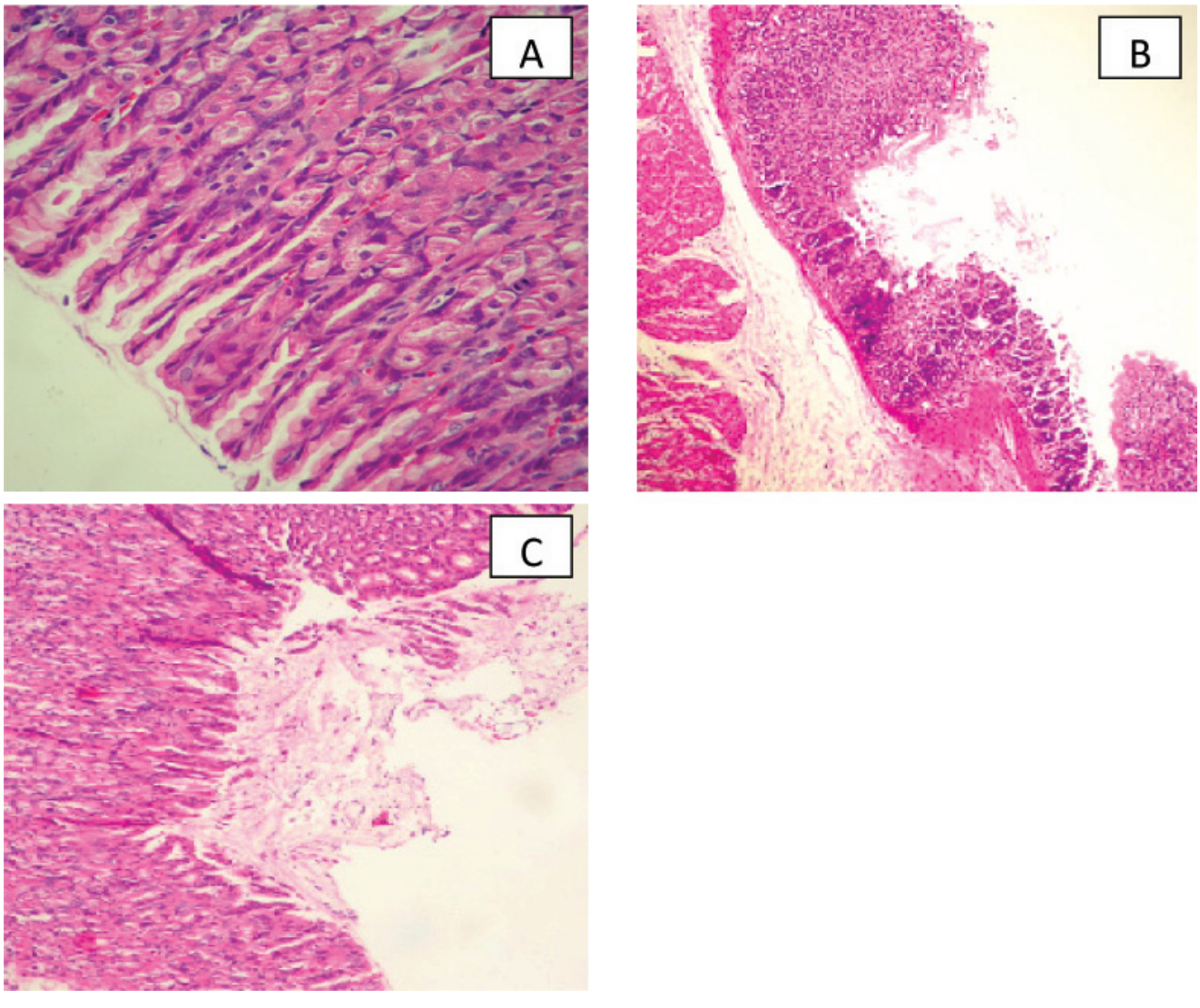
In this study, after administration of 150 mg/kg of indomethacin, erosions and ulcer were developed as well as increasing in polymorphonuclear infiltration around the gastric lesions. With *Aloe vera* treatment, stomach histopathology was improved compared with the NSAIDs group especially in the reduction of polymorphonuclear leukocyte infiltration score and reduction in gastric erosion severity score. Serum TNF-α level, serum CINC-1 level and gastric tissue MDA, which is a proinflammatory cytokine, a neutrophil chemoattractant and a metabolite of intracellular lipid peroxidation reaction respectively, level in the NSAIDs

Table 1. Summary of the gastric erosion and polymorphonuclear leukocyte infiltration score (n=6, each group).

Samples	Gastric erosion[a]			Ulcer	PMN infiltration[b]			
	0	1	2		0	1	2	3
Control	5	1	0		6	0	0	0
NSAIDs group	0	0	5	1	2	4	0	0
Aloe vera-treated group	1	4	1		4	2	0	0

[a] The gastric erosion score: score 0: no erosion, score 1: erosion 1/3 of epithelium depth, score 2: erosion 2/3 of epithelium depth, or develop ulcer.

[b] The gastric inflammation level was estimated and scored by the pathologist following the updated Sydney System.[12] PMN infiltration score: score 0: no infiltration, score 1: PMN infiltrate 1/3 of epithelium, score 2: PMN infiltrate 2/3 of epithelium, score 3: PMN infiltrate all depth of epithelium represented normal, mild, moderate, and marked gastric mucosal injury respectively.



A = The control group, stomach histology was normal.

B = The NSAIDs group developed 0.5 cm gastric ulcer.

C = The *Aloe vera*-treated group, stomach histopathology was improved.

Figure 1. Demonstrated the stomach histology in all group.

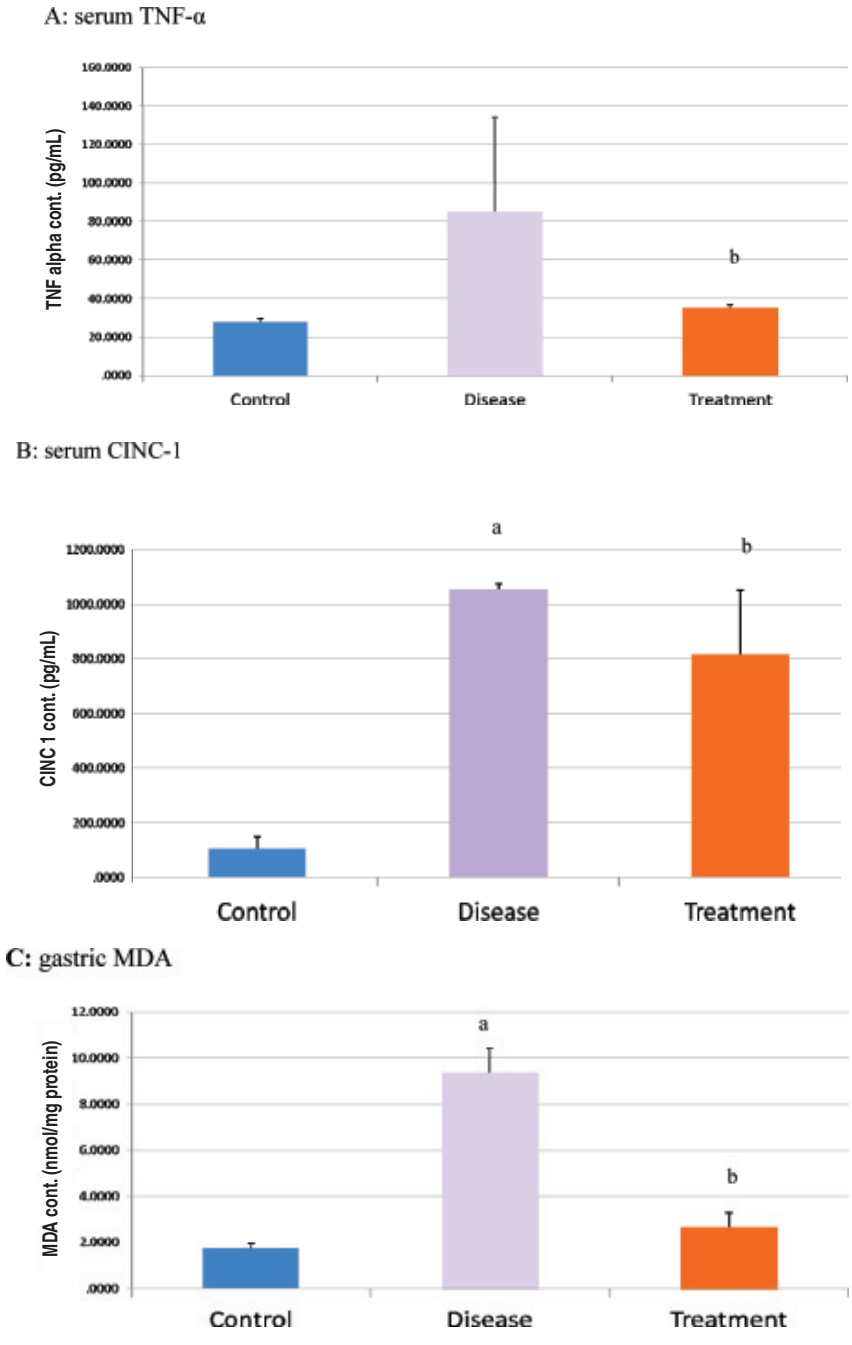


Figure 2. The effects of *Aloe vera* extract on serum TNF-α, CINC-1 and gastric MDA in Nonsteroidal anti-inflammatory drugs induced-gastropathy in rats. A: serum TNF-α level; B: serum CINC-1 level; C: gastric MDA. [a] $p < 0.05$ compare with control group and [b] $p < 0.05$ compare with NSAIDs group.

group were significantly higher than the control group and were significantly reduced in the *Aloe vera*-treated group.

TNF-α, the major proinflammatory cytokine released from the migrated macrophages, plays an important role in pathogenesis of gastric ulcer through stimulation of ICAM-1 expression on vascular endothelial cells which increasing leukocytes adhesion to the endothelial surface of post-capillary venules and promoting transendothelial migration of leukocytes to inflammatory sites[13]. TNF-α also increases intracellular oxidative stress and up-regulation of CINC-1 mRNA and protein in rat gastric epithelial cells[14]. *Aloe vera* treatment significantly reduced TNF-α level, in concordance with the previous studies, resulting in decrease of leukocyte adherence and promote gastric

ulcer healing[10,15].

The significantly higher level of serum CINC-1 in the NSAIDs group compared with the control group demonstrates the pathophysiologic mechanism of CINC-1 as a functional chemoattractant for neutrophils into the gastric ulcer area leading to a decrease in blood flow by their adhesion to microvessels and results in the production of reactive oxygen species, MPO, and proteases, which exert a noxious effect on the gastric mucosa. The CINC-1 level gradually decreases as the ulcers healed[6]. With *Aloe vera* treatment, there was significant reduction in serum CINC-1 compared with the NSAIDs group which may responsible for the reduced polymorphonuclear infiltration score.

Gastric tissue MDA, which is the main metabolite in the intracellular lipid peroxidation reaction, level represents the extent of the reactive oxygen species injuring the membrane structures of the cells which was reported to play a major role in pathomechanism of indomethacin-induced gastric lesions[17] and also play a key role in the mechanism of the gastric carcinogenesis[18]. *Aloe vera* treatment significant reduced the gastric MDA level compared with the NSAIDs group which may also reflect the reduced amount of the reactive oxygen species-mediated gastric tissues injury.

In conclusion, *Aloe vera* attenuated NSAIDs induced gastropathy in rats by the reduction of oxidative stress, inflammatory cytokines, and improvement of gastric injury.

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REFERENCES

1. Kaufman DW, Kelly JP, Rosenberg L, *et al*. Recent patterns of medication use in the ambulatory adult population of the United States: the Slone survey. JAMA : the journal of the American Medical Association 2002;287:337-44.
2. Wilairatana S, Kladchareon N, Israsena S, *et al*. Epidemiology of peptic ulcer disease in Thailand. Gastroen-terologia Japonica 1991;26 Suppl 3:265-6.
3. Lehmann FS, Stalder GA. Hypotheses on the role of cytokines in peptic ulcer disease. Eur J Clin Invest 1998;28:511-9.
4. Tytgat GN. Etiopathogenetic principles and peptic ulcer disease classification. Dig Dis 2011;29:454-8.
5. Brzozowski T, Konturek PC, Konturek SJ, *et al*. Role of prostaglandins in gastroprotection and gastric adaptation. J Physiol Pharmacol: an official journal of the Polish Physiological Society 2005;56 Suppl 5:33-55.
6. Tanaka A, Araki H, Komoike Y, *et al*. Inhibition of both COX-1 and COX-2 is required for development of gastric damage in response to nonsteroidal antiinflammatory drugs. J Physiol, Paris 2001;95:21-7.
7. Shelton RM. Aloe vera. Its chemical and therapeutic properties. International journal of dermatology 1991;30:679-83.
8. Ghannam N, Kingston M, Al-Meshaal IA, *et al*. The antidiabetic activity of aloes: preliminary clinical and experimental observations. Hormone Research 1986;24:288-94.
9. Park CH, Nam DY, Son HU, *et al.* Polymer fraction of Aloe vera exhibits a protective activity on ethanol-induced gastric lesions. Intern J Molec Med 2011; 27:511-8.
10. Eamlamnam K, Patumraj S, Visedopas N, *et al*. Effects of Aloe vera and sucralfate on gastric microcirculatory changes, cytokine levels and gastric ulcer healing in rats. World J Gastroenterol: WJG 2006;12:2034-9.
11. Ohkawa H, Ohishi N, Yagi K. Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. Analytical Biochemistry 1979;95:351-8.
12. Dixon MF, Genta RM, Yardley JH, *et al*. Classification and grading of gastritis. The updated Sydney System. International Workshop on the Histopathology of Gastritis, Houston 1994. Am J Surg Pathol 1996;20:1161-81.
13. Konturek PC, Duda A, Brzozowski T, *et al.* Activation of genes for superoxide dismutase, interleukin-1beta, tumor necrosis factor-alpha, and intercellular adhesion molecule-1 during healing of ischemia-reperfusion-induced gastric injury. Scand J Gastroenterol 2000;35:452-63.
14. Handa O, Naito Y, Takagi T, *et al.* Tumor necrosis factor-alpha-induced cytokine-induced neutrophil chemoattractant-1 (CINC-1) production by rat gastric epithelial cells: role of reactive oxygen species and nuclear factor-kappa B. J Pharmacol Experiment Ther 2004;309:670-6.
15. Duansak D, Somboonwong J, Patumraj S. Effects of Aloe vera on leukocyte adhesion and TNF-alpha and IL-6 levels in burn wounded rats. Clin Hemorheol Microcirc 2003;29:239-46.
16. Yamada H, Takahashi S, Fujita H, *et al*. Cytokine-induced neutrophil chemoattractants in healing of gastric ulcers in rats: expression of >40-kDa chemoattractant in delayed ulcer healing by indomethacin. Dig Dis Sci 1999; 44:889-95.
17. Kwiecien S, Brzozowski T, Konturek P, *et al*. The role of reactive oxygen species in action of nitric oxide-donors on stress-induced gastric mucosal lesions. J Physiol Pharmacol: an official journal of the Polish Physiological Society 2002;53:761-73.
18. Wang SH, Wang YZ, Zhang KY, *et al*. Effect of superoxide dismutase and malondialdehyde metabolic changes on carcinogenesis of gastric carcinoma. World J Gastroenterol: WJG 2005;11:4305-10.