

Curcumin Prevents Indomethacin-induced Acute Gastric Mucosal Damage in Rats

Murathanun R¹
Thong-Ngam D¹
Klaikaew N²

ABSTRACT

Background: Curcumin, a major constituent of tumeric powder, is renowned for its anti-inflammatory effect but the studies of its effects on nonsteroidal anti-inflammatory drugs (NSAIDs)-induced gastropathy are still limited.

Aims: To investigate the gastroprotective effect of curcumin on NSAIDs-induced acute mucosal injury and its influence on plasma tumor necrosis factor (TNF)- α level.

Methods: Male Sprague-Dawley rats, weighing 200-250 g, were randomly divided into 4 experimental groups of five rats. Group 1 (Control): Sterile water was given. Group 2: Indomethacin (100 mg/kg) was given. Group 3: Indomethacin (100 mg/kg) and curcumin (60 mg/kg) were given. Group 4: Indomethacin (100 mg/kg) and curcumin (200 mg/kg) were given. In groups 3 and 4, indomethacin was given at 30 minutes after curcumin administration. Four hours after sterile water or indomethacin administration, blood samples for the TNF assay were withdrawn. The stomach was then removed to study gastric histopathology. Serum level of TNF- α was determined using ELISA method.

Results: Pretreatment with curcumin (60mg/kg) at 30 minutes before administration of indomethacin resulted in a decrease in mean gastric erosion score ($p < 0.05$). In addition, there was a statistically significant difference on gastric erosion score between group 2 and group 4 ($p < 0.001$). However, there was no significant difference on TNF- α level among the four groups.

Conclusion: Curcumin accomplishes the protective effect on NSAIDs-induced gastric mucosal injury in a dose dependent manner without affecting serum TNF- α level.

Key words : NSAIDs, Curcumin, TNF- α

[*Thai J Gastroenterol* 2008; 9(3): 118-123.]

¹Department of Physiology, ²Department of Pathology,

Faculty of Medicine, Chulalongkorn University, Bangkok 10330, Thailand.

Address for Correspondence: Duangporn Thong-Ngam, M.D. Department of Physiology, Faculty of Medicine, Chulalongkorn University, Bangkok 10330, Thailand. E-mail: thongngam007@yahoo.com

INTRODUCTION

Although nonsteroidal anti-inflammatory drugs (NSAIDs) have numerous therapeutic and prophylactic roles, there are some adverse effects. A major limitation to the use of NSAIDs is the development of gastric mucosal injury. Many mechanisms have been proposed to describe the ulcerogenic action of NSAIDs. Previous studies have suggested that depletion of prostaglandin, neutrophil accumulation, impairment of mucosal blood flow and reduction of mucosal cell proliferation contribute to the pathogenic mechanisms.^(1,2)

There is evidence implicating that NSAIDs-induced gastric ulceration is a neutrophil-dependent process. NSAID administration to rats caused a rapid and significant increase in adhesion between neutrophils and vascular endothelial cells in both gastric and mesenteric venules.⁽³⁻⁶⁾ Indeed, using monoclonal antibodies that blocked NSAID-induced neutrophil adherence to vascular endothelium showed significantly reduced the NSAID-induced gastric mucosal injury.⁽⁶⁻⁸⁾ It is considered that an important signal for NSAID-induced neutrophil adherence within gastric microcirculation is tumor necrosis factor- α (TNF- α).⁽⁹⁾ Previous investigations have shown that indomethacin administration increases plasma TNF- α levels in both humans and animals (9,10) and that, in indomethacin-treated rats, there is a correlation between the degree of gastric damage, polymononuclear cell margination, and TNF- α release.⁽⁹⁾ Ding *et al.* demonstrated in rats that a reduction of prostaglandin E₂ after indomethacin administration was followed by an increase in TNF- α level and gastric mucosal neutrophil infiltration.⁽¹¹⁾ Prostaglandins are well recognized of their inhibitory effects on the release of TNF- α from macrophages and mast cells^(12,13), and TNF- α is a well-characterized stimulus for expression of adhesion molecules.⁽¹⁴⁾ Although there is definitely no information regarding the regulation of expression of endothelial adhesion molecules in experimental NSAIDs-induced gastric mucosal injury, nuclear factor- κ B (NF- κ B) may play an important role as a potential signal. Activation of NF- κ B requires proteasome-mediated proteolysis of I κ B that is complexed with NF- κ B in the cytoplasm, preventing translocation of NF- κ B into the nucleus⁽¹⁵⁾. Brand and colleagues have shown that proteasome inhibitors (MG341, lactocystin) inhibited TNF- α induced NF- κ B activation in human endothelial cells in vivo and significantly reduced indomethacin-induced gastric mucosal injury as well as gastric mucosal intercel-

lular adhesion molecule-1 (ICAM-1) expression in rat⁽¹⁶⁾.

Curcumin or diferuoylmethane is the major constituent of tumeric powder extracted from the rhizomes of the plant *Curcuma longa* L, found in southeast and south asia. It possesses a broad range of pharmacological activities including antioxidant⁽¹⁷⁾, anti-carcinogenic⁽¹⁸⁾, and anti-inflammatory^(19,20) effects. There are currently limited studies investigating the effect of curcumin on NSAIDs-induced gastric ulcer. A recent in vitro study demonstrated that curcumin blocked the TNF-mediated attachment of monocytes to endothelial cells by inhibiting the expression of the adhesion molecules ICAM-1, vascular cell adhesion molecule-1 (VCAM-1), and endothelial cell leukocyte adhesion molecule (ELAM-1 or also called E-selectin) on endothelial cell surface.⁽²¹⁾ This effect of curcumin might reduce the severity of NSAIDs-induced gastric mucosal damage. However, whether curcumin decreases serum TNF- α level in NSAIDs-induced gastric mucosal injury is still undefined.

In the present study, we examined the effects of curcumin on the serum TNF- α level and severity of indomethacin-induced gastric mucosal injury in rats.

MATERIALS AND METHODS

Materials

Male Sprague-Dawley rats, weighing 200-250 g, were obtained from the National Laboratory Animal Center, Mahidol University (Bangkok, Thailand). Indomethacin and curcumin were purchased from Cayman Chemical (Ann Arbor, MI, USA). The experimental protocol was approved by the Ethical Committee of Faculty of Medicine, Chulalongkorn University, Thailand. All rats were kept in 12-h light/dark cycles and housed at 25 °C room temperature.

Experimental Protocols

The animals were fasted, with free access to water ad libitum, for 22-24 hours before the experiment in wire-bottomed cages with cellulose bedding removed. Indomethacin was dissolved in sterile water while curcumin was dissolved in dimethylsulphoxide (DMSO). The total of animals were randomly divided into 4 experimental groups.

Group 1 (Control) : Sterile water was given via orogastric tube.

Group 2 (Indomethacin) : Indomethacin (100 mg/

kg) was given via orogastric tube.

Group 3 (Low dose curcumin + Indomethacin) : Indomethacin (100 mg/kg) and curcumin (60 mg/kg) were given via orogastric tube.

Group 4 (High dose curcumin + Indomethacin) : Indomethacin (100 mg/kg) and curcumin (200 mg/kg) were given via orogastric tube.

The animals in groups 3 and 4 received indomethacin at 30 minutes after curcumin administration. Four hours after sterile water administration in the first group and indomethacin administration in the other three groups, the rats were anesthetized with pentobarbital sodium (50 mg/kg body weight) by intraperitoneal injection. Blood sample for the TNF assay was withdrawn directly from the heart. The stomach was dissected and removed. The rats were then killed by the excess of intraperitoneal pentobarbital sodium administration.

TNF Assay

The blood samples were centrifuged at 2,500 g for 10 minutes at 4 °C. Serum samples for TNF assay were stored at -70 °C until analysis. Serum levels of TNF- α were determined using an ELISA kit for rat TNF- α (R&D Systems, Inc; Mineapolis, MN, USA)

Histological Examination

The stomach was cut along the greater curvature. The mucosal surface was exposed, rinsed with 0.9% NaCl and immersed in 10% formalin for 24 h. The stomach tissue was processed by a standard method, initially embedded in paraffin, sectioned, stained with hematoxylin-eosin (H&E) and then examined under magnification with a light microscope. Lesions were scored by an experienced pathologist unaware of the treatment received using a system described previ-

ously⁽¹⁶⁾ that takes into consideration both the number and size of the lesions.

Gastric lesions were scored according to the following system: 0 = no lesion; 1 = <5 lesions, all <2 mm; 2 = <5 lesions, at least one lesion >2 mm; 3 = 5-10 lesions, all <2 mm; 4 = 5-10 lesions, at least one lesion >2 mm; 5 = >10 lesions, all <2 mm; 6 = >10 lesions, at least one lesion >2 mm.

Statistical Analysis

All data are expressed as means \pm SE. The statistical significance of differences was determined by student's t-test. Statistical significance is based on probability of <5%.

RESULTS

Histological examination showed the reduced extent of gastric erosion in the rats received low dose and high dose curcumin compared to the control group. The summarized scores of gastric erosion were presented in Table 1. There was no gastric mucosal injury in the rats received sterile water. Most rats in indomethacin group experienced the gastric mucosal erosion with the score 2 out of 6. Three rats in the group of curcumin (60 mg/kg) had no erosion in their stomach, whereas five rats in the group received higher dose of curcumin (200 mg/kg) did not develop any gastric erosion.

Mean gastric erosion scores were demonstrated in Figure 1. Pretreatment with curcumin (60 mg/kg) 30 minutes before administration of indomethacin resulted in a decrease in mean gastric erosion score ($p < 0.05$). In addition, there was a statistically significant difference between the indomethacin and high dose curcumin groups ($p < 0.001$).

Table 1. Summarized scores of gastric erosion.

Experimental group	Erosion score*						
	0	1	2	3	4	5	6
Sterile water	6	-	-	-	-	-	-
Indomethacin (100 mg/kg)	-	-	4	1	1	-	-
Curcumin (60 mg/kg) and Indomethacin (100 mg/kg)	3	2	1	-	-	-	-
Curcumin (200 mg/kg) and Indomethacin (100 mg/kg)	5	1	-	-	-	-	-

Values are number of animals.

*Scoring system: 0, no lesion; 1: <5 lesions, all <2 mm; 2: <5 lesions, at least one lesion >2 mm; 3: 5-10 lesions, all <2 mm; 4: 5-10 lesions, at least one lesion >2 mm; 5: >10 lesions, all <2 mm; 6: >10 lesions, at least one lesion >2 mm.

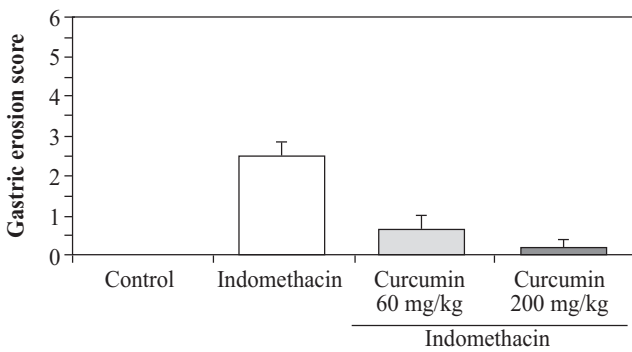


Figure 1. Mean erosion score \pm SE; n = 6 for each group.
 * Significant difference from the indomethacin group ($p < 0.05$).
 ** Significant difference from the indomethacin group ($p < 0.001$).

Figure 2 illustrated the histological findings of stomach from each group. Normal gastric histological finding was characterized in the control group. In contrast, marked edema with congestion of all layers, dispersed lymphocytic and plasmacytic infiltration, scattered hemorrhagic spots and focal erosion with a depth of 0.2-0.7 cm were indicated in the stomach of rats given indomethacin. In low dose curcumin group, its findings showed submucosal edema, sparse lymphocytic and plasmacytic infiltration with superficial focal erosion of 0.1 cm. Whereas merely submucosal edema, sparse plasma cells and lymphocytic infiltration were demonstrated in the group of high dose of curcumin.

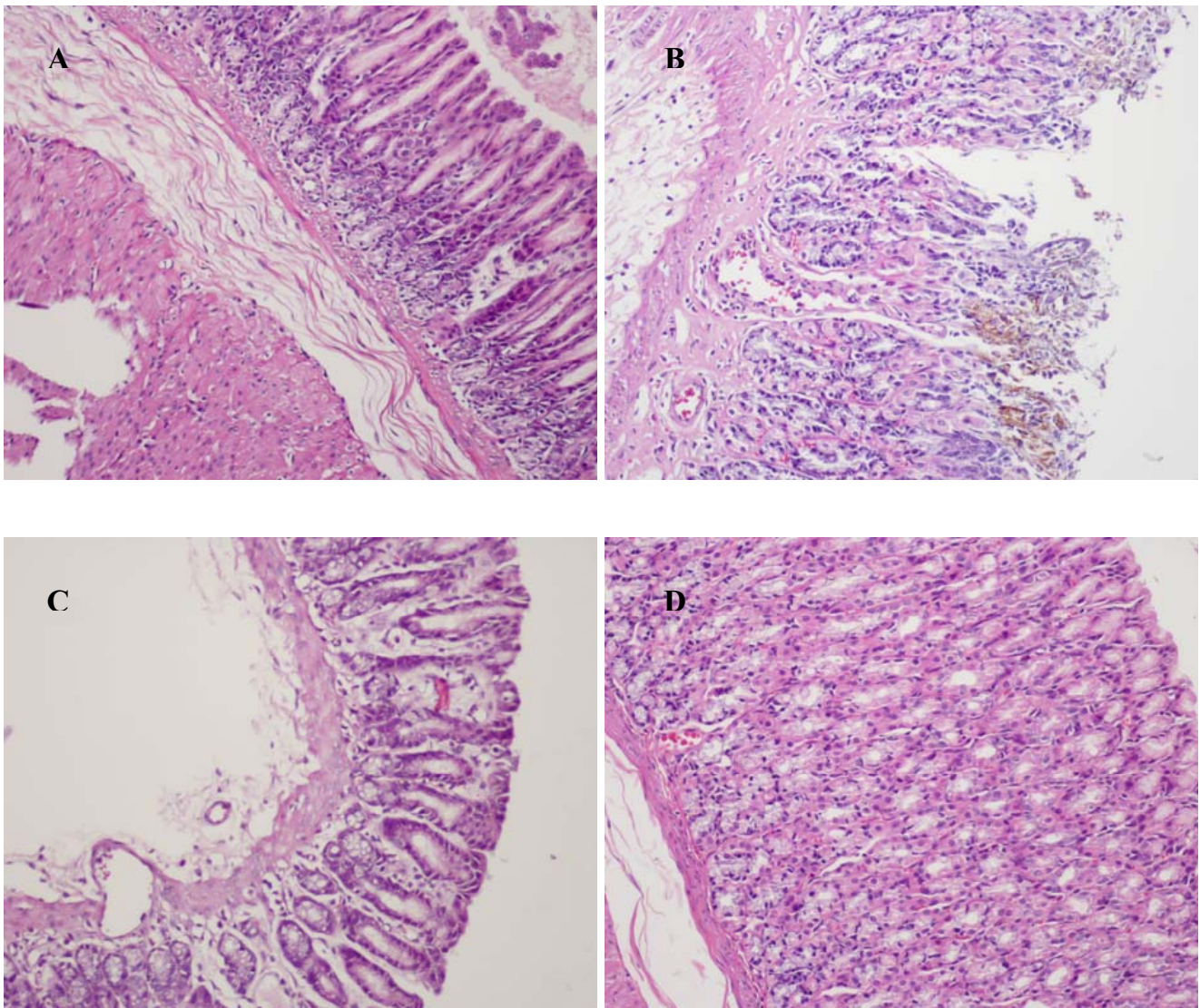


Figure 2. Histopathological findings of the stomach. A: Sterile water; B: Indomethacin 100 mg/kg; C: Curcumin 60 mg/kg and indomethacin 100 mg/kg; D: Curcumin 200 mg/kg and indomethacin 100 mg/kg

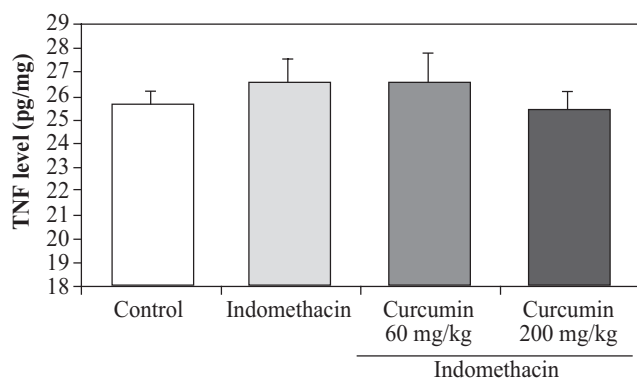


Figure 3. Comparison of serum TNF- α level.

** Results are expressed as mean \pm SE; n = 6 for each group

Serum levels of TNF- α were displayed in Figure 3. There was no significant difference on TNF- α level among the control, NSAID, low and high dose of curcumin groups.

DISCUSSION

In the present study, we investigated the effects of curcumin on indomethacin-induced gastric injury in rats and its influence on serum level of TNF- α . The results clearly demonstrate that pretreatment of curcumin prevents the ulcerogenic effect of indomethacin in dose dependent manner. Nonetheless, there is no significant change of TNF- α level.

Adherence of neutrophils to vascular endothelium is considered one of possible pathogenic mechanisms of NSAID-induced mucosal injury.^(7,21,22) A previous study evidenced that curcumin completely blocked TNF- α mediated adhesion of monocytes to endothelial cells, as well as the cell surface expression of ICAM-1, VCAM-1 and ELAM-1 in endothelial cells.⁽²³⁾ This suggests that the gastroprotective effect of curcumin in indomethacin-induced gastric mucosal injury which was found in our present study is possibly resulted from its inhibition of TNF- α mediated adhesion molecule expression in endothelial cells. However, whether curcumin inhibits the ulcerogenic activity of indomethacin by affecting TNF- α level has not been investigated and, if it does, this might be one of the possibilities explaining its gastroprotective effect in indomethacin-induced gastropathy. To prove this hypothesis we then further measured the level of TNF- α in the plasma. Our study revealed that curcumin does not change the plasma level of TNF- α at four

hours after oral administration of indomethacin. Consequently, it might be proposed that curcumin precludes the ulcerogenic action of NSAIDs without reducing TNF- α level.

Curcumin is renowned for its anti-inflammatory effect^(19,20) and broadly studied in many inflammatory conditions⁽²⁴⁻²⁶⁾; however, the studies of its anti-inflammatory effect on NSAIDs-induced gastric injury are very limited. A recent study by Swarnakar et al. has shown an anti-ulcerogenic effect of curcumin in indomethacin-induced gastric ulcer by regulating the expression and activity of matrix metalloproteinases 9.⁽²⁷⁾

Although in our study there was no significant difference of TNF- α level between the NSAID and curcumin groups, the same phenomenon was also observed in the control and NSAIDs groups. Because normally NSAIDs are taken by the patients along with water, in this study we therefore dissolved indomethacin by sterile water hoping to imitate physiologic conditions. Early in the experimental processes we observed that oral administration of indomethacin dissolved in sterile water failed to damage gastric mucosa when it was given at the usual dose used in the previous studies that dissolved it in 5% sodium bicarbonate. In stead of 20 mg/kg, indomethacin at a dose of 100 mg/kg dissolved in sterile water was given by orogastric tube and it was found that this created moderate gastric mucosal erosion as well as was still well tolerated by the rats. However this model is probably not enough to raise TNF- α level at four hours after indomethacin administration.

In conclusion, we provided evidence that curcumin attains the protective effect on NSAIDs-induced gastric mucosal injury without affecting TNF- α level.

ACKNOWLEDGEMENT

This study was supported by Ratchadapiseksompoj Grant of Faculty of Medicine, Chulalongkorn University. We also thank Dr. Mariem Yusuksawad for her technical assistance.

REFERENCES

1. Scarpigmento C. Nonsteroidal anti-inflammatory drugs: how do they damage gastroduodenal mucosa? *Digest Dis* 1995; 13 (Suppl 1):9-39

2. Wallace JL. Nonsteroidal anti-inflammatory drug gastropathy and cytoprotection: pathogenesis and mechanisms re-examined. *Scand J Gastroenterol* 1992; (Suppl 1) 3-8
3. Wallace JL, McCafferty D-M, Carter L, *et al.* Tissue-selective inhibition of prostaglandin synthesis in rat by tepoxalin: anti-inflammatory without gastropathy. *Gastroenterol* 1993; 105:1630-6.
4. Asako H, Kubes P, Wallace JL, *et al.* Indomethacin-induced leukocyte adhesion in mesenteric venules: role of lipoxigenase products. *Am J Physiol* 1992;262:G903-8.
5. Asako H, Kubes P, Wallace JL, *et al.* Modulation of leukocyte adhesion to rat mesenteric venules by aspirin and salicylate. *Gastroenterol* 1992;103:146-52.
6. Wallace JL, McKnight W, Miyasaka M, *et al.* Role of endothelial adhesion molecules in NSAID-induced gastric mucosal injury. *Am J Physiol* 1993;265:G993-8.
7. Wallace JL, Keenan CM, Granger DN. Gastric ulceration induced by nonsteroidal anti-inflammatory drugs is a neutrophil-dependent process. *Am J Physiol* 1990;259:G462-7.
8. Alican I, Coskun T, Corak A, *et al.* Role of neutrophils in indomethacin-induced gastric mucosal lesions in rats. *Inflamm Res* 1995;44:164-8.
9. Santucci L, Fiorucci S, Giansanti M, *et al.* Pentoxifylline prevents indomethacin-induced acute gastric mucosal damage in rats: role of tumor necrosis factor alpha. *Gut* 1994;35:909-15.
10. Spatafora M, Chiappara G, D'Amico D, *et al.* Effect of indomethacin on the kinetics of tumor necrosis factor alpha release and tumor necrosis factor alpha gene expression by human blood monocytes. *Pharmacol Res* 1991;23:247-57.
11. Ding SZ, Lam SK, Yuen ST, *et al.* Prostaglandin, tumor necrosis factor α and neutrophils: causative relationship in indomethacin-induced stomach injuries. *Eur J Pharmacol* 1998; 348:257-63.
12. Hogaboam CM, Bissonnette EY, Chin BC, *et al.* Prostaglandins inhibit inflammatory mediator release from rat mast cells. *Gastroenterol* 1993;104:122-9.
13. Kunkel SL, Wiggins RC, Chensue SW, *et al.* Regulation of macrophage tumor necrosis factor production by prostaglandin E₂. *Biochemical and Biophysics Research Communications* 1986; 137: 404-10.
14. Rothlein R, Czajkowski M, O'Neil MM, *et al.* Induction of intercellular adhesion molecule I on primary and continuous cell lines by pro-inflammatory cytokines. Regulation by pharmacologic agents and neutralizing antibodies. *J Immunol* 1988;141:1665-9.
15. Palombella VJ, Rando OJ, Goldberg AL, *et al.* The ubiquitin proteasome pathway is required for processing NF kappa B precursor and the activation of NF kappa B. *Cell* 1994;78: 773-85.
16. Brand SJ, Morise Z, Tagerud S, *et al.* Role of the proteasome in rat indomethacin-induced gastropathy. *Gastroenterol* 1999; 116:865-73.
17. Toda S, Miyase T, Arichi H, *et al.* Natural antioxidants. III . Antioxidative components isolated from rhizome of *Curcuma longa* L. *Chemic Pharmaceutic Bull* 1985;33:1725-8.
18. Kuttan R, Bhanumanthly P, Nirmala K, *et al.* Potential anticancer activity of tumeric (*Curcuma longa*). *Cancer Letter* 1985;129:197-202.
19. Srimal RC, Dhawan BN, Pharmacology of diferuloyl methane (curcumin), a non-steroidal anti-inflammatory agent. *J Pharm Pharmacol* 1973; 52(4): 519-25.
20. Satoskar RR, Shah SJ, Shenoy SG *et al.* Evaluation of anti-inflammatory property of curcumin (diferuloyl methane) in patients with postoperative inflammation. *International Journal of Clinical Pharmacology, Therapy and Toxicology* 1986; 24:651-4.
21. Wallace JL, Arfors K-E, McKnight GW. A monoclonal antibody against the CD18 leukocyte adhesion molecule prevents indomethacin-induced gastric damage in the rabbit. *Gastroenterol* 1991;100:878-83.
22. Lee M, Feldman M. Aspirin-induced acute gastric mucosal injury is a neutrophil-dependent process in rats. *Am J Physiol* 1992;26:G920-6.
23. Kumar A, Dhawan S, Hardegen NJ, *et al.* Curcumin (diferuloylmethane) inhibition of tumor necrosis factor (TNF)-mediated adhesion of monocytes to endothelial cells by suppression of cell surface expression of adhesion molecules and of nuclear factor-kB activation. *Biochem Pharmacol* 1998; 55:775-83.
24. Ammon HP, Wahl MA. Pharmacology of *Curcuma longa*. *Planta Medica* 1991;57:1-7.
25. Lim GP, Chu T, Yang F, *et al.* The curry spice curcumin reduces oxidative damage and amyloid pathology in an Alzheimer transgenic mouse. *J Neurosci* 2001;21:8370-7.
26. Gukovsky I, Reyes CN, Vaquero EC, *et al.* Curcumin ameliorates ethanol and nonethanol experimental pancreatitis. *Am J Physiol. Gastrointest Liver Physiol* 2003;284:G85-95.
27. Swarnakar S, Ganguly K, Kundu P, *et al.* Curcumin regulates expression and activity of matrix metalloproteinases 9 and 2 during prevention and healing of indomethacin-induced gastric ulcer. *J Biologic Chemistry* 2005;280:9409-15.