CHAPTER 3

RESULTS

1. Effect of curcumin on gastric acid secretion curcumin

Cimetidine, a histamine H_2 receptor antagonist (100 mg/kg, *i.d.*) exerted the most potent efficacy in reduction of the gastric juice volume, acid output and acid concentration (Table 3). On the other hand, aminoguanidine, an iNOS inhibitor (30 mg/kg, *s.c.*) significantly inhibited the acid output, whereas very little change was observed in the acid concentration. Curcumin (5-20 mg/kg, *i.d.*) significantly reduced the acid output and slightly but significantly depressed the acid concentration. The inhibitory potency reached almost the maximum at the dose of 20 mg/kg. The antisecretory activity of curcumin was rather weakened at 40 mg/kg. However, curcumin did not affect pepsin activity (Table 4).

Table 3 Effect of curcumin, cimetidine, and aminoguanidine on gastric acid secretion in pylorus-ligated rats

Treatment	Dose (mg/kg)	Route	N	Volume (ml)	Acid output (μEq/h)	Acid concentration (μEq/ml)
1% CMC (vehicle control)	-	*i.d.*	10	4.40 ± 0.44	0.063 ± 0.001	0.055 ± 0.006
Curcumin	5	*i.d.*	10	3.70 ± 0.50	0.043 ± 0.007*	0.046 ± 0.004
	10	*i.d.*	10	3.89 ± 0.39	0.035 ± 0.005*	0.037 ± 0.004*
	20	*i.d.*	10	3.04 ± 0.25*	0.030 ± 0.005*	0.040 ± 0.006*
	40	*i.d.*	10	3.34 ± 0.39*	0.043 ± 0.010*	0.048 ± 0.006
Cimetidine	100	*i.d.*	8	1.65 ± 0.17*	0.001 ± 0.001*	0.003 ± 0.001*
Aminoguanidine	30	*s.c.*	8	3.41 ± 0.26*	0.045 ± 0.007*	0.052 ± 0.006

Each value represents the mean ± S.E.M.

*$p<0.05$ compared to the vehicle control-treated rats (LSD test).

Table 4 Effect of curcumin on pepsin activity in pylorus-ligated rats

Treatment	N	Dose (mg/kg)	Route	Pepsin activity (mU/ml)
1% CMC	6	-	*i.d.*	1.42 ± 0.03
Curcumin	6	20	*i.d.*	1.48 ± 0.07

Each value represents the mean ± S.E.M.

2. Effect of curcumin on reflux esophagitis

2.1. Preventive effect of curcumin on acute acid reflux esophagitis

2.1.1. Induction of acute acid reflux esophagitis

The severity of esophageal damage in the esophagus increased with increasing duration of the ligations and a 6-h schedule was suitable for the evaluation of drug efficacy. In the water control-treated rats, macroscopic damage with haemorrhagic ulcer occurred in the middle part of the esophagus (Figure 16). The incidence of esophageal ulcer and perforation were 100% and 66.67 % respectively, while the mean severity of esophagitis (ulcer index) was 3.71 ± 0.61 (Table 5).

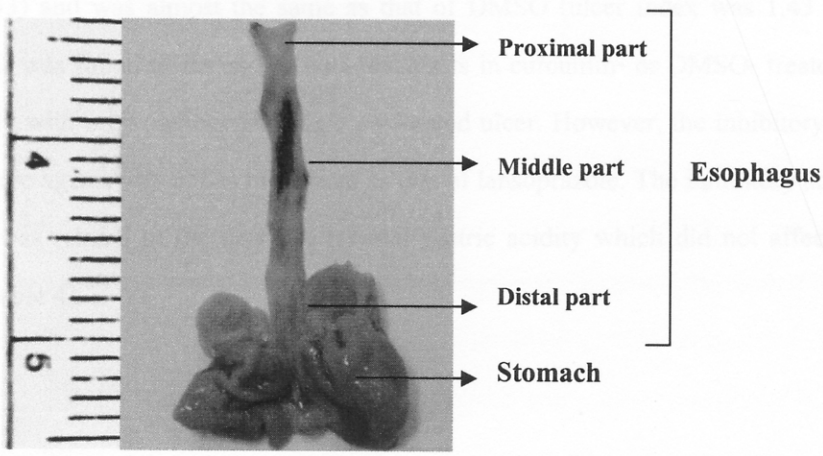


Figure 16 Macroscopic examination of acute acid reflux esophagitis

Table 5 Influence of time after simultaneous ligations of the pylorus and the limiting ridge on esophageal lesions in rats

Hours after ligations	N	Dead rats (%)	Ulcerated rats (%)	Ulceration perforated rats (%)	Lesion index (mm ± S.E.M)
4	6	0	50.00	16.67	2.00 ± 0.63
6	6	0	100.00	66.67	3.71 ± 0.61

2.1.2 Preventive effect of curcumin on acute acid reflux esophagitis

Intraduodenal injection of 1% CMC slightly reduced the incidence of perforation, but had no effect on the incidence or the severity of esophageal ulcer (ulcer index was 3.43 ± 0.57) (Table 6). In the lansoprazole (1 mg/kg, *i.d.*)-treated rats, neither severe haemorrhagic ulcer nor perforation was observed. Only scattered erosions or mild haemorrhagic spots were noticed in four of seven rats (57.14%). Aminoguanidine (30 mg/kg, *s.c.*) remarkably reduced the incidence of perforation, but it was not effective in decreasing the incidence or the severity of esophageal ulcer (ulcer index was 2.71 ± 0.75). Curcumin (10-40 mg/kg. *i.d.*) definitely inhibited the incidence of perforation and the incidence or the severity of esophageal ulcer although not in a dose dependent manner. The inhibitory potency reached almost the maximum at the dose of 20 mg/kg (ulcer index was 1.63 ± 0.53) and was almost the same as that of DMSO (ulcer index was 1.43 ± 0.65). Esophagitis was found in six and five of seven rats in curcumin- or DMSO- treated group respectively with only one haemorrhagic perforated ulcer. However, the inhibitory activity of these three agents was not as prominent as that of lansoprazole. The inhibitory activity of curcumin was related to the decrease in total gastric acidity which did not affect pepsin activity (Table 4).

Table 6 Preventive effect of curcumin, lansoprazole, and aminoguanidine on acute acid reflux esophagitis in rats

Treatment	Dose	Route	N	Perforated rats (%)	Ulcer index	Inhibition (%)
Water control	-	-	6	66.67	3.71 ± 0.61	-
1% CMC (vehicle control)	-	*i.d.*	7	42.85	3.43 ± 0.57	7.55
Curcumin	10 mg/kg	*i.d.*	7	28.57	2.43 ± 0.69	29.16
	20 mg/kg	*i.d.*	7	14.29**	1.63 ± 0.53**	52.48**
	40 mg/kg	*i.d.*	7	33.33	2.29 ± 0.75	33.24
Lansoprazole	1 mg/kg	*i.d.*	7	0.00**	1.00 ± 0.44**	70.85**
DMSO	1 ml/kg	*i.p.*	7	14.29*	1.43 ± 0.65*	61.46*
Aminoguanidine	30 mg/kg	*s.c.*	7	28.50*	2.86 ± 0.67	22.91

Each value represents the mean ± S.E.M.

* $p<0.05$ compared to the water control-treated rats (LSD test).

** $p<0.05$ compared to the vehicle control-treated rats (LSD test).

2.2 Preventive effect of curcumin on chronic acid reflux esophagitis

2.2.1 Induction of chronic acid reflux esophagitis

Chronic esophagitis was found in 100% of the rats in the water control-treated rats 2 weeks after the ligation of the limiting ridge and the induction of gastric outlet obstruction by covering the duodenum near the pylorus ring with a 18 Fr Nelaton catheter. The survival rate 2 weeks and 3 weeks after the start of the experiment was 83.33% and 66.67% respectively. The major causes of death were perforation of the ulceration area (occurred within 5-7 days after the start of the experiment), and misswallowing from the esophagostenosis of the ulceration area (occurred within 10-21 days after the start of the experiment). Grossly, the thickness of the esophageal wall was increased and the lower part of the esophagus had generally dilated with an approaching a diameter more than twice that of the normal esophagus in sham-operated control rats. The ulcer was located 1 or

2 cm above the esophagogastric junction (middle part of the esophagus) in most cases and near the esophagogastric junction (lower part of the esophagus) in some cases. The ulcers, in some severe cases, were observed at both parts of the esophagus or developed as a large coalesced longitudinal ulcer in the middle and lower part of the esophagus. There was also oval or round white folds intermingled with area of ulceration (Figure 17).

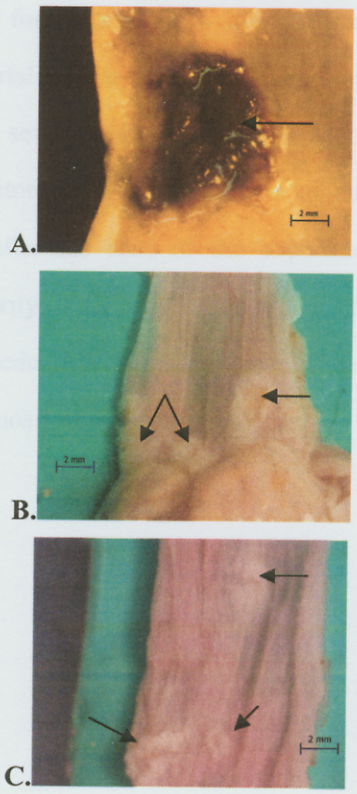


Figure 17 Macroscopic examination of chronic acid reflux esophagitis

Gross morphology of esophagus using stereoscope Olympus SZX 12 with all objective lens x7. The ulcers were observed at the middle part of the esophagus as a large coalesced longitudinal hemorrhagic ulcer **(A)**, or at the esophagogastric junction (lower part of the esophagus) as oval or round white folds intermingled with area of ulceration **(B)**, or at both parts of the esophagus **(C)**.

2.2.2 Preventive effect of curcumin on chronic acid reflux esophagitis

Administration of DMSO (1 ml/kg, *i.p.*) or aminoguanidine (30 mg/kg, *s.c.*) daily for 14 days after the start of the experiment significantly increased the mortality rate up to more than 80% of the total rats (Table 7). Lansoprazole (1 mg/kg, *p.o.*) at the same administration regimen, reduced significantly the mortality rate and inhibited the severity of esophagitis (ulcer index) by 53.85% (Table 7, Figure 18). The esophagitis was observed in 4 of 6 rats with one severe dilation of the lower part of the esophagus. Curcumin (10-40 mg/kg) administration orally daily for 14 or 21 days after the start of the experiment was not effective in reducing the mortality rate or inhibited the severity of esophagitis. In addition, the mortality rate and the severity of esophagitis tended to increase at the dose of 40 mg/kg. However, the intraperitoneal co-administration of curcumin (20 mg/kg) and DMSO (1 ml/kg) for 14 days after the start of the experiment, exerted comparable inhibitory potency against the severity of the esophagitis to that of lansoprazole by 56.52% (Table 7, Figure 18), with a slight reduction of mortality rate. The esophagitis was observed in 6 of 8 rats with one esophagostenosis of the ulceration area.

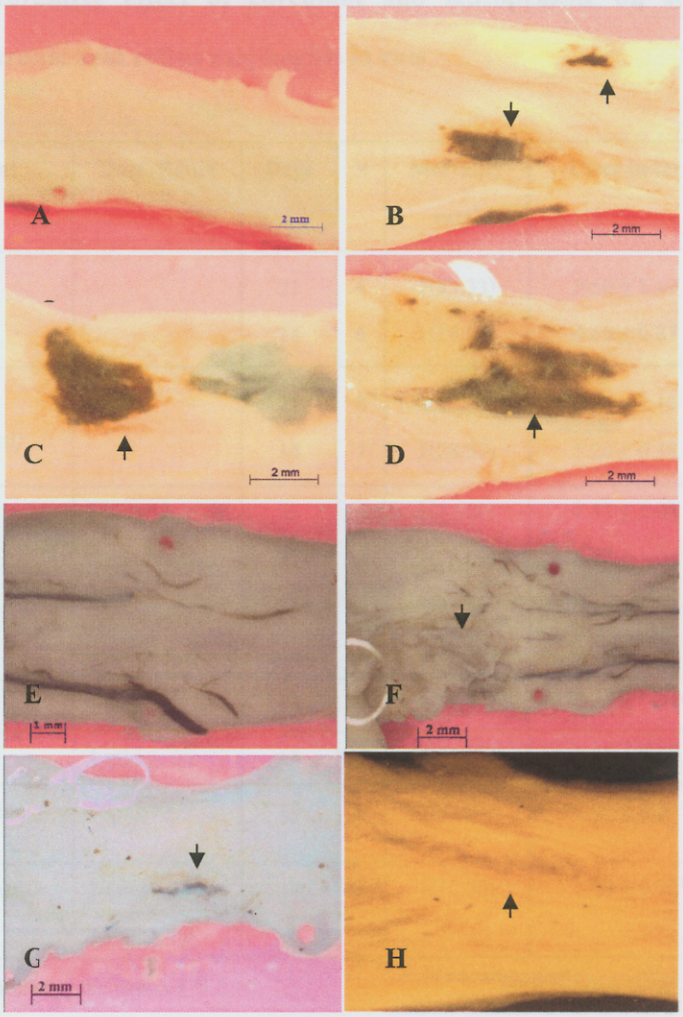


Figure 18 Preventive effect of curcumin and lansoprazole on surgically induced chronic acid reflux esophagitis in rats

Gross morphology of esophagus using stereoscope Olympus SZX12 with all objective lens x7. **A** = normal rat; **B**, **C** = water-treated rat; **D** = vehicle (1% CMC)-treated rat; **E**, **F** = lanzoprazole (1 mg/kg, *p.o*)-treated rat; **G**, **H** = curcumin (20 mg/kg in DMSO 1 ml/kg, *i.p*)-treated rat. All treatments were given for 14 days after the start of the experiment

Table 7 Preventive effect of curcumin and lansoprazole on surgically induced chronic acid reflux esophagitis in rats

Treatment	Dose, Route	Days	N	Mortality rate[a]	Ulcer index	Inhibition (%)
Water control	-	21	6	2(33.33)	3.50 ± 1.44	-
		14	6	1(16.67)	4.60 ± 0.87	-
1% CMC (vehicle control)	-	21	8	3(37.50)	3.20 ± 1.16	8.57
		14	7	2(28.57)	5.20 ± 0.58	-13.04
Curcumin	10 mg/kg, *p.o*	21	8	2(25.00)	2.83 ± 1.08	11.56
	20 mg/kg, *p.o*	21	8	2(25.00)	2.67 ± 1.09	16.56
	40 mg/kg, *p.o*	21	8	3(37.50)	3.60 ± 1.03	-12.50
	20 mg/kg, *p.o*	14	7	2(28.57)	4.20 ± 1.11	19.23
Lansoprazole	1 mg/kg, *p.o*	14	6	1(16.67)	2.40 ± 1.03**	53.85**
DMSO	1 ml/kg, *i.p.*	14	6	5(83.33)	1.00 ± 0.00	
Aminoguanidine	30 mg/kg, *s.c.*	14	6	5(83.33)	6.00 ± 0.00	
Curcumin+DMSO	20 mg/kg + 1 ml/kg, *i.p.*	14	8	1(12.50)	2.00 ± 0.72*	56.52*

Each value represents the mean ± S.E.M.

[a]Data show number of rats, with percentages in parentheses

* $p<0.05$ compared to the water control-treated rats (LSD test).

** $p<0.05$ compared to the vehicle control-treated rats (LSD test).

2.3 Preventive effect of curcumin on mixed reflux esophagitis

2.3.1 Induction of mixed reflux esophagitis

From macroscopic examination, only the control and the aminoguanidine-treated rats showed the occurrence of esophagitis in one of seven rats (14.30%). The noted haemorrhagic lesion was about 1 to 2 mm around the lower third of thoracic esophagus (Figure 19). From the histological examination, the thickness of the esophageal epithelium was markedly reduced in the control group and two main conditions were found to be

the criteria for acute reflux esophagitis. One was papillomatosis of mucosal epithelium observed on the top of the esophageal mucosa, the other was the infiltration of inflammatory cells, mainly of neutrophils, in lamina propria, in submucosa, and in muscularis externa. In addition, interruption of the lamina muscularis mucosae and numerous red blood cells were also noted in six and three of eight control rats respectively (Figure 20).

Figure 19 Macroscopic examination of acute mixed reflux esophagitis

Gross morphology of esophagus using stereoscope Olympus SZX 12 with all objective lens x7.

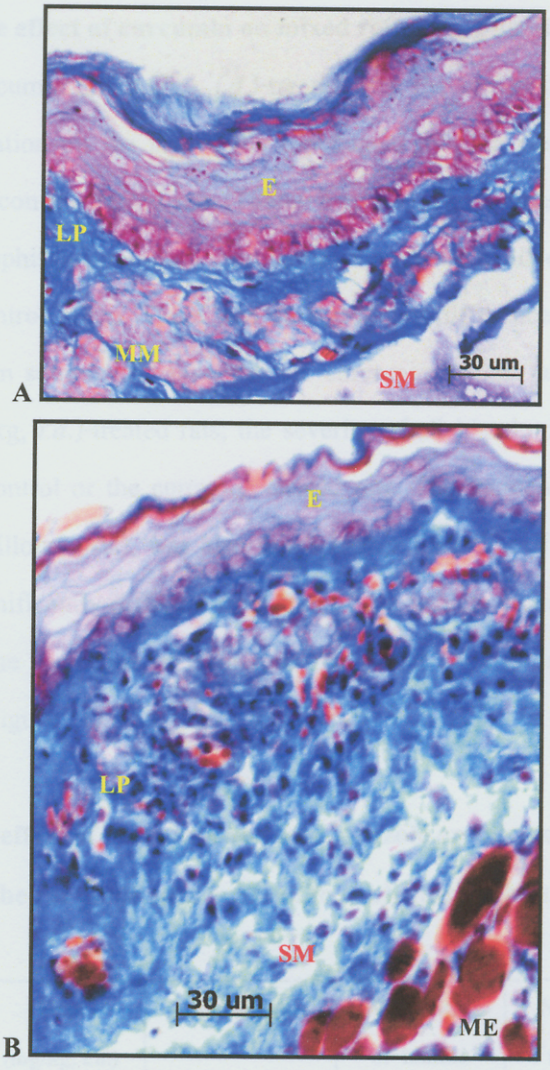


Figure 20 **Histological examination of acute mixed reflux esophagitis** (Masson trichrome staining, x132). **A:** Histologic features of esophagus in normal rat showing mucosal epithelium (E), lamina propria (LP), muscularis mucosae (MM), and submucosal layers (SM) The esophageal mucosa consisted of one to two basal cell layers and squamous cells. A parakeratotic layer was present on the esophageal epithelium. **B:** Histologic features of esophagus in the vehicle (1% CMC)-treated rat. Papillomatosis was clearly seen on the top of the mucosal epithelium. Inflammatory cell infiltration, mainly of neutrophils was markedly seen in lamina propria and in submucosa. Interruption of the lamina muscularis mucosae and numerous red blood cells were also noted. ME = muscularis externa.

2.3.2 Preventive effect of curcumin on mixed reflux esophagitis

In the curcumin (20 mg/kg, *i.d.*)-treated rats, the incidence and the severity of papillomatosis formation on the top of the esophageal mucosa were not different from those of the vehicle control-treated rats (Tables 8 and 9). Nevertheless, the incidence and the severity of neutrophil infiltration in muscularis externa and in adventitia were much less than those of the control group. There was no significant difference in red blood cells in lamina propria and in submucosal layers in the curcumin-treated rats (Figure 21). In the lansoprazole (1 mg/kg, *i.d.*)-treated rats, the severity of all histologic changes was much higher than in the control or the curcumin-treated rats. The incidence of most histologic changes (except papillomatosis), was also much higher than that of the curcumin-treated rats, but was not significantly different from that of the vehicle control-treated rats except for an increase in the incidence of numerous red blood cells in lamina propria and in submucosal layers (Figure 21).

Table 8 Preventive effect of curcumin and lansoprazole on the incidence of histologic changes in the rat esophagus exposed to acute mixed reflux esophagitis

Treatment	Dose (mg/kg, *i.d.*)	Papillomatosis[a]	Interruption of lamina propria[a]	No of red blood cells[a]	Neutrophil infiltration[a]
1% CMC (vehicle control) (n=8)	-	8 (100)	6 (75.00)	3 (37.50)	8 (100)
Curcumin (n=7)	20	7 (100)	3 (42.86)*	4 (57.14)	3 (42.86)*
Lansoprazole (n=8)	1	8 (100)	7 (87.50)	7 (87.50)	7 (87.50)

[a]Data show number of rats, with percentages in parentheses

* $p<0.05$ compared to the vehicle control-treated rats (LSD test).

Table 9 Preventive effect of curcumin and lansoprazole on the severity of pathologies of rat esophagus exposed to acute mixed-type reflux esophagitis

Treatment	Dose (mg/kg, *i.d.*)	Papillomatosis	Interruption of lamina propria	No of red blood cells	Neutrophil infiltration
1% CMC (vehicle control) (n=8)	-	+	+	+	++
Curcumin (n=7)	20	+	+	+	+*
Lansoprazole (n=8)	1	++	++	++	+++

* $p<0.05$ compared to the vehicle control-treated rats (Mann-Whitney U test).

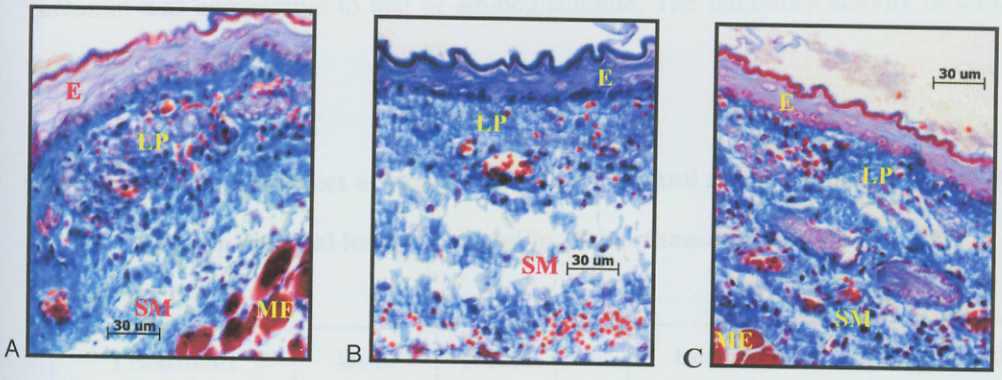


Figure 21 Histological examination of the effect of curcumin and lansoprazole on acute mixed reflux esophagitis **A:** In the vehicle (1% CMC)-treated rat, papillomatosis was clearly seen on the top of the mucosal epithelium. Inflammatory cell infiltration, mainly of neutrophils, was markedly seen in lamina propria (LP) and in submucosa (SM). Interruption of the lamina muscularis mucosae and numerous red blood cells were also noted. **B:** In the curcumin (20 mg/kg, *i.d*)-treated rat, papillomatosis was observed on the top of the mucosal epithelium. The mucosal epithelium was markedly thinner than that of normal control group. Inflammatory cell infiltration, mainly of neutrophils, was markedly seen in lamina propria and in submucosa, but much less than that of vehicle- or lansoprazole-treated rat. **C:** In the lansoprazole (1 mg/kg, *i.d*)-treated rat. Papillomatosis was observed on the top of the mucosal epithelium. The thickness of mucosal epithelium was markedly thinner than that of normal control group. Inflammatory cell infiltration, mainly of neutrophils, was markedly seen in lamina propria, in submucosa, and in muscularis externa (ME). Interruption of the lamina muscularis mucosae was noted.

3. Effect of curcumin on gastritis

3.1 Preventive effect of curcumin on acute gastric mucosal lesions induced by ethanol

Intragastric administration of 80% ethanol produced band-like hemorrhagic lesions in the glandular portion of the stomach. The lesion index in the vehicle control-treated group was 98.50 ± 10.16 mm (Table 10). Pretreatment of rats with either cimetidine (100 mg/kg, *p.o.*) or aminoguanidine (30 mg/kg, *i.p.*) significantly inhibited gastric mucosal injury by 75.46 % and 50.93 %, respectively. Pretreament with curcumin (5-20 mg/kg, *p.o.*) inhibited the gastric mucosal injury by no less than 50%, and the inhibitory potency of curcumin was comparable to that of aminoguanidine. The inhibitory activity of curcumin decreased at 40 mg/kg.

Table 10 Preventive effect of curcumin, cimetidine, and aminoguanidine on acute gastric mucosal lesions induced by 80% ethanol in rats

Treatment	Dose (mg/kg)	Route	N	Lesion index (mm)	Inhibition (%)
1% CMC (vehicle control)	-	*p.o.*	8	98.50 ± 10.16	-
Curcumin	5	*p.o.*	7	44.71 ± 6.98*	54.61*
	10	*p.o.*	8	45.50 ± 8.59*	53.81*
	20	*p.o.*	7	46.43 ± 12.4*	52.86*
	40	*p.o.*	6	73.17 ± 8.74	25.72
Cimetidine	100	*p.o.*	6	24.17 ± 4.70*	75.46*
Aminoguanidine	30	*i.p.*	6	48.33 ± 8.43*	50.93*

Each value represents the mean ± S.E.M.

* $p < 0.05$ compared to the vehicle control-treated rats (LSD test).

3.2 Preventive effect of curcumin on acute gastric mucosal lesions induced by serotonin

Repeated subcutaneous administration of serotonin once daily for 4 days caused varioliformed-like lesions in the whole corpus mucosa, with severe edema in the submucosa, and the lesion score was 65.14 ± 8.15 mm^2 (Table 11). Simultaneous administration of aminoguanidine (30 mg/kg, *i.p.*) significantly prevented the development of gastric lesions with 73.41 % inhibition, whereas cimetidine (100 mg/kg, *p.o.*) was without effect. In addition, the severity of serotonin-induced gastric lesions was significantly reduced by prior oral administration of curcumin (20 and 80 mg/kg) in a dose-related manner, with inhibition rates of 38.05 % and 67.79 %, respectively.

Table 11 Preventive effect of curcumin, cimetidine, and aminoguanidine on acute gastric mucosal lesions induced by repeated treatment with serotonin in rats

Treatment	Dose (mg/kg)	Route	N	Lesion index (mm^2)	Inhibition (%)
1% CMC (vehicle control)	-	*p.o.*	6	65.14 ± 8.15	-
Curcumin	20	*p.o.*	6	38.67 ± 8.79*	38.05*
	80	*p.o.*	6	20.11 ± 4.72*	67.79*
Cimetidine	100	*p.o.*	6	67.47 ± 13.10	-3.58
Aminoguanidine	30	*i.p.*	6	16.60 ± 5.72*	73.41*

Each value represents the mean ± S.E.M.

* $p < 0.05$ compared to the vehicle control-treated rats (LSD test).

3.3 Preventive effect of curcumin on acute gastric mucosal lesions induced by compound 48/80

A single intraperitoneal injection of compound 48/80, a mast-cell degranulator,

caused severe varioliformed-like lesions in the whole corpus mucosa at 3 h after the injection. Pretreatment with aminoguanidine (30 mg/kg, *s.c.*) significantly reduced the severity of gastric damage, whereas cimetidine was without effect (Table 12). Administration of curcumin (20-80 mg/kg, *p.o.*) also reduced the severity of gastric damage in a dose related manner.

Table 12 Preventive effect of curcumin, cimetidine, and aminoguanidine on acute gastric mucosal lesions induced by single treatment with compound 48/80 in rats

Treatments	Dose (mg/kg)	Route	N	Lesion index (%)								*P* value
				0	I	II	III	IV	V	VI	VII	
1% CMC (vehicle control)	-	*p.o.*	10					60	20	10	10	
Curcumin	20	*p.o.*	10			20	50	20	10			0.05
	40	*p.o.*	10			10	70	20				0.05
	80	*p.o.*	10			40	40	20				0.05
Cimetidine	100	*p.o.*	10					50	20	20	10	NS
Aminoguanidine	30	*s.c.*	10		40	30	20	10				0.05

The severity of gastric mucosal lesions was evaluated using the index of the following eight grades of lesions as: grade 0, no lesion (normal); grade I, edema only; grade II, damaged area of 1-10 mm^2; grade III, damaged area of 11-20 mm^2; grade IV, damaged area of 21-30 mm^2; grade V, damaged area of 31-40 mm^2; grade VI, damaged area of 41-50 mm^2 and grade VII, damaged area of more than 51 mm^2. (Ohta, *et al.*, 1999).

4. Curative effect of curcumin on chronic gastric ulcer induced by topical application of acetic acid

4.1 Induction of chronic gastric ulcer

Using the topical application of acetic acid method, the gastric antral ulcer on the lesser curvature was made. Well-defined, deep, round or oval gastric ulcer penetrating into the adjacent organs (mainly liver) was well established 4 days after the acetic acid application. In some cases, ulceration extended to the muscularis propia, but perforation

was not observed during the course of the study. The diameter of gastric ulcer was about 5-8 mm and the ulcer area was about 58.7 ± 5.03 mm^2 (Figure 22). Necrotic debris, food residue, and ingested hair were observed on the floor of the ulcer. The area of the ulcer decreased with time as shown in table 13. The original ulcer undergoes a healing process of approximately 3 weeks after ulcer induction and is found macroscopically healed in 60% of the animals.

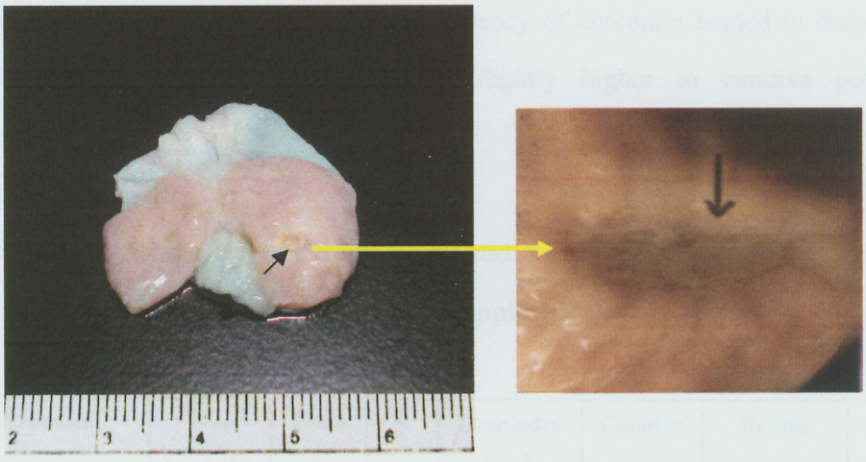


Figure 22 Macroscopic examination of chronic gastric ulcer induced by topical application of acetic acid in rats

Table 13 The healing process of acetic acid ulcer (by topical application) in rats

Days after ulcer induction	N	Ulcer index (mm^2)
4	10	58.70 ± 5.03
14	10	24.45 ± 5.81
21	10	8.50 ± 2.32

4.2 Curative effect of curcurmin on chronic gastric ulcer

Both cimetidine (100 mg/kg/day, *p.o.*, twice a day) and aminoguanidine (30 mg/kg/day, *s.c.* daily), significantly decreased the index of acetic acid-induced chronic gastric ulcer after 10 days treatment (the 14^{th} day after ulcer induction), and also promoted the mucosal

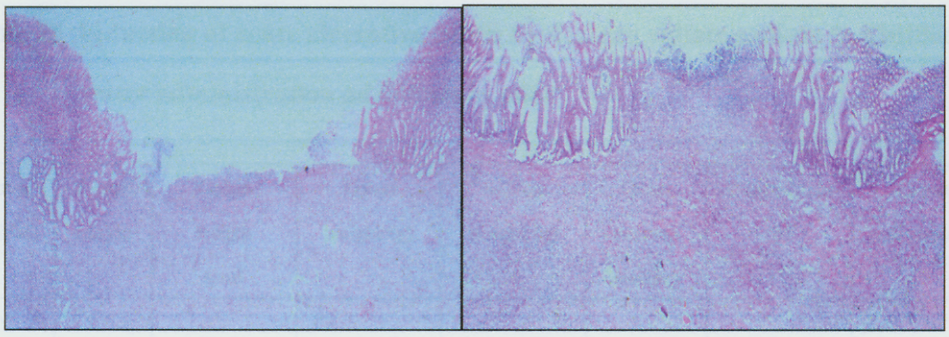
regeneration in the ulcerated portion (Table 14). Administration of curcumin (10-80 mg/kg/day) orally twice a day for 10 days also accelerated the healing of acetic acid-induced chronic gastric ulcer and promoted the mucosal regeneration in the ulcerated portion (Table 14, Figure 23). The ulcer scar had undergone very extensive healing and restoring when compared to the vehicle control-treated rats. Complete reepithelization of the ulcer crater was found in some stomaches. The potency of curcumin for the ulcer curation reached almost the maximum at the dose of 80 mg/kg/day, and was almost the same as that of cimetidine. The curative potency of curcumin tended to decrease at 160 mg/kg/day. In addition, curcumin was slightly higher in curative potency than aminoguanidine.

Table 14 Curative effect of curcumin, cimetidine, and aminoguanidine on chronic gastric ulcer induced by topical application of acetic acid in rats

Treatment	Dose (mg/kg/d)	Route	N	Ulcer index (mm^2)	Curation (%)	Healing index (%)	Mucosal regeneration index (%)
1% CMC (vehicle control)	-	*p.o.*	10	30.05±3.78	51.30	33.10±1.94	33.98±2.50
Curcumin	20	*p.o.*	10	11.73±1.97*	80.99*	35.10±3.85	43.20±3.88
	40	*p.o.*	10	10.18±1.04*	83.50*	36.30±4.06	43.53±2.90*
	80	*p.o.*	10	9.21±2.62*	85.07*	45.44±4.35*	57.99±4.52*
	160	*p.o*	10	11.80±2.11*	80.88*	39.50±2.73	41.53±2.37*
Cimetidine	200	*p.o.*	10	10.85±1.45*	82.42*	47.00±3.19*	49.37±2.95*
Aminoguanidine	30	*s.c.*	9	20.17±1.62*	66.69*	43.11±4.06*	45.57±2.69*

Each value represents the mean ± S.E.M.

$p < 0.05$ compared to the vehicle control-treated rats (LSD test).



A B

Figure 23 Microscopic appearances of acetic acid-induced gastric ulcer in rat after treatment with vehicle (1% CMC) (A) and curcumin 40 mg/kg, twice daily) (B) for 10 days (H&E)

Note that the size and depth of ulcer were reduced by curcumin. Thickening of the gastric wall, epithelization and mucosal regeneration were well advanced in the curcumin-treated rat.

5. Effect of curcumin on ulcerative colitis

5.1 Induction of ulcerative colitis

Clinical assessment of DSS-treated animals at two day intervals, showed drinking volume was similar in all groups (data not shown) and none of the untreated DSS mice exhibited any of the clinical signs normally associated with spontaneous intestinal inflammation (Table 15). Only mice given 4% DSS in drinking water for 7 or 10 days exhibited significant macroscopic changes which are classically associated with this mode of colitis with no mortality was observed. These changes included progressive body weight loss, bloody stools, shortening of the colon, and increase in weight of the spleen. The appearance of fecal blood started on day 4 and the significant changes in body weight started on day 5. The gross morphology score for evidence of inflammatory change in the colon in water and 1% CMC control group was 3.00 ± 0.58 and 3.50 ± 0.50 respectively. Since, the severe progress weight loss was observed in mice receiving 4% DSS for 10 days, 4% DSS for 7 days was chosen as an optimal dose for further studies.

Table 15 Induction of acute ulcerative colitis by dextran sulfate sodium in distilled water administration *ad libitum* in mice

Group (% DSS)	Period (days)	Colon length (cm)	Colon weight (g)	Spleen weight (g)	Gross rectum bleeding[a]	Weight gain (g)	Gross morphology
0 (n=4)	4	8.00±0.43	0.45±0.02	0.12±0.01	0/4	1.70±0.20	0
4 (n=4)	4	6.58±0.19	0.43±0.01	0.13±0.01	0/4	1.50±0.29	0
0 (n=4)	7	8.08±0.29	0.46±0.02	0.12±0.01	0/4	4.75±0.25	0
4 (n=6)	7	5.60±0.32*	0.40±0.02*	0.14±0.01*	4/6	3.00±0.37*	3.00±0.58*
5 (n=4)	7	5.03±0.24*	0.32±0.02*	0.11±0.01	4/4 (1)[b]	6.67±1.67*	3.67±0.33*
0 (n=4)	10	7.85±0.33	0.51±0.03	0.12±0.01	0/4	4.00±0.25	0
3 (n=4)	10	5.18±0.30*	0.40±0.04*	0.15±0.02*	0/4	2.00±0.50*	2.25±0.25*
4 (n=4)	10	5.13±0.19*	0.42±0.03*	0.19±0.02*	4/4	-5.25±0.60*	3.50±0.50*

Each value represents the mean ± S.E.M.

* $p < 0.05$ compared to the non-treated rats (LSD test),

[a] = no. of mice showing gross blood per rectum (blood clot around the anus), [b] = no. of mice death

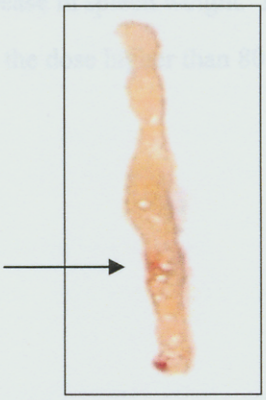


Figure 24 Macroscopic examination of acute ulcerative colitis induced by dextran sulfate sodium in distilled water administration *ad libitum* in mice

5.2 Preventive effect of curcumin on ulcerative colitis

Mesalazine (100 mg/kg) treated-mice showed only a significant decrease in the number of rats with diarrhea whereas the onset and severity of DSS colitis and other associated tissue damages remained unchanged (Table 16). In contrast to mesalazine, the attenuated disease activity was noted in sulfasalazine (100 mg/kg) treated-mice with the gross morphology score 1.50 $\pm$ 0.96. The number of rats with diarrhea or occult blood was reduced significantly. Minor increase in body weight gain and colon length was observed and no increase in the weight of spleen was seen. Curcumin, at the dose 20-320 mg/kg showed no difference in the onset and severity of DSS colitis and the associated tissue damage when compared with controls. In addition, the progressive DSS colitis severity tended to increase at the dose higher than 80 mg/kg (Table 16).

5.3 Curative effect of curcumin on ulcerative colitis

In non-treated mice, the mortality rate was about 40%. Neither diarrhea nor occult blood was seen in surviving mice (Table 17). The body weight reduced temporarily associated with development of colitis, followed by recovery. The colon and spleen weight recovered to nearly the same level as that of normal mice. However, no beneficial alteration was seen on colon length. The gross morphology score for water and vehicle control mice was 2.67 $\pm$ 0.88 and 2.33 $\pm$ 0.67 respectively. In curcumin-treated mice, only curcumin 40 mg/kg showed some beneficial treatment with decrease in mortality rate, increase in body weight gain and no increase in spleen weight. In addition, the progressive DSS colitis severity tended to increase at the dose higher than 80 mg/kg (Table 17).

Table 16 Preventive effect of curcumin on acute ulcerative colitis induced by 4 % dextran sulfate sodium in distilled water administration *ad libitum* for 7 days in mice

Treatment	Diarrhea	Occult blood[a]	Colon length (cm)	Colon weight (g)	Spleen weight (g)	Weight Gain (g)	Gross morphology
Normal mice (n=4)	0/4	0/4[a]	7.90±0.15	0.55±0.05	0.12±0.01	4.27±0.43	0
Control (water) (n=4)	4/4	4/4[a](1)[b]	5.03±0.78	0.33±0.05	0.16±0.03	0.73±1.07	3.00±0.58
Control (1%CMC) (n=4)	4/4	4/4[a](1)[b]	5.68±0.57	0.36±0.02	0.13±0.01	0.53±0.56	3.00±0.41
Curcumin 20 mg/kg (n=4)	4/4	4/4[a](2)[b]	5.68±0.21	0.40±0.03	0.15±0.02	0.25±0.63	2.25±0.63
40 mg/kg (n=4)	3/4	4/4[a](3)[b]	5.23±0.17	0.37±0.02	0.15±0.02	0.50±1.26	2.00±0.71
80 mg/kg (n=6)	2/6*	5/6[a](2)[b]	5.52±0.23	0.36±0.02	0.14±0.01	0.02±0.46	3.00±0.68
160 mg/kg (n=4)	4/4	4/4[a](2)[b]	5.58±0.74	0.37±0.02	0.16±0.01	2.38±1.14	3.50±0.65
320 mg/kg (n=4)	3/4	4/4[a](1)[b]	5.90±0.52	0.35±0.02	0.17±0.03	1.10±0.79	3.50±0.50
Mesalazine (n=4)	2/4*	4/4[a](1)[b]	5.58±0.18	0.39±0.02	0.14±0.02	0.50±0.06	3.00±0.71
Sulfasalazine (n=4)	2/4*	1/4[a](1)[b]*	6.43±0.36	0.40±0.02	0.11±0.01*	0.06±0.74	1.50±0.96*

Each value represents the mean ± S.E.M.

* $p < 0.05$ compared to the vehicle control-treated rats (LSD test)

[a] = no. positive/total, [b] = no. of mice with positive gross rectum bleeding

Table 17 Curative effect of curcumin on ulcerative colitis induced by 4 % dextran sulfate sodium in distilled water administration *ad libitum* for 7 days in mice

Treatment	Diarrhea	Occult blood[a]	Colon length (cm)	Colon weight (g)	Spleen weight (g)	Weight gain (g)	Gross morphology
Normal mice (n=5)	0/5	0/5	8.34±0.24	0.49±0.03	0.13±0.01	6.92±0.44	0
Control (water) (n=5)	0/5	0/5(2)[a]	6.83±0.32	0.39±0.06	0.12±0.01	0.79±3.25	2.67±0.88
Control (1%CMC) (n=5)	0/5	0/5(2)[a]	6.57±0.32	0.39±0.04	0.15±0.02	0.53±0.72	2.33±0.67
Curcumin 20 mg/kg (n=4)	0/4	0/4(2)[a]	6.40±0.30	0.39±0.05	0.11±0.01	0.50±0.33	1.50±0.50
40 mg/kg (n=4)	0/4	0/4(1)[a]	6.63±0.24	0.38±0.02	0.14±0.02	1.58±0.70	1.67±0.67
80 mg/kg (n=4)	0/4	0/4(2)[a]	6.45±0.65	0.41±0.03	0.16±0.05	2.15±2.53	2.50±1.50

Each value represents the mean ± S.E.M.

[a] = no. of mice death

6. Effect of curcumin on inflammatory cytokines on the gastric ulcer healing in rats

6.1 Effect of curcumin on the level of protein and mRNA expression of iNOS, COX-2, TNF-α or IL-1β in the gastric ulcerated mucosa

The protein expression of iNOS, COX-2, TNF-α and IL-1β was detected by Western blot analysis. Neither protein expression of iNOS, COX-2, TNF-α nor IL-1β protein was detected in the normal stomach. In rats with gastric ulcer, an increased expression of these cytokines was detected at the ulcer margin in the vehicle treated rat,as compared to that in the normal gastric mucosa. Treatment with curcumin significantly

reduced the increased protein expression level of iNOS and TNF-α into the normal level in a dose dependent manner (Figure 25, 27). In contrast, the level of COX-2 and IL-1β protein expression was significantly induced in a dose dependent manner after curcumin treatment (Figure 26, 28). Treatment with aminoguanidine significantly reduced the increased protein expression level of iNOS but induced the protein expression level of IL-1β. However, aminoguanidine had no any effect on the protein expression level of COX-2 or TNF-α. Using real time RT-PCR, no significant inhibition of iNOS mRNA expression was detected at the ulcer margin in aminoguanidine-treated rat, whereas significant inhibition of iNOS mRNA expression was detected in the curcumin-treated rat in a dose dependent manner (Figure 29).

6.2 Immunohistochemistry of iNOS and TNF-α

Immunoreactivity of iNOS or TNF-α was not detected in normal gastric mucosa not treated with acetic acid (sham-operated rat) (Figure 30). In the vehicle treated rats, iNOS-positive inflammatory cells were found in the submucosa around vessels at the margin of a gastric ulcer, in the muscularis mucosae and in epithelial cells at the basal portion of the regenerating mucosa. In the curcumin-treated rats, iNOS-positive inflammatory cells significantly decreased in a dose–dependent manner and were seen in epithelial cells at the basal portion of the regenerating mucosa. iNOS-positive inflammatory cells and epithelial cells were also decreased in the aminoguanidine-treated rats when compared to the vehicle-treated rats. However, a greater amount of iNOS-positive cells was seen when compared to the curcumin-treated rats. Immunoreactivity of TNF-α was also barely seen in the curcumin-treated rats when compared to the vehicle-treated rats.

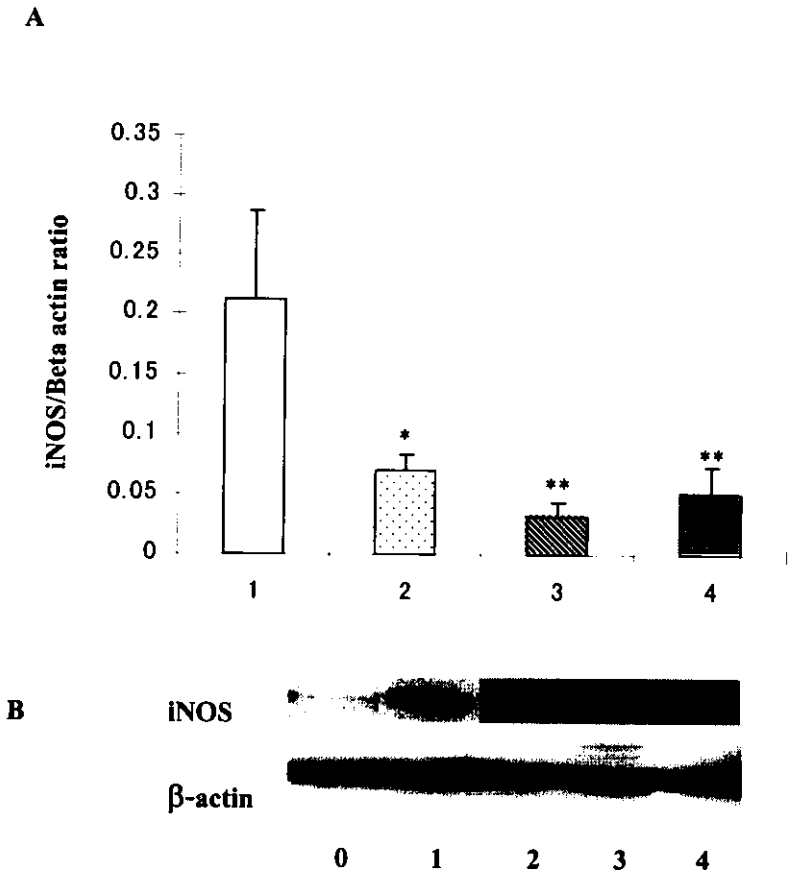


Figure 25 Protein expression of iNOS in the gastric mucosa of rats at the 14th day after ulcer induction

Densitometric analysis of iNOS protein expression assessed by Western blot and expressed as each cytokine per β-actin ratio (A) in normal gastric mucosa (lane 0); in mucosa around ulcer treated with vehicle (lane 1); in curcumin-treated rats (40 mg/kg *p.o*, bid) (lane 2); in curcumin-treated rat (80 mg/kg p.o, bid) (lane 3); or in aminoguanidine-treated rat (30 mg/kg s.c, od) (lane 4). Mean ± S.E.M of 6 rats.

*$p < 0.10$ compared to the vehicle control-treated rats. **$p < 0.05$ compared to the vehicle control-treated rats (Dunnett's test).

Representative Western blot analysis of iNOS or β-actin is presented in (B).

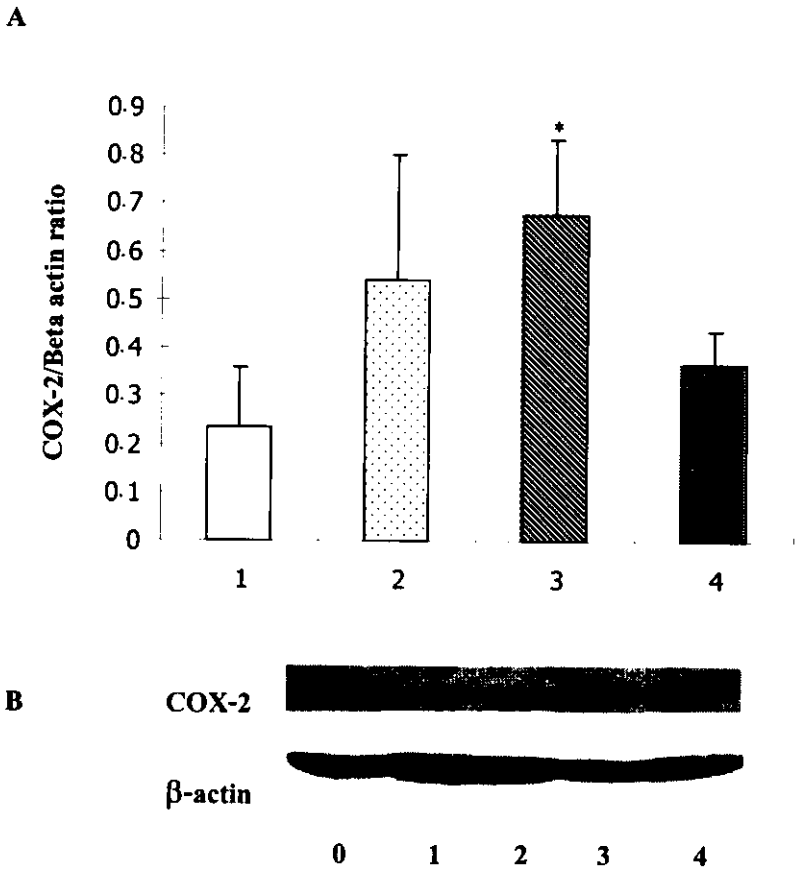


Figure 26 **Protein expression of COX-2 in the gastric mucosa of rats at the 14th day after ulcer induction**

Densitometric analysis of COX-2 protein expression assessed by Western blot and expressed as each cytokine per β-actin ratio (A) in normal gastric mucosa (lane 0); in mucosa around ulcer treated with vehicle (lane 1); in curcumin-treated rats (40 mg/kg *p.o*, bid) (lane 2); in curcumin-treated rat (80 mg/kg p.o, bid) (lane 3); or in aminoguanidine-treated rat (30 mg/kg s.c, od) (lane 4). Mean ± S.E.M of 6 rats.

*$p < 0.10$ compared to the vehicle control-treated rats. (Dunnett's test).

Representative Western blot analysis of COX-2 or β-actin is presented in (B).

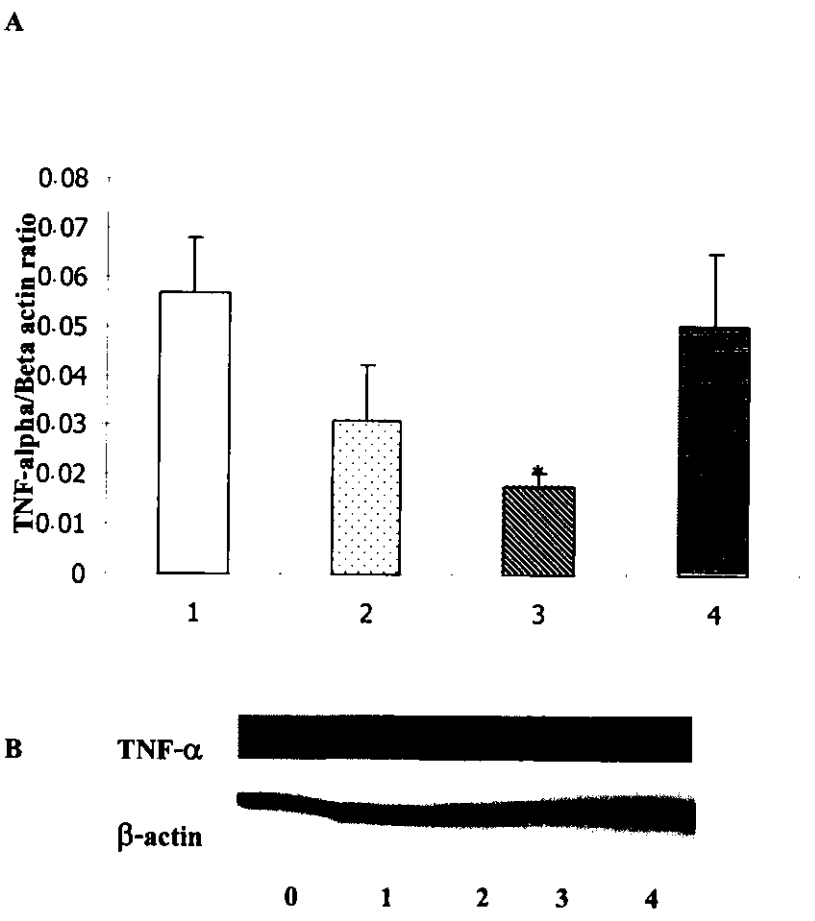


Figure 27 Protein expression of TNF-α in the gastric mucosa of rats at the 14th day after ulcer induction

Densitometric analysis of TNF-α protein expression assessed by Western blot and expressed as each cytokine per β-actin ratio (A) in normal gastric mucosa (lane 0); in mucosa around ulcer treated with vehicle (lane 1); in curcumin-treated rats (40 mg/kg *p.o*, bid) (lane 2); in curcumin-treated rat (80 mg/kg p.o, bid) (lane 3); or in aminoguanidine-treated rat (30 mg/kg s.c, od) (lane 4). Mean ± S.E.M of 6 rats.

*$p < 0.05$ compared to the vehicle control-treated rats (Dunnett's test).

Representative Western blot analysis of TNF-α or β-actin is presented in (B).

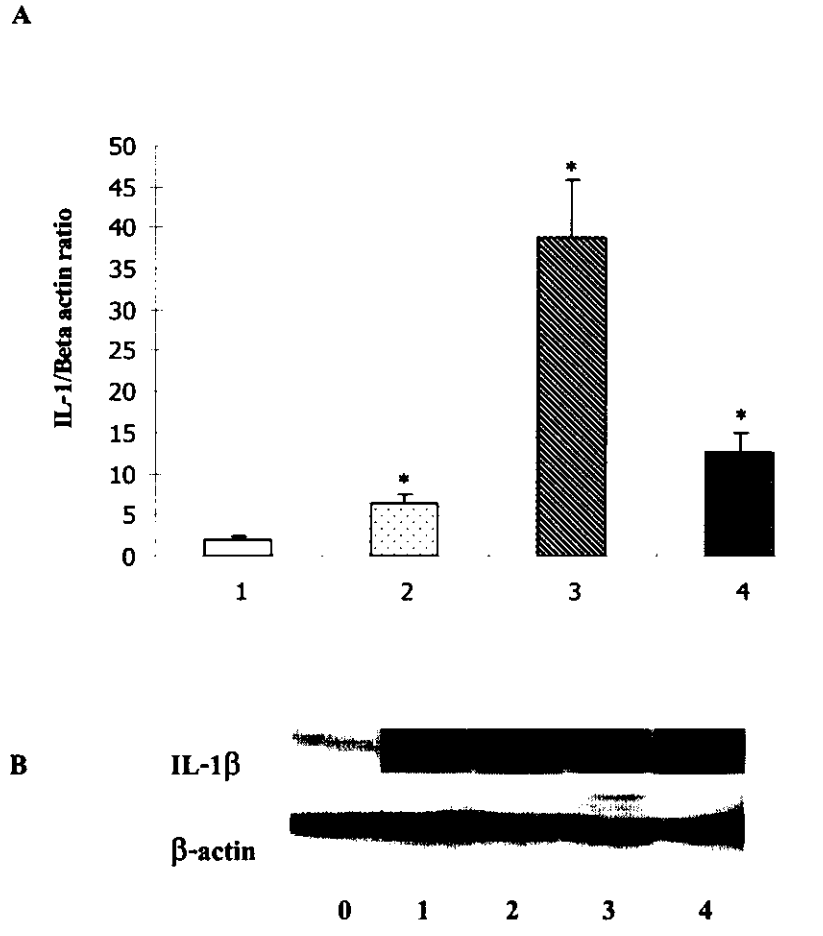


Figure 28 Protein expression of IL-1β in the gastric mucosa of rats at the 14th day after ulcer induction

Densitometric analysis of IL-1β protein expression assessed by Western blot and expressed as each cytokine per β-actin ratio (A) in normal gastric mucosa (lane 0); in mucosa around ulcer treated with vehicle (lane 1); in curcumin-treated rats (40 mg/kg *p.o*, bid) (lane 2); in curcumin-treated rat (80 mg/kg p.o, bid) (lane 3); or in aminoguanidine-treated rat (30 mg/kg s.c, od) (lane 4). Mean ± S.E.M of 6 rats.

*$p < 0.05$ compared to the vehicle control-treated rats (Dunnett's test).

Representative Western blot analysis of IL-1β or β-actin is presented in (B).

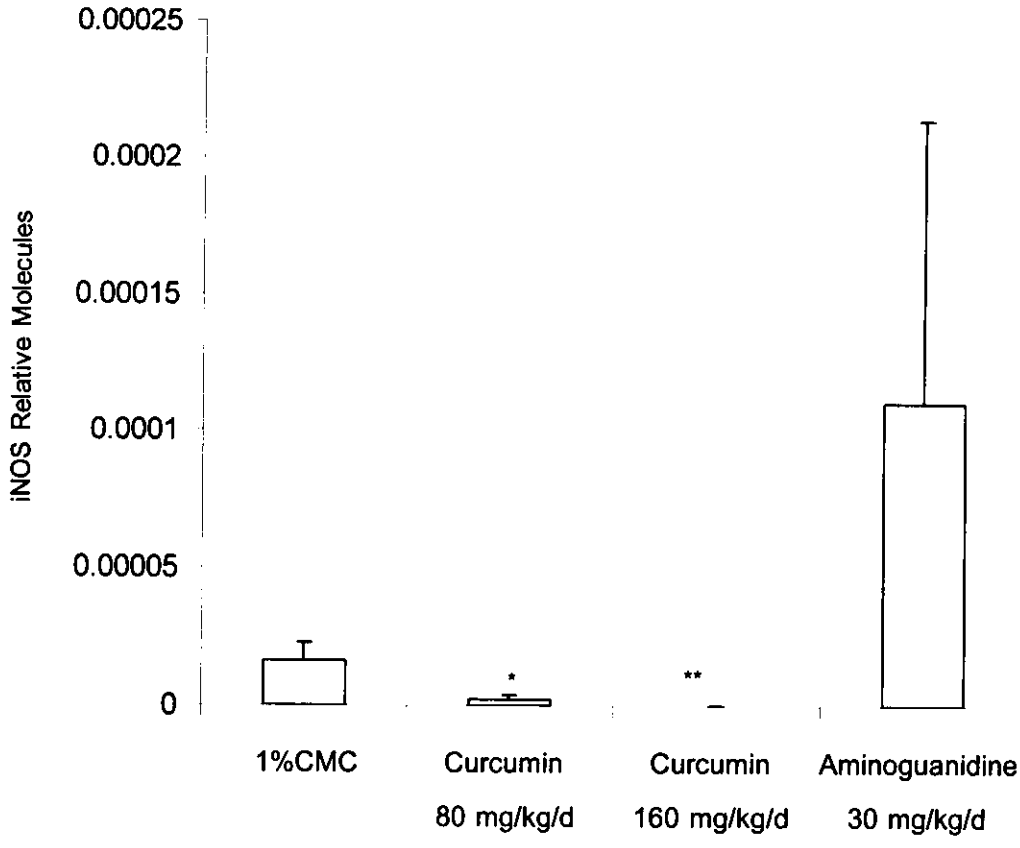


Figure 29 Expression of iNOS mRNA by realtime RT-PCR in the gastric mucosa of rats at the 14th day after ulcer induction

Values are expressed as mean ± S.E.M (N=6).

*$p < 0.10$ compared to the vehicle control-treated rats. (Dunnett's test).

**$p < 0.05$ compared to the vehicle control-treated rats (Dunnett's test).

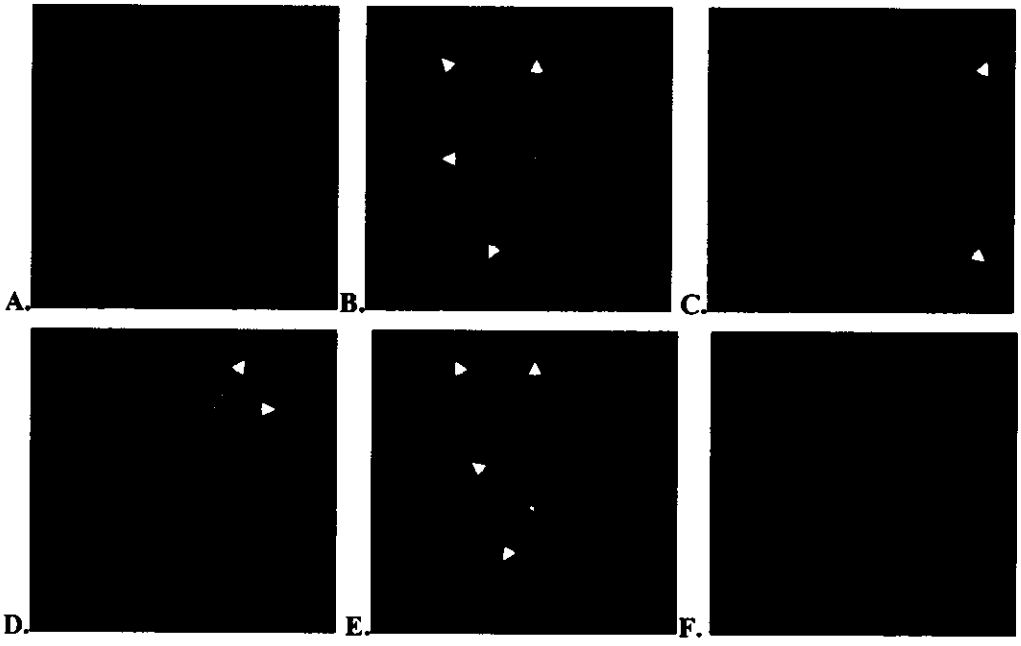


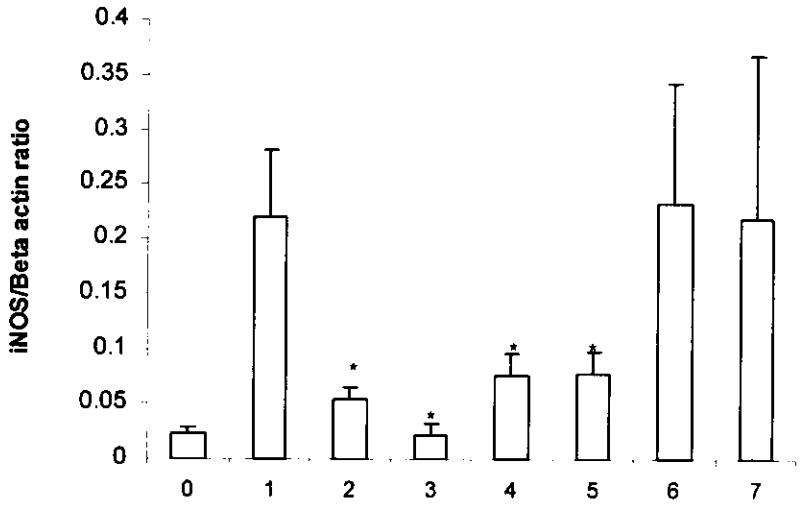
Figure 30 iNOS and TNF-α immunoreactivity in the gastric tissues of non-ulcer rat and ulcer rat treated with vehicle (1% CMC), curcumin (40 mg/kg *p.o*, bid), and aminoguanidine (30 mg/kg *s.c*, od) at the 14th day after ulcer induction

A: In the non-ulcer mucosa, iNOS or TNF-α immunoreactivity was not detected. **B:** In the vehicle control-treated mucosa, iNOS-positive inflammatory cells were found in the submucosa around vessels at the margin of a gastric ulcer. **C:** In the curcumin–treated mucosa, iNOS-positive inflammatory cells and epithelial cells decreased and were seen at the basal portion of the regenerating mucosa. **D:** In the aminoguanidine–treated mucosa, more amounts of iNOS-positive epithelial cells and inflammatory cells were seen at the ulcer bed than that of the curcumin–treated mucosa. **E:** In the vehicle control-treated mucosa, TNF-α positive inflammatory cells were found in the submucosa at the margin of a gastric ulcer. **F:** In the curcumin–treated mucosa, TNF-α immunoreactivity was barely seen.

7. Effect of curcumin, vanillin and tetrahydrocurcumin on LPS-induced iNOS, COX-2 and TNF-α protein and mRNA expression in RAW 264.7 cells

From Western blot analysis, either iNOS, COX-2 or TNF-α protein expression in unstimulated RAW 264.7 was not detectable. The protein expression level of these cytokines was markedly augmented in response to LPS (1 μg/ml). Co-incubation of macrophages with LPS plus curcumin significantly inhibited iNOS, COX-2 and TNF-α protein induction in a dose–dependent manner after 16 h of incubation (Figure 31-33). Only weaker suppression of iNOS protein induction was found in the presence of LPS plus vanillin. Tetrahydrocurcumin had no significant inhibitory effect on iNOS or COX-2 protein induction. However, low dose (3 μM) of tetrahydrocurcumin significantly inhibited TNF-α protein induction. RT-PCR and real time PCR analysis showed that the decrease in iNOS and COX-2 protein level by curcumin correlated with its decrease in iNOS and COX-2 mRNA expression (Figure 31-34). However, curcumin had no effect on the level of TNF-α mRNA expression.

A



B. iNOS

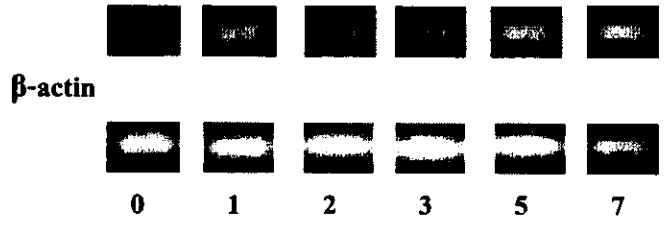


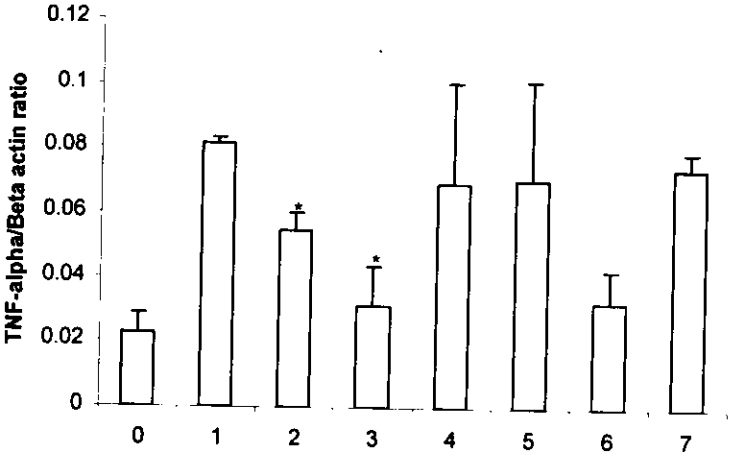
Figure 31 Effect of curcumin, vanillin and tetrahydrocurcumin on LPS-induced iNOS protein and mRNAexpression in RAW 264.7 cells

RAW 264.7 cells were cultured in the presence of LPS (1 μg/ml) with or without curcumin, vanillin and tetrahydrocurcumin for 16 h. **(A)** At the end of the incubation time, the cytosol protein fractions were subjected to Western blot analysis for iNOS protein analysis. **(B)** At the end of the incubation time, cells were lysed and total RNA was prepared for the RT-PCR analysis of gene expression. iNOS specific sequence (807bp) was detected by agarose gel electrophoresis. PCR of β-actin was performed to control similar initial cDNA content of samples. The values are expressed as mean ± S.E.M from three independent experiments.

*$p < 0.05$ compared to the LPS-treated group (Dunnett's test)

Lane 0: control; Lane 1: LPS; Lane 2: curcumin 3 μM; Lane 3: curcumin 10 μM; Lane 4: vanillin 3 μM; Lane 5: vanillin 10 μM; Lane 6: tetrahydro--curcumin 3 μM; Lane 7: tetrahydrocurcumin 10 μM.

A.



B. TNF-α

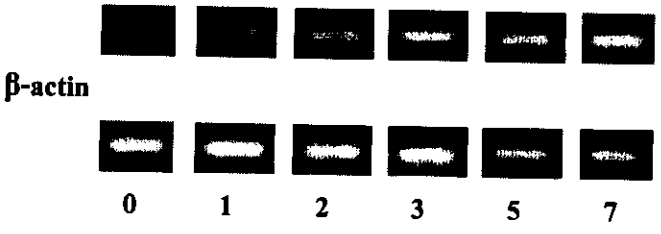


Figure 33 Effect of curcumin, vanillin and tetrahydrocurcumin on LPS-induced TNF-α protein and mRNA expression in RAW 264.7 cells

RAW 264.7 cells were ultured in the presence of LPS (1 μg/ml) with or without curcumin, vanillin and tetrahydrocurcumin for 16 h. **(A)** At the end of the incubation time, the cytosol protein fractions were subjected to Western blot analysis for TNF-α protein analysis. **(B)** At the end of the incubation time, cells were lysed and total RNA was prepared for the RT-PCR analysis of gene expression. TNF-α specific sequence (351bp) was detected by agarose gel ctrophoresis. PCR of β-actin was performed to control similar initial cDNA content of samples. The values are expressed as mean ± S.E.M from three independent experiments.

$^{**}p < 0.05$ compared to the LPS-treated group (Dunnett's test)

Lane 0: control; Lane 1: LPS; Lane 2: curcumin 3 μM; Lane 3: curcumin 10 μM; Lane 4: vanillin 3 μM; Lane 5: vanillin 10 μM; Lane 6: tetrahydro--curcumin 3 μM; Lane 7: tetrahydrocurcumin 10 μM.

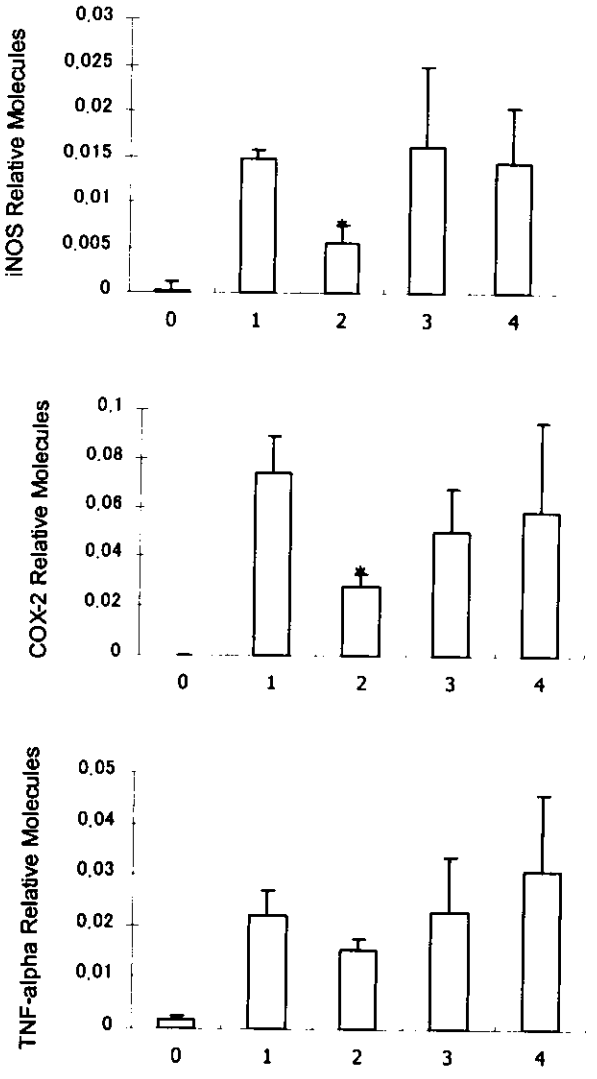


Figure 34 Effect of curcumin, vanillin and tetrahydrocurcumin on LPS-induced iNOS, COX-2 or TNF-α mRNA expression in RAW 264.7 cells

RAW 264.7 cells were cultured in the presence of LPS (1 μg/ml) with or without curcumin, vanillin and tetrahydrocurcumin for 16 h. At the end of the incubation time, cells were lysed and total RNA was prepared for the quantitative real time RT-PCR analysis of gene expression. Values are expressed as mean ± S.E.M. The experiment was repeated three times with similar results. *p< 0.05 compared to the LPS-treated group (Dunnett's test). Lane 0: control; Lane 1: LPS; Lane 2: curcumin 10 μM; Lane 3: vanillin 10 μM; Lane 4 : tetrahydrocurcumin 10 μM.