

GASTROPROTECTIVE EFFECT OF CURCUMINE ON ETHANOL-INDUCED GASTRIC MUCOSAL LESIONS IN RATS

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ABSTRACT

This study was conducted to investigate the protective effect of curcumine (50 mg/kg/day; 100 mg/kg/day and 200 mg/kg/day orally for 15 days) in ethanol-induced gastric mucosal lesions in male Wistar rats. Ulceration was induced in rats by administering 50 % ethanol orally. On day 16, the stomach was examined for ulcer by the severity of hemorrhagic erosions in acid secreting glandular mucosa. Total acid and peptic activity were determined in gastric juice using hemoglobin as substrate. Reduce glutathione (GSH) and glutathione peroxidase (GPX) were also estimated from gastric mucosa. Pretreatment with curcumine 100 mg/kg/day and 200 mg/kg/day for 15 days significantly reduced the incidence and severity of gastric erosions induced by ethanol. Curcumine treatment also favorably altered changes in volume and peptic activity of gastric juice in ethanol-treated animals. Furthermore, the levels of GSH and GPX were significantly decreased after treatment with ethanol, and this decrease was prevented by curcumine. The study provides evidence for possible involvement of glutathione in the curcumine-mediated gastroprotection against ethanol-induced ulceration.

Keywords: curcumine, ethanol, gastric mucosal lesions

INTRODUCTION

Gastric ulcers are caused due to imbalances between offensive and defensive factors of the gastric mucosa. The regulation of mucosal microcirculation is intimately involved in the maintenance of gastric integrity and endogenous nitric oxide (NO) has been established to have a role in this regulation (Pique, 1992; Tepperman, 1992). Reduced glutathione (GSH) is also important for mucosal integrity since depletion of GSH from the gastric mucosa by electrophilic compounds induces macroscopic mucosal ulceration (Boyd, 1981; Ghanayem, 1985). A number of antiulcer drugs like gastric anti secretory drugs-H₂ receptor antagonists, antimuscarinic agents, proton pump inhibitors, mucosal protective agents—carbenoxolone sodium, sucralfate and prostaglandin analogues are available which are shown to have side effects and limitations (Pique, 1992). Recent advances in curcumine research have revealed diverse pharmacotherapeutic effects like hypocholesterolemic, antihyperglycemic, anti carcinogenic and antiatherosclerotic effects (Sudjarwo, 2001). In order to ascertain whether the antiulcer effect is also present in curcumine, we evaluated in a rat model of gastric ulcer. The aim of the present study was to investigate the protective role of curcumine on ulceration induced by ethanol and to evaluate GSH and NO status in the gastroprotection afforded by curcumine.

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MATERIAL AND METHODS

Animals

Male Wistar rats weighing 120-180 g were fed with standard pellet diet (Comfeed, Indonesia) and were provided water ad libitum. Animals were housed under standard environmental conditions. All animal experiments were carried out according to the guidelines of the Institutional Animals Ethics Committee.

Antiulcer studies

Ulceration was induced in rats by the administration of 50 % ethanol orally at a dose of 5 ml/kg. The experiments used 30 rat were divided into negative control group (6 rat were given aquadest 5 ml/kg/day), positive control group (6 rat were given 50 % ethanol as described below) and treatment group (each dose consisted of 6 rats were given curcumine at 50 mg/kg BW, 100 mg/kg BW and 200 mg/kg BW) orally once daily for 15 days. At the end of 15 days the rats were fasted for 24 h and on day 16, the pylorus was ligated by the method of Shay et al in order to collect the gastric juice for analysis. In the ethanol-treated group, animals were administered with ethanol (50% at a dose of 5 ml/kg orally) once just after 24 h fasting on day 16, and then the pylorus was ligated 1 h after drug administration. The rats were sacrificed by exsanguination 3 h after pyloric ligation, the stomach was removed, and the gastric secretion was collected for

analysis. The stomach was cut open along the greater curvature and examined for ulcers.

Ulcer index

After sacrificing the rat, the stomach was removed and opened along the greater curvature, and the severity of hemorrhagic erosions in the acid secreting glandular mucosa was assessed on a scale of 0 to 3: 0= normal, 1= one to four petechiae, 2= five or more petechiae or hemorrhagic streaks up to 4 mm and 3= erosions longer than 5 mm or confluent haemorrhages (Maity, 1998).

Gastric juice

Three hours after pyloric ligation the rats were sacrificed by exsanguination and the stomach was removed. Gastric juice was collected, centrifuged for 5 min at 3000 X g and the supernatant was separated (Maity, 1986). Total acid was estimated by titrating with 0.01 N NaOH using phenolphthalein as an indicator and expressed as mEq/l. Peptic activity was determined using hemoglobin as substrate and expressed as moles of tyrosine/ml.

Assay of reduced glutathione (GSH) and glutathione peroxidase (GPX)

For the GSH assay, the stomach was perfused intraluminally with 5% sulphosalicyclic acid and then homogenized in the same solution (10%). The tissue homogenate was centrifuged for 5 min at 10,000 X g, and the amount of GSH was measured in the supernatant according to Griffith (Griffith, 1980). GPX in the gastric mucosa was assayed

spectrophotometrically by the method of Gunzler and Flohe (Gunzler, 1985) with t-butyl hydroperoxidase as the substrate. The assay is based on the oxidation of GSH by GPX coupled to the oxidation of NADPH by glutathione reductase. The rate of NADPH oxidation was monitored spectrophotometrically.

Statistical analyses

Data of independent observations are shown as the mean \pm SEM. Statistical analysis was performed using one-way analysis of variance (ANOVA) and Tukey-Kramer multiple comparisons test was used for post hoc analysis. $p < 0.05$ was considered as statistically significant.

RESULTS

Effect of Curcumine on Gastric Mucosal Lesions

Administration of ethanol (positive control) resulted in severe erosions in all the animals treated. However, curcumine (100 mg/kg BW and 200 mg/kg BW) pretreatment significantly reduced the severity and incidence of gastric erosions in ethanol-administered animals (Table 1). The antiulcer effect of curcumine was found to be increased with increasing doses with the maximum effect observed at 200 mg/kg body weight (Table 1). Therefore, this dose of curcumine was used in all subsequent experiments on gastric secretion and GSH estimation. While the stomach of rats not treated with curcumine prior to ethanol administration looked thin and fragile, those of rats treated with curcumine prior to ethanol administration looked relatively healthy.

Table 1. Effect of curcumine on ethanol-induced gastric ulceration in rats

Treatment	Gastric erosion (Mean \pm SEM)
Aquadest	0.22 \pm 0.15
Aquadest + Ethanol	3.4 \pm 0.41
Curcumine 50 mg/kg + Ethanol	2.9 \pm 0.53
Curcumine 100 mg/kg + Ethanol	1.8 \pm 0.77
Curcumine 200 mg/kg + Ethanol	1.1 \pm 0.65

Effect of Curcumine on Gastric Secretion

Treatment with ethanol significantly reduced the total acid and the volume of gastric secretion but markedly increased the peptic activity. Curcumine (100 mg/kg BW and 200 mg/kg BW) pretreatment significantly

increased the volume of gastric secretion and markedly reduced peptic activity in comparison with ethanol-treated rats (Table 2). Total acid, however, was marginally increased in the case of curcumine pretreatment as compared to ethanol-treated rats.

Table 2. Effect of curcumine on gastric secretion in rats subjected to ethanol treatment

Treatment	Volume (ml/h)	Total acid (mEq/l)	Peptic activity (mol/ml)
Aquadest	1.8 ± 0.04	143.8 ± 5.1	64.3 ± 2.7
Aquadest + Ethanol	0.9 ± 0.05	79.6 ± 4.3	77.2 ± 1.9
Curcumine 50 mg/kg + Ethanol	0.8 ± 0.03	82.9 ± 7.6	75.6 ± 3.8
Curcumine 100 mg/kg + Ethanol	1.2 ± 0.07	102.7 ± 6.9	69.7 ± 2.5
Curcumine 200 mg/kg + Ethanol	1.5 ± 0.05	133.2 ± 8.3	61.4 ± 2.1

Effect of Curcumine on Ethanol Induced Changes in GSH and GPX

Since GSH is intimately associated with the prevention of gastric erosions, it was thought worthwhile to measure GSH and GPX in gastric mucosa of ethanol-treated rats subjected to curcumine pretreatment. As shown in Table 3, ethanol reduced the GSH level in gastric mucosa. However, curcumine (100 mg/kg BW and 200 mg/kg BW) pretreatment was found to increase

GSH levels significantly in ethanol-treated rats (Table 3). There were significant differences between the ethanol-treated group and curcumine (100 mg/kg BW and 200 mg/kg BW) pretreatment group ($P < 0.01$). Similar to GSH, ethanol treatment resulted in a reduction in the GPX level, and the curcumine (100 mg/kg BW and 200 mg/kg BW) pretreatment affected a significant increase of this enzyme level as compared to ethanol-treated group (Table 3).

Table 3. Effect of curcumine on reduced glutathione (GSH) and glutathione peroxidase (GPX) activity in rats

Treatment	GSH (mol/g wet weight)	GPX (mU/mg protein)
Aquadest	1.52 ± 0.08	283.7 ± 10.1
Aquadest + Ethanol	0.76 ± 0.04	176.3 ± 9.7
Curcumine 50 mg/kg + Ethanol	0.83 ± 0.05	182.9 ± 7.6
Curcumine 100 mg/kg + Ethanol	1.14 ± 0.04	227.1 ± 13.4
Curcumine 200 mg/kg + Ethanol	1.35 ± 0.09	253.9 ± 12.6

DISCUSSION

It is evident from the results of the present investigation that curcumine possesses antiulcer activity in ethanol-induced acute ulcer model. It has shown a significant reduction in the gastric lesions of the ethanol treated pylorus ligated group of animals. Pyloric ligation was done only to collect the gastric content for analysis. Although the etiology of gastric ulcer is not known in most cases, it is generally accepted that it results from an imbalance between aggressive factors and the maintenance of mucosal integrity through the endogenous defense mechanisms (Piper, 1986). To regain the balance, different therapeutic agents including curcumine are used (in experimental animals) to inhibit the gastric acid secretion or to boost the mucosal defense mechanisms by increasing mucus production, or stabilizing the surface epithelial cells (Pal, 1991; Piper, 1986). In the present study, the marked

reduction in the volume of gastric secretion and total acidity associated with ethanol induced ulceration support the suggestion that acidity or gastric secretion (considered as aggressive factors for ulceration) may play a major role in the ulcer induced by ethanol. Decrease in mucosal resistance is also considered important in the etiology of gastric ulceration (Goel, 1985). It was observed that curcumine pretreatment alone could reverse the increased peptic activity associated with ethanol. The results also show that protein content of the gastric juice is significantly increased by ethanol. This could be due to leakage of plasma proteins into the gastric juice (Grossman, 1978) with weakening of the mucosal resistance/barrier of the gastric mucosa. Prior administration of curcumin to the group of rats treated with ethanol caused a significant decrease in protein content of the gastric juice which suggests that curcumine might primarily decrease the leakage of plasma proteins into the gastric juice with

strengthening of the mucosal barrier and increase in its resistance to the damaging effect of ethanol. However, the possibility of change in protein content as a result of change in gastric mucus components cannot be ruled out. Another major feature of curcumin-induced reversal of the effect of ethanol is the significant increase in GSH levels in gastric mucosa. The GSH level in gastric mucosa was significantly decreased in rats in which ulceration was induced by ethanol as compared to control rats. This suggests that GSH has a role to play in the ulcerogenesis induced by ethanol. The observation that curcumin could reverse the GSH level to a great extent provides evidence for the involvement of GSH in the antiulcer activity of curcumin. Such a conclusion is further strengthened by the observation that curcumin normalises the decreased gastric mucosal GPX activity in ethanol treated animals. The antioxidant activity of GPX is coupled with the oxidation of GSH to GSSG, which can subsequently be reduced by GSH reductase with NADPH as the reducing agent. GPX is important for the elimination of hydrogen peroxide and lipid hydroperoxides in the gastric mucosal cell (Gunzler, 1985). Thus, inhibition of this enzyme activity in the gastric mucosa by ethanol may result in the accumulation of hydrogen peroxide with subsequent oxidation of lipids. The reversal of GPX activity in ethanol treated animals by curcumin may therefore be due to the replenishment of GSH level.

Ethanol treatment caused a significant increase in the ulcer index whereas in curcumin pretreated rats there was a significant reduction in the ethanol effect. There are extensive experimental evidences that indicate that free radical scavengers protect the gastric mucosa (Goel, 1985). The reduction in the nucleic acid concentrations of the stomach in the ethanol-induced rats might be due to the accumulation of free radicals as free radicals induce significant damage to DNA (Szabo, 1981). Curcumin pretreatment offered protection against the action of ethanol on nucleic acid content showing that the presence of some antioxidant phyto constituents might have protected the gastric mucosa from free radical-induced damage. Curcumin, having antioxidant property (Rajakumar, 1994; Osawa, 1995). may therefore, exhibit the anti-ulcer action by scavenging free radicals generated in the injured mucosa or by inhibiting inactivation of endothelium derived NO by O₂. It may further be mentioned that in the present study, the NO mediated protection of curcumin pretreatment in ethanol-treated rats may be the result of an increase in the GSH level in gastric mucosa. GSH, present in high concentration in mammalian cells, has been suggested to play a possible role in NO synthesis, as a reducing cofactor for NO production (Mizui, 1987; Stamler, 1992) or more likely by preventing early inactivation of NOS by radical intermediates or NO

itself (Griscavage, 1994). The overall results presented here suggest that besides GSH involvement, NO also has an important role to play in the gastroprotective mechanism of curcumin on gastric ulceration induced by ethanol.

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