

Gastroprotective effect of *Cissus quadrangularis* extract in rats with experimentally induced ulcer

Mallika Jainu, K. Vijai Mohan & C.S. Shyamala Devi

Department of Biochemistry, University of Madras, Chennai, India

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Background & objectives: Most of the non-steroidal anti-inflammatory drugs (NSAIDs) including aspirin cause gastric ulcer. In order to study the gastroprotective effect of *Cissus quadrangularis* extract (CQE), this study was undertaken on aspirin-induced ulcerogenesis in pyloric ligated (ASP-PL) model in rats.

Methods: To assess the possible antiulcer effect of CQE, lesion index, gastric secretions glycoprotein levels, non-protein sulphhydryls (NPSH) and adherent mucus content were determined in ASP-PL induced gastric mucosal injury in rats.

Results: Pretreatment with CQE significantly prevented the gastric mucosal lesion development and decreased the gastric toxicity produced by ulcerogen. In addition, ulcerated rats showed depletion of gastric wall mucus, glycoproteins and NPSH levels whereas treatment with CQE reverted this decline in ASP-PL induced rats. Histological studies confirmed the results.

Interpretation & conclusion: The present finding suggests that CQE promotes ulcer protection by the decrease in ulcer index, gastric secretions and increase in the glycoprotein level, gastric mucin content and NPSH concentration. CQE may protect the gastric mucosa against ulceration by its antisecretory and cytoprotective property.

Key words Antiulcer activity - aspirin - *Cissus quadrangularis* extract - histopathology - mucus content

Non-steroidal anti-inflammatory drugs (NSAIDs) associated gastric ulceration occurs in 30 per cent of users that led to hospitalization and is also associated with high mortality¹. Aspirin (ASP) one of the most widely used NSAIDs, damages gastrointestinal mucosa by irritant action, causing alterations in mucosal permeability and/or suppression of prostaglandin synthesis².

Plant extracts are attractive sources of new drugs and have been shown to produce promising

results in the treatment of gastric ulcers³. *Cissus quadrangularis* Linn. (Vitaceae) commonly known as 'bone setter', is frequently used as a common food item in India⁴. The stout fleshy quadrangular stem of *C. quadrangularis*, is an edible plant found throughout the hotter parts of India, Malaya, West Africa and Ceylon⁵. The stem is used for the treatment of eye and ear diseases, irregular menstruation, asthma, piles, tumours, fractures of bones, wounds and scurvy⁶. Previous studies revealed that this plant possesses analgesic⁷,

antioxidant⁸ and fracture healing property⁹. The analysis showed that the stem of *C. quadrangularis* contains 398 mg of vitamin C, 267 mg of β -carotene and 0.73 per cent of calcium¹⁰. The stem has been used in the treatment of stomach ulcer and dyspepsia in traditional systems of medicine in India¹¹, which make it a potential antiulcer drug for evaluation. Preliminary studies have shown that *C. quadrangularis* extract (CQE) is well known to stimulate cell proliferation, gastric mucus synthesis and secretion in indomethacin-induced gastric ulcer model¹².

Experimental studies to determine the role of CQE in gastric ulcer are very limited. Apart from the antiulcerogenic activity of CQE, it is also important to monitor acid output and status of the mucosal barrier in gastric ulcer. The present study was therefore undertaken to describe the effect of CQE on acid-pepsin secretion, mucus content and sulphhydryl groups in aspirin-induced ulcerogenesis in pyloric ligated (ASP-PL) rat models, which might be useful for the treatment of gastric ulcer.

Material & Methods

Drugs and chemicals: Haemoglobin, alcian blue, Tris-EDTA, 5,5'-dinitrobenzoic acid (DTNB), sodium acetate, sucrose, trichloro acetic acid were all purchased from the Sigma Chemical Company, USA. Aspirin (ASP) was obtained from SRL, India. All other reagents used for the experiment were of analytical grade.

Preparation of *C. quadrangularis* extract: The stem of *C. quadrangularis* was purchased from Native Care and Cure Center, Chennai, India, and authenticated with the standard sample preserved in Pharmacognosy Department, Captain Srinivasa Murthy Drug Research Institute for Ayurveda, Chennai. Dried parts were coarsely powdered and 1 kg of this powdered plant material was soaked in 2 l methanol for 48 h, and the extract was filtered and distilled on a water bath. The last traces of the solvent were removed under vacuum drier and

the solid brown mass obtained was stored at -4°C until further use. The yield of the extract was 5.2 per cent w/w of powdered methanolic extract. For administration, the extract was suspended in distilled water.

Animals: Male albino rats weighing 175-200g purchased from Tamil Nadu University of veterinary and Animal Sciences, Chennai, were housed at 27 \pm 2°C, 55 per cent humidity, and a 12:12 h light - dark cycle. They were fed with standard laboratory chow (Hindustan Lever Foods, Bangalore, India) and provided with water *ad libitum*. Experimental protocols were approved by the institutional ethical committee for animal experimentation.

Toxicity studies: For acute oral toxicity studies, rats were divided into 5 groups of six animals each. Group I served as control received only distilled water while groups II, III, IV, and V were orally fed with CQE at the doses 0.5, 1.5, 3.0 and 5.0 g/kg b. w/day respectively for 14 days. On day 14, the animals were sacrificed, blood was collected and analyzed for red blood cell count (RBC), white blood cell count (WBC), haemoglobin (Hb), haematocrit (HCT), mean corpuscular volume (MCV), blood sugar, cholesterol and protein with the use of autoanalyzer (Hitachi 911, Germany).

Treatment protocol for antiulcer activity: Animals were divided into 4 groups of six animals each.

Group 1— received distilled water orally for 7 consecutive days.

Group 2— received distilled water orally for 7 consecutive days. On the last day rats were given aspirin (ASP) (200 mg/kg body weight) suspended in 1 per cent carboxymethyl cellulose orally¹³.

Group 3— received pretreatment with CQE (500 mg/kg body weight) orally for 7 consecutive days. On the last day 30 min after the extract treatment, rats received ASP (200mg/kg body weight).

Group 4— Received CQE (500 mg/kg body weight) alone orally for 7 consecutive days.

The dosage and duration of treatment for the above mentioned groups have been fixed based on the previous results¹⁴. At the end of the experimental period, the animals were fasted for 24 h and care was taken to avoid coprophagy. Animals were anaesthetized using pentobarbitone (35 mg/kg body weight, i p), the abdomen was opened and pyloric end of the stomach was ligated without causing any damage to its blood supply. The stomach was replaced carefully and the abdomen wall was closed in two layers with interrupted sutures. The animals were recovered fully from the anaesthetic agent and deprived of water during post-operative period. Aspirin was administered orally to the animals of groups 2 and 3 after pyloric ligation. After 4 h of aspirin induction, animals were sacrificed and stomach was dissected out after tying the oesophageal end. The stomach was cut open along the greater curvature and the contents were collected into tubes, centrifuged at 1000 rpm for 10 min and the sediment/supernatant used for the estimation of various biochemical parameters. The stomach was then inflated with normal saline and the inner surface is examined for ulceration as described by Szabo *et al*¹⁵. Erosion of at least 1mm in diameter were considered and the sum of the length of all lesions per stomach was calculated for each group of rats (in mm).

Total acid output of gastric juice was determined by titrating with 0.01N NaOH, using phenolphthalein as indicator and was expressed as $\mu\text{Eq}/4\text{h}$. Peptic activity was determined using haemoglobin as substrate and was expressed as μmol of tyrosine/4h¹⁶. Dissolved mucosubstances were estimated in 90 per cent alcoholic precipitate of the gastric juice. The precipitate thus obtained was either dissolved in 1 ml of 0.1N NaOH or 1 ml H_2SO_4 . The former was used for the estimation of protein¹⁷, total hexoses¹⁸, hexosamine¹⁹ and fucose²⁰, while the latter was used for the estimation of sialic acid²¹.

Assessment of adherent gastric mucus content: Alcian blue binding to gastric wall mucus was

determined by the method of Corn *et al*²². Animals from all groups were sacrificed, the gastric mucosal tissues were scrapped, weighed and incubated in tubes containing 1 per cent alcian blue solution (0.16M sucrose in 0.05M sodium acetate, pH 5.8) for 2 h. The alcian blue binding extract was centrifuged at 3000rpm for 10 min and the absorbance of supernatant was measured at 489nm.

Estimation of non-protein sulphydryl (NPSH) groups: Gastric mucosal NPSH was determined by the method of Sedlak and Lindsay²³. 200mg gastric mucosal tissues were homogenized in 2.0ml of 20 mM EDTA at 4°C in a homogenizer. To measure NPSH content, 2.0 ml of water was added to 1.0 ml of homotenate which was then treated with 1.0 ml of 10 per cent TCA and centrifuged. From this, 2.0 ml of supernatant was taken and treated with 4.0ml of Tris-EDTA (pH 8.0) and 0.1ml of DTNB in methanol. The contents were mixed well and absorbance read at 412 nm.

Histological studies: Gastric tissue samples from each group were fixed in 10 per cent formalin for 24 h. The formalin fixed specimens were embedded in paraffin, sectioned (3.5 μm) and stained with haematoxylin and eosin. The histochemical sections were evaluated by light microscopy.

Statistical analysis: The data were analysed using Dunnett's T3 multiple comparison test and ANOVA (one-way analysis of variance) using SPSS package version 7.0.

Results

ASP-PL induced animals showed extensive gastric lesions that were confined to the glandular portion of the stomach as compared to control rats ($P < 0.001$). In contrast, oral administration of CQE at 500 mg/kg body wt. for 7 days before ulcer induction lowered the lesion index values significantly ($P < 0.001$) in group 3 rats. No gastric mucosal injury was seen in either the control animals or in those given CQE alone orally (Table I).

Table I. Effect of *C. quadrangularis* extract (CQE) on gastric lesions in aspirin- induced ulcerogenesis in pyloric ligated (ASP-PL) rats

Treatment	Dose (mg/kg body wt.)	Lesion index (mm)
Control	-	-
ASP-PL	200	15.6 ± 1.07*
ASP-PL + CQE	500	3.18 ± 0.33†
CQE	500	-

Values are mean ± SD for 6 animals in each group. * $P < 0.001$ compared to control group; † $P < 0.001$ compared to ASP-PL group

Pretreatment with CQE showed significant antiulcer effect ($P < 0.001$) by reducing the volume of gastric juice, acid output, pepsin output, protein level and a significant increase ($P < 0.001$) in pH and ($P < 0.001$) glycoprotein levels such as total hexose, hexosamine, fucose, sialic acid and total carbohydrate: protein ratio when compared to group 2 animals. CQE *per se* in group 4 rats showed

non-significant changes in these parameters when compared with control (Table, II).

A significant decrease ($P < 0.001$) in adherent gastric mucosal content was seen in ASP-PL induced rats when compared to control rats. Pretreatment with CQE significantly attenuated these changes and showed a protective effect on gastric mucosa in ASP-PL induced rats. CQE *per se* in group 4 rats showed significant ($P < 0.005$) elevation in adherent gastric mucosal content as compared to control group (Fig.1). ASP-PL was found to significantly ($P < 0.001$) decrease NPSH concentration in the gastric mucosa of group 2 rats as compared to control group. Administration of CQE (500 mg/kg body weight) brought about a significant increase ($P < 0.001$) in NPSH concentration in ASP-PL induced rats (group 3) as compared to group 2 animals (Fig.1).

In the microscopic observation of control rats showed normal appearance of gastric mucosa (Fig.2a). ASP-PL induced rats (Fig.2b) showed ulcer

Table II. Effect of CQE on gastric mucosal factors in experimental gastric ulcer model

Parameters	Group 1	Group 2	Group 3	Group 4
Volume (ml/100g/4h) of gastric juice	2.89±0.18	2.47±0.17*	2.41±0.11†	2.01±0.08*
Acid output (mEq/100g/4h)	336. ±18.4	304.6±12.9**	297.3±15.3†	281.4±12.7*
pH	3.56±0.28	2.02±0.26**	2.94±0.18†	3.61±0.21*
Pepsin output (µmol/4h)	827.2±45.8	781.6±48.0*	799.2±42.4†	756.0±25.6*
Mucoproteins (µg/ml)				
Total hexoses	387.5±11.1	296.3±8.7**	318.9±9.9†	389.3±10.5
Hexosamine	186.2±11.1	134.2±6.9**	174.3±13.2†	190.8±16.8
Fucose	78.5±2.0	52.9±1.5**	65.6±1.8†	77.3±1.1
Sialic acid	40.5±1.6	27.1±1.4**	32.4±1.5†	42.55±1.7
Total carbohydrates (TC)	692.7±31.7	509.9±32.0**	599.2±33.8†	699.9±37.3
Protein (P)	405.2±10.2	540.6±12.9**	452.5±12.6†	394.6±14.7
TC:P	1.70±0.10	0.94±0.05**	1.32±0.07†	1.77±0.09*

Values are mean±SD for 6 animals in each group. * $P < 0.05$ and ** $P < 0.001$ compared to group 1; † $P < 0.001$ compared to group 2

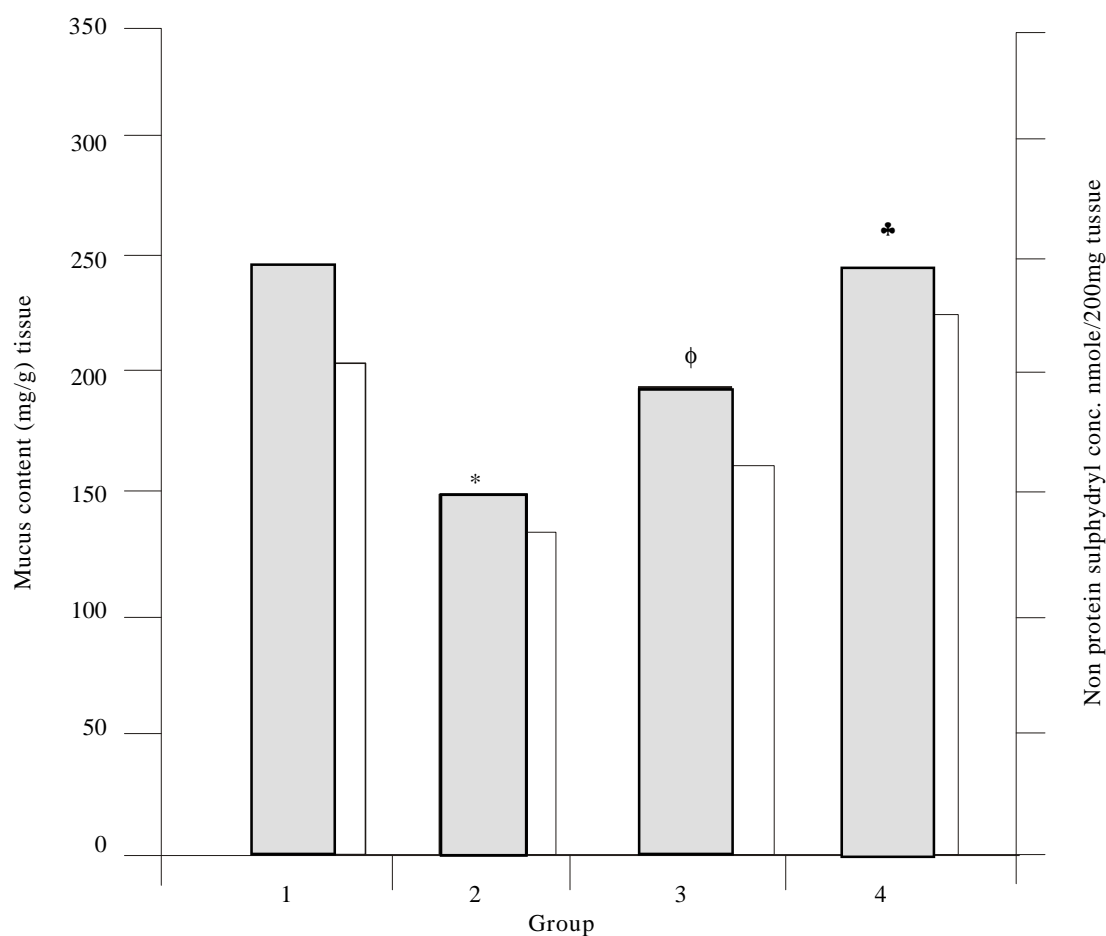


Fig.1. Effect of CQE on adherent mucus content (■) in control and experimental group of rats. Each values represents mean \pm SD for 6 animals. * $P < 0.05$ and $^{\#}P < 0.001$ significantly different from control rats (group 1); $^{\#}P < 0.001$ significantly different from ASP-PL induced group (group 2). Effect of CQE on non-protein sulphydryl (NPSH) (□) concentration in the gastric mucosa of control and experimental groups. Each value \pm SD for 6 animals in each group. * $P < 0.001$ significantly different from control rats (group 1); $^{\#}P < 0.001$ significantly different from ASP-PL induced group (group 2).

crater with distorted gastric glands, damaged mucosal epithelium, inflammatory exudates and cellular debris were found in ulcerated wall of the stomach. Protection against these histopathological changes was observed by apparent epithelializations, glandular organization, maintenance of mucularis mucosa and reduced size of ulcer crater in CQE pretreated rats (Fig. 2c). The histological picture of CQE alone treated rats showed normal cytoarchitecture of gastric mucosa with no pathological changes (Fig. 2d).

Discussion

The present results suggested that pretreatment with CQE markedly ameliorated the ulcer index, histological and biochemical changes of ASP-PL induced gastric ulceration in rats. Administration of aspirin produces severe gastric haemorrhagic erosions and has not been found to increase the aggressive factors (acid and pepsin) but significantly decreased the gastric output because of so called back diffusion of HCl through the broken barrier,

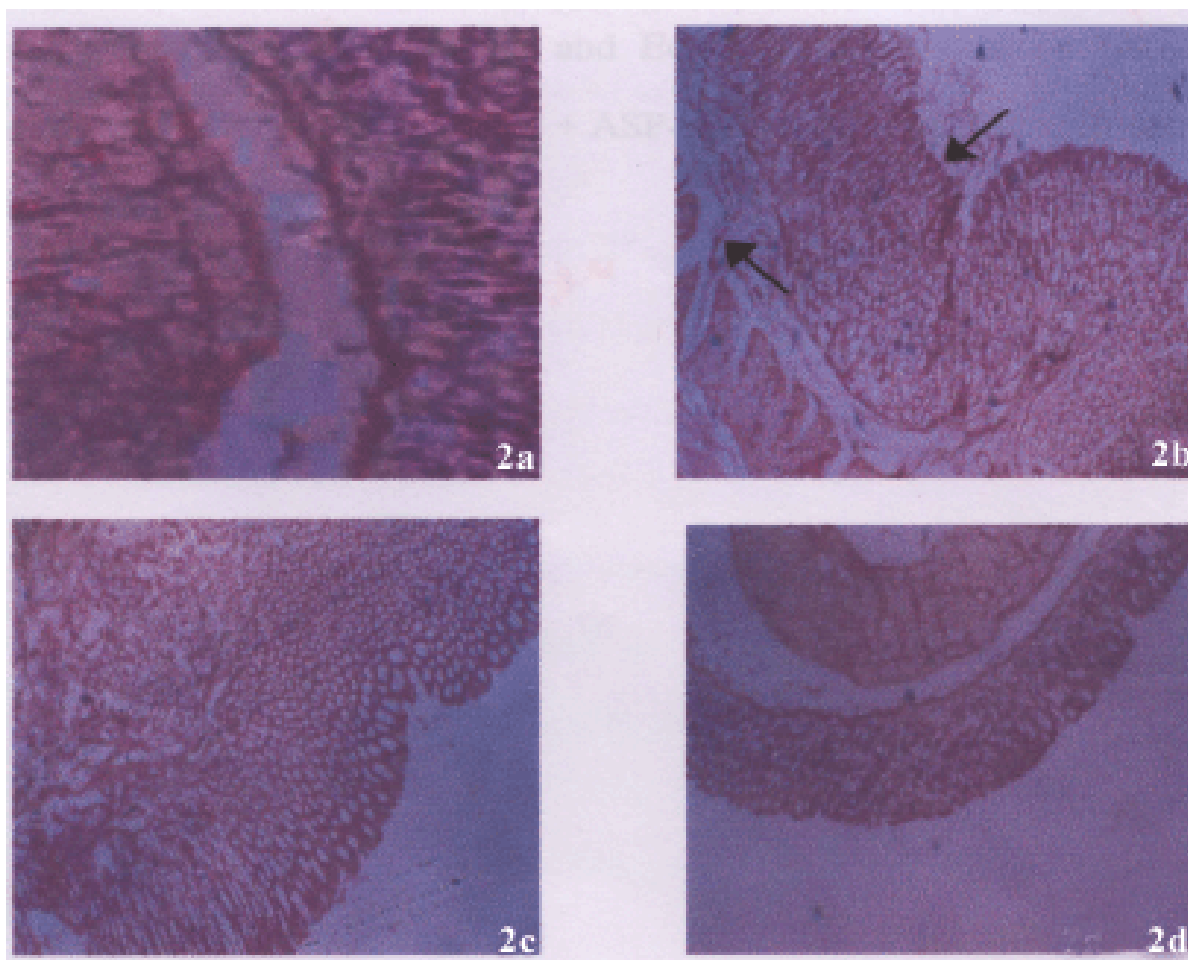


Fig.2. Histological examinations of gastric mucosal tissue sections of control and experimental rats (hematoxylin and eosin, 20×). Control rat (**2a**); ASP-PL-induced rats (**2b**); CQE pretreated + ASP-PL induced rats (**2c**); CQE alone treated rats (**2d**).

inhibition of mucosal blood flow and acute inflammation^{3,26} that is consistent with the present report. CQE demonstrated significant antiulcer effect by decreasing the ulcer lesions, volume of gastric juice, acid output and pepsin output and an increase in *pH*. The CQE not only reduced the volume of gastric secretion, but it also decreased total acid output and pepsin concentration indicating its antisecretory effect.

Mucus secretion is a crucial factor in the protection of gastric mucosa from the gastric lesions and has been regarded as an important defensive factor in the gastric mucus barrier. A decrease in

the synthesis of sulphated mucus glycoprotein has been implicated in the aetiology of gastric ulcer²⁴. The increase in total carbohydrate :protein (TC:P) ratio is the direct reflection of mucin activity, which is indicated by the enhanced level of individual mucopolysaccharides like hexose, hexosamine, fucose and sialic acid²⁵. Decrease in protein content in the gastric juice also signifies decrease in leakage from the mucosal cells indicating mucosal resistance. The wide distribution of adherent mucus content in the gastrointestinal tract plays a pivotal role in cytoprotection and repair of the gastric mucosa²⁶. The results showed increased levels of adherent mucus content of gastric tissue pretreated

with CQE indicating its cytoprotective action on experimentally induced gastric ulcer.

In the present study, the ulcerated region had less mucosal NPSH content than the intact region in normal rats. An increase in NPSH content limits the production of oxygen free radicals and could be related with gastric protection in ASP-PL model. In the present study, rats pretreated with CQE showed a significant increase in NPSH concentration, which might attribute to its direct cyroprotection and antioxidant activities.

In histological study, pretreatment with CQE was found to preserve the functional cytoarchitecture of the entire gastric mucosa. CQE treatment showed not only the maintenance but also the regeneration of gastric mucosa in the damaged regions. These findings confirm the cytoprotective nature of CQE¹².

The significant reduction in basal gastric secretion and ulcers formation by CQE after pylorus ligation suggests that the cytoprotective mechanism on the extract on gastric mucosa may involve direct reduction of gastric secretion. *C. quadrangularis* significantly reduced gastric secretion and acidity, which might be due to the enhancement of mucus and HCO₃ secretion by CQE²⁷. We have earlier reported that CQE prevented indomethacin-induced ulcer lesions by its cytoprotective property¹². The antiulcer activity of CQE might be attributed to the presence of biological compounds such as triterpenoids, glycosides, saponins, tannins, β -sitosterol and aminoacids²⁸ in the extract. Triterpenoids and glycosides have been shown to inhibit gastric acid secretion and enhancement in gastric mucus content against several experimental ulcer models^{29,30}. These plant constituents present in CQE might have the ability to protect against ulceration induced by ASP-PL.

In conclusion, CQE showed significant antiulcer activity in experimentally induced ulcer in rat model

by decreasing the gastric secretions and by enhancing glycoprotein levels. Further studies are needed to isolate and purify the active principle involved in the antiulcer activity of this plant.

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Reprint requests: Dr Mallika Jainu, Old No. 285, New No. 32, Royapettah High Road
Chennai 600014, India
e-mail: malsleo80@yahoo.co.in

