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The Anti-ulcerogenic Effect of an Alkaloidal Fraction from *Mikania cordata* on Diclofenac Sodium-induced Gastrointestinal Lesions in Rats

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Abstract

A decoction of *Mikania cordata* (Compositae) is commonly used for the treatment of gastric ulcer in the Rajbari district of Bangladesh. We have evaluated the anti-ulcerogenic effect of the alkaloidal fraction from the whole plant of *M. cordata* on diclofenac sodium-induced gastrointestinal lesion in rats.

Long Evan's rats were divided into five groups. The control group was kept undisturbed. The vehicle group received vehicle after a 48-h fast. The diclofenac group received diclofenac sodium suspension ($80\,mg\,kg^{-1}$) after a 48-h fast. The diclofenac-ranitidine group (anti-ulcer drug used as a standard) received $35\,mg\,kg^{-1}$ ranitidine hydrochloride suspension 1 h after diclofenac sodium administration, after a 48-h fast. The diclofenac-extract group received alkaloidal fraction ($50\,mg\,kg^{-1}$) 1 h after diclofenac administration, after a 48-h fast. The biochemical, morphological and histological changes were studied.

The data showed that the pH values of the stomach and duodenum were increased significantly ($P < 0{\cdot}001$) in the alkaloidal-administered group compared with the control group ($3{\cdot}09 \pm 0{\cdot}0429$ vs $2{\cdot}07 \pm 0{\cdot}0339$ and $6{\cdot}79 \pm 0{\cdot}1162$ vs $6{\cdot}19 \pm 0{\cdot}1273$, respectively). There were significant changes ($P < 0{\cdot}001$) detected in the morphological study. The ulcer index of the stomach ($0{\cdot}268 \pm 0{\cdot}0346$) and of the duodenum ($0{\cdot}050 \pm 0{\cdot}0129$) in the alkaloidal-administered group were significantly lower than the diclofenac-only administered group ($0{\cdot}691 \pm 0{\cdot}0184$ and $0{\cdot}093 \pm 0{\cdot}0138$, respectively). According to the grading of tissue damage in the histological study, there were less or no lesions on the gastrointestinal mucosa of the alkaloidal-administered group compared with the diclofenac-only group (0 vs 5, respectively). When the results of the alkaloid extract group where compared with the ranitidine hydrochloride group a similar or more potent effect was observed with the alkaloidal extract group.

The results of this study revealed that the bioactive principles of *M. cordata* have anti-ulcerogenic effects. The results validate the traditional use of this plant for the treatment of gastric ulcer in Bangladesh.

Gastric ailments, particularly ulcers are a worldwide problem. It is generally accepted that ulcers result from an imbalance between gastric aggressive factors and mucosal defensive factors (Sun 1974). For several decades, the main aggressive components reported have been acid and pepsin. Thus ulcers have been predominantly treated with antacids, H_2-receptor antagonists and proton pump inhibitors (Wallace et at 1990; Wallace & Granger 1996). However, conventional drugs such as ranitidine hydrochloride, cemitidine, pirenzepine and omeprazole are sometimes inadequate and have adverse effects such as hepatitis, constipation, photosensitivity, agranulocytosis, pancytopenia, diarrhoea, male breast tenderness and mental confusion (Douglas 1980; Satoskar et al 1986; Reminton's Pharmaceutical Sciences 1988; Craig & Stitzel 1997).

Therefore, it is necessary to search for effective and safe alternative drugs for the treatment of ulcer.

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Plants provide an alternative strategy in the search for new drugs for the treatment of gastrointestinal disorders (Lewis & Hanson 1991). Recent extensive phytochemical work and pharmacological screening of plant constituents has increased the acceptability of herbal medicine in the treatment of ulcer as found in India, Israel, China, Japan, Taiwan and even in Western countries (Craker & Simon 1986). Many natural products of herbs are used in the traditional medicine of Bangladesh for the treatment of gastrointestinal disorders. It was reported that in the village of Ramdia of Baliakandi Thana in the Rajbari district, *Mikania cordata* (Compositae), locally known as Paharilata or Asamilata, was used as a decoction for the treatment of gastric ulcers. This report prompted us to look for an anti-ulcer agent in the herb. The same plant is used for the treatment of skin diseases (Kirtikar & Basu 1980).

Ahmed (1990) described the isolation and identification of a flavonoid named mikanin (3,5 didydroxy-4′,6,7-trimethoxy flavone) from *M. cordata*. Previous studies have shown that *M. cordata* has several terpenoids such as friedelin, epifriedilinol, mikanolide, dihydromikanolide, deoxymikanolide, scandenolide, dihydroscandenolide, miscandinin, kaurenic acid, and 15 α-benzoyloxy kaurenic acid. Whether these compounds have anti-ulcer activity has not been reported. Moreover, the plant may have other constituents (alkaloids) responsible for anti-ulcer activity. Therefore, we have evaluated the anti-ulcerogenic effect of the alkaloidal fraction from the whole plant of *M. cordata* on diclofenac sodium-induced gastrointestinal lesions in rats using morphological and histological methods. Diclofenac sodium is used frequently as an inducer of experimental gastrointestinal lesions (Talukder 1993).

The anti-ulcerogenic properties of *M. cordata* were investigated by measuring the pH value of the gastric contents, the ulcer index and the state of tissue damage of gastrointestinal mucosa in rats. We also compared the anti-ulcerogenic activity of the alkaloidal fraction from *M. cordata* with that of ranitidine hydrochloride, an agent known to inhibit histamine-stimulated gastric acid secretion.

Materials and Methods

Plant materials

M. cordata is a climbing plant and occurs naturally in various districts of the Southern and Western regions of Bangladesh. Whole plant of *M. cordata* was collected from the Rajbari district of Bangladesh and authenticated by the Bangladesh National Herbarium, were a representative specimen of the plant has been preserved. The plants were sun dried and pulverized then stored as a powder in polythene bags.

Extraction of alkaloidal fraction

The plant material (1 kg) was extracted with 95% ethanol in a suitable airtight container for three days. The ethanol extract was then filtered off by cotton pad and concentrated at 50–60°C in a rotary evaporator under reduced pressure to obtain a deep greenish mass (25 g). The crude ethanol extract was treated with 3 M H_2SO_4 to convert the alkaloid to its water-soluble salts and then this acid-treated solution was filtered. The filtrate (alkaloid sulphate) was then basified with concentrated NH_4OH and the basic aqueous solution was extracted with chloroform to afford the alkaloidal fraction (Furniss 1978). The alkaloidal fraction was concentrated using a rotary evaporator under reduced pressure at 50°C to obtain the chloroform or alkaloidal extract (2 g).

Test of alkaloidal fraction

A dried sample (10 mg) of chloroform extract was dissolved in 1 M H_2SO_4 and filtered. A few drops of the clear aqueous solution were treated with two drops of Dragendroff's reagent in a watch glass. A brick-red participate developed. Similarly, when the clear aqueous solution was treated with Mayer's and Wagner reagent, a white and a brownish participate developed, respectively (Paech & Tracey 1970). The presence of an alkaloid in chloroform was confirmed further by TLC screening of the extract on silica gel using the solvent system ethanol : ethyl acetate (3 : 1), which developed one single brown spot (R_f 0·57) after sparing with Dragendroff's reagent (Harborne 1984).

Chemicals

Diclofenac sodium, ranitidine hydrochloride, Tween 80 and carboxy methyl cellulose were collected from Beximco Pharmaceuticals Ltd, Dhaka. The pH indicator papers were manufactured by Merck (Germany). Normal saline, haematoxylin and eosin (H & E), and Periodic acid schiff (PAS) stains were all analytical grade.

Animals

Long Evan's rats (180–290 g) were collected from the Animal Resources Branch of the International Center for Diarrhoeal Research, Bangladesh. The study was

performed at the Department of Pharmacology, Rajshahi Medical College, Rajshahi. The rats were kept individually in numbered iron cages for two weeks before treatment. They were fed a balanced diet (Hawk et al 1954) and tap water, under standard conditions of a 12-h dark–light cycle, 60 ± 10% humidity and a temperature of 21·5 ± 1·0°C. These protocols were approved by the Institutional Animal Care and Use Committee of UNICAMP, which follows the recommendations of the Canadian Council on Animal Care (Olfert et al 1993).

Grouping and administration

Ranitidine hydrochloride, diclofenac sodium or the test sample (alkaloid fraction) of *M. cordata* were dispersed in normal saline with 0·5% CMC (Shriver et al 1975) in such a way that required the dose of the drug to be present in 1 mL of the suspension. The drugs (or vehicle) were introduced into the oesophagus of the rat using a feeding tube. The rats were divided into five groups, four animals per group (two male and two female). Control group; the rats were kept undisturbed and allowed free access to food and water. Vehicle group; 1 mL vehicle (normal saline plus 0·5% CMC) was administrated to each rat after 48-h fasting. Diclofenac group; diclofenac sodium suspension ($80\,mg\,kg^{-1}$) was administrated to each rat after 48-h fasting with free access to water. Diclofenac-ranitidine group; each rat received $35\,mg\,kg^{-1}$ ranitidine hydrochloride suspension 1 h after diclofenac sodium ($80\,mg\,kg^{-1}$) administration, after 48-h fasting. Diclofenac-extract group; each rat was administered $50\,mg\,kg^{-1}$ alkaloidal fraction suspension after 48-h fasting and 1 h after $80\,mg\,kg^{-1}$ diclofenac sodium administration.

Determination of gastric and duodenal pH

The rats were killed 18 h after drug (or vehicle) administration by cervical dislocation under chloroform anaesthesia. Stomach and small intestine were dissected from the abdominal cavity and the duodenum was separated from the rest of the small intestine. Generally, stomach and duodenum were opened along the greater curvature and the antimesenteric side, respectively (Habib et al 1994). The pH value of the fluid on the gastric corpus mucosa and corpus mucosa of the duodenum was determined using pH indicator paper (Merck).

Measurement of morphological changes

After cleaning in 10% normal saline for 10 min, stomach and duodenum were pinned on a wax plate with the mucosa facing up. Gross lesions of the stomach and duodenum were measured under a dissecting microscope with a measuring eyepiece (Habib et al 1993). For the linear gastric corpus mucosal lesions the ulcer index was expressed as the sum total of the length (mm) of the individual lesions. For the rounder intestinal lesions the ulcer index was expressed as the sum total area (mm^2) of the individual lesion (Satoh et al 1981).

Histological examination

The tissues were fixed in 10% normal saline and histological slides of representative areas of stomach and duodenum were performed using haematoxylin and eosin, and Periodic acid schiff (PAS) stains (Begum 1993). Histological grading of damage was made following the method of Lacy & Ho (1982).

The stomach of a Long Evan's rat consists of a pro-ventricular and a glandular part. Structurally, the distal 5–10 mm of the glandular part is called the pyloric part and the remaining glandular part is called the corpus. The mucous membrane of the oesophagus continues into the pro-ventricular part.

Structurally the corpus of the stomach of a Long Evan's rat is homologous to the fundus and body of the human stomach. Like human small intestine, that of the Long Evan's rat consists of duodenum, jejunum and ileum with similar structure. Approximately 85% of the total length is the jejunum and the ileum is the smallest distal part (Hebel & Stromberg 1976).

Statistical analysis

Results are presented as the mean ± s.e.m or s.d. Student's *t*-test was used for comparison between the experimental and control groups. $P < 0{\cdot}001$ was considered to be statistically significant.

Results

pH measurements

The pH values of the gastric and duodenum content are shown in Table 1. The value was raised significantly in the diclofenac-extract group compared with the control group ($P < 0{\cdot}001$), diclofenac group ($P < 0{\cdot}001$) and diclofenac-ranitidine group ($P < 0{\cdot}001$).

Morphological changes

There was no ulceration on the mucosa of stomach or duodenum in the control and vehicle groups. In

Table 1. pH of the stomach and duodenum.

Animal group	Drug used	Dose ($mg\,kg^{-1}$)	Stomach pH (mean ± s.e.m.)	Duodenum pH (mean ± s.e.m.)
Control	Undisturbed	–	2·0 ± 0·039	6·2 ± 0·1273
Vehicle	Vehicle	–	2·0 ± 0·0293	6·1 ± 0·0826
Diclofenac	Diclofenac sodium	80	2·1 ± 0·0281	6·0 ± 0·0778
Diclofenac–ranitidine	Ranitidine hydrochloride	35 + 80	2·2 ± 0·0415**	6·0 ± 0·0580
Diclofenac–extract	Alkaloidal fraction	50 + 80	3·1 ± 0·0427**	6·8 ± 0·1162**

Data analysis was performed by Student's *t*-test for multiple companions. Data are expressed as means ± s.e.m for four animals per group. $^{**}P < 0{\cdot}001$ values are compared with control.

Table 2. Histomorphological changes of stomach and duodenum.

Animal group	Ulcer index of stomach (mean ± s.e.m.)	Ulcer index of duodenum (mean ± s.e.m.)	Grading S.T.D.	Grading D.T.D.
Control	0	0	0	0
Vehicle	0	0	0	0
Diclofenac	0·691 ± 0·0184**	0·093 ± 0·0138**	5	1
Diclofenac–ranitidine	0·289 ± 0·0306**	0·033 ± 0·0084**	3	0
Diclofenac–extract	0·268 ± 0·0346	0·050 ± 0·0129	0	0

Data analysis was performed by Student's *t*-test for multiple companions. Data are expressed as means ± s.e.m for four animals per group. $^{**}P < 0{\cdot}001$ values are compared with control. S.T.D. = stomach tissue damage; D.T.D. = duodenum tissue damage.

the diclofenac-only group the mucosa of the stomach and duodenum showed significant ulceration and perforation (ulcer index: 0·691 ± 0·0184 and 0·093 ± 0·0138, respectively).

In the diclofenac-ranitidine group, there was statistically significant ulceration in the stomach and in the duodenum (ulcer index: 0·289 ± 0·0306 and 0·033 ± 0·0084, respectively) compared with the control and vehicle groups. The ulcerative index for the stomach of the alkaloidal fraction group was insignificant against the diclofenac-ranitidine group (ulcer index: 0·268 ± 0·046 vs 0·289 ± 0·0306). The ulcerative index of the duodenum (0·050 ± 0·0129) was only significantly different compared with the control group. Morphological changes for stomach and duodenum are presented in Table 2.

Histological findings

In the stomach and duodenum of the diclofenac group there was erosion and inflammatory changes in the mucosa (Table 2), compared with the normal histological configuration (Table 3). The mucosa of the duodenum (grading state 1) was found to have less damage than that of stomach (grading state 5). A similar result was seen for stomach and duodenum of the diclofenac–ranitidine group, grading value 3 and 0, respectively. In the diclofenac–extract group, no perforation was observed in either gastric or duodenum mucosa. Duodenum mucosa was damaged less, relatively, than gastric mucosa.

Discussion

It is evident that the plant kingdom may be considered to be a potential source of anti-ulcer agents (Yao 1986). A variety of plant constituents, particularly the alkaloids, have been found to have anti-ulcer properties. Craker & Simon (1986) reported that atropine from *Atropa belladona*, 5-hydroxytryptamine from *Musa paradisiaca* and coryloid, and a mixture of alkaloids from *Corydalis bulbosa* had anti-ulcer activity. Moreover, Souza Brito et al (1997) showed that the lyophilized aqueous extract of *Dalbergia monetaria* had significant anti-ulcer activity against gastric ulcer lesion.

A variety of different methods has been adopted to evaluate the anti-ulcerogenic effect of a drug against induced gastrointestinal lesions. These have included gross morphological evaluation (Satoh et al 1982), biochemical techniques (Whittle & Steel 1985), computer based morphometric analysis (Guglietla et al 1990), and a group of miscellaneous methods such as urinary recovery index of phenol red (Nakamura et al 1983) or measurement of the tensile strength (Ezner 1987). Each of those methods has some limitation and fails to provide detailed information.

In this study diclofenac sodium, a non-steroidal anti-inflammatory drug (NSAID), was used to induce gastrointestinal damage. It is well known that

NSAIDs induce gastric mucosal damage and the "dual insult" hypothesis is the popularly accepted theory for induced gastric damage (Schoen & Vender 1989). The mechanism of small intestinal damage is still uncertain. Several precipitating factors are reported e.g. enterohepatic circulation of the drug, inhibition of prostaglandin synthesis, over growth of some bacteria in the intestinal lumen and inhibition of sodium active transport (Choudhury & Jacobson 1978; Bayless 1989).

Our results (Table 1) revealed that the pH of stomach and duodenum of the control, vehicle and diclofenac groups were identical, indicating no significant increase on acid secretion. Similar results were reported by Habib et al (1993). The diclofenac-ranitidine group was found to have a slight increase in gastrointestinal pH due to the inhibitory effect on HCl secretion through the H_2-receptor antagonism by ranitidine hydrochloride (Al-Ghamdi et al 1991). The diclofenac–extract group was found to have a similar effect as that of the diclofenac–ranitidine group. Thus the pH changes were noticed in the diclofenac–extract group only, which may be attributed to the effect of the alkaloidal fraction on the H_2-recptor mediated HCl secretion.

From the result of the morphological study (Table 2), the diclofenac group appeared to be the most affected by ulceration compared with the diclofenac–ranitidine group and diclofenac–extract group, whilst the control and vehicle groups were free of any gastrointestinal mucosal damage. The diclofenac–ranitidine group displayed a lesser degree of ulcerative damage in duodenum compared with the diclofenac–extract group, which in turn appeared to be more effective in the stomach than the diclofenac–ranitidine group. A lesser degree of ulcerative damage in the diclofenac–ranitidine group may be due to the effect of ranitidine hydrochloride, because this drug inhibits HCl secretion and makes it possible to maintain the gastrointestinal pH at a reasonably high level and thus reduces the damaging power of diclofenac sodium. Sachs et al (1994) reported that the mucosal damaging effect of diclofenac sodium appeared to be augmented in a low pH environment.

Histological analysis is essential for a precise evaluation of type and severity of damage, though it is expensive and time consuming (Lo et al 1988). In this study, histological examination revealed that diclofenac sodium induced acute haemorrhagic erosive gastritis, the extreme pattern of acute inflammation of gastric mucosa (Dayal & Delellis 1989). After microscopic examination of gastrointestinal mucosa it was shown that the alkaloidal fraction ($50\,mg\,kg^{-1}$) appeared to have an anti-ulcer effect when compared with the control. However, the effect was observed when an alkaloidal fraction was administrated before lesion induction, which means that the alkaloidal fraction had a preventive anti-ulcerogenic effect.

The mechanism by which the alkaloidal fraction reduced the degree of diclofenac sodium-induced ulceration as well as isolation and identification of the bioactive compound needs further investigation. The mechanism may be one or more of the following possibilities. Activation of cyclooxygenase enzyme and subsequent stimulation of prostaglandin synthesis, which is responsible for the formation of gastrointestinal mucosa and increasing bicarbonate level in the gastrointestinal lumen (Curtis et al 1995; Eberhart & Dubois 1995; Threvethick et al 1995). The alkaloidal fraction may inhibit the action of diclofenac sodium and stimulate the synthesis of prostaglandin. Like ranitidine hydrochloride, the alkaloidal fraction may increase gastrointestinal pH and/or shows anti-ulcer activity by H_2-receptor antagonism (Al-Ghamdi 1991). Finally, the mechanism of the anti-ulcer effect of the alkaloidal fraction may be an anticholinergic action evoked by muscarinic receptor blockers resulting in an inhibition of vagus stimulation. In some people the vagus may be stimulated by foods and may secrete high amounts of HCl (Culshaw 1988). This stimulation may be inhibited by the alkaloidal fraction.

Our results suggest that the alkaloidal fraction from the whole plant of *M. cordata* may have a beneficial preventative effect on gastric ulcers. The results support the use of this plant in Bangladeshi traditional medicine for the treatment of gastric ulcers.

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