Research Article

Gastroprotective activity of *Asystasia gangetica* stem aqueous extract against pylorus ligated gastric ulcer in rats

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ABSTRACT

Background: *Asystasia gangetica,* a medicinal plant commonly known as Maithal Kadi available in Udupi district in India. It is used as a fresh juice to treat gastric ulcers, rheumatism, inflammation, swelling by the folklore practitioners. The present studies were aimed to investigate the antiulcer activity of aqueous extract of *Asystasia gangetica* stem to provide scientific validation for its folklore use.

Materials and Methods: Healthy Wistar albino rats were divided into three different groups of six rats each. The rats were pre-treated with group specific drugs. Group I assigned as control, group II received the standard drug omeprazole 20 mg/kg, group III received aqueous extract of stem of the plant *Asystasia gangetica* 400 mg/kg once daily for seven consecutive days. On seventh day, one hour after drug administration, the gastric ulcer was induced by ligation of the pyloric part of the rat's stomach. This was followed by the macroscopic examination of the stomach, quantification of gastric juice, total and free acidity, peptic activity and histological examination of stomach tissue were performed. The test extract were subjected for preliminary phytochemical analysis as per standard methodology.

Results: The test drug treated group showed that there is a significant reduction in the total acidity and marked reduction in ulcer index, the volume of gastric juice and the effect was comparable with that of omeprazole. Histological examination revealed a normal cytoarchitecture with reduced gastric lesions in group III compared to group I.

Conclusion: The present finding suggests that *Asystasia gangetica* promotes ulcer protection as ascertained by the comparative decreases in total acidity, gastric lesions and peptic activity.

Key words: *Asystasia gangetica*, pylorus ligation, omeprazole, gastro protection, leukocyte, ulcer index.

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INTRODUCTION

Gastric ulcer affects a considerable number of people worldwide and in the United States approximately 500,000 people are affected by gastric ulcer each year.[1] The proton pump inhibitors have become classic anti ulcer therapy for the treatment of peptic ulcer, duodenal ulcer, gastroesophageal reflux disorders and other gastrointestinal infection, but there is still no definite cure for this disease.[2,3] Long term use of the drugs may lead to ineffectiveness of different drug regimen and emergence of drug resistance.[4] The wide ranges of synthetic pharmacological agents have been used to ameliorate gastric ulcer symptoms but have limited clinical efficacy. The medicinal plants constitute the cornerstone of traditional practice worldwide. They are easily available and are great reservoir of drugs with structurally diverse

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molecules. Thus plants make a valuable source of novel lead compounds.[5]

Asystasia gangetica (AG) is commonly known as Maithal kaddi in coastal Karnataka of India. Several medicinal uses have been documented on various parts of the plant in India and Africa. It has been used as a folk remedy to treat asthma, helminthiasis, inflammation, wound healing, rheumatism, intestinal astringent and in irritable bowel syndrome.[6] The stem of this species is squeezed in the water and used internally in the form of dishes in gastric ulcers, swelling, and rheumatism by traditional practitioners of Udupi district.[7]

Scientific evaluation on AG have been revealed several pharmacological activity posed by the plant AG leaves have been reported to exhibit significant antidiabetic, antiasthma and Anthelminthic activities.[8,9] The phytochemical analysis revealed the presence of flavonoids and glycosides in the plant.[10,11] The present studies was aimed to investigate the antiulcer activity of aqueous extract of *Asystasia gangetica* stem (AAGS) to provide scientific validation for its folklore use.

MATERIALS AND METHODS

Plant Material

AG plants were collected from SDM medicinal plant garden during December 2011. It was authenticated by the department of Pharmacognosy at SDM Centre for Research in Ayurveda and Allied Sciences, Udupi. A voucher specimen (No. 199/12121701) has been deposited for future reference.

Preparation of aqueous extract

The AG stem was shade dried and pulverized and finely sieved. The 500 g of plant stem powder was soaked in 2 L of distilled water for 24 h, after which it was filtered. The filtrate was evaporated in a rotator evaporator and used for the experimentation.

Phytochemical analysis

The preliminary phytochemical analysis of *A. gangetica* stem aqueous extract was carried out for coumarins, carbohydrates, saponins, flavonoids, triterpenoids, tannins and alkaloids.

Experimental Animals

Albino rats of wistar strains of either sex between 150 to 250 g body weights were obtained from animal house attached to department of Pharmacology, SDM Centre for Research in Ayurveda and Allied Sciences, Udupi. The experimental protocol was approved by the institutional animal ethical committee (Ref.no: SDMCRA/IAEC-2011-12DG-06). The animals were fed with normal rat diet and water *ad libitum* throughout the study. They were acclimatized in the laboratory condition for two weeks prior to the experimentation. The housing provided had the following conditions: controlled lighting of 12:12h light and dark cycle, temperature of 25ºC and relative humidity of approximately 50%.

Acute oral toxicity test

Acute oral toxicity was performed following OECD-425 guidelines using acute oral toxicity study (AOT) software. Albino rats of either sex selected by random sampling were used for acute toxicity study. The animal were kept fasting for overnight and provided only with water. The test drug was administered at a dose of 175, 550 up to 2000 mg/kg (up and down method) and observed for 14 days. If any mortality was observed the same dose repeated again to confirm its toxic potential. If mortality was not observed the procedure was repeated for higher doses.

Pylorus ligation induced ulceration

Pyloric ligation was carried according to the method described by Shay *et al.*[12,13] Eighteen Wistar albino rats weighing between 200 ± 40 g were selected for the study and divided into three groups of six animals each. Group I received tap water and served as pyloric ligated control group. Group II received omeprazole (20 mg/kg) served as reference standard and Group III received aqueous extract

of AG stem (400 mg/kg). The test drug, reference standard and vehicles were administered orally once daily for seven consecutive days. All the animals were fasted for 36 h by placing them in metabolic cages to prevent coprophagy, but provided free access to water *ad libitum* prior to pyloric ligation. On seventh day one hour after test drug administration pylorus were ligated by following the method of Shay *et al* (1945).[12,13]

Rats were anesthetized and the portion of abdomen was cut opened in layer by a small midline incision just below and lateral to the xiphoid process. Pyloric portion of the stomach was slightly lifted out avoiding traction to the pylorus or damage to its blood supply. The pylorus was ligated with cotton thread and stomach was replaced carefully. The incision was closed with interrupted sutures in layers. The animals were deprived of both food and water during the postoperative period and were sacrificed under anaesthesia at the end of ten hour of pyloric ligation procedure.

The abdomen was opened and a ligature was placed around the oesophagus; stomach was removed and the contents were drained into a graduated centrifuge tube after making a small nick along the greater curvature adjacent to the pyloric ligation and centrifuged at 3000 rpm for 15 min. Volume of gastric juice was noted; the volume of the supernatant was expressed as ml/100 g body weight and used for biochemical estimation. Further the stomach was carefully collected for assessment of ulcer index. For histopathological study full thickness biopsy specimen was fixed in 10% formalin solution.

Determination of volume, pH, free and total acidity of the gastric content.

The gastric content was subjected to centrifugation at 2500 rpm for 10 min. The volume and pH of the gastric juice were meassured and were subjected to free and total acidity estimation. The method described by (Srivastava et. al 2010) was employed in free and total acidity estimation.[14] The free acidity was determined by titration of with 0.01 N NaOH with methyl orange reagent until the colour of the solution became yellowish. The volume of the alkali added was noted. Immediately two or three drops of phenolphethline indicator were added and the solution was titrated until a definite red tinge appears. The total volume of NaOH was noted and this corresponds to total acidity. The acidity was calculated using following formula;

Acidity (mEq/L)

$$= \frac{\text{Volume of NaOH} \times \text{Normality of NaOH}}{0.1} \times 100$$

Peptic activity

Pepsin activity was estimated by the method described by Debnath PK et al.[15] One ml of diluted gastric juice was mixed with 2% haemoglobin solution in 0.06M HCl and incubated for 20 min. 0.6 M ice cold trichloroacetic acid was added to it. Later the solution was centrifuged and the supernatant fluid was mixed with reagent C and reagent E and optical density was measured at 610 nm against a blank of distilled water.

Total protein and carbohydrate

Dissolved mucosubstance was estimated in 90% alcoholic precipitate of the gastric juice. The precipitate obtained was dissolved in 1ml of 0.1 N NaOH and used for total protein estimation. Another part of precipitate was dissolved in 1ml of 0.01N H_2SO_4 used for total carbohydrate estimation.[16.17]

Ulcer index

The stomach was excised, cleaned and opened along its greater curvature. The inner surface was cleaned gently and rinsed with cold saline solution and spread on wax board with the mucous surface upwards avoiding corrugation and examined for ulceration with a magnifying lens. Ulcer index was calculated by following the method described by Kulkarni and Goel.[18. 19]

The following are descriptions of ulcer scores; 0.5 – red coloration, 1.0 – spot ulcers, 1.5 – hemorrhagic streaks, 2.0 – ulcers more than 3 mm and less than 5 mm and 3.0 – ulcers more than 5 mm. Mean ulcer scores for each experimental group were calculated and expressed as the ulcer index.

Histopathological study

Stomach tissue samples were collected after sacrificing the rats for histopathological examination. These tissue samples were fixed in 10% formalin solution embedded in paraffin wax, cut into 5µm thick sections and stained with H & E stain for examination under compound microscope.[20]

Statistical analysis

The experimental data were expressed as Mean ± SEM. Statistical analysis was carried out by one way analysis of variance followed by Dunnet's multiple comparison 't' test and p value < 0.05 implied statistical significance of results obtained.

RESULTS

In the acute oral toxicity test, no mortality was observed up to dose 2000 mg/kg and hence 1/5th of the highest dose was selected for the study.

In the present study, a decrease in the volume of gastric juice was observed in both, AAGS (51.75 %↓) and omeprazole (53.98 %↓) treated groups compared to control group. The decrease in the volume of the gastric juice may be one of the factors responsible for the observed anti-ulcer effect in the test formulation (table 1). Group II (Reference standard 94.1%) and Group III AAGS (38.75%) showed an increase in the pH of the gastric juice in comparison to control group (Table 1).

There was a significant decrease in the free acidity in both reference standard omeprazole treated group (100%) and in AAGS treated group (94.33%) while compared to control group. This indicates the gastric juice acidity neutralizing effect of the test drug (Table 1).

Total acidity was the sum total of free HCl, organic acids, combined acid and acid salts. It was observed that there is significant decrease in the total acidity in both omeprazole treated group (92.02%) and in AAGS treated group (87.73%) compared to control group ($p < 0.01$). The results were shown in table 2. There was a considerable decrease in the total carbohydrate in both omeprazole treated (33.29%) and in AAGS treated groups (17.76%) as compared to control group (Table 1).

There was a decrease in the total protein in omeprazole treated group (3.59%) but an increase was found in AAGS treated group (14.18%) compared to control group. The observed

Table 1: Effect of *Asystasia gangetica* stem aqueous extract on gastric juice volume, pH, total acidity, free acidity, total carbohydrate, total protein, peptic activity and total ulcer index

Group	Gastric volume in ml (% change)	Gastric juice pH (% change)	Total Acidity (mEq/dl)	Free acidity (mEq/dl)	Total carbohydrate (mg/ml)	Total protein (mg/ml)	Peptic activity (µ moles of tyrosine released /ml/ min)	Total ulcer index
Control	10.28 ± 1.86	2.4 ± 0.24	5.22 ± 0.45	3.53 ± 0.75	9.23 ± 3.30	7.29 ± 0.65	796.05 ± 158.6	22.83 ± 4.6
Omeprazole (20mg/kg)	4.73 ± 2.19 (53.98 ↓)	4.66 ± 0.33 (94.1 ↑)	0.42 ± 0.05*	0.0 ± 0.0*	6.15 ± 3.07	7.03 ± 0.20	782.35 ± 213.18	07.00 ± 2.0†
Asystasia gangetica (400mg/kg)	4.96 ± 2.03 (51.750 ↓)	3.33 ± 0.66 (38.75 ↓)	0.64 ± 0.07*	0.2 ± 0.0*	7.58 ± 1.26	8.33 ± 0.14	703.58 ± 189.67	11.66 ± 4.14

Values are expressed in Mean ± SEM, *$p < 0.01$ vs. control, $^{\dagger}P < 0.05$ vs. control.

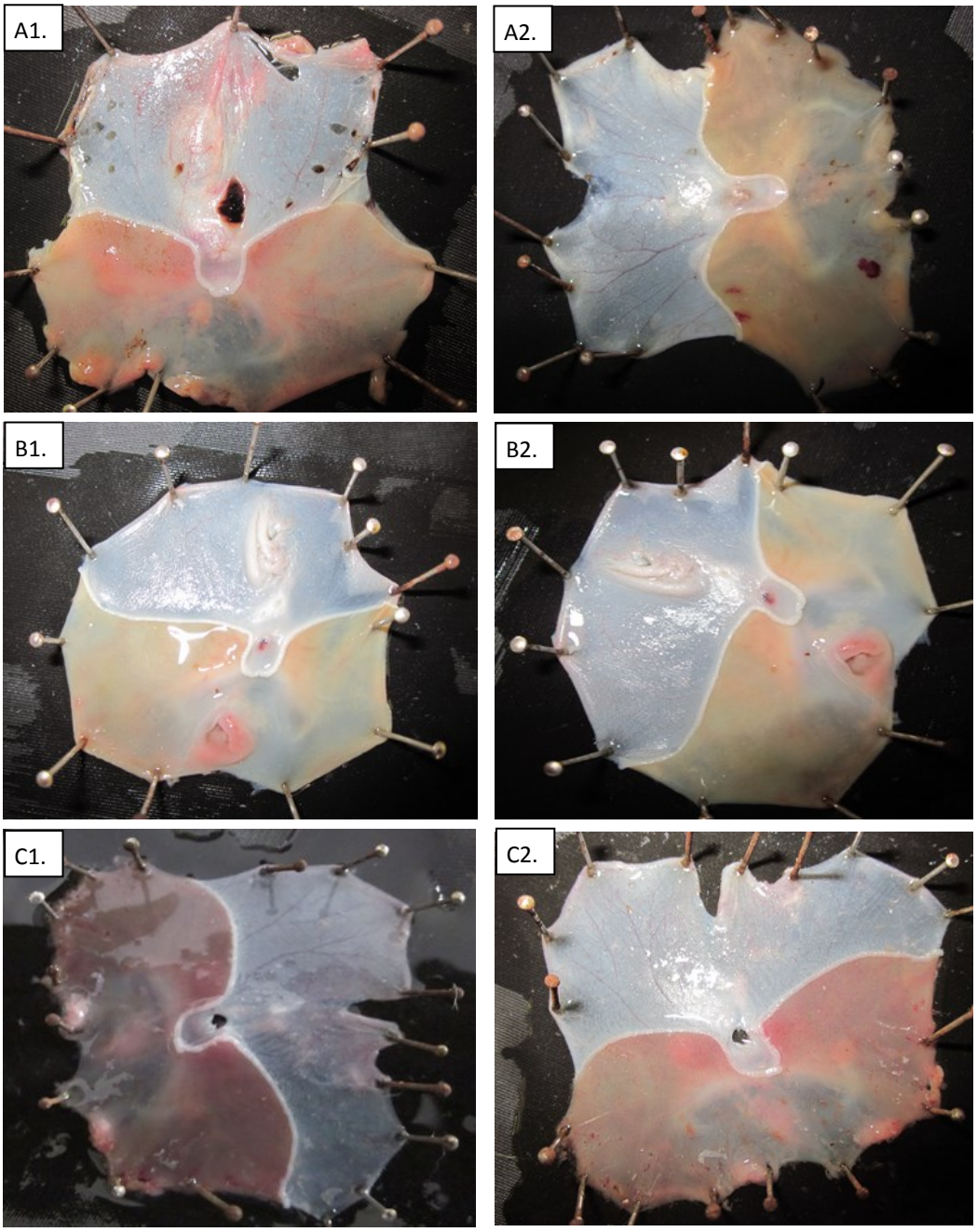
changes were only marginal hence were not of any significance. There was decrease in the peptic activity in both omeprazole (17.21%) and AAGS treated groups (11.61%) in comparison to control group. But the observed decrease was only marginal and hence may not be contributing significantly to the observed anti-ulcer effect. There was decrease in the total ulcer index in both omeprazole (69.33%) and AAGS treated groups (48.92%) while compared to control group (Table 1).

The total ulcer index in the standard group significantly decreased compared to control group (Table 1, Fig.1).

Effect of test drug on histopathological examination of stomach tissue:

In control group, there was marked destruction of epithelial layer (EL) with mixing of areas and loss of cytoarchitecture in some areas. Marked submucosal edema (ED) and cell infiltration was also observed (Fig. 2A). In omeprazole treated group, the severity of ulceration was much less in comparison to the section from the control group rats (Fig. 2B).

Figure 1: Effect of *Asystasia* gangetica stem aqueous extract on ulcer index (A - control, B - omeprazole group, C - *Asystasia gangetica group)*



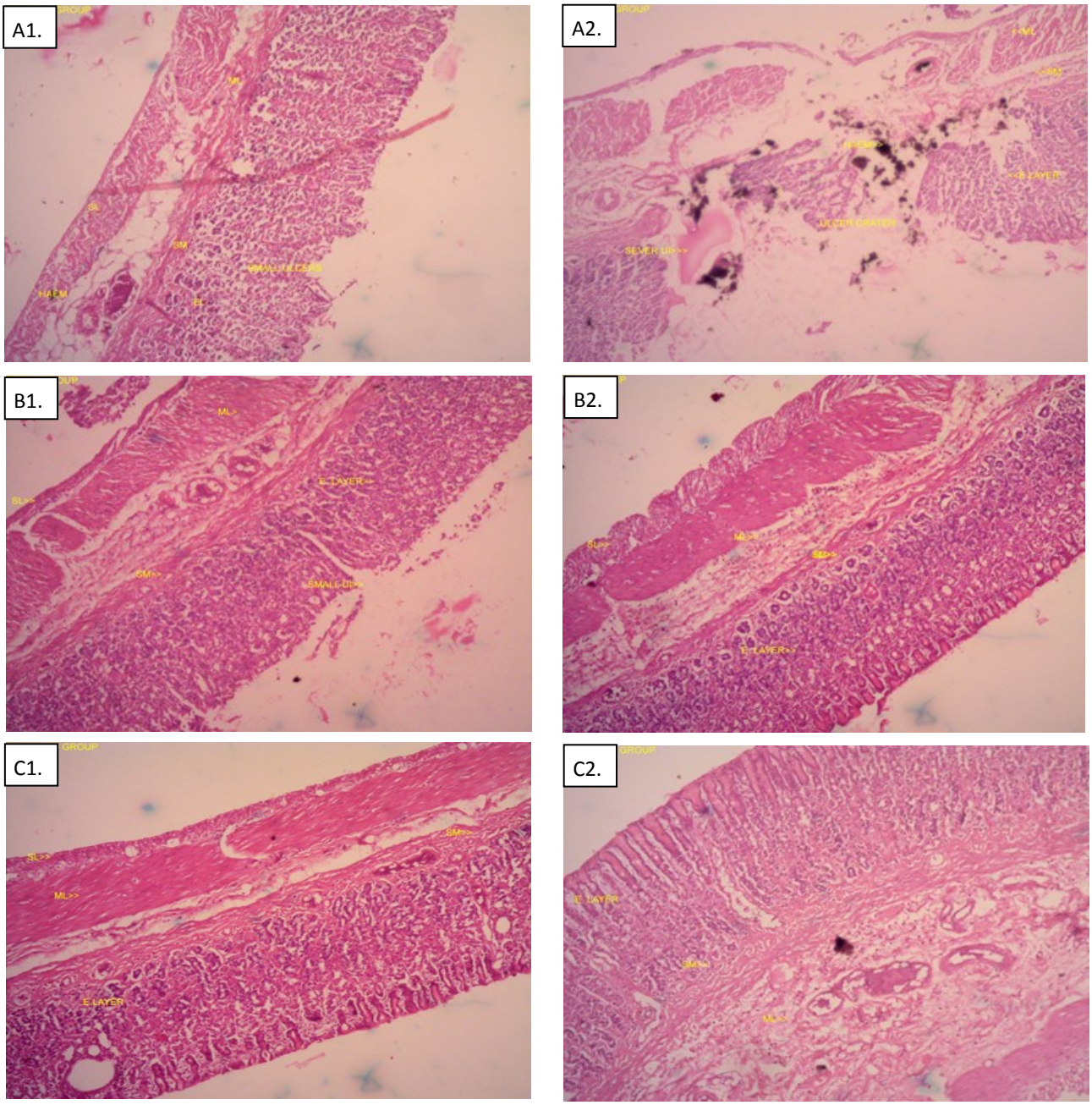
In *Asystasia gangetica* stem aqueous extract treated group, the severity of ulceration was much less in comparison to control group (Fig.2C).

DISCUSSION

Volume of secretion of gastric juice is an important factor in the formation of ulcer due to the exposure of the unprotected lumen of the stomach to the accumulating acid. If a drug is to be effective against the gastric ulcer it should have one or more than one of the following attributes; it should have an acidity decreasing effect or the drug should neutralize the gastric acidity.[21.22]

Ulcer area is one of the most reliable and accurate factors as the properties of ulcerated region regardless of its size and shape could be assessed. The ability of plant material to protect the stomach against ulcer without influencing acid secretion and neutralizing intra-gastric acidity can lead to the plant being classified as a cytoprotective agent. In this study it is observed that there is decrease in the total ulcer index in both omeprazole treated group (69.33%) and in aqueous extract of stem of AG treated groups (48.92%) when compared to control group. The total ulcer index in the standard

Figure 2: Effect of *Asystasia gangetica* stem aqueous extract on histology of gastric mucosa (A - control, B - omeprazole group, C - *Asystasia gangetica group)*



group significantly decreased compared to control group. Though test drug has shown highly significant result in decreasing the total and free acidity, and even decrease in peptic activity but failed to significantly reduce the intensity of the gastric ulceration. it could be due to its moderate effect on gastric acid secretion.

Test drug has shown significant cytoproective activity and reduction in ulcer severity by 68.92%. Histology of test drug showed reduction in depth and extent of ulceration in comparison to the control group rats. These indicate its anti ulcer potential. The preliminary phytochemical analysis of plant revealed the presence of polysaccharides, saponin and alkaloids. These phytochemicals might have influenced on decrease in the total and free acidity and also for the moderate cytoprotective activity of aqueous extract of stem of *Asystasia gangetica.*

The main possible mechanism through which the test formulation produces the observed moderate anti-ulcer effect is antisecretory and acidity neutralizing effects. The exact nature of the mechanism involved is not known. One of the mechanisms could be anticholinergic activity in the test formulation. Acidity neutralization may be a consequence of decreased gastric juice secretion. Other possibilities are antagonism of histamine receptors in the stomach and modulation of the activity of proton pump which is mainly involved in acid secretion. However, further studies are required to confirm them.

It can be concluded that the aqueous extract of *Asystasia gangetica* stem has anti-ulcer potential. It would be beneficial to assess the test formulation at different dose level and also against other models of gastric ulceration.

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