Contents lists available at ScienceDirect

Asian Pacific Journal of Tropical Medicine

journal homepage:www.elsevier.com/locate/apjtm

Document heading doi:

Pharmacological effects of aqueous-ethanolic extract of *Hibiscus rosasinensis* on volume and acidity of stimulated gastric secretion

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ARTICLE INFO

Article history:
Received 7 June 2011
Received in revised form 15 August 2011
Accepted 15 October 2011
Available online 20 November 2011

Keywords:
Verapamil
Cimetidine
Carbachol
Hibiscus rosasinensis
Rats

ABSTRACT

Objective: To explore the effects of extract of *Hibiscus rosasinensis (H. rosasinensis)* on the volume, free and total acidity of gastric secretion induced by carbachol. **Methods:** Animals were kept on fasting for 48 h, then the pylorus of each animal was ligated. They were randomly divided into 5 groups and treated by carbachol at 600 μg/kg. Then animals in group Ⅱ-Ⅴ were treated by *H. rosasinensis* extract at 250 and 500 mg/kg body weight, cimetidine at 2.5 mg/kg and verapamil at 10 mg/kg body weight intraperitoneally, respectively. The volume, free and total acidity of gastric secretion were observed and compared. **Results:** It was found that the extract significantly reduced the volume, free and total acidity of gastric secretion (P<0.01). These reductions were comparable to cimetidine and verapamil. And the reduction in the volume and free acidity were more significant in cimetidine and verapamil treated group indicating that cimetidine and verapamil were more effective. **Conclusions:** The extract of *H. rosasinensis* can reduced the volume, free and total acidity of gastric secretion, and can be used effectively in the treatment of peptic ulcer.

1. Introduction

Ulcers affect the gastrointestinal system, and are normally provoked by an imbalance between aggressive and protective factors in the stomach, which is affected by factors such as acid-pepsin secretion, mucosal barrier, mucus secretion, blood flow, cell regeneration, prostaglandins and epidermal growth factors[1-4]. Stress, smoking, nutritional deficiencies, ingestion of nonsteroidal anti-inflammatory drugs, hereditary predisposition and infection by *Helicobacter pylori* are all factors that can increase the incidence of gastric ulcer[5]. The drugs currently used in the treatment of gastric ulcers are antacids, anticholinergics, proton pump inhibitors and H_2-receptor antagonists[6,7]. In traditional medicines various herbal preparations are being used for treating ulcers. Plant extracts are some of the most attractive sources of new drugs and have shown promising results for the treatment of gastric ulcer[8]. The herb *Hibiscus rosa-sinensis (H. rosa-sinensis)* Linn. (Malvaceae) is a glabrous shrub widely cultivated in the tropics as an ornamental plant and has several forms with varying colours of flowers. In medicine, however, the red flowered variety is preferred [9]. The leaves and flowers are reported as promoters of hair growth and aid in healing of ulcers[10]. Aerial part of *H. rosasinensis* has calcium channel blocking action[11]. Recent reports have also shown anti-tumor, antioxidant[12], anti-ammonemic[13], anti-diabetic[14], hypolipidemic[15], post-coital anti-fertility, cardio protective and wound healing activities[16, 17]. The treatments of peptic ulcer disease are to relieve pain, heal the ulcer and prevent ulcer recurrence. Currently there is no cost-effective treatment that meets all these goals. Hence, efforts are needed to find a suitable treatment from natural product sources.

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2. Material and methods

2.1. Plant material

Aerial parts of *H. rosasinensis* were collected from the botanical garden of S.N. Institute of Pharmacy, Pusad, India. Identification and authentication of the samples was done by using standard botanical monographs. They were further confirmed with the Department of Botany, R.S.T.M University Nagpur, India.

2.2. Preparation of crude extract and fractionation

The plant material was cleaned off adulterants, shade dried and was coarsely grounded. The powdered material (1 kg) was soaked in 80% aqueous-ethanol for 3 days with occasional shaking. It was filtered through a muslin cloth and then through a filter paper. This procedure was repeated thrice and the combined filtrate was evaporated on a rotary evaporator under reduced pressure (-760 mmHg) to a thick, semi-solid mass of dark brown color, *ie*. the crude extract (Hr.Cr) with a yielding of approximately 10%[11].

For the purpose of fractionation, 20 g of the crude extract was dissolved in a minimum amount of 80% aqueous-ethanol and loaded on silica gel as inert support in the proportion of 1:20. Dried silica gel was packed in a chromatographic column and successively eluded with solvents of increasing polarity to get petroleum ether, ethyl acetate and aqueous fractions. Individually collected fractions were evaporated on rotary evaporator to give the fractions with yield of 8.5%, 5.4% and 64%, respectively.

2.3. Phytochemical screening

The preliminary phytochemical studies were conducted according to the methods of Adhirajan *et al*[9]and Gupta *et al*[18].

2.4. Animals

Wistar albino rats of either sex weighing between 160-180 g were used. Institutional Animal Ethics Committee approved the experimental protocol. Animals were maintained under standard conditions in an animal house approved by Committee for the Purpose of Control and Supervision on Experiments on Animals. Albino rats were used in this project was obtained from the Animal House of S.N. Institute of Pharmacy, Pusad. The animals were housed in poly propylene cages and maintained at (24± 2)℃ under 12 h light/ dark cycle and were feed *ad libitum* with standard pellet diet and had free access to water.

2.5. Experimental methods

All the animals were kept fasting for 48 h with free availability of water before they were subjected to experimental procedure. The animals were divided into 5 groups each containing 6 animals. Group Ⅰ were treated by carbachol only, Group Ⅱ were treated by low dose extract (250 mg/kg)+carbachol, Group Ⅲwere treated by high dose extract (500 mg/kg)+carbachol, Group Ⅳwere treated by cimetidine (2.5 mg/kg)+carbachol and Group Ⅴwere treated by verapamil (10 mg/kg)+carbachol. The operative procedure was according to method of Vischer *et al* and Mahmood *et al*[19, 20].

Animals were anaesthetized with ether, abdomen opened and pylorus was ligated with silk suture. Then abdominal wall was closed with suture clips and all animals had intraperitoneal injections of carbachol 600 μg/kg body weight. Then 250 mg/kg body weight of extract was administrated to Group Ⅱ, 500 mg/kg body weight of extract to Group Ⅲ, 2.5 mg/kg body weight of cimetidine to Group Ⅳ, verapamil 10 mg/kg to Group Ⅴ.

All rats were deprived of water for 4 h after administration of drugs. Then the rats were sacrificed, the thorax and abdomen were opened, esophagus was ligated and the stomach was removed quickly. The contents of the stomach were collected. The volume of the gastric juice was measured. Then the contents were centrifuged, filtered and subjected to titration for estimation of free and total acidity by the method described by Varley[21]. One mL of centrifuged and filtered gastric secretion was titrated against 0.1 N NaOH using Topfers reagent as indicator for determination of free acidty and 1% phenoplhthalein as indicator for combined acidity. The sum of the two titrations was total acidity. The data was analyzed statistically using student "*t*" test.

A score for the ulcer was studied for all the groups[22, 23] as follows: 0 for normal stomach, 0.5 for red coloration, 1 for spot ulcer, 1.5 for hemorrhagic streak, 2 for ulcer, 3 for perforation.

Mean ulcer score for each animal will be expressed as ulcer index. The percentage of ulcer protection was determined as follows:

2.6. Histopathological evaluation

The gastric tissue samples were fixed in neutral buffered formalin for 24 h. Sections of tissue from stomachs were examined histopathologically to study the ulcerogenic and/or anti-ulcerogenic activity of *H. rosasinensis*. The tissues were fixed in 10% buffered formalin and were processed using a tissue processor. The processed tissues were embedded in paraffin blocks and about 5-μm thick sections were cut using a rotary microtome. These sections were stained with hematoxylin and eosin using routine procedures. The slides were examined microscopically for pathomorphological changes such as congestion, hemorrhage, oedema and erosions using an arbitrary scale for the assessment of severity of these changes[22].

2.7. Statistical analysis

The statistical analysis of all the results was carried out using one-way ANOVA followed by Dunnets multiple comparisons using graph pad in stat 3 and all the results obtained in the study were compared with the carbachol

treated group.

3. Result

Phytochemical screening showed the presence of sterols, carbohydrates, glycosides, tannins and Flavonoid.

The volume, free acidity and total acidity of gastric secretion in Group Ⅰ were (29.24±0.54) mL, (6.90±0.34) mEq/dL and (8.20±0.50) mEq/dL, respectively. After treatment of extract, three indexes were significantly decreased ($P<0.01$), and these reductions were comparable to cimetidine and verapamil (Table 1). Cimetidine treated, verapamil treated and extract treated groups showed significant reduction in ulcer index as compared to Group Ⅰ ($P<0.05$). Extract had protection percentage of 76% and 82% at the dose of 250 and 500 mg/kg, respectively in comparison to Group Ⅰ whereas cimetidine and verapamil as reference standard drugs showed reduction of 88% and 81%, respectively. The pH of gastric juice was also significantly declined after treatment of extract, cimetidine and verapamil($P<0.05$).

Macroscopical and histopathological results showed congestion, degeneration, hemorrhage, edematous appearance of the gastric tissue in Group Ⅰ (Figure 1A & 2A); whereas extract treated, cimetidine and verapamil treated groups all showed regeneration and protection of muscosal layer (Figures 1B–1E, 2B–2E).

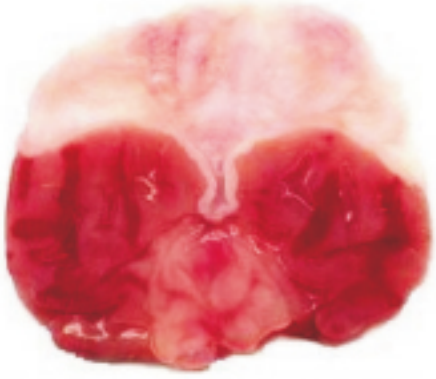

Figure 1 A. Carbachol treated group showing congestion, oedema, and mucosal damage.

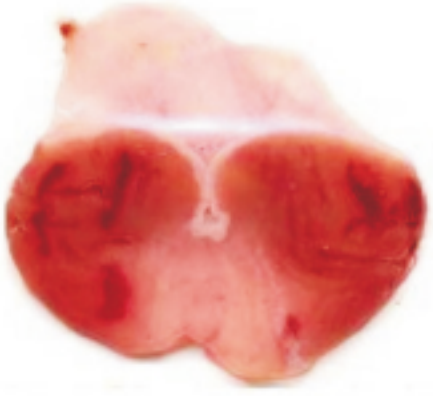

Figure 1 B. 500 mg/kg *H. rosasinesis* extract treated group showing protection of mucosal layer.

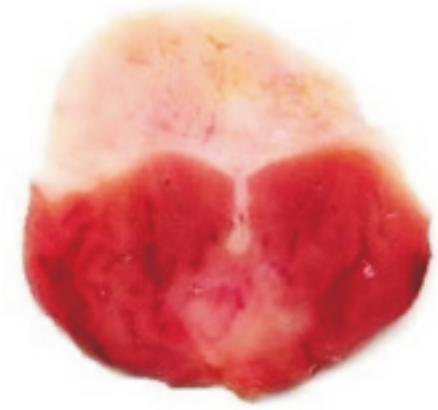

Figure 1 C. 250 mg/kg *H. rosasinesis* extract treated group showing protection of mucosal layer.

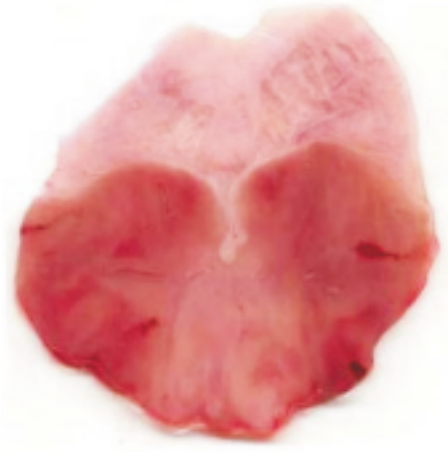

Figure 1 D. Cimetidine treated group showing protection of mucosal layer.

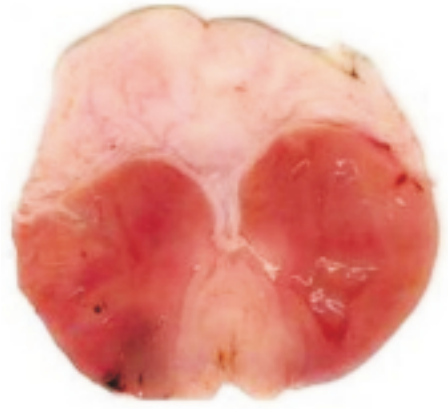

Figure 1 E. Verapamil treated group showing protection of mucosal layer.

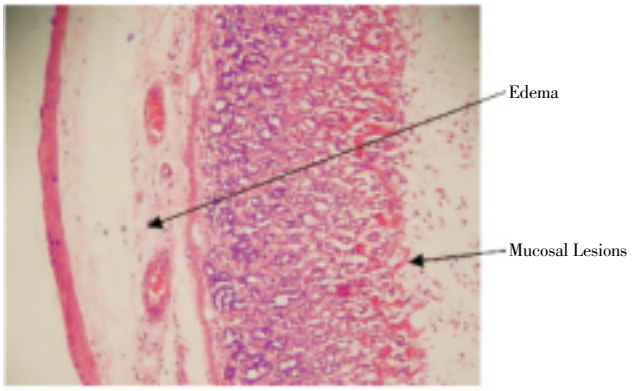

Figure 2A. Histological section of gastric mucosa in a rat pretreated with Carbachol 600 μg/kg showing severe disruption to the surface epithelium, and edema of the submucosal layer with leucocyte infiltration.

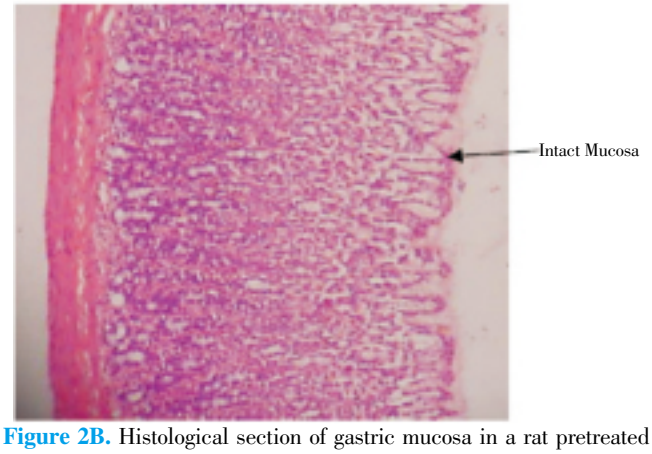

Figure 2B. Histological section of gastric mucosa in a rat pretreated with 500 mg/kg extract showing no disruption to the surface epithelium with no edema and no leucocytes infiltration of the submucosal layer.

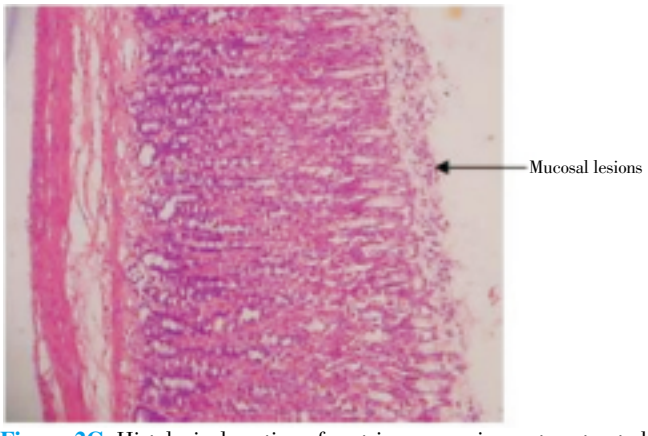

Figure 2C. Histological section of gastric mucosa in a rat pretreated with 250 mg/kg of extract showing mild disruption to the surface epithelium mucosa with no edema and no leucocytes infiltration of the submucosal layer.

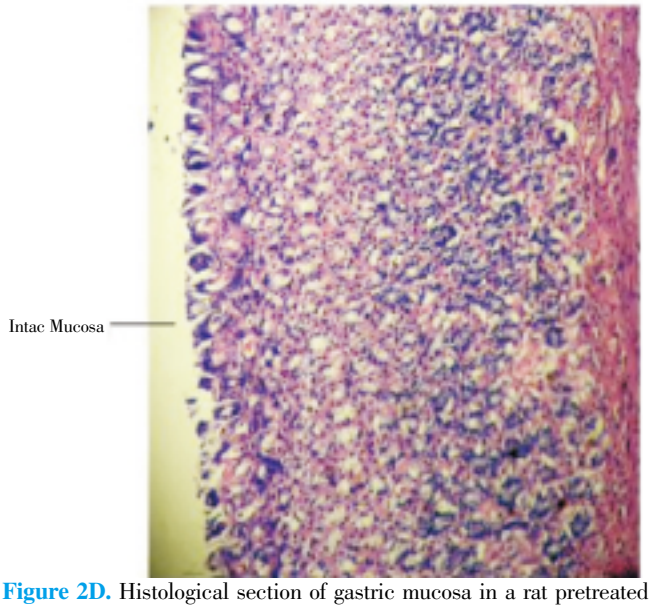

Figure 2D. Histological section of gastric mucosa in a rat pretreated with 2.5 mg/kg of cimetidine showing no disruptionto the surface epithelium with no edema and no leucocytes infiltration of the submucosal layer.

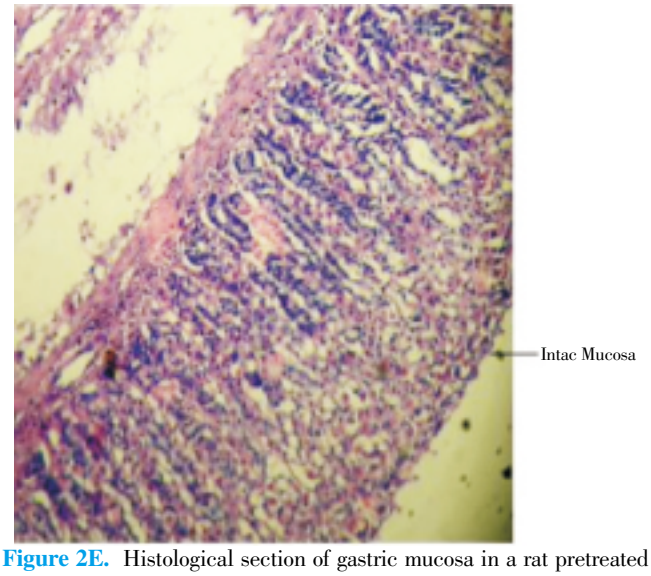

Figure 2E. Histological section of gastric mucosa in a rat pretreated with 10 mg/kg of Verapamil showing no disruption to the surface epithelium with no edema and no leucocytes infiltration of the submucosal layer.

4. Discussion

Acid secretion in the stomach is controlled at a variety of levels by neural, hormonal and paracrine mechanisms.

Table 1

Effect of extract of *H. rosasinensis* on volume, free acidity, total acidity, ulcer index, and pH of gastric juice (mean±SEM) (n=6).

Drug	Volume(mL)	Free acidity(mEq/dL)	Total acidity(mEq/dL)	Ulcer index	PH of gastric juice
Carbachol (600 μg/kg)	29.24±0.54	6.90±0.34	8.20±0.50	16.90±1.30	2.30±0.10
Extract (250 mg/kg)+Carbachol (600 μg/kg)	16.70±0.63**	3.40±0.55**	4.32±0.43**	4.00±0.10	4.00±0.20
Extract (500 mg/kg)+Carbachol (600 μg/kg)	15.20±0.44**	2.75±0.32**	3.95±0.25**	3.00±0.22*	4.50±0.12*
Cimetidine (2.5 mg/kg)+Carbachol (600 μg/kg)	13.25±0.26**	2.20±0.12**	3.25±0.30**	2.10±0.40*	5.10±0.12*
Verapamil(10 mg/kg)+Carbachol(600 μg/kg)	14.10±0.55**	2.40±0.14**	3.65±0.20**	3.20±0.15*	4.40±0.14*

**P<0.05, ** P<0.01 as compared to the Carbachol treated group.

When these regulatory mechanisms are in malfunction, acid and pepsin auto digest the mucosa resulting in the ulceration of esophagus, stomach and duodenum. Histamine, acetylcholine or carbachol are potent secretogogues for the parietal cells of gastric mucosa leading to the production of HCl[24, 25]. Acetylcholine and gastrin act through calcium ions. Carbachol being a cholinomimetic drug increases free intracellular calcium ions. This in turn activates protein kinase by phosphorylation and leads to increased production of HCl. In this study we observed that cimetidine reduced the volume free acidity and total acidity. All these reductions were significantly high when compared with the mean values in carbachol treated group. Our study is similar with the findings of other workers who observed that cimetidine significantly reduces the volume and acidity of gastric secretion[26, 27]. This is due to well known H_2-receptor antagonistic action of cimetidine which interacts with H_2-receptor and inhibits the activation of adenyl cyclase and as a result no cyclic AMP is formed which is required for HCl production[28,29]. Similar reduction was observed using the extract. Our study is consistent with other workers who concluded that verapamil significantly reduces gastric acid secretion[30,31]. Verapamil, a well known calcium channel blocker inhibits the calcium influx, which may be responsible for the observed reductions in volume and acidity of gastric secretion. Calcium channel blocker verapamil may interfere with H^+K^+ ATP ase due to its high affinity for the K^+ site H^+K^+ ATP ase system which is accessible from luminal side of the stomach[32]. Histamine release, from peritoneal mast cells, is critically dependent upon extra cellular Ca^{++} concentration, so non-availability of Ca^{++} may cause reduced effects of histamine on acid production in the stomach. Beside this, verapamil inhibits lipoxygenase pathway during metabolism of arachidonic acid. So leukotriene, the injurous substance is not formed and all the arachidonic acid is metabolized through cycloxygenase pathway. This will lead to the production of prostaglandin which couples with gastrointestinal protein and inhibits adenyl cyclase and thus decrease HCl production[33]. Similar effects may be due to the presence of natural calcium channel blocker present in the extract. Release of histamine from mast cells is critically dependent on external calcium ions, so calcium channel blocker by blocking calcium ions can inhibit histamine release, which is a potent agent for HCl secretion[34]. When we compared the differences in the mean values of reduction in volume, free and total acidity of gastric secretion, caused by *H. rosasinensis* and cimetidine, it was found that although the extract reduced the gastric acidity significantly but, was less effective than cimetidine. The extract can still be used as natural calcium channel antagonist. Calcium channel blockers are widely used in controlling contraction of cardiovascular smooth muscles, allergic reaction and prevention of premature labor[35].

It is concluded that the extract from *H. rosasinensis* show antiulcerogenic effect, which may be due to its calcium channel blocking activity. Further studies in this regard for evaluation of these effects are suggested in human subjects.

Conflict of Interest statement

We declare that we have no conflict of interest.

Acknowledgements

The authors are highly indebted to teaching and non teaching staff of S.N.Institute of Pharmacy, Pusad for their active participation throughout the study.

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