Antiulcer screening of *Carthamus tinctorius* on volume and acidity of stimulated gastric secretion in rats

Sir,

The ulcers that affect the gastrointestinal system 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] 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. [2] Moreover, calcium plays an important role in increased production of gastric acid. Induction of hypercalcemia through intravenous administration of calcium is usually associated with increased gastric volume and acidity. The acid stimulating ability of calcium is well known, and there is extreme sensitivity to calcium in patients with Z.E. syndrome.

It has been documented that *C. tinctorius* (Safflower) has natural calcium channel blocker activity.[3] *C. tinctorius* has long been used as Chinese medicine in clinics to treat cardiovascular disease, and has demonstrated anti-myocardial ischemia effects.[4] Safflower also possesses other pharmacological effects, including anti-coagulant, antioxidant, and neuroprotective. This study was planned to evaluate the effects of extract from *C. tinctorius* and to compare it with H_2-receptor antagonist cimetidine and calcium channel blocker verapamil on the volume and acidity of carbachol-induced gastric secretion.

An aerial part of *C. tinctorius* L. (Asteraceae) was collected in the month of April from the Hingoli district of Maharashtra, India. Identification and authentication of the *C. tinctorius* was done by a Botanist, Post Graduate Teaching Department of Botany, Rashtra Santa Tukadoji Maharaj Nagpur University, Nagpur (Voucher specimen no. 9715).

The plant materials were cleaned, shade dried, and coarsely ground. The powdered material was soaked in 70% aqueous-methanol 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 to a thick, semi-solid mass of dark brown color, i.e. the crude extract yielding approximately 6.1%.

Wistar albino rats of either sex weighing between 160 and 180 g were obtained from the Animal House, S.N. Institute of Pharmacy, Pusad. The animals were housed in polypropylene cages and maintained at 24 ± 2°C under 12 h light/dark cycle and were feed *ad libitum* with standard pellet diet and had free access to water. The study was approved by the Institute Animal Ethics Committee, and all the animal experiments were carried out as per CPCSEA guidelines.

Thirty Wistar rats were divided into six groups containing six animals and grouped as follows:

Group I:	Carbachol
Group II:	*C. tinctorius* 200 mg/kg
Group III:	*C. tinctorius* 400 mg/kg
Group IV:	Cimetidine 2.5 mg/kg + Carbachol
Group V:	Verapamil (10 mg/kg) + Carbachol

All the animals were kept fasting for 48 h with free availability of water before they were subjected to a experimental procedure. The operative procedure was the one adopted by Visscher *et al*.[5] Animals were anesthetized with ether, abdomen opened and pylorus was ligated with silk suture. Then the abdominal wall was closed with suture clips and intraperitoneally injections of Carbachol 600 µg/ kg body weight were administered to group I, 200 mg/kg body weight of extract to group II, 400 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 followed by Carbachol 600 µg/ kg body weight after 15 min to groups II, III, IV, and V.

The rats were deprived of water for 4 h after administration of drugs. Then, the rats were killed, 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. One milliliter of centrifuged and filtered gastric secretion was titrated against 0.1 N NaOH using the Topfers reagent as indicator for determination of free acidity and 1% phenolphthalein as indicator for combined acidity. The sum of the two titrations was total acidity. Mean ulcer score for each animal will be expressed as the ulcer index. The percentage of ulcer protection was determined as follows:

$$\% \text{ Protection} = \frac{\text{Control Mean Ulcer index - Test Mean Ulcer index}}{\text{Control Mean Ulcer index}} \times 100$$

The gastric tissue samples 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, edema, and erosions using an arbitrary scale for the assessment of severity of these changes. Data were expressed as mean ± SEM. The statistical analysis of all the results was carried out using one-way ANOVA followed by Dunnet's multiple comparisons.

The volume, free acidity, and total acidity of gastric secretion in all groups are shown [Table 1]. All these reductions were also found to be statistically significant when compared with the carbachol $P<0.001$). When compared the mean values of volume, free and total acidity for extract and cimetidine it was observed that the difference in mean values of volume was statistically significant ($P<0.001$), that of free acidity was significant ($P<0.05$) and total acidity was not significant. Cimetidine-treated, verapamil-treated and extract-treated groups show significant reduction in the ulcer index as compared to carbachol treated. Extract was showing a protection index of 78% and 83% at the dose of 200 and 400 mg/kg, respectively, in comparison to carbachol-treated whereas cimetidine and verapamil showed a protection index of 88% and 81%, respectively. Rat treated with *C. tinctorius* showed a normal architecture [Figure 1].

Acid secretion in the stomach is controlled at a variety of levels by neural, hormonal, and paracrine mechanisms. When these regulatory mechanisms 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.[6] Acetylcholine and gastrin act through calcium ions. Carbachol being a cholinomimetic drug increases the 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 statistically highly significant when compared with the mean values in the carbachol treated group. Our study correlates with the findings of other workers who observed that cimetidine significantly reduces the volume and acidity

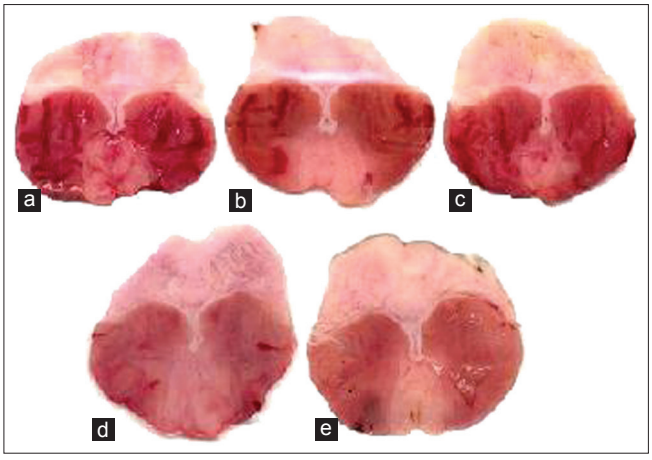

Figure 1: (a) Carbachol-treated group shows congestion, edema, and mucosal damage, (b) 400 mg/kg *Carthamus tinctorius* extract treated group shows protection of mucosal layer, (c) 200 mg/kg *Carthamus tinctorius* extract treated group shows protection of mucosal layer, (d) Cimetidine-treated group shows protection of mucosal layer, and (e) Verapamil-treated group shows protection of mucosal layer

Table 1: Effect of extract of *C. tinctorius* on volume, free acidity, total acidity, ulcer index, and pH of gastric juice

Drug	Volume of gastric secretion	Free acidity, mEq/dl of gastric secretion	Total acidity, mEq/dl of gastric secretion	Ulcer index	Protection (%)	pH of gastric juice
Carbachol (600 µg/kg)	29.24 ± 0.54	6.90 ± 0.34	8.20 ± 0.50	16.9 ± 1.3	00	2.3 ± 0.10
Extract (200 mg/kg) + carbachol	16.00 ± 0.55**	3.30 ± 0.50**	4.20 ± 0.36**	3.80 ± 0.2	78	3.9 ± 0.20
Extract (400 mg/kg) + carbachol (600 µg/kg)	14.80 ± 0.44**	2.65 ± 0.32**	3.70 ± 0.20**	2.9 ± 0.10*	83	4.5 ± 0.10*
Cimetidine (2.5 mg/kg) + carbachol (600 µg/kg)	13.25 ± 0.26**	2.20 ± 0.12**	3.25 ± 0.30**	2.1 ± 0.4*	88	5.1 ± 0.12*
Verapamil (10 mg/kg) + carbachol (600 µg/kg)	14.10 ± 0.55**	2.40 ± 0.14**	3.65 ± 0.20**	3.2 ± 0.15*	81	4.4 ± 0.14*

Values are expressed as mean ± SEM ($N=6$). *$P<0.05$ as compared to the carbachol-treated group. **$P<0.01$ as compared to the carbachol treated group

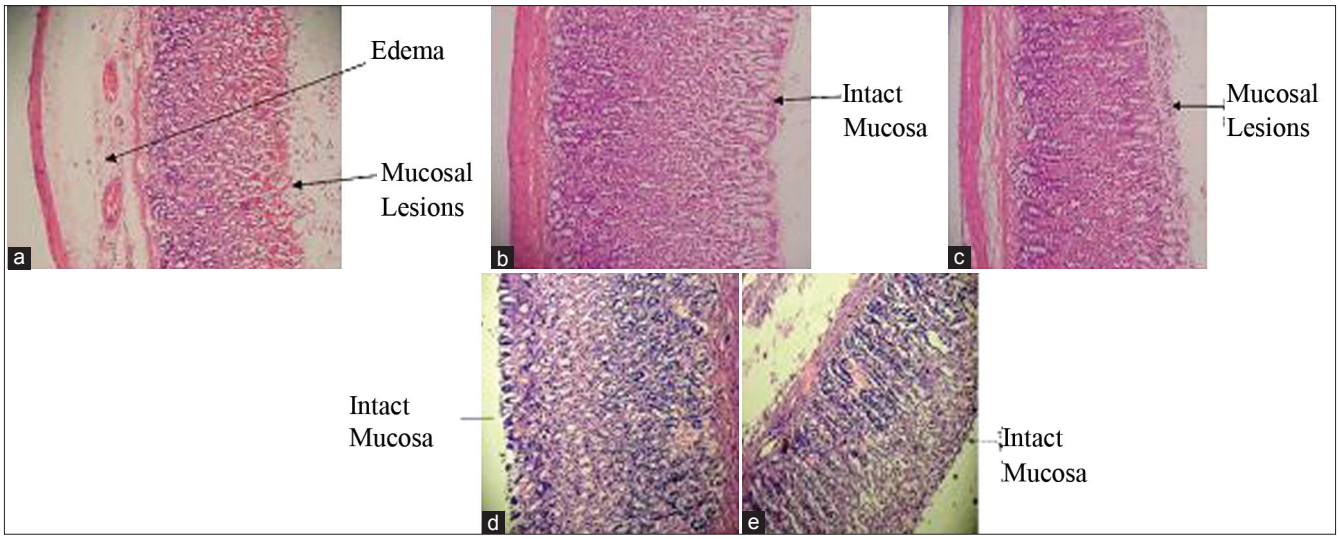

Figure 2: (a) Severe disruption to surface epithelium, and edema of submucosal layer with leucocytes infiltration. (b) No disruption to surface epithelium with no edema and no leucocytes infiltration of submucosal layer. (c) Mild disruption to surface epithelium mucosa with no edema and no leucocytes infiltration of submucosal layer. (d) No disruption to surface epithelium with no edema and no leucocytes infiltration of submucosal layer. (e) No disruption to surface epithelium with no edema and no leucocytes infiltration of submucosal layer

of gastric secretion.[7] This is due to well-known H_2-receptor antagonistic action of cimetidine which interacts with the 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.[8] A similar reduction was observed using the extract. All these reductions were found to be statistically highly significant when compared with carbachol alone. Our study is consistent with other workers who concluded that Verapamil significantly reduces gastric acid secretion.[9] Verapamil, a well-known calcium channel blocker, inhibits the calcium influx, which may be responsible for the observed reductions in the volume and acidity of gastric secretion. Calcium channel blocker *verapamil* may interfere with H+K+ ATPase due to its high affinity for the K+ site H+K+ ATPase system which is accessible from the luminal side of the stomach.[10] Histamine release, from peritoneal mast cells, is critically dependent upon extracellular Ca^{++} concentration, so non-availability of Ca^{++} may cause reduced effects of histamine on acid production in the stomach. Beside this, verapamil inhibits the lipoxygenase pathway during metabolism of arachidonic acid. So leukotriene, the injurious substance is not formed and all the arachidonic acid is metabolized through the cycloxygenase pathway. This will lead to the production of prostaglandin which couples with GI protein and inhibits adenyl cyclase and thus decrease HCl production. Similar effects may be due to the presence of the natural calcium channel blocker present in the extract. When compared the differences in the mean values of reduction in volume, free, and total acidity of gastric secretion caused by *C. tinctorius* and cimetidine, it was found that although the extract reduced the gastric acidity significantly but, was less effective than cimetidine [Figure 2].

It is concluded that the extract from *C. tinctorius* showed the 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.

Rajesh Mandade, S. A. Sreenivas,[1] Ravi Wanare[2]

Research Scholar, J.J.T. University, Rajasthan, [1]Guru Nanak Institute of Pharmacy, Ibrahimpattnam, Hyderabad, Andhra Pradesh, [2]Pharmaceutical Chemistry Department, S.N. Institute of Pharmacy, Pusad, Maharashtra, India

Address for correspondence:
Rajesh J. Mandade, Research Scholar, J.J.T. University, Rajasthan, India.
E-mail: raj_mandade@rediffmail.com

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