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. 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. 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. C. tinctorius has long been used as Chinese medicine in clinics to treat cardiovascular disease, and has demonstrated anti-myocardial ischemia effects. 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 H2-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 fed 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. Animals were anesthetized with ether, abdomen opened and pylorus was ligated with silk suture. Then the abdominal wall was closed with suture clips and intra-peritoneal 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:

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

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 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 secretagogues for the parietal cells of gastric mucosa leading to the production of HCl. 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 of gastric juice.

![Figure 1](image-url)
of gastric secretion. This is due to well-known H₂-receptor antagonistic action of cimetidine which interacts with the H₂-receptor and inhibits the activation of adenyl cyclase and as a result no cyclic AMP is formed which is required for HCl production. 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. 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. Histamine release, from peritoneal mast cells, is critically dependent upon extracellular Ca²⁺ concentration, so non-availability of Ca²⁺ 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 cyclooxygenase 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, Ravi Wanare
Research Scholar, J.J.T. University, Rajasthan, Guru Nanak Institute of Pharmacy, Ibrahimpattham, Hyderabad, Andhra Pradesh, 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

REFERENCES

1. Ishikawa T, Donatini RS, Diaz IE, Yoshida M, Bacchi EM, Kato ET. Evaluation of gastro protective activity of Pliniaedulis (Vell.) Sobral (Myrtaceae) leaves in rats. J Ethnopharmacol 2008;118:527-9.
2. Barros MP, Lemos M, Maistro EL, Leite MF, Sousa JP, Bastos JK, et al. Evaluation of antulcer activity of the main phenolic acids found in Brazilian Green Propolis. J Ethnopharmacol 2008;120:372-7.
3. Liu N, Yang Y, Mo S, Liao J, Jin J. Calcium antagonistic effects of Chinese crude drugs: Preliminary investigation and evaluation by ⁴⁵Ca. Appl Radiat Isot 2005;63:151-5.
4. Li XZ, Liu JX, Shang XH, Fu JH. Protective effects of hydroxyasafflor yellow A on acute myocardial ischemia in dogs. Chin Pharmacol Bull 2006;6:533-7.
5. Visscher FE, Sney PH, Tazelaar AP, Jr, Veldkamp W, Vander Broek MJ. Pharmacology of pamine bromide. J Pharmacol Exp Ther 1954;110:188-204.
6. Perez-Zoghbi JF, Mayora A, Ruiz MC, Michelangeli F. Heterogeneity of acid secretion induced by Carbachol and histamine along the gastric gland axis and its relationship to [Ca²⁺]. Am J Physiol Gastrointestin Liver Physiol 2008;295:671-81.
7. Zanatta F, Gandolfi RB, Lemos M, Ticona JC, Gimenez A, Clasen BK, et al. Gastro protective activity of alkaloid extract and 2-phenylethylamine obtained from C. tinctorius. J Ethnopharmacol 2008;118:527-9.
from the bark of Galipealongiflora Krause (Rutaceae). Chem Biol Interact 2009;180:312-7.
8. Hussain A, Syed SH. Effect of cimetidine on reduction of gastric secretion and pH in adult patients undergoing elective surgery and its impact on aspiration risk. Pak J Physiol 2007;3:8-13.
9. Kadalmi B, Saravana Kumar M, Revathi P, Prakash Shyam K. Gastric ulcer protective property of calcium channel blockers in male albino rats. Int J Pharm Bio Sci 2011;2:629-36.
10. Jan M, Mughal MA, Tanwani RK, Aamir K, Ali M. Evaluation of combined effect of verapamil and ranitidine on the volume and acidity of Carbachol induced gastric secretion. J Ayub Med Coll Abbottabad 2004;16;34-7.