Inhibition of gastric acid secretion by Ya-hom in isolated mouse whole stomach

Duangmate Chantharangsikula, Suwan Siriphaisarnpipat Thirawarapanb, Nuntavan Bunyapraphatsarab, Wisuda Suvitayavata
aDepartment of Physiology, bDepartment of Pharmacognosy, Faculty of Pharmacy, Mahidol University, 447 Sri-ayudhaya Rd, Bangkok 10400, Thailand

Background: Ya-hom, the traditional Thai formula for abdominal discomfort treatment has, been reported to inhibit gastric acid secretion in gastric fistula rats. However, the mechanism underlying its action remains unclear.

Objective: To investigate the gastric acid inhibitory action of Ya-hom and its mechanism of action by using an isolated mouse whole stomach model.

Methods: The gastric acid secretion of isolated mouse whole stomach was stimulated by histamine (5.0 μM) or bethanechol (10 or 100 μM) after adding the inhibitors (atropine 1 μM, ranitidine 10 μM, indomethacin 0.1 μM or L-NAME 300 μM) and/or Ya-hom to the serosal solution. The effluent perfusate was collected continuously in 10-minute fractions for 120 minutes after stimulation.

Results: Re-dissolved lyophilized Ya-hom extract at doses of 2.5, 5.0, 10.0, and 20.0 mg/mL inhibited histamine-stimulated gastric acid secretion in a dose-dependent manner. Inhibition of Ya-hom (10 mg/mL) was also observed in the presence of atropine (1 μM), which was used to eliminate effects of endogenous acetylcholine. Ya-hom inhibited bethanechol-stimulated gastric acid secretion in the presence and absence of ranitidine. While the inhibitory action of Ya-hom on histamine-stimulated gastric secretion was not affected by indomethacin, it was attenuated by concomitant treatment with the nitric oxide synthase inhibitor (L-NAME).

Conclusion: Ya-hom did not stimulate gastric acid secretion in the isolated mouse whole stomach. Ya-hom significantly inhibited gastric acid secretion after this was stimulated via histamine or bethanechol. Nitric oxide stimulation plays an important role in the inhibitory action of Ya-hom.

Keywords: Bethanechol, gastric acid secretion, histamine, isolated mouse stomach, Ya-hom

Thai traditional medicines have been widely used for the treatment of several diseases. Among Thai traditional medicines used currently, Ya-hom is one of the most popular formulas among the elderly. It is used for treatment of syncope, presyncope and abdominal discomfort, including nausea, vomiting, abdominal pain, gastrointestinal colic, and flatulence. The abdominal discomfort may be due to gastric function irregularity such as enhanced gastric acid secretion [1], dyspepsia, gastric ulceration, and stomach pain [2]. Although herbal formulations utilizing Ya-hom have been used for a long time, there is lack of scientific data on the efficacy and mechanism of action on gastrointestinal function. Jariyapongskul et al. [3] demonstrated that Ya-hom increased regional cerebral blood in rats. This only supports the treatment of syncope by Ya-hom [3]. In this study, we investigate the effect of Ya-hom in mice, to elucidate its mechanism of action in relieving abdominal discomfort.

The water-soluble extract of Ya-hom has been shown to inhibit the stimulatory effects of histamine and carbachol on the secretions of acid, pepsin, and soluble mucus but potentiate the visible mucus secretion in gastric fistula rats [4]. Attenuating gastric...
acid stimulation by secretagogues and modulating the gastric barrier contributes to the pharmacological effects of Ya-hom in relieving gastric discomfort [4]. However, the in vivo model used in the earlier study is not suitable for investigating the mechanisms of action due to the complication of endogenous regulation. To understand this mechanism, we use an isolated mouse whole stomach model to determine the effect of Ya-hom on gastric acid secretion. The advantage of this model is that the autonomic regulation of gastric secretion is absent. The secretagogues, test solutions, and inhibitors can be added directly to the serosal surface to evaluate drug effects without interference of endogenous mediators from the extrinsic nervous system. The mechanism of action of Ya-hom on histamine- and bethanechol-stimulated gastric acid secretion was evaluated using the specific inhibitors: atropine (acetylcholine antagonist), ranitidine (H2 receptor antagonist), indomethacin (cyclooxygenase inhibitor), and Nω-nitro-L-arginine methyl ester hydrochloride (L-NAME, the nitric oxide synthase inhibitor). Our results will provide a rigorous explanation of the gastric acid inhibitory action of Ya-hom when used to treat abdominal discomfort.

Materials and Methods

Chemicals

Urethane was obtained from Merck Chemicals Ltd (Nottingham, UK). Histamine dihydrochloride, atropine sulfate and Nω-nitro-L-arginine methyl ester hydrochloride (L-NAME) were purchased from Sigma Chemical Co (St Louis, USA). Bethanechol chloride was purchased from ICN Biomedicals Inc (Aurora, USA). Ranitidine hydrochloride was purchased from Neuland Laboratories Ltd (Hyderabad, India). Other reagents were of analytical grade.

Ya-hom

Ya-hom was obtained from Five Pagodas Pharmacy Co (Bangkok, Thailand). One hundred grams of Ya-hom contain Agastache rugosa (Fisch. Et Mey) O. Kuntze (whole plant, Labiatae) 7.1 g, Acorus gramineus Soland (rhizomes, Araceae) 3.5 g, Ligusticum wallichii Franch (rhizomes, Umbelliferae) 2.3 g, Glycyrrhiza glabra L (licorice, rhizomes, Leguminosae) 4.8 g, Eugenia caryophyllata Thunb (clove, flower-bud, Myrtaceae) 7.1 g, Saussurea lappa Clark (rhizomes, Compositae) 7.1 g, Aquilaria agallocha Roxb (wood, Thymelaeaceae) 7.1 g, Atractylis ovata Thunb (rhizomes, Compositae) 9.3 g, Menthol 4.7 g, Borneo camphor 1.4 g, and Angelica anomala Lallem (rhizomes, Umbelliferae) 3.5 g.

Lyophilized Ya-hom water extract

Ya-hom powder (1 kg) was dissolved in distilled water (10 L) and boiled. Ya-hom extract was filtered through cotton and muslin cloth. The filtrate was lyophilized and stored at -20°C. One gram of Ya-hom powder yielded 0.136 g of lyophilized powder. The test solution of Ya-hom was freshly prepared by dissolving the lyophilized product in serosal solution. The concentration of Ya-hom used in the study was expressed as equivalent to Ya-hom powder.

The HPLC analysis of this lyophilized Ya-hom extract was previously reported [4].

Animals

Male mice, Mus musculus, weighing between 25-35 g, were obtained from the National Laboratory Animal Center (Salaya, Mahidol University, Thailand). Mice were housed in the animal room with a controlled temperature (23±2°C) under 12-hour light/dark cycles. The animals were fed with standard pellet (CP Mice feed; SWT Co, Bangkok, Thailand) and distilled water ad libitum. Each experimental group consisted of 6-10 mice. The experimental protocol was approved by the Institutional Animal Care and Use Committee of the Faculty of Pharmacy, Mahidol University in accordance with Ethical Principles and Guidelines for the Use of Animals for Scientific Purposes by The National Research Council of Thailand.

Preparation of isolated mouse whole stomach

Isolated mouse whole stomach was obtained as previously described by Watanabe et al. [5]. Mice were anesthetized with urethane at a dose of 1.8 g/kg body weight intra-peritoneally. The abdomen was opened to expose the stomach. The esophagus and pyloroduodenal junction were exposed and ligated.
without damaging the blood vessels. A small incision was made at the fundus. The lumen was flushed with a warm mucosal solution (143 mM NaCl, 5.9 mM KCl, 1.18 mM MgSO₄, 1.3 mM CaCl₂, and 30 mM glucose at pH 5.0 adjusted with 0.1 N HCl and 0.1 N NaOH) and a dual cannula (internal; silicon diameter of 0.5 mm, external; polyethylene diameter of 3 mm) was inserted for the incision. After the ligation of the dual cannula with the fundus, the stomach was dissected out rapidly and placed in a 30 mL organ bath containing serosal solution (118 mM NaCl, 4.7 mM KCl, 1.15 mM KH₂PO₄, 25 mM NaHCO₃, 1.18 mM MgSO₄, 1.3 mM CaCl₂, and 30 mM glucose), which was kept at 37±1°C and gassed with 95% O₂ and 5% CO₂. The stomach lumen was perfused through the inlet tube of the dual cannula connected to the perfusion pump at a rate of one mL/min. The effluent perfusate from the stomach exiting from the outlet tube was introduced to a fraction collector placed 20 cm above the level of the stomach.

Collection of gastric acid secretion

The gastric sample solutions were collected as timed 10-minute fractions using a fraction collector. The first gastric sample was referred to as basal secretion. The inhibitors (atropine 1 μM, ranitidine 10 μM, indomethacin 0.1 μM or L-NAME 300 μM) and/or Ya-hom were added to the serosal solution after collecting the first control sample at -20 minutes. After the 0 minute collection, the secretagogue (histamine 5.0 μM or bethanechol 100 μM or bethanechol 10 μM) was added to stimulate gastric acid secretion. The effluent perfusate was collected continuously in 10 minute fractions for 120 minutes after stimulation. Acid output was determined by titrating with 2 mM NaOH to the end point of pH 5.0 and expressed as nEq HCl/10 min. After subtracting the basal gastric acid secretory rate, the amount of gastric acid secretion was also calculated as the area under the curve (AUC).

Statistical analysis

All results are presented as means±SEM. The gastric secretory rate was calculated by deducting the individual values for basal secretion. One way analysis of variance (ANOVA) was used to compare the difference among all experimental groups. Tukey’s Honestly significant difference (HSD) test was used to determine whether the difference between the experimental groups was statistically significant. A p-value of less than 0.05 was considered to be significantly different.

Results

Effect of Ya-hom on histamine-induced gastric acid secretion

Histamine treatment gradually increased the gastric acid secretory rate in the control group in a time-dependent manner with the maximum rate being achieved at 20 min. After that, the gastric secretions gradually decreased with time. Ya-hom (2.5, 5.0, 10.0 and 20.0 mg/mL) in the serosal solution inhibited the stimulatory effect of histamine on gastric acid secretory rate in a dose-dependent manner (Fig. 1).
The gastric acid secretory rates in all Ya-hom treated stomachs were significantly lower than those of histamine-stimulated gastric secretion. The AUC of Ya-hom at all doses (1887±297, 1561±312, 324±116, and 168±62 nEq HCl, respectively) was significantly lower than control (5424±545 nEq HCl). The inhibitory effect of Ya-hom was dose-dependent and the AUC of the 10 mg/mL and 20 mg/mL treated groups were significantly lower than the 2.5 mg/ml treated group (p <0.01) and the AUC of the 20 mg/mL treated group was significantly lower than that of the 5.0 mg/mL treated group. Ya-hom at a sub-maximal dose of 10 mg/mL was used in the remaining experiments.

**Effect of atropine on the action of Ya-hom**

Atropine (1 mM) significantly decreased the rate of histamine-induced gastric acid secretion. The gastric secretory rate of Ya-hom treated stomachs in the absence or presence of atropine was significantly lower than that of the histamine control group and significantly lower than the atropine-treated group, as shown in Fig. 2. The AUC of the atropine-treated group (2582±406 nEq HCl) was significantly lower than that of the histamine control (5424±545 nEq HCl) (p <0.01). The gastric secretory rates of Ya-hom-treated stomachs were similar, regardless of the presence or absence of atropine. There were no significant differences in AUC of Ya-hom-treated groups in the presence (81±66 nEq HCl) or absence of atropine (324±116 nEq HCl). These data indicated that atropine did not affect Ya-hom action on the histamine-stimulated gastric acid secretion.

**Effect of ranitidine on the action of Ya-hom**

The application of ranitidine (10 μM) almost completely inhibited histamine-stimulated gastric acid secretion. Ya-hom (10.0 mg/mL) significantly inhibited the stimulatory effect of histamine to the similar level as ranitidine at a dose of 10 μM. There were no significant differences in gastric acid secretory rates among the histamine-stimulated groups in the presence of ranitidine (H+R) or Ya-hom (10 mg/mL) without (H+YH) or with ranitidine (H+YH+R) (Fig. 3). The AUC of H+R (361±136 nEq HCl), H+YH (324±116 nEq HCl) and H+YH+R (132±60 nEq HCl) groups were significantly lower than that of histamine control (p <0.05) but not different between the groups. The data indicated that ranitidine did not alter the inhibitory effect of Ya-hom on histamine-stimulated gastric acid secretion in isolated mouse whole stomach.

![Fig. 2](image)

**Fig. 2** Effect of Ya-hom in the presence of atropine on histamine-induced gastric acid secretory rate (n=10). All values are expressed as means±SEM after subtracting the basal secretion (553±28 nEq HCl/10 min). The gastric acid secretory rates of atropine, Ya-hom and atropine + YH treated groups are significant lower than control (histamine alone) at the same time point from 10-90 minutes for atropine and from 10-110 minutes for YH and atropine +YH (p <0.05). YH and atropine + YH treated groups are significant lower than atropine treated group form 20-70 minutes (p <0.05).
Gastric acid secretion increased rapidly to a maximum rate at 20 minutes after adding bethanechol (100 \( \mu \text{M} \)) and decreased rapidly throughout the experimental period. Ya-hom and atropine significantly decreased the gastric acid secretory rate induced by bethanechol (Fig. 4). There was no significant difference in gastric secretory rate between atropine (1 \( \mu \text{M} \))-treated and Ya-hom-treated groups, as shown in Fig. 4. The AUC of Ya-hom-treated (109\pm51 \text{nEq HCl}) and atropine-treated (523\pm169 \text{nEq HCl}) groups was significantly lower than that of the bethanechol control group (2960\pm190 \text{nEq HCl}) (p <0.01). There was no significant difference in AUC of Ya-hom treated group and atropine-treated group. These data showed that Ya-hom inhibited high dose bethanechol-stimulated gastric acid secretion with potency similar to that of atropine at the concentration of 1 \( \mu \text{M} \).

**Action of Ya-hom on high dose bethanechol-induced gastric acid secretion**

Gastric acid secretion increased rapidly to a maximum rate at 20 minutes after adding bethanechol (100 \( \mu \text{M} \)) and decreased rapidly throughout the experimental period. Ya-hom and atropine significantly decreased the gastric acid secretory rate induced by bethanechol (Fig. 4). There was no significant difference in gastric secretory rate between atropine (1 \( \mu \text{M} \))-treated and Ya-hom-treated groups, as shown in Fig. 4. The AUC of Ya-hom-treated (109\pm51 \text{nEq HCl}) and atropine-treated (523\pm169 \text{nEq HCl}) groups was significantly lower than that of the bethanechol control group (2960\pm190 \text{nEq HCl}) (p <0.01). There was no significant difference in AUC of Ya-hom treated group and atropine-treated group. These data showed that Ya-hom inhibited high dose bethanechol-stimulated gastric acid secretion with potency similar to that of atropine at the concentration of 1 \( \mu \text{M} \).

**Fig. 3** Effect of Ya-hom in the presence of ranitidine on histamine-induced gastric acid secretory rate (n=10). All values are expressed as means \pm SEM after subtracting the basal secretion (553\pm28 \text{nEq HCl/10 min}). The gastric acid secretory rates of ranitidine, Ya-hom and ranitidine + YH treated groups are significant lower than control (histamine alone) at the same time point from 0-110 minutes (p <0.05).

**Fig. 4** Effect of Ya-hom and atropine on high dose (100 \( \mu \text{M} \)) bethanechol-induced gastric acid secretory rate (n=10). All values are expressed as means \pm SEM after subtracting the basal secretion (606\pm24 \text{nEq HCl/10 min}). The gastric acid secretory rates of atropine and Ya-hom treated groups are significant lower than control (bethanechol alone) at the same time point from 10-90 minutes (p <0.05).
Action of Ya-hom on low dose bethanechol-induced gastric acid secretion

Bethanechol (10 μM) rapidly increased gastric acid secretion to a maximum rate at 20 min. This was followed by a rapid time-dependent decline in gastric acid secretion, similar to the effect observed with the high dose. The gastric acid secretory rates of the bethanechol-stimulated stomach in the presence of atropine 1 μM (B+A), in the presence of ranitidine 10 μM (B+R), in the presence of Ya-hom (10 mg/ml) without (B+YH) and with ranitidine 10 μM (B+YH+R) groups were significantly lower than control (bethanechol alone). The gastric acid secretory rates of B+A, B+YH and B+YH+R groups were significantly lower than the ranitidine-treated group but there were no differences within these three groups (Fig. 5). The AUC of B+A (331±67 nEq HCl), B+R (1112±120 nEq HCl), B+YH (134±67 nEq HCl) and B+YH+R (35±16 nEq HCl) groups were significantly lower than control (2506±222 nEq HCl), (p <0.01). The AUC of B+A, B+YH, and B+YH+R groups were significantly lower than ranitidine-treated group as a whole but similar between subsets. These demonstrated that Ya-hom inhibited low dose bethanechol-stimulated gastric acid secretion and this was not affected by the inhibitors tested.

The roles of prostaglandin and nitric oxide in the action of Ya-hom

To determine the involvement of prostaglandins and nitric oxide on the gastric inhibitory action of Ya-hom in histamine-stimulated stomach, indomethacin, a cyclooxygenase inhibitor and L-NAME, a nitric oxide (NO) synthase inhibitor, were used. Indomethacin (0.1 μM) significantly increased the basal gastric secretion at –10 min and the rate of histamine-stimulated gastric acid at 70-120 minutes after addition of histamine. The gastric secretory rate of Ya-hom treated stomach was significantly lower than that of the histamine control group and significantly lower than the indomethacin-treated group (Fig. 6). The gastric secretory rates of Ya-hom-treated stomachs were similar, regardless of the presence or absence of indomethacin. The AUC of the indomethacin-treated group (7904±1137 nEq HCl) was significantly higher than that of control (5424±545 nEq HCl) (p <0.05). The AUC of Ya-hom-treated stomach with (383±149 nEq HCl) or without indomethacin (324±116 nEq HCl) was significantly lower than that of control (p <0.01) and indomethacin-treated groups (p <0.01). There were no significant differences in AUC of Ya-hom-treated groups in the presence or absence of indomethacin. The data indicates that inhibition of prostaglandin synthesis does not affect Ya-hom action on histamine-stimulated gastric acid secretion.

**Fig. 5** Effects of ranitidine, atropine and of Ya-hom (YH) alone and in the presence of ranitidine on low dose (10 mM) bethanechol-induced gastric acid secretory rate (n=10). All values are expressed as mean ± SEM after subtracting the basal secretion (588±21 nEq HCl/10 min). The gastric acid secretory rates of all treated groups are significant lower than control (bethanechol alone) at the same time point from 10-80 minutes (p <0.05). Atropine, YH and ranitidine + YH are significantly lower than ranitidine group from 20-40 minutes (p <0.05).
The gastric secretory rate in the L-NAME (300 \textmu M)-treated stomach was significantly different from that of histamine-stimulated gastric acid (control) 20 min and 120 min after addition of histamine. However, the AUC of the L-NAME-treated group (5885±1378 nEq HCl) was not significantly different from that of control (5424±545 nEq HCl). The gastric secretory rate of Ya-hom treated stomach in the absence or presence of L-NAME was significantly lower than that of the control group and significantly lower than the L-NAME-treated group (Fig. 7). The gastric secretory rate of Ya-hom-treated stomach in the
absence of L-NAME was significantly lower than that in the presence of L-NAME. The AUC of the Ya-hom-treated group (324±116 nEq HCl) was significantly lower (p <0.05) than that of control, L-NAME-treated and Ya-hom-treated with L-NAME groups (3127±416 nEq HCl). There were no significant differences in AUC between the following groups: Ya-hom-treated with L-NAME, control and L-NAME-treated. This demonstrates that inhibition of nitric oxide synthase attenuates the inhibitory effect of Ya-hom action on histamine-induced gastric acid secretion.

Discussion

The effect of Ya-hom has been studied previously in histamine- and carbachol-induced gastric acid secretion in gastric fistula rats. The maximal inhibition on histamine- and carbachol-stimulated gastric acid secretion was approximately 40 -50% [4]. Since the gastric secretion in animals is regulated by neuronal and hormonal factors, it is not possible to evaluate the action of Ya-hom specifically on gastric acid secretion in intact animals due to potential action on other cells and pathways. The isolated mouse whole stomach is a more relevant model to clarify the action of Ya-hom because it does not have the confounding effects of the autonomic nervous system and blood borne mediators influencing gastric secretory function. Moreover, the secretagogues, test solutions, and inhibitors can be added directly to the serosal surface to evaluate the effect of each substance without interference of endogenous mediators from the extrinsic nervous system. This model maintains endogenous mediators and remaining histamine secreting cells, making it possible to investigate the mechanism of Ya-hom action involving these regulators. The present study was designed to evaluate the inhibitory effect of Ya-hom on histamine- and bethanechol-stimulated gastric acid secretion in isolated mouse whole stomach and investigate the mechanism of action of Ya-hom.

The present result has demonstrated that Ya-hom does not directly stimulate gastric acid secretion. In fact, we observed marked inhibition of histamine- and bethanechol-stimulated gastric secretion, which was completely abolished at higher doses. The mechanism of inhibition was found to be at least partially mediated by nitric oxide (NO).

Histamine is a major physiological mediator of HCl secretion and stimulates H₂-receptors on parietal cells [6, 7]. Likewise, acetylcholine stimulates histamine secretion via muscarinic receptor on Enterochromaffin-like (ECL) cells and directly stimulates gastric acid secretion in parietal cells [8]. Bethanechol at a low dose (10 μM) can stimulate gastric acid secretion in parietal cells but at a high dose (100 μM) can also stimulate histamine release from ECL cells [9]. In this study, both concentrations were used to stimulate gastric acid secretion. Comparing the stimulatory effects of histamine and bethanechol, the AUC of bethanechol-stimulated gastric acid secretions, for low and high doses are 46% and 55% of histamine-stimulated value, respectively. Atropine (muscarinic antagonist) (1 μM) treatment results in greater than 90% inhibition of low and high dose bethanechol (10 and 100 μM) induced gastric secretion. The profile of gastric acid secretory rates of the two doses of bethanechol are similar, but the high dose has a longer acting effect than the low dose, possibly due to the stimulation of histamine secretion from the ECL cells.

Gastric acid secretion can be stimulated by histamine or bethanechol alone or by the synergistic effect of endogenous acetylcholine or histamine. The existence of endogenous acetylcholine and histamine was demonstrated by the partial inhibition of ranitidine (H₂ antagonist) and atropine (muscarinic antagonist) on bethanechol and histamine action, respectively. The mechanism of inhibition of gastric acid secretion by Ya-hom was analyzed in two gastric acid secretagogues. Ya-hom inhibited the gastric acid stimulatory effects of histamine and bethanechol and almost completely inhibited histamine-stimulated gastric acid secretion in the presence or absence of atropine or ranitidine. Ya-hom treatment did not result in direct gastric acid stimulation but did inhibit histamine-stimulated gastric acid secretion to the same level as ranitidine at a dose of 10 μM.

Acetylcholine is known to stimulate gastric secretion by two pathways: a direct effect on M₃ receptor in parietal cells and an indirect effect via M₁ receptor- stimulated histamine release on ECL cells [8]. It has been shown that at low doses, bethanechol directly stimulates only parietal cells, whereas at high doses it can also cause histamine release from ECL cells and potentiate the stimulation on parietal cells [9]. For these reasons, we used both a low and a high dose to identify the site of action of Ya-hom. Atropine (1 μM) inhibits gastric acid secretion by either dose of bethanechol to the same degree. Ya-hom almost
completely inhibits both low and high doses of bethanechol-induced gastric acid secretion. The present effect on AUC showed that Ya-hom and atropine inhibited low and high dose bethanechol-induced gastric acid secretion by more than 90% and 80%, respectively. Gastric acid secretion of Ya-hom-treated group was slightly lower than that of atropine-treated group, although the difference is not statistically significant.

High dose bethanechol caused 118% stimulation (AUC, 2960±190 vs. 2506±222 nEqHCl) compared to the low dose, perhaps due to the effect of the higher dose on histamine secretion from ECL cells. Ranitidine inhibited bethanechol-stimulated gastric acid secretion by 56% implying the presence of endogenous histamine. Moreover, Ya-hom inhibited low dose bethanechol-stimulated gastric acid secretion in the presence or absence of ranitidine to a similar extent (99% and 95%, respectively). These results demonstrate that Ya-hom at 10 mg/mL inhibits the effects of histamine as well as bethanechol with a similar potency as ranitidine (10 μM) or atropine (1 μM). Our studies demonstrate that Ya-hom inhibits gastric acid secretion via both the muscarinic receptor and H₂ receptor stimulated pathways. This indicates that the site of Ya-hom action is likely a common intracellular pathway that is distal to both these processes. It is also possible that Ya-hom treatment results in the release of secretory inhibitors such as somatostatin [6, 9, 10], NO [11-13] and prostaglandin [14]. To investigate this, we examined the effect of Ya-hom in the presence of L-NAME or indomethacin and found that L-NAME but not indomethacin attenuated the gastric acid inhibitory action of Ya-hom in histamine-stimulated stomach. This data demonstrate that the inhibitory action of Ya-hom might be mediated through activation of nitric oxide synthase (NOS).

The pharmacological activity of some of the individual ingredients of Ya-hom on gastric acid secretion has been well documented. For example, S. lappa [15-17], G. glabra [18-20], A. gramineus [21], and L. wallichii [22] have spasmylytic activity. G. glabra [23-25] inhibits gastric secretion, and E. caryophyllata [26], A. gramineus [21] and G. glabra [27-30] antagonize the effects of histamine and acetylcholine. These active ingredients likely mediate the pharmacological effects of Ya-hom on the gastric acid secretion.

In the gastric fistula model, carbachol (20 μg/kg, intravenously) maximally increased gastric acid secretion to 36.9% of that elevated by histamine (10 mg/kg, intramuscular injection). In a previous study [4], we showed that Ya-hom has only a partial inhibitory effect on histamine- and carbachol-stimulated gastric acid secretion [4]. By defining this further, we could show that histamine has a stronger stimulatory effect than bethanechol and the stimulatory effects of low and high doses of bethanechol are 46% and 55% of histamine stimulation, respectively. However, Ya-hom at 10 mg/mL almost completely inhibits histamine-stimulated gastric acid secretion by 93% and inhibits bethanechol-induced gastric acid secretion by 96% and 95% for low and high doses bethanechol, respectively.

The reasons for the different maximum effects of histamine and Ya-hom between gastric fistula model (in vivo) and isolated mouse whole stomach model (in vitro) are: a) the different period of sample collection; the in vivo model collects sample every one hour whereas the in vitro model samples every 10 minutes, b) the different route of drug administration as histamine administration is intramuscularly injection and Ya-hom’s is per oral, as it is directly added in the duodenum. While in the in vivo model, Ya-hom is absorbed by the intestinal mucosa and distributed through the blood to the stomach mucosa in the in vitro model drug is added directly in the serosal solution.

Additional mediators that are involved in the control of gastric acid secretion include gastrin, pituitary adenylate cyclase-activating peptide (PACAP), vasoactive intestinalpeptide (VIP), γ-aminobutyric acid (GABA) [8] and cholecystokinin [31, 32]. These mobilize histamine, somatostatin, prostaglandin E₂, galanin, or calcitonin gene-related peptide (CGRP) [8], while secretin [10, 33] inhibits histamine mobilization. Since the mediators can be secreted from organs other than gastric glands in an in vivo model, these may affect gastric acid secretion more so than in an in vitro model. It is also possible that the Ya-hom ingredients are metabolized to active gastric acid stimulating ingredients that attenuate the inhibitory effect of Ya-hom in an in vivo model. These may cause Ya-hom to have partial inhibition in the gastric fistula model and complete inhibition in the isolated whole stomach model. This is because the isolated whole stomach has multiple gastric regulators such as...
D-cells of the antrum and epithelial cells that produce the secondary inhibitors somatostatin and NO, respectively. Therefore, Ya-hom may have a direct inhibitory effect on gastric acid secretion and in addition, indirect effects that are attributable to the release of a second inhibitor. To characterize the pharmacology of Ya-hom, further studies should be focused on effects on isolated gastric gland and parietal cells.

**Conclusion**

Ya-hom potently inhibited the effects of histamine- and bethanechol-stimulated gastric acid secretion in the isolated mouse stomach. Ya-hom stimulated the release of NO thereby inhibiting gastric acid secretion. Ya-hom had no gastric acid stimulatory effect in this model. This study supports the claimed efficacy of Ya-hom ingestion for abdominal discomfort based on its attenuating gastric acid secretion.

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