EFFECT OF YA-HOM ON GASTRIC ACID SECRETION IN ISOLATED MOUSE WHOLE STOMACH

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ABSTRACT

The effects of Ya-hom, Thai traditional formula, on gastric acid secretion in isolated mouse whole stomach were evaluated to verify its use for stomach discomfort treatment. The action of Ya-hom on gastric acid secretion was studied in isolated mouse stomach was maintaining at the intragastric pressure at 20 cm H_2O. The actions of Ya-hom were studied in histamine- and bethanechol-induced gastric acid secretion in isolated mouse stomach. Gastric acid secretion was collected as 10 min interval fraction, combined to 10 min set time interval. After the first collection, redissolved lyophilized Ya-hom extract (2.5, 5.0, 10.0 or 20.0 mg/ml) or inhibitor (atropine 1 µM, muscarinic receptor antagonist or ranitidine 10 µM, H_2 receptor antagonist) were administrated into the serosal solution. After preincubate with Ya-hom or inhibitors at the certain period, the gastric acid secretion were induced by the secretagogues (histamine, 5.0 µM or bethanechol, 10, 100 µM).

In the present study, the preincubating Ya-hom for 20 min before adding secretagogue inhibited the gastric acid secretion more than preincubation period for 0 or 10 min. The presence of Ya-hom along the experiment inhibited the gastric acid secretion more than the washing out of Ya-hom before adding the secretagogues. Ya-hom inhibited histamine-induced gastric acid secretion in dose dependent manner. Ya-hom (10 mg/ml) inhibited histamine-induced gastric acid secretion in the presence of atropine to eliminate the effect of endogenous acetylcholine. The low dose (10 µM) bethanechol has only direct stimulation on parietal cell, whereas high dose (100 µM) bethanechol also causes a histamine release to potentiate the direct effect on parietal cell. Ya-hom inhibited both low dose (10 µM) and high dose (100 µM) bethanechol-induced gastric acid secretion in the presence and absence of ranitidine (10 µM).

The results suggest that Ya-hom inhibited gastric acid secretion both histamine-stimulating and bethanechol-stimulating pathways. Attenuating gastric acid secretion by secretagogues are parts of the Ya-hom action for the treatment of stomach discomfort.

KEY WORDS: ISOLATED MOUSE STOMACH / GASTRIC ACID SECRETION / YA-HOM

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ผลของยาหอมต่อการหลั่งกรดในกระเพาะอาหารหนูถีบจักรที่แยกออกมาจากตัว (EFFECT OF YA-HOM ON GASTRIC ACID SECRETION IN ISOLATED MOUSE WHOLE STOMACH)

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บทคัดย่อ

การประเมินผลของยาหอมซึ่งเป็นตำรับยาไทยที่มีผลต่อการหลั่งกรดในกระเพาะอาหารหนูถีบจักรที่แยกออกมาจากตัวเพื่อใช้เป็นข้อมูลสนับสนุนการใช้ยาหอมในการบรรเทาอาการไม่สบายท้อง การศึกษาการแสดงฤทธิ์ของยาหอมต่อการหลั่งกรดในกระเพาะอาหารหนูถีบจักรที่แยกออกมาจากตัวที่แขวนไว้ภายใต้ความดันภายในกระเพาะที่ 20 เซนติเมตรน้ำ ศึกษาการแสดงฤทธิ์ของยาหอมจากกระเพาะอาหารที่ถูกกระตุ้นด้วยฮีสตามีนและเบททาเนคอล ปริมาณกรดที่หลั่งออกมาจะถูกเก็บรวมกันทุก 10 นาที หลังจากเก็บตัวอย่างแรกแล้วจึงให้ยาหอม (2.5, 5.0, 10.0 และ 20.0 มก/มล) หรือสารยับยั้ง (อะโทรปีน 1 ไมโครโมล ซึ่งเป็นสารยับยั้งฤทธิ์ของตัวรับอะเซททิลโคลลีน และ รานิทิดีน 10 ไมโครโมล ซึ่งเป็นสารยับยั้งฤทธิ์ของตัวรับฮีสตามีนชนิด เอช2) ลงในสารละลายทางด้านซีโรซัล หลังจากยาหอมหรือสารยับยั้งตามเวลาที่กำหนดแล้วจึงให้ตัวกระตุ้น (ฮีสตามีน 5.0 ไมโครโมล หรือ เบททาเนคอล 10, 100 ไมโครโมล)

ผลการศึกษาพบว่า การให้ยาหอมก่อนเป็นเวลา 20 นาทีก่อนการให้สารกระตุ้นทำให้เกิดผลการยับยั้งการหลั่งกรดได้มากกว่าการให้ยาหอมพร้อมตัวกระตุ้นหรือ10 นาที ก่อนตัวกระตุ้น การไม่ล้างยาหอมออกให้ผลยับยั้งการหลั่งกรดมากกว่าการล้างยาหอมออกก่อนการให้สารกระตุ้น ยาหอมยับยั้งการหลั่งกรดที่กระตุ้นด้วยฮีสตามีนตามขนาดที่เพิ่มขึ้น ยาหอม 10 มก/มล ยับยั้งผลของฮีสตามีนในขณะที่มีอะโทรปีนเพื่อยับยั้งการออกฤทธิ์ของอะเซททิลโคลลีนภายใน นอกจากนั้น ยาหอมยับยั้งการหลั่งกรดที่กระตุ้นด้วยเบททาเนคอล ทั้งขนาด 10 และ 100 ไมโครโมล ซึ่ง เบททาเนคอล10 ไมโครโมล กระตุ้นการหลั่งกรดที่พาไรทัลเซลล์โดยตรง ในขณะที่เบททาเนคอล100 ไมโครโมล กระตุ้นการหลั่งฮีสตามีนจึงเสริมฤทธิ์การหลั่งกรดที่พาไรทัลเซลล์

การศึกษานี้แสดงให้เห็นว่ายาหอมมีผลลดการหลั่งกรดที่กระตุ้นด้วยฮีสตามีนและเบททาเนคอล ดังนั้นผลของยาหอมในการลดการหลั่งกรดที่เกิดจากการใช้สารกระตุ้นการหลั่งกรดจึงเป็นส่วนหนึ่งของฤทธิ์ของยาหอมในการบรรเทาอาการไม่สบายท้อง

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LIST OF ABBREVIATIONS

AUC	=	areas under the curve
AUC at peak	=	areas under the curve from 10 min before the peak to 10 min after the peak
AUC at 0 min-peak	=	area under the curve from 0 min to the peak
$CaCl_2$	=	calcium chloride
cAMP	=	cyclic adenosine monophosphate
cm	=	centimeter
ECL	=	enterochromaffin-like cell
PGE	=	prostaglandin
g	=	gram
kg	=	kilogram
L	=	liter
mg	=	milligram
min	=	minute
ml	=	milliliter
mm	=	millimeter
nEq	=	nano equivalent
HCl	=	hydrochloric acid
KCl	=	potassium chloride
HDC	=	histidine decarboxylase
KH_2PO_4	=	potassium dihydrogen phosphate
$MgSO_4$	=	magnesium sulfate
NaCl	=	sodium chloride
$NaHCO_3$	=	sodium hydrogen carbonate
NaOH	=	sodium hydroxide
PACAP	=	pituitary adenylase cyclase activating peptide

LIST OF ABBREVIATIONS (Continued)

YH	=	Ya-hom
^{o}C	=	degree celsius
μM	=	micromolar

CHAPTER I
INTRODUCTION

In Thailand, traditional medicine have been widely used and promoted to use for the treatment of several diseases, however, they also have some limitations for uses such as lack of scientific data on the efficacy, mechanism of actions and toxicity. The scientific studies were necessary to explain and support the efficacy and safety in traditional use. Among traditional medicines used nowadays, Ya-hom is one of the most popular formula among elderly and people used for fainted or feeling like fainting and stomach discomfort such as nausea, vomiting, stomachache and flatulent. The stomach discomfort may arrive from interfering on the gastric function such as increasing or decreasing gastric acid secretion, dyspepsia, gastric ulceration and stomach pain, thereby the effect of Ya-hom on gastric secretory function should be investigated to clarify its action on relieving stomach discomfort. However, Ya-hom preparations sold in market are under different trade names with various compositions and amount of medicinal plants, in this study only one preparation was selected.

One of the most popular brands of Ya-hom has been selected for this study. The pharmacological data of compositions of Ya-hom indicated that *Cinnamomum cassia* (1), *Saussurea lappa* (2), *Glycyrrhiza grabra* (3-10) and *Acorus gramineus* (11-12) have antiulcer activity. *S. lappa* (13-14), *G. grabra* (15-16) and *A. gramineus* (17) have spasmolytic activity. *G. grabra* (3, 5, 7, 18) have gastric secretion inhibitory effect. *Ligusticum wallichii* (19), *Eugenia caryophyllata* (20), *A. gramineus* (17) and *G. grabra* (21-22) have antagonized the effect of histamine and acetylcholine. *E. caryophyllata* (23-24) has gastroprotective effect and *G. grabra* (10) has anti-bacteria. Even if some Ya-hom's ingredients have no scientific data on their effect on the gastric function. Recently, the pharmacological data of water extract Ya-hom has showed the inhibition of the stimulatory effects of histamine and carbachol on the acid, pepsin and soluble mucus secretion but potentiating the visible mucus secretio

in the gastric fistula model. Attenuating gastric secretion by secretagogues and increasing gastric barrier effects are parts of the Ya-hom's action on stomach discomfort (25). However, *in vivo* model is not sufficient enough for investigation of mechanism of actions due to the complicated endogenous regulation. The isolated mouse whole stomach model has been used for this study.

In this model, the automatic nerve regulated gastric secretion has been removed. The secretagogues and tested solution are directly added into serosal solution to evaluate the effect of each substance without the interfering of endogenous mediators from the automatic nervous system. Since this model has never been set up in our laboratory, the optimal conditions for collecting the gastric acid secretion has been initially investigated before studying the effect of Ya-hom. Moreover, remained in the stomach and intragastric pressure may effect gastric secretion, the gastric secretion from fasted and non-fasted stomach with various intragastric pressure have been determined in order to establish the optimal condition for the experiment. Using isolated stomach, the mechanism of action of Ya-hom can be directly monitored by determining acid secretory rate after adding secretagogues and inhibitors. Gastric acid output was collected at set time intervals before and after addition of an acutely implanted secretagogue, inhibitor or Ya-hom in serosal solution by continuous intragastric perfusion of mucosal solution. The gastric acid secretion was stimulated by histamine and bethanechol. The acid output was determined to investigate the effects of Ya-hom on gastric acid secretion. The mechanism of action of Ya-hom on histamine- and bethanechol-induced gastric acid secretion was revealed by investigation the effect of Ya-hom in the presence of specific inhibitor; atropine, acetylcholine antagonist and ranitidine, H_2 receptor antagonist. The results will provide an explanation of the uses of Ya-hom on stomach discomfort treatment and a guide for further biological studies of other Ya-hom recipes and their ingredients.

CHAPTER II
LITERATURE REVIEW

I GASTRIC ACID SECRETION.

Gastric acid secretion by parietal cell is regulated by paracrine, endocrine, and neural pathways (26-28). The regulation of gastric acids secretion is achieved in the periphery by interplay between three major gastric endocrine cells: the enterochromaffin-like (ECL) cell, the gastrin or G cell, and the somatostatin or D cell. Regulation of these cell is via stimulatory or inhibitory paracrine, endocrine and neural pathways. The functional integration of these three cell types is the primary determinant of the degree of stimulation of the parietal cell (27). The parietal cells, located deep in the gastric glands of the stomach, are the only cells that secrete hydrochloric acid. However, secretion of this acid is under continuous control by both endocrine and nervous signals. Furthermore, the parietal cell operates in close association with another type of cell called the enterochromaffin-like cell, the primary function of which is to secrete histamine (26). Gastric acid secretion is a tightly regulated process triggered by ligand-receptor binding at the basolateral membrane with ultimate output of H^+, Cl^- and H_2O across the apical plasma membrane of the parietal cell. The physiological stimuli include acetylcholine, gastrin and especially, histamine, which operate via basolateral membrane receptors. Stimulation of acid secretion typically involves an initial elevation of intracellular calcium and cAMP, followed by activation of protein kinase cascades, which triggered the translocation and insertion of H^+, K^+-ATPase, the proton pump enzyme, into the apical plasma membrane of the parietal cell (28).

1. Functional and Anatomical Organization of the Stomach.

The anatomical subdivision of the stomach into the fundus, antrum and body (corpus) mirrors differences in motor function. The fundus functions as a reservoir for ingested meals, the body as the initial site of peristalsis and the antropyloric region as

the site of the greatest mechanical agitation and mixing of food with gastric secretion. In term of gastric mucosal function, however, the rat stomach can be divided into two majors regions: The exocrine or glandular, portion found in the mucosa of the fundus and body, and endocrine part located in the antral mucosa.

The mucosa of the exocrine portion of the stomach consists of columnar epithelial cells that line the luminal surface. These cells secrete mucus and an alkaline fluid, both necessary for protecting the stomach against its own potentially harmful juices. Opening into the mucosal surface are numerous gastric pits that serve as conduits for the secretions of 3-7 oxyntic gastric gland into the gastric lumen. The area occupied by the gastric pits is at least 50% of the total luminal surface area. The remainder of the gastric or oxyntic gland can be further divided into two regions. The neck of oxyntic gland contains parietal and mucous neck cells, the latter resembling intestinal goblet cells and secreting mucus. The neck region is also the site of germinal cell proliferation and differentiation, giving rise to surface cells that migrate up to the surface or glandular cells that move downward toward the base. Cells progressively mature as they reach their final destinations and then are continuously replaced by new cells, the turnover of various cell types ranging from days to weeks. The base of the gland contains chief cells in addition to some parietal and mucous neck cells. The oxyntic gland also contains a number of endocrine-type cells dispersed among chief and parietal cells that play a role in regulating their secretory functions (29).

Each cell type has different functions. Parietal cells are the acid-forming and acid-secreting cells (26, 28, 29). Chief cells make and secrete pepsinogen, which is converted by gastric acid to the active from pepsin (29, 30). Mucous cells make mucus, an essential component that lubricates and protects the gastric mucosa, and those at the surface and in gastric pits also secrete bicarbonate. Interspersed among these cells of the oxyntic gland are enterochromaffin cells that regulate their functions by secretion of bioactive amines and peptides (29). Finally, a number of ECL cells, histamine-containing endocrine in the gastric mucosa (27, 31), found in close apposition to epithelial cells of the gastric gland secrete histamine (26, 29, 30) indirect contact with the parietal cells of their gland themselves (31). Furthermore, ECL cells are rich in the histamine forming enzyme histidine decarboxylase (HDC) (26, 27, 30), allowing them to replace mobilized histamine quite readily (30, 32). The rate of

formation and secretion of hydrochloric acid by the parietal cells is directly related to the amount of histamine secreted by the ECL cell (26, 30).

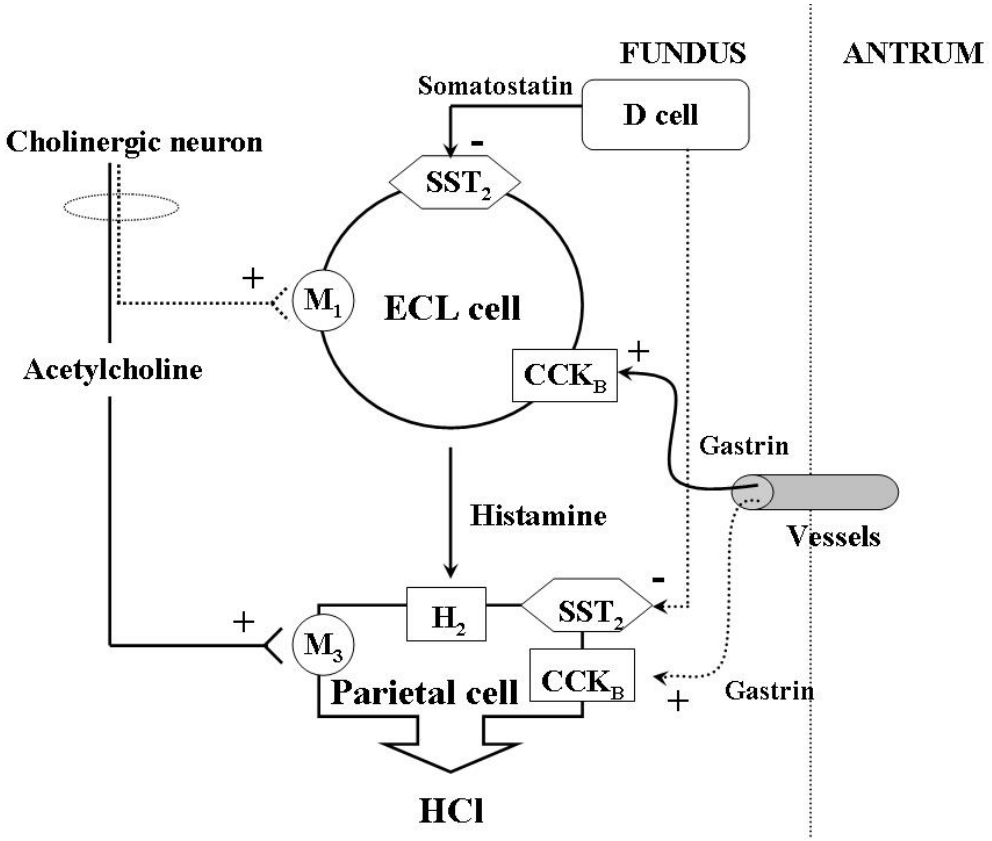


Figure 1 Diagram showing acetylcholine, histamine, somatostatin and gastrin interconnections in the regulatory pathways of gastric acid secretion. Acetylcholine (M), histamine (H), somatostatin (SST) and gastrin (CCK) receptors are indicated; - inhibitory signal; + stimulatory signal. (modified from Lindstrom E., 2001)

2. Control of Gastric Acid Secretion in the Level of ECL and Parietal Cell.

Acetylcholine, histamine and gastrin are the three physiological agonists of HCl secretion (26-28, 33-34). Each of these secretagogues binds to a distinct class of receptors on the plasma membrane of the parietal cell and directly stimulates the parietal cell to secrete HCl (33) as showed in Figure 1. Acetylcholine is released near parietal cells by cholinergic nerve terminals. Gastrin, a hormone, produced by G cells in the mucosa of the gastric antrum and the duodenum, reaches parietal cells via the blood stream. Histamine, a paracrine agonist, is released from cell in the gastric mucosa and diffuses to the parietal cells (26, 33). Thus, the control of HCl secretion provides a regulation by all three types of control mechanisms; neurocrine, endocrine and paracrine, (27, 33, 35) that regulate gastrointestinal secretion (33).

The receptors on parietal cell membrane for acetylcholine, gastrin and histamine as well as the intracellular second messengers by which these secretagogues act. Histamine, acetylcholine and gastrin potentiate one another's actions on the parietal cell. Histamine is a major physiological mediator of HCl secretion (33, 35-36). Histamine is synthesized from histidine by the enzyme HCD and is stored in enterochromaffine-like (ECL) cells (30, 33, 36-41), which are present in rat and mouse oxyntic mucosa (30, 33, 36). When gastric acid secretion was stimulated by acetylcholine or gastrin, the ECL cells release histamine, which diffuses to nearby parietal cells to stimulate HCl secretion (33). Gastrin stimulates histamine secretion and synthesis in the ECL cell via CCK_B receptors and that mobilized ECL-cell histamine stimulates the parietal cell to secrete HCl via an action on H_2 receptors. (27, 30, 36, 41-47). In addition, stimulation of ECL cell by acetylcholine also result in the release of histamine and is accompanied by a characteristic change in $[Ca]_i$. However, whereas all ECL cells response to gastrin, only about 10 to 30% of the cells response to acetylcholine (27).

The parietal cell is considered to have at least three types of activating receptors on its basolateral membrane, i.e., histamine H_2, acetylcholine M_3 and gastrin CCK_B, although much of the action of acetylcholine or gastrin is mediated by the release of endogenous histamine. It is believed that the H_2 receptor couples to Gs to activate adenylate cyclase (ACase) producing adenosine 3',5'-cyclic monophosphate (cAMP) and subsequent activation of cAMP-dependent protein kinase (PKA), whereas

both M_3- and CCK_B-type receptors couple to non-Gs/Gi systems, probably Gq, to activate phospholipase C (PLC) producing inositol1,4,5-triphosphate (IP_3) and diacylglycerol, with the former releasing Ca^{2+} from intracellular stores, and the latter activating protein kinase C (PKC). The final step of HCl production is operated by the gastric proton pump, the H^+, K^+-ATPase, that the enzyme consists of a catalytic α-subunit of approximately 100 kDa molecular size and a highly glycosylated β-subunit. The gastric enzyme uses the energy of ATP for the electroneutral countertransport of H^+ for K^+. The function of gastric proton pump is under dual-restriction while the parietal cell is in the resting state, i.e., the enzyme is sequestered within a population of cytoplasmic vesicles called tubulovesicles spatially insulating them from the gastric lumen, and the low permeability of tubulovesicular membranes to KCl limits the turn over of the pump even though there is ample ATP around the enzyme. Activation of acid secretion is achieved by two concomitant functional changes, namely, one; tubulovesicles fuse with the apical secretory membrane thus recruiting functional pumps to the expanded microvillar surface and two; the apical membrane acquires permeability to KCl. In other words, the pump molecule itself is not activated during the activation of acid secretion. It is thus concluded that the intracellular signal transduction pathways should be connected to the machinery leading to the activation of apical K^+ and Cl^- transporters (48).

Acetylcholine activates M_3 receptors on parietal cells, resulting in acid secretion (49). Acetylcholine binds to M_3 muscarinic receptors and opens Ca^{++} channels in the apical plasma membrane. Acetylcholine also elevates the intracellular Ca^{++} concentration by promoting release of Ca^{++} from intracellular stores. An elevated intracellular Ca^{++} concentration enhances HCl secretion by activating basolateral K^+ channels and by causing more H^+, K^+-ATPase molecules and Cl^- channels to be inserted into the apical plasma membrane (33).

3. Endogenous Antagonists of Acid Secretion

Somatostatin, prostagladins of the E (33, 49) and I series (33, 50), and epidermal growth factor (EGF) act on parietal cells to inhibit HCl secretion (33, 36) by inhibiting adenylyl cyclase and decreasing the cAMP concentration. Somatostatin released from D cell near the bases of gastric glands, is probably a physiological

regulator of acid secretion by parietal cells. Somatostatin may inhibit release of histamine by ECL cells (33).

Somatostatin is a major peptide inhibitor of gastric acid secretion (27, 34, 47, 51-52). Somatostatin binds at a somatostatin-2 receptor subtype, inhibiting both histamine release and calcium signaling in ECL cell. Somatostatin is present in both the fundus and the antrum in D cells, which are differentially activated. In the antrum, antral luminal acidity is thought to stimulate somatostatin release in order to inhibit gastrin release from the G cell. In the fundus, the D cell is stimulated by gastrin CCK-A and by acetylcholine, as well as by other neural peptides. This somatostatin pool is probably responsible for inhibition of ECL cell function (27).

4. *In Vivo* Control of Acid Secretion Rate.

When the stomach has been empty for several hours, HCl is secreted at a basal rate, which is approximately 10% of the maximal rate. After meal, the stomach promptly increases the rate of acid secretion. There are three phases of increased acid secretion in response to food: the cephalic phase, elicited before food reaches the stomach; the gastric phase, elicited by the presence of food in the stomach; and the intestinal phase, elicited by mechanisms that originate in the duodenum and upper jejunum.

The cephalic phase of gastric secretion is elicited by the sight, smell and taste of food. Cephalic phase secretion is entirely mediated by branches of the vagus nerves. Vagal fibers then stimulate enteric neurons that are predominantly cholinergic, and it is these enteric neurons that directly elicit cephalic phase secretion. Acetylcholine released from these neurons directly stimulates parietal cells to secrete HCl. Indirectly stimulates acid secretion by releasing gastrin from G cells in the antrum an duodenum, and by releasing histamine from ECl cells in the gastric mucosa.

The low pH in the antrum of the stomach inhibits HCl secretion by directly inhibiting parietal cells and by evoking inhibitory neural reflexes. In the absence of food in the stomach to buffer the acid secreted, the pH of the antral contents falls rapidly during the cephalic phase. The rate of acid secretion during the cephalic phase may be 40% of the maximum rate, but because of the inhibitory mechanisms evoked by low pH in the antrum, the amount of acid secreted is small.

The brain may also influence gastric acid secretion by other mechanisms, but their physiological importance remains uncertain. Low glucose level in cerebral blood, which occur, for example, during insulin-induced hypoglycemia, stimulate gastric acid secretion. Certain neuropeptides present in brain neurons, when injected into cerebrospinal fluid, stimulate or inhibit gastric secretion of HCl.

The gastric phase of gastric secretion is elicited by the presence of food in the stomach. The principle stimuli are distension of the stomach and the presence of amino acid and peptides that results from the actions of pepsins. Most of the acid secreted during the gastric phase (33).

Distension of either the body or the antrum of the stomach stimulates mechanoreceptors in the gastric wall. These mechanoreceptors are the afferent arms of local and central reflexes. Both the local and central reflexes are largely cholinergic. The central reflexes have their afferent and efferent fibers in the vagus nerves; thus they are called vagovagal reflexes. Activation of these local and central reflexes causes acetylcholine to be released onto parietal cells, which directly stimulates them to secrete HCl, and onto central G cells, which are stimulated to release gastrin (26, 33, 53). Distension of the stomach stimulates the secretion of the hormone gastrin and increase the activity of the vagus nerve, which stimulates stomach motility and further secretion of gastric juices (54).

The presence of amino acids and peptides in the antrum elicits HCl secretion by causing G cells in the antrum to release gastrin. Intact proteins do not have this effect. Other ingested substances that may enchance gastric acid secretion include calcium ions, caffeine, and alcohol. Gastric distension enhances the effect of chemical stimuli of HCl secretion.

Secretion of HCl elicited by any of the mechanisms just described is effectively blocked by bathing the mucosal surface with a solution that has a pH of 2 or less. Once the buffering capacity of the gastric contents is saturated, gastric pH falls rapidly and inhibits further acid release. In this way, the acidity of gastric contents regulates itself. The mechanism for this self-regulation stem from the antrum. Low pH in the antrum inhibits HCl secretion by parietal cells by evoking local inhibitory reflexes, by directly inhibiting parietal cell secretion, and by inhibiting the release of gastrin from G cells.

The intestinal phase, the presence of chyme in the duodenum brings about neural and endocrine responses that first stimulate and later inhibit acid secretion by the stomach. Early in gastric emptying, when the pH of gastric chyme is greater than 3, stimulation predominates. Later, when the buffer capacity of gastric chyme is empty into the duodenum falls to less than 3, inhibition prevails (33).

5. Chemical Mechanism of Hydrochloric Formation.

Different suggestions for the chemical mechanism of hydrochloric acid formation have been offered. One of these is shown in figure 2 and consists of 4 steps.

The first, Chloride ion is actively transported from the cytoplasm of the parietal cell into the lumen of the canaliculus, and sodium ions are actively transport out of the lumen. These two effects together create a negative potential of -40 to -70 millivolts in the canaliculus, which is turn causes diffusion of positively charged potassium ions and a small number of sodium ions from the cell cytoplasm also into the canaliculus. Thus, in effect, mainly potassium chloride and much smaller amounts of sodium chloride enter the canaliculus. Second, water becomes dissociated ions into hydrogen ions and hydroxyl ions in the cell cytoplasm. The hydrogen ions are then actively secreted into the canaliculus in exchange for potassium ions: This exchange process is catalyzed by H^+, K^+-ATPase. In addition, the sodium ions are actively reabsorbed by a separate sodium pump. Thus, most of the potassium and sodium ions that had diffused into the canaliculus are reabsorbed into the cell cytoplasm, and hydrogen ions take their place in the canaliculus, given a strong solution of hydrochloric acid in the canaliculus, which is then secreted outward through the open end of the canaliculus into the lumen of the gland. Third, water passes into the canaliculus by osmosis because of the secretion of the ion into the canaliculus. Thus, the final secretion from the canaliculus contains approximately hydrochloric acid at a concentration of 150-160 mEq/L, potassium chloride at a concentration of 15 mEq/L, and small amount of sodium chloride. Finally, carbon dioxide, either formed during metabolism in the cell or entering the cell from the blood, combines under the influence of carbonic anhydrase with the hydroxyl ions (formed in step 2 when water was dissociated) to form bicarbonate ions. This then diffuses out of the cell cytoplasm into the extracellular fluid in exchange for chloride ions that enter the cell from the extracellular fluid and are later secreted into the canaliculus (26).

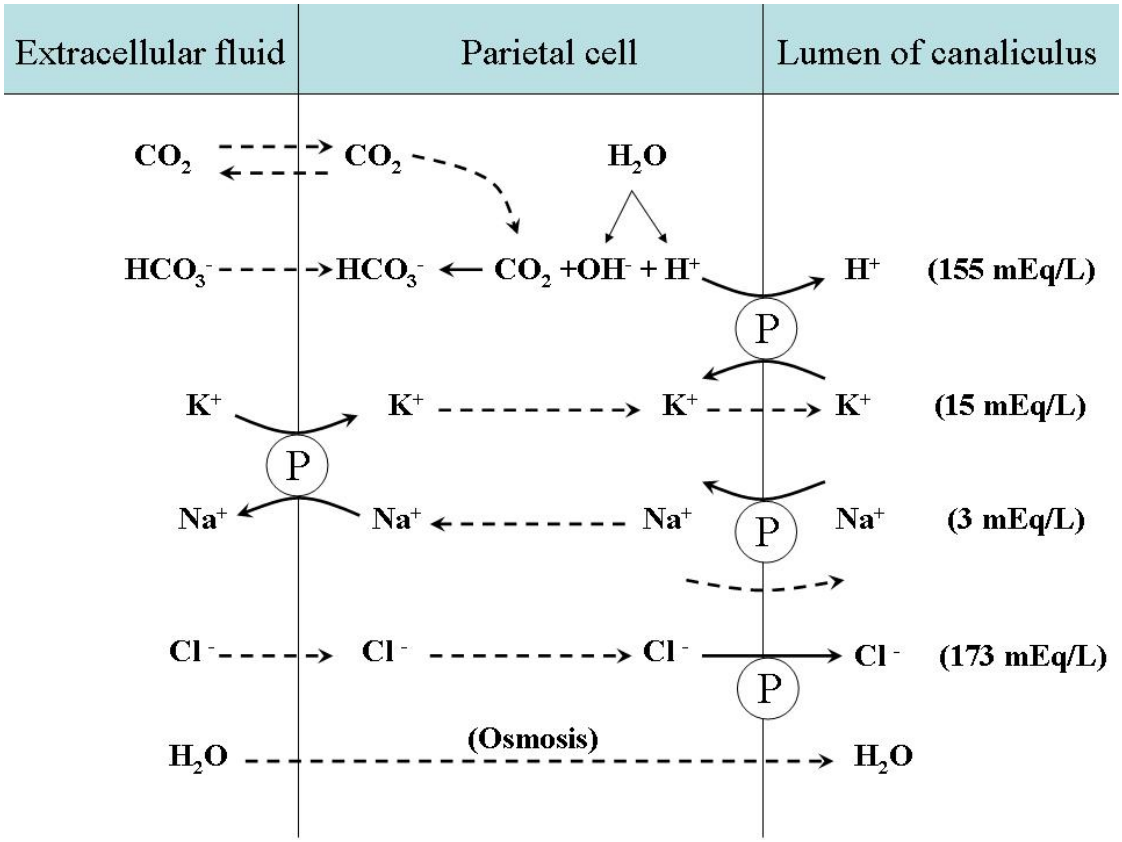


Figure 2 Postulated mechanism for the secretion of hydrochloric acid. The points labeled "P" indicate active pumps, and the dashed lines represent free diffusion and osmosis. (Guyton AC., 2000)

II. YA-HOM.

Ya-hom, the Thai traditional recipe, is composed of many plants. Ya-hom in market are under different trade names with various compositions and amount of medicinal plants. Ya-hom are usually used to treatment stomach discomfort in old people.

Ya-hom recipe selected for this study containing in 100 g are *Agastache rugosa* (Fisch. Et Mey) O. Kuntze (whole plant, Labiatae) 7.1 g, *Acorus gramineus* Soland (rhizomes, Araceae) 3.5 g, *Lysimachia foenum-graecum* Hance (whole plant, Primulaceae) 3.3 g, *Citrus nobilis* Lour. (outer yellow rind of the ripe fruit, Rutaceae) 7.1 g, *Magnolia officinalis* Rehd. et Wils (bark of stem, Magnoliaceae) 11.8 g, *Cinnamomum cassia* Presl (Chinese cinnamon, bark, Lauraceae) 7.1 g, *Mentha arvensis* L. (Japanese mint, whole plant, Lamiaceae) 3.5 g, *Asarum sieboldii* Miq. (whole plant, Aristolochiaceae) 2.3 g, *Ligusticum wallichii* Franch. (rhizomes, Umbelliferae) 9.3 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, Umbellaferae) 3.5 g.

1. Gastrointestinal Activity of the Ingredients of Ya-hom

1.1 *Acorus gramineus* Soland

The principle active components in *A. gramineus* were identified as the phenylpropanoids α- and β-asarone (55-56) and 1-allyl-2, 4, 5-trimethoxybenzene (17). These three compounds had a spasmolytic action on the isolated guinea pig trachea and ileum contracted by acetylcholine, histamine, serotonin and $BaCl_2$, in which α-asarone was the most active (17). *A. gramineus* have antiulcer avtivity (11-12)

1.2 *Eugenia caryophyllata* Thrub.

Some studies reported indicated that *E. caryophyllata* antagonized the effects of acetylcholine (20). Clove oil from *E. caryophyllata* has been reported to stimulate mucus secretion (23, 24).

1.3 *Cinnamomum cassia* Presl

Administration of the stem bark of the *C. cassia* compounds orally or parenterally at a remarkable low dose of 40 μg/kg body weight to rats also inhibited gastric ulcer induced by the ulcerogens such as phenylbutazone and ethanol (1).

1.4 *Ligusticum wallichii* Franch

Ethanol (95%) extract of dry rhizome of *L. wallichii* has antihistamine activity in geania pig. (19).

1.5 *Saussurea Lappa* Clark

There are some reports indicated that it caused intestinal, uterus and bronchial smooth muscle relaxation in which spasms had been induced by histamine and acetylcholine (13-14). Dried root of *S. lappa* showed anti-ulcer effect on HCl/ethanol-induced lesion in rats, and exhibited an inhibitory activity on stress-induced ulcer formation in mice (2).

1.6 Glycyrrhiza glabra Linn.

There are substantial evidences indicated that licorice has effect on gastrointestinal functions. It has been reported to have an antiulcer activity and inhibit gastric secretion (3-7). Some reports suggested that the extract of licorice had spasmolytic action and may help in the healing process of gastric ulcer (15-16). The licorice extract were effective in the treatment and prevention of the gastric and duodenal ulceration in rats (8-9, 57). The root of licorice showed synergistic effect on central nervous system depression, anti-inflammatory, inhibited gastric secretion and antidiuretic effect (18, 58). In addition, the roots of licorice have been showed to antagonize the effects of acetylcholine and histamine (21, 22). *G. grabra* have anti-*Helicobacter pylori*; a bacterium that lives in the stomach and duodenum as one of the etiological agent of peptic ulcer (59) and anti-ulcerogenic effect (10).

The biological information about some ingredients of Ya-hom on gastrointestinal function has not been reported. The summarize of gastrointestinal report has shown in the table 1 which some ingredients of Ya-hom has antiulcer activity (1-12, 57), spasmolytic activity (13-17), gastric secretion inhibitory effect (3, 5, 7, 18), antagonize effect of histamine and acetylcholine (17, 19, 20-22), gastroprotective effect (23-24) and anti- *Helicobacter pylori* (59).

Table 1 The summary of gastrointestinal activity of some ingredients of Ya-hom.

Gastrointestinal activity	Plants
Antiulcer activity	*C. cassia* (1), *S. lappa* (2), *G. grabra* (3-10, 57), *A. gramineus* (11-12)
Spasmolytic activity	*S. lappa* (13-14), *G. grabra* (15-16), *A. gramineus* (17)
Gastric secretion inhibitory effect	*G. grabra* (3, 5, 7, 18)
Antagonize effect of histamine	*L. wallichii* (19), *A. gramineus* (17), *G. grabra* (21-22)
Antagonize effect of acetylcholine	*E. caryophyllata* (20) *A. gramineus* (17), *G. grabra* (21-22)
Gastroprotective effect	*E. caryophyllata* (23-24)
Anti- *Helicobacter pylori*	*G. grabra* (59)

2. Gastrointestinal Effect of the Selected Ya-hom Recipe.

The *in vivo* study of the water extract of this studied Ya-hom has been reported to use for stomach discomfort treatment. The actions of Ya-hom on the gastric acid, pepsin and mucus secretion were studied in histamine- and carbachol-induced gastric fistula rats. Ya-hom inhibited both histamine- and carbachol-induced gastric acid, pepsin and soluble mucus secretions in a dose dependent manner. Ya-hom had lower minimum inhibition on the acid-stimulating effects of histamine than that of carbachol. Ya-hom also had a higher evaluated effect on histamine-induced visible mucous than that of carbachol. The study of Ya-hom actions reveal that Yahom inhibits the stimulatory effect of histamine and carbachol on the acid, pepsin and soluble mucus secretion but potentiates the visible mucus secretion. Attenuating gastric secretion by secretagogues and increasing gastric barrier effects are parts of Ya-hom's action on stomach discomfort treatment (25).

The *in vivo* study of Ya-hom exposed that Ya-hom inhibited gastric acid secretion and the others factor that decreased the stomach discomfort. The gastric acid secretion is one that caused stomach discomfort. However, the results are difficult for explanation of the mechanism of action of Ya-hom on gastric acid secretion because the *in vivo* study may be interfered by the other factors, for example, the endogenous regulation. To reveal the mechanism of action of Ya-hom, it may be studied in difference of the experimental models, the protocol or the methods for measurement of gastric acid secretion.

CHAPTER III
MATERIALS AND METHODS

MATERIALS.

1. Animals.

Male ICR (Institute of Cancer Research) mice, *Mus muscurus*, from the National Laboratory Animal Center, Salaya. Mahidol University.

2. Chemicals.

Atropine sulfate salt (sigma)

Bethanechol chloride (ICN Biomedicals Inc.)

Buffer solution ready for use pH 4.00±0.02 (Merck)

Buffer solution (phosphate) 7.00±0.02 (Merck)

Calcium chloride dihydrate (Merck)

Glucose (Merck)

Histamine dihydrochloride (Sigma)

Hydrochloric acid (Merck)

Magnesium sulfate heptahydrate (Merck)

Potassium chloride (Fluka)

Potassium dihydrogen phosphate (Merck)

Ranitidine hydrochloride (Neuland Laboratories Ltd, India)

Sodium chloride (Univar)

Sodium hydrogen carbonate (Unilab)

Sodium hydroxide (Carlo erba)

Urethane (Merck)

Ya-hom powder

3. Equipments.

Bigger 250, 500 ml

Cotton wool

Disposable syringe 1 ml, 50 ml

Dual cannula (internal; silicone diameter of 0.5 mm, external; polyethylene diameter of 3 mm)

Fraction collector DC-1200 (Eyela)

Gas (95% O_2 and 5% CO_2)

Magnetic stirrer (Ikamag)

Micropipette (Jencons sealpette)

Organ bath

pH electrode (Orion)

pH meter (Model 420A, Orion)

Plastic tube

Pump (Masterflex)

Thermometer

Water bath

METHODS.

1. Preparation of Test Solution of Ya-hom.

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 kept at –20^{o}C. One gram of Ya-hom powder yield 0.14376 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.

2. Preparation of Physiological Solution.

2.1 Mucosal solution.

The composition of mucosal solution were 143 mM NaCl, 5.9 mM KCl, 1.18 mM $MgSO_4$, 1.3 mM $CaCl_2$, and 30 mM glucose at pH 5.0 adjusted with 0.1 N HCl and 0.1 N NaOH.

2.2 Serosal solution.

The composition of serosal solution were 118 mM NaCl, 4.7 mM KCl, 1.15 mM KH_2PO_4, 25 mM $NaHCO_3$, 1.18 mM $MgSO_4$, 1.3 mM $CaCl_2$, and 30 mM glucose.

3. Preparation of Animal.

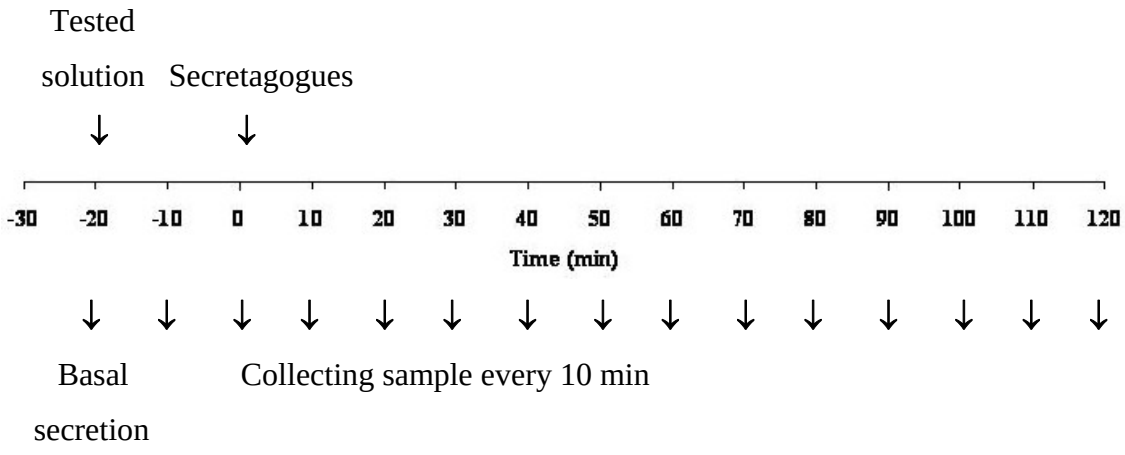
Male ICR mice, *Mus muscurus*, weighing between 25-35 g obtained from the National Laboratory Animal Center at Salaya, Mahidol University were used in all experiments. The mice were housed by putting 5 mice in one cage in the animal room at the Faculty of Pharmacy, Mahidol University for at least one week prior to the experiment. The animals were fed with commercial rat diet (C.P.Mice Feed; SWT.Co.,Ltd) and tab water *ad libitum*. The mice were given free access to food and water. The experimental protocol was approved by the Institutional Animal Care and Use Committee of Faculty of Pharmacy Mahidol University on 19 September 2004.

The animals were anesthetized with urethane at the dose of 1.8 mg/10 g body weight intraperitoneally. The abdomen was opened through a midline incision below the xiphoid cartilage. The stomach was exposed. The esophagus and pyloduodenum junction were exposed and ligated without damaging the blood vessels. The small incision was made at the fundic portion (non-glandular upper part with consisted of

the squamous epithelium). The lumen was flushed with warm mucosal solution (15-20 ml) and a dual cannula (internal; silicon diameter of 0.5 mm, external; polyethylene diameter of 3 mm) was inserted into the incision. After the ligation between a dual cannula with fundic portion, the stomach was rapidly dissected out and placed in a 30 ml organ bath containing serosal solution which was kept at 37 ± 1 ^{o}C and gassed with 95% O_2 and 5% CO_2. The stomach lumen was perfused through inlet tube of the dual cannula connected to the perfusion pump at the rate of 1 ml/min with gassed (95% O_2 and 5% CO_2) mucosal solution. The effluent perfusate from the stomach exited through the outlet tube was introduced to a fraction collector generally at a level of 20 cm except indicated in certain experiment above the stomach level to distend the stomach.

4. Collection of Gastric Acid Secretion.

The gastric sample solutions were collected as 10 min fraction with fraction collector in collecting tube. The first tube of gastric sample solution was referred as a basal secretion. The inhibitors (atropine 1 μM or ranitidine 10 μM) and Ya-hom were administrated into the serosal solution after collecting the first tube. The incubation time was indicated in each experiment. After the time 0 min 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 continuously collected in 10 minute fractions for 120 or 150 minutes after stimulation as shown in the following diagram. Acid output was determined by titrating with 2 mM NaOH to the end point of pH 5.0 and expressed as nEq HCl/10min. After subtracting the basal gastric acid secretory rate, the amount of gastric acid secretion was also calculated as AUC (area under the curve), the area under the curve from 10 minutes before the peak to 10 minutes after the peak of gastric secretory rate (AUC at peak), and the area under the curve from 0 minute to the peak of gastric secretory rate (AUC at 0 min – peak).



5. Experimental Protocol.

5.1 Study on the optimal conditions of gastric secretory response.

5.1.1 Comparing gastric secretory response to histamine between fasted and non-fasted mice.

The animals were separated into two groups. One group was fasted about 18 hours with free access to drinking water in order to ensure that the stomach was empty at the time of experiment. Another group was allowed to have free access of food and water. The first tube of gastric sample solution was referred as a basal secretion. After the first collection, both groups were stimulated with histamine 5.0 μM and samples were continuously collected in 10 minutes interval for 150 minutes. The condition with higher gastric acid secretion was used for the rest of the experiments.

5.1.2 Effect of stomach distension on histamine-induced gastric acid secretion.

The height in cm from the stomach to the opening of outlet tube is referred as H_2O hydrostatic pressure used to extend the stomach which is equivalent to cm water. The opening of the effluent perfusate outlet tube was connected to a fraction collector at a level of 18, 20 and 22 cm above the stomach level for study the effect of organ distension on the histamine-induced gastric acid secretion. The first tube of gastric sample solution was referred as a basal secretion. After the first collection, the gastric acid secretion was stimulated with histamine 5.0 μM and samples were

continuously collected in 10 minutes interval for 150 minutes. The level that gave highest gastric acid secretion was used in the following experiment.

5.2 Study the Effect of Ya-hom on Histamine-induced gastric acid secretion.

5.2.1 Study the effect of the Ya-hom preincubation period on the action of Ya-hom in histamine-induced gastric acid secretion.

Histamine 5.0 μM stimulated gastric acid secretion in the absence of Ya-hom was used as the control. The first tube of the sample collection was a basal secretion. In the first condition, Ya-hom at the concentration of 10 mg/ml was added simultaneously with histamine after collecting the sample at 0 min in serosal solution. In the second and third conditions, Ya-hom at the concentration of 10 mg/ml was added at -10 and -20 min in serosal solution before adding histamine. Each group was stimulated with histamine 5.0 μM and samples were continuously collected for 120 minutes. The preincubated period that showed the grestest gastric acid secretion of Ya-hom was used for the rest of the experiments.

5.2.2 To determine the effect of Ya-hom after washing out Ya-hom before adding histamine.

Ya-hom at the concentration of 10 mg/ml was added at -10 or -20 minutes in serosal solution and was washed out or not washed out before adding histamine. Each group was stimulated with histamine 5.0 μM and collections were continuously collected for 120 minutes. The condition that showed the greatest gastric acid secretion of Ya-hom was used for the rest of the experiments.

5.2.3 Dose response curve of Ya-hom on histamine-induced gastric acid secretion.

Ya-hom at the concentrations of 2.5, 5.0, 10.0 or 20.0 mg/ml was added in serosal solution after collection the first sample at -20 min. Ya-hom was preincubated for 20 minutes prior to histamine (according to the result of 5.1.3 and 5.1.4). Histamine was added after collecting the 0 min sample. Samples were continuously collected in a 10 minutes interval for 120 minutes.

The study in the isolated mouse whole stomach may be suitable for reveal the physiological action and mechanism of Ya-hom. Histamine directly stimulated parietal cells released gastric acid secretion. Using two secretogogue

antagonists, atropine (muscarinic antagonist) and ranitidine (H_2 receptor antagonist) would provide the mechanism of Ya-hom action and gastric acid secretion.

Since the gastric acid stimulation can be the synergistic effect of exogenous histamine and endogenous acetylcholine, it is possible that the effect of Ya-hom was on this pathway. To determine the involvement of muscarinic antagonist and H_2 antagonist on Ya-hom's action. The effect of Ya-hom was investigated in the presence and absence of atropine (muscarinic antagonist) or ranitidine (H_2 antagonist).

5.2.4 The effect of atropine, the muscarinic antagonist, on the action of Ya-hom.

Ya-hom 10 mg/ml with or without atropine 1 μM or atropine alone (after collecting sample at -20 min) was preincubated for 20 minutes before adding histamine 5.0 μM (after collecting sample at 0 min) and samples were continuously collected in a 10 minutes interval for 120 minutes.

5.2.5 The effect of ranitidine, the H_2 blocker, on the action of Ya-hom.

Ya-hom 10 mg/ml with or without ranitidine 10 μM or ranitidine alone (after collecting sample at -20 min) were preincubated for 20 minutes before adding histamine 5.0 μM (after collecting sample at 0 min) and samples were continuously collected in a 10 minutes interval for 120 minutes.

5.3 Study the effect of bethanechol-induced gastric acid secretion.

Bethanechol, the muscarinic against, at two doses: 10 μM and 100 μM have been suggested to stimulate gastric secretion by direct activation on parietal cell and by histamine release from enterochromaffine-like (ECL) cells (51). At low dose bethanechol (10 μM) may have only direct stimulation on acid secreting cells, where as at high dose bethanechol (100 μM), causes a histamine release to potentiate the direct effect on the parietal cells. This secreted histamine, in the combination with the direct effect of bethanechol on M_3 receptor to stimulate gastric acid secretion was investigated in this experiment. The effect of atropine and ranitidine were also used to determine the effects of Ya-hom on muscarinic and histamine receptor stimulation.

5.3.1 Effect of Ya-hom on high dose bethanechol (100 μM)-induced gastric acid secretion in the present and absence of atropine.

Ya-hom (10 mg/ml) with or without of atropine (1 μM) or atropine alone were added into the serosal solution after -20 min sample collection. Bethanechol 100 μM was used to stimulate gastric acid secretion by applying after 0 min sample collection. Samples were continuously collected in a 10 minutes interval for 120 minutes.

5.3.2 Effect of Ya-hom on low dose bethanechol (10 μM)-induced gastric acid secretion in the present and absence of atropine or ranitidine.

Ya-hom (10 mg/ml) with or without of atropine (1 μM) or ranitidine were preincubated at -20 minute before adding bethanechol 10 μM. Each group, the samples were continuously collected in a 10 minutes interval for 120 minutes.

6. Statistical Analysis.

The data was expressed as mean ± the standard error of mean (SEM). The gastric secretory rate was calculated by minus from its own basal secretion. Independent-sample T-test was used to compare the difference between two experimental groups. One way analysis of variance (ANOVA) was used to compare the difference more than two experimental groups. Tukey's Honesty Significant difference (HSD) test was used to determine the statistically significant difference between the experimental groups. The p-value of less than 0.05 ($p<0.05$) was considered to be significantly different.

CHAPTER IV
RESULTS

PART I. THE OPTIMAL CONDITIONS ON HISTAMINE-INDUCED GASTRIC ACID SECRETION.

1 Gastric Secretory Response to Fasted and Non-fasted Condition on Histamine-induced Gastric Acid Secretion in Isolated Mouse Whole Stomach.

The stimulatory effect of histamine on gastric acid secretion in isolated mouse whole stomach of fasted and non-fasted mice with intragastric pressure at 20 cm H_2O were shown in Table 2 and 3. The first tube was referred to the basal secretion. The gastric acid secretory rate rapidly increased to the maximum rate at 40 min after adding histamine and gradually decreased through out the experimental period (Figure 3). The gastric acid secretory rate of non-fasted mice from 0-110 min was not different from those of fasted mice. However, the gastric acid secretory rate at 120-150 min of non-fasted mice was significantly lower than the fasted condition (Table 4 and Figure 3). The cumulative amount of gastric acid secretion in histamine-induced isolated mouse whole stomach of the fasted and non-fasted condition were compared in term of AUC, AUC at peak and AUC at 0 min to peak (Table 5 and Figure 4). The AUC, AUC at peak and AUC at 0 min to peak of both groups were not significantly different. Since the gastric acid secretion of histamine-induced isolated mouse whole stomach from fasted and non-fasted mice were not significantly different, during 120 min experimental period. The isolated whole stomach from non-fasted mice was used in the other experiments.

Table 2 Stimulatory effect of histamine on gastric acid secretion in isolated mouse whole stomach of fasted mice with intragastric pressure at 20 cm H_2O.

Time (min)	Gastric acid secretion (nEq HCl/10 min)					
	Number of isolated mouse whole stomach					
	1	2	3	4	5	6
0	420	320	320	200	300	240
10	480	200	420	300	360	300
20	760	300	680	480	500	480
30	1080	420	940	660	800	640
40	1100	600	1020	680	860	680
50	1060	700	1040	660	840	660
60	1040	680	1020	660	840	660
70	900	680	1020	640	840	660
80	880	640	980	600	840	640
90	900	640	920	560	820	620
100	880	640	920	600	680	600
110	880	620	960	540	640	620
120	840	600	920	560	620	620
130	840	600	940	540	480	620
140	860	640	920	520	540	600
150	880	600	920	520	520	640

Adding histamine 5.0 μM after collecting the basal secretion at 0 min.

Table 3 Stimulatory effect of histamine on gastric acid secretion in isolated mouse whole stomach of non-fasted mice with intragastric pressure at 20 cm H_2O.

Time (min)	Gastric acid secretion (nEq HCl/10 min)					
	Number of isolated mouse whole stomach					
	1	2	3	4	5	6
0	520	380	400	480	480	460
10	700	300	400	680	520	600
20	1080	700	600	1080	560	1000
30	1140	880	740	1100	700	1040
40	1080	880	820	1140	780	1140
50	1080	920	800	1080	760	1100
60	1020	900	740	1080	700	1080
70	860	860	680	1060	700	1000
80	860	840	620	1040	720	1000
90	800	780	660	1040	720	900
100	680	820	620	920	680	800
110	580	820	620	840	680	720
120	560	800	620	800	640	600
130	540	720	620	780	660	540
140	480	720	600	800	680	520
150	480	700	620	780	620	540

Adding histamine 5.0 μM after collecting the basal secretion at 0 min.

Table 4 Effect of fasted and non-fasted state on histamine-induced gastric acid secretory rate in isolated mouse whole stomach (n=6).

Time (min)	Gastric acid secretion (nEq HCl / 10 min)	
	Fasted mice	Non-fasted mice
0	0	0
10	63 ± 15	93 ± 37
20	237 ± 53	383 ± 88
30	457 ± 82	480 ± 68
40	523 ± 65	520 ± 59
50	527 ± 54	503 ± 56
60	517 ± 52	467 ± 64
70	490 ± 49	417 ± 65
80	463 ± 49	403 ± 66
90	443 ± 44	363 ± 51
100	420 ± 41	300 ± 51
110	410 ± 51	257 ± 54
120	393 ± 46	217 ± 56*
130	370 ± 61	190 ± 51*
140	380 ± 51	186 ± 56*
150	380 ± 56	177 ± 52*

All values were expressed as mean ± SEM after subtracting the basal secretion.

Comparison at the same time point.

* $p<0.05$: significantly lower than fasted mice at the same time point.

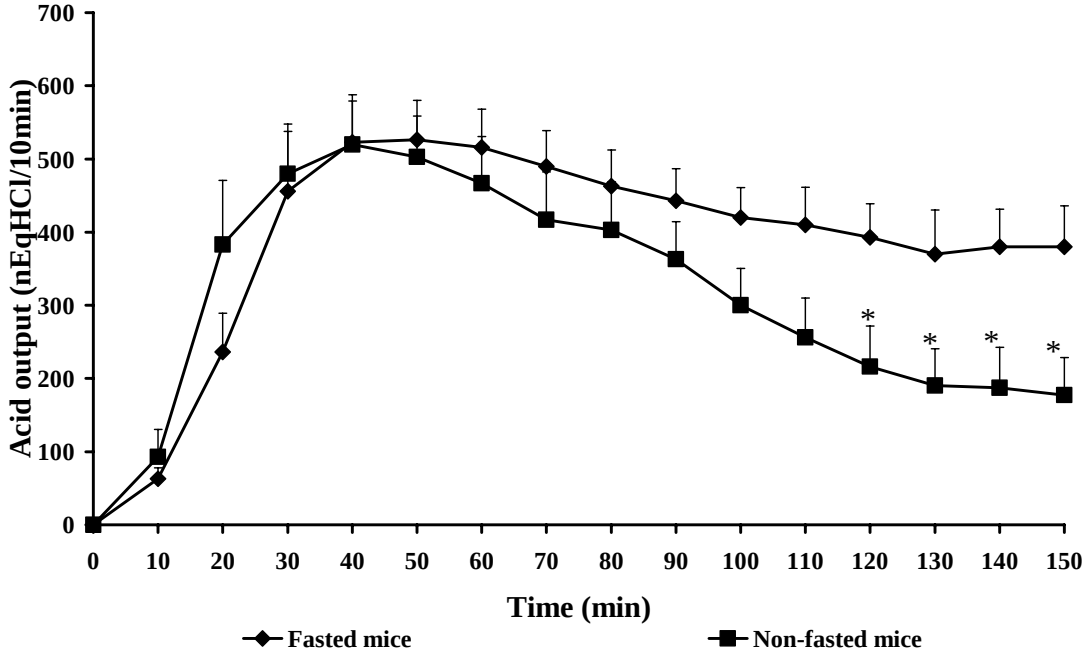


Figure 3 Effect of fasted and non-fasted state on histamine-induced gastric acid secretory rate in isolated mouse whole stomach (n=6).

All values were expressed as mean ± SEM after subtracting the basal secretion.
* $p<0.05$: significantly lower than fasted mice at the same time point.

Table 5 Effect of fasted and non-fasted state on cumulative amount of gastric acid secretion in histamine-induced isolated mouse whole stomach (n=6).

	Gastric acid secretion (nEq HCl)	
	Fasted mice	Non-fasted mice
AUC	6073 ± 686	4967 ± 638
AUC at peak	1563 ± 173	1507 ± 180
AUC at 0 min – peak	1463 ± 244	1473 ± 239

All values were expressed as mean ± SEM after subtracting the basal secretion.

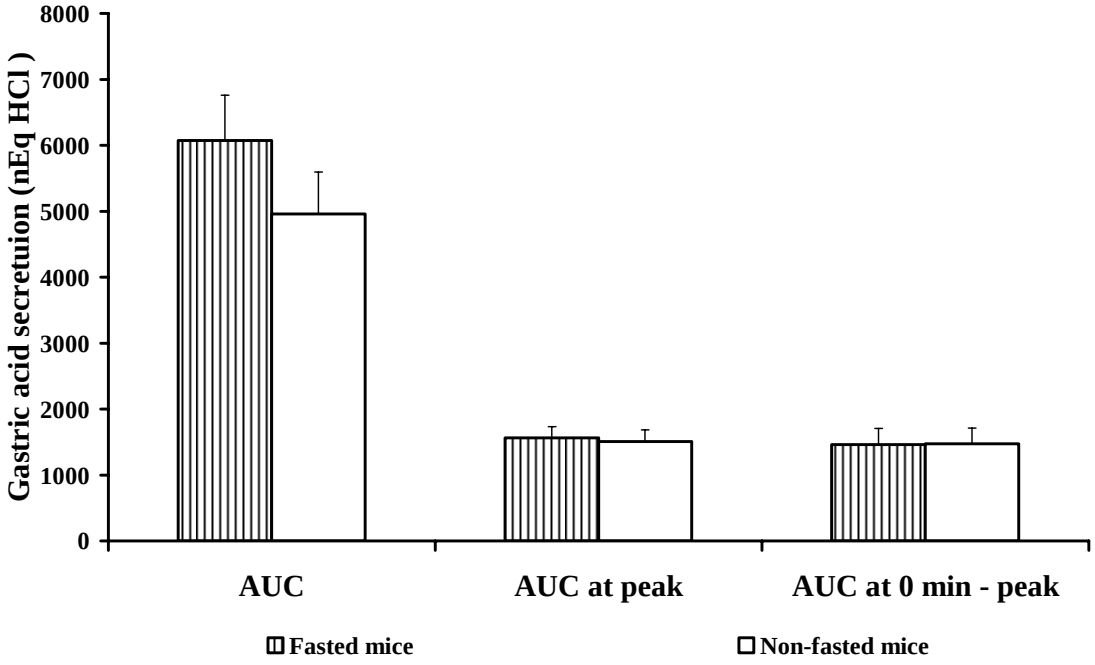


Figure 4 Effect of fasted and non-fasted state on cumulative amount of gastric acid secretion in histamine-induced isolated mouse whole stomach (n=6).

All values were expressed as mean ± SEM after subtracting the basal secretion.

2 Effect of Intragastric Pressure at 18, 20 and 22 cm H_2O on Histamine-induced Gastric Acid Secretion of Isolated Mouse Whole Stomach.

The stimulatory effect of histamine on gastric acid secretion in isolated mouse whole stomach with intragastric pressure at 18, 20, and 22 cm H_2O were shown in Table 6, 3, and 7, respectively. The gastric acid secretory rates after subtracting from their own basal secretion were shown in Table 8-9 and Figure 5-6. The cumulative amount of gastric acid secretion in histamine-induced isolated mouse whole stomach of each intragastric pressure condition were calculated as AUC, AUC at peak and AUC at 0 min – peak. There was no significant difference of histamine-induced gastric acid secretion among their groups of difference degree of stomach distension. Since the intragastric pressure at 20 cm H_2O was recommended by other reports (51, 60-65) and our result showed no difference among intragastric pressure at 18, 20 and 22 cm H_2O. From these results, the isolated mouse whole stomach from non-fasted mouse with intragastric pressure at 20 cm H_2O were used for the experiments in part II and part III.

Table 6 Stimulatory effect of histamine on gastric acid secretory rate in isolated mouse whole stomach of non-fasted mice with intragastric pressure at 18 cm H_2O.

Time (min)	Gastric acid secretion (nEq HCl/10 min)					
	Number of isolated mouse whole stomach					
	1	2	3	4	5	6
0	200	160	140	440	400	320
10	220	240	200	440	480	400
20	400	600	460	640	740	700
30	620	700	660	740	920	700
40	620	640	700	760	880	760
50	600	620	720	880	860	680
60	590	600	700	720	880	700
70	520	540	680	680	860	660
80	480	460	660	680	760	600
90	500	400	660	600	640	620
100	480	280	560	500	540	440
110	460	200	420	460	480	380
120	440	180	320	420	440	300
130	400	160	240	420	400	240
140	420	120	200	420	380	180
150	460	100	160	360	360	200

Adding histamine 5.0 μM after collecting the basal secretion at 0 min.

Table 7 Stimulatory effect of histamine on gastric acid secretory rate in isolated mouse whole stomach of non-fasted mice with intragastric pressure at 22 cm H_2O.

Time (min)	Gastric acid secretion (nEq HCl/10 min)					
	Number of isolated mouse whole stomach					
	1	2	3	4	5	6
0	200	420	420	320	400	580
10	320	400	520	280	400	560
20	520	520	1140	640	600	840
30	700	760	1300	820	800	1020
40	720	800	1200	740	800	860
50	680	720	1140	620	780	900
60	680	680	1060	600	720	880
70	640	640	1020	520	740	840
80	600	580	960	480	740	820
90	620	580	880	440	720	660
100	600	580	840	380	620	620
110	600	660	620	320	500	620
120	600	580	580	300	500	600
130	600	580	660	260	520	590
140	620	540	600	240	500	480
150	620	500	520	200	480	520

Adding histamine 5.0 μM after collecting the basal secretion at 0 min.

Table 8 Effect of intragastric pressure at 18, 20 and 22 cm H_2O on histamine-induced gastric acid secretory rate of isolated mouse whole stomach (n=6).

Time (min)	Gastric acid secretion (nEq HCl / 10 min)		
	Intragastric pressure (cm H_2O)		
	18	20	22
0	0	0	0
10	53 ± 14	93 ± 37	37 ± 23
20	313 ± 40	383 ± 88	320 ± 87
30	447 ± 39	480 ± 68	513 ± 78
40	433 ± 34	520 ± 59	463 ± 71
50	450 ± 30	503 ± 56	420 ± 66
60	422 ± 39	467 ± 64	380 ± 61
70	380 ± 44	413 ± 63	343 ± 63
80	330 ± 41	393 ± 61	273 ± 42
90	293 ± 50	363 ± 51	260 ± 66
100	190 ± 55	270 ± 68	217 ± 67
110	123 ± 47	257 ± 54	163 ± 60
120	80 ± 42	217 ± 56	140 ± 59
130	50 ± 34	190 ± 51	155 ± 62
140	47 ± 36	187 ± 56	137 ± 64
150	47 ± 43	177 ± 52	113 ± 64

All values were expressed as mean ± SEM after subtracting the basal secretion.
There was no significant difference among these groups at the same time point.

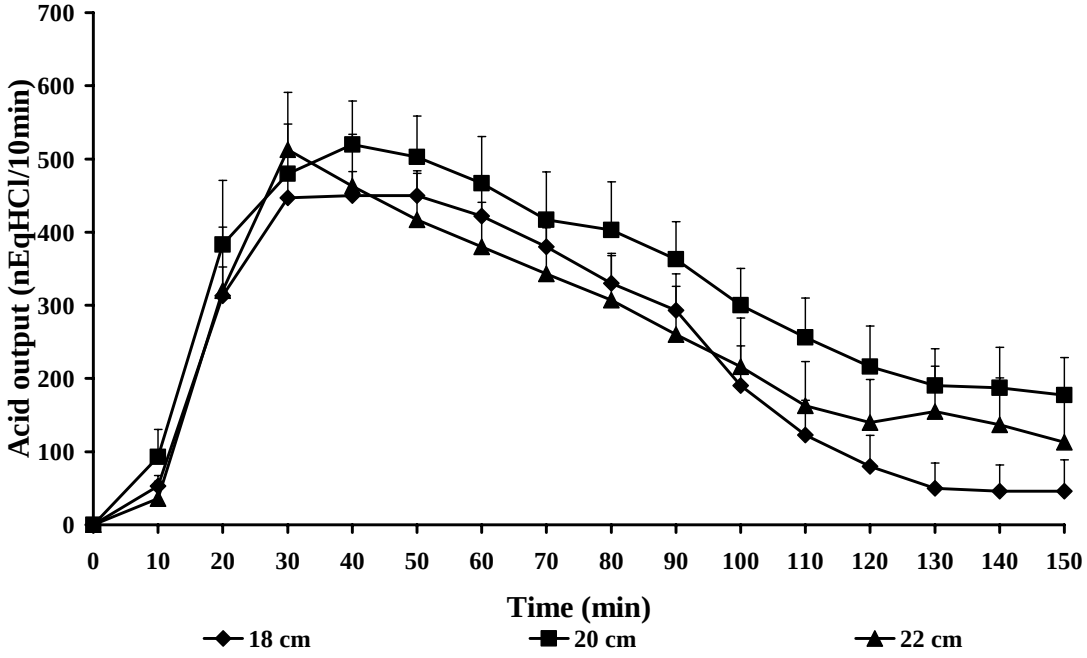


Figure 5 Effect of intragastric pressure at 18, 20 and 22 cm H_2O on histamine-induced gastric acid secretory rate of isolated mouse whole stomach (n=6).

All values were expressed as mean ± SEM after subtracting the basal secretion.

Table 9 Cumulative amount of histamine-induced gastric acid secretion of isolated mouse whole stomach with intragastric pressure at 18, 20, and 22 cm H_2O (n=6).

	Gastric acid secretion (nEq HCl)		
	Intragastric pressure (cm H_2O)		
	18	20	22
AUC	3658 ± 412	4913 ± 654	3935 ± 776
AUC at peak	1310 ± 91	1353 ± 181	1357 ± 220
AUC at 0 min - peak	1273 ± 162	1590 ± 314	1020 ± 183

All values were expressed as mean ± SEM after subtracting the basal secretion.
There was no significant difference among these groups at the same time point.

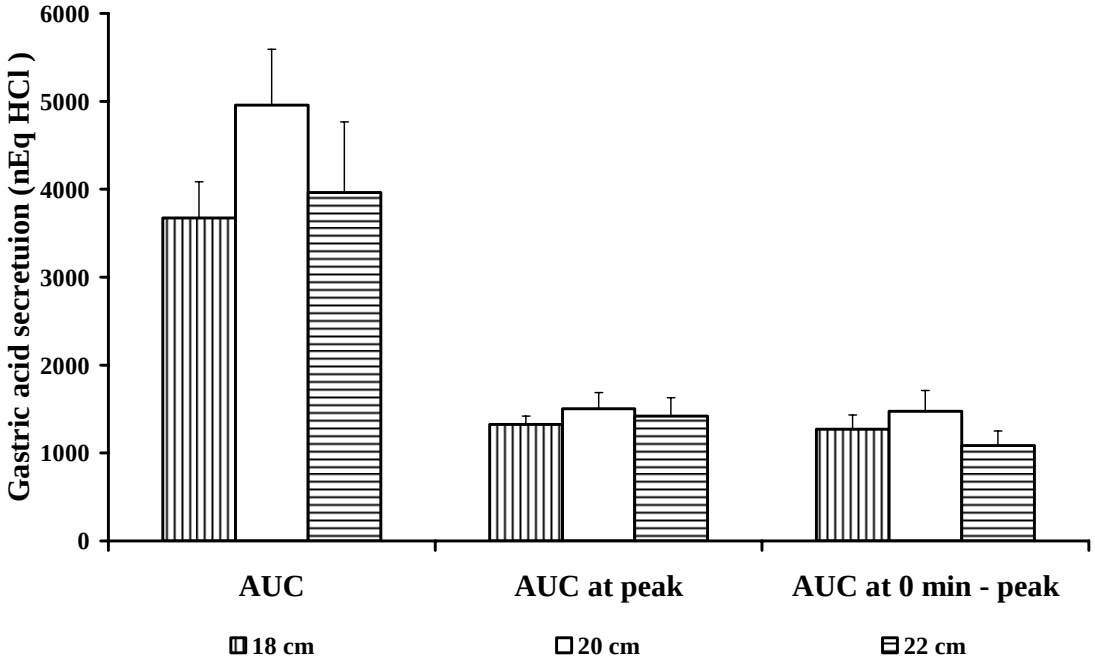


Figure 6 Cumulative amount of histamine-induced gastric acid secretion of isolated mouse whole stomach with intragastric pressure at 18, 20, and 22 cm H_2O (n=6).

All values were expressed as mean ± SEM after subtracting the basal secretion.

PART II EFFECT OF YA-HOM ON HISTAMINE-INDUCED GASTRIC ACID SECRETION.

1 Effect of Preincubation Period on Ya-hom Action in Histamine-induced Gastric Acid Secretion of Isolated Mouse Whole Stomach.

The stimulatory effect of histamine on gastric acid secretory rate of isolated mouse whole stomach used as the control group for all experiment in this part was shown in Table 10. The effect of Ya-hom on histamine-induced gastric acid secretion was shown in Table 11-14 and Figure 7. Ya-hom inhibited the stimulatory effect of histamine when it was simultaneously adding with histamine (Table 11), preincubated for 10 (Table 12) or 20 min (Table 13) before adding histamine. Simultaneously adding Ya-hom and histamine decreased gastric acid secretory rate at 20-110 min (Table 14 and Figure 7). Preincubation with Ya-hom increased the inhibitory effect of Ya-hom. The gastric secretory rates of 10 and 20 min preincubation of Ya-hom showed siginificantly lower than control at 10-110 min (Table 14). Preincubation of Ya-hom significantly lowered the gastric acid secretory rate than simultaneously adding Ya-hom and histamine at 10-30 min for 10 min preincubation and at 10-20 and 50 min for 20 min preincubation period as shown in Table 14 and Figure 7. The amount of gastric acid secretion of all four groups calculated as AUC, AUC at peak and AUC at 0 min to peak were shown in Table 15 and Figure 8. The AUC and AUC at peak of simultaneously adding Ya-hom and histamine were significantly lower than control whereas AUC, AUC at peak and AUC at 0 min to peak of 10 and 20 min preincubation of Ya-hom were all significantly lower than control. The simultaneous adding of Ya-hom and histamine group had significantly higher AUC at peak and AUC at 0 min to peak than those of 10 min preincubation group and significantly higher AUC, AUC at peak and AUC at 0 min to peak than those of 20 min preincubation group. These data showed that Ya-hom inhibited gastric acid secretion stimulatory effect of histamine and preincubation of Ya-hom potentiated its inhibitory effect.

Table 10 Stimulatory effect of histamine on gastric acid secretory rate of isolated mouse whole stomach.

Time (min)	Gastric acid secretion (nEq HCl/10 min)									
	Number of isolated mouse whole stomach									
	1	2	3	4	5	6	7	8	9	10
-20	240	920	450	540	720	270	360	700	300	270
- 10	300	960	500	360	800	300	440	660	340	300
0	420	880	640	440	780	260	460	780	480	310
10	620	1000	710	670	1300	330	780	1140	720	420
20	1480	1660	1140	1350	1640	740	880	1820	1160	1000
30	1360	1880	1090	1280	1480	820	1000	1760	1140	1040
40	1340	1720	1090	1240	1520	740	1000	1720	1040	910
50	1200	1640	1050	1230	1480	650	980	1300	980	930
60	1200	1500	940	1160	1200	630	860	1000	860	870
70	1040	1320	780	1100	1120	540	800	860	840	840
80	900	1100	700	1050	900	460	740	800	800	760
90	840	840	630	930	800	300	620	600	680	780
100	800	680	570	880	720	250	520	520	600	670
110	760	480	620	700	640	220	440	460	440	510
120	620	400	550	560	640	200	380	480	380	380

Collecting the basal secretion at -20 min.

Adding histamine 5.0 μM after collecting the sample at 0 min.

Table 11 Effect of Ya-hom (10.0 mg/ml) on histamine-induced gastric acid secretory rate by simultaneously adding Ya-hom and histamine.

Time (min)	Gastric acid secretion (nEq HCl/10 min)									
	Number of isolated mouse whole stomach									
	1	2	3	4	5	6	7	8	9	10
-20	420	440	500	240	280	420	380	420	270	260
- 10	460	520	460	280	240	420	260	480	460	320
0	620	720	480	240	320	440	300	500	590	300
10	620	840	540	360	440	540	460	540	710	400
20	680	1080	760	480	480	740	800	800	740	500
30	640	900	800	400	440	620	720	530	730	440
40	580	760	700	400	440	580	580	470	580	440
50	480	600	640	360	440	580	440	460	540	420
60	500	480	520	360	420	540	440	400	520	400
70	520	400	520	360	380	500	380	340	460	300
80	520	340	520	340	380	460	300	310	410	280
90	520	340	480	360	340	400	280	310	390	260
100	480	340	440	320	360	360	260	330	390	260
110	460	300	440	280	320	360	240	320	310	260
120	480	300	440	240	360	340	220	280	250	240

Collecting the basal secretion at -20 min.

Adding Ya-hom (10.0 mg/ml) and histamine 5.0 μM after collecting the sample at 0 min.

Table 12 Effect of Ya-hom (10 mg/ml) on histamine-induced gastric acid secretory rate by preincubating Ya-hom for 10 min before adding histamine.

Time (min)	Gastric acid secretion (nEq HCl/10 min)									
	Number of isolated mouse whole stomach									
	1	2	3	4	5	6	7	8	9	10
-20	720	560	530	580	500	340	850	810	680	580
- 10	800	530	440	430	510	420	880	890	790	520
0	820	550	460	490	600	560	810	840	780	490
10	810	560	430	430	520	490	600	640	680	450
20	870	630	520	490	570	660	800	880	700	490
30	770	680	590	590	670	670	660	900	690	490
40	730	630	560	570	700	660	550	750	630	450
50	720	610	510	510	540	580	480	680	550	400
60	680	560	470	490	390	520	420	580	500	390
70	610	520	460	450	350	480	360	520	510	330
80	570	500	400	400	260	360	360	470	470	290
90	520	480	420	440	270	320	320	420	490	230
100	530	470	380	380	340	290	330	380	440	200
110	530	460	370	410	330	250	330	390	460	160
120	490	400	360	360	300	230	330	400	400	160

Collecting the basal secretion at -20 min.

Adding Ya-hom (10.0 mg/ml) after collecting the sample at -10 min.

Adding histamine 5.0 μM after collecting the sample at 0 min.

Table 13 Effect of Ya-hom (10 mg/ml) on histamine-induced gastric acid secretory rate by preincubating Ya-hom for 20 min before adding histamine.

Time (min)	Gastric acid secretion (nEq HCl/10 min)									
	Number of isolated mouse whole stomach									
	1	2	3	4	5	6	7	8	9	10
-20	530	530	650	490	470	600	440	400	550	490
- 10	460	500	750	440	470	680	420	300	390	480
0	430	410	690	420	400	740	360	260	260	520
10	410	390	590	440	330	680	370	250	270	380
20	680	550	750	480	350	830	710	280	370	440
30	710	690	810	430	370	780	780	300	590	500
40	610	610	730	460	350	710	630	300	630	430
50	550	530	670	400	340	620	580	290	560	410
60	520	560	660	390	340	580	500	280	510	340
70	470	480	550	390	340	550	410	260	480	290
80	450	470	550	360	320	470	330	240	360	330
90	450	410	500	380	320	430	360	240	330	330
100	390	450	450	370	270	370	330	230	250	300
110	380	400	420	370	270	330	330	230	220	280
120	310	380	380	390	230	300	240	230	220	280

Adding Ya-hom (10.0 mg/ml) after collecting the basal secretion at -20 min.

Adding histamine 5.0 μM after collecting the sample at 0 min.

Table 14 Effect of preincubated period of Ya-hom (10 mg/ml) on histamine-induced gastric acid secretory rate (n=10).

Time (min)	Gastric acid secretion (nEq HCl / 10 min)			
	Control	Preincubation period of Ya-hom (min)		
		0	10	20
-20	0	0	0	0
-10	41 ± 9	47 ± 19	39 ± 14	18 ± 12
0	83 ± 24	98 ± 39	55 ± 23	21 ± 14
10	292 ± 57	182 ± 42	26 ± 16 ^{a**b}	8 ± 8 ^{a**b*}
20	810 ± 76	343 ± 43 ^{a**}	70 ± 32 ^{a**b*}	77 ± 33 ^{a**b*}
30	808 ± 59	259 ± 40 ^{a**}	84 ± 33 ^{a**b}	107 ± 36 ^{a**}
40	755 ± 59	190 ± 25 ^{a**}	63 ± 35 ^{a**}	62 ± 20 ^{a**}
50	667 ± 46	133 ± 21 ^{a**}	33 ± 24 ^{a**}	21 ± 14 ^{a**b}
60	544 ± 57	97 ± 23 ^{a**}	18 ± 18 ^{a**}	10 ± 6 ^{a**}
70	447 ± 57	65 ± 20 ^{a**}	14 ± 14 ^{a**}	0 ^{a**}
80	344 ± 60	52 ± 17 ^{a**}	2 ± 2 ^{a**}	0 ^{a**}
90	243 ± 69	40 ± 17 ^{a**}	0 ^{a**}	0 ^{a**}
100	188 ± 64	34 ± 15 ^{a*}	0 ^{a**}	0 ^{a**}
110	131 ± 51	16 ± 7 ^{a}	0 ^{a*}	0 ^{a*}
120	71 ± 37	14 ± 10	0	0

All values were expressed as mean ± SEM after subtracting the basal secretion.
Comparison at the same time point.
^{a}p<0.05, ^{a*}p<0.01, ^{a**}p<0.001 : significantly lower than control.
^{b}p<0.05 , ^{*b}p<0.01 : significantly lower than simultaneously adding Ya-hom with histamine.

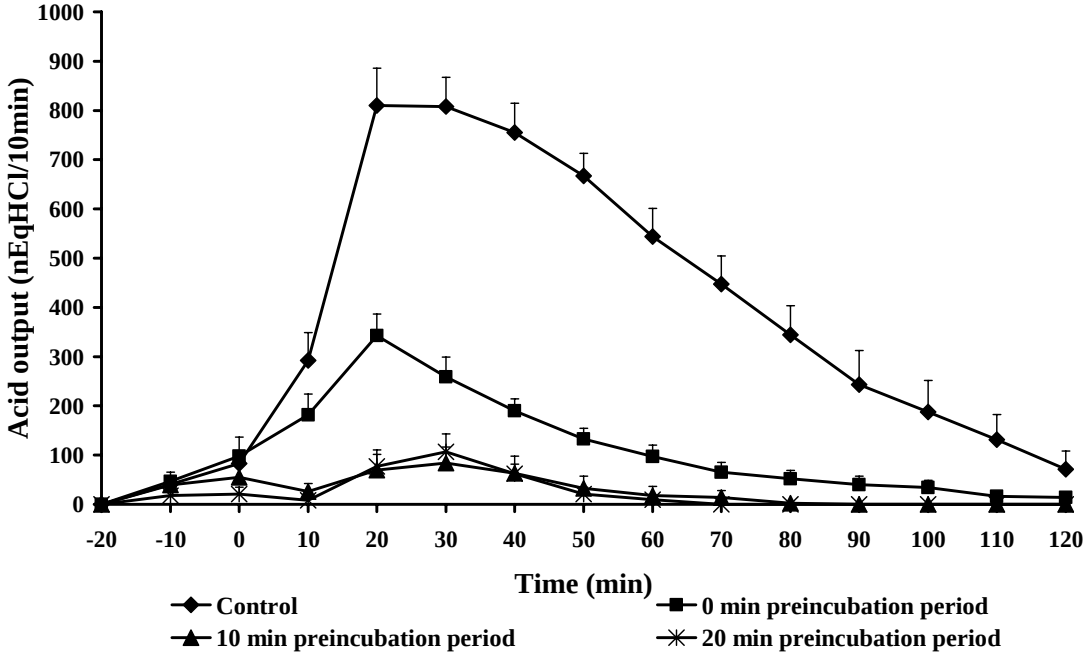


Figure 7 Effect of preincubated period of Ya-hom (10 mg/ml) on histamine-induced gastric acid secretoty rate (n=10).

All values were expressed as mean ± SEM after subtracting the basal secretion.

Table 15 Effect of preincubated period of Ya-hom (10 mg/ml) on histamine-induced gastric acid secretion (n=10).

Time (min)	Gastric acid secretion (nEq HCl)			
	Control	Preincubation period of Ya-hom (min)		
		0	10	20
AUC	5424 ± 545	1570 ± 237[a]	404 ± 189[a]	324 ± 116[ab]
AUC at peak	2094 ± 140	800 ± 111[a]	222 ± 94[ab]	244 ± 85[ab*]
AUC at 0 min - peak	1477 ± 99	653 ± 108	249 ± 102[ab*]	203 ± 688[ab*]

All values were expressed as mean ± SEM after subtracting the basal secretion.

[a] $p<0.001$: significantly lower than control.

[b] $p<0.05$, [b*] $p<0.01$: significantly lower than simultaneously adding Ya-hom with histamine.

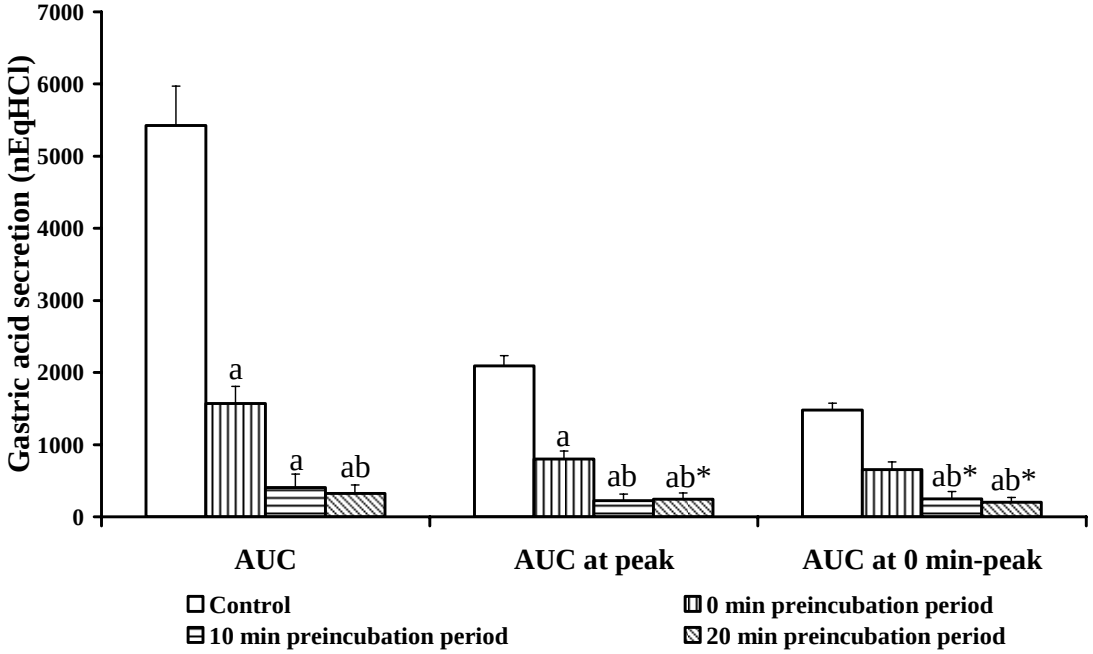


Figure 8 Effect of preincubated period of Ya-hom (10 mg/ml) on histamine-induced gastric acid secretion (n=10).

All values were expressed as mean ± SEM after subtracting the basal secretion.
[a] $p<0.001$: significantly lower than control.
[b] $p<0.05$, [b*] $p<0.01$: significantly lower than simultaneously adding Ya-hom with histamine.

2 Effect of Washing Out Ya-hom before Adding Histamine on Ya-hom Action.

The histamine-induced gastric acid secretory rate of isolated mouse whole stomach from the previous experiment (Table 10) was used as the control group. The effect of Ya-hom (10 mg/ml) on histamine-induced gastric acid secretory rate by preincubating Ya-hom for 10 and 20 min and washing out of Ya-hom before adding histamine were shown in table 16 and 17. The gastric secretory rate after subtracting the basal secretory rate was shown in table 18 and figure 9. Preincubated with Ya-hom for 10 and 20 min significantly decreased stimulatory effect of histamine on gastric acid secretion. The gastric secretory rates of 10 and 20 min preincubation groups were significantly lower than the control group at 10-40 min and 10-50 min, respectively. There was no significant difference in gastric acid secretion between 10 and 20 min preincubation of Ya-hom treated groups. The cumulative amount of gastric acid secretion as calculated as AUC, AUC at peak and AUC at 0 min – peak of preincubation period with Ya-hom were shown in table 19 and figure 10. Both AUC and AUC at peak of 10 and 20 min preincubation of Yahom were significantly lower than control whereas AUC at 0 min – peak was not significantly different from control.

Table 16 Effect of Ya-hom (10 mg/ml) on histamine-induced gastric acid secretory rate by preincubating Ya-hom for 10 min and washing out Ya-hom before adding histamine.

Time (min)	Gastric acid secretion (nEq HCl/10 min)									
	Number of isolated mouse whole stomach									
	1	2	3	4	5	6	7	8	9	10
-20	440	540	260	580	380	340	220	520	330	270
- 10	470	490	280	540	240	360	200	520	390	380
0	530	520	300	580	280	440	140	440	450	390
10	480	570	280	600	200	400	160	360	410	430
20	620	640	540	1120	300	540	620	640	720	580
30	790	850	720	1240	480	660	800	920	940	680
40	850	980	880	1360	580	680	800	960	1010	790
50	1100	1140	920	1320	600	680	720	880	900	790
60	810	880	960	1280	680	720	620	820	870	970
70	660	770	800	1200	600	700	620	800	730	750
80	610	700	700	1000	620	640	600	660	670	740
90	590	670	560	840	600	600	520	600	620	630
100	570	550	500	700	560	520	500	480	590	680
110	590	540	420	640	520	480	420	480	530	630
120	590	410	360	640	480	420	340	420	520	530

Collecting the basal secretion at -20 min.

Adding Ya-hom (10.0 mg/ml) after collecting the sample at -10 min.

Washing out Ya-hom after collecting the sample at 0 min and adding histamine 5.0 μM.

Table 17 Effect of Ya-hom (10 mg/ml) on histamine-induced gastric acid secretory rate by preincubating Ya-hom for 20 min and washing out Ya-hom before adding histamine.

Time (min)	Gastric acid secretion (nEq HCl/10 min)									
	Number of isolated mouse whole stomach									
	1	2	3	4	5	6	7	8	9	10
-20	340	260	420	520	240	520	400	280	500	400
- 10	320	320	480	520	280	440	460	260	480	400
0	400	280	460	480	180	420	440	320	450	390
10	400	200	540	400	180	380	440	280	460	360
20	400	680	900	660	520	700	660	320	910	670
30	540	720	920	720	760	800	780	440	1030	890
40	580	720	940	820	860	840	880	600	1110	1000
50	600	740	1020	820	880	840	800	560	1140	960
60	560	740	1040	880	720	840	760	540	1140	930
70	480	660	900	800	700	780	720	480	1040	760
80	460	620	820	700	640	780	620	420	970	710
90	400	580	720	700	540	740	520	360	910	650
100	420	460	700	680	480	700	460	340	840	570
110	420	460	600	600	400	720	440	300	740	480
120	400	420	560	620	380	640	400	280	620	450

Adding Ya-hom (10.0 mg/ml) after collecting the basal secretion at -20 min. Washing out Ya-hom after collecting the sample at 0 min and adding histamine 5.0 μM.

Table 18 Effect of preincubated period of Ya-hom (10 mg/ml) on histamine-induced gastric acid secretory rate by washing out of Ya-hom before adding histamine (n=10).

Time (min)	Gastric acid secretion (nEq HCl / 10 min)		
	Control	Preincubation period (min)	
		10	20
-20	0	0	0
-10	41 ± 9	24 ± 11	22 ± 9
0	83 ± 24	47 ± 17	20 ± 7[a]
10	292 ± 57	41 ± 16[a**]	22 ± 13[a**]
20	810 ± 76	252 ± 52[a**]	254 ± 48[a**]
30	808 ± 59	420 ± 53[a**]	372 ± 47[a**]
40	755 ± 59	501 ± 54[a*]	447 ± 45[a*]
50	667 ± 46	517 ± 52	448 ± 49[a*]
60	544 ± 57	473 ± 54	427 ± 46
70	447 ± 57	375 ± 45	344 ± 41
80	344 ± 60	306 ± 39	286 ± 38
90	243 ± 69	235 ± 28	224 ± 36
100	188 ± 64	181 ± 40	177 ± 30
110	131 ± 51	141 ± 34	128 ± 24
120	71 ± 37	106 ± 25	89 ± 18

All values were expressed as mean ± SEM after subtracting the basal secretion.

Comparison at the same time point.

[a] $p<0.05$, [a*] $p<0.01$, [a**] $p<0.001$: significantly lower than control.

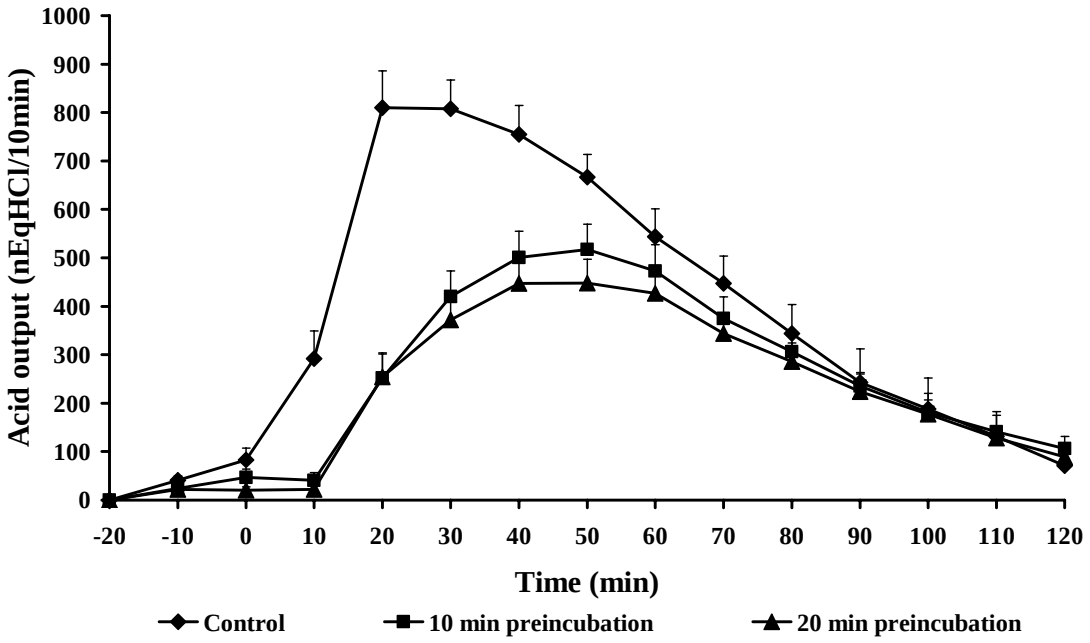


Figure 9 Effect of preincubated period of Ya-hom (10 mg/ml) on histamine-induced gastric acid secretory rate by washing out of Ya-hom before adding histamine (n=10).

All values were expressed as mean ± SEM after subtracting the basal secretion. Comparison at the same time point.

Table 19 Effect of preincubated period of Ya-hom (10 mg/ml) on histamine-induced gastric acid secretion by washing out of Ya-hom before adding histamine (n=10).

Time (min)	Gastric acid secretion (nEq HCl)		
	Control	Preincubation period (min)	
		10	20
AUC	5424 ± 545	3619 ± 395[a]	3260 ± 390[a*]
AUC at peak	2094 ± 140	1514 ± 137[a]	1304 ± 138[a**]
AUC at 0 min - peak	1477 ± 99	1769 ± 203	1537 ± 220

All values were expressed as mean ± SEM after subtracting the basal secretion.
[a] $p<0.05$, [a*] $p<0.01$, [a**] $p<0.001$: significantly lower than control.

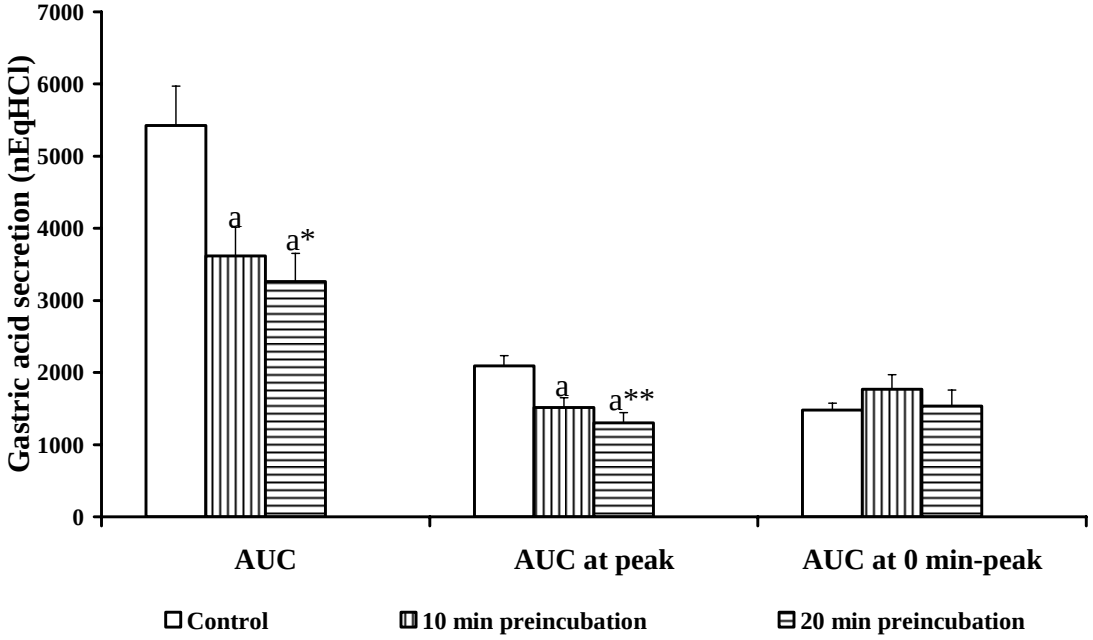


Figure 10 Effect of preincubated period of Ya-hom (10 mg/ml) on histamine-induced gastric acid secretion by washing out of Ya-hom before adding histamine (n=10).

All values were expressed as mean ± SEM after subtracting the basal secretion.
$^{a}p<0.05$, $^{a*}p<0.01$, $^{a**}p<0.001$: significantly lower than control.

The effects of Ya-hom on histamine-induced gastric acid secretory rate in washing out and without washing out of Ya-hom at the same preincubation periods were shown in Table 20, 21 and Figure 11, 12. Both 10 and 20 min preincubation period of Ya-hom with non-washing out of Ya-hom before stimulating with histamine showed significantly lower gastric secretory rates than the group with washing out of Ya-hom at 20-120 min (Table 20 and Figure 11) when comparing in the same preincubation period. The cumulative amount of gastric acid secretion in form of AUC, AUC at peak and AUC at 0 min – peak of without washing of Ya-hom were also significantly lower than those of washing out of Ya-hom (Table 21 and Figure 12). These data showed that washing Ya-hom out before adding histamine decreased the inhibitory effect of Ya-hom.

Table 20 Comparing the effect of Ya-hom (10 mg/ml) on histamine-induced gastric acid secretory rate between washing out and non-washing out of Ya-hom before stimulating with histamine (n=10).

Time (min)	Gastric acid secretion (nEq HCl / 10 min)				
	Control	Preincubation period (in)			
		10	20	10	20
		Washing out		Non-washing out	
-20	0	0	0	0	0
-10	41 ± 9	24 ± 11	22 ± 9	39 ± 14	18 ± 12.1
0	83 ± 24	47 ± 17	20 ± 7	55 ± 23	21 ± 14.0
10	292 ± 57	41 ± 16	22 ± 13	26 ± 16	8 ± 8.0
20	810 ± 76	52 ± 52	254 ± 48	70 ± 32 ^{a}	77 ± 33.2 ^{a}
30	808 ± 59	420 ± 53	372 ± 47	84 ± 33 ^{a**}	107 ± 36.3 ^{a**}
40	755 ± 59	501 ± 54	447 ± 45	63 ± 35 ^{a**}	62 ± 19.8 ^{a**}
50	667 ± 46	517 ± 52	448 ± 49	33 ± 24 ^{a**}	21 ± 13.5 ^{a**}
60	544 ± 57	473 ± 54	427 ± 46	18 ± 18 ^{a**}	10 ± 6.3 ^{a**}
70	447 ± 57	375 ± 45	344 ± 41	14 ± 14 ^{a**}	0 ^{a**}
80	344 ± 60	306 ± 39	286 ± 38	2 ± 2 ^{a**}	0 ^{a**}
90	243 ± 69	235 ± 28	224 ± 36	0 ^{a**}	0 ^{a**}
100	188 ± 64	181 ± 40	177 ± 30	0 ^{a**}	0 ^{a**}
110	131 ± 51	141 ± 34	128 ± 24	0 ^{a**}	0 ^{a**}
120	71 ± 37	106 ± 25	89 ± 18	0 ^{a**}	0 ^{a*}

All values were expressed as mean ± SEM after subtracting the basal secretion.
Comparison at the same time point.
^{a} $p<0.05$, ^{a*} $p<0.01$, ^{a**} $p<0.001$: significantly lower than without washing Ya-hom before adding histamine when comparing between the same preincubation period.

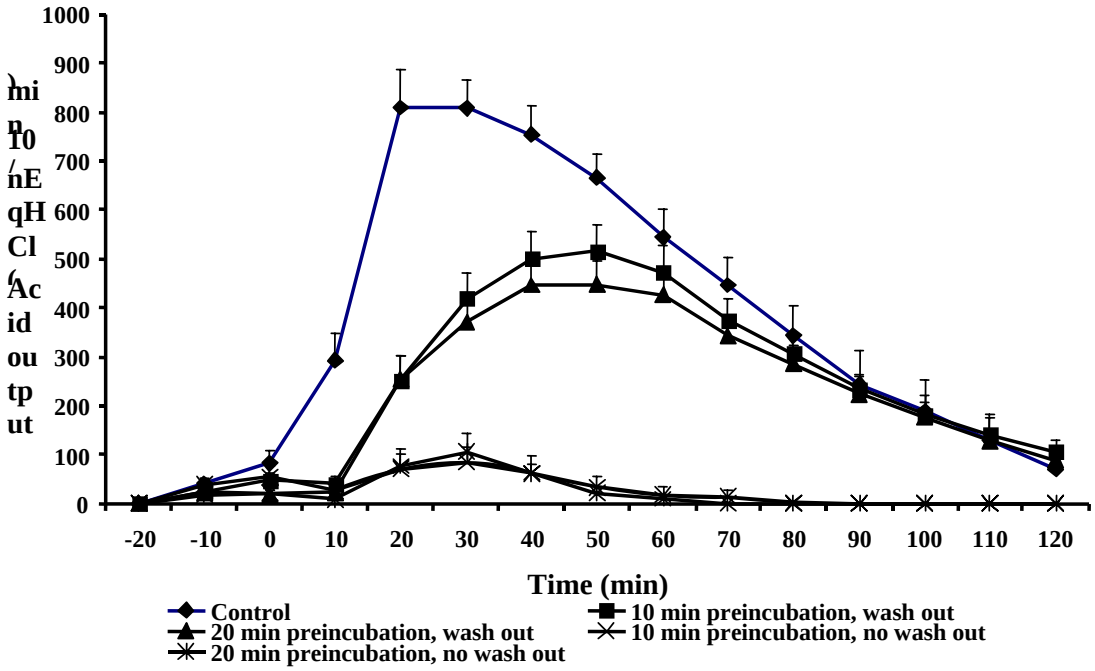


Figure 11 Comparing the effect of Ya-hom (10 mg/ml) on histamine-induced gastric acid secretory rate between washing out and non-washing out of Ya-hom before stimulating with histamine (n=10).

All values were expressed as mean ± SEM after subtracting the basal secretion.

Table 21 Comparing the effect of Ya-hom (10 mg/ml) on histamine-induced gastric acid secretion between washing out and non-washing out of Ya-hom before stimulating with histamine (n=10).

Time (min)	Gastric acid secretion (nEq HCl)				
	Control	Preincubation period (min)			
		10	20	10	20
		Washing out		Non-washing out	
AUC	5424 ± 545	3619 ± 395	3260 ± 390	404 ± 189[a]	324 ± 116[a]
AUC at peak	2094 ± 140	1514 ± 137	1304 ± 138	222 ± 94[a]	244 ± 85[a]
AUC 0 min - peak	1477 ± 99	1769 ± 203	1537 ± 220	249 ± 102[a]	203 ± 68[a]

All values were expressed as mean ± SEM after subtracting the basal secretion.

[a] $p<0.001$: Significantly lower than without washing Ya-hom before adding histamine when comparing between the same preincubation period.

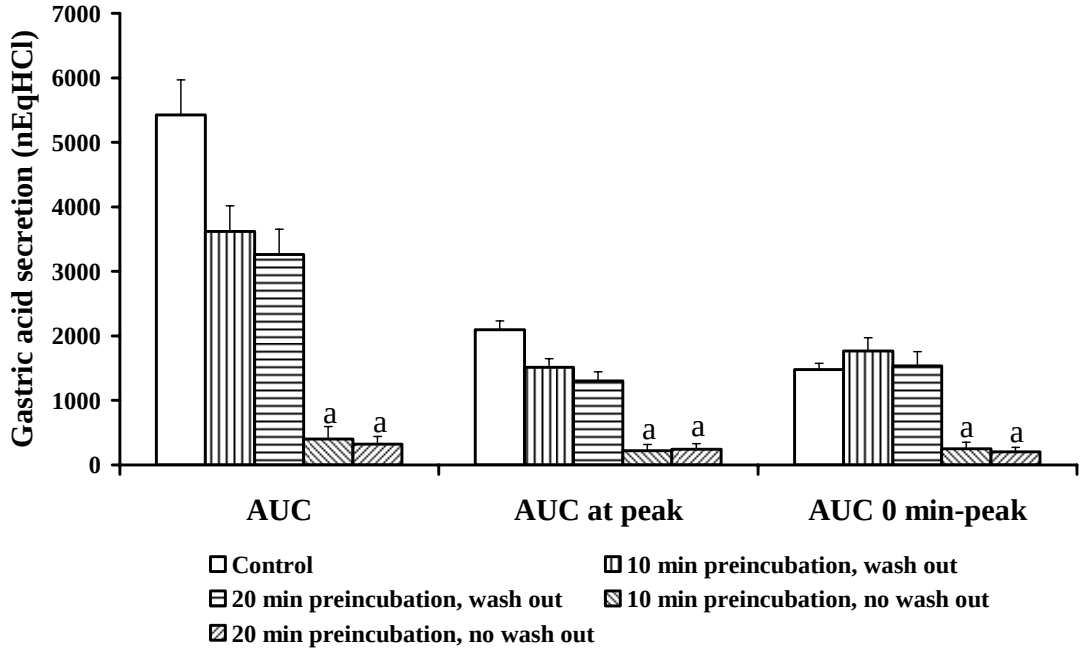


Figure 12 Comparing the effect of Ya-hom (10 mg/ml) on histamine-induced gastric acid secretion between washing out and non-washing out of Ya-hom before stimulating with histamine (n=10).

All values were expressed as mean ± SEM after subtracting the basal secretion.
[a] $p<0.001$: significantly lower than without washing Ya-hom before adding histamine when comparing between the same preincubation period.

3 Study the Dose Response of Ya-hom on Histamine-induced Gastric Acid Secretion.

The histamine-induced gastric acid secretory rate of isolated mouse whole stomach (Table 10) was the control group. Ya-hom was preincubated for 20 min by adding Ya-hom after collecting the basal secretion at -20 min. The effect of Ya-hom 2.5, 5.0, 10.0 and 20.0 mg/ml on histamine-induced gastric acid secretory rate of isolated mouse whole stomach were shown in Table 13, 22-26 and Figure 13-14. Ya-hom inhibited the stimulatory effect of histamine in the dose dependent manner (Table 25-26 and Figure 13-14) The gastric acid secretory rate in all Ya-hom treated stomachs were significantly lower than those of control at 10-110 min after adding histamine (Table 25). The inhibitory effect of Ya-hom at the doses of 10 and 20 mg/ml were significantly higher than that of 2.5 mg/ml of Ya-hom at 0-50 min and higher than that of 5.0 mg/ml of Ya-hom at 30-50 min and 20-50 min, respectively. The AUC, AUC at peak and AUC at 0 min – peak of all doses of Ya-hom were significantly lower than control ($p<0.001$). The AUC, AUC at peak and AUC at 0 min to peak of the Ya-hom treated groups also demonstrated the dose dependent of inhibitory effect of Ya-hom (Table 26 and Figure 14). The AUC, AUC at peak and AUC at 0 min to peak of Ya-hom 10 and 20 mg/ml treated group were significantly lower than Ya-hom 2.5 mg/ml treated group ($p<0.01$). The AUC at peak and AUC at 0 min to peak of Ya-hom 10 mg/ml treated group were significantly lower than those of Ya-hom 5.0 mg/ml treated group whereas all the calculated parameters of Ya-hom 20 mg/ml treated group were significantly lower than those of Ya-hom 5.0 mg/ml treated group.

Table 22 Stimulatory effect of histamine at the dose of Ya-hom 2.5 mg/ml on gastric secretory rate of isolated mouse whole stomach.

Time (min)	Gastric acid secretion (nEq HCl/10 min)									
	Number of isolated mouse whole stomach									
	1	2	3	4	5	6	7	8	9	10
- 20	320	430	740	490	580	640	560	330	530	790
- 10	590	430	800	500	660	740	650	430	570	750
0	510	370	810	630	810	960	680	490	500	750
10	520	400	950	750	980	830	650	510	490	740
20	930	580	1500	940	1170	1480	910	710	810	990
30	900	720	1360	760	860	1160	980	690	870	1080
40	770	670	1220	730	770	1020	850	530	840	900
50	630	520	1030	610	650	830	760	520	710	860
60	510	430	940	420	630	860	590	470	620	660
70	450	390	830	380	700	630	490	370	670	550
80	460	440	750	330	510	520	520	320	510	590
90	400	300	680	350	470	540	430	340	530	560
100	420	320	590	280	460	440	390	260	450	560
110	530	360	600	290	480	460	350	270	450	530
120	510	300	670	320	470	500	270	250	440	530

Adding Ya-hom (2.5 mg/ml) after collecting the basal secretion at -20 min.

Adding histamine 5.0 μM after collecting the sample at 0 min.

Table 23 Stimulatory effect of histamine at the dose of Ya-hom 5.0 mg/ml on gastric secretory rate of isolated mouse whole stomach.

Time (min)	Gastric acid secretion (nEq HCl/10 min)									
	Number of isolated mouse whole stomach									
	1	2	3	4	5	6	7	8	9	10
- 20	830	360	430	410	480	530	600	440	500	490
- 10	680	440	520	440	550	640	780	500	520	620
0	640	430	400	320	480	620	690	510	610	710
10	800	510	440	370	590	640	710	610	610	750
20	1090	570	560	540	1000	1000	920	650	750	820
30	1130	600	570	590	850	1150	1200	600	720	850
40	1110	530	510	650	850	1150	1080	590	660	750
50	1060	450	440	460	790	950	820	590	600	720
60	850	400	410	440	730	820	850	460	450	650
70	780	380	380	420	630	730	720	440	400	590
80	750	350	360	400	560	710	630	430	300	540
90	700	320	300	440	510	680	620	490	340	570
100	700	340	360	410	570	680	580	400	320	480
110	720	270	390	410	530	620	590	440	350	480
120	690	260	340	390	460	680	540	390	420	420

Adding Ya-hom (5.0 mg/ml) after collecting the basal secretion at -20 min.

Adding histamine 5.0 μM after collecting the sample at 0 min.

Table 24 Stimulatory effect of histamine at the dose of Ya-hom 20.0 mg/ml on gastric secretory rate of isolated mouse whole stomach.

Time (min)	Gastric acid secretion (nEq HCl/10 min)									
	Number of isolated mouse whole stomach									
	1	2	3	4	5	6	7	8	9	10
- 20	520	310	720	540	590	880	540	700	520	420
- 10	620	360	720	460	660	540	470	660	560	460
0	470	340	620	460	580	420	510	680	520	430
10	410	260	650	410	480	390	390	480	490	510
20	450	260	820	500	600	530	580	650	740	500
30	380	270	910	500	610	570	490	660	650	530
40	370	240	820	470	560	540	560	760	560	480
50	340	200	790	460	530	400	390	580	530	450
60	370	210	610	430	420	360	390	530	470	440
70	340	170	590	520	400	340	370	540	450	360
80	300	170	600	430	390	360	360	510	430	390
90	330	180	540	470	370	400	260	550	330	430
100	300	150	540	450	260	330	340	410	440	370
110	300	140	560	530	370	400	350	480	440	360
120	320	160	510	450	330	380	330	470	380	380

Adding Ya-hom (20.0 mg/ml) after collecting the basal seretion at -20 min.

Adding histamine 5.0 μM after collecting the sample at 0 min.

Table 25 Effect of Ya-hom on histamine-induced gastric acid secretory rate (n=10).

Time (min)	Gastric acid secretion (nEq HCl / 10 min)				
	Control	Ya-hom (mg/ml)			
		2.5	5.0	10.0	20.0
-20	0	0	0	0	0
-10	41 ± 9	75 ± 25	77 ± 17	18 ± 12	23 ± 11
0	83 ± 24	123 ± 34	65 ± 22	21 ± 14^{b}	4 ± 3^{b*}
10	292 ± 57	153 ± 41^{a}	103 ± 26^{a*}	8 ± 8^{a**b}	9 ± 9^{a**b}
20	810 ± 76	461 ± 74^{a**}	283 ± 41^{a**}	77 ± 33^{a**b**}	45 ± 23^{a**b**c}
30	808 ± 59	397 ± 42^{a**}	319 ± 55^{a**}	107 ± 36^{a**b**c}	45 ± 22^{a**b**c**}
40	755 ± 59	289 ± 37^{a**}	275 ± 52^{a**}	62 ± 20^{a**b*c*}	28 ± 11^{a**b**c**}
50	667 ± 46	171 ± 27^{a**}	175 ± 38^{a**}	21 ± 14^{a**b*c*}	11 ± 7^{a**b*c*}
60	544 ± 57	92 ± 28^{a**}	96 ± 34^{a**}	10 ± 6^{a**}	2 ± 2^{a**}
70	447 ± 57	52 ± 19^{a**}	53 ± 22^{a**}	0^{a**}	0^{a**}
80	344 ± 60	16 ± 14^{a**}	29 ± 18^{a**}	0^{a**}	0^{a**}
90	243 ± 69	8 ± 8^{a**}	42 ± 16^{a**}	0^{a**}	1 ± 1^{a**}
100	188 ± 64	10 ± 10^{a**}	20 ± 15^{a*}	0^{a**}	0^{a**}
110	131 ± 51	21 ± 21^{a}	9 ± 9^{a*}	0^{a*}	0^{a*}
120	71 ± 37	19 ± 19	15 ± 15	0	0

All values were expressed as mean ± SEM after subtracting the basal secretion.
Comparison at the same time point.
^{a}p<0.05, ^{a*}p<0.01, ^{a**}p<0.001: significantly lower than control.
^{b}p<0.05, ^{b*}p<0.01, ^{b**}p<0.001: significantly lower than Ya-hom 2.5 mg/ml.
^{c}p<0.05, ^{c*}p<0.01, ^{c**}p<0.001: significantly lower than Ya-hom 5.0 mg/ml.

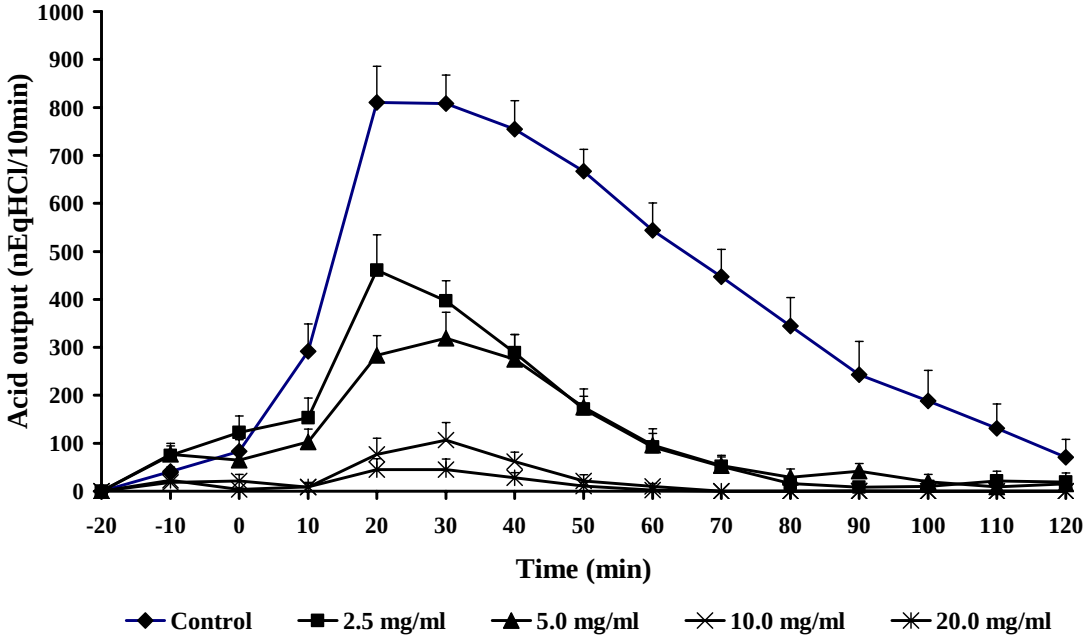


Figure 13 Effect of Ya-hom on histamine-induced gastric acid secretory rate (n=10).

All values were expressed as mean ± SEM after subtracting the basal secretion.

Table 26 Dose response of Ya-hom on histamine-induced gastric acid secretion (n=10).

Time (min)	Gastric acid secretion (nEq HCl)				
	Control	Ya-hom (mg/ml)			
		2.5	5.0	10.0	20.0
AUC	5424 ± 545	1887 ± 297[a]	1561 ± 312[a]	324 ± 116[ab]	168 ± 62[abc]
AUC at peak	2094 ± 140	1097 ± 109[a]	846 ± 137[a]	244 ± 85[ab*c*]	112 ± 49[ab*c**]
AUC 0 min - peak	1477 ± 99	871 ± 96[a]	719 ± 110[a]	203 ± 68[ab*c**]	96 ± 38[ab*c**]

All values were expressed as mean ± SEM after subtracting the basal secretion.

[a] $p<0.001$: significantly lower than control.

[b] $p<0.01$, [b*] $p<0.001$: significantly lower than Ya-hom 2.5 mg/ml.

[c] $p<0.05$, [c*] $p<0.01$, [c**] $p<0.001$: significantly lower than Ya-hom 5.0 mg/ml.

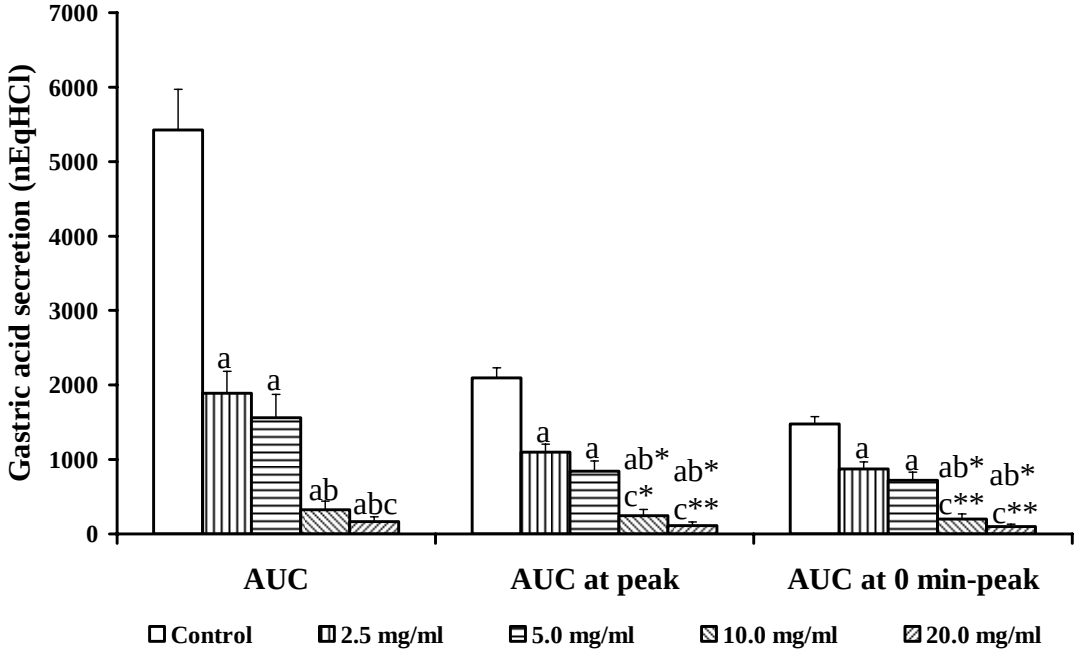


Figure 14 Dose response of Ya-hom on histamine-induced gastric acid secretion (n=10).

All values were expressed as mean ± SEM after subtracting the basal secretion.
[a] $p<0.001$: significantly lower than control.
[b] $p<0.01$, [b*] $p<0.001$: significantly lower than Ya-hom 2.5 mg/ml.
[c] $p<0.05$, [c*] $p<0.01$, [c**] $p<0.001$: significantly lower than Ya-hom 5.0 mg/ml.

4 Action of Ya-hom in the Present of Atropine (Acetylcholine Antagonist) on Histamine-induced Gastric Acid Secretion.

The histamine-induced gastric acid secretory rate of isolated mouse whole stomach (Table 10) was the control group. The effect of atropine (1 μM) on histamine-induced gastric acid secretory rate was shown in Table 27 and 29. Atropine significantly decreased the gastric acid secretory rate induced by histamine at 10-90 min after adding histamine (Table 29). The effect of Ya-hom (10 mg/ml) on histamine-induced gastric acid secretory rate in the absence of atropine (Table 13) and presence of atropine (Table 28) were compared with the control group as shown in Table 29-30 and Figure 15-16. The gastric secretory rate of Ya-hom treated stomach in the absence and presence of atropine (1 μM) was significantly lower than control group at 10-110 min and significantly lower than atropine treated group at 20-70 min. The gastric secretory rate of Ya-hom treated stomach in the presence of atropine was not significantly different from in the absence of atropine. The AUC, AUC at peak and AUC at 0 min to peak of all four groups were shown in Table 30 and Figure 16. The AUC and AUC at peak of atropine treated group was significantly lower than control group ($p<0.001$). The AUC, AUC at peak and AUC at 0 min to peak of groups of Ya-hom in the present and absence of atropine were significantly lower than control and atropine treated group ($p<0.001$). There was no significant difference in AUC, AUC at peak and AUC at 0 min to peak of Ya-hom treated groups between in the presence and absence of atropine. This data indicated that atropine did not affect Ya-hom action on histamine-induced gastric acid secretion.

Table 27 Effect of atropine (1 μM) on histamine-induced gastric acid secretory rate.

Time (min)	Gastric acid secretion (nEq HCl/10 min)									
	Number of isolated mouse whole stomach									
	1	2	3	4	5	6	7	8	9	10
- 20	400	730	400	520	790	810	440	420	420	400
- 10	420	720	600	680	800	760	540	580	430	420
0	460	590	650	710	780	760	580	570	460	440
10	450	630	700	720	830	820	750	650	540	480
20	660	940	1040	920	1030	1180	830	760	690	520
30	790	1240	1120	1200	1180	1390	740	820	780	880
40	720	1270	1010	1190	1210	1350	570	580	990	680
50	710	1240	920	1110	990	1080	470	510	660	610
60	670	1130	920	1050	870	1050	450	520	610	570
70	640	990	710	930	810	820	420	380	570	560
80	650	840	580	780	730	690	440	310	550	510
90	560	730	560	740	690	620	420	330	550	440
100	530	800	590	730	760	610	410	320	520	430
110	590	770	530	710	720	630	440	340	490	410
120	560	800	510	600	750	620	420	340	470	390

Adding atropine (1 μM) after collecting the basal secretion at -20 min.

Adding histamine 5.0 μM after collecting the sample at 0 min.

Table 28 Effect of Ya-hom (10 mg/ml) in the presence of atropine on histamine-induced gastric acid secretory rate.

Time (min)	Gastric acid secretion (nEq HCl/10 min)									
	Number of isolated mouse whole stomach									
	1	2	3	4	5	6	7	8	9	10
- 20	840	660	810	760	610	730	380	610	760	720
- 10	890	600	600	630	630	670	350	580	580	760
0	620	510	450	460	650	650	300	530	580	580
10	620	520	500	300	590	570	390	660	570	500
20	820	560	800	470	750	590	380	620	470	510
30	900	660	780	370	780	590	340	620	550	630
40	830	580	530	370	770	640	390	540	570	560
50	770	470	470	390	680	580	330	480	500	520
60	810	480	350	330	610	570	370	390	470	550
70	710	460	450	300	630	540	290	350	490	420
80	720	490	330	370	620	560	310	370	470	470
90	780	430	360	260	600	530	360	320	430	450
100	720	490	360	260	600	590	300	320	500	520
110	760	400	340	330	580	480	300	330	480	440
120	680	470	340	290	460	470	340	340	480	440

Adding atropine (1 μM) and Ya-hom (10.0 mg/ml) after collecting the basal secretion at -20 min.

Adding histamine 5.0 μM after collecting the sample at 0 min.

Table 29 Effect of Ya-hom (10 mg/ml) in the presence of atropine (1 μM) on histamine-induced gastric acid secretory rate (n=10).

Time (min)	Gastric acid secretion (nEq HCl / 10 min)			
	Control	Atropine	Ya-hom	Atropine + Ya-hom
-20	0	0	0	0
-10	41 ± 9	68 ± 25	18 ± 12	6 ± 4
0	83 ± 24	87 ± 28	21 ± 14	4 ± 4^{a,b}
10	292 ± 57	134 ± 37^{a}	8 ± 8^{a**}	10 ± 6^{a**}
20	810 ± 76	324 ± 45^{a**}	77 ± 33^{a**b*}	15 ± 14^{a**b**}
30	808 ± 59	481 ± 45^{a**}	107 ± 36^{a**b**}	19 ± 17^{a**b**}
40	755 ± 59	424 ± 61^{a**}	62 ± 20^{a**b**}	17 ± 16^{a**b**}
50	667 ± 46	297 ± 59^{a**}	21 ± 14^{a**b**}	7 ± 7^{a**b**}
60	544 ± 57	251 ± 57^{a**}	10 ± 6^{a**b**}	0^{a**b**}
70	447 ± 57	156 ± 47^{a**}	0^{a**b}	2 ± 2^{a**b}
80	344 ± 60	104 ± 33^{a**}	0^{a**}	1 ± 1^{a**}
90	243 ± 69	71 ± 27^{a*}	0^{a**}	0^{a**}
100	188 ± 64	73 ± 26	0^{a}	0^{a}
110	131 ± 51	65 ± 26	0^{a}	0^{a}
120	71 ± 37	47 ± 18	0	0

All values were expressed as mean ± SEM after subtracting the basal secretion.

Comparison at the same time point.

$^{a}p<0.05$, $^{a*}p<0.01$, $^{a**}p<0.001$: significantly lower than control.

$^{b}p<0.05$, $^{b*}p<0.01$, $^{b**}p<0.001$: significantly lower than atropine treated group.

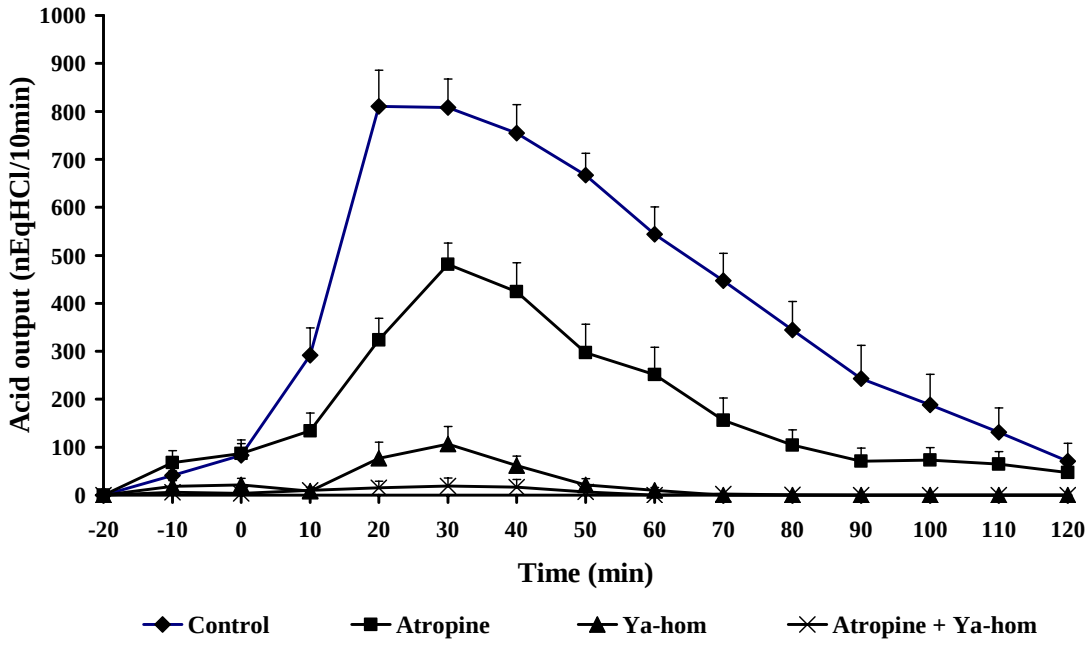


Figure 15 Effect of Ya-hom (10 mg/ml) in the presence of atropine (1 μM) on histamine-induced gastric acid secretory rate (n=10).

All values were expressed as mean ± SEM after subtracting the basal secretion.

Table 30 Effect of Ya-hom (10 mg/ml) in the presence or absence of atropine (1 μM) on histamine-induced gastric acid secretion (n=10).

Time (min)	Gastric acid secretion (nEq HCl)			
	Control	Atropine	Ya-hom	Atropine + Ya-hom
AUC	5424 ± 545	2582 ± 406[a]	324 ± 116[ab]	81 ± 66[ab]
AUC at peak	2094 ± 140	1270 ± 124[a]	244 ± 85[ab]	56 ± 47[ab]
AUC at 0 min - peak	1477 ± 99	1149 ± 116	203 ± 68[ab]	59 ± 54[ab]

All values were expressed as mean ± SEM after subtracting the basal secretion.

[a] $p<0.001$: significantly lower than control.

[b] $p<0.001$: significantly lower than atropine treated group.

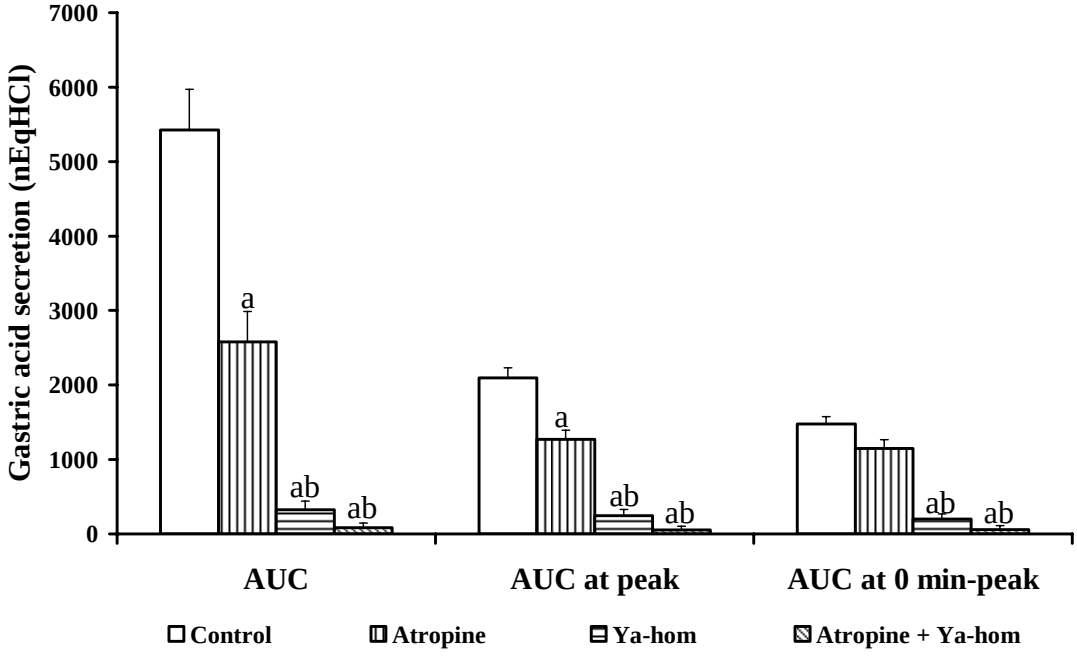


Figure 16 Effect of Ya-hom (10 mg/ml) in the presence or absence of atropine (1 μM) on histamine-induced gastric acid secretion (n=10).

All values were expressed as mean ± SEM after subtracting the basal secretion.
[a] $p<0.001$: significantly lower than control.
[b] $p<0.001$: significantly lower than atropine treated group.

5 Action of Ya-hom in the Presence of Ranitidine on Histamine-induced Gastric Acid Secretion.

The gastric acid secretory rate of histamine-induced stomach in the absence of Ya-hom and ranitidine, H_2 receptor antagonist; (control), in the presence of ranitidine (H+R) and in the presence of Ya-hom (10 mg/ml) without (H+YH) and with ranitidine (H+YH+R) were shown in Table 10, 31, 13 and 32, respectively. The gastric acid secretory rates subtracting their own basal secretion were summarized in Table 33. Ranitidine (10 μM) almost completely inhibited histamine-induced gastric acid secretory rate. Ya-hom of a concentration of 10.0 mg/ml significantly inhibited the stimulatory effect of histamine to the same level as ranitidine (10 μM) (Table 32 and Figure 17) There were no significant difference in gastric acid secretory rate among H+R, H+YH and H+YH+R groups. The AUC, AUC at peak and AUC at 0 min to peak of H+R, H+YH and H+YH+R groups were significantly lower than those of control and not different among groups (Table 34 and Figure 18) The data indicated that ranitidine did not altered the inhibitory effect of Ya-hom on histamine-induced gastric acid secretion in isolated mouse whole stomach.

Table 31 Effect of ranitidine (10 μM) on histamine-induced gastric acid secretory rate.

Time (min)	Gastric acid secretion (nEq HCl/10 min)									
	Number of isolated mouse whole stomach									
	1	2	3	4	5	6	7	8	9	10
- 20	500	380	520	370	380	430	720	700	850	850
- 10	480	400	450	350	400	460	680	680	810	890
0	390	370	510	380	380	470	590	580	700	810
10	380	380	410	420	360	450	540	550	710	760
20	410	430	440	480	540	430	580	590	860	790
30	420	390	490	460	570	430	640	580	940	920
40	490	370	490	470	500	420	610	670	890	960
50	510	390	410	480	480	420	640	650	900	880
60	520	300	370	460	500	450	660	610	980	880
70	480	290	410	470	490	410	610	580	900	840
80	570	300	480	480	420	370	640	550	910	850
90	610	320	360	450	420	430	560	510	920	870
100	540	270	440	420	440	350	580	510	940	770
110	520	270	410	460	420	360	600	560	930	820
120	480	310	440	520	370	330	690	510	890	770

Adding ranitidine (10 μM) after collecting the basal secretion at -20 min.

Adding histamine 5.0 μM after collecting the sample at 0 min.

Table 32 Effect of Ya-hom (10 mg/ml) in the presence of ranitidine (10 μM) on histamine-induced gastric acid secretory rate.

Time (min)	Gastric acid secretion (nEq HCl/10 min)									
	Number of isolated mouse whole stomach									
	1	2	3	4	5	6	7	8	9	10
- 20	860	560	570	500	730	600	580	870	750	490
- 10	880	670	550	600	610	550	520	900	580	460
0	600	500	490	530	560	530	470	660	630	440
10	510	470	440	480	500	420	450	600	630	480
20	450	520	430	490	520	440	480	710	840	490
30	480	510	460	520	510	470	430	910	770	500
40	430	520	460	510	570	440	490	760	790	570
50	490	590	430	510	520	420	470	770	730	520
60	420	510	460	590	500	460	490	810	780	570
70	430	540	430	510	480	380	530	730	640	460
80	410	540	460	560	500	380	450	760	590	450
90	460	540	520	550	440	430	460	700	680	550
100	430	580	460	560	500	310	470	690	700	470
110	450	600	510	590	440	340	420	770	550	490
120	390	430	450	560	360	300	420	630	530	480

Adding ranitidine (10 μM) and Ya-hom (10.0 mg/ml) after collecting the basal secretion at after collecting the sample at -20 min.

Adding histamine 5.0 μM after collecting the sample at 0 min.

Table 33 Effect of Ya-hom (10 mg/ml) in the presence of ranitidine (10 μM) on histamine-induced gastric acid secretory rate (n=10).

Time (min)	Gastric acid secretion (nEq HCl / 10 min)			
	Control	Ranitidine	Ya-hom	Ranitidine + Ya-hom
-20	0	0	0	0
-10	41 ± 9	11 ± 5	18 ± 12	26 ± 14
0	83 ± 24	4 ± 4 ^{a*}	21 ± 14 ^{a}	3 ± 3 ^{a*}
10	292 ± 57	7 ± 5 ^{a**}	8 ± 8 ^{a**}	0 ^{a**}
20	810 ± 76	33 ± 18 ^{a**}	77 ± 33 ^{a**}	9 ± 9 ^{a**}
30	808 ± 59	45 ± 20 ^{a**}	107 ± 36 ^{a**}	9 ± 4 ^{a**}
40	755 ± 59	37 ± 17 ^{a**}	62 ± 20 ^{a**}	13 ± 8 ^{a**}
50	667 ± 46	31 ± 13 ^{a**}	21 ± 14 ^{a**}	7 ± 4 ^{a**}
60	544 ± 57	41 ± 16 ^{a**}	10 ± 6 ^{a**}	20 ± 11 ^{a**}
70	447 ± 57	26 ± 14 ^{a**}	0 ^{a**}	1 ± 1 ^{a**}
80	344 ± 60	28 ± 13 ^{a**}	0 ^{a**}	6 ± 6 ^{a**}
90	243 ± 69	32 ± 13 ^{a**}	0 ^{a**}	11 ± 7 ^{a**}
100	188 ± 64	24 ± 11 ^{a*}	0 ^{a**}	8 ± 6 ^{a**}
110	131 ± 51	23 ± 11 ^{a}	0 ^{a**}	13 ± 9 ^{a}
120	71 ± 37	19 ± 15	0	6 ± 6

All values were expressed as mean ± SEM after subtracting the basal secretion.

Comparison at the same time point.

$^{a}p<0.05$, $^{a*}p<0.01$, $^{a**}p<0.001$: significantly lower than control.

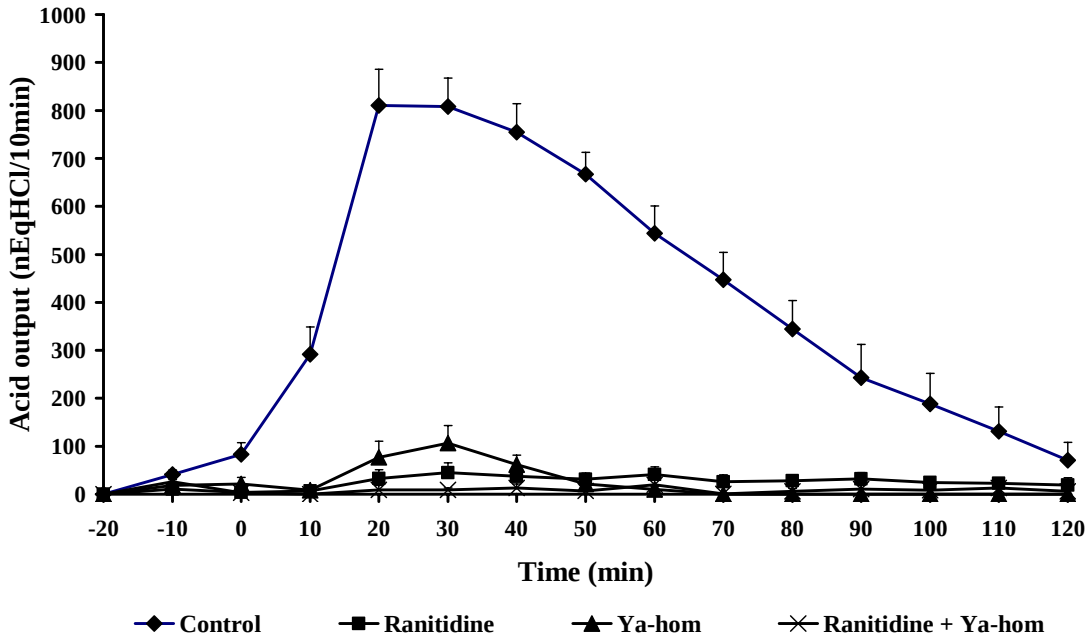


Figure 17 Effect of Ya-hom (10 mg/ml) in the presence of ranitidine (10 μM) on histamine-induced gastric acid secretory rate (n=10).

All values were expressed as mean ± SEM after subtracting the basal secretion.

Table 34 Effect of Ya-hom (10 mg/ml) in the presence or absence of ranitidine (10 μM) on histamine-induced gastric acid secretion (n=10).

Time (min)	Gastric acid secretion (nEq HCl)			
	Control	Ranitidine	Ya-hom	Ranitidine + Ya-hom
AUC	5424 ± 545	361 ± 136*	324 ± 116*	132 ± 60*
AUC at peak	2094 ± 140	113 ± 50*	244 ± 85*	28 ± 14*
AUC at 0 min – peak	1477 ± 99	90 ± 36*	203 ± 68*	61 ± 27*

All values were expressed as mean ± SEM after subtracting the basal secretion.

* $p<0.001$: significantly lower than control.

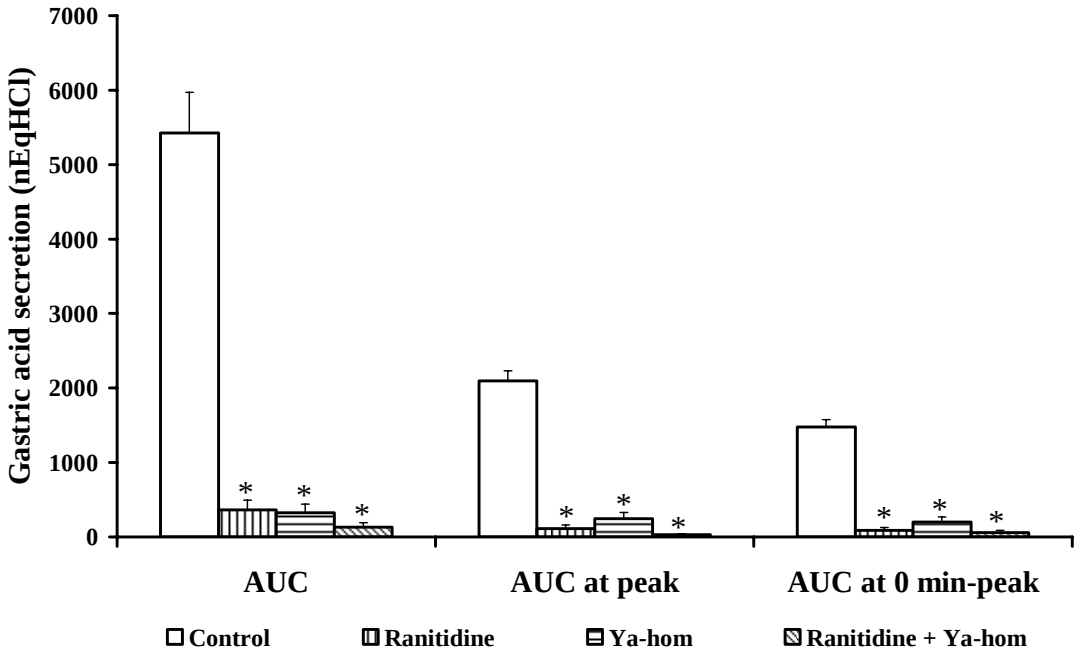


Figure 18 Effect of Ya-hom (10 mg/ml) in the presence or absence of ranitidine (10 μM) on histamine-induced gastric acid secretion (n=10).

All values were expressed as mean ± SEM after subtracting the basal secretion.

* $p<0.001$: significantly lower than control.

PART III EFFECT OF YA-HOM ON BETHANECHOL-INDUCED GASTRIC ACID SECRETION.

1 Action of Ya-hom on High Dose Bethanechol (100 μM)-induced Gastric Acid Secretion.

The effect of high dose bethanechol (100 μM) on gastric acid secretory rate of isolated mouse whole stomach was shown in Table 35 and reffered to as control. The effect of Ya-hom 10 mg/ml on high dose bethanechol-induced gastric acid secretory rate of isolated mouse whole stomach in the absence and presence of atropine (1 μM) were shown in Table 36 and 37, respectively. The gastric secretory rates of all groups subtracting their own basal secretion were summarized in Table 38-39 and Figure 19-20. The gastric acid secretory rate rapidly increased to the maximum rate at 20 min after adding bethanechol (100μM) and rapidly decreased through out the experimental period (Figure 19). Ya-hom treated group (table 35) and atropine treated group (Table 36) significantly decreased the gastric secretory rate induced by bethanechol at 10-100 min and 10-90 min after adding bethanechol. There were no significant difference in gastric secretory rate between atropine treated and Ya-hom treated groups as showed in Table 38. The AUC, AUC at peak and AUC at 0 min to peak of all groups were shown in Table 38 and Figure 20. The AUC, AUC at peak and AUC at 0 min to peak of Ya-hom treated group and atropine treated group were significantly lower than control group ($p<0.001$). There was no significant difference in AUC, AUC at peak and AUC at 0 min to peak of Ya-hom treated group and atropine treated group. These data showed that Ya-hom inhibited high dose bethanechol (100 μM)-induced gastric acid secretion in the absence and presence of inhibitor.

Table 35 Stimulatory effect of high dose bethanechol (100 μM)-induced gastric acid secretory rate of isolated mouse whole stomach.

Time (min)	Gastric acid secretion (nEq HCl/10 min)									
	Number of isolated mouse whole stomach									
	1	2	3	4	5	6	7	8	9	10
- 20	690	390	250	480	400	780	630	480	540	490
- 10	820	420	330	490	460	830	590	530	450	550
0	750	420	360	460	500	710	610	470	610	480
10	970	730	500	730	680	980	740	630	830	800
20	1360	970	860	1270	1140	1630	1320	1360	1410	1310
30	1050	1040	900	1070	1140	1450	1280	1360	1500	1180
40	830	830	710	850	900	1290	1060	840	1200	880
50	760	740	600	810	740	970	820	750	890	810
60	680	600	540	710	590	860	880	500	660	690
70	610	680	570	770	540	840	770	400	560	610
80	630	480	470	650	460	650	670	330	430	620
90	600	450	450	570	440	660	590	330	330	610
100	560	470	410	530	420	600	550	310	310	550
110	570	430	360	450	330	620	610	260	320	520
120	550	420	330	490	360	530	550	230	250	560

Collecting the basal secretion at -20 min.

Adding bethanechol 100 μM after collecting the sample at 0 min.

Table 36 Effect of Ya-hom (10.0 mg/ml) on high dose bethanechol (100 μM) -induced gastric acid secretory rate.

Time (min)	Gastric acid secretion (nEq HCl/10 min)									
	Number of isolated mouse whole stomach									
	1	2	3	4	5	6	7	8	9	10
- 20	580	690	770	620	810	540	630	650	660	650
- 10	530	730	710	610	580	530	600	530	560	620
0	370	610	600	590	500	490	520	420	680	600
10	400	550	620	590	670	490	590	430	720	560
20	500	850	750	830	800	510	530	510	740	580
30	450	750	780	810	670	530	590	530	700	720
40	410	630	670	690	550	490	550	430	640	730
50	390	540	560	570	540	490	400	350	530	650
60	260	530	450	530	440	430	310	330	500	570
70	250	500	320	440	410	440	420	340	490	520
80	250	450	340	380	390	470	380	330	520	550
90	240	360	300	410	390	420	370	340	590	410
100	220	430	280	330	370	500	270	290	580	450
110	200	380	300	310	350	470	260	330	620	410
120	180	410	230	320	460	490	320	350	590	370

Adding Ya-hom (10.0 mg/ml) after collecting the basal secretion at -20 min.

Adding bethanechol 100 μM after collecting the sample at 0 min.

Table 37 Effect of atropine (1 μM) on high dose bethanechol (100 μM)-induced gastric acid secretory rate.

Time (min)	Gastric acid secretion (nEq HCl/10 min)									
	Number of isolated mouse whole stomach									
	1	2	3	4	5	6	7	8	9	10
- 20	620	780	580	540	550	500	820	630	740	680
- 10	720	710	670	530	560	400	800	580	820	750
0	570	710	580	580	630	420	720	520	630	610
10	630	650	550	540	620	420	710	470	650	610
20	940	800	670	810	700	640	850	740	670	590
30	1080	940	710	720	680	520	920	590	770	750
40	1040	890	630	600	780	480	850	590	860	850
50	900	850	600	590	650	520	820	550	690	610
60	740	670	490	510	570	520	800	580	620	640
70	720	620	360	430	540	490	710	470	720	550
80	620	570	470	450	570	550	760	530	650	520
90	590	610	480	470	500	410	750	500	670	480
100	660	490	450	480	550	410	720	490	800	440
110	590	460	490	500	500	460	840	410	810	490
120	670	520	480	470	590	420	780	450	850	580

Adding Atropine (1 μM) after collecting the basal secretion at -20 min.

Adding bethanechol 100 μM after collecting the sample at 0 min.

Table 38 Effect of Ya-hom 10 mg/ml and atropine (1 μM) on high dose bethanechol (100 μM)-induced gastric acid secretory rate (n=10).

Time (min)	Gastric acid secretion (nEq HCl / 10 min)		
	Control	Atropine	Ya-hom
-20	0	0	0
-10	47 ± 13	35 ± 14	6 ± 4 [a]
0	37 ± 14	12 ± 9	6 ± 6
10	246 ± 23	10 ± 7 [a**]	29 ± 22 [a**]
20	740 ± 40	127 ± 34 [a**]	47 ± 23 [a**]
30	684 ± 51	123 ± 42 [a**]	21 ± 11 [a**]
40	427 ± 42	115 ± 41 [a**]	0 [a**]
50	276 ± 30	47 ± 28 [a**]	0 [a**]
60	159 ± 31	16 ± 12 [a**]	0 [a**]
70	138 ± 39	10 ± 10 [a*]	0 [a**]
80	81 ± 28	7 ± 5 [a*]	0 [a*]
90	51 ± 22	0 [a]	0 [a]
100	37 ± 17	4 ± 4	0 [a]
110	18 ± 12	7 ± 5	0
120	19 ± 10	10 ± 6	0

All values were expressed as mean ± SEM after subtracting the basal secretion.

Comparison at the same time point.

[a] $p<0.05$, [a*] $p<0.01$, [a**] $p<0.001$: significantly lower than control

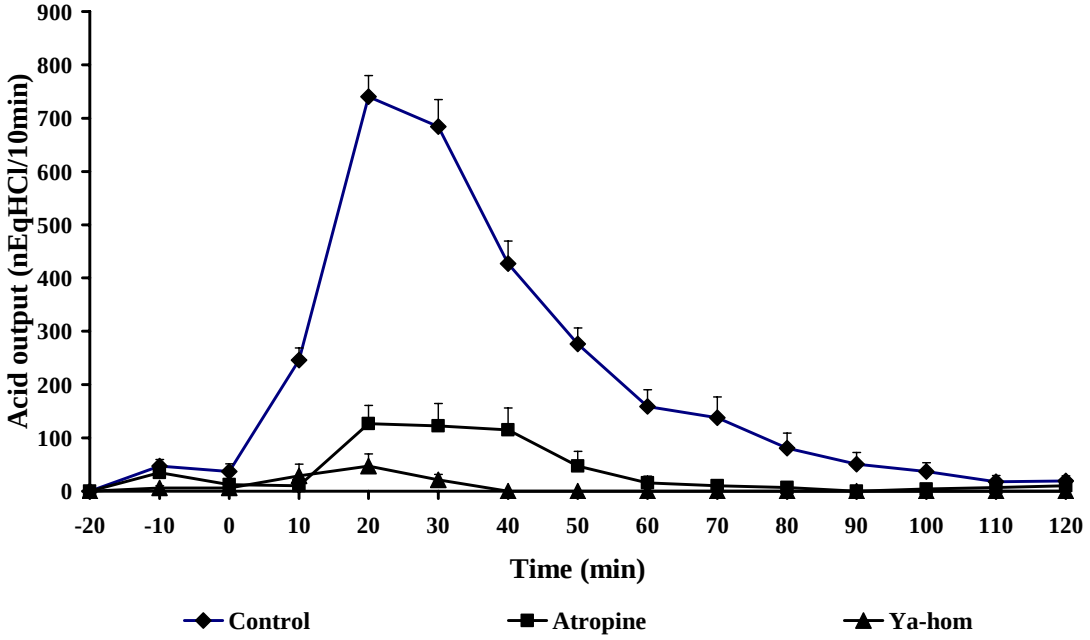


Figure 19 Effect of Ya-hom 10 mg/ml and atropine (1 μM) on high dose bethanechol (100 μM)-induced gastric acid secretory rate (n=10).

All values were expressed as mean ± SEM after subtracting the basal secretion.

Table 39 Effect of Ya-hom 10 mg/ml and atropine (1 μM) on high dose bethanechol (100 μM)-induced gastric acid secretion (n=10).

Time (min)	Gastric acid secretion (nEq HCl)		
	Control	Atropine	Ya-hom
AUC	2960 ± 190	523 ± 169*	109 ± 51*
AUC at peak	1739 ± 100	338 ± 101*	103 ± 49*
AUC at 0 min - peak	1249 ± 129	257 ± 63*	78 ± 41*

All values were expressed as mean ± SEM after subtracting the basal secretion.

* $p<0.001$: significantly lower than control.

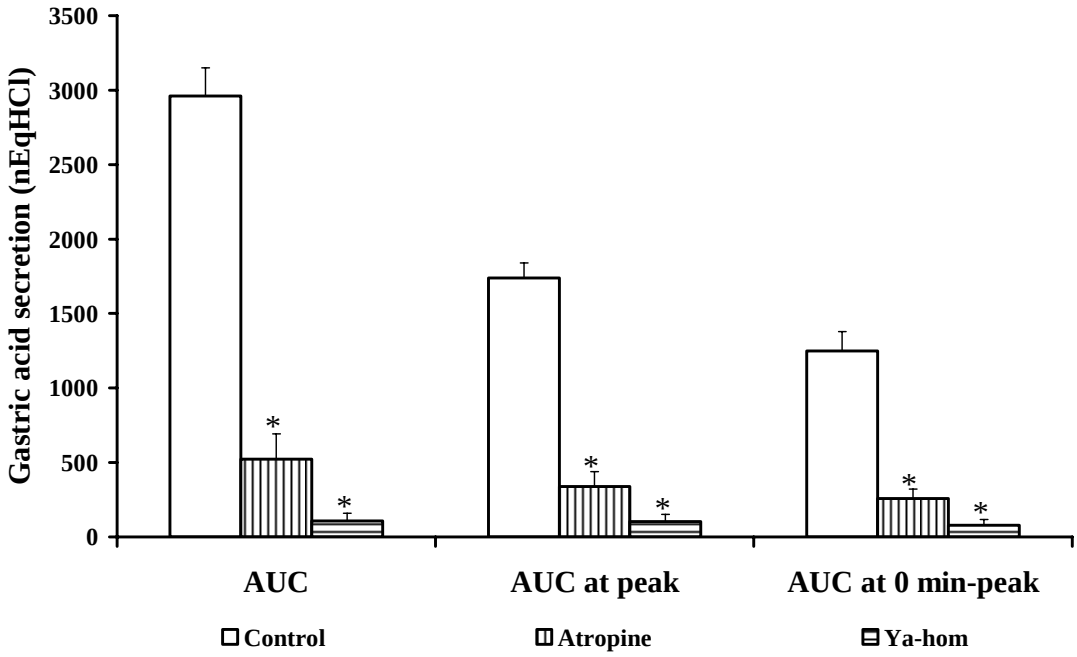


Figure 20 Effect of Ya-hom 10 mg/ml and atropine (1 μM) on high dose bethanechol (100 μM)-induced gastric acid secretion (n=10).

All values were expressed as mean ± SEM after subtracting the basal secretion.

*p<0.001: significantly lower than control.

2 Action of Ya-hom on Low Dose Bethanechol-induced Gastric Acid Secretion.

The gastric acid secretory rate of bethanechol (10 μM) -induced stomach in the absence of Ya-hom and inhibitor; atropine and ranitidine; (control), in the presence of ranitidine (10 μM) (B+R), in the presence of atropine (1 μM) (B+A) and in the presence of Ya-hom (10 mg/ml) without (B+YH) and with ranitidine (B+YH+R) were shown in Table 10-44, respectively. The gastric secretory rates subtracting their own basal secretion were compared with the control group as shown in Table 45 and Figure 21. The gastric acid secretory rate of B+A, B+R, B+YH and B+YH+R groups were significantly difference lower than control. The gastric acid secretory rate of B+A, B+YH and B+YH+R groups were significantly lower than ranitidine treated group and not different between groups. The AUC, AUC at peak and AUC at 0 min to peak of all five groups were shown in Table 46 and Figure 22. The AUC, AUC at peak and AUC at 0 min to peak of B+A, B+R, B+YH and B+YH+R groups were significantly difference lower than control. The AUC, AUC at peak and AUC at 0 min to peak of B+A, B+YH and B+YH+R groups were significantly lower than ranitidine treated group and not different between groups. These data showed that Ya-hom inhibited low dose bethanechol (10 μM)-induced gastric acid secretion in the absence and presence of inhibitors.

Table 40 Stimulatory effect of low dose bethanechol (10 μM)-induced gastric acid secretory rate of isolated mouse whole stomach.

Time (min)	Gastric acid secretion (nEq HCl/10 min)									
	Number of isolated mouse whole stomach									
	1	2	3	4	5	6	7	8	9	10
- 20	370	640	640	640	700	480	740	670	520	740
- 10	520	650	660	640	720	570	750	670	580	730
0	420	620	680	650	770	620	810	670	520	720
10	620	900	920	970	810	1090	1090	1010	600	1100
20	1140	1280	1350	1600	1430	1130	1540	1320	1180	1440
30	820	1280	1180	1240	1180	990	1350	1290	920	1200
40	810	1040	980	1090	1060	960	1000	1090	630	1040
50	620	840	820	860	920	910	920	850	600	950
60	510	770	750	640	630	800	790	740	540	820
70	460	740	740	620	680	750	720	640	530	750
80	540	760	760	510	520	680	700	670	510	600
90	380	720	600	560	520	590	520	570	480	590
100	480	780	510	580	500	600	670	530	460	550
110	370	700	600	540	470	530	690	550	460	560
120	340	780	490	630	530	550	720	500	470	500

Collecting the basal secretion at -20 min.

Adding bethanechol 10 μM after collecting the sample at 0 min.

Table 41 Effect of Ya-hom (10 mg/ml) on low dose bethanechol-induced gastric acid secretory rate of isolated mouse whole stomach.

Time (min)	Gastric acid secretion (nEq HCl/10 min)									
	Number of isolated mouse whole stomach									
	1	2	3	4	5	6	7	8	9	10
- 20	570	480	500	780	650	710	780	500	610	680
- 10	550	410	460	760	520	550	640	520	470	750
0	470	370	530	650	470	570	570	400	490	710
10	510	380	530	640	510	490	530	380	420	740
20	530	470	650	740	540	650	650	420	580	820
30	400	360	560	590	460	520	500	680	660	810
40	430	330	540	550	390	520	550	580	670	780
50	280	340	440	510	340	440	510	510	540	730
60	250	320	310	580	380	400	570	470	480	720
70	260	270	530	510	380	370	470	460	430	630
80	230	310	340	500	360	480	490	410	450	680
90	260	270	360	510	330	420	430	440	450	610
100	220	280	350	550	320	370	580	400	450	550
110	260	340	400	480	320	420	460	370	480	590
120	240	270	350	520	320	380	510	340	400	630

Adding Ya-hom (10.0 mg/ml) after collecting the basal secretion at -20 min.

Adding bethanechol 10 μM after collecting the sample at 0 min.

Table 42 Effect of atropine (1 μM) on low dose bethanechol (10 μM)-induced gastric acid secretory rate.

Time (min)	Gastric acid secretion (nEq HCl/10 min)									
	Number of isolated mouse whole stomach									
	1	2	3	4	5	6	7	8	9	10
- 20	610	630	780	410	710	450	760	420	580	430
- 10	600	540	740	430	610	410	740	490	470	420
0	500	490	710	460	620	400	680	500	560	370
10	500	470	690	420	630	350	730	560	520	360
20	780	600	890	390	690	370	710	700	600	510
30	890	670	990	350	740	410	710	560	700	490
40	810	650	860	350	630	360	770	470	600	510
50	610	440	720	420	560	320	810	520	560	520
60	640	450	640	340	590	350	780	470	550	510
70	630	480	570	360	680	360	740	450	450	400
80	620	440	600	320	630	290	700	450	410	450
90	660	430	630	360	630	300	600	530	400	410
100	550	460	600	320	640	300	580	510	480	370
110	620	540	680	360	670	280	550	500	380	480
120	660	490	710	330	610	270	500	450	390	450

Adding Atropine (1 μM) after collecting the basal secretion at -20 min.

Adding bethanechol 10 μM after collecting the sample at 0 min.

Table 43 Effect of ranitidine (10 μM) on low dose bethanechol (10 μM)-induced gastric acid secretory rate.

Time (min)	Gastric acid secretion (nEq HCl/10 min)									
	Number of isolated mouse whole stomach									
	1	2	3	4	5	6	7	8	9	10
- 20	630	580	520	420	390	280	450	320	470	330
- 10	610	630	440	380	300	370	480	340	470	450
0	650	670	450	320	300	260	430	290	470	430
10	640	590	610	410	390	350	490	440	520	420
20	1030	780	1060	810	830	660	850	1080	850	690
30	940	700	730	570	640	540	790	750	750	690
40	880	740	710	540	610	470	620	630	590	560
50	730	550	560	480	490	330	530	480	470	390
60	670	610	560	450	420	290	480	410	460	410
70	550	500	480	380	400	270	400	350	460	410
80	460	560	450	360	370	280	390	350	420	300
90	420	440	460	330	280	240	330	340	410	310
100	390	470	520	280	280	230	390	320	450	320
110	460	430	410	320	300	250	430	300	440	270
120	460	470	430	320	260	240	440	300	470	300

Adding ranitidine (10 μM) after collecting the basal secretion at -20 min.

Adding bethanechol 10 μM after collecting the sample at 0 min.

Table 44 Effect of Ya-hom (10 mg/ml) in the presence of ranitidine (10 μM) on low dose bethanechol (10 μM)-induced gastric acid secretory rate.

Time (min)	Gastric acid secretion (nEq HCl/10 min)									
	Number of isolated mouse whole stomach									
	1	2	3	4	5	6	7	8	9	10
- 20	650	790	790	840	790	830	510	520	400	700
- 10	630	710	690	800	810	760	450	540	400	660
0	500	670	730	740	730	740	430	500	340	620
10	500	680	840	650	630	720	400	460	300	560
20	690	740	880	650	660	800	480	600	420	540
30	650	690	710	670	600	750	440	530	420	520
40	590	730	640	590	630	700	300	460	380	500
50	570	650	580	610	510	730	380	320	360	480
60	620	640	600	550	540	610	320	340	360	500
70	540	620	540	540	500	560	330	420	340	480
80	500	550	570	510	490	630	340	410	360	460
90	500	550	500	580	660	540	330	400	300	440
100	490	520	580	470	520	510	350	370	280	440
110	500	580	550	510	520	490	380	350	280	480
120	480	470	590	510	680	470	360	330	280	460

Adding ranitidine (10 μM) and Ya-hom (10.0 mg/ml) after collecting the basal secretion at -20 min.

Adding bethanechol 10 μM after collecting the sample at 0 min.

Table 45 Effect of Ya-hom 10 mg/ml, ranitidine (10 μM), atropine (1 μM) and Ya-hom 10 mg/ml in the presence of ranitidine (10 μM) on low dose bethanechol (10 μM)-induced gastric acid secretoty rate (n=10).

Time (min)	Gastric acid secretion (nEq HCl / 10 min)				
	Control	Ranitidine	Atropine	Ya-hom	Ya-hom + ranitidine
-20	0	0	0	0	0
-10	36 ± 16	31 ± 14	9 ± 7	7 ± 7	4 ± 3
0	38 ± 15	21 ± 13	13 ± 9	6 ± 4	0
10	303 ± 47	48 ± 14 ^{a**}	15 ± 14 ^{a**}	9 ± 6 ^{a**}	5 ± 5 ^{a**}
20	727 ± 31	425 ± 46 ^{a**}	56 ± 23 ^{a**b*}	29 ± 19 ^{a**b*}	23 ± 11 ^{a**b*}
30	531 ± 26	271 ± 30 ^{a**}	89 ± 31 ^{a**b*}	42 ± 21 ^{a**b*}	3 ± 2 ^{a**b*}
40	356 ± 35	196 ± 19 ^{a**}	46 ± 20 ^{a**b*}	28 ± 12 ^{a**b*}	0 ^{a**b*}
50	215 ± 28	65 ± 15 ^{a**}	25 ± 13 ^{a**}	6 ± 5 ^{a**}	0 ^{a**b}
60	92 ± 30	38 ± 9	18 ± 9 ^{a*}	4 ± 4 ^{a**}	0 ^{a**}
70	58 ± 27	12 ± 8	5 ± 4 ^{a}	3 ± 3 ^{a}	0 ^{a}
80	61 ± 26	3 ± 3 ^{a*}	6 ± 4 ^{a*}	0 ^{a*}	0 ^{a*}
90	20 ± 13	2 ± 2	16 ± 12	0	0
100	37 ± 19	0	9 ± 9	0	0
110	11 ± 7	0	14 ± 9	0	0
120	21 ± 15	0	10 ± 6	0	0

All values were expressed as mean ± SEM after subtracting the basal secretion.
Comparison at the same time point.
^{a}p<0.05, ^{a*}p<0.01, ^{a**}p<0.001: significantly lower than control.
^{b}p<0.05, ^{b*}p<0.001: significantly lower than ranitidine.

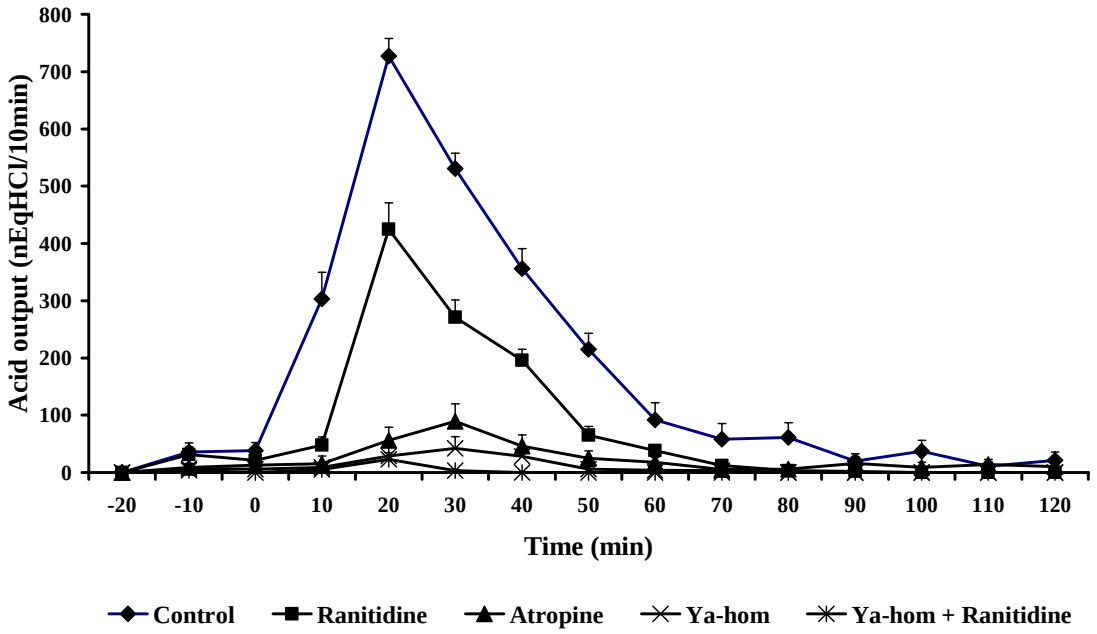


Figure 21 Effect of Ya-hom 10 mg/ml, ranitidine (10 μM), atropine (1 μM) and Ya-hom 10 mg/ml in the presence of ranitidine (10 μM) on low dose bethanechol (10 μM)-induced gastric acid secretoty rate (n=10).

All values were expressed as mean ± SEM after subtracting the basal secretion.

Table 46 Effect of Ya-hom 10 mg/ml, ranitidine (10 μM), atropine (1 μM) and Ya-hom 10 mg/ml in the presence of ranitidine (10 μM) on low dose bethanechol (10 μM)-induced gastric acid secretion (n=10).

Time (min)	Gastric acid secretion (nEq HCl)				
	Control	Ranitidine	Atropine	Ya-hom	Ya-hom + Ranitidine
AUC	2506 ± 222	1112 ± 120[a]	331 ± 125[ab]	134 ± 67[ab]	35 ± 16[ab]
AUC at peak	1561 ± 71	744 ± 79[a]	210 ± 71[ab]	94 ± 42[ab]	31 ± 15[ab]
AUC 0 min - peak	1068 ± 61	494 ± 51[a]	176 ± 53[ab]	67 ± 31[ab]	28 ± 15[ab]

All values were expressed as mean ± SEM after subtracting the basal secretion.

[a] $p<0.001$: significantly lower than control.

[b] $p<0.001$: significantly lower than ranitidine treated group.

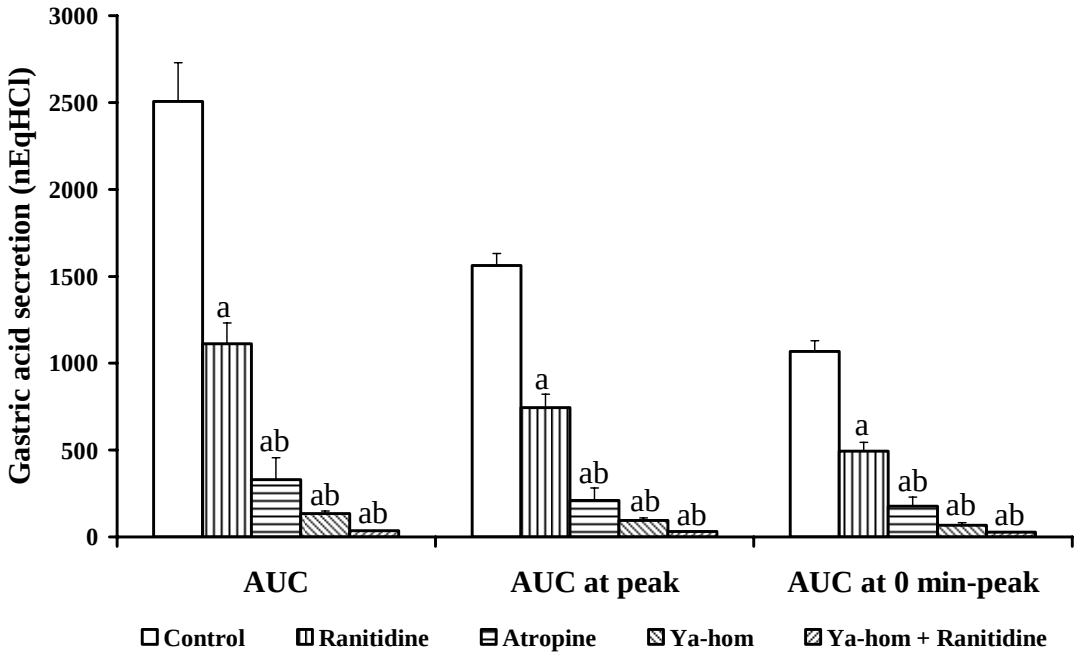


Figure 22 Effect of Ya-hom 10 mg/ml, ranitidine (10 μM), atropine (1 μM) and Ya-hom 10 mg/ml in the presence of ranitidine (10 μM) on low dose bethanechol (10 μM)-induced gastric acid secretion (n=10).

All values were expressed as mean ± SEM after subtracting the basal secretion.

[a] $p<0.001$: significantly lower than control

[b] $p<0.001$: significantly lower than ranitidine treated group

CHAPTER V
DISCUSSION

The objective of the present study is to determine the inhibitory effect of Ya-hom on gastric acid secretion in isolated mouse whole stomach. The results obtained in this study provide the evidence for the first time in the *in vitro* study mechanism action of Ya-hom on gastric acid secretion.

Food and stomach distension affect gastric acid secretion according to the composition of the food and degree of stomach distension. To avoid this variation, non-fasting condition and intragastric pressure of stomach distension were determined to investigate the suitable condition. First, the gastric acid secretion was compared between the fasting and non-fasting mice. In many reports, the fasting condition was used in the isolated mouse stomach model (51, 60-62), whereas some reports were used non-fasting mice (64-65). In *in vivo* study, the fasting animals need to ensure that the stomach didn't have food to interfere experiment. In this model, however, fasting may not necessary because in the preparation process, the stomach is flushed with mucosal solution before insert tube, thus there is no food to inferfere experiment. From the result, the stimulation gastric acid secretory rate by histamine between fasted and non-fasted mice were the same, even if the last 40 min (at 120-150 min after adding histamine) of gastric secretory rates were different. The gastric acid secretory rates at 120-150 min of fasted mouse stomach were higher than that of non-fasted mouse. The gastric secretory rate decreases within 2 hours after stimulating with histamine is preferable because it shows the ending of histamine action. The sustained gastric acid secretion after 2 hours of histamine stimulation in fasted mice may be due to unknown mediator released by fasted condition, which prolongs the effect of histamine. This condition may cause a complication to the rest of experiment. The other experiment, gastric acid secretory rate was kept 120 min after adding histamine, thus the gastric secretion of fasting and non-fasting condition would give the same result. On the other hand, the non-fasting animal was more similar to the natural

condition than fasting animal, so that the non-fasted condition is used in the rest experiment.

The effect of intragastric pressures were determined by keeping the intragastric pressure at 20 cm H_2O and comparison with higher and lower than that about 2 cm H_2O. Distension of stomach stimulates mechanoreceptor in gastric wall (5), thus deviation of stomach distension was determined to find the level that gave the highest gastric acid secretion. The gastric acid secretion as calculated as AUC, AUC at peak and AUC at 0 min to peak of stomach with the intragastric pressure at 18, 20 and 22 cm H_2O were not significantly difference (Table 9). However, the AUC, AUC at peak and AUC at 0 min-peak of stomach with the intragastric pressure at 20 cm H_2O were higher than the other groups. In spite of the results that the gastric acid secretory rate of three intragastric pressures was similar and other reports recommended the intragastric pressure at 20 cm H_2O, so it was used for investigation of Ya-hom effect.

The action of the drug is normally affected by the incubation period. The effect of incubation period on the inhibitory activity of Ya-hom was determined. The preincubation periods with Ya-hom at 10 and 20 min before adding histamine showed greater inhibitory effect than the simultaneously adding Ya-hom with histamine. The conditions that the preincubation periods with Ya-hom at 10 and 20 min were similar. However, AUC at 20 min preincubation period was significantly lower than simultaneously adding whereas AUC at 10 min preincubation period was not. Thus, the results indicated that the 20 min preincubation period of Ya-hom showed greatest inhibitory effect than the other conditions.

The existence of drug along the experimental period affects the absorption and activity of drug. Since, the 10 min and 20 min preincubation periods of Ya-hom had great inhibitory effect to gastric acid secretion, the comparing condition of washing out and non-washing out of Ya-hom before adding histamine were determined in both two preincubation periods. Washing out of Ya-hom before adding histamine was compared to non-washing condition with same preincubation times. For 10 min preincubation period, washing out of Ya-hom had gastric acid secretion 67% of histamine stimulation (AUC, 5424 ± 545 vs. 3619 ± 395 nEqHCl) and non-washing out of Ya-hom had gastric acid secretion 93% of histamine stimulation (AUC, 5424 ± 545 vs. 404 ± 189 nEqHCl). For 20 min preincubation period, washing out of Ya-hom

had gastric acid secretion 40% of histamine stimulation (AUC, 5424 ± 545 vs. 3260 ± 390 nEqHCl) and non-washing out of Ya-hom had gastric acid secretion 94% of histamine stimulation (AUC, 5424 ± 545 vs. 324 ± 116 nEqHCl). In the washing out condition, Ya-hom moderately inhibited gastric acid secretory rate both two preincubation periods. The gastric acid secretory rate was inhibited in the short periods (Table 18), that were only 40 min (the collection at 10-40 min) for 10 min preincubation period and 50 min (the collection at 10-40 min) for 20 min preincubation period. This result showed that the longer preincubation period (20 min) had greater inhibition than the shorter preincubation period (10 min). When the AUC, AUC at peak and AUC at 0 min to peak were analyzed (Table 19), the washing out condition revealed that both AUC and AUC at peak of 10 min and 20 min preincubation periods of Ya-hom showed the significantly inhibited gastric acid secretion, but AUC at 0 min to peak was not significantly difference than control. The gastric acid secretory rate of without washing out of Ya-hom was lower than those of washing out Ya-hom group (Table 20). The amount of gastric acid secretion in form of AUC, AUC at peak and AUC at 0 min to peak was also significantly lower than those of washing out of Ya-hom condition (Table 21). In addition, AUC of washing out of Ya-hom condition had inhibitory effect on histamine-induced gastric acid secretion with an inhibition of 33% of histamine stimulation for 10 min and 40% of histamine stimulation for 20 min preincubation periods, whereas non-washing out of Ya-hom condition had an inhibition of 93% of histamine stimulation for 10 min and 94% of histamine stimulation for 20 min preincubation periods. Comparing the washing out of Ya-hom and without washing out of Ya-hom before stimulating with histamine at the same period showed that the without washing out of Ya-hom was higher inhibitory effect than the condition that Ya-hom was washed out before adding histamine. These data illustrated that Ya-hom slowly absorbed into stomach and the existence of Ya-hom is needed to effectively inhibit gastric acid secretion. Although, the preincubation periods with Ya-hom at 10 and 20 min were similar but the preincubation periods at 20 min was higher inhibitory action than 10 min, hence, the preincubation periods at 20 min and non-washing out of Ya-hom before adding histamine were used as the condition for the other experiments.

Gastric acid secretion by parietal cell, the only cell that secrete acid (26) is regulated by paracrine, endocrine and neural pathway (26-28). The regulation occur from triggered process by ligand-receptor binding that operates directly on parietal cell; acid secreting and acid-forming cell (26, 28, 29), and indirectly on another cell type that is ECL cell; histamine-containing endocrine cell (29, 31). Histamine and acetylcholine are the agonists of HCl secretion (26-28, 33-34) which each secretagogue binds to a distinct receptor on parietal cell (26, 33).

The gastric secretory profile in histamine-induced and bethanechol-induced gastric acid secretory rate were similar, even if bethanechol-induced gastric acid secretory rate was more rapidly increased to the peak and rapidly decreased from the peak throughout the experiment than the gastric acid secretory rate inducing by histamine (Figure 17, 19, 21). Histamine is a major physiological mediator of HCl secretion (33, 35-36) that stimulates H_2-receptor on parietal cell (27, 30, 36, 41-47). Likewise, acetylcholine is another mediator, which stimulates HCl secretion via muscarinic receptor on ECL cell and parietal cell (49, 35). Comparing the stimulatory effect of histamine and bethanechol, the AUC of bethanechol-induced gastric acid secretion was 46% and 55% histamine stimulation of AUC (5424 ± 545 vs. 2906 ± 190 and 2506 ± 222 nEqHCl) of for low dose (10 µM) and high dose (100 µM) bethanechol. In spite of AUC at peak and AUC at 0 min to peak were 75% (2094 ± 140 vs. 1560 ± 71 nEqHCl) and 72% (1477 ± 99 vs. 1068 ± 61 nEqHCl) of histamine stimulation for low dose (10 µM) bethanechol, 83% (2094 ± 140 vs. 1739 ± 100 nEqHCl) and 84% (1477 ± 99 vs. 1249 ± 129 nEqHCl) of histamine stimulation for high dose (100 µM) bethanechol, respectively. These data showed stimulatory action of secretagogues that histamine had more stimulatory effect than low dose (10 µM) and high dose (100 µM) bethanechol. Atropine 1 µM (muscarinic antagonist) inhibited low dose (10 µM) bethanechol action for 97% (2506 ± 222 vs. 331 ± 125 nEqHCl) and high dose (100 µM) bethanechol action for 93% (2906 ± 190 vs. 523 ± 169 nEqHCl). The profile of gastric acid secretory rate of two doses of bethanechol were similar, but high dose (100 µM) was longer stimulating effect than low dose (10 µM). This may be due to high dose of bethanechol stimulating histamine secretion from the ECL cell.

Ya-hom at the concentrations of 2.5, 5.0, 10.0 and 20.0 mg/ml inhibited histamine's action in dose dependent manner. Ya-hom at the concentrations of 10.0 and 20.0 mg/ml completely inhibited gastric acid secretory rate. The AUC of Ya-hom 2.5, 5.0, 10.0 and 20.0 mg/ml (Table 26) had inhibitory effect on histamine-induced gastric acid secretion with an inhibition of 65%, 71%, 94% and 97%, respectively. The AUC at peak showed Ya-hom inhibited histamine-induced gastric acid secretion 48%, 60%, 88% and 95%, respectively. The AUC at 0 min to peak showed Ya-hom 2.5, 5.0, 10.0 and 20.0 mg/ml inhibited histamine-induced gastric acid secretion 40%, 51%, 86%, and 94%, respectively. These data suggested that Ya-hom at the concentration of 10 mg/ml was submaximum dose and will be used for determining the mechanism of action.

The mechanism of inhibitory gastric acid secretion of Ya-hom was analyzed by using two secretagogues induced gastric acid secretion. The gastric acid stimulatory effects of histamine and bethanechol were inhibited by Ya-hom 10 mg/ml. The gastric acid secretion may occur from histamine or bethanechol alone and the synergistic effect with endogenous acetylcholine or histamine. Ranitidine inhibited low dose (10 μM) bethanechol-stimulating gastric acid secretion for 44% (AUC, 2506 ± 222 vs. 1112 ± 120 nEqHCl) and atropine inhibited histamine-stimulating gastric acid secretion for 48% (AUC, 5424 ± 545 vs. 2582 ± 406 nEqHCl) showed the existence of endogenous histamine and acetylcholine. The action of Ya-hom on stimulatory effect of histamine and bethanechol were then investgate in the presence of ranitidine or atropine. The gastric acid stimulatory effect of histamine without the synergistic action of endogenous acetylcholine by using atropine was decreased (Table 29). In the presence action of atropine (1 μM), however, Ya-hom inhibited gastric acid secretion 97% (AUC, 2582 ± 406 vs. 81 ± 70 nEqHCl). The inhibitory action of Ya-hom on histamine-induced gastric acid secretion did not affect by atropine. On the other hand, ranitidine (10 μM), H_2 receptor blocker, was used to reveal the action of Ya-hom in the absence of the H_2 receptor action. Ranitidine (10 μM) almost completely inhibited histamine-induced gastric acid secretory rate (Table 31). The AUC (Table34) showed that ranitidine (10 μM) and Ya-hom (10 mg/ml) alone inhibited histamine-induced gastric acid secretion 93% and 94%, however, Ya-hom inhibited histamine-induced gastric acid secretion in the presence of ranitidine (10 μM) 98%. These data showed

Ya-hom inhibited histamine-stimulating gastric acid secretion at the same level of ranitidine (10 µM). The attempt to investigate the gastric acid secretion in the presence of H_2 receptor blocker caused by the endogenous acetylcholine or other mediator found ranitidine did not alter the inhibitory effect of Ya-hom on histamine-induced gastric acid secretion. Thus, the action of endogenous acetylcholine and other mediator may act via the action of histamine or directly action on parietal cell which is also inhibited by Ya-hom.

Ranitidine completely inhibited while atropine moderately inhibited gastric acid secretion stimulating by histamine, hence, histamine itself stimulate gastric acid secretion via H_2 receptor and synergistically stimulate gastric acid secretion via muscarinic receptor suggested that Ya-hom had no stimulatory effect on gastric acid secretion either by itself or endogenous secretagogues.

Bethanechol, acetylcholine derivative, another secretagogue was used to stimulate gastric acid secretion. Acetylcholine has been shown to stimulate gastric secretion by two pathways: a direct effect on M_3 receptor in parietal cell and indirect effect via M_1 receptor stimulating histamine release on ECL cells (35). Bethanechol two doses; low dose (10 µM) and high dose (100 µM), were used for revealing the site of action of Ya-hom. At low dose bethanechol (10 µM) has only direct stimulation on parietal cells, whereas at high dose (100 µM) can also cause a histamine release from ECL cell to potentiate its direct effect on parietal cells (51). In the present experiment, atropine (1 µM) inhibited both 10 and 100 µM bethanechol-induced gastric acid secretion.

Ya-hom completely inhibited high dose (100 µM) bethanechol-induced gastric acid secretory rate. The AUC (Table 39) showed Ya-hom inhibited high dose (100 µM) bethanechol-induced gastric acid secretion 96%, whereas atropine (1 µM) inhibited high dose (100 µM) bethanechol-stimulating gastric acid secretion 82%. Gastric acid secretion of Ya-hom treated group was lower than atropine treated group, however, there was no significant difference. Low dose (10 µM) bethanechol showed the stimulatory effect was similar with effect on high dose (100 µM) bethanechol-induced gastric acid secretion, even if low dose (10 µM) bethanechol was 85% (AUC, 2960 ± 190 vs. 2506 ± 222 nEqHCl) of high dose (100 µM) bethanechol.

The inhibitor was used to reveal the mechanism of action of Ya-hom on parietal by stimulating gastric acid secretion with low dose (10 μM) bethanechol (Table 46). Atropine (1 μM) completely inhibited bethanechol-induced gastric acid secretory rate stimulating by bethanechol 10 μM. The AUC of atropine treated group showed the inhibitory effect on gastric acid secretion with an inhibition of 87%. Ranitidine moderately inhibited gastric acid secretory rate. The AUC of ranitidine treated group showed the inhibitory effect on gastric acid secretion with an inhibition of 56%. This showed the presence of endogenous histamine. Ya-hom inhibited low dose (10 μM) bethanechol-stimulating gastric acid secretory rate both in the presence and absence of ranitidine with an inhibition of 99% and 95%, respectively. The result showed that Ya-hom inhibited bethanechol-stimulating gastric acid secretion at the same level of atropine. Ya-hom at the dose of 10 mg/ml inhibited both the stimulating effect of histamine and bethanechol as potent as ranitidine (10 μM) and atropine (1 μM). Ya-hom showed inhibitory effect on both muscarinic receptor and H_2 receptor stimulation on the parietal cell.

The present results illustrated that Ya-hom completely inhibited gastric acid secretion of both histamine-induced and bethanechol-induced gastric acid secretion. Ranitidine, the H_2 blocker, completely inhibited histamine-induced gastric acid secretion and moderately inhibited bethanechol-induced gastric acid secretion. Atropine, acetylcholine antagonist, moderately inhibited histamine-induced gastric acid secretion and completely inhibited bethanechol-induced gastric acid secretion. The moderately inhibitory effect of both inhibitors showed that the stimulating gastric acid secretion composed of both the stimulation on the H_2 receptor and muscarinic receptor. Ya-hom completely inhibited gastric acid secretion of both histamine-induced and bethanechol-induced gastric acid secretion in the presence of ranitidine and atropine; thus inhibitory action of Ya-hom may interfere both the histamine stimulating pathway on ECL cells and HCl stimulating pathway on parietal cells. It is possible that Ya-hom causes the release of inhibitor mediator such as somatostatin (46, 47, 49, 51, 52, 66) and prostaglandin (47, 49, 67) since then two mediator are stimulated to inhibit gastric acid secretion.

The effect of this Ya-hom recipe has been previously studied in histamine- and carbachol-induced gastric acid secretion in gastric fistula rat. Histamine (10

mg/kg, intramuscular injection) or carbachol (20 µg/kg, intravenous injection) were used to stimulate gastric secretion and water extract of Ya-hom 0.5, 1, 2 and 4 g/kg were administrated by intraduodenal injection. Ya-hom inhibited histamine- and carbachol-induced gastric acid secretion in dose dependent manner and higher inhibition on carbachol effect. Carbachol maximally increased gastric acid secretion to only 36.9% of that of histamine. Ya-hom had inhibitory effect on histamine- and carbachol-induced gastric acid secretion with maximum inhibition of dose 4 g/kg was 45.5% and 53.8% at one hour after stimulation (25). Similar to the previous experiment, this present study also showed that histamine had stronger stimulatory effect than bethanechol, that stimulatory action was 46% and 55% of histamine stimulation for low dose (10 µM) and high dose (100 µM) of bethanechol. Ya-hom 2.5, 5.0, 10.0 and 20.0 g/ml inhibited gastric acid secretion in dose dependent manner. However, Ya-hom 10 mg/ml completely inhibited histamine-induced gastric acid secretion with the inhibition of 93% and inhibited bethanechol-induced gastric acid secretion with the inhibition of 96% and 95% for low dose (10 µM) and high dose (100 µM) bethanechol, respectively.

Ya-hom completely inhibited gastric acid secretion in isolated mouse whole stomach, whereas the gastric fistula model had moderately inhibition. There are the others mediators that involved in the control of gastric acid secretion such as gastrin (44, 45, 47), galanin (46, 47), and PACAP (47, 49) stimulate gastric acid secretion or somatostatin (46, 47, 49, 51, 52, 66), prostaglandin (47, 49, 67), cholecystokinin (68, 69) and secretin (49) inhibit gastric acid secretion. *In vivo* model, these mediators may affect gastric acid secretion more than *in vitro* model. The control of gastric acid secretion may occur from the stimulating or inhibiting the inhibition pathway via the neural pathway or feed back from paracrine cells. In addition, Ya-hom composed of several ingredients which may have inhibitory effect of gastric acid secretion but some may have stimulatory effect. *A. gramineus* (17) and *G. grabra* (20-21) have been shown to antagonize the effect of both histamine and acetylcholine. *L. wallichii* has an antagonized effect of histamine (19) whereas *E. caryophylata* has an antagonized effect of acetylcholine (20). *G. grabra* (3, 5, 7, 18) has been also found to inhibit gastric secretion. The inhibitory effect of Ya-hom may cause by *A. gramineus, L. wallichii, E. caryophylata* and *G. grabra*. Some ingredients of Ya-hom may stimulate

the gastric acid secretion and only effective in *in vivo* model. Ya-hom partially inhibited gastric acid secretion in *in vivo* model whereas it completely inhibited gastric acid secretion, it may be due to the non-effective stimulatory ingredient by unclarified reason. Since the stomach discomfort can be cause by increasing gastric acid secretion, the inhibitory effect of Ya-hom on gastric acid secretion is suggested to decreasing stomach discomfort. The result from this study supports the claimed efficacy of Ya-hom ingestion to verify its use for stomach discomforts because it decreased gastric acid secretion.

CHAPTER VI
CONCLUSION

1. The suitable conditions for studied the action of Ya-hom on gastric acid secretion was isolated mouse whole stomach non-fasting mouse maintained at the intragastric pressure at 20 cm H_2O.
2. The water extract of Ya-hom inhibited gastric acid secretion on histamine and bethanechol stimulating isolated mouse whole stomach.
3. There were small amount of endogenous histamine and acetylcholine in isolated whole stomach preparation.
4. Inhibitory action of Ya-hom interferes with both the histamine and acetylcholine stimulating pathway on parietal cells.
5. Ya-hom may directly interfere the inhibiting cellular signaling pathway of the secretagogues stimulating gastric acid secretion or stimulating the release of inhibitor mediated to gastric acid secretion.

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