ANTI-GASTRIC ULCER ACTIVITY OF YA-HOM IN RATS

PATHAWEE INTAYOONG

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ABSTRACT

Gastric ulcers can be caused by interfering with the gastric function, for example by increasing gastric acid secretion and decreasing gastric mucus secretion. Ya-hom inhibited gastric acid secretion and stimulated gastric mucus secretion which supports its use for gastric ulcer protection and treatment. However, the effects of Ya-hom on gastric ulcer prevention and healing have not been reported. Thus, this study aims to assess anti-gastric ulcer activity and gastric ulcer healing effect of Ya-hom.

The effect of Ya-hom on gastric ulcer prevention in rats was evaluated by oral administration of Ya-hom (1, 2 and 4 g/kg) before induction of gastric ulcer by hydrochloric acid (0.6 N HCl, 6 ml/kg), aspirin (ASP, 200 mg/kg) and water immersion restraint stress (WIR, 16 + 2˚C) in the comparison to cimetidine (0.1 g/kg). Four, six and five hours after HCl-, ASP- and WIR-gastric ulcer induction, respectively, the rats were sacrificed for determination of gastric ulcer. The results showed that Ya-hom (1, 2 and 4 g/kg) inhibited HCl-, ASP- and WIR-induced gastric ulcer in a dose dependent manner with the maximum inhibition of 93.45, 54.5 and 61.79%, respectively. Cimetidine (0.1 g/kg) inhibited HCl-, ASP- and WIR-induced gastric ulcer with the inhibition of 78.44, 76.29 and 48.02%, respectively.

The effect of Ya-hom on gastric visible mucus secretion in gastric ulcer rats was evaluated by oral administration of Ya-hom (4 g/kg) before induction of gastric ulcer by hydrochloric acid and aspirin in the comparison to cimetidine (0.1 g/kg) and sucralfate (1 g/kg). Four and six hours after HCl- and ASP-gastric ulcer induction, respectively, the rats were sacrificed for determination of gastric visible mucus. The results showed that Ya-hom (4 g/kg) and sucralfate (1 g/kg) attenuated the effect of HCl-stimulated gastric visible mucus secretion. Ya-hom (4 g/kg) and cimetidine (0.1 g/kg) attenuated the effect of ASP-inhibited gastric visible mucus secretion.

The effect of Ya-hom on the inflammatory (0-3 days) and healing (more than 3 days after gastric ulcer induction) phase of gastric ulceration in gastric ulcer rats was evaluated by oral administration of Ya-hom (4 g/kg/day) after induction of gastric ulcer by water immersion restraint stress for five hours in the comparison to cimetidine (0.1 g/kg/day). One and four days after WIR-gastric ulcer induction, the rats were sacrificed for determination of gastric ulcer. The results showed that Ya-hom (4 g/kg/day) decreased the effect of WIR-induced gastric ulcers both at day 1 and day 4 with the curation of 57.67 and 58.18%. Cimetidine (0.1 g/kg/day) could not decrease the effect of WIR-induced gastric ulcer at day 1 but decreased the WIR-induced gastric ulcers at day 4 with the curation of 28.09%.

This study indicates that Ya-hom has an anti-gastric ulcer activity and a gastric ulcer healing effect which result from increased gastric visible mucus secretion, decreased inflammation and potentiated ulcer healing.

KEY WORDS: YA-HOM / GASTRIC ULCER / HCl-INDUCED GASTRIC ULCER / ASPIRIN-INDUCED GASTRIC ULCER / STRESS-INDUCED GASTRIC ULCER

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ฤทธิ์ยับยั้งแผลในกระเพาะอาหารของยาหอมในหนูขาว (ANTI-GASTRIC ULCER ACTIVITY OF YA-HOM IN RATS)

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บทคัดย่อ

แผลในกระเพาะอาหารเกิดจากภาวะที่มีกรดในกระเพาะอาหารเพิ่มขึ้นและเมือกเคลือบกระเพาะอาหารลดลง ยาหอมมีฤทธิ์ยับยั้งการหลั่งกรดในกระเพาะอาหารและกระตุ้นการหลั่งเมือกเคลือบกระเพาะอาหาร ซึ่งช่วยสนับสนุนผลของยาหอมต่อการป้องกันและรักษาแผลในกระเพาะอาหารได้ อย่างไรก็ตามยังไม่มีรายงานผลของยาหอมต่อการป้องกันและรักษาแผลในกระเพาะอาหาร ดังนั้นการศึกษาครั้งนี้จึงมีจุดประสงค์เพื่อทดสอบฤทธิ์ของยาหอมต่อการป้องกันและรักษาแผลในกระเพาะอาหาร

การประเมินผลของยาหอมต่อการป้องกันแผลในกระเพาะอาหารของหนูขาว ทำได้โดยการป้อนยาหอมขนาด 1, 2 และ 4 ก./กก. ก่อนการเหนี่ยวนำให้เกิดแผลในกระเพาะอาหารด้วยกรดเกลือ แอสไพริน และการแช่น้ำเย็นเพื่อกระตุ้นให้เกิดความเครียด เปรียบเทียบกับยาไซเมติดีน จากนั้น 4, 6 และ 5 ชม. ต่อมาตามลำดับจึงผ่ากระเพาะเพื่อวัดขนาดของแผลในกระเพาะอาหาร พบว่ายาหอมขนาด 1, 2 และ 4 ก./กก. สามารถยับยั้งแผลในกระเพาะอาหารของหนูขาวที่ถูกเหนี่ยวนำให้เกิดแผลด้วยกรดเกลือ แอสไพริน และการแช่น้ำเย็นเพื่อกระตุ้นให้เกิดความเครียดตามขนาดที่เพิ่มขึ้นได้สูงสุดถึง 93.45, 54.5 และ 61.79% ขณะที่ยาไซเมติดีนขนาด 0.1 ก./กก. สามารถยับยั้งแผลในกระเพาะอาหารของหนูขาวที่ถูกเหนี่ยวนำให้เกิดแผลด้วยกรดเกลือ แอสไพริน และการแช่น้ำเย็นเพื่อกระตุ้นให้เกิดความเครียดได้ถึง 78.44, 72.29 และ 48.02%

การประเมินผลของยาหอมต่อการหลั่งเมือกเคลือบกระเพาะอาหารของหนูขาวที่มีแผลในกระเพาะอาหาร ทำได้โดยการป้อนยาหอมขนาด 4 ก./กก. ก่อนการเหนี่ยวนำให้เกิดแผลในกระเพาะอาหารด้วยกรดเกลือ และแอสไพริน เปรียบเทียบกับยาไซเมติดีนและซูคราลเฟต จากนั้น 4 และ 6 ชม. ต่อมาตามลำดับจึงผ่ากระเพาะเพื่อวัดปริมาณเมือกเคลือบกระเพาะอาหาร พบว่ายาหอมขนาด 4 ก./กก. และยาซูคราลเฟตขนาด 1 ก./กก. มีผลลดการหลั่งเมือกเคลือบกระเพาะอาหารที่ถูกกระตุ้นด้วยกรดเกลือ ขณะที่ยาหอมขนาด 4 ก./กก. และยาไซเมติดีนขนาด 0.1 ก./กก. มีผลลดการหลั่งเมือกเคลือบกระเพาะอาหารที่ถูกยับยั้งด้วยแอสไพริน

การประเมินผลของยาหอมต่อการรักษาแผลในกระเพาะอาหารทั้งระยะที่มีการอักเสบ (0-3 วัน) และระยะที่มีการซ่อมแซมแผล (มากกว่า 3 วันหลังจากการเหนี่ยวนำให้เกิดแผล) ในกระเพาะอาหารของหนูขาว ทำได้โดยการป้อนยาหอมขนาด 4 ก./กก./วัน หลังจากการเหนี่ยวนำให้เกิดแผลในกระเพาะอาหารด้วยการแช่น้ำเย็นเพื่อกระตุ้นให้เกิดความเครียดนาน 5 ชั่วโมง เปรียบเทียบกับยาไซเมติดีน จากนั้น 1 และ 4 วันต่อมาจึงผ่ากระเพาะเพื่อวัดขนาดของแผลในกระเพาะอาหาร พบว่ายาหอมขนาด 4 ก./กก./วัน สามารถรักษาแผลในกระเพาะอาหารของหนูขาวที่ถูกเหนี่ยวให้เกิดแผลด้วยการแช่น้ำเย็นเพื่อกระตุ้นให้เกิดความเครียดทั้งระยะที่มีการอักเสบและระยะที่มีการซ่อมแซมแผลได้ถึง 57.67 และ 58.18% ขณะที่ยาไซเมติดีนขนาด 0.1 ก./กก./วัน ต่อวันไม่สามารถรักษาแผลในกระเพาะอาหารระยะที่มีการอักเสบ แต่สามารถรักษาแผลในกระเพาะอาหารระยะที่มีการซ่อมแซมแผลได้ถึง 28.09%

การศึกษานี้แสดงให้เห็นว่ายาหอมมีฤทธิ์ป้องกันและรักษาแผลในกระเพาะอาหาร อันเป็นผลเนื่องมาจากการเพิ่มการหลั่งเมือกเคลือบกระเพาะอาหาร การลดการอักเสบ และการกระตุ้นการซ่อมแซมแผล

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LIST OF ABBREVIATIONS

ACh	=	acetylcholine
ASP	=	aspirin
ATP	=	adenosine triphosphate
Ca^{2+}	=	calcium ion
cAMP	=	cyclic adenosine monophosphate
Ci	=	cimetidine
Cl^-	=	chloride ion
CMC	=	carboxy-methyl cellulose
COX-1	=	cyclooxygenase-1
COX-2	=	cyclooxygenase-2
DAG	=	diacylglycerol
ECL	=	enterochromaffin-like
g	=	gram
G	=	gastrin
GTP	=	guanine triphosphate
h	=	hour
H	=	histamine
H^+	=	hydrogen ion
HCl	=	hydrochloric acid
HCO_3^-	=	bicarbonate ion
IP_3	=	inositol triphosphate
K^+	=	potassium ion
kg	=	kilogram
M	=	molarity
mg	=	milligram
ml	=	milliliter
mm	=	millimeter

LIST OF ABBREVIATIONS (continued)

N	=	normality
nm	=	nanometer
NSAIDs	=	non-steroidal anti-inflammatory drugs
OH^-	=	hydroxide ion
PG	=	prostaglandin
PKC	=	protein kinase C
PLC	=	phospholipase C
rpm	=	round per minute
Su	=	sucralfate
TX	=	thromboxane
WIR	=	water immersion restraint stress
YH	=	Ya-hom
°C	=	degree celsius
µg	=	microgram
<	=	less than
>	=	more than

CHAPTER I
INTRODUCTION

The peptic ulcers are illness that affect a considerable number of people in the world and they are induced by several factors, for example, *Helicobactor pyroli* infection (1), ingestion of non-steroidal anti-inflammatory drugs (2) and psychological stress (3). The peptic ulcers result from an imbalance between increased aggressive factors, such as acid and pepsin secretion (4) and decreased defensive factors, such as mucus and bicarbonate secretion (5), mucosal barrier (6), mucosal blood flow (7) and endogenous prostaglandin production (8).

The goals of therapy for peptic ulcers are relief from pain, promotion of healing and prevention of recurrence. Therapeutic strategies are aimed at balancing aggressive factors against defensive factors. Anti-secretory agents as H_2-receptor antagonists and proton pump inhibitors and mucosal protective agents as sucralfate and prostaglandin analogs are very important drugs for the treatment of peptic ulcers. At the same time, each of these drugs confers simpler to severe side effects like gynaecomastia occur with cimetidine (H_2-receptor antagonist) (9) and enterochromaffin like cell (ECL) hyperplasia occur with omeprazole (proton pump inhibitor) (10). Therefore, it is interesting to investigate anti-ulcer effect of medicinal plants or traditional recipes possessing fewer side effects to have better and safer alternative for the treatment of peptic ulcers.

The medicinal plants are reported to have anti-ulcer activity, for example, banana (11), black tea (12), turmeric (13), aloe (14), roselle (15) and ginger (16). Futhermore, there are some traditional recipes of each countries which have been found to exhibit anti-ulcerogenic effect, such as Rhinax of India (17) and Makino of Japan (18). In Thailand, Ya-hom is one of the most popular folk formulas.

Ya-hom is Thai traditional medicine used for the treatment of fainting, nausea, vomiting and stomach discomfort. The action of Ya-hom in fainting treatment through the modification of cardiovascular function and in stomach discomfort treatment through the modification of gastrointestinal function. There are many recipes of Ya-hom according to different compositions and amount of medicinal plants. Although, some recipe has been studied the cardiovascular effect, most of them has not been done on gastrointestinal function. We selected one of the most popular recipes to study its effect on gastric function. The scientific reports of Ya-hom's ingredients on gastrointestinal function showed that *Saussurea lappa* Clark (19-21), *Eugenia caryophyllata* Thunb (22-23), *Cinnamomum cassia* Blame (24-25), *Glycyrrhiza glabra* Linn (26-29), *Mentha arvensis* Linn (30) and *Atractylodes ovata* DC (31) had anti-ulcer activity; *Saussurea lappa* Clark (32-33), *Eugenia caryophyllata* Thunb (34-36), *Glycyrrhiza glabra* Linn (37-38), *Acorus gramineus* Soland (39) and *Ligusticum wallichii* Franch (40) had anti-spasmodic activity; *Atractylodes ovata* DC (31) and *Eugenia caryophyllata* Thunb (41) possessed gastric mucus secretion stimulatory effect; *Glycyrrhiza glabra* Linn (42) possessed gastric acid secretion inhibitory effect.

At the present, the effect of the selected Ya-hom recipe has found to inhibit the stimulatory effect of histamine and carbachol on gastric acid, pepsin and soluble mucus secretion but potentiate gastric visible mucus secretion in gastric fistula rats (43) and inhibit histamine- and bethanechol-induced gastric acid secretion in isolated mouse whole stomach (44). Since the gastric ulcer can be caused by interfering on gastric function such as increasing gastric acid secretion and decreasing gastric mucus secretion. This selected Ya-hom recipe inhibited aggressive factor and potentiated defensive factor of gastric ulcer which supports its use for gastric ulcer protection and treatment. However, the effects of the selected Ya-hom recipe on gastric ulcer prevention and healing have not been reported.

According to the gastric acid secretion inhibition and gastric mucus stimulation actions of this recipe, it will be used for further studying an anti-gastric ulcer activity and a gastric ulcer healing effect. So, the objectives of this study are: 1) to assess the effect of Ya-hom on the gastric ulcer prevention in hydrochloric acid-, aspirin- and water immersion restraint stress-induced gastric lesion models in the comparison to cimetidine; 2) to assess the effect of Ya-hom on gastric visible mucus secretion in hydrochloric acid- and aspirin-induced gastric lesion models in the comparison to cimetidine and sucralfate and 3) to assess the effects of Ya-hom on inflammatory and healing phase of gastric ulceration in water immersion restraint stress-induced gastric lesion model in comparison to cimetidine. The result will lead to the scientific information for gastric ulcer protection and treatment of Ya-hom which are the new uses of this recipe.

CHAPTER II
LITERATURE REVIEW

I. PEPTIC ULCERS

1. Anatomy of the stomach

1.1 Human stomach

The J-shaped stomach is on the left side of the abdominal cavity, nearly hidden by the liver and diaphragm. The stomach has two curvatures: the convex lateral surface of the stomach is the greater curvature and its concave medial surface is the lesser curvature. The human stomach consists of four portions: cardia, fundus, corpus (body) and pyloric antrum. The cardia connects with esophagus through the cardiac orifice. The fundus is the portion of the stomach extending above the gastroesophageal junction. The corpus is the largest and central portion. The pyloric antrum is continuous with duodenum (the first part of the small intestine) through the pyloric sphinctor (45).

The stomach wall is composed of four layers: mucosa, submucosa, muscularis externa and serosa. The mucosa is the innermost layer, dividing into a surface epithelium, a loose connective tissue called lamina propria and a thin smooth muscle called muscularis interna or muscularis mucosae. The submucosa is the loose connective tissue, containing blood vessels, lymphatic vessels and nerve ending. The muscularis externa is the muscular layer, consisting of an inner oblique muscle, a middle circular muscle and an outer longitudinal muscle. The serosa is the outermost layer (46).

The stomach is richly innervated by extrinsic nerves from autonomic nervous system and by the intrinsic neurons of the enteric nervous system. The extrinsic nerves consist of the sympathetic nerve, which is derived from the celiac plexus and the parasympathetic nerve, which is derived from the right and left vagal trunks. Sympathetic activity inhibits gastric smooth muscle motility and gastric

secretion, whereas parasympathetic activity stimulates these functions. The enteric nervous system is composed mainly of two plexuses: the submucosal plexus or plexus of Meissner, an inner plexus that lies in the submucosa and the myenteric plexus or plexus of Auerbach, an outer plexus lying between the circular and longitudinal muscle layer. The submucosa plexus controls gastric secretion and local blood flow and the myenteric plexus controls gastric motility. The enteric nervous system can do these functions on its own, independently of the extrinsic nerves (47).

The stomach also has a very rich blood and lymphatic supply. The arterial supply is provided by gastric and splennic branches of the celiac trunk. Sympathetic stimulation has a direct effect on the entire stomach by causing intense vasoconstriction of the arterioles with greatly decreased blood flow. In contrast, stimulation of the parasympathetic nerves increases local blood flow at the same time that it increases gastric secretion. This increased blood flow probably result secondarily from the increased gastric activity and not as a direct effect of nervous stimulation (48).

The entire stomach is lined by a simple columnar epithelium and contains three types of gastric glands: the cardiac, oxyntic (parietal or fundic) and pyloric glands. The cardiac glands are present in a narrow zone near the cardiac orifice and have predominantly mucous cells. The oxyntic glands are found in the fundus and body, which contain mucous neck, parietal and chief cells. The pyloric glands occupy a large region adjacent to the duodenum and have mucous secreting and gastrin cells. These glands are divided into three areas: the isthmus, which contains surface mucous and parietal cells; the neck, which contains mainly parietal and mucous neck cells and the base, which contains chief and parietal cells. Endocrine cells are also present scattered throughout the glands (49).

1.2 Rat stomach

The rat stomach consists of two regions: non-glandular (forestomach) and glandular part. The forestomach is lined by cornified squamous epithelium. The glandular stomach, consisting of corpus, antrum and pyrolus, is lined by a simple columnar epithelium. The non-glandular part is separated from the glandular part of the stomach by the limiting ridge, which prevents a reflux of gastric juice from the glandular part into the non-glandular part when the stomach is empty.

The normal anatomy and physiology of the rat stomach is similar to the human stomach. Many studies used the rat stomach to investigate the gastric function, thus the results from rat model provide the fundamental data for human (50).

2. Physiology of the stomach

2.1 Gastric acid secretion

Hydrochloric acid is secreted from the parietal cells into the lumen where it establishes an extremely acidic environment. This acid is important for activation of pepsinogens and inactivation of ingested microorganisms such as bacteria, virus and parasite (51).

2.1.1 Regulation of gastric secretion

2.1.1.1 Regulation by acetylcholine

Acetylcholine, a neurocrine agent, is released from postganglionic fibers of the enteric nervous system that synapse with the efferent fibers of the vagus nerve. Acetylcholine has both direct action on parietal cell via M_3 receptor and indirect action on enterochromaffin (ECL) cell via M_1 receptor in stimulating acid secretion.

Acetylcholine binds to M_3 receptor and elevates the intracellular Ca^{2+} concentration. The M_3 receptor is coupled to phospholipase C (PLC) for activation of the transient calcium release pathway. An elevated intracellular Ca^{2+} concentration enhanced HCl secretion by activating basolateral K^+ channel and by insert into the apical plasma membrane (52).

2.1.1.2 Regulation by gastrin

Gastrin, a hormone, is produced by gastrin (G) cells in the mucosa of the gastric antrum. Gastrin acts directly on parietal cell via G receptor and indirect action on enterochromaffin (ECL) cell via G receptor in stimulating acid secretion.

Gastrin binds to G receptor and elevates the intracellular Ca^{2+} concentration. The G receptor is coupled to PLC for activation of the transient calcium release pathway. An elevated intracellular Ca^{2+} concentration enhanced HCl secretion by activating basolateral K^+ channel and by insert into the apical plasma membrane (53).

2.1.1.3 Regulation by histamine

Histamine, an amino derivative, is released from mast cells, neurons and ECL cells. In the rat, the ECL cells are very prominent in the lamina propria of the oxyntic mucosa, whereas in human and dog fundic mucosa mast cells are more prominent than ECL cells.

The binding of histamine to H_2 receptor on the parietal cells results in the activation of adenylate cyclase and the elevation of cyclic adenosine monophosphate (cAMP). This in turn activates cAMP-dependent protein kinases. Then, acid secretion is activated. These events stimulate HCl secretion by activating basolateral K^+ channel. They also cause more H^+-K^+-ATPase molecules and Cl^- channels to be inserted into the apical plasma membrane (54).

2.2 Pepsinogen secretion

Pepsinogens are secreted from the chief cells and the mucous neck cells. In the presence of gastric acid, the inactive pepsinogens are autocatalytically transformed into the active proteolytic enzyme pepsins. Optimal activity of pepsins are at pH of 1.8 to 3.5. They are reversibly inactivated at about pH 5 and irreversibly inactivated at pH 7 to 8. Pepsinogens can be separated electrophoretically on the basis of their physical properties into two major groups: Group I (Pepsinogen I) contains rapidly migrating proteins that secreted mostly in the oxyntic glandular mucosa and group II (Pepsinogen II) contains slowly migrating protein that secreted throughout the stomach and by Brunner's glands of the duodenum. Both groups occur in high concentration in the chief cells and in lower concentration in the mucous neck cells (55).

2.2.1 Regulation of pepsinogen secretion

Interaction of acetycholine, cholecystokinin (CCK) and gastrin with chief cell receptor result in the activation of PLC, thereby generating inositol triphosphate (IP_3) and diacylglycerol (DAG) and stimulating pepsinogen secretion. IP_3 stimulates releasing of calcium from intracellular stores that results in increasing cellular calcium concentration. DAG activates protein kinase C (PKC).

Adrenaline and noradenaline act via activation of adenylyl cyclase that increases the intracellular level of cAMP and stimulate pepsinogen secretion (56).

2.3 Gastric mucus secretion

Mucus is secreted by the mucous neck cells and by the surface epithelial cells. Mucus contains glycoprotein mucins. When hydrated, mucins form mucus. Both cells secrete mucus containing different mucins. The mucous neck cells secrete the clear mucus sometimes called soluble mucus. Soluble mucus is not present in the resting stomach. The surface epithelial cells secrete cloudy mucus is termed visible mucus. Visible mucus is secreted by the resting mucosa and lines the stomach with sticky, viscous and alkaline coat (57).

2.3.1 Regulation of mucus secretion

Secretion of soluble mucus is stimulated by sham feeding and by some of the same stimuli that enhance acid and pepsin secretion, especially by acetylcholine release from parasympathetic nerve endings (57). Secretion of visible mucus is stimulated by prostaglandin E_2 (58).

3. Pathogenesis of peptic ulcers

The peptic ulcers are lesions that occur in any part of gastrointestinal tract such as stomach “gastric ulcer” and duodenum “duodenal ulcer”. The peptic ulcers result from an imbalance between increased aggressive factors which breakdown the ability of gastrointestinal mucosa to protect itself and decreased defensive factors which maintain the mucosal integrity through the endogenous defense mechanisms. The aggressive and defensive factors and their roles in peptic ulcers are described as follow:

3.1 Aggressive factors

3.1.1 *Helicobactor pylori* (*H. pylori*)

H. pylori is a spiral-shaped, pH-sensitive, gram-negative, microaerophilic bacterium that resides between the mucous layer and surface epithelial cells in the stomach. *H. pylori* contributes to gastric mucosal injury by 1) direct mechanisms, 2) alterations in the immune/ inflammatory response and 3) hypergastrinemia leading to increased acid secretion.

Direct mucosal damage is produced by elaborating bacterial enzymes (lipases, proteases and urease), virulence factors (vacuolating cytotoxin, cytotoxin-associated gene protein and growth-inhibitory factor) and adherence. Lipases and proteases degrade gastric mucus, ammonia produced by urease may be

toxic to gastric epithelial cells and bacterial adherence enhances the uptake of toxins into gastric epithelial cells. *H. pylori* infection alters the inflammatory response and damages epithelial cells directly by cell-mediated immune mechanisms or indirectly by activated neutrophils or macrophages attempting to phagocytose bacteria or bacterial products (1).

3.1.2 Nonsteroidal anti-inflammatory drugs (NSAIDs)

NSAIDs cause gastric mucosal damage by two important mechanisms: 1) direct or topical irritation of the gastric epithelium and 2) systemic inhibition of endogenous prostaglandins (PGs) synthesis in gastric mucosa.

NSAIDs are weak acids and non-ionized at gastric pH that can penetrate the mucous layer, break the gastric mucosal barrier and diffuse into surface epithelial cells. During this diffusion, the cells of the surface epithelium loose their hydrophobicity and the ability to repel polarized substances such as HCl. The damage surface epithelium swells and forms, with exfoliated cells, the "mucoid cap", allowing the penetration of luminal H^+ into the mucosa to release various inflammatory mediators such as leukotriene B_4 and histamine, to damage the microvascular wall, increasing its permeability, and to decrease the mucosal blood flow. Systemic effects appear to be mediated through their ability to inhibit cyclooxygenase activity and thereby prostaglandin production. By inhibiting prostaglandin production, NSAIDs induce several changes in the gastric microenvironment, such as reduced gastric blood flow, reduced mucus and HCO_3^- secretion, decreased cell repair and replication, leading to breakdown of mucosal defense mechanisms (2).

3.1.3 Psychological stress

Stress increases gastric acid secretion and motility (3).

3.1.4 Acid and Pepsin

Acid and pepsin appears to play a critical role in ulcer formation. Hypersecretion of acid and pepsin can damages mucosal barrier (4).

3.2 Defensive factors

3.2.1 Gastric mucus and bicarbonate

Gastric mucus forms a thin viscoelastic gel that lines the entire mucosal surface and lubricates the stomach. Mucus may also form an unstirred water layer that traps secreted bicarbonate and retards the back diffusion of hydrogen ion from the luminal surface into the mucosa. Prostaglandin E_2 increases mucus gel thickness, whereas aspirin and nonsteroidal anti-inflammatory drugs reduce its thickness (5).

3.2.2 Gastric mucosal barrier

Normally, the luminal surfaces and intercellular tight junctions of the gastric epithelial cells create a gastric mucosal barrier that is almost completely impermeable to diffusion of hydrogen ions from the lumen. Gastric mucosal barrier can interrupt by bile salt, ethanol, salicylates and weak organic acids, permitting hydrogen ions to diffuse into gastric tissue. This results may be cell injury, release of histamine from mast cells, further stimulation of acid secretion, damage to small blood vessels, mucosal hemorrhage and erosion or ulceration (6).

3.2.3 Gastric mucosal blood flow

The maintenance of normal blood flow to the gastric mucosa is an essential component of mucosal resistance to injury. Rich blood flow supplies bicarbonate and nutrients and removes acid. Decrease mucosal blood flow, accompanied by diffusion of luminal hydrogen ions, is thought to be important in producing gastric mucosal damage (7).

3.2.4 Endogenous prostaglandins

Almost all mammalian cells contain cyclooxygenase, the first enzyme in the pathway converting arachidonic acid originating from membrane phospholipids to prostanoids. Cyclooxygenase exists, at the least, in two distinct isoforms, cyclooxygenase-1 (COX-1) and cyclooxygenase-2 (COX-2). COX-1 is considered a constitutive enzyme present in most body tissue including the stomach, kidney, intestine and platelets under normal physiologic conditions. COX-2 is overexpressed in inflammatory tissue, being an enzyme strictly inducible by cytokine, growth factors and tumor promoters. COX-1 is involved in the production of prostaglandin to maintain gastrointestinal mucosal integrity, vascular homeostasis and

renal function, while COX-2 is responsible for excesssive production of prostaglandin associated with inflammation and pain such as gastroduodenal ulcers and arthritis.

The prostanoids emcompasses prostacyclin (PGI_2), thromboxane A_2 (TXA_2), prostaglandin D_2 (PGD_2), prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) and prostaglandin E_2 (PGE_2). PGI_2, produced mostly by vascular endothelium, acts via I-prostanoid (IP) receptor that prevents platelet agggregation to cause vasodilation, stimulates natriuresis and inhibits gastric acid secretion. TXA_2, produced mostly by platelet, acts via T-prostanoid (TP) receptor that results in platelet aggregation and potent vasoconstriction. PGD_2 uses D-prostanoid (DP) receptor to increase gastric and renal blood flow, to inhibit gastric acid secretion and platelet aggregation. $PGF_{2\alpha}$ uses F-prostaniod (FP) receptor to induce uterus contraction, vasoconstriction, bronchospasm and reduction in gastric acid secretion. Binding of PGE_2 to E-prostanoid-1 (EP_1) receptor results in contraction of bronchial and gastrointestinal smooth muscle, to E-prostanoid-2 (EP_2) receptor results in relaxation of vascular, bronchial and gastrointestinal smooth muscle and to E-prostanoid-3 (EP_3) receptor results in deceased gastric acid secretion, increased gastric mucus secretion, contraction of uterus and gastrointestinal smooth muscle and inhibition of lipolysis and of autonomic neurotransmitter release (8).

4. Drugs therapy for peptic ulcers

At the present, Drugs used for the treatment of peptic ulcers are:

Antacids (aluminium hydroxide, magnesium hydroxide) are weak bases that neutralize gastric acid, inactivate pepsin and bind bile salts. Reduction in gastric acidity occurs when antacids chemically react with hydrochloric acid to form salt and water. Since pepsin is inactive in gastric juice above pH 4 to reduce peptic activity. Adverse effects of antacids are aluminium salts cause a constipation, whereas magnesium salts cause an osmotic diarrhea (59).

H_2-receptor antagonists (cimetidine, famotidine, nizatidine, ranitidine) competitively and reversibly bind H_2 receptors on gastric parietal cells, diminishing cytosolic concentration of cAMP and the secretion of histamine-stimulated gastric acid. Central nervous system effects, particularly drowsiness and headache, and thrombocytopenia, the most common hematologic effect, occur with all H_2-receptor antagonists (60).

Proton pump inhibitors (omeprazole, lansoprazole) irreversibly inhibit the gastric parietal cell proton pump (H^+/ K^+-ATPase). They produce only small and inconsistent changes in the volume of gastric juice and in the secretion of pepsin and intrinsic factor and do not affect gastric motility (61).

Sucralfate is an aluminum salt of sulfated disaccharide. When exposed to gastric acid, sucralfate forms a viscous adhesive gel that binds electrostatically to positively charged protein molecules in the ulcer crater, forming a protective barrier that inhibits back diffusion of hydrogen ions. Sucralfate also inhibits pepsin, adsorbs bile salts, stimulates endogenous prostaglandins and may suppress *H. pylori.* Although aluminum mediates some of these actions, the sucrose moiety plays an important role in ulcer healing. Sucralfate does not have an important effect on acid secretion (62).

Misoprostol, a methyl analog of PGE_1, is used for the prevention of ulcer induced by the administration of NSAIDs. It inhibits the secretion of acid and stimulates the secretion of mucus and bicarbonate (63).

II. YA-HOM

Ya-hom is a Thai traditional medicine used for the treatment of fainting, nausea, vomiting and stomach discomfort. There are many recipes of Ya-hom according to various compositions and amount of medicinal plants. The principal ingredients of the selected Ya-hom recipe in this study are *Agastache rugosa* (Fisch. et Mey) O. Kuntze, *Magnolia officinalis* Rend. et Wils., *Asarum sieboldii* Miq., *Angelica anomala* Ave-Lall., *Saussurea lappa* Clark, *Eugenia caryophyllata* Thunb., *Cinnamomum cassia* Blame, *Glycyrrhiza glabra* Linn, *Mentha arvensis* Linn, *Atractylodes ovata* DC., *Acorus gramineus* Soland, *Aquilaria agallocha* Roxb., *Citrus nobilis* Lour., *Ligusticum wallichii* Franch. and *Lysimachia foenum-graecum* Hance. The scientific reports of Ya-hom's ingredients and the selected Ya-hom recipe on gastrointestinal function as follows:

1. Gastrointestinal effects of Ya-hom's ingredients

1.1 *Saussurea lappa* Clark (Kote-kra-dook, costus, Compositae)

The methanolic (MeOH) extract of *Saussurea* radix possessed an ulcer inhibitory effect on restraint in water-induced gastric lesions in mice (19). The amino acid-sesquiterpene conjugates, isolated from Chinese *S. lappa* dried roots, named Sussureamines A, B and C showed a gastroprotective effect on acidified ethanol (EtOH)-induced gastric mucosal lesions in rat. Sussureamines A also exhibited an inhibitory effect on gastric mucosal lesions induced by water-immersion stress in mice (20-21).

Some reports indicated the extract of *S. lappa* caused ralaxation of the intestine, uterus and bronchial smooth muscle in rat with antagonism to the spasmogenic action of acetylcholine and histamine (32). In addition, The delactonized oil, isolated from Indian *S. lappa* dried roots, had a relaxant effect on the isolated guinea pig ileum contraction induced by acethylcholine (Ach), histamine and barium chloride ($BaCl_2$) (33).

1.2 *Eugenia caryophyllata* Thunb (Kan-plu, clove, Myrtaceae)

The mixture of 88% $FeSO_4$ and 12% clove extract given at 80 mg/ kg to rats with induced ulcer for 15 days showed 22.4% cure whereas one mixture of 68% $FeSO_4$, 9% clove extract and 23% licorice extract gave 55.7% cure. These mixtures had synergistic effects in the treatment of gastric ulcer (22-23).

Some studies indicated *E. caryophyllata* had spasmolytic activity. The extract of *E. caryophyllata* was found to relax isolated rat ileum motility and they also could inhibit Ach action (34). Clove oil showed an antispasmodic action on the isolated intestine of mice (35) and also had a musculotropic spasmolytic activity on rat, guinea pig and rabbit seminal vesicles, duodenum, ileum, aorta, or jejunum (36).

Clove oil and eugenol from *E. caryophyllata* was more effective stimulus for the collection of gastric mucus from Heidenhain-pouch dogs. The range of pH of gastric mucus secretion was 4.00 to 9.22 with a mean of 7.65 and a standard deviation of 1.08. Only aqueous emulsions of clove oil (5%) and eugenol (0.5 to 5%) yielded secretion with mean pH greater than 8.00. This study indicated *E. caryophyllata* possessed gastric mucus secretion stimulatory effect (41).

1.3 *Cinnamomum cassia* Blume (Op-chery-jin, Chinese cinnamon, Lauraceae)

Administration of the stem bark of *C. cassia* compounds orally or parenterally at a remarkable low dose of 40 μg/ kg body weight to rat inhibited gastric ulcer induced by the ulcerogens such as phenylbutazone and ethanol (24). In addition, administration of aqueous extract of *C.cassia* intraperitoneally at a dose of 100 mg/ kg body weight in rat prevented the occurrence of stress ulcer, strongly inhibited gastric ulcers induced by subcutaneous injection of serotonin and promoted gastric mucosal blood flow (25).

1.4 *Glycyrrhiza glabra* Linn (Cha-em-tet, licorice, Leguminosae)

Ibuprofen coated with licorice, deglycyrrhized licorice (DGL) or enoxolone (glycyrrhetinic acid) reduced the number and size of ulcers, lowering the ulcer index from 1.86 to 1 and the incidence from 100 to 59%. This study showed the protective effect of licorice or its derivatives against gastric ulcers induced by oral ibuprofen (26). The extract of licorice roots, given either orally or intraduodenally, inhibited Shay ulceration in rats (12 h ligation) in a dose-dependent manner. This extract at a dose of 1 g/ kg, given intraduodenally, also prevented the formation of aspirin-induced ulcers (27). The MeOH fraction of licorice (FM 100) had a significant repairing effect at doses of 200 and 400 mg/kg on chronic gastric ulcers in rat induced by injection of acetic acid into the gastric wall (28). Wistar strain rats were fed 40% glucose solution for 16 days to produce gastric ulcers and then treated with licorice

extract or water for 70 days, 98.8% of licorice-treated rats and 15.6% of the control rats did not have demonstrable ulcers (29).

Some reports suggested that the four known flavonoids from the roots of *G. glabra*: liquiritigenin, liquiritin, isoliquiritigenin and isoquiritin (37) and three new ones: neoisoliquiritin, neoisoliquiritin and licurazid (38) had a spasmolytic action on isolated sections of rat and guinea pig intestines, in which spasms had been induced by $BaCl_2$, Ach and histamine.

Intraperitoneal injection of deglycerrhizinized licorice preparation at a dose of 200 mg/ kg to rats markedly decreased the gastric acid output in rats with gastric fistula. This inhibition of acid secretion was not due to suppressed gastrin release or to histidine decarboxylase inactivation (42).

1.5 *Mentha arvensis* Linn (Sa-ra-nae-yee-pun, Japanese mint, Lamiaceae)

The petroleum ether, chloroform and ethanol extract of *M. arvensis* leaves at a dose of 1 g/kg has anti-ulcer activity in rats induced by aspirin at a dose of 200 mg/ kg suspended in 1% sodium CMC (30).

1.6 *Atractylodes ovata* DC. (Kote-ka-mao, atractylis, Compositae)

The oral administration of (6E, 12E)-tetradecadiene-8, 10-diyne-1, 3-diol diacetate (TDEYA), isolated from *Atractylodes* rhizomes, prevented lesion formation induced by HCl-ethanol or ethanol in a dose-dependent manner. TDEYA did not inhibit gastric ulcers induced by pyloric ligation nor did not reduce gastric juice output or pepsin activity. TDEYA significantly increased hexamine content in gastric juice and mucosa. This study showed TDEYA exerted protective action on the stomach by secreting mucus (31).

1.7 *Acorus gramineus* Soland (Wan-nam-lek, Araceae)

The principal active compounds in *A. gramineus* were identified as the phenylpropanoids α- and β-asarone and 1-allyl-2, 4, 5-trimethoxybenzene. These three compounds had a spasmolytic action on the isolated guinea pig trachea and ileum contracted by Ach, histamine, serotonin and $BaCl_2$, in which α-asarone was the most active (39).

1.8 *Ligusticum wallichii* Franch (Umbelliferae)

Butylidenephthalide, isolated from natural oil of *Ligusticum wallichii* Franch, inhibited guinea pig ileum contraction induced by ACh and $BaCl_2$ (40).

These Ya-hom' s ingredients has ulcer protective and repairing effects, spasmolytic effect, antagonistic effects of acetylcholine and histamine, gastric acid secretion inhibitory effect and gastric mucus secretion stimulatory effect as shown in Table 1.

Table 1 The summary of gastrointestinal effects of Ya-hom's ingredients.

Gastrointestinal effects	Plants
Ulcer protective effect	*S. lappa* (19-21) *C. cassia* (24-25) *G. glabra* (26-27) *M. arvensis* (30) *A. ovata* (31)
Ulcer repairing effect	*E. caryophyllata* (22-23) *G. glabra* (28-29)
Spasmolytic effect	*S. lappa* (32-33) *E. caryophyllata* (34-36) *G. glabra* (37-38) *A. gramineus* (39) *L. wallichii* (40)
Antagonistic effect of acetylcholine	*S. lappa* (32-33) *E. caryophyllata* (34) *G. glabra* (37-38) *A. gramineus* (39) *L. wallichii* (40)
Antagonistic effect of histamine	*S. lappa* (32-33) *G. glabra* (37-38) *A. gramineus* (39)
Gastric mucus secretion stimulatory effect	*A. ovata* (31) *E. caryophyllata* (41)
Gastric acid secretion inhibitory effect	*G. glabra* (42)

2. Effect of the selected Ya-hom recipe on gastric function

In vivo model, Suvitayawat W. *et al* (43) studied effect of Ya-hom on histamine- and carbachol-induced gastric acid, pepsin, soluble mucus and visible mucus secretion in gastric fistula rats. This study showed that Ya-hom (0.5, 1, 2 and 4 g/kg) inhibited both histamine- and carbachol-induced gastric acid, pepsin and soluble mucus secretion but potentiated visible mucus secretion in a dose dependent manner. Ya-hom had a lower maximum inhibition on the acid stimulating effects of histamine than that of carbachol. Ya-hom had a higher maximum inhibition on the pepsin and soluble mucus stimulating effects of histamine than that of carbachol. Ya-hom also had a higher evaluated effect on histamine-induced visible mucus than that of carbachol.

In vitro model, Chantharangsikul D. (44) studied effect of Ya-hom on histamine- and bethanechol-induced gastric acid secretion in isolated mouse whole stomach. This study showed that Ya-hom (2.5, 5, 10 and 20 mg/ml) inhibited histamine-induced gastric acid secretion in a 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. Since 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 (10 mg/ml) inhibited both low dose and high doses of bethanechol-induced gastric acid secretion in the presence and absense of ranitidine.

CHAPTER III
MATERIALS AND METHODS

MATERIALS

1. Animals

Male Wistar rats (National Laboratory Animal Center, Nakornpathom, Thailand)

2. Chemicals

Ya-hom powder

Cimetidine (Sigma Chemical Co., St Louis, USA)

Sucralfate (Siam Bheasach Co., Ltd., Bangkok, Thailand)

Hydrochloric acid (BDH Laboratory Supplies, Poole, England)

Aspirin granule (Rhone-Plulenc Chemical, Bangkok, Thailand)

Sodium carboxymethyl cellulose

Sodium chloride (Labscan Asia Co., Ltd., Bangkok, Thailand)

Formaldehyde, 37% solution (Mallinckrodt Baker, Inc., Phillipsburg, USA)

Carbondioxide gas, 95% (Thai Industial Gas, Chachoengsao, Thailand)

Alcian blue 8 GX (Sigma Chemical Co., St Louis, USA)

Magnesium chloride (Carlo Erba, Milano, Italy)

Sodium acetate (Ajax Chemicals, Sydney, Australia)

Acetic acid, glacial (Labscan Asia Co., Ltd., Bangkok, Thailand)

Sucrose (Carlo Erba, Milano, Italy)

Ether, anhydrous (Mallinckrodt Baker, Inc., Phillipsburg, USA)

3. Equipments

Disposable syringe 3 ml, 12 ml

Feeding tube No. 16 (National Laboratory Animal Center, Thailand)

Operating set

Beaker 10 ml, 25 ml, 100 ml, 250 ml

Cylinder 10 ml, 100 ml

Volumetric flask 100 ml, 500 ml

Macro pipette controller (BRAND GMBH + CO KG, Germany)

Micro pipette (Labmate, Poland)

Pipette 5 ml, 10 ml

Centrifuged tube

Magnetic stirrer (Ikamag)

pH electrode (Orion)

pH meter (Model 420A, Orion)

Centrifuge (Universal 16A, Hettich)

Spectrophotometer (Novaspec II, Pharmacia)

Thermometer 100 ºC

Stainless steel cage

Water bath

METHODS

1. Preparation of the test solution of Ya-hom

One kilogram of Ya-hom powder was mixed with boiled distilled water 10 liters and continuously boiled for 15 minutes. Ya-hom extract was filtered through cotton and muslin cloth. The filtrate was freeze dried by lyophilizer and kept in tight container at –20 ºC until use. One gram of Ya-hom power yielded 0.1278 g of lyophilized product. The test solution of Ya-hom was freshly prepared by redissolving the lyophilized product in distilled water. The concentration of Ya-hom used in this study was expressed as equivalent to Ya-hom powder.

2. Animal preparation

Male Wistar rats weighing between 200 and 250 g were obtained from the National Laboratory Animal Center at Salaya, Mahidol University. The rats were housed in hanging cages in the animal room at Faculty of Pharmacy, Mahidol University for the least 1 week prior to the experiment. The rats were maintained at 20 $\pm$ 2 ºC under a 12 h light-dark cycle and fed with a commercial diet (C.P. Mice Feed; SWT. Co., Ltd.) and tab water *ad libitum*. The rats were fasted 24 hours in wire mesh bottom cages to prevent coprophagy with free access to drinking water. The water was withdrawn 30 minutes in order to ensure that the stomach was empty before the experiment. The animal protocol was approved by the committee on animal use for research and education of Faculty of Pharmacy, Mahidol University

3. Experimental protocol

3.1 Gastric ulcer induction models

3.1.1 Gastric ulcer induction by hydrochloric acid (HCl)

This model was modified from the method of Robert *et al* (64). The fasted rats were administrated orally 5 or 6 ml/kg of 0.6 N HCl. Four hours later, the rats were sacrificed for determination of gastric ulcer. The dose that gave higher gastric ulcer induction would be used in the following experiment.

3.1.2 Gastric ulcer induction by aspirin (ASP)

This model was modified from the method of Goel *et al* (65). The fasted rats were administrated orally 100, 150 or 200 mg/5 ml/kg of aspirin suspended in 1% sodium carboxy-methyl cellulose (CMC) solution. Six hours later,

the rats were sacrificed for determination of gastric ulcer. The dose that gave higher gastric ulcer induction would be used in the following experiment.

3.1.3 Gastric ulcer induction by water immersion restraint stress (WIR)

This model was modified from the method of Takagi *et al* (66). The fasted rats were restrained in stainless steel cage and immersed up to their xiphoid in a water bath maintained at 4, 8, 12 or 16 $\pm$ 2 ºC. Five hours later, the rats were sacrificed for determination of gastric ulcer. The degree that gave higher gastric ulcer induction and did not kill rats during the experiment would be used in the following experiment.

3.1.4 Determination of gastric ulcer

The rats were killed with an overdose of 95% carbondioxide gas. The abdomen was opened, pylorus and cardia were ligated and the stomach was instilled with 7 ml of 0.5% formalin. The stomach was excised and then fixed in 0.5% formalin for 10 minutes. The stomach was cut along the greater curvature and rinsed with normal saline. The glandular portion of the stomach was examined under a magnifying lens. The lesion index was assessed using of the following methods:

3.1.4.1 Length of lesion

This method was used in evaluation of lesion index in HCl-induced and WIR-induced gastric lesion models. The length of lesion (mm) (67) was determined by measuring each lesion along its greatest diameter. The sum of the total lengths of lesion in each group divided by the number of rats in that group was expressed as the lesion index as follow:

$$\text{The lesion index} = \frac{\sum \text{Total lengths of lesion}}{\text{Number of rats}}$$

3.1.4.2 The severity score

This method was used in evaluation of lesion index in ASP-induced gastric lesion model. The severity score (67) was assigned according to the following scale: 0 = no pathology; 1 = mucosal edema and petechiae; 2 = 1-5 small ulcer (1-2 mm); 3 = more than 5 small ulcers or 1 medium ulcer (3-4 mm); 4 = two or more than 2 medium ulcers or 1 large ulcer (> 4 mm); 5 = perforated ulcer. The sum of

the total severity scores in each group divided by the number of rats in that group was expressed as the lesion index as follow:

$$\text{The lesion index} = \frac{\sum \text{Total severity scores}}{\text{Number of rats}}$$

3.1.4.3 The percent inhibition of gastric ulcer

The percent inhibition (68) was calculated as follow:

$$\% \text{ Inhibition} = \frac{(\text{lesion index}_{\text{control}} - \text{lesion index}_{\text{treated}}) \times 100}{\text{lesion index}_{\text{control}}}$$

3.2 Gastric ulcer prevention

3.2.1 Protocol for gastric ulcer prevention study

For each gastric ulcer induction model, the fasted rats were divided into 6 groups (6 rats per group). Each group was given distilled water or 1% sodium CMC solution or Ya-hom or cimetidine orally 30 min before induction of gastric ulcers as follow:

Group 1 water control group, received distilled water at the dose of 5 ml/kg

Group 2 vehicle control group, received 1% sodium CMC solution at the dose of 5 ml/kg

Group 3-5 treated group, received Ya-hom dissolved in distilled water at the dose of 1, 2 or 4 g/5 ml/kg, respectively.

Group 6 reference group, received cimetidine suspended in 1% sodium CMC solution at the dose of 100 mg/5 ml/kg

3.2.2 Induction of gastric ulcer

3.2.2.1 HCl-induced gastric lesion

Each rat was administrated orally 6 ml/kg of 0.6 N HCl. Four hours later, the rats were sacrificed for determination of gastric ulcer (Figure 1).

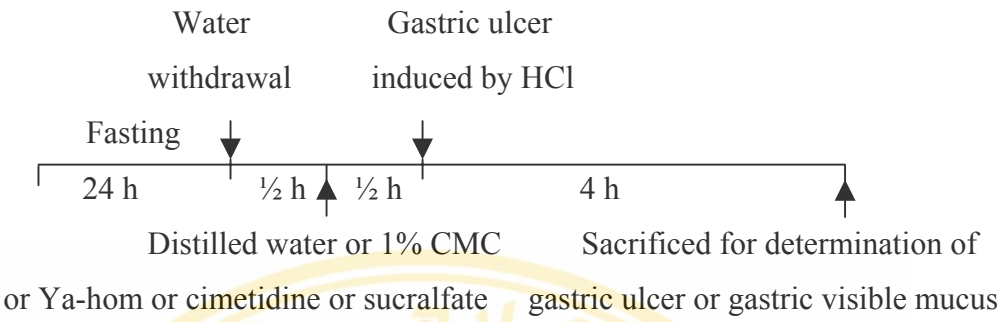

Figure 1 Diagram illustrated the protocol for gastric ulcer prevention and gastric visible mucus secretion studies: hydrochloric acid (HCl)-induced gastric ulcer in rats.

3.2.2.2 ASP-induced gastric lesion

Each rat was administrated orally 200 mg/5 ml/kg of aspirin suspended in 1% sodium CMC solution. Six hours later, the rats were sacrificed for determination of gastric ulcer (Figure 2).

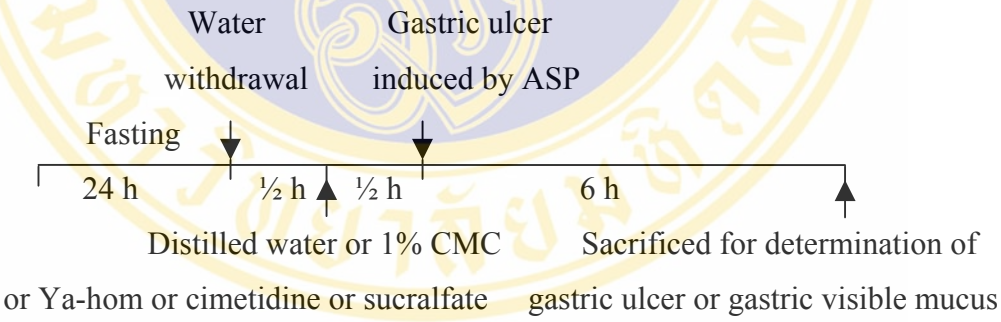

Figure 2 Diagram illustrated the protocol for gastric ulcer prevention and gastric visible mucus secretion studies: aspirin (ASP)-induced gastric ulcer in rats.

3.2.2.3 WIR-induced gastric lesion

Each rat was restrained in stainless steel cage and immersed up to their xiphoid in a water bath maintained at 16 $\pm$ 2 ºC. Five hours later, the rats were sacrificed for determination of gastric ulcer (Figure 3).

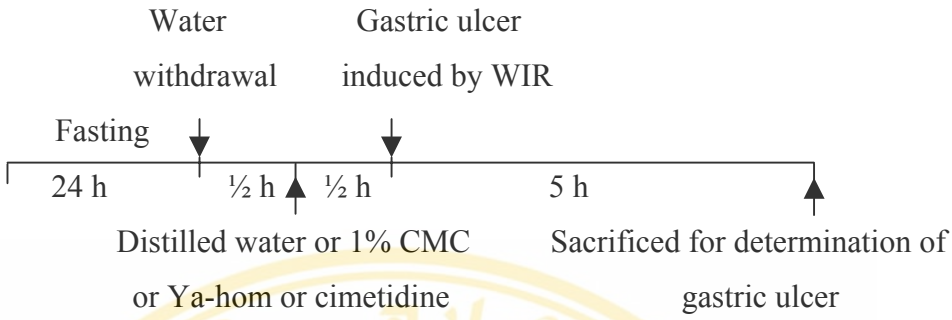

Figure 3 Diagram illustrated the protocol for gastric ulcer prevention study: water immersion restraint stress (WIR)-induced gastric ulcer in rats.

3.3 Gastric visible mucus secretion

3.3.1 Protocol for gastric visible mucus secretion study

For each gastric ulcer induction model, the fasted rats were divided into 6 groups (6 rats per group). Each group was given distilled water or 1% sodium CMC solution or Ya-hom or cimetidine or sucralfate orally 30 min before induction of gastric ulcers as follow:

Group 1 normal group, received distilled water at the dose of 5 ml/kg (No induction of gastric ulcer)

Group 2 water control group, received distilled water at the dose of 5 ml/kg

Group 3 vehicle control group, received 1% sodium CMC solution at the dose of 5 ml/kg

Group 4 treated group, received Ya-hom dissolved in distilled water at the dose of 4 g/5 ml/kg

Group 5-6 reference group, received cimetidine or sucralfate suspended in 1% sodium CMC solution at the dose of 100 mg or 1 g/5 ml/kg, respectively

3.3.2 Induction of gastric ulcer

3.3.2.1 HCl-induced gastric lesion

Each rat was administrated orally 6 ml/kg of 0.6 N HCl. Four hours later, the rats were sacrificed for determination of gastric visible mucus (Figure 1).

3.3.2.2 ASP-induced gastric lesion

Each rat was administrated orally 200 mg/5 ml/kg of aspirin suspended in 1% sodium CMC solution. Six hours later, the rats were sacrificed for determination of gastric visible mucus (Figure 2).

3.3.3 Determination of gastric visible mucus

Visible mucus on the gastric mucosal surface was determined according to the method described by Corne *et al* (69). The rats were killed with an overdose of 95% carbondioxide gas. The abdomen was opened and the stomach was removed. The stomach was cut along the lesser curvature, rinsed with normal saline and blotted with filter paper. The glandular portion of stomach was excised, weighed and immersed in 8 ml of 0.1% Alcian blue 8 GX dissolved in 0.16 M sucrose buffered with 0.05 M sodium acetate, adjusted to pH 5.8 with acetic acid for 2 hours. The excess of uncomplexed dye was rinsed from the stomach twice with 0.25 M sucrose for 30 and 15 minutes, respectively. The dye complexed with the gastric visible mucus was extracted in 10 ml of 0.5 M magnesium chloride for 2 hours. The blue extract was shaken vigorously with an equal volume of diethyl ether and then centrifuged at 4,500 rpm for 10 minutes. The aqueous layer was separated and measured the absorbance at 605 nm by a spectrophotometer and using the Alcian blue 8 GX dissolved in 0.5 M magnesium chloride at the concentrations of 10, 20, 40 and 60 µg/ml as a standard. The amount of gastric visible mucus was expressed as µg Alcian blue/g stomach.

3.4 Gastric ulcer healing

3.4.1 Protocol for gastric ulcer healing study

The fasted rats were divided into 4 groups (12 rats per group). Each group was restrained in stainless steel cage and immersed up to their xiphoid in a water bath maintained at 16 $\pm$ 2 ºC for 5 hours before oral administration of distilled water or 1% sodium CMC solution or Ya-hom or cimetidine as follow:

Group 1 water control group, received distilled water at the dose of 5 ml/kg

Group 2 vehicle control group, received 1% sodium CMC solution at the dose of 5 ml/kg

Group 3 treated group, received Ya-hom dissolved in distilled water at the dose of 4 g/5 ml/kg

Group 4 reference group, received cimetidine suspended in 1% sodium CMC solution at the dose of 100 mg/5 ml/kg

The 6 rats of each group were given distilled water or 1% sodium CMC solution or Ya-hom or cimetidine immediately after 5 h of gastric ulcer induction and then sacrificed after 24 h of the first oral administration for determination of gastric ulcer. The other 6 rats of each group were given distilled water or 1% sodium CMC solution or Ya-hom or cimetidine immediately after 5 h of gastric ulcer induction and repeated given every 24 h for three days and then sacrificed after 24 h of the forth oral administration for determination of gastric ulcer (Figure 4).

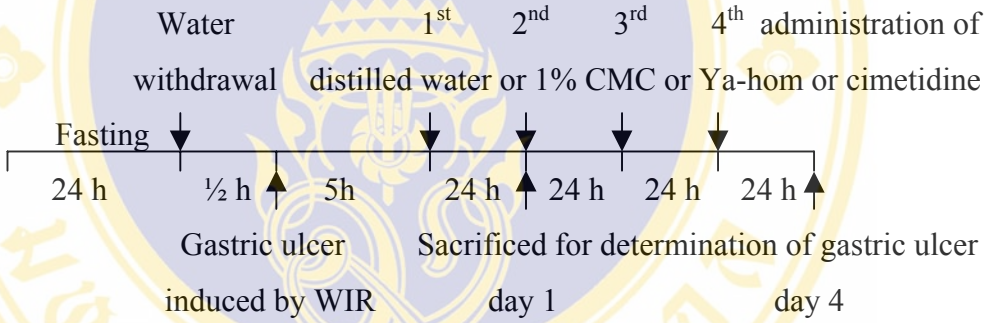

Figure 4 Diagram illustrated the protocol for gastric ulcer healing study: water immersion restraint stress (WIR)-induced gastric ulcer in rats.

3.4.2 Determination of gastric ulcer

The rats were killed with an overdose of 95% carbondioxide gas on day 1 and day 4 after induction of gastric ulcer. The abdomen was opened, pylorus and cardia were ligated and the stomach was instilled with 7 ml of 0.5% formalin. The stomach was excised and then fixed in 0.5% formalin for 10 minutes. The stomach was cut along the greater curvature and rinsed with normal saline. The glandular portion of the stomach was examined under a magnifying lens. The ulcer index was assessed using of the following methods:

3.4.2.1 The ulcer area

This method was used in evaluation of ulcer index in the ulcer development and ulcer healing phase of gastric ulceration. The ulcer development (inflammatory) phase was observed at day 1 to day 3 after gastric ulcer induction and characterized by an expansion of ulcer area. The ulcer healing (proliferative) phase was observed at more than 3 days after gastric ulcer induction and characterized by a reduction of ulcer area. The ulcer area (mm^2) (70) was determined by counting the numbers of 1 mm^2 square of grid paper it covered. The sum of the total ulcer areas in each group divided by the number of rats in that group was expressed as the ulcer index as follow:

$$\text{The ulcer index} = \frac{\sum \text{Total the total ulcer areas}}{\text{Number of rats}}$$

3.4.2.2 The percent curation of gastric ulcer

The percent curation (68) was calculated as follow:

$$\% \text{ Curation} = \frac{(\text{ulcer index}_{\text{control}} - \text{ulcer index}_{\text{treated}}) \times 100}{\text{ulcer index}_{\text{control}}}$$

3.5 Statistical analysis

The data were expressed as mean $\pm$ the standard error of mean (SEM). One way analysis of variance (ANOVA) was used to compare the values for each of experimental groups. Tukey's honestly significant difference (HSD) test was used to differentiate the difference between two experimental groups. The P-value of less than 0.05 ($P<0.05$) was considered to be statistical significant difference.

CHAPTER IV
RESULTS

PART I GASTRIC ULCER INDUCTION MODELS

1.1 HCl-induced gastric lesion model

Four hours after oral administration of 0.6 N HCl induced elongated bands of necrotic mucous membrane in the glandular portion of the stomach as shown in Figure 5. Hydrochloric acid 0.6 N at doses of 5 and 6 ml/kg produced gastric ulcer as shown in Table 2. The 6 ml/kg of 0.6 N HCl that gave higher gastric ulcer induction would be used in the following experiment.

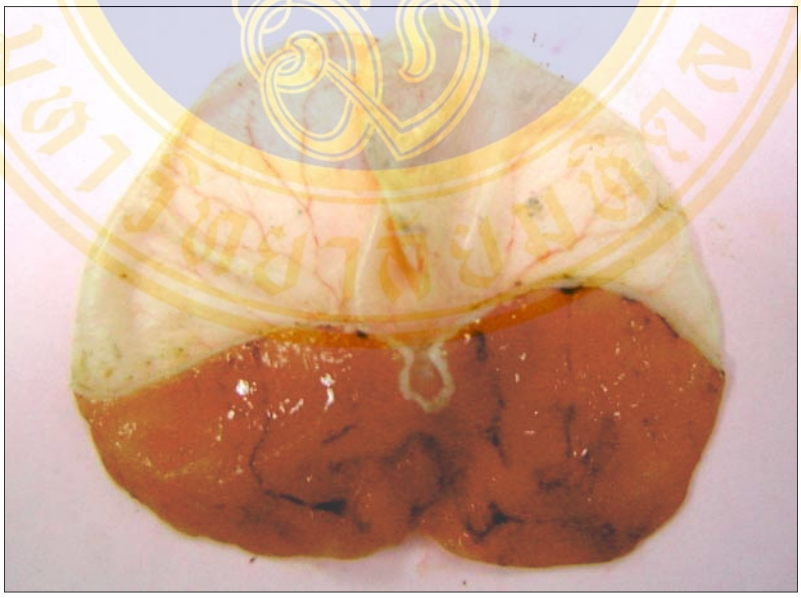

Figure 5 Gastric ulcer induced by 0.6 N hydrochloric acid (HCl)

Table 2 Effect of 0.6 N hydrochloric acid (HCl) at the doses of 5 or 6 ml/kg on gastric ulcer induction for 4 hours

Rat No.	Lengths of lesion (mm)	
	0.6 N HCl, 5 ml/kg	0.6 N HCl, 6 ml/kg
1	35	70
2	40	68
3	32	83
4	28	91
5	45	75
6	43	60
mean $\pm$ SEM	37.17 $\pm$ 2.70 *	74.50 $\pm$ 4.54

All values were expressed as mean $\pm$ SEM.

* $p<0.05$: significantly lower than the 0.6 ml/kg of 0.6 N HCl

1.2 ASP-induced gastric lesion model

Six hours after oral administration of ASP suspended in 1% sodium CMC solution induced round lesions or petichiae throughout the glandular portion of the stomach as shown in Figure 6. Aspirin suspended in 1% sodium CMC solution at doses of 100, 150 and 200 mg/5 ml/kg produced gastric ulcer as shown in Table 3. The 200 mg/5 ml/kg of ASP suspended in 1% sodium CMC solution that gave the highest gastric ulcer induction would be used in the following experiment.

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Figure 6 Gastric ulcer induced by aspirin (ASP) suspended in 1% sodium carboxy-methyl cellulose (CMC) solution

Table 3 Effect of aspirin (ASP) suspended in 1% sodium carboxy-methyl cellulose (CMC) solution at the doses of 100, 150 or 200 mg/5 ml/kg on gastric ulcer induction for 6 hours

Rat No.	Severity scores		
	ASP, 100 mg/kg	ASP, 150 mg/kg	ASP, 200 mg/kg
1	0	2	3
2	1	2	3
3	2	3	4
4	0	1	3
5	1	2	3
6	0	2	4
mean $\pm$ SEM	0.67 $\pm$ 0.33 *, **	2.00 $\pm$ 0.26 **	3.33 $\pm$ 0.21

All values were expressed as mean $\pm$ SEM.

* $p<0.05$: significantly lower than the 150 mg/5ml/kg of aspirin suspended in 1% CMC

** $p<0.05$: significantly lower than the 200 mg/5ml/kg of aspirin suspended in 1% CMC

1.3 WIR-induced gastric lesion model

Restraint in stainless steel cage and immersion in cold water for 5 hours induced hemorrhagic lesions in the glandular portion of the stomach as shown in Figure 7.Water immersion restraint stress maintained at 4, 8, 12 or 16 $\pm$ 2 ºC produced gastric ulcer as shown in Table 4. The 16 $\pm$ 2 degree celsius of WIR that gave higher gastric ulcer induction and did not kill rats during the experiment would be used in the following experiment.

Figure 7 Gastric ulcer induced by water immersion restraint stress (WIR)

Table 4 Effect of water immersion restraint stress (WIR) maintained at 4, 8, 12 or 16 ± 2 degree celsius (°C) on gastric ulcer induction for 5 hours

Rat No.	Lengths of lesion (mm)			
	WIR, 4 ± 2 °C	WIR, 8 ± 2 °C	WIR, 12 ± 2 °C	WIR, 16 ± 2 °C
1	Death	Death	Death	28
2	30	35	25	36
3	Death	27	40	30
4	28	Death	34	40
5	36	38	26	48
6	Death	28	32	32
mean ± SEM	31.33 ± 2.40	32.00 ± 2.68	31.40 ± 2.75	35.67 ± 3.03

PART II EFFECT OF YA-HOM ON GASTRIC ULCER PREVENTION

2.1 HCl-induced gastric lesion

The effect of CMC (1%), Ya-hom (1, 2 and 4 g/kg) and cimetidine (0.1 g/kg) on gastric ulcer induced by hydrochloric acid (0.6 N HCl, 6 ml/kg) for 4 hours in rats were shown in Table 5 and Figure 8, 9. There was no difference in gastric lesion index between water control group and vehicle (CMC) control group. The 1, 2 and 4 g/kg of Ya-hom and 0.1 g/kg of cimetidine treated groups had significantly lower gastric lesion indexes than those of water and vehicle (CMC) control groups.

These results showed that Ya-hom treated groups at doses of 1, 2 and 4 g/kg inhibited HCl-induced gastric ulcer in a dose-dependent manner with the maximum inhibition of 93.45%. Cimetidine treated group at dose of 0.1 g/kg inhibited HCl-induced gastric ulcer with an inhibition of 78.44%.

Table 5 Effect of 1% of sodium carboxy-methyl cellulose solution (CMC), Ya-hom 1 g/kg (YH1), 2 g/kg (YH2), 4 g/kg (YH4) and cimetidine 0.1 g/kg (Ci) on gastric induced by hydrochloric acid (HCl) in rats

Rat No.	Lengths of lesion (mm)					
	Water	CMC	YH1	YH2	YH4	Ci
1	85	87	63	40	8	17
2	81	78	72	51	9	28
3	90	85	67	45	6	10
4	108	96	58	34	11	25
5	86	102	79	59	0	21
6	100	90	81	65	2	15
Lesion index (mean ± SEM)	91.67 ± 4.20	89.67 ± 3.45	70.00 ± 3.68 [a, b, cc, #, ##]	49.00 ± 4.77 [a, b, cc, ##]	6.00 ± 1.73 [a, b]	19.33 ± 2.72 [a, b]
% Inhibition	-	2.18	23.64	46.55	93.45	78.44

All values were expressed as mean ± SEM.

[a] $p<0.05$: significantly lower than the water control group

[b] $p<0.05$: significantly lower than the vehicle (CMC) control group

[cc] $p<0.05$: significantly higher than the cimetidine treated group

[#] $p<0.05$: significantly higher than the 2 g/kg of Ya-hom treated group

[##] $p<0.05$: significantly higher than the 4 g/kg of Ya-hom treated group

% Inhibitions of Ya-hom were calculated from water control.

% Inhibition of cimetidine was calculated from CMC control.

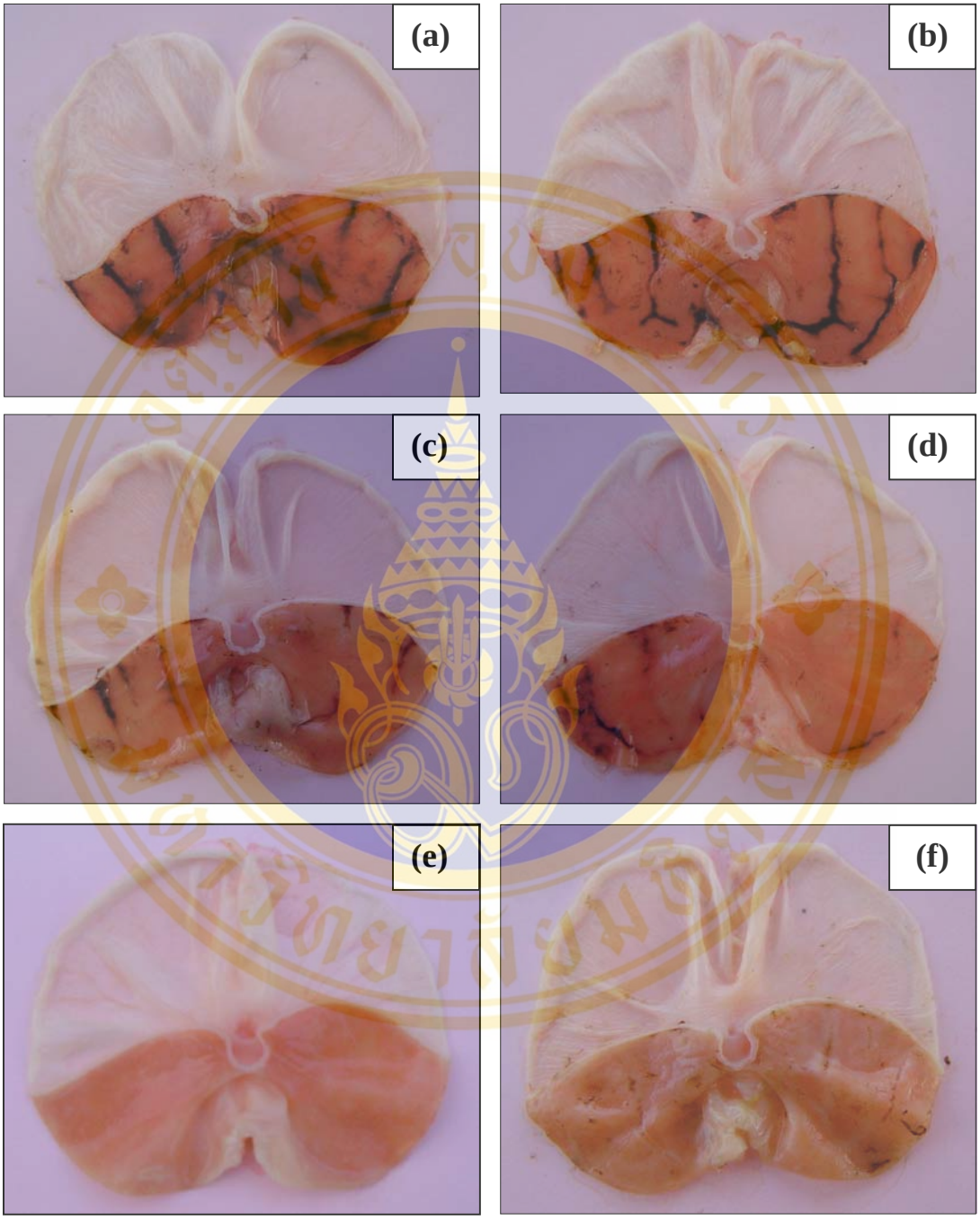

Figure 8 Effect of distilled water (a), 1% of sodium carboxy-methyl cellulose solution (b), Ya-hom 1 g/kg (c), 2 g/kg (d), 4 g/kg (e) and cimetidine 0.1 g/kg (f) on gastric ulcer induced by hydrochloric acid (HCl) in rats (n = 6)

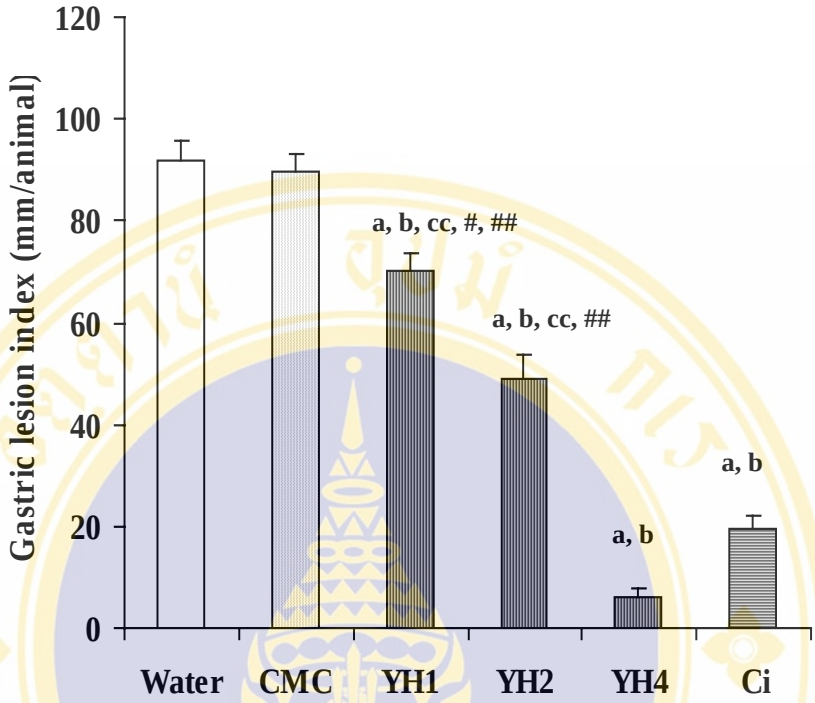

Figure 9 Effect of 1% of sodium carboxy-methyl cellulose solution (CMC), Ya-hom 1 g/kg (YH1), 2 g/kg (YH2), 4 g/kg (YH4) and cimetidine 0.1 g/kg (Ci) on gastric ulcer induced by hydrochloric acid (HCl) in rats (n = 6)

[a] $p<0.05$: significantly lower than the water control group

[b] $p<0.05$: significantly lower than the vehicle (CMC) control group

[cc] $p<0.05$: significantly higher than the cimetidine treated group

[#] $p<0.05$: significantly higher than the 2 g/kg of Ya-hom treated group

[##] $p<0.05$: significantly higher than the 4 g/kg of Ya-hom treated group

2.2 ASP-induced gastric lesion

The effect of CMC (1%), Ya-hom (1, 2 and 4 g/kg) and cimetidine (0.1 g/kg) on gastric ulcer induced by aspirin (ASP, 200 mg/kg) for 6 hours in rats were shown in Table 6 and Figure 10, 11. There was no difference in gastric lesion index between water control group and vehicle (CMC) control group. The gastric lesion index of Ya-hom 1 g/kg treated group was not different from that of water and vehicle (CMC) control groups. The 2 and 4 g/kg of Ya-hom and 0.1 g/kg of cimetidine treated groups had significantly lower gastric lesion indexes than those of water and vehicle (CMC) control groups.

These results showed that Ya-hom treated groups at doses of 2 and 4 g/kg inhibited ASP-induced gastric ulcer in a dose-dependent manner with the maximum inhibition of 54.50%. Cimetidine treated group at dose of 0.1 g/kg inhibited ASP-induced gastric ulcer with an inhibition of 76.29%.

Table 6 Effect of 1% of sodium carboxy-methyl cellulose solution (CMC), Ya-hom 1 g/kg (YH1), 2 g/kg (YH2), 4 g/kg (YH4) and cimetidine 0.1 g/kg (Ci) on gastric ulcer induced by aspirin (ASP) in rats

Rat No.	Severity scores					
	Water	CMC	YH1	YH2	YH4	Ci
1	4	4	2	3	2	0
2	4	3	3	3	2	1
3	3	3	4	2	1	2
4	3	4	3	2	2	1
5	4	3	2	2	2	1
6	4	4	3	2	1	0
Lesion index (mean ± SEM)	3.67 ± 0.21	3.50 ± 0.22	2.83 [cc, ##] ± 0.31	2.33 [a, b, cc] ± 0.21	1.67 [a, b] ± 0.21	0.83 [a, b] ± 0.31
% Inhibition	-	1.70	22.89	36.51	54.50	76.29

All values were expressed as mean ± SEM.

[a] $p<0.05$: significantly lower than the water control group

[b] $p<0.05$: significantly lower than the vehicle (CMC) control group

[cc] $p<0.05$: significantly higher than the cimetidine treated group

[##] $p<0.05$: significantly higher than the 4 g/kg of Ya-hom treated group

% Inhibitions of Ya-hom were calculated from water control.

% Inhibition of cimetidine was calculated from CMC control.

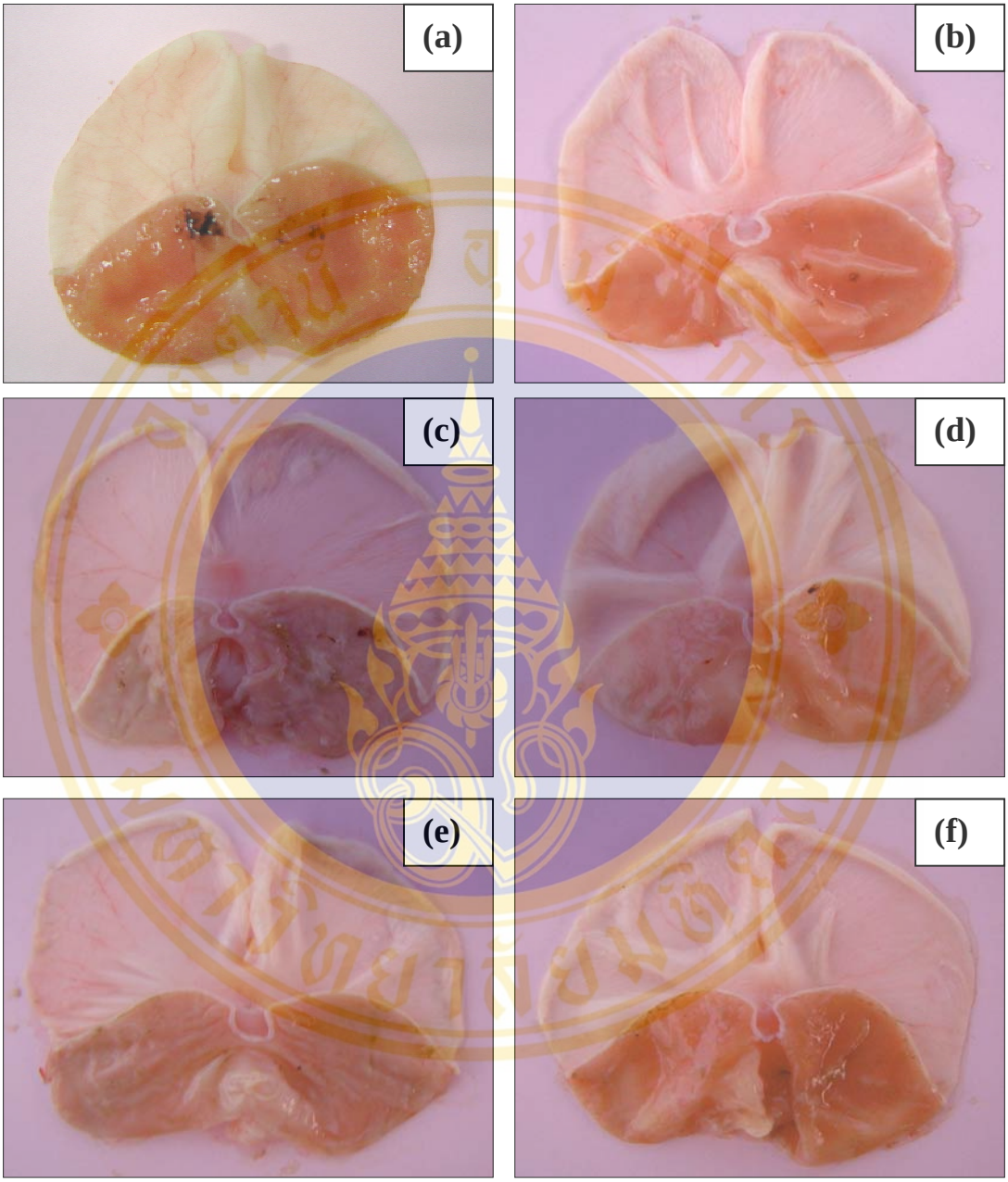

Figure 10 Effect of distilled water (a), 1% of sodium carboxy-methyl cellulose solution (b), Ya-hom 1 g/kg (c), 2 g/kg (d), 4 g/kg (e) and cimetidine 0.1 g/kg (f) on gastric ulcer induced by aspirin (ASP) in rats (n = 6)

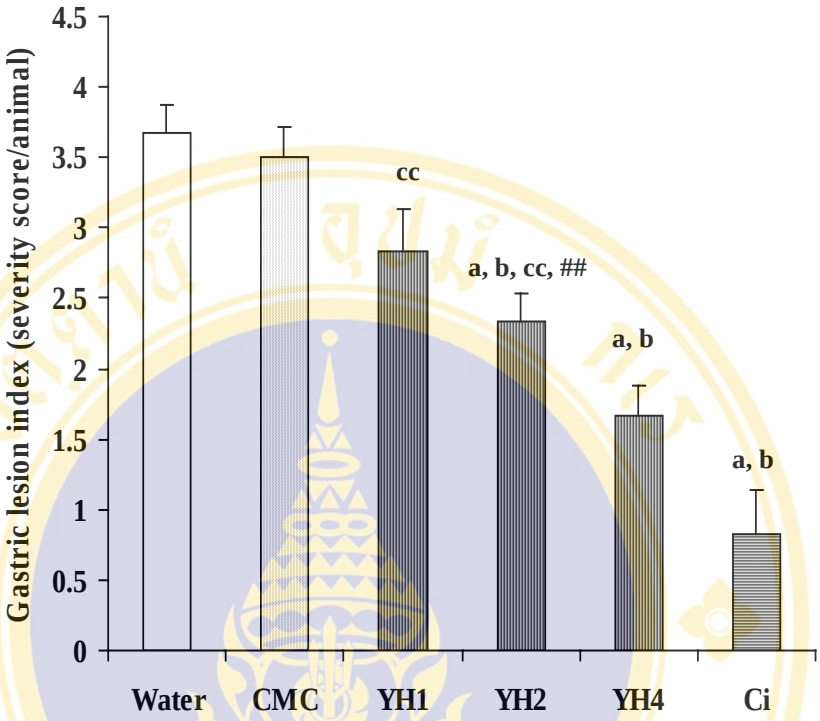

Figure 11 Effect of 1% of sodium carboxy-methyl cellulose solution (CMC), Ya-hom 1 g/kg (YH1), 2 g/kg (YH2), 4 g/kg (YH4) and cimetidine 0.1 g/kg (Ci) on gastric ulcer induced by aspirin (ASP) in rats (n = 6)

[a] $p<0.05$: significantly lower than the water control group

[b] $p<0.05$: significantly lower than the vehicle (CMC) control group

[cc] $p<0.05$: significantly higher than the cimetidine treated group

[##] $p<0.05$: significantly higher than the 4 g/kg of Ya-hom treated group

2.3 WIR-induced gastric lesion

The effect of CMC (1%), Ya-hom (1, 2 and 4 g/kg) and cimetidine (0.1 g/kg) on gastric ulcer induced by water immersion restraint stress (WIR, 16 $\pm$ 2 ºC) for 5 hours in rats were shown in Table 7 and Figure 12, 13. There was no difference in gastric lesion index between water control group and vehicle (CMC) control group. The gastric lesion index of Ya-hom 1 g/kg treated group was not different from that of water and vehicle control groups. The 2 and 4 g/kg of Ya-hom and 0.1 g/kg of cimetidine treated groups had significantly lower gastric lesion indexes than those of water and vehicle (CMC) control groups.

These results showed that Ya-hom treated groups at doses of 2 and 4 g/kg inhibited WIR-induced gastric ulcer in a dose-dependent manner with the maximum inhibition of 61.79%. Cimetidine treated group at dose of 0.1 g/kg inhibited WIR-induced gastric ulcer with an inhibition of 48.02%.

Table 8 demonstrated the effect of Ya-hom 1, 2 and 4 g/kg and cimetidine 0.1 g/kg on gastric ulcer prevention in HCl-, ASP- and WIR-induced gastric lesion models.

Table 7 Effect of 1% of sodium carboxy-methyl cellulose solution (CMC), Ya-hom 1 g/kg (YH1), 2 g/kg (YH2), 4 g/kg (YH4) and cimetidine 0.1 g/kg (Ci) on gastric ulcer induced by water immersion restraint stress (WIR) in rats

Rat No.	Lengths of lesion (mm)					
	Water	CMC	YH1	YH2	YH4	Ci
1	68	61	53	37	19	26
2	51	57	50	36	13	28
3	60	63	60	39	27	35
4	54	50	48	45	32	38
5	66	65	62	32	25	30
6	57	58	54	42	20	27
Lesion index (mean ± SEM)	59.33 ± 2.73	59.00 ± 2.18	54.50 [cc, #, ##] ± 2.25	38.50 [a, b, cc, ##] ± 1.88	22.67 [a, b] ± 2.74	30.67 [a, b] ± 1.96
% Inhibition	-	0.56	8.14	35.11	61.79	48.02

All values were expressed as mean ± SEM.

[a] $p<0.05$: significantly lower than the water control group

[b] $p<0.05$: significantly lower than the vehicle (CMC) control group

[cc] $p<0.05$: significantly higher than the cimetidine treated group

[#] $p<0.05$: significantly higher than the 2 g/kg of Ya-hom treated group

[##] $p<0.05$: significantly higher than the 4 g/kg of Ya-hom treated group

% Inhibitions of Ya-hom were calculated from water control.

% Inhibition of cimetidine was calculated from CMC control.

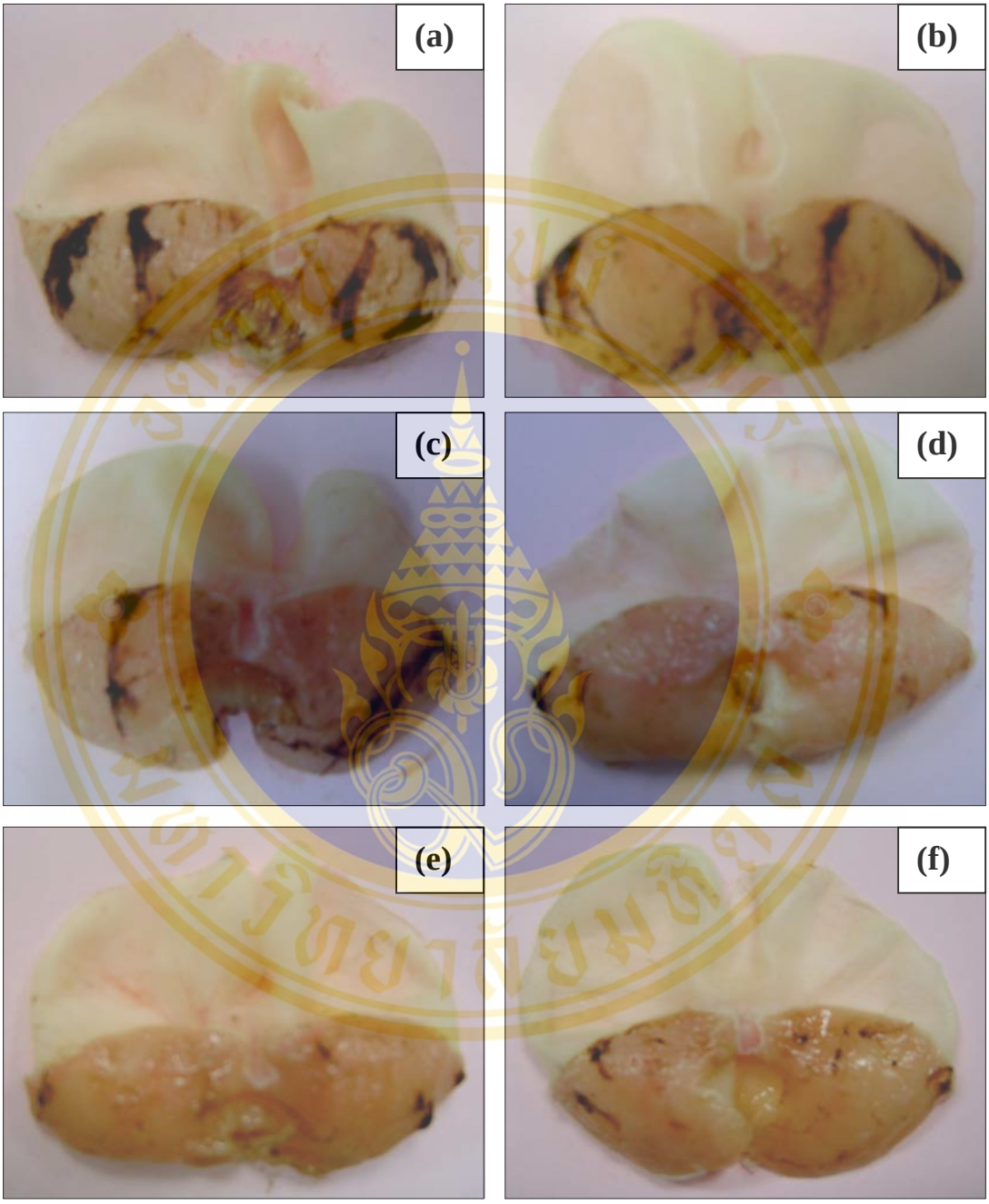

Figure 12 Effect of distilled water (a), 1% of sodium carboxy-methyl cellulose solution (b), Ya-hom 1 g/kg (c), 2 g/kg (d), 4 g/kg (e) and cimetidine 0.1 g/kg (f) on gastric ulcer induced by water immersion restraint stress (WIR) in rats (n = 6)

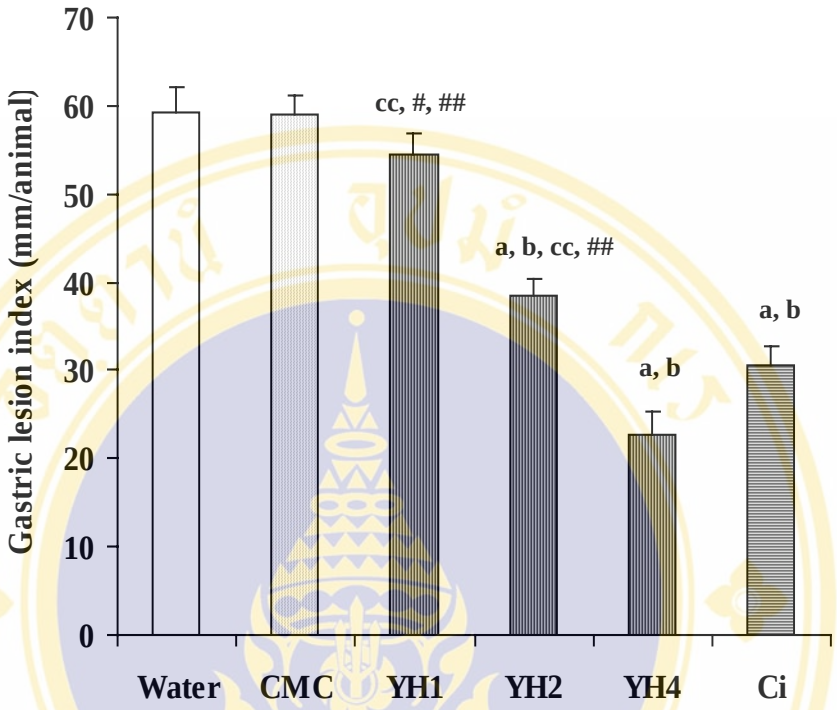

Figure 13 Effect of 1% of sodium carboxy-methyl cellulose solution (CMC), Ya-hom 1 g/kg (YH1), 2 g/kg (YH2), 4 g/kg (YH4) and cimetidine 0.1 g/kg (Ci) on gastric ulcer induced by water immersion restraint stress (WIR) in rats (n = 6)

[a] $p<0.05$: significantly lower than the water control group

[b] $p<0.05$: significantly lower than the vehicle (CMC) control group

[cc] $p<0.05$: significantly higher than the cimetidine treated group

[#] $p<0.05$: significantly higher than the 2 g/kg of Ya-hom treated group

[##] $p<0.05$: significantly higher than the 4 g/kg of Ya-hom treated group

Table 8 Effect of Ya-hom and cimetidine on gastric ulcer prevention in HCl-, ASP- and WIR-induced gastric lesion models (n = 6)

Group	Gastric lesion index, (% Inhibition)		
	HCl (mm/animal)	ASP (severity score/ animal)	WIR (mm/animal)
Water control	91.67 ± 4.20	3.67 ± 0.21	59.33 ± 2.73
Vehicle (CMC)	89.67 ± 3.45	3.50 ± 0.22	59.00 ± 2.18
control	(2.18)	(1.70)	(0.56)
Ya-hom (1 g/kg)	70.00 ± 3.69 [a, b, cc, #, ##]	2.83 ± 0.31 [cc, ##]	54.50 ± 2.25 [cc, #, ##]
	(23.64)	(22.89)	(8.14)
Ya-hom (2 g/kg)	49.00 ± 4.77 [a, b, cc, ##]	2.33 ± 0.21 [a, b, cc]	38.50 ± 1.88 [a, b, cc, , ##]
	(46.55)	(36.51)	(35.11)
Ya-hom (4 g/kg)	6.00 ± 1.73 [a, b]	1.67 ± 0.21 [a, b]	22.67 ± 2.74 [a, b]
	(93.45)	(54.50)	(61.79)
Cimetidine	19.33 ± 2.72 [a, b]	0.83 ± 0.31 [a, b]	30.67 ± 1.96 [a, b]
(0.1 g/kg)	(78.91)	(77.38)	(48.31)

All values were expressed as mean ± SEM, comparing in the same model.

[a] $p<0.05$: significantly lower than the water control group

[b] $p<0.05$: significantly lower than the vehicle (CMC) control group

[cc] $p<0.05$: significantly higher than the cimetidine treated group

[#] $p<0.05$: significantly higher than the 2 g/kg of Ya-hom treated group

[##] $p<0.05$: significantly higher than the 4 g/kg of Ya-hom treated group

% Inhibitions of Ya-hom were calculated from water control.

% Inhibition of cimetidine was calculated from CMC control.

PART III EFFECT OF YA-HOM ON GASTRIC VISIBLE MUCUS SECRETION IN GASTRIC ULCER RATS

3.1 HCl-induced gastric lesion

The effect of CMC (1%), Ya-hom (4 g/kg), cimetidine (0.1 g/kg) and sucralfate (1 g/kg) on gastric visible mucus secretion in gastric ulcer rats induced by hydrochloric acid (HCl) were shown in Table 9 and Figure 14. The amount of gastric visible mucus of both water and CMC (referred to as water and vehicle control groups) in HCl-induced gastric ulcer rats were significantly higher than the normal rats. The amount of gastric visible mucus of cimetidine treated group was not significant different from water and vehicle control groups. Sucralfate treated group had a significant lower amount of gastric visible mucus than that of water and vehicle control groups. Ya-hom treated group was not significant different from all groups.

These results showed that HCl stimulated gastric visible mucus secretion. Cimetidine treated group at dose of 0.1 g/kg had no inhibitory effect, whereas sucralfate treated group at dose of 1 g/kg and Ya-hom treated group at dose of 4 g/kg attenuated effect of HCl-stimulated gastric visible mucus secretion.

Table 9 Effect of 1% of sodium carboxy-methyl cellulose solution (CMC), Ya-hom 4 g/kg (YH4), cimetidine 0.1 g/kg (Ci) and sucralfate 1 g/kg (Su) on gastric visible mucus in HCl-induced gastric ulcer rats

Rat No.	Gastric visible mucus (μg/g stomach)					
	Normal	Water	CMC	YH4	Ci	Su
1	114.69	169.35	170.30	122.70	193.85	72.03
2	130.72	138.97	140.83	134.46	164.45	94.96
3	102.45	167.86	156.45	172.33	180.71	115.26
4	110.81	169.85	155.77	179.96	166.83	146.57
5	118.88	179.66	185.82	123.40	175.91	164.45
6	132.31	187.11	173.17	117.87	114.36	130.79
mean	118.31	168.80 **	163.73 **	141.79	166.02 **	120.68 a, b, c
± SEM	4.73	6.70	6.48	11.13	11.20	13.84

All values were expressed as mean ± SEM.

** $p<0.05$: significantly higher than the normal group

a $p<0.05$: significantly lower than the water control group (Water, HCl-induced gastric ulcer)

b $p<0.05$: significantly lower than the vehicle control group (CMC, HCl-induced gastric ulcer)

c $p<0.05$: significantly lower than the cimetidine treated group

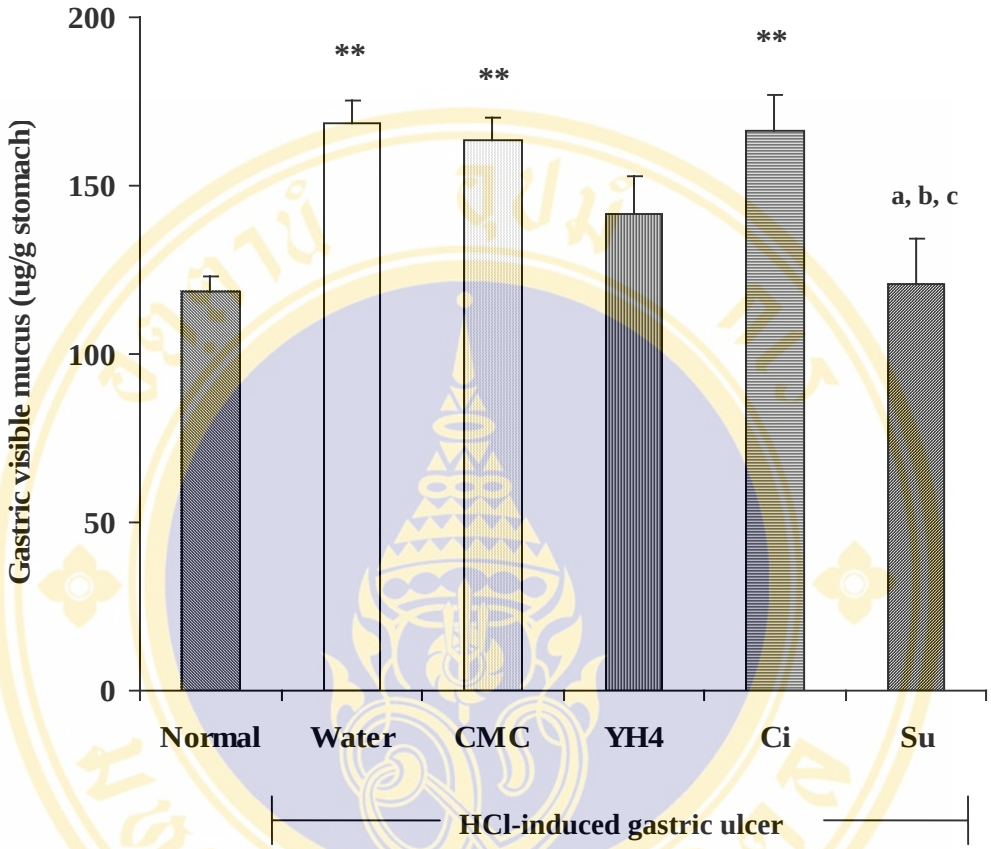

Figure 14 Effect of Ya-hom 4 g/kg (YH4), cimetidine 0.1 g/kg (Ci) and sucralfate 1 g/kg (Su) on gastric visible mucus secretion in HCl-induced gastric ulcer rats (n = 6)

** $p<0.05$: significantly higher than the normal group

[a] $p<0.05$: significantly lower than the water control group

[b] $p<0.05$: significantly lower than the vehicle control group (CMC group)

[c] $p<0.05$: significantly lower than the cimetidine treated group

3.2 ASP-induced gastric lesion

The effect of CMC (1%), Ya-hom (4 g/kg), cimetidine (0.1 g/kg) and sucralfate (1 g/kg) on gastric visible mucus secretion in gastric ulcer rats induced by aspirin (ASP) were shown in Table 10 and Figure 15. The amount of gastric visible mucus of both H_2O and CMC (referred to as water and vehicle control groups) in ASP-induced gastric ulcer rats were significantly lower than the normal rats. The amount of gastric visible mucus of sucralfate treated group was not significant different from water and vehicle control groups. Cimetidine treated group had a significant higher amount of gastric visible mucus than that of water and vehicle control groups. Ya-hom treated group was not significant different from all groups.

These results showed that ASP inhibited gastric visible mucus secretion. Sucralfate had no stimulatory effect, whereas cimetidine treated group at dose of 0.1 g/kg and Ya-hom treated group at dose of 4 g/kg and attenuated effect of ASP-inhibited gastric visible mucus secretion.

Table 11 demonstrated the effect of Ya-hom, cimetidine and sucralfate on gastric visible mucus secretion in HCl- and ASP-induced gastric lesion models.

Table 10 Effect of 1% of sodium carboxy-methyl cellulose solution (CMC), Ya-hom 4 g/kg (YH4), cimetidine 0.1 g/kg (Ci) and sucralfate 1 g/kg (Su) on gastric visible mucus in ASP-induced gastric ulcer rats

Rat No.	Gastric visible mucus (μg/g stomach)					
	Normal	Water	CMC	YH4	Ci	Su
1	114.69	43.52	68.89	70.97	93.80	50.36
2	130.72	75.49	81.91	67.68	80.59	44.90
3	102.45	65.83	53.51	100.46	138.60	80.59
4	110.81	62.09	74.10	133.33	153.94	129.37
5	118.88	44.45	50.42	92.65	166.74	68.57
6	132.31	62.30	67.14	105.10	117.06	64.74
mean	118.31	58.95 *	65.99 *	95.03	125.12 aa, bb, dd	73.09 *
± SEM	4.73	5.13	4.92	9.88	13.87	12.42

All values were expressed as mean ± SEM.

* $p<0.05$: significantly lower than the normal group

aa $p<0.05$: significantly higher than the water control group (Water, ASP-induced gastric ulcer)

bb $p<0.05$: significantly higher than the vehicle control group (CMC, ASP-induced gastric ulcer)

dd $p<0.05$: significantly higher than the sucralfate treated group

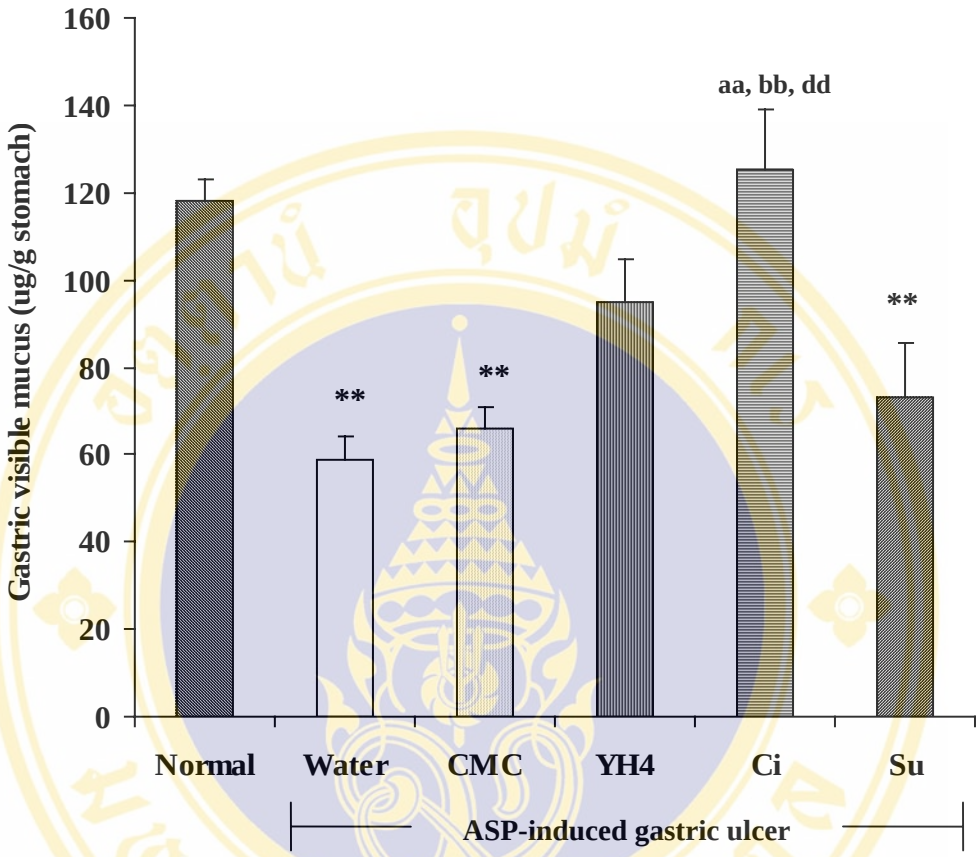

Figure 15 Effect of Ya-hom 4 g/kg (YH4), cimetidine 0.1 g/kg (Ci) and sucralfate 1 g/kg (Su) on gastric visible mucus secretion in ASP-induced gastric ulcer rats (n = 6)

[*] $p<0.05$: significantly lower than the normal group

[aa] $p<0.05$: significantly higher than the water control group

[bb] $p<0.05$: significantly higher than the vehicle control group (CMC group)

[dd] $p<0.05$: significantly higher than the sucralfate treated group

Table 11 Effect of Ya-hom, cimetidine and sucralfate on gastric visible mucus secretion in HCl- and ASP-induced gastric lesion models (n = 6)

Group	Gastric visible mucus (μg/g stomach)	
	HCl	ASP
Normal	118.31 ± 4.73	118.31 ± 4.73
Water control	168.80 ± 6.70 **	58.95 ± 5.13 *
Vehicle (CMC) control	163.73 ± 6.48 **	65.99 ± 4.92 *
Ya-hom (4 g/kg)	141.79 ± 11.13	95.03 ± 9.88
Cimetidine (0.1 g/kg)	166.02 ± 11.20 **	125.12 ± 13.87 aa, bb, dd
Sucralfate (1 g/kg)	120.68 ± 13.84 a, b, c	73.09 ± 12.42 *

All values were expressed as mean ± SEM, comparing in the same model.

* p<0.05: significantly lower than the normal group

a p<0.05: significantly lower than the water control group

b p<0.05: significantly lower than the vehicle (CMC) control group

c p<0.05: significantly lower than the cimetidine treated group

** p<0.05: significantly higher than the normal group

aa p<0.05: significantly higher than the water control group

bb p<0.05: significantly higher than the vehicle (CMC) control group

dd p<0.05: significantly higher than the sucralfate treated group

PART IV EFFECT OF YA-HOM ON GASTRIC ULCER HEALING

4.1 WIR-induced gastric lesion

The effect of CMC (1%), Ya-hom (4 g/kg) and cimetidine (0.1 g/kg) at day 1 after gastric ulcer induced by water immersion restraint stress (WIR) in rats were shown in Table 12 and Figure 16, 17. The 1% CMC had no significantly curative effect on gastric ulcer induced by WIR. Ya-hom treated group had a significantly lower gastric ulcer index than that of water and vehicle (CMC) control groups. Cimetidine treated group was not different from water and vehicle (CMC) control groups.

These results showed that Ya-hom treated group at a dose of 4 g/kg decreased WIR-induced gastric ulcer at day 1 with the curation of 57.67%.

The effect of CMC (1%), Ya-hom (4 g/kg) and cimetidine (0.1 g/kg) at day 4 after gastric ulcer induced by water immersion restraint stress (WIR) in rats were shown in Table 13 and Figure 18, 19. The 1% CMC had no significantly curative effect on gastric ulcer induced by WIR. Ya-hom treated group had a significantly lower gastric ulcer index than that of water and vehicle (CMC) control groups. Cimetidine treated group had a significantly lower gastric ulcer index than the water control group but was not different from the vehicle (CMC) control group.

These results showed that Ya-hom treated group at a dose of 4 g/kg decreased WIR-induced gastric ulcer at day 4 with the curation of 58.18%. Cimetidine at dose of 0.1 g/kg decreased WIR-induced gastric ulcer at day 4 with the curation of 28.09%.

Table 14 demonstrated the effect of Ya-hom and cimetidine on gastric ulcer healing at day 1 and day 4 after WIR-induced gastric lesion.

Table 12 Effect of 1% of sodium carboxy-methyl cellulose solution (CMC), Ya-hom 4 g/kg (YH4) and cimetidine 0.1 g/kg (Ci) at day 1 after gastric ulcer induced by water immersion restraint stress (WIR) in gastric ulcer rats

Rat No.	Ulcer area (mm^2)			
	Water	CMC	YH4	Ci
1	38	37	18	32
2	42	35	12	30
3	53	46	20	48
4	50	43	25	45
5	45	39	17	37
6	46	42	24	40
Ulcer index (mean ± SEM)	45.67 ± 2.20	40.33 ± 1.67	19.33 [a, b, c] ± 1.96	38.67 ± 2.89
% Curation	-	11.69	57.67	4.11

All values were expressed as mean ± SEM.

[a] $p<0.05$: significantly lower than the water control group

[b] $p<0.05$: significantly lower than the vehicle (CMC) control group

[c] $p<0.05$: significantly lower than the cimetidine treated group

% Curation of Ya-hom was calculated from water control.

% Curation of cimetidine was calculated from CMC control.

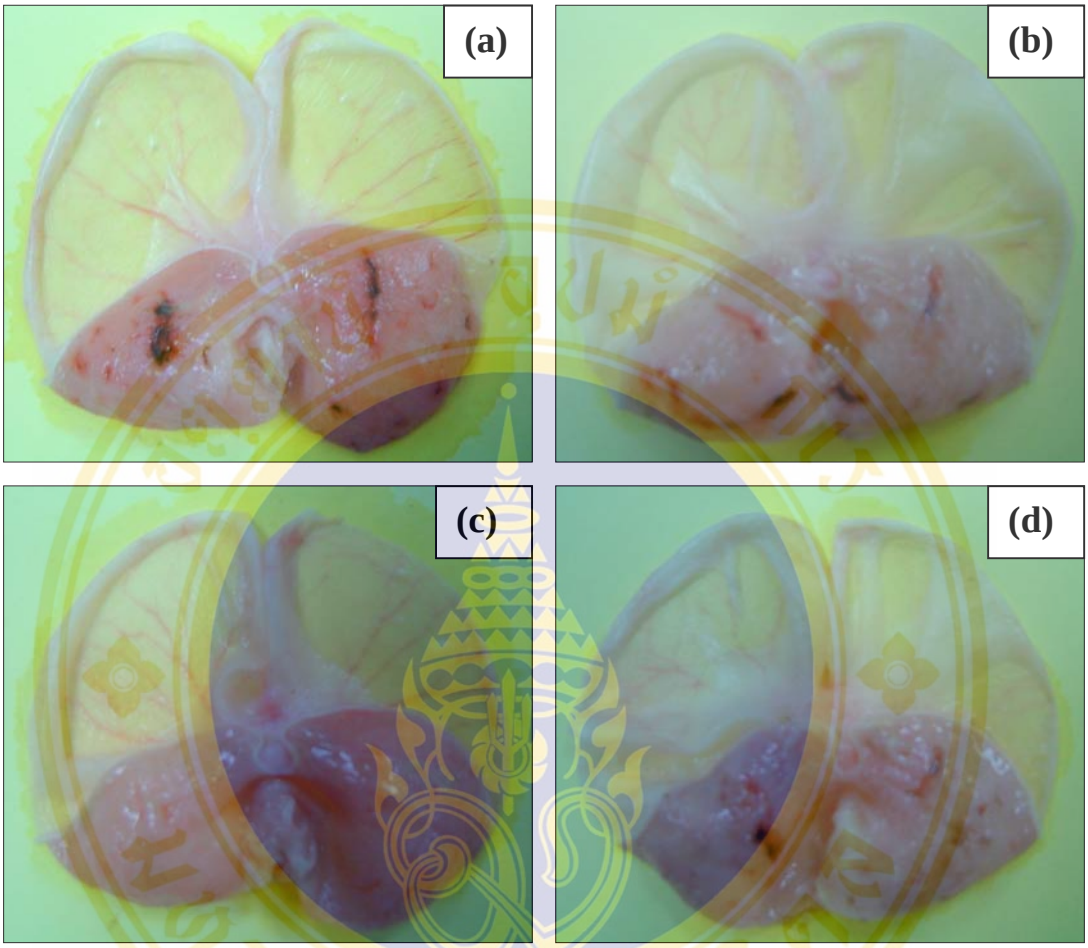

Figure 16 Effect of distilled water (a), 1% of sodium carboxy-methyl cellulose solution (b), Ya-hom 4 g/kg (c) and cimetidine 0.1 g/kg (d) at day 1 after gastric ulcer induced by water immersion restraint stress (WIR) in rats (n = 6)

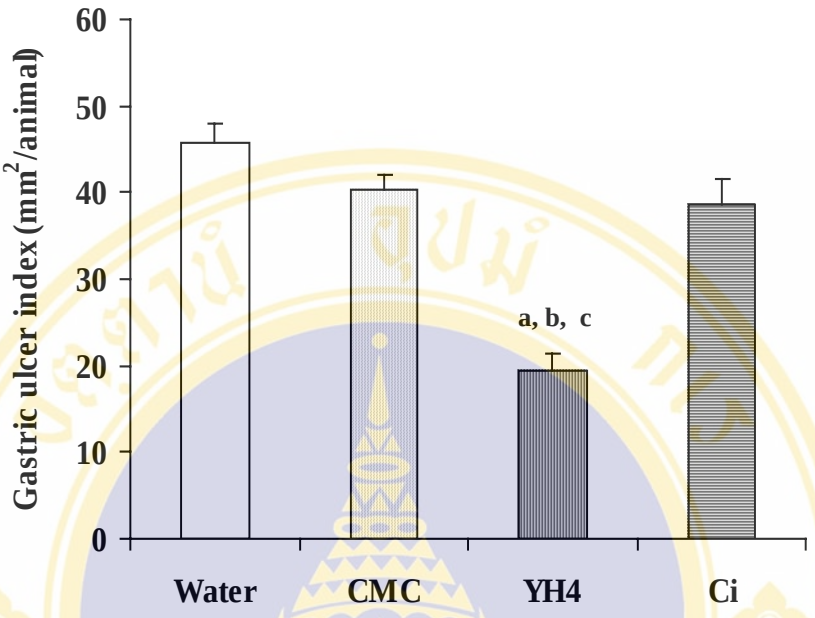

Figure 17 Effect of 1% of sodium carboxy-methyl cellulose solution (CMC), Ya-hom 4 g/kg (YH4) and cimetidine 0.1 g/kg (Ci) at day 1 after gastric ulcer induced by water immersion restraint stress (WIR) in rats (n = 6)

[a] $p<0.05$: significantly lower than the water control group

[b] $p<0.05$: significantly lower than the vehicle (CMC) control group

[c] $p<0.05$: significantly lower than the cimetidine treated group

Table 13 Effect of 1% of sodium carboxy-methyl cellulose solution (CMC), Ya-hom 4 g/kg (YH4) and cimetidine 0.1 g/kg (Ci) at day 4 after gastric ulcer induced by water immersion restraint stress (WIR) in rats

Rat No.	Ulcer area (mm^2)			
	Water	CMC	YH4	Ci
1	20	15	8	13
2	17	18	10	7
3	18	12	5	15
4	16	15	4	8
5	12	10	6	6
6	15	12	8	10
Ulcer index (mean ± SEM)	16.33 1.12	13.67 1.17	6.83 [a, b] 0.91	9.83 [a] 1.45
% Curation	-	16.29	58.18	28.09

All values were expressed as mean ± SEM.

[a] $p<0.05$: significantly lower than the water control group

[b] $p<0.05$: significantly lower than the vehicle (CMC) control group

% Curation of Ya-hom was calculated from water control.

% Curation of cimetidine was calculated from CMC control.

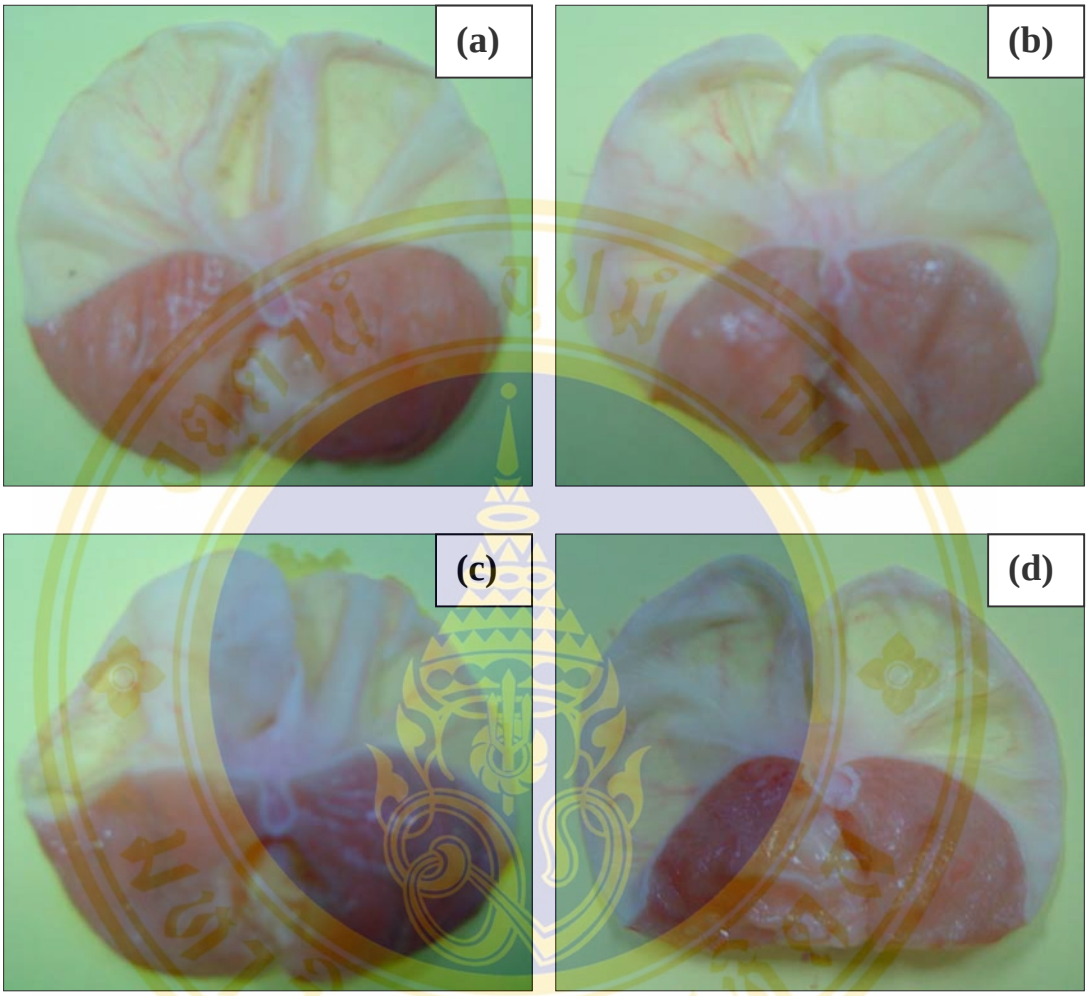

Figure 18 Effect of distilled water (a), 1% of sodium carboxy-methyl cellulose solution (b), Ya-hom 4 g/kg (c) and cimetidine 0.1 g/kg (d) at day 4 after gastric ulcer induced by water immersion restraint stress (WIR) in gastric ulcer rats (n = 6)

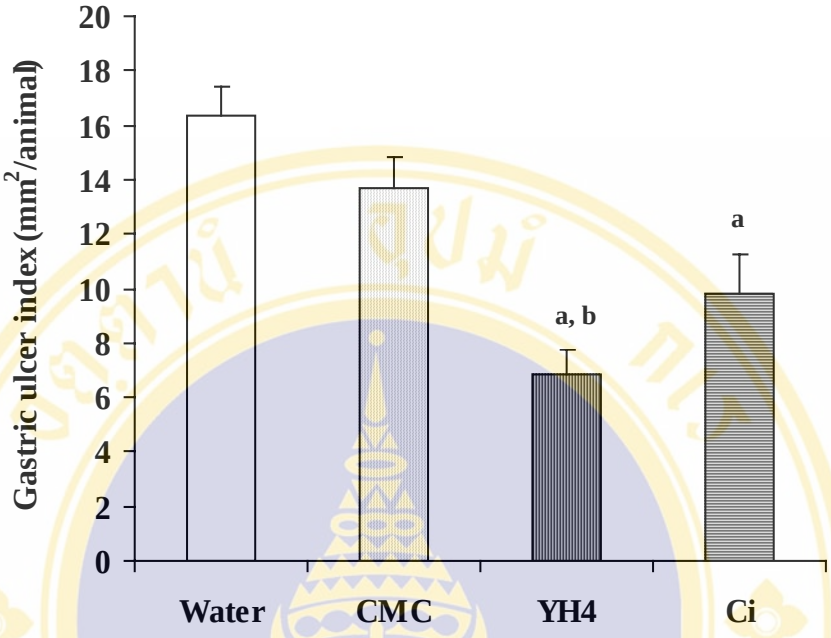

Figure 19 Effect of 1% of sodium carboxy-methyl cellulose solution (CMC), Ya-hom 4 g/kg (YH4) and cimetidine 0.1 g/kg (Ci) at day 4 after gastric ulcer induced by water immersion restraint stress (WIR) in rats (n = 6)

[a] $p<0.05$: significantly lower than the water control group

[b] $p<0.05$: significantly lower than the vehicle (CMC) control group

Table 14 Effect of Ya-hom and cimetidine on gastric ulcer healing at day 1 and day 4 after WIR-induced gastric lesion (n = 6)

Group	Gastric ulcer index (mm^2/animal), (% Curation)	
	at day 1	at day 4
Water control	45.67 ± 2.20	16.33 ± 1.12
Vehicle (CMC) control	40.33 ± 1.67	13.67 ± 1.17
	(11.69)	(16.29)
Ya-hom (4 g/kg)	19.33 ± 1.96 [a, b, c]	6.83 ± 0.91 [a, b]
	(57.67)	(58.18)
Cimetidine (0.1 g/kg)	38.67 ± 2.89	9.83 ± 1.45 [a]
	(4.11)	(28.02)

All values were expressed as mean ± SEM, comparing in the same day.

[a] $p<0.05$: significantly lower than the water control group

[b] $p<0.05$: significantly lower than the vehicle (CMC) control group

[c] $p<0.05$: significantly lower than the cimetidine treated group

% Curation of Ya-hom was calculated from water control.

% Curation of cimetidine was calculated from CMC control.

CHAPTER V
DISCUSSION

The selected Ya-hom brand used in the present study has been showed to inhibit gastric acid secretion both in gastric fistula rats (43) and isolated mouse whole stomach (44) and potentiate gastric visible mucus secretion (43). Decreasing aggressive factor and increasing defensive factor for gastric ulcer of Ya-hom provide the possibility of using Ya-hom for treatment of gastric ulcer. This indication of Ya-hom has never been claimed and verified. The present study then attempts to investigate the gastric ulcer protective effect of Ya-hom by using three gastric ulcer induction models. The effect of Ya-hom on gastric visible mucus secretion in hydrochloric acid- and aspirin-induced gastric ulcer rats was determined. In addition, the effects of Ya-hom on inflammatory and healing phase of gastric ulceration were assessed in water immersion restraint stress-induced gastric ulcer rats. The result will lead to the scientific information for gastric ulcer protection and treatment by Ya-hom which are the new uses of this recipe.

Hydrochloric acid (HCl)-, aspirin (ASP)- and water immersion restraint stress (WIR)-induced gastric lesion models were used in this experiment. Three models induced different characteristics of gastric ulcer by different mechanisms of gastropathogenesis. Hydrochloric acid caused necrosis of superficial epithelial cells on gastric mucosa with its direct necrotizing action (64). Aspirin produced round lesion or petichiae by depleting of prostaglandin production, leading to decreased gastric visible mucus secretion and gastric mucosal blood flow (2, 65). Stress induced hemorrhagic lesion through histamine release causing an increase in gastric acid secretion and gastric motility (3, 66). Using these three different gastric ulcer induction models to investigate the anti-gastric ulcer effect of Ya-hom will provide both the effects and some mechanisms of action. The dose of hydrochloric acid, the dose of aspirin and degree of water immersion affected gastric ulcer induction

were firstly determined to investigate the suitable conditions. The dose that gave a higher gastric ulcer induction was chosen for HCl- and ASP-induced gastric lesion models. For WIR-induced gastric lesion model, the degree that gave a higher gastric ulcer induction and did not kill the rats during the experiment was selected for this study. The selected conditions for studied the anti-gastric ulcer activity were an oral administration of 0.6 N HCl at the dose of 6 ml/kg for four hours, an oral administration of ASP suspended in 1% sodium carboxy-methyl cellulose (CMC) solution at the dose of 200 mg/5 ml/kg for six hours and WIR maintained at 16 $\pm$ 2 °C for five hours.

HCl-induced gastric ulcer (Fig. 5) had a highest severity (91.67 $\pm$ 4.2 mm) because HCl had direct damaging effect on gastric mucosa, whereas ASP-induced gastric ulcer (Fig. 6) had a lowest severity according to the ulcer morphology (Fig. 5, 6, 7) and WIR-induced gastric ulcer (Fig. 7) gave an intermediate degree of severity (59.33 $\pm$ 2.73 mm). 1%CMC used as solvent for aspirin and cimetidine had no protective effect in all three gastric ulcer induction models. Ya-hom (1, 2 and 4 g/kg) inhibited HCl-, ASP-and WIR-induced gastric ulcer in a dose dependent manner with the maximum inhibition of 93.45, 54.5 and 61.79%. Cimetidine inhibited HCl-, ASP- and WIR-induced gastric ulcer with the inhibition of 78.44, 76.29 and 48.02%. Both Ya-hom and cimetidine had a highest maximum inhibition on HCl-induced gastric ulcer. The inhibitory effect of Ya-hom on HCl- and WIR-induced gastric ulcer were higher than cimetidine. However, the inhibitory effect of Ya-hom on ASP-induced gastric ulcer was lower than cimetidine. These indicated that Ya-hom protected gastric mucosa against HCl, ASP and WIR damage by different mechanisms. Since cimetidine decreases gastric ulcer by inhibiting gastric acid secretion via blocking H_2 receptor at parietal cell and has lower gastric ulcer protective effect than Ya-hom on HCl- and WIR-induced gastric ulcer rats. Ya-hom should have another action besides gastric acid inhibition as previously report for its antiulcer action. Ya-hom has been shown to inhibit the stimulatory effect of histamine and carbachol on gastric acid secretion in gastric fistula rats (43) and inhibit histamine- and bethanechol-induced gastric acid secretion in isolated mouse whole stomach (44). Morever, some of Ya-hom's ingredients such as *Glycyrrhiza glabra* Linn (42) possesses gastric acid secretion inhibitory effect and *Saussurea lappa* Clark (19-21), *Cinnamomum cassia*

Blame (24-25), *Glycyrrhiza glabra* Linn (26-27), *Mentha arvensis* Linn (30) and *Atractylodes ovata* DC (31) have a gastric ulcer protective effect. So, the anti-gastric ulcer effect of Ya-hom may be due to these ingredients by decreasing gastric acid secretion. In addition to gastric acid secretion inhibition, Ya-hom has been found to increase gastric mucus secretion (43) making Ya-hom show the higher gastric ulcer protective effect than cimetidine in both HCl- and WIR-induced gastric ulcer models. In contrast to the HCl- and WIR-induced gastric ulcer models, Ya-hom has lower gastric ulcer protective effect than cimetidine on ASP-induced gastric ulcer rats. Since aspirin inhibits prostaglandin synthesis resulting in a decreasing of gastric mucosa barrier (decrease mucus and bicarbonate secretion and mucosal blood flow) (2), gastric acid then damages the gastric mucosa causing gastric lesion. Ya-hom has been shown to inhibit gastric acid secretion with the maximum inhibition of 45.5% and 53.8% when induced by histamine and carbachol, respectively (43). The low gastric acid inhibitory effect of Ya-hom may cause the lower gastric ulcer protective activity than cimetidine in ASP-induced gastric ulcer rats.

In gastric visible mucus secretion study, HCl induced an increase in mucus secretion whereas aspirin caused a decrease in mucus secretion. An increase in gastric visible mucus secretion after treated with HCl should be due to the direct irritation by acidity of HCl (64). In contrast to HCl, aspirin inhibits prostaglandin synthesis (2) causing a decrease in gastric visible mucus secretion making the gastric mucosa susceptible to be damaged by gastric acid. 1%CMC did not have any effect on mucus secretion in both gastric lesion induction models. Sucralfate, an aluminum salt of sulfated disaccharide, forms a viscous adhesive gel to protect the gastric mucosa form the gastric corrosive substances. Even through, sucralfate has some mucus stimulation effects, it showed completely protective effect on HCl-induced gastric mucus secretion (168.8 ± 6.7 vs 120.68 ± 13.84 as compared to normal, 118.31 ± 4.73 μg/g stomach) whereas it has almost no effect on aspirin decreasing mucus secretion (58.95 ± 5.13 vs 73.09 ± 12.42 as compared to normal, 118.31 ± 4.73 μg/g stomach). Sucralfate slightly increased the mucus secretion in aspirin-treated rats but it was not a significant change. From the observation of the gastric mucosa after gastric ulcer induction, sucralfate-treated gastric mucosa was intact and no gastric lesion. This showed that sucralfate is not be able to increase the gastric mucus inhibitory effect of aspirin but it

seemed to be able to protect the ASP-induced ulcer. Since aspirin not only inhibits mucus secretion but also reduces gastric mucosal blood flow, bicarbonate secretion, cell repair and replication leading to gastric ulcer (2), sucralfate has a gastric ulcer protective effect by protecting the mucosa from the aggressive factors. In contrast to sucralfate, cimetidine has no effect of HCl increasing mucus secretion (168.8 $\pm$ 6.7 vs 166.02 $\pm$ 11.2 as compared to normal, 118.31 $\pm$ 4.73 μg/g stomach) but is able to increase mucus secretion (58.95 $\pm$ 5.13 vs 125.12 $\pm$ 13.87 μg/g stomach) to the normal level (118.31 $\pm$ 4.73 μg/g stomach) in aspirin-treated rats.

Ya-hom showed an inhibitory effect on HCl-induced gastric visible mucus secretion but it has a lower inhibitory effect than sucralfate. Ya-hom probably has an inhibition on irritation effect of HCl. Ya-hom (4 g/kg) has been shown to increase gastric visible mucus secretion in normal gastric mucosa (43) which causes the high level of gastric mucus in Ya-hom-treated rat. Even through, Ya-hom-treated gastric mucosa had a slightly decrease in mucus secretion compared with water control group (HCl-treated group) (from 168.8 $\pm$ 6.7 to 141.79 $\pm$ 11.33 μg/g stomach), this effect was enough to decrease the mucus secretion to the level that was not significantly different to the normal gastric mucosa (non-HCl treated rats) (118.31 $\pm$ 4.73 μg/g stomach). In contrast to the HCl-treated gastric ulcer model, Ya-hom (4 g/kg) had a higher inhibitory effect on aspirin-decreased mucus secretion than sucralfate. Ya-hom increased the mucus secretion (from 58.95 + 5.13 to 95.03 μg/g stomach) to the non-significant different level to normal rats (118.31 $\pm$ 4.73 μg/g stomach). However, Ya-hom had lower effect than cimetidine to increase mucus secretion in aspirin-induced gastric ulcer rats. In this study, cimetidine is the best drug to recover the mucus secretion in the aspirin-induced gastric ulcer model. Since cimetidine inhibited the effect of histamine resulting in a decrease of the aggressive factors (gastric acid and pepsin) for damaging the gastric mucosa, the gastric mucosa will not develop gastric ulcer when the defensive factors are decreased by aspirin treatment. Even through, cimetidine has no direct effect on mucus secretion, it may attenuate the effect of aspirin-induced ulcer by several mechanisms such as decreasing gastric acid secretion, increasing mucosal blood flow and bicarbonate secretion which are able to protect the decrease of mucus secretion by aspirin. The result corresponds to the

gastric ulcer protective effect of Ya-hom and cimetidine determining by gastric lesion index (Table 8) which shows that Ya-hom had a higher protective effect than cimetidine in HCl gastric ulcer induction rats but had a lower protective effect than cimetidine in aspirin gastric ulcer induction rats.

The period of gastric ulcer development and healing of ulcer process has been previously described (70-71). At day 1 after WIR-induced gastric lesion, the gastric ulcer expands and then the ulcer reduces after gastric ulcer induction by water immersion for five hours at day 4. An expansion of ulcer area results from the tissue necrosis, accumulation of neutrophil and prominent inflammatory infiltration. This phase was observed at day 1 to day 3 after gastric ulcer induction and defined as the inflammatory phase of gastric ulceration. The intensive proliferation of epithelial cells, the development of granulation tissues and the formation of new blood vessels leads to a reduction of ulcer area. This phase was observed at more than 3 days after gastric ulcer induction and defined as the healing phase of gastric ulceration. The effect of all treatments were investigated at day 1 and day 4 after WIR-gastric ulcer induction and oral administration of 1%CMC, cimetidine and Ya-hom. 1%CMC had no curative effect in both days. Ya-hom (4 g/kg/day) decreased the effect of WIR-induced gastric ulcer at day 1 and at day 4 with the curation of 57.67 and 58.18%. Cimetidine could not decrease the effect of WIR-induced gastric ulcer at day 1 but decreased the WIR-induced gastric ulcer at day 4 with the curation of 28.09%. In addition, some of Ya-hom's ingredients such as *Eugenia caryophyllata* Thunb (22-23) and *Glycyrrhiza glabra* Linn (28-29) have a gastric ulcer repairing effect and *Glycyrrhiza glabra* Linn (72-74) and *Cinnamomum cassia* Blame (75) have an anti-inflammatory effect. Thus, the gastric ulcer healing effect of Ya-hom may be due to these ingredients by decreasing inflammation of gastric ulcer and potentiation of gastric ulcer healing.

As previously mention, some of Ya-hom ingredients have been shown to have an ulcer protective effect (19-21, 24-27, 30-31), an ulcer repairing effect (22-23, 28-29), a gastric mucus secretion stimulatory effect (31, 41) and a gastric acid secretion inhibitory effect (42). This recipe also has been shown to inhibit gastric acid secretion (43, 44) and increase visible mucus secretion (43). In addition, the present study found that this Ya-hom recipe had an anti-gastric ulcer activity and a gastric ulcer healing effect results from increased gastric visible mucus, decreased

inflammation and potentiated gastric ulcer healing. These result lead to the scientific information for gastric ulcer protection and treatment of Ya-hom which are the new uses of this recipe. Since most of Ya-hom recipe composed of some similar ingredients, it is possible that other recipes possess the same anti-ulcer activity. However, each Ya-hom formula also contains some different ingredients with a different ratio, the antiulcer effect should be individually investigated before claiming to use for gastric ulcer treatment.

CHAPTER VI
CONCLUSION

1. Ya-hom (1, 2 and 4 g/kg) had an anti-gastric ulcer activity in a dose dependent manner. The anti-gastric ulcer activities of Ya-hom (4 g/kg) were 119.1, 71.4 and 128.7% of those of cimetidine (0.1 g/kg) on hydrochloric acid-, aspirin- and water immersion restraint stress-induced gastric lesion, respectively, in rats.

2. Ya-hom (4 g/kg) with the potency lower than sucralfate (1 g/kg) decreased the effect of hydrochloric acid-stimulated gastric visible mucus secretion. Ya-hom with the potency lower than cimetidine (0.1 g/kg) decreased the effect of aspirin-inhibited gastric visible mucus secretion in gastric ulcer rats.

3. Ya-hom (4 g/kg/day) had both anti-inflammatory and gastric ulcer healing effect on water immersion restraint stress-induced gastric lesion in rats. Ya-hom had a two-fold higher ulcer healing effect than cimetidine (0.1 g/kg/day).

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