ฤทธิ์ป้องกันการเกิดแผลในกระเพาะอาหารของน้ำมันขมิ้นชัน

นางสาว นิตยา กิจสุภา

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GASTROPROTECTIVE EFFECT OF TURMERIC OIL

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การศึกษาครั้งนี้ มีวัตถุประสงค์เพื่อศึกษาฤทธิ์ในการป้องกันการเกิดแผลในกระเพาะอาหาร และ คุณสมบัติการเป็นสาร antioxidant ของน้ำมันขมิ้นชัน โดยการทดสอบประสิทธิภาพของน้ำมันขมิ้นชันในหนูขาว โดยการป้อนทางปาก ขนาด 0.075, 0.15, 0.3 กรัม/น้ำหนัก 1 กิโลกรัม เป็นเวลา 30 นาที ก่อนการให้สารเคมีที่ชักนำให้เกิดแผลในกระเพาะอาหาร ได้แก่ 0.6 N HCl, indomethacin 50 mg/Kg และทำการตรวจสอบผลการป้องกันการเกิดแผล จากการตรวจสอบ Ulcer index พบว่ามีผลในการป้องกัน จึงเก็บตัวอย่าง เพื่อทดสอบหากลไกที่เกี่ยวข้อง เช่น ทดสอบความเป็นกรด, การทำงานของเปปซิน, ตรวจสอบ mucus และในทางด้าน antioxidant นั้น ทดสอบโดยวิธี TLC และ spectrophotometric assay ตลอดถึงตรวจสอบ lipid peroxidation และ superoxide dismutase ผลการทดลองพบว่า น้ำมันขมิ้นชันสามารถป้องกัน การเกิดแผลได้ โดยที่ปริมาณ 0.3 กรัม/กิโลกรัมให้ผลดี ในการป้องกันการเกิดแผล ในกระเพาะอาหารที่เหนี่ยวนำด้วยกรดเกลือ และ อินโดเมทาซิน ได้ถึง 97.4 และ100 เปอร์เซ็นต์ ตามลำดับ ใกล้เคียงกับยามาตรฐาน atropine sulfate 1.25 mg/kg และ misoprostol 100 μg/kg โดยความเป็นกรดในน้ำย่อยลดลง เมื่อป้อนน้ำมันขมิ้นชัน ขนาด 0.075, 0.15 และ 0.3 กรัม/กิโลกรัมน้ำหนักตัว วัดค่าความเป็นกรด-ด่าง ได้ค่า pH 2.7±0.48, 3.0±0.00 และ 3.5±0.52 ทำให้การงานของเปปซินลดลง 4.8±25.21, 12.7±27.82 และ 62.6±12.94 เปอร์เซ็นต์ตามลำดับ พบว่า gastric wall mucus content เพิ่มขึ้น 23.3±2.61 และ 59± 6.95 เปอร์เซ็นต์ใกล้เคียงกับยามาตรฐาน การศึกษาคุณสมบัติการเป็นสาร antioxidant ทั้งวิธี TLC และ spectrophotometric assay พบว่ามีค่าต่ำ เพียง 36.7% ที่ 1000 ppm. เมื่อเทียบกับสารมาตรฐาน BHA และ Gallic acid ซึ่งมีค่าเท่ากับ 94.8% และ 95.1% ตามลำดับ การทดลองในหนูขาวพบว่า น้ำมันขมิ้นชันสามารถยับยั้งการเกิด lipid peroxidation จากการชักนำ ให้เกิดแผลโดยกรดไฮโดรคลอริก พบว่าปริมาณ MDA ในซีรัม มีค่าเท่ากับ 8.69±0.28, 7.65±0.76 และ 6.69±0.32 นาโนโมลต่อมิลลิกรัม โปรตีน ซึ่งลดลงเมื่อเทียบกับกลุ่มควบคุมที่เป็นแผลที่มีปริมาณ MDA เท่ากับ 10.97±2.01 นาโนโมลต่อมิลลิกรัมโปรตีน และในเนื้อเยื่อ ปริมาณ MDA มีค่าเท่ากับ 10.20±0.65, 9.59±1.69 และ 8.10±0.92 นาโนโมลต่อมิลลิกรัมโปรตีน ซึ่งลดลงเมื่อเทียบเท่ากับกลุ่มควบคุม ที่เป็นแผล ที่มีปริมาณ MDA เท่ากับ 13.39±1.38 นาโนโมลต่อมิลลิกรัมโปรตีน และนอกจากนี้พบว่ามีปริมาณ Superoxide dismutase (SOD) ในซีรัม เพิ่มขึ้นเป็น 12.98±0.86, 15.13±0.115 และ 16.84±1.53 ยูนิตต่อมิลลิกรัมโปรตีน เมื่อเปรียบเทียบกับกลุ่มควบคุมที่เป็นแผล 10.90±0.70 ยูนิตต่อมิลลิกรัมโปรตีน และในเนื้อเยื่อมีค่าเท่ากับ 22.90±2.03, 23.13±0.98 และ 23.9±1.40 ยูนิตต่อมิลลิกรัมโปรตีน เพิ่มขึ้นเมื่อเทียบกับกลุ่มควบคุมที่เป็นแผลที่มีค่าเท่ากับ 18.65±1.86 ยูนิตต่อมิลลิกรัมโปรตีน นอกจากนี้น้ำมันขมิ้นชันสามารถยับยั้งการเกิด lipid peroxidation จากการชักนำให้เกิดแผลโดยใช้อินโดเมทาซิน 50 มิลลิกรัมต่อกิโลกรัม พบว่า ในซีรัมมีปริมาณ MDA เท่ากับ 6.49±0.52 นาโนโมลต่อมิลลิกรัม ซึ่งลดลงเมื่อเทียบกับกลุ่มควบคุมที่ทำให้เกิดแผลที่มีปริมาณ MDA เท่ากับ 12.14±0.83 นาโนโมลต่อมิลลิกรัมโปรตีน และมีค่าลดลงเทียบเท่ากับยามาตรฐาน Misoprostol 100 ไมโครกรัมต่อกิโลกรัม และในเนื้อเยื่อมีค่าเท่ากับ 4.56±0.71 ซึ่งลดลง เมื่อเทียบกับกลุ่มควบคุม ที่เป็นแผลที่มีปริมาณ MDA เท่ากับ 9.53±0.44 นาโนโมล/มิลลิกรัมโปรตีน นอกจากนี้ยังพบว่ามีปริมาณ SOD ในซีรัมในกลุ่มที่ให้น้ำมันขมิ้นชันในปริมาณ 0.3 กรัมต่อกิโลกรัม เพิ่มขึ้นเป็น 6.48±0.52 จากกลุ่มควบคุมที่ทำให้เกิดแผล 8.71±2.48 และในเนื้อเยื่อเพิ่มขึ้น 26.15±2.84 จากกลุ่มควบคุมที่ทำให้เกิดแผล 9.92±1.93 ยูนิตต่อมิลลิกรัมโปรตีน

ภาควิชา.....ชีวเคมี.....ลายมือชื่อนิสิต.....

สาขาวิชา.....ชีวเวชเคมี.....ลายมือชื่ออาจารย์ที่ปรึกษา.....

ปีการศึกษา.... 2545.....ลายมือชื่ออาจารย์ที่ปรึกษาร่วม.....

4376584133: MAJOR BIOMEDICINAL CHEMISTRY
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NITAYA KITSUPA : GASTROPROTECTIVE EFFECT OF TURMERIC OIL. THESIS ADVISOR: ASST. PROF. NIYADA KIATYING-AUNGSULEE, Ph.D., THESIS CO-ADVISOR: ASSOC. PROF. PIYAWAN SURINRAT, Ph.D., 97 Page. ISBN 974-17-3320-8

This research aims to study the gastroprotective and antioxidant effects of turmeric oil. Gastroprotective effect was done in rat's model by using 0.6N HCl and indomethacin as ulcer agents. Turmeric oil was given orally to rat at doses of 0.075, 0.15, and 0.3 g/kg 30 minutes prior to experimental induced ulcer. Gastroprotective mechanism of turmeric oil was examined by using ulcer index as measuring gastric pH, pepsin activity and gastric wall mucus content. Antioxidant of turmeric oil was studied by using Thin Layer Chromatography (TLC) method and spectrophotometric assay of 2, 2-diphenyl-1-picrylhydrazyl (DPPH) at λ= 517 nm. Measuremet of superoxide dismutase (SOD) and lipid peroxidation in same groups of ulcer inducing rats were also determined. Turmeric oil 0.3 g/kg exhibited a significant gastroprotection against ulcerative condition. The values of 97.41% and 100% ulcer inhibition were obtained in HCl model and indomethacin model respectively, compared to control. These doses were comparable to atropine sulfate 1.25 mg/kg and misoprostol 100 µg/kg respectively. Three oral doses of turmeric oil could reduce pH of gastric acid from 2.5±0.53 to 2.7±0.48, 3.0±0.00, 3.5±0.52 respectively, reduce pepsin activity 4.8± 25.21, 12.7±27.82, 62.6±12.94% and increase gastric wall mucus content 23.3±2.61, 59±6.95% accordingly. Turmeric oil showed less antioxidation property by TLC method, which conformed to the spectrophotometric assay of 36.7% radical scavenging properties compared to 95.1% of gallic acid and 94.8% of 3-tert-butyl-4-hydroxyanisole (BHA) standard reference at 1000 ppm. Treatment of turmeric oil could reduce lipid peroxidation on HCl induced ulcer to range 8.67±0.28, 7.65±0.76 and 6.69±0.32 nmol/mg protein in serum compared to control 10.97±2.01 nmol/mg protein and 10.20±0.65, 9.59±1.69 and 8.10±0.92 nmol/mg protein in tissue compared to control 13.39±1.38 nmol/mg protein. Increased SOD 12.98±0.86, 15.13±0.12 and 16.84±1.53 units/mg protein in serum compared to control 10.90±0.70 units/mg protein and 22.90±2.04, 23.13±0.98 and 23.91±1.40 units/mg protein in tissue compared to control 18.65±1.86 units/mg protein. Turmeric oil could reduce lipid peroxidation in serum on indomethacin induced ulcer 6.49 ±0.52 nmol/mg protein compared to control 12.14±0.83 nmol/mg protein and 4.56±0.71 nmol/mg protein compared to control 9.53±0.44 nmol/mg protein in tissue, and the turmeric oil treated at dose of 0.3 g/kg could increase SOD in indomethacin induced ulcer 11.05±0.94 units/mg protein in serum compared to control 8.71±2.48 units/mg protein and 26.15±2.84 units/mg protein in tissue compared to control 9.92± 1.93 units/mg.

Department ….Biochemistry…….Student's signature……..
Field of study…Biomedicinal Chemistry…..Advisor's signature……..
Academic year……2002…….……Co-advisor's signature……..……

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ABBREVIATIONS

Ach	=	Acetylcholine
°C	=	Degree cellsius
etc.	=	et cetera
DPPH	=	2,2-diphenyl-1-picrylhydrazyl
DU	=	Duodenal ulcer
et al.	=	et alii (and other)
EP receptors	=	Epithelial receptors
EtOH	=	Ethanol
Fig.	=	Figure
g	=	Gram
GC-MS	=	Gas chromatography-mass spectroscopy
GERD	=	Gastroesophageal reflux disease
GI	=	Gastrointestinal
UGIB	=	Upper gastrointestinal bleeding
GU	=	Gastric ulcer
HCl	=	Hydrocholic acid
hr	=	hour
H_2RA	=	H_2-receptor antagonists
H.Pylori	=	*Helicobacter pylori*
kg	=	Kilogram (s)
LPO	=	Lipid peroxide
M	=	Molar
MDA	=	Malondialdehye
m	=	Meter (s)
MHA	=	Mega hertz
mg	=	Milligram (s)
min	=	Minute
ml	=	Millimeter (s)
mM	=	Millimolar
m.p.	=	Melting point

MW	=	Molecular weight
nm	=	Nanometer
NBT	=	Nitroblue tretrazolium
NMR	=	Nuclear magnetic resonance spectroscopy
NSAIDs	=	Nonsteroidal anti-inflammatory drugs
No.	=	Number
ppm	=	Part per million
PPIs	=	Proton pump inhibitions
RNS	=	Reactive nitrogen species
ROS	=	Reactive oxygen species
rpm	=	Round per million
SOD	=	Superoxide dismutase
TBA	=	Thiobarbituric acid
TLC	=	Thin layer chromatography
TTRF	=	Tocotrienol-rich fraction
μg	=	microgram (s)
μl	=	micro liter (s)

CHAPTER I

GENERAL BACKGROUND

1. Introduction

1.1 Rationale for the study of anti-gastric ulcer activity of *Curcuma longa* L.

Gastric ulcer patients has been a high trend recently. Statistics of data records in Chulalongkorn Hospital showed 266 hyperacid patients in the year 1999 (Sirisamut, Tangkiatkumjai and Kiattisaksakorn, 1999), while Sawanpracharak Hospital had 324 peptic ulcer from 969 patients admitted to general medical ward in 2001 (Thungwilai, 2001).

Major natural factors and social factors contributing to gastric ulcer disease are stress and non steroidal anti-inflammatory drugs (NSAIDs).

NSAIDs are one of the most commonly used medications in many countries including Thailand. They are used mostly for the treatment of musculoskeletal and arthritic syndromes. Elderly people used these drugs more frequent (Larkai *et al.*, 1987). In Thailand, many investigative studies showed that NSAID induced gastrointestinal (GI) effects, for example almost half (45.5%) of the patients with peptic ulcer bleeding (Sriussadaporn, Tanphiphat and Vilairat, 1988), NSAIDs is known as a cause of upper gastrointestinal bleeding (UGIB), risk a factors of UGIB in NSAIDs users are age, a history of dyspepsia, peptic ulcer, high dose of NSAIDs use and *Helicobacter pylori* (*H. pylori*) infection. 30% UGIB patients used NSAIDs was found in Chulalongkorn Hospital during January 1 to December 31, 2000 (Tangkiatkumjai and Ratisoontorn, 2001). In Pra Mong Kut Hospital, during February 1 and 28,1999 from about 1132 patients, 69.64% used NSAIDs and 31.36% used NSAIDs and gastroprotective drug (Tang and Suppakitkosol, 1999).

The period of August 1, 2001 to January 31, 2002, their were 324 patients prescribed with drugs for the treatment of peptic ulcer in about 519 times (30.60%), gastric ulcer 53 times (10.20%), eradication of *H. pylori* as well as NSAIDs prophylaxis 32 times each (6.20%). Eight drugs for treatment of peptic ulcer were found. Cimetidine was most frequent used (64%), followed by antacid (13.90%),

omeprazole (8.90%), ranitidine (8.30%), sucralfate (3.00%), famotidine (0.80%) and lansoprazole or rabeprazole (0.6%) (Thungwilai, 2001).

NSAIDs have been among the most widely used drugs and although the non selective NSAIDs have been notorious in causing gastrointestinal lesions, the mechanism of how these lesion are generated have remained obscure. The emergence of the selective NSAIDs is indeed a breakthrough as it ameliorates the gastrointestinal side effects. The use of the non selective NSAIDs is still popular due to its relatively cheap cost.

Due to high cost of available chemical drugs for treatment peptic ulcer, efforts were made to find a suitable agent from natural products of plant for the treatment of peptic ulcer. Some medicinal plants have been studied such as Plau-noi (Hiruma *et al.,* 1999), Kaprau (Singh and Majumdar, 1999), Banana (Costa, Amtonio and Souza, 1997) and Aloe vera (Reynolds and Dweck, 1999). They exerted protective action against experimental induced gastric ulcer in various models of lesion production, e.g. stress, HCl, NSAIDs, ligature, ethanol, ischemia-reperfusion, reserpine, serotonin and histamine as well as the production of free radicals and lipid peroxidation in the development of ulcers (Gutteridg, 1995).

In Thai traditional medicine, *Curcuma longa* L. ("Khamin Chan" or "Turmeric") is a perennial herb in the Zingiberaceae family. The rhizome is used not only as a coloring agent and spices in Thai cuisine but also as herbal medicine for the treatment of various diseases and symptoms especially in dyspepsia and stomachache (Sutisri, 2001).

There have been a number of reports indicating that turmeric rhizome possessed anti-gastric ulcer action (Rafatullah *et al.*, 1990). Controversial data on antipeptic ulcer activity of curcumin, the major component of *Curcuma longa* L. have been reported. The antiulcerogenic activity of curcumin was reported by Sinha *et al,* (1975), whereas Bhatia *et al*. (1964) indicated that curcumin did not show any protective action against histamine-induced gastric ulceration in guinea pigs. Many clinical studies on antiulcerative and antidyspepsia effects of turmeric powders were reported (Prucksunand *et al*., 1987 and 1997, Thamlikitkul *et al.,* 1989) and phase II clinical trial on effect of the long turmeric (*Curcuma longa* L.) on healing of peptic ulcer (Prucksunand *et al*., 2001). Animal studies suggested that hexane soluble fraction showed such healing effects (Permpipat *et al., 1996*). Turmeric oil was found in hexane soluble fraction, but there was no report on turmeric oil regarding this ulcer

protective effect produced yet. It is this report to investigate into more details to explain it's mechanism.

1.2 Objectives

1.2.1 To study the gastroprotective effect of turmeric oil

1.2.2 To study the antioxidant property of turmeric oil

2. Review of literature

2.1 Mechanism of gastric ulcer disease

2.1.1 Peptic ulcer disease

The stomach is a distensible organ located in the left upper quadrant of the abdomen, it's major function is to ingest food to provide controlled emptying into the duodenum. The stomach can be divided into four anatomic regions, the cardiac region, the fundus, the body and antrum or pyloric region. Five types of cell are found within the stomach. The surface mucous cells produce mucus and bicarbonate, the mucous neck cells produce mucus, the parietal cell secret HCl and intrinsic factor, the Zymogenic (Chief) cell secret pepsinogen and the endocrine cell (G cell) produce gastrin (Tangaumnoy, 1997). The abnormal function of stomach induced to disease.

Peptic ulcer disease refers to ulcerations in the mucosal lining of the lower esophagus, stomach, or small intestine. The two most common types of peptic ulcers are duodenal ulcers and gastric ulcers. Ulcers may by acute or chronic and shallow or deep. They are differentiated from the erosions characteristic of gastritis by depth of penetration (Dorothy and Debra, 1993).

The development of peptic ulcer requires acid, peptic activity and a break down of mucosal defense (Aly, 1987 cited in Rao *et al.*, 1998).

Peptic ulcer, an ulcer involving the mucsa, submucosa and muscular layer on the lower esophagua, stomach, or duodenum, due in part at leat to action of acid-pepsin gastric juice (McGraw-Hill, 2003)

2.1.2 The etiologic factors in peptic ulcer disease

The etiologic factors in peptic ulcer disease are nature factors and social factors. Nature factors or some factors shows injurious factors and protective factors. Both factors must be balance (Figure 1). Details are described below.

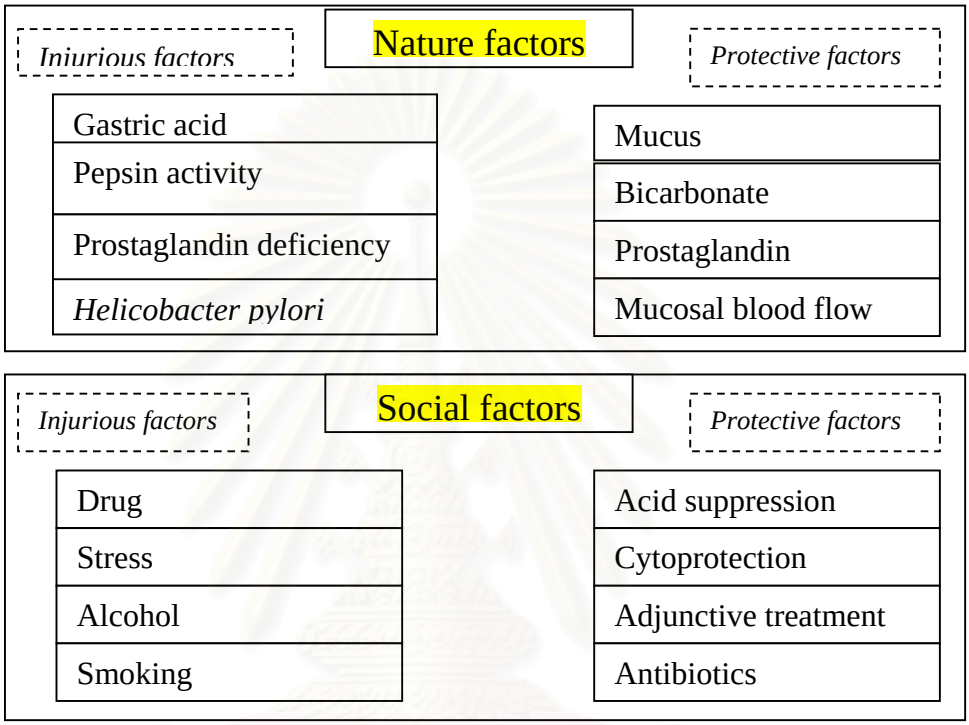


Figure 1. Injurious and protective factors in peptic ulceration
(Source adapted from: Feldman, Scharschmidt and Sleisenger, 1998)

2.1.2.1 Nature factors: injurious

- **Gastric acid**

The stomach is famous for its secretion of acid, but acid is only one of four major secretory products such as mucus, acid, proteases and hormones. The key player in acid secretion is a H^+/K^+ ATPase or "proton pump" located in the cannalicular membrane and secreted hydrochloric acid from parietal cells in to the lumen, (http://arbl.cvmbs.colostate.edu). Abnormal hyper-secretion of acid will increase pepsin activity. The reported showed that hydrochloric acid regulates the pepsin activity of gastric juice. Short-term studies in rats fed for 16 day with basal

diets and drinking water containing 0.3 % acid. 0.3 % acid plus 20 % pepsin, one set of groups was fasted for 48 hours before, being allowed access to food and fluid on the third day all groups receiving acid in their drinking water developed peptic ulcer like lesions (Matzner and Windwer, 1937).

- **Pepsin activity**

Pepsinogen, an inactive zymogen, is secreted into gastric juice from chief cells. Gastric acid could cleavage pepsinogen to proteolytically active pepsin. Both gastric acid and pepsin activity destroy gastric mucus, the defensive of stomach, induced to gastric ulcer. The optimum pH of pepsin activity is between 2 and 3. Secretion of gastric acid plus effects of pepsin promote tissue injury (Matzner and Windwer, 1937). High volume and activity of pepsin and the contract to acid contributed to the damage of gastric mucus, the results slim gastric mucus, may be induced gastric ulcer.

- **Prostaglandin deficiency**

Prostaglandin E_2 (PGE_2) and I_2 (PGI_2) were produced by polyunsaturated fatty acid “arachidonic acid” PGE_2 and PGI_2 are cytoprotective agent by increasing mucus production. The inhibition of enzyme involved in prostaglandin production by NSAIDs such as aspirin, ibuprofen, and etc. Slim gastric mucus easy to gastric damage from acid and pepsin contraction, red or brown spot was characteristic of gastric ulcer from prostaglandin deficiency. Nonsteroid anti-inflammatory agent well known to cause gastrointestinal damage in many species including rat and humans. Administration of compounds such as indomethacin leads to the acute formation of gastric mucosal erosions, indomethacin caused marked inhibition of prostacyclin. Aspirin caused a prolonged inhibition of small-intestinal cyclooxygenase activity (Whittle, 1981)

- ***Helicobacter pylori*** (*H. pylori*)

Helicobacter pylori are either gram-negative or gram-positive bacteria with aerobic or microaerophilic, spiral-shaped bacilli that are motile by way of flagella at one end of the cell. Generally, gram-positive bacteria are more susceptible to the damaging effects of fatty acids than gram-negative bacteria. *H*. pylori is sensitive to unsaturated free fatty acids through their incorporation into phospholipids and membrane destruction (Khulusi *et al.,* 1995). The bacterium lives deep in the gastric mucus layer adjacent to the gastric mucosa. The immune response consists of the recruitment of leukocytes, polymorphonuclear cells and plasma cells into the gastric mucosa and this leads to the development of gastritis. *H. pylori* infection and NSAIDs used independently and significantly increase the risk of peptic ulcer and ulcer bleeding (Huang, Sridhar and Hunt, 2002).

Moreover, 75-85% of chronic ulcer in human are due to *H. pylori* infection in the stomach-wall which potential the polymorphic nuclear leucocyte oxidative burst leading to considerable production of reactive oxygen metabolites which degenerate the tissue causing peptic ulcer (Banerjee *et al.,* 1994)

2.1.2.2 Nature factors: protective factors

- **Mucus**

The surface mucous cells and the mucous neck cells at gastric antrum are responsible for secreting a viscous, alkaline mucus that coats the surface epithelial cells of stomach. This mucous which normally is about 1 to 1.5 mm thick, provides a barrier against the proteolytic and highly acidic gastric contents. Any gastric irritation causes a tremendous increase in the production of mucus. If the barrier was damaged, ulcers may occur (Dorothy and Debra, 1993).

Mucus layer can be cleaved by pepsin, so that continual production was required. Prostaglandins are also cytoprotective by increasing mucus production and blood flow. Deficiency in prostaglandin may reduce HCO^-_3 due to mucosal damage induced gastric ulcer. The first line defense was the mucus and bicarbonate barrier

deficiency that made slim mucus and damage. The second line was the damage of epithelial mechanism and plasma membrane (as shown in Figure 2).

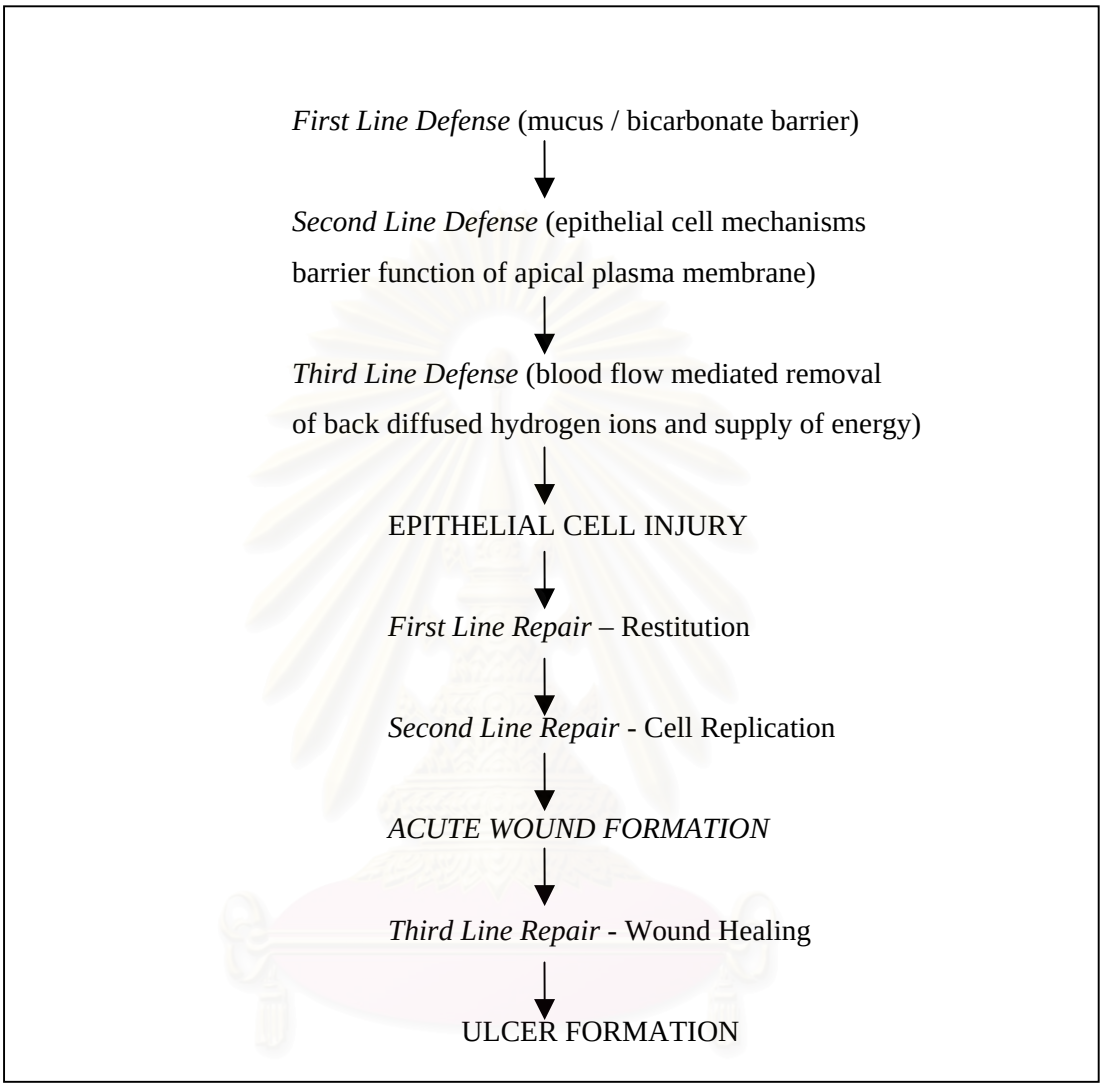


Figure 2. Mucosal defence
(Source adapted from: Feldman, Scharschmidt and Sleisenger, 1998)

- **Bicarbonate**

Bicarbonate is transported out of the basolateral membrane in exchange for chloride. The outflow of bicarbonate into blood results in a slight elevation of blood pH known as the “alkaline tide”. This process serves to maintain intracellular pH in the parietal cell (http://arbl.cvmbs.colostate.edu).

Bicarbonate content creates a micro-environment around surface cells to prevent acid damage, bicarbonate secretion is inhibited by stress.

- **Prostaglandins**

Arachidonic acid metabolism possess the ability to synthesize several prostaglandins. These compounds function as local regulatory agents. PGE_2 and PGI_2 are stimulate the secretion of mucus and bicarbonate in the gastric epithelial cells and decrease secretion of gastric juice in stomach (http://arbl.cvmbs. colostate.edu). Several models were proposed to explain mechanism of prostaglandin to protect peptic ulcer such as relationship between the prevention of rat gastric erosions and the inhibition of acid secretion by prostaglandins (Whittle, 1976) and the report of antiulcerogenic activity of crude hydroalcoholic extract of *Rosmarinus officinalis* L., the studied used indomethacin induced ulcer that relate to prostaglandin (Dias *et al.,* 2000).

- **Mucosal blood flow**

The blood flow to the mucosa is obviously important to the normal functioning of defence mechanisms. Like any organ system, oxygen delivery is impaired, function suffers. Hypotensis or any other cause of impaired oxygen delivery will affect gastric mucosal blood flow adversely (http://arbl.cvmbs. colostate.edu). Mucosal blood flow is one mechanism that protected gastric ulcer by increase mucosal blood flow and remove a high concentrate of H+ from gastric mucosal.

2.1.2.3 Social factors: injurious factors

- **Nonsteroidal anti-Inflammatory drugs (NSAIDs)**

Nonsteroidal anti-inflammatory drugs (NSAIDs) are drugs that reduce swelling, pain and fever. They are used to treat mild to moderate pain, arthritis, ankylosing spondylitis, bursitis, headaches, tendonitis and other conditions.

There are two components in NSAIDs induced ulceration. First, there is a local acid effect of dissolved drug. Most NSAIDs are weak acidic, lipid-soluble compounds. Since the cell membranes on the stomach wall contain lipids for protection against strong acid, they offer little resistance to the lipid-soluble NSAIDs. The NSAIDs act against the cell membrane, increasing its permeability. This results in cell swelling and death (Arthritis, Society, 1996). The local acid effect of NSAIDs has been reduced by enteric-coating, the drug delaying dissolution until later in the digestive process.

The second much more significant component to NSAID-induced ulceration is the systemic effect after being absorbed into the bloodstream. NSAIDs can be associated with inhibition of cyclo-oxygenase (COX-2) whereas their harmful side effects are associated with inhibition of cyclo-oxygenase (COX-1)(Warner, 1999). There are 2 isoforms, COX-1 and COX-2. COX-1 presents in stomach tissue produces prostaglandins which increase mucous and bicarbonate production, inhibit stomach acid secretion and increase blood flow within the stomach wall. COX-2 has enzyme induced inflammatory. The ideal objective of NSAIDs is to inhibit COX-2, but general NSAIDs was non specific and these both COX were inhibited, led to COX-1 reducing prostaglandin PGE_2 and PGI_2 production reduce HCO^-_3 and mucus compromised gastroduodenal mucosal defence (Figure 3). They are specific COX-2 inhibiters are produced to show less ulcerative effect while still maintain anti-inflammatory action.

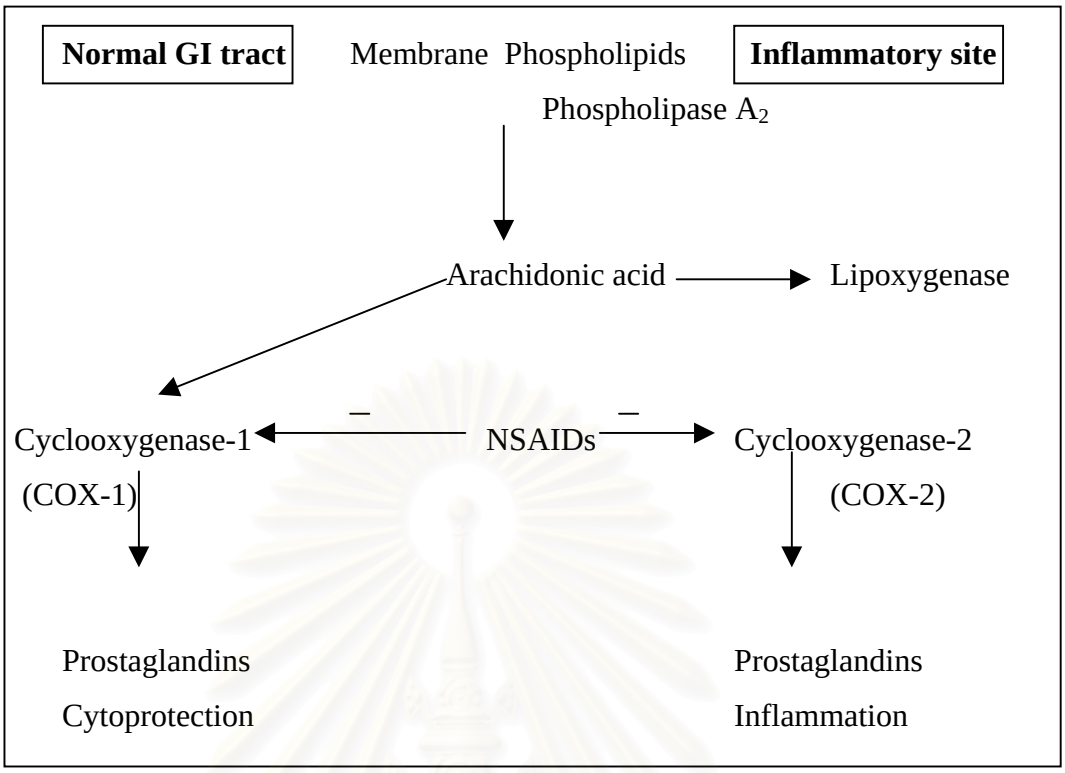


Figure 3. COX-1 and COX-2 in normal tissue and inflamed tissue
(Source adapted from: Wolfe, Lichtenstein and Singh, 1999)

Some example of NSAIDs are aspirin, indomethacin, diclofenac, ibuprofen, and etc. Indomethacin is used to reduce pain/swelling involved in osteoarthritis, rheumatoid arthritis, bursitis, tendinitis, gout, ankylosing spondylitis and headaches. Indomethacin more than 20 mg/kg were used to induce ulcer in animal experimental model of peptic ulcer (Singh and Majumdar, 1999, Rafatullah *et al.*, 1990).

NSAIDs are weak acid hence, high volume of NSAIDs can destroy gastric mucosa barrier by local acid and induced ion trapping and back diffusion, the describe follow (Figure 4).

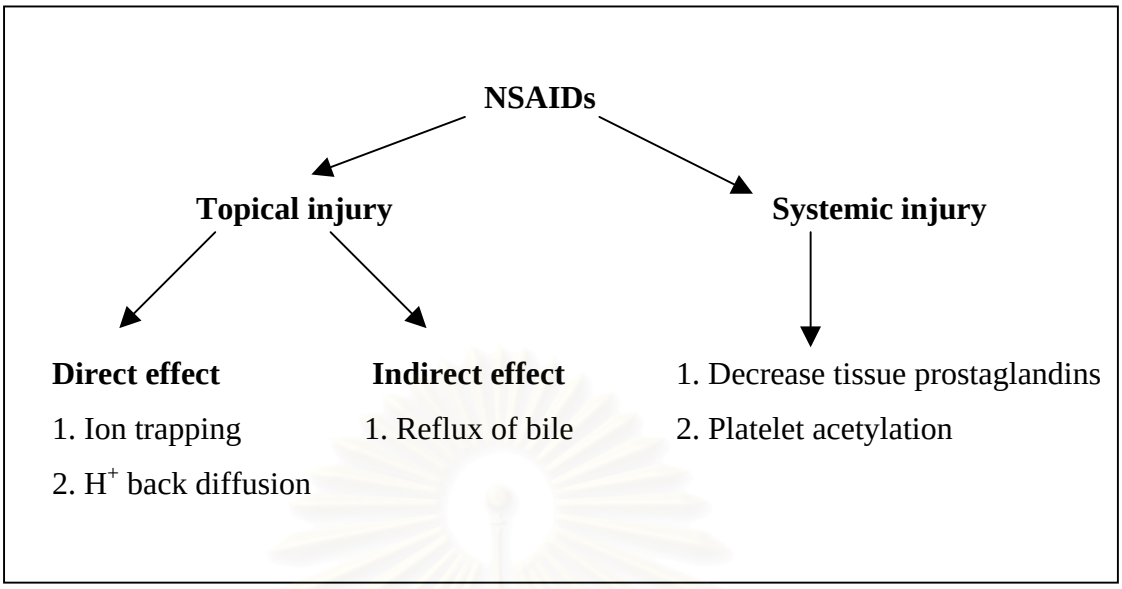


Figure 4. Mechanisms of NSAIDs-induced gastroduodenal mucosal damage. (Source: Schoen and Vender, 1989)

- **Acute Stress ulcer**

Hypothermic restraint stress ulcers have been widely used experimentally for the evaluation of anti-ulcer activity in rats because of data reproducibility (Murakami *et al.,* 1985). Disturbances of gastric mucosal microcirculation, alteration of gastric secretion and abnormal motility have been considered to be the pathogenic mechanism responsible stress induce gastric mucosal lesions and gastric mucus depletion (Koo *et al.,* 1986).

Stress produced acute haemorrhagic lesions along with discrete ulcerations of the gastric mucosa. Stress affects the target organ primarily, the anterior hypothalamus cholinergic pathways (Vagus) secreted acetylcholine (Ach) to the parietal cell. The stress ulcer was used in the experimental model, for the production of chronic gastric ulcer in Rhesus monkey (Dasupta and Mukherjee, 1974).

When the tissue injured there is increase in oxidative stress induced lipid peroxidation and the malondialdehyde the last production were detected (Figure 5).

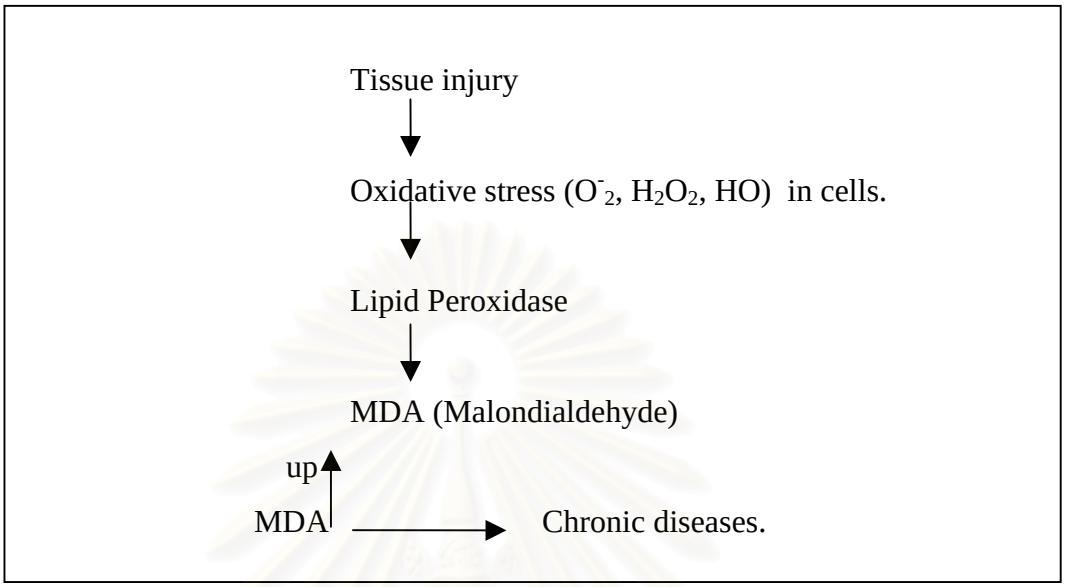


Figure 5. Relationships of stress and ulcer

- **Other factors contributing to ulcer disease**

- Smoking cause delay in healing of both DU and GU. Ulcer development related to number of cigaretter smoked to more 10 cigarettes per day. Nicotine is one of the active components in cigarette smoke, exhibited an inhibitory effect on both cell migration and cell proliferation (Shin, 2001).

- Alcohol has been shown to increase gastric acid secretion by direct stimulation of parietal cells. Ethanol induced gastric injury is associated with the significant production of free radicals leading to increased lipid peroxidation which caused damage to cell and cell membreans (Fridouich, 1978). Ethanol delayed gastric emptying, administration of ethanol produced more apoptotic cell and vascular damages in the gastric mucosa (Edgar, 2000).

Conclusion of the factors contributing to gastric ulcer were gastric mucus deficiency. It was major factors contributing to gastric ulcer. The damages of gastric mucus may be explained from (1) highly acidic gastric contents induce pepsin activity which mucus layer could be cleaved by pepsin. (2) Inhibition of COX-1 from NSAIDs which COX-1 imported for normal stomach. (3) The stress induced gastric ulcer in several mechanism such as promote gastric acid, pepsin activity, reduced gastric mucus (Rafatullah *et al.,* 1990) and free radical induced lipid peroxidation.

2.1.3 Contemporary approach to ulcer therapy

Since etiology of gastric ulcer were composed of 2 factors (social factor and natural factor) studies on mechanism to develop to new drugs target on two point: acid suppression and cytoprotective).

2.1.3.1 Acid suppressions

- **H_2-blocker**

A major function of gastric acid is secreted in response to the activation of H_2-histamine receptors on parietal cell membranes and the subsequent activation of adenylate cyclase. H2-antihistamines (e.g. cimetidine, ranitidine, famotidine) have pronounced effects on both acid and pepsin secretion elicited by histamine, gastrin and Vagus stimulation, thus consistent with a major role of histamine in acid secretion

-Cimetidine not only inhibits basal and stimulated acid secretion, it also decreases pepsin output and secretion to heal peptic ulcer. But **cimetidine has side effect,** (3%) side effect of cimetidin more than the other H_2-antihistamines (1%). The most commonly seen side effects consist of headache, dizziness, diarrhea and muscular pain etc. (http://rxweb.nlu.edu/pharmacy/414/DIDrugs.htm).

-Ranitidine inhibits gastric secretion and more powerful inhibitor of pepsin secretion. It has the advantage of not having the antiandrogenic or the prolactin stimulating, but also has some side effects.

-Famotidine competitive H2-receptor antagonist, famotidine slowly dissociates from its active site on the parietal cell. Side effects like ranitidine, famotidine has very low binding affinity for either cytochrom P-450 or for the androgen receptors.

- **Proton pump inhibitors (PPI)**

The proton pump involves an H^+/K^+-ATPase, used energy from the hydrolysis of ATP drives H+ into the canaliculi of the parietal cell, H+ diffuses into the lumen of the stomach to form HCl. Proton pump inhibitors (PPI) are most potent inhibitor of acid secretion. Some example drugs Omeprazole and Lansoprazole.

- Omeprazole, the prototype inhibitor, is a substituted benzimidazole prodrug that is activated at acid pH to a sulfenamide that binds to and irreversibly inhibition the ATPase. Because of this irreversible inhibition of enzyme, profound inhibition of gastric secretion (95-100%). **Side effects** are headache, diarrhea and abdominal pain.

2.1.3.2 Cytoprotective agent

Superficial gastric epithelial cell secrete mucus and bicarbonate forming a protective layer. This mucus layer normally inhibits diffusion of H+ and large protein such as pepsin (e.g. Sucralfate, Misoprostol, Bismuth Compounds).

- **Sucralfate** is a complex aluminum salt of sucrose containing eight sulfate groups. Mechanism of action is local effect to selectively bind to free protein in the base of ulcer craters. It forms a complex, which protects from acid, pepsin and bile salts, absorbs acid.
- **Misoprostol (Cytotec®)** is methyl analog of prostaglandin E1 that binds to epithelial receptors resulting in increase of mucus and bicarbonate production and inhibits gastric acid secretion. This drug protects gastric mucosa, reduces risk of gastric bleeding and ulcers. The clinical use is for prophylaxis for NSAIDs side effects and peptic ulcer (http://cim.ucdavis.edu).

- **Bismuth Compounds** appear to selectively bind to ulcers providing a coating and protection from acid and pepsin. Black stools, black tongue are main side effects. It has weak anti-microbial actions.

2.1.3.3 Adjunctive Treatment

- **Antacid**

Antacids are substances that neutralize hydrogen ion and provide mucosal protection. Antacids are weak bases that react with acid to form salt and water. Their action is to increase the pH of gastric content to 3.5 – 4.5 at which pepsin activity is diminished. These agents consist of metal salts, such as sodium bicarbonate, calcium carbonate, magnesium hydroxide, aluminum hydroxide.

- **Na salt (sodium bicarbonate)** is called baking soda and has been used in households for generation. Sodium bicarbonate reacts with hydrochloric acid to liberate carbon dioxide. Sodium bicarbonate over neutralizes the gastric acid and elevates the pH of the stomach contents above the optimal pH 4–5 this results in a rapid increase in acid secretion or “acid rebound” and a short duration of pain relief.

- Ca salt (calcium carbonate) calcium carbonate, a chalky taste compound, is a potent and effective antacid with a rapid onset and long duration of action. It creates rapid, prolonged, effective acid neutralization, disadvantages: acid rebound, milk-alkali syndrome, tendency to constipate.

- Mg salt (magnesium oxide, magnesium hydroxide) has a higher acid neutralizing capacity than aluminum hydroxide gel and does not produce systemic alkalosis. Diarrhea is the most common side effect associated with their use.

- Al salt (aluminum hydroxide, aluminum carbonate, aluminum phosphate, aluminum amino acetate)

2.1.3.4 Antibiotics

Since *H. pylori* proposed to be one caused of ulcer, so antibiotic are proposed.

- Amoxicillin is the most common form of penicillin. It is inexpensive, but many people are allergic to it.
- Tetracycline is effective, but tetracycline has unique side effect among antibiotics, including skin reactions to sunlight, possible burning in the throat, and tooth discoloration.

US FDA has approved therapies for *H. pylori* infection in gastric ulcer (Appendix A).

2.2 Turmeric

Turmeric (*Curcuma longa* L. or *Curcuma domestica* Val.) is a medicinal plant of Southeast Asia. This plant has been used to treat abdominal pain, which sounds like the pain caused by peptic ulcer. It has been used in empirical medicine for centuries by Thai traditional healers (Thamlikitkul *et al.* 1989).

Several studies about turmeric indicated the gastroprotective mechanism properties such as the studies of spiecs & gastric function: part I-effect of *Curcuma longa* on the gastric secretion in rabbits, *Curcuma longa* test meal does not show any change in the volume, free acid of peptic activity, but appreciably increase the mucus content of the gastric juice, it has been suggested that the beneficial effect of *Curcuma longa* as a therapeutic agent in gastric disorder and possibly be due to its mucous stimulatory effect (Mukerji, Zaidi and Singh, 1961). Fresh extract of *C. longa* was administered orally to guinea pigs pre-treated with phenylbutazone, an ulcerogenic agent. *Curcuma longa* L. was found to afford almost complete protection against phenylbutazone induced gastric ulcers (Dasgupta *et al.,* 1969). Turmeric increase the resistance of gastric mucosal cells to the necrotizing effect of strong irritant such as 0.6 N HCl used (Prucksunand *et al.,* 1997). Ethanol extracts of turmeric rhizome anti-gastric ulcer action (Rafatullah *et al.,* 1990). A major compound of ethanol extract was curcumin. Curcumin was reported to protect gastric ulcer (Sinha *et al.,* 1975*)* whereas curcumin did not show any protective action against histamine-induced gastric ulceration in guinea pigs (Bhatia *et al.,* 1964*)* and high dose of curcumin could reduce the mucin content (Gupta *et al.,* 1980). Animal studies suggested that hexane soluble fraction showed gastroprotective (Permpipat, Chuthaputti and Kiatying-Angsulee, 1996). Turmeric oil is main fraction in hexane soluble fraction, but no report of turmeric oil regarding this effect was produced yet. This thesis focuses on medicinal properties of turmeric oil.

Many clinical studies on anti-ulcerative and anti-dyspepsia effects of turmeric powders were reported (Prucksunand *et al.,* 1987 and Thamlikitkul *et al.* 1989), phase II clinical trial on effect of the long turmeric (*Curcuma longa* L.) on healing of peptic ulcer (Prucksunand *et al.,* 2001).

2.2.1 Botanical description of *Curcuma longa* L.

Curcuma longa L. (Khamin chan or Thai common names) is a parennial herb of Zingiberaceae family.

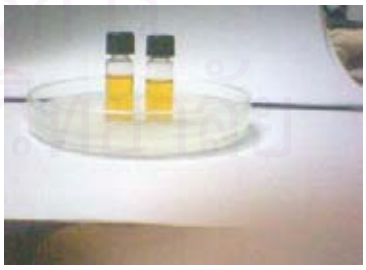
(a) Turmeric plant

(b) Flower of turmeric
(http://Kanchanapisek.or.th)

(c) Rhizomes of turmeric
(http://Kanchanapisek.or.th)

(d) Rhizomes of turmeric
(http://www-ang.kfunigraz.ac.at)

(e) The dry section of rhizomes



(f) Turmeric oil

Figure 6. Characteristic of *Curcuma longa* L.

- **Macroscopic characteristic of *Curcuma longa* rhizomes**

Dried rhizome is ovate, oblong or pear-shaped, often short-branched. Externally is yellowish to yellowish-brown, with root scars and annulations, fracture horny. It internally orange-yellow to orange, waxy, cortex separated from a central cylinder by a distinct endodermis (Jirawongse, 1982)

- **Microscopic characteristic of *Curcuma longa* rhizomes**

The transverse section of the rhizome is characterized by the presence of mostly thin-walled rounded parenchyma cells, scattered vascular bandles, definite endodermis, a few layers of cork developed under the epidermis and scattered oleoresin cells with brownish contents. The cells of the ground tissue are also filled with many starch grains. Epidermis is thin walled, consisting of cubical cells of various dimensions. The cork cambium is developed from the subepidermal layers and even after the development of the cork, the epidermis is retained. Cork is generally composed of 4-6 layers of thin-walled brick-shaped parenchymatous cells. The parenchyma of the pith and cortex contains curcumin and is filled with starch grains. Cortical vascular bundles are scattered and are of collateral type. The vascular bundles in the pith region are mostly scattered and they form discontinuous rings just under the endodermis. The vessels have mainly spiral thickening and only a few have reticulate and annular structure (available from: http://www.islamet.com).

- **Powdered plant material**

Powder plant material show deep yellow color with fragments of parenchymatous cells contain numerous altered, pasty masses of starch grains color yellow by curcumin, fragments of vessels; cork fragments of cell in sectional view; scattered unicellular trichomes; abundant starch grains; fragments of epidermal and cork cells in surface view and scattered oil droplets.

2.2.2 Chemical constituents of *Curcuma longa* rhizome

Thai pharmacopoeia has specified the chemical constituents as follows:

Foreign organic matter	: not more than 2%w/w
Acid-insoluble ash	: not more than 1%w/w
ethanol-soluble extractive	: not less than 10%w/w
Water-soluble extractive	: not less than 9.0%w/w
Total ash	: not more than 8.0%w/w
Water	: not more than 10%v/w
Curcuminoids content	: not less than 5.0%w/w
Volatile oil	: not less than 6.0%v/w

(Source: Jirawongse, 1995)

(Available from: http://www.islamset.com/sc/plants/curcumae.html)

Curcuma longa rhizome was reported to contain a lot of chemical groups such as curcuminoids, volatile oil, oleoresin, and etc. as shown in Table 1. Chemical structure of curcumin, turmerone and ar-turmerone were listed in Appendix A.

Table 1. Chemical constituents of *Curcuma longa* rhizome

Chemical group	Composition
Curcuminoids (3-5%)	Curcumin Demethoxycurcumin Bisdemethoxycurcumin Dihydrocurcumin
Volatile oil (3-7.2%)	**Monoterpenes** α-phellandrene, 1,8-cineol, p-cymenene, terpinolene, minor components **Sesquiterpenes** β-caryophyllene, α-caryophyllene, α-curcumene + α-Zingiberene, β-bisabolene, β-sesquiphellandrene, minor components **Sesquiterpene ketones** 17-26%Ar-turmerone, 15–18%β-turmerone , 30-32%α-turmerone, Turmeronal A, Turmeronal B minor compoments.
Carbohydrates	Arabinose Fructose Glucose Starch
Oleoresin	Oleoresin

(Source adapted from: Tuatakul, 1992, Sharma *et al.*, 1997, Imai *et al*., 1999)

2.2.3 Pharmacological and clinical activity of turmeric

Several reports about active compounds of turmeric have been published. Turmeric had been shown to exert various pharmacological activities as well as clinical activities such as anti-gastric ulcer and clinical, anti-oxidant, anti-cancer, anti-inflammatory, anti-microbial and toxicity (Table 2).

Table 2. Pharmacological activity and clinical of turmeric

Pharmacological activity / Clinical	Active compounds/ fractions	Reference
1. Clinical		
Dyspepsia Anti-peptic ulcer	Capsule of turmeric powder	Thamkikitkul *et al.,* 1989 Prucksunand *et al.,* 1987 Prucksunand *et al.,* 2001
2. Pharmacology		
Anti-gastric ulcer	Turmeric powder	Mukerji, Zaidi and Singh 1961
	Aqueous extract	Dasupta *et al.,* 1969 Prucksunand *et al.,* 1997
	Ethanol extract Haxane extract	Rafatullah *et al.,* 1990 Permpipat, Chuthaputti and Kiatying-Angsulee, 1996
	Curcumin	Sinha *et al.,* 1975
Induced gastric ulcer	Curcumin	Bhatia *et al.,* 1964 Gupta *et al.,* 1980

Table 2. Pharmacological activity and clinical of turmeric (Cont.)

Pharmacological activity / Clinical	**Active compounds/ fractions**	**Reference**
3. <u>Others</u>		
Antioxidant	Curcumin Demethoxycurcumin Bisdemethoxycurcumin Curcuminoids	Dikshit, 1995 Ramsewak, Dewitt and Nair 2000
	Volatile oil	Jayaprakasha *et al.*, 2002
Anti-cancer	Curcumin	Kuttan *et al.*, 1985 Singh *et al.*, 1998 Polasa *et al.*, 1991 Huang *et al.*, 1994
Anti-inflammatory (Acute inflammation)	Curcumin	Chandra and Gupta, 1972 Chuthaputti and Permpipat, 1994
	Volatile oil	Ammon, 1993
(Chronic inflammation)	Volatile oil Sodium curcuminate Curcumin	Chandra and Gupta, 1972 Ghatak and Basu, 1972

Table 2. Pharmacological activity and clinical of turmeric (Cont.)

Pharmacological activity / Clinical	**Active compounds/fractions**	**References**
Anti-microbial		
- Anti-bacterial Anti H. pylori	Curcuminoids Curcumin Volatile oil	Gritsanapan, 2000 Mahady *et al.,* 2002 Munzenmaier, 1997 Benerjee and Nigam, 1978
- Anti-fungi	Curcumin Volatile oil	Jayaprakasha *et al.*, 2001 Benerjee and Nigam, 1978
Toxicity	Ethanol extract Curcumin Oleoresin	Sittisomwong *et al.,* 1990 Qureshi, Shah and Ageel, 1992 Bille *et al.*, 1985

The active compounds of anti-gastric ulcer had been studied to search for active agent that protected the stomach from ulcer.

Pharmacological activity

Mukerji, Zaidi and Singh (1961) studied effect of *Curcuma longa* L. (turmeric) on gastric secretion in rabbits, that found the beneficial effect of *Curcuma longa* L. as a therapeutic agent in gastric disorders may possibly be due to its mucous stimulatory effect. Dasupta *et al.* (1969) studies fresh extract of *Curcuma longa* L. on experimental gastric ulcers in guinea pigs. *Curcuma longa* L was found to afford almost complete protection against phenylbutazone-induced gastric ulcer. Turmeric increase the resistance of gastric mucosal cells to the necrotizing effect of strong irritant such as 0.6 N HCl used (Prucksunand *et al.*, 1997). Ethanol extracts of turmeric rhizome anti-gastric ulcer action (Rafatullah *et al.*, 1990). A major

compound of ethanol extract was curcumin. Curcumin was reported to protect gastric ulcer (Sinha *et al.,* 1975*)* whereas curcumin did not show any protective action against histamine-induced gastric ulceration in guinea pigs (Bhatia *et al.,* 1964*)* and high dose of curcumin could reduce the mucin content (Gupta *et al.,* 1980). Animal studies suggested that hexane soluble fraction showed gastroprotective (Permpipat *et al.,* 1996). A major fraction of hexane was turmeric oil, turmeric oil has not reported to studied antipeptic ulcer effect of turmeric oil.

Clinical

Prucksunand *et al.* (1987) studied effect of the long turmeric on healing of peptic ulcer, a preliminary report of 10 case study, they used capsule filled turmeric on healing of peptic ulcer was studied endoscopically in gastric and duodenal ulcer patients with abdominal pain. The drug was given orally with the dose of 2 capsules (250 mg each) four times daily, one half to an hour before meal and at bed time. Endoscopic examinations, the ulcer complete healing in 4 weeks or 12 week and the bood chemistry showed no change in hematologic system, liver and renal function. While a double-blind trial has found turmeric helpful for people with indigestion (Thamlikitkul *et al.,* 1989), resulted in people with stomach or intestinal ulcers have not shown it to be superior to a placebo and have demonstrated it to be less effective than antacids (Kositchaiwat,1993). Prucksunand *et al.* (2001) studied of phase II clinical trial on effect of the long turmeric on healing of peptic ulcer by used capsule filled turmeric, it was found that the erosion, gastritis and dyspepsia were absent 48% after 4 week, they abdominal pain and discomfort satisfactorily subsided in the first and second week and blood chemistry has no significant changes in hematological system, liver and renal function.

2.2.4 Turmeric oil from *Curcuma longa* rhizomes

The quality of turmeric rhizomes could be varied by ages of plants. At about 5 month-old (Chavalittumrong and Jirawattanapong, 1992) or 7 or 9 month-old the rhizomes gave the highest percent of volatile oil. The sample was cleaned, sliced and dried in hot air oven at 50 °C. Dried rhizomes were distilled with hydro distillation apparatus to produce turmeric oil.

Dried ground rhizomes of *Curcuma longa* L. containing high yield of volatile oil (8 –16 % v/w) are found in Phisanulok, Tak, Chiang Rai, Nakhon Phanom and Chumpron (Tuatakul, 1992). While turmeric with five-month-old from Nakorn pathom and Prachuap Khiri Khan contain volatile oil 9.79% and 11.98% (v/w), respectively (Chavalittumrong and Jirawattannapon, 1992).

The volatile oil from steam distillation of turmeric has characteristic odor and slighytly fluorescence. The physico-chemical properties of turmeric oil can be described as follows:

Specific gravity at 15 °C	-0.938 to 0.967
Optical rotation	-13 °O' to - 25°O'
Refractive index at 20 °	1.512 to 1.517
Acid number	0.6 to 3.1
Ester number	0.5 to 16
Ester number after acetylation	28 to 53
Solubility	soluble in 4 to 5 vol. of 80% alcohol;
Solubility	soluble 0.5 to 1 vol. of 90% alcohol

The active ingredients in turmeric oil from various location are different such as turmeric grown in Sukhothai contain 15.8% pinene, 3.23% borneol, 1.30% cineole, 0.15% turmerone and 0.77% phellandrene (Unjitwatana and Sengvanich, 1998). Dried turmeric rhizomes grown in Chumporn contain 54.62% β-turmerone, 9.09% α-turmerone, 16.18% ar-turmerone, 4.73% terpinolene, 2.37% α-phyllandrene and others, while the fresh rhizomes contain 93.24% β-turmerone, 1.67% α-turmerone, 2.76% ar-turmerone, terpinolene and Other (Caichompoo, 1999).

Studies of turmeric oil showed antifungi, antibacterial, antioxidant, and etc. Properties described as follows:

Antifungi: The activity of essential oil against pathogenic fungi such as *Aspergillus niger, Aspergillus flavus, Penicillium lilacinum, Penicillium javanieum, Trichoderma viride, Curvularia oryzae, Helminthosporium oryzae, Pestalotia lapagericola, Microsprorum gypseum, Trichophyton mentarophytes* (Banerjee and Nigam, 1978), *A. parasiticus, Fusarium moniliforme* and *Penicillium digitatum (*Jayaprakasha *et al.,* 2001).

Antibacterial: The activity of essential oil against human pathogenic bacteria gram negative such as *Bacillus subtilis, Escherichia coli, Klebsiell aerogenes, Salmonella paratyphi, Salmonella typhi, Straphylococus aureas, Erwinia carotovora, Pseudomonas solanacearum, Xanthomonas citri* and *Xanthomonas malroccarum* (Banerjee and Nigam, 1978).

Antioxidant: Jayaprakasha *et al.* (2002) studied antioxidant activity of turmeric oil by removing curcumin from turmeric oleoresin, and extracted oleoresin with hexane and concentrated to get turmeric oil and pass to silica gel column chromatography to obtaine three fractions and analyzed by GC and GC-MS. They found that turmeric oil contained aromatic turmerone (31.32%), turmerone (15.08%) and curlone (9.7%) whereas the fraction III has aromatic turmerone (44.5%), curlone (19.22%) and turmerone (10.88%). All fractions were tested on antioxidant by using the β-carotene-linoleate model system and the phosphomolybdenum method. Fraction III showed maximum antioxidant capacity.

Antimutagenicity: Jayaprakasha *et al.* (2002) studied antimutagenicity of turmeric oil. The protective action of turmeric oil and all fraction against the mutagenicity of sodium azide was evaluated by the Ames test using *S. typhimurium* TA100. Turmeric oil and all fractions showed an antimutagenicity ranging from weak to strong.

Anticarcinogenic: Synthetic turmerone has been reported to act as neoplasm inhibitior and anticarcinogenic (Baik *et al.*, 1993).

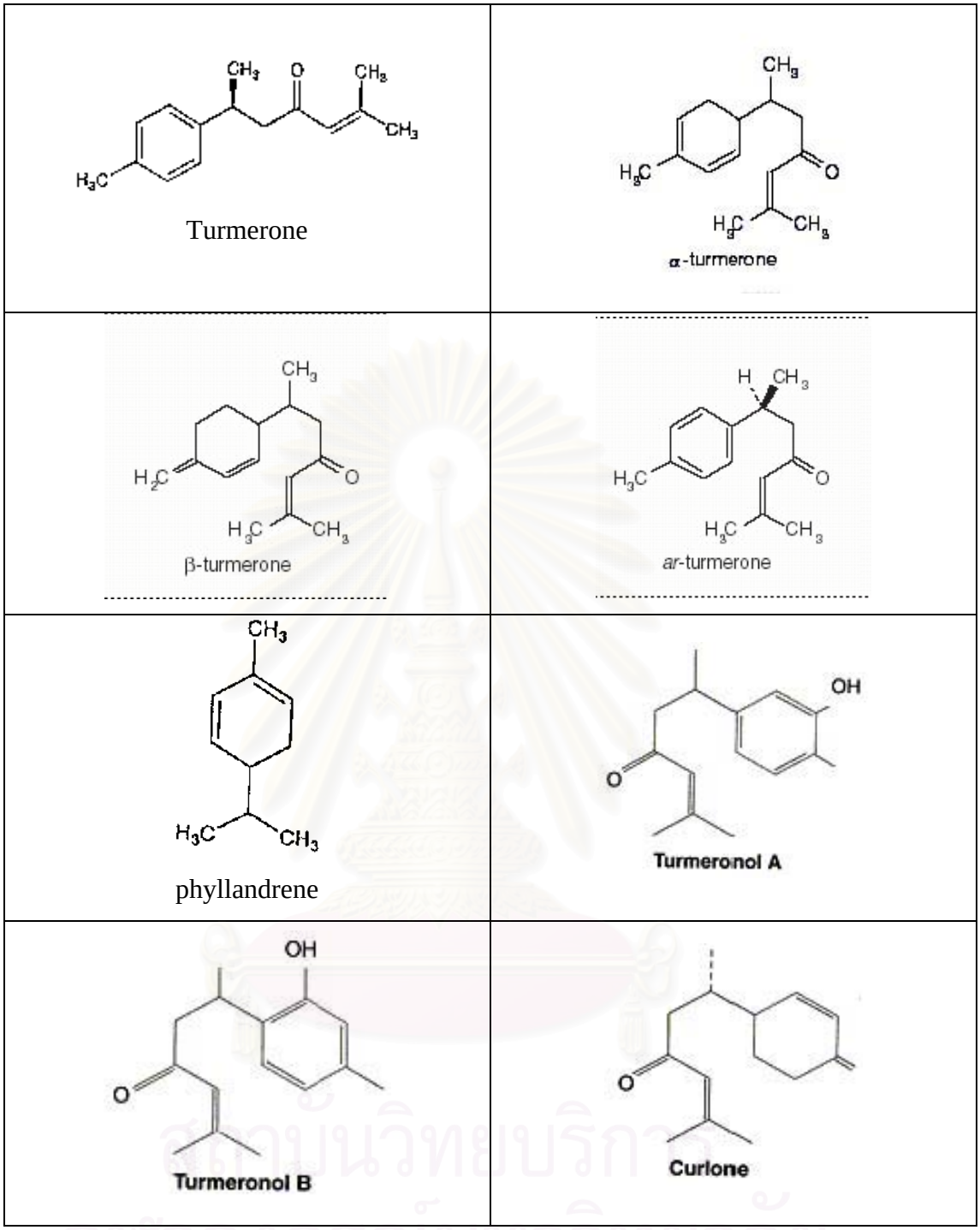


Figure 7. Chemical structure of main compounds found in turmeric oil
(Available from: http://www.curcuminoids.com)

2.3 Methods to study gastric ulcer

Several methods were used to study gastric ulcer. In general there are use the models of stress, pyloric ligation and ulcer inducing agents and biochemistry tests.

2.3.1 Water-immersion and restraint-induced stress ulcer

Stress induced gastric ulcer are probably mediated by histamine release with enhancement in acid secretion and reduction in mucous production (Parmar and Desai, 1993). The animals were placed individually in each compartment of a restraint cage and immersed vertically up to the level of the xiphoid in a water-bath (19-22°C) to induce stress ulcer (Rujjanawate *et al.*, 2001). Hypothermic restraint stress ulcers have been widely used experimentally for the evaluation of anti-ulcer activity in rats because of data reproducibility disturbances of gastric mucosal microcirculation, alteration of gastric secretion and abnormal motility of have been considered to be the pathogenic mechanisms responsible for stress-induced gastric mucosal lesions and gastric mucus depletion (Koo *et al.*, 1986). Yesilada *et al.* (2000) modification of the method described by Takagi and Okabe, (1968) was employed. They studies anti-ulcerogenic effects of *Spartium junceum* flowers on in vivo test models in rats. A groups of rats was used for each treatment. The animals were placed individually in each compartment of a restraint cage and immersed vertically up to the level of the xiphoid in a water-bath (19-20°C) to induce stress ulcer. Test sample was administered either orally or intraperitoneally to rats just before immobilization. After 7 hours immersion, rats were sacrificed by an overdose of ether. Their stomachs were removed, inflated with 10 ml of physiological saline solution and immersed in 1% formalin solution overnight to fix to outer layer of the stomach. Each stomach was opened along the greater curvature, rinsed with physiological saline to remove gastric content and blood clots, and examined under a dissecting microscope. Hiruma-lima *et al.* (2000), used restraint hypothermic stress ulcer model to studies gastroprotective effect of essential oil from *Croton cajucara* Benth. in mices. Mice were divided into groups, after 24 hours of starvation, the animals received an oral administration of sample test. One hour after the treatment ulceration was induced by immobilizing. The animals inside a closed cylindrical cage maintained at 4° C, after 3 hours the animal were sacrificed with ether. The stomach

removed and examined for ulcers. The method of Hiruma-Lima *et al*. (2000) used for studies, used lower temperature and shorter-term to studied than Yesilada *et al.* (2000).

2.3.2 Pyloric ligation – induced ulcer

Rats were slightly anesthesiaed by ether. The abdomen was opened and the pylorus was ligated. The abdomen was closed by suturing as described by Parmar *et al.* (1984), Rujjanawate *et al.* (2001)

Singh and Majumdar (1999) used pylorus ligated rats to studies antigastric ulcer of *Ocimum sanctum* by divided groups of rats and fasted for 24 hours were subjected to pylorus ligation. The sample and control vehicle 3 ml/kg was administered 30 minutes prior to pylorus ligation, 4 hours later the animals were sacrificed and the abdomen was opened. The cardiac end of the stomach was ligated. The stomach was then removed and gastric juice was evacuated into a centrifuge tube. After centrifugation the gastric contents of each animals were individually assayed for volume of gastric secretion and for the total acidity.

Yesilada *et al.* (2000) modification of the method described from Shay *et al.* (1945) they studies anti-ulcerogenic effects of *Spartium junceum* flowers on in vivo test models in rats. Rats were used in each group. Under ethereal anesthesia, the pyloric sphincter was ligated surgically. Each test sample was administered at a volume of 3 ml/kg immediately following pylorus ligation. The animal were then placed in cages without food and water. After 4 hours, the animals were sacrificed, their stomachs removed by clamping the esophagus at the cervical portion, and the inner surface of each stomach was examined for ulceration.

Dias *et al.* (2000) The method of this model by prepared animal, fasted for 24 hours with free access to water, were divided in groups according to the respective treatment, the animal abdomens were incised under ether anesthesia and the pylorus was ligated. Immediately after this procedure, the abdominal, the saline solution, the crude alcoholic extract and cimetidine were administrated intraduodenally to the respective animal groups. The abdominal wall was sutured and after 4 hours, the animals were sacrificed. Their were removed after cardia ligature. The gastric content was centrifuged and subsequently, the gastric juice volume and the pH was

measured. The pyloric ligature method gastric volume, for measurement of volume, pH and increased of its contents.

2.3.3 Inducing agents

- **Aspirin** inhibited prostaglandin as indomethacin induced ulcer. (descripted by Parmar and Desai, 1993, Permpipat *et al.*, 1996) by prepared animals for test, fasted for 24 hours with free access to water, animals were divided in groups according to the respective treatment, after 30 minutes animals were given 100 mg/kg aspirin suspending in1% methyl cellulose. After 4 hours, the animals were sacrificed and removed stomach to determined.

- **Ethanol** (Robert *et al.*, 1979) oral administration of 75% ethanol for 1 hour, produced severe haemorrhagic bands of lesions in the corpus mucosal, along the axis of the stomach (Yesilada *et al.*, 2000). Gurbuz *et al.* (2000), test sample was administered orally 15 minute before the oral administration of ethanol 96% (1 ml) to a group. Later 1 hour, the animals were sacrificed with overdose of ether. The stomachs were then removed and inflated with 10 ml of 1% formalin solution and immersed in the same solution to fix the outer layer of stomach. Each stomach was opened, rinsed with tap water to remove gastric content and blood clots and examined under a dissecting microscope. The ethanol produced more apoptotic cell and vascular damage in the gastric mucosa. Ethanol induces solubilization of mucus constituents in the stomach with a concomitant fall in the transmucosal potential difference and increase Na^+ and K^+ flux into the lumen, pepsin secretion, the loss of H^+ ions and the histamine content in the lumen. The drug also depresses tissue levels of DNA, RNA and proteins, leading to flow stasis in injured areas (Szabo, 1987)

- **HCl** (Permpipat *et al.*, 1996) gastric acid cleavage of pepsinogen to proteolytically active pepsin. Both gastric acid and pepsin activity destroy gastric mucus induced gastric ulcer. Each groups of animal were fasted and received water for 24 hour and cage 18 hour without water. After these, each group were given samples test. After 30 minutes, animals were given 0.6 N HCl at volume of 5 ml/Kg orally. The rats were sacrificed with ether 1 hour later. Their stomachs were removed to examined ulcerative index. Hiruma-Lima *et al.* (2000) and Rujjanawate

et al. (2001) used HCl mixture with ethanol to increase more injury. The HCl and ethanol induced gastric injury were produced free radical leading to increased lipid peroxidation. HCl/EtOH are due to the direct necrotizing action on the gastric mucosa. Oates and Hakkinen (1988) reported the perturbation produced by ethanol induces histamine release from mast cells. The method of this model by given sample orally to 48 hours, fasted rats 60 minutes prior to induction of gastric ulcers by 1.0 ml HCl-EtOH or 0.2 ml of solution containing 0.3 M HCl/60% ethanol (Hiruma-Lima *et al.,* 2000). The animals were sacrificed and examined for gastric ulcers 60 minutes later.

- **Histamine**-induced gastric lesions, histamine is known to be mediated by enhanced gastric acid secretion, more gastric secretion destroy gastric mucus develop to gastric ulcer (Parmar and Desai, 1993). Singh and Majumdar (1999) studies evaluation of the gastric anti-ulcer activity of fixed oil of *Ocimum sanctum* by Histamine-induced gastric ulcers in guinea pigs, guinea pigs fasted for 36 hours were divided into two groups. Gastric ulceration was induced by intraperitoneal administration of histamine acid phosphate 50 mg base. To protect the animal against histamine toxicity, 5 mg promethazine hydrochloride was injected intraperitoneally to each animal, 15 minutes prior to and 15 minute after histamine administration. The sample test was given orally 45 min prior to histamine administration. The animals were sacrificed after 4 hours following histamine administration and the stomach was dissected out to determine the ulcer index.

- **Indomethacin**-induced gastric lesions (West, 1982, Rujjanawate, *et al.,* 2001), indomethacin reduce gastric cyclooxygenase activity and decrease endogenous prostaglandin levels (Konturek *et al.,* 1984) and these agents break the mucosal barrier, provoke an increase in gastric mucosal permeability to H^+ and Na^+ ions and a drop in the transmucosal potential difference and induce the formation of erosions and ulcers.

Singh and Majumdar (1999) used indomethacin induced gastric lesions to studies anti-ulcer activity of *Ocimum sanctum* by divided groups of rats, fasted for 24 hours were treated with sample and control vehicle. After 30 minute each animal received 20 mg/kg indomethacin orally. The rats were killed 6 hours later and their stomachs removed and examined for ulcer index.

Dias *et al.* (2000) studies the antiulcerogenic of medicinal plants were used this model such as antiulcerogenic activity of crude hodroalcoholic extract of *Rosmarinus officinalis* L. by used male wistar rats (200-250g), fasted for 24 hours with free access to water, were divided in a least three groups according to the respective treatment employed (saline, cimitidine 100 mg/kg and crude hydro alcoholic extract 1000 mg/kg. After 30 minutes of oral treatment, indomethacin (30 mg/kg) was administrated subcutaneously, after 4 hours the animals were sacrificed, their stomach were removed, and opened along the greater curvature. The ulcerative lesion index.

- **Reserpine**-induced gastric ulcer by degranulation of the gastric mast cells and the degranulation of the gastric mast cells (Parmar *et al.*, 1985). The machanism of reserpine in the production of acute gastric lesions has not been delineated. It has been shown to stimulate gastric acid secretion in acute doses, reserpine produces the gastrointestinal effects mainly due to parasympathetic stimulation and single doses of reserpine consistently stimulate gastric secretion (Emas and Fyro, 1965 cited in Tariq, Parmar and Ageel, 1985).

Singh and majumdar (1999) studies the anti-gastric ulcer of *Ocimum sanctum* They used reserpine induced gastric ulcers by rats were divided into groups and fasted for 24 hours, reserpine 5 mg/kg was administered intramuscularly 30 minutes after treatment with sample or control vehicle. All the animals were sacrificed after 18 hours and degree of ulceration was expressed as lesion index.

Dias *et al.* (2000) The studies of anti-gastric ulcer, they used reserpine induced gastric ulcer model by prepared animals for treatment, animals were fasted for 24 hours with free access to water and divided in groups according to the respective treatment (saline, atropine 10 mg/kg and crude hydro alcoholic extract 1000 mg/kg. After 30 minutes of oral treatment, reserpine (20 mg/kg) was administrated intraperitoneally to all animal groups. After 2 hours the animal were sacrificed and their stomachs were removed and opened along the greater curvature. The ulcerative lesion index was determined.

- **Serotonin**-induced gastric ulcers is believed to arise from a dis-balance of gastric mucosal microcirculation (Main and Whittle, 1975).

Singh and Majumdar (1999) studied anti-gastric ulcer property of *Ocimum sanctum* by divided groups of rats, fasted for 24 hours, after this administration with sample and control vehicle. After 30 min, ulceration was induced by subcutaneous injection of serotonin creatinine sulphate 20 mg/kg. The animals were sacrificed after 18 hours and the ulcer index was determined.

2.3.4 Biochemistry studies

- **Determination of gastric and duodenal pH**

Mosaddik and Alam (2000) studies the anti-ulcerogenic effect of an alkaloidal fraction from *Mikania cordata* on diclofenac sodium-induced gastrointestinal lesions in rats. They measured gastric and duodenal pH , the rats were killed 18 hours after drug administration by cervical dislocation under chloroform anaesthesia. Stomach and small intestine were dissected form the abdominal cavity and the duodenum was separated from the rest of the small intestinal. Generally, stomach and duodenum were opened along the greater curvature and antimesenteric side. The pH value of the fluid on the gastric corpus mucosal and copus mucosal of the gastric corpus mucosal and corpus mucosal of the duodenum was determined using pH indicator paper (Merck).

- **Determination of pepsin activity**

Mukerji *et al.* (1960) studies effect of *Curcuma longa* L. on the gastric secretion in rabbits and determined effect on pepsins activity. Roy and Bandyopadhyay (1998) studies on Indian traditional medicine, *Picrorhiza kurrooa* and peptic ulcer. They determined the effect on pepsin content in the gastric secretion. Measured activity of pepsin follows description of USP (2000) by prepared pepsin solution by transfer about 2.5 mg of purified pepsin, accurately measured, to a 100 ml. Volumetric flask, dilute with 10 mM hydrochloric acid to volume, and mix. Prepared 2.0% hemoglobin solution by dissolve and dilute 2.5 g of bovine hemoglobin with water to 100 ml. Dilute 80 ml of this solution with 0.3 M hydrochloric acid to a volume of 100 ml. Prepared trichloroacetic acid solution by dilute 5 g of trichloroacetic acid with water to 100 ml. Test solution by transfer 5 ml

of 0.2% hemoglobin solution to a suitable container equilibrated at 37°C add 1 ml of pepsin solution, mix by swirling, and incubate at 37°C for 10 minutes. Immediately add 10 ml of trichloroacetic acid solution, mix by swirling and incubate at 37°C for 5 minutes. Pass through a filter. Control solution by transfer 5 ml of 2% hemoglobin solution to a suitable container equilibrated at 37°C mix by swirling, and incubate at 37°C for 10 minutes. Immediately add 10 ml of trichloroacetic acid solution and 1 ml of pepsin solution, mix by swirling, and incubate at 37°C for 5 minutes. Pass through a filter. Determine the absorbances of the test solution and control solution, in 1-cm cell, at a wavelength of about 280 nm. Using water as the reference.

- **Determination of gastric wall mucus contents**

Gastric wall mucus was determined by the alcian blue method. Rafatullah *et al.* (1999), Rujjanawate *et al.* (2001) and Saad *et al.* (2002) modified procedure of Corne *et al.* (1974). The glandular segments from stomachs which had been opened along their greater curvature were removed and weighed. Each segment was transferred immediately to 10 ml of 0.1% w/v alcian blue solution in 0.16 M sucrose solution, buffered with 0.05 M sodium acetate pH 5. after immersion for 2 hours, excess dry was removed by two successive rinses with 10 ml of 0.25 M sucrose, first for 15 and then for 45 minutes. Dry complex with the gastric wall mucus was extracted with 10 ml of 0.5 M magnesium chloride by shaking intermittently for 2 hours. Four milliliters of blue extract were then shaken vigorously with an equal volume of diethyl ether. The resulting emulsion was centrifuged at 3600 rpm for 10 min and the absorbance of the aqueous layer was recorded at 580 nm. Using a spectrophotometer, the quantity of alcian blue extracted per gram wet stomach was then calculated from a standard curve.

Okwari *et al.* (2000) obtained gastric mucus by scraping the mucosal with a glass slide and was immediately homogenized in 4 ml of weighed distilled water. The weight of mucus was the difference between the weight of homogenate and that of the original 4 ml of water.

2.3.5 Methods to study gastric ulcer in this project

Two models were used

(1) HCl-induced ulcers, high acid secretion activated pepsin activity to destroy mucus layer induced gastric ulcer, pH of gastric juice and pepsin activity were used to investigation mechanism to protection.

(2) Indomethacin-induced ulcer, non-steroidal anti-inflammatory drugs (NSAIDs) such as indomethacin was known to induced gastric ulceration. People used this drug frequency. Determination of gastric wall mucus content was used to investigation mechanism to protection.

Both model are natural factor induced gastric ulcer. Currently, this factor has been a high trend.

2.4 Antioxidant mechanism and technique

2.4.1 Antioxidants

Antioxidants are group of substances which, when present at low concentrations, in relation to oxidizable substrates, significantly inhibit or delay oxidative processes, while often being oxidized themselves (http://courses.che. umn.edu). Example of antioxidants are vitamin E, β-carotene, ascorbic acid (Vitamin C) and polyphenolic compound, and etc.

In recent years there has been an increased interest in the application of antioxidant to medical treatment as information is constantly gathered linking the development of human diseases to oxidative stress. The generally accepted hypothesis is that in any biological system an important balance must be maintained between the formation of reactive oxygen and nitrogen species (ROS and RNS, respectively) and their removal ROS and RNS are formed regularly as a result of normal organ function, or as a result of excess oxidative stress. The reactive species superoxide (O^{-}_{2}), hydrogen peroxide (H_2O_2), hydroxyl radical (HO•), nitrogen oxide (NO•), peroxynitrite (ONOO-) and hypochlorous acid (HOCl), are all products of normal metabolic pathways of the human organs, but under certain conditions, when in excess they can exert an harmful compounds. Superoxide, the most important source of initiating radicals in vivo, is produced in mitochondria during eletron chain

transfer and it regularly leaks outside of the mitochondia. To maintain an oxido/redox balance, organs protect themselves from the toxicity of excess ROS/RNS in different ways, including the use of endogenous and exogenous antioxidants.

Mechanism of action have been proposed for antioxidants. The first is a chain-breaking mechanism, by which the primary antioxidant donates an electron to the free radical present in the system (e.g. lipid radical).

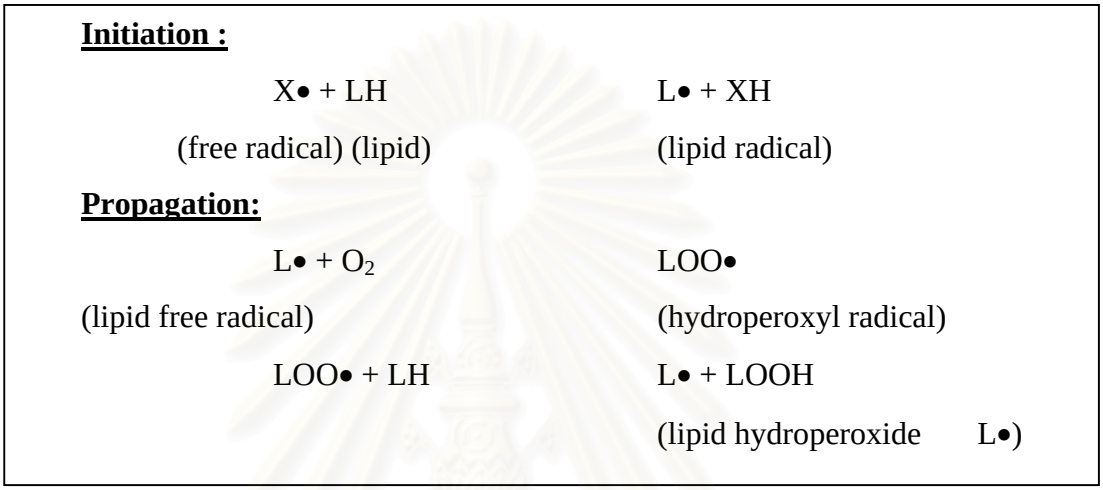


Figure 8. Free radical initiation & propagation

(Available from: http://courses.che.umn.edu)

The second mechanism involves the removal of radical oxygen spices (ROS)/ radical nitrogen species (RNS) initiators (secondary antioxidants) by quenching chain-initiating catalysts.

ROS, strong oxidants like the various ROS can damage other molecules and the cell structures of with they are a part. Various ROS are show in Figure 9.

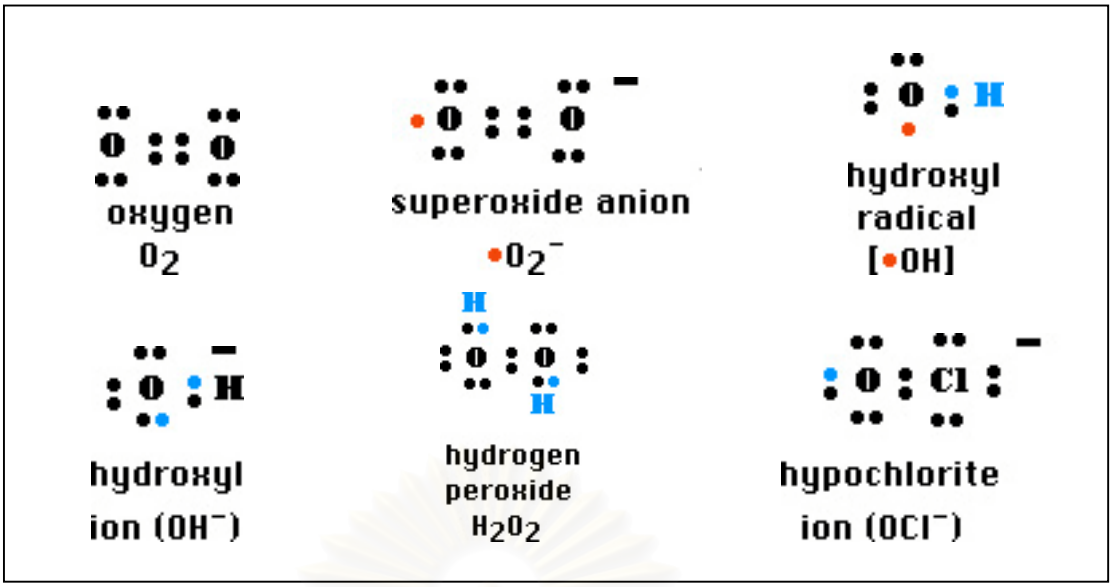


Figure 9. Reactive oxygen species

(Available from: http://users.rcn.com)

Reactive oxygen species are formed by different mechanisms such as

a) The interaction of ionizing radiation with biological molecules

b) An unavoidable byproduct of cellular respiration and go directly to reduce oxygen molecules to the superoxide anion,

c) Synthesized by dedicated enzymes in phagocytic cell like neutrophil and machophages, NADPH oxidase in both type of phagocytes and myeloperoxidase in neutrophils.

Superoxide dismutase (SOD) catalyzes the destruction of the O2- free radical. It protects oxygen metabolizing cells against harmful effect of superoxide free radical (Figure 10). SOD is inactivated by H_2O_2 and may be protected by catalase.

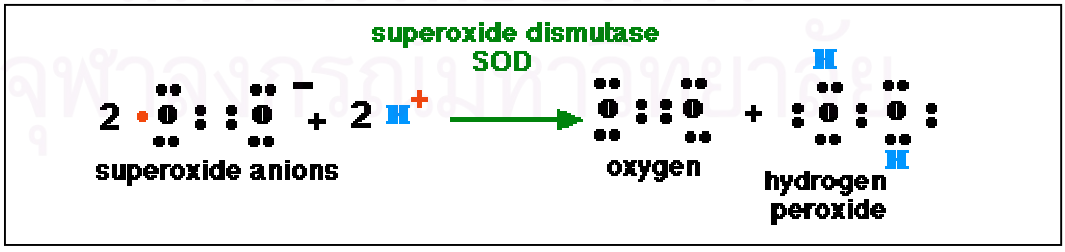


Figure 10. Defenses against reaction oxygen species

(Available from: http://users.rcm.com)

2.4.2 Oxidative stress and gastric ulcer

Gastric ulcer, like many other tissue damages, may be considered to be caused by anions, O^-_2, apart from its in situ interaction between protective and aggressive factors (Sandip *et al.,* 2000), superoxide dismutase are the first contraction with anions.

Superoxide dismutase (SOD) are metalloenzymes which catalyzes the dismutation of superoxide anion (O_2^-) into hydrogen peroxide and molecular oxygen.

Three classes of SODs have been described, each characterized by the catalytic metal at the active site, namely, Cu/Zn-SOD, Mn-SOD and Fe-SOD. Fe-SOD is found mainly in prokaryotes, Mn-SOD crosses the entire range from prokaryotes to eukaryotes. The Cu/Zn-SOD is localized in the cytosol and nucleus, which Mn-SOD is located in the mitochondrial matrix. In human the oxygen taken in by the body is always converted to O_2 , H_2O_2, hydroxy radical (OH) and other molecules by various enzymatic metabolism systems. In order to protect the body from highly toxic Free radical oxygen species (ROS), the body acquired anti-oxidative stress mechanisms including SOD. These antioxidative stress mechanisms are localized in tissue and inside the cells. SOD is on of the most important enzymes in the front line of defense against oxidative stress.

In currently, several experiment model of medicinal plant extract on protective peptic ulcer, all most investigated antioxidant enzyme and lipid peroxidation. Such as Studies on indian traditional medicine, *Picrorhiza kurrooa* (Katuki) and peptic ulcer, unveil that ulcerated rats treated with the extracts lowered the lipid peroxidation (malondialdehye level) and catalase but increase SOD content in gastric mucosa (Roy and Bandyopadhyay, 1998). The role of antioxidant activity of *Phyllanthus emblica* fruits on prevention from indomethacin induced gastric ulcer, the extract could significant decrease the level of malondialdehyde (MDA) and increase high level of SOD in tissue (Bandyopadhyay *et al.,* 2000).

SOD is the first enzyme to contact with superoxide anion. It is interesting to study and investigate mechanism to protect peptic ulcer.

2.4.3 Method to study antioxidant activity of medicinal plants

2.4.3.1 Screening test of antioxidant

Three methods to determination of antioxidant

1) TLC method, this method to observe the reduction of 1,1 diphenyl-2-picrylhydrazyl (DPPH) by looked for clear zone. If the samples have antioxidant properties. The clear zone appeared. The clear zone is circle and the color was white or yellow. Time in observe clear zone show activity properties (Hostettmann *et al.,* 1997).

2) Spectrophotometric assay, this method measured with spectrophotometer at 517 nm to observe the reduction of 2,2 diphenyl-1-picrylhydrazyl (DPPH). The color of DPPH changed from purple to yellow or white, this method according to Moreno *et al.* (2000) and Fejes *et al.* (2000).

3) The carotene-linoleic acid system, determination of the coupled oxidation of carotene and linoleic acid. This assay is simple, reproducible and time efficient for a rapid evaluation of antioxidant properties (Jayaprakasha *et al.,* 2002).

2.4.3.2 Lipid peroxidation

Malondialdehyde (MDA) is the production of lipid peroxidation (Yagi, 1998). Lipid peroxides are formed, with a subsequent formation of peroxyl radicals, followed by a decomposition phase to yield the aldehydes such as hexanal, MDA and 4-hydroxynonenal. This assay is based on the detection of a stable product which is form between aldehydes and thiobarbituric acid (TBA) in the aqueous phase.

Vanisree, Mitra and Shyamala Devi (1996) studied antiulcerogenic effect of UL-409 against experimentally induced gastric ulcer in rats, they used the method of Hara and Okabe (1985) to induced gastric ulcer in rats, 1.5 ml of HCl ethanol mixture was given orally for the induction of gastric mucosal damage. Aqueous suspension of UL-409 was administered by oral intubation at a dose of 600 mg/kg for a period of 30 days. The experimental animals were divided into three groups of six animals in each group. At the end of the experiment period, on thirty first day, the rat were killed 1 hour after the administration of necrotizing agent (HCl-ethanol) by overdose of

chloroform vapours and stomach was removed. The stomach was inflated with normal saline and the incised through the greater curvature and examined for the number of lesions under a dissection microscope. The mucosal tissue was scrapped from the stomach an was used for the estimation of different parameters. Lipid peroxidation followed Okhawa, Onoshi and Yagi (1979) by thiobarbutiric acid reaction and assay superoxide dismutase used method of Misra and Fridovich (1972) by epinephrine and spectrophotometric assay.

Pandit *et al.* (2000) studies anti-ulcer effect of *Shankha bhasma* in rats : a preliminary study by used male wistar rats and used drug induced gastric ulcer in rats, animals of control group received saline (5 ml/kg) and test group received *Shankha bhasma* (25 mg/kg and 50 mg/kg) for 6 days. From day 6, the animals received saline or test drug, 2 hours prior to the administration of indomethacin (20 mg/kg, orally). Overnight fasted animals were sacrificed by cervical dislocation 3 hours after the last dose of ulcerogen. The stomach was incised along the greater curvature and examined for ulcers. The gastric lesions were counted and the mean ulcerative index was calculated and biochemical estimations, gastric tissue lipid peroxidation were estimated in rats the developed ulcers due to indomethacin. The stomach homogenates were prepared in chilled 0.15 M KCl and lipid peroxidation (thiobarbituric acid reacting substances of TBARS).

Bandyopadhyay *et al.* (2000) studies the role of antioxidant activity of *Phyllanthus emblica* Fruits on prevention from indomethacin induced gastric ulcer by used Sprague-dawley (180-210g) rats of both sexes. Rats were divided into three groups of five each, normal control group A, ulcerated control group B and experimental group C, group A and B were gave with gum acacia solution (2%), group C were fed with the drug at the dosages of 60, 80,100 and 120 mg/kg in a suspension of gum acacia for 10 consecutive days. Thereafter, all the groups were kept fasting for around 48 hours. Indomethacin (20 mg/kg) was then given to group B and C for 2 consecutive days and the animals were sacrificed under ether anaesthesia 3 hours after the last dose. The ulcer index was determined. And biochemical estimations, blood was collected immediately after the sacrifice of the animals and the serum was separated in the usual way. Gastric tissue was also taken from the antral portion of stomach. Lipid peroxide was estimated by thiobarbituric acid (TBA) method using MDA as described by Das *et al.* (1994). Thus, 1 ml each of each of serum/properly homogenized gastric tissue in 2 ml of normal saline was mixed with

24% trichloroacetic acid (TCA) and centrifuged at 2000 rpm for 20 min. to 2 ml of protein-free supernatant, 1 ml of fresh TBA (0.67%) reagent was added, mixed thoroughly and heated at 95°C for 1 hour on a water bath. The suspension was then cooled to room temperature, centrifuged at 2000 rpm for 10 minutes and the pink colored supernatant was taken for spectroscopic measurements at 532 nm for the assay of MDA.

Saad *et al.* (2002) studied tocotrienol-rich fraction (TTRF) and its effects on parameters affecting gastric mucosal integrit after a single exposure to indomethacin, by used forty-eight male rats of the Sprague-Dawley (200-250 g) species were randomly assigned into two groups (N and T). The N group was fed with a commercially prepared normal rat diet and the T group was fed with an identical diet enriched with TTRF 150 mg/kg diet. Each group was further subdivided into two subgroups that was either challenged (NI and TI) or not challenged (NX and TX) with indomethacin. After eight weeks of treatment the NX and TX rats were killed and the stomachs isolate whereas the NI and TI rats were challenged with a single dose of indomethacin (80 mg/kg) orally and after six hours the rats were killed. Measurements for malondialdehyde (MDA), glutathione content, PGE_2, gastric acid concentration and gastric adherent mucous (GAM). Gastric tissue MDA content was measured using a modified method described by Ledwozyw *et al.* (1988), the gastric tissue was homogenize in distilled water, centrifuged and the diluted supernatent was add with thiobarbituric acid. After 15 minutes at room temperature, thiobarbituric acid was added and the samples were incubated in 100°C water bath for 30 minutes. After cooling, n-butanol was add and the absorbency of the upper phase was read.

2.4.3.3 Superoxide dismutase

Yoshikawa *et al.* (1993) studies role of active oxygen, lipid peroxidation, and antioxidants in the pathogenesis of gastric mucosal injury induced by indomethacin in rats. They used male sprague-dawley rats, weight range 190-210 g, the animals were deprived of food but allowed free access to water for 24 hours before the experiment. Gastric haemorrhagic damage was induced by oral administration of indomethacin at a dose of 20 mg/kg, suspended in 0.5% carboxymethylcellulose solution with a few drops of tween 80 in volume of 0.5 ml/100 g. In the control group, the rats received

and equivalent volume of the vehicle. Time course studies of indomethacin induced gastric mucosal injury by the groups of indomethacin treated rats were killed three or six hours after administration of indomethacin. Animals were killed by exsanguinations via the abdominal aorta under inhaled ether anaesthesia. The stomachs were removed, opened along the greater curvature, and examined under a dissecting microscope with a square grid for lesions developed in the glandular portion. The extent of the gastric damage was expressed as the total area (mm^2) of haemorrhage erosion. The gastric mucosal was scraped off by means of two glass slides on ice, and homogenized with 1-5 ml of 10 mM potassium phosphate buffer (pH 7-8) containing 30 mM KCl in a potter-elvehjem homogenizer, to measure concentrations of lipid peroxides and tocopherols. To measure the activity of SOD and glutathione peroxidase, the homogenates were sonicated over ice for two minites. The sonicated samples were centrifuged at 20000 g for 20 minutes and selection of supernatants for test.

There are several methods in detecting superoxide dismutase. It could division by equipments, such as spectrophotometer, chemiluminometric method. Spectrophotometric detection, the most typical SOD detection method is the one based on spectrophotometric detection. The nitroblue tretrazoliume (NBT) method is based on the generation of water-insoluble blue formazan dye (λ=560 nm) by a reaction with O_2^-. Bandyopadlhyay *et al.* (2000) used the method of Mishra and Fridovich (1972) by involving inhibition of epinephrine auto-oxidation in an alkaline medium at 480 nm in a UV-VIS spectrophotometer. Tian Xing Zhou *et al.* (2000) use adrenaline auto oxidation method. McEwan and Huss-Danell (1997), Tsekova and Todorova (2000) used methionine, as donation of an electron resulted in the production of a superoxide molecule. The superoxide molecule is able to reduce the nitroblue tetrazolium (NBT), giving an insoluble purple formazan, the color change is measured spectrotometrically at A_{560} nm.

CHAPTER II

MATERIALS AND METHODS

1. Materials

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1. Materials

1.1 Turmeric oil

Turmeric oil in this experiment was supplied by Chalalai shop. Properties of a commercial sample of turmeric oil for uesd was yellow in color. pH of turmric oil was measured that found at pH 5 and 1 ml of turmeric oil was weight, that found 0.4 g/ml. Stored at refigerature until used.

1.2 Animals

Male Sprague – Dawley rats (150 – 180 g) and animal feed Nakronpathom province was purchased from the National Laboratory Animal Center, Mahidol University, Salaya, Thailand. Rats were acclimatized for at least 7 days prior to the day of experiment in an animal room where the temperature was maintained at 25±3 ° C and 12 hours light-dark cycle. The bedding was autocaved. Food and water were given ad libitum. All animals received approval from committee of the Faculty of Pharmaceutical Sciences, Chulalongkorn University on November 27, 2001.

Animals were randomly divided into 3 treatment groups, namely, those treated with 1%targacanth was suspending agent (control), standard drug plus suspending agent and turmeric oil plus suspending agent. The turmeric oil group consisted of three subgroups in HCl model and two subgroups in indomethacin model, the standard drug group consisted of two subgroups. Each subgroup was composed of 10 animals.

1.3 Chemicals

- 0.9% normal saline
- 2,2-diphenyl-1-picrylhydrazyl (DPPH) (Fluka, Switzerland)
- Alcian blue, analytical grade (Sigma, USA)
- Atropine Sulfate ($C_{17}H_{23}NO_3$)• H_2SO_4, analytical grade (Sigma, USA)
- Diethyl ether, analytical grade (Merck)
- Ether, analytical grade (J.T. baker, USA)
- Ethyl acetate (J.T. Baker, USA)

- Ethylene diamine tetraacetic acid (Merck)
- Formalin (Vitayasom)
- Gum tragacanth, analytical grade (Sigma, USA)
- Hemoglobin, analytical grade (Sigma, USA)
- Hexane, analytical grade (J.T. Baker, USA)
- Indomethacin ($C_{19}H_{16}CINO_4$), analytical grade (Sigma,USA)
- Magnesium chloride, analytical grade (Merck)
- Malonylaldehyde (MDA) assay kit set (OXI-TEK, New york)
- Misoprostol (Sigma, USA)
- Nitroblue tretrazolium, analytical grade (Sigma, USA)
- Pepsin standard (Sigma, USA)
- Potassium phosphate, analytical grade (Merck)
- Riboflavin (Sigma, USA)
- Silica gel60 GF254, precoated TLC plants (Merck, Germany)
- Silica gel60 No.1.07734 (Merck, Germany)
- Sodium acetate, analytical grade (Merck)
- Sucrose, analytical grade (Fisher chemicals, Leics)
- Sulfuric acid, analytical grade (J.T. Baker, USA)
- Superoxide dismultase (Sigma, USA)
- Toluene, analytical grade (J.T. Baker, USA)
- Trichloroacetic acid, analytical grade (Merck)
- Vanillin, analytical grade (Fluka, Switzerland)
- Coomassie brilliant-blue G-250, analytical grade (Sigma)
- 95% ethanol, analytical grade (Merck)
- 85% Phosphoric acid, analytical grade (Merck)
- Bovine serum albumin (Merck)

1.4 Equipments

- Hot air oven (Memmert, USA) and Water bath (Memmert, USA)
- Hydro distillation apparatus
- Magnetic stirrers (Heidolph, Germany)
- pH meter (Orion Research Incorporated, USA)
- pH paper (Merck)

- Refrigerated centrifuge (Hettich, Germany)
- Rotary evaporator (Eyela, Japan)
- Shaker water bath (Heto)
- Stereo microscope (Olympus, Germany)
- TLC tank
- UV/VIS spectrophotometer (Shimadzu)
- Vortex-Mixer (Scientific Industries, USA)

2. Methods

2.1 Determination of gastric ulcer

The objective of these experiments was to investigate the anti-gastric ulcer mechanism of turmeric oil on the naturals factor and social induced ulcer factor. So we used HCl induced ulcer agent and NSAIDs induced gastric ulcer, (indomethacin).

2.1.1 Preparing drugs

- Atropine sulfate was dissolved in 1% tragacanth, suspending agent immediately to oral feeding at dose 0.16 and 1.25 mg/kg. Atropine blocks the action of acetylcholine reduce gastric secretion.
- Indomethacin was dissolved in 1% tragacanth, suspending agent immediately to oral feeding at dose 50 mg/kg
- Misoprostol was dissolved in 1% tragacanth, suspending agent, immediately to oral feeding at dose 50 and 100 μg/kg
- Turmeric oil was dissolved in 1% tragacanth, suspending agent, immediately to oral feeding at does 0.075, 0.15, 0.2 and 0.3 g/kg

2.1.2 HCl induce ulcer

This method was adapted from Permpipat, Chuthaputti and Kiatying-Angsulee (1996) each groups of animal were fasted and received water for 24 hour and cage 24 hour without water. After these, the control group were given 1% tragacanth (suspending agent), the treatment groups with turmeric oil plus 1% tragacanth were given at dose of 0.075, 0.15, 0.3 g/kg for three subgroups and the treatment groups of drug standard, Atropine sulfate plus 1% tragacanth at dose 0.16 and 1.25 mg/kg at volume of 3 ml/kg, 30 minute later. rats were then given 0.6 N HCl at volume of 5 ml/kg orally. The rats were sacrificed with ether vapours, 1 hour later.

2.1.3 Indomethacin induced ulcer

Each group of animal were fasted and received water for 24 hour, cage 24 hour without water. After these, the control group were given 1% tragacanth (suspending agent), the treatment groups with turmeric oil plus 1% tragacanth were given at dose 0.2, 0.3 g/kg for two subgroups and the treatment groups of drug standard was Misoprostol plus 1% tragacanth at dose 50 and 100 μg /kg, each animal was given 3 ml/kg 30 minutes. After these, animal was given 50 mg/kg indomethacin suspending in 1%targacanth, 5 ml/kg orally. The rats were sacrificed with ether vapours, 3 hours later. This model was selected blood from heart and gastric tissues for measure lipid peroxidation and superoxide dismutase, adapted from Bandyopadhyay *et al.* (2000)

2.1.4 Collection of sample for test

- **Collection of blood from heart**

Blood from heart was drawn by using 0.8 x 25 mm needle. Blood of sample were kept at temperature for 1 hour, until clot and centrifuged at 1200 gravity for 10 minutes. Serum was collected and kept at –20 °C until the experiment (measured lipid peroxidation and Superoxide dismutase).

- **Collection of gastric juices**

Collection of gastric juice was perfermed, after stomach was removed, sewn at pyloric and the connecter of esophageal. Used needle fill 1 ml water to stomach, draws gastric juices and centrifuge at 30 gravity, 10 minute. Selected of the supernatant, kept at –20 °C for measured pepsin activity.

- **Collection of gastric tissue**

After stomach was removed and measurement of gastric lesion. The gastric antrum was section and weighted about 0.2 g, to homoginized and centrifuged at 30 gravity for 10 minutes. The supernatant were collected and kept at –20 °C for measurement of lipid peroxidation and superoxide dismutase.

2.1.5 Determination and examination of ulcer (HCl and indomethacin model)

After each rat was sacrificed, the stomach was removed and selected sample (blood, gastric juice and tissue) for measured in other method. Opened along the greater curvature in HCl model and lesser curvature in indomethacin model. And fix stomach with tack before measured. The wide and length in mm of each lesion was measured under a dissecting microscope and the sum of the areas of all lesions was designated as the ulcer index.

Calculations Ulcer index (UI) = $\sum(mm)^2$ of ulcer areas / N

(N=number of rat used in each group)

$$\%\text{Inhibition} = \frac{\text{Lesion}_{control} - \text{Lesion}_{test}}{\text{Lesion}_{control}} \times 100$$

2.1.6 Determination of gastric pH (HCl model)

After collection of gastric juice, the pH value of the fluid on the gastric corpus was determined by using pH indicator paper follow as the method of Mosaddik and Alam (2000)

2.1.7 Determination of pepsin activity (HCl model)

The sample were stand in room temperature to dissolve for 10 minutes.

Prepare solution for test: pepsin solution, 2% hemoglobin solution, trichloroacetic acid solution.

Test solution : pipette 5 ml of 2% hemoglobin solution warmed as 37 °C into series of tubes. Add 1 ml of pepsin solution or sample, mix by swirling incubate at 37 °C for 10 minutes. Approximately add 10 ml trichloroacetic acid solution into each tubes incubate at 37 °C for 5 minutes after this pass through filter.

Control solution : pipette 5 ml of 2% hemoglobin solution 37 °C into series tubes. Incubate at 37 °C for 10 minutes add 10 ml trichloroacetic acid solution and 1 ml pepsin solution, mix by swirling and incubate at 37 °C for 5 minutes after this pass through filter. The aborbance of test solution (At) and control solution (Ac) were detected at A_{280} nm. Recorded data and calculation follows the method of USP24 (2000).

Calculation Activity = 10,000(At-Ac)

2.1.8 Determination of gastric wall mucus content (indomethacin model)

The stomach body was weighted and immersed in 0.1 % alcian blue solution for 2 hour. After that, rinsed with 0.25 M sucrose solution for 15 minutes. Dye complexed with gastric wall mucus was extracted with 0.5 M magnesium chloride for 2 hour. The blue extrated was then shaken vigorously with an equal volume of diethyl ether and the resulting emulsion was centrifuged. The optical density of alcian bule in the aqueous layer was read against a buffer blank at 580 nm using a spectrophotometer. The quantity of alcian bule extracted per gram wet stomach was then calculated from a standard curve, applied from Rafatullah *et al.* (1990), Rujjanawate *et al.* (2001).

2.2 Determination of antioxidation screening test (Turmeric oil)

Two methods of antioxidant activity were used and compare each method with two standard antioxidants.

2.2.1 TLC screening for radical scavengers

This method applied from Hostettmann *et al.* (1997) by prepare solvent system, prepare 0.2 mM 2,2-diphenyl-1-picrylhydrazyl (DPPH), spot sample extract (turmeric oil, BHA and gallic acid standard) and run TLC after this spray DPPH on TLC plate. The plates were examined 30 min after spraying look for clear zone. Proved also to be well suited for the detection of antioxidants in turmeric oil. DPPH is a stable radical with a purple color. Upon reduction by a scavenger, the extensive conjugation is disrupted and the compound turns yellow (clear zone).

2.2.2 Spectrophotometric assay

This method was applied from Beavo and Reifsngder (1990) by prepare sample extract (turmeric oil and BHA, gallic acid standard) 10–1000 ppm, prepare 0.2 mM 2,2-diphenyl-1-picrylhydrazyl (DPPH), pipette 0.5 ml of sample extract to series tubes, pipette 1 ml of DPPH solution into each tube mix and place in dark box for 30 minutes. After this, read the aborbance at wavelength 517 nm and calulate % radical scavenging.

Calculation $\%\text{radical scavenging} = \left[1 - \frac{\text{A sample}}{\text{A control}}\right] \times 100$

A sample = absorbance value of the sample mixture DPPH.

A control = absorbance value of DPPH solution.

2.3 Determination of lipid peroxidation (HCl and indomethacin model)

Lipid peroxidation was estimated by thiobarbituric acid (TBA) method using malondialdehyde (MDA) assay kit set. Thus, 1 ml each of serum properly homogenized gastric tissue in 2 ml of normal saline was mixed 24% TCA and centrifuged at 3000 rpm for 20 minutes collected protein-free supernatant for test.

Triobabituric assay (TBARS) kit was used. Add 100 μL sample or standard to series of tubes, add 100 μL SDS solution and swirl to mix, Add 2.5 ml TBA/Buffer Reagent forcefully down the side of each tube, incubate at 95 °C for 60 min. After time, cool at room temperature in an ice bath for 10 minutes. The tubes were centrifuge at 30xg for 15 minutes and remove supernatant of each tube for spectrophotometer analysis at A_{530} nm followed Yagi (1998)

The total protein of each sample was determined by prepareing reagents bovine serum albumin 0.1, 0.2, 0.3, 0.4 and 0.5 mg/ml. Prepare Bradford stock solution, Bradford working buffer and Standard Bovine serum albumin 1 mg/ml stock solution followed Bradford method, pipette 100 μl of standard BSA or sample into tubes. Add 2 ml Bradford working buffer, stand for 10 minutes after time read A_{595} nm

2.4 Determination of superoxide dismutase (SOD) (HCl and indomethacin model)

This method was applied from Winterbourn *et al.* (1975) by prepareing reagents (A) 0.067 M potassium phosphate buffer pH 7.8, (B) 0.1 M ethylene diamine tetraacetic acid (EDTA) containing 0.3 mM sodium cyanide, (C) 0.12 mM riboflavin (store cold in a dark bottle) and (D) 1.5mM nitroblue tetrazolium (NBT) and prepare stock solution of enzyme (SOD) at one mg/ml

Pipette 0.2 ml of ethylene diamine tetraacetic acid containing 0.3 mM sodium cyanide, 0.1 ml of nitroblue tetrazolium, 13 mM methionin, enzyme 20 microlite or sample and phosphate buffer into tubes. After pipette reagent and enzyme into a series of tubes. Place the tubes in a light box 36 w fluorescent 10 minutes to achieve

a standard temperature. At timed intervals add 0.05 ml riboflavin. Incubate the tubes in the light box 15 miniutes after time read A_{560} nm.

The total protein of each sample was measured by prepare reagents bovine serum albumin 0.1, 0.2, 0.3, 0.4 and 0.5 mg/ml. Prepare Bradford stock solution, Bradford working buffer and standard bovine serum albumin 1 mg/ml stock solution followed Bradford method, pipette 20 μl of standard BSA or sample into tubes. Add 2 ml Bradford working buffer at room temperature for 10 minutes after time read A_{595} nm.

2.5 Statistical analysis

Data were subjected to statistical analysis by using ANOVA (descriptive and least-significant different (LSD) test) from SPSS version 9.05, The value exceeding 95% or 99.9% confident limits were considered to be significant according to the test.

CHAPTER III

RESULTS

1. Determination of gastric ulcers
   1.1 HCl induced ulcer model
   1.2 Indomethacin induced ulcer model
   1.3 Determination of gastric pH
   1.4 Determination of pepsin activity
   1.5 Determination of gastric wall mucus content
2. Determination of antioxidant of turmeric oil
   2.1 TLC screening of radical scavengers
   2.2 Spectrophotometric assay
3. Determination of lipid peroxidation
4. Determination of superoxide dismultase (SOD)

1. Determination of gastric ulcers

1.1 HCl induced ulcer model

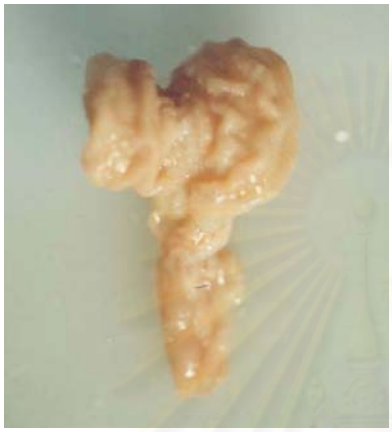
Turmeric oil possessed gastric ulcer protection. The result showed dose dependent reduction of ulcer index in HCl treated rats (Table 3). Percent inhibition was calculation as compared to control (1% tragacanth 3 ml/kg). At oral dose of 0.3 g/kg of turmeric oil exerted 97.41% inhibition of gastric ulcer comparable to 1.25 mg/kg of atropine sulfate. The treatment of turmeric oil at doses of 0.075 g/kg and 0.15 g/kg showed only 5.44% and 22.8% inhibition of gastric ulcer.

Table 3. Effect of turmeric oil protected 0.6 N HCl induced ulcer

Group	N	Ulcer index Mean± SD	%Inhibition
Control (1%targacanth)	10	38.60 ± 24.89	0.00
Turmeric oil 0.075 g/kg	10	36.50 ± 7.61	5.44
Turmeric oil 0.150 g/kg	10	29.80 ± 7.13	22.80
Turmeric oil 0.30 g/kg	10	1.00 ± 2.21**	97.41
Atropine sulfate 0.16 mg/kg	10	5.20 ± 6.63**	86.53
Atropine sulfate 1.25 mg/kg	10	2.55 ± 6.87**	93.39

** Significant difference at $p \leq 0.001$, each group compared to control, N=number of rats,
SD = standard deviation, %inhibition calculation from mean of ulcer index

The gastric tissues showed different characteristic form of gastric ulcer induced by 0.6 N HCl compared with normal gastric tissue. The normal gastric tissue had more mucus and smooth surface than gastric ulcer tissue. The ulcer was red or black 1-3 mm wide and 1-10 mm long and a lot on gastric corpus.



Gastric normal

Gastric ulcer induced by 0.6 N HCl

Figure 11. Characteristic of ulcered gastric compare to normal gastric

The result showed that turmeric oil could protect gastric ulcer formation from hydrocholic acid. So, in this next step we observed the relation of mechanism of ulcer healing such as pH of gastric juice, pepsin activity and antioxidant properties.

1.2 Indomethacin induced ulcer

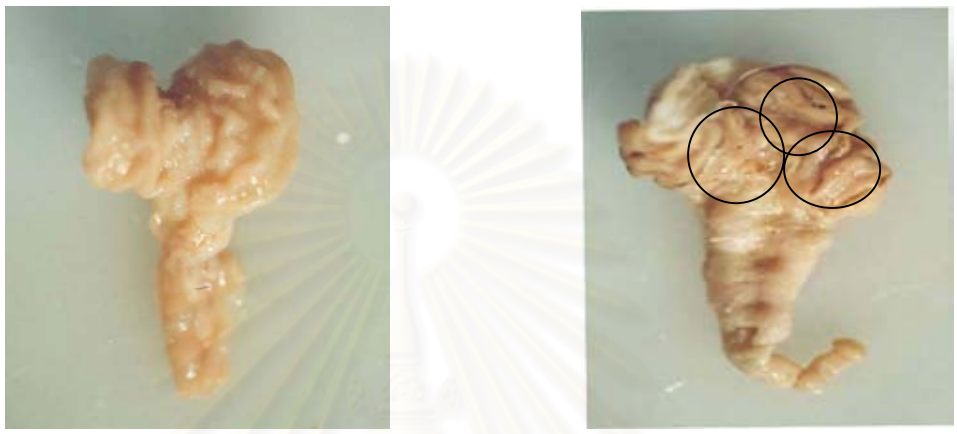
Turmeric oil could protect gastric ulcer, the result showed dose dependent reduction of ulcer index in indomethacin treated rats (Table 4). Turmeric oil treated at oral dose of 0.2, 0.3 g/kg exhibited 87.97%, 100% inhibition of gastric dose comparable the dose of 100 μg/kg of misoprostol produced 98.22% inhibition.

Table 4. Effect of turmeric oil protected indomethacin induced ulcer

Group	**N**	**Ulcer index Mean± SD**	**% Inhibition**
Control (1%tragacanth)	10	44.90 ± 28.33	0.00
Turmeric oil 0.20 g/kg	10	5.40 ± 2.79**	87.97
Turmeric oil 0.30 g/kg	10	0.00 ± 0.00**	100.00
Misoprostrol 50 μg/kg	10	7.55 ± 7.02**	83.18
Misoprostrol 100 μg/kg	10	0.8 0± 2.52**	98.22

** Significant difference at $p \leq 0.001$, each group compared to control, N=number of rats,
SD = standard deviation, %inhibition calculation from mean of ulcer index

The gastric tissues showed different characteristic of gastric ulcer induced by 50 mg/kg of indomethacin compared with normal gastric tissue. The normal gastric tissues had more mucus and smooth surface. The ulcer was red, brown and black spot or long and a lot on gastric corpus.



Gastric normal Gastric ulcer induced by indomethacin

Figure 12. Characteristic of ulcer gastric compare to normal gastric

This result showed that turmeric oil could protect gastric ulcer formation from NSAIDs (indomethacin). We then tried to look for mechanism to healing ulcer, such as gastric wall mucus content and in vivo antioxidant properties.

1.3 Determination of gastric pH

Effect of turmeric oil on pH of gastric juices in HCl induced ulcer model (Table 5) showed increase gastric pH. The treatment of turmeric oil at dose of 0.15 and 0.3 g/kg increase gastric juices compared control while pH of turmeric oil (crude oil) was found at pH 5. The relative of pH and pepsin activity were observed later.

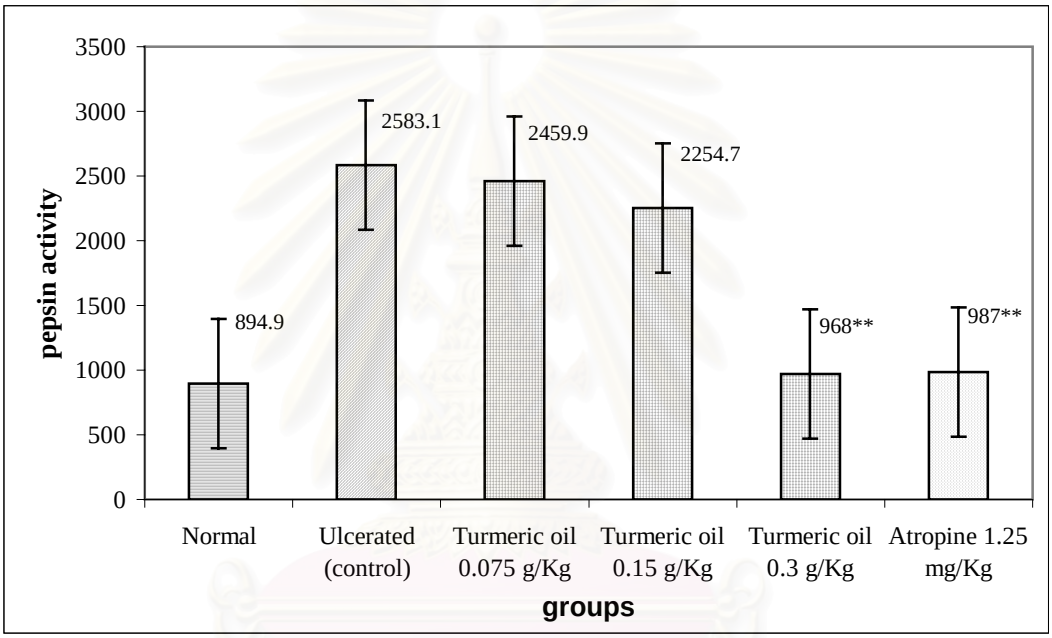
Table 5. Effect of turmeric oil on pH of gastric juices in HCl induced ulcer model

Group	N	pH of Gastric juices Mean±SD
Normal	10	3.3 ± 0.67**
Control (1%tragacanth)	10	2.5 ± 0.52
Turmeric oil 0.075 g/kg	10	2.7 ± 0.48
Turmeric oil 0.15 g/kg	10	3.0 ± 0.00**
Turmeric oil 0.3 g/kg	10	3.5 ± 0.52**
Atropine sulfate 0.16 mg/kg	10	2.7 ± 0.67
Atropine sulfate 1.25 mg/kg	10	2.7 ± .067

** Significant difference at $p \leq 0.001$, each group compared to control, N=number of rats, SD = standard deviation

1.4 Determination of pepsin activity

Pepsin activity of the turmeric oil treated on HCl induced ulcer was reduced. At oral dose of 0.3 g/kg of turmeric oil was significantly to inhibition 62.6% of pepsin activity comparable to control and 61.8% inhibition by 1.25 mg/kg of atropine sulfate. The turmeric oil treated at dose 0.075 and 0.15 g/kg was induced 4.8% and 12.7% inhibition respectively, which was not significantly reduced pepsin activity compared ulcerated (1% tragacanth, control)(Figure 13).



** Significant difference at $p \leq 0.001$, each group compared to ulcerated (control)

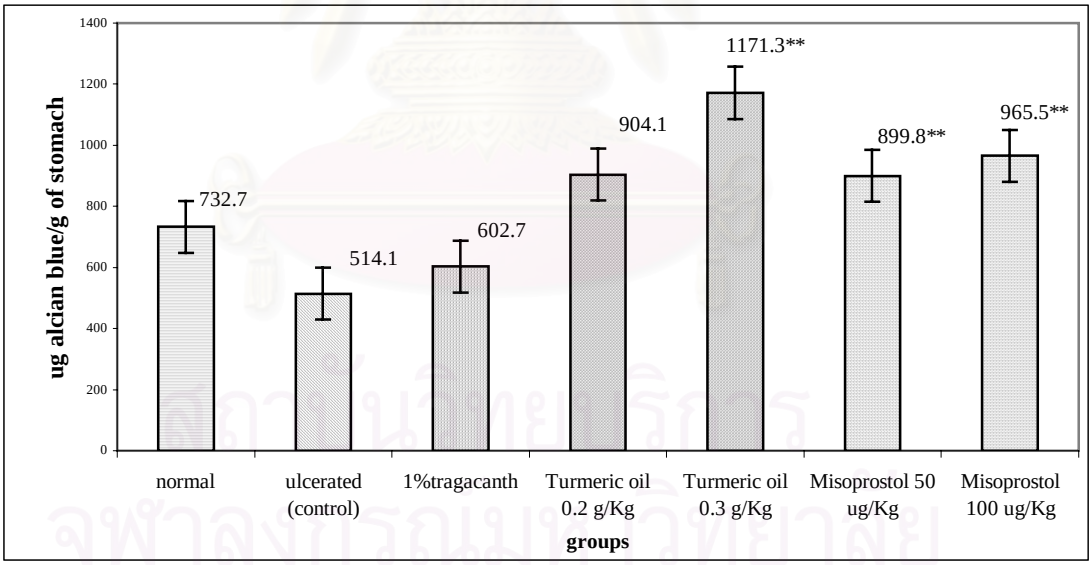
Figure 13. Pepsin activity in HCl induced ulcer rats after turmeric treated and atropine treated

Conclusion of this method: turmeric oil at dose of 0.3 g/kg could significantly reduce pepsin activity.

1.5 Determination of gastric wall mucus content

The measurement of gastric wall mucus content on indomethacin induced gastric ulcer model. It was found that the control (ulcerated group) was decrease gastric wall mucus content 30% (514.1 μg alcian blue) from normal group (732.7 μg alcian blue). The treatment of 1% tragacanth was increase gastric wall mucus content 12.3% (602.7 μg alcian blue) from control (ulcerated group), it was not significant to increase gastric wall mucus content.

The treated of turmeric oil at dose of 0.2 and 0.3 g/kg could increase gastric wall mucus content 53% (904.1 μg alcian blue) and 89.8% (1171.3 μg alcian blue) comparable control (ulcerated group). And the doses of turmeric oil were significant to increase gastric wall mucus 23.3% (904.1 μg alcian blue), 59% (1171.3 μg alcian blue) comparable normal rats. The turmeric oil treated at dose 0.2 g/kg could increase gastric wall mucus content 53% comparable 52.8% in 50 μg/kg of misoprostol. The 1% tragacanth not significant compared to control (ulcerated group).



** Significant difference at $p \leq 0.001$, each group compared to control (ulcerated group)

Figure 14. Gastric wall mucus content in indomethacin induced ulcer after treated with turmeric oil and misoprostol

Conclusion of this method: turmeric oil cloud significantly increase gastric wall mucus content.

2. Determination of antioxidant of turmeric oil

2.1 TLC screening of radical scavengers

The TLC method was found that turmeric oil has low antioxidant by looked for clear zone after spray DPPH on TLC plate. The plates are examined 30 min after spraying look for clear zone. Proved also to be well suited for the detection of antioxidants in turmeric oil. DPPH is a stable radical with a purple color. Upon reduction by a scavenger, the extensive conjugation is disrupted and the compound turns yellow (clear zone). The turmeric oil was slowly to showed 4 clear zone, the purple color changed to yellow, it contrast in 10 minutes and two standard control was saw the contrast clear zone in suddenly.

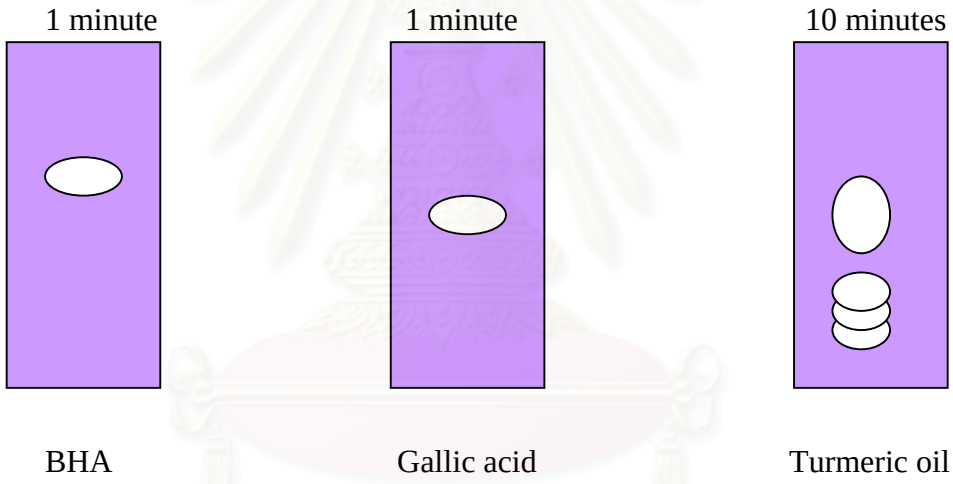


Figure 15. Antioxidant clear zone of the BHA, gallic acid and turmeric oil

Conclusion of this method: turmeric oil had antioxidant properties, with slower effect. There were four clear zones in TLC plate of turmeric oil showing possibly many antioxidant chemicals. When compared with two standards turmeric oil showed lower antioxidant properties.

2.2 Spectrophotometric assay

The spectrophotometric assay showed that turmeric oil at 1000 ppm possessed 36% radical scarvenging compared to 95% of gallic acid and 94.8% of BHA (Table 6). In other word, IC_{50} of gallic acid = 0.6 ppm while the BHA = 1.7 ppm, while IC_{50} for turmeric oil was more than 1,000 ppm. IC_{50} = The concentration of sample to reduce concentration of DPPH 50%

Table 6. Effect of turmeric oil on antioxidant activity comparison with gallic acid and BHA standard references

Group	**% radical scarvenging**		
Conc (ppm)	**Gallic acid**	**BHA**	**Turmeric oil**
0.00	0.00	0.00	0.00
1.95	49.390	37.449	32.089
3.90	69.945	42.994	32.311
7.81	93.937	58.484	32.384
15.63	94.048	85.065	32.458
31.25	94.196	94.048	32.569
62.50	94.307	94.159	32.828
125.00	94.566	94.270	33.420
250.00	94.824	94.455	33.789
500.00	95.009	94.677	34.196
1,000.00	95.120	94.824	36.747

Conclusion of this method: turmeric oil had lower antioxidant properties than gallic acid and BHA standard.

3. Determination of lipid peroxidation

Study on free radical induced gastric injury and the protection effect of turmeric oil was done by using 0.6 N HCl and indomethacin 50 mg/kg as gastric ulcer inducer. MDA is a production of lipid peroxidation. The treatment of inducing agent increased MDA from normal (untreated group) 77.80% in serum and 108.57% in tissue (Table 7). The serum MDA of turmeric oil treated at dose of 0.075, 0.15 and 0.3 g/kg on HCl induced gastric ulcer decreased by 37.28%, 53.81% and 69.37% significantly compared with ulcerated group (control). The MDA in tissue of turmeric oil treated at dose of 0.075, 0.15 and 0.3 g/kg on HCl induced gastric ulcer decreased by 49.69%, 59.32% and 82.4% compared to ulcerated group (control). At dose of 0.3 g/kg of turmeric oil decreased MDA 69.37% in serum and 82.4% in gastric tissue compared to 72.13% and 104% by 0.125 mg/kg of atropine sulfate respectively.

Table 7. Effect of turmeric oil on lipid peroxidation of serum and gastric tissue compared to control (HCl induced ulcer model)

Group	**MDA level (nmol/ mg protein)**	
	Serum	**Tissue**
	Mean ± SD	**Mean ±SD**
Normal	6.17 ± 0.42**	6.42 ± 0.65**
Control (1% tragacanth)	10.97 ± 2.01	13.39 ± 1.38
Turmeric oil 0.075 g/kg	8.67 ± 0.28**	10.20 ± 0.65**
Turmeric oil 0.15 g/kg	7.65 ± 0.76**	9.59 ± 1.69**
Turmeric oil 0.3 g/kg	6.69 ± 0.32**	8.10 ± 0.92**
Atropine sulfate 1.25 mg/kg	6.52 ± 0.63**	6.70 ± 0.67**

** Significant difference at $p \leq 0.001$, each group compared to control (ulcerated group),

SD = standard deviation

The turmeric oil treated at dose of 0.3 g/kg on indomethacin induced gastric ulcer could decrease MDA inserum by 96.58% compared to control (1%tragacant) and 95.21% of 100 μg/kg misoprostol. The gastric tissue MDA of the turmeric oil treated at dose of 0.3 g/kg on indomethacin induced gastric ulcer could decrease by 112.95% compared to control (1% tragacanth) and 113.18% by 100 μg/kg misoprostol.

Table 8. Effect of turmeric oil on lipid peroxidation of serum and gastric tissue compared to control (indomethacin induced ulcer model)

Group	**MDA level (nmol/ mg protein)**	
	Serum	**Tissue**
	Mean ±SD	**Mean ±SD**
Normal	5.85 ± 0.64**	4.40 ± 0.26**
Control (1%tracanth)	12.14 ± 0.83	9.53 ± 0.44
Turmeric oil 0.3 g/kg	6.49 ± 0.52**	4.56 ± 0.71**
Misoprostol 50 μg/kg	8.32 ± 0.39**	4.95 ± 0.71**
Misoprostol 100 μg/kg	6.57 ± 0.52**	4.55 ± 0.45**

** Significant difference at $p \leq 0.001$, each group compared to control (ulcerated group),

SD = standard deviation

4. Determination of superoxide dismultase (SOD)

The superoxide anion scavenging enzymes, i.e. superoxide dismutase in serum and tissue were increased compared to control and untreated group of rats (normal) (Table 9). It was found that the SOD of ulcerated group (HCl induced gastric ulcer) was decrease 64.7% (10.90 ± 0.704) from normal group (30.92 ± 1.891). The turmeric oil treated at dose of 0.075, 0.15 and 0.3 g/kg were increased 41.98 % (12.98 ± 0.861), 48.93 % (15.13 ± 0.115) and 54.64 % (16.84 ± 1.531) of SOD from the normal group (30.92 ± 1.891). The turmeric oil treated at dose of 0.3 g/kg was increased 54.46% (16.84 ± 1.531) of SOD comparable to 57.6% (17.81 ± 1.171) by 1.25 mg/kg of atropine sulfate.

SOD in gastric tissue of the control group was decreased 42.76% (18.65 ± 1.862) from 100% of normal group (32.58 ± 4.500). The turmeric oil treated at dose of 0.075, 0.15 and 0.3 g/kg could increase the SOD 70.29% (22.90 ± 2.035), 70.99% (23.13 ± 0.983) and 73.4% (23.91 ± 1.403) respectively compared to the normal group. Atropine sulfate could increase SOD 86.89% (28.31 ± 1.194) compared to the normal group (32.58 ± 4.500).

Table 9. Effect of turmeric oil on SOD of serum and gastric tissue of HCl induced ulcer model

Group	SOD units/ mg of protein in rat serum Mean ± SD	SOD units/ mg of protein rats gastric tissue Mean ± SD
Normal (non-ulcer)	30.92 ± 1.891**	32.58 ± 4.500**
Control (1%tragacanth)	10.90 ± 0.704	18.65 ± 1.862
Turmeric oil 0.075 g/Kg	12.98 ± 0.861**	22.90 ± 2.035**
Turmeric oil 0.15 g/Kg	15.13 ± 0.115**	23.13 ± 0.983**
Turmeric oil 0.3 g/Kg	16.84 ± 1.531**	23.91 ± 1.403**
Atropine sulfate 1.25 mg/Kg	17.81 ± 1.171**	28.31 ± 1.194**

** Significant difference at $p \leq 0.001$, each group compared to normal, SD = standard deviation

The effect of turmeric oil on SOD in gastric serum, it was found that the control was decreased SOD 70.98% (8.71 ± 2.479) from 100% of the normal group (30.01 ± 2.406). The treatment of turmeric oil at dose of 0.3 g/kg increased SOD 36.82% (11.05 ± 0.943) from normal group. Misoprostol 50 and 100 μg/kg could increase 34.52% (10.36 ± 1.656) and 44.15% (13.25 ± 1.421) SOD from the normal group.

SOD in ulcerated gastric tissue (control) decreased 70.54% from the normal group. The turmeric oil treated at dose of 0.3 g/kg increased SOD by 48.2% (26.15 ± 2.836) from the control group (9.92± 1.931). Misoprostol 50 and 100 μg/kg could increase SOD in gastric tissue by 75.71 % (25.49 ± 3.046) and 81.62% (27.48 ± 7.679) SOD from the normal group.

Table 10. Effect of turmeric oil on SOD in serum and gastric tissue compared to control (indomethacin induced ulcer model)

Group	**SOD units/ mg of protein in rat serum Mean ± SD**	**SOD units/ mg of protein in rat gastric tissue Mean ± SD**
Normal (non-ulcer)	30.01 ± 2.406**	33.67 ± 1.484**
Control (1%tragacanth)	8.71 ± 2.479	9.92 ± 1.931
Turmeric oil 0.3 g/kg	11.05 ± 0.943*	26.15 ± 2.836**
Misoprostol 50 μg/kg	10.36 ± 1.656	25.49 ± 3.046**
Misoprostol 100 μg/kg	13.25 ± 1.421**	27.48 ± 7.679**

* Significant difference at $p \leq 0.05$, ** Significant difference at $p \leq 0.001$, each group compared to normal, SD = standard deviation

CHAPTER IV

Discussions and Conclusions

1. Gastroprotective effect
   1.1 HCl induced gastric ulcer
   1.2 Indomethacin induced gastric ulcer
2. Antioxidant properties in vitro
3. Lipid peroxidation
4. Superoxide dismutase (SOD)
5. Conclusion
6. Propose for future studies

It is generally accepted that gastric ulcer results from an imbalance between aggressive factors and the maintenance of the mucosal integrity through the endogenouse defence mechanism. Several studies have indicated that gastroduodenal protection by prostaglandin include both increase in mucosal resistance as well as decrease in aggressive factors, mainly acid and pepsin. Inhibition of prostaglandin synthesis by indomethacin coincides with the earlier stages of damage to the cell membranes of mucosal, parietal and endothelial cells (Rainsford, 1984 cited in Pandit, 2000).

Turmeric received high attention from researchers because of its wide range of activities noted by traditional healers. At the early phase of development much attention was made to curcuminoids the major components. However, later various pharmacological and clinical effects were reviewed (Ammon and Wahl, 1990, Grant and Schneider, 2000) and amongst them are anti-peptic ulcer actions. This thesis emphasized the anti-gastric ulcer and anti-oxidation actions of turmeric oil. Anti-oxidation was already confirmed to link to its gastro-protective effect. Furthermore, anti-oxidation effects not only involve in gastroprotection activity but also involve in number of activities such as lipid lowering, anti-carcinogen, anti-mutagens, and etc.

1. Gastoprotective effect

This study showed that volatile oil fraction of turmeric can exert anti-ulcer effect both from injurous factors (HCl) and protective social factors (indomethacin). The action was dose dependent as same as *Musa paradisiacal* L. (banana)(Costa and Atonio, 1997), the *Ocimum sanctum* (Holy Basil) (Singh and Majumdar, 1999), the crude hydro-alcoholic extract of *Rosmarinus officinalis* L. (Dias *et al.*, 2000), alkaloidal fraction from *Mikania cordata* (Mosaddisk and Alam, 2000), Thai propolis (Rujjanawate, 2001), the essential oil of the *Croton cajucara* Benth. (Euphorbiaceae) (Hiruma-Lime *et al.*, 2000) and *Shankha bhasma* (Pandit *et al.*, 2000).

The result of this study confirmed the previous reports on antipeptic ulcer activity of hexane extract (Permpipat, Chuthaputti and Kiatying-Angsulee, 1996) and also to verify the model of crude turmeric suspension against HCl-induced gastric necrosis in rat (Prucksunand *et al.*, 1997). Ethanol extracts of turmeric rhizome had anti-gastric ulcer action (Rafatullah *et al.*, 1990).

While turmeric oil possessed consistent result in gastroprotective action curcumin showed unclear data. One report showed that curcumin could protect gastric ulcer (Sinha *et al.*, 1975) whereas Bhatia *et al. (*1964) indicated that curcumin did not show any protective action against histamine-induced gastric ulceration in guinea pigs, and high dose of curcumin could even reduce the mucin content (Gupta *et al.*, 1980). Hexane soluble fraction showed antipeptic ulcer (Permpipat, Chuthaputti and Kiatying-Angsulee, 1996). Turmeric oil was found in hexane soluble fraction, but there was not report on turmeric oil regarding this ulcer protective effect produced yet. This project focused on the gastroprotective effect of turmeric oil and antioxidant property.

1.1 HCl-induced gastric ulcer

Hydrochloric acid regulates the pepsin activity of gastric juice. Secreted HCl plus effects of pepsin promote tissue injury (Matzner, 1939). Gastric acid cleavages pepsinogen to the active pepsin. Both gastric acid and pepsin activity destroys gastric mucus, the defensive of stomach. Induced to gastric ulcer. The optimum pH of pepsin activity is between 2 –3. In this study turmeric oil could protect gastric ulcer as shown by dose dependent reduction of ulcer index in HCl treated rats (Table 3). At 0.3 g/kg the oil exhibited significant 97.41% inhibition of gastric ulcer which were comparable to atropine sulfate (dose 50 mg/kg). We investigated the mechanism to protect them by measuring pH of gastric juice and pepsin activity, and found that pH of gastric juice increased from 2.5 to 3.5 (Table 5). This effect was similar to those gastric ulcer induced by phenylbutazone and treated with *Curcuma longa* (Dasgupta *et al.,* 1969). High gastric pH reduced activity of pepsin (Figure 13).

1.2 Indomethacin induced gastric ulcer

There are various experiment models on indomethacin induced gastric ulcer to study the effect of the plant extract such as *Turmeric* (Rafatullah *et al.*, 1990), *Croton cajucara* (Hiruma-Kima *et al.*, 1999*), Ocimum sanctum* (Singh *et al.*, 1999), *Phyllanthus emblica* (Bandyopadhyay *et al.,* 2000), *Rosmarinus officinalis* L. (Dias *et al.,* 2000), Thai propolis (Rujjanawate *et al.,* 2001) and tocotrienol-rich fraction of palm oil (Saad *et al.,* 2000).

This experiment with turmeric oil showed an anti-ulcer activity against non steroidal anti-inflammatory drugs (NSAIDs) induced ulcer. NSAIDs, such as indomethacin, aspirin ibuprofen, and etc. are known to induce ulcer during the course of their anti-inflammatory action. These drugs inhibit both constitutive (COX-1) and inducible (COX-2) isoforms of cyclooxygenase. The induction of COX-2 after inflammatory stimuli has led to the hypothesis that it is the inducible isoform (COX-2) which generates the exogenous prostaglandins, involved in fever, pain and inflammation while the constitutive isoform (COX-1) generates prostaglandins stimulates mucus release, thus increases the mucosal barrier. Turmeric oil induced protection of gastric mucus (Figure14) may be involved in some of the pathway of prostaglandin synthesis. However, these assumptions need further confirmation.

Mucus contents of gastric tissues of rats pretreated with turmeric oil indicated the gastroprotection in this indomethacin model (Table 4). This effect was similar to the studies of spices & gastric function: part I-effect of *Curcuma longa* on the gastric secretion in rabbits (Mukerji *et al.,* 1960). This result was corroborated with the morphological observation (Figure 12).

The properties of turmeric oil in the indomethacin induced gastric ulcer is good, since NSAIDs are extensively used in arthritis and inflammatory led to the rise in chemical anti-peptic ulcer used.

In conclution, turmeric oil could protect ulcerated from two inducing agent. At dose of 0.3 g/kg turmeric exerted ulcer 100% inhibition. Thai pharmacopoeia have specified the chemical constituents of turmeric that it showed contain not less than 6.0%v/w of turmeric oil (Jirawonge, 1995), 1 ml of turmeric oil was weight, that found 0.4 g/ml. Since 0.3 g/kg of turmeric oil can exert activity 100% inhibition for both HCl-induced and indomethacin induced, it was assumed that it may be equivalent to 12.5 g/kg powder, calculating from 6.0% v/w of turmeric oil in dried powder rhizome (Jirawonge, 1995). 0.3 g of turmeric oil is aqueous to 0.75 ml. So from the calculation of this results it is lightly that 625 g of dried powder of turmeric will be used to show the action. From the study of Permpipat, Chuthaputti and Kiatying-Angsulee (1996) and Prucksunand *et al.* (1997). The dose used to show of 1.25 g/kg of powder drug that 83.67% inhibition. So dried powder is more powerful than this result probably by the suspension of different mode of action, for example the fibre of the powder, antiulcer action of turmeric oil, and antioxidation activity of curcuminoid fraction.

2. Antioxidant properties in vitro

The antioxidant effects may provide an explanation for many etiologies such as antimutagenic action, stress ulcer, and etc.

We thus looked for another mechanism to explain the healing effect toward gastric ulcer. Antioxidant was one interesting mechanism. The gastric ulcer protection effect of turmeric oil may be partly explained by its antioxidant properties.

Turmeric oil showed clear zone in TLC method after 10 minutes, which was slower than the standard gallic acid (1 minute) and BHA (1 minute) (Figure 15). The quantitative of antioxidant by Spectrophotometric assay revealed that turmeric oil had 36.7% radical scavenging properties (Table 6), lower than the standard BHA 94.8% and gallic acid (95.1%). So turmeric oil did not show strong anti-oxidation action in the in vitro study. Gastro-protective action of turmeric oil may be involve antioxidant properties of turmeric oil could be protected gastric ulcer, but not mainly to protect gastric ulcer.

This research showed different result from the others that turmeric oil exerted antioxidant action by the beta-carotence-linoleate model system and the phosphomolybdenum method (Jayaprakasha *et al.,* 2002). The research used DPPH to detect so that the results were different. Chemical structure of active constituents in turmeric oil supported these findings of antioxidant. Nonetheless, it is likely that anti-oxidant effect of turmeric in general may be due to the presence of curcumin in another fraction rather than the direct effect of turmeric oil. Anti-inflammatory action of curcumins was believed to relate to this anti-oxidant activity (Ramsewak *et al*., 2000).

3. Lipid peroxidation

Gastric mucosal damage induced by 0.6 N HCl and 50 mg/kg of indomethacin may be due to free radicals production leading to increase in lipid peroxidation. This causes damage to cell and cell membranes. The decrease in level of lipid peroxidation in serum and gastric tissue of the turmeric oil treated, when compared to ulcerated group (control), suggested the ability of turmeric oil to protect gastric mucosal against the ulcer agent thus preventing cell damage. The protective action may be via the increase and maintenance near normalcy in the activity of

superoxidase dismutase as same antiulcerogenic effect of an aqueous suspension of herbal drug formation (UL-409), the decrease in level of lipid peroxidation and increase in the activities of free radical scavenging enzymes (Vanisree, Mitra and Shyamala Devi, 1996). Tian Xing Zhou *et al.* (2000) investigated lipid peroxide (LPO) according to Yagi, (1998) found that LPO levels were significantly higher in patient of gastric ulcer, gastric cancer, colon cancer and esophagus cancer than normal.

The treatment of turmeric oil significantly decreased the level of lipid peroxidation product, malondialdehyde (MDA), both in serum and gastric tissue of ulcerated rats (Table 7-8). The lipid peroxidation of HCl induced gastric ulcer. Turmeric oil could be protected lower than indomethacin induced gastric ulcer in gastric tissue and serum, MDA produced more than indomethacin induced gastric ulcer.

4. Superoxide dismutase (SOD)

SOD activity were compared ulcer inhibition Table 3 and Table 9 the rate of increasing in SOD in turmeric oil treated group were increased the same potter as increasing of ulcer inhibition. The treated of turmeric oil at dose of 0.75, 0.15 and 0.30 g/kg could increased SOD 41.98% (12.98±0.861), 48.93% (15.13±0.115) and 54.46%(16.84±1.531) in serum and 70.29% (22.90±2.035), 70.99% (23.13±0.983) and 73.4% (23.91±1.403) in gastric tissue could inhibition of ulcer 5.4%, 22.8% and 97.4% compared to 100% of the normal group. In model of indomethacin induced ulcer model found that the treated of turmeric oil at dose of 0.3 g/kg could increase SOD 36.82% (11.05±0.943) in serum and 77.66% (25.49±3.046) in gastric tissue that could inhibition of ulcer 100%.

It is currently known that superoxide anion, O_2 is involved in the ulcer formation apart from the interactive process like many other tissue degeneration situation. This experiment showed the effect of turmeric oil on the free radical scavenging enzymes in relation to the peptic ulcer. The superoxide dismutase (SOD) content in serum and gastric tissue in the experimental increases the SOD content in gastric mucosa and serum compare to the untreated group (normal) and ulcerated group of rats (control) by reducing the reactive oxygen species, O_2^- (Table 9-10).

Turmeric oil treated could protect indomethacin induced gastric ulcer (Table 10) by increasing SOD in gastric tissue more than HCl induced ulcer, but lower than serum.

The relationship between SOD increased and anti-peptic ulcer activity in this studies was similar to two studies in other plants.

The protective action may be via the increase and maintenance to near normal activity of superoxide dismutase as same antiulcerogenic effect of an aqueous suspension of herbal drug formation, increase in the activities of free radical scavenging enzymes (SOD) (Vanisree, Mitra and Devi, 1996). Zhou *et al.* (2000). Were found that SOD activity in tissue and blood was significantly decrease patient of gastric ulcer, gastric cancer, colon cancer and esophagus cancer. The SOD activity was determined by using adrenaline auto oxidation method.

This research showed different data from the others that turmeric oil exert increase of SOD by the methionine and spectrophotometric assay detected at λ=560 nm model and the other plant such as *Phyllantus emblica* fruits that used epinephrine auto-oxidation and spectrophotometric assay detected at λ= 480 nm method (Bandyopadhay *et al.,* 2000).

5. Conclusions

The data from this study led to the conclusion that turmeric oil could protect gastric ulcer from two inducing agents in rat model (0.6N HCl, and indomethacin 50 mg/kg). This was supported by reasonable antioxidant activity of SOD and MDA techniques.

In HCl induced gastric ulcer rat model the treatment of turmeric oil at dose of 0.075, 0.15 and 0.3 g/kg could produce 5.44%, 22.80% and 97.41% protection of ulcer in a dose dependent pattern. Turmeric oil at dose of 0.3 g/kg showed equivalent result to atropine sulfate 1.25 mg/kg (93.39%). The pH of gastric juices in treated rats was increased from 2.5 to 2.7, 3, 3.5 respectively and pepsin activity reduced to 4.8%, 12.7% and 62.6% from ulcerated control.

In indomethacin induced gastric ulcer rat model the treatment of turmeric oil at dose of 0.2 and 0.3 g/kg showed 87.97% and 100% protection. These doses were comparable to misoprostol 50 and 100 μg/kg (83.18%, 98.22% protection), and increased gastric wall mucus content to 23.3% and 59%

The antioxidant properties of Turmeric oil in vitro was lower (36% at 1000 ppm) than standard BHA and gallic acid. However it could increase SOD in serum in HCl induced ulcer model from 41.98% to 54.46% compared to normal group and increased SOD in gastric tissue from 70.29% to 73.4% compared to normal group. In indomethacin induced ulcer model, turmeric oil could significantly increase SOD 36.82% in serum and 77.67% compared to normal group.

Turmeric oil showed the combination of various mechanisms in protective gastric ulcer; e.g. increasing mucin of gastric wall and SOD while decreasing of pepsin activity and lipid peroxidation activity.

6. Propose for future studies

1) It is important to further study on anti-*H. pylori* of Turmeric oil which will complement the anti-peptic ulcer already studied here. In fact turmeric oil was quite well known in showing anti-microbial activities in various pathogens. But there was only one report that revealed the anti- *H pylori* effect of turmeric (against 19 strains) and curcumin but with relation to anti-mutagen action (Mahady *et al.,* 2002).
2) The active ingredients of Turmeric oil that exert anti-peptic ulcer property should be studied for drug development. Some components of turmeric oil were analyzed as aromatic turmerone, turmerone, ar-turmerone, curione, and oxygenated compounds (Jayaprakasha *et al.,* 2002) while hexane fraction denoted the ar-turmerone as major compound (Nutakul, 1994).
3) The toxicity test of active compounds of turmeric oil in animal models needed to be clarified before introducing to patients to ensure the safety. Some tests had already made for various parts of turmeric such as powder (Sittisomwong *et al.,* 1990), ethanol extract (Deshpande *et al*., 1998), and oleoresin (Bille *et al*., 1985), but not yet on turmeric oil.
4) The mechanism of turmeric oil to reduction lipid peroxidation should be studied.

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APPENDICES

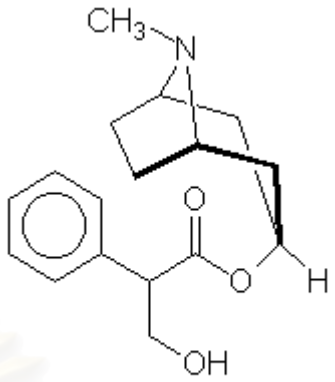
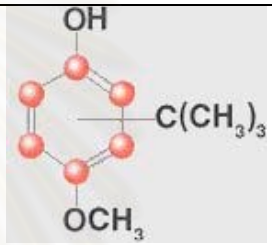
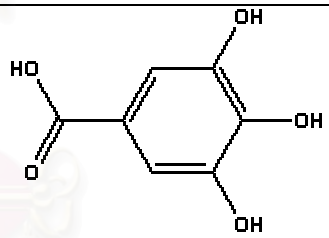
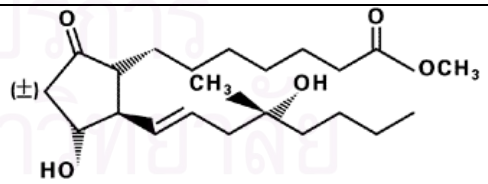
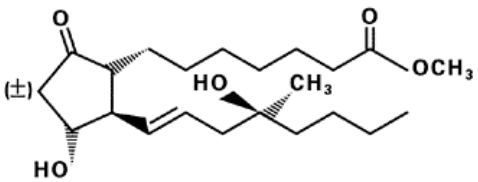
APPENDIC A

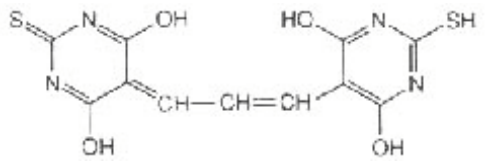
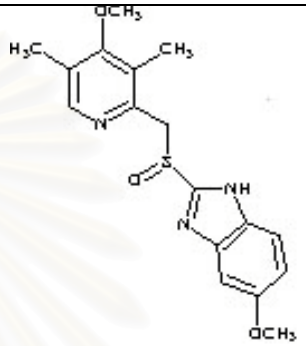
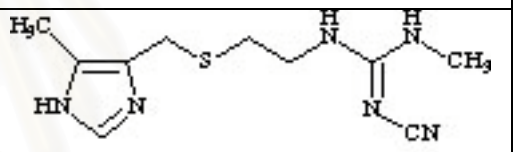
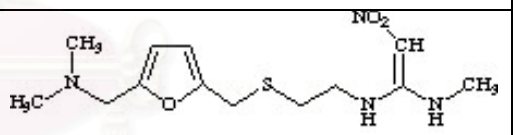
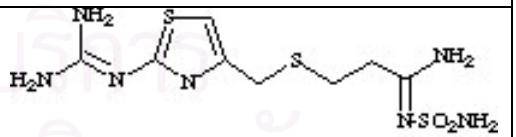
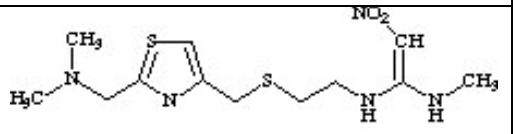
FDA-Approved Therapies for *H. pylori* infection
From Drug Facts and Comparison, St. Louis 2002
50th edition volume 2. A Wolters Kluwer Company.
Page A-10 to A-11

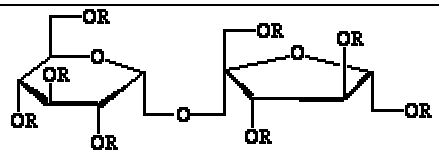
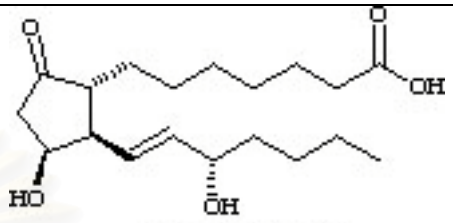
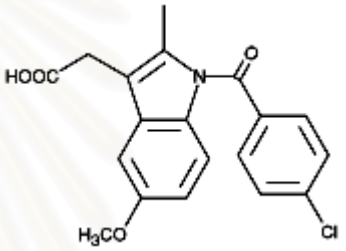
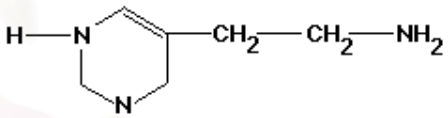
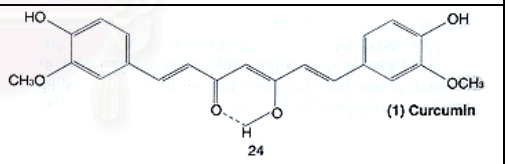
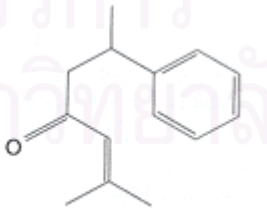
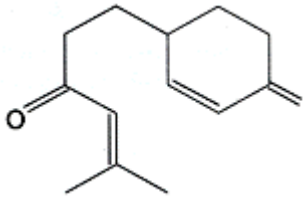
Regimens	Dosing
Lansoprazole +	30 mg. bid for 10-14 days
Clarithromycin +	500 mg. bid for 10-14 days
Amoxicillin	1 g. bid for 10-14 days
Omeprazole +	20 mg. bid for 10 days
Clarithromycin +	500 mg. bid for 10 days
Amoxicillin	1 g. bid for 10 days
Omeprazole +	40 mg. O.D. for 2 weeks
Clarithromycin	500 mg. tid for 2 weeks
Ranitidine bismuth citrate	Follow by 20 mg. O.D. for additional 2 weeks
Ranitidine bismuth citrate +	400 mg. bid for 2 weeks
Clarithromycin	500 mg. tid for 2 weeks
Ranitidine bismuth citrate	Follow by 400 mg. bid for additional 2 weeks
Bismuth subsalicylate +	525 mg. qid for 2 weeks
Metronidazole +	250 mg. qid for 2 weeks
Tetracycline) +	500 mg. qid for 2 weeks
Histamine-2 (H2) receptor antagonist	Dose as directed for 2 weeks + additional 2 weeks
Lansoprazole +	30 mg. tid for 2 weeks
Amoxicillin	1 g. tid for 2 weeks

APPENDIC B

Chemical structures

Atropine	 Atropine (1)
BHA Synonym name (s) : 3-tert-buryl-4-hydroxyanisole Molecular formula : $C_{11}H_{16}O_2$ Formula weight : 180.25	
Gallic acid Chemical name **:** Gallic acid Synonym name (s) : 3,4,5Trihydroxybenzoic acid Molecular formula : $C_7H_6O_5$ Formula weight : 170.12	
Misoprostol	 

Malondialdehyde (MDA) form a 1:2 adduct with thiobarbituric acid	
<u>Omepazole</u>	 omeprazole
Cemitidine	 cimetidine (Tagamet®)
Ranitidine	 ranitidine (Zantac®)
Famotidine	 famotidine (Pepcid®)
Nizatidine	 nizatidine (Axid®)

Sucralfate	 sucralfate (Carafate®) $R = SO_3Al(OH)_5 . (H_2O)_2$
Prostaglandin E1	 prostaglandin E1
Indomethacin	
Histamine	Histamine 
Curcumin	 (1) Curcumin 24
ar-Turmerone	 (7) ar-Turmerone
Turmerone	

APPENDIX C

Potassium phosphate buffer 0.1 M

Solution A : 27.2 g KH_2PO_4 per liter (0.2M)

Solution B : 45.6 g K_2HPO_4 per liter (0.2M)

Referring to table A1.3 for desired pH, mix the indicated volume of solutions A and B, then dilute with H_2O to 200 ml. This may be made as a5 – or 10 – fold concentrate by scaling up the amount of potassium phosphate in the same volume.

Phosphate buffer show concentration dependent pH changes, so check concentrate pH by diluting an aliquot to the final concentration.

Potassium phosphate buffer, pH 8.0, 1 M

A : 1 M K2HPO4

B : 1 M KH2PO4

Add A to B until pH = 8.0

Preparation of 0.1 M sodium and potassium phosphate buffers

Desired pH	Solution A (ml)	Solution B (ml)	Desired PH	Solution A (ml)	Solution B (ml)
5.7	93.5	6.5	6.9	45.0	55.0
5.8	92.0	8.0	7.0	39.0	61.0
5.9	90.0	10.0	7.1	33.0	67.0
6.0	87.7	12.3	7.2	28.0	72.0
6.1	85.0	15.0	7.3	23.0	77.0
6.2	81.5	18.5	7.4	19.0	81.0
6.3	77.5	22.5	7.5	16.0	84.0
6.4	73.5	26.5	7.6	13.0	87.0
6.5	68.5	31.5	7.7	10.5	90.5
6.6	62.5	37.5	7.8	8.5	91.5
6.7	56.5	43.5	7.9	7.0	93.0
6.8	51.0	49.0	8.0	5.3	94.7

APPENDIX D Determination of major compounds of turmeric oil

The two main constituents of the turmeric rhizome are the yellow colouring matter, curcuminoids, and the volatile oils (Chavalittumrong and Jirawattanapong, 1992). The turmeric oil from the zhizome contained more than 40 constituents. There compounds were alpha-turmerone (30-32%), ar-turmerone (17-26%) and beta-turmerone (15-18%) (Sharma *et al.*, 1997). An anti-peptic ulcer principle of *Curcuma longa* L. were isolated and identified as ar-turmerone, one of major components in the essential oil (Nutakul, 1994).

In the experiment we used 3 ways analyze the main constituents of turmeric oil.

1.1 GC- MS pattern of turmeric oil and major compounds

- SGS (Thailand) limited. Analysis of major compound of turmeric oil, separated major compound were prepared in chloroform solution (1:50) then analyzed by GC-MS. The system was connected with a 30 m x 0.25 mm (i.d.) capillary coated with DB-WAX, J&W (film thickness 0.25 μm). The operating parameters were as follows: (R.P. Adams “The analysis of essential oil by GC-MS”)

Helium carrier gas flow rate	: 1 ml/min
Injector temperature	: 220 °C
Initial column temperature	: 60 °C
Final column temperature	: 220 °C
Increasing temperature rate	: 10 °C/min
Injection volume	: 1μl

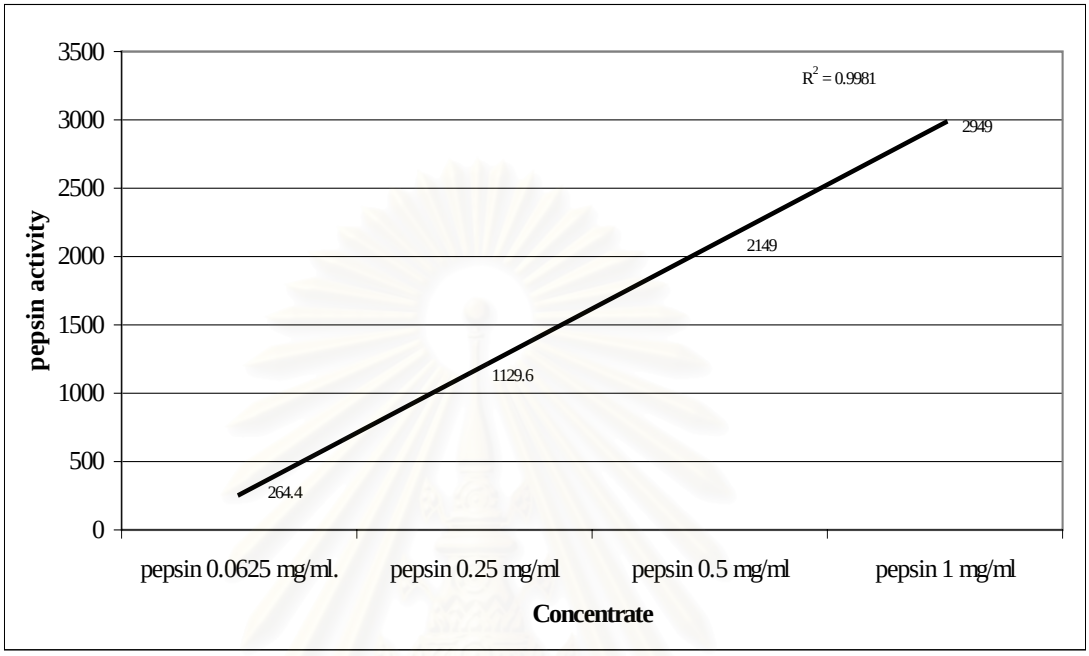
- Analysis of turmeric oil to finding major compound in turmeric oil at the Faculty of Medicine, Department of Biochemistry Chiang Mai University.
- Analysis of turmeric oil by NMR Technique at Institute of Medicinal plant Research and Development.

1.2 Major compound of turmeric oil

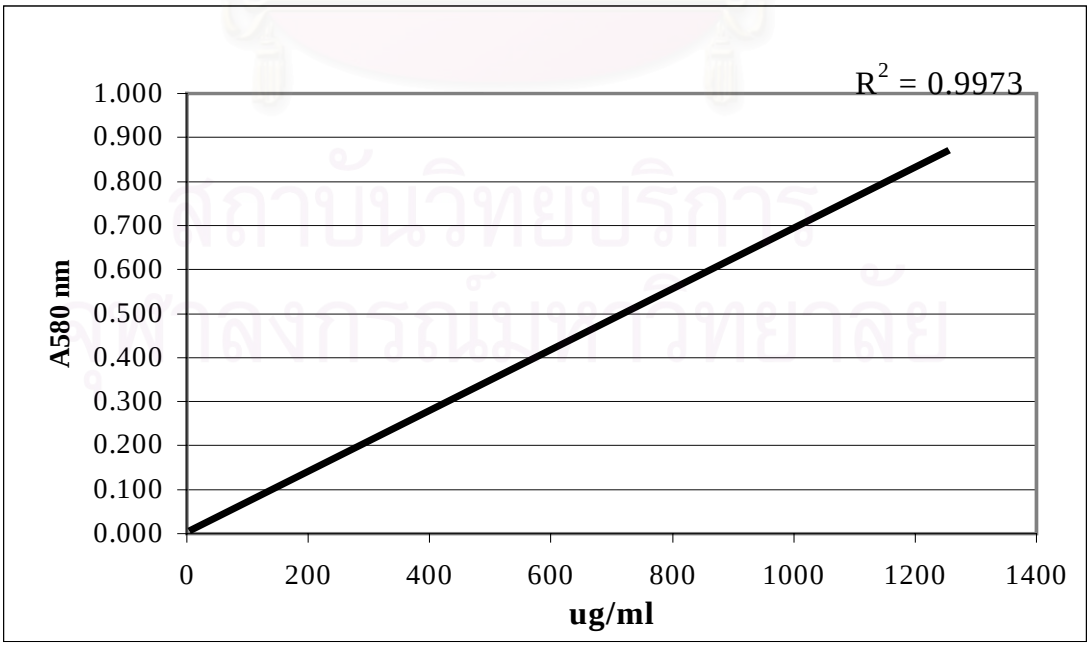
GC/MS could detect 53.78% of ar-turmerone in turmeric oil sample (Kungtaweelead, 2003) and 19.89% β-turmerone in the sample Turmeric oil. (Faculty of Medicine, Department of Biochemistry Chiang Mai University, Institute of Medicinal plant Research and Development, Laboratory of SGS (Thailand) Limited, 2002).

APPENDIX E Standard curve

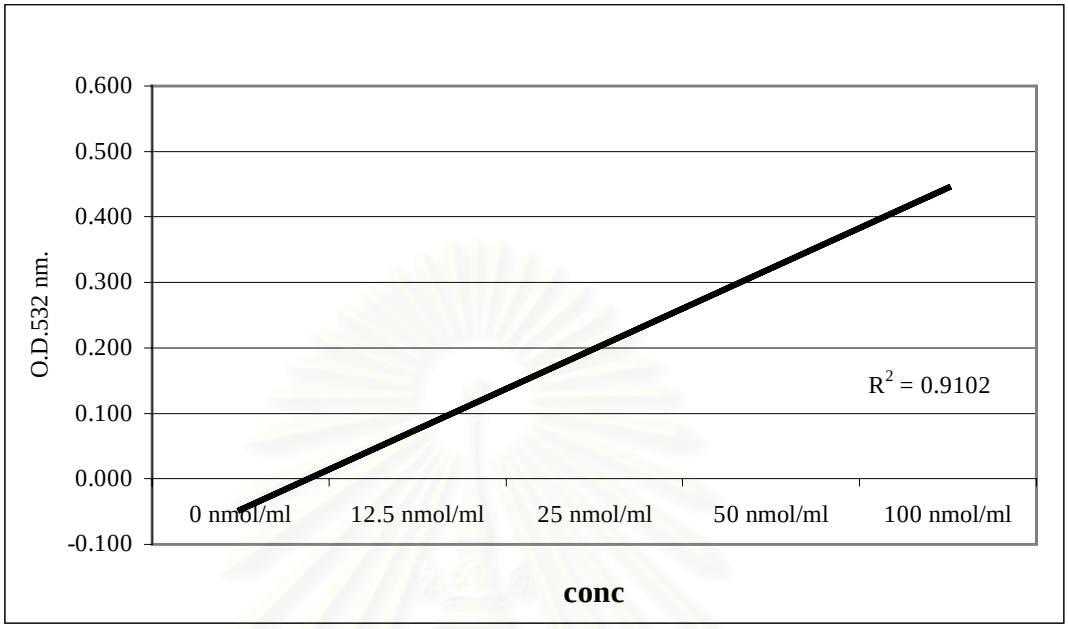
1. Standard curve of pepsin



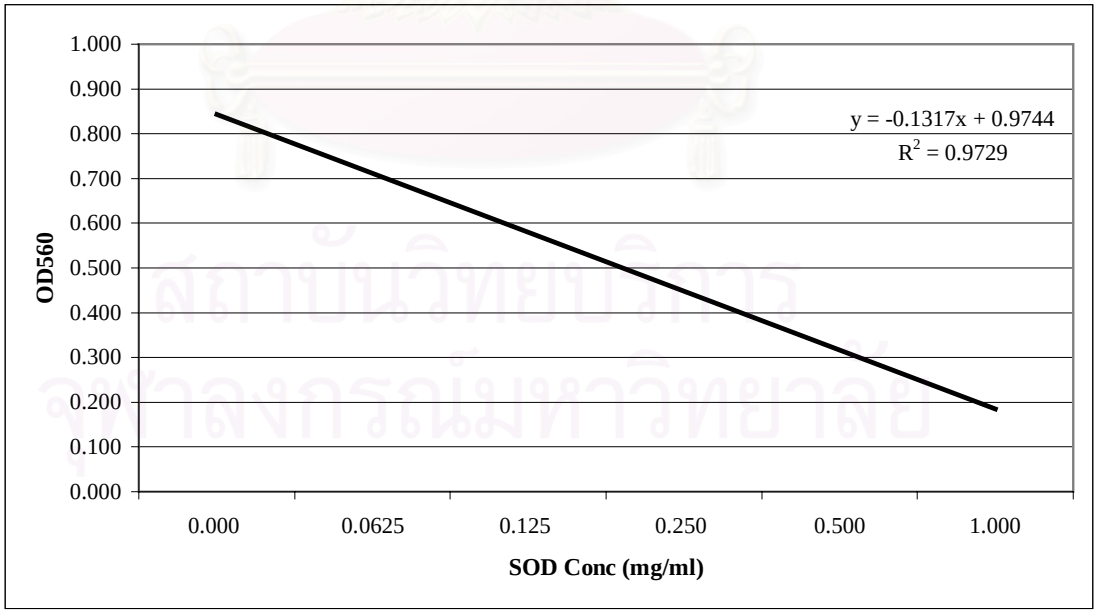
2. Standard curve of alcian blue



3. Standard curve of malondialdehyde



4. Superoxide dismutase inhibited formazam



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