ANTI-INFLAMMATORY, ANALGESIC, ANTIPYRETIC AND ANTI-GASTRIC ULCER ACTIVITIES OF EXTRACT FROM *Pseuderanthemem palatiferum* IN ANIMALS

KANNIKA INCHAB

DOCTOR OF PHILOSOPHY

IN PHARMACOLOGY

GRADUATE SCHOOL

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IN PHARMACOLOGY

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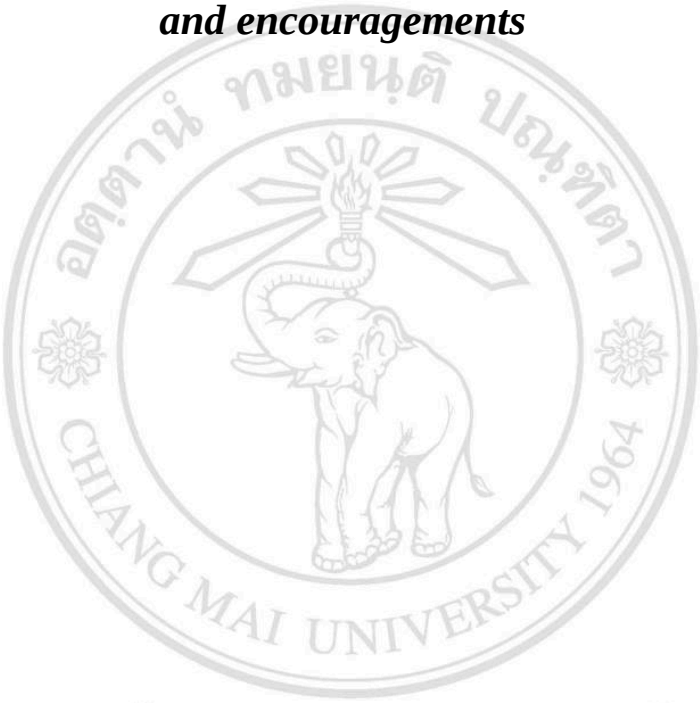
24 April 2019

To

Mr.Satapat Racha, my parent, and my advisor for their support and encouragements



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Kannika Inchab

หัวข้อดุษฎีนิพนธ์	ฤทธิ์ต้านการอักเสบ ระงับปวด ลดไข้ และฤทธิ์ต้านการเกิดแผลในกระเพาะอาหารของสารสกัดจากพญาวานร (*Pseuderanthemum palatiferum*) ในสัตว์	
ผู้เขียน	นางสาว กรรณิการ์ อินจับ	
ปริญญา	วิทยาศาสตรดุษฎีบัณฑิต (เภสัชวิทยา)	
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	ผศ.ดร.พญ. ศรัณยภิญ โพธิกานนท์	อาจารย์ที่ปรึกษาร่วม

บทคัดย่อ

พญาวานรเป็นพืชสมุนไพรที่มีการใช้ทั่วไปในการแพทย์แผนโบราณของเวียดนาม และไทยสำหรับรักษาโรคต่างๆ โดยใช้ใบสด หรือใบต้มในการป้องกันหรือรักษาโรคความดันโลหิตสูง ท้องเสีย ข้ออักเสบ และกระเพาะอาหารอักเสบ เป็นต้น แต่ทว่ายังไม่มีรายงานการศึกษาฤทธิ์ทางเภสัชวิทยาของสารสกัดน้ำจากใบของพญาวานรที่ใช้ตามการรักษาในไทย การศึกษานี้เพื่อประเมินฤทธิ์ต้านการอักเสบ ฤทธิ์ระงับปวด ฤทธิ์ลดไข้ และฤทธิ์ต้านการเกิดแผลในกระเพาะอาหารของสารสกัดน้ำจากพญาวานร ผลการประเมินฤทธิ์เบื้องต้นพบว่าสารสกัดน้ำจากพญาวานรมีฤทธิ์ยับยั้งการอักเสบเฉียบพลันซึ่งพบได้ในการเหนี่ยวนำการบวมของใบหูหนูขาวด้วยเอทิลฟีนิลโพรพิโอเลท การเหนี่ยวนำการบวมของอุ้งเท้าหนูขาวด้วยคาราจีแนน และการเหนี่ยวนำการบวมของอุ้งเท้าหนูขาวด้วยกรดอะราชิโดนิก ผลการศึกษาเสนอว่าฤทธิ์ต้านการอักเสบของสารสกัดน้ำจากพญาวานรอาจเป็นผลจากการยับยั้งผ่านทางวิถีไซโคลออกซิจิเนสและไลปอกซิจิเนส อย่างไรก็ตามในแบบจำลองของการเหนี่ยวนำการบวมของอุ้งเท้าหนูด้วยคาราจีแนน สารสกัดน้ำจากพญาวานร (ขนาด 600 มิลลิกรัมต่อกิโลกรัม) พบว่าความเข้มข้นของไนไตรท์ลดลงแต่การทำงานของไมอีโลเพอรอกซิเดสของหนูไม่เปลี่ยนแปลงเมื่อเทียบกับกลุ่มควบคุม นอกจากนี้เซลล์อักเสบในอุ้งเท้าหนูที่ได้รับสารสกัดน้ำจากพญาวานรมีมากกว่าที่พบในอุ้งเท้าของกลุ่มไอบูโพรเฟน ในแบบจำลองการอักเสบเรื้อรังพบว่าหนูที่ได้รับสารสกัดน้ำจากพญาวานรไม่มีการลดลงของน้ำหนักแกรนูโลมา ระดับของอัลคาไลน์ฟอสฟาเทสในซีรั่ม การเพิ่มขึ้นของน้ำหนักตัวและน้ำหนักแห้งของไทมัสในแบบจำลองการฝังก้อนสำลี ผลดังกล่าวแสดง

ว่าสารสกัดน้ำจากพญาวานรไม่มีฤทธิ์เหมือนสเตียรอยด์ ในการทดสอบฤทธิ์ระงับปวดพบว่าสารสกัดน้ำจากพญาวานรแสดงฤทธิ์ระงับปวดในหนูถีบจักรที่ถูกเหนี่ยวนำให้เกิดความเจ็บปวดโดยการฉีดกรดอะซิติก แต่ผลต่อการทดสอบการสะบัดหางหนีของหนูขาวน้อยกว่าโคเดอิน แสดงว่ากลไกการระงับปวดของสารสกัดน้ำจากพญาวานรผ่านทางระบบประสาทส่วนปลายเป็นหลัก ส่วนการศึกษาฤทธิ์ลดไข้พบว่าหนูที่ได้รับสารสกัดน้ำจากพญาวานรไม่สามารถลดอุณหภูมิร่างกายของหนูขาวที่ถูกเหนี่ยวนำให้เกิดไข้โดยการฉีดยีสต์ การทดสอบฤทธิ์ต้านการเกิดแผลในกระเพาะอาหารพบว่าการให้สารสกัดน้ำจากพญาวานรก่อนสามารถลดการเกิดแผลในกระเพาะอาหารได้ในแบบจำลองการเกิดแผลในกระเพาะอาหารแบบเฉียบพลัน ได้แก่ การเหนี่ยวนำให้เกิดแผลในกระเพาะของหนูด้วยการให้สารผสมเอทานอลและกรดไฮโดรคลอริก ยาอินโดเมทาซิน และความเครียดที่เหนี่ยวนำโดยการขังกรงและแช่ในน้ำเย็น กลไกการต้านแผลในกระเพาะอาหารอาจเกี่ยวข้องกับการป้องกันการทำลาย และ/หรือการสร้างเมือกที่กระเพาะอาหาร แต่ไม่เกี่ยวกับการยับยั้งการหลั่งกรด สรุปว่าสารสกัดน้ำจากพญาวานรมีฤทธิ์ต้านการอักเสบในการอักเสบแบบเฉียบพลัน ฤทธิ์แก้ปวด และฤทธิ์ต้านการเกิดแผลในกระเพาะอาหาร

Dissertation Title	Anti-inflammatory, Analgesic, Antipyretic and Anti-gastric Ulcer Activities of Extract from *Pseuderanthemum palatiferum* in Animals	
Author	Ms. Kannika Inchab	
Degree	Doctor of Philosophy (Pharmacology)	
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ABSTRACT

Payawanorn (*Pseuderanthemum palatiferum*) is a herbal plant commonly used in Vietnamese and Thai traditional medicine for the prevention or treatment of many disorders. The fresh leaves or boiled leaves are widely used for prevention or treatment of blood pressure, diarrhea, arthritis, and gastritis disorders. Yet, no study has been performed on the pharmacological activity of the water extract of *P. palatiferum* according to Thai traditional remedy. In the present study, the *P. palatiferum* water extract was investigated for anti-inflammatory, analgesic, antipyretic, and anti-gastric activities. In preliminary investigation, the *P. palatiferum* water extract was found to exert an inhibitory activity on the acute phase of inflammation as seen in ethyl phenylpropiolate-induced ear edema as well as in carrageenan-induced hind paw edema in rats and arachidonic acid-induced hind paw in rats. The results suggest that the anti-inflammatory activity of the *P. palatiferum* water extract may be due to an inhibition via cyclooxygenase pathway and lipoxygenase pathway. However, in carrageenan-induced hind paw edema experiment, the rat receiving *P. palatiferum* water extract (600 mg/kg), a reduced plasma nitrite content but without a reduced MPO activity was found when

compared to the control group. Additionally, the inflammatory cells in the paw of rats receiving the water extract of *P. palatiferum* were greater than those found in the ibuprofen group. In the chronic inflammatory model, the rats received *P. palatiferum* water extract did not reduce granuloma weight, serum alkaline phosphatase activity, body weight gain and thymus dry weight on cotton pellet-induced granuloma formation. These results pointed the non-steroidal like action of the *P. palatiferum* water extract. In the analgesic tests, the *P. palatiferum* water extract showed an analgesic effect on acetic acid-induced writhing response in mice, but the effect on tail-flick test in rats was less than that of codeine. The mechanism of analgesic activity of the *P. palatiferum* water extract may act majorly via the peripheral nervous system. In antipyretic test, the rats received *P. palatiferum* water extract did not show a decrease of body temperature on brewer's yeast-induced hyperthermia rats. In anti-gastric test, the pretreatment with *P. palatiferum* water extract can reduce gastric lesion in acute gastric ulcer models (ethanol and hydrochloric acid-, indomethacin-, and restraint water immersion stress-induced gastric lesions in rats). The mechanism of anti-gastric ulcer effect may involve the preservation and/or synthesis of the gastric mucus but was not involved the inhibition of gastric acid secretion. In conclusions, *P. palatiferum* water extract possesses anti-inflammatory activity on acute inflammation, analgesic, and anti-gastric ulcer activities.

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LIST OF ABBREVIATIONS

µL	Microlitre
µM	Micrometer
µmol	Micromole
µ-opioid	Mu-opioid
5HT	Serotonin
AA	Arachidonic acid
ALP	Alkaline phosphatase
ANOVA	One-way analysis of variance
ASA	Aspirin
BBB	Blood brain barrier
BSA	Bovine serum albumin
CNS	Central nervous system
COX	Cyclooxygenase
COX-1	Cyclooxygenase-1
COX-2	Cyclooxygenase-2
ECL	Enterochromaffin-like
eNOS	Endothelial nitric oxide synthase
EtOH/HCl	Ethanol/Hydrochloric acid
GFP	Green fluorescent protein
GI	Gastrointestinal
h	Hour
HIF-1	Hypoxia-induced factor-1
H_2O_2	Hydrogen peroxide
H. pylori	*Helicobacter pylori*
IFN-α	Interferon-alpha
IL	Interleukin
iNOS	Inducible nitric oxide synthase

LIST OF ABBREVIATIONS (Continued)

LOX	Lipoxygenase
LTB_4	Leukotriene B4
LTs	Leukotrienes
LX	Lipoxins
MAC	Membrane attack complex
min	Minute
mL	Milliliter
mm	Millimetre
MPO	Myeloperoxidase
mU	Milliunit
NADPH	Nicotinamide adenine dinucleotide phosphate
nM	Nanometre
nNOS	Neuronal nitric oxide synthase
NO	Nitric oxide
NOS	Nitric oxide synthase
NSAIDs	Nonsteroidal anti-inflammatory drugs
ORL-1	Opioid receptor-like subtype 1
PAF	Platelet activating factor
PG	Prostaglandin
PGD_2	Prostaglandin D_2
PGE_2	Prostaglandin E_2
PGI_2	Prostacyclin
PPIs	Proton-pump inhibitors
PPWE	Water crude extract of *P. palatiferum*
ROS	Reactive O_2 species
sec	Second
S.E.M.	Standard error of the mean

LIST OF ABBREVIATIONS (Continued)

TNF	Tumor necrosis factor
TXA_2	Thromboxane A_2

LIST OF SYMBOLS

%	percent
°C	degree Celsius
μ	micro, 10^{-6}
k	kilo, 10^{3}
m	milli, 10^{-3}
n	nano, 10^{-9}
κ	kappa
δ	delta
α	alpha
β	beta
γ	gramma
λ	lambda

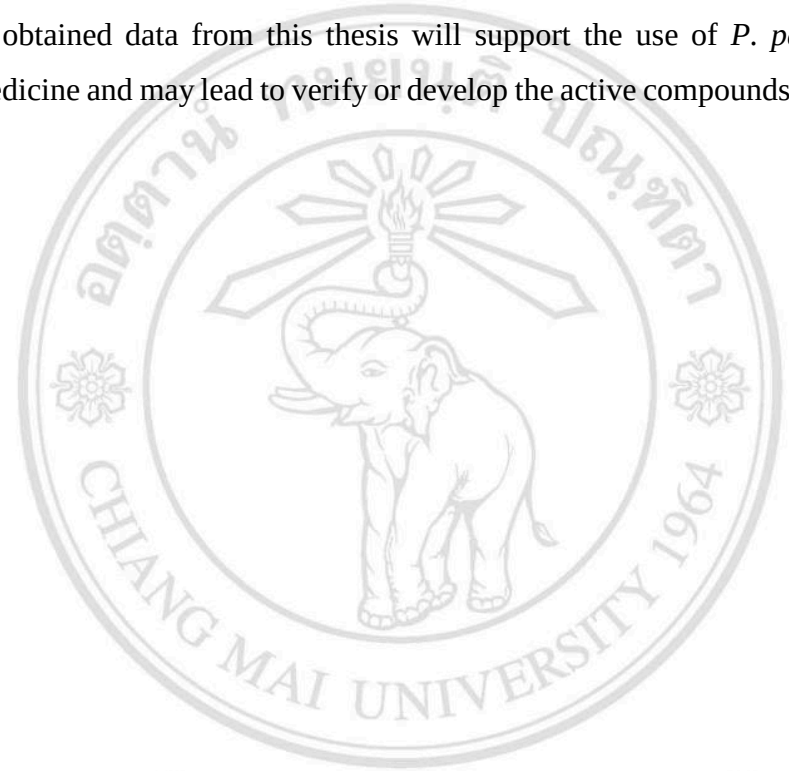
ข้อความแห่งการริเริ่ม

พญาวานร จัดเป็นพืชสมุนไพรพื้นบ้านที่มีการนำมาใช้รับประทานเป็นอาหาร มีสรรพคุณในการบรรเทาโรคต่างๆ เช่น ความดันโลหิตสูง อุจจาระร่วง การอักเสบในโรคข้ออักเสบ และโรคกระเพาะอาหารอักเสบ อย่างไรก็ตามสารสกัดน้ำของพญาวานรยังขาดการศึกษาเกี่ยวกับฤทธิ์ต้านการอักเสบ ระงับปวด และฤทธิ์ในการป้องกันการเกิดแผลในกระเพาะอาหารในสัตว์กัดแทะ

ผลที่ได้รับจากวิทยานิพนธ์นี้เป็นข้อมูลสนับสนุนการใช้พญาวานรเป็นสมุนไพรพื้นบ้าน และอาจนำไปสู่การพัฒนาหาสารสำคัญในการออกฤทธิ์ต่อไปในอนาคต

STATEMENT OF ORIGINALITY

P. palatiferum is use food and a folk medicine for relieving high blood pressure, diarrhea, inflammation in arthritis, and gastritis. However, there is no scientific data for anti-inflammatory, analgesic, and anti-gastric effects using the *P. palatiferum* water extract in rodents. The obtained data from this thesis will support the use of *P. palatiferum* in traditional medicine and may lead to verify or develop the active compounds in the future.



CHAPTER 1

Introduction

1.1 Principles, Theory, Rationale and Hypothesis

The term of Inflammation is basically a protective response to reduce the basic condition of cellular damage as well as the necrosis and tissues or organs resulting from the original insult. Inflammation performs its protective purpose by reducing, otherwise neutralizing dangerous substances (for example, toxins or microbes) [1]. Inflammation is dived into two essential patterns; 1) acute inflammation and 2) chronic inflammation. Acute inflammation is extremely fast in onset (seconds to minutes) and is approximately short duration, remaining from a few minutes up to a few days; its main features are exudation of fluid and plasma protein and neutrophilic leukocyte aggregation. Chronic inflammation is acknowledged to be inflammation of prolonging duration (weeks to months to years) and is linked histologically with the occupation of macrophages and lymphocytes, tissue necrosis, fibrosis, and the proliferation of blood vessels. The ultimate type of chronic inflammation is the event of cell injury in some of the most prevalent and disabling inflammatory diseases, such as chronic lung diseases, tuberculosis, atherosclerosis, and rheumatoid arthritis. However, these basic patterns of inflammation can overlap [2].

The fundamental role of eicosanoids in inflammatory manners is indicated by the clinical efficacy of agents that block eicosanoid synthesis. Corticosteroids are the strongest anti-inflammatory medicines that are currently available. They inhibit the aggregation of neutrophils at localities of inflammation but also have broad effects on other inflammatory cells and inflammatory processes. This is the reason for overflowing side effects ranging from osteoporosis to disruption of the hypothalamic-hypophyseal axis.

Nonsteroidal anti-inflammatory drugs (NSAIDs) can inhibit cyclooxygenase (COX) enzyme, which is associated with prostaglandin (PG) production. There are mainly two isoforms of this enzyme. COX-1 is detected in platelets and catalyzes the production of thromboxane A_2 (TXA_2). COX-1 inhibition influences to diminished blood clotting, some COX-1 inhibitors are applied as an anticoagulant and antiplatelet drugs. COX-2 is shown in blood vessels and macrophages in response to inflammation and leads to the generation of prostacyclin (PGI_2). Drugs that specifically block COX-2 can also reduce inflammatory pain. However, both COX-1 and COX-2 are normally present in several areas, which is a reason for the rich pharmacologic side effects of COX inhibitors. More than 20,000 persons per year passed away in the United States as the main result of adverse events linked to NSAIDs treatment. Long-term use of NSAIDs leads to dyspepsia and nausea in approximately half of the patients. Peptic ulceration happens in up to 25% of patients who remedy NSAIDs for long-term, with perforation or gastrointestinal (GI) bleeding happening in approximate 1.5% of patients who develop ulcers. NSAIDs-related peptic ulcers normally happen in the stomach whereas duodenal ulcers are much less common. Alternative therapies include a wide variety of natural substances that have antioxidative and anti-inflammatory features but often have poorly understood their mechanisms of action [2, 3].

In recent years, a wide-spread search has been launched new anti-inflammatory drugs from natural resources and synthetic [4]. It has been reported that many plants with anti-inflammatory activity have been found to lack anti-gastric ulcer effects for examples *Asparagus cochinchinensis* Merrill, *Chrysanthemum indicum* Linn and *Terminalia paniculata* Roxb.

Pseuderanthemum palatiferum is originated in Vietnam and widely used as a traditional medicine in Thailand. It is used to treat or prevent many diseases and symptoms including high blood pressure, diarrhea, constipation, gastritis, colitis, hemorrhoids, flu, rhinitis, pharyngitis, arthritis, nephritis, wound, and colon cancer. Since the *P. palatiferum* water extract is used according to Thai remedy, however the anti-inflammatory, analgesics, antipyretic and anti-gastric ulcer activities have not been studied yet. Thus, the present work was carried out to evaluate the *P. palatiferum* water extract for these pharmacological activities [3, 5].

1.2 Literature review

1.2.1 Inflammation

The inflammatory manner is the immune system's protecting response to a damaging stimulus. It can be evoked by infections, physical injuries and noxious agents, which release pathogen- and damage-associated molecules that are known by cells charged with immune surveillance [6]. The ability to mount an inflammatory response is crucial for endurance in the appearance of injury and environmental pathogens. In some conditions and diseases, inflammation may be excessive and provided without obvious benefit and even with severe adverse outcomes such as chronic inflammation, autoimmune diseases, and hypersensitivity. The inflammatory response is defined mechanistically by increased vascular permeability and vasodilation; infiltration of phagocytic cells and leukocytes; and resolution with or without fibrosis and tissue degeneration [7].

Several molecules are associated with the development and resolution of the inflammatory process. Histamine, bradykinin, serotonin (5HT), prostanoids, leukotrienes (LTs), platelet activating factor (PAF), and an array of cytokines are important mediators. Prostanoid biosynthesis is significantly progressed in inflamed tissue. Prostaglandin E_2 (PGE_2) and PGI_2 are the main prostanoids that interfere with inflammation. They enhance vascular permeability, local blood flow, and leukocyte infiltration for activation of their corresponding receptors, EP_2 receptor and IP receptor. PGD_2 is the main product of mast cells, it usually provides inflammation in allergic responses, especially in the lung. Activation of endothelial cells represents an essential function in recruiting the circulating cells to inflammatory sites. Endothelial activation effects in leukocyte rolling and adhesion as the leukocytes recognize newly displayed selectins, integrins, and adhesion molecules. PGE_2 and TXA_2 enhance leukocyte chemoattraction and endothelial adhesion [8].

The recruitment of inflammatory cells to localities of trauma also includes the concerted interactions of the complement factors PAF, and eicosanoids such as leukotriene B4 (LTB_4). There can perform as chemotactic agents. Cytokines work crucial roles in organizing the inflammatory process, particularly tumor necrosis factor (TNF)

and interleukin-1 (IL-1). Several biological anti-inflammatory therapeutics targets these cytokines or their signaling pathways. Other growth factors and cytokines (for example, granulocyte macrophage colony-stimulating factor, IL-2, IL-6, IL-8) contribute to displays of the inflammatory response. The concentrations of numerous of these factors have risen in the inflammatory condition such as in the synovia of subjects with arthritis [9]

1.2.2 Chemical mediators of inflammation

1) Vasoactive amines

Histamine and 5HT are vasoactive amines, it is collected as preformed molecules in mast cells and other cells and is among the primary mediators to be released in acute inflammatory responses.

Histamine is produced by several cell types, especially mast cells adjacent to vessels, as well as distributing basophils and platelets. Preformed histamine is normally released from mast cell granules in response to a variety of stimuli: (1) cytokines (for example, IL-1, IL-8); (2) neuropeptides (for example, substance P); (3) physical injury such as heat or wound (4) C3a and C5a parts of complement that called anaphylatoxins; (5) immune responses including the interaction of IgE antibodies to Fc receptors on mast cells; and (6) leukocyte-derived histamine-releasing proteins. In humans, histamine effects on arteriolar dilation and fast increases vascular permeability by influencing venular endothelial contraction and formation of inter-endothelial gaps. After there release, histamine is normally inactivated by histaminase enzyme [10, 11].

5HT acts as vasoactive mediator located within platelet granules that are released when platelet aggregation. It regularly shows vasoconstriction during clotting. 5HT is produced mainly in some enterochromaffin cells and neurons, and is a neurotransmitter and controls intestinal motility [12].

2) Plasma Protein

A difference of appearances in the inflammatory responses is arbitrated by plasma proteins that belong to three interrelated systems 1) complement, 2) kinin, and 3) clotting systems.

The complement system consists of plasma proteins that perform an essential function in inflammation and host protection response (immunity). Upon activation, various complement proteins coat particles, such as microbes, for destruction and phagocytosis, and contribute to the inflammatory response by promoting leukocyte chemotaxis and vascular permeability. The activation of complement creates a porelike membrane attack complex (MAC) that produces holes in the membranes of microbes. Complement components, numbered C1 to C9, are located in plasma in inactive forms, and several of them are activated by proteolysis to acquire their own proteolytic activity, thus setting up an enzymatic cascade. The complement-derived factors that are produced along the way contribute to a variance of events in acute inflammation. C3a and C5a enhance vascular permeability (capillary permeability or microvascular permeability) and influence vasodilation by effecting mast cells to release histamine. C5a also stimulates the lipoxygenase (LOX) pathway of arachidonic acid (AA) metabolism in macrophages and neutrophils, making the release of numerous chemical inflammatory mediators [13-15].

Some of the molecules stimulated during blood clotting are responsible for triggering multiple features of the inflammatory response. Hageman factor (also acknowledged as factor XII) is a protein produced by the liver that shows in an inactive form until it encounters collagen, basement membrane, or activated platelets (for example, at a site of endothelial lesion). Activated Hageman factor (factor XIIa) initiates four systems that may contribute to the inflammatory response: First, the kinin system, creating vasoactive kinins; Second, the clotting system, affecting the activation of fibrinopeptides, factor X, and thrombin, all with inflammatory features; Third, the fibrinolytic system, producing plasmin and inactivating thrombin; Finally, the complement system, simulating the anaphylatoxins (both C3a and C5a) [16].

3) Arachidonic acid metabolites

Products obtained from the metabolism of AA influence a variety of biologic processes, including inflammation and hemostasis. AA metabolites, also called eicosanoids, can interfere essentially all step of inflammation; their synthesis is developed at sites of the inflammation, and substances that inhibit their synthesis also relieve inflammation. Mast cells, leukocytes, platelets, and endothelial cells are the important sources of AA metabolites. These AA-derived mediators represent regionally at the site of formation and then turn instinctively or are enzymatically destroyed. AA is an unsaturated fatty acid (consist of four double bonds) produced originally from dietary linoleic acid and detect in the body essentially in its esterified form as a part of cell membrane phospholipids. It is commonly released from these phospholipids through the action of cellular phospholipases that have been stimulated by physical, mechanical, or chemical stimuli, or by chemical mediators of inflammation such as C5a [17, 18].

The metabolism of AA advances with one of two important enzymatic pathways: COX stimulates the synthesis of TXs and PGs, and LOX is accountable for the production of lipoxins (LX) and LTs [19].

TXA_2, PGI_2, $PGF_{2\alpha}$, PGD_2, and PGE_2, each obtained by the operation of a particular enzyme on an intermediate. Some of those enzymes have a restricted tissue distribution. For example, platelets comprise the enzyme TX synthase, TXA_2 is a vasoconstrictor and platelet-aggregating agent. Endothelial cells, on the other hand, lack TX synthase but contain PGI_2 synthase, which is capable for the establishment of PGI_2, a potent inhibitor of platelet aggregation and a vasodilator. PGD_2 is the primary metabolite of the COX pathway in mast cells; along with $PGF_{2\alpha}$ and PGE_2 (which are more broadly distributed), they cause vasodilation and potentiate edema formation. The PGs also contribute to the pain and illness that accompany inflammation; PGE_2 increases pain sensibility to a variety of other stimuli and associates with cytokines to create a fever [20].

LTs are generated by the action of 5-LOX, the important AA-metabolizing enzyme in neutrophils. The synthesis of LTs includes multiple steps. The first step generates LTA_4, which in turn contributes an increase to LTC_4 or LTB_4. LTB_4 is created by some macrophages and neutrophils and is a strong chemotactic substance for

neutrophils. LTC_4 and its consequent metabolites, LTE_4 and LTD_4, are created principally in mast cells and caused bronchoconstriction and increased amounts of vascular permeability [21].

Once leukocytes access to tissues, they regularly modify their primary LOX-derived AA products from LTs to anti-inflammatory mediators called LX, which inhibit neutrophil chemotaxis and adhesion to endothelium and thus serve as endogenous antagonists of LTs. Platelets that are generally activated and adhered to leukocytes also are major sources of lipoxins. Platelets alone cannot integrate lipoxins A4 (LXA_4) and lipoxins B4 (LXB_4), but they can form these mediators from an intermediate obtained from adjacent neutrophils, by a transcellular biosynthetic pathway. By this mechanism, AA products can cross from one cell type to others. [22].

4) Platelet-activating factor (PAF)

Basically mentioned for its facility to aggregate platelets and cause their degranulation, PAF is a phospholipid-derived mediator with inflammatory effects. PAF is acetyl glycerol ether phosphocholine. It is created from the membrane phospholipids of basophils, neutrophils, endothelial cells, monocytes, and platelets (and other cells) by the operation of phospholipase A2. PAF plays directly on target cells through the effects of a particular G protein-coupled receptor. In addition to stimulating platelets, PAF effects bronchoconstriction and more effective than histamine in influencing vasodilation and increasing vascular permeability. It also generates the synthesis of mediators, such as eicosanoids and cytokines, from platelets and other cells. Thus, PAF can elicit several of the responses of inflammation, including increased leukocyte adhesion, leukocyte degranulation, chemotaxis, and the respiratory burst [23].

5) Cytokines and Chemokines

Cytokines are polypeptide produced by several cell types that perform as mediators of inflammatory and immune responses. Various cytokines are involved in the initial immune and inflammatory responses to noxious stimuli and in the later adaptive (specific) immune responses to microbes. Some cytokines mainly stimulate bone marrow precursors to generate more leukocytes, thus replacing the ones that are utilized during inflammatory and immune responses. Molecularly characterized cytokines are called ILs, assigning to their ability to mediate communication between leukocytes [24, 25]. The important cytokines in the acute phase of inflammatory are TNF, IL-1, IL-6, and a group of chemoattractant cytokines named chemokines. Other cytokines that are more necessary in chronic inflammation involve interferon-γ (IFN-γ) and IL-12. A cytokine called IL-17, generated by T lymphocytes and other cells performs an essential function in recruiting neutrophils and is included in host protection against infections and in inflammatory diseases. TNF and IL-1 are generated by stimulated macrophages, as well as endothelial cells, mast cells, and some other cell types. Their secretion is normally stimulated by microbial outcomes, such as bacterial endotoxin, immune complexes, and products of T lymphocytes created during adaptive immune responses. IL-1 is also the cytokine-induced by activation of the inflammation. The primary function of cytokines in inflammatory process is in endothelial activation. TNF and IL-1 activate the expression of adhesion molecules on endothelial cells, resulting in enhanced leukocyte binding and recruitment, and improve the production of further eicosanoids and cytokines. TNF also raises the thrombogenicity of endothelium. IL-1 stimulates tissue fibroblasts, following in enhanced proliferation and production of extracellular matrix. Although IL-1 and TNF are generally secreted by macrophages and other cells at localities of inflammation, they may reach the circulation and play at distant places to influence the systemic acute-phase response that is usually correlated with inflammatory diseases and infection. Components of this response include lethargy, fever, hepatic synthesis of various acute phase proteins (also stimulated by IL-6), the release of neutrophil into the circulation, and decrease of blood pressure [26-29].

The chemokines are a group of small (8 to 10 kDa) proteins, structurally related proteins that play essentially as chemoattractants for different subsets of leukocytes. The two main functions of chemokines are to obtain leukocytes to the site of inflammation and to control the natural anatomic organization of cells in lymphoid tissues and others. Chemokines also stimulate leukocytes; one result of such activation, as mentioned earlier, is ordinarily improved the affinity of leukocyte to integrins on endothelial cells. Some chemokines are displayed constitutively in tissues and are responsible for the anatomic segregation of different cell populations in tissues (e.g., the discrimination of T and B lymphocytes in various domains of lymph nodes and spleen) [30-32].

6) Nitric oxide and myeloperoxidase

Nitric oxide (NO) is a short-lived, soluble, free radical gas generated by several cell types and is capable of mediating a variety of functions. In the central nervous system (CNS), NO controls neurotransmitter release. When produced by endothelial cells it relaxes vascular smooth muscle and causes vasodilation. NO is normally synthesized de novo from NADPH, molecular oxygen, and L-arginine by the enzyme nitric oxide synthase (NOS). There are three types of NOS, with various tissue distributions. Type I, neuronal NOS (nNOS), is constitutively displayed in neurons and does not perform a significant part in inflammation. Type II, inducible NOS (iNOS), is induced in macrophages and endothelial cells by a number of inflammatory mediators and cytokines, most notably by bacterial endotoxin and by IL-1, TNF, and IFN-γ, , and is accountable for the result of NO in inflammatory reactions. This inducible form is also present in many other cell types, including hepatocytes, cardiac myocytes, and respiratory epithelial cells. Type III, endothelial NOS, (eNOS), is constitutively synthesized originally in endothelium. An important function of NO is as a microbicidal (cytotoxic) agent in activated macrophages. NO has dual actions in inflammation: it promotes vasodilation but it antagonizes of all stages of platelet activation (adhesion, aggregation, and degranulation), and reduces of leukocyte recruitment at inflammatory places [33, 34].

Recently studies give insight regarding the dichotomous nature of NO responses under different microenvironmental situations and propose that they are context dependent. The primary level of signaling comprises cGMP where low concentrations of NO (<1–30 nM) interfere proliferative as well as defensive effects. These levels may be

lower in endothelial cells as steady-state NO fluxes of less than 1 nM can induce cellular proliferation. As NO levels develop, protein kinase B phosphorylation provides protection against apoptosis through the phosphorylation and inactivation of Bad and caspase-9. At higher NO levels, stabilization of hypoxia-induced factor-1 (HIF-1) can initiate cell proliferative and angiogenic responses and is acknowledged to confer protection against tissue injury. Levels of NO that are high enough to affect p53 phosphorylation result in apoptosis and cytostasis. These studies recommend that low concentrations of NO manage to support pro-growth and anti-apoptotic responses while higher levels promote pathways influencing to cell cycle arrest or apoptosis [35].

Myeloperoxidase (MPO), a protein in monocytes and neutrophils, performs a vital function in host defense mechanisms and immune surveillance. This hemeprotein is regularly collected in granules and secreted from leukocytes following activation into the phagolysosomal compartment and extracellular space. MPO employs hydrogen peroxide (H_2O_2) produced during a respiratory burst to form cytotoxic oxidants and diffusible radical species. For example, MPO catalyzes the 2-electron oxidation of halides (I_2, Cl_2, and Br_2) and pseudo-halides, creating their respective hypohalous acids. MPO also oxidizes various organic substrates, generating free radical intermediates by the peroxidase cycle. Reactive intermediates produced by the MPO/H_2O_2 system occupy strong virucidal and microbicidal activities and are thought to act a crucial function in typical leukocyte function. Current reports with animal models containing targeted gene disruption for MPO showed enhanced susceptivity to yeast and fungal infections, and similar weaknesses in immune responses have been reported in patients with MPO insufficiency. However, the advantageous roles of MPO proceed at a price: the oxidants obtained from MPO also have the ability to injure host tissues. Indeed, MPO has been associated with oxidative damage and inflammatory tissue damage in a variety of ailments. To perform host protection and inflammatory tissue damage roles, an order of diffusible radicals and reactive oxidant species may be produced through oxidation reactions catalyzed at the heme core of the enzyme [36].

MPO and the iNOS are co-localized in primary granules of neutrophils. During leukocyte activation such as during phagocytic ingestion of bacteria, MPO and iNOS are secreted into the phagolysosome and extracellular space. NO reacts with MPO, leading to nitro-oxidative damage at the inflammatory area [37].

7) Lysosomal constituents of leukocytes

The lysosomal granules of monocytes and neutrophils contain many enzymes that destroy phagocytosed substances and are capable of causing tissue damage. Lysosomal granule contents also may be released from activated leukocytes, as described earlier. Acid proteases generally are active only in the low-pH environment of phagolysosomes, whereas neutral proteases, including collagenase, cathepsin, and elastase, are active in extracellular locations and cause tissue injury by degrading basement membrane, collagen, elastin, and other matrix proteins. Neutral proteases also can divide the complement proteins C3 and C5 directly to produce the vasoactive mediators C3a and C5a and can produce bradykinin-like peptides from kininogen. The potentially damaging results of lysosomal enzymes are limited by antiproteases display in the plasma and tissue fluids, such as α1-antitrypsin, the major inhibitor of neutrophil elastase. Deficiencies of these inhibitors may result in maintained activation of leukocyte proteases, following in tissue destruction at localities of leukocyte aggregation. For instance, α1-antitrypsin deficiency in the lung can cause a severe panacinar emphysema [38-40].

8) Oxygen-derived free radicals

Reactive O_2 species (ROS) are manufactured via the nicotinamide adenine dinucleotide phosphate (NADPH) oxidase (for example, phagocyte oxidase) pathway and are normally released from macrophages and neutrophils that are generally stimulated by cytokines, immune complexes, microbes, and a difference of other inflammatory inducements. When the ROS are generated within lysosomes they operate to kill phagocytosed microbes and necrotic cells. When secreted at low levels, ROS can increase chemokine, cytokine, and adhesion molecule expression, thus amplifying the cascade of inflammatory mediators. At higher levels, these mediators are also effective for cell damage by numerous mechanisms of action, including (1) endothelial dysfunction, with thrombosis and vascular endothelial permeability; (2) antiprotease inactivation and

protease activation, with a net increase in breakdown of the extracellular metrix; and (3) direct injury to other cell types (for example, parenchymal cells, tumor cells, red cells). Fortunately, various antioxidant protective mechanisms (for example, mediated by glutathione, catalase, and superoxide dismutase) presenting in tissues and blood can decrease the harmful toxicity of the oxygen metabolites [41].

1.2.3 Pain

Nociceptors, peripheral terminals of main afferent fibers that sense pain signaling, can be initiated by dangerously various inducements, such as pressure, acids, or heat. Inflammatory mediators released from nonneuronal cells during tissue damage improve the sensitivity of nociceptors and probably increase the effect of pain perception. Among these mediators including H^+, bradykinin, 5HT, neurotrophins (nerve growth factor), ATP, PGs, and LTs. PGI_2 and PGE_2 decrease the threshold to stimulation of nociceptors, producing peripheral sensitization. Reversal of peripheral sensitization is considered to describe the mechanistic explanation for the peripheral part of the analgesic (antinociceptive) activities of NSAIDs. NSAIDs may also have major basic operations in the spinal cord and brain. COX-1 and COX-2 are normally exposed in the spinal cord under basal conditions and then PGs are regularly synthesized and released in response to peripheral pain inducements [2].

Central action of PGE_2 and perhaps also $PGF_{2\alpha}$, PGD_2, and PGI_2 provide to central sensitization, an increasing improvement in the excitatory system of spinal dorsal horn neurons that causes allodynia and hyperalgesia in part by disinhibition of neural pathways glycinergic signaling. Central sensitization exhibits the plasticity of the nociceptive system that is invoked by injury. This normally is reversible within hours to days following satisfactory responses of the nociceptive system. Nevertheless, chronic inflammatory diseases may induce persistent modification of the structure of the nociceptive system, which may influence long-lasting shifts in its responsiveness. These mechanisms of action contribute to chronic pain.

1.2.4 Fever

The hypothalamus controls the set point at which body temperature is controlled. This set point is raised in fever, following an infection, or resulting from malignancy, graft rejection, tissue damage, or inflammation. These conditions effectively improve the formation of cytokines such as IFN, TNF-α, IL-1β, and IL-6, which play as endogenous pyrogens. The first phase of the thermoregulatory response to such pyrogens may be mediated by ceramide release in neuron cells of the preoptic region in the anterior hypothalamus. The second phase is mediated by correspondent initiation of COX-2 and formation of PGE_2. PGE_2 can cross the blood-brain barrier and plays on thermosensitive neurons. This triggers the hypothalamus to promote body temperature by developing an increase in heat generation and a reduction in heat loss. Some NSAIDs can suppress this response by inhibiting COX-2-dependent PGE_2 synthesis in the hypothalamus [2].

1.2.5 Drugs used in inflammation, pain, and fever

There are many anti-inflammatory, analgesic, and antipyretic agents available in the market. This section focused on the drugs used in this study; NSAIDs, corticosteroids, and opioids.

1) Nonsteroidal Anti-Inflammatory Drugs (NSAIDs)

NSAIDs are mechanistically classified as isoform nonselective NSAIDs, which inhibit both COX-1 and COX-2, and COX-2–selective NSAIDs. They are competitive, noncompetitive, or mixed reversible inhibitors of the COX enzymes. Aspirin (ASA) is a noncompetitive, irreversible inhibitor because it acetylates the isozymes in the AA-binding channel. Acetaminophen, which possesses antipyretic and analgesic effects but largely devoid of anti-inflammatory activity, acts as a noncompetitive reversible inhibitor by reducing the peroxide site of the enzymes [42].

The most of NSAIDs are organic acids with relatively low pKa values. As natural acid compounds commonly are quite absorbed orally, normally interact to plasma proteins, and excreted or eliminated either by tubular secretion or by glomerular filtration. They also are accumulated in localities of the inflammatory site, where the pH is acidic, potentially interfering the association between plasma concentrations and duration of

action of medicines. All COX-2–selective NSAIDs have a bulky group substitution, which regulates with a large binding pocket in the AA-binding domain of COX-2 but limits its optimal adjustment in the smaller binding domain of COX-1. Both isoform nonselective NSAIDs and the COX-2-selective NSAIDs commonly are hydrophobic drugs, a feature that provides them to reach the hydrophobic AA-binding channel and results in shared pharmacokinetic characteristics. However, acetaminophen and ASA are differences to this rule.

The principal pharmacotherapy of NSAIDs obtain from their ability to inhibit PG production. The primary enzyme in the PG synthetic pathway also known as COX. This enzyme transforms AA to the unstable intermediates form of PGG_2 and PGH_2 and lead to the production of the prostanoids, TXA_2, and a classical class of PGs. COX-1 displayed constitutively in several cells or organs, is the predominant origin of prostanoids for "housekeeping" roles, such as effective pharmacologic hemostasis. COX-2 also induced by stress, cytokines, and tumor cell or cancer, is the more valuable source of prostanoid development in inflammation and perhaps in cancer. However, both enzymes contribute to the general formation of autoregulatory and homeostatic prostanoids with important functions in normal physiology. The NSAIDs reduce the sensation of vessels to bradykinin and histamine, normally affect lymphokine production from T lymphocytes, and reform the vasodilation of inflammation. To varying degrees, all newer NSAIDs are anti-inflammatory, antipyretic, and analgesic agents, and all (except the nonacetylated salicylates and the COX-2–selective agents) inhibit platelet aggregation. The indiscriminate inhibition of both inflammatory and homeostatic prostanoids by NSAIDs explains mechanistically most adverse reactions to this drug class. For example, inhibition of COX-1 accounts largely for the gastric adverse events and bleeding that complicate therapy because COX-1 is the dominant cytoprotective isoform in gastric epithelial cells and forms TXA_2 in platelets, which amplifies platelet activation and constricts blood vessels at the site of injury. Similarly, COX-2-derived products play important roles in blood pressure regulation and act as endogenous inhibitors of hemostasis. Inhibition of COX-2 can cause or exacerbate hypertension and increases the likelihood of thrombotic events [42, 43].

2) Corticosteroids

The action of corticosteroids is described as electrolyte balance-regulating (mineralocorticoid) and carbohydrate metabolism-regulating (glucocorticoid). The effects of corticosteroids such as prednisolone and dexamethasone are numerous, with widespread anti-inflammatory and immunosuppressive actions. Most effects of corticosteroids are not immediate but become obvious after several hours because of the time required for changes in protein synthesis and gene expression. Glucocorticoids inhibit transcription of genes for synthesis of inducible COX-2, the iNOS and of inflammatory cytokines such as IL-1, IL-2, and IL-5 and TNF-α and many others. In addition corticosteroids stimulate the production of the intracellular protein lipocortin-1 (or annexin-1). Lipocortin-1 inhibits phospholipase A_2 and therefore reduces the synthesis of PGs and LTs. It may also impair leukocyte migration in response to cytokine IL-1. The pharmacological effects of corticosteroids in various tissues and several of their physiological effects seem to be arbitrated by the same receptor. Thus, the various corticosteroid derivatives used currently as pharmacological substances have numerous side effects on physiological processes that belong to their therapeutic effectiveness. Adverse effects of long-term use with high dosages of corticosteroids include atrophy of lymphoid tissue and reduction of numbers of white blood cells leading to an increased risk of infection and reduction of immune response, retarded growth in children, retention of sodium and water, often leading to edema [44].

3) Opioids

The name of opioid represents all substances that act at opioid receptors. The term of opiate definitely defines the generally occurring alkaloid compounds such as morphine, codeine, papaverine, and thebaine. Opioid drugs constitute partial agonists, antagonists, and agonists. Morphine is a full agonist operates at the μ (mu)-opioid receptor, the main analgesic opioid receptor which are located in the brain, in the spinal cord, on peripheral neurons, and digestive tract. Morphine presents an excellent binding affinity at the μ-opioid receptor more than codeine. Other opioid receptor subtypes comprise κ (kappa) and δ (delta) nociception or opioid receptor-like subtype 1 (ORL-1) receptors. The substitution of an allyl group of morphine plus the addition of a hydroxyl group results in

naloxone, a μ-receptor antagonist. Some synthetic opioids such as nalbuphine (N-cyclobutylmethyl-4, 5α-epoxy-3,6α,14-morphinantriol), a mixed agonist-antagonist, are capable of producing a partial agonist or agonist influence at one opioid receptor subtype and an antagonist effect at another. The Affinities of opioid analgesics and receptor-activating properties can be manipulated by molecular modeling; in addition, certain opioid analgesics are metabolized in the liver, producing in compounds with greater analgesic action [45].

1.2.6 Acid-peptic diseases

Acid-peptic diseases cover stress-related mucosal injury, peptic ulcer (duodenal and gastric ulcer), and gastroesophageal reflux. In all of these conditions, ulceration or mucosal erosions occur when the erosive effects of aggressive factors (pepsin, acid, bile) overwhelm the defensive factors of the gastrointestinal mucosa (bicarbonate secretion and mucus, blood flow, PGs, and the processes of restitution and regeneration after cellular injury). Over 80% of peptic ulcers are caused by infection with the gram-negative bacterium *Helicobacter pylori* or by long-term using of NSAIDs. Medicines used in the treatment of acid-peptic diseases may be classified into two classes: agents that increase mucosal defense and agents that decrease intragastric acidity [46, 47].

1.2.7 Mucosal increasing agents

The gastro mucosa has emerged a number of protection mechanisms to defend itself against the harmful effects of pepsin and acid. Both the tight junctions of epithelial cells and mucus decrease diffusion of pepsin and acid. Epithelial bicarbonate secretion authenticates a pH gradient within the mucous membrane layer in which the ranges from pH 7 at the mucosal surface to 1-2 in the gastric lumen. Blood flow transports vital nutrients and bicarbonate to surface cells. Domains of wounded epithelium are speedily repaired by restoration, a process in which movement of cells from gland neck cells seals small erosions to restore intact epithelium. Mucosal PGs seem to be necessary for bicarbonate secretion and stimulating mucus and mucosal blood flow. A number of compounds that potentiate these mucosal protection mechanisms are possible for the treatment or prevention of acid-peptic disorders [48].

1) Sucralfate

A description of advantageous outcomes have been assigned to sucralfate, but the exact mechanism of action is also unknown. It is guessed that the strongly negatively charged of sucrose sulfate interacts with positively charged of proteins in the base of erosion or ulcers, creating a physical barrier (cytoprotective properties) that decreases further caustic injury and stimulates bicarbonate secretion and mucosal PG. Because sucralfate is not well absorbed, it is practical without adverse effect in the systemic circulation. [49].

2) Prostaglandin analogs

Misoprostol is a methyl analog compound of PGE_1. It has been approved for treatment of gastrointestinal conditions. Misoprostol has both mucosal protective and acid inhibitory properties. It is considered to enhance mucosal blood flow and stimulates mucus and bicarbonate secretion. Misoprostol can decrease the incidence rate of NSAID-induced ulcers to less than 3% and the prevalence of ulcer complications by 50%. This medicine is approved for the prevention or treatment of NSAID-induced ulcers in high-risk patients; however, misoprostol has nevermore reached popular use owing to its required for multiple daily dosing and high adverse-effect profile [50].

3) Bismuth compounds

The exact mechanisms of action of this medicine are also unknown. Bismuth coats erosions and ulcers, forming a shielding layer against pepsin and acid. It may additionally activate mucus stimulation, PG, and bicarbonate releasing. Bismuth subsalicylate reduces liquidity and defecation frequency in acute infectious diarrhea, due to salicylate interference of intestinal chloride and PG secretion. Bismuth has direct antimicrobial effects and binds enterotoxins, considering its advantage in treating and preventing traveler's diarrhea. In addition, this medicine has direct antimicrobial activity against *H. pylori* [49, 51].

1.2.8 Intragastric acidity reducing agents

The parietal cell consists of receptors for gastrin (CCK-B), acetylcholine (muscarinic M_3), and histamine (H_2). When gastrin and acetylcholine bind to their receptors on the parietal cells, they induce a rise in cytosolic calcium, which in aid stimulates protein kinases that increasingly stimulate acid secretion from the proton pump (H^+/K^+-ATPase) on the canalicular surface area [52].

In close nearness to the parietal cells are enterochromaffin-like (ECL) cells. ECL cells also have receptors for acetylcholine and gastrin, which can stimulate histamine release from cells. Histamine binds into the H_2 receptor on the parietal cell, following by activation of adenylyl cyclase, which stimulates protein kinases that increase acid secretion by the H^+/K^+-ATPase and increases intracellular cyclic adenosine monophosphate (cAMP). In *in vivo*, it is considered that the main influence of gastrin upon acid secretion is mediated indirectly through the release of histamine from ECL cells than through direct parietal cell stimulation. In contrast, acetylcholine presents dominant direct parietal cell stimulation [53, 54].

1) Antacids

This medicine have been used for eras in the practice of patients with acid-peptic disorders and dyspepsia. Antacids were the backbone of medicine for acid-peptic disorders until the advent of H_2-receptor antagonists and proton-pump inhibitors (PPIs). They remain to be used generally by patients as over-the-counter medicines for the treatment of dyspepsia and intermittent heartburn. All antacids may influence the pharmacokinetics of other medications by interacting the drug (reducing absorption) or by developing pH so that the drug's dissolution or solubility (especially acidic or weakly basic drugs) is changed. Consequently, antacids should not be administered within 2 h of doses of iron, itraconazole, fluoroquinolones, and tetracyclines [46, 55].

2) H_2 blockers

H_2 blockers (generally called H_2-receptor antagonists) were the most generally prescribed medications in the world. With the realization of the function of *H. pylori* in gastric disease (which may be treated with suitable antibacterial treatment) and the arrival of PPIs, the use of prescription H_2 blocking agents has decreased markedly. This medicine presents competitive inhibition at the parietal cell H_2 receptor and represses basal and meal-stimulated acid secretion in a linear, dose-dependent manner. The concentration of pepsin and the volume of gastric secretion are also decreased. H_2 blockers decrease acid secretion stimulated by histamine as well as by cholinomimetic agents and gastrin by two mechanisms. First, the primary stimulation of the parietal cell of gastric glands by acetylcholine or gastrin has a diminished affect acid secretion in the presence of H_2-receptor blockade. Second, histamine generally released from ECL cells by vagal stimulation or gastrin is blocked from binding to the H_2 receptor of parietal cell such as cimetidine, ranitidine, and famotidine. They are extremely selective and do not affect other histamine receptors [56].

3) Proton-pump inhibitors (PPIs)

Since PPIs debut in the 1980s, these efficient acid inhibitory agents have considered the important function for the treatment of gastric acid production. PPIs are now among the numerous popularly prescribed drugs global. In contrast to H_2 blocking agents, PPIs inhibit both meal-stimulated acid secretion and fasting because PPIs block the terminal basic pathway of acid secretion, the proton pump such as omeprazole, lansoprazole, and rabeprazole [57].

1.2.9 *Pseuderanthemum palatiferum*

P. palatiferum belongs to the Acanthaceae family; its vernacular names are Hoanngoc and Xuan-hoa in Vietnam, and Payawanorn in Thailand (Figure 1.2.9). It was discovered in the latter of the 1990s in Cuc Phuong national park in the north of Vietnam. *P. palatiferum* grows very fast as a bush with an extent of 1 to 2 m and produces 700-1,000 leaves/plant. The young caulis is green, but the brownish wood and multi branches spread. The leaves are 12-17 cm long and 3.5-5.0 cm.

Figure 1.2.9 Characteristic of *P. palatiferum*, family Acanthaceae

1) Phytochemical and pharmacological studies of *P. palatiferum*

Phytochemistry of the leaf of *P. palatiferum* has been reported about many active compounds including flavonoids, β-sitosterol, stigmasterol, kaempferol, apigenin, phytol, triterpenoid saponin, and salicylic acid. It is expected that active phytochemical compounds are found in *P. palatiferum* might support using this plant as a medicinal plant. Pharmacological activities of the leaf of *P. palatiferum* have been reported and summarized in Table 1.2.9.1.

Table 1.2.9.1 Summary of the pharmacological activity of the leaf of *P.palatiferum*

Pharmacological activity	Extract preparation	References
Antidiabetic effect	water, 80%ethanol	[58, 59]
Antioxidant activity	water	[58]
Antiproliferative effect	water, 80%ethanol	[60]
Growth performances of piglets	dried leaf-powder	[61]
Antidiarrhea of piglets	dried leaf-powder	[61]
Hypotensive effect	water	[5]
Acetylcholinesterase inhibitory effect	water	[62]

There is scientific data on acute toxicity and sub-acute toxicity of *P. palatiferum*. The study assessed the toxicity of an 80% ethanol extract of *P. palatiferum* leaf both in *in vitro* and *in vivo* experiments. In *in vitro* study, the 80% ethanol extract of *P. palatiferum* was tested in vero cells (african green monkey kidney) by a green fluorescent protein (GFP)-based assay. It was found that no cytotoxicity was detected at the concentration of 50 μg/mL, which was the highest concentration that could be prepared. In *in vivo* study, the 80% ethanol extract was administered once orally to adult Wistar rats in various doses (500, 1,000, 1,500 and 2,000 mg/kg). The results showed that no doses of extract produced any signs or symptoms of toxicity during the first 24 h and no rat died within 14 days. Moreover, the body weight of control and treatment groups were not different. Longer acute toxicity was tested on Wistar rats in various doses of 250, 500 and 1,000 mg/kg by daily oral administration for 14 days. None of the dose levels showed any signs or symptoms of toxicity and no rat died. Compared to the control group, the clinical chemistry values such as creatinine, albumin, total protein, triglycerides, and total cholesterol were not different, except BUN of groups received was significantly lower the doses of 500 and 1,000 mg/kg. The alkaline phosphatase (ALP) of 250 mg/kg received group was significantly lower than the dose of 500 mg/kg. The gain in body weight of all treated groups was significantly higher than the control group [63].

1.2.10 Hypothesis

The hypothesis of this study was that the water extract of the leaves of *P. palatiferum* prepared according to the Thai traditional remedy possessed anti-inflammatory, analgesic, and antipyretic activities and anti-ulcerogenic effects.

1.2.11 Purposes of the study

The purposes of the present study were:

1) To investigate the anti-inflammatory effect and the possible mechanism of action of the water extract of *P. palatiferum* in animal models.

2) To investigate the analgesic and antipyretic effects of the water extract of *P. palatiferum* in animal models.

3) To investigate anti-gastric ulcer activity and the possible mechanism of action of the water extract of *P. palatiferum* in animal models.

CHAPTER 2

Materials and Methods

2.1 Plant material and preparation of the extract

P. palatiferum leaves were collected from Chiang Mai (north region, Thailand) and authenticated by a botanist at Queen Sirikit Botanic Garden Herbarium, Chiang Mai, Thailand (The voucher specimen number 42335).

The water crude extract of *P. palatiferum* (PPWE) was prepared as follows: fresh leaves were washed, shredded and soaked in distilled water for 24 h, after that the extract was filtered and evaporated at 60-70 °C, and then lyophilized. The yield was 6% (w/w).

2.2 Laboratory Animals

All animals (male Swiss albino mice and Sprague Dawley rats) were purchased from the National Laboratory Animal Center, Mahidol University (NLAC-MU), Thailand. Experimental animals were kept at 24 ± 1°C, 12 h light-12 h dark cycle. Food pills and drinking water were accessible ad libitum. At least 1 week before the trials, the animals were kept in the animal room for adaptation. All procedures comply with Laboratory Animal Center Chiang Mai University Organization and approved by the ethical committee for research animals at Faculty of Medicine, Chiang Mai University, Thailand (20/2557).

2.3 Preparation of test drugs and drug administration

All drugs were prepared immediately before use by being dissolved in distilled water but in the ear edema test dissolved in chloroform. All drugs were given in an equal volume of 0.1 mL/10 g of the mice and in a volume of 0.5 mL/100 g of the rats.

2.4 Experimental models

2.4.1 Anti-inflammatory studies

1) Ethyl phenylpropiolate (EPP)-induced ear edema in rats test [64]

Rats (40-60 g) were randomly divided into 3 groups each consisting of three rats.

Group 1: Control group, applied chloroform (20 µL/ear)

Group 2: Reference group, applied ibuprofen (1 mg/20 µL/ear)

Group 3: Test group, applied PPWE (2 mg/20 µL/ear)

The chloroform, ibuprofen, or PPWE was applied to outer and inner sides of both ears prior to EPP (50 mg in 1 mL of chloroform, 20 µL/ear). The ear thickness was estimated with the digital vernier calipers prior to and at 15 min, 30 min, 1 h and 2 h after the induction of inflammation. The ear edema thickness and percent inhibition were calculated using the formula:

$$ED_n = ET_n - ET_0$$

$$\%ED = \frac{ED_c - ED_t}{ED_c} \times 100$$

When; ED_n = edema thickness (mm) at time n

ET_n = ear thickness (mm) at time n

ET_0 = ear thickness (mm) before application of EPP

ED_c = edema thickness (mm) of the control group at time n

ED_t = edema thickness (mm) of test group at time n

%ED = percent edema inhibition of test substance at time n

2) Carrageenan-induced hind paw edema in rats test [65-67]

This test was carried out by injecting 0.05 mL of 1% carrageenan in sterile normal saline solution (NSS) intraplantarly into the right side of the hind paw. Except, group 1 was induced by NSS instead of carrageenan.

Rats (100-120 g) were randomly divided into 6 groups, each consisting of ten rats.

Group 1: Normal group, received vehicle (distilled water)

Group 2: Control group, received vehicle (distilled water)

Group 3: Reference group, received ibuprofen (100 mg/kg)

Groups 4-6: Test groups, received PPWE (150, 300 and 600 mg/kg, respectively)

Distilled water, ibuprofen or PPWE was orally given 1 h before carrageenan injection. The edema of the right hind paw was evaluated using a plethysmometer (model no. 7150, Ugo Basile, Italy) prior to and at 1, 3 and 5 h after induction of carrageenan injection. The edema volume of the paw and the percent edema inhibition were calculated using the formula:

$$EV_n = PV_n - PV_0$$

$$\%EI_n = \frac{EV_c - EV_t}{EV_c} \times 100$$

When; EV_n = edema volume (mL) at time n

PV_n = paw volume (mL) at time n

PV_o = paw volume (mL) measured before carrageenan injection

% EIn = percent edema inhibition of test substance at time n

After 6 h, the rats were normally anesthetized with sodium pentobarbital. The samples of blood tests were generally receieved from the heart for determination the NO concentration. After receiving the blood sample of each rat, the carrageenan-inflamed right hind paws were cut 0.5 cm above the paw. Then the four paws of each group were randomized for determination of histopathologic examination. The remainder paw was regularly homogenized in a solution comprising 0.5% hexa-decyll-trimethyl-ammonium bromide mixed in potassium phosphate buffer (10 mM at pH 7) and then centrifuged for 30 min at 20,000 g at 4 °C. Finally, the supernatant was determined for MPO activity.

2.1) Determination of NO concentration [68]

2.1.1) Protein concentration in plasma [68]

Protein content in each of the plasma was estimated by the Bradford method. The Coomassie-brilliant blue G-250 (working dye reagent) was provided by diluting the stock solution with distilled water. A standard curve was made by using various dilutions (0.1-1 μg/μL) of bovine serum albumin fraction V (BSA). Test samples and standards were transferred into 96-well microplate together with the Coomassie-brilliant blue G-250. The plate was incubated for 15 min and then analyzed at 650 nm using microplate reader (Synergy H4 Hybrid, BioTek Instruments, Inc., USA).

2.1.2) NO concentration [68]

The nitrite concentration was utilized as an indication of NO generation. The method for NO determination was based on the Griess reaction. Sodium nitrite standard (7.5-500 μM) or 100 μL of plasma was mixed with an equivalent volume of Griess reagent. After 20 min at room temperature, the light absorbance was estimated at 540 nm. The nitrite contents were normalized to the protein content.

2.2) Determination of myeloperoxidase activity [69]

An aliquot of the supernatant of homogenized paw tissue was then allowed to react with a solution of tetra-methyl-benzidine (1.6 mM) and 0.1 mM H_2O_2. The rate of change in absorbance was assessed by spectrophotometry at 650 nm. MPO activity was

established as the quantity of enzyme reducing 1 μmol of peroxide/min at 37 °C and was displayed in mU per weight of wet tissue (g).

3) Arachidonic acid (AA)-induced hind paw edema in rats test [67, 70]

Rats (100-120 g) were randomly divided into 6 groups, each consisting of six rats.

Group 1: Control group, received vehicle (distilled water)

Group 2: Reference group, received ibuprofen (100 mg/kg)

Group 3: Reference group, received prednisolone (5 mg/kg)

Group 4-6: Test groups, received PPWE (150, 300 and 600mg/kg, respectively)

The procedure to induce hind paw edema in rats was similar to the previous method by intradermal injection of AA (0.1 mL, 0.5% w/v in 0.2 M carbonate buffer) instead of carrageenan. Distilled water, ibuprofen, prednisolone, and PPWE were orally given to the rats 2 h prior to AA injection. The same equipment was used to evaluate the edema volume before and at 1 h after AA injection. [67, 70]

4) Cotton pellet-induced granuloma formation in rats test [71, 72]

Rats (200-250 g) were randomly divided into 5 groups each consisting of six rats.

Group 1: Normal group, received vehicle (distilled water)

Group 2: Control group, received vehicle (distilled water)

Group 3: Reference group, received ibuprofen (100 mg/kg)

Group 4: Reference group, received prednisolone (5 mg/kg)

Group 5: Test group, received PPWE (600 mg/kg)

In groups 2 to 5, the rats under anesthesia stage were implanted subcutaneously with 2 sterilized cotton pellets (20 ± 1 mg), one on each side of the abdomen of the rat. The rats in each group were orally given distilled water (0.5 mL/100 g), ibuprofen (100 mg/kg/day), prednisolone (5 mg/kg/day) or PPWE (600 mg/kg/day), once a day for 7 days. Each rat was anesthetized on the eighth day and the blood was obtained from the heart for determination of total protein and alkaline phosphatase (ALP) activity. The implanted pellets were inspected and measured for wet and dry weights (dried at 60 °C

for 18 h). The body weight and dry weight of the thymus gland were also recorded. The transudative weight and the granuloma weight were calculated. The percentage granuloma inhibition was calculated using the formula:

$$\text{Transudative weight} = \text{Wt}_\text{w} - \text{Wt}_\text{d}$$

$$\text{GW(mg/mg cotton)} = \frac{\text{Wt}_\text{d} - \text{Wt}_\text{i}}{\text{Wt}_\text{i}}$$

$$\%\text{GI} = \frac{\text{GW of control} - \text{GW of the test}}{\text{GW of control}} \times 100$$

When; Wt_w = wet weight of granuloma pellet (mg)

Wt_d = dry weight of granuloma pellet (mg)

Wt_i = initial dry weight of cotton pellet determined before implantation (mg)

GW = granuloma weight (mg)

% GI = percent granuloma inhibition

2.4.2 Analgesic studies

1) Acetic acid-induced writhing response in mice test [67, 72, 73]

Mice (30-40 g) were randomly divided into 5 groups each consisting of six mice.

Group 1: Control group, received vehicle (distilled water)

Group 2: Reference group, received ibuprofen (100 mg/kg)

Group 3-5: Test groups, received PPWE (150, 300 and 600 mg/kg, respectively)

An acetic acid-induced writhing response in mice experiment was normally invested by injecting acetic acid (0.1 mL/10 g body weight, 0.75% v/v in NSS) into the peritoneal cavity. Distilled water (0.1 mL/10 g, p.o.), ibuprofen (100 mg/kg, p.o.) or PPWE (150, 300, and 600 mg/kg, p.o.) was given 1 h before acetic acid injection. The number of writhes was recorded for 15 min beginning from 5 min after the acetic acid injection. The percentage inhibition was calculated using the formula:

$$\%WI = \frac{NWcontrol - NWtest}{NWcontrol} \times 100$$

When; %WI = Percent writhing response inhibition

NW = Number of writhe

2) The tail-flick test [74, 75]

Rats (180-220 g) were randomly divided into 4 groups each consisting of six rats.

Group 1: Control group, received vehicle (distilled water)

Group 2: Reference group, received ibuprofen (100 mg/kg)

Group 3: Reference group, received codeine (200 mg/kg)

Group 4: Test group, received PPWE (600 mg/kg)

Each group was orally pretreated with distilled water (0.5 mL/100 g), codeine (200 mg/kg), ibuprofen (100 mg/kg) or PPWE (600 mg/kg), respectively. Then 1 h later, the tail-flick response was determined using a photocell window of the tail-flick apparatus (model 7360, Ugo Basile, Italy). The reaction time was recorded when rat flicked it tail off the heat generator. A cut-off time of 10 sec was set to avoid tissue damage. The reaction time was recorded before and at 1 h after the substance administration. The percentage inhibition was calculated using the formula:

$$\%MT = \frac{T_t - T_c}{10 - T_c} \times 100$$

When; %MT = Percent maximum possible response time

T_t = Reaction time after received test drugs

T_c = Reaction time of control group

10 = Cut-off time of 10 sec

2.4.3 Antipyretic study

1) Yeast-induced hyperthermia in rats test [76]

Rats (180-220 g) were randomly divided into 3 groups, each consisting of six rats.

Group 1: Control group, received vehicle (distilled water)

Group 2: Reference group, received ibuprofen (100 mg/kg)

Groups 3: Test group, received PPWE (600 mg/kg)

The baseline rectal temperatures were assessed by using a "12-channel electric thermometer (LETICA, model TMP 812 RS, Panlab S.L., Spain)".Yeast-induced hyperthermia in rats test was carried out by injection yeast (1 mL/100 g body weight, 25% w/v in NSS) subcutaneously. Rats which had an equal or more than a 1°C rise from the baseline temperature at 18 h after yeast injection was given with distilled water, ibuprofen (100 mg/kg, p.o.) or PPWE (600 mg/kg, p.o.) depended on the groups. The rectal temperatures were repeatedly measured before and at 60, 90, and 120 min after treatment.

2.4.4 Anti-gastric ulcer studies

1) Preparation of rats for gastric ulceration activity models [77, 78]

Rats (200-250 g) were randomly divided into 5 groups each consisting of six rats.

Group 1: Control group, received vehicle (distilled water)

Group 2: Reference group, received cimetidine (100 mg/kg)

Group 3-5: Test groups, received the PPWE (150, 300 and 600 mg/kg, respectively)

Rats fasted 48 h and water ad libitum. The water was starved 1 h before the study. The vehicle, reference drug or PPWE was administrated orally 1 h before induction of gastric ulcers.

The gastric ulcers were induced in rats by 3 methods as follows:

1.1) Ethanol/Hydrochloric acid (EtOH/HCl)-induced gastric ulcer

Each rat was administrated orally with 1 mL of EtOH/HCl (60 mL absolute EtOH + 1.7 mL 36.5% HCl + 38.3 mL distilled water). One hour later, the rats were sacrificed for determination of gastric ulcers.

1.2) Restraint water immersion stress-induced gastric ulcer

The rats were placed individually in each compartment of stainless iron cages and drowned up to the level of the xiphoid in a water-bath controlled at 22 ± 2 °C for 5 h to induce stress ulcer. After that, the rats were sacrificed for determination of gastric ulcers.

1.3) Indomethacin-induced gastric ulcer

The rats were orally administrated with indomethacin (suspended in 0.5% carboxymethylcellulose) at the dose 100 mg/kg. After 5 h, the rats were sacrificed for determination of gastric ulcers.

2) Evaluation of gastric ulcer

All the rats were generally sacrificed by an intraperitoneal injection of pentobarbital sodium (100 mg/kg). The stomach was removed and opened along the larger curvature. After washing with normal saline, pinned out on a wax plate and the gastric lesion was quantified using a dissecting microscope (10X). Gastric ulcer was normally evaluated by estimating the length of the lesion in mm and then the ulcer index and percent inhibition of gastric ulcer were determined as follows:

$$\text{Ulcer index} = \frac{\sum \text{Total length of the lesion in each rat}}{\text{Number of rats in that group}}$$

$$\% \text{ Inhibition} = \frac{\text{Ulcer index}_{\text{Control}} - \text{Ulcer index}_{\text{Treated}}}{\text{Ulcer index}_{\text{Control}}} \times 100$$

3) Investigation the mechanism of anti-gastric ulcer activity

3.1) Pylorus ligation [73]

The rats weighing 200 to 250 g had fasted 48 h and water ad libitum. The water was abstained 1 h before starting the trial. The rats were divided into 3 groups of 6 animals as follows:

Group 1: Control group, received vehicle (distilled water)

Group 2: Reference group, received cimetidine (100 mg/kg)

Group 3: Test group, received the PPWE (600 mg/kg)

The rats were given orally vehicle, reference drug, or PPWE 1 h before pylorus ligation. The abdomen of anesthesia rats was opened and the pylorus was ligated then an incision was closed by suturing. Five hours after the ligation, the rats were sacrificed by pentobarbital sodium (100 mg/kg, i.p.). The stomach was excised and the gastric juice was collected, centrifuged at 2,500 rpm for 5 min and its volume was estimated. The total acidity of the supernatant was analysed by titration with 0.1 N NaOH to an endpoint of pH 7.4 using phenolphthalein as an indicator.

Total acidity of gastric juice was calculated as follows:

$$N_1V_1 = N_2V_2$$

When; N_1 = normality of gastric juice

N_2 = normality of NaOH

V_1 = volume of gastric juice (mL)

V_2 = volume of NaOH (mL)

3.2) Gastric-wall mucus determination experiment [78-80]

The rats were fasted 48 h, but had water ad libitum. Before starting the experiment for 1 h the water was withdrawn. The rats were divided into 7 groups of 6 rats as follows:

Group 1: Normal group without ulcer induction, administered distilled water

Group 2: Extract group without ulcer induction, administered PPWE (600 mg/kg)

Group 3: Control group, administered distilled water

Group 4: Reference group, administered misoprostol (100 μg/kg)

Group 5-7: Test groups, administered PPWE (150, 300 and 600 mg/kg, respectively)

After drug administration for 1 h, the rats in groups 3, 4, 5, 6, and 7 were orally given 1 mL of EtOH/HCl (60 ml absolute EtOH + 1.7 mL 36.5% HCl + 38.3 mL distilled water) to produce a gastric ulcer. One hour later all rats were normally sacrificed and the stomach was separated. The stomach was generally opened along the minor curvature, cleaned with normal saline and weighed then dipped in 0.1% w/v alcian blue solution for 2 h. The excessive dye was also removed by two successive rinses with 0.25 M sucrose solution for 15 and 45 min, respectively. The dye complex with gastric wall mucus of the stomach was ordinarily obtained after immersion in 10 mL of 0.5 M magnesium chloride for 2 h. The blue extract was thrilled quickly with an equal volume of diethyl ether and the resulting emulsion was centrifuged at 2,500 rpm for 15 min. The optical density of alcian blue in the aqueous layer was read against a buffer blank at 580 nm using a microplate reader (Synergy H4 Hybrid, BioTek Instruments, Inc., USA). The number of alcian blue extract per the wet stomach weight (g) was calculated from a standard curve of concentration and absorbance of alcian blue. Gastric wall mucus was calculated as follows:

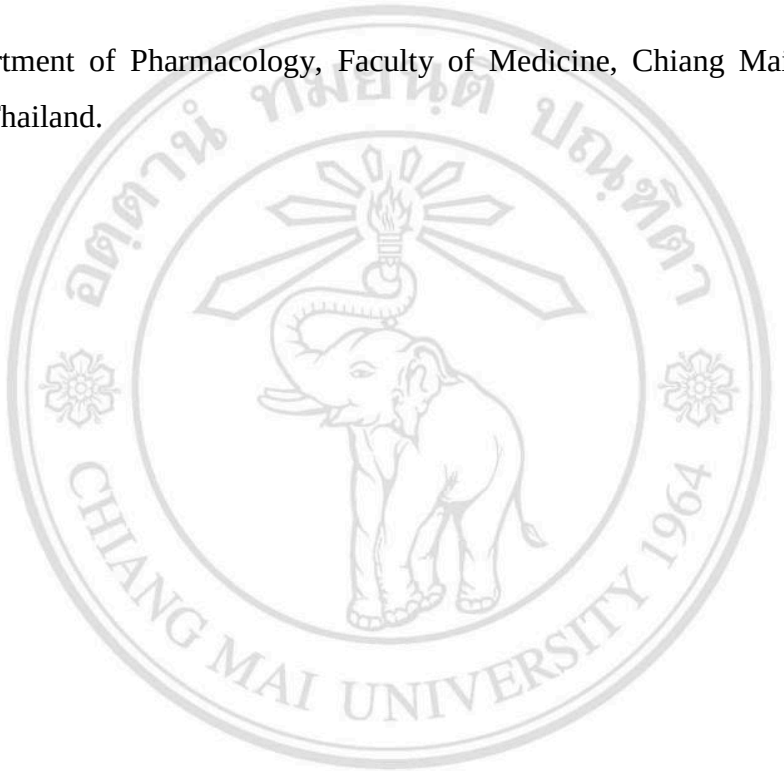
$$\text{Gastric wall mucus} = \frac{\text{Concentration of alcian blue}}{\text{Weight of wet stomach}}$$

2.5 Statistical Analysis

The data from the research was revealed as “the mean ± standard error of the mean (S.E.M)”. Statistical comparison between groups was assessed by using “one-way analysis of variance (ANOVA)” test and “p” values less than 0.05 was considered significant.

2.6 Research Location

Department of Pharmacology, Faculty of Medicine, Chiang Mai University, Chiang Mai, Thailand.



CHAPTER 3

Results

3.1 Anti-inflammation

3.1.1 EPP-induced ear edema in rats

PPWE (2 mg/ear) significantly inhibited ear edema caused by EPP at all assessment times (Table 3.1.1). However, ibuprofen (1 mg/ear) was more efficient than PPWE (2 mg/ear) in reducing ear edema.

Table 3.1.1 Effects of PPWE and ibuprofen on EPP-induced ear edema in rats

Group	Dose (mg/ear)	Edema thickness in mm (% inhibition)			
		15 min	30 min	1 h	2 h
Control	-	0.24 ± 0.01	0.32 ± 0.01	0.38 ± 0.01	0.28 ± 0.02
Ibuprofen	1	0.04 ± 0.01* (83%)	0.06 ± 0.01* (81%)	0.14 ± 0.01* (63%)	0.11 ± 0.01* (61%)
PPWE	2	0.06 ± 0.01* (75%)	0.10 ± 0.01* (69%)	0.18 ± 0.01* (53%)	0.12 ± 0.01* (57%)

Note: *Significantly different from the control group ($p < 0.05$).

Values are shown as mean ± S.E.M. (n=6).

3.1.2 Carrageenan-induced hind paw edema in rats

The effects of PPWE and ibuprofen on carrageenan-induced hind paw edema in rats is shown in Table 3.1.2. PPWE (300 and 600 mg/kg) and ibuprofen (100 mg/kg) significantly inhibited hind paw edema formation at all assessment times. The lower dose at 150 mg/kg of PPWE significantly inhibited the paw edema of the rats only at the 1st and the 3rd h of the evaluating period. The highest percent inhibition of paw edema of ibuprofen and PPWE groups was evident at the 3rd h and the 1st h after carrageenan injection, respectively.

Carrageenan caused the significant increases in plasma nitrite content and MPO activity of the control group when compared to that of normal group (Figs. 3.1.2.1 and 3.1.2.2). Pre-administration with ibuprofen (100 mg/kg) or PPWE (600 mg/kg) resulted in a significantly lower nitrite content when compared to that of control group (Fig. 3.1.2.1). Additionally, only the ibuprofen group showed significantly lower MPO activity when compared to that of the control group (Fig. 3.1.2.2).

Table 3.1.2. Effects of PPWE and ibuprofen on carrageenan-induced hind paw edema in rats

Group	Dose (mg/kg)	Edema volume in mL (% inhibition)		
		1 h	3 h	5 h
Control	-	0.31 ± 0.02	0.61 ± 0.05	0.46 ± 0.02
Ibuprofen	100	0.16 ± 0.02* (48%)	0.26 ± 0.02* (56%)	0.24 ± 0.03* (54%)
PPWE	150	0.17 ± 0.01* (45%)	0.44 ± 0.03* (27%)	0.41 ± 0.02 (10%)
PPWE	300	0.15 ± 0.03* (51%)	0.40 ± 0.05* (33%)	0.36 ± 0.05* (21%)
PPWE	600	0.13 ± 0.02* (57%)	0.35 ± 0.03* (41%)	0.29 ± 0.01* (36%)

Note: *Significantly different from the control group ($p < 0.05$)

Values are shown as mean ± S.E.M. (n=6)

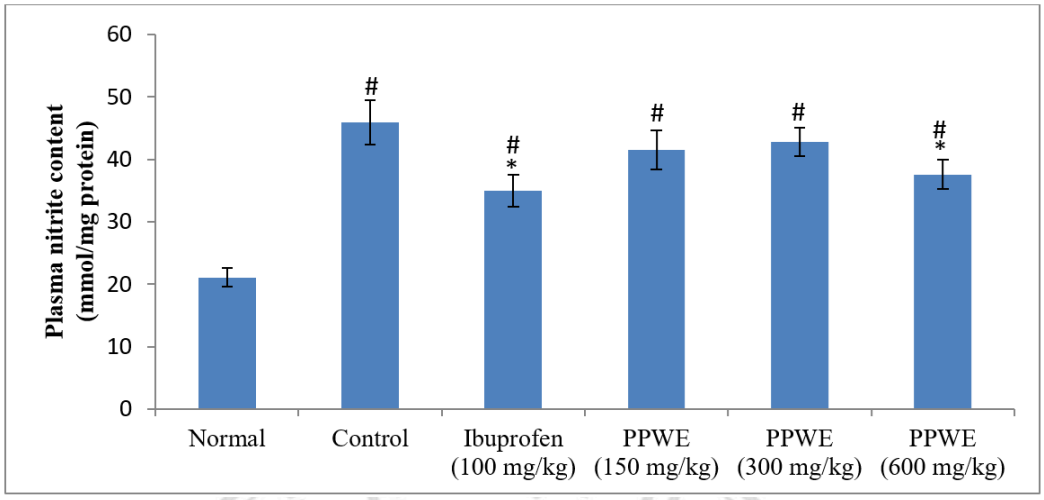


Figure 3.1.2.1 Plasma nitrite content of PPWE and ibuprofen in carrageenan-induced hind paw edema model

Note: * Significantly different from control group: ($p < 0.05$)

Significantly different from normal group: ($p < 0.05$)

Data are shown as mean ± S.E.M. (n = 6)

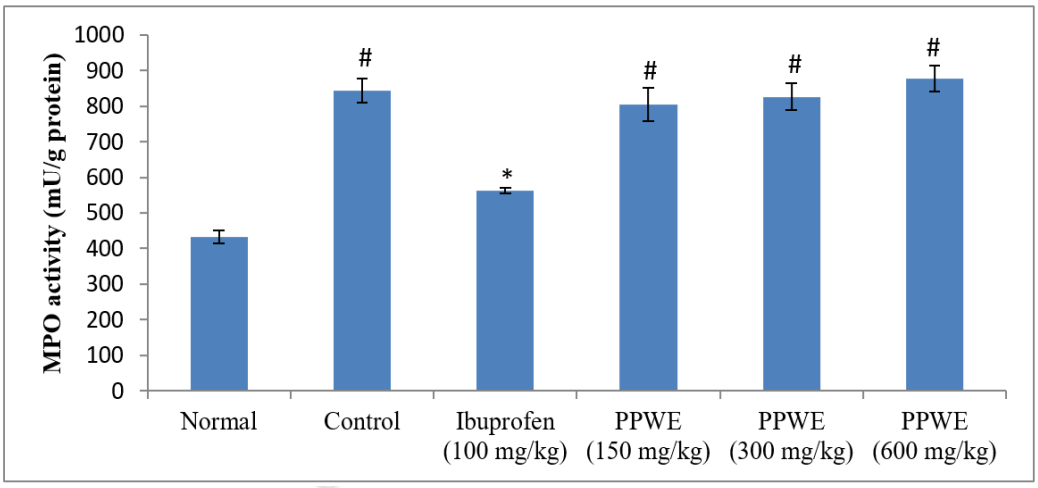


Figure 3.1.2.2 MPO activity of PPWE in carrageenan-induced hind paw edema model

Note: *Significantly different from control group: ($p < 0.05$)

#Significantly different from normal group: ($p < 0.05$)

Data are shown as mean ± S.E.M. (n = 6)

3.1.3 Histopathologic examination

Histopathologic examination of the inflamed paw of rats is shown in Figure 3.1.3. The paw of control rats showed a number of inflammatory cells compared to that of the normal rats. The number of inflammatory cells of paw of ibuprofen and PPWE groups (600 mg/kg) was fewer than that of the control rats.

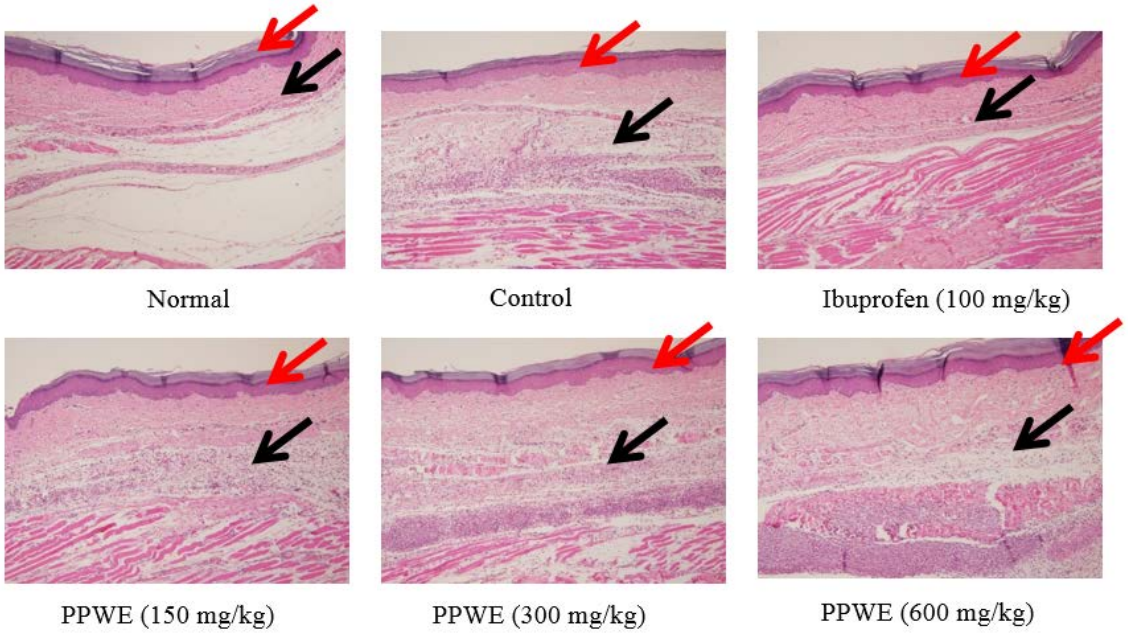


Figure 3.1.3 Histology of the right hind paw of rats 6 h after carrageenan injection. Images magnified 40x, the red arrows point the squamous epithelium and the black arrows point the inflammatory cells.

3.1.4 AA-induced hind paw edema in rats

A significantly lower paw edema volume compared to the control group was shown in rats pretreated with prednisolone (5 mg/kg) and PPWE (600 mg/kg) (Table 3.1.4). The inhibitory effect on AA-induced paw edema of ibuprofen (100 mg/kg) and PPWE (150 and 300 mg/kg) was less than those of prednisolone and PPWE (600 mg/kg).

Table 3.1.4 Effects of PPWE, ibuprofen and prednisolone on AA-induced hind paw edema in rats.

Group	Dose (mg/kg)	Edema volume at 1 h (mL)	% inhibition
Control	-	0.16 ± 0.02	-
Ibuprofen	100	0.11 ± 0.01	31
Prednisolone	5	0.07 ± 0.01*	59
PPWE	150	0.15 ± 0.03	9
PPWE	300	0.13 ± 0.02	18
PPWE	600	0.09 ± 0.01*	47

Note: Values are shown as mean ± S.E.M. (n=6)

*Significantly different from the control group ($p < 0.05$)

3.1.5 Cotton pellet-induced granuloma formation test

As presented in Table 3.1.5.1, the transudative weight and the granuloma weight of rats received PPWE (600 mg/kg/day) or ibuprofen (100 mg/kg/day) were similar to those of the control rats. Both parameters of rats received prednisolone (5 mg/kg/day) were significantly different from those of control group. The percent inhibition of granuloma formation of prednisolone was marked. It was also discovered that the dry thymus weight, the body weight gain, and ALP activity were not significantly changed among groups (control, ibuprofen, and PPWE groups), except in the prednisolone group which evidenced a significant decrease both the dry thymus weight and the body weight gain as well as ALP activity from the control group (Table 3.1.5.2).

Table 3.1.5.1. Effect of PPWE, ibuprofen and prednisolone on cotton pellet-induced granuloma formation in rats.

Group	Dose (mg/kg)	TW (mg)	GW (g/g cotton)	% GI
Control	-	222.78 ± 0.02	2.54 ± 0.24	-
Prednisolone	5	94.92 ± 0.01*	1.09 ± 0.10*	57.09
Ibuprofen	100	206.60 ± 0.02	1.96 ± 0.20	22.83
PPWE	600	171.67 ± 0.01	2.25 ± 0.12	11.42

Note: Values are shown as mean ± S.E.M. (n=6).
*Significantly different from the control group ($p < 0.05$)
TW = Transudative weight
GW = Granuloma weight
GI = Granuloma inhibition

Table 3.1.5.2. Effect of PPWE, ibuprofen and prednisolone on thymus weight, body weight gain, and ALP activity on cotton pellet-induced granuloma formation in rats.

Group	Dose (mg/kg)	Dry thymus weight (g/100 g BW)	Body weight gain (g)	ALP activity (U/g protein)
Control	-	0.05 ± 0.00	26.67 ± 2.79	37.88 ± 1.57
Prednisolone	5	0.03 ± 0.00*	9.17 ± 4.55*	27.76 ± 1.37*
Ibuprofen	100	0.04 ± 0.00	30.83 ± 5.23	34.36 ± 1.41
PPWE	600	0.05 ± 0.00	30.00 ± 2.24	31.94 ± 3.16

Note: Values are shown as mean ± S.E.M. (n=6)
*Significantly different from the control group ($p < 0.05$)
ALP = Alkaline phosphatase

3.2 Analgesic

3.2.1 Acetic acid-induced writhing response in mice

As shown in Table 3.2.1, a significantly reduced number of the writhes of the abdomen caused by acetic acid injection compared to that of the control group was observed in rats which received PPWE (150, 300 or 600 mg/kg,) or ibuprofen (100 mg/kg). The writhing response inhibition of PPWE was a dose-dependent manner.

Table 3.2.1 Effects of PPWE and ibuprofen on acetic acid-induced writhing response in mice.

Group	Dose (mg/kg)	Number of writhes	% inhibition
Control	-	28.00 ± 1.41	-
Ibuprofen	100	10.00 ± 0.67*	64
PPWE	150	22.00 ± 0.99*	21
PPWE	300	12.00 ± 0.63*	57
PPWE	600	6.00 ± 0.54*	79

Note: Values are shown as mean ± S.E.M. (n=6)

*Significantly different from the control group ($p < 0.05$)

3.2.2 Tail-flick test

Pretreatment the rats with a high dose of PPWE (600 mg/kg), ibuprofen (100 mg/kg), or codeine (200 mg/kg) significantly extended the reaction time (Table 3.2.2). The highest percent maximum possible response time was observed in the rats received codeine.

Table 3.2.2. Effect of PPWE, ibuprofen and codeine on tail-flick test in rats.

Group	Dose (mg/kg)	Baseline reaction time (sec)	Test reaction time (sec)	% MT
Control	-	3.05 ± 0.10	3.30 ± 0.10	-
Ibuprofen	100	3.08 ± 0.07	4.42 ± 0.17*	17
Codeine	200	3.05 ± 0.06	7.60 ±0 .06*	64
PPWE	600	3.06 ± 0.05	5.00 ± 0.29*	25

Note: Values are shown as mean ± S.E.M. (n=6)

*Significantly different from the control group ($p < 0.05$)

3.3 Antipyretic

3.3.1 Yeast-induced hyperthermia in rats

The body temperature of the hyperthermic rats received ibuprofen (100 mg/kg) significantly reduced at 90 and 120 min after drug administration, whereas the antipyretic effect of PPWE (600 mg/kg) was not observed in this model (Table 3.3.1).

Table 3.3.1 Effect of PPWE and ibuprofen on Yeast-induced hyperthermia in rats.

Group	Rectal temperature (°C)				
	Baseline	18 h after yeast inj.	Time after medication (min)		
			60	90	120
Control	37.65 ± 0.06	39.17 ± 0.15	38.88 ± 0.24	38.75 ± 0.22	38.62 ± 0.17
Ibuprofen (100 mg/kg)	37.95 ± 0.10	39.15 ± 0.17	38.00 ± 0.21	37.85 ± 0.20*	37.80 ± 0.20*
PPWE (600mg/kg)	37.91 ± 0.09	39.15 ± 0.10	38.60 ± 0.11	38.50 ± 0.11	38.30 ± 0.10

Note: Values are shown as mean ± S.E.M. (n=6)

*Significantly different from the control group ($p < 0.05$)

3.4 Anti-gastric ulcer studies

3.4.1 Anti-gastric ulcer activity

Pretreatment with PPWE (150, 300, and 600 mg/kg) or cimetidine (100 mg/kg) significantly diminished gastric ulcer formation generated by every inducers compared to those of control group (Table 3.4.1). The ulcer index of rats pretreated with PPWE (600 mg/kg) resembled that of cimetidine in the EtOH/HCl- and indomethacin-induced gastric ulcer tests ($p > 0.05$). Inhibition of gastric ulcer by PPWE was a dose-dependent manner in three models (Fig. 3.4.1).

Table 3.4.1 Effects of PPWE on gastric ulcer formation in rats in three models.

Group	Dose (mg/kg)	Ulcer index (mm)		
		EtOH/HCl	Stress	Indomethacin
Control	-	110.58 ± 6.46	15.60 ± 1.16	8.57 ± 0.74
Cimetidine	100 mg/kg	49.75 ± 1.45*	0.62 ± 0.07*	1.28 ± 0.11*
PPWE	150 mg/kg	88.22 ± 1.31*	9.20 ± 0.29*	4.42 ± 0.16*
PPWE	300 mg/kg	64.17 ± 2.02*	7.27 ± 0.32*	2.75 ± 0.29*
PPWE	600 mg/kg	59.50 ± 4.37*	4.37 ± 0.33*	1.95 ± 0.41*

Note: Data are shown as mean ± S.E.M. (n = 6)

*Significantly different from control group ($p < 0.05$)

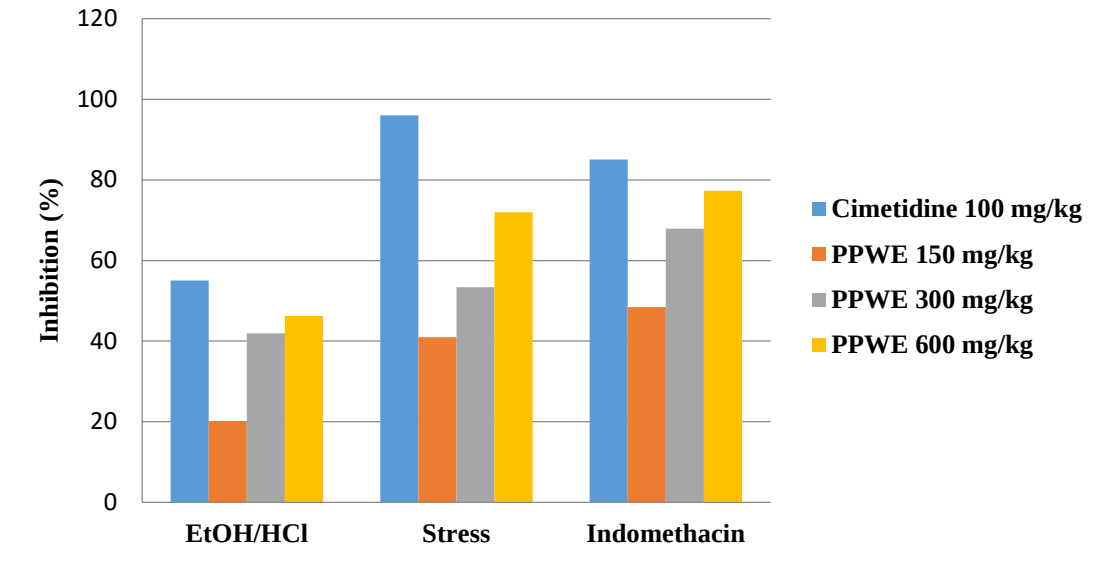


Figure 3.4.1 Gastric ulcer inhibition of PPWE and cimetidine in EtOH/HCl-, stress-, and indomethacin-induced gastric ulcer in rats.

3.4.2 Investigation of the anti-gastric ulcer activity mechanism

In this experiment, the result of PPWE at a high dose (600 mg/kg) and a dose of cimetidine (100 mg/kg) on gastric pH, gastric volume, gastric acid secretion, and total acidity are presented in Table 3.4.2. In the cimetidine group, gastric volume, gastric acid secretion rate, and total acidity were decreased whereas gastric pH was raised when compared to those of the control group. In the PPWE group, gastric volume, gastric acid secretion rate, and total acidity did not differ from the control group. Pretreatment with PPWE significantly reduced only gastric volume when compared to the control group.

Table 3.4.2 Effects of PPWE on gastric secretion in rats.

Group	Gastric volume (mL/100g)	Gastric pH	Total acidity (mEq)	Gastric acid secretion rate (mL/100g/h)
Control	2.80 ± 0.10	1.15 ± 0.01	120 ± 4.47	0.56
Cimetidine (100 mg/kg)	1.22 ± 0.10*	7.28 ± 0.31*	18 ± 4.01*	0.24
PPWE (600 mg/kg)	2.36 ± 0.12*	1.57 ± 0.13	107 ± 8.03	0.47

Note: Values are shown as mean ± S.E.M. (n = 6)

*Significantly different from control group ($p < 0.05$)

The result of PPWE and misoprostol on the quantity of gastric wall mucus is exposed in Figure 3.4.2. Administration of EtOH/HCl caused a significant decrease of gastric wall mucus of the control group compared to that of normal group (without EtOH/HCl). Pretreatment with misoprostol (100 µg/kg, a PGE1 analog) or PPWE (600 mg/kg) significantly defended gastric wall mucus from the influence of EtOH/HCl. Additionally, the gastric wall mucus content of rats pretreated with PPWE (600 mg/kg) without gastric ulcer induction was nearly to that of the normal rats.

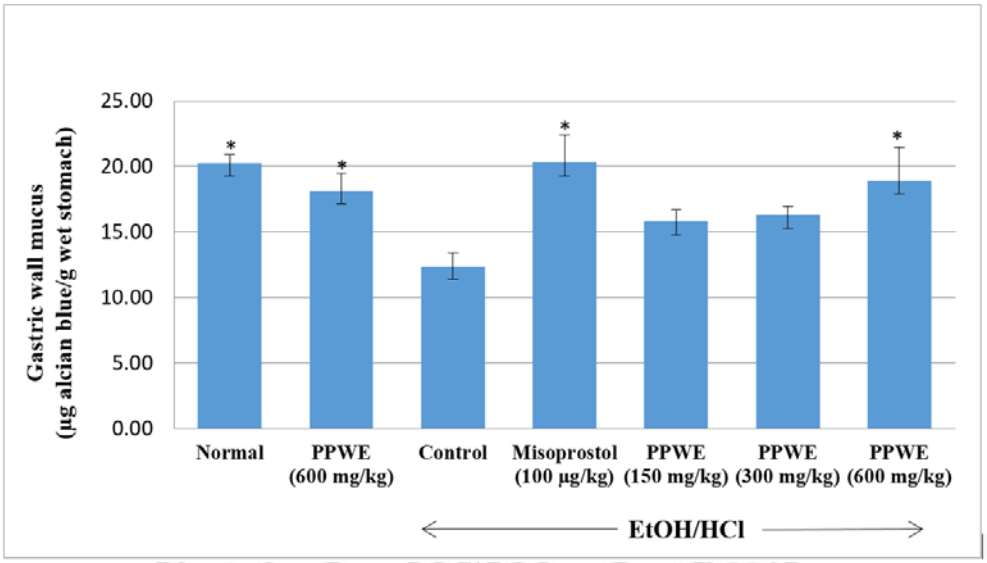


Figure 3.4.2 Effects of PPWE on gastric wall mucus content in rats

Note: Data are shown as mean ± S.E.M. (n = 6)

*Significantly different from control group ($p < 0.05$)

CHAPTER 4

Discussion

Anti-inflammatory models in animal are designed to evaluate drugs that affect vascular permeability, chemotaxis, and leukocyte migration. In a topical model of acute inflammation, ear edema of rats is induced by various inflammatory agents such as EPP, AA, mustard oil, bee venom, and zymosan [81, 82].

EPP-induced ear edema is a popular experiment for investigating and screening the anti-inflammatory action of test remedy. This model is a fast and easy experiment, requires a small number of compounds and presents good results [83]. EPP is a strong stimulant to activate the release of pro-inflammatory mediators, histamine, 5HT, bradykinin, and PGs which increase the vascular permeability and inflammatory cell infiltration [84]. Moreover, the application of EPP has been reported to cause inflammation and epidermal hyperplasia [85]. In this study, PPWE and ibuprofen showed a significant inhibitory activity on edema formation evoked by EPP, suggesting that PPWE may possess the anti-inflammatory effect. The possible mechanism of action may relate to the interference of the synthesis, release or action of pro-inflammatory mediators found in the acute phase of inflammation.

The carrageenan-induced hind paw edema test is also popularly used to produce an acute inflammation. This test, originally explained by Winter and co-workers in 1962, is an acute, non-immune, well-researched and very reproducible method. This inflammatory model is normally used for evaluating the anti-inflammatory activity of a test compound of which its mechanism involves the inhibition of COX [66, 86]. The carrageenan injection includes a three-phase process of inflammation that causes the paw

edema: an initial phase (0-1.5 h) is attributed to release of histamine and 5HT; a second phase (1.5-2.5 h) is attributed to release of bradykinin; a third phase (2.5-6 h) is attributed to the synthesis of PGs [87]. Normally, test substances are estimated for acute anti-inflammatory action by analyzing their efficacy to decrease or block the development of carrageenan-induced paw swelling. In this study, the edema volume of rat paw in the control group (received distilled water) was increased gradually and reached a maximum increase at the 3 h after carrageenan injection. PPWE (300 and 600 mg/kg) and ibuprofen exhibited a significant paw edema inhibition at all recorded times except the lower dose (150 mg/kg) of PPWE significantly inhibited the paw edema of the rats only at 1 and 3 h. The duration of action of the low dose of PPWE was less than 5 h since it may be excreted or metabolized from the body before 5 h. Moreover, the percent inhibition of PPWE on the edema formation was progressively increased as the dose increased. It is suggested that the mechanism of anti-inflammatory effect of PPWE may partly involve with the release or the synthesis of the predominant pro-inflammatory mediators synthesized and/or released during these periods i.e. COX pathway products.

MPO activity and plasma nitrite content evaluated in this study indicated the inflammation caused by carrageenan. NO is a signaling molecule that quickly diffuses across cell membranes and manages a wide area of pathophysiologic and physiologic processes involving inflammation, neuronal functions, and cardiovascular system. Moreover, it gives an anti-inflammatory effect under normal physiological conditions. On the other hand, NO is considered as a pro-inflammatory mediator that induces inflammation due to over production in abnormal situations. MPO is known as a sign of cell infiltration (mainly neutrophils) in inflammatory process. When host response to damages, neutrophils will release MPO to catalyze halogenation effect to form cytotoxic oxidants and diffusible radical species. After carrageenan injection, an increase of plasma nitrite content and MPO activity was observed in the control group which its histological examination of paw showed enormously of the inflammatory cells. When the inflammation process, plasma nitrite concentration of PPWE group (600 mg/kg) and ibuprofen group was lower than that of the control group that cause by anti-inflammatory effect of PPWE and ibuprofen. However, a reduction of MPO activity was observed in the only ibuprofen group, which was consistent with a few amounts of the inflammatory cells of the paw. In the histopathologic examination of the group received PPWE at a

dose of 600 mg/kg showed more the number of inflammatory cells than ibuprofen group lead to the higher MPO activity level which makes the probability of anti-inflammation of PPWE is lower than that of ibuprofen.

AA-induced hind paw edema in rat test is used to evaluate the anti-inflammatory effect of LOX inhibitors or the dual inhibitors (COX and LOX inhibitors) [88]. AA has previously been reported to produce significant inflammatory edema. Moreover, edema produced by AA is extremely sensitive to inhibition by prednisolone (a steroid drug), a dual inhibitor of AA metabolism and LOX inhibitor but is insensitive to COX inhibitors. In this study, oral administration of prednisolone and PPWE (600 mg/kg) significantly inhibited AA-induced hind paw edema. Moreover, the percent inhibition of PPWE at all doses on the edema formation was gradually increased in a dose dependent manner. Both results from carrageenan-induced hind paw edema and AA-induced hind paw edema, indicate that the anti-inflammatory effect of PPWE may relate to inhibition of both LOX and COX pathway.

The cotton pellet-induced granuloma formation in rat test is a typical model for chronic inflammation [89]. The ability of test substances to reduce the deposition of granulation tissue around the implanted cotton pellets reveals the inhibitory effect on chronic stage of the inflammatory process. The response to subcutaneously implanted cotton pellet in rats has been classified into three phases; transudative, exudative and proliferative phases, respectively. The first, transudative phase is defined as the leakage of liquid from blood vessels affected by an increase in vascular permeability. This phase is expressed as the rise in the wet weight of the granuloma that happened during the first 3 h. The second, exudative phase is defined as the leakage of protein from bloodstream around granuloma caused by the intensive maintenance in vascular permeability change. This phase is expressed as the rise of the dry weight of the granuloma occurred between 3-6 days after implantation. The final, proliferative phase is defined as the product of granulomatous tissues made by continuous pro-inflammatory mediator release. The reduction of a transudative weight by anti-inflammatory drugs involves in the inhibition of the permeability response of the blood vessels around the cotton pellet whereas the inhibition of granuloma formation is probably via the interference with a proliferative component such as mucopolysaccharide, collagen, and fibroblasts during granuloma

tissue formation [71, 90]. The present results revealed that PPWE and ibuprofen could slightly reduce granuloma weight. In addition, Swingle and Shideman, 1972, explained that NSAIDs show a slight inhibition whereas steroids show to be potent inhibitors on both the transudative and proliferative phases [71]. In this study, prednisolone showed the superior inhibitory effects on both phases than those of the ibuprofen and PPWE.

Steroids such as prednisolone can inhibit or suppress the inflammatory reaction. Prolonged treatment of steroids induces the loss of body weight gain and thymus weight of rodents. These effects may be due to an increase in protein catabolism and lymphoid tissue injury. In this study, PPWE and ibuprofen did not affect body weight gain and thymus weight of the rats whereas prednisolone-treated rats presented a marked lowering of both parameters. All results clearly demonstrated the anti-inflammatory property of PPWE on the acute phase and not on the chronic phase of inflammation. Accordingly, the anti-inflammatory activity of PPWE that showed in acute inflammation did not participate in the steroidal-like activity.

The migration of leukocytes into the tissue damage site reaches to the release of oxygen radicals and lysosomal enzymes at the local site of inflammation [91]. In cotton pellet-induced granuloma formation model, the activity of a lysosomal enzyme such as ALP in serum is particularly raised on the day 7 after implantation and can be normalized by steroids and NSAIDs through the stabilization of lysosomal membrane and interference of the migration of the inflammatory cells into local site of inflammation [91]. In this study, the serum ALP activity of prednisolone group was significantly reduced when compared with that of the control group. In PPWE group, the serum ALP activity was not statistically different from that of the control group. The results confirmed that PPWE had no influence on chronic phases of inflammation.

The analgesic activity of PPWE was estimated using both chemical (acetic acid-induced writhing response) and radiant heat (tail-flick test) methods [92]. The acetic acid-induced writhing response in mice is generally examined for exposure of peripheral and central analgesic acting of drugs. Intraperitoneal injection of acetic acid induces tissue damage and generates the release of various endogenous substances such as bradykinins, 5HT, and PGs, and provides to the manner of inflammation and developed the sensitivity

of nociceptors. These endogenous substances sensitize peripheral nerve terminal (peripheral sensitization), leading to phenotypic alteration of the sensory neurons and developed excitability of the spinal cord dorsal horn neurons (central sensitization)[92]. Results of peripheral sensitization are dropping of the activation threshold of nociceptors and an improvement in their firing rate. These changes result in the formation of allodynia and hyperalgesia associated with chronic pain. In addition, peripheral sensitization also performs a crucial part in the maintenance and development of central sensitization. The writhing responses elicited by intraperitoneal injection of noxious chemical stimuli (acetic acid) consist of abdominal wall contraction, pelvic rotation and followed by hind limb extension. The results from the study revealed that pre-treatment with ibuprofen and different doses of PPWE significantly reduced the writhing response produced by an acetic acid injection in a dose-dependent manner. The obtained data indicate the analgesic activity of PPWE. To clarify the mechanism of analgesic activity involving CNS pain pathway, the tail flick test was conducted.

The tail-flick test with radiating heat is a remarkably clarified version of the experimental model applied to human subjects by the research group of Hardy and co-workers, 1940. Indeed, Hardy and his collaborators ultimately applied the technique in the rat. Fundamentally, the whole body without the tail of an animal is clothed with Kim Towel or sterilized cloth taking responsibility not to wrap the animal too tightly. Radiant heat is utilized on the tail located at the designated point on a tail-flick analgesia meter. When the animal seems to ache, its response by the removal of the tail by a slight dynamic movement (tail flick), which automatically stops the stimulation. The analysis of the animal response time as “tail-flick latency” (the time from the start of the stimulation until exposure of the animal’s response) is accomplished by starting a timer at the same time as the utilization of the heat source. Tail-flick latency is estimated three times and exerted the average of the information. It is recommended not to continue the expression to radiant heat exceeding 10-20 s, otherwise, the surface skin may be burned or ignited. The benefits of this technique are simplicity and possess the small inter-animal variability in response time measurements under a given set of controlled circumstances. This pain is created by the continuous activation of Aδ and C-nociceptors in response to a harmful inducement. It is well-known that pain produced in the tail-flick test is sensitive and

inhibited by centrally acting analgesic drugs such as codeine, which was also evidenced in this study. The maximal dose of PPWE (600 mg/kg) used in the present study and reference drug ibuprofen show the analgesic effect in this model. On the other hand, codeine showed a marked inhibitory effect on the tail-flick response in rats. Codeine is a centrally acting agent that is administered orally and can be used for mild to moderate pain. It is a weak opioid analgesic with weak affinity to μ opioid receptors which found primarily in the brainstem and medial thalamus. Opioid receptors are found on the presynaptic terminals of the nociceptive C-fibers and nociceptive Aδ-fibers. Once the receptors are activated, they will indirectly inhibit the voltage-dependent calcium channels, decrease the cAMP levels and block the release of pain neurotransmitters such as substance P, glutamate, and calcitonin gene-related peptide from the nociceptive fibers, resulting in analgesia. The mechanisms of action of ibuprofen to relieve nociceptive pain and induce analgesia at central level, which results in a decreased production of PGs. These PGs are potent mediators of pain that act directly at nociceptors to increase nociceptor sensitivity as well as act indirectly by enhancing the pain-producing effect of other agents such as 5-HT or bradykinins, hence, inhibition of PGs production results in analgesia. PPWE and ibuprofen increased the reaction time but the percent inhibition of PPWE and ibuprofen was lower than that of codeine. The results suggest that the analgesic activity of PPWE probably mediated through majorly peripheral mechanism.

Yeast-induced hyperthermia in rat test is used to evaluate antipyretic activity. The result of yeast injection is an increased the body temperature by inducing cytokine and increasing PGs [93]. Ibuprofen can pass through the blood brain barrier (BBB) of the CNS to inhibit PGs synthesis in hypothalamic area which exhibits its antipyretic effect. Moreover, ibuprofen exert antipyretic effect on cytokine production. In this study, ibuprofen could significantly reduce the body temperature of the hyperthermic rats, whereas PPWE could not show this effect. The results from yeast-induced hyperthermia test assumed that PPWE might not be able to inhibit cytokine production that causes hyperthermia in animals.

It is well known that the peptic ulcer develops when aggressive factors overcome defensive factors. It is now clear that NSAIDs induce injury to account for the majority cause of peptic ulcer. Different therapeutic agents including plant extracts are used to

treat peptic ulcer. The study on new gastroprotective agent commonly uses the different models with different mechanisms of ulcerogenesis. In the present study, PPWE showed anti-gastric ulcer activity in experimental models, included EtOH/HCl-, indomethacin-, and stress-induced gastric lesions [3].

PPWE and cimedtidine, an H_2-receptor antagonist, significantly decreased gastric ulcer formation in the EtOH/HCl-induced gastric ulcer in rats. The effect of EtOH/HCl on GI tract depends on both the concentration and the duration of exposure. The lesions are characterized by multiple-hemorrhage red bands of different sizes along the long axis of the glandular stomach [3]. The gastric lesion produced by EtOH is due to its direct necrotizing action causing a reduction of gastric mucosal defensive factors. EtOH causes disruption of the physiological function of the gastric mucosa throughout the glandular stomach in rats, resulting in a reduction of the transmucosal potential difference [3]. This phenomenon leads to an increase net flux of Na^+ and K^+ across the membranes into the lumen, and an increased H^+ back-diffusion through the gastric mucosa. The diffusion of acid into the gastric mucosa is increased when HCl is used in combination with EtOH. Additionally, EtOH can cause acute gastric mucosal damage by disturbance of gastric microvascular circulation resulting in an increase of microvascular permeability and followed by microvascular stasis. The microvascular stasis results in the failure of oxygen and nutrition delivery, as well as dilution and carries away back-diffusion of H^+. The possible mechanisms of PPWE to reduce gastric lesions in this model could be the stimulation of mucus and/or bicarbonate secretion or reduction of acid secretion [3]. The exact mechanism of action of PPWE was further investigated.

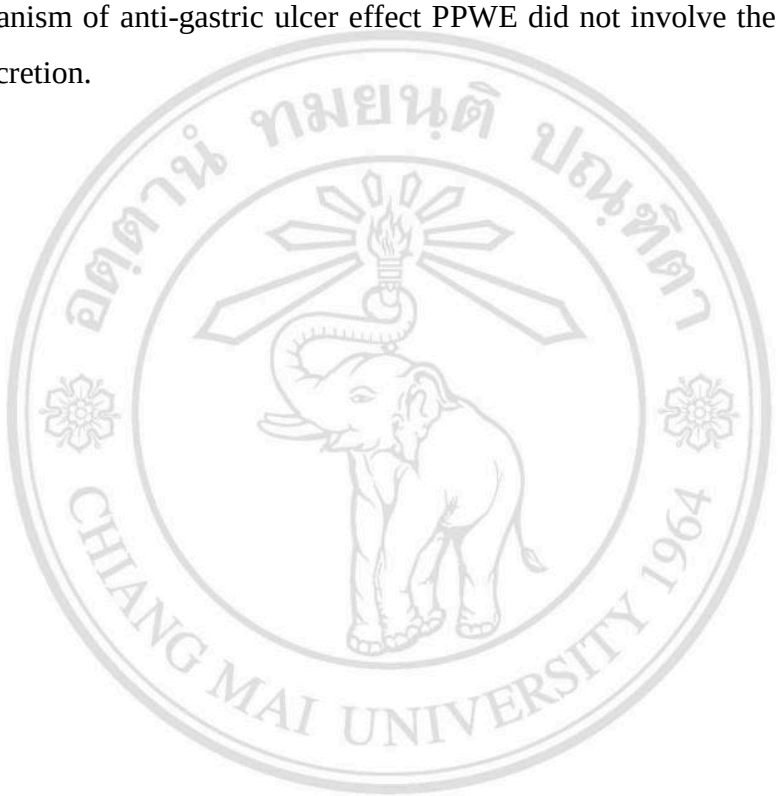
The most common adverse effect of the use of indomethacin is the development of gastric ulcer. Indomethacin inhibits PG biosynthesis by inhibiting both COX-1 and COX-2 resulting in over production of LTs. PGs, especially PGI_2 and PGE_2, have a cytoprotective effect on gastric mucosa, which possibly mediated via increased mucosal blood flow, promotion of gastric mucus and bicarbonate secretion. LTB_4, an active metabolite from 5-LOXs, which plays roles in the production of proinflammatory cytokines, contributing the gastric damage by stimulating of the polymorphonuclear leukocytes aggregation and adhesion of neutrophils to vascular endothelial cells causing gastric vasoconstriction and generation of reactive oxygen metabolite resulting in gastric

ulcer. Because the anti-inflammatory effect of PPWE involved the inhibition of LOX pathway that proved in AA-induced paw edema experiment, the significant inhibitory effect of PPWE on indomethacin-induced gastric ulcer indicates that the gastroprotective effect of PPWE may involve the inhibition of LTs biosynthesis and/or LTs effects. Additionally, the decrease of gastric acidity or the induction of mucus of PPWE may be involved [3].

The mechanism of stress-induced gastric ulceration suggests the involvement of sympathetic as well as parasympathetic nervous function which innervates the digestive organs [3]. Many endogenous substances are known to affect HCl secretion by the parietal cell including with ACh from the vagus nerve, histamine from mast cell, and gastrin from G cell. Moreover, stress-induced gastric ulcers can be prevented partly or entirely by vagotomy, anti-cholinergic agents, and anti-secretory agents such as H_2-receptor antagonists and PPIs. PPWE and cimetidine significantly inhibited stress-induced gastric ulcer formation. Thus, PPWE was tested in the pylorus ligation model for investigation of the possible mechanism of anti-secretory effect. The ligation of the pyloric end of the stomach causes stimulation of secretion and accumulation of gastric acid in the stomach which leading to auto-digestion of gastric mucosa and breaking down of the gastric mucosal barrier. The results showed an anti-secretory activity of cimetidine at the dose of 100 mg/kg by significant decreases of both gastric volume and total acidity in the cimeitidine-treated group. PPWE, on the contrary, did not show any effect on total acidity.

The effect of the PPWE on gastric wall mucus was then studied in another the EtOH/HCl-induced gastric ulcer model. EtOH/HCl is a necrotizing agent causes a decrease of gastric wall mucus after administration. The amount of gastric wall mucus was found to decrease in the control groups, whereas those of the PPWE at the dose of 600 mg/kg and misoprostol (PGE_1 analog) groups were higher than that of the control group. The results showed that PPWE possibly enhances the synthesis and/or secretion of the gastric mucus and may preserve the construction of gastric mucus interrupted by a necrotinizing agent such as EtOH/HCl.

The finding obtained from the present study discloses that PPWE prevented gastric ulcer formation in EtOH/HCl-, indomethacin, and stress-induced gastric lesions models. The possible mechanisms mediating the gastric ulcer protective activity may relate to preservation and/or synthesis of the gastric mucus and other cytoprotective effects such as the inhibition of LTs biosynthesis and/or LTs effects, and an increase mucosal blood flow. However, PPWE did not show any effect in pylorus ligation model, thus the mechanism of anti-gastric ulcer effect PPWE did not involve the inhibition of gastric acid secretion.



CHAPTER 5

Conclusions

The water extract of *P. palatiferum* leaves holds anti-inflammatory effect on acute inflammation and analgesic effect. The mechanism of anti-inflammatory effect may be via the inhibition of COX and LOX pathways and does not share steroid-like action and the mechanism of analgesic effect may be due to the inhibition of peripherally mediated nociception. Moreover, *P. palatiferum* water extract exhibits a gastroprotective effect via preservation and/or synthesis of the gastric mucus. Our results suggest that *P. palatiferum* has the potential to be used as traditional medicine and may lead to verify and develop the active compounds possess anti-inflammatory and anti-gastric ulcer activity in the future.

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