Studies on Analgesic, Antipyretic and Anti-inflammatory Activities of *Abroma augusta* Linn. Seed Oil Extract in Experimental Animals

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ชื่อวิทยานิพนธ์ การศึกษาฤทธิ์ระงับปวด ลดไข้ และต้านการอักเสบของน้ำมันสกัดจากเมล็ดเทียนดำในสัตว์ทดลอง
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บทคัดย่อ

เทียนดำ มีชื่อทางวิทยาศาสตร์ว่า *Abroma augusta* Linn. อยู่ในวงศ์ Sterculiaceae มีชื่อสามัญว่า devil's cotton สามารถพบได้ในอินเดีย จีน ปากีสถาน และไทย มีการนำมาใช้ในการผสมอาหารและน้ำมันเมล็ดเทียนดำนำมาใช้ทาในการรักษาอาการปวด ซึ่งนิยมมากในชาวไทยมุสลิมใน 3 จังหวัดของภาคใต้ เทียนดำเป็นไม้พุ่มขนาดเล็กสูง 2-3 เมตร และใบเป็นวงรีเรียงสลับกัน ยาว 10-15 เซนติเมตร กว้าง 10-12 เซนติเมตร องค์ประกอบทางเคมีจากส่วนต่างๆ ของพืช ได้แก่ choline, betaine, β-sitosterol, stigmasterol, L-rhamnose, L-aribinose, D-xylose, D-mannose, D-galactose, D-glucose, D-galacturonic acid และ D-glucuronic acid น้ำมันเทียนดำเป็นสารสกัดสำคัญจากเมล็ดเทียนดำซึ่งมีสรรพคุณระบุในการแพทย์แผนไทย คือ ใช้ในการรักษา โรคปวดตามข้อ หอบหืด ไซนัสอักเสบ ต่อมทอนซินอักเสบ ปวดหลัง ท้องเสีย และไข้หวัดใหญ่ แต่ยังขาดข้อมูลการศึกษาทางวิทยาศาสตร์สนับสนุน ดังนั้นวัตถุประสงค์ในการศึกษาครั้งนี้เพื่อ ประเมินฤทธิ์ระงับปวด ลดไข้ และต้านการอักเสบของน้ำมันสกัดจากเมล็ดเทียนดำในสัตว์ทดลอง โดยทดสอบฤทธิ์ระงับปวดด้วยวิธี writhing, formalin, hot plate และ tail flick ในหนูถีบจักร ทดสอบฤทธิ์ต้านการอักเสบโดยการฉีด carrageenan บริเวณอุ้งเท้าหลังเพื่อเหนี่ยวนำให้อุ้งเท้าบวม และใช้ cotton pellet เหนี่ยวนำให้เกิด granuloma ในหนูขาวใหญ่ ส่วนการศึกษาฤทธิ์ลดไข้ใช้ brewer's yeast เหนี่ยวนำให้เกิดไข้ในหนูขาวใหญ่

เมื่อให้สารสกัดเฮกเซนจากเมล็ดเทียนดำในขนาด 100, 200 และ 400 มก./กก. ทางปาก พบว่าสามารถลดการเกิด writhing ได้ 35.65%, 49.74% และ 57.99% ตามลำดับเมื่อเปรียบเทียบกับกลุ่มควบคุมและลดการการเลียอุ้งเท้าได้ทั้งช่วง early phase และ late phase โดยในช่วง early phase ลดได้ 33.84%, 42.46% และ 54.58% และ late phase ลดได้ 36.79%, 63.71% และ 80.83% ตามลำดับ ฤทธิ์ในการระงับปวดของสารสกัดจากเมล็ดเทียนดำเมื่อทดสอบด้วยวิธี writhing และ formalin พบว่าขึ้นกับขนาดของสารสกัด สารสกัดจากเมล็ดเทียนดำทุกขนาดที่ใช้ในการทดลองทำให้ระยะเวลาของการตอบสนองต่อความเจ็บปวดของสัตว์

ทดลองเพิ่มขึ้นทั้งในการทดลองด้วยวิธี hot plate และ tail flick โดยยืดระยะเวลาการตอบสนองต่อความเจ็บปวดทุกช่วงเวลา ยา naloxone (2 มก./กก.) สามารถต้านฤทธิ์ระงับปวดของ morphine (5 มก./กก.) และสารสกัด (400 มก./กก.) ในการทดลองด้วยวิธี hot plate และ tail flick ได้ ในการทดสอบฤทธิ์ต้านการอักเสบเมื่อเหนี่ยวนำให้เกิดการบวมของอุ้งเท้าหนูขาวใหญ่ด้วย carrageenan พบว่า สารสกัดจากเมล็ดเทียนดำในขนาดสูงเท่านั้น (400 มก./กก.) สามารถลดการบวมของอุ้งเท้าได้ทุกช่วงเวลาในการทดลอง แต่พบว่าสารสกัดไม่สามารถยับยั้งการเกิดการอักเสบแบบเรื้อรังจากการเหนี่ยวนำด้วย cotton pellet ที่ทำให้เกิด granuloma ได้ ในการทดสอบฤทธิ์ในการลดไข้ของสารสกัดเฮกเซนจากเมล็ดเทียนดำ พบว่าสารสกัดในขนาดสูง (400 มก./กก.) เท่านั้นสามารถลดไข้จากการเหนี่ยวนำด้วย brewer's yeast ในหนูขาวใหญ่ได้ในทุกช่วงเวลาตั้งแต่ 1 ชั่วโมงถึง 5 ชั่วโมง ส่วนในการทดสอบความเป็นพิษเฉียบพลัน เมื่อให้สารสกัดเฮกเซนเมล็ดเทียนดำในขนาดสูง 5 ก./กก.ทางปาก พบว่าไม่ทำให้สัตว์ทดลองตายและไม่แสดงอาการจากความเป็นพิษของสารสกัดจากเมล็ดเทียนดำ ดังนั้นค่าประมาณของ LD_{50} ของสารสกัดเฮกเซนของเมล็ดเทียนดำในสัตว์ทดลองมีค่ามากกว่า 5 ก./กก.

โดยสรุปสารสกัดเฮกเซนจากเมล็ดเทียนดำที่ใช้ในการทดลอง พบว่ามีฤทธิ์ระงับปวดได้ ซึ่งกลไกการออกฤทธิ์ในการระงับปวดอาจจะออกฤทธิ์ทั้งในระบบประสาทส่วนกลางระดับสมองและไขสันหลังคล้ายกับยา morphine และระบบประสาทส่วนปลายคล้ายกับยา aspirin ฤทธิ์ในการต้านการอักเสบของสารสกัดอาจเกิดจากผลจากสารสกัดสามารถยับยั้งการหลั่งสารตัวกลางในกระบวนการอักเสบ ส่วนกลไกในการลดไข้ของสารสกัดอาจเกิดจากการยับยั้งการสังเคราะห์ prostaglandins จากผลการทดลองในการศึกษาครั้งนี้แสดงให้เห็นว่า สารสกัดเฮกเซนจากเมล็ดเทียนดำมีฤทธิ์ระงับปวด ลดไข้ และต้านการอักเสบ ซึ่งแสดงให้เห็นว่าสนับสนุนการนำน้ำมันสารสกัดจากเมล็ดเทียนดำมาใช้ในการแพทย์แผนไทยโดยใช้ในการระงับปวด ลดไข้ และต้านการอักเสบ

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ABSTRACT

Abroma augusta (*A. augusta*) Linn., Family Sterculiaceae, commonly known as "devil's cotton", but in Thai it is called "Teandum". This plant is widely distributed in India, China, Pakistan and Thailand. *A. augusta* has been brought to be a part of food in local people, especially, it is very popular among Thai Muslim people in three southern provinces of Thailand since. *A. augusta* seed oil has been used for treatment of pain. Teandum is a shrub or small tree 2 to 3 meters in height and the leaves are alternate, ovate, 10 to 15 centimeters in length and 10 to12 centimeters in width. Several parts of this plant contain biologically active compounds such as choline, betaine, β-sitosterol, stigmasterol, L-rhamnose, L-aribinose, D-xylose, D-mannose, D-galactose, D-glucose, D-galacturonic acid and D-glucuronic acid. According to the traditional medicine, seed oil of *A. augusta* has been used to treat joint pain, asthma, sinusitis, tonsillitis, back pain, diarrhea and influenza, but lack of scientific data of the experiments to prove. Thus, the aims of the present study are to evaluate the analgesic, antipyretic and anti-inflammatory activities of *A. augusta* seeds oil in experimental animal models. The nociceptive models included acetic acid-induced writhing, formalin, hot plate and tail flick tests. Carrageenan induced rat hind paw edema and cotton pellet-induced granuloma formation in rats were used to examine the anti-inflammatory activity of *A. augusta*, whereas brewer's yeast induced fever in rats was used to evaluate antipyretic activity.

The hexane extract of *A. augusta* Linn. seeds (HEAA) at the doses of 100, 200 and 400 mg/kg, po significantly reduced the number of writhing induced by acetic acid in mice by 35.65%, 49.74% and 57.99%, respectively and decreased the duration

of licking time in early phase by 33.84%, 42.46% and 54.58%, and late phase by 36.79%, 63.71% and 80.83%, respectively. The decrease in the number of writhing and licking time was dose-related. HEAA at doses of 100, 200 and 400 mg/kg, po significantly increased the latency of nociceptive responses both in hot plate and tail flick tests at all time intervals measured. Naloxone (2 mg/kg, ip) could antagonize antinociceptive activity of both morphine (5 mg/kg, sc) and HEAA (400 mg/kg, po) in hot plate and tail flick tests. Based on analgesic test models, the results indicated that HEAA exhibited analgesic activity and possible analgesic mechanisms were likely to be mediated peripherally and centrally. In carrageenan-induced rat paw edema model, HEAA at only the dose of 400 mg/kg, po significantly reduced rat paw edema at all time interval measured. However, HEAA did not exhibit ant-inflammatory activity on cotton pellet-induced granuloma in rats. In antipyretic test, only HEAA 400 mg/kg, po significantly decreased rectal temperature at 1 h and continued up to 5 h after brewer's yeast injection. In oral acute toxicity test, the estimated LD_{50} in mice or rats is more than 5 g/kg.

In conclusion, the hexane extract of *A. augusta* Linn. seeds possesses antinociceptive activity. The analgesic mechanism is probably mediated peripherally and centrally (spinal and supraspinal levels) in similar to aspirin and morphine, respectively whereas anti-inflammatory action may be due to its inhibition of inflammatory mediator release during inflammatory process. The possible antipyretic action of HEAA is probably due to inhibition of prostaglandin synthesis. The overall results in this study demonstrated that hexane extract of *A. augusta* exhibited significant antinociceptive, antipyretic and anti-inflammatory activities. Therefore, the *A. augusta* seeds oil used in traditional medicine is proved to possess analgesic, antipyretic and anti-inflammatory activities confirming than traditional use of this plant.

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Sineenat Kuadkaew

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LIST OF ABBREVIATIONS AND SYMBOLS

AA	=	Arachidonic acid
AMPA	=	Alpha-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid
B_2	=	Bradykinin 2
cAMP	=	Cyclic-adnosine monophosphate
CGRP	=	Calcitonin gene-related peptide
CNS	=	Central nervous system
COX	=	Cyclooxygenase
etc.	=	Et cetera
g	=	Gram
GC-MS	=	Gas Chromatography-Mass Spectrometric
h	=	Hour
HEAA	=	Hexane extract of *Abroma augusta* Linn.
IL-1β	=	Interleukin-1β
ip	=	Intraperitoneal
kg	=	Kilogram
L	=	Liter
LD_{50}	=	Lethal dose at 50% of deadly animal
LPS	=	Lipopolysaccharide
m	=	Meter
mg	=	Milligram
min	=	Minute
ml	=	Milliliter
mM	=	Micro molar
NGF	=	Nerve growth factor
NK_1	=	Neurokinin 1
NMDA	=	N-methyl-D-aspartic acid
No.	=	Number
NO	=	Nitric oxide
NSAIDs	=	Non-steroidal anti-inflammatory drugs

PGE_2	=	Prostaglandin E_2
PGH_2	=	Prostaglandin H_2
pH	=	Potential of hydrogen
PGI_2	=	Prostacyclin
PKC	=	Protein kinase C
PKA	=	Protein kinase A
po	=	*Per os*
POAH	=	Preoptic nuclei of the anterior hypothalamus
sec	=	Second
sc	=	Subcutaneously
S.E.M	=	Standard error of the Mean
TNF	=	Tumor necrosis factor
TXA_2	=	Thromboxane A_2
w/v	=	Weight by volume
w/w	=	Weight by weight
°C	=	Degree Celsius
°F	=	Degree Fahrenheit
/	=	Per
µL	=	Microliter
µg	=	Microgram
%	=	Percentage

CHAPTER 1

INTRODUCTION

The enormous floral diversity of Thailand has provided local traditional health practitioners with an impressive array of plant material from which to select ingredients for use in herbal medicines. Today, medicinal plants are important sources of new chemical compounds that potentially have strong therapeutic effects. Most people living in developing countries are almost completely dependent on traditional medical practices for their primary health care needs and higher plants are know to be the main source for drug therapy in traditional medicine (Calixto, 2005). Pre-clinical assays are essential to guarantee the efficacy and safety of natural products and commonly requested by regulatory agencies to approve marketing of these products (Remirez, 2008). In Thailand, there are many plant species that possess medical values. Several medicines used today are derived straight from many natural products including medicinal plants. However, the potential used of plants as a source of new drug is still poorly explored.

Today, various Thai herbal plants are used widely for preparing of herbal medicine. One of which is *Abroma augusta* (*A. augusta*) Linn. (Sterculiaceae) has a common name as Devil's Cotton, but in Thai it is called Teandum. *A. augusta* is one of the most interesting medicinal plants because it is used as topical and massage to relieve pain, and as food by mixer of food and the plants is populated of Thai Muslim in three southern provinces. Teandum is a shrub or small tree 2 to 3 meters in height and lowly distributed throughout Thailand. The leaves are alternate, ovate, 10 to 15 centimeters in length and 10 to12 centimeters in width. The flowers are bisexual and yellowish with purple bases. The seeds are subellipsoid, numerous and finely punctuate (Encyclopedia of Plants in Thailand, 2006). In addition, it was used as a medicinal herb because various parts of this plant, such as fruit, root or leave, contains many chemical constituents e.g. choline, betaine, β-sitosterol, stigmasterol, L-rhamnose, L-aribinose, D-xylose, D-mannose, D-galactose, D-glucose, D-galacturonic acid and D-glucuronic acid (Dasgupta and Basu, 1970; Majurnder *et al*.,

1994). The pharmacological activities of the aqueous extract of the whole plant of *A. augusta* and rhizome of *Curcuma longa* have been reported to reduce blood glucose and increase in total hemoglobin in streptozotocin-induced diabetes in rats (Eshrat, 2002). The water extract of the whole root of *A. augusta* has shown a hypoglycemic and hypocholesterolemic effects in rats (Halim and Ali, 2001). The combined aqueous extract of the roots of *Abroma augusta* and *Cocinia indica* leaves was found to reduce fasting blood sugar to almost normal value in streptozotocin-induced diabetic rats (Eshrat, 2003). Additionally, the studied of biological and pharmacological properties of *A. augusta* seed oil has significantly demonstrated antifungal activities against *Trichophyton schoenleinii* and *Microsporum canis* (Khan and Ahmad, 2003).

Several kinds of medicinal plants in Thailand have been studied to investigate the analgesic, anti-inflammatory and antipyretic activities in various experimental animal models, for examples, *Premna herbacea* (Karw-Yen-Nueun) root, *Piper sarmentosam* (Cha-Plu) leaves, *Kaempferia galanga* L. (Proh-Hom) rhizomes, *Aegle marmelos* (Bua-Tunm) leaves, *Tabernaemontana pandacaqui* (Pud-Fa-Rung) stems and *Bauhinia racemosa* (Puk-Seuw) stem barks. In Thai traditional medicine, *A. augusta* of aerial parts has been used in traditional medicine to treat joint pain, asthma, sinusitis, tonsillitis, back pain, diarrhea and influenza (Ahmad *et al*, 2003).

In the present study, *A. augusta* seeds was selected to examine for its analgesic, anti-inflammatory and antipyretic activities since it has been used in Thai traditional medicine to treat joint pain, asthma, sinusitis, tonsillitis, back pain, diarrhea and influenza. However, systematically scientific pharmacological studies on the analgesic, antipyretic and anti-inflammatory activities of this plant have not been reported. Thus, this study was aimed to evaluate the analgesic, antipyretic and anti-inflammatory effect of *A. augusta* seeds in animal models.

CHAPTER 2

LITERATURE REVIEW

Abroma augusta Linn. (Sterculiaceae)

Abroma augusta Linn. (*A. augusta*), Family Sterculiaceae, commonly known as devils cotton, but in Thai it is called Teandum. The plant is found in India, China, Pakistan and Thailand.

A. augusta, is a shrub or small tree 2 to 3 meters in height. The branches and branchlets are downy. The leaves are alternate, ovate, 10 to 15 centimeters in length, and 10 to 12 centimeters in width, with pointed tip, heart-shaped base, and toothed margins. The flowers are bisexual, about 5 centimeters across, and yellowish with purple bases. The fruit (capsule) is obpyramidal, about 3.5 centimeters in diameter, covered with irritating hairs, and ultimately smooth, with five prominent angles or wings, which are 4 by 7 centimeters across. The seeds are subellipsoid, numerous, and finely punctuate. (Figure 1)

Flower

Leaves

Fruit and seeds

Dry seeds

Figure 1. Aerial parts of *Abroma augusta* Linn.

Aerial parts of *Abroma augusta* Linn. have been used in traditional medicine and reported that it is used to treat joint pain, asthma, sinusitis, tonsillitis, back pain, diarrhea, influenza, and to be as antifungal (Ahmad *et al*., 2003).

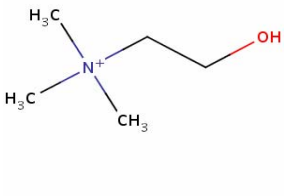
Chemical constituents

Ahmad *et al*., (2003) studied the chemical composition of *A. augusta* Linn. seed oil. The composition of oil was determined using Gas Chromatography-Mass Spectrometric (GC-MS) technique, and in the n-hexane fraction was found to be the most abundant fatty acid which comprised of 33% of the total peak areas for the *A. augusta* Linn. seed oil. (Table 1)

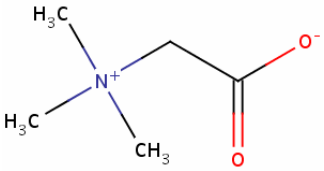
Dasgupta and Basu, (1970) reported that chemical constituents have been identified from the various parts of this plant included choline, betaine, β-sitosterol and stigmasterol (Figure 2). The various parts of this plant also contain L-rhamnose, L-aribinose, D-xylose, D-mannose, D-galactose, D-glucose, D-galacturonic acid and D-glucuronic acid (Majumder *et al*., 1994) (Figure 2).

Table 1. Fatty acids identified in the seed oil *A. augusta* Linn.

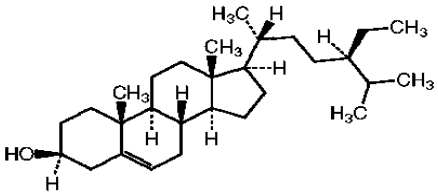
Fatty acids	Molecular Wt.	% of Whole oil
Saturated fatty acids		
n-Hexadecanoate, methyl ester		
CH_3-$(CH_2)_{14}$-CO_2CH_3	270	33
2-Methly hexadecanoate, methyl ester		
$CH_3(CH_2)_{13}$-CH-CO_2CH_3	284	28
n-Octadecanoate, methyl ester		
CH_3-(CH_2)15-CO_2CH_3	298	13
2-Methyl octadecanoate, methyl ester		
$CH_3(CH_2)_{15}$-CH-CO_2CH_3	312	01
Unsaturated fatty acids		
9-Octadecnoate, methyl ester		
CH_3-$(CH_2)_7$-CH=CH-$(CH_2)_7$-CO_2CH_3	294	23
10-Nonadecenoate, methyl ester		
CH_3-$(CH_2)_7$-CH=CH-$(CH_2)_8$-CO_2CH_3	310	02



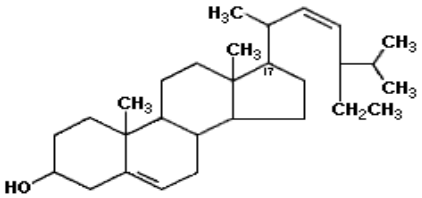
Choline



Betaine



β-sitosterol



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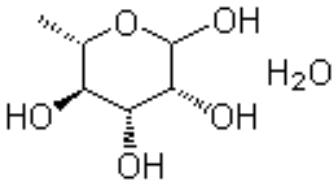

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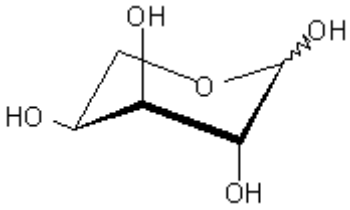
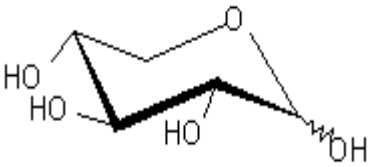
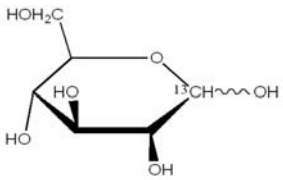


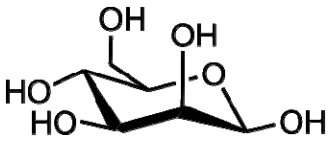
L-arabinose



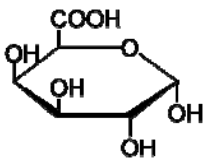
D-xylose



D-glucose

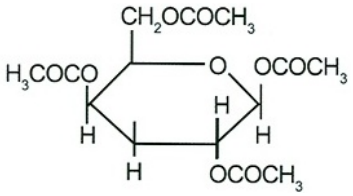


D-mannose

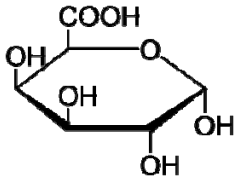


D-galacturonic acid

Figure 2. The molecular structure of various compounds found in *Abroma augusta* Linn.



D-galactose



D-glucuronic acid

Figure 2. The molecular structure of various compounds found in *Abroma augusta* Linn. (continued)

Pharmacological activity

The preliminary studies on the hypoglycaemic effect of *Abroma augusta* in alloxan-induced diabetic rats were indicated that the water extract of *A. augusta* has both hypoglycemic and hypocholesterolemic effects (Halim and Hussain, 2001).

Eshrat and Hussain (2002) studied on hypoglycemic, hypolipidemic and antioxidant properties of combination of curcumin from *Curcuma longa* Linn. and partially purified product from *A. augusta* Linn. in streptozotocin-induced diabetes in rats. The results showed that there was a significant reduction in blood glucose and an increase in total hemoglobin.

Eshrat (2003) studied on the effect of *Cocinia indica* (L.) and *A. augusta* on glycemia and lipid profiles in streptozotocin-induced diabetic rats. The results after 8 weeks of treatment in streptozotocin (STZ) diabetic rats, the fasting blood sugar decreased to almost normal value, and there was an improvement in glucose tolerance.

Khan and Ahmad (2003) studied on the biological and pharmacological properties of *A. augusta* Linn. seed oil. The oil was also screened for various in *vitro* biological and pharmacological activities including antifungal, antibacterial, insecticidal, phytotoxic and brine-shrimp cytotoxic activities. The results have been shown a significant antifungal activity against *Trichophyton schoenleinii* (56%) (human pathogens) and *Microsporum canis* (50%) (animal pathogen).

Pain

Pain is defined as an unpleasant sensory and emotional experience associated with actual or potential tissue damage. The detection of noxious chemical, thermal, and mechanical stimuli is mediated by receptors on specialized sensory neurons. Recent technical advances in molecular genetics and applications of cellular physiology to the study of pain have led to considerable progress in our understanding of pain signaling mechanisms. Identification of novel channels expressed in nociceptive sensory neurons (Lee and Oh, 2005).

According to the IASP definition, the relation between pain and degree of injury is not obligatory. Thus, the alert function applies only to an acute manifestation, i.e., the one that follows damage to the tissue. The pain experience is divided into 2 types as acute and chronic pain. Both of these experiences alter the comfort level of the patient and cause different pain reaction behaviors. Acute pain is characterized by the fact of being delimited in time and disappearing with the resolution of the pathological process. Chronic pain that persists for an extended period of time is associated with chronic pathological processes and causes suffering in multiple systems (Merskey and Bogduk, 1994; Nathan, 1977).

Acute pain is a warning to possible or real danger. The onset of the pain is sudden and a temporary duration. The behavioral response is based on the activation of the sympathetic nervous system with release of epinephrine-norepinephrine. Acute pain is usually accompanied by physiological and behavioral response. Example of physiological responses are increase in heart rate, respiration, blood pressure, peripheral blood flow, muscle tension, sweating, dilated pupils and behavioral responses include distress, restlessness and inability to concentrate.

Chronic pain is persist beyond the normal healing time as pain signals are repeatedly being generated marking neural pathway hypersensitive to pain signals and resistant to antinociceptive input. There are no physiological responses in chronic pain. But the behavioral response is based on the long-term activation of the autonomic nervous system. The patient can exhibit sleep and appetite disturbances, irritability, decreased libido, loss of interests and increased preoccupation with body

sensation. The patient can also experience apathy, withdrawal, hopelessness and depression (Beyers, 1991).

Chemical sensitivity of nociceptors

This review focuses on the molecular aspects of pain signaling pathway in the primary sensory neurons and provides insight into the roles of key molecules in pain pathways.

Bradykinin signaling

Tissue damage and inflammation lead to the activation of proteolytic kallikreins. Kallikreins generate the kinins including bradykinin from kininogen substrates. Bradykinin, consisting of nine amino acids, is the initial mediator of inflammation and induces pain. Bradykinin is also known to act as a potent en dothelium-dependent vasodilator. The actions of bradykinin are mediated through the activation of two types of G-protein coupled receptors, B_1 and B_2. The B_1 receptors is only expressed as a result of tissue damage and inflammatory signals such as nerve growth factor (NGF) and the cytokines, tumor necrosis factor (TNF) and interleukin-1β (IL-1β), whereas the B_2 receptor is constitutively expressed. The B_1 and B_2 receptor knockout mice show hypoalgesia against painful stimuli (Pesquero *et al*., 2000; Rupniak *et al*., 1997), suggesting the role of bradykinin and the B_1 and B_2 receptors in pain signaling pathways in response to tissue damage and inflammation.

COX-2 and prostaglandins signaling

Non-steroidal anti-inflammatory drugs (NSAIDs) such as aspirin and ibuprofen act as inhibitors of COX and are used primarily to treat pain, inflammation, and fever. COX-1 is constitutively expressed throughout the body and of particular importance for gastrointestinal protection, and COX-2 can be induced by proinflamatory cytokines such as TNF-α and IL-Iβ at the site of local inflammation. COX mediates the conversion of arachidonic acid (AA) into the prostaglandin precursors PGG_2 and PGH_2. Prostaglandins bind specific G-protein coupled receptors and stimulate second messengers such as protein kinase A (PKA) and PKC, and mediate an increase in intracellular Ca^{2+}. For example, PGE_2 acts on EP_2 receptor linked to Gs protein and causes activation of adenylyl cyclase and PKA (An *et al*., 1993).

Serotonin (5-hydroxytryptamine, 5-HT) signaling

5-HT is released from platelets and acts on no less than 14 distinct receptor types that are heterogeneously distributed throughout tissues. Of these receptors, only the 5-HT_3 receptor is not linked to a G-protein mediated second messenger pathway. Rather, it forms a ligand gated ion channel and causes Na^+ influx and neuronal excitation in response to ligand binding. 5-HT_3 receptor antagonists are known to have antinociceptive effects with in the dorsal root ganglion and peripherally, whereas intrathecal administration of 5-HT_3 receptor antagonists blocks 5-HT-induced algesia and produces a moderate hyperalgesic response (Giordano and Dyche, 1989).

Adenosine triphosphate (ATP) signaling

ATP can elicit pain upon infusion into the skin via ATP-gated ion channels of the P2X family. There have been seven P2X subunits identified so far. Each subunit has two transmembrane segments separated by a large ectodomain. ATP is believed to bind the ectodomain and cause significant membrane Ca^{2+} permeability (Bouvier *et al*., 1991).

Voltage-gated ion channels

Ca^{2+} channels

Activation of voltage-gated Ca^{2+}channels directly affects membrane potential and contributes to electrical excitability of neurons. Voltage-gated Ca^{2+} channels also play a crucial role in neurotransmitter release from the presynaptic terminals in the dorsal horn in response to incoming action potentials. (Cheng and Chiou, 2006).

Na^+ channels

Voltage-gated Na^+ channel subunits, similar to voltage-gated Ca^{2+} channel α1 subunits, comprise four repeated domains of six transmembrane segments. Voltage-gated Na^+ channels confer excitability on neurons in pain pathway by responding to membrane depolarization with transient opening to allow influx of Na^+. (Renganathan *et al*., 2001).

Glutamatergic signaling

Excitation of the nociceptive sensory neurons by tissue damage and nerve injury evokes a continuous release of glutamate from central terminals of noxious sensory neurons. The released glutamate acts on postsynaptic glutamate receptors such as N-methyl-D-aspartic acid (NMDA) receptors, leading to central sensitisation.

Glutamate receptors also localize on central terminals of primary afferent sensory neurons and they are involved in controlling neurotransmitter release from primary afferents.

Phases of pain

Phase 1 pain (Acute nociceptive pain)

The mechanisms subserving the processing of brief noxious stimuli (phase 1 pain) can be viewed as a fairly simple and direct of transmission centrally toward the thalamus and cortex and thus the conscious perception of pain, with possibilities for modulation occurring at synaptic relays along the way. The relative simplicity of this model reflects the experimental observation that, in humans undergoing phase 1 pain, there is a close correlation between the discharges in peripheral nociceptors and the subjective expression of the pain. Phase 1 pain is the type that has been most study experimentally, and on the basis of the large body of data from both humans and animals, it is reasonably easy to construct plausible and detailed neuronal circuits to explain the features of phase 1 pain (Cervero and Laird, 1991) (Figure 3).

Phase 2 pain (Inflammatory pain)

The situation changes to what we have called phase 2 pain if a noxious stimulus is very intense, or prolonged, leading to tissue damage and inflammation. The pain state under these conditions is different from that in phase 1 pain, because the response properties of various components of the nociceptive system change. There is a greatly increased afferent inflow to the CNS from the injured area as a result of the increased activity and responsiveness of sensitized nociceptors. In addition, nociceptive neurons in the spinal cord modify their responsiveness in way that are not merely an expression of the changes in their inputs from the periphery. These changes mean that the CNS has moved to a new, more excitable state as a result of the noxious input generated by tissue injury and inflammation. Phase 2 pain is characterized by its central drive, a drive that is triggered and maintained by peripheral input (Cervero and Laird, 1991) (Figure 3).

Phase 3 pain (Neuropathic pain)

Phase 3 pains are abnormal pain state and are generally the consequence of damage to peripheral nerves or to the CNS itself. These are neuropathic pain states characterized by a lack of correlation between injury and pain. In clinical terms, phase1 and phase 2 pains are symptoms of peripheral injury, whereas phase 3 pain is a symptom of neurological diseases that include lesions of peripheral nerves or damage to any portion of the somatosensory system within the CNS. Phase 3 pains are spontaneous, triggered by innocuous stimuli, or are exaggerated responses to minor noxious stimuli. These sensations are expressions of substantial alteration in the normal nociceptive system induced by peripheral or central damage (Figure 3). The particular combination of mechanisms responsible for each one of the various phase 3 pain state is probably unique to the individual disease, or to a particular subgroups of patients. Phase 3-type syndromes are seen only in a few people. Even patients with seemingly identical damage to their nervous system may or may not complain of pain. Thus, the development of phase 3 pain may involve genetic, cognitive, or emotional factors that have yet to be identified (Cervero and Laird, 1991).

The loss of sensory function as a result of damage to the nervous system is conceptually easy to understand as the interruption of connections either within the CNS or between the CNS and the peripheral sensory receptors. However, the abnormal sensory symptoms that accompany many cases of neuronal damage require more explanation. Two groups of mechanisms probably account for these sensory symptoms:

1. Pathological changes in the damaged neurons
2. Reactive changes in response to nociceptive (damage-related) afferent input, and to the loss of portions of the normal afferent input.

The pathological changes in damaged neurones are almost certainly unique to phase 3 pain, some the reactive changes to nociceptive input may well be the expression of normal mechanisms also seen in type 2-type pain. In phase 3 pain, the activation of these mechanisms may be abnormally prolonged or intense owing to the abnormal input from damaged neuron, or simply because the regenerative properties of neurons are very poor, healing never occurs.

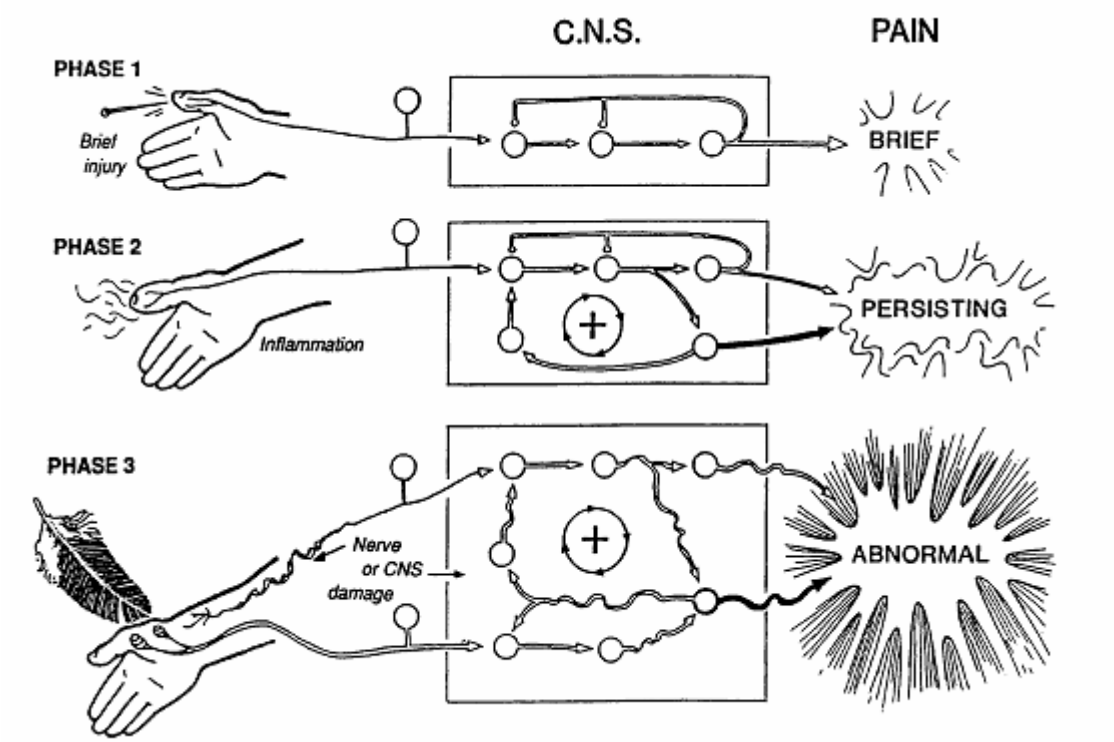


Figure 3. Schematic representation of the three phases of pain (Cervero and Laird, 1991)

Pain mechanisms

Peripheral receptor

The propagation of pain is initiated with the activation of physiological receptors, called nociceptors, widely found in the skin, mucosa, membranes, deep fascias, connective tissues of visceral organs, ligaments and articular capsules, periosteum, muscles, tendons, and arterial vessels. The receptors correspond to free nerve endings and represent the more distal part of a first-order afferent neuron consisting of small-diameter fibers, with little or unmyelinated, of the A-Delta or C type, respectively (Table 2). Their receptor fields can consist of areas ranging from punctiform regions to regions measuring several millimeters in diameter, or even of more than one site in distant territories (Lynn, 1992) (Figure 4).

The nociceptors found in the skin originate from small nervous stems that, when approaching the epidermis, lose their myelin, ramifying into extensive plexuses. Two types of free nervous endings exist: the ramified ones originating from 1 or 2

myelinated fibers forming intraepithelial terminations and the nonencapsulated glomerular bodies, deriving from a single unmyelinated fiber and organized in a densely spiral manner below the epidermis or the mucosa. In other organs, this organization may vary because the type of propagated stimulation, the form of propagation, and the quality of the painful sensation depend on the receptor nervous fiber complex and the innerved organ (Millan, 1999; Willis and Westlund, 1997).

Normally, the painful sensation results from specific activation of the nociceptors by mechanical, thermal, or chemical stimulus, and not by the hyperactivity of other sensory modality receptors. They present higher thresholds than the other receptors and respond progressively according to the intensity of the stimulus. However, the sensitization of the nociceptors causes reduction of the thresholds and, in some cases, spontaneous activity (Mense, 1983; Millan, 1999).

Table 2. Classification of sensory neurons (Wang, 1995)

Classification		Fiber type	Velocity (m/s)	Propagation velocity
Sensory neuron	Destination			
Aβ	Laminar II and IV	Myelinated	6-12	35-75
Aδ	Laminar I and V	Myelinated	1-6	5-30
C	Laminar II	Unmyelinated	< 1	0.5-2

Peripheral sensitization

After tissue damage which may be mechanical, chemical or thermal (below 15°C, above 45°C) on nociceptor, biological molecules are produced such as substance P, histamine, hydrogen ions, bradykinin, prostaglandin, nitric oxide, ATP. Bradykinin is a main substance that stimulates nociceptives (act on Bradykinin 2, BK_2 receptor) via protein kinase C (PKC), and prostaglandin (mainly E_2, I_2), sensitize nociceptor (act on EP/IP receptor) to noxious stimuli by lowering of nociceptive activation thresholds via cyclic-adnosine monophosphate (cAMP). It causes the afferent neuron to discharge, sending impulse (action potential) to the spinal cord. This state is called peripheral sensitization (Guyton, 1992; Mutschler and Derendorf, 1995a) (Figure 5).

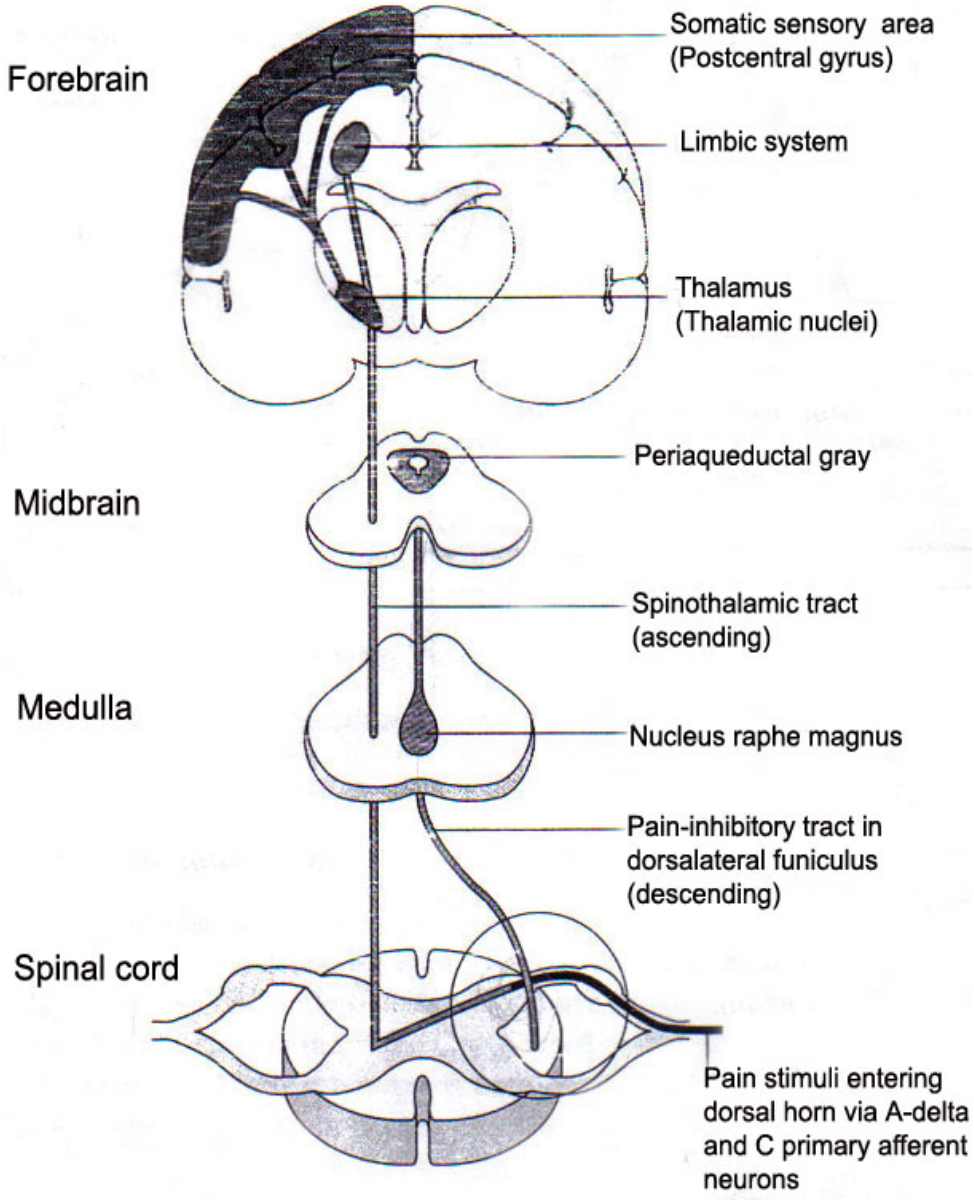


Figure 4. Pain pathway (Stephen, 1998)

Central sensitization

The repeated afferent impulse to the spinal cord as a result of the sensitizing biological molecules at the site of tissue damage, cause the dorsal hone neurons within the spinal cord to become hyperexcitable (William, 1998). When A-delta fibers produce the acute sensation of sharp and bright pain, their neurotransmitter in the dorsal horn is glutamate acting on alpha-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid (AMPA) receptors. The C fiber can respond to a broad range of painful stimuli, including mechanical, thermal or metabolic factors. The pain produced is slow, burning, and long lasting. The neurotransmitter in the dorsal horn is glutamate along with certain peptides such as substance P (act on neurokinin 1, NK_1 receptor). The receptor for glutamate are not only AMPA, but also N-methyl-D-aspartate (NMDA). When AMPA receptor was activated and open following prolonged depolarization, continue stimulation of C fiber eventually causes greater excitation in the postsynaptic neurons in the dorsal horn as the NMDA receptor start added to the response.

Spontaneous activity of C fiber results in increased dorsal horn excitatory. C fiber release not only glutamate but also substance P, which acts through the neurokinin-1 (NK_1) receptor to increase dorsal horn intracellular calcium and enhance N-methyl-D-aspartate (NMDA) sensivity to glutamate. This up-regulation of glutamate signaling is one aspect of central sensitization (Singleton, 2005). When glutamate releases from primary afferent neuron and binds to NMDA, there is an influx of Ca^{2+} into the postsynaptic neuron. The resulting influx of Ca^{2+} could activate enzyme such as nitric oxide (NO) syntheses or trigger other long lasting cellular changes, so signal transduction coming to sensory projection fields in the cortex (postcentral gyrus). This part of the cortex, together with the thalamus, is responsible for the conscious perception of pain and particularly localizing and registering the intensity of the pain. The ascending reticular activating system has an influence on evaluation. The limbic system is responsible for emotional reactions triggered by pain while autonomic reactions are controlled by the hypothalamus (Mutschler and Derendorf, 1995b). This state of hyperexcitability is called central sensitization.

Recall that these only open with prolonged depolarization, such as would occur with prolonged pain. The resulting influx of Ca^{2+} cause trigger other long

lasting cellular changes, so signal transduction coming to sensory projection fields in the cortex (postcentral gyrus). This part of the cortex, together with the thalamus, is responsible for the conscious perception of pain and particularly localizing and registering the intensity of the pain. The ascending reticular activating system has an influence on evaluation. The limbic system is responsible for emotional reactions triggered by pain while autonomic reactions are controlled by the hypothalamus (Mutscheler and Derendorf, 1995b; William, 2000). (Figure 6)

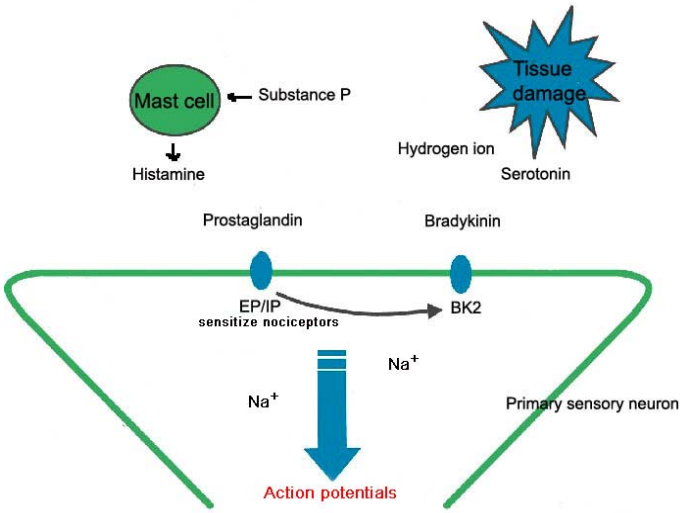


Figure 5. Pain pathway in peripheral sensitization (Adapted from Samad, 2002)

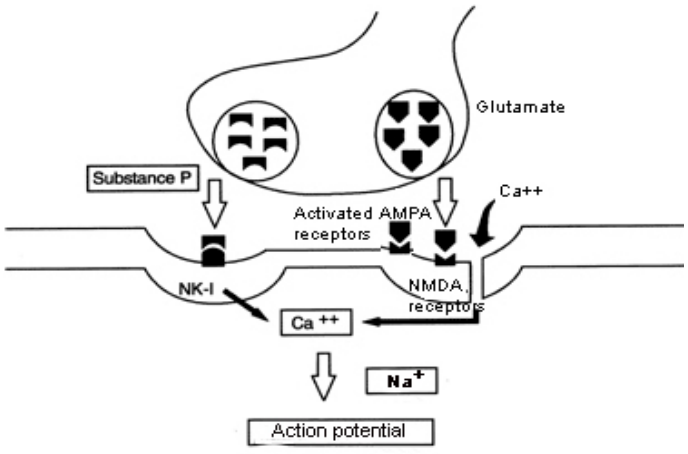


Figure 6. Pain pathway in central sensitization (DeVane, 2001)

Inflammation

Inflammation is defined as the reaction of a tissue and its microcirculation to a pathogenic insult. It is characterized by the generation of inflammatory mediators and movement of fluid and leukocytes from the blood into extravascular tissues (Fantone and Ward, 1999) or when tissue injury occurs, whether caused by bacteria, trauma, chemicals, heat or other phenomena, multiple substances that causes dramatic secondary changes in the tissue are released by the injured tissue (Mariab, 2001). This is frequently an expression of the host's attempt to localize and eliminate metabolically altered cells, foreign particles, microorganisms or antigens.

Cause of inflammation

Inflammation is instigated when pro-inflammatory hormones (cytokines, prostaglandins) signal the white blood cells to isolate and clear out antigens, damaged tissue and infected areas of the body. The inflammatory process then moves into high gear, neutralizing and eliminating the intruder and then beginning the healing process. If such inflammation is acute, and ebbs and flows according to specific needs, then the immune system is working efficiently. However, sometimes the inflammatory response does not switch off when the danger has passed. This leads to chronic inflammation. Inflammation has been divided into 2 types, acute inflammation and chronic inflammation.

I. Acute inflammation

The inflammatory responses consist of changes in blood flow, increased permeability of blood vessels and escape of cells from the blood into the tissues. Acute inflammation is of short duration, which could be anything from a few minutes to a few days. Such as inflammation is caused by foreign substances entering the body, or by physical damage. A viral infection may also precipitate acute inflammation. The cardinal sings of acute inflammation:

1. Redness (rubor): An acutely inflamed tissue appears red, for example skin affected by sunburn, cellulites cause by bacterial infection or acute conjunctivitis. This is due to dilatation of small blood vessels within the damaged area.

2. Heat (calor): Increase of temperature on the skin is seen only in peripheral parts of the body. It is due to increased blood flow (hyperemia) through the region, resulting in vascular dilatation and the delivery of warm blood to the area.

3. Swelling (tumor): Swelling results from edema, the accumulation of fluid in the extra vascular space as part of the fluid exudates, the physical mass of the inflammatory cells migrating into the area.

4. Pain (dolor): For the patient, pain is one of the best known features of acute inflammation. It results partly from the stretching and distortion of tissues due to inflammatory edema and, in particular, from pus under pressure in an abscess cavity. Some of the chemical mediators of acute inflammation, including bradykinin, prostaglandins and serotonin, are known to induce pain.

5. Loss of function: Loss of function is a well-known consequence of inflammation. Movement of an inflamed area is consciously and reflexly inhibited by pain, while severe swelling may physically immobilize the tissue (Macfalane *et al*., 2000). These in turn give rise so called fifth sign of inflammation (Norris, 2004).

The chemical mediators

1. Histamine : This is the best-known chemical mediator in acute inflammation. It causes vascular dilatation and the immediate transient phase of increased vascular permeability. It is stored in mast cells, basophil and eosinophil leukocytes and platelets (Pflanzer, 1992). Histamine release from those sites such as mast cell degranulation is stimulated by complement components C3a and C5a.

2. Lysosomal compounds : These are released from neutrophils and include cationic protein, which may increase vascular permeability and neutral proteases, which may activate complement (Brestel and Dyke, 1990).

3. Prostaglandins : Inflammatory cells contain specific cyclooxygenase enzyme that generate endoperoxide derivatives of arachidonic acid, including prostaglandin G_2 (PGG_2) and prostaglandin H_2 (PGH_2). The endoperoxide are unstable and depending on the specific inflammatory cell or tissue are further metabolized to more stable prostaglandins. The latter include prostacyclin (PGI_2), $PGF_{2\alpha}$, PGE_2, PGD_2 and thromboxane A_2 (TXA_2) (Goodman, 1992). The primary

cyclooxygenase metabolite in platelet is TXA_2. Endothelial cells secrete principally PGI_2. Monocyte or macrophages, depending on their state of activation, produce any or all of these derivative products. The PGI_2 and PGE_2 having the vasodilation effects. However, vasodilation can enhance vascular permeability at sites of inflammation. PGI_2 and PGE_2 bind to specific receptors on inflammatory cells, there by activating adenylyl cyclase and increasing intracellular cyclic-adenosine monophosphate (cAMP) level.

4. Leukotrienes : These are also synthesis from arachidonic acid, especially in neutrophils and appear to have vasoactive properties. Slow reacting substance of anaphylaxis (SRS-A) involved in type I hypersensitivity is a mixture of leukotrienes.

5. Serotonin (5-hydroxytryptamine) : This is present in high concentration in mast cells and platelets. It is a potent vasoconstrictor.

6. Lymphokines : This family of chemical messengers released by lymphocytes. Apart from their major role in type IV hypersensitivity, lymphokines may also have vasoactive or chemotactic properties (Rang and Dale, 1991).

The following chemical mediators were found from plasma.

1. Complement system: The complement system is a cascade system of enzymatic proteins. It can be activated during the acute inflammatory reaction in various ways.

2. Kinin system: The kinins are peptides of 9-11 amino acid; the most important vascular permeability factor is bradykinin. The kinin system is activated by coagulation factor XII. Bradykinin is also a chemical mediator of the pain which is a cardinal feature of acute inflammation (Rang and Dale, 1991).

3. Coagulation system : The coagulation system is responsible for the conversion of soluble fibrinogen into fibrin a major component of the acute inflammation exudates

4. Fibrinolytic system: Plasmin is responsible for the lysis of fibrin degradation products which may have local effects on vascular permeability.

Role of NO in acute inflammation

NO may be pro- or anti-inflammatory depending upon the disease state, the tissue being examined, the time-point under investigation and/or its enzymatic source (i.e. nNOS, eNOS or iNOS). Together, these variables confound efforts to label NO as simply 'pro-' or 'anti-inflammatory' and may account for the literary discrepancies often seen in this field of research. The relationship between NO and the acute inflammatory response is therefore discussed with reference to these considerations and categorized according to functional indices of relevance to cutaneous burn injury (e.g.microvascular hemostasis and leukocyte-endothelial cell interactions) (Rawlingson, 2003).

NO and microvascular blood flow

NO is a potent vasodilator in the microvasculature, it regulates basal tone and has the ability to increase localized blood flow in response to inflammatory stimuli. Falcone and Bohlen, (1990) demonstrated that NO scavengers profoundly attenuate Ach-induced relaxation in the arterioles of rat smooth muscle (Falcone and Bohlen, 1990) and more recently, Figueroa *et al*., (2001) reported that NOS inhibition could increase basal arteriolar and venula tone in a manner that was reversible with L-arginine (Figueroa *et al*., 2001) NOS inhibition has also been shown to prevent increases in blood flow caused by direct application of bradykinin (Khalil and Helme, 1992), substance P (Ralevic *et al.*, 1995), calcitonin gene-related peptide (CGRP) and histamine to microvascular preparations *in vivo*. From these studies, it can be inferred that a variety of monoamine/peptide receptors mediate NOS and guanylate cyclase activation either directly, or indirectly via secondary pathway (e.g. prostaglandin production) to cause microvascular dilation. Furthermore, there is strong evidence to suggest that as part of the neurogenic inflammatory response, nNOS-derived NO can potentiate the release of substance P and CGRP from peripheral sensory neuron (Kajekar *et al.*, 1995; Towler *et al.*, 1998). Thus, it would appear that NO can act both pre- and post-junctionally to increase microvascular blood flow. With respect to more generalized models of skin inflammation, NO has also been implicated in mediating ultraviolet light, Lipopolysaccharide (LPS), IL-1 and zymosan-induced hyperemia.

The sequelae of acute inflammation dependupon the type of tissue involved and the amount of tissue destruction which depend in turn upon the nature of the injurious agent. The possible outcomes of acute inflammation are as follows.

1. Resolution of acute inflammation: The term resolution means the complete restoration of the tissues to normal after acute inflammation. The sequence of events leading to resolution is usually to phagocytosis of bacteria by neutrophils and intracellular killing fibrinolysis, phagocytosis of debris, especially by macrophages.

2. Suppuration: Suppuration is the formation of pus a mixture of living dying and dead neutrophils and bacteria. Once pus begins to accumulate in a tissue, it becomes surrounded by a "pyogenic membrane" consisting of sprouting capillaries, neutrophils and occasional fibroblasts. Such s collection of pus is called an abscess and bacteria within the abscess cavity are relatively inaccessible to antibodies and to antibiotic drugs (Guyton and Hall, 2000).

3. Organisation: Organisation of tissue is their replacement by granulation tissue. When the large amounts of fibrin are formed which cannot be removed completely by fibrinolytic enzymes from the plasma or from neutrophil polymorphs, substantial volumes of tissue become necrotic or dead tissue, it is not easily digested, exudate and debris cannot be removed or discharged.

During organization new capillaries grow into the inert material (inflammatory exudate) macrophages migrate into the zone and fibroblasts proliferate, resulting in fibrosis.

4. Progression to chronic Inflammation: If the agent causing acute inflammation is not removed, the acute inflammation may progress to the chronic state. In addition to organization of the tissue, the character of the cellular exudate changes, with lymphocytes, plasma cell and macrophages replacing the neutrophil polymorphs. However, chronic inflammation occurs as a primary event, there being no proceeding period of acute inflammation (Wynsberghe *et al*., 1995).

The local effects are usually clearly beneficial, the fluid and cellular exudates may have useful effects. Beneficial effects of the fluid exudate are as dilution of toxins, entry of antibodies, drug transport, fibrin formation, delivery of nutrients and oxygen and stimulation of immune response. But the release of lysosomal enzymes by

inflammatory cells may also have harmful effects as digestion of normal tissues, swelling and inappropriate inflammatory response.

II. Chronic inflammation

Inflammation that has a slow onset and persists for weeks or more is classified as being chronic. The symptoms are not as severe as with acute inflammation, but the condition is insidious and persistent. Chronic inflammation may follow on from acute inflammation or exist by itself. An acute inflammation will become chronic if the immune system is unable to rid the body of the offending foreign agent or if the agent is constantly able to re-enter the body. In the case of persistent infections, such as tuberculosis and autoimmune diseases, chronic fatigue will arise without the person first going through the acute inflammation stage.

The main cells involved in chronic infection are macrophages and lymphocytes. Because both these cells have a single nuclei, they are known as mononuclear cells. With the aid of chemical mediators such as lymphokines, macrophages do an excellent job of engulfing and neutralizing or killing foreign antigens. Lymphocytes are the predominant cell in chronic inflammation. There are two types, labeled T and B. T-lymphocytes are produced in the thymus gland. They ensure cell based immunity from infection. B-lymphocytes originate in the bone marrow and ensure humoral (bodily fluid) immunity. The activation of B-lymphocytes produces plasma cells, which manufacture and secrete antibodies to fight specific types of antigens.

Macrophages and lymphocytes are interdependent in that the activation of one stimulates the actions of the other. Certain chronic infections cells known as eosinophils accumulate. Within their cytoplasms are bright red granules. These granules contain a substance called ‘major basic protein’ which has the ability to destroy certain antigens. In cases of chronic inflammation involving foreign particulate matter, such as splinters, macrophages cells can fuse together to form multinucleated giant cells. Tuberculosis may also cause macrophage cells to unite in this manner.

A key feature of chronic inflammation is collagen production. If too much collagen is formed, this can lead to the condition known as fibrosis. Connective tissue cells known as fibroblasts enter the area of tissue injury and then go to work to

produce collagen which is necessary to replace the tissue lost during long term inflammation. The dilated blood vessels which are characteristic of acute inflammation are not evident in cases of chronic inflammation.

The two major complications associated with chronic infection are fibrosis leading to scarring and persistence. The over abundance of collagen production over time can lead to scarring that can cause permanent distortion of the tissue, interfering with its function. Chronic inflammation can be continually stimulated by substances with low antigenic properties or by auto-immunity. The cellular components of the chronic response are described as following :

The macrophage is the pivotal cell in regulating the reaction that lead to chronic inflammation. The accumulation of macrophages mainly reflects the recruitment of circulating monocytes by chemotactic stimuli and their differentiation in tissues. The local proliferation of resident tissue macrophage may also contribute. In addition, macrophages regulate lymphocyte response to antigen and secrete other mediators that modulate the proliferation and function of fibroblasts and endothelial cells (Stanier and Forsling, 1990).

Plasma cells also participate in the chronic inflammatory response. These lymphoid cells, which are rich in rough endoplasmic reticulum are the primary source of antibodies. The production of antibody to specific antigens at site of chronic inflammation is important in antigen neutralization, clearance of foreign antigens and particles and antibody dependent cell-meadiated cytotoxicity (Fantone and Ward, 1999).

Lymphocytes are prominent feature of chronic inflammation reaction and perform vital functions in both humoral and cell-madiated immune response. T lymphocytes not only function in the regulation of macrophage activation and recruitment through the secretion of specific mediators (lymphokines) but also modulate antibody production and cell-mediated cytotoxicity (Rang and Dale, 1991). Recently, natural killer cells and specifialized forms of lymphocytes as T cells, CD5 B cells, have been implicated as participants in the defense against viral and bacterial infections. Their activity dose not require previous sensitization to foreign antigens.

Eosinophils are occasionally a conspicuous component of the chronic inflammation response. They are particularly evident during allergic-type reaction and

parasitic infestations. Eosinophils share many functional features with the neutrophil. Their rhomboid, crystalloid granules are rich in acid phosphate and have a specific peroxidase activity. The precise role of eosinophils in chronic inflammatory reactions is less clear (Robert, 2003).

Polymorphonuclear leukocytes, although characteristic of acute inflammation may also be observed at sites of chronic inflammation.

Fever

Fever is a complex physiologic response triggered by infectious or aseptic stimuli. Elevations in body temperature occur when concentrations of prostaglandin E_2 (PGE_2) increase within certain areas of the brain. These elevations alter the firing rate of neurons that control thermoregulation in the hypothalamus. Although fever benefits the nonspecific immune response to invading microorganisms, it is also viewed as a source of discomfort and is commonly suppressed with antipyretic medication. Antipyretics such as aspirin have been widely used since the late 19^{th} century, but the mechanisms by which they relieve fever have only been characterized in the last few decades. It is now clear that most antipyretics work by inhibiting the enzyme cyclooxygenase and reducing the levels of PGE_2 within the hypothalamus. (David. *et al*., 2001)

Normal thermoreguration

Normal body temperature is circadian and varies from an approximate low of 36.48°C (97.68°F) in the morning to a high of 36.98°C (98.58°F) in the late afternoon (Mackowiak, 1997). At the heart of thermoregulation is an integrated network of neural connections involving the hypothalamus, limbic system, lower brainstem, the reticular formation, spinal cord, and the sympathetic ganglia (Boulant, 1997). An area in and near the rostral hypothalamus is also important in orchestrating thermoregulation. This region, the “preoptic area”, includes the preoptic nuclei of the anterior hypothalamus (POAH) and the septum.

Regulation of body temperature

The regulation of body temperature in human is a biological control system that has evolved to maintain thermal homeostasis. The control of body temperature within narrow limits is dependent upon the coordinated activities of several subsystems of the body (Seeley *et al*., 1996). The physiological control system that coordinates the responses for effective regulation of body temperature is a negative feedback system with in least three major components such as vasomotor response, metabolism, sweating response and shivering (Wenger, 1995). The effector organ system that determines heat production or heat loss and an integrator or controller that compares the sensed temperature to a "normal" or "reference" temperature to determine whether it is high or low. It activates the appropriate effector systems, which return the body temperature back to normal (Figure 7).

The balance that maintains a constant total body heat content can be expressed by the relation as follows.

Heat output = Heat input + Heat production

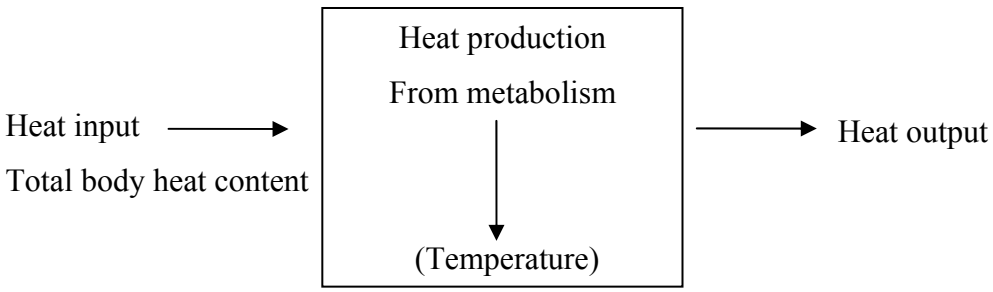


Figure 7. Balance of heat input, output and production (Seeley *et al*., 1996).

Temperature receptors are divided into 2 pathways.

1) Peripheral thermoreceptor in temperature regulation

Temperature receptors that measure skin temperature are located beneath the skin. Peripheral temperature receptors are naked nerve ending that are very sensitive to temperature and classified as cold receptors or warm receptors (Rhoades and Pflanzen, 1992). Cold receptors are characterized by increasing steady-state discharge rate as the skin is cooled (10-38°C) and afferents for cold are Aδ and C

fiber. In contrast, warm receptors are those which steady state discharge rates increase in response to warming of the skin (30-45°C) and afferents for warm are C fiber, but above 45°C or below 15°C causes tissue damage begin and the sensation becomes one of pain (Julius, 2001). Nerve impulses from peripheral receptors enter the spinal cord at all levels and ascend to the brain.

2) Central thermoreceptor in temperature regulation

Central thermoreceptors are found in deep body areas including the hypothalamus, spinal cord, abdominal viscera and great veins. Central thermoreceptors include both cold receptors and warm receptors that are uniquely sensitive to decrease in body core temperature. Nerve impulses from core receptors feed into the brain, when they are integrated with thermal information from peripheral nerves (Julius, 2001).

So, sensors that detect changes in body temperature are located in body peripheral areas and in the central nervous system. However, the important sensory activity and the integrated of the various feedback loops involved in the maintenance of a constant body temperature that take place in the preoptic hypothalamus

When the body temperature changes to the higher or lower set-point, new balance of heat loss and heat production is achieved in other responses that called “adaptation”. Below a skin temperature of 20°C and above 40°C, there is no adaptation, but between 20°C and 40°C, there is adaptation. In the 43-50°C, capsaicin receptor mediates warmth response (Ganong, 2001).

Response to heat

1. Vasodilation : Skin vasodilation causes the increase heat loss. During vasodilation venous blood returns near to the skin hence increasing the availability of heat loss from the skin to the environment. Arterio-venous anastomoses deep to the skin capillaries can open and reduce the fall in temperature along the length of the artery, hence increasing arterial temperature, raising skin temperature and increasing heat loss.

2. Sweating : When the body temperature raises, sweat is secreted over the body to allow cooling by evaporation. There are two types of sweat glands:

Apocrine glands, found in the armpits and pubic regions are generally vestigial and are responsible for the distributed odour in these regions.

Eccrine glands distributed through out body (mainly on the forehead, neck, trunk, back of forearm, hand and fewer on things soles, and palms). It is the eccrine glands that perform the thermoregulatory function (Table 3).

Table 3. Summary of thermoregulatory effector responses to increase skin temperature (Rhoades and Pflanzen, 1992).

Response	Mechanism	Effect on heat gain or loss
Skin vasodilation	Decreased sympathetic outflow to skin resistance vessels	Warmer skin increases heat loss to environment; increased convective heat transfer from core to skin reduce core temperature
Sweating	Sympathetic stimulation of sweat glands	Increased evaporation of water from the skin increases heat loss
Behavior	Voluntary skeletal muscle contraction	Shelter from the sun reduces radiant heat gain; reduced clothing increases conduction and convection of heat from the skin

Response to cold

1. Vasoconstriction : To reduce heat loss. Cold vasoconstriction still allows some blood for the required small amount of oxygen to reach the cell. In the limbic, a countercurrent heat exchange occurs due to constriction of superficial veins so that cool blood from the skin returns along the venae comitans close to the artery, hence gaining heat and returning to the body core.

2. Shivering : Both skin temperature and core temperature effect the onset of shivering which can be both voluntary and involuntary. Shivering describes as the stimultaneous asynchronous contraction of the muscle fibers in both the flexor and exterior muscle: i.e. activity producing heat with no net external muscular work. If the

body temperature falls then metabolic rate begins to increase, first due to an increase in muscle tone (causing stiffness) and then due to shivering (Parsons, 2003) (Table 4).

Table 4. Summary of thermoregulatory effector responses to decrease skin temperature (Rhoades and Pflanzen, 1992).

Response	Mechanism	Effect on heat gain or loss
Skin vasoconstriction	Increased sympathetic outflow to skin resistance vessels	Cooler skin reduce heat loss to environment; reduce convective heat transfer from core to skin maintains core temperature
Shivering	Involuntary skeletal muscle contraction	Increased energy expenditure increases heat production
Behavior	Voluntary skeletal muscle contraction	Posture changes reduce surface exposed to cold; movement to warmer environment reduce heat loss ; increased clothing traps air near the skin, reducing heat loss by convection

The Pathophysiology of Fever

The febrile response is a complex reaction to disease that is characterized by activation of numerous physiologic, endocrinologic, immunologic (Mackowiak, 1997) and behavioral systems. The preoptic area of the anterior hypothalamus functions as the body's thermostat by controlling thermoregulatory mechanisms that balance heat loss with heat production. The body's normal metabolic rate produces more heat than necessary to keep the set-point euthermic. Therefore, at baseline, the hypothalamic temperature control is regulating the amount of heat loss via vasodilation. When the set point rises above the body temperature the hypothalamus activates the sympathetic system to induce vasoconstriction, increase skeletal muscle activity either as an insensible increase in muscle tone or as frank shivering, and to increase cell metabolism. This cascade of responses in turn increases core body temperature to reach the set point. Conversely, if the set point is lowered below the body

temperature, hypothalamic signals cause vasodilation, sweat formation, decreased metabolism and behavioral responses such as taking off clothing or moving to cooler environments to decrease body temperature back to its set point.

Infection by microorganisms triggers a series of events that ultimately increase the body's set point to produce fever. The invading organism releases exogenous pyrogens including lipopolysaccaride (LPS), superantigens, peptidoglycans and muramyldipeptides that in turn activate leukocytes to release endogenous pyrogens including interleukin1, interleukin 6, interferon-α and tumor necrosis factor (TNF). These endogenous pyrogens, which are produced both centrally and peripherally, signal receptors in endothelial cells of the hypothalamic vascular organs to activate phospholipase A_2, which subsequently liberates prostaglandin E_2 (PGE_2) from the cyclooxygenase pathway. Prostaglandin E_2 then raises the thermoregulatory set point in the anterior hypothalamus and the sympathetic response that ensues raises the core temperature to the febrile set-point (Figure 8).

The role of prostaglandin E_2

PGE_2 is synthesized from arachidonic acid, which is released from cell membrane lipid by phospholipase. Arachidonic acid is metabolized by two isoforms of the COX enzyme, COX-1 and COX-2. COX-1 usually is expressed constitutively and generates prostanoids important to housekeeping functions supporting homeostasis. COX-2, on the other hand, is inducible by inflammatory signals such as the pyrogenic cytokines, IL-1β, TNF and IL-6, and bacterial lipopolysaccharide (Simon, 1999). Genetically engineered mice that lack either the COX-1 or COX-2 gene demonstrate that the inducible isoform is responsible for hypothalamic PGE_2 production during a febrile response (Wang, 1999). As COX-2 is the key provider of PGE_2 during pyrexia, it is not surprising that the selective COX-2 antagonist, rofecoxib, is an effective antipyretic in humans.

Many cells, including synoviocytes, macrophages, endothelial cells and chondrocytes, have the capacity to rapidly up-regulate the expression of the COX-2 during inflammation (Simon, 1999). The most likely cell type in the central nervous system responsible for producing PGE_2 is the microvascular endothelial cell, which expresses COX-2 exuberantly after stress.

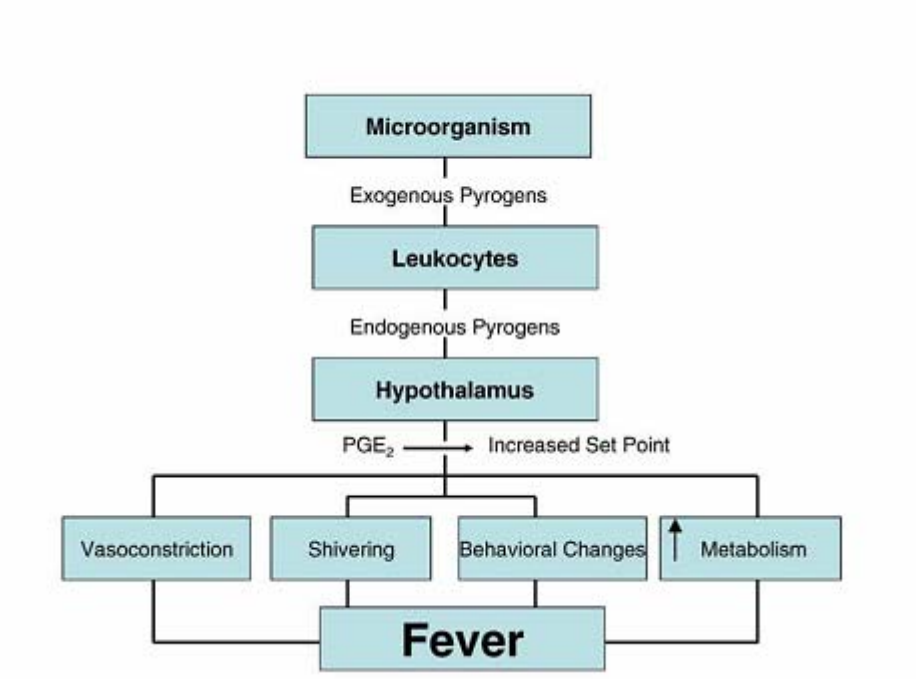


Figure 8. Pathophysiology of Fever (Zomorrodi, 2008)

Aspirin

This drug is the prototype of non-steroidal anti-inflammatory drug (NSAIDs) and used for the treatment of mild to moderate pain. Aspirin (Figure 9), is more useful in the treatment of headache, neuralgia, arthralgia and other pain arising from integumental structures than in acute severe pain of visceral origin. However, it may relieve moderate postoperative, postpartum or other visceral pain (Ross and Dehoratius, 1989). If the pain is mild to moderate, aspirin may provide adequate relief and be tried prior to use of opioid analgesics. Large dose a has an anti-inflammatory action, which may contribute to relief of pain when inflammation is a factor. When therapy is indicated to reduce fever, aspirin is one of the most effective drug (Rang and Dale, 1999a).

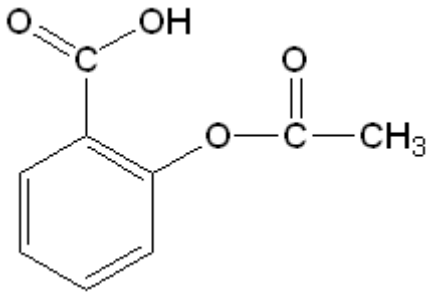


Figure 9. Structure of Aspirin

1. Pharmacokinetics

Aspirin is absorbed primary from the small intestine and secondary from stomach. Absorption is rapid following oral administration of conventional tablets or capsules, but the rate is affected by gastric emptying time and the release characteristics of the dosage from. Absorption is most rapid when aspirin is given in solution. Appreciable concentrations are found in plasma in less than 30 minutes after a single dose and a peak value is reached in about 1 h. Aspirin is rapidly hydrolyzed to salicylic acid before entering the systemic circulation and CNS (Ross and Dehoratius, 1989). Hydrolysis by plasma esterase is rapid and cleared by renal extraction. It is conjugated with glycine (forming salicyluric acid) and glucuronic acid (forming salicylphenolic glucoronide and salicylacyl glucoronide). A small fraction of

salicylic acid is oxidized to gentisic acid. The enzyme forming salicyuric acid and salicylphenolic glucoronide are saturable and follow Michaelis-Menten kinetics. Therefore, the pharmacokinetics of salicylate elimination are complex, since both the ratio of metabolites and clearance are dose-dependent. Approximately 70% to 90% of salicylic acid is bound to serum albumin and apparent volume of distribution ranges from 0.1 to 0.35 L/kg, dependent on drug concentration. The half-life of salicylate increases with the dose 3.1 to 3.2 h with 300 to 650 mg, 5 h with 1 g and 9 h with 2 g. As the dose and half-life increase, a larger portion is excreted unchanged (Jonh and Bonald, 1993).

2. Pharmacodynamics

Data from animal studies have shown that the analgesic effect of aspirin on induced pain is principally peripheral (blockade of pain impulse generation). The primary clinical effect of aspirin appears to be related to inhibit cyclooxygenase (prostaglandin synthesis) (Jonh and Bonald, 1993), since the action of the prostaglandin has been reported to include hyperalgesia (pain), fever, edema (inflammation) and erythema. They do not inhibit 5-lipoxygenase and therefore, do not affect the formation of leukotrienes (Mutschler and Derendorf, 1995b)(Figure10).

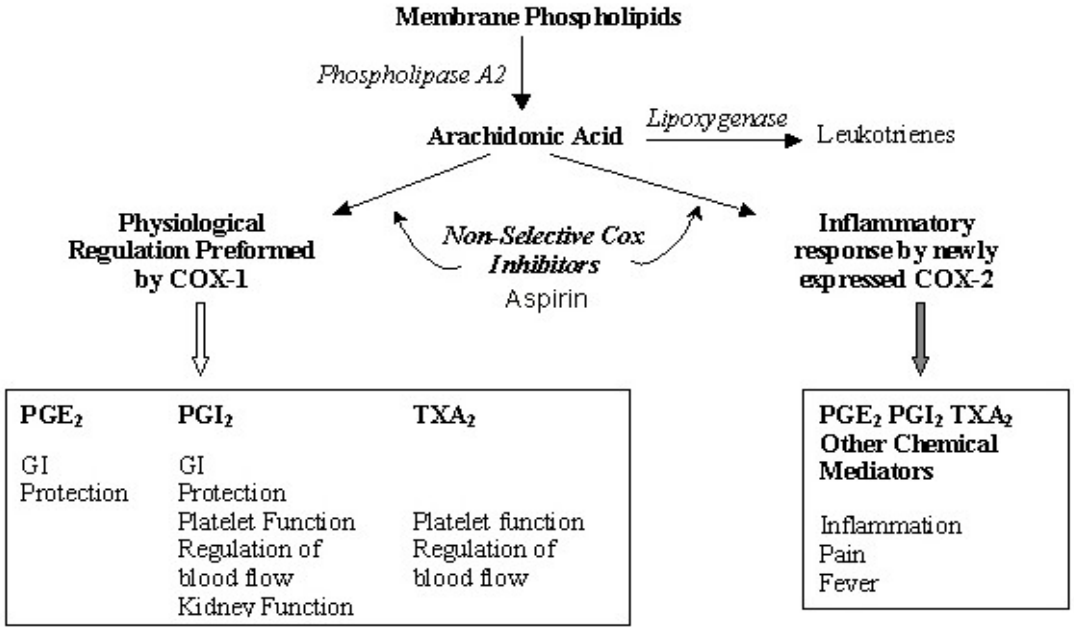


Figure 10. Mechanism of aspirin to inhibit cyclooxgenase (Vander, 2001).

3. Indication

Aspirin is used for the relief of mind to moderate pain such as headache, toothache, dysmenorrhoea and myalgias. It is also used in acute and chronic inflammatory disorders such as rheumatoid arthritis and osteoarthritis. In the treatment of minor febrile condition, such as colds or influenza, aspirin is of value for the reduction of temperature. It is also used in the prevention of arterial and venous thrombosis (Aichison *et al*., 1993a).

4. Adverse Reaction

Although some of the therapeutic action of these drugs may be related to the inhibition of prostaglandin synthesis, many adverse reaction also are due to this pharmacologic property. Generally, serious adverse responses are associated with long-term drug use, but a few life-threatening reaction have been reported after a single exposure to the drug (Brestel and Dyke, 1997).

4.1 Gastrointestinal

Aspirin has been shown to increase the risk of gastrointestinal bleeding (Mellemkjaer, 2000). Although some enteric coated formulations of aspirin are advertised as being "gentle to the stomach", in one study enteric coating did not seem to reduce this risk. Combining aspirin with other NSAIDs has also been shown to further increase this risk. Using aspirin in combination with clopidogrel or warfarin also increases the risk of upper gastrointestinal bleeding.

4.2 Hepatic

Aspirin administered regularly in dose greater than 50 mg/kg can produce mild and reversible hepatic damage. This is usually manifested as an increase in aminotransferase values but biopsies reveal focal hepatocellular necrosis, hepatocytic swelling, intracellular and extracellular acidophilic bodies and portal inflammation. A small number of patient experience is more severe hepatic damage with jaundice, prolonged prothrombin time with bleeding or intravascular coagulation. Aspirin also may precipitate hepatic encephalopathy in patients with chronic liver disease. The hepatotoxicity generally occurs only after several months of treatment and appears as cholestatic jaundice with markedly elevated values in hepatic function tests and

histologic evidence of necrosis, portal infiltrates and cholestasis (Foegh and Ramwell, 2001).

4.3 Pregnancy and lactation

Aspirin has been examined extensively for their potential adverse effects on the pregnant woman, the fetus and on the nursing neonate whose mother is receiving one of these drugs. It is presumed that, like aspirin, the other NSAIDs prolong gestation and labor, increase maternal blood loss during delivery and may cause fetal intracranial hemorrhage. Fetal growth retardation may be related to inhibition of glucose-induced insulin release. No teratogenic effects have been substiated. Large dose of aspirin in the mother can induce bleeding or rash in nursing infant (Foegh and Ramwell, 2001).

4.4 Hypersensitivity

Two distinct immunologic syndromes characterized by bronchospasm and rhinitis or angioedema and urticaria may follow a single dose or may occur in patients who previously received this drug without incident.

4.5 Hematologic

Aspirin exerts a pronounced inhibitory platelet aggregation. This effect is more prominent in patients with inborn disorders of platelet function in Von Willebrand or Bernard-Soulier disease and in patients receiving heparin or oral anticoagulants. For most NSAIDs were revesibles inhibited platelet aggregation is reversible, but inhibition by aspirin irreversible (Schuna and Coulter, 1993).

5. Drug Interactions

Because aspirin is widely used, its interaction with other drugs must be considered. Large dose of aspirin taken for several days could inhibit platelet aggregation, patient receiving oral anticoagulants should be instructed to avoid. In noninsulin-dependent diabetics, aspirin decreases the blood glucose concentration by inhibiting prostaglandins synthesis and may enchance the effect of the oral hypoglycemics. So, this drug should not be given hypoglycemic agent. In addition, it has a uricosuric effect, cause uric acid retention. Patients with gout should avoid aspirin (Jonh and Bonald, 1993).

Morphine

Morphine is the prototype opioid agonist to which all other opioids are compared. In human, morphine produces analgesia, euphoria, sedation and a diminished ability to concentrate. Other sensations include nausea, a felling the body warmth, heaviness of the extremities, dryness of the mouth and especially in the cutaneous areas around the nose. The cause of pain persists, but even low doses of morphine increase the threshold to pain and modify the perception of noxious stimulation so that it is no longer experienced as pain. In the absence of pain, however, morphine may produce dysphoria rather than euphoria (Stoelting and Hillier, 2006) (Figure 11).

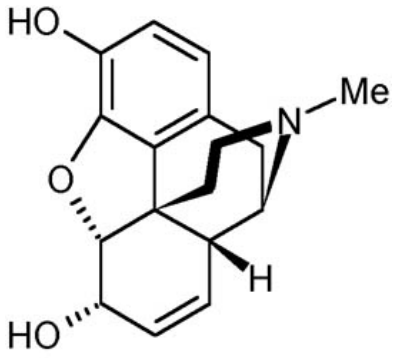


Figure 11. Structure of morphine

1. Pharmacokinetic

Absorption of morphine from the gastrointestinal tract is relative slow. The mean T_{max} is 3.7 h and mean C_{max} is 9.9 to 27.4 ng/mL (dose dependent) for sustained- release form. Bioavailability is approximately 40%, distributes to skeletal muscle, kidneys, liver, intestinal tract, lungs, spleen, and brain; crosses the placental membrane and is found in breast milk. Metabolism of virtually all converted into glucuronide metabolites; small fraction is demethylated in the liver. Major metabolite is morphine-3-glucuronide (55% to 75%) and the $t_{½}$ is approximately 2 to 4 h. The response to morphine may be enhanced in patients with uremia, various renal disorders and renal ischemia. This may be due to accumulation of an active

metabolite, morphine-6-glucoronide that is eliminated by kidney (Jaffe and Martin, 1990). The mean elimination half-life and clearance of the parent drug are similar in patients with renal failure and in normal subjects.

2. Pharmacodynamics

2.1 Mechanism of action

Opioid receptors are found in the central nervous system and gastrointestinal tract and lesser degree, in peripheral tissue. Opioid drugs manifest analgesic effects primarily by binding to and activating (agonizing) opioids receptor in the nervous system (CNS). The interaction of exogenous opioids, for example, morphine and opioid receptors, mimics the interaction seen when endogenous opioid peptides (dynorphins, endophins, enkephalins) bind with these same receptors.

The three generally recognized classes of opioid receptor are the mu (μ), delta (δ) and kappa (κ) receptor and epsilon (ε) receptors were formerly classified as opioid receptors because opioid can bind to them (Lipman and Jackson, 2004).

Mu-opioid receptor : Morphine acts mainly on the μ-receptor, which is primarily responsible for the analgesic action. Most of the common opioid are full agonists at the μ-receptor. This receptor is also responsible for the often unwanted effect of opioid, such as respiratory depression, constriction of pupils (miosis), sedation and reduction in motility of the gastrointestinal system, as well as euphoric effects.

Delta-opioid receptor : This opioid receptor occurs at different location within the brain to the μ-receptor but also mediated analgesia, respiratory depression, euphoria and dependence. The functional significance of this receptor is less clear but seem to be involved more with peripheral pain control. Etrophine is the best agonist.

Kappa-opioid receptor : This receptor occurs in the spinal cord and mediates spinal analgesia and sedation and support only low physical dependence. Pentazocine and etorphine are the best opioid drug of this site (Drummer, 2001).

Opioid receptors are composed of glycoproteins found in cellular membranes. These receptors are coupled to G-protein that modulate potassium and calcium ion conduction. When opioid agonists occupy either μ or δ receptor, they

open the potassium ion channel that permits an increase in potassium conductance. The hyperpolarlization inhibits neuronal activity. In contrast, κ receptor activation inhibits calcium entry via a calcium ion channel. Activation of the opioid receptors decreases transmission of signals from the primary peripheral afferent nerves to higher CNS centers, as well as the processing of the pain stimulus (Lipman and Jackson, 2004).

3. Adverse effects

3.1 Ventilation

All opioid agonists produce a dose-dependent and gender specific depression of ventilation, primarily through an agonist effect at μ_2 receptor, which lead to a direct depressant effect on brainstem ventilation centers.

3.2 Sedation

The postoperative titration morphine freguently induces a sedation that precedes the onset of analgesia.

3.3 Cardiovascular system

Morphine can also evoke decreases in systemic blood pressure due to drug induce bradycardia or histamine release. The administration of morphine in the preoperative medication or before the induction of anesthesia tends to slow heart rate during exposure to volatile anesthetics, with or without surgical stimulation.

3.4 Nausea and vomiting

Nausea and vomiting induced by opioid reflects their direct stimulation of the chemoreceptor trigger zone in the floor of the fourth ventricle.

3.5 Cutaneous changes

Morphine causes the cutaneous blood vessels of the face, neck and upper chest to dilate.

3.6 Gastrointestinal tract

Commonly used opioids can produce spasm of the gastrointestinal smooth muscles, resulting in a variety of side effects includind constipution, and delayed gastric emptying.

4. Drug interaction

The ventilatory depressant effect of some opioids may be exaggerated by amphetamines, phenothiazines, mono-amine oxidase inhibitors and tricyclic anti-depressants (Stoelting and Hillier, 2006).

Naloxone

The pure opioid antagonist drug, naloxone (Figure 12) is a morphine derivative with bulkier substituents at the N position. One structure change that converts a narcotic agonist to an antagonist is alkylation of the piperidine nitrogen. For example, when the methyl group on the piperidine nitrogen of morphine is replaced by the unbranched three-carbon side chain (such as propyl, allyl or isopropyl), the compound becomes a narcotic antagonist. Naloxone has a relatively high affinity for opioid binding sites of the μ receptor type. Their affinity for the other receptors such as κ and δ receptor are found in both supraspinal and spinal sites (Colasanti, 1990 and Smith, 1995).

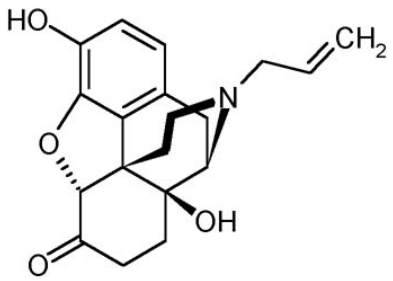


Figure 12. Structure of naloxone

1. Pharmacokinetics

Naloxone has poor efficacy when given by the oral route and a short duration of action about 1-4 hours when given by injection. It is usually given intravenously and its effects are produced immediately. It is rapidly metabolismed by the liver. Metabolic disposition is chiefly by glucoronide conjugation (Rang *et al*., 1999b) (Table 5).

2. Pharmacodynamics

Naloxone is capable of antagonizing the effect produced by narcotics at μ, κ and δ receptors. The affinity of the antagonist for the μ receptor is 10-20 fold greater than for the κ and δ receptors sites (Colasanti, 1990). When given to a morphine-treated subject, the antagonist will completely and dramatically reverse the opioid effects within 1-3 minutes. The patients who are acutely depressed by overdose of an opioid, the antagonist will effectively normalize respiration, level of consciousness, pupil size and bowel activity (Way and Way, 1992).

3. Indication

Naloxone is the drug of choice for narcotic overdose. It is very important that the relatively short duration of action of naloxone be born in mild because a severely depressed patient may recover after a single dose of naloxone and appear normal, only to relapse into coma after 1-2 hours. The usual dose of naloxone is 0.1-0.4 mg intravenously, which can be repeated as necessary (Way and Way, 1992).

4. Adverse effects

Nausea and vomiting have occurred and have been individual reports of hypotension, hypertension, cardiac arrhythmias and pulmonary edema, generally in patients given naloxone postoperatively. Seizures have been reported infrequently (Aichison *et al*., 1993b).

Table 5. Narcotic analgesic pharmacokinetics (Terry, 1993)

Agent	Time to peak (h)	Half-life (h)	Analgesic onset (min)	Analgesic duration (h)
Morphine	0.5-1	2-4	15-60	4-5
Levorphanol	0.5-1	12-16	30-90	4-5
Pentazocine	0.25-1	4-5	15-20	3
Naloxone*	0.5-2	0.5-1.5	2-5	0.5-1
Naltrexone*	1	4-13	15-30	24-72

Based on intramuscular data unless other wise indicated

* Drug antagonist

CHAPTER 3

MATERIALS AND METHODS

Materials

1. Plant material

The plant, *Abroma augusta* Linn. (Sterculiaceae), has a common name as Devil's Cotton, but in Thai called “Teandum”. The seeds of *Abroma augusta* Linn. purchased from the local Thai medicinal plant drugstore in Hat Yai, Songkhla, Thailand, were used in this study. The 3 kg of seeds of *Abroma augusta* was cleaned and air-dried at room temperature. The dried seeds were pulverized by an electric blender to give 2.9 kg of coarsely powder and stored in airtight containers.

2. Extraction procedure

The coarsely powdered seeds of *A. augusta* (2.9 kg) was macerated with 6 L of n-hexane for 7 days at room temperature. The extraction process was repeated 2 times and the combined extracts were filtrated. The solvent was then evaporated under reduced pressure to give oil-like brownish extract (yield 23.02% w/w). The obtained extract was stored in a closed bottle and kept in a refrigerator at temperature below 4 °C until use. Before testing of the extract for the experiments, the extract at different concentrations was freshly prepared by dissolving in cosolvent (Propylene glycol: Tween 80: Distilled water at the ratio 3:1:6, respectively).

3. Animals

Male ICR mice, weighing ranging from 30-40 g, were used for analgesic activity tests (writhing, formalin, hot plate and tail flick tests). Male Wistar rats weighing ranging 180-220 g were used for testing of antipyretic and anti-inflammatory activities. All animals used in this study were obtained from the Southern Laboratory Animal Facility, Prince of Songkla University, Hat Yai, Songkhla, Thailand and kept in a room maintained under the condition of 25 ± 1°C and 12 h light-12 h dark cycles. All animals had free access to water and standard diets. The A animal Ethics Committees, Prince of Songkla University approved all experimental protocols (No. 0521.11/179).

4. Chemicals and instruments

The drugs used in this study included morphine sulfate (Sigma), naloxone hydrochloride (Troikaa), brewer's yeast (Sigma), carrageenan (Sigma), aspirin (Sigma), urethane (Sigma), propylene glycol (Vidhyasom Co., LTD), tween 80 (Srichand Co., LTD), acetic acid A.R grade (JT Baker) and 37% formalin A.R. grade and n-hexane A.R. grade (Bright Chem SDN BHD).

The instrument used included the tail flick apparatus (Socrel model DS20, Ugo basile, Milan, Italy), plethysmometer (Ugo basile, Milan, Italy), hot plate (Harvard Apparatus Ltd., UK) and digital thermometer (YSI 4600 Precision Thermometer Yellow Springs, Ohio 45387, USA).

5. Drugs and extract administration

The cosolvent, extract and reference drug aspirin were administered by oral gavage. Animals were divided into 5-8 groups. Each group comprised of 6 animals of mice or rats for analgesic, antipyretic and anti-inflammatory studies.

Number	Group	Details	Tests
1	Control	Cosolvent composed of Propylene glycol: Tween 80: Distilled water at the ratio of 3:1:6, respectively at doses of 10 ml/kg (mice) and 5 ml/kg (rats), orally (po).	Analgesic, antipyretic and anti-inflammatory
2,3,4	HEAA	Hexane extract of *Abroma augusta* Linn. seeds (HEAA) at doses of 100, 200 and 400 mg/kg, orally, respectively.	Analgesic, antipyretic and anti-inflammatory
5,6	Reference drugs	1) Aspirin at the dose of 200 mg/kg, orally.	Writhing, formalin, Carrageenan-induce, Cotton-pellet-induce granuloma formation Yeast-induce,

		2) Morphine sulfate at the dose of 5 mg/kg, subcutaneously (sc).	Formalin, Hot plate and tail flick
		3) Naloxone at the dose of 2 mg/kg, intraperitoneally (ip).	Hot plate and tail flick
7,8	Antagonist effect	1) Naloxone at the dose of 2 mg/kg, intraperitoneally before morphine 5 mg/kg, subcutaneously 2) Naloxone at the dose of 2 mg/kg, intraperitoneally before HEAA at dose of 400 mg/kg, orally.	Hot plate and tail flick

Methods

1. Acute toxicity

The up-and-down method described by Bruce (1985) was used in this study. This method used for acute toxicity testing has been developed and statistically evaluated, and permits a reduction in the number of animal used. In the procedure, the animals used were male mice and rats. The animal is dosed one at a time. If animal survived, the dose for the next was increased, if it died the dose was decreased. Each animal was observed for 1 or 2 days before dosing the next animal. The first dose was begun at 5 g/kg, orally. The n-hexane extract of *Abroma augusta* Linn. was orally administered to a mouse and a rat. Behavior parameters such as convulsion, hyperactivity, sedation, loss of righting reflex and increased or decreased respiration during a period of 8 h and 14 days were not found. Food and water were given *ad libitum*.

2. Analgesic activity

2.1 Writhing test

The experiment was done according to the previous described by Koster *et al.* (1959). Male mice weighing 30-40 g were divided into 5 groups of 6 animals.

Group 1: Control (cosolvent 10 ml/kg, po)

Group 2: Aspirin 200 mg/kg, po

Group 3: HEAA 100 mg/kg, po

Group 4: HEAA 200 mg/kg, po

Group 5: HEAA 400 mg/kg, po

The control group (group 1) received cosolvent (10 ml/kg, po). Group 2 received a reference drug aspirin at the dose of 200 mg/kg, po. Group 2, 3 and 4 received the extract at doses of 100, 200 and 400 mg/kg, po, respectively. After 30 min of treatment, each mouse of each group was intraperitoneally injected with 0.6% (v/v) acetic acid in normal saline at the dose of 10 ml/kg. The mice were then observed for the number of abdominal constriction and stretching, and counted over a period of 0-20 minutes. The schematic plan of the writhing test was illustrated in Figure 13.

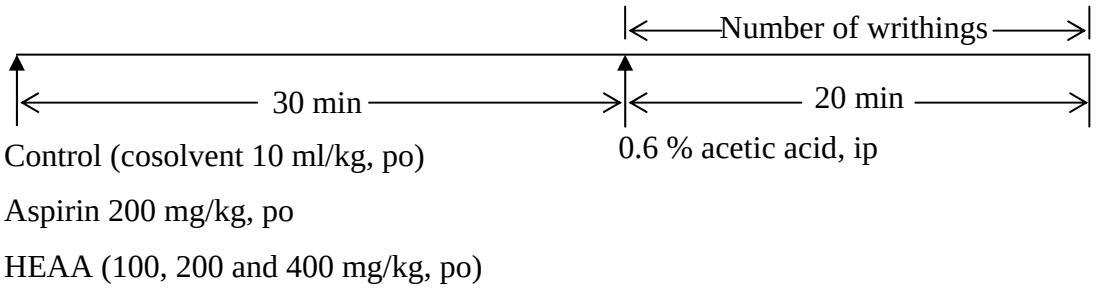


Figure 13. Schematic plan of the writhing test

A reduction in the writhing number compared to a control group was evaluated for analgesia, and was expressed as % inhibition of writhings. The percentage of inhibition was determined for each experimental group as a following formula.

$$\text{Inhibition (\%)} = \frac{\text{No. of writhing (control group)} - \text{No. of writhing (experimental group)}}{\text{No. of writhing (control group)}} \times 100$$

2.2 Formalin test

The experiment was done according to the previous described by Hunskaar *et al.* (1985). Male mice weighing 30-40 g were divided into 6 groups of 6 animals.

Group 1: Control (cosolvent 10 ml/kg, po)

Group 2: Aspirin 200 mg/kg, po

Group 3: Morphine sulphate 5 mg/kg, sc

Group 4: HEAA 100 mg/kg, po

Group 5: HEAA 200 mg/kg, po

Group 6: HEAA 400 mg/kg, po

The control group received cosolvent (10 ml/kg, po). Group 2 and 3 received reference drug aspirin (200 mg/kg, po) and morphine sulphate (5 mg/kg, sc), respectively. Group 4, 5 and 6 received the extract at doses of 100, 200 and 400 mg/kg, po., respectively. After 30 min of treatment (except only 15 min for morphine), 20 µl of 2.5% formalin was subcutaneously injected into a hind paw of each mouse. The time spent in licking of the injected paw was recorded. The total licking times in early phase (0-5 min) and late phase (15-30 min) after formalin injection were recorded. The schematic plan of the formalin test was illustrated in Figure 14.

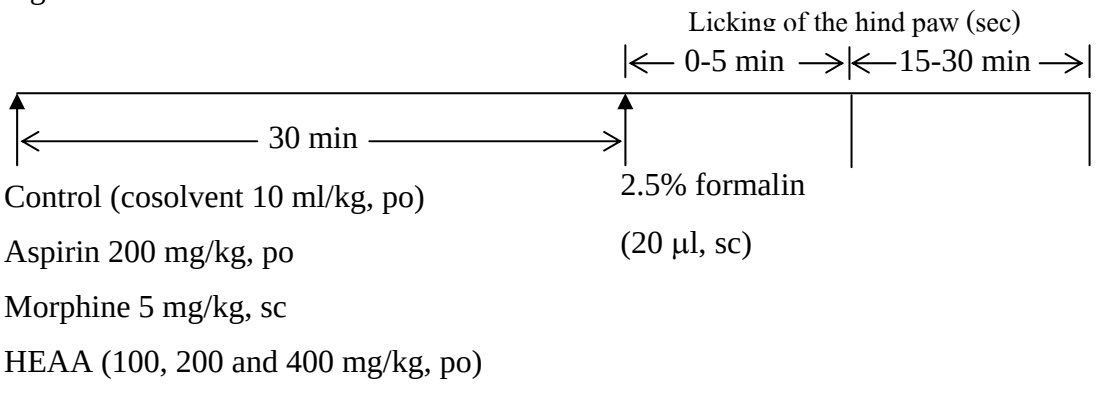


Figure 14. Schematic plan of the formalin test

The time spent licking the injected paw was evaluated for analgesia which was expressed as % inhibition of the time spent licking. The percentage of inhibition was determined for each experimental group as a following formula.

$$\text{Inhibition (\%)} = \frac{\text{Licking time (control gr.)} - \text{Licking time (experimental gr.)}}{\text{Licking time (control gr.)}} \times 100$$

2.3 Hot plate test

The hot plate test was carried out according to the method described by Woolfe and MacDonald (1944). Male mice weighing 30-40 g were divided into 8 groups of 6 animals.

Group 1: Control (cosolvent 10 ml/kg, po)

Group 2: Morphine sulphate 5 mg/kg, sc

Group 3: Naloxone 2 mg/kg, ip

Group 4: HEAA 100 mg/kg, po

Group 5: HEAA 200 mg/kg, po

Group 6: HEAA 400 mg/kg, po

Group 7: Naloxone 2 mg/kg, ip + Morphine sulphate 5 mg/kg, sc

Group 8: Naloxone 2 mg/kg, ip + HEAA 400 mg/kg, po

The control group received cosolvent (10 ml/kg, po). Group 2 and 3 received reference drug morphine sulphate (5 mg/kg, sc) and naloxone (2 mg/kg, ip), respectively. Group 4, 5 and 6 received the extract at doses of 100, 200 and 400 mg/kg, po., respectively. Group 7 and 8 received naloxone (2 mg/kg, ip) 10 min before morphine (5 mg/kg, sc) and the extract at the dose of 400 mg/kg, po, respectively. After 30 min of each treatment (except only 15 min for morphine and 10 min for naloxone), each mouse was placed on a hot plate maintained a temperature at 55 ± 1 °C. The latency of nociceptive responses such as licking of the hind limb or jumping were recorded at 30, 45, 60, 75 and 90 min after administration. The cut-off time of observation was 45 seconds. Only the mouse showed nociceptive responses within 15 seconds was used in the experiments. The schematic of the hot plate was illustrated in Figure 15.

2.4 Tail flick test

The tail flick test was carried out according to the method described by D'Amour and Smith (1941). Male mice weighing 30-40 g were divided into 8 groups of 6 animals.

Group 1: Control (cosolvent 10 ml/kg, po)

Group 2: Morphine sulphate 5 mg/kg, sc

Group 3: Naloxone 2 mg/kg, ip

Group 4: HEAA 100 mg/kg, po

Group 5: HEAA 200 mg/kg, po

Group 6: HEAA 400 mg/kg, po

Group 7: Naloxone 2 mg/kg, ip + Morphine sulphate 5 mg/kg, sc

Group 8: Naloxone 2 mg/kg, ip + HEAA 400 mg/kg, po

The control group received cosolvent (10 ml/kg, po). Group 2 and 3 received reference drug morphine sulphate (5 mg/kg, sc) and naloxone (2 mg/kg, ip), respectively. Group 4, 5 and 6 received the extract at doses of 100, 200 and 400 mg/kg, po, respectively. Group 7 and 8 received naloxone (2 mg/kg, ip) 10 min before morphine (5 mg/kg, sc) and the extract at the dose of 400 mg/kg, po, respectively. After 30 min of each treatment (except only 15 min for morphine and 10 min for naloxone), each mouse was placed on the tail flick apparatus, and the tail flick responses were measured by gently placing the mice tail on a center position of light beam. The reaction time was measured by focusing an intensity controlled beam of light on the distal about 2 centimeters from tip. The time taken by the animal to withdraw (flick) its tail from heat induced by the light beam was recorded as the reaction time. Only the mice that showed nociceptive responses within 15 seconds were used for the experiments. A cut-off time was 20 seconds to prevent any injury to the mice tail. The nociceptive responses were measured at 30, 45, 60, 75 and 90 min after administration. The schematic of the tail flick test was illustrated in Figure 15.

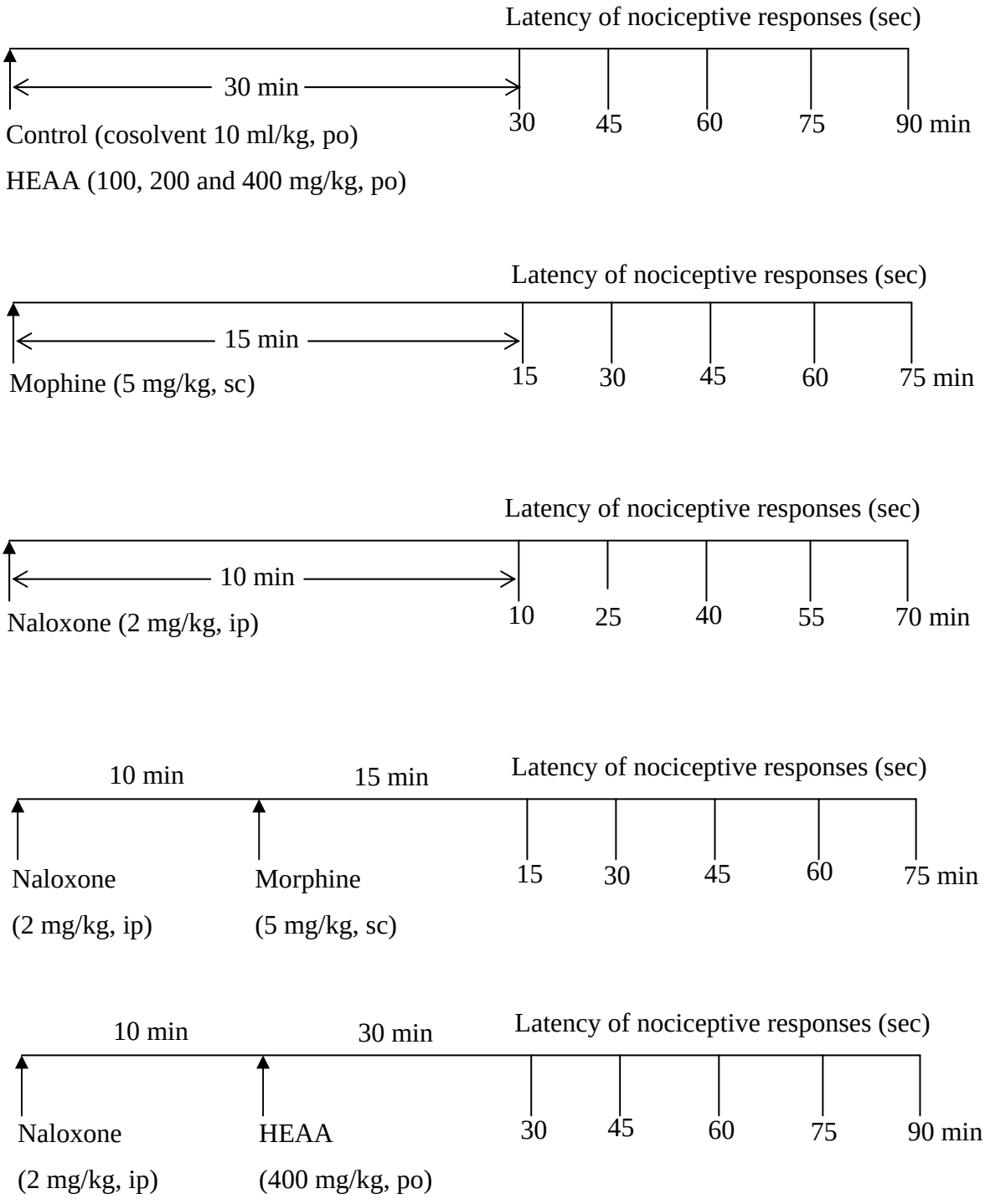


Figure 15. Schematic plan of the hot plate test and tail flick test

3. Anti-inflammatory activity

3.1 Acute inflammation (carrageenan-induced paw edema test)

The experiment was done according to the previous described by Winter *et al.* (1962). The initial right hind paw volume of the rat was measured using a plethysmometer (Ugo basile). Male Wistar rats, weighing 180-220 g, were divided into 5 groups of 6 rats each.

Group 1: Control (cosolvent 5 ml/kg, po)

Group 2: Aspirin 200 (mg/kg, po)

Group 3: HEAA 100 (mg/kg, po)

Group 4: HEAA 200 (mg/kg, po)

Group 5: HEAA 400 (mg/kg, po)

The control group received cosolvent (5 ml/kg, po). Group 2 received a reference drug aspirin at the dose of 200 mg/kg, po. Group 3, 4 and 5 received the extract at dose of 100, 200 and 400 mg/kg, po., respectively. After 30 min of treatment, each animal of all group was subcutaneously injected with 0.1 ml of 1% (w/v) carrageenan in 0.9% normal saline into the subplantar region of the right hind paw. The volume of right hind paw was measured at 0.5,1, 2, 3, 4 and 5 h after carrageenan injection and edema volume was determined. The data were expressed of swelling compared with initial hind paw volume of each rat. The schematic of the carrageenan-induce paw edema experiment was illustrated in Figure 16.

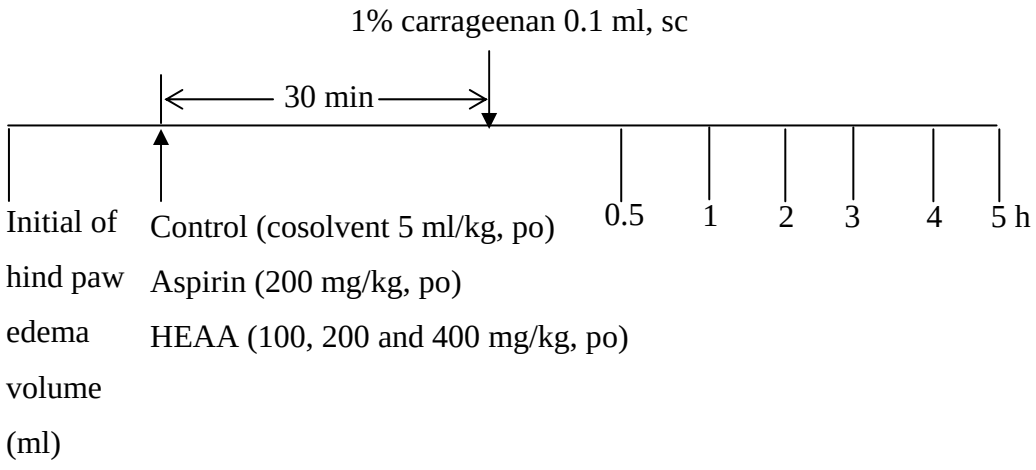


Figure 16. Schematic plan of the carrageenan-induced paw edema

The volume of hind paw was evaluated for anti-inflammatory activity and was expressed as % inhibition of the hind paw volume. The percentage of inhibition was determined for each experimental group as following formula.

$$\text{Inhibition (\%)} = \frac{A - B}{A} \times 100$$

A = Edema volume of hind paw of control group

B = Edema volume of hind paw of treated group

3.2 Chronic inflammation (cotton pellet-induced granuloma test)

Cotton pellet-induced chronic inflammation and anti-inflammatory activity study were evaluated by slightly modifying the method described by Swingle and Shideman, (1972). Male Wistar rats, weighing 180-220 g, were divided into 5 groups of 6 rats each.

Group 1: Control (cosolvent 5 ml/kg, po)

Group 2: Aspirin 200 mg/kg, po

Group 3: HEAA 100 mg/kg, po

Group 4: HEAA 200 mg/kg, po

Group 5: HEAA 400 mg/kg, po

Subcutaneous implantation of pellets of compressed cotton provokes foreign body granuloma. Cotton rolls were cut and made into pellets weighting 20 ± 1 mg each and sterilized in an hot air oven at 160 °C for 2 h. The animals were anesthetized using urethane dose 150 mg/100g and all processes were done under the sterile condition. After recovery from anesthesia, the animals were treated with cosolvent, aspirin and HEAA, orally for 7 days. On the eighth day, animals were sacrificed and the cotton pellet granulomas were carefully removed from extraneous tissues and their wet weights were determined, and then dried the cotton pellet by hot air oven at 60°C for 18 h and weighed to obtain a constant weight. The average weight of the pellets of the control group as well as of the test group was calculated. The percent change of granuloma weight relative to control group was determined.

Inhibition of granuloma was evaluated for anti-inflammation which was expressed as % inhibition of granuloma. The percentage of inhibition was determined for each experimental group as following formula.

$$\text{Granuloma inhibition (\%)} = \frac{A - B}{A} \times 100$$

A = Mean granuloma weight for the control group

B = Mean granuloma weight for the treated group

4. Antipyretic activity

Antipyretic activity was tested using the slightly modifying method described by Adam *et al.* (1986). Male Wistar rats, weighing 180-220 g, were divided into 5 groups of 6 rats each.

Group 1: Control (cosolvent 5 ml/kg, po)

Group 2: Aspirin 200 mg/kg, po

Group 3: HEAA 100 mg/kg, po

Group 4: HEAA 200 mg/kg, po

Group 5: HEAA 400 mg/kg, po

The animals were fasted overnight with water *ad libitum* before experiments. Pyrexia was induced by subcutaneous injection of 20% (w/v) brewer's yeast suspension (10 ml/kg) into animal's dorsum region. Nineteen hours after injection, the rectal temperature of each rat was measured using a digital thermometer. The probe was attached to a digital display and was inserted 1.5 cm into the rectum. Only rats that showed an increase in temperature of at least 0.7 °C were used for the experiments and the initial rectal temperature was recorded. The control group received cosolvent (5 ml/kg, po). Group 2 received a reference drug aspirin at the dose of 200 mg/kg, po. Group 3, 4 and 5 received the extract at doses of 100, 200 and 400 mg/kg, po., respectively. The rectal temperature was measured at 1, 2, 3, 4 and 5 h after each agent administration. The schematic plan of the brewer's yeast-induced pyrexia was illustrated in Figure 17.

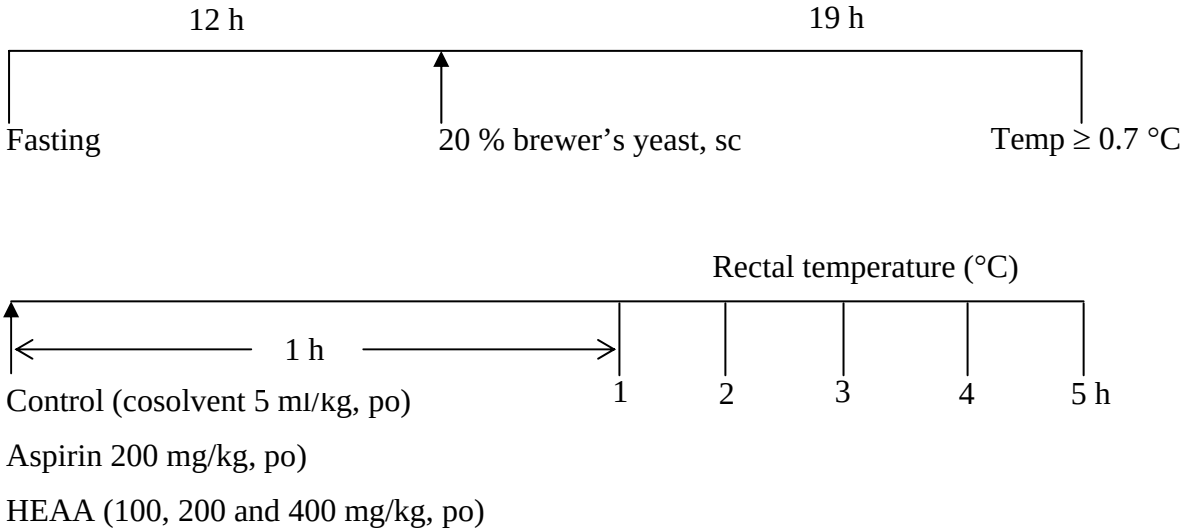


Figure 17. The schematic plan of the brewer's yeast-induced pyrexia

Statistical analysis

The data obtained were analysed using SPSS software program and expressed as mean ± S.E.M. Statistical significance between groups was performed by the application of analysis of variance (one-way ANOVA) followed by LSD test. *P* values less than 0.05 ($p< 0.05$) were used as the significant level.

CHAPTER 4

RESULTS

1. Acute toxicity

The hexane extract of *Abroma augusta* seeds (HEAA) at the dose of 5 g/kg was given orally to a male mouse or rat had no effect on behavioral responses. The abnormal symptom and mortality in each mouse or rat were not found. The LD_{50} value of the extract in mice or rats was therefore estimated to be more than 5 g/kg, po. Thus, the HEAA at doses of 100, 200 and 400 mg/kg used in this study and given orally to animals were assumed to be safe.

2. Analgesic activity

2.1 Writhing

The HEAA at doses of 100, 200 and 400 mg/kg, po significantly decreased the number of writhing induced by 0.6% acetic acid compared with control. The reference drug aspirin at the dose of 200 mg/kg, po significantly inhibited the number of writhing induced by 0.6% acetic acid compared with control by 55.32% (28.00±0.77 vs 62.67±1.84, $p<0.05$). The HEAA at doses of 100, 200 and 400 mg/kg, po significantly decreased the number of writhing induced by 0.6% acetic acid (40.33±1.94 vs 62.67±1.84, $p<0.05$; 31.50±0.56 vs 62.67±1.84, $p<0.05$; 26.33±0.67 vs 62.67±1.84, $p<0.05$, respectively) with the percentage of inhibition by 35.65, 49.74 and 57.99, respectively when compared with control. The activity of HEAA to reduce the number of writhing was likely to be dose-dependent manner. Moreover, the results also showed that the HEAA at the dose of 400 mg/kg, po reduced the number of writhing which could be comparable to the reference drug aspirin at the dose of 200 mg/kg, po which % inhibition by 57.99 and 55.32, respectively. (Table 6 and Figure 18).

Table 6. Effects of aspirin (200 mg/kg, po) and the hexane extract of *Abroma augusta* seeds (HEAA) at doses of 100, 200, and 400 mg/kg on the number of writhing induced by 0.6% acetic acid in mice.

Treatment	Dose (mg/kg, po)	Number of writhing	Inhibition (%)
Control (cosolvent 10 ml/kg, po)		62.67±1.84	0
Aspirin	200	28.00±0.77*	55.32
HEAA	100	40.33±1.94*	35.65
HEAA	200	31.50±0.56*	49.74
HEAA	400	26.33±0.67*	57.99

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

*$p < 0.05$, significantly different compared with the control (LSD test)

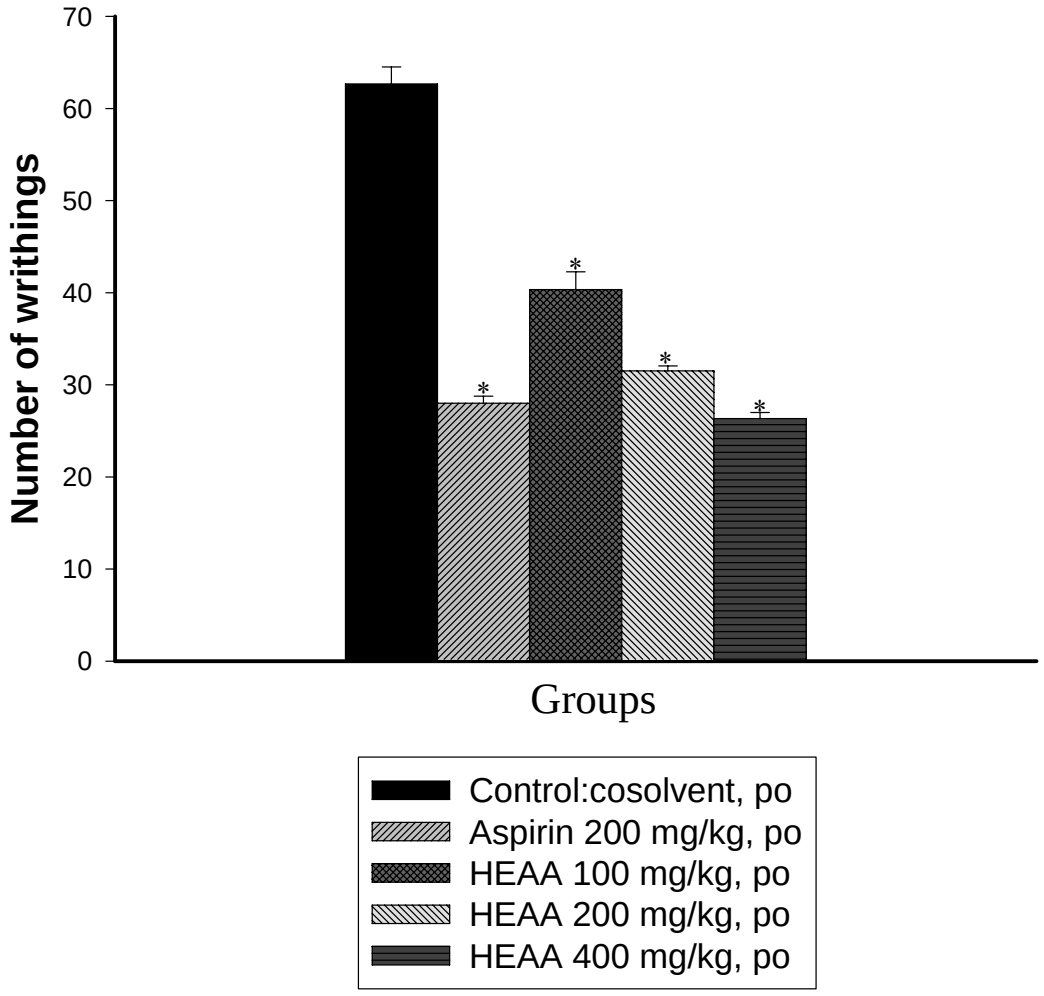


Figure 18. Effects of aspirin (200 mg/kg, po) and the hexane extract of *Abroma augusta* seeds (HEAA) at doses of 100, 200, and 400 mg/kg on the number of writhing induced by 0.6% acetic acid in mice.

$^{*}p < 0.05$, significantly different compared with control (LSD test)

2.2 Formalin test

After the reference drug morphine (5 mg/kg, sc) and aspirin (200 mg/kg, po) given before injection of 20 µl of 2.5% formalin for 15 min (morphine) and 30 min (aspirin), the licking time in early phase (0-5 min) significantly decreased by 65.31% (37.15±3.52 vs 107.10±2.27, $p<0.05$) and 59.54% (43.33±3.64 vs 107.10±2.27, $p<0.05$), respectively when compared with the control group. In early phase (0-5 min), the HEAA at doses of 100, 200 and 400 mg/kg, po significantly decreased the licking time (s) by 33.84%, 42.46% and 54.58%, respectively (70.86±1.43 vs 107.10±2.27, $p<0.05$; 61.62±2.53 vs 107.10±2.27, $p<0.05$ and 48.65±1.75 vs 107.10±2.27, $p<0.05$, respectively) when compared with control. In late phase (15-30 min), morphine (5 mg/kg, sc) and aspirin (200 mg/kg, po) significantly reduced the licking time induced by 20 µl of 2.5% formalin by 88.18% (24.90±2.14 vs 210.70±1.01, $p<0.05$) and 79.50% (43.20±1.05 vs 210.70±1.01, $p<0.05$), respectively when compared with control whereas the HEAA at doses of 100, 200 and 400 mg/kg, po significantly decreased the licking time by 36.79%, 63.71% and 80.83%, respectively (132.58±2.34 vs 210.70±1.01, $p<0.05$; 76.46±6.51 vs 210.70±1.01, $p<0.05$ and 40.39±2.46 vs 210.70±1.01, $p<0.05$, respectively) when compared with control. The results indicated that the HEAA at doses of 100, 200 and 400 mg/kg, po significantly reduced the licking time both in early and late phase when compared with control. This activity was likely to be dose-related. (Table 7 and Figure 19)

Table 7. Effects of aspirin (200 mg/kg, po), morphine (5 mg/kg, sc) and the hexane extract of *Abroma augusta* seeds (HEAA) at doses of 100, 200, and 400 mg/kg, po on the nociceptive responses induced by 2.5% formalin in mice.

Treatment	Dose (mg/kg, po)	Licking of the hind paw (sec)			
		Early phase (0-5 min)	Inhibition (%)	Late phase (15-30 min)	Inhibition (%)
Control		107.10±2.27	0	210.70±1.01	0
Morphine	5, sc	37.15±3.52*	65.31	24.90±2.14*	88.18
Aspirin	200	43.33±3.64*	59.54	43.20±1.05*	79.50
HEAA	100	70.86±1.43*	33.84	132.58±2.34*	36.79
HEAA	200	61.62±2.53*	42.46	76.46±6.51*	63.71
HEAA	400	48.65±1.75*	54.58	40.39±2.46*	80.83

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

*p<0.05, significantly different compared with the control (LSD test)

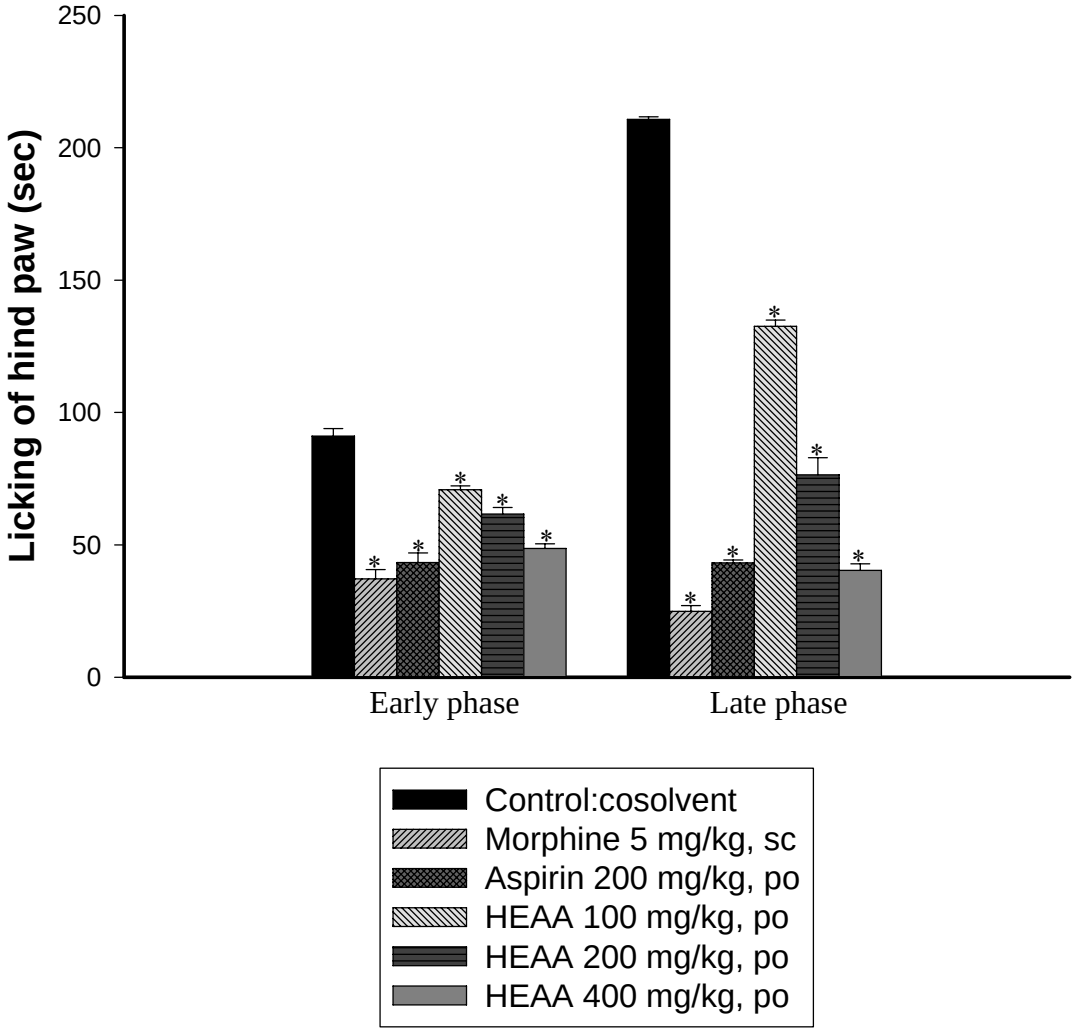


Figure 19. Effects of aspirin (200 mg/kg, po), morphine (5 mg/kg, sc) and the hexane extract of *Abroma augusta* seeds (HEAA) at doses of 100, 200, and 400 mg/kg, po on the nociceptive response induced by 2.5% formalin in mice.

*$p<0.05$, significantly different compared with control (LSD test)

2.3 Hot plate test

The result showed that morphine at the dose of 5 mg/kg, sc markedly increased the pain latency at all the time intervals measured at 30, 45, 60, 75 and 90 minutes after administration. The HEAA at doses of 100, 200 and 400 mg/kg, po significantly increased the latency of nociceptive response at the time intervals measured at 30, 45, 75 and 90 minutes. This increased pain latency activity of the HEAA was likely to be dose-related. The HEAA at the dose of 400 mg/kg exhibited the most potent effect to delay the latency of nococeptive response in hot plate test. All results were shown in Table 8 and Figure 20.

The antagonistic action of naloxone on the effect of morphine or HEAA at the dose 400 mg/kg on the latency of nociceptive response was also investigated. The results showed that naloxone (2 mg/kg, ip) given before morphine (5 mg/kg, sc) could antagonize the effect of morphine at the time 30, 45, 60, 75 and 90 minutes compared to the morphine administered alone. Furthermore, naloxone (2 mg/kg, ip) given before the HEAA (400 mg/kg, po) significantly decreased the latency of nociceptive response at the time 30, 45, 60, 75 and 90 minutes compared to the HEAA at the dose of 400 mg/kg, po given alone. The results were shown in Table 9 and Figure 21.

Table 8. Effects of morphine (5 mg/kg, sc) and the hexane extract of *Abroma augusta* seeds (HEAA) at doses of 100, 200, and 400 mg/kg, po on the heat-induced pain in mice.

Treatment	Dose (mg/kg, po)	Latency of nociceptive response (sec)					
		Initial response (min)	30 min	45 min	60 min	75 min	90 min
Control (cosolvent 10 ml/kg)		8.60±0.81	10.01±0.95	10.78±0.93	10.27±0.41	10.03±0.68	10.43±0.28
Morphine	5, sc	10.50±0.81	15.43±0.28**	17.40±0.74**	18.38±0.41**	16.53±0.34**	15.36±0.31**
HEAA	100	8.90±0.36	11.50±0.71*	12.20±1.25*	11.00±0.28	11.30±0.53*	12.20±0.59*
HEAA	200	12.10±0.47	12.00±0.52*	15.17±0.75**	14.65±0.62**	12.90±0.48**	12.70±1.15**
HEAA	400	10.60±0.18	14.30±0.75**	16.28±0.41**	16.68±0.22**	14.40±0.49**	13.20±0.67**

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

*p<0.05, **p<0.01, significantly different compared with control (LSD test)

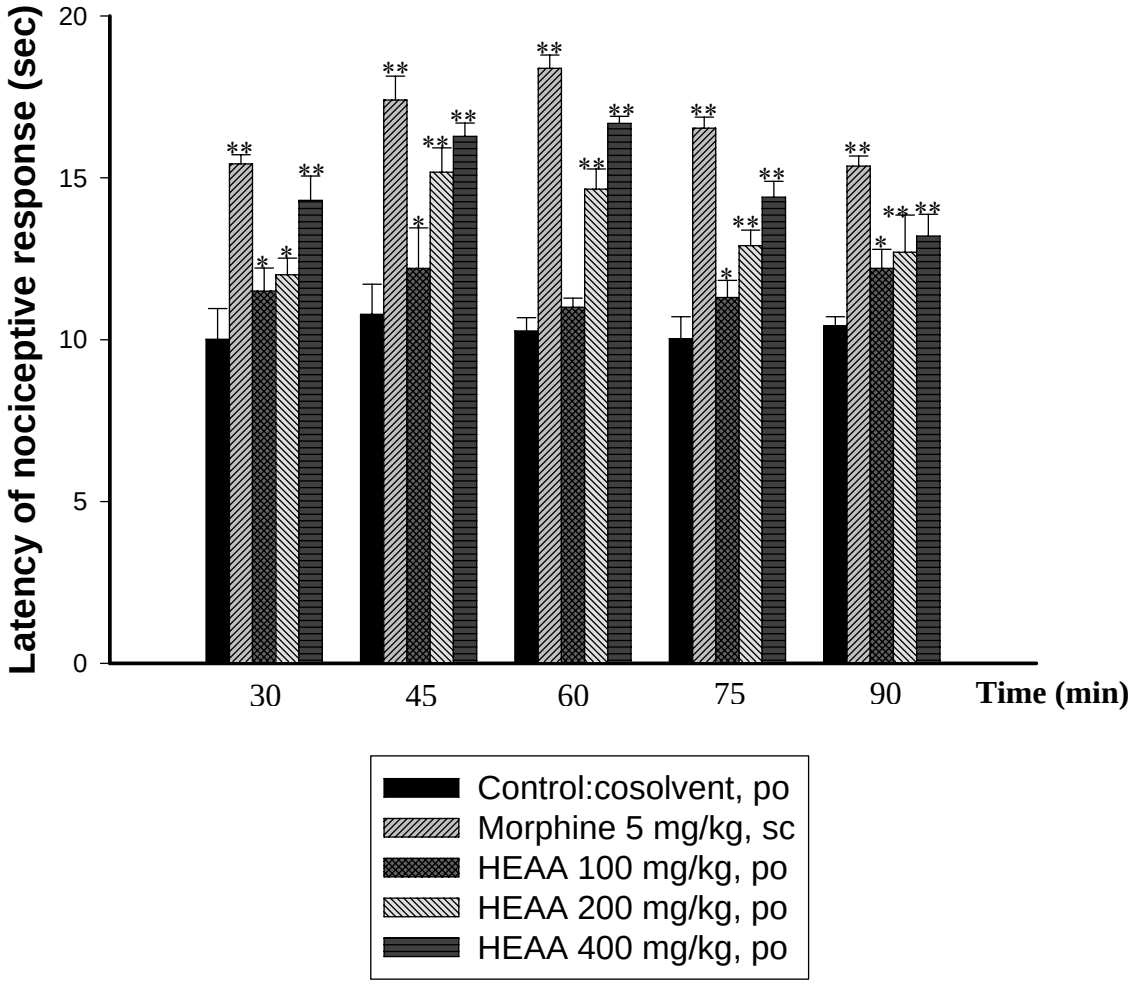


Figure 20. Effects of morphine (5 mg/kg, sc) and the hexane extract of *Abroma augusta* seeds (HEAA) at doses of 100, 200, and 400 mg/kg, po on heat-induced pain in mice.

$^{*}p<0.05$, $^{**}p<0.01$, significantly different compared with control (LSD test)

Table 9. Antagonistic effects of naloxone (2 mg/kg, ip) on the action of morphine (5 mg/kg, sc) or the hexane extract of *Abroma augusta* seeds (HEAA) at the dose 400 mg/kg, po on heat-induced pain in mice.

Treatment	Dose (mg/kg)	Latency of nociceptive response (sec)					
		Initial response (min)	30 min	45 min	60 min	75 min	90 min
Morphine	5, sc	10.50±0.81	15.43±0.28	17.40±0.74	18.38±0.41	16.53±0.34	15.36±0.31
Naloxone	2, ip	11.00±0.61	11.70±0.36	11.50±0.48	11.30±0.51	11.00±0.36	10.70±0.39
Naloxone + Morphine	2, ip 5, sc	11.70±0.44 **	13.60±1.33 **	11.60±0.83 **	9.20±1.06 **	9.10±0.65 **	8.10±0.96 **
HEAA	400, po	10.60±0.18	14.30±0.75	16.28±0.41	16.68±0.22	14.40±0.49	13.20±0.67
Naloxone + HEAA	2, ip 400, po	11.50±0.92 #	11.10±0.67 #	12.10±0.74 #	11.20±0.39 #	10.00±0.67 #	10.50±0.49 #

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

# p<0.01, significantly different compared with HEAA at the dose of 400 mg/kg, po

** p<0.01, significantly different compared with morphine (5 mg/kg, sc)

(LSD test)

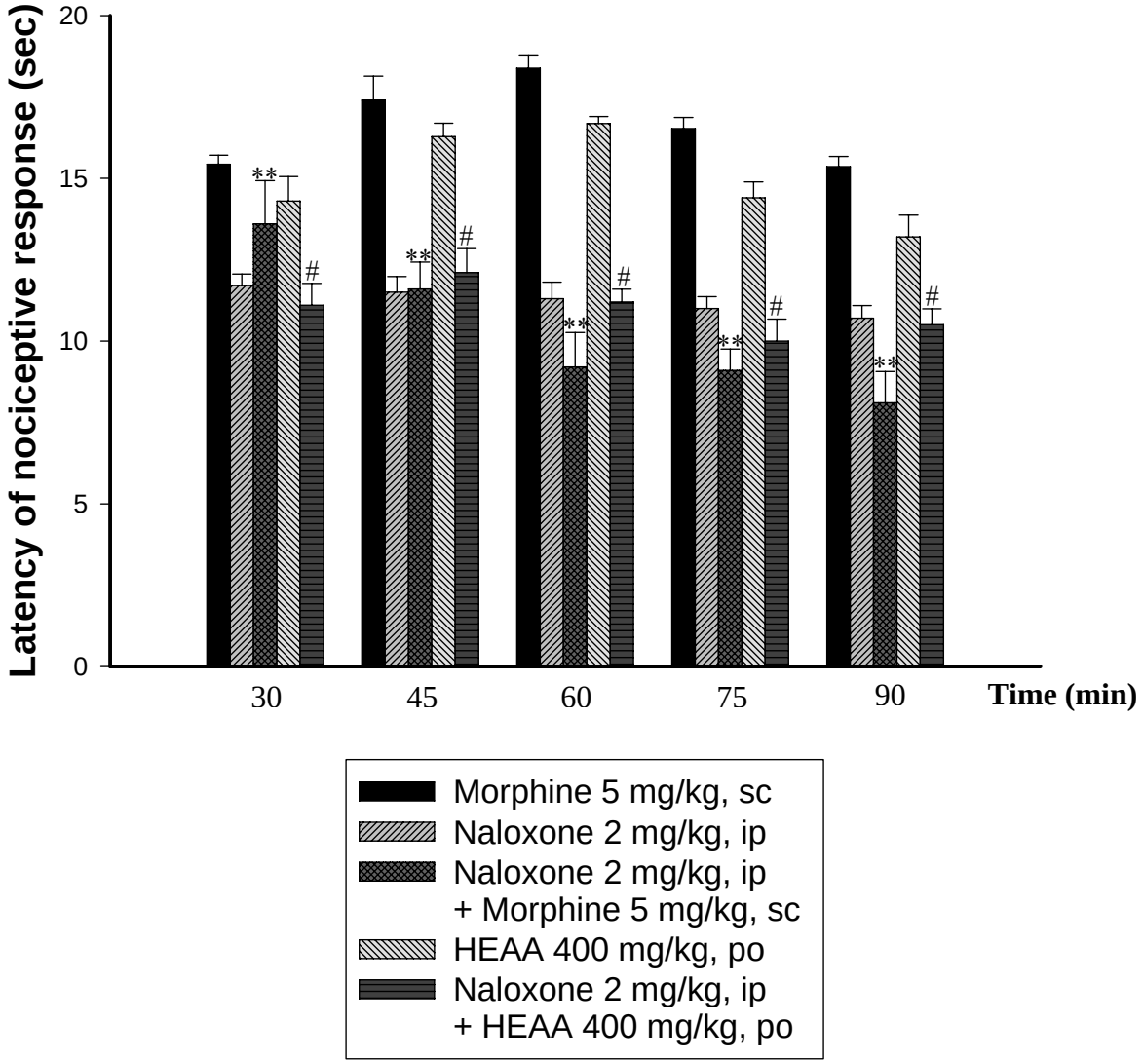


Figure 21. Antagonistic effects of naloxone (2 mg/kg, ip) on the action of morphine (5 mg/kg, sc) or the hexane extract of *Abroma augusta* seeds (HEAA) at the dose 400 mg/kg, po on heat-induced pain in mice.

$^{\#}p<0.01$, significantly different compared with HEAA at the dose of 400 mg/kg, po

$^{**}p<0.01$, significantly different compared with morphine (5 mg/kg, sc)

(LSD test)

2.4 Tail flick test

Morphine at the dose of 5 mg/kg, sc markedly increased the reaction time at all the time intervals measured at 30, 45, 60, 75 and 90 minutes after administration. The HEAA at doses of 100, 200 and 400 mg/kg**,** po significantly increased the reaction time to the nociceptive responses at all the time intervals measured at 30, 45, 60, 75 and 90 minutes after administration. The HEAA at the dose 400 mg/kg**,** po showed the effect which could be comparable with morphine (5 mg/kg, sc). The results were shown in Table 10 and Figure 22.

In antagonistic studies using naloxone as an antagonist, the results showed that naloxone (2 mg/kg, ip) antagonized the effect of morphine (5 mg/kg, sc) on the latency of nociceptive responses at the time 45, 60, 75 and 90 minutes. Furthermore, naloxone (2 mg/kg, ip) give before the HEAA at the dose 400 mg/kg, po significantly decreased the latency of nociceptive responses at the time 45, 60, 75 and 90 minutes when compared to the HEAA (400 mg/kg, po) given alone (Table 11 and Figure 23).

Table 10. Effects of morphine (5 mg/kg, sc) and the hexane extract of *Abroma augusta* seeds (HEAA) at doses of 100, 200 and 400 mg/kg, po on nociceptive responses in the tail flick tests in mice.

Treatment	Dose (mg/kg, po)	Latency of nociceptive response (sec)					
		Initial response (min)	30 min	45 min	60 min	75 min	90 min
Control (cosolvent 10 ml/kg)		5.58±0.31	6.28±0.12	6.87±0.07	8.22±0.20	8.88±0.18	8.45±0.20
Morphine	5, sc	5.40±0.29	7.38±0.18*	8.98±0.06$^{\#}$	10.25±0.14$^{\#}$	11.17±0.16$^{\#}$	11.98±0.18$^{\#}$
HEAA	100	6.07±0.17	7.20±0.17*	8.30±0.18$^{\#}$	8.98±0.13*	9.53±0.13*	10.05±0.15$^{\#}$
HEAA	200	5.43±0.22	7.25±0.20*	8.80±0.13$^{\#}$	9.22±0.08$^{\#}$	10.28±0.17$^{\#}$	10.70±0.17$^{\#}$
HEAA	400	6.05±0.29	7.35±0.17*	8.82±0.15$^{\#}$	9.33±0.20$^{\#}$	10.43±0.15$^{\#}$	11.25±0.17$^{\#}$

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

$^{*}p<0.05$, $^{\#}p<0.01$, significantly different compared with control (LSD test)

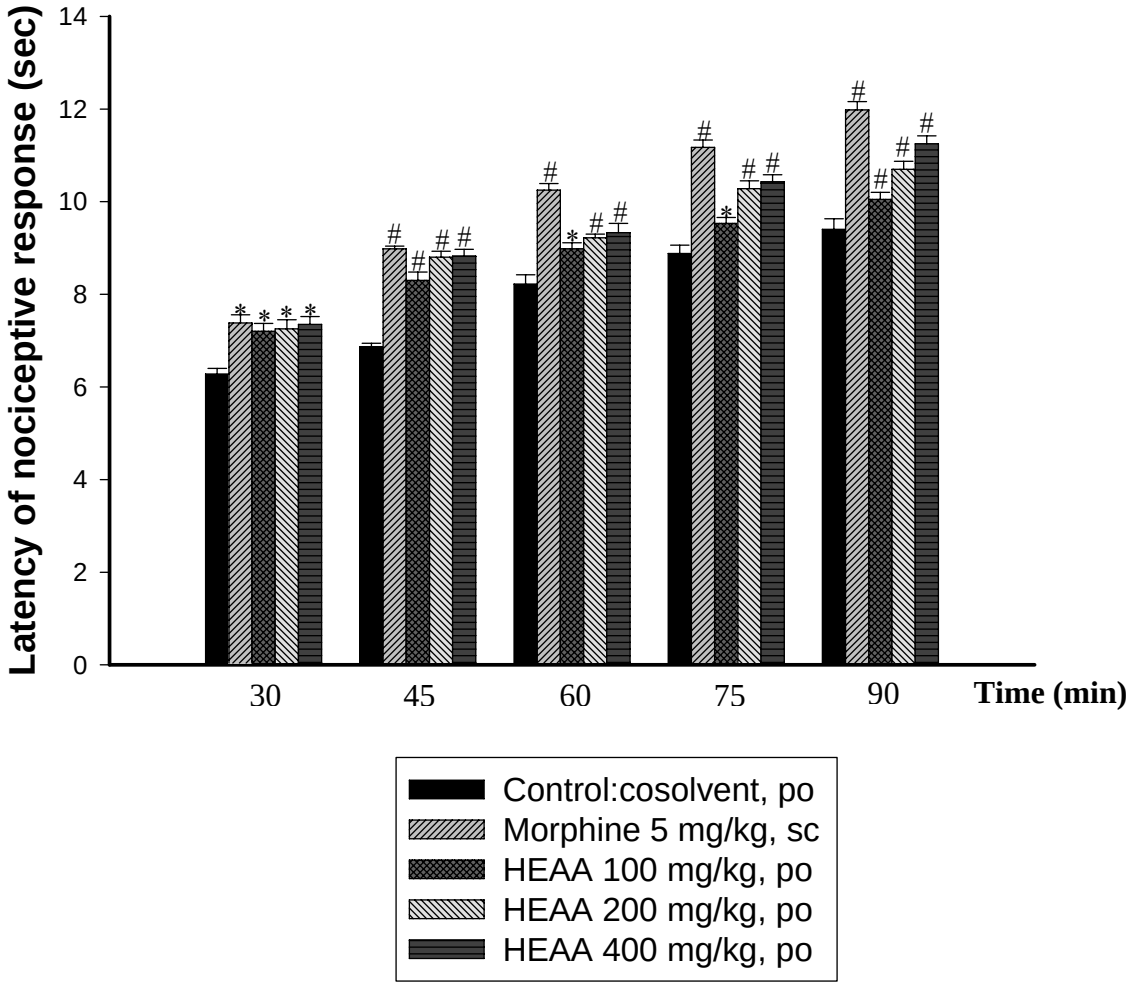


Figure 22. Effects of morphine (5 mg/kg, sc) and the hexane extract of *Abroma augusta* seeds (HEAA) at doses of 100, 200 and 400 mg/kg, po on nociceptive responses in the tail flick tests in mice.

$^{*}p<0.05$, $^{\#}p<0.01$, significantly different compared with control (LSD test)

Table 11. Antagonistic effects of naloxone (2 mg/kg, ip) on the action of morphine (5 mg/kg, sc) or the hexane extract of *Abroma augusta* seeds (HEAA) on nociceptive responses in the tail flick tests in mice.

Treatment	Dose (mg/kg)	Latency of nociceptive response (sec)					
		Initial response (min)	30 min	45 min	60 min	75 min	90 min
Morphine	5, sc	5.40±0.29	7.38±0.18	8.98±0.06	10.25±0.14	11.17±0.16	11.98±0.18
Naloxone	2, ip	5.41±0.29	5.98±0.22	6.68±0.21	7.60±0.20	7.43±0.27	6.62±0.12
Naloxone + Morphine	2, ip 5, sc	5.60±0.42	6.70±0.30**	7.23±0.21**	7.80±0.15**	8.02±0.10**	8.18±0.08**
HEAA	400, po	6.05±0.29	7.35±0.17	8.82±0.15	9.33±0.20	10.43±0.15	11.25±0.17
Naloxone + HEAA	2, ip 400, po	6.93±0.31	7.07±0.18	7.43±0.22#	8.00±0.13#	8.45±0.29#	8.35±0.22#

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

#p<0.01, significantly different compared with HEAA at the dose of 400 mg/kg, po.

**p<0.01, significantly different compared with morphine (5 mg/kg, sc). (LSD test)

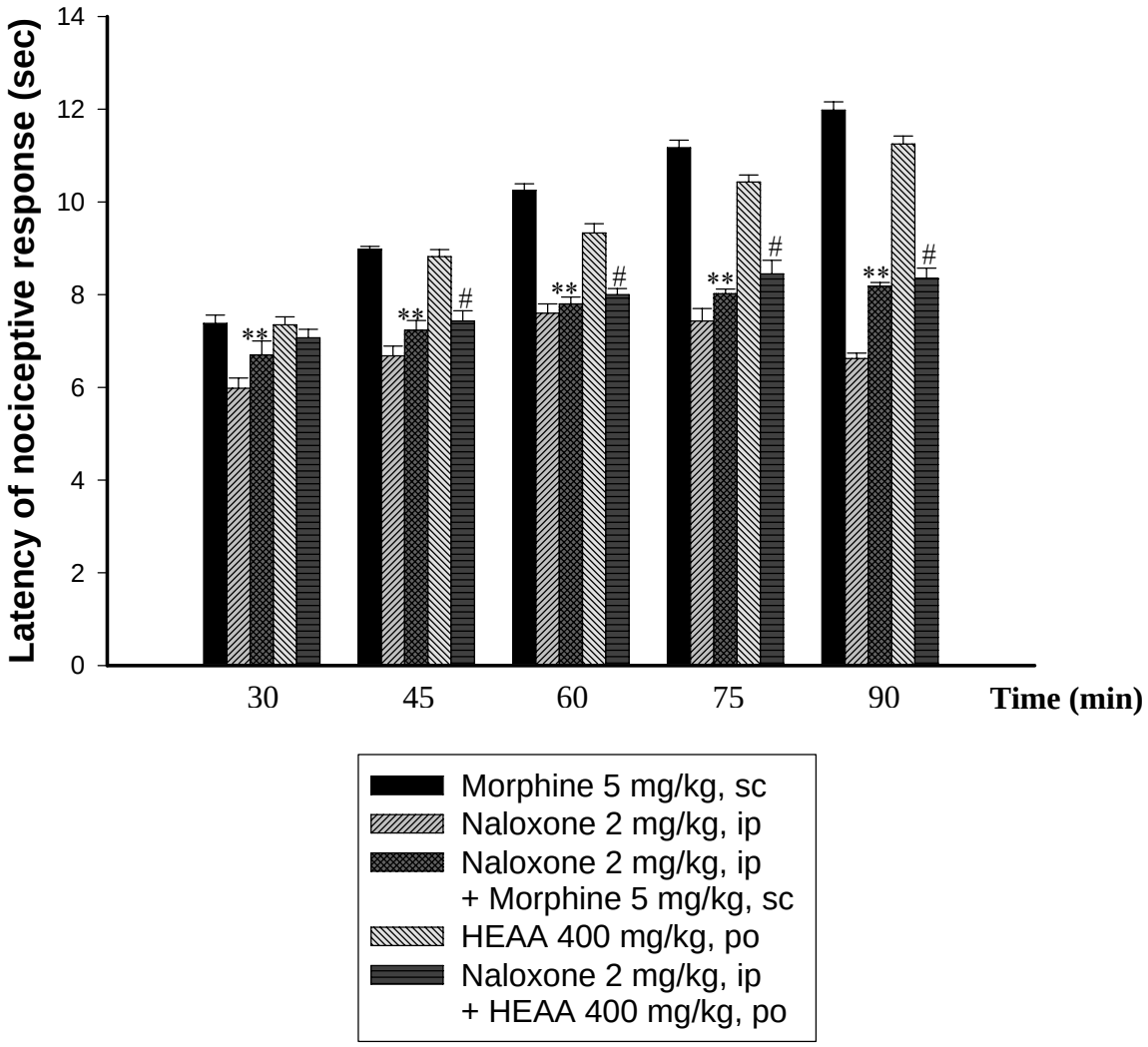


Figure 23. Antagonistic effects of naloxone (2 mg/kg, ip) on the action of morphine (5 mg/kg, sc) or the hexane extract of *Abroma augusta* seeds (HEAA) at the dose 400 mg/kg, po on nociceptive responses in the tail flick tests in mice.

$^{\#}p<0.01$, significantly different compared with HEAA at the dose of 400 mg/kg, po

$^{**}p<0.01$, significantly different compared with morphine (5 mg/kg, sc)

(LSD test)

2. Anti-inflammatory activity

2.1 Carrageenan-induced rat paw edema

In the carrageenan-induced rat paw edema test, the average right hind paw volumes and percentages of inhibition by the HEAA and aspirin are shown in Table 12 and Figure 24. The standard drug aspirin at the dose of 200 mg/kg significantly decreased the volume of hind paw edema at 0.5, 1, 2, 3, 4 and 5 h (p<0.05) with percentage of inhibition by 16.52, 7.51, 16.00, 15.23, 14 and 14.27, respectively when compared with control. The HEAA at only the dose of 400 mg/kg significantly reduced the volume of paw edema at 0.5, 1, 2, 3, 4 and 5 h (p<0.05) with percentage of inhibition by 15.63, 4.47, 12.81, 12.82, 10.40 and 10.21, respectively when compared with control. Therefore, the result indicated the HEAA at the dose of 400 mg/kg, po used in this study possessed anti-inflammatory activity.

Table 12. Effects of the hexane extract of *Abroma augusta* Linn. seeds (HEAA) and aspirin on the carrageenan-induced paw edema in rats.

Treatment	Dose (mg/kg, po)	Initial paw volume (ml)	Paw edema volume (ml)						Inhibition of paw edema (%)					
			0.5 h	1 h	2 h	3 h	4 h	5 h	0.5 h	1 h	2 h	3 h	4 h	5 h
Control (cosolvent 5 ml/kg, po)		4.66±0.19	5.63±0.09	5.59±0.09	6.40±0.03	6.63±0.05	6.83±0.02	7.15±0.05						
Aspirin	200	4.25±0.11	4.70±0.08*	5.17±0.12*	5.38±0.09*	5.62±0.08*	5.88±0.10*	6.13±0.14*	16.52	7.51	16	15.23	14	14.27
HEAA	100	4.28±0.24	5.56±0.03	5.89±0.03	6.26±0.05	6.61±0.03	6.76±0.05	7.06±0.03	1.24	-5.37	2.19	0.3	1.02	1.26
HEAA	200	4.27±0.13	5.20±0.04	5.62±0.04	5.84±0.05	6.45±0.06	6.62±0.08	6.92±0.08	7.64	-0.54	8.75	2.71	3.07	3.21
HEAA	400	3.86±0.21	4.75±0.10*	5.34±0.05*	5.58±0.07*	5.78±0.07*	6.12±0.04*	6.42±0.03*	15.63	4.47	12.81	12.82	10.4	10.21

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

*p<0.05, significantly different compared with control (LSD test)

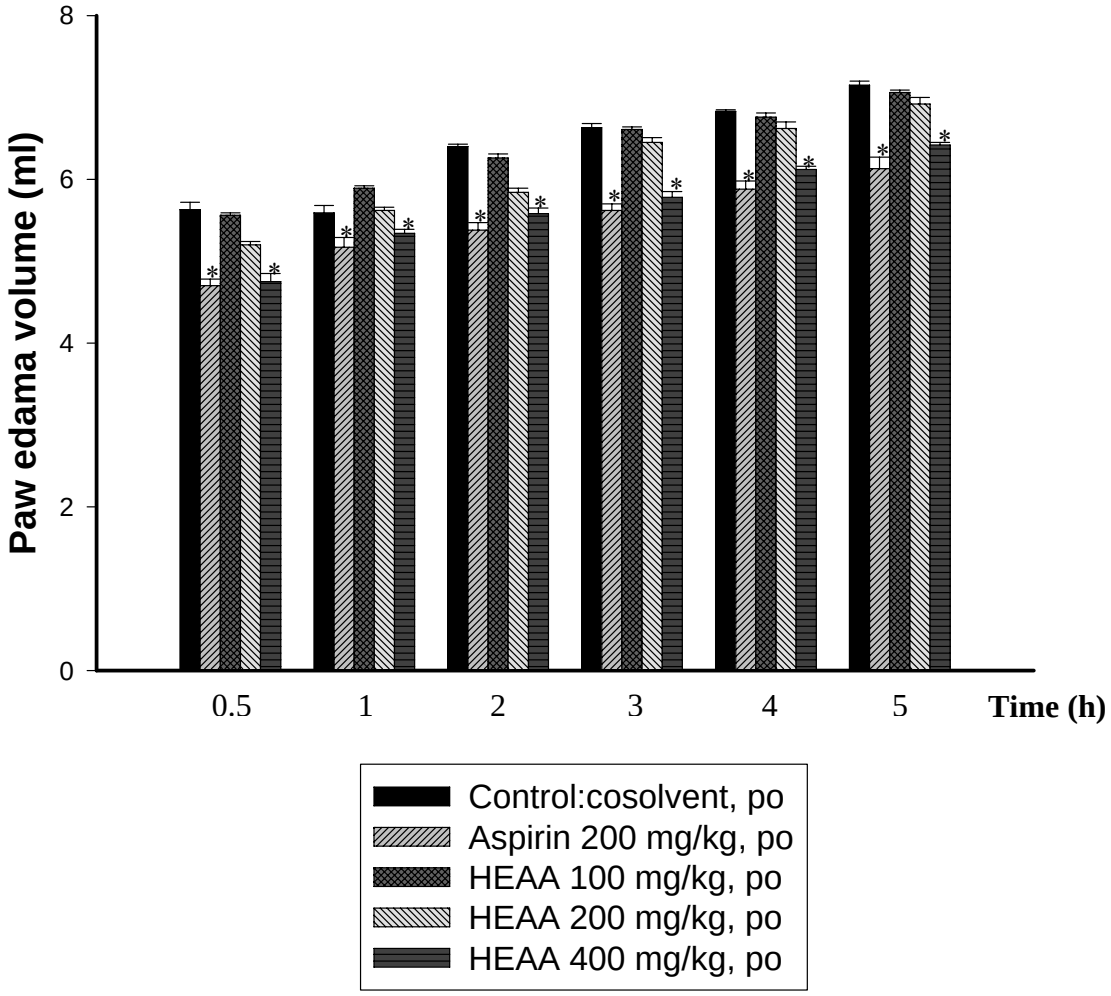


Figure 24. Effects of aspirin (200 mg/kg, po) and the hexane extract of *Abroma augusta* seeds (HEAA) at doses of 100, 200 and 400 mg/kg, po on the carrageenan-induced paw edema in rats.

$^{*}p<0.05$, significantly different compared with control (LSD test)

2.2 Cotton pellet-induced granuloma formation in rats

The results of aspirin (200 mg/kg, po) and the HEAA at doses of 100, 200 and 400 mg/kg, po on cotton pellet-induced granuloma in rats were presented in Table 13 and Figure 25. The aspirin (200 mg/kg, po) exhibited a significantly reduced both in transudative weight (21.33±0.29 vs 49.00±2.35 mg, $p<0.05$) and granuloma weight (1.09±0.02 vs 2.45±0.11 mg/mg cotton, $p<0.05$) when compared to the control. The percentage of granuloma inhibition (% GI) of aspirin (200 mg/kg, po) was 55.51. The HEAA at doses of 100, 200 and 400 mg/kg, po did not reduce both in transudative weight and granuloma weight. The result indicated that the HEAA at doses used in this study did not exhibit ant-inflammatory activity on cotton pellet-induced granuloma in rats.

Table 13. Effects of aspirin (200 mg/kg, po) and the hexane extract of *Abroma augusta* seeds (HEAA) on cotton pellet-induced granuloma formation in rats.

Treatment	Dose (mg/kg, po)	Granuloma wet weight (mg)	Granuloma dry weight (mg)	Transudative weight (mg)	Granuloma weight (mg/mg cotton)	GI (%)
Control (cosolvent 5 ml/kg, po)		85.67±2.08	36.68±1.26	49.00±2.35	2.45±0.11	
Aspirin	200	48.52±2.15*	27.18±1.94*	21.33±0.29*	1.09±0.02*	55.51
HEAA	100	87.68±3.13	40.27±1.73	47.42±4.38	2.37±0.23	3.26
HEAA	200	85.60±3.38	39.07±4.00	46.53±3.96	2.35±0.20	4.08
HEAA	400	86.21±2.25	39.70±2.17	46.52±3.59	2.30±1.81	6.12

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

GI; granuloma inhibition

*p<0.05, significantly different compared with control (LSD test)

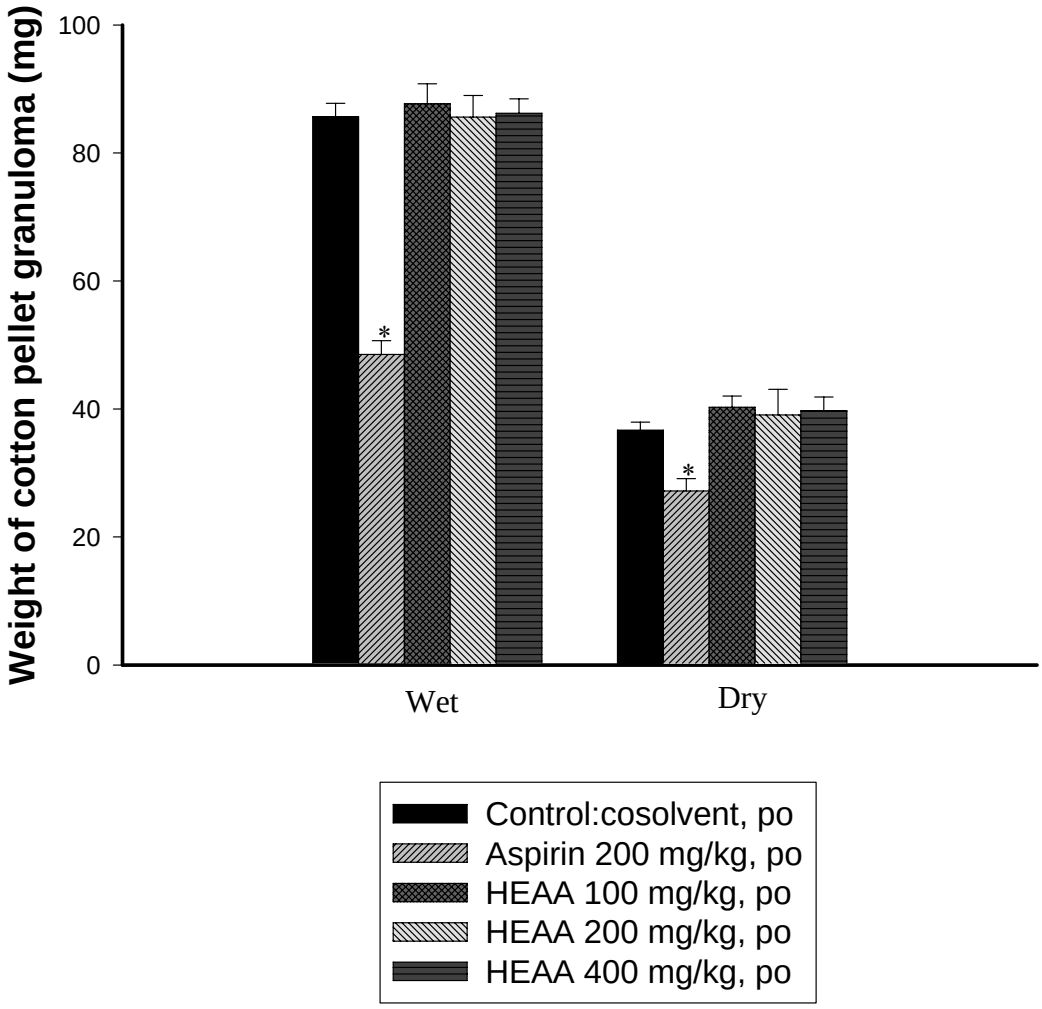


Figure 25. Effects of aspirin (200 mg/kg, po) and the hexane extract of *Abroma augusta* seeds (HEAA) at doses of 100, 200 and 400 mg/kg, po on cotton pellet-induced granuloma formation in rats.

$^{*}p<0.05$, significantly different compared with control (LSD test)

3. Antipyretic activity

The results of the antipyretic effects of aspirin and the HEAA were shown in Table 14 and Figure 26. Administration of the brewer's yeast to the rat produced an increase of rectal temperature in 19 hours after the yeast injection. Aspirin at the dose of 200 mg/kg, po caused a significant decreased in rectal temperature beginning at 1 h after aspirin administration and continued up to 5 h. The HEAA at the dose 200 mg/kg, po significant decreased in rectal temperature at 5 h (36.82±0.24 vs 37.22±0.17, $p<0.05$) while the HEAA (400 mg/kg, po) significantly decreased of rectal temperature at 1 h and continued up to 5 h (37.00±0.23 vs 37.81±0.22; 36.85±0.20 vs 37.57±0.23; 36.62±0.18 vs 37.40±0.21; 36.38±0.07 vs 37.25±0.21 and 36.31±0.05 vs 37.22±0.17, $p<0.05$), respectively when compared with control. The results indicated that the HEAA at the dose of 400 mg/kg exhibited antipyretic activity in the rat induced fever by brewer's yeast injection.

Table 14. Effects of aspirin and the hexane extract of *Abroma augusta* seeds (HEAA) on the brewer's yeast induced pyrexia in rats.

Treatment	Dose (mg/kg, po)	Before yeast Injection (-19h)	After yeast Injection (0 h)	Rectal temperature (°C) Time after treatment (h) 1	2	3	4	5
Control		36.52±0.13	37.48±0.25	37.81±0.22	37.57±0.23	37.40±0.21	37.25±0.21	37.22±0.17
Aspirin	200	36.29±0.05	37.29±0.12	36.92±0.16*	36.73±0.13*	36.49±0.12*	36.36±0.08*	36.29±0.06*
HEAA	100	36.61±0.23	37.55±0.30	37.68±0.21	37.52±0.15	37.34±0.17	37.16±0.15	37.04±0.12
HEAA	200	36.49±0.21	37.44±0.27	37.49±0.26	37.33±0.26	37.17±0.24	37.02±0.27	36.82±0.24*
HEAA	400	36.64±0.17	37.50±0.20	37.00±0.23*	36.85±0.20*	36.62±0.18*	36.38±0.07*	36.31±0.05*

Values are presented as mean ± S.E.M (*N*=6)

*$p<0.05$, significantly different compared with control (LSD test)

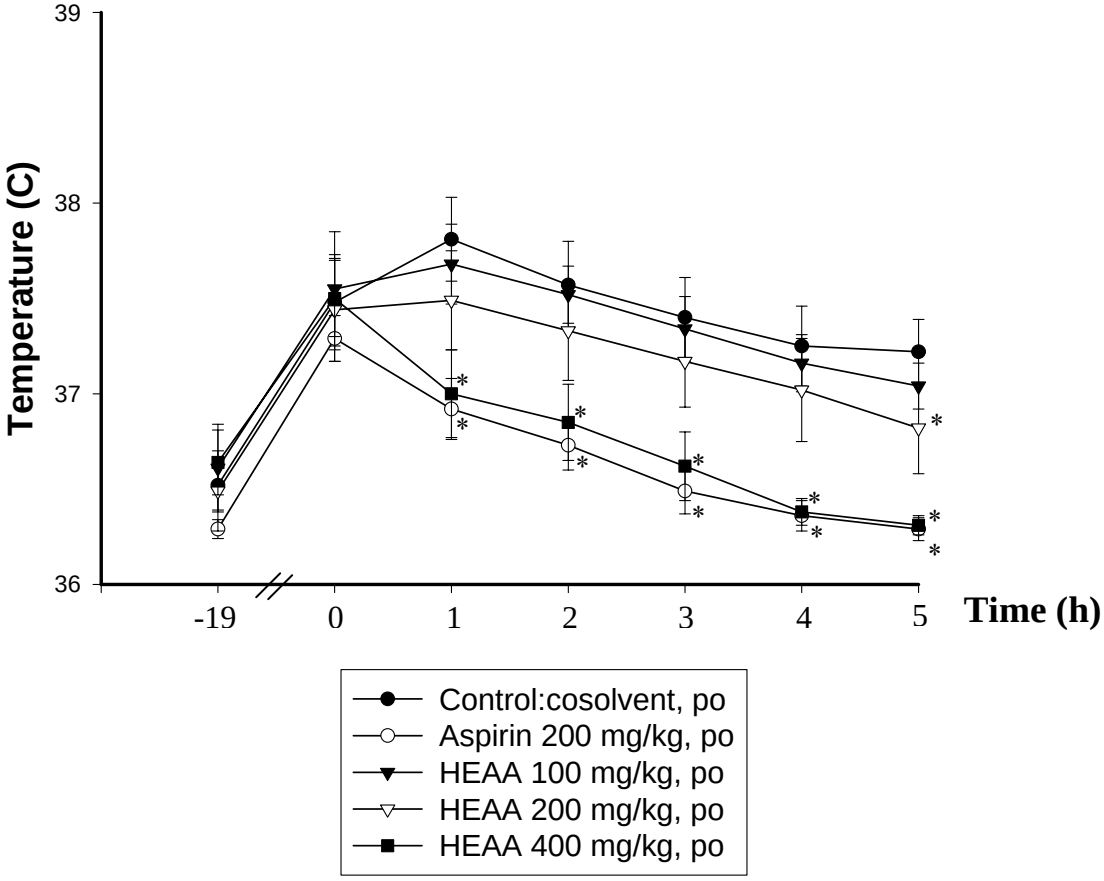


Figure 26. Effects of aspirin (200 mg/kg, po) and the hexane extract of *Abroma augusta* seeds (HEAA) at doses of 100, 200 and 400 mg/kg, po on the brewer's yeast induced fever in rats.

$^{*}p<0.05$, significantly different compared with control (LSD test)

CHAPTER 5

DISCUSSION AND CONCLUSION

Abroma augusta Linn., Family Sterculiaceae, is commonly known in Thai name as "Teandum" or its generic name as "Devil's cotton". The plant is distributed in India, China, Pakistan and Thailand. This plant contains several biologically active compounds including of choline, betaine, β-sitosterol and stigmasterol (Dasgupta and Basu, 1970), L-rhamnose, L-arabinose, D-xylose, D-mannose, D-galactose, D-glucose, D-galacturonic acid and D-glucuronic acid (Majumder *et al*., 1994). A number of pharmacological activities has been reported such as antifungal (Khan and Ahmad, 2003) and antidiabetic activity (Eshrat, 2003). In traditional medicine, it is claimed that aerial parts of *A. augusta* has been used to treat joint pain, asthma, sinusitis, tonsillitis, back pain, diarrhea and influenza. Based on these data, the plant may possess analgesic, antipyretic and anti-inflammatory activities. Thus, the study on these pharmacological activities is designed to support medical uses in folklore medicine.

Acute toxicity test

In the present study, oral acute toxicity was carried out in mice and rats to evaluate the toxicities of the hexane extract of *Abroma augusta* Linn. seeds (HEAA). The results revealed that the extract at the dose up to 5 g/kg given orally did not affect on behaviors. Signs of toxicities such as convulsion, hyperactivity, sedation, loss of righting reflex and mortality were not observed in the first 8 hour period and every 24 h for 7 days. Since the HEAA at the dose of 5 g/kg, po did not produce any signs of toxicities and mortality in mice and rats, it could be assumed that the HEAA up to at this dose was to be safe. The estimated LD_{50} of the HEAA in mice or rats is more than 5 g/kg, po. The HEAA at doses of 100, 200 and 400 mg/kg, po used in the present study was also to be safe.

Analgesic activity

Analgesic activity of HEAA at doses of 100, 200 and 400 mg/kg, po was assessed in mice using writhing, formalin, hot plate and tail flick tests. In writhing test, HEAA at doses of 100, 200 and 400 mg/kg significantly reduced writhings induced by acetic acid with percentage of inhibition by 35.65, 49.74 and 57.99, respectively, whereas a reference drug aspirin (200 mg/kg, po) inhibited number of writhings by 55.32. The analgesic activity of HEAA in writhing test was dose-related, and the potency of HEAA at the dose of 400 mg/kg could be comparable to a reference drug aspirin. In formalin test, HEAA at doses of 100, 200 and 400 mg/kg, po significant dose-dependently decreased the licking time both in early phase by 33.84%, 42.46% and 54.58%, respectively and in late phase by 36.79%, 63.71% and 80.83%, respectively, whereas a reference drug morphine and aspirin significantly reduced the licking time in early phase by 65.31% and 59.54%, and in late phase by 88.18% and 79.50%, respectively. In hot plate test, HEAA at doses of 100, 200 and 400 mg/kg, po significantly increased the latency of nociceptive responses, and naloxone (2 mg/kg), an opioid antagonist, could antagonized the action of morphine or HEAA (400 mg/kg) on nociceptive responses in mice. In tail flick test, HEAA at doses of 100, 200 and 400 mg/kg significantly increased the reaction time of the nociceptive responses, and naloxone (2 mg/kg) given before morphine or HEAA (400 mg/kg) significantly decreased the latency of nociceptive responses in mice.

These data indicated that HEEA clearly possessed analgesic activity in mice using writhing, formalin, hot plate and tail flick tests. Take all these data together, suggesting that HEAA had an analgesic activity via central and peripheral mediated mechanisms. The details of its possible analgesic mechanisms of HEAA will be later discussed.

Pain is an unpleasant sensory and emotional experience associated with actual or potential tissue damage. Although pain is natural warning and protection mechanisms, patients often seek medical intervention to relieve pain. Pain is generated when mechanical, thermal, chemical or electrical stimuli exceed a certain threshold value. After tissue damage, many chemical mediators are released such as substance P, histamine, bradykinin and prostaglandins. Bradykinin is an important substance to stimulate nociceptors and prostaglandins sensitize nociceptor which

causes impulses sending to spinal cord. This state is called peripheral sensitization. When spinal cord becomes hyperexcitable, excitatory amino acid or substance P from A delta (Aδ) fiber and/or C fiber were released and depolarized on its receptor. Signal transduction coming to postcentral gyrus in thalamus which is responsible for the conscious perception of pain. This state is called central sensitization (Guyton, 1997; William, 2000; Mutschler and Derendorf, 1995b).

In analgesic activity test, we designed different tests to measure different types of pain; chemical stimulus by writhing and formalin test; thermal stimulus by hot plate and tail flick test. Moreover, the different features involved in the process, such as intensity, location, quality and duration of noxious stimulus must be considered in the effect of the antinociceptive substance.

In acetic acid induced writhings, a visceral pain model, is a chemical stimulus widely used for the evaluation of general analgesic activity (Koster *et al.*, 1959). Intraperitoneal injection of 0.6% acetic acid that was irritating to serous membranes provokes a stereotypical behavior in rodents that is characterized by abdominal contractions, whole body movement, contortions of the abdominal muscle and reduced motor activity and incoordination. The writhing test can predict effective analgesic doses for agents that can be used in humans (Dubinsky *et al*., 1987; Eaton, 2003).

Acetic acid cause, pain is generated indirectly via endogenous mediators like bradykinin, serotonin, histamine, substance P and prostaglandins, acting on stimulating peripheral nociceptive neurons, which are sensitive to narcotic analgesic and non-steroid anti-inflammatory drugs (NSAIDs) (Collier *et al*., 1986; Deraedt and Jouquey, 1980). Acetic acid induced writhings are highly sensitive and useful test for analgesic drug development, but the test is not selective pain test, therefore, it is used as a screening test for both peripheral and central acting analgesic activity (Raj, 1996).

HEAA at the dose of 100, 200 and 400 mg/kg significantly decreased the number of writhing in mice induced writhing response by 0.6% acetic acid, it could be assumed that HEAA decreased visceral pain induced by acetic acid and sites of action for analgesic activity would be mediated both peripheral and central mechanisms. It has been suggested that acetic acid stimulates the vanilloid receptor (VR1) and

bradykinin B_2 receptor in the pathway comprising sensory afferent C-fiber (Ikeda *et al.*, 2001). The prostaglandin biosynthesis by arachidonic acid via cyclooxygenase plays a role in the nociceptive mechanism (Franzotti *et al.*, 2002). The quantification of prostaglandins by radioimmunoassay in the peritoneal exudates of mice obtained after the intraperitoneal injection of acetic acid demonstrated high level of PGE_2 and $PGF_{2\alpha}$ during 30 minutes after stimulus (Deraedt and Jouquey, 1980). Of the prostanoids, PGI_2 has been mainly responsible for the causation of pain following acetic acid administration (Murata *et al*., 1997). Thus, the abdominal constriction is related to the sensitization of nociceptors to prostaglandins.

The results of writhing suggested that HEAA at the dose of 100, 200 and 400 mg/kg was likely to inhibit the number of writhing induced by acetic acid as a standard drug aspirin at the dose of 200 mg/kg. Aspirin and other NSAIDs can inhibit cyclooxygenase in peripheral tissues, thus, interfering with the mechanism of transduction in primary afferent nociceptive via inhibiting the synthesis of prostaglandins (Fields, 1987). The mechanism of analgesic action of HEAA could probably be due to interfere with the synthesis, release of endogenous substances, blockade of the effect or desensitization of nerve fiber (Collier *et al*., 1986), that excite pain nerve endings similarly to aspirin and other NSAIDs.

The formalin test is another pain model of nociception that it can discriminate pain in central and peripheral components (Tjolsen *et al*., 1992) termed early and late phases. In the present study, the formalin test was conducted to confirm and study the possible analgesic mechanisms of action of HEAA, animal presented two distinct nociceptive behavior phases. Drugs that act primarily on the central nervous system such as morphine inhibit both phases and NSAIDs such as aspirin acting supraspinally which inhibit prostaglandin synthesis can reduce the pain in both phases (Martindale et al., 2001). Thus the formalin test is best used to examine opioid mimetics (Eaton, 2003). Inflammatory pain is selectively inhibited by NSAIDs because these agents reduce inflammation. However, it has also been proposed that NSAIDs may have analgesic effects independent of their anti-inflammatory activity (Hunskaar *et al*., 1985). The formalin test consists of two different phases which can be separated in time; the early phase initiated immediately after formalin injection and lasted about 3-5 min, resulting from chemical stimulation of nociceptors; the late phase initiated 15-

20 min after formalin injection, lasted about 20-40 min. There has been reported that bradykinin was involved in the early phase, while histamine, 5-hydroxytryptamine (5-HT), bradykinin and prostaglandins were involved in the late phase (Tjolsen *et al.*, 1992). The direct stimulation of nerve ending caused substance P release with cooperation with bradykinin, while late phase which is also termed as inflammatory pain, is a pain due to inflammation and mediated by the peripheral effect via the release of some chemical mediators such as histamine, serotonin, bradykinin and prostaglandins (Shibata *et al*., 1989). Licking activity of the late phase is due to inflammation with release of serotonin, histamine, bradykinin, and prostaglandins, which to some can cause the sensitization of central nociceptive neurons (Verma *et al*., 2007)

The HEAA showed an inhibitory action on nociceptive responses in formalin test both in early and late phases, so it was indicated that *A. augusta* has central and peripheral analgesic properties. It has been widely agreed that nociceptive behaviors manifested during the acute early phase may be caused by the direct stimulation of the sensory nerve fiber by formalin, so the extract might reduce the licking time in the early phase of formalin test via blockade on nociceptor and/or inhibited releasing of substance P and bradykinin. The HEAA (100, 200 and 400 mg/kg) had strong analgesic activity, which was commonly associated with inflammatory pain. Thus, it might be concluded that analgesic effect on the early phase of the HEAA was due to the direct effect on the nociceptor via blockade on the nociceptor or the inhibit releasing the substance P or bradykinin. The analgesic effect on the late phase of HEAA*A* indicates its inhibitory activity on pain arise from inflammation, which reflects the effect on the synthesis and/or release of prostaglandin, serotonin, histamine and bradykinin (Trongsakul e*t al*., 2002). Morphine, a centrally acting analgesic drug, can inhibit nociception in both phases (Shibata *et al.*, 1989). In this study, results revealed that the HEAA provides analgesia for both phases, and the late phase is more sensitive. The mechanism of action of HEAA was opioidmimetics, and also possessed its action similar to aspirin. So, the active component of HEAA was believed to have more than one.

To check for possible central antinociceptive effect of the HEAA, the hot plate and tail flick models are considered specific tests for evaluation of the central pain

(Marchioro *et al*., 2005) at the supraspinal and spinal levels (Wong *et al*., 1994), respectively, possible acting on either ascending and descending inhibitory pain pathway (Richardson *et al*., 1998).

In the hot plate test, it is widely used to assess analgesic activity of drugs. The paw-licking hot plate responses are complex supraspinally organized behavior (Chapman, 1985). This test does not directly measure the intensity of the noxious stimulus perceived by the animal, but only the animal's response to it, and so may be affected by non-analgesic drugs. In the present investigation, HEAA considerably increased the reaction time to the heat stimulus in hot plate test. Only mice showed nociceptive response in hot plate test such as jumping or licking the pain less than 15 seconds were used. The HEAA at the dose of 100, 200 and 400 mg/kg significantly increased latency of nociceptive responses at all time interval measured when compared to control. The HEAA at the high dose (400 mg/kg) exhibited more potent effects to delay the latency of nociceptive responses than the extract at lower doses (100 and 200 mg/kg). To investigate mechanism the central analgesic effect of the extract, the antagonistic action of naloxone on effects of morphine or HEAA (400 mg/kg) on the latency of nociceptive responses was also investigated. Naloxone given before morphine or HEAA (400 mg/kg) could antagonize the effect of morphine or the extract compared to the morphine or the extract administered alone. The hot plate test is usually provided to determine the involvement of central nociceptive mechanism, and at least to confirm the central mechanism as seen in formalin test. The antinociceptive action of the extract was blocked by naloxone, an opioid receptor antagonist, suggesting that the antinociception was partly mediated via opioid mechanisms. The mu (μ) opioid receptor has generally been regarded as the receptor type associated with pain relief, and shown to be potent in regulating thermal pain (Dhawan, 1996). This could be due to the direct agonist activity of opioidmimetic constituents in the extract and/or due to increase release of endogeneous opioid peptides (Deraniyagala *et al*., 2003).

Another model, a tail flick test, is often used to investigate for the centrally mediated mechanisms of drugs or substances. The tail flick model is considered specific test for evaluation of the central pain (Marchioro *et al*., 2005) as spinal level (Wong *et al*., 1994). The analgesic effect reflected in the tail flick test is dependent on

centrally acting opioid like analgesics (Conner *et al*., 2000; Yonathan *et al*., 2006). Oipoid receptors are widely distributed in several peripheral tissues and cutaneous nerves as well as the CNS, especially in the pain transmission pathways including in the dorsal horn at the spinal cord (Jin *et al*., 2006). Descending control of spinal nociception is a major determinant of acute pain in different behavioral and emotional states (McMullan and Lumb, 2006). There are strong suggestions that analgesic system is heavily related to the endogenous opioid system (Yaksh, 1999; Brooks and Tracey, 2005).

The present results obtained from a tail flick test indicated that HEAA at doses of 100, 200 and 400 mg/kg significantly increased the reaction time to the nociceptive responses at all the time intervals measured when compared to control. Morphine (5 mg/kg), a centrally acting analgesic drug, significantly increased the tail flick latency at all time interval measured in tail flick test in mice. In antagonistic study, naloxone (2 mg/kg) given before morphine or HEAA (400 mg/kg) could antagonize the analgesic effects of morphine or HEAA (400 mg/kg) on the latency of nociceptive responses at all time interval measured. Based on the results obtained from hot plate and tail flick tests in this study, all tested doses of the HEAA prolong latency of nociceptive responses especially the high dose (400 mg/kg). The hexane extract of HEAA had an analgesic activity-like morphine via central mechanism through the opioid receptor at the supraspinal level than the spinal level.

This HEAA has a relatively high affinity for μ opioid binding sites. It has lower affinity for the δ and κ receptors (Barber, 1997) but can also reverse antagonists at the δ and κ sites (Way *et al*., 1992). In animal models, the opioid receptor μ, δ and κ receptors are responsible for supraspinal and spinal analgesics (Gutstein and Akil, 2001). All three opioid receptor types have known subtypes. Two μ subtypes have been best elucidated. Activation of μ_1 leads to supraspinal analgesic, whereas μ_2 activation leads to spinal analgesic (Stoelting and Hillier, 2006), and are commonly thought to be responsible for the advance effects such as sedation, respiratory depression, euphoria and constipation. Activation of κ (κ_1 and κ_2) receptor leads to the spinal analgesia and κ_3 leads to the supraspinal analgesia (Lipman and Jackson, 2004).

According to Rossi and Colleagues (1993), antinociceptive effect could be due to a direct decrease in the activity evoked by C fiber in ascending axon, or decrease in

the production of prostaglandins responsible for the C fiber stimulation. The mechanism of action of HEAA in hot pate test (supraspinal analgesia) and tail flick test (spinal analgesia) could be involved in the pathway or direct action on opioid receptor on the peripheral terminals of thinly myelinated and unmyelinated cutaneous sensory fibers as well as the morphine-like substances (Conggreshall and Carlton, 1997).

In conclusion, the results evaluated from the hot plate and tail flick test indicated that HEAA had an analgesic activity-like morphine via central mechanism, in the level of spinal and supraspinal mechanisms to produce analgesic property.

Anti-inflammatory activity

In the present study, only HEAA at the dose of 400 mg/kg significantly reduced rat paw edema at 0.5, 1, 2, 3, 4 and 5 h with percentage of inhibition by 15.63, 4.47, 12.81, 12.82, 10.40 and 10.21, respectively when compared with control. However, the extract at tested doses did not exhibit ant-inflammatory activity on cotton pellet-induced granuloma formation in rats.

Inflammation is defense reaction of the organism and its tissue to injurious stimuli. Although it is a defense mechanism, the complex events and mediators involved in the inflammatory reaction can be included, maintained or aggravated by many diseases (Gupta *et al*., 2003). Causes of inflammation include direct damage (cuts, sprains), chemicals such as acid, ischemia and cell necrosis or infarction, allergic reaction, physical agents (thermal injury or burns, radiation), foreign bodies (splinters or dirt) and infection (Gould, 2002).

In acute inflammation, when tissue injured (damaged cells), mast cells and platelets release chemical mediators such as histamine, serotonin, prostaglandins and leukotrienes into the interstitial fluid and blood. These chemicals affect the blood vessels and the nerves in the area. First neutrophils and later monocytes and macrophages collect along the capillary wall and then move through the wider separations in the well into the interstitial area. There they destroy and remove foreign materials, microorganisms and cell debris by phagocytosis (Gould, 2002).

The carrageenan-induced paw inflammation is a useful phlogistic tool for investigation of systemic anti-inflammatory agents. This test is sensitive to most

clinically effective anti-inflammatory drugs and comprises two phases. The initial phase, which occurs within 1-2 h after carrageenan injection, is due to the release of serotonin and increase of prostaglandin, histamine and bradykinin in the inflammatory area. The second phase occurs 3-5 h after carregeenan injection which is correlated with production and release of bradykinin, leukotrienes and prostaglandins produced by the macrophages and sustained by released prostaglandins (Garcia and Hamamura, 1973; Vinegar *et al*., 1987; Crunkhon and Meacock, 1971; Brito, 1998). In addition, one of the most important features of inflammation is edema which is caused by the action of some inflammatory autocoids like kinins, prostaglandins especially the E series, leukotrienes, ect. resulting in vasodilation, enhancement of capillary permeability, plasma exudation and these mediators also cause pain and fever (Silbernagl and Lang, 2000). So, inhibition of these mediators from injured sites or from bringing out their pharmacological effects will normally ameliorate the inflammation and other symptoms (Asongalem *et al*., 2004).

In the present results, HEAA at only the dose of 400 mg/kg significantly reduced rat paw edema at all time interval measured, suggesting that the extract can inhibit both phases. The mechanism of action of *A. augusta* extract might be due to its ability to inhibit the release of histamine, bradykinin, serotonin, kinin substance and biosynthesis of prostaglandins which is consistent with the observation from anti-inflammation activity of a reference drug aspirin.

The anti-chronic inflammatory activity of the extract was also investigated in subcutaneous implantation of cotton pellets that provoked foreign body granuloma. The response to subcutaneously implanted cotton pellet in the rat has been divided into three phases: a transudative phase, an exudative phase and a proliferative phase and is a typical feature of established chronic inflammation reaction. The fluid absorbed by the pellet greatly influenced the wet weight of the granuloma and the dry weight correlates well with amount of granulomatous tissues formed (Swingle and Shideman, 1972). Tissue granulation, one of the distinctive features of chronic inflammation, which is composed of marked infiltration macrophages and neovascularization, was induced by subcutaneous implantation of biomaterials. The implanted material induces a host inflammatory response and modulates the release of inflammatory mediators which finally leads to tissue proliferation and granuloma

formation (Rames and Williams, 1992; Tang and Eaton, 1995; Hu *et al*., 2001). Monocyte infiltration and fibroblast proliferation rather than neutrophil infiltration and exudates take place in chronic inflammation. This proliferation becomes widespread by the proliferation of small vessels or granuloma (Hosseinzadeh *et al*., 2000). Nonsteroidal anti-inflammatory drugs (NSAIDs) decrease the size of granuloma which resulted from cellular reaction by inhibiting granulocyte infiltration, preventing generation of collagen fiber and suppressing mucopolysaccharides (Suleyman *et al*., 1999; Ramprasath *et al*., 2004). Activated monocytes can release a series of pro-inflammatory cytokines, inducing tumor necrosis factor-α (TNF-α) (Laupattarakasem *et al*., 2006). TNF-α facilitates inflammatory cell infiltration by promoting the adhesion of neutrophils and lymphocytes to endothelial cell (Dore and Sirois, 1996). Additionally, TNF-α stimulates neutrophils to transcribe and release cytokines and chemokines biosynthesis (Marucha *et al*., 1990; Fernandez *et al*., 1996). Interaction between these mediators thus enhances further inflammatory reactions (Gouwy e*t al*., 2005) and inhibition of TNF-α release can reduce the severity of inflammation. Cellular accumulations and pro-inflammatory cytokines, TNF-α and IL-1β were demonstrated within the first 2 week (Dalu *et al*., 2000).

In the present study, the results showed that hexane extract of *A. augusta* seeds at oral doses of 100, 200 and 400 mg/kg did not significantly inhibit granuloma formation, both dry and wet weight of granuloma, on cotton pellet-induced granuloma formation in rats. These results suggest that the extract can not inhibit the transudative, exudative and proliferative phases of inflammation as well as monocytes infiltration and fibroblast proliferation.

Antipyretic activity

Fever may be resulted of infection or one of the sequelae of tissue damages, inflammation, graft injection or other disease states. Antipyretics are drugs which reduce the elevated body temperature. Regulation of body temperature requires a delicate balance between production and loss of heat and the hypothalamus regulates the set point at which body temperature is maintained. In fever, this set point elevates and a drugs like paracetamol dose not influence body temperature when it is elevated by the factor such as exercise or increase in ambient temperature. Prostaglandins,

mainly PGE_2 alters metabolism of thermoregulatory cells in the hypothalamus resulting in an increase of the set point for thermoregulation to a higher temperature (Wemgerl, 1995; Vander, 2001; Guyton and Hall, 2000a).

Brewer's yeast is the most important microorganism for producing fermented beverages. It is a eukaryote and belongs to the fungi. All beer-brewing stains of yeast are placed in the genus *Saccharomyces* and species *cerevisiae*. Viewed under the light microscope, a single cell is spherical or ellipsoidal in shape and is 5-13 μm across (Lewis and Young, 1995). Yeast-induced fever is called pathogenic fever. Its etiology includes production of prostaglandins, which set the thermoregulatory center at a higher set point. It could stimulate phagocytes to release endogenous pyrogens which circulates in blood to act on the thermoregulatory center in the hypothalamus. The endogenous pyrogens produce and activate IL-1 and prostaglandins, mainly PGE_2 which alter metabolism of thermoregulatory cells via cAMP secondary messenger-mediated mechanism. The result is an increase in set point for thermoregulation to a higher temperature. So, inhibition of prostaglandin synthesis could be the possible mechanism of antipyretic action such as acetylsalicyclic acid (Howard, 1993; Rawlins and Postgrad, 1973).

In brewer's yeast induced pyrexia test, the present results in this study showed that *A. augusta* extract at the dose 400 mg/kg and a reference drug aspirin suppressed fever induced by yeast in rats and possible mechanisms to reduce fever may be due to inhibition of the synthesis of prostaglandin E_2 within the hypothalamus in the CNS which is the final common pathway responsible for fever induction.

As previously mentioned in the experiments, aspirin and morphine were used as reference drugs to investigate the possible antinociceptive, anti-inflammatory and antipyretic activities of *A. augusta* extract incorporated with various essential tests for analgesic (writhing, formalin, hot plate and tail flick tests in mice), anti-inflammatory (carrageenan-induced rat paw edema and cotton pellet-induced granuloma formation in rats) and antipyretic (brewer's yeast-induced fever in rats) activities. It could be suggested that *A. augusta* extract showed analgesic effect similar to both morphine and aspirin, whereas the effect of anti-inflammatory and antipyretic activity is seemed similar to aspirin.

In summary, in the present investigation it could be concluded that the hexane extract of *A. augusta* at tested doses exhibited the pharmacological activities as follows:

1. In analgesic activity, the hexane extract of *A. augusta* showed the antinociceptive responses in acetic acid-induced writhings, formalin, hot plate and tail flick tests, and analgesic mechanisms were likely to aspirin via peripheral mechanism and/or morphine via central mechanism depending on the test used. So, the extract exhibited the analgesic activity via peripheral and central mechanisms both at spinal and supraspinal levels.

2. In anti-inflammatory activity, the hexane extract of *A. augusta* exhibited the anti-inflammatory activity in acute phase by inhibition of the rat paw edema, the possible mechanism of the observed anti-inflammatory activity might be related to its ability to inhibit the release of bradykinin or prostaglandins synthesis, but the extract did not have anti-inflammatory activity in chronic inflammation using cotton pellet-induced granuloma formation in rats.

3. In antipyretic activity, the hexane extract of *A. augusta* suppressed fever induced by yeast in rats. This antipyretic mechanism is probably due to inhibition of prostaglandin E_2 synthesis within the hypothalamus.

Therefore, the hexane extract of *A. augusta* seeds used in traditional medicine is proved to have an analgesic, anti-inflammatory and antipyretic activities.

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APPENDIX

APPENDIX-1

Extraction procedure

The seeds of *Abroma augusta* (3 kg)

↓

Air-dried at room temperature

↓

Pulverized to powder (2.9 kg)

↓

Macerated with 6 L of n-hexane for 7 days for 2 times

↓

Filtration

↓

Evaporated by Rotary Evaporator at 40-60 °C

↓

Oil-like brownish extract (yield 23.02% w/w)

APPENDIX-2

Agent preparation

1. Normal saline (0.9% NaCl)
   Dissolve 0.9 g of sodium chloride with 100 ml of distilled water
2. 2.5% (v/v) formalin
   Dilute 0.676 ml of 37% formalin with normal saline to produce 10 ml
3. 0.6% (v/v) acetic acid
   Dilute 0.06 ml of acetic acid with normal saline to produce 10 ml
4. 1% (w/v) carrageenan
   Dissolve 0.1 g of carrageenan with 10 ml of normal saline
5. 20% (w/v) Brewer's yeast
   Dissolve 2 g of Brewer's yeast with 10 ml of normal saline
6. Morphine sulfate and naloxone
   Dissolve Morphine or naloxone with normal saline
7. Aspirin, hexane extract of *Abroma augusta* (HEAA) at the dose of 100, 200, 400 mg/kg
   Dissolve aspirin or HEAA with cosolvent (Propylene glycol: Tween 80: Distilled water at the ratio 3:1:6, respectively)

Dosing for oral administration

Hexane extract of *Abroma augusta* (HEAA) and aspirin administered orally in a constant volume, 10 ml/kg for mice and 5 ml/kg for rats.

APPENDIX-3

Effect of the hexane extract of *Abroma augusta* (HEAA) and aspirin on acetic acid induced writhing in mice.

Group	**Number**	**Weight (g)**	**Number of writhing**	**Group**	**Number**	**Weight (g)**	**Number of writhing**
Control (cosolvent, po)	1	34	65	Aspirin 200 mg/kg, po	1	38	25
	2	30	67		2	34	30
	3	37	57		3	34	30
	4	39	57		4	33	28
	5	35	64		5	32	27
	6	38	66		6	32	28
	Mean±S.E.M		62.67±1.84		Mean±S.E.M		28.0±0.77

Group	Number	Weight (g)	Number of writhing	Group	Number	Weight (g)	Number of writhing
HEAA 100 mg/kg, po	1	30	39	HEAA 200 mg/kg, po	1	38	32
	2	40	36		2	38	33
	3	39	48		3	31	30
	4	33	42		4	40	33
	5	37	35		5	34	30
	6	32	42		6	35	31
	Mean±S.E.M		40.33±1.94		Mean±S.E.M		31.50±0.56

Group	Number	Weight (g)	Number of writhing
HEAA 400 mg/kg, po	1	39	24
	2	34	28
	3	39	26
	4	33	25
	5	31	28
	6	33	27
	Mean±S.E.M		26.33±0.67

Effect of the hexane extract of *Abroma augusta* (HEAA), morphine and aspirin on 2.5% formalin induced paw licking in mice.

Group	**Number**	**Weight (g)**	**Licking of hind paw (sec)**	
			Early phase (0-5 min)	**Late phase (15-30 min)**
Control (cosolvent, po)	1	37	102.69	195.42
	2	32	110.87	208.51
	3	35	103.49	207.80
	4	35	109.04	175.45
	5	33	115.40	233.96
	6	33	101.09	243.06
	Mean±S.E.M		107.10±2.27	210.70±1.01

Group	Number	Weight (g)	Licking of hind paw (sec)	
			Early phase (0-5 min)	Late phase (15-30 min)
Morphine (5 mg/kg, sc)	1	33	46.07	32.98
	2	40	29.33	21.13
	3	36	39.57	23.98
	4	40	48.11	29.69
	5	30	30.59	20.20
	6	30	29.25	21.43
	Mean±S.E.M		37.15±3.52	24.90±2.14

Group	Number	Weight (g)	Licking of hind paw (sec)	
			Early phase (0-5 min)	Late phase (15-30 min)
Aspirin (200 mg/kg, po)	1	36	35.42	40.88
	2	35	43.99	42.45
	3	33	35.11	40.47
	4	34	37.42	43.11
	5	37	52.69	47.21
	6	31	55.33	45.09
	Mean±S.E.M		43.33±3.64	43.20±1.05

Group	Number	Weight (g)	Licking of hind paw (sec)	
			Early phase (0-5 min)	Late phase (15-30 min)
HEAA (100 mg/kg, po)	1	36	65.39	133.78
	2	31	71.39	138.32
	3	36	69.11	122.75
	4	38	73.49	138.04
	5	33	70.31	130.88
	6	33	75.46	131.71
	Mean±S.E.M		70.86±1.43	132.58±2.34

Group	Number	Weight (g)	Licking of hind paw (sec)	
			Early phase (0-5 min)	Late phase (15-30 min)
HEAA (200 mg/kg, po)	1	38	63.07	98.96
	2	39	58.18	92.39
	3	32	69.39	71.12
	4	34	61.30	74.03
	5	32	66.06	59.10
	6	32	51.73	63.15
	Mean±S.E.M		61.62±2.53	76.46±6.51

Group	Number	Weight (g)	Licking of hind paw (sec)	
			Early phase (0-5 min)	Late phase (15-30 min)
HEAA (400 mg/kg, po)	1	36	50.04	39.74
	2	35	43.21	30.11
	3	33	51.03	38.84
	4	34	52.40	42.41
	5	37	52.22	48.31
	6	31	43.02	42.93
	Mean±S.E.M		48.65±1.75	40.39±2.46

Effect of the hexane extract of *Abroma augusta* (HEAA), morphine and naloxone on nociceptive responses in hot plate test in mice.

Group	**Number**	**Weight (g)**	**Latency of nociceptive response (sec)**					
			Initial response	**30 min**	**45 min**	**60 min**	**75 min**	**90 min**
Control (cosolvent, po)	1	30	7.5	9.7	10.6	9.6	10.0	10.3
	2	38	7.8	13.3	10.8	11.9	12.3	11.7
	3	33	11.8	10.7	11.5	9.5	11.5	10.5
	4	34	6.2	8.3	9.8	11.1	9.8	9.6
	5	32	8.4	11.4	14.5	9.9	8.8	10.3
	6	31	9.9	6.7	7.5	9.6	7.8	10.2
		Mean±S.E.M	8.60±0.81	10.01±0.95	10.78±0.93	10.27±0.41	10.03±0.68	10.43±0.28

Group	Number	Weight (g)	Latency of nociceptive response (sec)					
			Initial response	**30 min**	**45 min**	**60 min**	**75 min**	**90 min**
Morphine (5 mg/kg, sc)	1	34	12.3	15.1	17.0	17.5	16.3	15.2
	2	32	8.4	15.2	20.4	19.9	17.4	14.1
	3	32	10.9	16.4	15.9	17.3	15.2	15.8
	4	36	13.2	16.1	15.4	18.9	17.3	15.4
	5	34	9.8	14.5	17.3	17.9	16.1	16.4
	6	33	8.5	15.3	18.4	18.8	16.9	15.3
		Mean±S.E.M	10.5±0.81	15.43±0.28	17.4±0.74	18.38±0.41	16.53±0.34	15.36±0.31

Group	Number	Weight (g)	Latency of nociceptive response (sec)					
			Initial response	30 min	45 min	60 min	75 min	90 min
HEAA (100 mg/kg, po)	1	38	8.9	8.5	10.6	10.0	12.3	12.8
	2	35	8.3	11.4	13.3	11.7	10.2	10.6
	3	33	8.6	11.4	10.3	11.3	11.5	13.4
	4	29	8.8	13.9	18.0	11.1	10.4	12.9
	5	33	8.1	11.5	10.2	10.3	10.0	10.2
	6	32	10.6	12.1	10.8	11.5	13.2	13.4
		Mean±S.E.M	8.9±0.36	11.5±0.71	12.2±1.25	11.0±0.28	11.3±0.53	12.2±0.59

Group	Number	Weight (g)	Latency of nociceptive response (sec)					
			Initial response	**30 min**	**45 min**	**60 min**	**75 min**	**90 min**
HEAA (200 mg/kg, po)	1	34	11.0	12.3	13.1	14.0	12.1	8.6
	2	36	11.2	13.9	13.3	13.7	13.0	12.6
	3	37	11.2	12.5	17.6	14.0	12.1	14.7
	4	34	13.8	11.4	16.5	13.3	12.4	10.4
	5	36	13.0	11.3	14.3	17.3	12.6	14.3
	6	33	12.2	10.2	16.2	15.6	15.2	16.0
		Mean±S.E.M	12.1±0.47	12.0±0.52	15.17±0.75	14.65±0.62	12.9±0.48	12.7±1.15

Group	Number	Weight (g)	Latency of nociceptive response (sec)					
			Initial response	30 min	45 min	60 min	75 min	90 min
HEAA (400 mg/kg, po)	1	33	10.7	13.2	15.9	16.8	14.2	11.9
	2	35	10.4	13.8	16.3	16.4	12.8	11.3
	3	35	10.4	14.5	15.3	17.4	15.3	13.2
	4	38	11.4	13.3	15.6	17.0	16.2	14.9
	5	32	10.1	13.1	16.5	15.8	13.9	12.6
	6	33	10.5	17.9	18.1	16.7	13.8	15.4
		Mean±S.E.M	10.6±0.18	14.3±0.75	16.28±0.41	16.68±0.22	14.4±0.49	13.2±0.67

Group	Number	Weight (g)	Latency of nociceptive response (sec)					
			Initial response	**30 min**	**45 min**	**60 min**	**75 min**	**90 min**
Naloxone (2 mg/kg, ip)	1	31	11.9	10.9	12.7	13.3	10.0	10.7
	2	32	12.6	11.4	10.5	10.2	10.2	11.8
	3	37	8.9	11.1	10.5	10.0	12.4	9.3
	4	35	12.4	12.4	12.8	12.1	11.4	9.9
	5	35	9.8	13.2	12.0	11.3	10.6	11.5
	6	35	10.6	11.6	10.2	10.9	11.2	10.9
		Mean±S.E.M	11.0±0.61	11.7±0.36	11.5±0.48	11.3±0.51	11.0±0.36	10.7±0.39

Group	Number	Weight (g)	Latency of nociceptive response (sec)					
			Initial response	**30 min**	**45 min**	**60 min**	**75 min**	**90 min**
	1	35	10.9	8.7	9.7	9.2	9.6	8.7
Naloxone	2	31	11.8	17.8	10.0	4.7	7.3	6.0
(2 mg/kg, ip)	3	31	11.7	13.8	11.6	10.6	11.4	6.9
+	4	33	13.5	11.0	10.5	12.5	9.0	5.5
Morphine	5	35	10.4	14.6	12.3	9.7	9.9	10.3
(5 mg/kg, sc)	6	34	11.1	15.5	15.2	8.4	7.3	11.2
		Mean±S.E.M	11.7±0.44	13.6±1.33	11.6±0.83	9.2±1.06	9.1±0.65	8.1±0.96

Group	Number	Weight (g)	Latency of nociceptive response (sec)					
			Initial response	**30 min**	**45 min**	**60 min**	**75 min**	**90 min**
Naloxone (2 mg/kg, ip) + HEAA (400 mg/kg, po)	1	36	9.2	10.2	12.4	11.1	9.2	10.0
	2	31	8.5	10.5	11.5	10.6	12.6	10.0
	3	33	13.6	13.3	9.1	10.5	10.3	10.2
	4	35	10.9	13.1	12.7	11.0	10.9	9.9
	5	32	13.7	10.2	14.7	13.1	8.0	10.2
	6	33	12.9	9.5	12.3	10.9	8.9	13.0
		Mean±S.E.M	11.5±0.92	11.1±0.67	12.1±0.74	11.2±0.39	10.0±0.67	10.5±0.49

Effect of the hexane extract of *Abroma augusta* (HEAA), morphine and naloxone on nociceptive responses in tail flick test in mice.

Group	**Number**	**Weight (g)**	**Latency of nociceptive response (sec)**					
			Initial response	**30 min**	**45 min**	**60 min**	**75 min**	**90 min**
Control (cosolvent, po)	1	34	4.2	5.8	6.9	7.5	8.7	7.7
	2	36	5.3	6.1	7.1	8.1	8.5	8.1
	3	34	5.9	6.3	6.8	8.4	8.9	9.0
	4	32	6.0	6.6	6.7	7.9	8.4	8.9
	5	39	6.3	6.5	6.7	8.5	9.3	8.6
	6	36	5.8	6.4	7.0	8.9	9.5	8.4
		Mean±S.E.M	5.58±0.31	6.28±0.12	6.87±0.07	8.22±0.20	8.88±0.18	8.45±0.20

Group	Number	Weight (g)	Latency of nociceptive response (sec)					
			Initial response	30 min	45 min	60 min	75 min	90 min
Morphine (5 mg/kg, sc)	1	34	6.1	6.8	8.9	9.8	10.7	11.9
	2	31	5.4	7.4	9.1	10.3	11.4	12.5
	3	35	5.0	7.7	9.0	10.4	11.4	12.1
	4	32	6.4	6.9	8.9	9.9	11.0	11.7
	5	40	4.8	7.7	9.2	10.4	10.8	11.3
	6	35	4.7	7.8	8.8	10.7	11.7	12.4
		Mean±S.E.M	5.40±0.29	7.38±0.18	8.98±0.06	10.25±0.14	11.17±0.16	11.98±0.18

Group	Number	Weight (g)	Latency of nociceptive response (sec)					
			Initial response	**30 min**	**45 min**	**60 min**	**75 min**	**90 min**
HEAA (100 mg/kg, po)	1	35	6.4	6.8	8.1	8.6	9.7	10.3
	2	38	6.0	7.2	8.4	8.9	9.8	10.4
	3	32	5.8	7.5	7.9	9.0	9.4	9.8
	4	35	6.7	7.8	8.8	9.3	9.8	10.4
	5	32	6.0	7.2	8.8	9.4	9.5	9.9
	6	30	5.5	6.7	7.8	8.7	9.0	9.5
		Mean±S.E.M	6.07±0.17	7.20±0.17	8.30±0.18	8.98±0.13	9.53±0.13	10.05±0.15

Group	Number	Weight (g)	Latency of nociceptive response (sec)					
			Initial response	30 min	45 min	60 min	75 min	90 min
HEAA (200 mg/kg, po)	1	40	5.7	7.8	8.7	9.0	10.2	10.7
	2	38	6.3	7.4	8.9	9.2	10.5	10.8
	3	34	5.2	6.8	9.3	9.5	9.8	10.5
	4	33	4.8	6.7	8.9	9.4	10.4	11.0
	5	38	5.5	7.0	8.7	9.2	10.9	11.2
	6	37	5.1	7.8	8.3	9.0	9.9	10.0
		Mean±S.E.M	5.43±0.22	7.25±0.20	8.80±0.13	9.22±0.08	10.28±0.17	10.70±0.17

Group	Number	Weight (g)	Latency of nociceptive response (sec)					
			Initial response	**30 min**	**45 min**	**60 min**	**75 min**	**90 min**
HEAA (400 mg/kg, po)	1	34	6.6	7.3	8.4	9.4	10.4	10.7
	2	31	6.1	7.7	8.5	9.7	10.5	10.8
	3	34	7.1	7.6	8.8	8.9	9.8	11.5
	4	32	5.3	7.8	9.3	9.6	10.7	11.3
	5	37	5.4	6.8	8.7	8.6	10.3	11.8
	6	34	5.8	6.9	9.2	9.8	10.9	11.4
		Mean±S.E.M	6.05±0.29	7.35±0.17	8.82±0.15	9.33±0.20	10.43±0.15	11.25±0.17

Group	Number	Weight (g)	Latency of nociceptive response (sec)					
			Initial response	30 min	45 min	60 min	75 min	90 min
Naloxone (2 mg/kg, ip)	1	32	4.7	5.4	6.2	7.3	8.3	7.0
	2	32	6.3	6.2	6.9	7.6	7.4	6.5
	3	35	5.1	5.7	6.8	7.8	8.2	6.3
	4	31	5.7	6.4	7.1	7.9	6.9	6.5
	5	34	4.6	5.5	5.9	6.8	6.9	6.4
	6	31	6.1	6.7	7.2	8.2	6.9	7.0
		Mean±S.E.M	5.41±0.29	5.98±0.22	6.68±0.21	7.60±0.20	7.43±0.27	6.62±0.12

Group	Number	Weight (g)	Latency of nociceptive response (sec)					
			Initial response	**30 min**	**45 min**	**60 min**	**75 min**	**90 min**
	1	35	4.6	6.4	6.8	7.4	7.7	8.2
Naloxone	2	34	7.1	7.4	7.8	8.0	8.3	8.4
(2 mg/kg, ip)	3	36	5.2	6.8	7.1	7.5	7.8	7.9
+	4	35	6.6	7.4	7.9	8.4	8.3	8.4
Morphine	5	35	4.7	5.4	6.6	7.7	8.1	8.0
(5 mg/kg, sc)	6	40	5.4	6.8	7.2	7.8	7.9	8.2
		Mean±S.E.M	5.60±0.42	6.70±0.30	7.23±0.21	7.80±0.15	8.02±0.10	8.18±0.08

Group	Number	Weight (g)	Latency of nociceptive response (sec)					
			Initial response	**30 min**	**45 min**	**60 min**	**75 min**	**90 min**
	1	37	6.4	6.5	6.6	7.6	7.8	7.5
Naloxone	2	37	7.6	7.8	8.1	8.4	8.3	8.4
(2 mg/kg, ip)	3	32	7.4	7.0	7.6	7.9	8.8	9.2
+	4	32	6.0	7.2	7.8	8.3	9.7	8.4
HEAA	5	37	7.8	6.8	7.1	8.1	8.1	8.4
(400 mg/kg, po)	6	34	6.4	7.1	7.4	7.7	8.0	8.2
		Mean±S.E.M	6.93±0.31	7.07±0.18	7.43±0.22	8.00±0.13	8.45±0.29	8.35±0.22

Effect of the hexane extract of *Abroma augusta* (HEAA) and aspirin on carrageenan induced paw edema in rat.

Group	**Number**	**Weight (g)**	**Paw edema volume (ml)**						
			Initial	**0.5 h**	**1 h**	**2 h**	**3 h**	**4 h**	**5 h**
Control (cosolvent, po)	1	200	3.60	5.67	5.80	6.43	6.51	6.88	7.29
	2	200	3.76	5.42	5.90	6.51	6.73	6.84	7.31
	3	191	4.60	5.39	5.51	6.33	6.57	6.91	7.18
	4	180	4.80	5.93	6.03	6.42	6.74	6.82	7.21
	5	180	4.08	5.84	6.01	6.33	6.75	6.78	6.97
	6	188	4.13	5.55	6.10	6.42	6.53	6.80	7.14
		Mean±S.E.M	4.66±0.19	5.63±0.09	5.59±0.09	6.40±0.03	6.63±0.05	6.83±0.02	7.15±0.05

Group	Number	Weight (g)	Paw edema volume (ml)						
			Initial	**0.5 h**	**1 h**	**2 h**	**3 h**	**4 h**	**5 h**
Aspirin (200 mg/kg, po)	1	200	3.95	4.81	5.41	5.53	5.68	5.89	6.23
	2	188	4.36	4.64	5.08	5.21	5.57	5.93	6.33
	3	189	4.41	4.52	5.01	5.14	5.25	5.41	5.42
	4	180	4.64	4.84	5.68	5.72	5.83	6.05	6.22
	5	180	4.05	4.95	4.97	5.33	5.61	6.03	6.31
	6	212	4.10	4.45	4.91	5.37	5.77	5.98	6.28
		Mean±S.E.M	4.25±0.11	4.70±0.08	5.17±0.12	5.38±0.09	5.62±0.08	5.88±0.10	6.13±0.14

Group	Number	Weight (g)	Paw edema volume (ml)						
			Initial	**0.5 h**	**1 h**	**2 h**	**3 h**	**4 h**	**5 h**
HEAA (100 mg/kg, po)	1	200	3.50	5.62	5.83	6.11	6.58	6.94	7.19
	2	200	4.38	5.48	5.92	6.43	6.55	6.63	6.98
	3	187	5.02	5.57	5.83	6.23	6.70	6.81	7.08
	4	182	4.05	5.48	5.91	6.33	6.63	6.69	7.01
	5	180	3.88	5.55	6.03	6.31	6.70	6.81	7.11
	6	189	4.83	5.63	5.83	6.18	6.55	6.70	6.97
		Mean±S.E.M	4.28±0.24	5.56±0.03	5.89±0.03	6.26±0.05	6.61±0.03	6.76±0.05	7.06±0.03

Group	Number	Weight (g)	Paw edema volume (ml)						
			Initial	**0.5 h**	**1 h**	**2 h**	**3 h**	**4 h**	**5 h**
HEAA (200 mg/kg, po)	1	200	4.37	5.11	5.78	5.83	6.25	6.34	6.59
	2	185	4.51	5.20	5.53	5.71	6.34	6.42	6.68
	3	180	3.75	5.14	5.69	5.70	6.48	6.83	6.95
	4	182	4.67	5.41	5.59	5.87	6.65	6.80	7.03
	5	180	4.18	5.16	5.51	5.96	6.40	6.60	6.97
	6	185	4.14	5.15	5.62	5.98	6.59	6.75	7.09
		Mean±S.E.M	4.27±0.13	5.20±0.04	5.62±0.04	5.84±0.05	6.45±0.06	6.62±0.08	6.92±0.08

Group	Number	Weight (g)	Paw edema volume (ml)						
			Initial	**0.5 h**	**1 h**	**2 h**	**3 h**	**4 h**	**5 h**
HEAA (400 mg/kg, po)	1	205	3.32	4.85	5.11	5.25	5.55	5.93	6.43
	2	200	3.41	4.66	5.42	5.72	5.98	6.15	6.41
	3	180	4.67	4.87	5.39	5.58	5.61	6.08	6.33
	4	180	4.16	4.39	5.35	5.61	5.88	6.19	6.53
	5	182	3.67	5.11	5.35	5.59	5.79	6.18	6.37
	6	191	3.93	4.62	5.47	5.70	5.92	6.19	6.43
		Mean±S.E.M	3.86±0.21	4.75±0.10	5.34±0.05	5.58±0.07	5.78±0.07	6.12±0.04	6.42±0.03

Effect of the hexane extract of *Abroma augusta* (HEAA) and aspirin on cotton pellet induced granuloma formation in rats.

Group	Number	Weight (g)	Cotton (mg/cotton)	Granuloma wet weight (mg)	Granuloma dry weight (mg)	Transudative weight (mg)	Granuloma weight (mg/mg cotton)
Control (cosolvent, po)	1	193	19.7	77.9	32.4	45.5	2.31
	2	189	20.0	82.4	34.1	48.3	2.42
	3	203	19.8	78.5	38.4	40.1	2.03
	4	189	20.0	92.5	35.8	56.7	2.84
	5	200	19.9	92.3	40.1	52.2	2.62
	6	195	20.6	90.4	39.3	51.1	2.48
		Mean±S.E.M	20.00±0.13	85.67±2.08	36.68±1.26	49.00±2.35	2.45±0.11

Group	Number	Weight (g)	Cotton (mg/cotton)	Granuloma wet weight (mg)	Granuloma dry weight (mg)	Transudative weight (mg)	Granuloma weight (mg/mg cotton)
Aspirin (200 mg/kg, po)						21.6	
	1	206	20.1	44.1	22.5	21.7	1.07
	2	198	20.0	55.3	33.6	21.6	1.09
	3	200	19.5	50.4	28.8	21.1	1.11
	4	187	19.8	49.4	28.3	22.0	1.07
	5	186	19.3	51.2	29.2	20.0	1.14
	6	191	19.5	40.7	20.7	21.33±0.29	1.03
		Mean±S.E.M	19.70±0.13	48.52±2.15	27.18±1.94		1.09±0.02

Group	Number	Weight (g)	Cotton (mg/cotton)	Granuloma wet weight (mg)	Granuloma dry weight (mg)	Transudative weight (mg)	Granuloma weight (mg/mg cotton)
HEAA (100 mg/kg, po)	1	185	20.9	73.0	44.2	28.8	1.38
	2	198	20.2	92.4	32.8	59.6	2.95
	3	187	19.9	91.7	43.3	48.4	2.43
	4	186	19.5	93.5	38.1	55.4	2.84
	5	201	19.5	85.9	42.2	43.7	2.24
	6	204	20.5	89.6	41.0	48.6	2.37
		Mean±S.E.M	20.08±0.23	87.68±3.13	40.27±1.73	47.42±4.38	2.37±0.23

Group	Number	Weight (g)	Cotton (mg/cotton)	Granuloma wet weight (mg)	Granuloma dry weight (mg)	Transudative weight (mg)	Granuloma weight (mg/mg cotton)
HEAA (200 mg/kg, po)					56.2		
	1	200	18.9	87.5	42.2	31.3	1.67
	2	204	20.2	83.4	38.2	41.2	2.04
	3	198	19.8	96.2	38.3	58.0	3.00
	4	199	20.6	92.7	29.4	54.4	2.64
	5	107	19.9	73.7	30.1	44.3	2.23
	6	203	20.0	80.1	39.07±4.00	50.0	2.50
		Mean±S.E.M	20.00±0.23	85.60±3.38		46.53±3.96	2.35±0.20

Group	Number	Weight (g)	Cotton (mg/cotton)	Granuloma wet weight (mg)	Granuloma dry weight (mg)	Transudative weight (mg)	Granuloma weight (mg/mg cotton)
HEAA (400 mg/kg, po)				91.7			
	1	188	20.1	82.3	35.4	56.3	2.80
	2	189	20.7	84.1	43.0	39.3	1.90
	3	200	20.3	88.3	30.2	53.9	2.66
	4	199	19.8	92.4	37.0	51.3	2.60
	5	186	20.5	78.5	48.1	44.3	2.16
	6	192	19.8	86.21±2.25	44.5	34.0	1.72
		Mean±S.E.M	20.20±0.15		39.70±2.17	46.52±3.59	2.30±1.81

Effect of the hexane extract of *Abroma augusta* (HEAA) and aspirin on Brewer's yeast induced pyrexia in rats.

Group	**Number**	**Weight (g)**	**Rectal temperature (°C)**						
			Before yeast Injection(-19)	**After yeast Injection(0)**	**After treatment**				
					1	**2**	**3**	**4**	**5**
Control (cosolvent, po)	1	180	36.80	38.00	38.07	37.93	37.86	37.70	37.47
	2	220	36.78	38.45	38.37	38.11	37.82	37.63	37.41
	3	209	36.84	37.39	37.82	37.92	37.74	37.59	37.36
	4	207	36.32	37.12	37.79	37.68	37.48	37.38	37.29
	5	196	36.20	36.97	37.01	36.94	36.75	36.55	36.38
	6	199	36.18	36.92	37.12	36.81	36.77	36.63	37.40
		Mean±S.E.M	36.52±0.13	37.48±0.25	37.81±0.22	37.57±0.23	37.40±0.21	37.25±0.21	37.22±0.17

Group	Number	Weight (g)	Rectal temperature (°C)						
			Before yeast Injection(-19)	After yeast Injection(0)	After treatment				
					1	2	3	4	5
Aspirin (200 mg/kg, po)	1	180	36.50	37.65	37.32	37.24	37.02	36.63	36.58
	2	190	36.23	37.21	37.01	36.67	36.23	36.20	36.19
	3	204	36.36	37.22	37.04	36.88	36.55	36.44	36.33
	4	191	36.21	37.65	37.29	36.73	36.52	36.47	36.23
	5	209	36.26	36.97	36.35	36.38	36.20	36.17	36.23
	6	210	36.16	37.01	36.61	36.52	36.44	36.23	36.19
		Mean±S.E.M	36.29±0.05	37.29±0.12	36.92±0.16	36.73±0.13	36.49±0.12	36.36±0.08	36.29±0.06

Group	Number	Weight (g)	Rectal temperature (°C)						
			Before yeast Injection(-19)	After yeast Injection(0)	After treatment				
					1	2	3	4	5
HEAA (100 mg/kg, po)	1	180	36.80	37.99	37.86	37.66	37.68	37.53	37.32
	2	194	36.66	37.39	37.42	37.44	37.41	37.39	37.20
	3	196	37.05	38.45	38.32	37.98	37.65	37.14	36.93
	4	194	37.11	37.90	37.84	37.63	37.51	37.31	37.28
	5	203	36.46	37.25	37.82	37.56	37.27	37.10	36.98
	6	214	35.59	36.33	36.82	36.84	36.54	36.50	36.51
		Mean±S.E.M	36.61±0.23	37.55±0.30	37.68±0.21	37.52±0.15	37.34±0.17	37.16±0.15	37.04±0.12

Group	Number	Weight (g)	Rectal temperature (°C)						
			Before yeast Injection(-19)	After yeast Injection(0)	After treatment				
					1	2	3	4	5
HEAA (200 mg/kg, po)	1								
	2	190	36.30	37.50	37.84	37.76	37.48	37.38	37.14
	3	194	37.09	38.14	37.98	37.52	37.60	37.43	37.19
	4	200	36.73	38.00	38.03	37.82	37.74	37.59	37.20
	5	198	36.95	37.69	37.75	37.84	37.30	37.32	37.23
	6	212	35.77	36.48	36.53	36.52	36.32	35.95	35.95
		201	36.09	36.81	36.83	36.52	36.57	36.42	36.18
		Mean±S.E.M	36.49±0.21	37.44±0.27	37.49±0.26	37.33±0.26	37.17±0.24	37.02±0.27	36.82±0.24

Group	Number	Weight (g)	Rectal temperature (°C)						
			Before yeast Injection(-19)	After yeast Injection(0)	After treatment				
					1	2	3	4	5
HEAA									
(400 mg/kg, po)	1	206	37.30	38.00	37.81	37.61	37.37	36.36	36.29
	2	195	36.71	37.76	37.23	37.05	36.73	36.52	36.39
	3	192	36.83	37.81	37.24	36.93	36.57	36.50	36.38
	4	186	36.59	37.58	36.84	36.75	36.66	36.55	36.45
	5	210	36.14	36.87	36.42	36.27	36.17	36.12	36.11
	6	206	36.24	36.96	36.44	36.46	36.21	36.20	36.21
		Mean±S.E.M	36.64±0.17	37.50±0.20	37.00±0.23	36.85±0.20	36.62±0.18	36.38±0.07	36.31±0.05

ที่ ศธ 0521.11/ ๑๗๙

สำนักวิจัยและพัฒนา
มหาวิทยาลัยสงขลานครินทร์
อ.หาดใหญ่ จ.สงขลา 90110

Ref.18/52

หนังสือรับรอง

โครงการวิจัย เรื่อง **การศึกษาฤทธิ์ระงับปวด ลดไข้ และต้านการอักเสบของน้ำมันสกัดจากเมล็ดเทียนดำในสัตว์ทดลอง**

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ให้ไว้ ณ วันที่ 26 พฤษภาคม 2552

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