Evaluation of Analgesic, Antipyretic and Anti-inflammatory Activities of the Ethanol and Dichloromethane Extract from *Scaphium lychnophorum* Fruit in Experiment Animals

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ชื่อวิทยาศาสตร์	การประเมินฤทธิ์ระงับปวด ลดไข้ และต้านการอักเสบของสารสกัดเอธานอลและไดคลอโรมีเธนจากลูกสำรองในสัตว์ทดลอง
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บทคัดย่อ

สำรอง มีชื่อทางวิทยาศาสตร์ว่า *Scaphium lychnophorum* (Hance) Pierre อยู่ในวงศ์ Sterculiaceae พืชชนิดนี้ส่วนใหญ่กระจายอยู่ในประเทศเวียดนาม ไทย (อุบลราชธานี และจันทบุรี) มาเลเซีย อินโดนีเซีย และตอนล่างของจีน องค์ประกอบทางเคมีในสำรองส่วนใหญ่ประกอบด้วยสารกลุ่ม polysaccharides เช่น glucose, rhamnose, arabinose, galactose, galacturonic acid และ xylose สรรพคุณทางสมุนไพรของสำรองมีรายงานว่า ใช้ในการรักษาอาการปวด แก้ไอและขับเสมหะ และใช้ลดไข้ วัตถุประสงค์ในการศึกษาครั้งนี้เพื่อประเมินฤทธิ์ในการระงับปวด ลดไข้ และต้านการอักเสบของสารสกัดเอธานอลและไดคลอโรมีเธนจากลูกสำรองในขนาด 50, 100 และ 200 มก/กก ในหนูถีบจักรและหนูขาวใหญ่เพศผู้

ในการทดสอบความเป็นพิษเฉียบพลันในหนูถีบจักรหรือหนูขาวใหญ่เพศผู้ พบว่า สัตว์ทดลองที่ได้รับสารสกัดเอธานอลหรือไดคลอโรมีเธนจากลูกสำรองในขนาดสูงสุด 5 กรัมต่อกก.ทางปาก จะไม่เกิดอาการแสดงถึงความเป็นพิษและไม่ทำให้สัตว์ทดลองตาย ดังนั้น ค่าประมาณของ LD_{50} เมื่อบริหารทางปากในหนูถีบจักรและหนูขาวใหญ่มีค่ามากกว่า 5 ก/กก ในการศึกษาผลของสารสกัดเอธานอล และไดคลอโรมีเทนจากลูกสำรองต่อการตอบสนองต่อความเจ็บปวดจะใช้วิธี writhing, formalin, hot plate และ tail flick ในหนูถีบจักร และฤทธิ์ลดไข้ของสารสกัดจะศึกษาฤทธิ์ในการลดไข้โดยใช้ yeast เหนี่ยวนำให้เกิดไข้ในหนูขาวใหญ่ ส่วนการทดสอบฤทธิ์ต้านการอักเสบจะใช้โมเดล ที่ทำให้เกิดการอักเสบจากการฉีด carrageenan บริเวณอุ้งเท้าหลังของหนูขาวใหญ่เพื่อเหนี่ยวนำให้เกิดการบวมและ cotton pellet เหนี่ยวนำการสร้าง granuloma ในหนูขาวใหญ่ ผลการศึกษาฤทธิ์ระงับปวด ลดไข้ และต้านการอักเสบของสารสกัดเอธานอลและไดคลอโรมีเทนจากลูกสำรอง พบว่าสารสกัดเอธานอลจากลูกสำรอง (ethanol extract of *Scaphium lychnophorum*, EESL) ในขนาดที่ใช้ในการศึกษามีฤทธิ์ระงับปวด ลดไข้ และต้านการอักเสบในสัตว์ทดลอง แต่สารสกัดไดคลอโรมีเทนจากลูกสำรอง (dichloromethane extract of *Scaphium lychnophorum*, DESL) ในขนาดที่ใช้ในการศึกษาครั้งนี้ไม่มีฤทธิ์ระงับปวด ลดไข้ และต้านการอักเสบในสัตว์ทดลอง

ในการทดสอบฤทธิ์ระงับปวดโดย writhing test พบว่า EESL ในขนาด 50, 100 และ 200 มก/กก ทางปาก สามารถลดจำนวนการเกิด writhings จากการฉีดกรดอะซิติคเข้าทางช่องท้อง อย่างมีนัยสำคัญทางสถิติ โดยมีฤทธิ์ในการยับยั้งการเกิด writhings ร้อยละ 13.95, 35.4 และ 62.79 ตามลำดับเมื่อเปรียบเทียบกับกลุ่มควบคุมและขึ้นกับขนาดของสารสกัด เมื่อเปรียบเทียบฤทธิ์ระงับปวดกับยา aspirin ซึ่งใช้เป็นยาอ้างอิงในการทดสอบ พบว่ายา aspirin ยับยั้งจำนวน การเกิด writhings ได้ร้อยละ 64.6 ดังนั้น EESL ในขนาด 200 มก/กก ทางปาก จึงมีความแรงในการระงับปวดเทียบเคียงได้กับยา aspirin ขนาด 200 มก/กก ทางปาก ในการทดสอบฤทธิ์ระงับปวดโดยใช้ formalin test เป็นโมเดล ในการศึกษา พบว่า EESL ในขนาด 50, 100 และ 200 มก/กก ทางปาก ลด total licking time ทั้งใน early และ late phases อย่างมีนัยสำคัญทางสถิติ ส่วนในการทดสอบฤทธิ์ระงับปวดโดยใช้ hot plate test พบว่า EESL ในขนาด 50, 100 และ 200 มก/กก ทางปาก มีฤทธิ์ระงับปวดโดยทำให้สัตว์ทดลองทนต่อความเจ็บปวดที่เหนี่ยวนำด้วยความร้อนได้นานขึ้นอย่างมีนัยสำคัญทางสถิติ ยา naloxone ซึ่งเป็นยาต้านฤทธิ์ของยา morphine เมื่อให้ก่อนยา morphine (5 มก/กก ฉีดเข้าใต้ผิวหนัง) หรือ EESL (200 มก/กก ทางปาก) สามารถต้านฤทธิ์ในการระงับปวดของทั้ง morphine และ EESLได้เมื่อเปรียบเทียบกับกลุ่มที่ได้รับยา morphine หรือ DESL อย่างเดียว ในทำนองเดียวกันในกรณีทดสอบฤทธิ์ระงับปวดโดยใช้ tail flick test ผลการทดลองพบว่า EESL ในขนาด 50, 100 และ 200 มก/กก ทางปาก มีฤทธิ์ระงับปวดอย่างมีนัยสำคัญทางสถิติ โดยทำให้สัตว์ทดลองทนต่อความเจ็บปวดจาก tail flick test ได้เมื่อเปรียบเทียบกับกลุ่มควบคุม และฤทธิ์ในการระงับปวดขึ้นกับขนาดของสารสกัดซึ่งถ้าขนาดของสารสกัดสูง (100 และ 200 มก/กก ทางปาก) จะออกฤทธิ์ระงับปวด (onset) ได้เร็วโดยมีฤทธิ์ระงับปวดในทุกช่วงเวลา แต่ถ้าขนาดของสารสกัดต่ำ (50 มก/กก ทางปาก) จะออกฤทธิ์ในการระงับปวดได้ช้ากว่าโดยพบว่ามีฤทธิ์ระงับปวดในช่วงเวลา 45, 60, 75 และ 90 นาทีเมื่อเปรียบเทียบกับกลุ่มที่ได้รับยา morphine หรือ EESL เพียงอย่างเดียว และในทำนองเดียวกันยา naloxone ซึ่งเป็นยาต้านฤทธิ์ของยา morphine สามารถต้านฤทธิ์การระงับปวดของทั้งยา morphine (5 มก/กก ฉีดเข้าใต้ผิวหนัง) และ EESL (200 มก/กก ทางปาก) ใน tail flick test ได้ ในการทดสอบฤทธิ์ลดไข้ของEESLในหนูขาวใหญ่โดยทำให้หนูขาวใหญ่เกิดไข้ด้วยการฉีดสารสกัดyeast พบว่า EESL ในขนาด 50, 100 และ 200 มก/กก ทางปาก สามารถลดไข้ได้อย่างมีนัยสำคัญทางสถิติ ส่วนการทดสอบฤทธิ์ต้านการอักเสบของ EESL พบว่า EESL ในขนาด 100 และ 200 มก/กก ทางปาก ลดการอักเสบใน carrageenan-induced rat paw edema ได้อย่างมีนัยสำคัญทางสถิติ เมื่อเปรียบเทียบกับกลุ่มควบคุม ในขณะที่ EESL ในขนาด 200 มก/กก ทางปาก สามารถลดการเกิด granuloma ใน cotton pellet-induced granuloma model ได้

จากผลการทดลองทั้งหมดนี้ กลไกการออกฤทธิ์ในการระงับปวดของ EESL น่าจะออกฤทธิ์ที่ระบบประสาทส่วนกลางบริเวณ peripherally และ centrally ในระดับ spinal และ supraspinal คล้ายกับยา morphine และ aspirin ส่วนกลไกในการลดไข้ของ EESL ในหนูขาวใหญ่คล้ายกับยา aspirin กล่าวคือ อาจยับยั้งการสังเคราะห์ prostaglandins และกลไกในการต้านการอักเสบของ EESL อาจเกี่ยวข้องกับการยับยั้งการหลั่งสารที่ทำให้เกิดการอักเสบที่เกิดขึ้นในกระบวนการการอักเสบ เช่น histamine, serotonin, bradykinin, prostaglandins และ TNF-α

โดยสรุป ผลจากการทดลองในครั้งนี้แสดงให้เห็นว่า สารสกัดเอธานอลจากลูกสำรองมีฤทธิ์ระงับปวด ลดไข้ และต้านการอักเสบ ซึ่งสนับสนุนในการนำสารสกัดเอธานอลจากลูกสำรองมาใช้ในแพทย์แผนไทยโดยใช้ระงับปวด ลดไข้ และต้านการอักเสบ

Thesis Title	Evaluation of Analgesic, Antipyretic and Anti-inflammatory Activities of the Ethanol and Dichloromethane Extract from *Scaphium lychnophorum* Fruit in Experiment Animals
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ABSTRACT

Scaphium lychnophorum (*S. lychnophorum*) (Hance) Pierre is in the Sterculiaceae family which is commonly known in Thai as Samrong. This plant is mainly distributed in Vietnam, Thailand (Ubon Ratchathani and Chanthaburi), Malaysia, Indonesia, as well as South China. The major chemicals of *S. lychnophorum* are polysaccharides such as glucose, rhamnose, arabinose, galactose, galacturonic acid, and xylose. The traditional use of *S. lychnophorum* has been claimed to treat pain, cough and clear phlegm, and used as antipyretics. The aim of this study was therefore to evaluate the analgesic, antipyretic and anti-inflammatory activities of the ethanol (EESL) and dichloromethane (DESL) extract from *S. lychnophorum* fruit at doses of 50, 100 and 200 mg/kg in male mice and rats.

In acute toxicity test, when the extract (EESL, DESL) was given up to 5 g/kg orally in male mice and rats, the animals did not show any signs of toxicity and mortality. Thus, the estimated oral LD_{50} of EESL and DESL was more than 5 g/kg. The effects of ethanol and dichloromethane extracts of *Scaphium lychnophorum* fruit were investigated on nociceptive responses using writhing, formalin, hot plate, and tail flick tests in mice, and antipyretic activity was determined in yeast-induced pyrexia in rats. The anti-inflammatory activity was also assessed using carrageenan-induced hind paw edema and cotton pellet-induced granuloma in rats. The results indicated that only the ethanol extract from *S. lychnophorum* fruit (EESL) exhibited analgesic, antipyretic and anti-inflammatory activities in all experimental animal models used in this investigation, whereas dichloromethane extract fraction from *S. lychnophorum* fruit (DESL) did not demonstrat these activities.

In writhing test, EESL (50, 100 and 200 mg/kg, po) significantly produced a dose-related in inhibition of writhing responses induced by acetic acid by

13.95 %, 35.40 % and 62.79%, respectively when compared to control, whereas a reference drug aspirin significantly inhibited writhing responses by 64.6%. The potency of analgesic activity of EESL at the dose of 200 mg/kg could be comparable to a reference drug aspirin (200 mg/kg, po). In formalin test, EESL (50, 100 and 200 mg/kg, po) significantly showed a dose-dependent in reducing of total licking time both in early and late phases. In hot plate test, EESL at doses of 50, 100 and 200 mg/kg significantly increased the latency of nociceptive responses. Naloxone, an opioid antagonist, given before morphine (5 mg/kg, sc) or EESL (200 mg/kg, po) significantly suppressed the action of morphine or EESL on the latency of nociceptive responses when compared to morphine or EESL alone. In tail flick test, EESL at doses of 100 and 200 mg/kg, po significantly increased the tail flick latency at all the interval times measured in this experiment, whereas a low dose of EESL (50 mg/kg, po) significantly increased the reaction time at only the time measured at 45, 60, 75 and 90 minutes. In similar to the hot plate test, naloxone given before morphine (5 mg/kg, sc) or EESL (200 mg/kg, po) significantly decreased latency of nociceptive responses at the time 45, 60, 75 and 90 min when compared to morphine or EESL alone. In antipyretic activity screening, EESL (50, 100 and 200 mg/kg, po) significantly reduced fever induced by yeast in rats. In anti-inflammatory activity models, only EESL at doses of 100 and 200 mg/kg, po significantly decreased carrageenan-induced rat paw edema, whereas EESL (200 mg/kg, po) reduced granuloma formation in cotton pellet-induced granuloma model in rats.

Based on these results, analgesic mechanisms of EESL are likely to be mediated both peripherally and centrally (spinal and supraspinal levels) in the central nervous system in similar to a reference drug aspirin and morphine, whereas the antipyretic action of EESL is proposed to inhibit prostaglandin biosynthesis in similar to a reference drug aspirin. The possible anti-inflammatory mechanisms of action of EESL may involve inhibition of inflammatory mediators release such as histamine, serotonin, bradykinin, prostaglandins and TNF-α during inflammatory processes.

In conclusion, these findings suggest that ethanol extract of *S. lychnophorum* fruit possesses analgesic, antipyretic and anti-inflammatory activities. These results provide evidence to support their traditional use in folklore medicine.

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LIST OF ABBREVIATIONS AND SYMBOLS

AC	=	Adenylate cyclase
ASP	=	Acidic polysaccharide
ATP	=	Adenosine triphosphate
cAMP	=	Cyclic adenosine monophosphate
CNS	=	Central nervous system
COX	=	Cyclooxygenase enzyme
CYP	=	Cytochrome P450
DESL	=	Dichrolomethane extract of *Scaphium lychnophorum*
DRG	=	Dorsal root ganglion
EESL	=	Ethanol extract of *Scaphium lychnophorum*
g	=	Gram
GI	=	Granuloma inhibition
h	=	Hour
IL-1β	=	Interleukin- 1β
IL-6	=	Interleukin-6
IL-8	=	Interleukin-8
IM	=	Intramuscular
ip	=	Intraperitoneal
IV	=	Intravenous
kg	=	Kilogram
L	=	Liter
LD_{50}	=	Lethal dose at 50% of motality animal
m	=	Meter
mg	=	Milligram
min	=	Minute
ml	=	Milliliter
mM	=	Millimolar
M3G	=	Morphine-3-glucuronide
M6G	=	Morphine-6-glucuronide

NO	=	Nitric oxide
NK_1	=	Neurokinin-1 receptor
NMDA	=	N-methyl-D-aspartate
NSP	=	Neutral polysaccharide
PAF	=	Platelet-activating factor
PGE_2	=	prostaglandin E_2
pH	=	Potential of hydrogen
po	=	*Per os*
RBC	=	Red blood cell
sec	=	Second
sc	=	Subcutaneous
S.E.M	=	Standard error of the mean
TNF	=	Tumor necrosis factor
UGT2B7	=	UDP-glucuronosyl transferase-2B7
WSP	=	Water soluble polysaccharides
w/v	=	Weight by volume
w/w	=	Weight by weight
ºC	=	Degree Celsius
ºF	=	Degree Fahrenheit
/	=	Per
µl	=	Microliter
µg	=	Microgram
%	=	Percentage

CHAPTER 1

INTRODUCTION

Traditional medicine is as old as human kind and is practised by virtually all cultures, each one with its own indigenous knowledge, health practices and benefits. Scientific and non-scientific knowledge generally has been transmitted by oral tradition from generation to generation since antiquity until it finally became a significant part of the foundation of today's school of medicine. As people practically only had limited access to hospitals, these institutions could not seriously develop as an alternative to traditional medical practices. However, knowledge about the medical benefits of plants still persisted in the population.

Natural products are believed to be important sources of new chemical substances which have potential therapeutic effects. Medicinal plants, one of the important sources, are extensively investigated both *in vitro* and *in vivo* to examine for their potential activities. Most people living in developing countries are almost completely dependent on traditional medical practices for their primary health care needs and higher plants are known to be the main source for drug therapy in traditional medicine (Calixto, 2005). Many biologically active natural products continue to be useful in the exploration and control of intracellular signaling processes. For example, the sesquiterpene lactone parthenolide from the anti-inflammatory medicinal herb Feverfew (*Tanacetum parthenium*) appears to inhibit the pro-inflammatory signaling pathway (Benjamin *et al.,* 2001). The available analgesic drugs exert a wide range of side effects and either too potent or too weak, however, the search for new analgesic compound has been a priority of pharmacologists and pharmaceutical industries (Mattison *et al.,* 1998). In Thailand, there are many plant species that possess medical values. Several medicines used today are derived straight from many plants. The plant kingdom represents a virtually reservoir of new and exciting chemical compounds, and many of them show extraordinarily biodynamics (Deraniyagala *et al*., 2003).

Drugs of natural origin continue to be important for the treatment of many diseases worldwide and are believed to be an important source of new compounds. Many medicinal plants are undergoing investigated for their antinociceptive, antipyretic and anti-inflammatory activities. Among many species of medicinal plants used in traditional medicine, *Scaphium lychnophorum* (Hance) Pierre (Family Sterculiaceae) was claimed to relieve pain, cough and clear phlegm, and used as antipyretics (Anjaneyulu and Raju, 1987).

The composition of chemical compounds in *S. lychnophorum* has been reported. The crude polysaccharides isolated from the fruit of *S. lychnophorum* include glucose (22.6%), rhamnose (10.0%), arabinose (7.9%), galactose (5.0%), galacturonic acid (11.8%) and xylose (0.8%). Furthermore, *in vitro* study of the anti-inflammatory bioactivity of water soluble polysaccharides fraction from boat-fruited sterculia seed has been clearly demonstrated (Wu *et al.*, 2007). Since the *S. lychnophorum* has been claimed to treat pain, cough and clear phlegm, and used as antipyretics in folklore medicine, however, any studies and reports of *in vivo* study of the antinociceptive, antipyretic and anti-inflammatory activities of *S. lychnophorum* have not been systemically scientific investigated.

In the present study, a crude ethanol extract of *S. lychnophorum* was tested for its antinociceptive, antipyretic and anti-inflammatory activities in experimental animals because it is commonly used in folklore medicine. In addition, the dichoromethane fraction of *S. lychnophorum* was also tested and evaluated for these activities. Therefore, in this investigation it might be of great values to evaluate its effect on analgesic, anti-inflammatory and antipyretic activities in experimental animals to confirm its therapeutic efficacy of both ethanol and dichloromethane fraction of *S. lychnophorum*.

CHAPTER 2

LITERATURE REVIEW

Scaphium lychnophorum (Hance) Pierr

S. lychnophorum (Hance) Pierre is in the Sterculiaceae family which is commonly known in Thai as "Samrong". Boat-fruited sterculia seed is a traditional Chinese drug and specified as the seeds of *Sterculia lychnophora* Hance in the Chinese pharmacopoeia (The Pharmacopoeia Commission of PRC, 2000). This plant is mainly distributed in Vietnam, Thailand (Ubon Ratchathani and Chanthaburi), Malaysia, Indonesia, as well as South China (Wu *et al*., 2007). This is a large, evergreen broad-leaved tree species that can reach up to 18-25 m in height and produce boles from 50-75 cm. The wood is rather hard and heavy, but easy to saw and to work. Leaves are 12-15 cm long and 8-10 cm wide. The fruit is egg-shaped and from 8-15 mm in diameter. The color of young fruit is green-yellow, turning to dark-brown when mature (Polsena, 2007) (Figure 1).

This traditional drug is reputed for its prevention of, and as a remedy against pharyngitis. It has also been used for the treatment of constipation since ancient times in China (Anjaneyulu and Raju, 1987). In the method of preparation as remedy to use in folk medicine included fruit of *S. lychnophorum*.

Figure 1. The fruit of *Scaphium lychnophorum* (Hance) Pierre

In the compound of *S. lychnophorum*, monosaccharide analysis of the polysaccharides from *Sterculia lychnophora* reveal rhamnose, xylose, arabinose, galactose and galacturonic acid as the main constituents (Torabi *et al*., 2009).

The crude polysaccharides of *Scaphium lychnophorum* has been reported in the fruit of *S. lychnophorum* included glucose (22.6%), rhamnose (10.0%), rrabinose (7.9%), galactose (5.0%), galacturonic acid (11.8%), and xylose (0.8%), and the study on anti-inflammatory bioactivity of polysaccharides from boat-fruited sterculia seed has been reported that the water soluble polysaccharides (WSP) from boat-fruited sterculia seed showed clearly anti-inflammatory activity in rats. Bioactivity of neutral polysaccharide (NSP) and acidic polysaccharide (ASP) was tested using ear edema induced by dimethylbenzene and cotton pellet-induced granuloma tissue in murine models. The results showed ASP possessed a potent dose-dependent anti-inflammatory activity (Wu *et al*., 2007).

Klinsukon *et al.* (2009) has reported that the chemical compositions of gum from Malva nut seeds (*S. lychnophorum*) composed of 45.2% crude fiber, 38.9% carbohydrates, 4.9% protein and 1.4% ash. The seeds gum also contained 32.3% total sugar content and 4.6% uronic acid. Under these optimum conditions (adjusted pH to 7 before precipitation with 95% ethanol) the highest yield of gum was 21.2 %.

The isolation and structure determination of two new alkaloids, named sterculinine I and sterculinine II from the ethanol extract of this traditional drug. In addition, 13 known compounds, soya-cerebroside II , 1-*O*-β-D-glucopyranosyl-(2S,3R,4E,8Z)-2-[(2-hydroxy-icosanoyl)amido]-4,8-octadecadiene-1,3-diol, kaempferol-3-*O*-β-D-glucoside, uracil, succinic acid, 2, 4-dihydroxy benzoic acid, daucosterol, b-sitosterol, sucrose, isorhamnetin-3- *O*-β-D -rutinoside , kaempferol- 3-*O*-β-D-rutinoside, n-butyl-a-d-mannopyranside, and adenosine were obtained from the extract (Wang *et al.,* 2003).

The aqueous extracts from boat-fruited sterculia seeds were reported to exhibit potent anti-inflammatory property as shown by the inhibition of croton oil-induced acute inflammation in rats and antibacterial activity against the *Escherichia coli* and *Bacillus dysenteriae* (Du *et al*., 1995).

Pain

Pain is defined as “an unpleasant sensory and emotional experience associated with actual or potential tissue damage, or described in terms of such damage”. Pain involves a significant psychological component which can alter its perception and therefore, undergoes extensive processing through the nervous system, and particularly in the brain (Mello and Dickenson, 2008). Nociception is important for being aware of and reacting to potentially or actually damaging stimuli in the environment (Woolf and Costigan, 1999). The detection of noxious chemical, thermal, and mechanical stimuli is mediated by receptors on specialized sensory neurons (Lee *et al.*, 2005). Electrical signals generated at these sites are common amplified and transmitted further to the higher centers in the central nervous system (CNS) in order to generate a systemic response aimed at self-preservative (Coderre *et al.*, 1993). Pain is a body defense mechanism and is a warning of a problem particularly when it is acute (Gould, 2002) and may become chronic where it outlasts any potential for healing and becomes modified centrally (Peck, 2002). Pain has adverse effect on well-being in reduced physical and emotion function (Niv and Kreitler, 2001)

Cause of pain

The painful may occur for many reasons. Pain may be felt because of inflammation, infection, tissue necrosis, stretching of tissue, chemicals, or burn. In skeletal muscle, pain may result from ischemia or hemorrhage. In the stomach and intestines, pain may result from inflammation of the mucosa or from distention or muscle spasm. Depending on the cause, pain may sudden and short-term, marked primary by a reflex withdrawal (Gould, 2002).

Pain can be classified as acute or chronic. Acute pain is usually of short duration and the cause often identifiable (disease, trauma). Chronic pain persists after healing is expected to be complete, or is caused by a chronic disease. Pain may be modified by psychological factors and attention to these is essential in pain management (Rahman and Dickenson, 2008)

Fast pain is also described by many alternate name such as sharp pain, acute pain, electric pain, and others. The processing of acute and prolonged painful stimulation is depicted, and provides the physiological basis for two major characteristics of clinical pain: hyperalgesia and allodynia. An acute stimulus will trigger a series of events leading to excitatory pain signals reaching the brain via the spinal cord; as the stimulus is short lived, so is the neuronal response. However, given a longer, more chronic stimulus, sensitization may occur at either the peripheral and/or the central level (Brooks and Tracey, 2005)

Peripheral mechanisms of sensory transmission

The sensory experience begins in the periphery, where the peripheral terminals of primary afferent fibers respond to a myriad of stimuli and translate this information into the dorsal horn of the spinal cord, where the central ends of these fibers terminate. There are three main types of sensory fiber in the peripheral nervous system, Aß-fibers, Aδ fibers, and C-fibers. Each has different properties allowing them to respond to and transmit different types of sensory information. Aß-fibers are large in diameter and highly myelinated, thus allowing them to quickly conduct action potentials from their peripheral to central terminals. These fibers have low activation thresholds and normally respond to light touch and are responsible for conveying tactile information. Aδ fibers are smaller in diameter and thinly myelinated, and they also possess higher activation thresholds. They respond to both thermal and mechanical stimuli. C-fibers are the smallest type of primary afferents and are unmyelinated, thus making them the slowest conducting (Park *et al.,* 2000) (Table 1). They have the highest thresholds for activation and therefore detect selectively nociceptive or “painful” stimuli. Collectively, both Aδ - and C-fiber can be termed as nociceptors or “pain fibers”, responding to noxious stimuli which may be mechanical, thermal, or chemical (Mello and Dickenson, 2008).

Table 1. Classification of sensory neurons (Wang,1995)

Classification		**Fiber Type**	**Velocity (m/s)**	**Propagation Velocity**
Sensory neuron	**Destination**			
Aß	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

Chemical sensitivity of nociceptors

The results of injury in the local release of neuron chemicals which mediated or facilitated the inflammatory process. Such chemical mediators include leukotrienes, serotonin, nitric oxide, bradykinin, prostaglandins, histamine, substance P, thromboxanes, platelet activating factor, protons and free radicals. Some of these chemicals activate nociceptors and therefore are directly involved in producing pain, while others lead to a sensitization of the nociceptor response to natural stimuli and therefore play a role in primary hyperalgesia.

Bradykinin is released upon tissue injury, and present in inflammatory exudates.

Serotonin can also potentiate the pain induced by bradykinin and enhance the response of nociceptors to bradykinin. Mast cells, upon degranulation release platelet activating factor which in turn lead to serotonin release from platelets.

Proton is selectively activated nociceptors and produces a sensitization of nociceptors to mechanical stimuli.

Histamine can lead to a variety of response including vasodilation and edema. Substance P releases from nociceptor terminals can cause the release of histamine from mast calls.

Arachidonic acid metabolites (prostagrandins, thromboxanes and leukotrienes) are collectively known as eicosanoids. The eicosaniods are generally considered not to active nociceptors. This sensitizing effect of eicosaniods may play

an important role in hyperalgesia associated with inflammation (Wall and Melzack, 1994).

Pain-producing (algesic) substance

Algesic substances are released by damaged tissues and either directly or indirectly evoke pain, including substances such as H^+, K^+, acetylcholine, histamine, serotonin (5-HT), bradykinin (Peck, 2000) which can directly stimulate the sensory nerve endings.

Sensitization of nerve ending

Algesic substances are released from damaged tissues in localized area. This phenomenon is known as peripheral sensitization. As healing starts, nerve ending of the polymodal C-fibers show increased sensitivity to stimuli. Thus any stimuli in a winder area than the initial site of injury become painful. This process is a result of increases sensitization of nerve endings and is known as central sensitization (Park *et al.,* 2000).

Pathophysiology of pain

After the chemical messengers released during tissue injury in turn stimulate nociceptors to release neuropeptides such as substance P, neurokinin A, calcium gene-related peptides and nerve growth factor. These neuropeptides lead to peripheral sensitization.

Under the guidance of the sympathetic nervous system after painful injury, the release of norepinephrine stimulates release of excitatory transmitters at the level of the spinal cord. These excitatory transmitters have been implicated in the process of central sensitization and work to prolong the response of dorsal horn neurons, resulting in persistent changes in the excitability of the cell, also referred to "wind-up". Central sensitization refers to sensory changes in the undamaged tissue surrounding the injury owing to hypersensitive spinal neurons, which produce secondary pain. Tenderness in a zone surrounding the injury is due to central sensitization.

Nociception is conveyed from the periphery to the brain by an adaptable and dynamic pathway. The pathway is transmitted and modulated at three level: the peripheral nociceptor, the spinal (dorsal horn of the cord), and the supraspinal (brain) (Serpell, 2006) (Figure 2.)

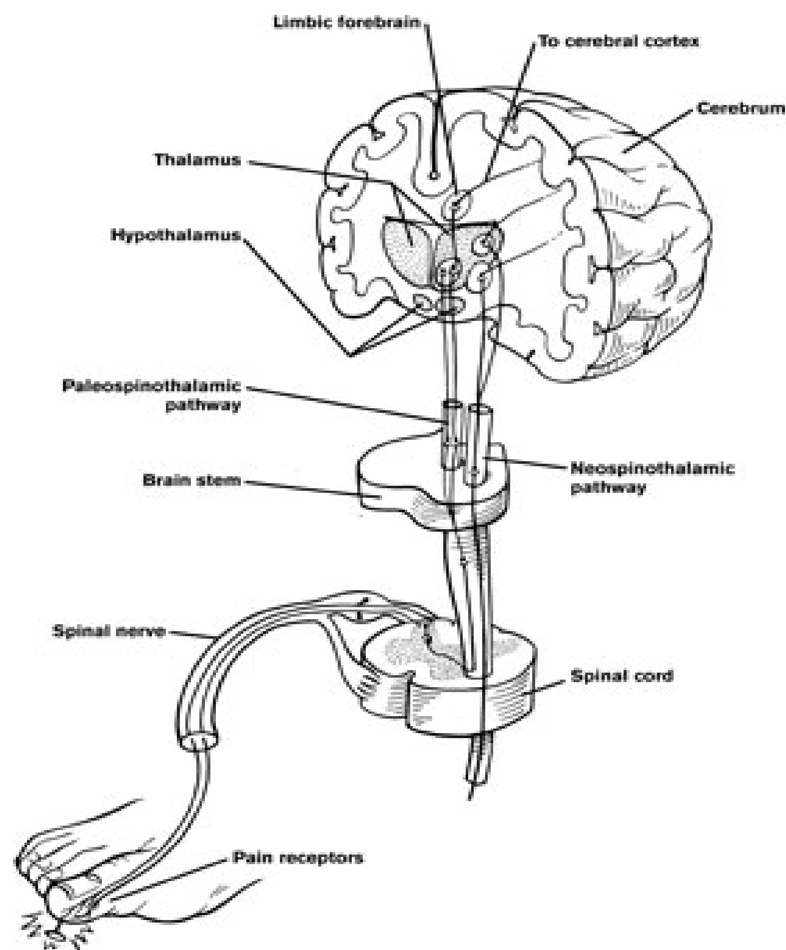


Figure 2. Pain pathway (Howard, 2007)

Peripheral sensitization

The conduction of nociceptive information from the periphery to the CNS is carried out by neurons that have a bipolar structure. The cell body lies within the dorsal root ganglion (DRG), with the proximal axon running into the dorsal aspect of the spinal cord. Due to the uneven distribution of ions within and outside the cell membrane, the resting membrane potential of these cells is 50-100 mV, either the

position charge being on the outside of the cell. An adenosine triphosphatase (ATPase) pump continuously keeps the concentration of sodium outside the cell at 20 times of that with in the cell. Conversely, this pumping system maintains a potassium concentration inside the cell at 35 times of that outside charge becomes either neutral or negative. This result in an action potential, and it can only run in one direction due to the refractory phase that follows membrane depolarization in nociceptors that is from the periphery towards the spinal cord. There are two types of nociceptor: fast conducting Aδ fibers and the more slowly conducting C fibers.

Aδ fibers associated with cold and pressure, and as nociceptors they convey fast pain information. They are thinly myelinated, so conduct signals more rapidly than unmyelinated C fibers. These fibers are rapid in transfer of information from the periphery to the spinal cord, and these fibers are high-threshold receptors which respond to mechanical stimulation such as firm pinch. Some of the fibers also respond to noxious heat (> 45ºC). Aδ fibers activation result in sharp pain.

C fibers are unmyelinated unlike most other fibers in the nervous system. This lack of myelination is the cause of their slow conduction velocity, which is on the order of no more than 2 m/s. C fibers are on average 0.2-1.5 µm in diameter. The other main classification of nociceptors is Aδ fibers. These fibers have axons that are larger (1-5 µm), in diameter, are myelinated, and have a higher conduction velocity, which is on the order of about 20 m/s. Overall, the C-fiber population is responsible for 80% of the nociceptive primary afferents and the perception of this information tends to be associated with poorly localized, aching and burning pain.

When the tissue injury occurs, potassium and kinins are released from the damage cells. These substances stimulate the receptor directly, resulting in the release of neuropeptides such as substance P from the receptor. This in turn causes the degranulation of adjacent mast calls with the production of platelet-activating factor (PAF) which in turn releases serotonin from the platelets. Histamine is also released from the mast call, starting an inflammatory reaction within the tissue with vasidilation, lowered pH and the release of ecosaniods such as leukottienes and prostagrandins (PGs) (Campbell, 2004)

Central sensitization

Nociceptive formation within the Aδ and C fibers arrives at the dorsal horn of spinal cord. C fibers terminate in the superficial dorsal horn at the luminar II, and Aδ fibers terminate in the laminar I and V. Aδ fibers terminate mainly in the laminar I and V with some of their high-threshold fibers ending directly in laminar II. Most cutaneous C fibers terminate in the laminae II. However, visceral C fibers terminate in the laminar I, II, IV, V, and X (Campbell, 2004).

Peripheral nerve injury leads to an increase in the general excitability of multireceptive spinal cord neurons. This hyperexcitability is manifested by increased neuronal activity in response to noxious stimuli, expansion of neuronal receptive fields and spreads of a spinal hyperexcitability to other segments (Baron, 2006)

The results of C fibers in the increased dorsal horn excitatory. C fibers release not only glutamate but also substance P, which acts through the neurokinin-1 (NK_1) receptor to increase dorsal horn intracellular calcium and enchance N-methyl-D-aspartate (NMDA) sensitivity to glutamate (Singleton, 2005). When glutamate release from primary afferent neuron and bind to NMDA, there is an in flux of Ca^{2+} into the post synaptic neuron (Basbaum and Jessell, 2000). These results could activate enzyme such as nitric oxide (NO) synthases or trigger other long lasting cellular changes, so signal transduction coming to sensory projection fields in the cortex. This part of the cortex 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 evolution. This state of hyperexcitability is called central sensitization. (Mutscheler and Derendorf, 1995).

Regulation of body temperature

Body temperature is controlled deep in the hypothalamus, a primitive part of the brains tem that regulates the so-called vegetative functions (such as sleep and appetite) and secretes several neuroendocrine-releasing factors. The ability to thermoregulate and to maintain a relatively constant body temperature is manifested by all vertebrates, including "cold-blooded" animals. The thermoregulatory center of the hypothalamus (specifically, the preoptic/ anterior hypothalamus) contains thermosensitive neurons whose rate of discharge is affected by the temperature of the area's blood supply and also via direct neural connections with receptors of cold and warmth that are distributed in the skin and muscle (Charle *et al.,* 1988). The human heat balance equation describes how the body (homeotherm) can maintain an internal body temperature near to 37°C in terms of heat generation and heat exchange with the environment. In practice, what is achieved is not a steady state (constant temperatures) but a dynamic equilibrium: as external conditions continually change, so the body responds to 'regulate' internal body temperature (Parsons, 2003).

Fever describes a regulated rise in body temperature after an increase in the hypothalamic set point. Under the influence of the hypothalamus, physiologic and behavioral functions favoring heat production and heat retention are stimulated until arriving at a newly elevated set point temperature. Typical early behavioral changes prior to fever include seeking a warmer environment or adding clothing (Aronoff and Neilson, 2001).

It's generally accepted that temperature sensors inside the body are situated in the hypothalamus as well as the medulla, spinal cord and other sites. There are two types of thermal sensor distributed across the skin of the body, so called warm and cold receptors. Signals from these sensors as well as from core sensors are integrated at the hypothalamus. Core temperatures higher than 42 ºC are associated with a breakdown of cellular proteins and death (Rhaodes and Pflazer, 2003).

The thalamus contains the central coordinating center for temperature regulation. This group of specialized neurons at the floor of brain acts as a thermostat which usually set and carefully regulated at 37ºC that continually markes thermoregulatory adjustments to deviations from a temperature norm. Unlike the

home thermostat, the hypothalamus cannot turn off the heat; it can only initiate response to protect the body from either a buildup or loss of heat.

Two ways activate the body's heat-regulating mechanism:

1. Thermal receptors in the skin provide input to the central control center.
2. Changes in the temperature of blood that perfuse the hypothalamus directly stimulate this area (Drinkwater, 2007)

Temperature- sensitive neurons within the body core and found in the hypothalamus, spinal cord, abdominal viscera and great veins. Specific thermoreceptors are integrated with peripheral thermal information at the hypothalamus.

1. Thermosensors

In the skin, thermoregulators are free nerve endings widely distributed over and within the epidermis. Their signals are carried by either non-myelinated C fibers or small myelinated A fibers and the main afferent spinal pathway is the lateral spinothalamic tract. Thermoreceptors (skin and central) are either warm and cold types, according to the response to stimuli (Person, 2003) Temperature sensation is encoded by thermoreceptors located on the free endings of small myelinated (Aδ) and unmyelinated (C) fibers. Separate receptors with discrete receptive fields exist for encoding warm and cold sensations (Wang, 1995)

2. Peripheral thermoreceptor regulation of body temperature

Peripheral receptors measure temperature in the skin. These receptors are naked, temperature-sensitive nerve ending and selective respond to cold or warm stimuli (Figure 3). While both types of temperature receptors are found throughout the body surface, cold receptors are ten times more numerous. Nerve impulses from peripheral receptor enter the spinal cord and ascend to the brain, to be integrated in the hypothalamus with temperature information from the body core (Rhoades and Pflazer, 2003).

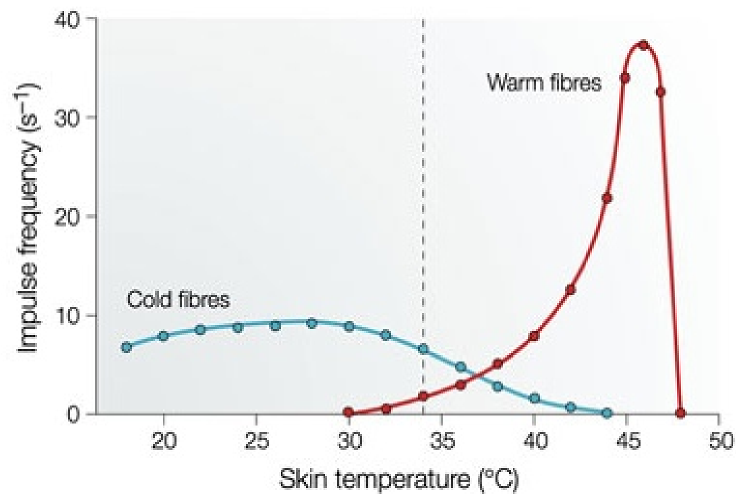


Figure 3. Static discharge frequency of cold and warm nerve fibers as a function of skin temperature (Patapoutian *et at.,* 2003).

3. Central integration of thermal information

Core and skin temperature are integrated in the hypothalamus. Because the hypothalamus itself is maintained at core temperature, changes in hypothalamic temperature are the single most important input determining thermoregulatory responses. When skin and core temperature deviate from a regulated value, the hypothalamus initiates a number of physiological responses that modify heat loss or heat gain. These responses include regulation of sympathetic neural control of sweat shivering. This pathway modifies voluntary skeletal muscle activity through an influence on the cerebral cortex (Rhoades and Pflazer, 2003).

Response to heat

1. Vasodilation

Skin vasodilation causes the increase in heat loss. During vasodilation venous blood returns near to the skin hence increasing the avaibility of heat loss from the skin to the environment.

2. Sweating

When the body temperature raises, sweat is excreted 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 theses regions.

Eccrine glands distributed through out the body. It is the eccrine glands that perform the thermoregulatory function.

Response to cold

1. Vasoconstriction

To reduce heat loss. Cold vasoconstriction still allows some blood flow the required small amount of oxygen to reach the cells. In the limbs, a countercurrent heat exchange occurs due to constriction of superficial vein so that cool blood from the skin returns along the venae comitans close to the artery, hence gaining heat and return to the body core.

2. Shivering

Both skin temperature and core temperature affect the onset of shivering which can be both voluntary and involuntary. Shivering describes as the simultaneous asynchronous constriction of the muscle fibers in both the flexor and exterior muscle (Parsons, 2003)

Pyrexia or fever

Pyrexia or 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 (Aronoff and Neilson, 2001).

Fever (also known as pyrexia or controlled hyperthermia) is a common medical sign characterized by an elevation of temperature above the normal range of 36.5–37.5 °C (98–100 °F) due to an increase in the body temperature regulatory set-point. This increase in set-point triggers increased muscle tone and shivering. Fever describes a regulated rise in body temperature after an increase in the hypothalamic

set point. Under the influence of the hypothalamus, physiologic and behavioral functions favoring heat production and heat retention are stimulated until arriving at a newly elevated set point temperature. Typical early behavioral changes prior to fever include seeking a warmer environment or adding clothing. Physiologic alterations include cutaneous vasoconstriction, shivering, and nonshivering thermogenesis through enhanced release of thyroid hormones, glucocorticoids, and catecholamines. Upon reaching the elevated set point of fever, an increase or decrease in core temperature will stimulate thermoregulatory mechanisms similar to those evoked at normal body temperature. In other words, normal thermoregulation modulates at this higher set point. (Aronoff and Neilson, 2001).

The pathogenesis of fever

Many of the mediators underlying pyrexia have been described in recent years (Figure 4). The critical "endogenous pyrogens" involved in producing a highly regulated inflammatory response to tissue injury and infection are polypeptide cytokines. Pyrogenic cytokines, such as interleukin- 1β (IL-1β), tumor necrosis factor (TNF), and interleukin-6 (IL-6), are those that act directly on the hypothalamus to effect a fever response. Exogenous pyrogens, such as microbial surface components, evoke pyrexia most commonly through the stimulation of pyrogenic cytokines. The gram-negative bacterial outer membrane lipopolysaccharide (endotoxin), however, is capable of functioning at the level of the hypothalamus, in much the same way as IL-1β.

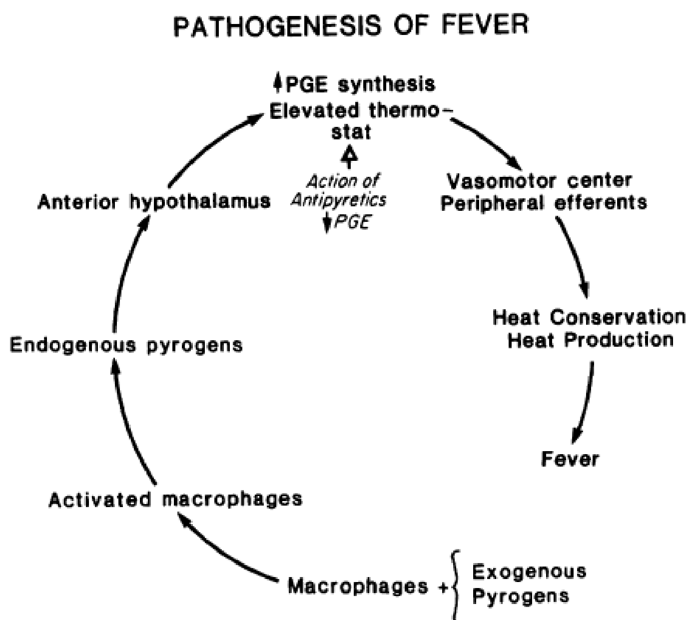


Figure 4. Pathogenesis of fever (Dinarello and Wolff, 1978)

Inflammation

The definition of inflammation is the body's response to tissue injury (Gould, 2002). Inflammation is a defense reaction of the organism and its tissue to injurious stimuli that lead to the local accumulation of plasmatic fluid and blood cells. 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)

Cause of inflammation

Causes include direct damage (cuts, sprains), chemical such as acid, ischemia and cell necrosis, allergic reaction, physical agent (thermal injuries or burn)

Inflammation can be classified based on duration of inflammation as acute and chronic inflammation.

1. Acute inflammation

Acute inflammation begins within seconds to minutes following the injury of tissues. The damage may be purely physical, or it may involve the activation of an immune response (Steven and Lowe, 2000). Three main processes occur:

- **Increased blood flow** due to dilation of blood vessels (arterioles) supplying the region
- **Increased permeability** of the capillaries, allowing fluid and blood proteins to move into the interstitial spaces
- **Migration of neutrophils** (and perhaps a few macrophages) out of the venules and into interstitial spaces

The cardinal signs of acute inflammation

1. **Redness (rubor):** An acute inflamed tissue appears red, for example skin affected by sunburn.
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 through the region, resulting in vascular dilation and the delivery of warm blood to the area.
3. **Swelling (tumor):** Swelling result from edema, The accumulation of fluid in the extra vascular space as a part of the fluid exudates.
4. **Pain (dolor):** It results partly from the stretching and distortion of tissue due to inflammatory edema and, in particular, from pus under pressure in an abcess cavity.
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 (Macfarlane *et al.,* 2000).

Process of acute inflammation

The process of acute inflammation is initiated by cells already present in all tissues, mainly resident macrophages, dendritic cells, histiocytes, Kuppfer cells and mastocytes. At the onset of an infection, burn, or other injuries, these cells

undergo activation and release inflammatory mediators responsible for the clinical signs of inflammation. Vasodilation and its resulting increased blood flow causes the redness (rubor) and increased heat (calor). Increased permeability of the blood vessels results in an exudation (leakage) of plasma proteins and fluid into the tissue (edema), which manifests itself as swelling (tumor). Some of the released mediators such as bradykinin increase the sensitivity to pain (hyperalgesia). The mediator molecules also alter the blood vessels to permit the migration of leukocytes, mainly neutrophils, outside of the blood vessels (extravasation) into the tissue. The neutrophils migrate along a chemotactic gradient created by the local cells to reach the site of injury. The loss of function is probably the result of a neurological reflex in response to pain.
In addition to cell-derived mediators, several a cellular biochemical cascade systems consisting of preformed plasma proteins act in parallel to initiate and propagate the inflammatory response. These include the complement system activated by bacteria, and the coagulation and fibrinolysis systems activated by necrosis, e.g. a burn or a trauma.

The acute inflammatory response requires constant stimulation to be sustained. Inflammatory mediators have short half lives and are quickly degraded in the tissue. Hence, inflammation ceases once the stimulus has been removed.

Chemical mediators of inflammation

- **Histamine** is the main pre-formed mediator of inflammation. Released from mast cells, basophils, and platelets, it cause transient dilatation of arterioles, increases permeability in venules, and is the primary cause of increased vascular permeability in the first hour after injury.
- **Prostaglandins:** Released from mast cells. It is derived by local synthesis by arachidonic acid. A group of lipids which can cause vasodilation, fever, and pain.
- **Leukotriene B4** is able to mediate leukocyte adhesion and activation, allowing them to bind to the endothelium and migrate across it. In neutrophils, it is also a potent chemoattractant, and is able to induce the formation of reactive oxygen species and the release of lysosome enzymes by these cells.

- **Nitric oxide** is a small molecule that is locally synthesized by endothelium and macrophages through the activity of the enzyme, nitric oxide synthase.

NO in acute inflammatory

A variety of chemical mediators, operating sequentially in different phases of the inflammatory response, mediate the associated changes in microvascular caliber and permeability. In addition, cell injury/necrosis and neutrophil infiltration are also principal changes. NO is inhibited in the intermediate phase of vasodilatation and increased vascular permeability in skin inflammation after injection of carrageenan or exposure to ultraviolet light, suggesting that NO involved in skin inflammation of some hours duration. However, it is unclear whether eNOS has a role in the immediate acute phase of inflammation; it is possible that the transitory initial vasoconstriction seen initially may be due, at least in part, to an acute loss of endothelial NO synthesis as a result of cell dysfunction. Thus, the increase in vascular permeability is a key, early event in an acute inflammatory process (Wu, 2004).

NO in chronic inflammation

Macrophage infiltration, proliferative and synthetic connective tissue reactions, and, in processes where hypersensitivity is involved, T lymphocyte accumulation are all regarded as the hallmarks of chronic inflammation. Usually, macrophage infiltration is demonstrated by processes similar to those for neutrophils acting on circulating blood monocytes, but with certain crucial differences in the chemoattractants and adhesion molecules involved. In addition, the response is highly dependent on the release of cytokines (Wu, 2004).

- **Platelet activating factor (PAF)** is synthesized by mast cell or basophils and can be stimulated by IgE-mediated release. It is a specialized phospholipids compound, which causes vasoconstriction, increased vascular permeability and platelet aggregation.
- **Cytokines** are polypeptide products of activated lymphocytes and monocytes. The main cytokines participating in acute inflammation are interleukin-1 (IL-1), interleukin-8 (IL-8)**,** and tumor necrosis factor alpha (TNFα).

- **The chemokines** are a family of factors secreted by leukocytes and endothelial cells in response to tissue damage and other inflammatory mediators. Specific chemokine receptors are activated and these signals for activation of leukocyte integrins, mediating adhesion and migration.
- **The complement system:** There is a cascade of activation, with production of numerous intermediary activated peptides. The main products with roles in acute inflammation are as follow:
    - C3a increases vascular permeability by liberating histamine from mast cells or platelet.
    - C5a: stimulates histamine release by mast cells, thereby producing vasodilation. It is also able to act as a chemoattractant to direct cells via chemotaxis to the site of inflammation.
    - C345 is chemotatic to neutrophils.
    - C3b opsonizes bateria and facilitates neutrophils phagocytosis.
- **The kinins** are small peptides derived from plasma precursors by proteolytic cleavage. The system is activated by one of the coagulation proteins, activated Hageman factor (factor XII) (Vane and Botting, 1987)**.**
- **The clotting pathway** is responsible for coagulation of blood by formation of fibrin from fibrinogen. Factor XII is activated the inflammatory exudates when it comes into with collagen outside the vessel. These cause increased vascular permeability, as well as being chemotactic for neutrophils.
- **The thrombolytic pathway:** The enzyme plasmin is a proteolytic enzyme with several role in inflammation.

Pathophysiology of acute inflammation

The acute response to tissue injury occurs in the microcirculation at the site of injury. Initially there is a transient constriction of arterioles; however, within several minutes, chemical mediators released at the site cause relaxation of arteriolar smooth muscle, vasodilation, and increased capillary permeability. Protein-rich fluid then exudes from capillaries into the interstitial space. This fluid contains many of the

components of plasma, including fibrinogen, kinins, complement, and immunoglobulins that mediate the inflammatory response. The subacute phase is characterized by movement of phagocytic cells to the site of injury. In response to adhesion molecules released from activated endothelial cells, leukocytes, platelets, and RBC in injured vessels become sticky and adhere to the endothelial cell surfaces. Polymorphonuclear leukocytes such as neutrophils are the first cells to infiltrate the site of injury. Basophils and eosinophils are more prevalent in allergic reactions or parasitic infections. As the inflammatory process continues, macrophages predominate, actively removing damaged cells or tissue. If the cause of injury is eliminated, acute inflammation may be followed by a period of tissue repair. Blood clots are removed by fibrinolysis, and damaged tissues are regenerated or replaced with fibroblasts, collagen, or endothelial cells. However, inflammation may become chronic, leading to further tissue destruction and fibrosis (Gould, 2002).

2. 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 that the activation of one stimulates the actions of the other. Certain chronic infections cells known as eosinophils accumulate. Within their cytoplasm are bright red granules. These granules contain a substance called 'major basic protein' which has the ability to destroy certain antigens (Steven and Lowe, 2000). 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.

Pathophysiology of chronic inflammation

Characteristics of chronic inflammation include less swelling but the presence of more lymphocytes, and fibroblasts than in acute inflammation and macrophage have been unable to complete clear (debride) the area of foreign substances. This material may be dead cells, extracellular blood, or sand or dirt in some cases. Either way, the material is surrounded by collagen to isolate it from the body. This mass of encapsulating scar is called a granuloma (Norris, 2004).

Aspirin (acetylsalicylic acid)

Aspirin is a nonsteroidal anti-inflammatory agent whose pharmacologic activity includes anti-inflammatory, analgesic, and antipyretic effects. In addition, aspirin exerts antiplatelet effects, which are clinically detectable as a prolongation of the bleeding time. (Neal, 1992). The anti-inflammatory effects of aspirin are related to inhibition of cyclooxygenase with consequent inhibition of prostaglandin synthesis. The antithrombotic effects of aspirin are related to the permanent inactivation of platelet cyclooxygenase activity (mediated by irreversibly acetylating the enzyme cyclooxygenase). Because platelets are incapable of protein biosynthesis, the inactivation of platelet cyclooxygenase is permanent for the life of the platelet (8 to 10 days). The structure of aspirin is shown in figure 5.

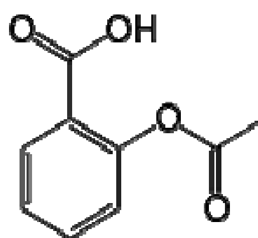


Figure 5. Structure of aspirin

1. Pharmacokinetics

The bioavailability of aspirin averages 42% from an oral solution compared to an intravenous dose. The bioavailability of immediate release aspirin was found to be complete when comparing relative concentrations of salicylate from oral and intravenous doses. The absorption of enteric coated aspirin from the GI tract is erratic. Following rectal dosing, aspirin absorption has been described as slow and unreliable.

The protein binding of salicylate is concentration dependent (nonlinear) and averages 95% at low salicylate concentrations of 1.4 mg/dL. At high doses approximately 75% is bound and a disproportionate increase in the concentration of unbound drug may occur. The volume of distribution of aspirin averages 0.20 L/kg in patients with normal renal and hepatic function. The plasma clearance of aspirin averages 9.3 mL/min/kg in patients with normal renal and hepatic

function. The plasma clearance (Cl/F) of salicylate ranges from 0.64 to 1.2 mL/min/kg.

The elimination half-life of aspirin averages 0.46 hours and that of salicylate averages 1.6 to 2.2 hours in patients with normal renal and hepatic function. Because salicylate metabolism is saturable, clearance decreases at higher serum concentrations. Toxic doses of aspirin (10 to 20 grams) can result in an elimination half-life of >20 hours.

2. Pharmacodynamics

The anti-inflammatory activity of the NSAIDs is similar in mechanism to that the aspirin and is mediated chiefly through inhibition of biosynthesis of prostaglandins. Inflammation is reduce by decreasing the release of the mediators from granulocytes, and reverse vasodilatation (Donald and Payan, 1992).

Mechanism of action

Aspirin's ability to suppress the production of prostaglandins and thromboxanes is due to its irreversible inactivation of the cyclooxygenase (COX) enzyme. Cyclooxygenase is required for prostaglandin and thromboxane synthesis. Aspirin acts as an acetylating agent where an acetyl group is covalently attached to a serine residue in the active site of the COX enzyme. This makes aspirin different from other NSAIDs (such as diclofenac and ibuprofen), which are reversible inhibitors. However, other effects of aspirin, such as uncoupling oxidative phosphorylation in mitochondria, and the modulation of signaling through NF-κB, are also being investigated.

3. Adverse effects

- **Cardiovascular side effects** includ salicylate-induced variant angina, ventricular ectopy, conduction abnormalities, and hypotension, particularly during salicylate toxicity. In addition, at least one case of fluid retention simulating acute congestive heart failure has been reported during aspirin therapy. Antiplatelet therapy has also been associated with acute deterioration of intracerebral hemorrhage.

• **Endocrine side effects** have included hypoglycemia (which has been reported in children) and hyperglycemia.

• **Gastrointestinal side effects** includ epigastric distress (in as many as 83% of patients treated with regular aspirin), abdominal discomfort or pain, endoscopically identifiable gastric mucosal lesions, nausea, and vomiting. More serious gastrointestinal effects include hemorrhage, peptic ulcers, perforation, small bowel enteropathy, and esophageal ulcerations.

• **Hematologic side effects** includ increased blood fibrinolytic activity. In addition, hypoprothrombinemia, thrombocytopenia, thrombocyturia, megaloblastic anemia, and pancytopenia have been reported rarely.

• **Renal side effects** includ reduction in glomerular filtration rate (particularly in patients who are sodium restricted or who exhibit diminished effective arterial blood volume, such as patients with advanced heart failure or cirrhosis), interstitial nephritis, papillary necrosis, elevations in serum creatinine, elevations in blood urea nitrogen, proteinuria, hematuria, and renal failure.

• **Respiratory side effects** includ hyperpnea, pulmonary edema, and tachypnea. Aspirin desensitization has been used to decrease disease activity and reduce the need for systemic corticosteroids in patients with aspirin-exacerbated respiratory disease.

• **Other side effects** includ Reye's syndrome with aspirin use in children with an acute viral illness. Reye's syndrome has also been reported even more rarely in adults. Reye's syndrome typically involves vomiting, neurologic dysfunction, and hepatic dysfunction during or shortly after an acute viral infection.

4. Drug interaction

Drugs that enhance salicylate intoxication include acetazolamide and ammonium chloride. Alcohol increases gastrointestinal bleeding produced by salicylate. Aspirin displaces a number of drugs from protein binding sites in the blood. These include tolbutamide, chlorpropamide, NSAIDs, methotrexate, phenytoin and probenecid.

Morphine

Morphine is a potent opiate analgesic psychoactive drug and is considered to be the prototypical opioid. In clinical medicine, morphine is regarded as the gold standard, or benchmark, of analgesics used to relieve severe or agonizing pain and suffering. Like other opioids, e.g. oxycodone (OxyContin, Percocet, Percodan), hydromorphone (Dilaudid, Palladone), and diacetylmorphine (heroin), morphine acts directly on the central nervous system (CNS) to relieve pain. Morphine has a high potential for addiction; tolerance and both physical and psychological dependence develop rapidly. The structure of morphine is shown in figure 6.

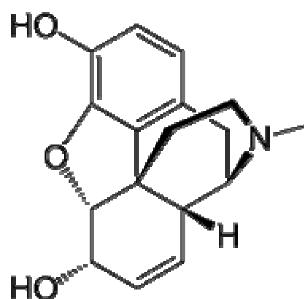


Figure 6. Structure of morphine

1. Pharmocakinetics

Morphine can be taken orally, rectally, subcutaneously, intravenously, intrathecally or epidurally (Gutstein and Akill, 2001). On the streets, it is becoming more common to inhale ("chasing the dragon"), but for medicinal purposes, intravenous (IV) injection is the most common method of administration. Morphine is subject to extensive first-pass metabolism (a large proportion is broken down in the liver), so if taken orally, only 40–50% of the dose reaches the central nervous system. Resultant plasma levels after subcutaneous (SC), intramuscular (IM), and IV injection are all comparable. After IM or SC injections, morphine plasma levels peak in approximately 20 minutes, and after oral administration levels peak in approximately 30 minutes. Morphine is metabolised primarily in the liver and approximately 87% of a dose of morphine is excreted in the urine within 72 hours of administration. Morphine is primarily metabolized into morphine-3-glucuronide (M3G) and

morphine-6-glucuronide (M6G) via glucuronidation by phase II metabolism enzyme UDP-glucuronosyl transferase-2B7 (UGT2B7). About 60% of morphine is converted to M3G, and 6–10% is converted to M6G (Stoelting and Hillier, 2006). The cytochrome P450 (CYP) family of enzymes involved in phase I metabolism plays a lesser role. Not only does the metabolism occur in the liver but it may also take place in the brain and the kidneys. M3G does not undergo opioid receptor binding and has no analgesic effect. M6G binds to mu-receptors and is a more potent analgesic than morphine. Morphine may also be metabolized into small amounts of normorphine, codeine, and hydromorphone. Metabolism rate is determined by gender, age, diet, genetic makeup, disease state (if any) and use of other medications. The elimination half-life of morphine is approximately 120 minutes, though there may be slight differences between men and women. Morphine can be stored in fat, and thus can be detectable even after death. Morphine is able to cross the blood-brain barrier but because of poor lipid solubility, protein binding, rapid conjugation with glucuronic acid and ionization, it does not cross easily. Diacetylmorphine, which is derived from morphine, crosses the blood-brain barrier more easily, making it more potent.

2. Pharmacodynamics

The three generally recognized class of opioid receptors are the mu (μ), delta (δ), and kappa (k) receptors were formerly classified as opioid receptors because opioids can bind to them (Lipman and Jackson, 2004)

Mu-opioid receptor: Morphine acts mainly on the μ-receptor, which is primary responsible for the analgesic actions. Most of the common opioid are full agonists at the μ-receptor. This receptor is also responsible for the often unwanted effects of opioids, 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 locations within the brain to the μ-receptor but also mediates 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. Etorphine is the best agonist.

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

Opioid inhibition of neurotransmitter releasese

Neurotransmitter release from neurons is normally preceded by depolarisation of the nerve terminal and Ca^{2+} entry through voltage-sensitive Ca^{2+} channels. Drugs may inhibit neurotransmitter release by a direct effect on Ca^{2+} channels to reduce Ca^{2+} entry, or indirectly by increasing the outward K^+ current, thus shortening repolarisation time and the duration of the action potential. Opioids produce both of these effects because opioid receptors are apparently coupled via G-proteins directly to K^+ channels and voltage-sensitive Ca^{2+} channels. Opioids also interact with other intracellular effector mechanisms, the most important being the adenylate cyclase system. Opioids have been proposed to inhibit neurotransmitter release by inhibiting calcium entry, by enhancing outward movement of potassium ions, or by inhibiting adenylate cyclase (AC), the enzyme which converts adenosine triphosphate (ATP) to cyclic adenosine monophosphate (cAMP) (Chahl, 1996) (Fig. 7).

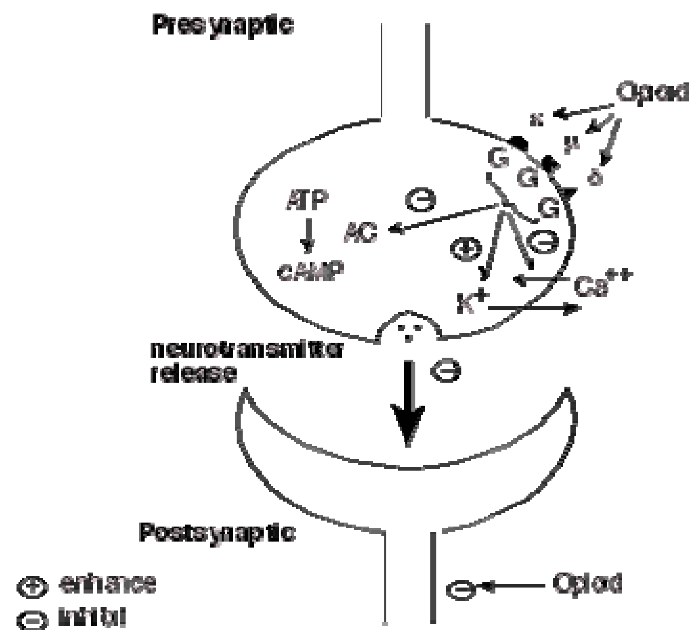


Figure 7. Mechanism of opioid (Chahl, 1996)

3. Adverse effect

- **Cardiovascular system**

Morphine can also evoke decreases in systemic blood pressure due to drug-induced 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.

- **Ventilation**

All opioid agonists produce a dose-dependent and gender specific depression of ventilation, primarily through an agonist effect at μ2 receptors, which lead to a direct depressant effect on brainstem ventilation centers.

- **Sedation**

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

- **Gastrointestinal tract**

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

- **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.

- **Cutaneous changes**

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

4. Drug interaction

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

Naloxone

Naloxone is a drug used to counter the effects of opioid overdose, for example heroin or morphine overdose. Naloxone is specifically used to counteract life-threatening depression of the central nervous system and respiratory system**.** The pure opioid antagonist drugs naloxone is morphine derivative with bulkier substituents at the N_{17} position. These agent have a relatively high affinity for μ opioid binding sites and have lower affinity for the receptors but can also reverse agonists at δ and k sites. The structure of naloxone is shown in figure 8.

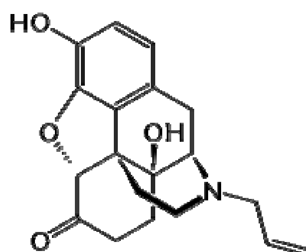


Figure 8. Structure of naloxone

1. Pharmacokinetics

Naloxone has poor efficacy when given by the oral route and short duration of action (1-2 hour) when given by injection. Metabolic disposition is chiefy by glucuronide conjugation, like that of the agonist opioids with free hydroxyl groups (Way *et al.,* 1998). Naloxone is almost completely metabolized by the liver before reaching the systemic circulation and thus must be administered parenterally. The drug is absorbed rapidly from parenteral sites of injection and is metabolized in the liver, primarily by conjugation with glucuronic acid, other metabolites are produced in small amounts. Subcutaneous dose of naloxone produces no discernible subjective effects in human begins and 24 mg cause only slight drowsiness. Small dose (0.4-0.8 mg) of naloxone given intramuscularly or intravenously prevents or promptly reverses the effect of μ receptor agonists (Gutstein and Akil, 2001)

2. Pharmacodynamics

Naloxone has an extremely high affinity for μ-opioid receptors in the central nervous system. Naloxone is a μ-opioid receptor competitive antagonist, and its rapid blockade of those receptors often produces rapid onset of withdrawal symptoms. Naloxone also has an antagonist action, though with a lower affinity, at κ- and δ-opioid receptors.

3. Adverse effect

Major side effect derive directly from its mechanism of action-antagonism of opioid agonists at all of known opioid receptors. This agent was available for use as an antagonist. It was mixed agonist-antagonist, such that if given after opioid, the opioid effects were reversed. However, when given alone, it produced respiratory depression, cough suppression, miosis and analgesia (Smith, 1995).

CHAPTER 3

MATERIALS AND METHODS

Materials

1. Plant material

Dried fruits of *Scaphium lychnophorum* (Hance) Pierre, common name called in Thai as Samrong, were collected in April 2009 from Chanthaburi Province, Thailand. The plant material was previously identified by Associate Professor Choathip Purintavarakul, Department of Biology, Faculty of science, Prince of Songkla University. The 3.0 kg of dried fruits of *S. lychnophorum* was cleaned by removing contaminated materials, and then were pulverized by an electric blender to give 2.6 kg of coarsely powder and stored in the airtight container.

2. Extract procedure

The dried and coarsely powdered fruits of *S. lychnophorum* (2.6 kg) were macerated 2 times with 7.5 L and 5.5 L of ethanol, respectively for 7 days at room temperature, and then filtrated, evaporated at room temperature in airflowed hood and subsequent evaporation under reduced pressure. The final filtrate was lyophilized to give a total brownish-green semisolid residue of 41.57 g (yield 1.6%, w/w) which was stored and kept in temperature below 4 ºC until tested. The ethanol extract of *S. lychnophorum* (EESL) at doses of 50, 100 and 200 mg/kg were prepared in cosolvent (distilled water : Tween 80: propylene glycol; 5:1:4, respectively). The dichloromethane extract of *S. lychnophorum* (DESL) was prepared by the same extract procedure of the ethanol extract. After lyoplhilized to give a total oily-green residue of 8.15 g (yield 0.36%), the extract residue was stored and kept at the temperature below 4 ºC until tested. DESL at doses of 50, 100 and 200 mg/kg were prepared in cosolvent (distilled water: Tween 80: propylene glycol; 5:1:4, respectively).

3. Animals

Male ICR albino mice, weighing 30-40 g, were used for testing of antinociceptive activities (writhing, formalin, hot plate and tail flick tests). Male Wistar rats, weighing 180-220 g, were used for testing of antipyretic and anti-inflammatory activities (brewer's yeast-induced fever, carrageenan-induced paw edema and cotton pellet-induced granuloma). All animals were obtained from the Southern Laboratory Animal Facility, Prince of Songkla University, Hat Yai, Songkhla, Thailand. The animals were housed in the room of controlled conditions of 24 ± 1ºC and 12 h light – 12 h dark cycles. Food and water were given *ad libitum* unless otherwise specified. All experiments were approved by Animal Ethics Committees, Prince of Songkla University, Thailand. (No. 0521.11/ 178)

4. Chemicals and instruments

The drugs used in the present study included morphine sulfate (Sigma), naloxone hydrochloride (Sigma), carrageenan (Sigma), acetylsalicylic acid or aspirin (Sigma). Urethane (Sigma), propylene glycol (Vidhyasom co., Ttd), Tween 80 (Srichand co., Ltd), acetic acid and 37 % formalin (A.R. grade), ethanol and dichloromethane (A.R. grade) (BDH).

The instruments 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. Drug and extract administration

The cosolvent, extracts and reference drug aspirin were administered by oral gavage where as morphine and naloxone were injected subcutaneously and intraperitoneally, respectively. Animals were divided into 8-12 groups. Each group comprised of 6 animals of mice or rats for analgesic, antipyretic and anti-inflammatory studies.

Group	Name group	Detail	Test
1	Control	Cosolvent composed of Tween 80 : Propylene glycol: Distilled water at the ratio of 1:4:5 at doses of 10 ml/kg (mice) and 5 ml/kg (rats).	Analgesic, antipyretic and anti-inflammatory
2, 3	Reference drugs	1) Aspirin at the dose of 200 mg/kg, orally (po). 2) Morphine sulfate at the dose of 5 mg/kg, subcutaneously (sc). 3) Naloxone at the dose of 2 mg/kg, intraperitoneally (ip)	Writhing, formalin, antipyretic and anti-inflammatory Formalin, hot plate and tail flick Hot plate and tail flick
4, 5, 6	EESL	Ethanol extract of *S. lychnophorum* (EESL) at doses of 50, 100 and 200 mg/kg, orally, respectively.	Analgesic, antipyretic and anti-inflammatory
7, 8, 9	DESL	Dichloromethane extract of *S. lychnophorum* (DESL) at doses of 50, 100 and 200 mg/kg, orally, respectively.	Analgesic, antipyretic and anti-inflammatory
10, 11,12	Antagonist	1). Naloxone at dose 2 mg/kg, given intraperitoneally before morphine 5mg/kg, subcutaneously. 2). Naloxone at dose 2 mg/kg, given intraperitoneally before EESL at the dose 200 mg/kg, orally 3). Naloxone at the dose 2 mg/kg, given intraperitoneally before DESL at the dose 200 mg/kg, orally	Hot plate and tail flick

Methods

1. **Acute toxicity**

The up and down procedure for acute toxicity (LD_{50}) testing was carried out as previously described by Bruce (1985). Using strategy for acute toxicity testing, the animal is dosed one at a time. If an animal survives, the dose of next animal is increased. But if it died, the dose is decreased. The observed symptoms and behavioral changes after the extract administration were convulsion, hyperactivity, sedation, grooming and loss of righting reflex. The first dose was begun at 5 g/kg, po. The ethanol and dichloromethane extracts of *S. lychnophorum* were orally administered to a group of male mice and rats. After administration, behavioral changes and abnormal symptoms were observed during a period of 8 h and 7 days. Food and water was given *ad libitum*.

2. **Antinociceptive activity**

2.1 Writhing test

The writhing test was performed as described by Koster *et al.* (1959). Male ICR mice, weighting 30-40 g, were used in the experiments. The animals were divided into 8 groups and each group consisted of 6 mice.

Group 1 : Control (cosolvent 10 ml/kg, po)
Group 2 : Aspirin (200 mg/kg, po)
Group 3 : EESL (50 mg/kg, po)
Group 4 : EESL (100 mg/kg, po)
Group 5 : EESL (200 mg/kg, po)
Group 6 : DESL (50 mg/kg, po)
Group 7 : DESL (100 mg/kg, po)
Group 8 : DESL (200 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 3, 4 and 5 received the EESL at doses of 50, 100 and 200 mg/kg, po, and group 6, 7 and 8 received the DESL at doses of 50, 100 and 200 mg/kg, po, respectively. After 30 min of treatment, each mouse of each group was administered intraperitoneally with 0.6%

acetic acid in normal saline at the dose 10 ml/kg. The mice were observed and counted for the number of abdominal constrictions and stretchings in a period of 0-20 min. The schematic plan of the writhing test was illustrated in Figure 9.

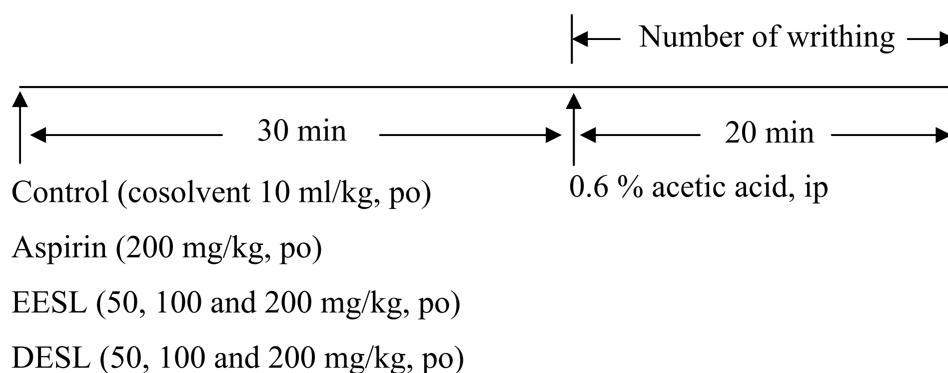


Figure 9. Schematic plan of the writhing test

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

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

2.2 Formalin test

The method was done as previous described by Hunskarr *et al.* (1985). Male ICR male, weighing 30- 40 g, were divided in to 9 groups of 6 mice each.

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

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

Group 3 : Morphine sulfate (5 mg/kg, sc)

Group 4 : EESL (50 mg/kg, po)

Group 5 : EESL (100 mg/kg, po)

Group 6 : EESL (200 mg/kg, po)

Group 7 : DESL (50 mg/kg, po)

Group 8 : DESL (100 mg/kg, po)

Group 9 : DESL (200 mg/kg, po)

The control group (group 1) received cosolvent (10 ml/kg, po) where as the mice in group 2 and 3 received reference drug aspirin and morphine sulfate at the dose of 200 mg/kg, po and 5 mg/kg, sc. Group 4, 5 and 6 received the ethanol extract at doses of 50, 100 and 200 mg/kg, po, and group 7, 8 and 9 received the dichloromethane extract at doses of 50, 100 and 200 mg/kg, po, respectively. After 30 min of treatment (except 15 min for morphine), 20 µl of 2.5% formalin in normal saline was injected subcutaneously to a hind paw of each mouse. The times spent in the licking hind paw in early phase (0-5 min) and late phase (15-30 min) were recorded. The schematic plan of the formalin test was illustrated in Figure 10.

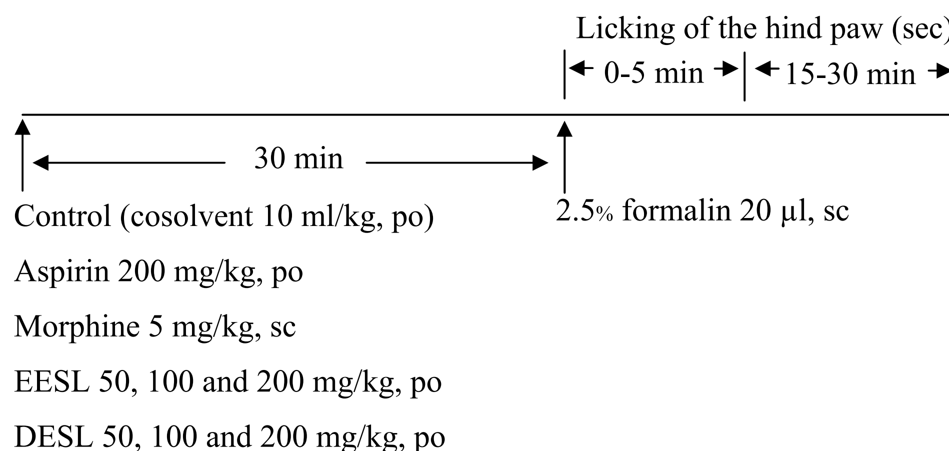


Figure 10. Schematic plan of the formalin test

The times spent in the licking hind 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 following formula.

$$\text{Inhibition (\%)} = \frac{\text{Licking time (Control group)} - \text{Licking time (exp. gr)} \times 100}{\text{Licking time (Control group)}}$$

2.3 Hot plate test

The hot plate test was conducted according to the method previously described by Woolfe and MacDonald (1994). Male ICR mice, weighing 30-40 g, were divided in 12 groups of 6 mice each.

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

Group 2 : Morphine sulfate (5 mg/kg, sc)

Group 3 : Naloxone (2 mg/kg, ip)

Group 4 : EESL (50 mg/kg, po)

Group 5 : EESL (100 mg/kg, po)

Group 6 : EESL (200 mg/kg, po)

Group 7 : DESL (50 mg/kg, po)

Group 8 : DESL (100 mg/kg, po)

Group 9 : DESL (200 mg/kg, po)

Group 10 : Naloxone (2 mg/kg, ip) + Morphine (5 mg/kg, sc)

Group 11 : Naloxone (2 mg/kg, ip) + EESL (200 mg/kg, po)

Group 12 : Naloxone (2 mg/kg, ip) + DESL (200 mg/kg, po)

The control group (group 1) received cosolvent (10 ml/kg, po). Group 2 and 3 received the reference drug morphine sulfate (5 mg/kg, sc) and naloxone (2 mg/kg, ip), respectively. Group 4, 5 and 6 received the ethanol extract at doses of 50, 100 and 200 mg/kg, po, and group 7, 8 and 9 received the dichloromethane extract at doses of 50, 100 and 200 mg/kg, po, respectively. Group 10, 11 and 12 received naloxone (2 mg/kg, ip) 10 min before morphine (5 mg/kg, sc.), EESL and DESL at the dose 200 mg/kg, po, respectively. After 30 min of treatment with each extract

dose or cosolvent (except 15 min for morphine and 10 min for naloxone), mice were placed on a hot plate at temperature of 55 ± 1 ºC for a maximum time of 45 sec. Reaction times were recorded when the animals licked and flicked of hind paw or jumping at 30, 45, 60, 75 and 90 min. The cut off time of observation was 45 seconds. Only the mouse showed nociceptive response within 15 seconds was used in the experiments. The schematic plan of the hot plate test was illustrated in Figure 3.

2.4 Tail flick test

The method was done as previous described by D'Amour and Smith (1941). The experiment was carried out in the same manner as the hot plate test. Male ICR mice, weighing 30-40 g, were divided in 12 groups of 6 mice each.

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

Group 2 : Morphine sulfate (5mg/kg, sc)

Group 3 : Naloxone (2 mg/kg, ip)

Group 4 : EESL (50 mg/kg, po)

Group 5 : EESL (100 mg/kg, po)

Group 6 : EESL (200 mg/kg, po)

Group 7 : DESL (50 mg/kg, po)

Group 8 : DESL (100 mg/kg, po)

Group 9 : DESL (200 mg/kg, po)

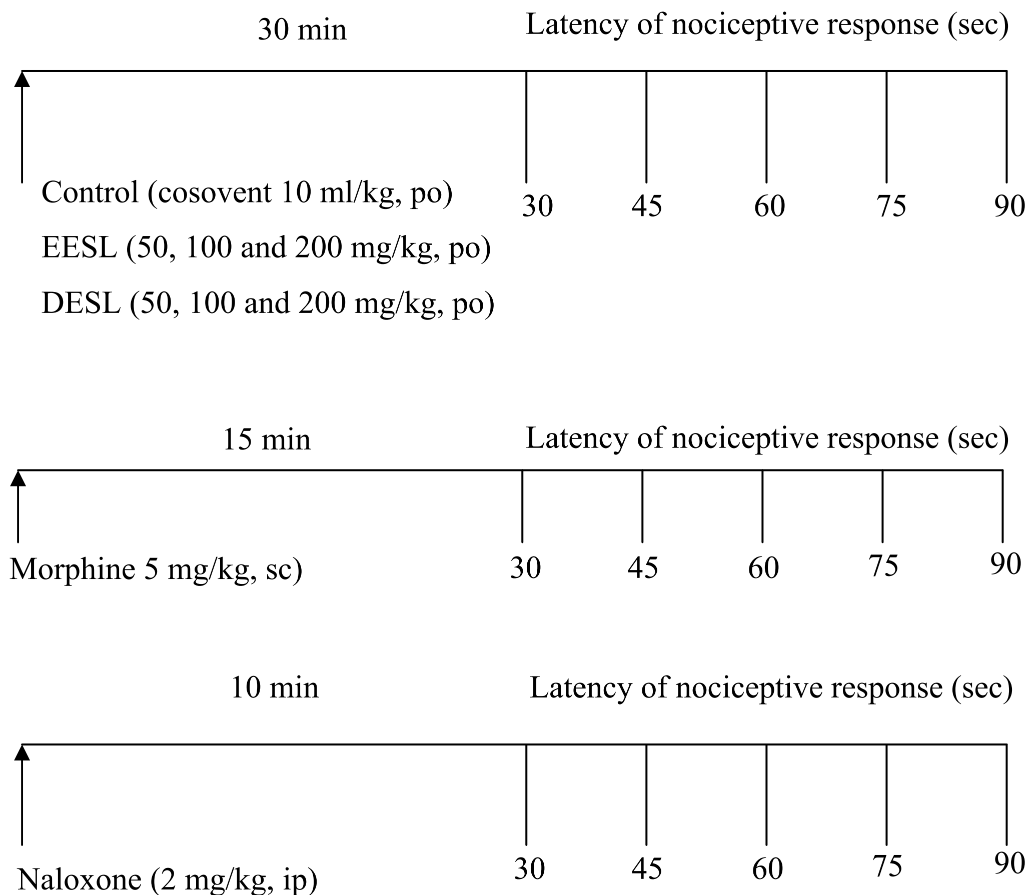
Group 10 : Naloxone (2 mg/kg, ip) + Morphine (5 mg/kg, sc)

Group 11 : Naloxone (2 mg/kg, ip) + EESL (200 mg/kg, po)

Group 12 : Naloxone (2 mg/kg, ip) + DESL (200 mg/kg, po)

The control group (group 1) received cosolvent (10 ml/kg, po). Group 2 and 3 received the reference drug morphine sulfate (5 mg/kg, sc) and naloxone (2 mg/kg, ip), respectively. Group 4, 5 and 6 received the ethanol extract at dose of 50, 100 and 200 mg/kg, po, and group 7, 8 and 9 received the dichloromethane extract at dose of 50, 100 and 200 mg/kg, po, respectively. Group 10, 11 and 12 received naloxone (2 mg/kg, ip) 10 min before morphine (5 mg/kg, sc), EESL and DESL at the dose 200 mg/kg, po, respectively. After 30 min of treatment with each extract dose or cosolvent (except 15 min for morphine and 10 min for naloxone), the reaction time

was determined. In all groups in this experiment, each mouse was placed on the tail flick apparatus and the tail flick response was measured by gently placing the mouse tail on center position of light beam. The reaction time was measured by focusing an intensity controlled beam of light on the detail one-third portion of the animal tail (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 mice that showed nociceptive response 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 response was measured at 30, 45, 60, 75, and 90 min after administered agents. The schematic plan of the tail flick test was illustrated in Figure 11.



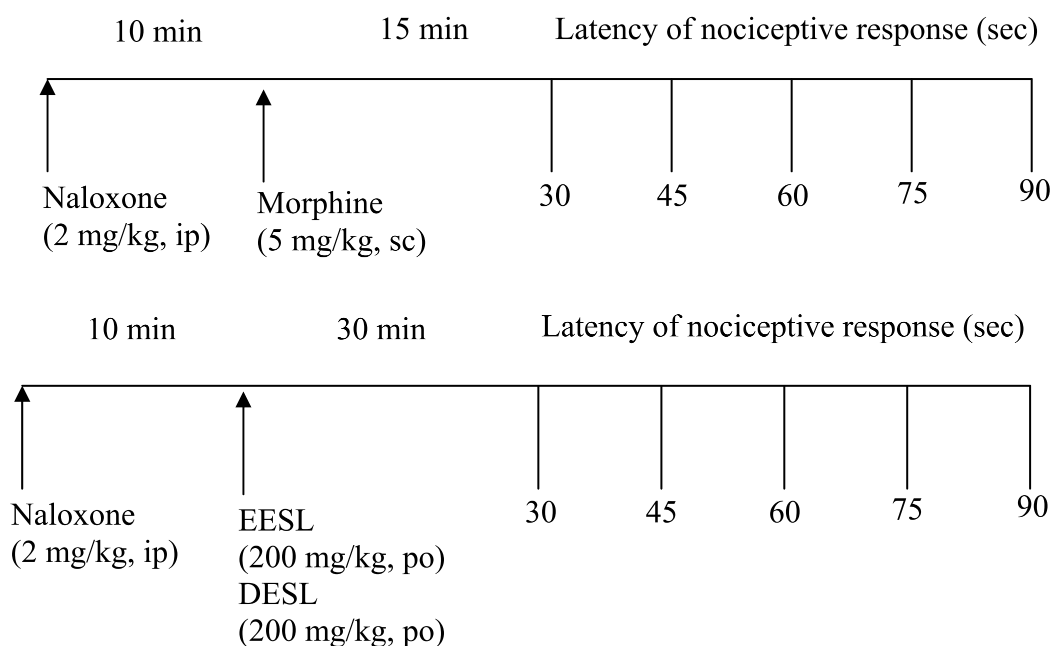


Figure 11. Schematic plan of the hot plate and tail flick tests

3. Antipyretic activity

Antipyretic activity of drug was measured by slightly modifying the method described by Adams *et al.* (1968). Male Wistar rats, weighing 180-220 g, were fasted overnight (12 h) with water *ad libitum* before the experiments. Pyrexia was induced by subcutaneously injecting 20% (w/v) brewer's yeast suspension (10 ml/kg) into the rat's dorsum region. nineteen hours after the 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 ºC were used for the experiments. The selected rats were divided into 8 groups of 6 rats each. The control group (group 1) 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 ethanol extract at doses of 50, 100 and 200 mg/kg, po, and group 6, 7 and 8 received the dichloromethane extract at doses of 50, 100 and 200 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 12.

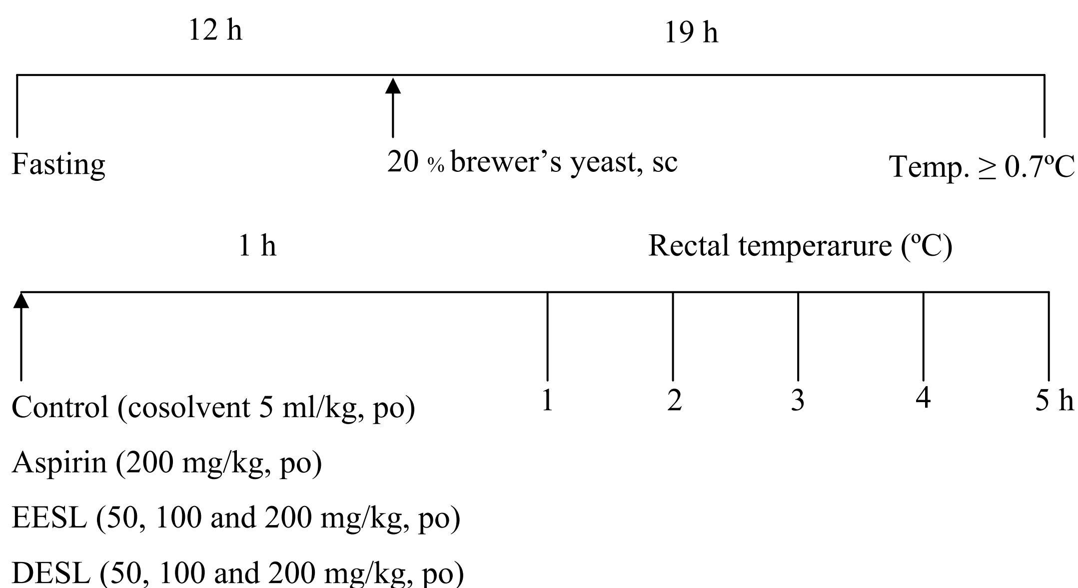


Figure 12. Schematic plan of the brewer's yeast-induced pyrexia

4. Anti-inflammatory activity

4.1. Acute inflammation (carrageenan-induced paw edema in rats)

This method was performed as previously described by Winter *et al*. (1962). The initial right hind paw volume of the rats was measured using a plethysmometer. Male Wistar rats, weighing 180-220 g, were divided into 8 groups of 6 rats each.

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

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

Group 3 : EESL (50 mg/kg, po)

Group 4 : EESL (100 mg/kg, po)

Group 5 : EESL (200 mg/kg, po)

Group 6 : DESL (50 mg/kg, po)

Group 7 : DESL (100 mg/kg, po)

Group 8 : DESL (200 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 3, 4 and 5 received the ethanol extract at doses of 50, 100 and 200 mg/kg, po, and group 6, 7 and 8 received the dichloromethane extract at doses of 50, 100 and 200 mg/kg, po, respectively. After 30 minutes, each rat in all groups was subcutaneously injected with 0.1 ml of 1% (w/v) carrageenan in normal saline into subplantar region of the right hind paw. The volume of right hind paw was measured at 1, 2, 3, 4, and 5 h after carrageenan injection, and the edema volume was determined. The data were expressed as percentage of swelling compared with initial hind paw volume of each rat. The schematic plan of the carrageenan-induced paw edema experiment was illustrated in Figure 5.

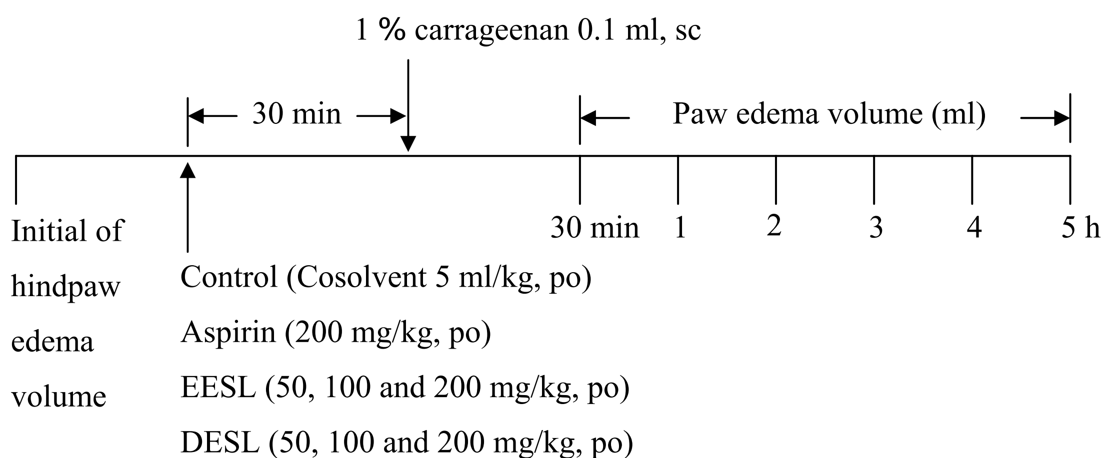


Figure 13. Schematic plan of the carrageenan-induced paw edema in rats

The volume of hind paw was evaluated for anti-inflammatory activity and 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= volume of hind paw before carrageenan injection

B = volume of hind paw after carrageenan injection

4.2.Chronic inflammation (cotton pellet-induced granuloma in rats)

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 8 groups of 6 rats each.

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

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

Group 3 : EESL (50 mg/kg, po.)

Group 4 : EESL (100 mg/kg, po.)

Group 5 : EESL (200 mg/kg, po.)

Group 6 : DESL (50 mg/kg, po.)

Group 7 : DESL (100 mg/kg, po.)

Group 8 : DESL (200 mg/kg, po.)

Subcutaneous implantation of pellets of compressed cotton provokes foreign body granuloma. Cotton roll were cut and made into pellets weighing 20 ± 1 mg each and sterilized in a hot air oven at 160 ºC for 2 h. The rat was anesthetized using urethane, ip and making small subcutaneous incisions in both sides of groin regions. Afterward, under sterile conditions, cotton pellets were implanted subcutaneously in the groin area. The incisions were sutured by sterile catgut and all process were done under sterile conditions. After recovery from anesthesia, the animals were treated with cosolvent, aspirin, EESL and DESL, orally for 7 days. On the eighth day, animals were sacrificed and the cotton pellet granulomas were freed from extraneous tissue and their wet weights were determined and then dried the cotton pellet by hot air oven at 60 ºC for 18 h and weighed to obtain a constant weight. The average weight of the control group as well as of the test groups was

calculated. The percent change of granuloma weight relative to control group was determined.

Inhibition of granuloma was evaluated for anti-inflammatory activity 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.

Statistical analysis

The data obtained were analysed as a mean ± SEM. Statistically significant differences between groups were calculated by the application of analysis of variance (one –way ANOVA) following by LSD test. *P* Values less than 0.05 ($p<0.05$) were used as the significance level.

CHAPTER 4

RESULTS

1. Acute toxicity

Both the EESL and DESL at the dose of 5 g/kg given orally did not affect on general behaviors, and did not cause any abnormal symptoms, signs of toxicity and mortality in the animals during the observation period of 8 h and 7 days. Therefore, the estimated LD_{50} in mice and rats was more than 5 g/kg when administered orally.

2. Analgesic activity

1.1 Writhing test

The effects of EESL and DESL extract of *S. lychnophorum* on 0.6% acetic acid-induced writhings in mice were summarized in Table 2 and Figure 14. Oral administration of the EESL extract at doses of 50, 100 and 200 mg/kg significantly decreased (p<0.05) the number of writhings and stretchings when compared to the control (55.50 ± 2.93 vs 64.50 ± 3.85, 41.67 ± 3.31 vs 64.50 ± 3.85 and 24 ± 1.81 vs 64.50 ± 3.85, respectively) with the percentage of inhibition being 13.95, 35.40 and 62.79, respectively. This activity was dose-related. The reference drug aspirin at the dose of 200 mg/kg, po significantly decreased the number of writhings when compared to the control (22.83 ± 1.14 vs 64.50 ± 3.85; p<0.05), and the percentage of inhibition was 64.60. The results indicated that the EESL at the dose of 200 mg/kg, po showed a similar analgesic activity to aspirin (200 mg/kg, po).

The DESL at doses of of 50, 100 and 200 mg/kg, po did not significantly decrease the number of writhings and stretchings induced by 0.6% acetic acid in mice (Table 2 and Figure 14).

Table 2. Effect of aspirin, ethanol and dichloromethane extract of *S. lychnophorum* (EESL, DESL) on 0.6% acetic acid-induced writhing in mice.

Treatment	**Dose (mg/kg, po)**	**Number of writhings**	**Inhibition (%)**
Control		64.50 ± 3.85	0
Aspirin	200	22.83 ± 1.14*	64.60
EESL	50	55.50 ± 2.93*	13.95
EESL	100	41.67 ± 3.31*	35.40
EESL	200	24.00 ± 1.81*	62.79
DESL	50	58.50±0.99	9.30
DESL	100	57.33±0.92	11.11
DESL	200	56.50±0.67	12.40

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

*$p<0.05$, significant different compared to the control (LSD test).

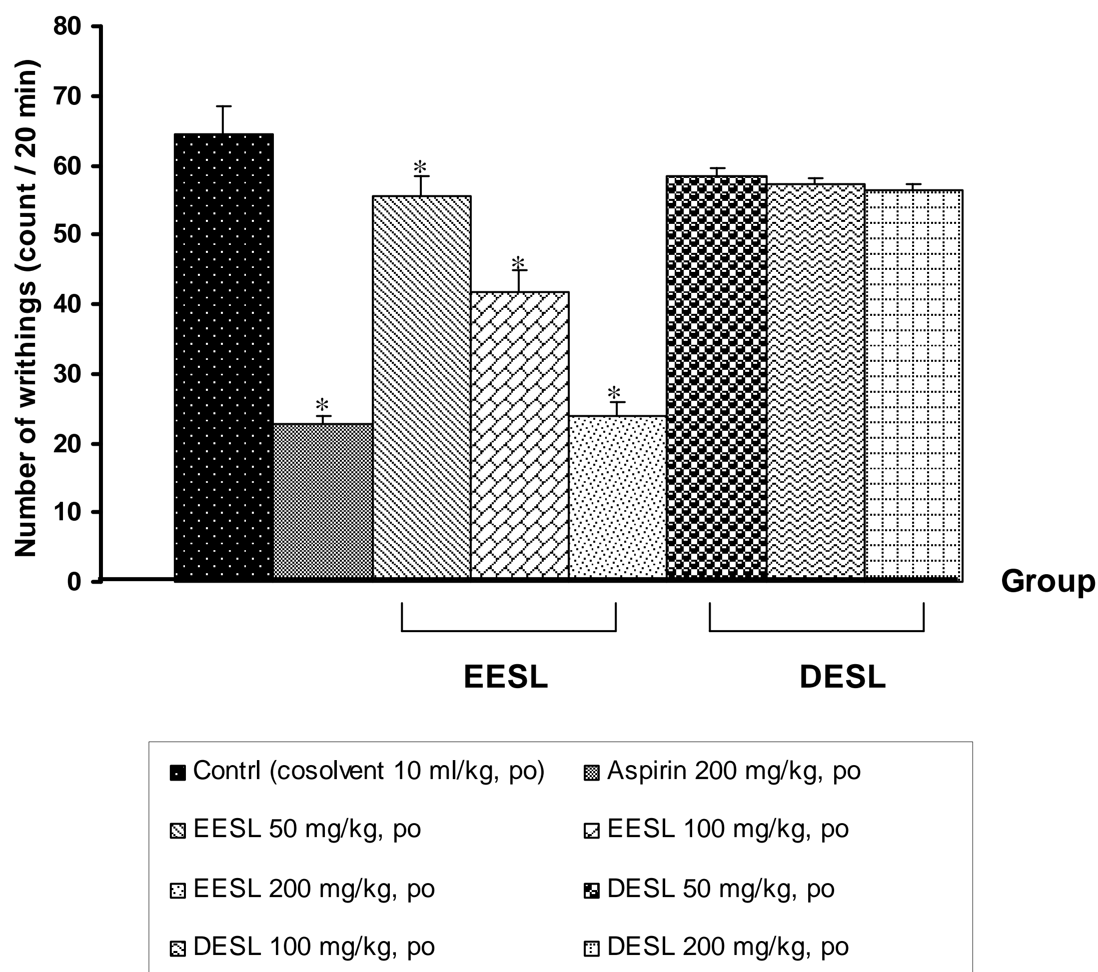


Figure 14. Effect of aspirin, ethanol and dichloromethane extract of *S. lychnophorum* (EESL, DESL) on 0.6% acetic acid-induced writhing in mice.

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

1.2 Formalin test

The results (Table 3 and Figure 15) showed that the times spent in licking the paw after the reference drug aspirin (200 mg/kg, po), morphine (5 mg/kg, sc) and the EESL (50, 100 and 200 mg/kg, po) given before the injection of 20 μl of 2.5% formalin at the right hind paw of the mice were significantly decreased both in the early and late phase compared to the control (p<0.05) where as the DESL (50, 100 and 200 mg/kg, po) did not significantly reduced. The percentage of inhibition after treatment with aspirin and morphine in early and late phase was 50.98, 56.13 and 57.51, 69.40, respectively. In the early phase, the EESL at doses of 50, 100, 200 mg/kg, po significantly decreased the total licking time when compared to the control with the inhibition by 11.77, 23.58 and 26.43%, respectively. In the late phase, the EESL at doses of 50, 100 and 200 mg/kg significantly reduced the total licking time with the percentage of inhibition by 12.61, 30.37 and 67.61, respectively.

Table 3. Effect of aspirin, morphine, ethanol and dichloromethane extract of *S. lychnophorum* (EESL, DESL) on 2.5% formalin-induced paw licking in mice.

Treatment	Dose (mg/kg, po)	Licking of the hind paw (sec)			
		Early phase	Inhibition (%)	Late phase	Inhibition (%)
Control		57.92±2.79	0	78.44±3.59	0
Aspirin	200	28.39±1.67*	50.98	33.33±2.87*	57.51
Morphine	5 mg/kg, sc	25.41±1.12*	56.13	24.00±1.23*	69.40
EESL	50	51.10±1.83*	11.77	68.55±0.55*	12.61
EESL	100	44.26±2.97*	23.58	54.62±1.52*	30.37
EESL	200	42.61±1.96*	26.43	25.40±1.12*	67.61
DESL	50	53.47±0.63	7.68	71.83±0.84	8.43
DESL	100	53.03±0.57	8.44	71.63±0.86	8.68
DESL	200	51.84±0.86	10.49	71.91±0.88	8.32

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

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

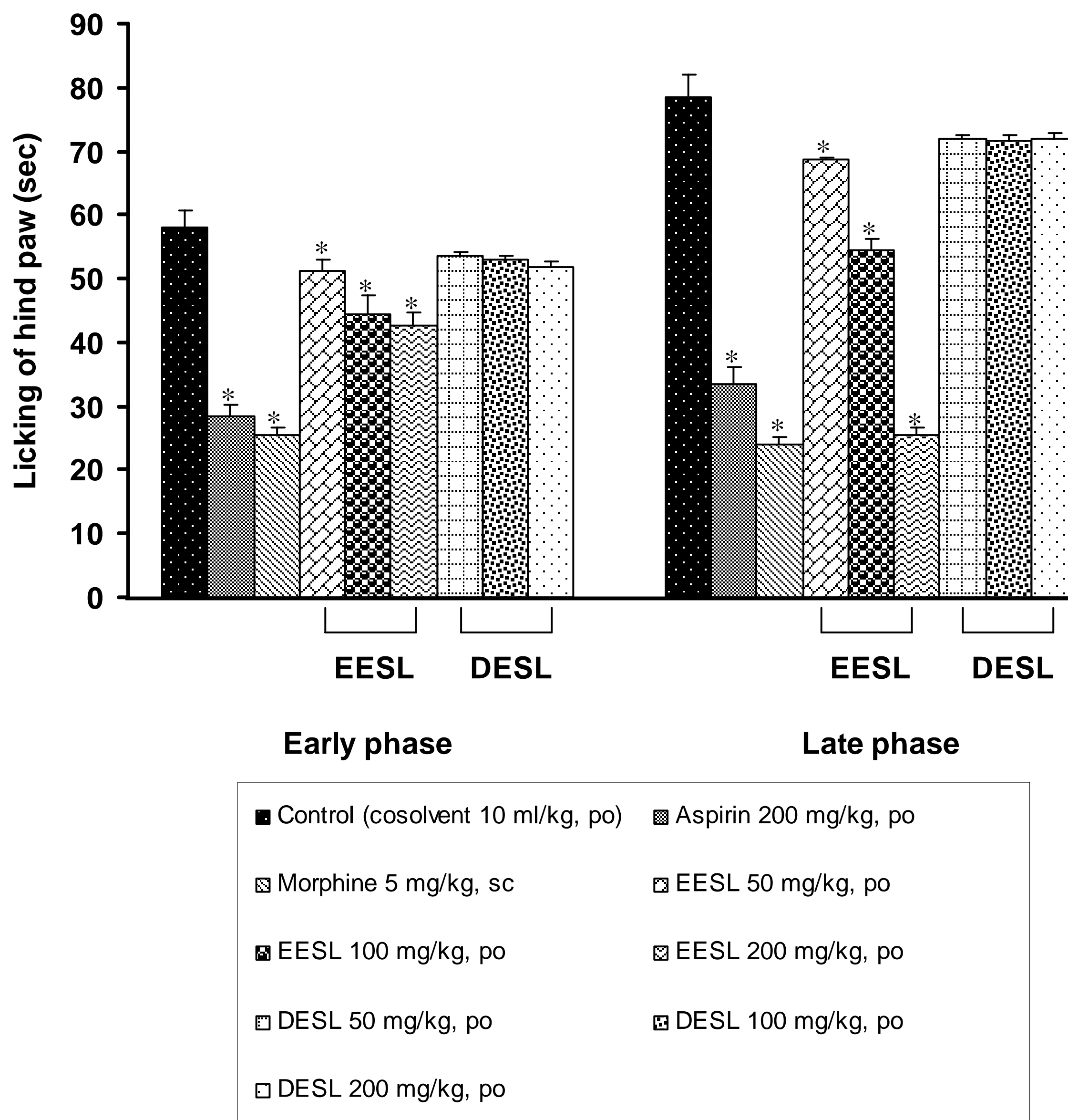


Figure 15. Effect of aspirin, morphine, and the ethanol and dichloromethane extract of *S. lychnophorum* (EESL, DESL, respectively) on 2.5% formalin-induced paw licking in mice.

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

1.3 Hot plate test

The results showed that the reference drug morphine at the dose of 5 mg/kg, sc markedly increased the pain latency in mice at all the time intervals measured (30, 45, 60, 75 and 90 minutes, $p<0.05$) after administration. The EESL at doses of 50, 100 and 200 mg/kg, po significantly increased latency of nociceptive response at different time measured. The EESL at the dose of 50 mg/kg, po significantly increased the latency of nociceptive response at 60, 75 and 90 min when compared to the control whereas the EESL at doses of 100 and 200 mg/kg, po significantly increased the latency of nociceptive response at 45, 60 and 75 min, respectively. The DESL at doses of 50, 100 and 200 mg/kg, po did not have a significant effect on the latency of nociceptive response in mice. The results were summarized in Table 4 and Figure 16.

The antagonistic action of naloxone (2 mg/kg, ip) on effects of morphine (5 mg/kg, sc), EESL (200 mg/kg, po) and DESL (200 mg/kg, po) on the latency of nociceptive response in mice was also investigated. The results indicated that naloxone given before morphine could antagonize the effect of morphine at 60, 75 ($p<0.05$) and 90 minutes ($p<0.01$) when compared to the morphine administered alone. Furthermore, naloxone given before the EESL (200 mg/kg, po) decreased latency of nociceptive response at 30 and 45 minutes ($p<0.05$) when compared to the EESL 200 mg/kg alone. The results were summarized in Table 5 and Figure 17.

Table 4. Effect of morphine, naloxone, ethanol and dichloromethane extract of *S. lychnophorum* (EESL, DESL) on heat-induced pain in mice.

Treatment	Dose (mg/kg, po)	Latency of nociceptive (sec)					
		Initial response	**30 min**	**45 min**	**60 min**	**75 min**	**90 min**
Control		7.65 ± 0.74	10.48 ± 0.62	11.51 ± 0.42	11.28 ± 0.45	10.93 ± 0.55	11.21 ± 0.54
Morphine	5 mg/kg, sc	10.25±0.79	14.22±0.37*	14.48±0.72*	15.62±0.85*	14.31±0.70*	14.27±0.67*
Naloxone	2 mg/kg, ip	11.33±0.63	11.15±0.51	12.55±0.87	13.08±0.53	10.46±0.56	10.02±0.43
EESL	50	10.00±0.73	10.18±0.67	12.27±0.78	12.42±0.59*	12.77±0.97*	12.83±0.64*
EESL	100	10.13±0.93	11.92±0.48	12.52±0.42*	13.02±0.63*	13.17±0.55*	11.57±0.39
EESL	200	10.59±0.59	12.72±0.61	13.63±0.51*	14.03±0.38*	12.10±0.36*	11.68±0.34
DESL	50	10.03±0.15	10.60±0.28	11.55±0.15	11.37±0.17	10.96±0.14	11.25±0.24
DESL	100	9.90±0.13	10.92±0.14	11.52±0.11	11.36±0.24	11.23±0.14	11.25±0.19
DESL	200	10.25±0.16	10.95±0.19	11.52±0.09	11.48±0.34	11.27±0.23	11.26±0.11

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

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

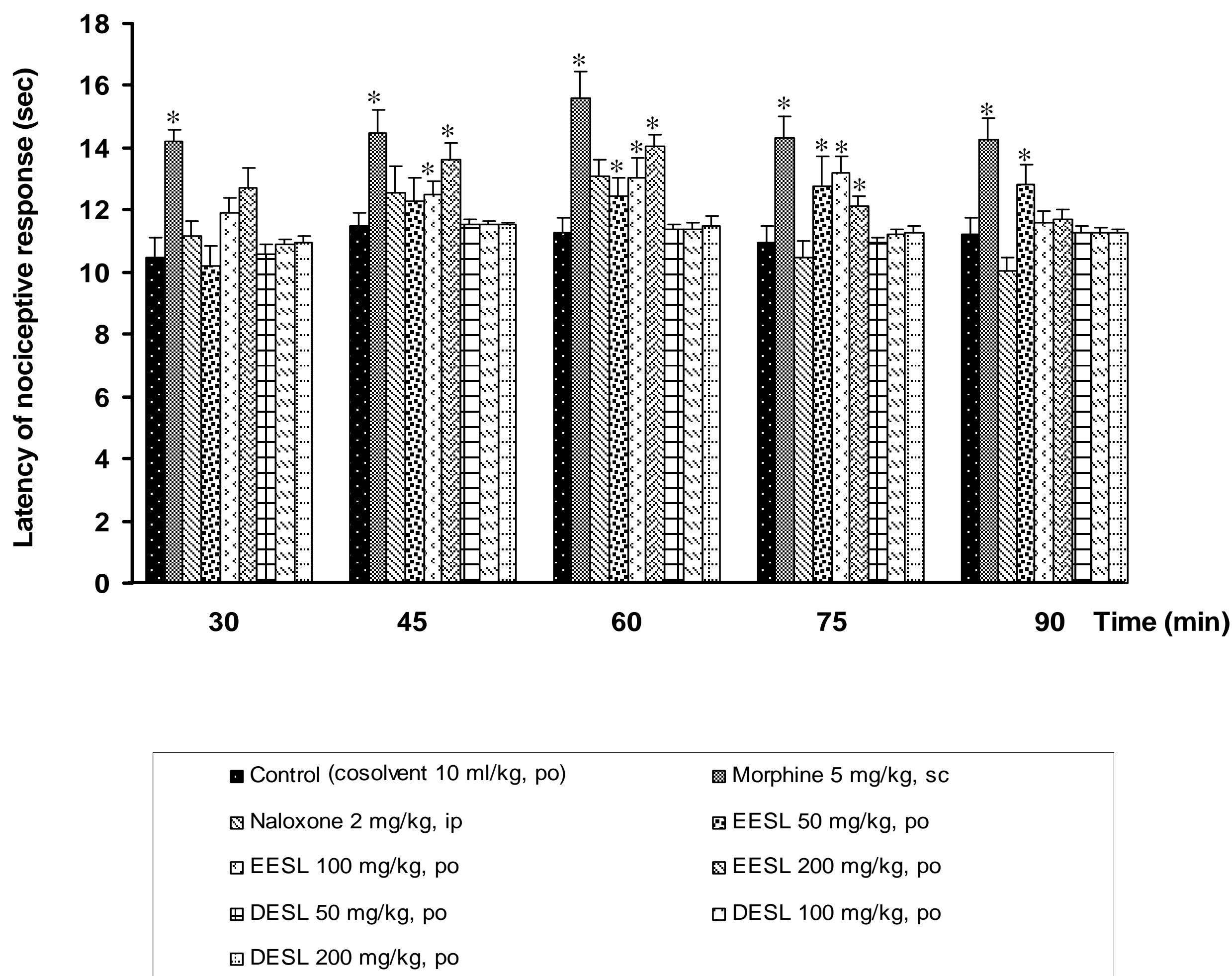


Figure 16. Effect of morphine, naloxone, ethanol and dichloromethane extract of *S. lychnophorum* (EESL, DESL) on heat-induced pain in mice.
* $p<0.05$, significantly different compared to the control (LSD test).

Table 5. Antagonistic effect of naloxone on the analgesic activity of morphine, ethanol and dichloromethane extract of *S. lychnophorum* (EESL, DESL) on heat-induced pain in mice.

Treatment	**Dose (mg/kg, po)**	**Latency of nociceptive (sec)**					
		Initial response	**30 min**	**45 min**	**60 min**	**75 min**	**90 min**
Control		7.65 ± 0.74	10.48 ± 0.62	11.51 ± 0.42	11.28 ± 0.45	10.93 ± 0.55	11.21 ± 0.54
Morphine	5 mg/kg, sc	10.25±0.79	14.22±0.37*	14.48±0.72*	15.62±0.85*	14.31±0.70*	14.27±0.67*
EESL	200	10.59±0.59	12.72±0.61	13.63±0.51*	14.03±0.38*	12.10±0.36*	11.68±0.34
DESL	200	10.25±0.16	10.95±0.19	11.52±0.09	11.48±0.34	11.27±0.23	11.26±0.11
Naloxone + Morphine	2 mg/kg, ip 5 mg/kg, sc	10.92±0.85	13.12±0.52	13.57±0.54	12.05±0.37**	10.62±0.48**	10.91±0.36**
Naloxone +EESL	2 mg/kg, ip 200 mg/kg, po	11.80 ± .85	10.96 ± 0.29$^{\#}$	10.90 ± 0.51$^{\#}$	12.79 ± 0.87	11.35 ± 0.49	11.43 ± 0.59
Naloxone + DESL	2 mg/kg, ip 200 mg/kg, po	10.40±0.23	11.60±0.55	11.52±0.14	11.30±0.13	10.95±0.14	11.22±0.12

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

*p<0.05, significant different compared to the control (LSD test),

**$p<0.05$, significant different compared to the morphine (LSD test),

$^{\#}$ $p<0.05$ significant different compared to the EESL 200 mg/kg (LSD test).

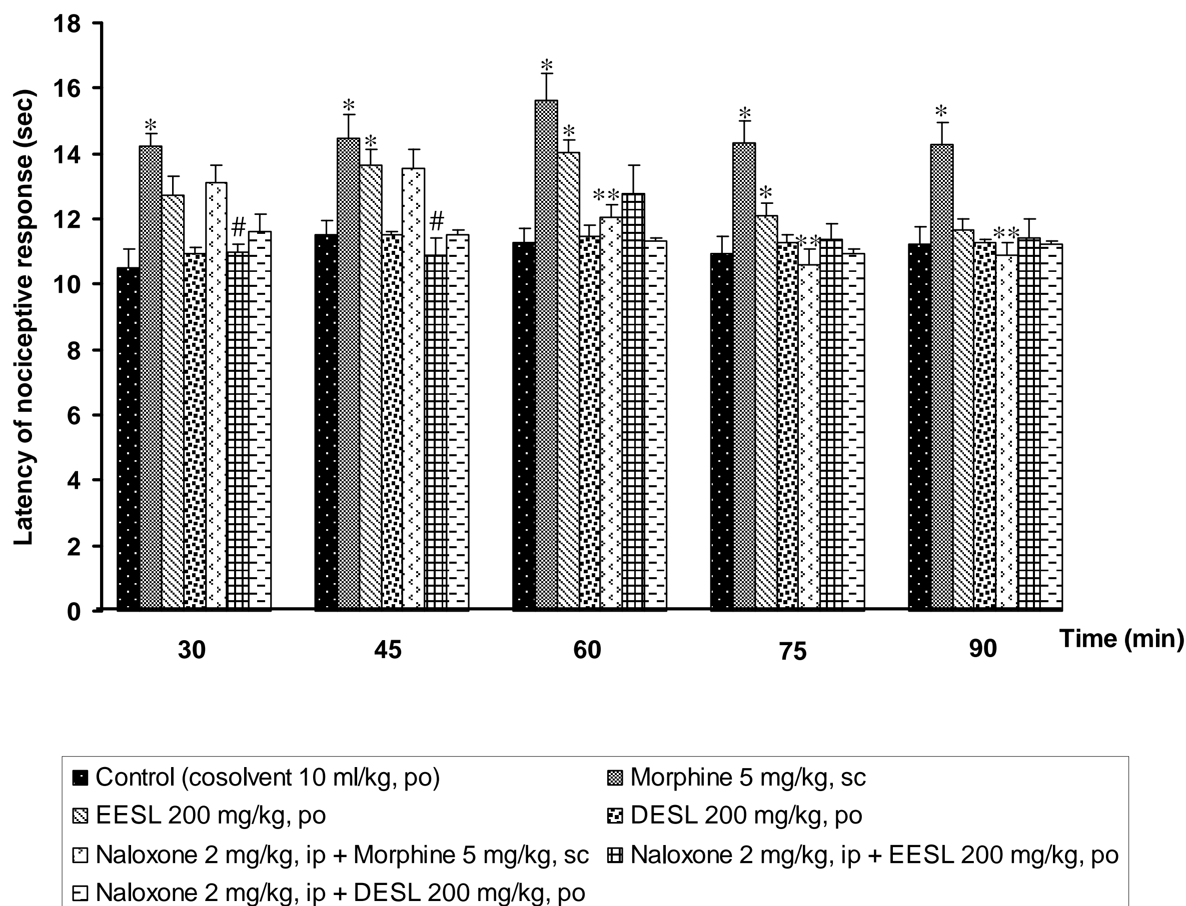


Figure 17. Antagonistic effect of naloxone on the analgesic activity of morphine, ethanolic and dichloromethane extract of *S. lychnophorum* (EESL, DESL) on heat-induced pain in mice.

*p<0.05, significant different compared to the control (LSD test),

**$p<0.05$, significant different compared to the morphine (LSD test),

$^{\#}p<0.05$, significant different compared to the EESL 200 mg/kg (LSD test).

1.4 Tail flick test

The EESL extract (50, 100 and 200 mg/kg, po) and morphine (5 mg/kg, sc) significantly increased the reaction time to the nociceptive responses. The antinociceptive effect of the EESL extract at doses of 50, 100 and 200 mg/kg, po began at the time 45, 30, 30 and 30 minutes, respectively after administration. Morphine (5 mg/kg, sc) and the EESL extract at doses of 100 and 200 mg/kg, po significantly increased the tail flick latency at all the interval times measured in the tail flick test in mice and its antinociceptive activity still remained for 90 minutes while the EESL extract at the dose of 50 mg/kg, po significantly increased the reaction time at only the time measured at 45, 60, 75 and 90 minutes. However, the DESL at doses of 50, 100 and 200 mg/kg, po did not show the antinociceptive activity in tail flick test. All results were summarized in Table 6 and Figure 18.

In antagonistic studies using naloxone as an antagonist, the results showed that naloxone (2 mg/kg, ip) completely antagonized the effect of morphine (5 mg/kg, sc) on the latency of nociceptive response at all the time intervals measured. Furthermore, naloxone at the dose of 2 mg/kg, ip given before the EESL of *S. lychnophorum* at the dose of 200 mg/kg, po significantly decreased the latency of nociceptive response produced by the extract in the tail flick test at the time 45, 60, 75 and 90 ($p<0.05$) minutes when compared to the EESL at the dose of 200 mg/kg, po alone. The results were shown in Table 7 and Figure 19.

Table 6. Effect of morphine, naloxone, ethanol and dichloromethane extract of *S. lychnophorum* (EESL, DESL) on nociceptive response in the tail flick test in mice.

Treatment	**Dose (mg/kg, po)**	**Latency of nociceptive response (sec)**				
		30 min	**45 min**	**60 min**	**75 min**	**90 min**
Control		6.43±0.13	6.35±0.10	6.20±0.09	6.32±0.09	6.25±0.06
Morphine	5 mg/kg, sc	9.78±0.14*	11.62±0.08*	13.52±0.13*	13.42±0.05*	11.72±0.08*
Naloxone	2 mg/kg, ip	6.43±0.08	6.50±0.07	6.43±0.06	6.45±0.11	6.22±0.09
EESL	50	6.43±0.08	6.67±0.10*	6.88±0.12*	7.23±0.09*	6.85±0.08*
EESL	100	6.65 ±0.08*	6.83±0.06*	7.12±0.14*	7.55±0.11*	6.95±0.11*
EESL	200	6.95±0.10*	7.53±0.13*	8.25±0.14*	7.93±0.09*	7.71±0.09*
DESL	50	6.51±0.04	6.36±0.03	6.32±0.06	6.32±0.08	6.26±0.06
DESL	100	6.47±0.08	6.45±0.10	6.40±0.05	6.33±0.05	6.27±0.04
DESL	200	6.42±0.13	6.43±0.11	6.40±0.08	6.32±0.07	6.27±0.07

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

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

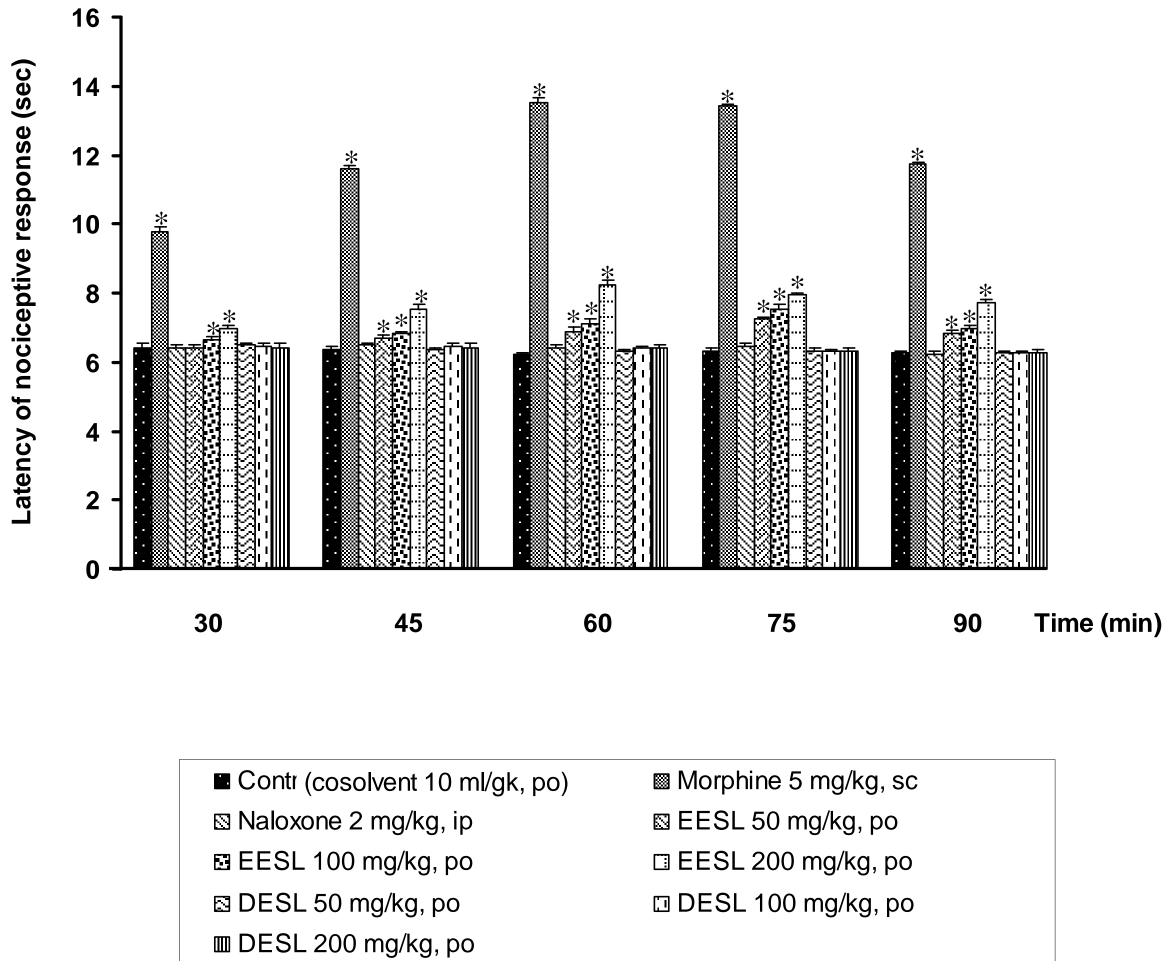


Figure 18. Effect of morphine, naloxone, ethanol and dichloromethane extract of *S. lychnophorum* (EESL, DESL) on nociceptive response in the tail flick test in mice. * $p<0.05$, significantly different compared to the control (LSD test).

Table 7. Antagonistic effect of naloxone on analgesic activity of morphine, ethanol and dichloromethane extract of *S. lychnophorum* (EESL, DESL) on nociceptive response in the tail flick test in mice.

Treatment	Dose	Latency of nociceptive response (sec)				
	(mg/kg, po)	30 min	45 min	60 min	75 min	90 min
Control		6.43±0.13	6.35±0.10	6.20±0.09	6.32±0.09	6.25±0.06
Morphine	5 mg/kg, sc	9.78±0.14*	11.62±0.08*	13.52±0.13*	13.42±0.05*	11.72±0.08*
Naloxone	2 mg/kg, ip	6.43±0.08	6.50±0.07	6.43±0.06	6.45±0.11	6.22±0.09
EESL	200	6.95±0.10*	7.53±0.13*	8.25±0.14*	7.93±0.09*	7.71±0.09*
DESL	200	6.42±0.13	6.43±0.11	6.40±0.08	6.32±0.07	6.27±0.07
Naloxone	2 mg/kg, ip					
+ Morphine	5 mg/kg, sc	7.52±0.15**	7.87±0.13**	7.95±0.08**	7.32±0.14**	7.15±0.19**
Naloxone	2 mg/kg, ip					
+ EESL	200 mg/kg, po	6.85±0.15	6.95±0.11#	7.31±0.06#	6.98±0.10#	6.85±0.07#
Naloxone	2 mg/kg, ip					
+ DESL	200 mg/kg, po	6.42±0.07	6.42±0.05	6.35±0.04	6.22±0.11	6.25±0.11

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

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

** $p<0.05$, significantly different compared to the morphine (LSD test).

#$p<0.05$, significantly different compared to the EESL 200 mg/kg (LSD test).

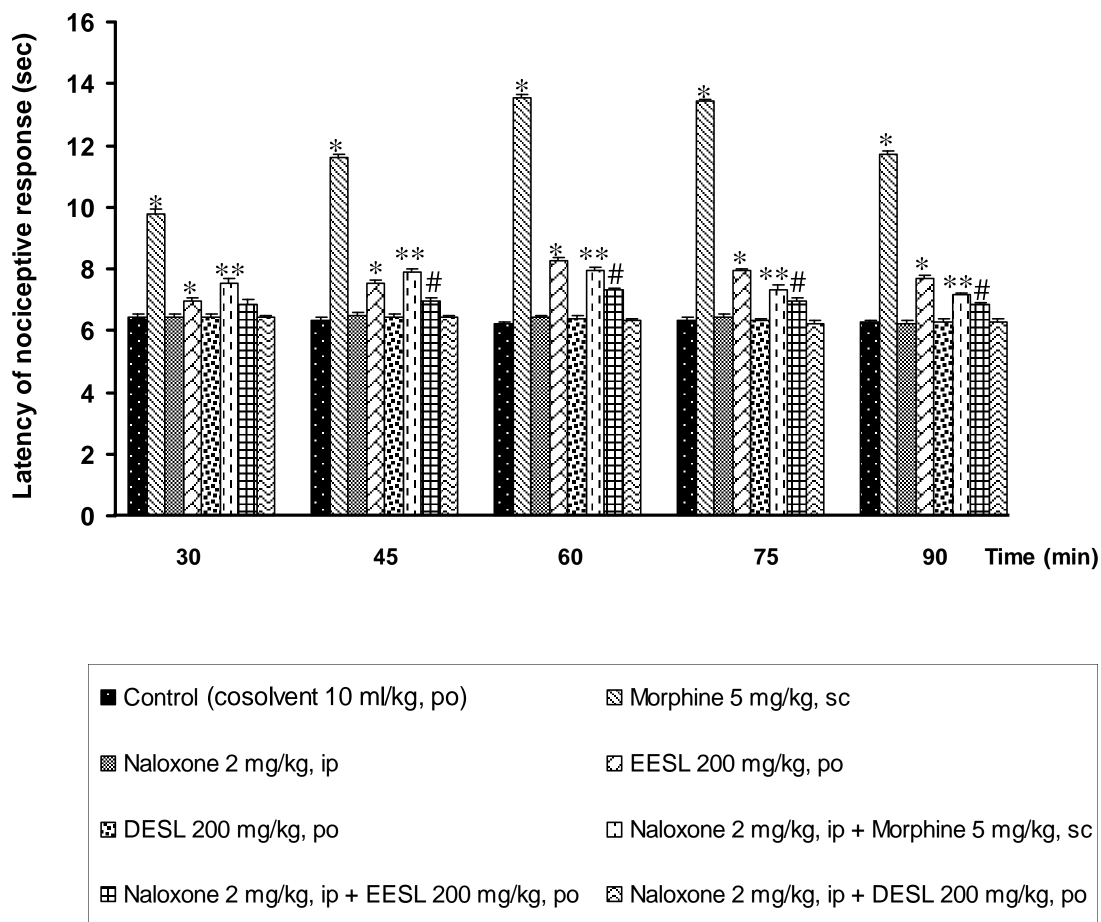


Figure 19. Antagonistic effect of naloxone on analgesic activity of morphine, ethanol and dichloromethane extract *S. lychnophorum* (EESL, DESL) on nociceptive response in the tail flick test in mice.

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

** p<0.05, significantly different compared to the morphine (LSD test).

\# p<0.05, significantly different compared to the EESL 200 mg/kg (LSD test).

2. Antipyretic activity

The result showed the effect of the antipyretic effect of reference drug aspirin (200 mg/kg, po), the EESL (50, 100 and 200 mg/kg, po) and DESL (50, 100 and 200 mg/kg, po) as presented in Table 8 and Figure 20. After the brewer's yeast injection to the rat for 19 h, the rectal temperature was increased > 0.7 ºC. Aspirin at the dose of 200 mg/kg, po significantly decreased in rectal temperature at 2 and 3 h after administration. The EESL at the dose of 50 mg/kg, po significantly reduced the rectal temperature of rats induced by the brewer's yeast injection at 3 h while the EESL at the dose of 100 mg/kg, po significantly decreased the rectal temperature at 2 and 3 h. The EESL at the dose of 200 mg/kg, po significantly decreased the rectal temperature at 3 and 4 h. The DESL at doses of 50, 100 and 200 mg/kg, po, could not reduced the rectal temperature at the time interval measured.

Table 8. Effects of aspirin, the ethanol and dichloromethane extract of *S. lychnophorum* (EESL and DESL) on the brewer's yeast induced pyrexia in rats.

Treatment	Dose (mg/kg,po)	Rectal temperature (ºC)						
		Before yeast injection	0 h	1 h	2 h	3 h	4 h	5 h
Control		35.57±0.16	36.47±0.17	36.42±0.15	36.08±0.19	36.11±0.12	36.10±0.07	35.91±0.22
Aspirin	200	35.73±0.08	36.47±0.09	36.26±0.03	35.87±0.14*	35.71±0.15*	35.86±0.19	35.81±0.22
EESL	50	35.49±0.18	36.40±0.20	35.98±0.19	35.86±0.10	35.61±0.17*	35.75±0.14	35.72±0.19
EESL	100	35.27±0.23	36.20±0.27	36.02±0.11	35.81±0.10*	35.51±0.18*	35.69±0.13	35.93±0.31
EESL	200	35.51±0.10	36.41±0.05	36.42±0.11	36.08±0.14	35.63±0.10*	35.49±0.07*	35.67±0.16
DESL	50	36.09±0.12	36.81±0.13	36.78±0.06	36.28±0.19	36.07±0.10	36.05±0.06	36.09±0.03
DESL	100	35.86±0.14	36.82±0.11	36.76±0.07	35.87±0.10	35.94±0.12	36.06±0.04	36.21±0.08
DESL	200	35.85±0.17	36.87±0.11	36.86±0.05	36.14±0.03	36.14±0.02	36.06±0.05	36.31±0.03

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

Rectal temperature was measured after the yeast injection 19 h

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

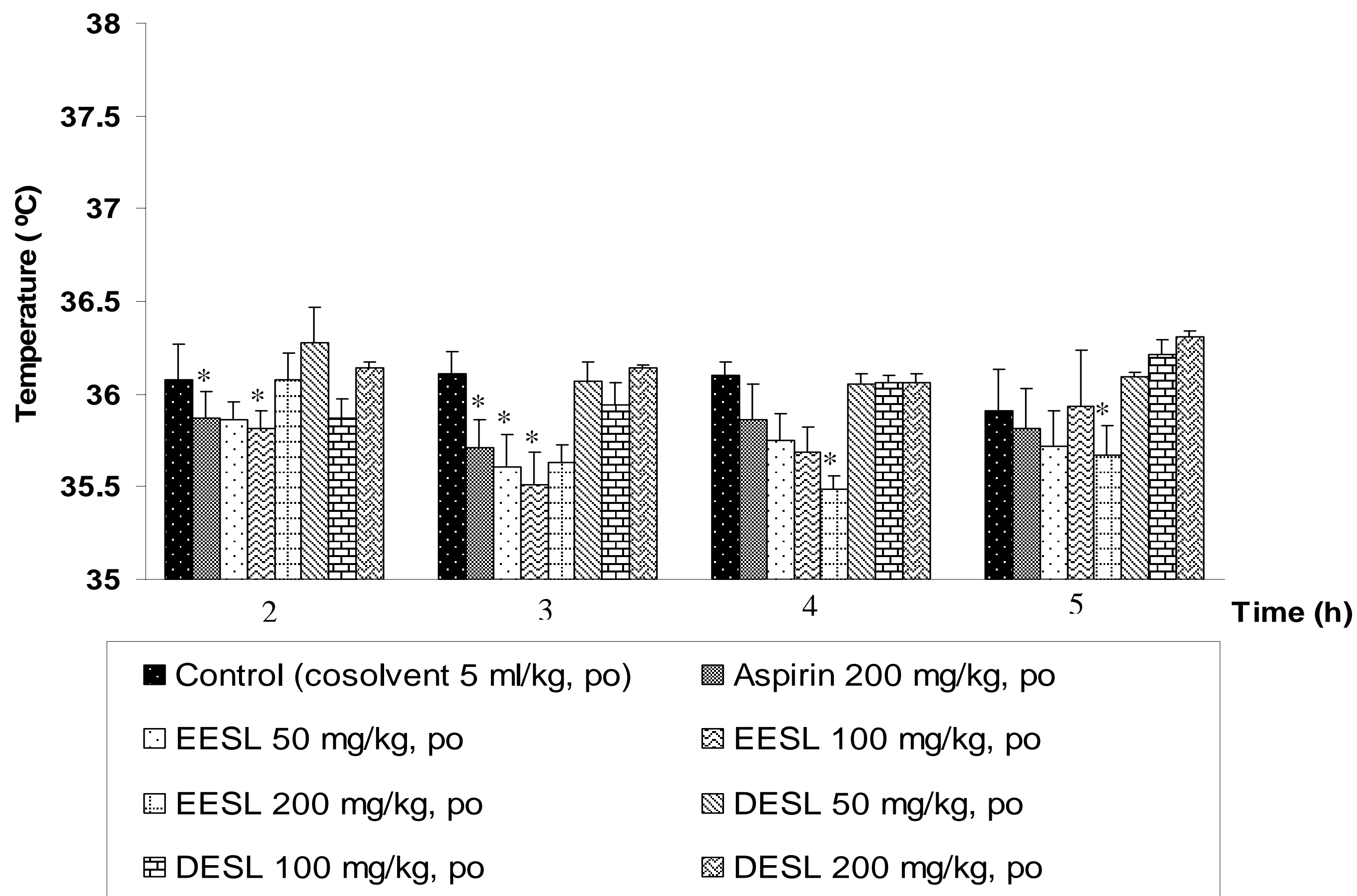


Figure 20. Effects of aspirin, the ethanol and dichloromethane extract of *S. lychnophorum* (EESL, EDSL) on the brewer's yeast induced pyrexia in rats.

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

3. Anti-inflammatory activity

3.1 Carrageenan-induced rat paw edema

The injection of carrageenan, the phlogistic agent, caused localized edema starting at 0.5 h after injection. The swelling increased progressively to a maximum volume of 6.67±0.11 ml at 5 h after the carrageenan injection. In pre-treated with aspirin (200 mg/kg, po) had a significant reduction of rat paw edema at 0.5 h after the aspirin administration and continued up to 5 h with the percentage of inhibition of 25.39, 26.88, 26.28 and 28.64, respectively compared to the control. Only the EESL extract at dose of 100 and 200 mg/kg, po exhibited the anti-inflammatory effect to reduce the rat paw edema at 1 h after administration and continued up to 5 h. The inhibition of rat paw edema of the EESL at the dose of 100 mg/kg, po at 2, 3, 4 and 5 h was 23.07, 25.23, 23.26 and 23.69%, respectively while the EESL at the dose of 200 mg/kg, po decreased the rat paw edema at 2, 3, 4 and 5 h by 23.99, 25.83, 25.08 and 25.19%, respectively when compared to the control (Table 9 and Figure 21).

Table 9. Effect of aspirin, the ethanol and dichloromethane extract of *S. lychnophorum* (EESL, DESL) on carrageenan-induced paw edema in rats.

Treatment	Dose (mg/kg, po)	Paw edema volume (ml)						Inhibition of paw edema (%)			
		0.5 h	1 h	2 h	3 h	4 h	5 h	2 h	3 h	4 h	5 h
Control		4.94±0.14	5.77±0.07	6.46±0.13	6.66±0.08	6.62±0.09	6.67±0.11				
Aspirin	200	4.51±0.12*	4.72±0.11*	4.82±0.07*	4.87±0.06*	4.88±0.05*	4.76±0.08*	25.39	26.88	26.28	28.64
EESL	50	4.96±0.18	5.15±0.17	5.74±0.11	6.03±0.11	6.11±0.15	6.23±0.18	11.15	9.46	7.70	6.60
EESL	100	4.93±0.15	4.94±0.15*	4.97±0.09*	4.98±0.08*	5.08±0.05*	5.09±0.05*	23.07	25.23	23.26	23.69
EESL	200	4.91±0.14	4.93±0.14*	4.91±0.11*	4.94±0.09*	4.96±0.09*	4.99±0.08*	23.99	25.83	25.08	25.19
DESL	50	4.80±0.04	5.15±0.09	5.38±0.14	6.17±0.16	6.57±0.14	6.67±0.18	16.72	7.36	0.75	0
DESL	100	4.79±0.05	5.03±0.04	6.10±0.27	6.51±0.12	6.57±0.08	6.65±0.05	5.57	2.25	0.75	0.30
DESL	200	4.90±0.02	5.07±0.05	5.54±0.07	6.21±0.12	6.46±0.07	6.42±0.07	14.24	6.76	2.42	3.15

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

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

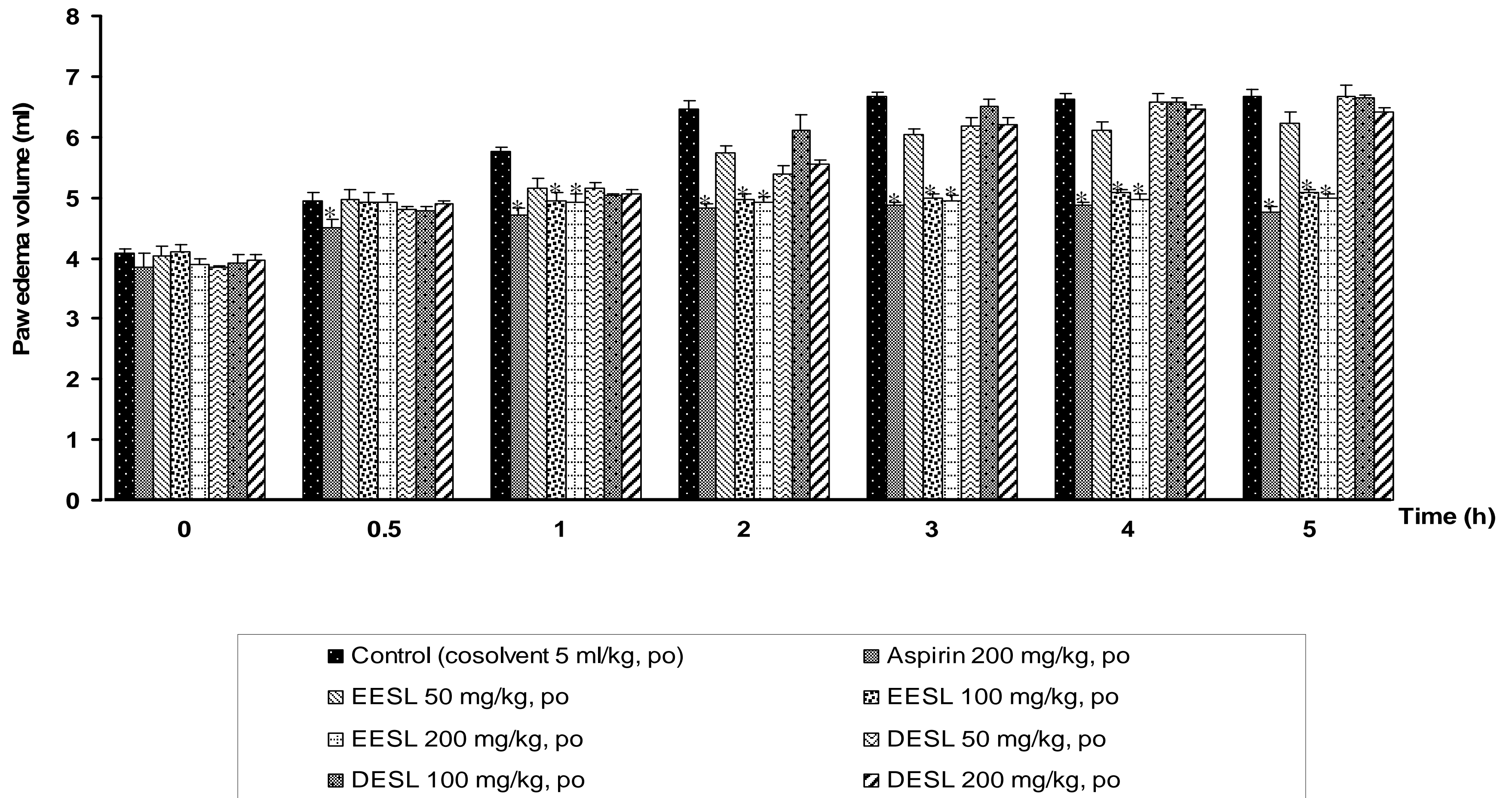


Figure 21. Effect of aspirin, the ethanol and dichloromethane extract of *S. lychnophorum* (EESL, DESL) on carrageenan-induced paw edema in rats.* $p<0.05$, significantly different compared to the control (LSD test).

3.2 Cotton pellet-induced granuloma formation in rats

The EESL at doses 200 mg/kg, po exhibited granuloma inhibition (GI) by 7 23.06 %. Only the DESL at doses of 50 and 100 mg/kg, po demonstrated GI by 13.26 and 11.76 %, respectively. However, the reference drug aspirin (200 mg/kg, po) exhibited GI by 37.29%. The results were summarized in Table 10 and Figure 22.

Table 10. Effect of aspirin, the ethanol and chloromethane extract of *S. lychnophorum* (EESL, DESL) on cotton pellet-induced granuloma formation in rats.

Treatment	Dose (mg/kg, po)	Granuloma dry weight (mg)	Granuloma wet weight (mg)	Transdative weight (mg)	Granuloma weight (mg/mg cotton)	GI (%)
Control		63.10±0.28	123.2±0.68	60.1±0.56	3.32±0.02	-
Aspirin	200	39.47±0.72*	70.25±1.38*	30.78±0.85*	1.93±0.15*	41.87
EESL	50	58.5±1.06*	104.65±1.90*	46.15±1.37*	2.93±0.06	11.75
EESL	100	54.78±0.67*	115.33±1.08*	60.55±0.94	2.81±0.04	15.36
EESL	200	48.55±0.35*	115.85±1.05*	67.3±1.00	2.44±0.02*	26.51
DESL	50	58.9±0.15	121.55±0.95	62.65±0.42	2.84±0.04	14.46
DESL	100	58.68±0.23	121.46±1.4	62.78±1.27	2.86±0.08	13.86
DESL	200	58.78±0.10	120.43±1.61	61.65±1.57	2.79±0.04	15.96

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

GI; granuloma inhibition

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

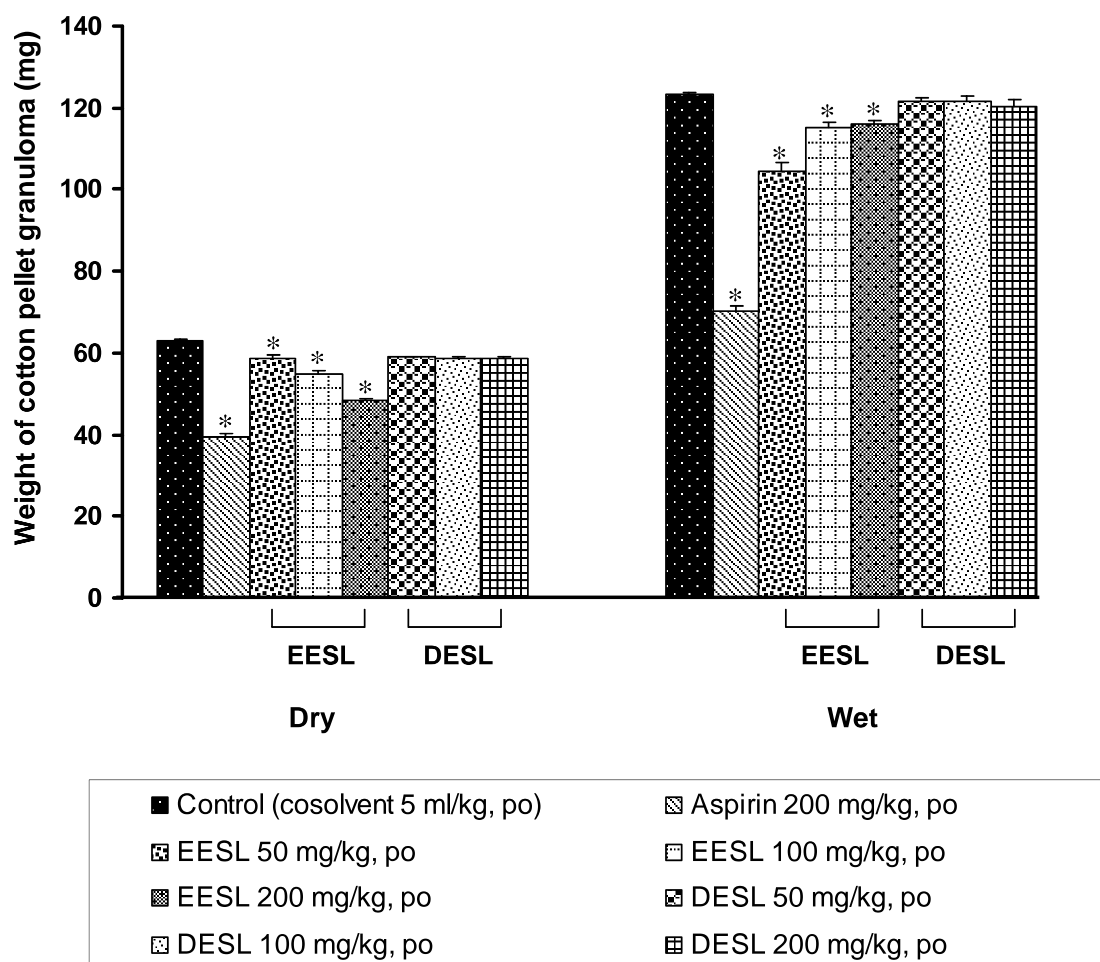


Figure 22. Effect of aspirin, the ethanol and chloromethane extract of *S. lychnophorum* (EESL, DESL) on cotton pellet-induced granuloma formation in rats.

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

CHAPTER 5

DISCUSSION AND CONCLUSION

In the present study, only the EESL clearly exhibited the analgesic, antipyretic and anti-inflammatory activities in experimental animal models (writhing, formalin, hot plate, tail flick, carrageenan-induced rat paw edema, cotton pellet induced granuloma and brewer's yeast induced pyrexia), where as the DESL did not possess these activities.

Scaphium lychnophorum (Hance) Pierre, locally known as "Samrong", belongs to the family Sterculiaceae. This plant is mainly distributed in Vietnam, Thailand (Ubon Ratchathani and Chanthaburi provinces), Malaysia, Indonesia as well as South China (Wu *et al*., 1998). This plant fruit, "Samrong" was chosen to study for its analgesic, antipyretic and anti-inflammatory activities because it was claimed to possess its use in folklore medicine such as relieve pain, cough and clear phlegm, and used as antipyretics.

Acute toxicity test

In oral acute toxicity test, the up-and-down method described by Bruce (1985) was used in this study. This method consumed a fewer animals than conventional method. Mortality, signs of toxicity and abnormalities were observed during the experimental period. The estimated LD_{50} of both the EESL and DESL was more than 5 g/kg, po since this dose failed to produce any clinical signs of toxicities such as convulsion, hyperactivity, sedation, respiratory depression and loss of righting reflex. These results show that the EESL and DESL are toxicologically safe by oral administration in male mice and rats. The test substance in oral acute toxicity which is indicated to be practically non-toxic or non-lethal after an acute exposure is generally considered to be 5.0 g/kg body weight. If mortality was not observed at this dose level, a more high dosage is generally not necessary to test (Hayes, 1989).

Analgesic activity

In analgesic activity test of the extract fraction of *S. lychnophorum* fruit, experimental animal models included the writhing, formalin, hot plate and tail flick tests were used in this study. The results in this study indicated that only the EESL at tested doses markedly exhibited a dose-related analgesic activity. The potency of the EESL at the dose of 200 mg/kg, po could be comparable to a reference standard aspirin at the dose of 200 mg/kg, po. However, in the present study the analgesic activity of the DESL was not seen at tested doses. The experimental animal models used in this study are sufficient to evaluation of antinociceptive mechanisms of the extract both peripherally and centrally mediated effects. The acetic acid and formalin tests (chemical stimuli) are used to elucidate the peripherally and central mediated action, while hot plate and tail flick tests (thermal stimuli) are applied for investigation of the central mediated mechanism. The EESL clearly demonstrated analgesic activity in all experimental animal models used in this study. Therefore, these results could be implied that the EESL had analgesic mechanisms in central nervous system both peripherally and centrally mediated.

Pain is ‘an unpleasant sensory and emotional experience associated with actual or potential tissue damage, or described in terms of such damage’ (Nicholas and Moore, 2009). Pain may felt because of inflammation, infection, ischemia, tissue necrosis, chemical or burn. In the stomach and intestines, pain may result from inflammation of the mucosa of from distension or muscle spasm. Depending of the cause, pain may be sudden and short-term marked primary by reflex withdrawal (Gould, 2002). The concept of a distinct class of peripheral nerve fibers conducting pain signals was first. That convey information about the type of painful stimulus being experienced. For example, thinly myelinated Aδ fibers respond to changes in temperature and to mechanical stimuli; however, one may further classify Aδ nociceptors to reflect whether they are fast or slowly adapting, and whether they have a high or low threshold for activity (so-called type I or II A-fiber nociceptors). The other major class of nociceptors are C-fibers, or C mechano-heat receptors; these are unmyelinated, and thus are relatively slow conducting, and

convey a sensation of burning (Brooks and Tracey, 2005). When an injury occurs, pain is first evoked by stimulation of the nociceptor (Aδ fibers and C fibers) causing potassium and kinins to be released from the damaged cells. These stimulate the receptor directly resulting in the release of the neuropeptides such as substance P from nociceptive terminals and the release of the histamine from the mast cells with the production of the platelet-activating factor (PAF) which in turn releases serotonin from the platelets. Histamine is also released from the mast cells, starting an inflammatory reaction leading to vasodilation and edema (Cambell, 2004). Bradykinin is released upon tissue injury. Bradykinin is an important substance to stimulate nociception and prostaglandins sensitize nociceptor sending impulses to the spinal cord. This state is called peripheral sensitization. When signal transduction comes to postcentral gyrus in thalamus, which is responsible for the conscious perception of pain. This state is called central sensitization (Mutschelor and Derendorf, 1995).

In the present study, results indicated that the EESL showed an analgesic effect against pain induced by experimental animal models included chemical stimuli in the acetic acid-induced writhing and the formalin test, and the thermal stimuli in the hot plate and tail flick tests. The DESL did not show analgesic action in this study. In the writhing test, acetic acid, a chemical stimuli, is used to screen both peripherally and central acting analgesic activity (Panthong *et al.,* 2007). The writhing test can predict effective analgesic doses for agents that can be used in humans (Dubinky *et al.,* 1987; Eaton, 2003). Acetic acid cause pain by liberating endogenous substance including serotonin, histamine, prostaglandins, bradykinin and subsatance P and many others that excite pain nerve ending (Collier *et al.,* 1968; Raj, 1996; Intahphuak *et al.,* 2004) leading to the abdominal writhing. The writhing response of the mouse to an injection of noxious chemical is not very specific nociceptive model, it is important to reveal a general antinociceptive effect of the extract under study (Marchioro *et al.,* 2005). The EESL at all doses significantly reduced the number of writhing in mice. Acetic acid-induced writhing is related to the increase in the peritoneal fluid levels of PGE_2 and $PGF_{2\alpha}$ (Derardt *et al.,* 1980). The analgesic action of nonsteroidal anti-inflammatory drugs (NSAIDs)

is exerted both peripherally and centrally. In this study, the reference drug aspirin (200 mg/kg) also exhibited significant protective effects towards the acetic-induced pain in mice. In comparison, the analgesic potency of the EESL could be comparable with aspirin in mice. Aspirin is usually associated with anti-inflammatory action and results from the inhibition of prostaglandin synthesis via cyclooxygenase. Protaglandins produce little pain by themselves, but potentiate the pain caused by other mediators (such as histamine and bradykinin) (Neal, 1992).

The formalin test of nociception can discriminate pain in central and peripheral components (Tjolsen *et al.,* 1992), termed early and late phase. The formalin test is used to confirm the possible analgesic mechanism of action of the extract. Drug that act primary on the central nervous system such as morphine inhibits both the early and late phases, whereas peripherally acting drugs such as aspirin only inhibits the second phase (Dubuisson and Dennis, 1977; Hunskaar and Hole, 1987; Shibata *et al*., 1989). However, it has been reported that NSAIDs such as aspirin acting supraspinally which inhibit prostaglandin synthesis can reduce the pain in both phase (Martindale *et al.,* 2001). The formalin test consists of two different phases which can be separated in time; the early phase (0-5 min) is neurogenic phase which is also known as non-inflammatory pain. This phase is mediated by central effect through a direct activation of the nociceptors by C fibers activation. The direct stimulation of nerve ending caused substance P release with cooperation with bradykinin, while the 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 transmitter such as histamine, serotonin, bradykinin and prostaglandins (Shibata *et al.,* 1989). Inhibition of the late phase is due to inflammation with release of serotonin, histamine, bradykinin and protaglandins, which to some degree can cause the sensitization of central nociceptive neuron (Verma *et al.,* 2007).

The EESL showed an inhibitory effect in both early and late phases, so it was indicated that EESL had central and peripheral analgesic properties. The extract might reduce the licking time in the early phase of formalin test via blockade on nociceptor or inhibited releasing of substance P and bradykinin. Then, it is

followed by the second phase which might be resulted from chronic inflammatory nociception responses. The EESL could reduce the licking time in the late phase, it might be due to the inhibition of the inflammatory mediators or blockade on receptors. Thus, it might be conclude that analgesic effect on the early phase of the EESL was due to the direct effect on the nociceptor via blockade on the nociceptor or the inhibition of releasing the substance P or bradykinin. The formalin test is best to examine opioid mimetics because opipoid analgesics provides analgesia for both phase of the behavioral response but the late, delayed phase is more sensitive (Eaton, 2003).

Pain induced by thermal stimuli included hot plate and tail flick tests is known to be selective to centrally but not peripherally acting analgesics (Chau, 1989). The hot plate and tail flick tests 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. The EESL significantly increased the latency of nociceptive responses in hot plate test. The lower dose of the EESL (50 mg/kg) was less potent in analgesic activity than the higher doses (100 and 200 mg/kg) as indicated by a significant delay responses against pain at lower dose in mice, while higher doses of the EESL (100 and 200 mg/kg) exhibited more potent effects and had a rapid analgesic action. To confirm the central analgesic effect of the EESL , the antagonist action of naloxone on the effect of morphine or EESL (200 mg/kg) on the latency of nociceptive response was also investigated. The results showed that naloxone given before morphine also antagonized the effect of morphine at 60, 75 and 90 min when compared to the morphine alone. Furthermore, naloxone given before the EESL (200 mg/kg) significantly decreased the latency of nociceptive response at 30 and 45 min when compared to the EESL alone. These results indicated that the EESL had a central analgesic action in similar to morphine. The hot plate method is one of the most common tests used for evaluating the analgesic efficacy of drugs in rodents (Somchit *et al.,* 2004). The drug that reduces the nociceptive response indicated by cutaneous thermic stimuli in the hot plate test might exhibit central analgesic properties or supraspinal analgesia (Matheus *et al.,* 2005). Thermic painful stimuli are known to be selective to centrally, but not

peripherally acting analgesic drugs (Chau, 1989). Therefore, the hot plate test which is usually used to determine the involvement of central nociceptive mechanism has, at least, confirmed the ability of the EESL to influence the central mechanism as seen with the formalin test. The antinociceptive action of the EESL was inhibited by naloxone, an opiod receptor antagonist, indicating that the antinociception was partly mediated via opioid mechanism. This could be due to the direct agonist activity of opioidiomimetric constituents in the EESL, and due to increase release of endogeneous opiod peptides (Deranigagala *et al.,* 2003).

In tail flick test, the EESL at low dose (50 mg/kg) produced more slow onset of antinociceptive action than the higher doses (100 and 200 mg/kg). Therefore, the antinociceptive action of the EESL was likely to be dose-related. Morphine, a centrally acting analgesic drug, significantly increased the tail flick latency at all time interval measured. Naloxone, an opioid antagonist, completely antagonized the effect of morphine on the latency of nociceptive response in this model. Furthermore, naloxone given before the EESL (200 mg/kg) also significantly decreased the latency of nociceptive response in the tail flick test when compared to the EESL alone. These results indicated that the EESL had a similar action of antinociceptive action to morphine.

The tail flick model is considered specific test for evaluation of the central pain (Marchioro *et al.,* 2005) at spinal levels (Wong *et al*., 1994). The analgesic effect reflected in the tail flick test is dependent on centrally acting opiod like analgesics (Conner *et al.,* 2000; Yonathan *et al.,* 2005). Opioid 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). Opioids have the excitatory effect in multiple regions of the nervous system and this effect is generally attributed to the reduction on inhibitory pathways (Charles and Hales, 2004). Descending control of spinal nociception is a major determinant of acute pain in different behavioral and emotional states (McMullan and Lumb, 2006).

Opioid receptors of the μ-, δ- and κ-subtypes mediate the potent analgesic and addictive actions of opioid drugs (Kieffer, 1999). Opioid receptors are

composed of glycoproteins found in cellular membranes. These receptors are couple to G proteins that modulate potassium and calcium ion conduction. When opioid agonists occupy either μ or δ opioid receptors, they open potassium ion channels that permit an increase in potassium conductance. The hyperpolarization inhibits neuronal activity. In contrast, κ receptors activation inhibits calcium entry via a calcium ion channel. Activation of the opioid receptors decreases transmission of the signals from the primary peripheral afferent nerves to higher CNS centers, as well as the processing of the pain stimulus. Activation of opioid receptors leads to analgesic action as well as adverse effects (Lipman and Jackson, 2004).

All results obtained from hot plate and tail flick tests used in this study indicated that the EESL had an analgesic activity. The analgesic mechanisms of the EESL were centrally mediated in both spinal and supraspinal level like morphine.

Antipyretic activity

Pyrexia or fever is caused as a secondary impact of infection, malignancy or other diseased states. It is the body's natural function to create an environment where infectious agents or damaged tissues can not survive. Normally, the infected or damaged tissue initiates the enhanced formation of pro-inflammatory mediators (cytokines such as interleukin 1β, α, β, and TNF- α), which increase the synthesis of prostaglandin E_2 (PGE_2) near hypothalamic area and thereby trigger the hypothalamus to elevate the body temperature. When the body temperature becomes high, the temperature regulatory system which is governed by a nervous feedback mechanism dilates the blood vessels and increases sweating to reduce the temperature. When the body temperature becomes low, hypothalamus protects the internal temperature by vasoconstriction (Khan *et al*., 2008).

Yeast-induced fever is called pathogeneic fever. Its etiology includes production of prostaglandins which set the thermoregulatory center at a higher set point. It could stimulate phagocytes to release endogenous pyrogen which circulates in blood to act on the thermoregulatory center in the hypothalamus. Endogeneous pyrogen activates IL-1 and prostaglandins, mainly PGE_2 which alters metabolism of

thermoregulatory cells via cAMP secondary messenger-mediated mechanism. The result is an increase set point for thermoregulation to a high temperature. So, inhibition of prostaglandin synthesis could be the possible mechanism of antipyretic action of acetylsalicyclic acid (Howard, 1993; Rawlins and Postgrad, 1973)

The results in this study showed that the EESL posseses antipyretic activity in yeast induced pyrexia in rats whereas the DESL did not demonstrate this activity. The reference drug aspirin also decreased the yeast induced fever in rats by inhibition of the synthesis of prostaglandin E2 (Dascombe, 1985; Vane, 1987). Therefore, the antipyretic effect of the EESL might be due to the inhibition of prostaglandins (PGE_2) synthesis in prostaglandin pathways in similar to aspirin. These results support the use *S. lychnophorum* fruit as an antipyretic for the treatment of fever in traditional medicines.

Anti-inflammatory activity

Inflammation is part of the complex biological response of vascular tissues to harmful stimuli such as pathogens, damaged cells, or irritants. Although it is an 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). The aim of the inflammation is to repair the damage or at least to limit it and also to remove the cause. Causes of inflammation include direct damage, chemical such as acids, ischemia and cell necrosis or infarction and infections (Gould, 2002).

In acute inflammation, the process of acute inflammation is initiated by cells already presented in all tissues, mainly resident macrophages, dendritic cells, histiocytes, Kuppfer cells and mastocytes. At the onset of an infection, burn, or other injuries, these cells undergo activation and release inflammatory mediators responsible for the clinical signs of inflammation. Vasodilation and its resulting increased blood flow causes the redness (rubor) and increased heat (calor) (Gould, 2002).

Carragenan-induced rat paw edema is an acute inflammatory model involved several mediators released in sequence (DiRosa *et al*., 1971; Olajide *et al.,*

1999). An initial phase during the first 1.5 h, is caused by the release of histamine and serotonin; the second phase is mediated by bradykinin from 1.5 to 2.5 h and the third phase, in which the mediator is likely to be prostaglandin, occurs from 2.5 to 6 h after carragenan injection (DiRosa, 1972). The carragenan-induced hind paw edema in rats is known to be sensitive to cyclooxygenase inhibitors, but not to lipoxygenase inhibitors. This test has been used to evaluate the effect of NSAIDs which primarily inhibit the cyclooxygenase involved in prostaglandins synthesis (DiRosa, 1972). In the present study, the EESL at doses of 100 and 200 mg/kg significantly decreased the rat paw edema induced by carragenan in all phases, suggesting the possible mechanism of action of the EESL may involve inhibition of these inflammatory mediators release in all phases.

The cotton pellet-induced granuloma formation in rats is a model involved with chronic inflammation. The inflammatory responses have been divided into 3 phases; transdutive, exudative and proliferative phases. The fluid absorbed by the pellet greatly influenced the wet weight of the granuloma, and the dry weight correlates well with amount of granulomatous tissue formed (Swingle and Shideman, 1972). In the present study, the EESL at all tested doses significantly reduced cotton pellet-induced granuloma formation in rats, indicating that the transdutive, exudative and proliferrative phases of inflammation were partially inhibited. It is possible that the EESL may inhibit monocytes infiltration and fibroblast proliferation. 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 the endothelial cell (Dore and Sirois, 1996). It has been reported that the water soluble polysaccharides (W-SP) from boat-fruited Sterculia seed by hot water extraction and ethanol precipitation were fractionated into a neutral polysaccharide (NSP) and an acidic one (ASP) by anion-exchange chromatography, showed clearly anti-inflmmatory activity. Bioactivity of NSP and ASP was tested using ear edema induced by dimethylbenzene and cotton pellet-induced granuloma tissue in murine models. The results showed ASP possessed a potent dose-dependent anti-inflammatory activity

(Wu *et al*., 2007). In the present study, only the ethanolic fraction of *S. lychnophorum* (EESL) clearly exhibited a dose-dependent anti-inflammatory activity whereas dichoromethane fraction (DESL) failed to demonstrate anti-inflammatory action. Therefore, active constituents that produced anti-inflammatory effect are most likely to present only in the ethanolic fraction of *S. lychnophorum* (EESL).

In summary, in the present investigation it could be concluded that the EESL at tested doses exhibited the pharmacological activities as follow:

1. In analgesic activity, the EESL exhibits the analgesic activity, in which its mechanism of action is peripherally and centrally mediated (spinal and supraspinal levels).

2. In antipyretic activity, the EESL can reduce fever induced by brewer's yeast injection. The antipyretic effect of the EESL may be due to the inhibition of prostaglandins (PGE_2) biosynthesis.

3. In anti-inflammatory activity, the EESL shows the anti-inflammatory activity both in acute and chronic inflammation. The possible mechanism of action may involve inhibition of inflammatory mediators release (histamine, serotonin, bradykinin, prostaglandins and TNF-α) in both phases.

Based on these results, it is clearly that the EESL possesses analgesic, antipyretic and anti-inflammatory activities in experimental animal models which support the traditional uses of *S. lychnophorum* fruit, commonly called in Thai name as "Samrong", in folklore medicine.

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APPENDIX

APPENDIX-A

Extraction procedure

Dry fruit of *Scaphium lychnophorum* (2.6 kg)

Macerated with 7.5 L and 5.5 L ethanol 2 times for 7 days

Evaporated at room temperature in air-flowed hood
and subsequent evaporation under reduced pressure.

Lyophilized by freeze-dry

semisolid residue for the ethanol extract of *S. lychnophorum*
1.6% (w/w) from dry fruit of *S. lychnophorum*

Note:

The dichrolomethane extract of *S. lychnophorum* was extracted using the same method, and oily-green residue 0.36% (w/w) yield was obtained.

APPENDIX-B

Agents 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 0.625 ml of 40% 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 surfate and naloxone
   Dissolve Morphine or naloxone with normal saline
7. Aspirin, ethanol and dichrolomethane extract of *Scaphium lychnophorum* (EESL, DESL) at 50, 100 and 200 mg/kg
   Dissolve aspirin or the extract with cosolvent (Propylene glycol: Tween 80: Distill water; 4: 1: 5)

Dosing for oral administration

EESL, DESL and aspirin administered orally in a constant volume, 10 ml/kg for mice and 5 ml/kg for rats.

APPENDIX-C

Effect of the ethanol and dichloromethane extract of *Scaphium lychnophorum* (EESL, DESL) and aspirin on acetic-induced writhing in mice.

Group	Number	Weight (g)	Number of writhing	Group	Number	Weight (g)	Number of writhing
Control (cosolvent), po	1	40	75	Aspirin 200 mg/kg, po	1	36	20
	2	30	56		2	36	23
	3	37	78		3	38	20
	4	38	59		4	39	25
	5	39	59		5	37	22
	6	39	60		6	38	27
	Mean ± S.E.M	37.17 ± 1.49	64.50 ± 3.85		Mean ± S.E.M	37.33 ± 0.49	22.83 ± 1.14

Group	Number	Weight (g)	Number of writhing	Group	Number	Weight (g)	Number of writhing
EESL 50 mg/kg, po	1	36	62	EESL 100 mg/kg, po	1	36	48
	2	33	61		2	38	48
	3	33	63		3	36	32
	4	33	48		4	35	31
	5	37	49		5	38	43
	6	30	50		6	40	48
	Mean ± S.E.M	33.67 ± 1.02	55.50 ± 2.93		Mean ± S.E.M	37.17 ± 0.75	41.67 ± 3.31

Group	Number	Weight (g)	Number of writhing	Group	Number	Weight (g)	Number of writhing
EESL 200 mg/kg, po	1	37	22	DESL 50 mg/kg, po	1	36	57
	2	34	28		2	37	59
	3	39	20		3	34	62
	4	34	21		4	32	55
	5	36	22		5	35	58
	6	34	31		6	37	60
	Mean ± S.E.M	35.67 ± 0.84	24 ± 1.81		Mean ± S.E.M	35.17±0.79	58.50±0.99

Group	Number	Weight (g)	Number of writhing	Group	Number	Weight (g)	Number of writhing
DESL 100 mg/kg, po	1	32	55	DESL 200 mg/kg, po	1	34	49
	2	35	56		2	36	52
	3	34	57		3	34	55
	4	38	59		4	32	56
	5	37	61		5	37	54
	6	32	56		6	39	49
	Mean ± S.E.M	34.67±1.02	57.33±0.92		Mean ± S.E.M	35.33±1.02	52.50±1.23

Effect of aspirin, morphine, ethanol and dichloromethane extract of *S. lychnophorum* (EESL, DESL) on 2.5% formalin-induced paw licking in mice.

Group	Number	Weight (g)	Licking of the hind paw (sec)		Group	Number	Weight (g)	Licking of the hind paw (sec)	
			Early phase	Late phase				Early phase	Late phase
Control (cosolvent, 10 ml/kg, po)	1	32	53.72	85.45	Aspirin 200mg/kg, po	1	35	6.53	32.42
	2	32	55.72	89.55		2	35	30.95	38.53
	3	30	59.60	80.57		3	35	27.94	35.70
	4	30	57.68	78.74		4	30	35.07	42.53
	5	34	70.32	68.19		5	32	23.36	24.35
	6	39	50.49	68.19		6	34	26.53	26.42
	Mean±S.E.M	32.83±1.38	57.92±2.79	78.44±3.59		Mean±S.E.M	33.5±0.85	28.39±1.67	33.33±2.87

Group	Number	Weight (g)	Licking of the hind paw (sec)		Group	Number	Weight (g)	Licking of the hind paw (sec)	
			Early phase	Late phase				Early phase	Late phase
Morphine 5 mg/kg, sc	1	32	23.65	22.33	EESL 50 mg/kg, po	1	33	52.63	68.02
	2	32	23.65	23.76		2	35	56.53	70.25
	3	31	29.31	26.10		3	36	51.61	69.97
	4	30	21.87	29.10		4	32	54.15	67.03
	5	33	27.14	21.59		5	40	44.43	68.67
	6	30	26.82	21.09		6	37	47.27	67.35
	Mean±S.E.M	31.33±0.49	25.41±1.12	24±1.23		Mean±S.E.M	35.50±1.18	51.10±1.83	68.55±0.55

Group	Number	Weight (g)	Licking of the hind paw (sec)		Group	Number	Weight (g)	Licking of the hind paw (sec)	
			Early phase	Late phase				Early phase	Late phase
EESL 100 mg/kg, po	1	32	46.96	55.78	EESL 200 mg/kg, po	1	37	43.85	23.65
	2	32	56.84	56.10		2	34	45.64	25.95
	3	33	45.69	49.13		3	33	37.25	28.37
	4	33	40.05	51.05		4	33	37.09	22.89
	5	30	38.73	58.85		5	33	49.31	22.71
	6	33	37.28	56.78		6	31	42.53	28.83
	Mean±S.E.M	32.67±0.48	44.26±2.97	54.62±1.52		Mean±S.E.M	33.50±0.81	42.61±1.96	25.40±1.12

Group	Number	Weight (g)	Licking of the hind paw (sec)		Group	Number	Weight (g)	Licking of the hind paw (sec)	
			Early phase	Late phase				Early phase	Late phase
DESL 50 mg/kg, po	1	32	52.39	69.05	DESL 100 mg/kg, po	1	34	53.09	68.99
	2	34	51.12	70.24		2	33	51.86	70.52
	3	35	54.67	72.43		3	36	55.63	69.87
	4	32	54.23	71.89		4	32	53.33	73.56
	5	33	53.19	72.34		5	31	52.21	74.12
	6	36	55.20	75.04		6	33	52.08	72.69
	Mean±S.E.M	33.67±0.67	53.47±0.63	71.83±0.84		Mean±S.E.M	33.17±0.70	53.03±0.57	71.63±0.86

Group	Number	Weight (g)	Licking of the hind paw (sec)	
			Early phase	Late phase
DESL 200 mg/kg, po	1	32	49.98	73.89
	2	32	51.37	74.25
	3	34	53.54	71.20
	4	33	55.21	73.11
	5	31	51.07	69.08
	6	35	49.87	69.94
	Mean±S.E.M	32.83±0.60	51.84±0.86	71.91±0.88

Effect of morphine, naloxone, ethanol and dichloromethane extract of *S. lychnophorum* (EESL, DESL) on heat-induced pain in mice.

Group	Number	Weight (g)	Initial response (s)	Latency of nociceptive response (sec)				
				30 min	45 min	60 min	75 min	90 min
Control (cosovent, po)	1	34	9.7	11.6	12.6	11.6	11.3	10.6
	2	31	9.7	12.4	10.8	9.71	9.3	11.8
	3	38	8.3	8.8	11.5	12.1	10.6	9.8
	4	35	6.4	8.7	10.4	10.2	9.6	9.8
	5	34	5.9	10.3	11	11.5	12.2	12.5
	6	34	5.9	11.1	12.8	12.6	12.6	12.8
	Mean±S.E.M	34.33 ± 0.92	7.65 ± 0.74	10.48 ± 0.62	11.51 ± 0.42	11.28 ± 0.45	10.93 ± 0.55	11.21 ± 0.54

Group	**Number**	**Weight (g)**	**Initial response (s)**	**Latency of nociceptive response (sec)**				
				30 min	**45 min**	**60 min**	**75 min**	**90 min**
Morphine (5 mg/kg, sc)	1	31	8.2	14.6	16.5	17.7	16.85	16.9
	2	37	12.1	15.6	16.7	15.6	12.2	13.6
	3	30	13.1	13	12.4	13	13.4	13.9
	4	30	9.4	14	13.5	17.8	13.9	12.08
	5	35	9.9	14.5	14.4	16.3	15.8	15.24
	6	37	8.8	13.6	13.4	13.3	13.7	13.87
	Mean±S.E.M	33.33 ± 1.38	10.25±0.79	14.22±0.37	14.48±0.72	15.62±0.85	14.31±0.70	14.27±0.67

Group	**Number**	**Weight (g)**	**Initial response (s)**	**Latency of nociceptive response (sec)**				
				30 min	**45 min**	**60 min**	**75 min**	**90 min**
Naloxone (2 mg/kg, ip)	1	32	10.2	10.9	10.7	14	9.9	9.8
	2	30	12.4	9.8	12.3	13.8	10.8	11.18
	3	36	11.2	11.3	11.01	12.8	9.17	8.91
	4	32	9.9	12.5	15.2	11.1	10.7	11.3
	5	34	13.9	9.8	10.9	14.6	9.3	8.85
	6	30	10.4	12.6	15.2	12.2	12.89	10.1
	Mean±S.E.M	32.33±0.95	11.33±0.63	11.15±0.51	12.55±0.87	13.08±0.53	10.46±0.56	10.02±0.43

Group	Number	Weight (g)	Initial response (s)	Latency of nociceptive response (sec)				
				30 min	45 min	60 min	75 min	90 min
EESL (50 mg/kg, po)	1	33	8.2	8.9	10	10.9	14.5	14.5
	2	33	7.9	8.5	14.5	12.5	12.2	13.9
	3	36	10.1	12.5	13.7	13.1	10.9	10.6
	4	36	12.8	11.9	12.8	10.5	10.2	12.6
	5	31	10.5	10	12.7	14.2	12.2	11.4
	6	32	10.5	9.3	9.89	13.3	16.6	14
	Mean±S.E.M	33.50±0.85	10.00±0.73	10.18±0.67	12.27±0.78	12.42±0.59	12.77±0.97	12.83±0.64

Group	Number	Weight (g)	Initial response (sec)	Latency of nociceptive response (sec)				
				30 min	45 min	60 min	75 min	90 min
EESL (100 mg/kg, po)	1	39	8.8	13.3	14.3	15.4	14.7	11.7
	2	30	9.7	10.2	12.2	12.9	12.8	11.81
	3	34	10.3	12.7	11.7	11.9	10.9	9.93
	4	36	7.1	12.2	12.8	10.9	13.3	11.1
	5	34	11	10.8	11.4	13.3	14.4	12.6
	6	33	13.9	12.3	12.7	13.7	12.9	12.28
	Mean±S.E.M	34.33±1.23	10.13±0.93	11.92±0.48	12.52±0.42	13.02±0.63	13.17±0.55	11.57±0.39

Group	Number	Weight (g)	Initial response (sec)	Latency of nociceptive (sec)				
				30 min	45 min	60 min	75 min	90 min
EESL (200 mg/kg, po)	1	33	10.6	13.3	13.2	14.9	10.7	12.4
	2	34	10.4	12.8	13	12.8	13.1	11.5
	3	32	9.2	14.6	15.4	14.3	11.6	11.4
	4	31	12.6	13.5	14.9	15.2	12.87	11.3
	5	30	11.8	11.8	12.2	13.3	12.4	10.6
	6	31	8.9	10.3	13.1	13.7	11.9	12.9
	Mean±S.E.M	31.83±0.60	10.59±0.59	12.72±0.61	13.63±0.51	14.03±0.38	12.10±0.36	11.68±0.34

Group	Number	Weight (g)	Initial response (sec)	Latency of nociceptive response (sec)				
				30 min	45 min	60 min	75 min	90 min
Naloxone (2 mg/kg, ip) + Morphine (5 mg/kg, sc)	1	32	14.2	14.4	12.2	10.7	9.87	11.6
	2	34	12.5	12.3	15.5	11.7	10.6	10.2
	3	31	8.4	12.3	13.1	12.8	12.87	12.1
	4	32	9.7	11.6	14.9	12.5	10.65	10.98
	5	33	10.1	14.8	12.9	13.1	9.8	9.7
	6	33	10.6	13.3	12.8	11.5	9.92	10.9
	Mean±S.E.M	32.50±0.43	10.92±0.85	13.12±0.52	13.57±0.54	12.05±0.37	10.62±0.48	10.91±0.36

Group	Number	Weight (g)	Initial response (sec)	Latency of nociceptive response (sec)				
				30 min	45 min	60 min	75 min	90 min
Naloxone (2 mg/kg, ip) + EESL (200 mg/kg, po)	1	36	12.8	11.7	9.89	9.91	10.2	13.4
	2	30	13.5	9.86	11.5	15.7	11.1	10.6
	3	36	11.5	10.9	9.7	10.8	10.9	12.8
	4	30	8.2	10.5	9.8	12.7	10.4	10.2
	5	30	11	11.7	11.9	14.1	13.27	9.9
	6	31	13.8	11.1	12.6	13.5	12.2	11.7
	Mean±S.E.M	32.17±1.22	11.8000 ± .85	10.96 ± 0.29	10.90 ± 0.51	12.79 ± 0.87	11.35 ± 0.49	11.43 ± 0.59

Group	Number	Weight (g)	Initial response (sec)	Latency of nociceptive response (sec)				
				30 min	45 min	60 min	75 min	90 min
DESL (50 mg/kg, po)	1	32	9.9	11.6	11.8	11.6	11.3	11.7
	2	31	10.2	10.4	11.8	11.7	10.8	11.5
	3	34	9.7	9.9	11.4	11.5	11.3	11.6
	4	30	9.6	9.9	10.9	11.0	10.7	10.4
	5	32	10.5	11.1	11.5	11.7	11.2	11.5
	6	35	10.3	10.7	11.9	10.9	10.5	10.8
	Mean±S.E.M	32.33±0.76	10.03±0.15	10.60±0.28	11.55±0.15	11.37±0.17	10.96±0.14	11.25±0.24

Group	Number	Weight (g)	Initial response (sec)	Latency of nociceptive response (sec)				
				30 min	45 min	60 min	75 min	90 min
DESL (100 mg/kg, po)	1	32	10.3	10.8	11.3	11.4	11.5	11.7
	2	34	9.5	11.6	11.5	11.6	11.5	11.3
	3	36	9.7	10.8	11.8	12.4	11.6	11.9
	4	37	9.7	10.6	11.9	11.0	10.9	11.0
	5	35	10.2	10.9	11.2	10.7	10.9	10.9
	6	33	10.0	10.8	11.4	11.1	11.0	10.7
	Mean±S.E.M	34.50±0.76	9.90±0.13	10.92±0.14	11.52±0.11	11.36±0.24	11.23±0.14	11.25±0.19

Group	Number	Weight (g)	Initial response (sec)	Latency of nociceptive response (sec)				
				30 min	**45 min**	**60 min**	**75 min**	**90 min**
DESL (200 mg/kg, po)	1	32	10.6	11.0	11.3	12.9	11.7	11.6
	2	35	9.9	10.6	11.6	11.6	11.6	11.4
	3	34	10.5	11.7	11.2	11.8	11.4	11.2
	4	33	10.2	11.3	11.5	11.2	11.8	11.5
	5	37	9.7	10.6	11.8	10.5	10.5	11.0
	6	36	10.6	10.5	11.7	10.9	10.6	10.9
	Mean±S.E.M	34.50±0.76	10.25±0.16	10.95±0.19	11.52±0.09	11.48±0.34	11.27±0.23	11.26±0.11

Group	Number	Weight (g)	Initial response (sec)	Latency of nociceptive response (sec)				
				30 min	45 min	60 min	75 min	90 min
Naloxone (2 mg/kg, ip) + DESL (200 mg/kg, po)	1	32	10.6	13.5	11.3	11.3	11.1	11.2
	2	33	9.8	10.4	11.8	11.6	10.8	11.1
	3	35	10.9	11.5	11.6	11.7	10.5	10.9
	4	31	11.1	12.1	11.8	11.2	11.5	11.8
	5	34	10.3	12.3	11.7	11.2	10.9	11.1
	6	33	9.7	9.8	10.9	10.8	10.9	11.2
	Mean±S.E.M	33.00±0.58	10.40±0.23	11.60±0.55	11.52±0.14	11.30±0.13	10.95±0.14	11.22±0.12

Effect of morphine, naloxone, ethanol and dichloromethane extract of *S. lychnophorum* (EESL, DESL) on nociceptive response in the tail flick test in mice.

Group	Number	Weight (g)	Latency of nociceptive response (sec)					
			Initial response(sec)	**30 min**	**45 min**	**60 min**	**75 min**	**90 min**
Control (cosovent, 10 ml/kg, po)	1	32	6.4	6.5	6.3	6.1	6.2	6.0
	2	38	6.6	6.4	6.0	6.1	6.6	6.2
	3	36	6.5	6.0	6.5	5.9	6.5	6.4
	4	34	6.7	6.2	6.7	6.3	6.4	6.3
	5	36	6.8	6.6	6.4	6.5	6.1	6.2
	6	34	6.4	6.9	6.2	6.3	6.1	6.4
	Mean±S.E.M	35.00±0.86	6.57±0.07	6.43±0.13	6.35±0.10	6.20±0.09	6.32±0.09	6.25±0.06

Group	Number	Weight (g)	Latency of nociceptive response (sec)					
			Initial response(sec)	**30 min**	**45 min**	**60 min**	**75 min**	**90 min**
Morphine 5 mg/kg, sc	1	37	6.4	10.2	11.9	13.8	13.4	11.4
	2	36	6.5	9.6	11.8	14.0	13.3	11.8
	3	33	6.3	9.4	11.5	13.2	13.5	11.7
	4	33	6.3	9.6	11.6	13.2	13.6	11.9
	5	36	6.6	9.7	11.5	13.5	13.4	11.9
	6	37	6.8	10.2	11.4	13.4	13.3	11.6
	Mean±S.E.M	35.33±0.76	6.48±0.08	9.78±0.14	11.62±0.08	13.52±0.13	13.42±0.05	11.72±0.08

Group	**Number**	**Weight (g)**	**Latency of nociceptive response (sec)**					
			Initial response(sec)	**30 min**	**45 min**	**60 min**	**75 min**	**90 min**
Naloxone 2 mg/kg, ip	1	37	7.1	6.6	6.5	6.3	6.3	6.1
	2	36	6.9	6.6	6.6	6.7	6.6	6.5
	3	34	6.5	6.3	6.4	6.4	6.5	6.4
	4	36	6.3	6.3	6.2	6.3	6.0	5.9
	5	35	6.1	6.2	6.6	6.5	6.7	6.1
	6	33	7.4	6.6	6.7	6.4	6.6	6.3
	Mean±S.E.M	35.16±0.60	6.72±0.20	6.43±0.08	6.50±0.07	6.43±0.06	6.45±0.11	6.22±0.09

Group	Number	Weight (g)	Latency of nociceptive response (sec)					
			Initial response(sec)	30 min	45 min	60 min	75 min	90 min
EESL 50 mg/kg, po	1	34	6.3	6.4	6.8	7.2	7.1	7.1
	2	32	6.8	6.3	6.1	6.5	7.3	6.7
	3	36	6.2	6.2	6.3	6.7	7.1	6.6
	4	35	7.0	6.6	6.6	7.3	7.3	6.8
	5	34	7.1	6.7	6.4	6.8	7.0	6.9
	6	34	6.7	6.4	6.6	6.8	7.6	7.0
	Mean±S.E.M	34.17±0.54	6.68±0.15	6.43±0.08	6.67±0.10	6.88±0.12	7.23±0.09	6.85±0.08

Group	Number	Weight (g)	Latency of nociceptive response (sec)					
			Initial response(sec)	30 min	45 min	60 min	75 min	90 min
EESL 100 mg/kg, po	1	38	6.6	6.8	7.0	7.1	7.4	7.2
	2	37	6.4	6.3	6.7	6.8	7.9	7.0
	3	38	6.5	6.8	6.8	7.0	7.4	6.7
	4	39	6.7	6.7	6.8	7.1	7.4	6.6
	5	35	6.8	6.8	7.0	7.8	7.3	6.9
	6	34	6.2	6.5	6.7	6.9	7.9	7.3
	Mean±S.E.M	36.83±0.79	6.53±0.09	6.65 ±0.08	6.83±0.06	7.12±0.14	7.55±0.11	6.95±0.11

Group	Number	Weight (g)	Latency of nociceptive response (sec)					
			Initial response(sec)	**30 min**	**45 min**	**60 min**	**75 min**	**90 min**
EESL 200 mg/kg, po	1	36	6.8	7.2	7.9	8.3	8.0	7.8
	2	36	6.6	6.8	7.3	7.8	7.6	7.7
	3	35	6.5	7.0	7.7	8.5	7.9	7.7
	4	34	6.1	6.9	7.8	8.7	8.3	7.8
	5	34	6.1	6.6	7.1	8.3	7.9	7.3
	6	36	6.6	7.2	7.4	7.9	7.9	8.0
	Mean±S.E.M	35.17±0.40	6.45±0.12	6.95±0.10	7.53±0.13	8.25±0.14	7.93±0.09	7.71±0.09

Group	**Number**	**Weight (g)**	**Latency of nociceptive response (sec)**					
			Initial response(sec)	**30 min**	**45 min**	**60 min**	**75 min**	**90 min**
	1	34	6.8	6.9	7.3	7.8	7.6	7.6
Naloxone	2	36	7.4	7.6	7.9	7.9	7.4	7.4
5 mg/kg, ip	3	38	7.3	7.3	7.8	7.9	7.0	7.0
+	4	34	7.0	7.6	8.2	8.0	7.5	7.4
Morphine	5	38	7.4	7.9	8.0	7.8	6.8	6.3
2 mg/kg, sc	6	34	7.0	7.8	8.0	8.3	7.6	7.2
	Mean±S.E.M	35.67±0.80	7.15±0.10	7.52±0.15	7.87±0.13	7.95±0.08	7.32±0.14	7.15±0.19

Group	Number	Weight (g)	Latency of nociceptive response (sec)					
			Initial response(sec)	30 min	45 min	60 min	75 min	90 min
Naloxone 5 mg/kg, ip + EESL 200 mg/kg, po	1	38	7.1	7.1	7.3	7.4	6.8	7.0
	2	35	6.8	7.0	6.8	7.4	7.1	7.0
	3	37	6.9	7.0	7.2	7.3	7.2	6.9
	4	39	6.3	6.3	6.7	7.2	6.8	6.7
	5	36	7.2	7.2	7.0	7.5	7.3	6.6
	6	35	6.4	6.5	6.7	7.1	6.7	6.9
	Mean±S.E.M	36.67±0.67	6.78±0.15	6.85±0.15	6.95±0.11	7.31±0.06	6.98±0.10	6.85±0.07

Group	Number	Weight (g)	Latency of nociceptive response (sec)					
			Initial response(sec)	30 min	45 min	60 min	75 min	90 min
DESL 50 mg/kg, po	1	31	6.6	6.5	6.3	6.4	6.2	6.1
	2	30	6.8	6.5	6.4	6.3	6.6	6.5
	3	33	6.5	6.4	6.3	6.5	6.4	6.3
	4	34	6.6	6.5	6.4	6.2	6.0	6.1
	5	33	6.8	6.7	6.5	6.1	6.3	6.3
	6	34	6.5	6.5	6.3	6.4	6.4	6.3
	Mean±S.E.M	32.5±0.67	6.63±0.06	6.51±0.04	6.36±0.03	6.32±0.06	6.32±0.08	6.26±0.06

Group	Number	Weight (g)	Latency of nociceptive response (sec)					
			Initial response(sec)	30 min	45 min	60 min	75 min	90 min
DESL 100 mg/kg, po	1	36	6.7	6.3	6.6	6.5	6.4	6.3
	2	35	6.5	6.7	6.6	6.4	6.3	6.4
	3	31	6.6	6.7	6.7	6.6	6.5	6.3
	4	32	6.1	6.3	6.4	6.3	6.2	6.1
	5	36	6.1	6.3	6.0	6.3	6.4	6.2
	6	32	6.4	6.5	6.4	6.3	6.2	6.3
	Mean±S.E.M	33.67±0.92	6.40±0.10	6.47±0.08	6.45±0.10	6.40±0.05	6.33±0.05	6.27±0.04

Group	Number	Weight (g)	Latency of nociceptive response (sec)					
			Initial response(sec)	30 min	45 min	60 min	75 min	90 min
DESL 200 mg/kg, po	1	32	6.6	6.7	6.9	6.7	6.5	6.4
	2	31	6.7	6.1	6.2	6.3	6.4	6.3
	3	37	6.2	6.4	6.3	6.5	6.3	6.1
	4	36	6.4	6.0	6.6	6.4	6.3	6.5
	5	36	6.8	6.5	6.3	6.1	6.0	6.2
	6	35	6.8	6.8	6.3	6.4	6.4	6.1
	Mean±S.E.M	34.5±0.99	6.58±0.10	6.42±0.13	6.43±0.11	6.40±0.08	6.32±0.07	6.27±0.07

Group	Number	Weight (g)	Latency of nociceptive response (sec)					
			Initial response(sec)	30 min	45 min	60 min	75 min	90 min
Naloxone 2 mg/kg, ip + DESL 200 mg/kg, po	1	35	6.6	6.4	6.3	6.4	6.6	6.2
	2	34	6.5	6.7	6.5	6.2	5.8	5.9
	3	31	6.6	6.4	6.5	6.3	6.3	6.5
	4	34	6.6	6.4	6.6	6.5	6.2	6.5
	5	37	6.0	6.2	6.3	6.4	6.3	6.4
	6	31	6.2	6.4	6.3	6.3	6.1	6.0
	Mean±S.E.M	33.67±0.95	6.42±0.10	6.42±0.07	6.42±0.05	6.35±0.04	6.22±0.11	6.25±0.11

Effects of aspirin, ethanol and dichloromethane extract of *S. lychnophorum* (EESL and DESL) on the brewer's yeast induced pyrexia in rats

Group	Number	Weight (g)	Rectal temperature (ºC)						
			Before yeast injection	0 h	1 h	2 h	3 h	4 h	5 h
Control (Cosolvent), po	1	193	35.2	35.99	36.0	35.75	36.06	36.20	35.17
	2	197	35.3	36.45	36.26	36.29	36.22	36.02	35.30
	3	186	36.18	37.19	36.98	35.32	35.55	35.82	36.43
	4	178	35.31	36.24	36.31	36.22	36.25	36.26	36.07
	5	204	35.52	36.28	36.26	36.25	36.24	36.24	36.15
	6	190	35.91	36.68	36.73	36.65	36.36	36.03	36.35
	Mean±S.E.M	191.33±3.67	35.57±0.16	36.47±0.17	36.42±0.15	36.08±0.19	36.11±0.12	36.10±0.07	35.91±0.22

Group	Number	Weight (g)	Rectal temperature (ºC)						
			Before yeast injection	0 h	1 h	2 h	3 h	4 h	5 h
Aspirin 200 mg/kg, po	1	202	35.4	6.12	36.29	35.55	35.32	35.32	34.93
	2	193	35.7	36.44	36.37	35.61	35.40	35.21	35.35
	3	183	36.04	36.79	36.17	35.87	35.66	36.28	36.17
	4	193	35.81	36.57	36.32	35.73	35.57	36.18	36.24
	5	179	35.69	36.45	36.18	36.05	36.22	35.99	36.12
	6	201	35.75	36.46	36.25	36.44	36.08	36.18	36.05
	Mean±S.E.M	191.83±3.80	35.73±0.08	36.47±0.09	36.26±0.03	35.87±0.14	35.71±0.15	35.86±0.19	35.81±0.22

Group	Number	Weight (g)	Rectal temperature (ºC)						
			Before yeast injection	**0 h**	**1 h**	**2 h**	**3 h**	**4 h**	**5 h**
EESL 50 mg/kg, po	1	186	35.1	36.21	35.30	35.83	35.52	35.59	35.44
	2	205	36.1	36.89	35.82	35.55	36.19	35.94	35.32
	3	183	34.9	35.77	35.88	35.70	35.36	35.24	35.20
	4	180	35.84	37.05	36.72	36.21	35.13	35.53	36.36
	5	191	35.40	36.18	36.05	36.09	36.08	36.11	35.88
	6	202	35.58	36.29	36.16	35.78	35.40	36.10	36.11
	Mean±S.E.M	191.17±4.19	35.49±0.18	36.40±0.20	35.98±0.19	35.86±0.10	35.61±0.17	35.75±0.14	35.72±0.19

Group	**Number**	**Weight (g)**	**Rectal temperature (ºC)**						
			Before yeast injection	**0 h**	**1 h**	**2 h**	**3 h**	**4 h**	**5 h**
EESL 100 mg/kg, po	1	187	34.4	35.34	35.61	35.62	35.28	35.38	35.16
	2	200	35.0	35.82	35.94	35.90	35.12	35.33	35.15
	3	180	35.82	37.23	36.30	36.07	35.24	35.59	36.59
	4	188	35.96	36.67	36.37	35.47	35.31	36.10	37.03
	5	192	35.19	36.00	35.92	35.75	35.92	35.95	35.62
	6	201	35.22	36.11	35.99	36.07	36.19	35.81	36.01
	Mean±S.E.M	191.33±3.30	35.27±0.23	36.20±0.27	36.02±0.11	35.81±0.10	35.51±0.18	35.69±0.13	35.93±0.31

Group	Number	Weight (g)	Rectal temperature (ºC)						
			Before yeast injection	0 h	1 h	2 h	3 h	4 h	5 h
EESL 200 mg/kg, po	1	180	35.2	36.32	36.35	36.22	35.59	35.20	35.20
	2	195	35.8	36.53	36.72	36.15	35.89	35.56	35.30
	3	185	35.5	36.33	36.34	36.31	35.92	35.51	35.56
	4	182	35.46	36.39	36.46	35.42	35.61	35.52	36.23
	5	187	35.78	36.60	36.68	36.41	35.39	35.42	36.00
	6	204	35.29	36.27	35.98	35.99	35.38	35.73	35.83
	Mean±S.E.M	188.83±3.7	35.51±0.10	36.41±0.05	36.42±0.11	36.08±0.14	35.63±0.10	35.49±0.07	35.67±0.16

Group	Number	Weight (g)	Rectal temperature (ºC)						
			Before yeast injection	0 h	1 h	2 h	3 h	4 h	5 h
DESL 50 mg/kg, po	1	209	35.79	36.51	36.77	36.19	36.15	36.26	36.23
	2	191	36.39	37.10	36.53	36.67	36.51	35.81	36.02
	3	210	36.40	37.19	36.86	36.40	36.01	35.99	36.06
	4	193	36.06	36.81	36.83	36.84	35.84	36.15	36.07
	5	186	35.67	36.36	36.75	35.52	35.96	36.07	36.09
	6	194	36.20	36.91	36.96	36.06	35.95	36.04	36.08
	Mean±S.E.M	197.17±4.06	36.09±0.12	36.81±0.13	36.78±0.06	36.28±0.19	36.07±0.10	36.05±0.06	36.09±0.03

Group	Number	Weight (g)	Rectal temperature (ºC)						
			Before yeast injection	0 h	1 h	2 h	3 h	4 h	5 h
DESL 100 mg/kg, po	1	205	36.23	37.02	36.82	36.20	35.98	35.95	36.03
	2	186	35.82	36.60	36.86	35.52	36.21	36.19	35.98
	3	181	36.13	37.11	36.83	35.81	36.12	36.07	36.23
	4	189	36.06	37.07	36.94	36.03	36.08	36.14	36.52
	5	185	35.54	36.58	36.49	35.96	35.82	35.94	36.32
	6	190	35.35	36.54	36.61	35.71	35.43	36.05	36.15
	Mean±S.E.M	189.33±0.39	35.86±0.14	36.82±0.11	36.76±0.07	35.87±0.10	35.94±0.12	36.06±0.04	36.21±0.08

Group	Number	Weight (g)	Rectal temperature (ºC)						
			Before yeast injection	**0 h**	**1 h**	**2 h**	**3 h**	**4 h**	**5 h**
DESL 200 mg/kg, po	1	192	35.45	36.80	36.90	36.08	36.19	36.14	36.33
	2	200	36.42	37.11	36.93	36.20	36.11	36.08	36.31
	3	194	35.98	37.13	36.97	36.06	36.15	35.84	36.37
	4	186	35.58	36.45	36.68	36.06	36.07	36.06	36.16
	5	191	36.18	37.05	36.74	36.17	36.16	36.14	36.32
	6	190	35.48	36.65	36.91	36.24	36.16	36.10	36.39
	Mean±S.E.M	192.17±1.90	35.85±0.17	36.87±0.11	36.86±0.05	36.14±0.03	36.14±0.02	36.06±0.05	36.31±0.03

Effect of aspirin, ethanol and dichloromethane extract of *S. lychnophorum* (EESL, DESL) on carrageenan-induced paw edema in rats

Group	Number	Weight (g)	Paw edema volume (ml)						
			Initial response	0.5 h	1 h	2 h	3 h	4 h	5 h
Control (Cosolvent), po	1	176	3.92	5.41	5.92	6.21	6.33	6.32	6.34
	2	175	3.91	4.35	5.86	6.26	6.52	6.49	6.45
	3	180	4.07	5.07	5.80	6.21	6.64	6.45	6.48
	4	179	4.38	4.94	5.95	6.35	6.82	6.91	6.94
	5	189	4.23	4.98	5.57	6.89	6.84	6.72	6.94
	6	185	4.04	4.86	5.54	6.83	6.82	6.81	6.87
	Mean±S.E.M	180.67±2.20	4.09±0.07	4.94±0.14	5.77±0.07	6.46±0.13	6.66±0.08	6.62±0.09	6.67±0.11

Group	Number	Weight (g)	Paw edema volume (ml)						
			Initial response	0.5 h	1 h	2 h	3 h	4 h	5 h
Aspirin 200 mg/kg, po	1	186	4.03	4.14	4.78	4.83	4.93	4.94	4.82
	2	181	4.79	4.95	4.97	5.05	4.98	4.92	4.96
	3	187	3.40	4.55	4.85	4.86	4.98	4.98	4.91
	4	179	4.15	4.57	4.91	4.93	4.89	4.93	4.77
	5	184	3.33	4.18	4.25	4.53	4.59	4.67	4.63
	6	187	3.48	4.65	4.57	4.73	4.87	4.86	4.45
	Mean±S.E.M	184±1.37	3.86±0.23	4.51±0.12	4.72±0.11	4.82±0.07	4.87±0.06	4.88±0.05	4.76±0.08

Group	Number	Weight (g)	Paw edema volume (ml)						
			Initial response	0.5 h	1 h	2 h	3 h	4 h	5 h
EESL 50 mg/kg, po	1	193	3.95	4.86	5.20	5.70	5.97	5.88	5.88
	2	183	3.67	4.87	5.39	5.89	6.10	6.61	6.78
	3	176	3.72	4.54	4.70	5.36	5.87	5.83	5.87
	4	175	4.75	5.85	5.85	6.06	6.37	6.42	6.70
	5	187	4.17	4.83	4.86	5.90	6.23	6.20	6.31
	6	180	3.95	4.82	4.90	5.52	5.63	5.72	5.86
	Mean±S.E.M	182.30±2.80	4.03±0.16	4.96±0.18	5.15±0.17	5.74±0.11	6.03±0.11	6.11±0.15	6.23±0.18

Group	Number	Weight (g)	Paw edema volume (ml)						
			Initial response	**0.5 h**	**1 h**	**2 h**	**3 h**	**4 h**	**5 h**
EESL 100 mg/kg, po	1	189	4.22	5.10	5.06	5.06	4.99	5.04	5.14
	2	186	4.09	5.25	5.29	5.17	5.19	5.21	5.22
	3	180	4.30	5.10	5.25	5.19	5.19	5.20	5.23
	4	182	4.42	5.17	5.07	4.98	4.99	5.09	5.07
	5	186	3.74	4.58	4.59	4.75	4.77	4.93	4.95
	6	181	3.84	4.38	4.36	4.65	4.77	4.99	4.92
	Mean±S.E.M	184±1.44	4.10±0.11	4.93±0.15	4.94±0.15	4.97±0.09	4.98±0.08	5.08±0.05	5.09±0.05

Group	Number	Weight (g)	Paw edema volume (ml)						
			Initial response	**0.5 h**	**1 h**	**2 h**	**3 h**	**4 h**	**5 h**
EESL 200 mg/kg, po	1	189	3.71	4.58	5.02	5.09	5.11	5.13	5.14
	2	189	4.06	4.73	5.22	5.22	5.16	5.19	5.20
	3	177	4.15	4.97	4.75	4.70	4.82	4.87	4.98
	4	182	4.12	5.13	5.29	5.10	5.08	5.05	5.04
	5	185	3.68	4.64	4.38	4.48	4.57	4.56	4.62
	6	180	3.65	5.46	4.93	4.92	4.94	4.94	4.89
	Mean±S.E.M	183.67±1.99	3.90±0.10	4.91±0.14	4.93±0.14	4.91±0.11	4.94±0.09	4.96±0.09	4.99±0.08

Group	Number	Weight (g)	Paw edema volume (ml)						
			Initial response	0.5 h	1 h	2 h	3 h	4 h	5 h
DESL 50 mg/kg, po	1	184	3.78	4.82	5.43	5.53	6.54	6.73	6.72
	2	194	3.88	4.62	4.95	4.78	5.91	6.12	5.96
	3	197	3.81	4.73	5.02	5.41	6.06	6.50	6.44
	4	194	3.91	4.93	5.46	5.33	5.57	6.23	6.67
	5	190	3.93	4.82	5.06	5.82	6.37	6.99	7.22
	6	187	3.87	4.85	4.98	5.42	6.56	6.85	7.02
	Mean±S.E.M	191.00±2.00	3.86±0.02	4.80±0.04	5.15±0.09	5.38±0.14	6.17±0.16	6.57±0.14	6.67±0.18

Group	Number	Weight (g)	Paw edema volume (ml)						
			Initial response	0.5 h	1 h	2 h	3 h	4 h	5 h
DESL 100 mg/kg, po	1	177	3.67	4.65	4.88	5.28	6.10	6.30	6.69
	2	186	4.36	4.84	5.43	6.71	6.75	6.85	6.83
	3	176	3.39	4.85	5.02	6.73	6.65	6.69	6.60
	4	181	4.24	4.70	5.03	6.59	6.87	6.64	6.69
	5	173	3.79	4.75	4.48	5.44	6.39	6.47	6.49
	6	179	3.98	4.96	5.34	5.86	6.33	6.44	6.60
	Mean±S.E.M	178.67±1.84	3.91±0.15	4.79±0.05	5.03±0.04	6.10±0.27	6.51±0.12	6.57±0.08	6.65±0.05

Group	**Number**	**Weight (g)**	**Paw edema volume (ml)**						
			Initial response	**0.5 h**	**1 h**	**2 h**	**3 h**	**4 h**	**5 h**
DESL 200 mg/kg, po	1	175	4.12	4.89	4.98	5.37	6.51	6.35	6.28
	2	176	3.63	4.97	5.12	5.32	6.44	6.78	6.36
	3	177	4.07	4.90	5.21	5.65	6.44	6.51	6.52
	4	187	4.21	4.86	4.95	5.78	6.01	6.46	6.73
	5	181	3.81	4.82	4.99	5.55	5.87	6.27	6.27
	6	182	3.98	4.96	5.18	5.62	5.97	6.39	6.36
	Mean±S.E.M	179.67±1.86	3.97±0.09	4.90±0.02	5.07±0.05	5.54±0.07	6.21±0.12	6.46±0.07	6.42±0.07

Effect of aspirin, ethanol and chloromethane extract of *S. lychnophorum* (EESL, DESL) on cotton pellet-induced granuloma formation in rats.

Group	Number	Granuloma dry weight (mg)	Granuloma wet weight (mg)	Transudative weight (mg)	Granuloma weight (mg/mg cotton)
Control	1	62.4	122.4	60	3.25
	2	63.5	122.0	58.5	3.38
	3	62.2	122.1	59.9	3.29
	4	64.0	126.1	62.1	3.32
	5	63.1	124.3	61.2	3.34
	6	63.4	122.3	58.9	3.34
	Mean± S.E.M	63.10±0.28	123.2±0.68	60.1±0.56	3.32±0.02

Group	Number	Granuloma dry weight (mg)	Granuloma wet weight (mg)	Transudative weight (mg)	Granuloma weight (mg/mg cotton)
Aspirin, 200 mg/kg,po	1	42.5	75.5	33.0	2.20
	2	38.7	68.6	29.9	1.97
	3	39.0	66.2	27.2	1.97
	4	37.2	67.8	30.6	1.22
	5	40.1	72.1	32.0	2.14
	6	39.3	71.3	32.0	2.06
	Mean± S.E.M	39.47±0.72	70.25±1.38	30.78±0.85	1.93±0.15

Group	Number	Granuloma dry weight (mg)	Granuloma wet weight (mg)	Transudative weight (mg)	Granuloma weight (mg/mg cotton)
	1	57.4	100.1	42.7	2.83
EESL	2	54.5	99.0	44.5	2.76
50 mg/kg, po	3	59.7	102.5	42.8	3.0
	4	62.3	109.3	47.0	3.21
	5	59.1	108.3	49.2	2.87
	6	58.0	108.7	50.7	2.91
	Mean± S.E.M	58.5±1.06	104.65±1.90	46.15±1.37	2.93±0.06

Group	Number	Granuloma dry weight (mg)	Granuloma wet weight (mg)	Transudative weight (mg)	Granuloma weight (mg/mg cotton)
EESL 100 mg/lkg, po	1	55.9	115.4	59.5	2.81
	2	52.8	113.0	60.2	2.74
	3	54.1	118.1	64.0	2.77
	4	57.2	118.8	61.6	2.99
	5	53.4	114.3	60.9	2.74
	6	55.3	112.4	57.1	2.82
	Mean± S.E.M	54.78±0.67	115.33±1.08	60.55±0.94	2.81±0.04

Group	Number	Granuloma dry weight (mg)	Granuloma wet weight (mg)	Transudative weight (mg)	Granuloma weight (mg/mg cotton)
EESL 200 mg/kg, po	1	48.9	119.7	70.8	2.51
	2	48.9	118.4	69.5	2.45
	3	49.6	114.5	64.9	2.40
	4	47.6	113.6	66.0	2.34
	5	47.4	115.1	67.7	2.47
	6	48.9	113.8	64.9	2.47
	Mean± S.E.M	48.55±0.35	115.85±1.05	67.3±1.00	2.44±0.02

Group	Number	Granuloma dry weight (mg)	Granuloma wet weight (mg)	Transudative weight (mg)	Granuloma weight (mg/mg cotton)
DESL 50 mg/kg, po	1	59.3	121.6	62.3	2.89
	2	59.2	120.3	61.1	2.70
	3	58.4	122.1	63.7	2.72
	4	58.9	121.8	62.9	2.90
	5	59.1	121.2	62.1	2.89
	6	58.5	122.3	63.8	2.95
	Mean± S.E.M	58.9±0.15	121.55±0.95	62.65±0.42	2.84±0.04

Group	**Number**	**Granuloma dry weight (mg)**	**Granuloma wet weight (mg)**	**Transudative weight (mg)**	**Granuloma weight (mg/mg cotton)**
DESL 100 mg/kg, po	1	59.3	123.6	64.3	2.82
	2	58.1	121.3	63.2	2.99
	3	58.5	118.5	60.0	2.82
	4	59.3	121.5	62.2	3.17
	5	58.0	117.2	59.2	2.66
	6	58.9	126.7	67.8	2.68
	Mean± S.E.M	58.68±0.23	121.46±1.4	62.78±1.27	2.86±0.08

Group	Number	Granuloma dry weight (mg)	Granuloma wet weight (mg)	Transudative weight (mg)	Granuloma weight (mg/mg cotton)
DESL 200 mg/kg, po	1	58.5	117.1	58.6	2.71
	2	59.1	126.8	67.7	2.77
	3	59.0	117.1	58.1	2.74
	4	58.9	119.3	60.4	2.84
	5	58.7	123.7	65	2.68
	6	58.5	118.6	60.1	2.98
	Mean± S.E.M	58.78±0.10	120.43±1.61	61.65±1.57	2.79±0.04

ที่ ศธ 0521.11/ ๑ๆ๕

สำนักวิจัยและพัฒนา
มหาวิทยาลัยสงขลานครินทร์
อ.หาดใหญ่ จ.สงขลา 90110

Ref.17/52

หนังสือรับรอง

โครงการวิจัย เรื่อง **การประเมินฤทธิ์ระงับปวด ลดไข้ และต้านการอักเสบของสารสกัดเอธานอลและไดคลอโรมีเธนจากลูกสำรอง**

หัวหน้าโครงการวิจัย **รศ.ดร.วิบูลย์ ฤทธิทิศ**

ได้ผ่านการพิจารณาและเห็นชอบจาก คณะกรรมการจรรยาบรรณการใช้สัตว์ทดลอง มหาวิทยาลัยสงขลานครินทร์

ให้ไว้ ณ วันที่ ๒๖ พฤษภาคม 2552

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