Studies on Analgesic, Antipyretic and Anti-inflammatory Activities of Ethanol Extract of *Garcinia cowa* Roxb. in Experimental Animals

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ชื่อวิทยาศาสตร์	การศึกษาฤทธิ์ระงับปวด ลดไข้ และต้านการอักเสบของสารสกัดเอธานอลจากใบชะมวงในสัตว์ทดลอง
ผู้เขียน	นางสาวสุวิพร วรรณโชติ
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บทคัดย่อ

ชะมวง มีชื่อวิทยาศาสตร์คือ *Garcinia cowa* Roxb เป็นพืชอยู่ในวงศ์ Guttiferae พบได้ทั่วไปในประเทศไทย สารสำคัญที่พบในชะมวงได้แก่ cowanin, cowanol, cowaxanthone, 1,3,6-trihydroxy-7-methoxy-2,5-bis(3-methyl-2-butenyl)xanthone, 7-O-methylgarcinone E และ β –mangostin ชะมวงมีสรรพคุณที่ระบุในตำราแพทย์แผนไทย คือ ส่วนของเปลือกไม้ใช้ลดไข้และเป็นยาต้านจุลชีพ สารสกัดหยาบจากใบมีฤทธิ์ต้านการเจริญของเซลล์มะเร็งและต้านอนุมูลอิสระ วัตถุ ประสงค์ในการศึกษาครั้งนี้เพื่อ ประเมินฤทธิ์ระงับปวด ลดไข้ และต้านการอักเสบของสารสกัดเอธานอลจากใบชะมวงในสัตว์ทดลอง โดยทดสอบฤทธิ์ระงับปวดด้วยวิธี writhing, formalin, tail flick และ hot plate ในสัตว์ทดลอง ทดสอบฤทธิ์ลดไข้โดยใช้ brewer's yeast เหนี่ยวนำให้เกิดไข้ในหนูขาวใหญ่ ในขณะที่ทดสอบฤทธิ์ต้านการอักเสบแบบเฉียบพลันโดยการฉีด carrageenan บริเวณอุ้งเท้าหลังของหนูขาวใหญ่เพื่อเหนี่ยวนำให้อุ้งเท้าบวมและใช้ cotton pellet เหนี่ยวนำให้เกิด granuloma ในหนูขาวใหญ่เพื่อทดสอบฤทธิ์ต้านอักเสบแบบเรื้อรัง

ในการทดสอบฤทธิ์ระงับปวด เมื่อให้สารสกัดเอธานอลจากใบชะมวงในขนาด 300 และ 200 มก./กก. ทางปาก ในหนูถีบจักร พบว่าสามารถลดการเกิด writhing ได้ 41.20% และ 32.57% ตามลำดับ เมื่อเทียบกับกลุ่มควบคุม ในขณะที่ aspirin (200 มก./กก.) สามารถลดการเกิด writhing ได้ 66.45% และในการทดสอบด้วย formalin พบว่าสารสกัดในขนาดเดียวกันสามารถลดการเลียอุ้งเท้าในช่วง early phase ได้ 40.62% และ 23.53% และในช่วง late phase ได้ 33.44% และ 17.39% ตามลำดับ ในขณะที่ morphine (5 มก./กก.) และ aspirin สามารถลดการเลียอุ้งเท้าในช่วง early phase ได้ 81.07% และ 76.51% ตามลำดับ และ ในช่วง late phase ลดการเลียอุ้งเท้าได้ 84.07% และ73.17% ตามลำดับ ในการทดสอบโดยวิธี tail flick และ hot plate พบว่า morphine และสารสกัดเอธานอลจากใบชะมวงในขนาด 300 มก./กก. ทำให้ระยะเวลาของการตอบสนองต่อความเจ็บปวดของหนูขาวใหญ่ช้าลงระหว่างเวลา 30-90 นาที หลังให้ยา และ naloxone (2 มก./กก.) สามารถต้านฤทธิ์ระงับปวดของ morphine (5 มก./กก.) ในการทดสอบโดยวิธี tail flick และ hot plate ในขณะที่ naloxone สามารถต้านฤทธิ์ระงับปวดของสารสกัดเอธานอลจากใบชะมวงได้เฉพาะ

ในการทดสอบโดยวิธี tail flick แต่ไม่ได้ผลในการทดสอบด้วย hot plate จากผลในการทดสอบฤทธิ์ระงับปวดทั้งหมด แสดงว่าสารสกัดเอธานอลจากใบชะมวงสามารถลดความเจ็บปวดได้ทั้งที่ระบบประสาทส่วนปลายและในระบบประสาทส่วนกลาง โดยออกฤทธิ์ทั้งในระดับไขสันหลังและเหนือไขสันหลัง โดยกลไกการออกฤทธิ์อาจจะเกิดจากการยับยั้งการสังเคราะห์สาร prostaglandins และไม่ได้ออกฤทธิ์กระตุ้น opioid receptor ในการทดสอบฤทธิ์ลดไข้ที่เกิดจากการฉีด brewer's yeast พบว่าสารสกัดในขนาด 300 มก./กก. เท่านั้นที่สามารถลดไข้ในหนูขาวใหญ่ได้ในระหว่าง 1-5 ชั่วโมง ในขณะที่ aspirin ลดไข้ได้ที่เวลา 3-5 ชั่วโมง และในการทดสอบฤทธิ์ต้านการอักเสบแบบเฉียบพลัน โดยเหนี่ยวนำให้เกิดการบวมของอุ้งเท้าหนูขาวใหญ่ด้วย carrageenan พบว่าสารสกัดในขนาด 100, 200 และ 300 มก./กก. สามารถลดการบวมของอุ้งเท้าได้ในช่วงเวลา 1-3 ชั่วโมง, 0.5-4 ชั่วโมง และ 0.5-5 ชั่วโมงตามลำดับ ในขณะที่ aspirin ลดการอักเสบได้ระหว่าง 3-5 ชั่วโมง แสดงว่าสารสกัดมีฤทธิ์ระงับการอักเสบแบบเฉียบพลันได้และสารสกัดในขนาด 300 มก./กก. และ aspirin สามารถยับยั้งการอักเสบเรื้อรังจากการเหนี่ยวนำด้วย cotton pellet ที่ทำให้เกิด granuloma ในหนูขาวใหญ่ได้ 53.43% และ 94.03% ตามลำดับ ส่วนในการทดสอบความเป็นพิษเฉียบพลันในหนูถีบจักรและหนูขาวใหญ่ พบว่าเมื่อให้สารสกัดเอธานอลจากใบชะมวงในขนาด 5 ก./กก. ทางปาก ไม่ทำให้สัตว์ทดลองตาย และสัตว์ทดลองไม่แสดงอาการใดๆที่เกิดจากความเป็นพิษของสารสกัด ดังนั้นค่าประมาณของ LD_{50} ของสารสกัดเอธานอลจากใบชะมวงเมื่อให้ทางปากในหนูถีบจักรและหนูขาวใหญ่มีค่ามากกว่า 5 ก./กก. ซึ่งแสดงว่าสารสกัดไม่มีพิษ

โดยสรุปจากผลการทดลองครั้งนี้แสดงให้เห็นว่าสารสกัดเอธานอลจากใบชะมวงมีฤทธิ์ระงับปวดทั้งในระบบประสาทส่วนปลายและระบบประสาทส่วนกลาง โดยออกฤทธิ์ในระดับไขสันหลังและเหนือไขสันหลังคล้ายกับ aspirin มากกว่า morphine โดยอาจเกิดจากการยับยั้งการสังเคราะห์สาร prostaglandins มากกว่าออกฤทธิ์กระตุ้น opioid receptor กลไกในการลดไข้อาจเกิดจากการยับยั้งการสังเคราะห์ prostaglandins ใน hypothalamus นอกจากนั้นสารสกัดเอธานอลจากใบชะมวงยังมีฤทธิ์ในการต้านการอักเสบทั้งการอักเสบแบบเฉียบพลันและการอักเสบแบบเรื้อรัง โดยอาจยับยั้งการหลั่งสารตัวกลางที่ก่อให้เกิดการอักเสบและยับยั้งการสร้าง granuloma ในกระบวนการอักเสบ จากผลการทดลองทั้งหมดในการศึกษาครั้งนี้แสดงให้เห็นว่าสารสกัดเอธานอลจากใบชะมวงมีฤทธิ์ในการระงับปวด ลดไข้ และต้านการอักเสบ ซึ่งข้อมูลที่ได้ยืนยันสรรพคุณของใบชะมวงที่ใช้ในการแพทย์แผนไทยในการระงับปวด ลดไข้ และต้านการอักเสบ อย่างไรก็ตามควรมีการศึกษาเพิ่มเติมเกี่ยวกับสารสำคัญที่มีฤทธิ์แก้ปวด ลดไข้และต้านการอักเสบในใบชะมวงต่อไป

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ABSTRACT

Garcinia cowa Roxb., Family Guttiferae, is widely distributed in Thailand where it is known as "Cha-muang". Several parts of this plant contain biologically active compounds such as cowanin, cowanol, cowaxanthone, 1,3,6-trihydroxy-7-methoxy-2,5-bis(3-methyl-2-butenyl)xanthone, 7-O-methylgarcinone E and β--mangostin. The plant has been used in the folk medicine for various purposes. The bark has been used as an antipyretic and antimicrobial agent. Some pharmacological properties of the crude extracts of leaves, e.g., anti-tumor-promoting activity and antioxidant have been reported. The aims of the present study are to evaluate the analgesic, antipyretic and anti-inflammatory activity of *G. cowa* leaves in experimental animal models. The nociception models, including acetic acid-induced writhing, formalin, tail flick and hot plate tests, were used to evaluate analgesic activity in mice and rats. Brewer's yeast induced fever in rats was used to evaluate antipyretic activity, whereas carrageenan-induced rat paw edema and cotton pellet-induced granuloma formation in rats were used to examine the acute and chronic anti-inflammatory activity, respectively.

In analgesic tests, the ethanal extract of *G. cowa* (EEGC) leaves at the dose of 200 and 300 mg/kg significantly reduced the number of writhing induced by acetic acid in mice by 32.57% and 41.20%, respectively, while aspirin (200 mg/kg) showed 66.45% reduction. In formalin test, EEGC at the same dose decreased the duration of licking time in early phase by 40.62% and 23.53%, and in late phase by 33.44% and 17.39%, respectively. Morphine (5 mg/kg) and aspirin showed 81.07% and 76.51% inhibition in early phase and 84.07% and 73.17% inhibition in late phase,

respectively. In tail flick and hot plate tests, morphine and EEGC (300 mg/kg) significantly increased the reaction time to the nociceptive responses in rats during 30-90 min after drug administration. Naloxone (2 mg/kg) antagonized antinociceptive activity of morphine in tail flick and hot plate tests, whereas the antagonistic effect of naloxone on EEGC was observed only slightly in tail flick test but not in hot plate test. Base on the analgesic tests, the results indicated that EEGC exhibited analgesic activity and the possible analgesic mechanisms were likely to be both peripherally and centrally mediated at both spinal and supraspinal levels via the inhibition of prostaglandins (PGs) synthesis but not involved opioid receptor. In brewer's yeast-induced pyresis, EEGC (300 mg/kg) and aspirin (200 mg/kg) significantly decreased rectal temperature during 1-5 and 3-5 h, respectively, after drug administration. In carrageenan-induced rat paw edema model, EEGC at the dose of 100, 200 and 300 mg/kg and aspirin (200 mg/kg) significantly reduced the thickness of hind paw edema during 1-3, 0.5-4, 0.5-5 and 0.5-5 h, respectively, which suggested that EEGC could inhibit acute inflammation. In cotton pellet-induced granuloma model in rats, EEGC (300 mg/kg) and aspirin (200 mg/kg) exhibited a significant reduction both in transudative weight and granuloma weight. The granuloma inhibition of EEGC and aspirin were 53.43% and 94.03%, respectively. This suggested that EEGC could inhibit chronic inflammation. In acute oral toxicity test, the estimated LD_{50} of EEGC in mice and rats was more than 5 g/kg which indicated that was is practically non toxic.

In conclusion, the *G. cowa* ethanolic leaves extract possesses antinociceptive activity. The analgesic activity is probably peripherally and centrally mediated at the spinal and supraspinal levels with the possible mechanism by inhibition of PGs synthesis more than via opioid receptor. The possible antipyretic action of EEGC is probably due to inhibition of prostaglandin synthesis in hypothalamus. In addition, the EEGC showed acute and chronic anti-inflammatory activity which might be due to the inhibition of inflammatory mediators release and granuloma formation during inflammatory process. The overall results of this study demonstrated that ethanol extract of *G. cowa* leaves exhibited significant antinociceptive, antipyretic and anti-inflammatory activities. Therefore, this study support the traditional use of *G. cowa* for the treatment of pain, fever and

inflammation. Further investigation are of value to identify the active compounds in EEGC responsible for analgesic, antipyretic and anti-inflammatory activity.

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Suviporn Wannachote

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LIST OF ABBREVIATIONS AND SYMBOLS

EEGC	=	Ethanol extract of *Garcinia cowa* Roxb
etc.	=	Et cetera
g	=	Gram
h	=	Hour
i.p.	=	Intraperitoneal
kg	=	Kilogram
L	=	Liter
LD_{50}	=	Lethal dose at 50% of dead animal
m	=	Meter
mg	=	Milligram
min	=	Minute
ml	=	Milliliter
mM	=	Micro molar
No.	=	Number
p	=	*p*-value
p.o.	=	Per oral
sec	=	Second
s.c.	=	Subcutaneous
S.E.M.	=	Standard error of the mean
w/v	=	Weight by volume
°C	=	Degree Celsius
°F	=	Degree Fahrenheit
/	=	Per
µL	=	Microliter
µg	=	Microgram
%	=	Percentage

CHAPTER 1

INTRODUCTION

Herbal medicine involves the use of plants for medicinal purposes. The term 'herb' includes leaves, stems, flowers, fruits, seeds, roots, rhizomes, and bark, although other naturally occurring substances including animal and mineral products are also used in many traditions. There can be little doubt that the use of plants for healing purposes is the most ancient form of medicine known. Herbal Medicine, sometimes referred to as Herbalism or Botanical Medicine, is the use of herbs for their therapeutic or medicinal value. A herb is a plant or plant part valued for its medicinal, aromatic or savory qualities. Herb plants produce and contain a variety of chemical substances that act upon the body. The pharmacological treatment of disease began long ago with the use of herbs (Schulz *et al.,* 2001). Methods of folk healing throughout the world commonly used herbs as part of their tradition.

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 the traditional medicine (Calixto, 2005). 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, *Garcinia cowa* (*G. cowa*) Roxb (Family Guttiferae) is widely distributed in Thailand where it is known as Cha-muang. Several active

compounds have been isolated including of cowanin, cowanol, cowaxanthone, 1,3,6-trihydroxy-7-methoxy-2,5-bis(3-methyl-2-butenyl) xanthone, 7-O-methylgarcinone E and β–mangostin (Suwanna *et al.*, 2009). It has been used in the folk medicine for various purposes. The bark has been used in traditional medicine as an antipyretic and antimicrobial agent (Na Pattalung *et al.*, 1994). Some pharmacological properties of the crude extracts of leaves, e.g., anti-tumor-promoting activity (Murakami *et al.*, 1995) has been reported.

In the present study, ethanol leave extract of *G. cowa* was tested for its antinociceptive, antipyretic and anti-inflammatory activities in experimental animals because it is commonly used in folk medicine but still lack of scientific information.

CHAPTER 2

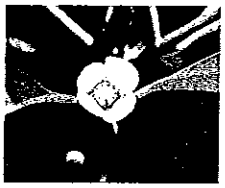
LITERATURE REVIEW

Garcinia cowa Roxb. (Guttiferae)

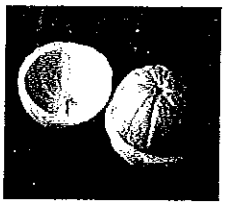
G. cowa Roxb., belongs to the family Guttiferae, is commonly known in Thai as "Cha-muang". It is a tropical tree indigenous to monsoon rain forests in Southwest Asia (Richards 1990; Zhu 1993). *G. cowa* is a small to medium-sized tree 15-30 m high. The trees are found scattered in lowland and peat swamp forests. The leaves are opposite, 7-15 cm long and 2-3.5 cm wide. The flower is white to yellow in colour and is found in clusters in the leaf axis and also below the leaves. The flower has 4 sepals and 4 petals. The subglobose fruits are large (3–6 cm in diameter; 40–122 g) and ripen with a bright yellow thick husk. The interior of the fruit is divided into several segments, which hold a rough and resinous seed with juicy flesh. The exalbuminous seed is relatively large (approximately 2.4 g) and has high moisture content (approximately 50% fresh weight) at maturity (Liu *et al.* 2002). Both the young shoots and fruit are edible. Young leaves and shoots are used in many Thai soups (Figure 1).

Tree

Leaves



Flower



Fruit

The fruits and leaves are used for the improvement of blood circulation, as an expectorant for the treatment of cough and indigestion and as a laxative, while the root is used for fever relief (Poomipamorn and Kumkong, 1997). Some pharmacological properties of the crude extracts of leaves have been reported, such as anti-tumor promoting activity (Murakami *et al.*, 1995), anti-inflammatory activity and antioxidant activity (Panthong, *et al.*, 2009), antimalarial activity (Likhitwitayawuid, *et al.*, 1997).

Chemical constituents isolated from *G. cowa* Roxb. were reported according to information from NAPRALERT database developed by University of Illinois at Chicago, Chemical Abstracts and Dictionary of Natural Products. Several types of compounds isolated from some *G. cowa* were demonstrated in Table 1.

Table 1. Compounds isolated from the *G. cowa* Roxb. and their pharmacological effects

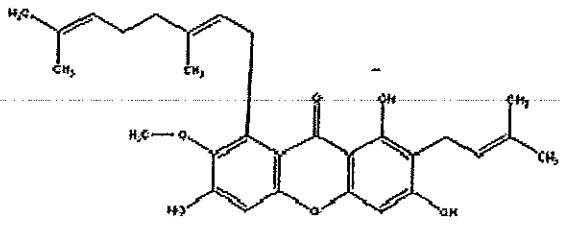
Investigated part	Compound	Pharmacological properties / Ethnological uses	References
Fruits	- cowaxanthones A – E - *β*-mangostin - 1-hydroxy-3,6,7trimethoxy-2,8-*bis*(3-methyl-2-butenyl) xanthone - 1,3-dihydroxy-6,7-dimethoxy-2,8-*bis* (3-methyl-2-butenyl)xanthone - 7-*O*-methylgarcinone E - 1,6-dihydroxy-7-methoxy-5,8-*bis*(3-methyl- 2butenyl) -6′,6′-dimethylpyrano (2′,3′:3,2) -xanthone		Panthong, *et al.*, 2006

	- 1,6-dihydroxy-7-methoxy-8-(3-methyl-2-butenyl)-6′,6′-dimethylpyrano (2′,3′:3,2)xanthone		
Fruits	- cowaxanthones A -D - cowanin - α-mangostin - mangostanin - cowanol	Anti-inflammatory activity	Panthong, *et al.*, 2009
Fruits and leaves	-	Expectorant Laxative Indigestion	Poomipamorn and Kumkong, 1997
Latex	- cowanin - cowanol - cowaxanthone -1,3,6-trihydroxy-7-methoxy-2,5-bis(3-methyl-2-butenyl) xanthone - norcowanin	Antimicrobial activity	Na Pattalung, *et al.*, 1994
Latex	- cowagarcinone A - E		Mahabusarakam *et al.*, 2005
Leave, Fruits	(-)-hydroxycitric acid (-)-hydroxycitric acid lactone,		Jena, *et al.*, 2002
Leave	-	Anti-tumor promoting activity	Murakami *et al.*, 1995
Twigs	- cowaxanthone F - morelloflavone - volkensiflavone - fukugiside - 1,6-dihydroxyxanthone	Antioxidant activity	Panthong, *et al.*, 2009

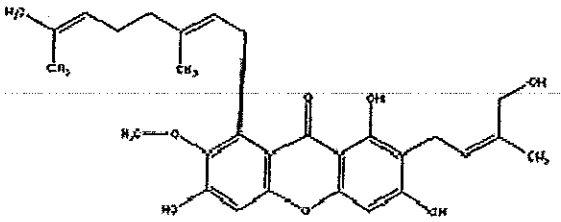
Root	- 3-geranyloxy-1,7 dihydroxyxanthone - cowaxanthone - cowanin - cowanol - mangostin - β –mangostin - 1,3,6-trihydroxy-7-methoxy-2,5-bis(3-methyl-2-butenyl) xanthone - 7-geranyloxy-1,3-dihydroxyxanthone - cochinchinone A - cratoxycochinchinone C - macluraxanthone -10-*o*-methylmacluraxanthone - isocudraniaxanthone B - cowagarcinone B - stigmasterol		Deachathai, *et al.*, 2005
Stem	- 1,3,6-trihydroxy-7-methoxy-8-(3,7-dimethyl-2,6-octadienyl) xanthone or rubraxanthone		Lee, *et al.*, 1977
Stem	- 1,5,6-trihydroxy-3-methoxy-4-(3-hydroxyl-3-methylbutyl) xanthone - 1,5-dihydroxy-3-methoxy-6',6'-dimethyl-2*H*-pyrano (2_,3_:6,7)-4-(3-methylbut-		Shen and Yang, 2006

	2-enyl)xanthone		
Stem bark	- 7-*O*-methylgarcinone E - cowanin - cowanol - cowaxanthone - *β*-mangostin	Antimalarial activity Antioxidant activity	Likhitwitayawuid, *et al.*, 1997-1998
Stem bark	- (2E,6E,10E)-(+)-4 *β*-hydroxy-3-methyl-5*β*-(3,7,11,15-tetramethyl-hexadeca-2,6,10,14-tetraenyl)-cyclohex-2-en-1-one - 4-(1,1-dimethyl-2-propenyl)-1,5,6-trihydroxy-3-methoxy-2-(3-methyl-2-butenyl) xanthone or nigrolineaxanthone E - rubraxanthone		Wahyuni, *et al.*, 2004

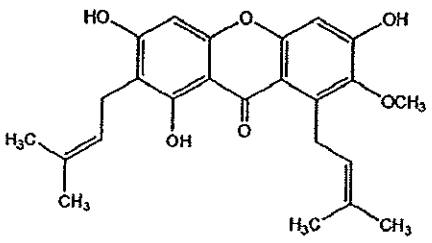
Figure 2. Chemical constituents isolated from *G. cowa* Roxb.



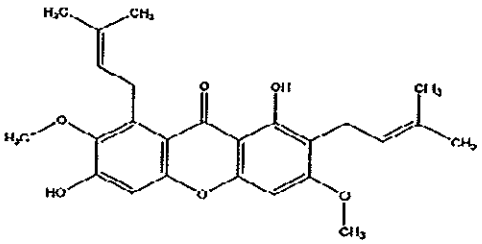
Cowanin



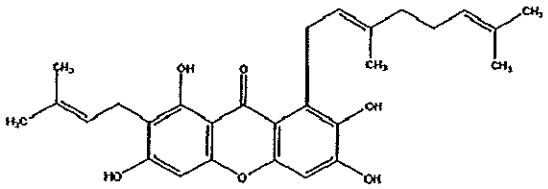
Cowanol



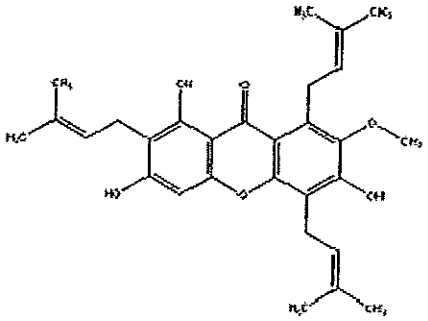
Mangostin



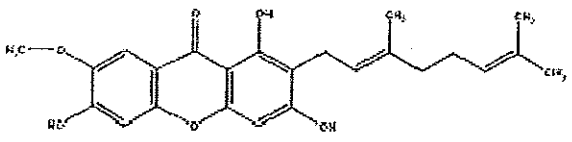
β-mangostin



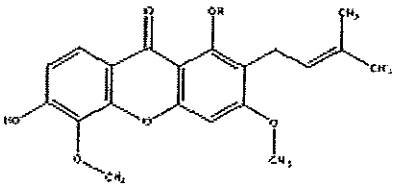
Norcowanin



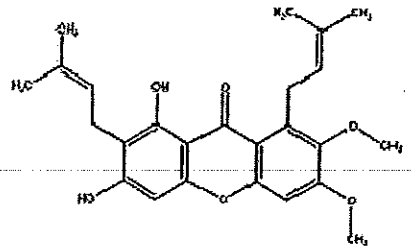
7-*O*-methylgarcinone E



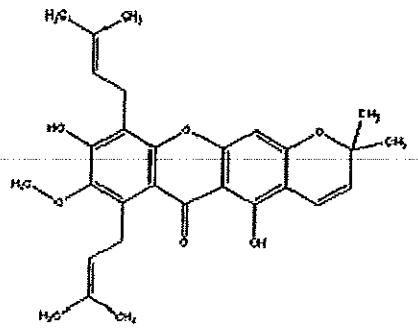
Cowaxanthone



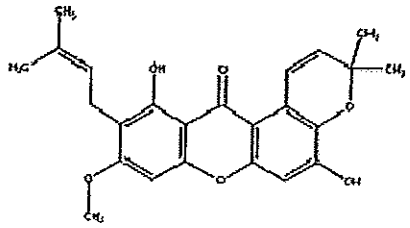
Cowaxanthone A



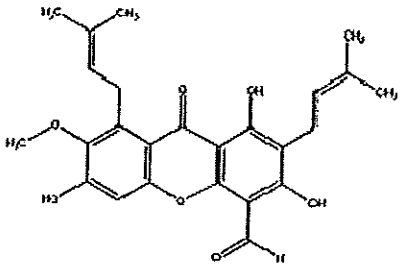
Cowaxanthone B



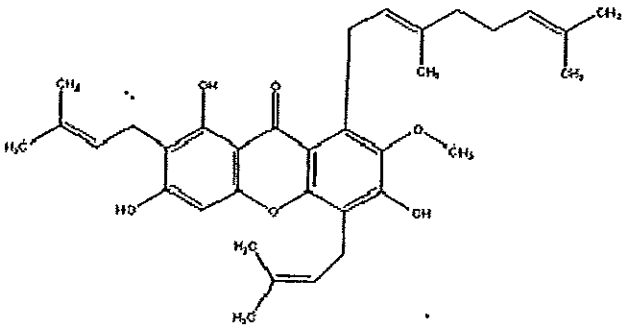
Cowaxanthone C



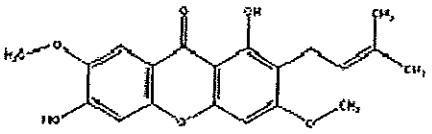
Cowaxanthone D



Cowaxanthone E



Cowagarcinone A



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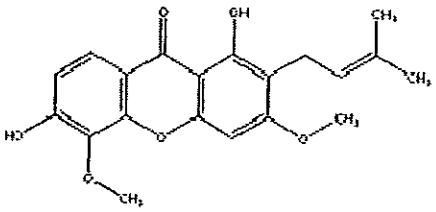

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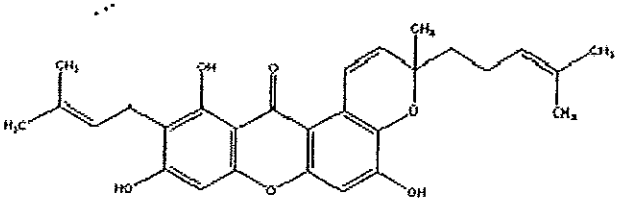
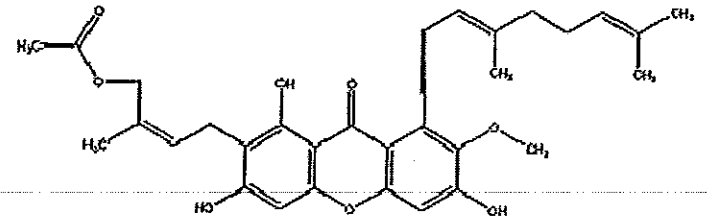


Cowagarcinone D



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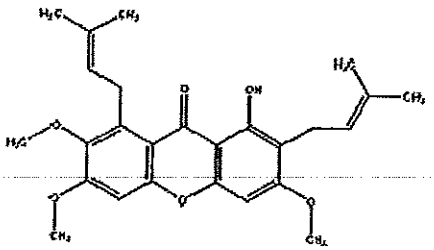
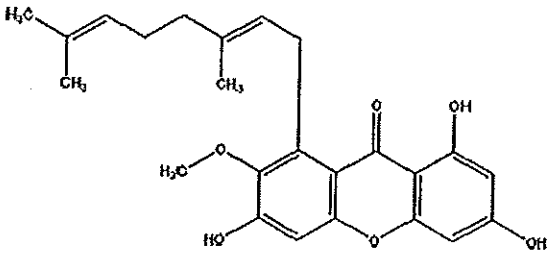
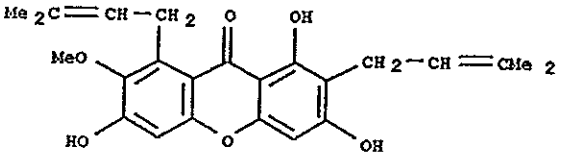


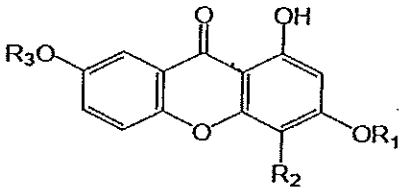
Fuscaxanthone C



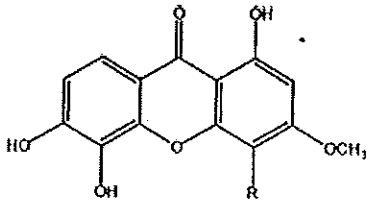
Rubraxanthone

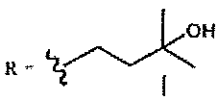


1-hydroxy-3,6,7-trimethoxy-2,8-bis (3-methyl-2-butenyl) xanthone

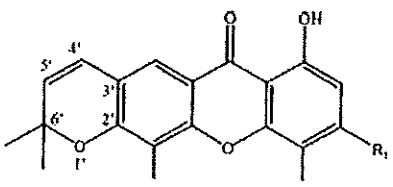


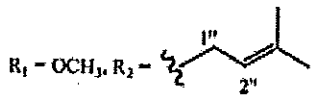
7-geranyloxy-1,3-dihydroxyxanthone (R1 = H , R2 = H, R3 = 
)



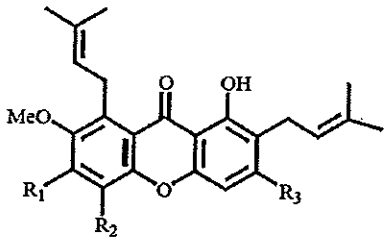


1,5,6- trihydroxy-3-methoxy-4-(3-hydroxyl-3-methylbutyl)xanthone



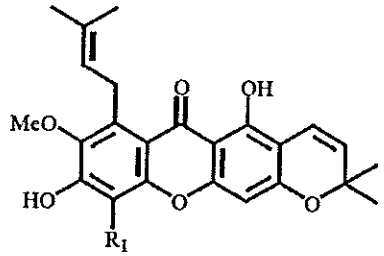


1,5-dihydroxy-3-methoxy-6',6'-dimethyl-2*H*pyrano(2',3':6,7)-4-(3-methylbut-2-enyl)xanthone



R_1 = OMe, R_2 = H, R_3 = OH

1,3-dihydroxy-6,7-dimethoxy-2,8-*bis*(3-methyl-2-butenyl)xanthone (1)

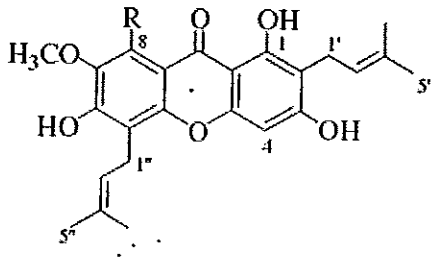


(1) R_1 = prenyl

(2) R_1 = H

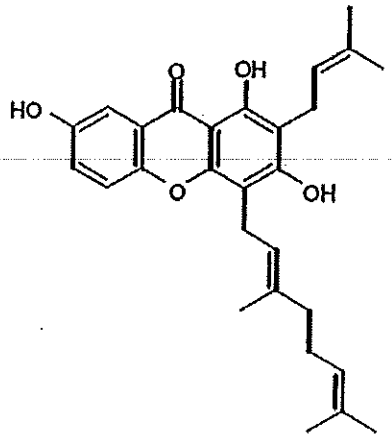
1,6-dihydroxy-7-methoxy-5,8-*bis*(3-methyl-2-butenyl)-6',6' dimethylpyrano (2',3':3,2) xanthone (1)

1,6-dihydroxy-7-methoxy-8-(3-methyl-2-butenyl)-6',6'-dimethylpyrano(2',3':3,2) xanthone (2)

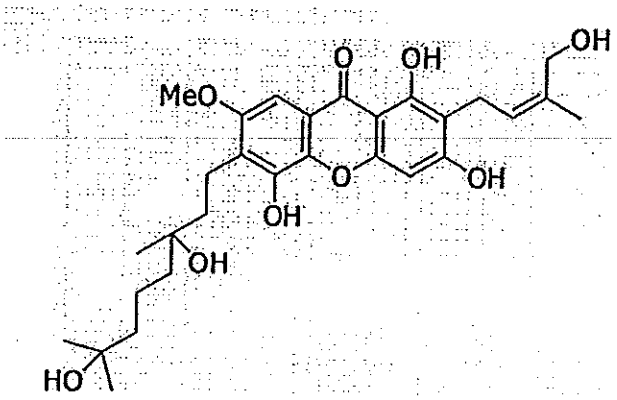


R = H

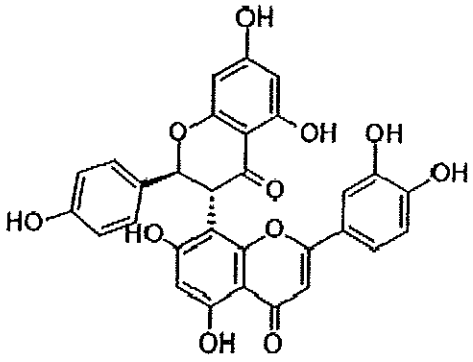
1,3,6-trihydroxy-7-methoxy-2,5-bis(3-methyl-2-butenyl)xanthone



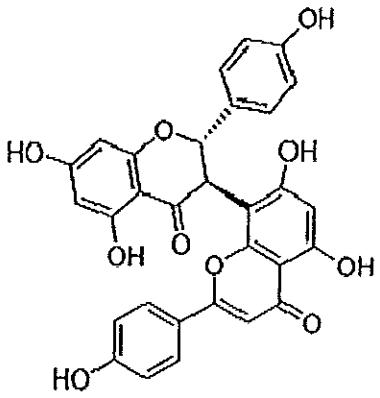
Cochinchinones A



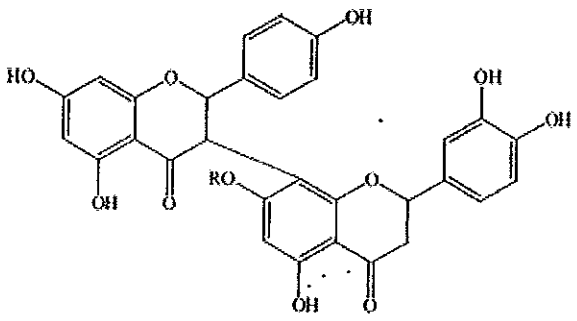
Cowaxanthone F



Morelloflavone



Volkensiflavone



R = glucose

fukugiside (morelloflavone-7″-*O*-glucoside)

I. Pain

Pain is defined as an unpleasant sensory and emotional experience associated with actual or potential tissue damage (Balestrieri and Fisher, 1994). It 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 Dickinson, 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 chornic 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). The pain experience is divided into 2 types as acute and chronic pain. Both of these experiences alter the comfort level of the patient and cause different pain reaction behaviors.

- **Acute pain** is a warning to possible or real danger. The onset of the pain is

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

- **Chronic pain** is persist beyond the normal healing time as pain signals are repeatedly being generated making neural pathway hypersensitive to pain signals and resistant to antinociceptive input. There are no physiological responses in chornic pain. But the behavioral response is based on the long-term activition of the

autonomic nervous system. The patient can exhibit sleep and appetite disturbances, irritability, decreased libido, loss of interests and increased preoccupation with body sensation. The patient can also experience apathy, withdrawal, hopelessness and depression (Beyers, 1991).

Quality of pain has been classified into 2 different major causes as fast pain and slow pain.

- **Fast pain** is also described by many alternate names such as sharp pain, pricking pain, electric pain and acute pain. This type of pain is felt when a needle is stuck into the skin or when the skin is cut with a knife. This pain is also felt when the skin is subjected to electric shock. The fast pain is transmitted through type Aδ pain fibers.

- **Slow pain** is also described as burning pain, aching pain, throbbing pain, nauseous pain and chronic pain. This type of pain is usually associated with tissue destruction. It can become excruciating and lead to prolonged, unbearable suffering. It can occur both in the skin and in almost any deep tissue or organ. The slow pain results from stimulation of the more primitive type C fiber (Moffett and Moffett, 1993; Guyton, 1992).

Injury to tissues initiates the production and release substances that interact with each other and stimulate the nerve endings of pain fibers. These chemicals also increase the pain response produce by heat or pressure. In addition, the chemicals released are mediators in inflammation and allergic reactions and also participate in many other functions of the body.

These algesic substances consist of histamine released from mast cellls and basophils, serotonin from damaged platelets, bradykinin and other kinins synthesized from cell membranes and probably many other compounds.

Two groups of chemicals that interact together, the kinins and prostaglandins, appear to be particularly important in mediating pain. Bradykinin is a member of the first group, considered to be one of the more important compounds for the initiating of pain. It can produce immediate pain when injected even in small amounts. However, the second group, the prostaglandins, can produce pain only when injected in high concentration. At low doses these compounds do not produce pain, but potentiate the action of bradykinin and other substances. In addition,

bradykinin stimulates an increase in the synthesis of prostaglandins (Brechter and Lerner, 2007).

Substance P (SP), which is considered to be a neurotransmitter in pain fibers, is released at the site of injury. Although SP is not itself an algesic, it increases the permeability of blood vessels. This produces a leakage of fluid into the surrounding tissue which provides for wider diffusion of the algesics. In this manner a larger area becomes painful.

Prostaglandins are associated particularly with the development of pain that accompanies injury or inflammation. Large doses of prostaglandin E_2 (PGE_2) or prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$), given to women by intramuscular or subcutaneous injection to induce abortion, cause intense local pain. Prostaglandins can also cause headache and vascular pain when infused intravenously. Although the doses of prostaglandins required to elicit pain are high in comparison with the concentrations expected *in vivo*, sensitization to painful stimuli (hyperalgesia) occurs when even minute amounts of PGE_1 are given intradermally. Moreover, subdermal infusion of mixtures of PGE_1 with small, subtheshold amounts of either bradykinin or histamine cause marked pain (Insel, 1991).

- **Pain mechanisms**

Four steps occur along the pain pathway: transduction, transmission, modulation and perception (Figure 2).

Transduction is the process by which afferent nerve endings participate in translating noxious stimuli into nociceptive impulses. There are three types of primary afferents: A-beta, A-delta and C fibers. Of these, the A-delta and C fibers are involved in nociception. Fibers that respond maximally to noxious stimulation are classified as pain fibers or nociceptors. These are generally A-delta and C fibers. Silent nociceptors, also involved in transduction, are afferent nerves that do not respond to external stimulation unless inflammatory mediators are present.

Transmission is the process by which impulses are sent to the dorsal horn of the spinal cord and then along the sensory tracts to the brain. The primary afferent neurons are active senders and receivers of chemical and electrical signals. Their axons terminate in the dorsal horn of the spinal cord where they have connections with many spinal neurons.

Modulation is the process of dampening or amplifying the pain-related neural signals. Modulation has been extensively characterized in the dorsal horn of the spinal cord, but probably takes place at multiple other levels as well. Rich arrays of opioid receptors (mu, kappa and delta) are present in the dorsal horn. In addition to an ascending tract, the nociceptive system contains descending pathways that send neurons from the frontal cortex, hypothalamus and other brain areas to the midbrain and medulla, and also down to the spinal cord. The result of the descending inhibitory input is that incoming nociceptive signals from the periphery are dampened, or even blocked entirely at the "gate" in the dorsal horn.

The perception of pain is the conscious awareness of the experience of pain. Perception results from the interaction of transduction, transmission, modulation, psychological aspects and other characteristics of the individual. All four steps along the pain pathway are tied together by the Gate Control Theory.

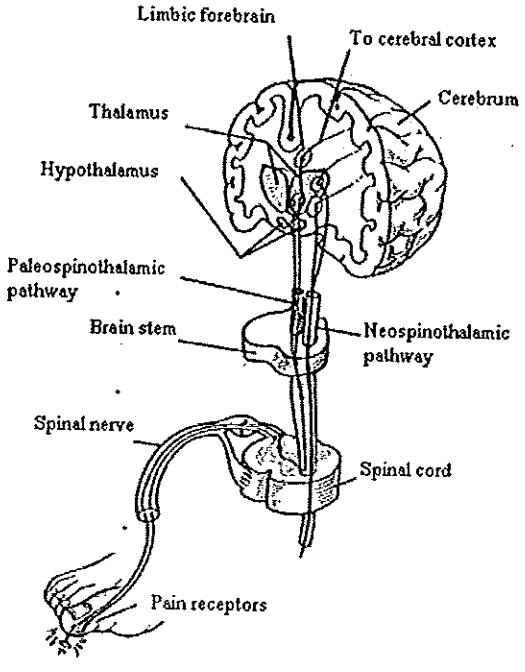


Figure 3. Pain mechanisms (Howard, 2007)

➢ **Peripheral sensitization**

After tissue damage which may be mechanical, chemical or thermal (below 15°C, above 45°C) on nociceptor, biological molecules are produced such as substance P, histamine, hydrogen ions, bradykinin, prostaglandin, nitric oxide. Bradykinin is a main substance that stimulates nociceptives (act on Bradykinin2, BK2 receptor) via protein kinase C (PKC), and prostaglandin (mainly E_2, I_2), sensitize nociceptor (act on EP/IP receptor) to noxious stimuli by lowering of nociceptive activation thresholds via cyclic-adnosine monophosphate (cAMP). Its causes the afferent neuron to discharge, sending impulse (action potential) to the spinal cord. This state is called peripheral sensitization (Guyton, 1992; Mutschler and Derendorf, 1995) (Figure 4).

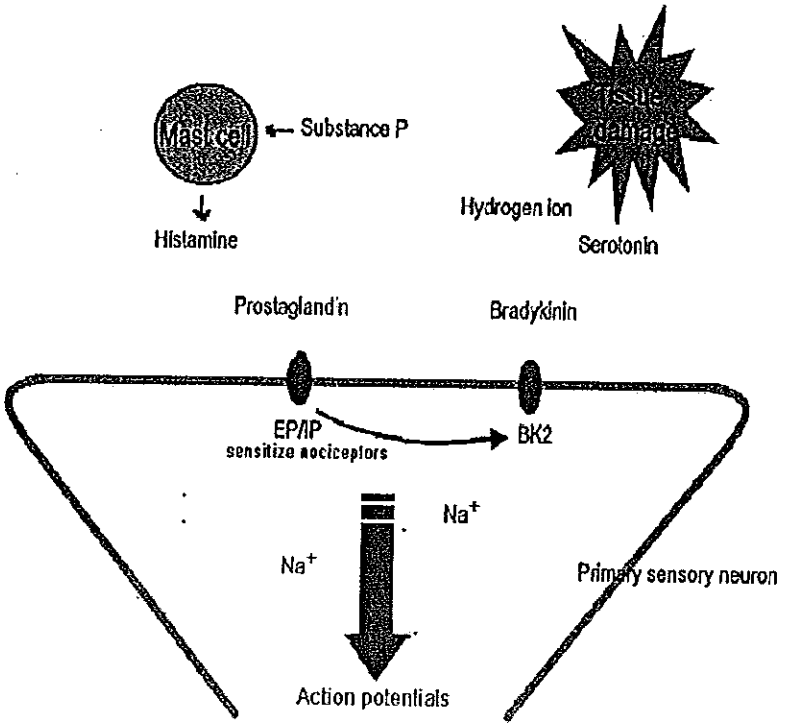


Figure 4. Pain pathway in peripheral sensitization (Samad, 2002)

➢ **Central sensitization**

Nociceptive formation within the Aδ and C fibers arrives at the dorsal horn of spinal cord. 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 laminar II. However, visceral C fibers terminate in the larminar 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).

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-aspatate (NMDA) sensitivity to glutamate (Singleton, 2005). When glutamate release from primary afferent neuron and bind to NMDA, there is an influx of Ca^{2+} into the post synaptic neuron (Basbaum and Jessell, 2000). These results could activate enzyme such as nitric oxide (NO) synthase 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).

II. Normal Thermoregulation

The human heat balance equation describes how the body can maintain an internal body temperature at approximately to 37 °C in terms of heat generation and heat exchange with the environment. At the heart of thermoregulation is an integrated network of neural connections involving the hypothalamus, limbic system, lower brainstem, the reticular formation, spinal cord, and the sympathetic ganglia (Boulant, 1997). An area in and near the rostal hypothalamus is also important in orchestrating thermoregulation. This region, the "preoptic area", includes the preoptic nuclei of the anterior hypothalamus (POAH) and the septum.

➢ **Regulation of Body Temperature**

The human body has the remarkable capacity for regulating its core temperature between 98°F and 100°F when the ambient temperature is between approximately 68°F and 130°F (Guyton *et al.*, 1972.) This presumes a nude body and dry air. The external heat transfer mechanisms are a radiation, conduction and convection and evaporation of perspiration. The process is far more than the passive operation of these heat transfer mechanisms. The body takes a very active role in temperature regulation. The temperature of the body is regulated by neural feedback mechanisms which operate primarily through the hypothalmus. The hypothalmus contains not only the control mechanisms, but also the key temperature sensors. Under control of these mechanisms, sweating begins almost precisely at a skin temperature of 37°C and increases rapidly as the skin temperature rises above this value. The heat production of the body under these conditions remains almost constant as the skin temperature rises. If the skin temperature drops below 37°C, a variety of responses is initiated to conserve the heat in the body and to increase heat production. These include

- Vasoconstriction to decrease the flow of heat to the skin.
- Cessation of sweating.
- Shivering to increase heat production in the muscles.
- Secretion of norepinephrine, epinephrine, and thyroxine to increase heat production
- In lower animals, the erection of the hairs and fur to increase insulation.

The balance that maintains a constant total body heat content can be expressed by the relation as follows (Figure 5).

Heat output = Heat input + Heat production

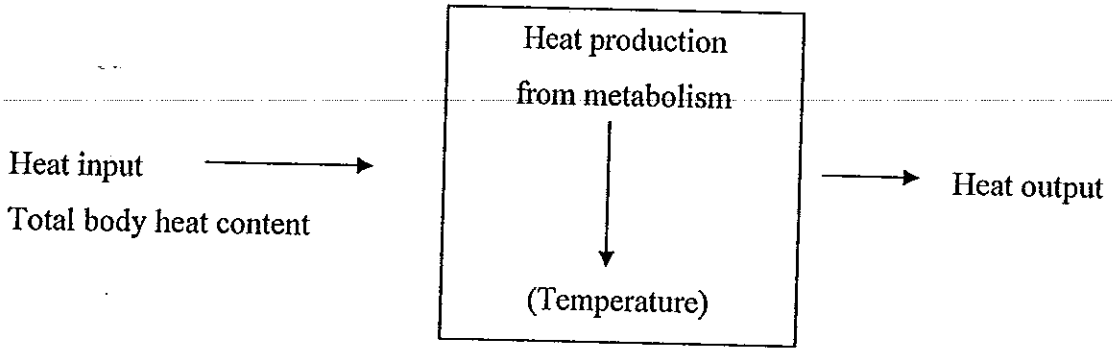


Figure 5. Balance of heat input, output and production (Seeley *et al.*, 1996)

➢ **Peripheral thermoreceptor regulation of body temperature**

Peripheral receptors measure temperature in the skin. These receptors are naked nerve ending that are very sensitive to temperature and selective respond to cold receptors or warm receptors (Figure 6). While both types of temperature receptors are found everywhere 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).

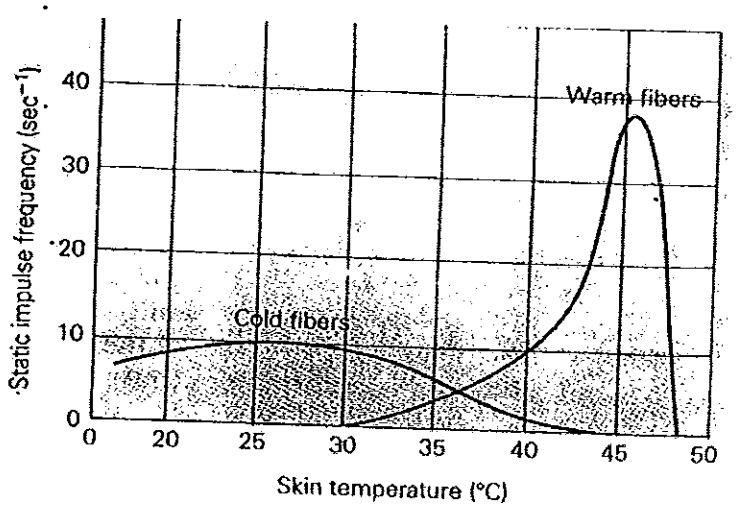


Figure 6. Static impulse frequency of cold and warm nerve fibers as a function of skin temperature (Patapoutian *et al.*, 2003)

➢ **Central thermoreceptor in temperature regulation**

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 response include regulation of sympathetic neural control of sweating, shivering. This pathway modifies voluntary skeletal muscle activity through an influence on the cerebral cortex (Rhoades and Pflazer, 2003).

Response to heat

1. Vasodilation

Arterioles leading to skin capillaries dilate and shunt vessels are closed leading to increase blood flow to the skin. As a result, more heat is lost to the environment. Alongwith sweating, vasodilation is an efficient cooling mechanism.

2. Sweating

In hot temperature, excessive sweating occurs. Evaporation of sweat from the surface of skin leads to the cooling.

Response to cold

1. Vasoconstriction

The arterioles leading to the blood capillaries in the surface layers of skin constrict, reducing the flow of blood to skin. This cuts down the amount of heat lost through the skin. Vasoconstriction is controlled by sympathetic nerves passing from brain.

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).

III. Fever

Fever also known as pyrexia 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. As a person's temperature increases, there is, in general, a feeling of cold despite an increasing body temperature. Once the new temperature is reached, there is a feeling of warmth. A fever can be caused by many different conditions ranging from benign to potentially serious. There are arguments for and against the usefulness of fever, and the issue is controversial. With the exception of very high temperatures, treatment to reduce fever is often not necessary; however, antipyretic medications can be effective at lowering the temperature, which may improve affected person's comfort.

Temperature is ultimately regulated in the hypothalamus. A trigger of the fever, called a pyrogen, causes a release of prostaglandin E_2 (PGE_2). PGE_2 then in turn acts on the hypothalamus, which generates a systemic response back to the rest of the body, causing heat-creating effects to match a new temperature level. These elevations alter the firing rate of neurons that control thermoregulation in the hypothalamus. Although fever benefits the nonspecific immune response to invading microorganisms, it is also viewed as a source of discomfort and is commonly suppressed with antipyretic medication. Aspirin is an antipyretic which have been widely used since the late 19^{th} century, but the mechanism by which they relieve fever have only been characterized in the last few decades. It is now clear that most antipyretics work by inhibiting the enzyme cyclooxygenase and reducing the levels of PGE_2 within the hypothalamus.

➢ **The pathogenesis of fever**

Cytokines released by monocytic cells play a central role in the genesis of fever. The cytokines primarily involved in the development of fever include interleukin (IL) 1, IL-6, and tumor necrosis factor (TNF)-α (Kackowiak, 1998). The interaction between these cytokines is complex, with each being able to up-regulate and down-regulate their own expression as well as that of the other cytokines. These cytokines bind to their own specific receptors located in close proximity to the preoptic region of the anterior hypothalamus (Kackowiak, 1998). Here, the cytokine receptor interaction activates phospholipase A_2, resulting in the liberation of plasma membrane arachidonic acid as substrate for the cyclo-oxygenase pathway. Some cytokines appear to increase cyclo-oxygenase expression directly, leading to liberation of prostaglandin E_2. This small lipid mediator diffuses across the blood brain barrier, where it acts to decrease the rate of firing of preoptic warm-sensitive neurons, leading to activation of responses designed to decrease heat loss and increase heat production. (Kackowiak, 1997; Mackowiak, 1998). In a small proportion of hospitalized patients, hyperthermia may result from increased sympathetic activity with increased heat production (Figure 7).

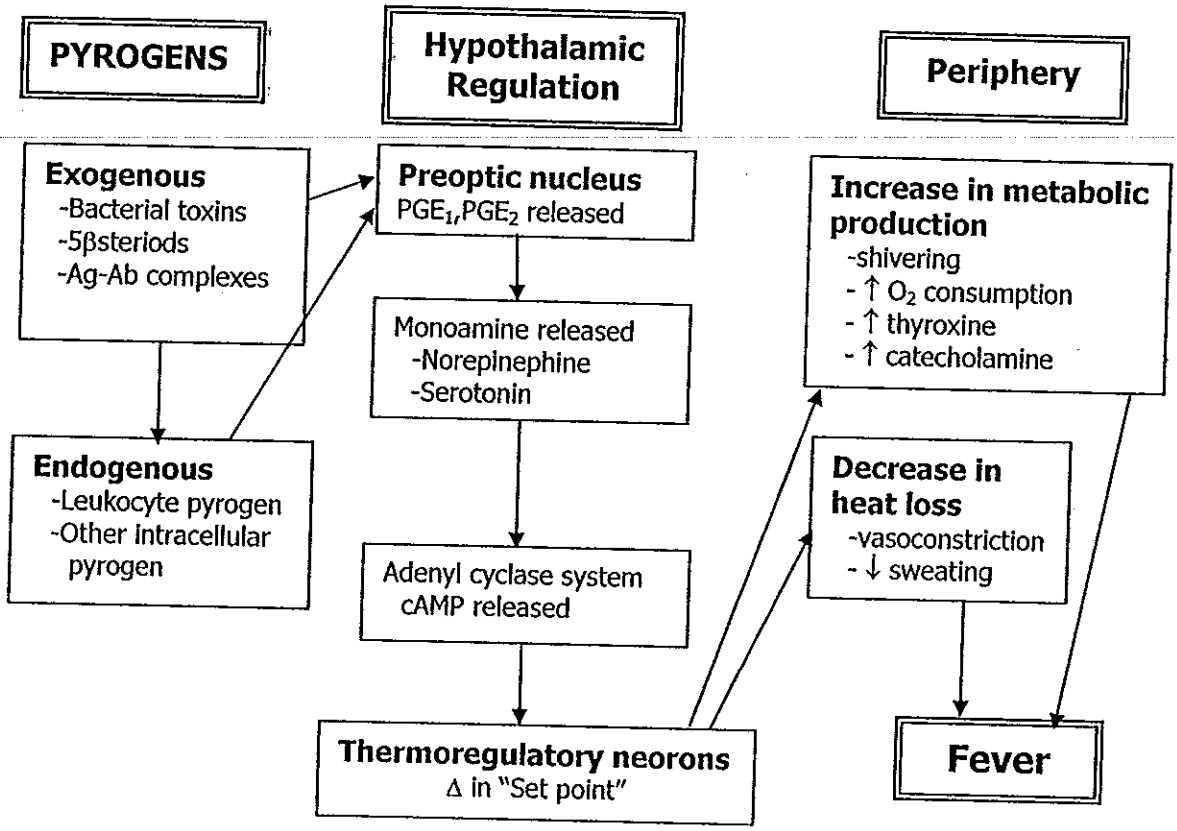


Figure 7. Pathogenesis of fever

IV. Inflammation

Inflammation is part of the complex biological response of vascular tissues to harmful stimuli, such as pathogens, damaged cells, or irritants. (Ferrero-Miliani *et al.*, 2007). It is a protective attempt by the organism to remove the injurious stimuli as well as initiate the healing process for the tissue. Inflammation is not a synonym for infection. Even in cases where inflammation is caused by infection it is incorrect to use the terms as synonyms: infection is caused by an exogenous pathogen, while inflammation is the response of the organism to the pathogen.

➢ Cause of inflammation

Causes include direct damage (cut, 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.

➢ **Acute inflammation**

Acute inflammation is a short-term process, usually appearing within a few minutes or hours and ceasing upon the removal of the injurious stimulus (Cotran *et al.,* 1998). The damage may be purely physical, or it may involve the activation of an immune response (Steven and Lowe, 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, Kupffer 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, *dolor*). 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 (Cotran *et al.,* 1998). The loss of function is probably the result of a neurological reflex in response to pain. In addition to cell-derived mediators, several acellular 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, helping to quickly cease the inflammatory response once the stimulus has been removed.

❖ The classic signs and symptoms of acute inflammation

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

2. Heat (calor): Increase in temperature is seen only in peripheral parts of the body, such as the skin. It is due to increased blood flow (hyperaemia) through the region, resulting in vascular dilatation and the delivery of warm blood to the area. Systemic fever, which results from some of the chemical mediators of inflammation, also contributes to the local temperature.

3. Swelling (tumor): Swelling results from edema, the accumulation of fluid in the extravascular space as part of the fluid exudate and to a much lesser extent, from the physical mass of the inflammatory cells migrating into the area.

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

5. Loss of function: Loss of function is a well-known consequence of inflammation. Movement of an inflamed area is consciously and reflexly inhibited by pain, while severe swelling may physically immobilize the tissue (Macfarlane *et al.*,2000).

❖ Chemical mediators of inflammation

• **Histamine:** It causes vascular dilatation and the immediate transient phase of increased vascular permeability. It is stored in mast cells, basophil and eosinophil leukocytes, and platelets (Pflazer, 1992). Histamine release from these sites (for example, mast cell degranulation) is stimulated by complement components C3a and C5a, and by lysosomal proteins released from neutrophils.

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

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

• **Prostaglandins:** A group of lipids released from mast cells. It is derived by local synthesis from arachidonic acid, which can cause vasodilation, fever, and pain.

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

• **Lymphokines:** This family of chemical messengers released by lymphocytes have a major role in type IV hypersensitivity but may also have vasoactive or chemotactic properties.

❖ **The following chemical mediators were found from plasma**

• **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 follows:

- C5a: chemotactic for neutrophils; increases vascular permeability; releases histamine from mast cells.

- C3a: similar properties to those of C5a, but less active.

-- C345 is chemotactic to neutrophils.

- C4b, 2a, 3b: opsonisation of bacteria (facilitates phagocytosis by macrophages).

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

• **Coagulation system:** It is responsible for the conversion of soluble fibrinogen into fibrin, a major component of the acute inflammatory exudate.

• **Fibrinolytic systems:** Plasmin is responsible for the lysis of fibrin into fibrin degradation products, which may have local effects on vascular permeability.

• **Nitric Oxide (NO):** This inflammatory mediator is released by macrophages. NO has been shown to contribute to swelling, hyperalgesia (heightened reaction to pain) and pain. NO localized in high amounts in inflamed tissues has been shown to induce pain locally and enhances central as well as peripheral stimuli. Inflammatory NO is thought to be synthesized by the inducible isoform of nitric oxide synthase (iNOS).

❖ **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 red blood cells (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 tissue are regenerated or replaced with fibroblasts, collagen or endothelial cell. However, inflammation may become chronic, leading to further tissue destruction and fibrosis (Gould, 2002).

➢ **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 engulf, neutralize or kill foreign antigens. Lymphocytes are the predominant cell in chronic inflammation. There are two types, labeled T and B. T-lymphocytes which are produced in the thymus gland. They ensure cell based immunity from infection. B-lymphocytes originate in the bone marrow and ensure humoral (body fluid) immunity. The activation of B-lymphocytes produces plasma cells, which manufacture and secrete antibodies to fight specific types of antigens. Macrophages and lymphocytes are interdependent in that the activation of one stimulates the actions of the other. Certain chronic infections cells known as eosinophils accumulate. Within their cytoplasm are bright red granules. These granules contain a substance called 'major basic protein' which has the ability to destroy certain antigens. In cases of chronic inflammation involving foreign particulate matter, such as splinters, macrophages cells can fuse together to form multinucleated giant cells. Tuberculosis may also cause macrophage cells to unite in this manner. A key feature of chronic inflammation is collagen production. If too much collagen is formed, this can lead to the condition known as fibrosis. Connective tissue cells known as fibroblasts enter the area of tissue injury and then go to work to produce collagen which is necessary to replace the tissue lost during long term inflammation. The dilated blood vessels which are characteristic of acute inflammation are not evident in cases of chronic inflammation. The two major complications associated with chronic infection are fibrosis leading to scarring and

persistence. The overabundance of collagen production over time can lead to scarring that can cause permanent distortion of the tissue, interfering with it's function. Chronic inflammation can be continually stimulated by substances with low antigenic properties or by auto-immunity. The cellular components of the chronic response are described as followeing:

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

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

• **Lymphocytes** are prominent feature of chronic inflammation reaction and perform vital functions in both humoral and cell-mediated immune response. T lymphocytes not only function in the regulation of macrophage activation and recruitment through the secretion of specific mediators (lymphokines) but also modulate antibody production and cell-mediated cytotoxicity (Rang and Dale, 1991).

• **Eosinophils** are occasionally a conspicuous component of the chronic inflammation response. They are particularly evident during allergic-type reaction and parasitic infestations. Eosinophils share many functional features with the neutrophil. Their rhomboid, crystalloid granules are rich in acid phosphatase and have a specific peroxidase activity. The precise role of eosinophils in chronic inflammatory reactions is less clear (Robert, 2003).

• **Polymorphonuclear leukocytes**, although characteristic of acute inflammation many also be observed at sites of chronic inflammation.

➢ **Pathophysiology of chronic inflammation**

Charracteristics of chronic inflammation include less swelling but the presence of more lymphocytes, and fibroblasts than in acute inflammation and macrophage have been unable to completely clear (debride) the area of foreign substances. This material may be dead cell, 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).

V. Aspirin

Aspirin is in a group of drugs called salicylates. This drug is the prototype of non-steroidal anti-inflammatory drug (NSAIDs). It works by reducing substances in the body that cause pain, fever, and inflammation. Aspirin is used to treat mild to moderate pain, and also to reduce fever or inflammation. It is sometimes used to treat or prevent heart attacks, strokes, and angina. In addition, it is more useful in the treatment of neuralgia, arthralgia and other pain arising from integumental structures than in acute severe pain of visceral origin. However, it may relieve moderate postoperative, postpartum or other visceral pain (Ross and Dehoratius, 1989). If the pain is mild to moderate, aspirin may provide adequate relief and tried prior to use of opioid analgesics. The structure of aspirin is shown in Figure 8.

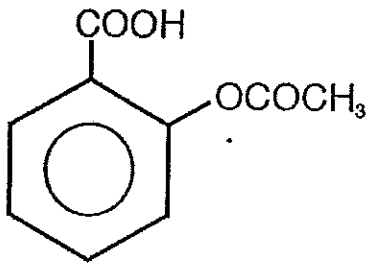


Figure 8. Structure of aspirin

➢ **Pharmacokinetics**

Aspirin is absorbed primary from the small intestine and secondary from stomach. Absorption is rapid following oral administration of conventional tablets or

capsules, but the rate is affected by gastric emptying time and the release characteristics of the dosage form. Absorption is most rapid when aspirin is given in solution. Appreciable concentrations are found in plasma in less than 30 minutes after a single dose and a peak value is reached in about 1 hour. Aspirin is rapidly hydrolyzed to salicylic acid before entering the systemic circulation and central nervous system (CNS) (Ross and Dehoratius, 1989). Hydrolysis by plasma esterase is rapid and cleared by renal extraction. It is conjugated with glycine (forming salicyluric acid) and glucuronic acid (forming salicylphenolic glucuronide and salicylacyl glucoronide). A small fraction of salicylic acid is oxidized to gentisic acid. The enzyme forming salicyuric acid and salicylphenolic glucuronide are saturable and follow Michaelis-Menten kinetics. Therefore, the pharmacokinetics of salicylate elimination are complex, since both the ratio of metabolites and clearance are dose-dependent. Approximately 70% to 90% of salicylic acid is bound to serum albumin and the apparent volume of distribution ranges from 0.1 to 0.35 L/kg, depending on drug concentration. The half-life of salicylate increases with the dose, 3.1 to 3.2 h with 300 to 650 mg, 5 h with 1 g and 9 h with 2 g. As the dose and half-life increase, a larger portion is excreted unchanged (Jonh and Bonald, 1993).

- **Pharmacodynamics**

The anti-inflammatory activity of the NSAIDs is similar in mechanism to that of aspirin which is mediated chiefly through inhibition of biosynthesis of prostaglandins. Inflammation is reduced 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.

Aspirin acts peripherally through its effect on inflammation but probably also depress pain stimuli at a subcortical site (Donald and Payan, 1992). The primary clinical effects of aspirin appear to be related to inhibit cyclooxygenase (prostaglandin synthesis) (Jonh and Bonald, 1993), since the action of the prostaglandins have been reported to include hyperalgesia (pain), fever, edema (inflammation) and erythema. They do not inhibit 5- lipoxygenase and therefore, do not affect the formation of leukotrienes (Mutschler and Derendorf, 1995) (Figure 9).

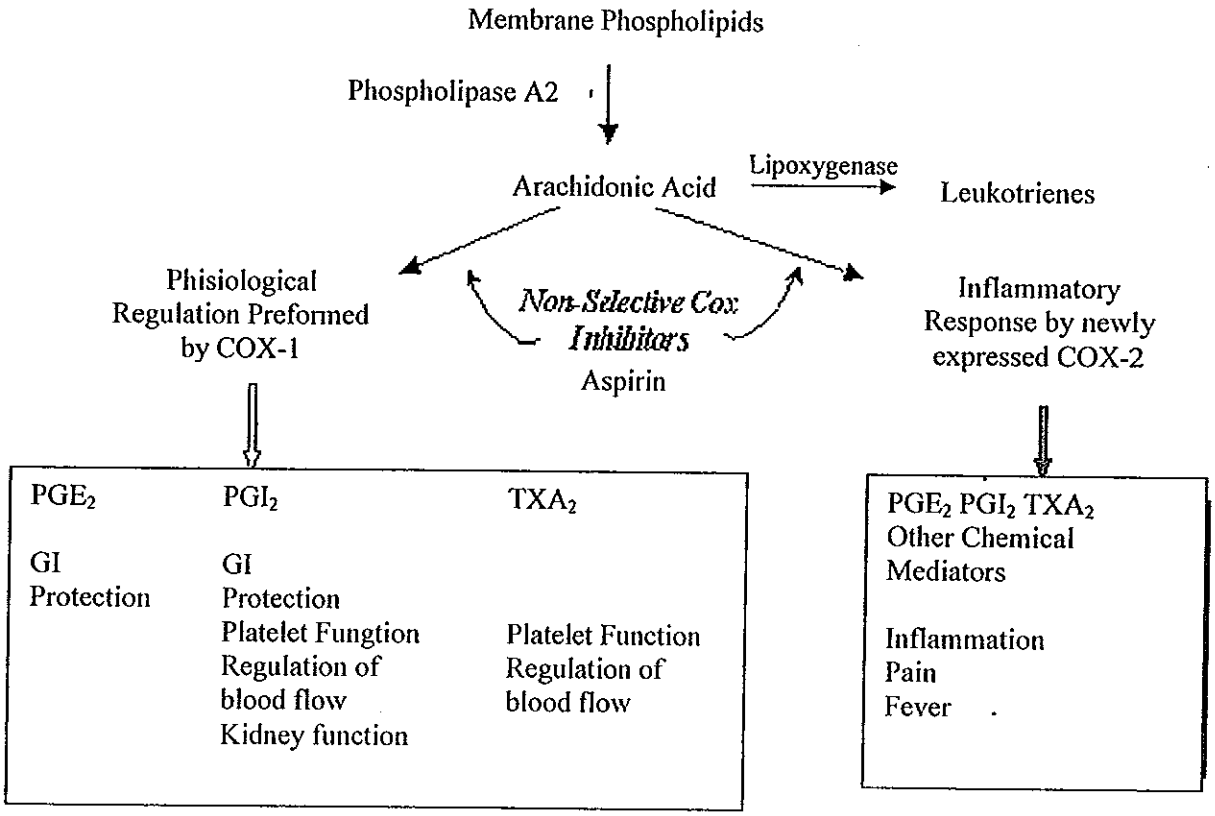


Figure 9. Mechanism of aspirin to inhibit cyclooxygenase (Vander, 2001)

➢ **Indication**

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

➢ **Adverse effects**

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

• **Hepatic:** Aspirin administered regularly in dose greater than 50 mg/kg can produce mild and reversible hepatic damage. This is usually manifested as an increase in aminotransferase values but biopsies reveal focal hepatocellular necrosis, hepatocytic swelling, intracellular and extracellular acidophilic bodies and portal inflammation. A small number of patient experience more severe hepatic damage with jaundice, prolonged prothrombin time with bleeding or intravascular coagulation. Aspirin also may precipitate hepatic encephalopathy in patients with chronic liver disease. The hepatotoxicity generally occurs only after several months of treatment and appears as cholestatic jaundice with markedly elevated values in hepatic function tests and histologic evidence of necrosis, portal infiltrates and cholestasis (Foegh and Ramwell, 2001).

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

• **Hematologic:** Aspirin exerts a pronounced inhibitory platelet aggregation. This effect is more prominent in patients with inborn disorders of platelet

function in Von Willebrand or Bernard-Soulier disease and in patients receiving heparin or oral anticoagulants. For most NSAIDs inhibited platelet aggregation is reversible, but inhibition by aspirin is irreversible (Schuna and Coulter, 1993).

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

➢ **Drug interactions**

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.

VI. Morphine

Morphine is the prototype opioid agonist to which all other opioids are compared. In human, morphine produces analgesia, euphoria, sedation and diminished ability to concentrate. Other sensations include nausea, a feeling of the body warmth, heaviness of the extremities, dryness of the mouth and especially in the cutaneous areas around the nose. If the cause of pain persists, but even low doses of morphine increase the threshold to pain and modify the perception of noxious stimulation so that it is no longer experienced as pain. In the absence of pain, however, morphine may produce dysphoria rather than euphoria (Stoelting and Hillier, 2006). The structure of morphine is shown in Figure 10.

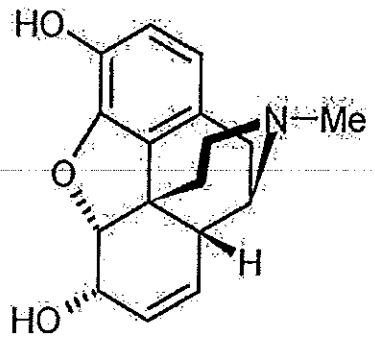


Figure 10. Structure of morphine

➢ **Pharmacokinetics**

Absorption of morphine from the gastrointestinal tract is relative slow. The mean T_{max} is 3.7 h and mean C_{max} is 9.9 to 27.4 ng/mL (dose dependent) for sustained-release form. Bioavailability is approximately 40%, distributes to skeletal muscle, kidneys, liver, intestinal tract, lungs, spleen, and brain; crosses the placental membrane and found in breast milk. Metabolism of virtually all converted into glucuronide metabolites; small fraction is demethylated in the liver. Major metabolite is morphine-3-glucuronide (55% to 75%) and the $t_{1/2}$ is approximately 2 to 4 h. The response to morphine may be enhanced in patients with uremia, various renal disorders and renal ischemia. This may be due to accumulation of an active metabolite, morphine-6-glucuronide that is elimanated by kidney (Jaffe and Martin, 1990). The mean elimination half-life and clearance of the parent drug are similar in patients with renal failure and in normal subjects.

➢ **Pharmacodynamics**

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

kappa (κ) 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 common opioid, and 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 to the μ-receptor within the brain but also mediates analgesia, respiatory 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 receptors are composed of glycoprotines found in cellular membranes. These receptors are coupled to G-protein that modulate potassium and calcium ion conduction. When opioid agonists occupy either μ or δ receptor, they open the potassium ion channel that permits an increase in potassium conductance. The hyperpolarlization inhibits neuronal activity. In contrast, κ receptor activation inhibits calcium entry via a calcium ion channel. Activation of the opioid receptors decreases transmission of signals from the primary peripheral afferent nerves to higher CNS centers, as well as the processing of the pain stimulus (Lipman and Jackson, 2004).

➢ **Indication**

• **Acute pain:** Morphine is indicated for relief of moderate to severe acute pain. When administered systemically, it alters the psychological response to pain as well as the nociceptive sensation. It acts on higher centers of the CNS to produce analgesia without loss of consciousness. Morphine is used for short-term therapy for severe, acute pain (trauma, burns, surgery) (Bonica, 1980).

• **Chronic pain:** The treatment of chronic pain of nonmalignant origin depends on its cause and severity. The selection of opioid should be based on the severity of the pain and patient's response. Usually, therapy is initiated with an opioid such as codeine plus a salicylate or acetaminophen. The morphine preparation should not be used primarily to relieve anxiety or depression. Neuropatic pain may follow injury to peripheral nerves, spinal cord, soft tissue or visceral structure. The injury may be caused by compression, surgery, viral infection or neoplastic disease (Meed *et al.*, 1987). Neuropatic pain is typically a dysesthesia (abnormal burning, shooting pain) that is less responsive to opioid analgesics than other types of pain. Nonopioid drugs, including selected antidepressants and anticonvulsants, may be useful.

➢ **Adverse effects**

• **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 cause the cutaneous blood vessels of the face, neck and upper chest to dilate.

• **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 μ_2 receptors, which lead to direct depressant effect on brainstem ventilation centers.

➢ **Drug interactions**

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).

VII. Naloxone

The pure opioid antagonist drug, naloxone is a morphine derivative with bulkier substituents at the N- position. One structure change that converts a narcotic agonist to an antagonist is alkylation of the piperidine nitrogen. For example, when the methyl group on the piperidine nitrogen of morphine is replaced by the unbranched three-carbon side chain (such as propyl, allyl or isopropyl), the compound becomes a narcotic antagonist. Naloxone has a relatively high affinity for opioid binding sites of the μ- receptor type. Their affinity for the other receptors such as κ- and δ-opioid receptors is found in both supraspinal and spinal sites (Colasanti, 1990, Smith, 1995). The structure of naloxone is shown in Figure 11.

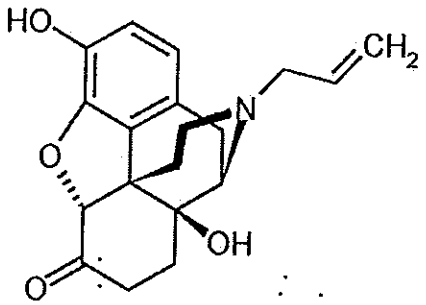


Figure 11. Structure of naloxone (Colasanti, 1990)

➢ **Pharmacokinetics**

Naloxone is only minimally absorbed when given orally as it is rapidly destroyed in the GI tract. Much higher doses are required if using this route of administration for any pharmacologic effect. When given IV, naloxone has a very rapid onset of action (usually 1-2 minutes). If given IM, the drug generally has an onset of action within 5 minutes of administration. The duration of action usually persists from 45-90 minutes, but may act for up to 3 hours.

Naloxone is distributed rapidly throughout the body with high levels found in the brain, kidneys, spleen, skeletal muscle, lung and heart. The drug also readily crosses the placenta.

Naloxone is metabolized in the liver, principally via glucuronide conjugation and excreted into the urine. In humans, the plasma half-life is approximately 60-100 minutes (Rang *et al.,* 1999).

- **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 these 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. The affinity of the antagonist for the μ receptor is 10-20 fold greater than for the κ and δ receptors sites (Colasanti, 1990).

- **Indication**

Naloxone is the drug of choice for narcotic overdose. It is very important that naloxone has short duration of action. A severely depressed patient may recover after a single dose of naloxone and appear normal, only to relapse into coma after 1-2 hours. The usual dose of naloxone is 0.1-0.4 mg intravenously, which can be repeated as necessary (Way and Way, 1998).

- **Adverse effect**

This agent was available for use as an antagonist. So there are few adverse effects associated with its use. The abrupt reversal of an opioid by naloxone may produce symptoms of acute withdrawal such as agitation, nausea and vomiting, diarrhea, diaphoresis, tachycardia, hypertension, shivering, tremors, or seizures in patients with physiologic dependence, including neonates whose mothers were chronic opioid users. In postoperative patients, particularly those with underlying cardiac disease, the administration of naloxone has been associated with changes in blood pressure, ventricular tachycardia or fibrillation and pulmonary edema. (Milanes *et al.*, 2001).

CHAPTER 3

MATERIALS AND METHODS

Materials

1. Plant material

Fresh leaves of *Garcinia cowa* (*G. cowa*) Roxb. were collected in July, 2010 from Singhanakon District, Songkhla Province, Thailand. The taxonomical identification of this plant was made by Associate Professor Chuotip Purintaworakul, Botany setion, Department of Biology, Prince of Songkla University, Thailand. The 25 kg of fresh leaves were cleaned and air-dried at room temperature for 2 days. The dried leaves were pulverized by an electric blender to give 5 kg of coarsely powder and stored in airtight containers.

2. Extraction procedure

The coarsely powder of *G. cowa* leaves (5 kg) was macerated with 22 L of ethanol (95%) for 7 days at room temperature. This extraction process was repeated 2 times and the combined extracts were filtered through Whatman No.1. The solvent was then evaporated by rotary evaporator at 40-60 °C to give oil-like green extract (EEGC) (yield 21.4% w/w) which was stored in a closed bottle and kept in a refrigerator at temperature below 4 °C until testing. The extract at different concentrations were prepared by dissolving in cosolvent (Propylene glycol : Tween 80 : Distilled water at the ratio of 4:1:5, respectively).

3. Animals

Male ICR mice, weighing 29-47 g, were used for analgesic and acute toxicity testing (writhing, formalin, tail flick and hot plate tests). Male Wistar rats weighing 159 – 240 g were used for testing of antipyretic and anti- inflammatory activities and acute toxicity. The animals were obtained from the Southern Labolatory Animal Facility, Prince of Songkla University, Hatyai, Songkla, Thailand. They were kept in the room maintained under the condition of 25 ± 1 °C and 12 h light/12 h dark cycles, free access to water and standard diets. The animal

Ethics Committees, Prince of Songkla University approved all experimental protocols (MOE. 0521.11/694).

4. Chemicals and Instruments

The drugs used in this study included morphine sulfate (Sigma), naloxone hydrochloride injection USP (400 µg/ml) (Troikaa), brewer's yeast (Sigma), carrageenan (Sigma), aspirin (Sigma), urethane (Sigma), propylene glycol (Vidhyasom Co., LTD), tween 80 (Srichand Co., LTD), acetic acid A.R. grade (JT Baker) and 40% formalin A.R. grade and ethanol A.R. grade.

The instrument used included the tail flick apparatus (Socrel model DS20, Ugo basile, Milan, Itary), digital vernier caliper (S2 instrument Co.,Ltd), hot plate (Harvard Apparatus Ltd., UK) and digital thermometer (YSI 4600 Precision Thermometer Yellow Springs, Ohio 45387 USA).

Methods

1. Acute toxicity

The up-and-down method (Bruce, 1985) was used to study the acute toxicity. In the procedure, three mice and rats were used, the animal is dosed once. If animal survives, the dose for the next experiment is increased, if it dies the dose was decreased. The dose was adjusted by a constant multiplicative factor, viz. 1.5 folds, for the experiment. The ethanol extract of *G. cowa* at the dose of 5 g/kg was orally administered to male mice and rats. Behavior parameters were observed every hour for 8 h and once a day for 7 days after administration such as convulsion, hyperactivity, sedation, loss of righting reflex, increased or decreased respiration. Food and water was given *ad libitum.*

2. Anagesic activity

2.1 Writhing test

The method was described by Koster *et al.* (1959). Male ICR mice weighing 32-47 g were divided into 5 groups (each group consisted of 6 mice)

Group 1 : Control (cosolvent 10 ml/kg, p.o.)
Group 2 : Aspirin (200 mg/kg, p.o.)
Group 3 : EEGC (100 mg/kg, p.o.)
Group 4 : EEGC (200 mg/kg, p.o.)
Group 5 : EEGC (300 mg/kg, p.o.)

After 30 minutes, 0.6% acetic acid (v/v) in normal saline (10 ml/kg body weight) was injected intraperitoneally and the number of writhing and stretching was counted over a period of 0-20 minutes. The schematic plan of the writhing test was illustrated in Figure 12.

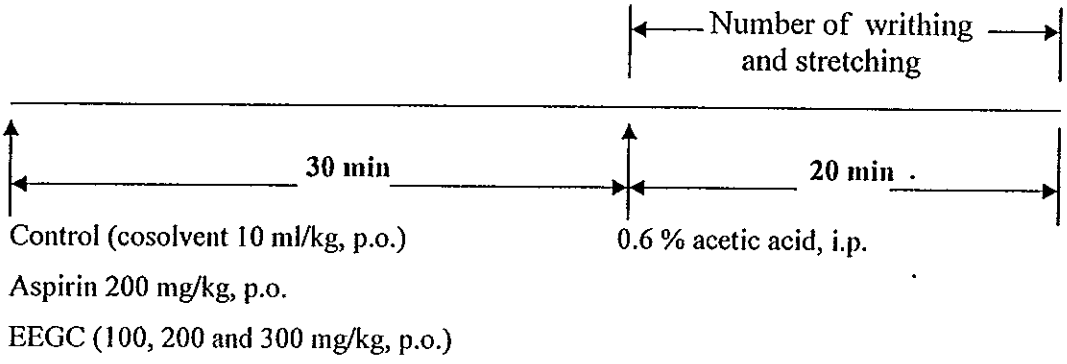


Figure 12. Schematic plan of the writhing test

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

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

2.2 Formalin test

The experiment was done according to the previous described method by Hunskaar *et al.* (1985). Male ICR mice weighing 31-43 g were divided into 6 groups.

Group 1 : Control (cosolvent 10 ml/kg, p.o.)

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

Group 3 : Morphine sulphate (5 mg/kg, s.c.)

Group 4 : EEGC (100 mg/kg, p.o.)

Group 5 : EEGC (200 mg/kg, p.o.)

Group 6 : EEGC (300 mg/kg, p.o.)

Thirty minutes after oral administration of the extract (100, 200 and 300 mg/kg), aspirin (200 mg/kg) or cosolvent except morphine (15 min after subcutaneously administration), 20 µl of 2.5% formalin in saline was injected subcutaneously to a hind paw of the mice. Morphine sulfate was injected subcutaneously. The time spent licking the injected paw was recorded and the data were expressed as total licking time in the early phase (0-5 min) and the late phase (15-30 min) after formalin injection. The schematic plan of the hot plate test was illustrated in Figure 13.

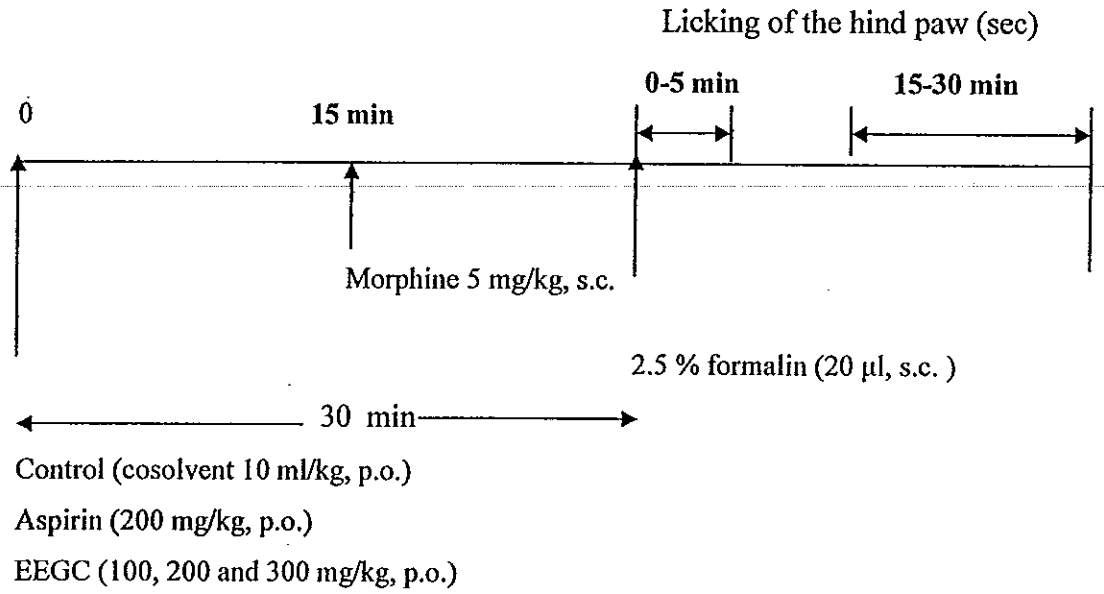


Figure 13. Schematic plan of the formalin test

2.3 Tail flick test

The tail flick test used in this experiment was described by D' Amour and Smith (1941). Male Wistar rats weighting 169-240 g were divided into 8 groups (each group consisted of 6 rats).

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

Group 2 : Morphine sulphate (5 mg/kg, s.c.)

Group 3 : Naloxone (2 mg/kg, i.p.)

Group 4 : EEGC (100 mg/kg, p.o.)

Group 5 : EEGC (200 mg/kg, p.o.)

Group 6 : EEGC (300 mg/kg, p.o.)

Group 7 : Naloxone (2 mg/kg, i.p.) + Morphine sulphate (5 mg/kg, s.c.)

Group 8 : Naloxone (2 mg/kg, i.p.) + EEGC (300 mg/kg, p.o.)

After 30 min (15 min for morphine and 10 min for naloxone), rats were placed on the tail flick apparatus, and the tail flick responses were measured by gently placing the rats tail on a center position of light beam. The reaction time measured by focusing an intensity controlled beam of light on the distal about 2 centimeters from tip. The time taken by the animal to withdraw (flick) its tail from heat induced by the

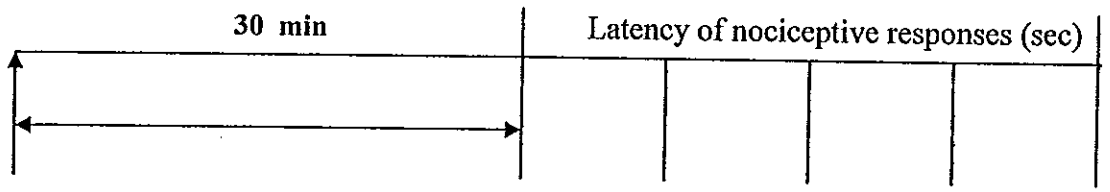
light beam was recorded as the reaction time. Only the rats that showed nociceptive responses within 5 seconds were used for the experiments. A cut-off time was 10 seconds to prevent any injury to the rats tail. The nocicepive responses was measured at 30, 45, 60, 75, and 90 min after administration. The schematic plan of the tail flick test was illustrated in Figure 14.

2.4 Hot plate test

The hot plate test was carried out according to the method described by Woolfe and MacDonald (1994). Male ICR mice weighting 29-45 g were divided into 8 groups (each group consisted of 6 mice)

Group 1 : Control (cosolvent 10 ml/kg, p.o.)
Group 2 : Morphine sulphate (5 mg/kg, s.c.)
Group 3 : Naloxone (2 mg/kg, i.p.)
Group 4 : EEGC (100 mg/kg, p.o.)
Group 5 : EEGC (200 mg/kg, p.o.)
Group 6 : EEGC (300 mg/kg, p.o.)
Group 7 : Naloxone (2 mg/kg, i.p.) + Morphine sulphate (5 mg/kg, s.c.)
Group 8 : Naloxone (2 mg/kg, i.p.) + EEGC (300 mg/kg, p.o.)

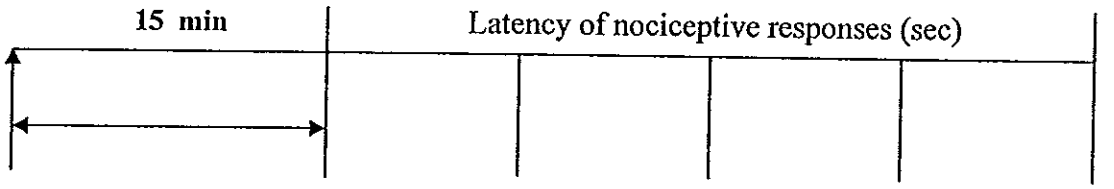
After 30 min (15 min for morphine and 10 min for naloxone), mice were placed on a hot plate maintained the temperature at 55 °C ± 1 °C. Latency of nociceptive responses such as licking of the hind limb or jumping was recorded at 30, 45, 60, 75, and 90 min after administration. The cut-off time of observation was 45 seconds. Only the mouse showed nociceptive responses within 15 seconds was used in the experiments. The schematic plan of the hot plate was illustrated in Figure 14.



Control (cosolvent 10 ml/kg, p.o.) 0 15 30 45 60

min

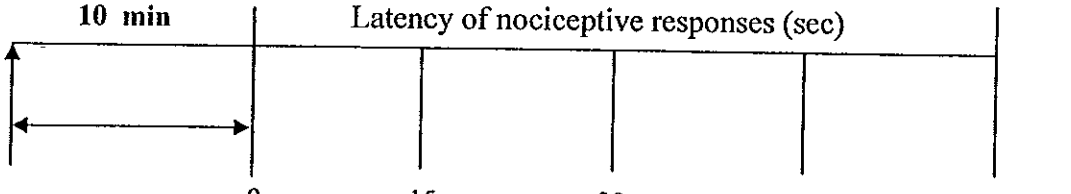
EEGC (100, 200 and 300 mg/kg, p.o.)



Morphine 0 15 30 45 60

min

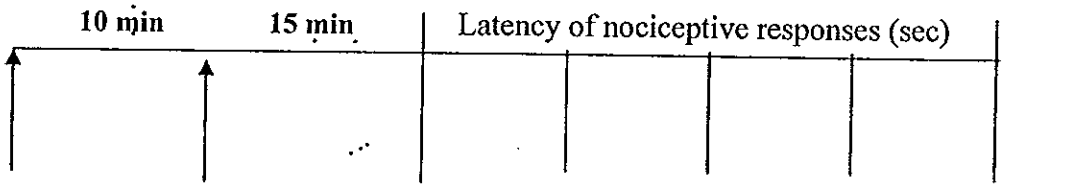
(5 mg/kg, s.c.)



0 15 30 45 60 min

Naloxone

(2 mg/kg, i.p.)



Naloxone Morphine 0 15 30 45 60 min

(2 mg/kg, i.p.) (5 mg/kg, s.c.)

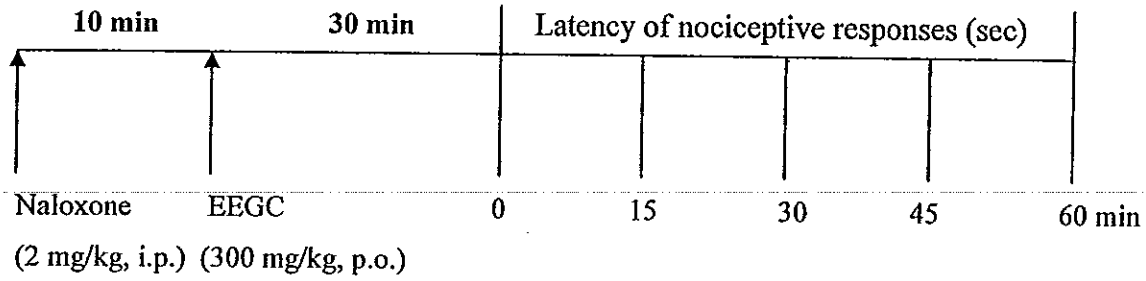


Figure 14. Schematic plan of the tail flick test and hot plate test

3. Antipyretic activity

Antipyretic activity was tested using the slightly modifying method described by Adam *et al.* (1968). The animals were fasted overnight with water *ad libitum* before experiments. Pyrexia was induced by subcutaneously injecting 20% (w/v) brewer's yeast suspension (10 ml/kg) into animals dorsal region. Eighteen hours after injection, the rectal temperature of each rat was measured using a digital thermometer. The probe was attached to a digital display and was inserted 2 cm into rectum. Only rats that showed an increase in temperature of at least 0.7 ° C was used for the experiments, an initial rectal temperature was recorded. Male Wistar rats, weighing 207-237 g, were divided into 5 groups (each group consisted of 6 rats).

Group 1 : Control (cosolvent 5 ml/kg, p.o.)
Group 2 : Aspirin (200 mg/kg, p.o.)
Group 3 : EEGC (100 mg/kg, p.o.)
Group 4 : EEGC (200 mg/kg, p.o.)
Group 5 : EEGC (300 mg/kg, p.o.)

The rectal temperature was measured at 1, 2, 3, 4 and 5 h after agent administration. The schematic plan of antipyretic study was shown in Figure 15.

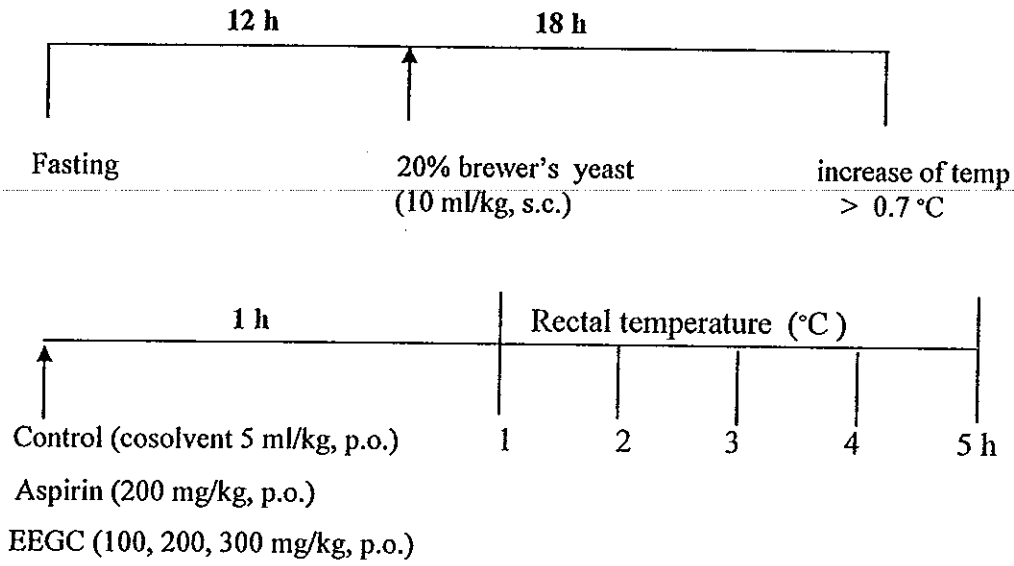


Figure 15. Schematic plan of the brewer's yeast-induced pyrexia test

4. Anti-inflammatory activity

4.1 Acute inflammation (carrageenan – induced paw edema test)

The anti-inflammatory activity was studied according to the method described by Winter *et al.* (1962). The initial right hind paw thickness of the rats was measured and recroded using digital vernier callipers. Male Wistar rats weighing 203-231 g were divided into 5 groups (each group consisted of 6 rats).

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

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

Group 3 : EEGC (100 mg/kg, p.o.)

Group 4 : EEGC (200 mg/kg, p.o.)

Group 5 : EEGC (300 mg/kg, p.o.)

After 30 min, 0.1 ml of 1% (w/v) carageenan in normal saline was subcutaneously injected into the subplantar region of the right hind paw. The thickness of the right hind paw was measured at 0.5, 1, 2, 3, 4 and 5 hour after carrageenan injection.

The data were expressed as percentage of swelling compared with the initial hind paw thickness of each rat. The schematic plan of the carrageenan- induced paw edema test was illustrated in Figure 16.

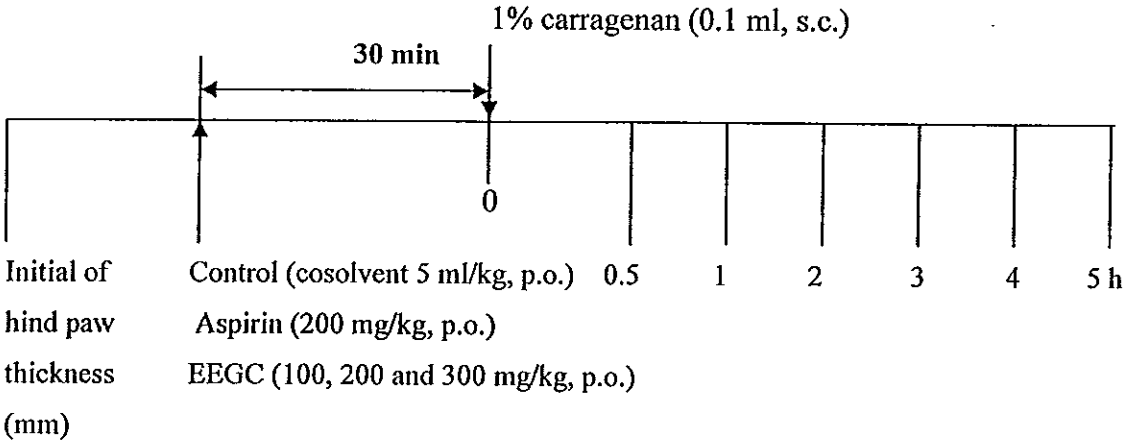


Figure 16. Schematic plan of the carrageenan- induced paw edema test

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

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

A = Mean hind paw thickness of the control group

B = Mean hind paw thickness of the treated group

4.2 Chronic inflammation (cotton pellet – induced granuloma test)

The cotton pellet-induced chronic inflammation and anti-inflammatory activity were evaluated by slightly modifying the method described by Swingle and Shideman (1972). Male Wistar rats weighing 159-227 g were divided into 5 groups (each group consisted of 6 rats).

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

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

Group 3 : EEGC (100 mg/kg, p.o.)

Group 4 : EEGC (200 mg/kg, p.o.)

Group 5 : EEGC (300 mg/kg, p.o.)

Subcutaneous implantation of pellets of compressed cotton provokes foreign body granuloma. Cotton rolls were cut and made into pellets weighting 20 ± 1 mg each and sterilized in an hot air oven at 160 °C for 2 h. The animals were received anesthetized (Urethane 1.5 g/kg) each cotton pellet was inserted into both side of groin regions by making small subcutaneous incisions. The incisions were sutured by sterile catgut and all processes were done under the sterile condition. After recovery from anesthesia, the animals were treated with cosolvent, aspirin and EEGC, orally for 7 days. On the eight day, animals were sacrificed and the cotton pellet granulomas were carefully removed from extraneous tissues and their wet weight were determined, and then dried the cotton pellet by hot air oven at 60°C for 18 h and weighted to obtain a constant weight. The average weight of the pellets of the control group as well as of the test group was calculated. The percent change of granuloma weight relative to control group was determined.

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

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

A = Mean of granuloma weight of the control group

B = Mean of granuloma weight of the treated group

Statisical analysis

The data were analysed using SPSS softwere program version 11.5 and result were expressed as mean $\pm$ S.E.M. Statistical significance between treatment groups were compared to control, accept for antipyretic test which compare to initial value of each group, using one- way ANOVA followed by student's t- test. *P*-values less than 0.05 ($p< 0.05$) were used as the significant level.

CHAPTER 4

RESULTS

1. Acute toxicity

The ethanol extract of *G. cowa* leaves (EEGC) at the dose of 5 g/kg given orally to 3 male mice and rats did not have any effect on their behavioral responses. No abnormal symptoms or mortality were observed. Therefore, the estimated LD_{50} in mice and rats was more than 5 g/kg when administered orally. Thus, the EEGC at the doses of 100, 200, and 300 mg/kg were used in this study.

2. Analgesic activity

2.1 Writhing test

The EEGC (200 and 300 mg/kg, p.o.) and aspirin (200 mg/kg, p.o.) significantly decreased ($p < 0.05$) the number of writhing induced by 0.6% acetic acid when compared with control (29.50±1.98 vs 50.17 ± 3.91; 33.83±3.80 vs 50.17 ± 3.91 and 16.83 ± 1.25 vs 50.17 ± 3.91, respectively) with the percentage of inhibition of 32.57%, 41.20% and 66.45 %, respectively. While the EEGC at the dose of 100 mg/kg, p.o. did not significantly suppress writhing induced by 0.6% acetic acid in mice. (Table 2 and Figure 17).

Table 2. Effect of aspirin and an ethanol extract of *G. cowa* leaves (EEGC) on the number of writhing induced by 0.6% acetic acid in mice.

Treatment	Dose (mg/kg, p.o.)	Number of writhing	Inhibition (%)
Control (cosolvent)	10 ml/kg	50.17±3.91	0
Aspirin	200	16.83±1.25*	66.45
EEGC	100	47.33±3.05	5.66
EEGC	200	33.83±3.80*	32.57
EEGC	300	29.50±1.98*	41.20

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

*$p < 0.05$, significantly different compared with the control (cosolvent group)

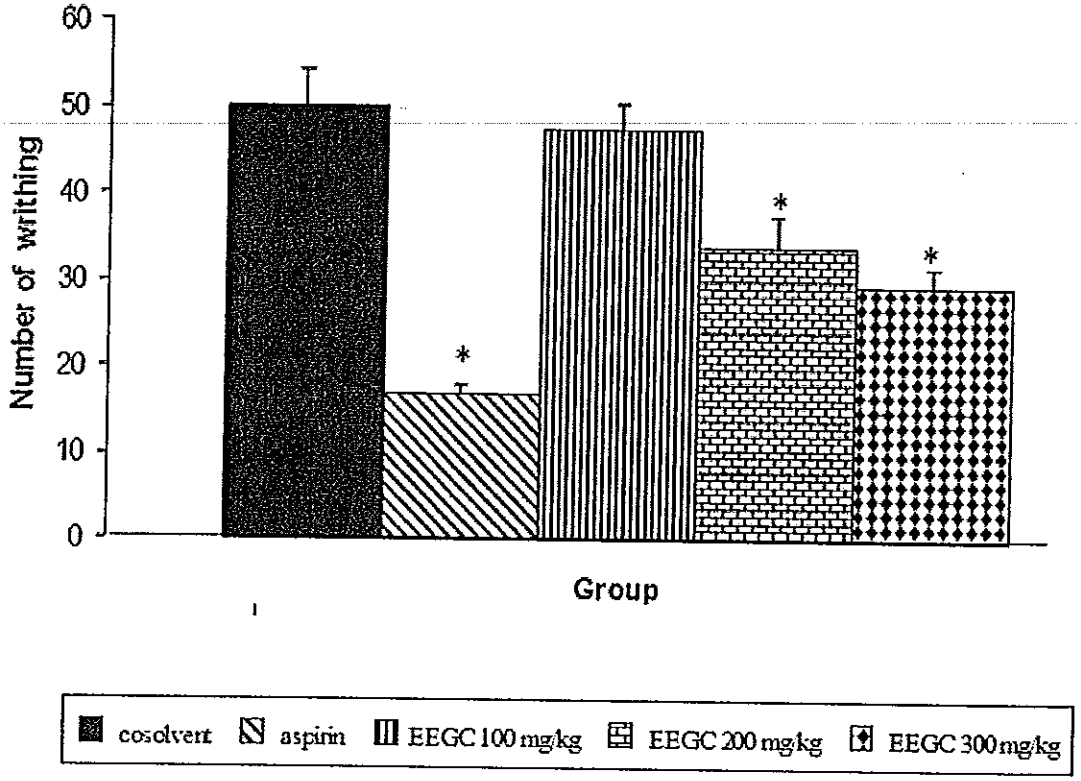


Figure 17. Effect of aspirin and an ethanol extract of *G. cowa* leaves (EEGC) on the number of writhing induced by 0.6% acetic acid in mice.
* $p < 0.05$, significantly different compared to the control group.

2.2 Formalin test

When the reference drug morphine (5 mg/kg, s.c.) and aspirin (200 mg/kg, p.o.) were given before injection of 20 µl of 2.5% formalin for 15 minutes (morphine) and 30 minutes (aspirin), the licking time in early phase (0-5 minutes) significantly decreased by 81.07% (32.02±1.31 vs 169.16±1.60, $p<0.05$) and 76.51% (39.73±0.83 vs 169.16±1.60, $p<0.05$), respectively when compared with the control group. In early phase (0-5 minutes), the EEGC at the doses of 200 and 300 mg/kg, p.o. significantly decreased the licking time (sec) of 23.53 % (129.39±0.85 vs 169.16±1.60, $p<0.05$) and 40.62 % (100.44±0.99 vs 169.16±1.60, $p<0.05$), respectively when compared with control. In late phase (15-30 minutes), morphine (5 mg/kg, s.c.) and aspirin (200 mg/kg, p.o.) significantly reduced the licking time induced by 2.5% formalin by 84.07% (27.73±1.11 vs 174.03±1.27, $p<0.05$) and 73.17% (46.70±1.47 vs 174.03±1.27, $p<0.05$), respectively when compared with control whereas the EEGC at the doses of 200 and 300 mg/kg, p.o. significantly decreased the licking time by 17.39% (143.78±0.75 vs 174.03±1.27, $p<0.05$) and 33.44% (115.83±0.96 vs 174.03±1.27, $p<0.05$), respectively. The results (Table 3 and Figure 18) showed that the time spent in paw licking after administration of the standard drug morphine (5 mg/kg, s.c.), aspirin (200 mg/kg, p.o.) and EEGC (200 and 300 mg/kg, p.o.) before the injection of 20 µl of 2.5% formalin at the right hind paw of the mice, significantly decreased both in the early phase and late phase when compared with control ($p<0.05$) whereas the EEGC (100 mg/kg, p.o.) did not significantly reduce licking activity.

Table 3. Effect of aspirin, morphine and an ethanol extract of *G. cowa* leaves (EEGC) on licking activity induced by 2.5% formalin in mice.

Treatment	Dose (mg/kg, p.o.)	Licking of the hind paw (sec)			
		Early phase (0-5 min)	Inhibition (%)	Late phase (15-30 min)	Inhibition (%)
Control	10 ml/kg	169.16±1.60	0	174.03±1.27	0
Morphine	5, s.c.	32.02±1.31*	81.07	27.73±1.11*	84.07
Aspirin	200	39.73±0.83*	76.51	46.70±1.47*	73.17
EEGC	100	164.61±1.51	2.69	171.74±0.56	1.32
EEGC	200	129.39±0.85*	23.53	143.78±0.75*	17.39
EEGC	300	100.44±0.99*	40.62	115.83±0.96*	33.44

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

*$p < 0.05$, significantly different compared with the control (cosolvent group)

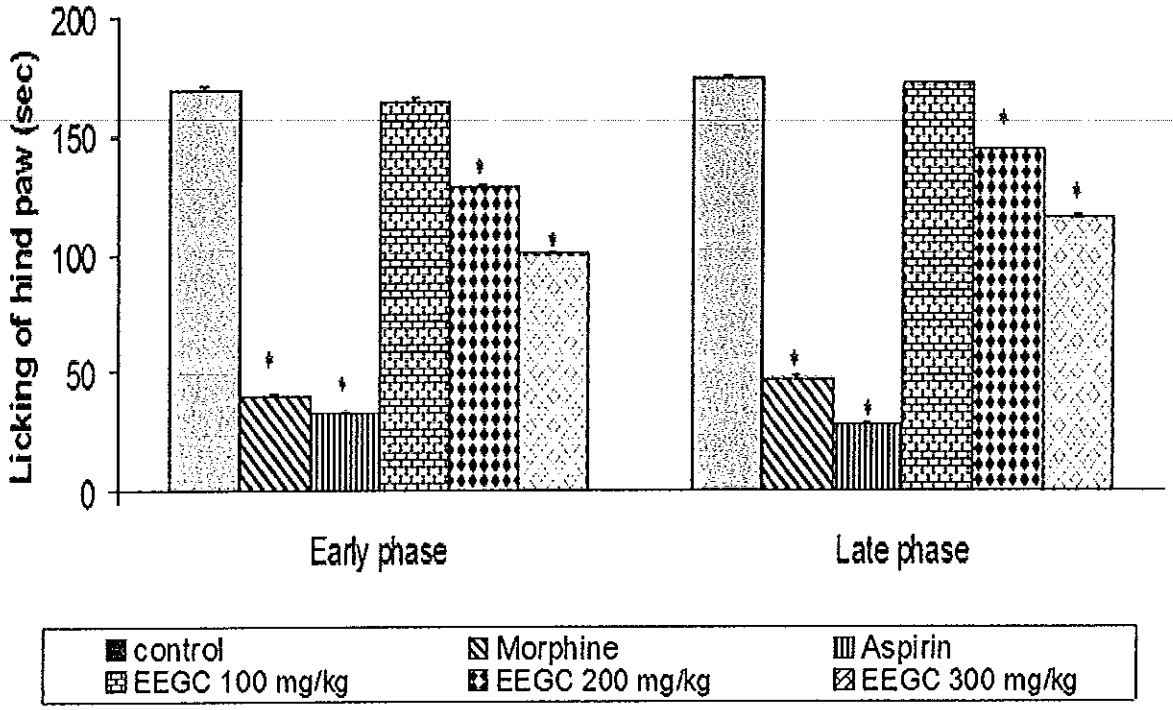


Figure 18. Effect of aspirin, morphine and an ethanol extract of *G. cowa* leaves (EEGC) on nociceptive response induced by 2.5% formalin in mice.
* $p < 0.05$, significantly different compared to the control group.

2.3 Tail flick test

The EEGC at the dose of 300 mg/kg, p.o. and morphine at the dose of 5 mg/kg, s.c. significantly increased the reaction time to the nociceptive responses at all the time intervals measured at 30, 45, 60, 75 and 90 minutes after administration. However, the EEGC at the doses of 100 and 200 mg/kg, p.o. did not show the antinociceptive activity in tail flick test. The results were shown in Table 4 and Figure 19.

In antagonistic study, naloxone (2 mg/kg, i.p.) given before morphine and the EEGC (300 mg/kg) could antagonize the analgesic effects of morphine on the latency of nociceptive responses at all time interval measured when compared with morphine alone and antagonize the analgesic effects of EEGC (300 mg/kg) only during 60-90 min when compared with EEGC (300 mg/kg) alone. The results were shown in the Table 5 and Figure 20.

Table 4. Effect of morphine and an ethanol extract of *G. cowa* leaves (EEGC) on nociceptive responses in the tail flick test in rats.

Treatment	Dose (mg/kg, p.o.)	Latency of nociceptive response (sec)				
		30 min	45 min	60 min	75 min	90 min
Control (cosolvent)	5 ml/kg	2.67±0.16	2.67±0.16	2.67±0.12	2.68±0.09	2.63±0.12
Morphine	5, s.c.	10±0.00*	9.77±0.17*	9.73±0.10*	9.40±0.20*	9.23±0.13*
EEGC	100	3.03±0.09	3.03±0.08	2.97±0.05	2.88±0.06	2.78±0.06
EEGC	200	3.05±0.08	3.03±0.04	2.90±0.10	2.88±0.04	2.82±0.10
EEGC	300	4.12±0.11*	4.27±0.18*	5.07±0.11*	4.95±0.12*	4.88±0.12*

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

*$p < 0.05$, significantly different compared with the control (cosolvent group)

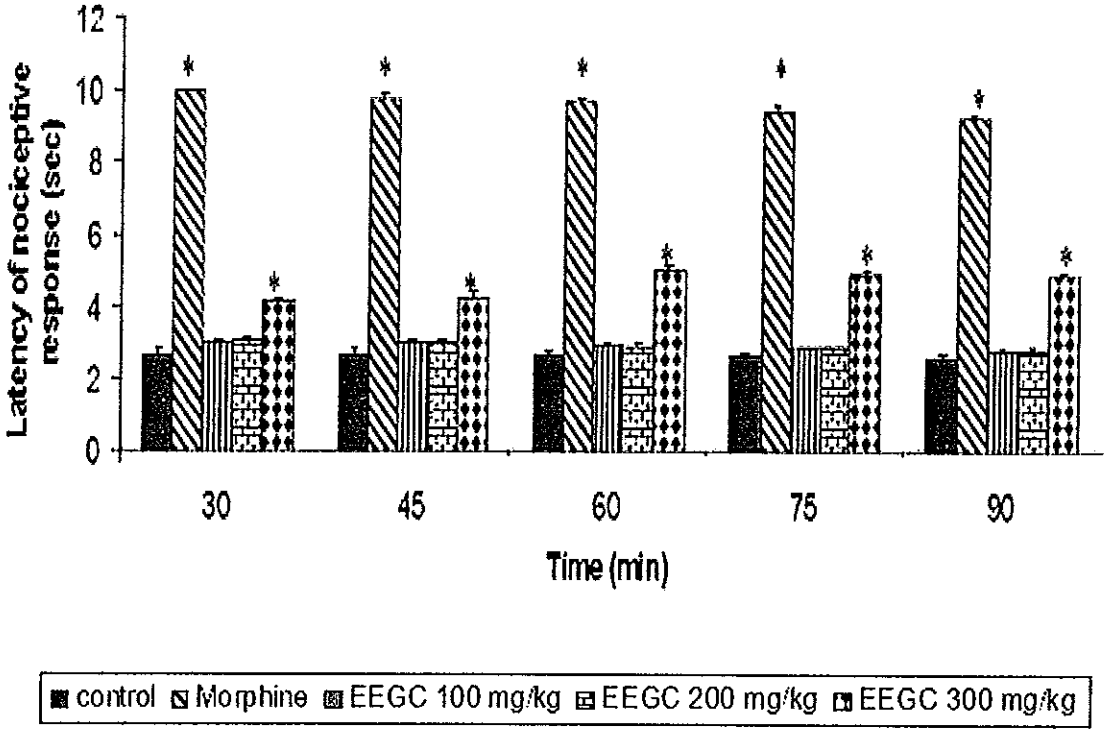


Figure 19. Effect of morphine and an ethanol extract of *G. cowa* leaves (EEGC) on nociceptive responses in the tail flick test in rat.

* $p < 0.05$, significantly different compared to the control group.

Table 5. Antagonistic effects of naloxone on the action of morphine and ethanol extract of *G.cowa* leaves (EEGC) in the tail flick test in rats.

Treatment	Dose (mg/kg, p.o.)	Latency of nociceptive response (sec)				
		30 min	45 min	60 min	75 min	90 min
Morphine	5, s.c.	10±0.00	9.77±0.17	9.73±0.10	9.40±0.20	9.23±0.13
Naloxone	2, i.p.	3.20±0.19	3.10±0.12	3.03±0.12	2.98±0.10	2.85±0.10
Naloxone+ Morphine	2, i.p. 5, s.c.	4.78±0.11*	4.98±0.14*	5.12±0.11*	5.12±0.22*	4.92±0.11*
EEGC	300, p.o.	4.12±0.11	4.27±0.18	5.07±0.11	4.95±0.12	4.88±0.12
Naloxone+ EEGC	2,i.p. 300, p.o.	4.00±0.09	4.18±0.14	4.47±0.15**	4.23±0.09**	4.25±0.12**

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

*$p < 0.05$, significantly different compared with the morphine

**$p < 0.05$, significantly different compared with the EEGC 300 mg/kg

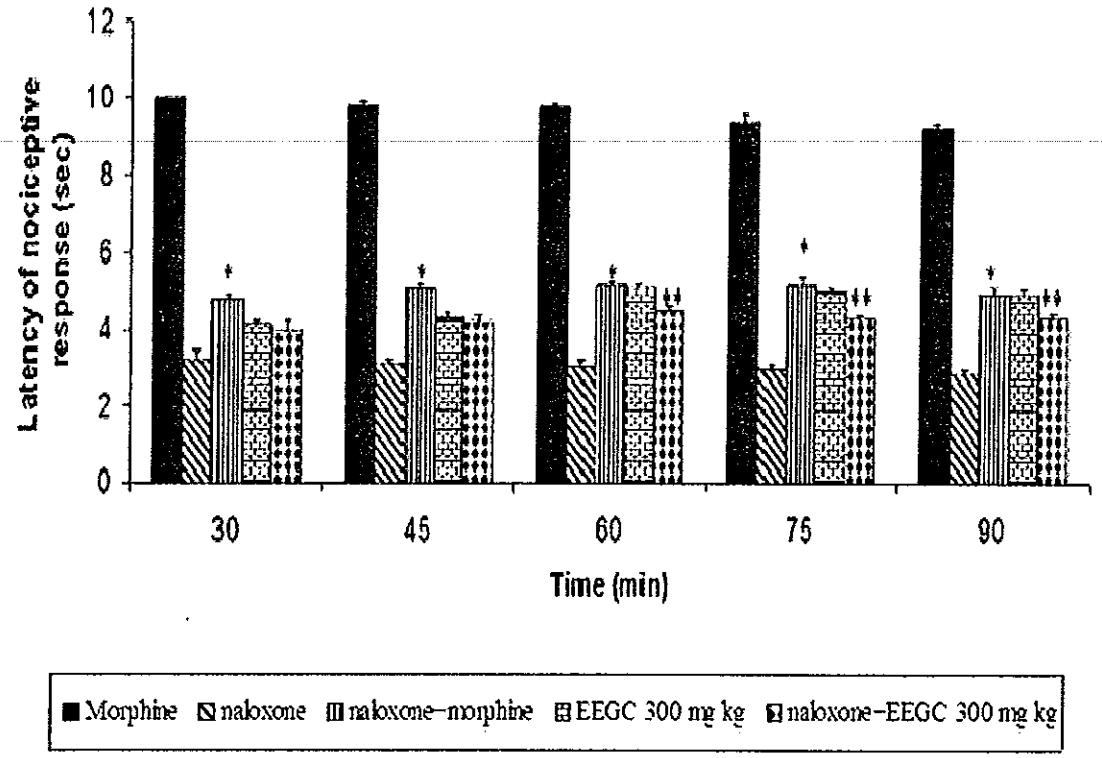


Figure 20. Antagonistic effects of naloxone on the action of morphine and ethanol extract of *G. cowa* leaves (EEGC) in the tail flick test in rat.

* $p < 0.05$, significantly different compared to the morphine.

2.4 Hot-plate test

The results showed that the reference drug morphine (5 mg/kg, s.c.) and EEGC 300 mg/kg, p.o. 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 EEGC 200 mg/kg, p.o. significantly increased the latency of nociceptive response only at 75 and 90 minutes when compared to the control, whereas the EEGC 100 mg/kg, p.o. did not have a significant effect on the latency of nociceptive response in mice (Table 6 and Figure 21).

In the antagonistic study, the results showed that naloxone (2 mg/kg, i.p.) given before morphine (5 mg/kg, s.c.) could antagonize the effect of morphine at 30, 45, 60, 75 and 90 minutes ($p< 0.05$) compared to the morphine administered alone, while naloxone (2 mg/kg, i.p.) given before the EEGC (300 mg/kg, p.o.) did not significantly decrease the latency of nociceptive response when compared with the EEGC (300 mg/kg, p.o.) given alone. The results were shown in the Table 7 and Figure 22.

Table 6. Effect of morphine and an ethanol extract of *G. cowa* leaves (EEGC) on the hot plate test in mice.

Treatment	Dose (mg/kg, p.o.)	Latency of nociceptive response (sec)				
		30 min	45 min	60 min	75 min	90 min
Control (cosolvent)	10 ml/kg	6.98±0.92	6.97±0.40	7.58±0.41	7.82±0.63	7.85±0.33
Morphine	5, sc	17.45±0.59*	17.27±0.92*	18.33±0.85*	18.33±0.74*	17.78±0.62*
EEGC	100	8.75±0.28	7.55±0.15	7.70±0.18	8.70±0.35	8.02±0.38
EEGC	200	9.00±0.37	8.05±0.26	8.57±0.13	10.12±0.27*	10.43±0.28*
EEGC	300	10.27±0.70*	11.38±0.41*	11.32±0.49*	11.05±0.47*	11.62±0.76*

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

*$p < 0.05$, significantly different compared with the control (cosolvent group)

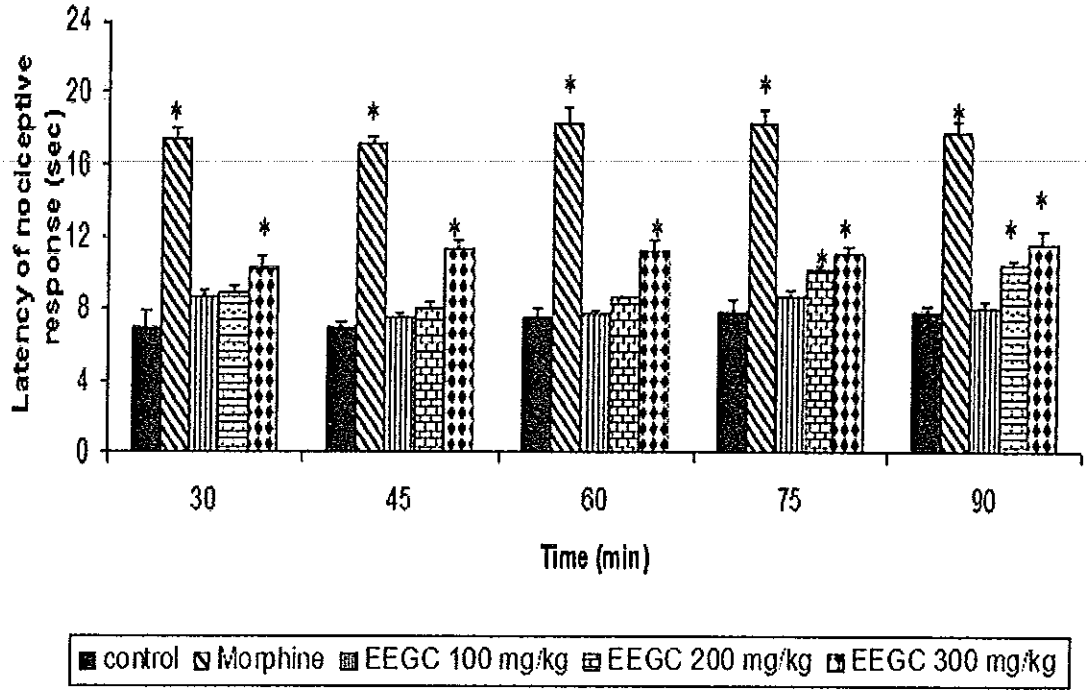


Figure 21. Effect of morphine and the ethanol extract of *G. cowa* leaves (EEGC) on the hot plate test in mice.

* $p < 0.05$, significantly different compared to the control group.

Table 7. Antagonistic effects of naloxone on the action of morphine and ethanol extract of *G. cowa* leaves (EEGC) on the hot plate test in mice.

Treatment	Dose (mg/kg, p.o.)	Latency of nociceptive response (sec)				
		30 min	45 min	60 min	75 min	90 min
Morphine	5, s.c.	17.45±0.59	17.27±0.92	18.33±0.85	18.33±0.74	17.78±0.62
Naloxone	2, i.p.	8.20±0.79	7.35±0.70	7.63±0.38	7.17±0.51	7.78±0.33
Naloxone+	2, i.p.					
Morphine	5, s.c.	10.63±0.66*	11.48±0.96*	11.50±0.21*	10.87±0.54*	11.43±0.24*
EEGC	300, p.o.	10.27±0.70	11.38±0.41	11.32±0.49	11.05±0.47	11.62±0.76
Naloxone+	2, i.p.					
EEGC	300, p.o.	10.27±0.70	10.47±0.66	10.78±0.56	10.73±0.55	12.90±0.50

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

*$p < 0.05$, significantly different compared with the control (cosolvent group)

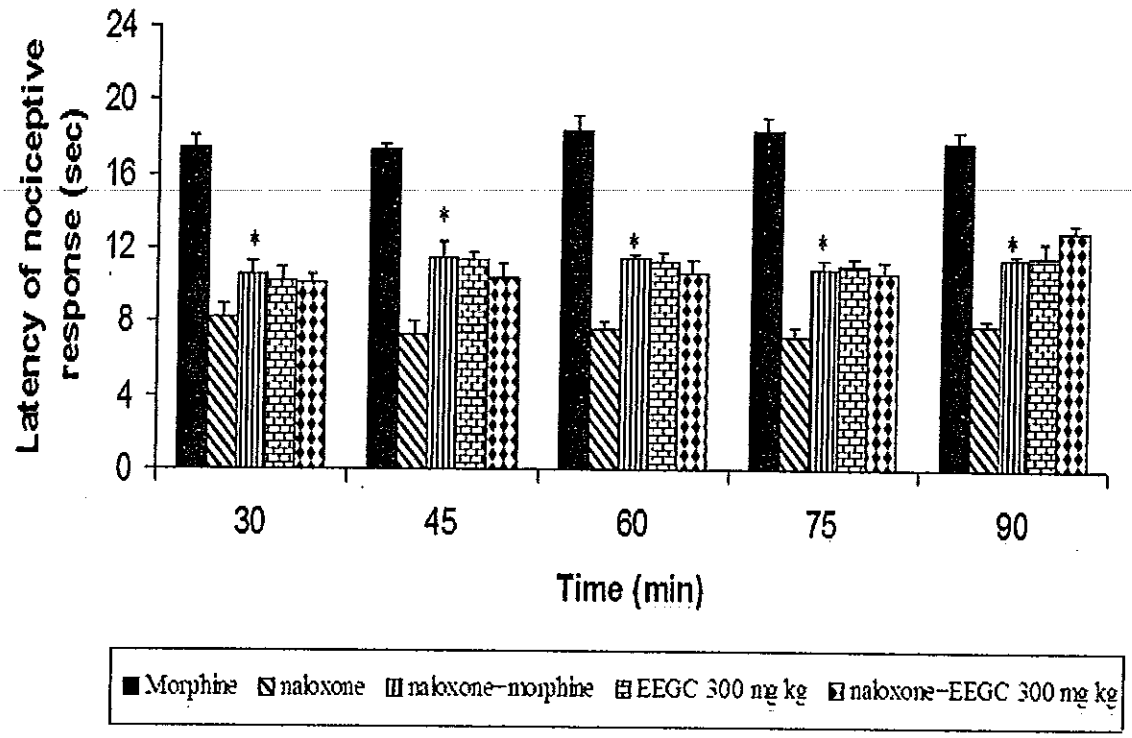


Figure 22. Antagonistic effects of naloxone on the action of morphine and ethanol extract of *G. cowa* leaves (EEGC) on the hot plate test in mice.
* $p < 0.05$, significantly different compared to the morphine.

3. Antipyretic activity

The results showed antipyretic effect of standard drug aspirin (200 mg/kg, p.o.) and the EEGC (100, 200 and 300 mg/kg, p.o.) (Table 8 and Figure 23). After the brewer's yeast injection to the rat for 18 hours, the rectal temperaturre was increased more than 0.7 °C (> 0.7 °C). Aspirin (200 mg/kg, p.o.) significantly decreased in rectal temperature at 3 hours after administration and continued up to 5 hour. The EEGC at the dose of 100 and 200 mg/kg, p.o. could reduced the rectal temperature only at 3 hours after administration, while the EEGC 300 mg/kg, p.o. significantly decreased of rectal rectal temperature at 1 hour after administration and continued up to 5 hours when compeared with control.

Table 8. Effects of aspirin and an ethanol extract of *G. cowa* leaves (EEGC) on the brewer's yeast induced pyrexia in rats.

Treatment	Dose (mg/kg, p.o.)	Before yeast Injection (-18 h)	After yeast Injection (0 h)	Rectal temperature (° C) Time after treatment (h) 1	2	3	4	5
Control	5 ml/kg	35.53±0.16	36.33±0.17	36.35±0.18	36.25±0.19	35.83±0.18	35.98±0.16	36.18±0.20
Aspirin	200	34.95±0.30	35.78±0.26	35.37±0.21	35.03±0.27	34.92±0.26*	35.00±0.21*	34.63±0.20*
EEGC	100	35.48±0.24	36.23±0.25	35.78±0.25	35.78±0.15	35.45±0.10*	35.60±0.15	35.40±0.38
EEGC	200	35.38±0.21	36.12±0.20	35.80±0.17	35.70±0.17	35.30±0.18*	35.48±0.20	35.82±0.25
EEGC	300	35.520.34	36.27±0.12	35.77±0.17*	35.42±0.14*	35.23±0.12*	35.33±0.21*	35.53±0.24*

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

Rectal temperature was measured after the yeast injection 18 h

*$p < 0.05$, significantly different compared with T_0 (after yeast injection)

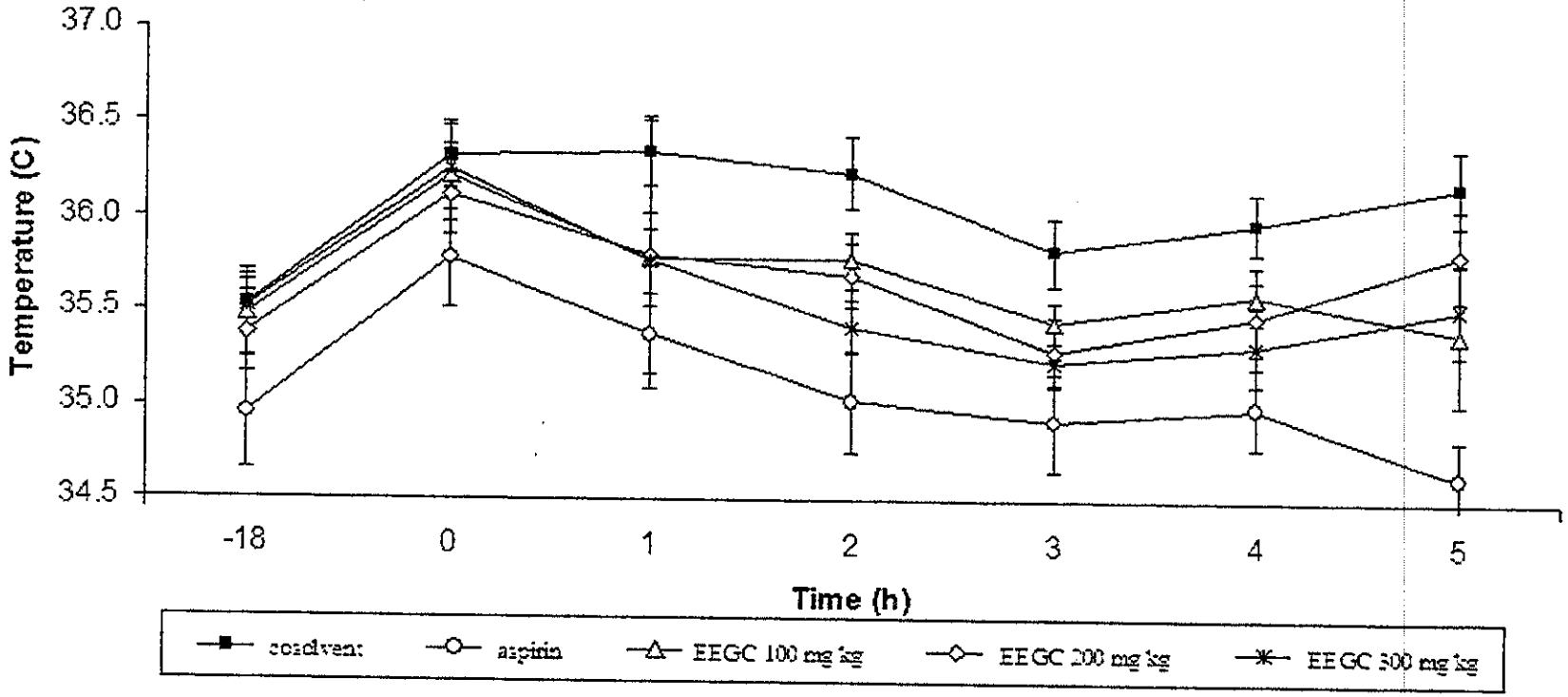


Figure 23. Effects of aspirin and an ethanol extract of *G. cowa* leaves (EEGC) on the brewer's yeast induced pyretic in rats.

* $p < 0.05$, significantly different compared to the T_0 (after yeast injection).

4. Anti-inflammatory activity

4.1 Carrageenan-induced rat paw edema (Acute anti-inflammatory activity test)

In the carrageenan-induced rat paw edema test, the average right hind paw thickness and the percentages of inhibition by the extracts and standard drug are shown in Table 9 and Figure 24. The standard drug aspirin (200 mg/kg, p.o.) and the EEGC (300 mg/kg, p.o.) significantly decreased the thickness of hind paw edema at 0.5 hour after administration and continued up to 5 hour ($p<0.05$). The percentage inhibition of aspirin were 13.03, 11.81, 13.24, 13.53, 12.21 and 12.07%, respectively and those of EEGC (300 mg/kg, p.o.) was 9.50, 11.81, 12.31, 12.18, 10.44 and 9.23%, respectively when compared to the control. The inhibition of rat paw edema of the EEGC (200 mg/kg, p.o.) at 0.5, 1, 2, 3 and 4 hour were 6.15, 8.97, 9.50, 7.37 and 5.29 %, respectively, while those of EEGC (100 mg/kg, p.o.) at 1, 2 and 3 hour were 6.15, 3.43 and 1.65%, respectively when compared to the control.

Table 9. Effect of aspirin and an ethanol extract of *G. cowa* (EEGC) on the carrageenan- induced paw edema in rats

Treatment	Dose (mg/kg,,p.o.)	Initial paw thickness (mm)	Paw edema thickness (mm)						Inhibition of paw edema (%)					
			0.5 h	1 h	2 h	3 h	4 h	5 h	0.5 h	1 h	2 h	3 h	4 h	5 h
Control	5 ml/kg,p.o.	4.42±0.12	5.37±0.09	6.02±0.09	6.42±0.07	6.65±0.04	6.80±0.03	7.04±0.03	0	0	0	0	0	0
Aspirin	200	4.42±0.13	4.67±0.11*	5.30±0.05*	5.57±0.06*	5.75±0.06*	5.97±0.04*	6.19±0.05*	13.03*	11.81*	13.24*	13.53*	12.21*	12.07*
EEGC	100	4.44±0.13	5.19±0.09	5.65±0.10*	6.20±0.06*	6.54±0.03*	6.73±0.03	6.95±0.04	3.35	6.15*	3.43*	1.65*	1.03	1.28
EEGC	200	4.45±0.07	5.04±0.04*	5.48±0.07*	5.81±0.06*	6.16±0.06*	6.44±0.03*	6.91±0.05	6.15*	8.97*	9.50*	7.37*	5.29*	1.85
EEGC	300	4.47±0.11	4.86±0.06*	5.30±0.07*	5.63±0.04*	5.84±0.07*	6.09±0.05*	6.39±0.04*	9.50*	11.81*	12.31*	12.18*	10.44*	9.23*

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

*$p < 0.05$, significantly different compared with the control (cosolvent group)

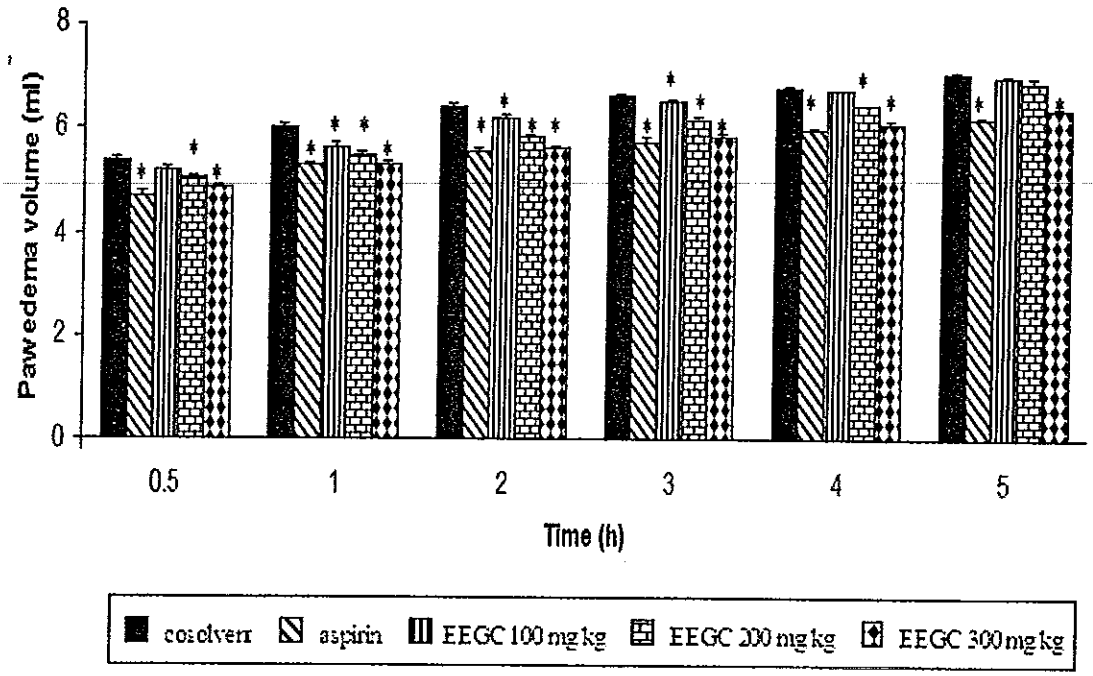


Figure 24. Effect of aspirin and an ethanol extract of *G. cowa* leaves (EEGC) on the carrageenan- induced paw edema in rats.

* $p < 0.05$, significantly different compared to the control group.

4.2 Cotton pellet-induced granuloma formation (chronic anti-inflammatory activity test)

The standard drug aspirin (200 mg/kg, p.o.) exhibited a significant reduction both in transudative weight (36.83±2.20 vs 60.60±0.86 mg) and granuloma weight (1.40±0.72 vs 23.45±0.39 mg) with the percentage of granuloma inhibition (% GI) of 94.03 when compared to the control, while those of EEGC (300 mg/kg, p.o.) were 50.73±0.82 mg, 10.92±0.71 mg and 53.43%, respectively. Whereas the EEGC at the dose of 100 and 200 mg/kg, p.o. did not significantly reduce both transudative weight and granuloma weight. The results were shown in the Table 10 and Figure 25.

Table 10. Effect of aspirin and an ethanol extract of *G. cowa* leaves (EEGC) on cotton pellet-induced granuloma formation in rats.

Treatment	Dose (mg/kg, p.o.)	Granuloma wet weight (mg)	Granuloma dry weight (mg)	Transudative weight (mg)	Granuloma weight (mg)	GI (%)
Control	5 ml/kg, p.o.	123.92±0.77	63.32±0.59	60.60±0.86	23.45±0.39	0
Aspirin	200	78.37±1.59*	41.53±1.10*	36.83±2.20*	1.40±0.72*	94.03*
EEGC	100	122.62±0.64	62.77±0.56	59.85±0.70	22.43±0.98	4.35
EEGC	200	122.12±1.05	61.70±0.53	60.42±1.01	21.67±1.03	7.59
EEGC	300	101.28±0.92*	50.55±0.60*	50.73±0.82*	10.92±0.71*	53.43*

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

*$p < 0.05$, significantly different compared with the control (cosolvent group)

Transudative weight = Granuloma wet weight - Granuloma dry weight

Granuloma weight = Granuloma dry weight – cotton weight

% GI : Granuloma inhibition $= \frac{A - B}{A} \times 100$

A = granuloma weight of the control group

B = granuloma weight of the treated group

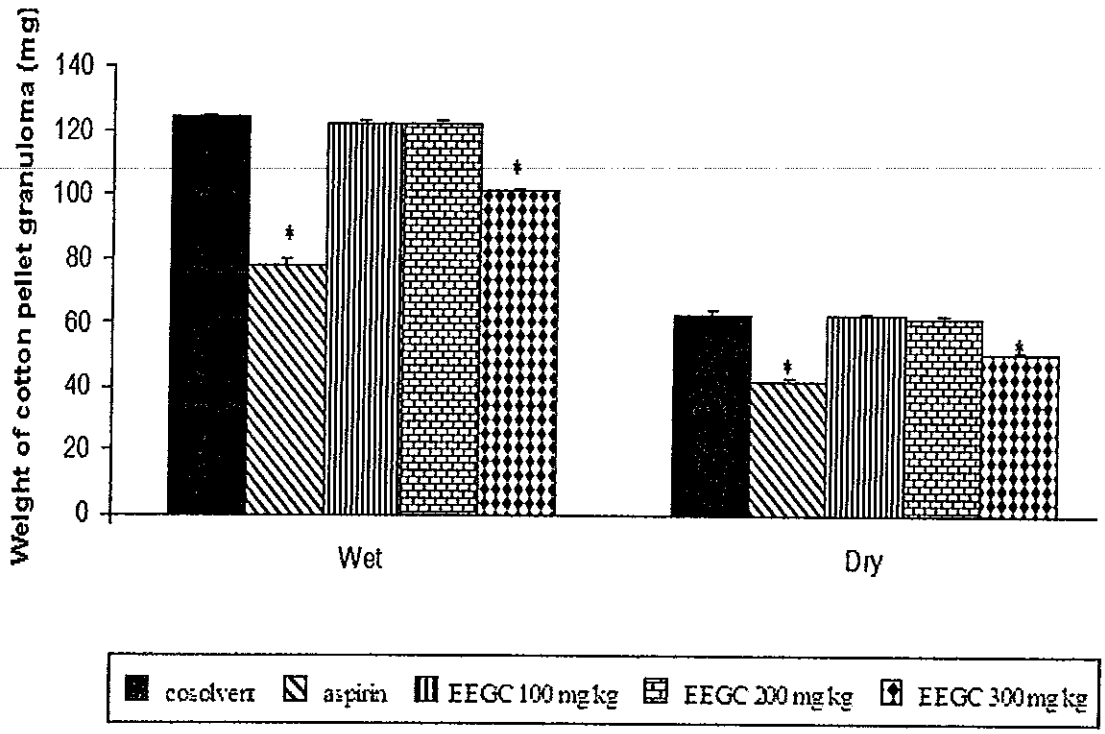


Figure 25. Effect of aspirin and an ethanol extract of *G. cowa* leaves (EEGC) on cotton pellet-induced granuloma formation in rats.
* $p < 0.05$, significantly different compared to the control group.

CHAPTER 5

DISCUSSION AND CONCLUSION

Garcinia cowa Roxb. (Guttiferae) is widely distributed in Thailand where it is known as Cha-muang. Several active compounds have been isolated including of cowanin, cowanol, cowaxanthone, 1,3,6-trihydroxy-7-methoxy-2,5-bis(3-methyl-2-butenyl) xanthone, 7-O-methylgarcinone E , β –mangostin (Deachathai, *et al.*, 2005). It has been used in the folk medicine for various purposes. The bark has been used in traditional medicine as an antipyretic and antimicrobial agent (Na Pattalung *et al.*, 1994). Some pharmacological properties of the crude extracts of leaves, e.g., anti-tumor-promoting activity (Murakami *et al.*,1995) has been reported. Base on these data, the extract from leaves of the plant may possess analgesic, antipyretic and anti-inflammatory activities.

Acute toxicity test

In the present study, oral acute toxicity test, the up-and-down method described by Bruce (1985), was used. It was carried out in three mice and rats to evaluate the toxicities of the ethanol extract of *G. cowa* leaves (EEGC). The results showed that EEGC at the dose up to 5 g/kg given orally did not affect on behaviour. No signs of toxicities such as convulsion, sedation, hyperactivity, respiratory depression, loss of righting reflex and mortality were shown during the experimental period of 14 days. According to Hayes (1989), the test substance which is shown non-toxic or non-lethal after an acute exposure of 5 g/kg body weight, a higher dosage is generally not necessary to test. Thus, EEGC at the dose of 100, 200 and 300 mg/kg, p.o. used in the present study was supposed to be safe to the animals.

Analgesic activity

In analgesic activity testing of EEGC, 4 models were used, such as writhing, formalin, hot plate and tail flick tests. The methods for investigating antinociception were selected for both peripherally and centrally mediated. The acetic acid induced writhing

test is the screening test for antinociceptive activity, whereas the formalin tests (chemical stimuli) elucidated peripherally or/and the central activity, while the hot plate and tail flick tests (thermal stimuli) investigated the central activity.

The EEGC showed the antinociceptive activity. This was evident in all the nociceptive models which indicates that it possesses both central and peripheral activities.

Pain is defined as an unpleasant sensory and emotional experience associated with actual or potential tissue damage (Balestrieri and Fisher, 1994). It occurs when tissue damage activates the free nerve endings (pain receptors or nociceptors) of peripheral nerves. When cell injured, it releases a number of chemicals that initiate the nociceptive response. The examples of these chemicals are kinins, histamine, substance P, bradykinin and prostaglandins. Bradykinin is an important mediator to stimulate nociceptors and prostaglandins sensitize nociceptor which causes impulses sending to spinal cord. This state is called peripheral sensitization.

The writhing test is used to screen for analgesic activity. Writhing response was induced in mice by an intraperitoneal injection of 0.6% acetic acid, which causes algesia by liberating endogenous substance including serotonin, histamine, prostaglandins, bradykinin, substance P and many others that excite pain nerve ending (Raj, 1996). Acetic acid-induced writhing is related to the increase in PGE_2 and $PGF_{2\alpha}$ in peritoneal fluid (Derardt *et al.*, 1980).

In this study, EEGC (200 and 300 mg/kg) and the reference drug, aspirin (200 mg/kg), significantly decreased the number of writhing in mice. It is known that aspirin and other nonsteroidal anti-inflammatory drugs (NSAIDs) can decrease the number of writhing by inhibiting enzyme cyclooxygenase (COX), the essential enzyme in the synthesis of prostaglandins (PGs), in peripheral tissue (Fields, 1987). The analgesic property of EEGC may be due to the inhibition of the effect or the synthesis and/or release of endogenous substances that excite pain nerve ending similarly to aspirin.

The formalin test is another pain model. This model can be used to discriminate pain in central and peripheral components (Tjolsen *et al.*, 1992). In the

present study, the formalin test was conducted to confirm and study the possible analgesic mechanisms of EEGC.

The formalin test consists of two distinct phases that possibly reflecting different types of pain mechanism (Dubuisson and Dennis, 1977; Tjolsen *et al.,* 1992; Hunskaae *et al.,* 1985). The first phase (early phase) starts immediately after injection of formalin and lasts about 5 min. This is due to direct peripheral chemical stimulation of nociceptors (Dubuisson and Dennis, 1977; Hunskaae *et al.,* 1985) that seems to be caused predominantly by C fiber activation. In this phase, the first response is evoked by the direct formalin stimulation of the nerve endings followed by substance P release, which may play a role through cooperation with bradykinin in this phase. The second phase (late phase) starts approximately 15-20 min after formalin injection and lasts for 20-40 min (Tjolsen *et al.,* 1992). This phase appears to be dependent on the combination of an inflammatory reaction in the peripheral tissue and functional changes in the dorsal horn of the spinal cord (Tjolsen *et al.,* 1992; Ito *et al.*, 2001). Several chemical mediators such as histamine, serotonin, prostaglandins and bradykinin are involved in the second phase. These mediators take part in the inflammatory response and are also able to stimulate nociceptors and induce pain (Rosland, 1991).

Formalin test is often used to examine opioid mimetics (Eaton, 2003). The response of the early phase can be inhibited by centrally acting analgesics such as morphine and codeine. In contrast, the late phase which seems to be due to an inflammatory response is partly mediated by prostaglandins and can be inhibited by NSAIDs (e.g., aspirin), as well as the centrally acting analgesics (Chen *et al.,* 1959). However, it is now well accepted that the anti-nociceptive efficacy of aspirin depends not only upon the inhibition of PG synthesis at the site of injury but also on the prevention of a nociception-induced by PGs, especially PGE_2 release in the spinal cord.

In the present study, EEGC (200 and 300 mg/kg) showed an inhibitory effect in both early and late phases, so it was indicated that EEGC had both 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. In the late phase, EEGC also exerted its inhibitory effect on inflammatory pain in the peripheral tissue, which may be due to reduction of the synthesis and/or release of PGs like the mechanism of action of NSAIDs and aspirin. Morphine, a centrally acting analgesic drug caused marked reduction of licking time in both phases. Opioids exert their actions by interfering pain transmission in the central nervous system by acting on μ and κ receptors in spinal cord and thalamus (Samad *et al.*, 2001; 2002).

Pain induced by thermal stimuli included tail flick and hot plate 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 at the supraspinal and spinal levels, respectively (Wong *et al.*, 1994; Marchioro *et al.*, 2005).

The tail flick model is considered a specific test for evaluation of the central pain at spinal level (Wong *et al.*, 1994, Marchioro *et al.*, 2005). The analgesic effect reflected in the tail flick test is dependent on centrally acting opioid like analgesic property (Conner *et al.*, 2000; Yonathan *et al.*, 2006). Opioid receptors are widely distributed in the CNS, especially in the pain transmission pathways including in the dorsal horn at the spinal cord (Jin *et al.*, 2006). Opioids have inhibitory effects at the cellular level (Charles and Hales, 2004), possibly acting on a descending inhibitory pain pathway (Richardson *et al.*, 1998). 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 receptor activation inhibits calcium entry via 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.

In tail flick test in rat, the EEGC only at the dose of 300 mg/kg significantly increased the reaction time to the nociceptive responses at all the time intervals measured during 30-90 min by about 2 folds when compared to control. Morphine (5 mg/kg), a centrally acting analgesic drug, significantly increased the tail flick latency at all time interval measured by about 5 folds. The results indicated that the EEGC had a weak antinociceptive action at spinal level.

In antagonistic study, naloxone (2 mg/kg) given before morphine could antagonize the analgesic effects of morphine by about 50% on the latency of nociceptive responses at all time interval measured (30-90 min) when compared with morphine alone. While naloxone showed weak antagonistic effect on EEGC (300 mg/kg). So, it is suggested that the mechanism of action of EEGC was only partially mediated by the opioid receptor.

The hot plate test is widely used to estimate analgesic activity of drugs at the supraspinal level. The paw-licking response are complex supraspinally organized behavior (Chapman, 1985). This test does not directly measure the intensity of the noxious stimulus perceived by the animal, but only the animal's response to it, and so may be affected by non-analgesic drugs such as muscle relaxant or local anaesthetics. The drugs that reduced the nociceptive response induced by cutaneous thermic stimuli in hot plate test may exhibited supraspinal analgesiac properties (Matheus *et al.*, 2005).

In this study, EEGC considerably increased the reaction time to the heat stimulus in hot plate test. This study used only mice that showed nociceptive responses such as licking or jumping to pain less than 15 seconds. The EEGC (300 mg/kg) and morphine (5 mg/kg) significantly increased latency of nociceptive responses at all time interval measured during 30-90 min when compared to control group by about 1.5 and 3 folds, respectively. To elucidate the effect on opioid receptor, the antagonistic action of naloxone on effects of morphine and EEGC on the latency of nociceptive responses was also investigated. The results showed that naloxone given before morphine significantly

decreased the latency of nociceptive response at all time interval measured when compared to morphine alone. While naloxone did not affect the latency of nociceptive responses of EEGC. These data suggested that the supraspinal analgesic activity of EEGC might not involve opioid receptor, but might be due to an inhibition of prostaglandin synthesis and/or release other mediators involving supraspinal level.

In conclusion, all the results evaluated from the writhing, formalin, tail flick and hot plate test indicated that EEGC had an analgesic activity through both peripheral and central mechanism, both at spinal and supraspinal levels, by which the mechanisms may involve inhibition of prostaglandin synthesis rather than via opioid receptor.

Antipyretic activity

Body temperature is regulated by the thermoregulatory center in anterior hypothalamus. Its function is to control the balance between heat production and heat loss. In fever, the set point in thermoregulatory center is elevated. Fever may be provoked by many stimuli including bacteria and their endotoxins, viruses, yeasts, protozoa, immune reactions, several hormones and medications. These substances are commonly called exogenous pyrogens. Cells stimulated by exogenous pyrogens formed and produced cytokines called endogenous pyrogens. The most important endogenous pyrogens are IL-1 and TNF-α. These endogenous pyrogens produce their effect by activating PG synthesis in the hypothalamus and the PGE_2 produced causes a rise in body temperature.

Yeast-induced fever is called pathogenic fever. Its etiology includes production of prostaglandins which set the thermoregulatory center at a higher set point. It could stimulate phagocytes to release endogenous 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 acetylsalicylic acid (Howard, 1993; Rawlins and Postgrad, 1973).

In brewer's yeast induced pyrexia test, the results of this study showed that the EEGC (300 mg/kg) and a reference drug aspirin suppressed fever in rats and the possible mechanisms to reduce fever may be due to inhibition of the synthesis of prostaglandin (PGE_2) within the hypothalamus in the CNS which is the final common pathway responsible for fever induction.

Anti-inflammatory activity

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

Acute inflammation is an immediate and early response to the injurious agents. It has relatively short duration, lasting from a few minutes up to a few days. This inflammation is most frequently caused by infectious agents, heat and cold or physical trauma. It may also be a response to immunologic injury. In acute inflammation, the process of inflammation is initiated by cells already presented in all tissue, mainly resident macrophages, dendritic cells, Kuppfer cells and mastocytes. At the onset of an infestion, 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 and increased heat (Gould, 2002).

Carrageenan-induced rat paw edema formation is one of the widely used model for verification of the ability of anti-inflammatory effect of test compounds (Winter *et al.,* 1962). This experiment is an excellent test for assessment of cyclooxygenase inhibitors (Sedgwick and Willoughby, 1989). The edema formation induced by carrageenan is mediated by the initial release of histamine and serotonin

followed by the release of bradykinin during the 1-2 h after carrageenan injection (Crunkhorn and Meacock, 1971). The second phase of inflammation is due to the release of prostaglandins which occurs 2-2.5 h after carrageenan injection and lasts about 6 h (Winter *et al.*, 1962; Vinegar *et al.*, 1969). The release of prostaglandins is closely associated with leucocytes migration to the inflamed site. The carrageenan-induced hind paw edema in rats is known to be sensitive to cyclooxygenase inhibitors, but not to lipoxygenase inhibitors, and has been used to evaluate the effect of non-steroidal anti-inflammatory agents which primarily inhibit the cyclooxygenase involved in prostaglandins synthesis. It has been demonstrated that the suppression of carrageenan-induced hind paw edema after the third hour correlates reasonably with therapeutic doses of most clinically effective anti-inflammatory agents (Salmon and Higgs, 1987). In the present study, EEGC (100 mg/kg) significantly reduced rat paw edema during 1-3 h, EEGC (200 mg/kg) significantly reduced rat paw edema during 0.5 - 4 h while EEGC (300 mg/kg) significantly reduced rat paw after edema induction at all assessment times during 0.5-5 h. Base on the inhibitory effect of the EEGC extract seen at the 3 h, it suggests that the main mechanism of action probably may be due to the inhibition on the cyclooxygenase pathway. Moreover, the inhibitory effect of the EEGC may partly involve other acute inflammatory mediators e.g. histamine, serotonin, bradykinin and pro-inflammatory cytokines which are released during 1 h after carrageenan injection. The results from this model support the possible mechanisms of anti-inflammatory action of EEGC on the cyclooxygenase pathway and on other inflammatory mediators involved in paw edema caused by carrageenan injection, which is consistent with the observation from anti-inflammation activity of a reference drug aspirin.

Cotton pellet-induced granuloma formation in animals is a typical method to provoke chronic inflammatory reactions (Spector, 1969). The inflammatory responses have been divided into 3 phases; transudative, exudative and proliferative phase. 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). Tissue granulation, one of the distinctive features of chronic

inflammation, which is composed of marked infiltration of macrophages and neovascularization, was induced by subcutaneous implantation of biomaterials. The implanted materials induce a host inflammatory response and modulate the release of inflammatory mediators which finally lead to tissue proliferation and granuloma formation (Rames and Williams, 1992; Tang and Eaton, 1995; Hu *et al.,* 2001). Monocyte infiltration and fibroblast proliferation rather than neutrophil infiltration and exudates take place in chronic inflammation. This proliferation becomes widespread by the proliferation of small vessels or glanuloma (Hosseinzadeh *et al.*, 2000). Nonsteroidal anti-inflammatory drugs (NSAIDs) decrease the size of glanuloma which resulted from cellular reaction by inhibiting granulocyte infiltration, preventing generation of collagen fiber and suppressing mucopolysaccharides (Suleyman *et al.,* 1999; Ramprasath *et al.*, 2004). Activated monocytes can release a series of pro-inflammatory cytokines, tumor necrosis factor-α (TNF-α) (Laupattarakasem *et al.*, 2006). TNF-α facilitates inflammatory cell infiltration by promoting the adhesion of neutrophils and lymphocytes to endothelial cell (Dore and Sirois, 1996). Additionally, TNF-α stimulates neutrophils to transcribe and release cytokines and chemokines (Marucha *et al.,* 1990). Interaction between these mediators thus enhances further inflammatory reactions and inhibition of TNF-α release can reduce the severity of inflammation (Gouwy *et al.,* 2005).

In the present study, the results of the extract on cotton pellet- induced granuloma formation in rats indicated that the extract at an oral dose of 300 mg/kg significantly inhibited the granuloma dry weight, granuloma wet weight and granuloma formation. These results suggest that the extract inhibits the transudative and proliferative phase of inflammation. It is possible that the EEGC may inhibit monocyte infiltration and fibroblast proliferation. Although this effect was not so strong as those of the reference drug aspirin.

In summary, the results obtained in the present study suggest that EEGC possesses analgesic, antipyretic and anti-inflammatory activities as follows:

1. The analgesic effects of EEGC may be due to an inhibition of prostaglandin synthesis and/or release other mediators involving both peripherally and centrally at both spinal and supraspinal levels.

2. The EEGC can reduce fever induced by brewer's yeast injection. The antipyretic mechanism is probably due to inhibition of prostaglandin E_2 (PGE_2) synthesis within the hypothalamus.

3. The anti-inflammatory effect of EEGC was found in both acute and chronic phases of inflammation. In acute phase inflammation, EEGC significantly reduced paw edema induced by carrageenan. It seems likely that the extract reduces inflammatory reaction by inhibiting only the cyclooxygenase pathway and/or the synthesis or release of other mediators e.g. histamine, serotonin and bradykinin. In the chronic inflammation, EEGC reduced the transudative weight and granuloma formation, which may be due to the inhibition of prostaglandin synthesis as well as inhibition of fibroblast proliferation and cell migration after tissue injury.

Therefore, the ethanol extract of *G. cowa* leaves used in traditional medicine is proved to have an analgesic, antipyretic and anti-inflammatory activities.

BIBLIOGRAPHY

Adam, S.S., Hebborn, P., Nichols J.S. 1968. Pharmacology of ibufenac a nonsteriodal anti-inflammatory. *Journal of Phamacy and Pharmacology*, 20: 305-312.

Aitchison, E.J., Blarke, P.S., Byrne, J.O., Eager, K., Farenden, W.M., Funnell, S.T., Jefferson,. S.L., Mcglashan, J.M., Moore, D.D., Neathercoat, G.C., Rourke, A.O., Qureshi, S.J., Riley, K.S. 1993. Analgesics and Anti-inflammatory Agents. Reynolds E.F. (ed.). Martindale: The extra pharmacopoeia (13th ed), London, England. The pharmaceutical press, pp. 3-7.

Balestrieri, F., Fisher,S. 1994. Analgesic. Chernow B. (ed.). The pharmacologic approach to the critically ill patients (3nd ed), Maryland, USA. William and Wilkins. pp. 640-643.

Barber, D. 1997. The Physiology and Pharmacology of pain: A Review of opioids: *Journal of PeriAnesthesia Nursing*, 12(2): 95-99.

Baron, R. 2006. Mechanism of disease: neuropathic pain: a clinical perspective. Neurology, 2(2): 95-106.

Beyers, V.L., 1991. Drugs that provide pain relief. Kunn M.M. (ed.). pharmaco-therapeutics: A nursing process approach (2nd ed), philadelphia, USA. F.A. David, pp. 452-455.

Bonica, J.J. 1980. Pain research and therapy: Past and current status and future needs. Bonica J.J. (ed.). Pain discomfort and humanitarian, New York, USA. Elsevier, pp. 20-46.

Boulant, JA. 1997. Thermoregulation. In: Mackowaik PA, (ed). Fever: Basic Mechanisms and Management (2nd ed), Philadelphia: Lippincott Reven: 35-58.

Brechter, A.B., Lerner, U.H. 2002. Characterization of bradykinin receptors in a human osteoblastic cell line. Regul Pept. 103: 39–51.

Brestel, E.P., Dyke, K.V. 1990. Lipid mediators of Homeostasis and Inflammation. Craig C.R., Stitzel R.E. (eds). Modern pharmacology (3rd ed), Boston, USA. Little Brown, pp 561-569.

Bruce, R.D. 1985. An up-and-down procedure for acute toxicity. *Fundamental and Applied Toxicology*, 5: 151-157.

Calixto, J.B. 2005. Twenty-five years of research on medicinal plants in Latin America: a personal view. *Journal of Ethnopharmacology* 100: 131-134.

Campbell, W.I. 2004. Mechanism of Pain. Fee J.H., Bovill J.G. (eds.). Physiology for anaesthesiologists, London, England. Taylor and Francis, pp. 59-68.

Chapman, C.R., Casey, K.L., Dubner, R., Foley, K.M., Gracely, R.H., Reading, A.E., 1985. Pain measurement: an overview. *Pain,* 22: 1-31.

Charles, A.C., Hales, T.G. 2004. From inhibition to excitation: functional effects of interaction between opioid receptors. *Life Science*, 76: 479-485.

Chau, T. 1989. Pharmacology methods in the control of inflammation. In Modern method in pharmacology, Vol. V, New York, USA. Alan R. Liss, pp. 195-212.

Chen, G., Ensor, C., Russell, D., Bohner, B. 1959. The pharmacology of 1-(1-phenylcyclohexyl) piperidine-HCl. *Journal of Pharmacology and Experimental Therapeutics*. 127: 241-250.

Coderre, T.J, Katz J., Vaccarino, A.L., Melzack, R. 1993. Contribution of central neuroplasticity to pathological pain: review of clinical and experimental evidence. *Pain*, 52:259–85.

Colasanti, B.K. 1990. Non-nacrotic analgesics and antipyretic. Craig C.R., Stitzel R.E. (eds.). modern pharmacology (3nd ed), New York , USA. Littie Brown, pp. 509-514.

Connor, J., Makonnen, E., Rostom, A. 2000. Comparison of analgesic effects of Khat (Catha edulis Forsk) extract, D-amphetamine and ibuprofen in mice. *Journal of Pharmacy and Pharmacology*, 52: 107-110.

Cotran, R.S., V. Kumar, T. Collins and S.L. Robbins, 1998. Robbins Pathologic Basis of Disease. 6th Edn., Elsevier Science Health Science, London, ISBN-13: 9780721673356.

Crunkhon, P., Meacock, S.E. 1971. Mediators of the inflammation induced in the rat paw by carrageenan. *British Journal of Pharmacology*, 42: 392-402.

D' Amour, F.E., Smith, D.L. 1941. A method for determining loss of pain sensation. *Journal of pharmacology and Experimental Therapeutics*, 72: 74-79.

Deachathai, S., Mahabusarakam, W., Phongpaichit, S. and Taylor, W.C. 2005. Phenolic compounds from thefruit of Garcinia dulcis. *Phytochemistry*, 66(19), 2368–2375.

Deraniyagala, S.A., Ratnasooriya, W.D., Goonasekara, C.L. 2003. Antinociceptive effect and toxicological study of the aqueous bask extract of Barringtonia racemosa on rat. *Journal of Ethnopharmacology*, 86: 21-26.

Derardt, R., Jouquey, S., Delevallee, F., Flahaut, M. 1980. Release of prostaglandins E and F in an algogenic reaction and its inhibition. *European* Journal of *Pharmacology*, 61: 17-24.

Donald, G., Rayan. 1992. Nonsteroidal Anti-inflammatory Drugs; Nonopioid Analgesics; Drugs. Used in Gout. Katzung B.G (ed.). Basic and Clinical Pharmacology, America, Appleton and Lange. pp. 491-510.

Dore, M., Sirois, J. 1996. Regulation of P-selectin expression by inflammatory mediators in canine jugular endothelial cells. *Verterinary Pathology*, 33: 662-671.

Drummer, O.H. 2001. Opioids. Drummer O.H. (ed.). The Forensic Pharmacology of Drugs of. Abuse, London, England. Arnold, pp. 227-239.

Dubuisson, D., Dennis, S. G. 1977. The formalin test: a quantitative study of the analgesic effects of morphine, meperidine, and brain stem stimulation in rats and cats. *Pain,* 4: 161-174.

Fantone, J.C., Ward, P.A. 1999. Inflammation. Rubin E., Farber J.L. (eds.). Pathology, (3rd ed), New York, USA. Lippincott-Raven, pp. 37-75.

Ferrero-Miliani, L., O.H. Nielsen, P.S. Andersen and S.E. Girardin. 2007. Chronic inflammation: Importance of NOD2 and NALP3 in interleukin-1beta generation. *Clinical & Experimental Immunology* 147: 227-235.

Fields, H.L. 1987. Analgesic drugs. Day W. (ed.). Pain (1st ed). USA. MacGraw-Hill, pp. 272.

Foegh, M.L. and Ramwell, P.W. (2001) The eicosanoids: prostaglandins, thromboxanes, leukotrienes, and related compounds, in Basic & Clinical Pharmacology (8th ed). (Katzung BG ed). pp. 311–325, McGraw-Hill Medical Publishing Division, New York.

Gould, B.E., 2002. Inflammation and Healing. Gould B.B. (ed). Pathophysiology of the Health Professions (2nd ed), Philadelphia, USA. W.B. Saunder, pp.192-199.

Gupta, M., Mazumder, U.K., Kumar, R.S., Kumar T.S. 2003. Studies in anti-inflammatory, analgesic and antipyretic properties of methanol extract of Caesalpinia bonducella leaves in experiment animal models. *Iranian Journal of Pharmacology and Therapeutics*, 2: 30-34.

Guyton, A.C. 1971. Pain. Guyton A.C. (ed.). Textbooks of Medical Physiology (2nd ed), Philadelphia, USA. Saunders, pp. 571-581.

Guyton, A.C. 1992. Pain, Headache and Thermal sensations. Guyton A.C. (ed.). Human physiology and mechanisms of disease (5th ed), Philadelphia, USA. Saunders, pp. 357-362.

Haayes, A.W. 1989. Principle and methods of Toxicology (2nd ed), New York, USA.

Hosseinzadeh, H., Ramezani, M., Salmani, G.H. 2000. Antinociceptive, anti-inflammatory and acute toxicity effects of Zataria multiflora Boiss extracts in mice and rats. *Journal of Ethnopharmacology*, 73: 379-385.

Howard, M. 1993. Fever: cause and consequences. *Neuroscience Biobehavioral Review*, 17: 237-269.

Howard L. Fields. 2007. Pain Perception. The Dana Guide to Brain Health. A Practical Family Reference From Medical Experts.

Hu, W.J., Eaton J.W., Ugarova, T.P., Tang, L. 2001. Molecular basis of biomaterial-mediated foreign body reactions. *Bloods*, 98: 1231-1238.

Hunskaar, S., Fasmer, O.B., Hole, K. 1985. Formalin test in mice, a useful technique in evaluating mild analgesics. *Journal of Neurosci Methods* 14:69-76.

Insel, P.A. 1991. Analgesic, antipyretics and anti-inflammatory agents: drugs employed in treatment of rheumatic arthritis and gout. In: Goodman AG and Gilman, AG (Eds), 'The Pharmacological Basis of Therapeutics', 9th Edn, Pergamon Press, Oxford, pp. 638-681.

Ito, S., Okuda-Ashitaka, E., Minami, T. 2001. Central and peripheral roles of prostaglandins in pain and their interactions with novel neuropeptides nociceptin and nocistatin. *Neuroscience Research*. 41(4): 299-332.

Jaffe, J.H., Martin, W.R. 1990. Opioid analgesics and antagonists. Gilman A.G, Rall T.W., Nies A.S., Taylor P. (eds). The pharmacological basic of therapeutics (8th ed), New York, USA. Pergamon, pp. 485-521.

Jena, B.S., Jayaprakasha, G.K., Singh, R.P. and Sakariah, K.K. (2002) Chemistry and biochemistry of (–)-hydroxycitric acid from Garcinia. *Journal of Agricultural and Food Chemistry*, 50: 10–22.

Jin, Y.H., Nishioka, H., Yashi, W., Fujita, T., Yonehara, N. 2006. Effect of morphine on the release of excitatory amino acids in the rat hind instep: Pain is modulated by the interaction between the peripheral opioid and glutamate system. Neuroscience, 138: 1329-1339.

Jonh, J.A., Bonald, R.B. 1993. Analgesic. Ambre J.J., Bennett D.R. (eds.). Drug evaluations annual 1994. American medical association, New York USA. De Press, pp. 118-119.

Kieffer, B.L. 1999. Opioids: first lessons from knockout mice. Elsevier Science, 20: 19-25.

Koster, R., M. Anderson and E.J. De Beer, 1959. Acetic acid analgesic screening. *Federation Proceedings*, 18: 412-417.

Laupattarakasem, P., Wangsrimongkol, T., Surarit, R., Hahnvajanawong, C. 2006. In vitro and in vivo anti-inflammatory potenial of Cryptoleisis buchanani. *Journal of Ethnopharmacology*, 108(3): 349-354.

Lee, H-H., Chan, H.-K., 1977. 1,3,6-Trihydroxy-7-methoxy-8-(3,7-dimethyl-2,6-octadienyl)xanthone from *Garcinia cowa. Phytochemistry* 16, 2038–2040.

Lee, Y., Lee, C. H., Oh, U. 2005. Painful channels in sensory neurons. *Molecular Cells,* 20(3), 315-324.

Lipman, A.G., and Jackson, K.C. 2004. Opioid pharmacotherapy. Warfield C.A., Bajwa Z.H. (eds.). Principle and Practice of pain Medicine (2nd ed), New York, USA. McGraw Hill, pp. 586-588.

Liu, Y., Chen, J., Bai, Z.L., Deng, X.B., Zhang, L. 2002. Seed dispersal, seed predation, and seedling spatial pattern of *Garcinia cowa* (Guttiferae). Acta Phytoecol Sin 26, 427–434 (in Chinese with an English abstract).

Likhitwitayawuid, K., Phadungcharoen, T., Manhidol, C., Ruchirawat,S., 1997. 7-O-Methylgarcinone E from *Garcinia cowa. Phytochemistry* 45 (6), 1299–1301.

Likhitwitayawuid, K., Phadungcharoen, T., Krungkrai, J., 1998. Antimalarial xanthones from *Garcinia cowa. Planta Medica.* 64, 70–72.

Macfalane, P.S., Reid, R., Callander, R. 2000. Inflammation. Mcfalane P.S., Reid R., Callander R. (eds.). Pathology Illustrated (15th ed). Edinburgh, Scotland. Churchill Livingstone, pp. 32-42.

Mackowiak PA, Bartlett JG, Borden EC, et al. 1997.Concepts of fever: recent advances and lingering dogma. *Clinical Infectious Diseases*, 25:119–138.

Mackowiak, PA. 1998. Concepts of fever. *Archives of Internal Medicine.* 158:1870–1881.

Marchioro, M., Blank, M.F.A., Mourao, R.H.V., Antoniolli, A.R. 2005. Antinociceptive activity of the aqueous extract of *Ertheina velutina* leaves. *Fitoterapia*, 76: 637-642.

Marucha, P.T., Zeff, R.A., Kreutzer, D.L. 1990. Cytokine regulation of IL-1 beta gene expression in the human polymorphonuclear leukocyte. *Journal of Immunology*, 145: 2932-2937.

Matheus, M.E, Berrondo, L.F., Vietas, E.C., Menzes, F.S., Fernandes, P.D. 2005. Evaluation of the antinociceptive propoties from *Brillantaisia palisotii* Lindau stems extract. *Journal of Ethnopharmacology*, 102: 377-381.

McMullan, S., Lumb, B.M. 2006. Midbrain control of spinal nociception discriminates between response evoked by myelinated and unmyelinated heat nociceptors in the rat. *Pain*. 124: 59-68.

Meed, S.D., Kleinmann, P.M., Kantor, T.G. 1987. Management of cancer pain with oral controlled-release morphine sulfate. *Journal of Clinical Pharmacology*, 24: pp. 155-161.

Mello, R.D., Dickinson, A.H. 2008. Spinal cord mechanisms of pain. *British Journal of Anaesthesia*, 101(1): 8-16.

Milanes, M.V., Martinez, M.D., Gonzalez-Cuello, A. 2001. Evidence for a peripheral mechanism in cardiac opioid withdrawal. *Naunyn-Schmiedeberg's Archives of Pharmacology*; 364:193-198.

Moffett, D.F., Moffett, S.B. 1993. Action potentials: Pain perception. Callanan R.J. (ed.). Human physiology (2nd ed), St. Louis Missouri, USA. Mosby, pp. 233-236.

Murakami, A., Jiwajiinda, S., Koshimizu, K., Ohigashi, H., 1995. Screening for in vitro anti-tumor promoting activities of edible plants from Thailand. *Cancer Lett*ers. 95, 137–146.

Mutschler, E., Derendorf, H. 1995. Pathophysiology of Pain. Mutschler E., Derendorf H., Korting M.S., Elrod K. (eds). Drug action: Basic principle and Therapeutic, Stuttgart, Germany. Mesphram CRC, pp. 8-10.

Na Pattalung, P., Thongtheeraparp, W., Wiriyachitra, P., Taylor, W.C., 1994. Xanthones of Garcinia cowa. *Planta Medica*. 60, 365–368.

Niv, D., Kreitler, S. 2001. Pain and quality of life. Pain Practice: *The Official Journal of World Institute of Pain*, 1(2): 61-150.

Norris, C. 2004. Diagnosis and Management: Healing. Norris (ed.). Sports Injuries (3rd ed). Edinburgh , Scotland. Butterworth Heine mann. pp. 30-38.

Panthong, K., Pongcharoen, W., Phongpaichit, S., Taylor, WC. 2006. Tetraoxygenated xanthones from the fruits of *Garcinia cowa*. *Phytochemistry* 67: 999-1004.

Panthong K., Hutadilok-Towatana N., Panthong A. 2009. Cowaxanthone F, a new tetraoxygenated xanthone, and other anti-inflammatory and antioxidant compounds from *Garcinia cowa*. *Canadian Journal of Chemistry*. 87: 1636-1640.

Parson, K. 2003. Human thermal physiology and thermoregulation. Parson K. (ed.). Human Thermal Environments: The effect of hot, moderate, and cold environments on human health, comfort and performance (2nd ed), London, England. Taylor and Francis, pp. 31-48.

Patapoutian, A., Peier, A.M., Story, G.M., Viswanath, V. 2003. ThermoTRP channels and beyond: mechanism of temperature sensation. *Nature Reviews Neuroscience*. pp. 529-539.

Peck, T.E. 2002. Analgesics Peck, T.E. (ed). Pharmacology for analgesia and intensive care, London, England. Greenwich Ltd., pp. 101-112.

Pflanzer, R.G. 1992. Function of the Blood. Rhoades R., Pflanzer R.G. (eds.). Human physiology (2nd ed), Florida, USA. Saunder, pp. 607-611.

Poomipamorn, S., Kumkong, A., 1997. Edible Multipurpose Tree Species. FaungFa Printing (in Thai), Bangkok, p. 486.

Raj, P.P. 1996. Pain mechanism. Raj P.P. (ed.), Pain Medicine: A Comprehensive Review (1st ed). Missouri, USA. Mosby-Year, pp. 12-23.

Ramprasath, V.R., Shanthi, P., Sachdanandam, P. 2004. Anti-inflammatory effect of *Semecarpus anacardium* Linn. Nut extract in acute and chronic inflammatory conditions. *Biological and pharmacology Bulletin*, 27(12): 2028-2031.

Rang, H.P., Dale, M.M. 1991. Local hormone, Inflammation and Allergy. Anderson and. Critchley (eds.). Pharmacology (2nd ed), Hongkong. Churchill Livingstone, pp. 284-290.

Rang, H.P., Dale, M.M. 1999. Drug disposition. Anderson and Critchley (eds.). Pharmacology (4th ed), Hongkong. Churchill Livingstone, pp. 600-603.

Rawlins, M.D., Postgrad, R. 1973. Mechanism of salicylate-induce antipyresis. *Pharmacology Thermoregulatory Proceeding Satellite Symposium*, 311-324.

Remes, A., Williums, D.F. 1992. Immune response in biocompatility. *Biomaterials,* 13: 731-734.

Richards, AJ. 1990. Studies in *Garcinia*, dioecious tropical forest trees, Agamospermy. *The Botanical Journal of the Linnean Society* 103, 233–250.

Rhaodes, R., Pflazer, R. 2003. Regulatory of body temperature. Rhaodes R. (ed.). Human Physiology (4th ed), Australia. Thomson, pp. 830-840.

Robert, V.C. 2003. The adrenal gland. Rhoades R.A., Tanner G.A. (eds.). Medical physiology (2nd ed), Philadelphia, USA. Lippincott Williams and Wilkins, pp. 619-620.

Rosland, J.H., 1991. The formalin test in mice: the influence of ambient temperature. *Pain.* 45, 211–216.

Ross, J.M., Dehoratius, R.J. 1989. Nonnarcotic analgesics. DiPiro J.T., Talbert R.L., Hayes. P.E.,Yee G.C., Matzke G.R., Posey L.M.(eds.). Basic pharmacology in Medicine (3rd ed), Philadelphia, USA. McGraw Hill, pp. 309-315.

Salmon JA, Higgs GA. Prostaglandins and leukotrienes as inflammatory mediators. *British Medical Bulletin* 1987; 43(2): 285-296.

Samad, T.A., Moore, K.A., Sapirstein, A., Billet, S., Allchorne, A., Poole, S., Bonventre, J., Woolf, C.J. 2001. Interleukin-1beta-mediated induction of Cox-2 in the CNS contributes to inflammatory pain hypersensitivity. *Nature.* 410: 471–475.

Samad, T.A., 2002. Peripheral mechanism of inflammaion pain. *Trends in Molecular Medicine,* 8: 309-396.

Schuna, A., Coulter, L. 1993. Rheumatoid arthritis and the seronegative spondy loarthropathies. DiPiro J.T., Talbert R.L., Hayes P.E., Yee G.C., Matzke G.R., Posey L.M. (eds.). Pharmacotherapy: A pathophysiologic approach (2nd ed), New Jersey, USA. Appleton and Lange, pp. 1623-1636.

Sedgwick AD, Willoughby DA. 1989. Animal models for testing drugs on inflammatory and hypersensitivity reactions. In: Dale MM, Foreman JC, editors. Textbook of immunology. 2nd ed. Oxford, Blackwell Scientific Publications, p 253-261.

Seeley, R.R., Stephens, T.D., Tate P. 1996. Nutrition, metabolism and body temperature. Seeley R.R., Stephens T.D., Tate P. (eds.). Essential of anatomy physiology (2nd ed), St. Louis Missouri, USA. Mosby, pp. 474-476.

Shen, J. and Yang, J-S. 2006. "Two New Xanthones from the Stems of *Garcinia cowa*", *Chemical & Pharmaceutical Bulletin,* Vol. 54, 126-128.

Singleton, J.R. 2005. Evaluation and treatment of painful peripheral polyneuropathy. Seminar in Eurology. 25(2): 185-193.

Spector, W.G. 1969. The granulomatous inflammatory exudate. *International Research in Experimental Pathology.* 8: 1-55.

Stanier, M.W., Forsling, M.L. 1990. Defense Mechanisms of the body. Stanier M.W., Forsling M.L. (eds.). Physiology process, Great Britain. McGraw Hill, pp. 263-265.

Steven, S.A., Lowe, J. 2000. Tissue responses to damage. Steven S.A.,Lowe J. (eds). Pathology (2nd ed). Edinburgt, *Scotland. Butterworth Heine mann.* pp. 30-38.

Stoelting, R.K., Hillier, S.C. 2006. Opioid agonists and antagonists. In: Pharmacology and physiology in anesthetic practice. 4th ed. Philadelphia: Lippincott Williams & Wilkins; p. 87-126.

Suleyman, H., Derirezer, L.O., Kuruuzum, A., Banoglu, Z.N., Gocer, F., Ozbakir, G., Gepdiremen, A. 1999. Anti-inflammatory effect of the aqueous extract from *Rumex patientia L.* roots. *Journal of Ethnopharmacology*, 65: 141-148.

Swingle, K.F., Shiderman, F.E. 1972. Phases of the inflammatory response to subcutaneous implantation of a cotton pellet and their modification by certain anti-inflammatory agents. *Journal of* Pharmacology *and Experimental Therapeutics*. 13: 226-234.

Tang, L., Eaton, J.W. 1995. Inflammatory responses to biomaterials. *American Journal of Clinical Pathology,* 103: 466-471.

Tjolsen, A., Berge, O.G., Hunskaar, S., Rosland, J.H. and Hole, K. 1992. The formalin test: an evaluation of the method. *Pain,* 51: 5-17.

Vander, A.J. 2001. The mechanism of body temperature. Vander A.J., Sherman D.L. (eds.). Human physiology (8th ed), Singapore. McGraw Hill, pp. 630-632.

Vinegar, R., Truax, J.F. 1987. Pathway to carrageenan-induced inflammation in the hind limb of the rat. *Federation proceedings*, 46(1): 118-126.

Wahyuni, F.S., Byrne, L.T., Dianita, R., Jubahar, J., Lajis, N.H., Sargent, M.V. 2004. "A New Ring-Reduced Tetraprenyltoluquinone and a Prenylated Xanthone from Garcinia cowa", *Australian Journal of Chemistry*, 57, pp. 223-226.

Way, W.L., Fields, H.L., Way, E.L. 1998. Opioid analgesic and antagonists. Katzung, B.G. (ed.). Basic and Clinical Pharmacology (7th ed), USA. Prentice Hall, pp. 512-513.

Winter, C.A., Risley, E.A., Nuss, G.W. 1962. Carrageenan induced edema in hind paw of the rat as an assay for anti-inflammatory drug. *Proceeding of the Society for experimental Biology and medicine*, 111: 544-547.

Wong, C.H., Day, P., Yarmush, J., Wu, W., Zbuzek, U.k. 1994. Nufedipine-induced analgesic after eoidural injections in rats. *Aneasthesia and Analgesic*, 79: 303-306.

Woolfe, G. and A.D. MacDonald, 1944. The evaluation of the analgesic action of pethidin hydrochloride (DEMEROL). *Journal of* Pharmacology *and Experimental Therapeutics*, 80: pp. 300-307.

Woolf, C.J., Costigan, M. 1999. Transcriptional and posttranslational plasticity and the generation of inflammatory pain. *Proceedings of the National Academy of Sciences*, 96: 7723-7730.

Yonathan, M., Asves, K., Assefa, A., Bucar, F. 2006. In vivo anti-inflammatory and anti-nociceptive activity of Chelanthes farinosa, *Journal of Ethnopharmacology*, 108: 462-470.

Zhu, H. 1993. Flora characters on Xishuangbanna tropical forest. *Trop Geogr* 13, 149-155 (in Chinese with an English abstract).

APPENDIX

APPENDIX-1

Extraction procedure

The leaves of *Garcinia cowa* Roxb (11 kg)

↓

Air-dried at room temperature

↓

Pulverized to powder (5 kg)

↓

Macerated with 5 L of 95% ethanol for 7 days for 2 times

↓

Filtration

↓

Evaporation by Rotary Evaporator at 40 – 60 °C

↓

oil-like dark green viscous extract (yield 21.4%, w/w)

APPENDIX-2

Reagents preparation

1. Normal saline (0.9% NaCl)
   Dissolve 0.9 g of sodium chloride with distilled water to make 100 ml total solution
2. 0.6% (v/v) acetic acid
   Dilute 0.06 ml of acetic acid with normal saline solution to make 10 ml solution
3. 2.5% (v/v) formalin
   Dilute 0.625 ml of 40% formalin with normal saline solution to make 10 ml solution
4. 20% (w/v) brewer's yeast
   Dissolve 2 g of brewer's yeast with normal saline solution to make 10 ml solution
5. 1% (w/v) carrageenan
   Dissolve 0.1 g of carrageenan with normal saline solution to make 10 ml solution
6. Morphine sulfate 5 mg and naloxone 2 mg
   Dissolve morphine sulfate 5 mg or naloxone 2 mg with normal saline solution to make 10 ml solution
7. Aspirin 200 mg , ethanol extract of *G.* cowa leaves (EEGC) at the dose of 100, 200 and 300 mg/kg
   Dissolve aspirin 200 mg or EEGC 100, 200 and 300 mg with cosolvent (Propylene glycol: Tween 80: Distill water; 4:1:5) 10 ml solution

Dosing for oral administration

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

APPENDIX-3

Effect of the ethanol extract of *G.cowa* (EEGC) and aspirin on acetic acid-induced writhing in mice

Group	Number	Weight (g)	Number of writhing	Group	Number	Weight (g)	Number of writhing
Control (cosolvent, p.o.)	1	44	48	Aspirin 200 mg/kg, p.o.	1	41	15
	2	41	67		2	42	18
	3	44	53		3	48	17
	4	38	44		4	31	21
	5	32	50		5	36	12
	6	38	39		6	38	18
	Mean± S.E.M	39.50 ±1.86	50.17 ±3.91		Mean± S.E.M	39.33 ±2.36	16.83 ±1.25
EEGC 100 mg/kg, p.o.	1	47	49	EEGC 200 mg/kg,po	1	39	23
	2	47	44		2	36	41
	3	46	40		3	35	25
	4	35	59		4	44	45
	5	35	52		5	34	29
	6	38	40		6	43	40
	Mean± S.E.M	41.33 ±2.43	47.33 ±3.05		Mean± S.E.M	38.50 ±1.73	33.83 ±3.80

Group	Number	Weight (g)	Number of writhing
EEGC 300 mg/kg, p.o.	1 2 3 4 5 6 Mean ± S.E.M	36 38 37 39 39 38 37.83 ±0.48	29 36 27 22 30 33 29.50 ±1.98

Effect of the ethanol extract of *Garcinai cowa* (EEGC) and aspirin on 2.5% formalin induced paw licking in mice

Group	Number	Weight (g)	Licking of hind paw (sec)	
			Early phase (0-5 min)	Late phase (15-30 min)
Control (cosolvent, p.o.)	1	43	165.13	172.56
	2	39	168.7	171.20
	3	36	170.06	172.57
	4	39	164.64	175.13
	5	41	171.52	179.81
	6	38	174.92	172.92
	Mean± S.E.M	39.33 ±0.99	169.16 ±1.60	174.03 ±1.27
Morphine (5 mg/kg, s.c.)	1	33	32.17	27.62
	2	37	31.68	29.96
	3	35	35.15	26.58
	4	36	28.41	26.87
	5	33	36.12	31.53
	6	41	28.61	23.80
	Mean± S.E.M	35.83 ±1.22	32.02 ±1.31	27.73 ±1.11

Group	Number	Weight (g)	Licking of hind paw (sec)	
			Early phase (0-5 min)	Late phase (15-30 min)
EEGC (100 mg/kg, p.o.)	1 2 3 4 5 6 Mean± S.E.M	35 39 39 39 31 42 37.50 ±1.59	164.15 162.58 161.37 168.91 169.42 161.21 164.61 ±1.51	170.50 173.49 170.64 172.91 170.42 172.46 171.74 ±0.56
EEGC (200 mg/kg, p.o.)	1 2 3 4 5 6 Mean± S.E.M	40 36 34 36 38 36 36.67 ±0.84	132.39 131.17 127.17 128.35 127.55 129.68 129.39 ±0.85	143.04 145.57 145.73 141.63 141.90 144.78 143.78 ±0.75

Group	Number	Weight (g)	Licking of hind paw (sec)	
			Early phase (0-5 min)	Late phase (15-30 min)
EECG (300 mg/kg, p.o.)	1 2 3 4 5 6 Mean± S.E.M	35 40 32 39 34 36 36.00 ±1.24	104.41 100.47 100.26 101.46 98.52 97.51 100.44 ±0.99	114.42 113.71 118.60 114.09 115.17 118.99 115.83 ±0.96

Effect of the ethanol extract of *Garcinai cowa* (EEGC), morphine and naloxone on nociceptive responses in tail flick test in rats

Group	Number	Weight (g)	Latency of nociceptive response (sec)					
			Initial response	30 min	45 min	60 min	75 min	90 min
Control (cosolvent, p.o.)	1	184	3.3	2.9	2.4	2.6	2.8	2.5
	2	203	3.6	2.2	2.3	2.3	2.4	2.3
	3	216	3.4	2.5	3.3	3.2	2.8	2.7
	4	196	3.4	2.3	2.4	2.5	2.5	2.4
	5	206	3.5	3	2.9	2.7	2.6	3.1
	6	210	3.1	3.1	2.7	2.7	3.0	2.8
	Mean ± S.E.M	202.50 ±4.60	3.38 ±0.07	2.67 ±0.16	2.67 ±0.16	2.67 ±0.12	2.68 ±0.09	2.63 ±0.12
Morphine (5 mg/kg, sc)	1	189	3.3	10	10	10	8.8	9.1
	2	206	3.3	10	10	9.6	8.9	8.7
	3	199	3.4	10	10	10	10	9.6
	4	211	3.8	10	9	9.7	9.5	9.5
	5	208	3.6	10	9.6	9.4	9.4	9.2
	6	230	3.7	10	10	9.7	9.8	9.3
	Mean± S.E.M	207.17 ±5.58	3.52 ±0.09	10 ±0.00	9.77 ±0.17	9.73 ±0.10	9.40 ±0.20	9.23 ±0.13

Group	Number	Weight (g)	Latency of nociceptive response (sec)					
			Initial response	30 min	45 min	60 min	75 min	90 min
Naloxone (2 mg/kg, ip)	1	201	2.8	2.5	2.6	2.5	2.6	3.0
	2	197	3.4	3.0	3.2	3.0	2.8	2.6
	3	215	4.2	3.8	3.5	3.2	3.3	3.2
	4	169	3.8	3.2	3.1	3.1	3.1	2.7
	5	217	3.9	3.1	3.0	3.3	3.1	3
	6	222	4.0	3.6	3.2	3.1	3.0	2.6
	Mean± S.E.M	203.50 ±7.95	3.68 ±0.21	3.20 ±0.19	3.10 ±0.12	3.03 ±0.12	2.98 ±0.10	2.85 ±0.10
EEGC (100 mg/kg, p.o.)	1	182	3.4	3.0	3.1	2.9	2.7	2.6
	2	220	4.1	3.2	3.2	3.1	2.9	2.7
	3	215	2.8	2.6	2.7	2.9	3.1	3.0
	4	208	3.6	3.1	3.2	3.0	2.8	2.8
	5	190	4.0	3.2	3.0	3.1	2.8	2.9
	6	208	3.3	3.1	3.0	2.8	3.0	2.7
	Mean± S.E.M	203.83 ±6.02	3.53 ±0.20	3.03 ±0.09	3.03 ±0.08	2.97 ±0.05	2.88 ±0.06	2.78 ±0.06

Group	Number	Weight (g)	Latency of nociceptive response (sec)					
			Initial response	30 min	45 min	60 min	75 min	90 min
EEGC (200 mg/kg, p.o.)	1	197	3.1	3.0	3.2	3.0	2.8	2.8
	2	181	3.1	3.0	3.0	2.6	2.9	2.7
	3	206	3.2	3.1	3.1	2.8	2.8	3.2
	4	199	3.2	2.9	3.0	3.1	3.0	2.5
	5	213	3.4	3.4	2.9	3.2	3.0	2.7
	6	216	3.3	2.9	3.0	2.7	2.8	3.0
	Mean± S.E.M	202.00 ±5.19	3.22 ±0.05	3.05 ±0.08	3.03 ±0.04	2.90 ±0.10	2.88 ±0.04	2.82 ±0.10
EEGC (300 mg/kg, p.o.)	1	215	3.8	4.4	4.5	4.7	5.1	4.9
	2	212	3.4	4.0	4.1	5.2	5.3	5.1
	3	221	3.5	4.5	4.7	5.1	5.2	5.3
	4	200	3.4	3.9	4.7	5.4	4.9	4.8
	5	240	3.4	3.8	3.6	5.2	4.6	4.5
	6	228	3.2	4.1	4.0	4.8	4.6	4.7
	Mean± S.E.M	219.33 ±5.63	3.45 ±0.08	4.12 ±0.11	4.27 ±0.18	5.07 ±0.11	4.95 ±0.12	4.88 ±0.12

Group	Number	Weight (g)	Latency of nociceptive response (sec)					
			Initial response	30 min	45 min	60 min	75 min	90 min
Naloxone (2 mg/kg, ip) + Morphine (5 mg/kg, sc)	1	231	3.6	4.8	4.7	5.2	4.8	4.8
	2	217	3.9	4.5	4.7	4.8	4.4	4.6
	3	220	4.2	5.2	5.5	4.8	4.7	4.7
	4	192	4.0	4.5	4.7	5.4	5.6	5.2
	5	220	3.4	4.9	5.1	5.1	5.5	4.9
	6	228	3.8	4.8	5.2	5.4	5.7	5.3
	Mean± S.E.M	218.00 ±5.64	3.82 ±0.12	4.78 ±0.11	4.98 ±0.14	5.12 ±0.11	5.12 ±0.22	4.92 ±0.11
Naloxone (2 mg/kg, ip) + EEGC (300 mg/kg, p.o.)	1	202	4.1	4.2	4.5	4.6	4.2	4.4
	2	218	3.6	3.8	3.6	4.2	3.9	3.9
	3	202	3.4	3.9	4.2	4.4	4.1	4.0
	4	215	3.8	3.8	4.3	4.8	4.5	4.5
	5	213	3.5	4.0	4.0	3.9	4.3	4.1
	6	217	4.2	4.3	4.5	4.9	4.4	4.6
	Mean± S.E.M	211.17 ±2.98	3.77 ±0.13	4.00 ±0.09	4.18 ±0.14	4.47 ±0.15	4.23 ±0.09	4.25 ±0.12

Effect of the ethanol extract of *Garcinai cowa* (EEGC), morphine and naloxone on nociceptive responses in hot plate test in mice

Group	Number	Weight (g)	Latency of nociceptive response (sec)					
			Initial response	30 min	45 min	60 min	75 min	90 min
Control (cosolvent, p.o.)	1	33	6.2	8.7	7.2	8.3	9.8	8.2
	2	34	6.7	10.5	8.7	8.9	9.1	8.7
	3	35	7.7	5.6	6.4	6.9	5.8	7.4
	4	36	8.7	6.4	6.9	8.2	6.4	6.8
	5	30	8.4	4.3	5.8	6.4	7.5	7.3
	6	32	6.8	6.4	6.8	6.8	8.3	8.7
	Mean± S.E.M	33.33 ±0.88	7.42 ±0.41	6.98 ±0.92	6.97 ±0.40	7.58 ±0.41	7.82 ±0.63	7.85 ±0.33
Morphine (5 mg/kg, sc)	1	34	8.3	17.2	17.0	19.1	16.2	18.4
	2	35	7.8	18.7	17.0	21.3	16.4	17.2
	3	35	8.4	18.7	16.1	18.3	20.0	17.6
	4	33	9.2	17.4	17.4	18.9	19.8	19.6
	5	30	8.6	17.9	18.9	15.0	17.6	18.7
	6	32	9.1	14.8	17.2	17.4	20.0	15.2
	Mean± S.E.M	33.17 ±0.79	8.57 ±0.21	17.45 ±0.59	17.27 ±0.92	18.33 ±0.85	18.33 ±0.74	17.78 ±0.62

Group	Number	Weight (g)	Latency of nociceptive response (sec)					
			Initial response	30 min	45 min	60 min	75 min	90 min
Naloxone (2 mg/kg, ip)	1	36	8	10.8	6.6	8.7	9.4	9
	2	33	12.3	9.3	4.3	6.1	5.9	7.7
	3	33	8.9	8.8	7.5	8.4	6.3	6.5
	4	34	8.3	8.3	8.2	7.4	6.8	7.9
	5	35	6.2	6.6	8.6	7.4	7.6	8.0
	6	32	8.4	5.4	8.9	7.8	7	7.6
	Mean± S.E.M	33.38 ±0.60	8.68 ±0.82	8.20 ±0.79	7.35 ±0.70	7.63 ±0.38	7.17 ±0.51	7.78 ±0.33
EEGC (100 mg/kg, po)	1	37	8.1	7.9	7.4	7.5	9.4	8.2
	2	36	8.8	8.4	7.2	7.5	7.4	7.5
	3	35	7.6	9.8	8.0	8.3	9.6	7.8
	4	34	8.3	8.3	7.3	7.5	9.1	7.6
	5	32	9.5	8.9	7.4	7.2	8.7	9.8
	6	36	8.9	9.2	8	8.2	8.0	7.2
	Mean± S.E.M	35.00 ±0.73	8.53 ±0.27	8.75 ±0.28	7.55 ±0.15	7.70 ±0.18	8.70 ±0.35	8.02 ±0.38

Group	Number	Weight (g)	Latency of nociceptive response (sec)					
			Initial response	30 min	45 min	60 min	75 min	90 min
EEGC (200 mg/kg, p.o.)	1	32	10.5	9.8	8.4	8.5	11.2	10.8
	2	35	10.9	7.4	6.9	8.2	9.4	9.1
	3	35	10.4	8.9	8.2	8.7	9.6	10.4
	4	34	7.8	8.7	7.8	8.4	10.5	10.8
	5	33	8.9	9.4	8.3	8.5	9.8	10.5
	6	29	8.6	9.8	8.7	9.1	10.2	11.0
	Mean± S.E.M	33.00 ±0.93	9.52 ±0.51	9.00 ±0.37	8.05 ±0.26	8.57 ±0.13	10.12 ±0.27	10.43 ±0.28
EEGC (300 mg/kg, p.o.)	1	32	12.3	9.0	10.1	10.3	10.4	11.7
	2	33	11.5	11.5	10.2	10.8	10.0	9.1
	3	34	9.3	9.5	12.6	11.4	12.7	14.9
	4	45	8.2	9.1	12.0	10.3	10.6	11.2
	5	34	10.4	13.2	11.8	11.6	10.3	11.4
	6	30	9.3	9.3	11.6	13.5	12.3	11.4
	Mean± S.E.M	33.00 ±0.73	10.17 ±0.63	10.27 ±0.70	11.38 ±0.41	11.32 ±0.49	11.05 ±0.47	11.05 ±0.47

Group	Number	Weight (g)	Latency of nociceptive response (sec)					
			Initial response	30 min	45 min	60 min	75 min	90 min
Naloxone (2 mg/kg, ip) + Morphine (5 mg/kg, sc)	1	38	8.6	8.6	8.3	12.3	11.6	11.5
	2	35	12.3	10.3	14.8	11.5	10.4	10.4
	3	33	10.7	11.7	10.9	11.5	13.2	12.1
	4	34	11.7	11.8	12.9	11.8	10.2	11.6
	5	30	10.2	12.5	12.4	10.8	9.6	11.2
	6	37	9.6	8.9	9.6	11.1	10.2	11.8
	Mean± S.E.M	34.50 ±1.18	10.52 ±0.55	10.63 ±0.66	11.48 ±0.96	11.50 ±0.21	10.87 ±0.54	11.43 ±0.24
Naloxone (2 mg/kg, i.p.) + EEGC (300 mg/kg, p.o.)	1	35	9.7	9.8	11	9.0	8.4	13.6
	2	33	10.7	8.4	9.5	9.9	10.3	14.6
	3	34	10.2	10.0	8.9	11.7	12.1	12.2
	4	29	9.3	9.5	12.7	11.5	11.8	12.6
	5	30	7.0	12.3	11.8	12.6	11.3	13.3
	6	35	7.5	10.4	8.9	10.0	10.5	11.1
	Mean± S.E.M	32.67 ±1.05	9.07 ±0.61	10.27 ±0.70	10.47 ±0.66	10.78 ±0.56	10.73 ±0.55	12.90 ±0.50

Effect of the ethanol extract of *Garcinai cowa* (EEGC) and aspirin on Brewer's yeast induced pyrexia in rats

Group	Number	Weight (g)	Rectal temperature (°C)						
			Before yeast injection (-18 h)	After yeast injection (0 h)	After treatment (h)				
					1	2	3	4	5
Control (cosolvent, p.o.)	1	224	35.1	36.0	36.1	36.2	36.2	35.9	35.9
	2	237	35.3	36.0	36.0	36.0	35.8	35.7	36.1
	3	225	35.7	36.6	36.5	36.1	36.2	35.9	35.8
	4	220	35.2	35.9	35.8	35.6	35.1	35.5	35.4
	5	229	36.0	36.7	36.9	36.8	35.6	36.5	36.5
	6	227	35.9	36.8	36.8	36.8	36.1	36.4	36.4
	Mean± S.E.M	227.00 ±2.35	35.53 ±0.16	36.33 ±0.17	36.35 ±0.18	36.25 ±0.19	35.83 ±0.18	35.98 ±0.16	36.18 ±0.20
Aspirin (200 mg/kg, p.o.)	1	217	35.6	36.4	35.4	35.2	35.0	35.5	34.9
	2	212	34.7	35.5	35.3	34.8	35.0	35.3	34.8
	3	225	34.3	35.5	35.7	35.9	35.8	35.5	34.4
	4	233	35.3	36.0	35.5	35.6	35.3	34.7	33.9
	5	216	34.0	34.8	34.4	34.5	34.3	34.2	34.5
	6	226	35.8	36.5	35.9	34.2	34.1	34.8	35.3
	Mean± S.E.M	221.50 ±3.19	34.95 ±0.30	35.78 ±0.26	35.37 ±0.21	35.03 ±0.27	34.92 ±0.26	35.00 ±0.21	34.63 ±0.20

Group	Number	Weight (g)	Rectal temperature (°C)						
			Before yeast Injection (-18 h)	After yeast Injection (0 h)	After treatment (h)				
					1	2	3	4	5
EEGC (100 mg/kg, p.o.)	1	215	35.6	36.3	36.0	35.6	35.3	35.3	34.2
	2	207	35.1	35.8	35.3	36.3	35.8	36.1	35.8
	3	225	34.8	35.6	34.8	35.6	35.2	35.4	36.7
	4	217	36.5	37.3	36.2	36.2	35.7	35.7	35.9
	5	224	35.2	35.9	36.0	35.6	35.4	35.9	34.6
	6	220	35.7	36.5	36.4	35.4	35.3	35.2	35.2
	Mean± S.E.M	218.00 ±2.71	35.48 ±0.24	36.23 ±0.25	35.78 ±0.25	35.78 ±0.15	35.45 ±0.10	35.60 ±0.15	35.40 ±0.38
EEGC (200 mg/kg, p.o.	1	221	35.5	36.2	36.2	35.8	35.4	35.7	35.0
	2	211	36.2	36.9	36.1	36.0	35.6	35.8	36.5
	3	212	35.0	35.8	35.1	35.0	35.1	35.2	36.4
	4	227	34.7	35.4	35.5	35.5	34.5	34.6	35.5
	5	216	35.5	36.2	36.0	35.7	35.5	35.8	36.1
	6	225	35.4	36.2	35.9	36.2	35.7	35.8	35.4
	Mean± S.E.M	218.67 ±2.74	35.38 ±0.21	36.12 ±0.20	35.80 ±0.17	35.70 ±0.17	35.30 ±0.18	35.48 ±0.20	35.82 ±0.25

Group	Number	Weight (g)	Rectal temperature (° C)						
			Before yeast Injection (-18 h)	After yeast Injection (0 h)	After treatment (h)				
					1	2	3	4	5
EEGC (300 mg/kg, p.o.)	1	238	35.5	36.2	35.4	35.4	34.8	35.0	35.0
	2	225	35.3	36.1	35.3	35.8	35.0	34.5	34.8
	3	214	36.1	36.8	36.4	35.8	35.5	35.8	36.1
	4	220	35.3	36.1	35.8	35.4	35.2	35.5	35.3
	5	233	35.7	36.4	36.1	35.1	35.6	35.8	35.9
	6	227	35.2	36.0	35.6	35.0	35.3	35.4	36.1
	Mean± S.E.M	226.17 ±3.54	35.52 ±34	36.27 ±0.12	35.77 ±0.17	35.42 ±0.14	35.23 ±0.12	35.33 ±0.21	35.53 ±0.24

Effect of the ethanol extract of *Garcinai cowa* (EEGC) and aspirin on carrageenan induced paw edema in rat

Group	Number	Weight (g)	Paw edema thickness (mm)						
			Initial response	0.5 h	1 h	2 h	3 h	4 h	5 h
Control (cosolvent, p.o.)	1	212	4.93	5.76	5.88	6.53	6.61	6.83	7.12
	2	221	4.15	5.43	6.02	6.64	6.73	6.88	7.06
	3	229	4.58	5.35	5.81	6.38	6.57	6.84	7.08
	4	205	4.27	5.26	6.38	6.55	6.74	6.70	6.96
	5	227	4.19	5.27	6.13	6.24	6.72	6.73	6.93
	6	203	4.38	5.16	5.87	6.19	6.35	6.79	7.11
	Mean± S.E.M	216.17 ±4.55	4.42 ±0.12	5.37 ±0.09	6.02 ±0.09	6.42 ±0.07	6.65 ±0.04	6.80 ±0.03	7.04 ±0.03
Aspirin (200 mg/kg, p.o.)	1	231	4.53	4.82	5.41	5.56	5.68	5.89	6.13
	2	228	4.73	4.98	5.17	5.35	5.58	5.93	6.21
	3	223	4.83	4.86	5.24	5.64	5.72	5.81	5.95
	4	226	4.14	4.57	5.48	5.58	5.83	6.03	6.23
	5	206	4.20	4.45	5.33	5.51	5.72	6.05	6.31
	6	214	4.06	4.31	5.16	5.77	5.98	6.08	6.28
	Mean± S.E.M	221.33 ±3.88	4.42 ±0.13	4.67 ±0.11	5.30 ±0.05	5.57 ±0.06	5.75 ±0.06	5.97 ±0.04	6.19 ±0.05

Group	Number	Weight (g)	Paw edema thickness (mm)						
			Initial response	0.5 h	1 h	2 h	3 h	4 h	5 h
EEGC (100 mg/kg, p.o.)	1	216	4.65	5.62	5.83	6.11	6.58	6.84	7.01
	2	225	4.55	5.23	5.90	6.43	6.56	6.63	6.88
	3	215	4.82	5.02	5.84	6.22	6.48	6.81	7.0
	4	214	4.06	5.11	5.51	6.33	6.63	6.69	6.84
	5	208	4.02	5.08	5.38	6.01	6.48	6.66	6.91
	6	222	4.57	5.06	5.43	6.12	6.49	6.73	7.06
	Mean± S.E.M	216.67 ±2.47	4.44 ±0.13	5.19 ±0.09	5.65 ±0.10	6.20 ±0.06	6.54 ±0.03	6.73 ±0.03	6.95 ±0.04
EEGC (200 mg/kg, p.o.)	1	208	4.66	5.12	5.78	5.83	6.25	6.34	6.89
	2	222	4.47	5.20	5.53	5.81	6.14	6.48	6.94
	3	204	4.45	4.96	5.34	5.67	5.93	5.93	6.92
	4	211	4.17	4.94	5.51	5.92	6.34	6.34	7.01
	5	219	4.58	5.01	5.46	5.64	6.21	6.21	7.04
	6	207	4.38	4.99	5.28	5.98	6.11	6.11	6.68
	Mean±	211.83	4.45	5.04	5.48	5.81	6.16	6.44	6.91
	S.E.M	±2.92	±0.07	±0.04	±0.07	±0.06	±0.06	±0.03	±0.05

Group	Number	Weight (g)	Paw edema thickness (mm)						
			Initial response	0.5 h	1 h	2 h	3 h	4 h	5 h
EEGC (300 mg/kg, p.o.)	1	213	4.73	4.92	5.11	5.56	5.65	6.02	6.46
	2	205	4.67	5.03	5.42	5.71	5.98	6.05	6.31
	3	218	4.72	4.92	5.09	5.48	5.61	5.93	6.23
	4	217	4.35	4.76	5.52	5.71	5.98	6.16	6.49
	5	209	4.15	4.86	5.40	5.63	5.92	6.28	6.37
	6	211	4.21	4.64	5.25	5.70	5.87	6.12	6.45
	Mean± S.E.M	212.17 ±2.01	4.47 ±0.11	4.86 ±0.06	5.30 ±0.07	5.63 ±0.04	5.84 ±0.07	6.09 ±0.05	6.39 ±0.04

Effect of the ethanol extract of *Garcinai cowa* (EEGC) and aspirin on cotton pellet induced granuloma formation in rat

Group	Number	Weight (g)	Cotton (mg/2 cotton pellet)	Granuloma wet weight (mg)	Granuloma dry weight (mg)	Transudative weight (mg)	Granuloma weight (mg)
Control (cosolvent, p.o.)	1	216	40.4	121.1	63.5	57.6	23.1
	2	200	40.0	122.7	62.3	60.4	22.3
	3	215	39.4	124.4	63.1	61.3	23.7
	4	182	39.6	123.6	64.0	59.6	24.4
	5	189	41.0	126.3	65.6	60.7	24.6
	6	190	38.8	125.4	61.4	64.0	22.6
	Mean± S.E.M	198.67 ±5.82	39.87 ±0.32	123.92 ±0.77	63.32 ±0.59	60.60 ±0.86	23.45 ±0.39
Aspirin (200 mg/kg, p.o.)	1	174	42.0	74.5	43.2	31.3	1.2
	2	227	41.4	77.7	45.8	31.9	4.4
	3	202	39.0	84.1	40.4	43.7	1.4
	4	159	38.6	82.2	39.3	42.9	0.7
	5	203	40.2	76.3	41.9	34.4	1.7
	6	165	39.6	75.4	38.6	36.8	-1.0
	Mean± S.E.M	188.33 ±10.81	40.13 ±0.55	78.37 ±1.59	41.53 ±1.10	36.83 ±2.20	1.40 ±0.72

Group	Number	Weight (g)	Cotton (mg/2 cotton pellet)	Granuloma wet weight (mg)	Granuloma dry weight (mg)	Transudative weight (mg)	Granuloma weight (mg)
EEGC (100 mg/kg, p.o.)	1	209	41.6	124.2	61.4	62.8	19.8
	2	196	41.8	120.1	60.9	59.2	19.1
	3	187	40.4	121.4	63.6	57.8	23.2
	4	160	39.8	123.4	64.5	58.9	24.7
	5	189	39.0	123.7	63.4	60.3	24.4
	6	185	39.4	122.9	62.8	60.1	23.4
	Mean± S.E.M	187.67 ±6.58	40.33 ±0.47	122.62 ±0.64	62.77 ±0.56	59.85 ±0.70	22.43 ±0.98
EEGC (200 mg/kg, p.o.)	1	199	41.2	125.2	62.7	62.5	21.5
	2	214	39.0	123.7	61.3	62.4	22.3
	3	203	37.8	121.4	63.8	57.6	26.0
	4	198	40.8	119.9	61.1	58.8	20.3
	5	180	42.0	118.6	60.4	58.2	18.4
	6	178	39.4	123.9	60.9	63.0	21.5
	Mean± S.E.M	195.33 ±5.67	40.03 ±0.64	122.12 ±1.05	61.70 ±0.53	60.42 ±1.01	21.67 ±1.03

Group	Number	Weight (g)	Cotton (mg/2 cotton pellet)	Granuloma wet weight (mg)	Granuloma dry weight (mg)	Transudative weight (mg)	Granuloma weight (mg)
EEGC (300 mg/kg, p.o.)	1	218	40.2	101.4	50.1	51.3	9.9
	2	209	40.6	100.7	51.6	49.1	11.0
	3	167	41.4	105.1	50.7	54.4	9.3
	4	180	37.8	99.7	48.9	50.8	11.1
	5	173	39.2	98.6	49.2	49.4	10.0
	6	163	38.6	102.2	52.8	49.4	14.2
	Mean± S.E.M	185.00 ±9.38	39.63 ±0.55	101.28 ±0.92	50.55 ±0.60	50.73 ±0.82	10.92 ±0.71

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Education Attainment

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Bachelor of Science (Biology)	Prince of Songkla University	2007

List of Publication and Proceeding

1. Suviporn Wannachote, Malinee Wongnawa, Wantana Reanmongkol, Wibool Ridtitid. 2011. Study on Analgesic and Antipyretic Activities of Ethanol Extract of *Garcinia cowa* Roxb. in Eperimental Animals. Proceeding of the 3rd NPRU NATIONAL CONFERENCE (August 10-11, 2011).
2. Suviporn Wannachote, Malinee Wongnawa, Wantana Reanmongkol, Wibool Ridtitid. 2011. Anti-inflammatory Activity of *Garcinia cowa* Roxb. Leaves Extract in Animal models. 7th Joint Seminar on Biomedical Sciences, (October 13-16, 2011); pp. 105.