ฤทธิ์ระงับปวดและฤทธิ์ต้านการอักเสบของสิ่งสกัดจากรากสมุนไพรทั้งห้าชนิดของตำรับยาเบญจมูลใหญ่

นางสาวชญานิน กีรติไพบูลย์

วิทยานิพนธ์นี้เป็นส่วนหนึ่งของการศึกษาตามหลักสูตรปริญญาเภสัชศาสตรมหาบัณฑิต
สาขาวิชาเภสัชวิทยา ภาควิชาเภสัชวิทยาและสรีรวิทยา
คณะเภสัชศาสตร์ จุฬาลงกรณ์มหาวิทยาลัย
ปีการศึกษา 2554

ลิขสิทธิ์ของจุฬาลงกรณ์มหาวิทยาลัย
บทคัดย่อและแฟ้มข้อมูลฉบับเต็มของวิทยานิพนธ์ตั้งแต่ปีการศึกษา 2554 ที่ให้บริการในคลังปัญญาจุฬาฯ (CUIR)
เป็นแฟ้มข้อมูลของนิสิตเจ้าของวิทยานิพนธ์ที่ส่งผ่านทางบัณฑิตวิทยาลัย
The abstract and full text of theses from the academic year 2011 in Chulalongkorn University Intellectual Repository(CUIR)
are the thesis authors' files submitted through the Graduate School.

ANTINOCICEPTIVE AND ANTI-INFLAMMATORY EFFECTS OF FIVE ROOT EXTRACTS OF BEN-CHA-MOON-YAI REMEDY

Miss Chayanin Kiratipaiboon

A Thesis Submitted in Partial Fulfillment of the Requirements
for the Degree of Master of Science in Pharmacy Program in Pharmacology
Department of Pharmacology and Physiology
Faculty of Pharmaceutical Sciences
Chulalongkorn University
Academic Year 2011
Copyright of Chulalongkorn University

Thesis Title	ANTINOCICEPTIVE AND ANTI-INFLAMMATORY EFFECTS OF FIVE ROOT EXTRACTS OF BEN-CHA-MOON-YAI REMEDY
By	Miss Chayanin Kiratipaiboon
Field of Study	Pharmacology
Thesis Advisor	Assistant Professor Flight Officer Pasarapa Towiwat, Ph.D.
Thesis Co-advisor	Associate Professor Nijsiri Ruangrungsi, Ph.D.

Accepted by the Faculty of Pharmaceutical Sciences, Chulalongkorn University in Partial Fulfillment of the Requirements for the Master's Degree

……………………………………..Dean of the Faculty of Pharmaceutical Sciences
(Associate Professor Pintip Pongpech, Ph.D.)

THESIS COMMITTEE

……………………………………………………Chairman
(Associate Professor Police Lieutenant Colonel Somsong Lawanprasert, Ph.D.)

……………………………………………………Thesis Advisor
(Assistant Professor Flight Officer Pasarapa Towiwat, Ph.D.)

……………………………………………………Thesis Co-advisor
(Associate Professor Nijsiri Ruangrungsi, Ph.D.)

……………………………………………………Examiner
(Assistant Professor Rataya Luechapudiporn, Ph.D.)

……………………………………………………External Examiner
(Associate Professor Siriporn Fungwithaya, M.S.)

ชญานิน กีรติไพบูลย์ : ฤทธิ์ระงับปวดและฤทธิ์ต้านการอักเสบของสิ่งสกัดจากรากสมุนไพรทั้งห้าชนิดของตำรับยาเบญจมูลใหญ่. (ANTINOCICEPTIVE AND ANTI-INFLAMMATORY EFFECTS OF FIVE ROOT EXTRACTS OF BEN-CHA-MOON-YAI REMEDY) อ.ที่ปรึกษาวิทยานิพนธ์หลัก : ผศ. ร.ท.หญิง ดร. ภัสราภา โตวิวัฒน์, อ. ที่ปรึกษาร่วม : รศ. ดร. นิจศิริ เรืองรังษี, 210 หน้า.

ตำรับยาเบญจมูลใหญ่เป็นตำรับยาแพทย์แผนไทยโบราณมีสรรพคุณในการลดไข้และแก้อักเสบ ประกอบไปด้วยรากมะตูม รากเพกา รากลำไย รากแคแตร และรากคัดลิ้น การทดลองครั้งนี้เริ่มต้นศึกษาฤทธิ์ระงับปวด ของสิ่งสกัดรวมของตำรับยาเบญจมูลใหญ่ (BMY) สิ่งสกัดจากรากของ *Aegle marmelos* (AM), *Oroxylum indicum* (OI), *Dimocarpus* longan (DL), *Dolichandrone serrulata* (DS) และ *Walsura trichostemon* (WT) เมื่อให้โดยการป้อนด้วยวิธี hot-plate ในหนูเมาส์ โดยจับเวลาที่หนูเมาส์สามารถทนอยู่บนแผ่นความร้อนได้ (hot-plate latencies) ก่อนให้น้ำเกลือ และมอร์ฟีน (10 มก./กก.) ทางช่องท้อง หรือ 2% Tween 80, BMY ขนาดต่างๆ (125, 250 และ 500 มก./กก.) AM, OI, DL, DS และ WT ขนาดต่างๆ (25, 50, 100, 200 และ 400 มก./กก.) โดยการป้อน พบว่า BMY ทุกขนาดที่ให้ AM (400 มก./กก.), DS (200 และ 400 มก./กก.) และ WT (100-400 มก./กก.) มีฤทธิ์ระงับปวดอย่างมีนัยสำคัญทางสถิติ ซึ่งฤทธิ์ระงับปวดนั้นถูกยับยั้งได้ด้วยนาลอกโซน แสดงว่ากลไกการออกฤทธิ์ระงับปวดน่าจะเกี่ยวข้องกับตัวรับ opioid ในการทดลองที่เหนี่ยวนำให้หนูเมาส์เกิดความเจ็บปวดด้วยฟอร์มาลิน ทำการฉีด 2.5% ฟอร์มาลิน ปริมาตร 20 ไมโครลิตร เข้าที่บริเวณอุ้งเท้าหลังด้านซ้ายของหนูเมาส์หลังจากให้น้ำเกลือและมอร์ฟีนทางช่องท้อง หรือหลังจากให้ 2% Tween 80 อินโดเมทาซิน (IND; 10 มก./กก.) BMY, AM, OI, DL, DS และ WT ขนาดต่างๆ โดยการป้อน พบว่า BMY (250 มก./กก.), AM, DS และ WT (400 มก./กก.) ทำให้หนูใช้เวลาเลียอุ้งเท้าลดลงอย่างมีนัยสำคัญทางสถิติในระยะแรก ในขณะที่ BMY ทุกขนาดที่ให้ AM (400 มก./กก.), OI (100-400 มก./กก.), DL, DS และ WT (200 และ 400 มก./กก.) ทำให้หนูใช้เวลาเลียอุ้งเท้าลดลงอย่างมีนัยสำคัญทางสถิติในระยะหลัง ในการทดสอบฤทธิ์ระงับปวดโดยเหนี่ยวนำให้หนูเมาส์เกิดความเจ็บปวดจนเกิดอาการบิดงอลำตัว (writhing) ด้วยกรดอะซิติก ทำการฉีดกรดอะซิติก 0.6 % (10 มล./กก.) เข้าทางช่องท้องหลังจากให้ 2% Tween 80 อินโดเมทาซิน BMY, AM, OI, DL, DS และ WT ขนาดต่างๆ โดยการป้อน พบว่า BMY ทุกขนาดที่ให้ AM, DS และ WT (200 และ 400 มก./กก.) OI และ DL (100-400 มก./กก.) สามารถลดจำนวนครั้งของการบิดงอลำตัวของหนูได้อย่างมีนัยสำคัญทางสถิติเมื่อเปรียบเทียบกับกลุ่มควบคุม ในการทดสอบด้วย rota-rod พบว่า BMY, AM, OI, DL, DS และ WT ในขนาดสูงสุดไม่มีผลต่อการทรงตัวของหนูเมาส์หลังจากให้สิ่งสกัดโดยการป้อน

หลังจากนั้นทำการประเมินฤทธิ์ต้านการอักเสบของ BMY, AM, OI, DL, DS และ WT ที่ให้โดยการป้อน ด้วยการเหนี่ยวนำให้อุ้งเท้าหนูเมาส์บวมด้วยคาราจีแนน พบว่า BMY ทุกขนาดที่ให้ AM (400 มก./กก.) OI, DL, DS (200 และ 400 มก./กก.) และ WT (25-400 มก./กก.) สามารถลดการบวมของอุ้งเท้าหนูเมาส์ได้อย่างมีนัยสำคัญทางสถิติในระยะที่ 2 ของการบวม แสดงถึงการยับยั้งพรอสตาแกลนดิน (PGs) ส่วนการเหนี่ยวนำให้อุ้งเท้าหนูเมาส์บวมด้วยพรอสตาแกลนดินอี 2 พบว่าสิ่งสกัดจากรากทั้งห้าชนิดในขนาดสูงสุด สามารถลดการบวมของอุ้งเท้าที่เวลา 0.5-1.5 ชั่วโมงหลังจากฉีดพีจีอี 2 ได้อย่างมีนัยสำคัญทางสถิติ จากผลการทดสอบฤทธิ์ระงับปวดทั้งหมดสนับสนุนว่า BMY, AM, DS และ WT ออกฤทธิ์ระงับปวดได้ทั้งในระบบประสาทส่วนกลางและระบบประสาทส่วนปลาย กลไกการออกฤทธิ์ระงับปวดมีความเกี่ยวข้องกับตัวรับ opioid ส่วนกลไกในการต้านการอักเสบของสิ่งสกัดจากรากทั้งห้าชนิดอาจเกี่ยวข้องกับการรบกวนการหลั่งพรอสตาแกลนดินหรือการยับยั้งผลของพีจีอี 2

ภาควิชา	เภสัชวิทยาและสรีรวิทยา	ลายมือชื่อนิสิต	
สาขาวิชา	เภสัชวิทยา	ลายมือชื่อ อ. ที่ปรึกษา	
ปีการศึกษา	2554	ลายมือชื่อ อ. ที่ปรึกษาร่วม	

5376585033 : MAJOR PHARMACOLOGY

KEYWORDS : BEN-CHA-MOON-YAI / *AEGLE MARMELOS* / *OROXYLUM INDICUM* / *DIMOCARPUS LONGAN* / *DOLICHANDRONE SERRULATA* / *WALSURA TRICHOSTEMON* / ANTINOCICEPTION / ANTI-INFLAMMATORY / HOT-PLATE TEST / FORMALIN TEST / ACETIC ACID-INDUCED WRITHING / CARRAGEENAN-INDUCED PAW EDEMA / PROSTAGLANDIN E_2-INDUCED PAW EDEMA

CHAYANIN KIRATIPAIBOON: ANTINOCICEPTIVE AND ANTI-INFLAMMATORY EFFECTS OF FIVE ROOT EXTRACTS OF BEN-CHA-MOON-YAI REMEDY. ADVISOR: ASST. PROF. FLG. OFF. PASARAPA TOWIWAT, Ph.D., CO-ADVISOR: ASSOC.PROF. NIJSIRI RUANGRUNGSI, Ph.D., 210 pp.

Ben-Cha-Moon-Yai remedy is an antipyretic and anti-inflammatory drug in Thai traditional medicine which includes roots of Ma-tum, Phe-ka, Lam-yai, Chare-tare and Khad-lin. We initially determined the antinociceptive property of orally administered the root extracts of Ben-Cha-Moon-Yai remedy (BMY), *Aegle marmelos* (AM), *Oroxylum indicum* (OI), *Dimocarpus longan* (DL), *Dolichandrone serrulata* (DS) and *Walsura trichostemon* (WT) in the mouse hot-plate test. Hot-plate latencies were determined in male ICR mice prior to the intraperitoneal administration of normal saline solution, morphine (MO; 10 mg/kg) or oral administration of 2% Tween 80, various doses of BMY (125, 250 and 500 mg/kg), AM, OI, DL, DS and WT (25, 50, 100, 200 and 400 mg/kg. All doses of BMY, AM (400 mg/kg), DS (200 and 400 mg/kg) and WT (100-400 mg/kg) produced significant analgesic responses that were naloxone-sensitive suggesting opioid-mediated mechanism. In the formalin-induced nociception test, 2.5% formalin (20 µl) was injected into the plantar surface of the left hind paw of each mouse after intraperitoneal administration of NSS and MO or oral administration of 2% Tween 80, indomethacin (IND; 10 mg/kg), various doses of BMY, AM, OI, DL, DS and WT. BMY (250 mg/kg), AM, DS and WT (400 mg/kg) significantly decreased time spent on paw licking during the early phase, while BMY at all doses tested, AM (400 mg/kg), OI (100-400 mg/kg), DL, DS and WT (200-400 mg/kg) significantly decreased time spent on paw licking during the late phase. In the acetic acid-induced writhing response in mice, animals were induced with intraperitoneal injection of 0.6% acetic acid (10 ml/kg) after oral administration of 2% Tween 80, IND, various doses of BMY, AM, OI, DL, DS and WT. All doses of BMY, AM, DS and WT (200 and 400 mg/kg), OI and DL (100-400 mg/kg) significantly decreased the number of writhes compared to vehicle controls. In rota-rod test, the highest doses of BMY, AM, OI, DL, DS and WT did not produce any motor dysfunction in mice after oral administration.

Studies then determined the anti-inflammatory property of orally administered BMY, AM, OI, DL, DS and WT using carrageenan-induced paw edema model in mice. All doses of BMY, AM (400 mg/kg), OI, DL and DS (200 and 400 mg/kg) and WT (25-400 mg/kg) significantly reduced mouse paw edema during the second phase of edema suggesting inhibition of prostaglandins (PGs). In prostaglandin E_2–induced mouse paw edema test, the highest dose of AM, OI, DL, DS and WT significantly reduced paw edema during 0.5-1.5 hr after PGE_2 administration. Altogether, these results support the antinociceptive effects through both central and peripheral mechanisms of BMY, AM, DS and WT. The analgesic mechanism of action seems to be partly related to opioid receptors. The mechanism of anti-inflammatory effect may be due to the interference of all five root extracts on the liberation of PGs or inhibition of PGE_2 effects.

Department: Pharmacology and Physiology Student's Signature ....

Field of Study : Pharmacology Advisor's Signature ....

Academic Year : 2011 Co-advisor's Signature ....

ACKNOWLEDGEMENTS

The author wish to express her sincere gratitude and appreciation to her advisor, Assistant Professor Flight Officer Dr. Pasarapa Towiwat, for her intensive advice, guidance, suggestions and encouragement throughout her study. The author wish to thank Associate Professor Dr. Nijsiri Ruangrungsi, her thesis co-advisor for his valuable advice and providing all five herbal roots extracts for this study. Thanks are also extended to the committee members.

The author wish to thank Ms. Rawiwan Manohan for her help with the extractions and identification of the herbal extracts for this study and Mr. Somkit Bansuttee for his advice. The author would like to thank all staff members in the Department of Pharmacology and Physiology, Faculty of Pharmaceutical Sciences, Chulalongkorn University for their help and support.

The author also would like to thank her lab partners especially Anusara Jongchanapong and Jutirat Wongsrisakul, and her friends for their help, advice and encouragement throughout her thesis study.

Finally, the author would like to thank her parents and her family for everything in her life.

CONTENTS

	Page
ABSTARCT (THAI)…………………………………………………………………	iv
ABSTRACT (ENGLISH)...	v
ACKNOWNLEDGEMENTS...	vi
CONTENTS..	vii
LIST OF TABLES………………………………………………………………	ix
LIST OF FIGURES……………………………………………………………	xvi
LIST OF ABBREVIATIONS…………………………………………………....	xxii
CHAPTER	
I INTRODUCTIONS…………………………………........................	1
Background and Rationale…………………………………..	1
Purpose of the study……………………………………........	3
Hypothesis…………………………………………………......	3
Research design……………………………………………	3
Expected Benefit and Application....................................	3
Key words……………………………………………………	3
II LITERATURE REVIEW……………………………………………	4
Pain..	4
Pharmacological management of pain……………………..	14
Inflammation....................................	19
Pharmacological management of inflammation……………	27
Animal models……………………………………………...	28
Ben-Cha-Moon-Yai remedy…………………………………..	35
Aegle marmelos Corr…………………………………….	36
Oroxylum indicum Vent…………………………………...	43
Dimocarpus longan Lour………………………………….	50

CHAPTER		Page
	Dolichandrone serrulata (DC) Seem…………………………..	57
	Walsura trichostemon Miq…………………………………	59
III	MATERIALS AND METHODS………………………………………	61
	Experimental Animals………………………………………..	61
	Drugs……………………………………………………………..	61
	Experimental methods................................ ………………	62
	Data treatment and statistical analysis.......……………….	69
IV	RESULTS………………………………………………….........	70
	Mouse Hot-plate Test………………………………………..	70
	Formalin Test in mice………………………………………..	91
	Acetic acid induced writhing in mice……………………...	109
	Rota-rod test in mice………………………………………...	123
	Carrageenan-induced paw edema Test…………….......	125
	Prostaglandin E_2-induced paw edema Test………………	136
V	DISSUSION & CONCLUSION...	145
REFERENCES………………………………………………………………...		154
APPENDICES..		170
	APPENDIX A .... ………	171
	APPENDIX B..	173
	APPENDIX C..	181
	APPENDIX D...	200
	APPENDIX E..	207
VITAE.. …………………………………		210

.

LIST OF TABLES

Table		Page
1	Functional effects associated with the main types of opioids receptor …..	18
2	Time spent on paw licking after intraperitoneal administration 0.9% normal saline solution (NSS; 10 ml/kg) and morphine sulfate (MO; 10 mg/kg)……………………………………………………………….	102
3	Time spent on paw licking after oral administration of 2% Tween 80 (10 ml/kg), indomethacin (IND; 10 mg/kg) and various doses of Ben-Cha-Moon-Yai remedy (BMY; 125-500 mg/kg)………………………………	103
4	Time spent on paw licking after oral administration of 2% Tween 80 (10 ml/kg), indomethacin (IND; 10 mg/kg) and various doses of *Aegle marmelos* root extract (AM; 25-400 mg/kg)…………………………	104
5	Time spent on paw licking after oral administration of 2% Tween 80 (10 ml/kg), indomethacin (IND; 10 mg/kg) and various doses of *Oroxylum indicum* root extract (OI; 25-400 mg/kg)…………………………	105
6	Time spent on paw licking after oral administration of 2% Tween 80 (10 ml/kg), indomethacin (IND; 10 mg/kg) and various doses of *Dimocarpus longan* root extract (DL; 25-400 mg/kg)……………………….	105
7	Time spent on hind paw licking after oral administration of 2% Tween 80 (10 ml/kg), indomethacin (IND; 10 mg/kg) and various doses of *Dolichandrone serrulata* root extract (DS; 25-400 mg/kg)………………….	107
8	Time spent on paw licking after oral administration of 2% Tween 80 (10 ml/kg), indomethacin (IND; 10 mg/kg) and various doses of *Walsura trichostemon* root extract (WT; 25-400 mg/kg)………………………………	108
9	Change of edema volume (ml) of oral administration of 2% Tween 80, indomethacin (IND; 10 mg/kg) and various doses of the root extract of Ben-Cha-Moon-Yai remedy (BMY; 125-500 mg/kg) from 1-6 hr after carrageenan administration…………………………………………………....	128

Table		Page
10	Change of edema volume (ml) of oral administration of 2% Tween 80, indomethacin (IND; 10 mg/kg) and *Aegle marmelos* root extract (AM; 25-400 mg/kg) from 1-6 hr after carrageenan administration……………..	129
11	Change of edema volume (ml) of oral administration of 2% Tween 80, indomethacin (IND; 10 mg/kg) and various doses of *Oroxylum indicum* root extract (OI; 25-400 mg/kg) from 1-6 hr after carrageenan administration…………………………………………………………………	130
12	Change of edema (ml) volume of oral administration of 2% Tween 80, indomethacin (IND; 10 mg/kg) and various doses of *Dimocarpus longan* root extract (DL; 25-400 mg/kg) from1-6 hr after carrageenan administration………………………………………………………………….	131
13	Change of edema volume (ml) of oral administration of 2% Tween 80 (10 ml/kg), indomethacin (IND; 10 mg/kg) and various doses of *Dolichandrone serrulata* root extract (DS; 25-400 mg/kg) from1-6 hr after carrageenan administration…………………………………………………	132
14	Change of edema volume (ml) of oral administration of 2%Tween 80 (10 ml/kg), indomethacin (IND; 10 mg/kg) and various doses of Walsura trichostemon root extract (WT; 25-400 mg/kg) from 1-6 hr after carrageenan administration………………………………………………....	133
15	Change of edema volume (ml) at the maximum percentage of inhibition of Ben-Cha-Moon-Yai remedy (BMY; 125, 250 and 500 mg/kg), Aegle marmelos root extract (AM; 25, 50 and 100 mg/kg), Oroxylum indicum root extract (OI; 25, 50 and 100 mg/kg), Dimocarpus longan root extract (DL; 25, 50 and 100 mg/kg), Dolichandrone serrulata root extract (DS; 25, 50 and 100 mg/kg) and Walsura trichostemon root extract (WT; 25, 50 and 100 mg/kg)……………………………………………………………	135

Table Page

16 Change of edema volume (ml) of oral administration of 2% Tween 80 (10 ml/kg), indomethacin (IND; 10 mg/kg) and Aegle marmelos root extract (AM; 400 mg/kg) from 0.5-4 hr after prostaglandin E2 administration……. 138

17 Change of edema volume (ml) of oral administration of 2% Tween 80 (10 ml/kg), indomethacin (10 mg/kg) and Oroxylum indicum root extract (OI; 400 mg/kg) after prostaglandin E_2administration…………………………... 139

18 Change of edema volume (ml) of oral administration of 2% Tween 80 (10 ml/kg), indomethacin (IND; 10 mg/kg) and Dimocarpus longan root extract (DL; 400 mg/kg) from 0.5-4 hr after prostaglandin E_2 administration………………………………………………………………… 140

19 Change of edema volume (ml) of oral administration of 2% Tween 80 (10 ml/kg), indomethacin (10 mg/kg) and Dolichandrone serrulata root extract (DS; 400 mg/kg) from 0.5-4 hr after prostaglandin E_2 administration………………………………………………………………... 141

20 Change of edema volume (ml) of oral administration of 2% Tween 80 (10 ml/kg), indomethacin (10 mg/kg) and Walsura trichostemon root extract (WT; 400 mg/kg) from 0.5-4 hr after prostaglandin E_2 administration…….. 142

21 Summary of the effective doses of the root extract of Ben-Cha-Moon-Yai remedy (BMY), Aegle marmelos root extract (AM), Oroxylum indicum root extract (OI), Dimocarpus longan root extract (DL), Dolichandrone serrulata root extract (DS) and Walsura trichostemon root extract (WT) in various of antinociceptive and anti-inflammatory activity testing models……………………………………………………………………………… 144

22 Latency (sec) in the mouse hot-plate test from 0-240 min after oral administration of the root extract of Ben-Cha-Moon-Yai remedy (BMY; 125-500 mg/kg)………………………………………………………………… 182

23 Latency (sec) in the mouse hot-plate test from 0-240 min after oral administration of Aegle marmelos root extract (AM; 25-400 mg/kg)……... 183

Table Page

24 Latency (sec) in the mouse hot-plate test from 0-240 min after oral administration of Oroxylum indicum root extract (OI; 25-400 mg/kg)…….. 184

25 Latency (sec) in the mouse hot-plate test from 0-240 min after oral administration of Dimocarpus longan root extract (DL; 25-400 mg/kg)….. 185

26 Latency (sec) in the mouse hot-plate test from 0-240 min after oral administration of Dolichandrone serrulata root extract (DS; 25-400 mg/kg)………………………………………………………………………… 186

27 Latency (sec) in the mouse hot-plate test from 0-240 min after oral administration of Walsura trichostemon root extract (WT; 25-400 mg/kg).. 187

28 %MPE-Time in the mouse hot-plate test from 0-240 min after oral administration of various doses of the root extract of Ben- Cha-Moon-Yai remedy (BMY; 125-500 mg/kg)……………………………………………….. 188

29 %MPE-Time in the mouse hot-plate test from 0-240 min after oral administration of various doses of Aegle marmelos root extract (AM; 25-400 mg/kg)………………………………………………………………………. 189

30 %MPE-Time in the mouse hot-plate test from 0-240 min after oral administration of various doses of Oroxylum indicum root extract (OI; 25-400 mg/kg)………………………………………………………………………. 190

31 %MPE-Time in the mouse hot-plate test from 0-240 min after oral administration of various doses of Dimocarpus longan root extract (DL; 25-400 mg/kg)…………………………………………………………………... 191

32 %MPE-Time in the mouse hot-plate test from 0-240 min after oral administration of various doses of Dolichandrone serrulata root extract (DS; 25-400 mg/kg)…………………………………………………………….. 192

33 %MPE-Time in the mouse hot-plate test from 0-240 min after oral administration of various doses of Walsura trichostemon root extract (WT; 25-400 mg/kg)…………………………………………………………….. 193

Table		Page
34	Latency (sec) in the mouse hot-plate test from 0-240 min after administration of naloxone (5 mg/kg; i.p.), 2% Tween 80 (10 ml/kg; p.o.), Aegle marmelos root extract (AM; 400 mg/kg; p.o.) and the combination of naloxone and Aegle marmelos root extract (5/400 mg/kg)……………..	194
35	Latency (sec) in the mouse hot-plate test from 0-240 min after administration of naloxone (5 mg/kg; i.p.), 2% Tween 80 (10 ml/kg; p.o.), Dolichandrone serrulata root extract (DS; 200 mg/kg; p.o.) and the combination of naloxone and Dolichandrone serrulata root extract (5/200 mg/kg)……………………………………………………………………….	195
36	Latency (sec) in the mouse hot-plate test from 0-240 min after administration of NSS (10 ml/kg, i.p.), naloxone (5 mg/kg; i.p.), 2% Tween 80 (10 ml/kg; p.o.), Walsura trichostemon root extract (WT; 400 mg/kg, p.o.) and the combination of naloxone and Walsura trichostemon root extract (5/400 mg/kg)…………………………………………………......	196
37	%MPE-Time in the mouse hot-plate test from 0-240 min after administration of naloxone (5 mg/kg; i.p.), 2% Tween 80 (10 ml/kg; p.o.), Aegle marmelos root extract (AM; 400 mg/kg, p.o.) and the combination of naloxone and Aegle marmelos root extract (5/400 mg/kg)……………..	197
38	%MPE-Time in the mouse hot-plate test from 0-240 min after administration of naloxone (5 mg/kg; i.p.), 2% Tween 80 (10 ml/kg; p.o.), Dolichandrone serrulata root extract (DS; 200 mg/kg; p.o.) and the combination of naloxone and Dolichandrone serrulata root extract (5/200 mg/kg)………………………………………………………..........………	198
39	%MPE-Time in mouse hot-plate test from 0-240 min after administration of NSS (10 ml/kg, i.p.), naloxone (5 mg/kg; i.p.), 2% Tween 80 (10 ml/kg; p.o.), Walsura trichostemon root extract (WT; 400 mg/kg; p.o.) and the combination of naloxone and Walsura trichostemon root extract (5/400 mg/kg)……………………………………………………………………….	199

Table		Page
40	Dose-response and time-course effect of 2%Tween 80, indomethacin (IND; 10 mg/kg) and various doses of the root extract of Ben-Cha-Moon-Yai remedy (BMY) on acetic acid-induced writhing in mice……………….	201
41	Dose-response and time-course of 2%Tween 80, indomethacin (IND; 10 mg/kg) and various doses of Aegle marmelos root extract (AM) on acetic acid-induced writhing in mice……………………………………...	202
42	Dose-response and time-course effect of 2%Tween 80, indomethacin (IND; 10 mg/kg) and various doses of Oroxylum indicum root extract (OI) on acetic acid-induced writhing in mice………………………………..	203
43	Dose-response and time-course effect of 2%Tween 80, indomethacin (IND; 10 mg/kg) and various doses of Dimocarpus longan root extract (DL) on acetic acid-induced writhing in mice………………………………..	204
44	Dose-response and time-course effect of 2%Tween 80, indomethacin (IND; 10 mg/kg) and various doses of Dolichandrone serrulata root extract (DS) on acetic acid-induced writhing in mice……………………….	205
45	Dose-response and time-course effect of 2%Tween 80, indomethacin (IND; 10 mg/kg) and various doses of Walsura trichostemon root extract (WT) on acetic acid-induced writhing in mice……………………………….	206
46	Effect of the root extract of Ben-Cha-Moon-Yai remedy (BMY; 500 mg/kg; p.o.) in the rota-rod test……………………………………………….	208
47	Effect of Aegle marmelos root extract (AM; 400 mg/kg; p.o.) in the rota-rod test………………………………………………………………………..	208
48	Effect of Oroxylum indicum root extract (OI; 400 mg/kg; p.o.) in the rota-rod test……………………………………………………………………….	208
49	Effect of Dimocarpus longan root extract (DL; 400 mg/kg; p.o.) in the rota-rod test………………………………………………………………..	209
50	Effect of Dolichandrone serrulata root extract (DS; 400 mg/kg; p.o.) in the rota-rod test…………………………………………………………….	209

Table Page

51 Effect of Walsura trichostemon root extract (WT; 400 mg/kg; p.o.) in the rota-rod test…………………………………………………………………….. 209

LIST OF FIGURES

Figure		Page
1	Primary afferents with cell bodies in the dorsal root ganglion, include those with large-diameter myelinated (Aβ), small-diameter myelinated (Aδ) and unmyelinated (C) axons…………………………………………	7
2	Chemical mediators of injured and inflamed tissues……………………	8
3	Modulation of pain at several different central nervous system levels....	13
4	The gate control theory of pain…………………………………………..	14
5	The multistep process of leukocyte migration through blood vessels…	22
6	The process of phagocytosis and production of reactive oxygen intermediates within phagocytic vesicles…………………………………	23
7	Biosynthesis of the prostaglandins, thromboxanes and leukotrienes by the cyclooxygenase and lipooxygenase pathway………………………..	25
8	Fruit and root of Aegle marmelos Corr……………………………………	36
9	Fruit, flowers and root of Oroxylum indicum Vent………………………...	43
10	Fruit and root of Dimocarpus longan Lour………………………………..	50
11	Flower, fruit and leaves of Dolichandrone serrulata DC. Seem…………	57
12	Fruits and flowers of Walsura trchostemon Miq…………………………..	59
13	Hot-Plate Analgesiometer……………………………………………………	63
14	Formalin-induced paw licking in mouse…………………………………...	65
15	Writhing response in mouse……………………………………………...	66
16	Rota-rod test in mice………………………………………………………	67
17	Plethysmometer…………………………………………………………….	68
18	Area of analgesia (%MPE-min) from 0-240 minutes after intraperitoneal administration of normal saline solution (NSS; 10 ml/kg) and morphine sulphate (MO; 10 mg/kg)……………………………………..………..	72

Figure		Page
19	Area of analgesia (%MPE-min) from 0-240 minutes after oral administration of 2% Tween 80 (10 ml/kg) and various doses of the root extract of Ben-Cha-Moon-Yai remedy (BMY; 125-500 mg/kg)…...	73
20	Individual time courses of the response (%MPE versus time (min)) after oral administration of various doses of the root extract of Ben-Cha-Moon-Yai remedy (BMY; 125-500 mg/kg)…………………………	74
21	Area of analgesia (%MPE-min) from 0-240 minutes after oral administration of 2% Tween 80 and various doses of Aegle marmelos root extract (AM; 25-400 mg/kg)…………………………………………..	75
22	Individual time courses of the response (%MPE versus time (min)) after oral administration of various doses of Aegle marmelos root extract (AM; 25-400 mg/kg)………………………………………………..	76
23	Area of analgesia (%MPE-min) from 0-240 minutes after oral administration of 2% Tween 80 and various doses of Oroxylum indicum root extract (OI; 25-400 mg/kg)………………………………….	77
24	Individual time courses of the response (%MPE versus time (min)) after oral administration of various doses of Oroxylum indicum root extract (OI; 25-400 mg/kg)………………………………………………...	78
25	Area of analgesia (%MPE-min) from 0-240 minutes after oral administration of 2% Tween 80 and various doses of Dimocarpus longan root extract (DL; 25-400 mg/kg)………………………………….	79
26	Individual time courses of the response (%MPE versus time (min)) after oral administration of various doses of Dimocarpus longan root extract (DL; 25-400 mg/kg)………………………………………………...	80
27	Area of analgesia (%MPE-min) from 0-240 minutes after oral administration of 2% Tween 80 and various doses of Dolichandrone serrulata root extract (DS; 25-400 mg/kg)………………………………..	81

Figure

28 Individual time courses of the response (%MPE versus time (min)) after oral administration of various doses of Dolichandrone serrulata root extract (DS; 25-400 mg/kg)………………………………………………….... 82

29 Area of analgesia (%MPE-min) from 0-240 minutes after oral administration of 2% Tween 80 and various doses of Walsura trichostemon root extract (WT; 25-400 mg/kg)……………………….……. 83

30 Individual time courses of the response (%MPE versus time (min)) after oral administration of various doses of Walsura trichostemon root extract (WT; 25-400 mg/kg)……………………………………………………………….. 84

31 Area of analgesia (%MPE-min) from 0-240 minutes after administration of 2%Tween 80 (10 ml/kg, p.o.), naloxone (NAL; 5 mg/kg, i.p.), Aegle marmelos root extract (AM; 400 mg/kg, p.o.) and the combination of naloxone and AM (5/400 mg/kg)…………………………………..……….. 85

32 Individual time courses of the response (%MPE versus time (min)) after administration of 2% Tween 80 (p.o.), naloxone 5 mg/kg (i.p.), Aegle marmelos root extract 400 mg/kg (AM, p.o.) and the combination of naloxone and AM (5/400 mg/kg)…………………………………………….... 86

33 Area of analgesia (%MPE-min) from 0-240 minutes after administration of 2% Tween 80 (10 ml/kg, p.o.), naloxone (NAL; 5 mg/kg, i.p.), Dolichandrone serrulata root extract (DS; 200 mg/kg, p.o.) and the combination of naloxone and DS (5/200 mg/kg)……………………………. 87

34 Individual time courses of the response (%MPE versus time (min)) after administration of 2% Tween80 (p.o.), naloxone 5 mg/kg (i.p.), Dolichandrone serrulata root extract 200 mg/kg (DS, p.o.) and the combination of naloxone and DS (5/200 mg/kg)…………………….……... 88

Figure

35 Area of analgesia (%MPE-min) from 0-240 minutes after administration of 2% Tween 80 (10 ml/kg, p.o.), naloxone (NAL; 5 mg/kg, i.p.), Walsura trichostemon root extract (WT; 400 mg/kg, p.o.) and the combination of naloxone and WT (5/400 mg/kg)………………………………………...……. 89

36 Individual time courses of the response (%MPE versus time (min)) after administration of 2% Tween 80 (p.o.), naloxone 5 mg/kg (i.p.), Walsura trichostemon root extract 400 mg/kg (WT, p.o.) and the combination of naloxone and WT (5/400 mg/kg)…………………………………………..…. 90

37 Time spent on paw licking after intraperitoneal administration of 0.9% normal saline solution (NSS; 10 ml/kg) and morphine sulphate (MO; 10 mg/kg)……………………………………………………………………………. 95

38 Time spent on paw licking after oral administration of 2% Tween 80 (10 ml/kg), indomethacin (IND; 10 mg/kg) and various doses of the root extract of Ben-Cha-Moon-Yai remedy (BMY; 125-500 mg/kg)………….... 96

39 Time spent on paw licking after oral administration of 2% Tween 80 (10 ml/kg), indomethacin (IND; 10 mg/kg) and various doses of Aegle marmelos root extract (AM; 25-400 mg/kg)………………………………….. 97

40 Time spent on paw licking after oral administration of 2% Tween 80 (10 ml/kg), indomethacin (IND; 10 mg/kg) and various doses of Oroxylum indicum root extract (OI; 25-400 mg/kg)……………………………….….. 98

41 Time spent on paw licking after oral administration of 2% Tween 80 (10 ml/kg), indomethacin (IND; 10 mg/kg) and various doses of Dimocarpus longan root extract (DL; 25-400 mg/kg)……………………………………... 99

42 Time spent on hind paw licking after oral administration of 2% Tween 80 (10 ml/kg), indomethacin (IND; 10 mg/kg) and various doses of Dolichandrone serrulata root extract (DS; 25-400 mg/kg)…….………….. 100

Figure

43	Time spent on paw licking after oral administration of 2% Tween 80 (10 ml/kg), indomethacin (IND; 10 mg/kg) and various doses of Walsura trichostemon root extract (WT; 25-400 mg/kg)……………………………...	101
44	Mean number of writhes after oral administration of 2% Tween 80 (10 ml/kg), indomethacin (IND; 10 mg/kg) and various doses of the root extract of Ben-Cha-Moon-Yai remedy (BMY; 125-500 mg/kg)……..……...	111
45	Mean number of writhes after oral administration of 2% Tween 80 (10 ml/kg), indomethacin (IND; 10 mg/kg) and various doses of Aegle marmelos root extract (AM; 25-400 mg/kg)……………………….…………	112
46	Mean number of writhes after oral administration of 2% Tween 80 (10 ml/kg), indomethacin (IND; 10 mg/kg) and various doses of Oroxylum indicum root extract (OI; 25-400 mg/kg)…………………..…………………	113
47	Mean number of writhes after oral administration of 2% Tween 80 (10 ml/kg), indomethacin (IND; 10 mg/kg) and various doses of Dimocarpus longan root extract (DL; 25-400 mg/kg)………………………………….…..	114
48	Mean number of writhes after oral administration of 2% Tween 80 (10 ml/kg), indomethacin (IND; 10 mg/kg) and various doses of Dolichandrone serrulata root extract (DS; 25-400 mg/kg)……………..….	115
49	Mean number of writhes after oral administration of 2% Tween 80 (10 ml/kg), indomethacin (IND; 10 mg/kg) and various doses of Walsura trichostemon root extract (WT; 25-400 mg/kg)…………………………….….	116
50	Area of analgesia (%MPE-min) from 0-240 minutes of the root extract of Ben-Cha-Moon-Yai remedy (BMY), Aegle marmelos root extract (AM), Oroxylum indicum root extract (OI), Dimocarpus longan root extract (DL), Dolichandrone serrulata root extract (DS) and Walsura trichostemon root extract (WT)………………………………………………..	118

Figure Page

Figure		Page
51	Time spent on paw licking of the root extract of Ben-Cha-Moon-Yai remedy (BMY), Aegle marmelos root extract (AM), Oroxylum indicum root extract (OI), Dimocarpus longan root extract (DL), Dolichandrone serrulata root extract (DS) and Walsura trichostemon root extract (WT)……………………………………………………………………………….	120
52	Mean number of writhes of the root extract of Ben-Cha-Moon-Yai remedy (BMY), Aegle marmelos root extract (AM), Oroxylum indicum root extract (OI), Dimocarpus longan root extract (DL), Dolichandrone serrulata root extract (DS) and Walsura trichostemon root extract (WT)……………………………………………………………………………….	122
53	Rota-rod latency of each extract after oral administration compared to 2% Tween 80 (10 ml/kg)……………………………………………………….	124
54	Thin-layer chromatogram of the methanolic extract of Aegle marmelos root……………………………………………………………………………….	176
55	Thin-layer chromatogram of the methanolic extract of Oroxylum indicum root……………………………………………………………………………….	177
56	Thin-layer chromatogram of the methanolic extract of Dimocarpus longan root……………………………………………………………………...	178
57	Thin-layer chromatogram of the methanolic extract of Dolichandrone serrulata root………………………………………………………………….…	179
58	Thin-layer chromatogram of the methanolic extract of Walsura trichostemon root………………………………………………………………	180

LIST OF ABBREVIATIONS

α	=	alpha
β	=	beta
κ	=	kappa
δ	=	delta
µl	=	microliter
µM	=	micro molar
/	=	per
%	=	percent
%MPE	=	percentage of the maximum possible effect
ºC	=	Celsius degree
ABTS	=	2, 2-azinobis-3-ethylbenzothiazoline-6-sulphonate
AM	=	*Aegle marmelos* root extract
AP-1	=	activator protein
AUC	=	area under the curves (area of analgesia)
BDNF	=	Brain-derived neurotrophic factor
BMY	=	the extract of Ben-Cha-Moon-Yai remedy
cm	=	centimeter
CNCP	=	Chronic noncancer pain
CNS	=	central nervous system
Co.	=	company
CPS	=	chronic pain syndrome
CR	=	corticosteroid receptor
CREB	=	cAMP response element binding protein
DL	=	*Dimocarpus longan* root extract
DPPH	=	diphenyl-picryl-hydrazyl
DRG	=	dorsal root ganglia
DS	=	*Dolichandrone serrulata* root extract
ED_{50}	=	median effective dose
e.g.	=	example gratia
et al.	=	et alii (and other)

FRAP	=	ferric-reducing antioxidant power assay
g	=	gram
GFPMA	=	green fluorescent protein microplate assay
GRE	=	glucocorticoid response element
Gy	=	Gray
hr	=	hour
IASP	=	International Association for the Study of Pain
IC_{50}	=	median inhibition dose
i.e.	=	id est
IL	=	interleukin
IND	=	indomethacin
i.p.	=	intraperitoneal
LPS	=	Lipopolysaccharide
m	=	meter
MES	=	maximal electroshock
mg/kg	=	milligram per kilogram
mg/ml	=	milligram per milliliter
min	=	minute
ml	=	milliliter
ml/kg	=	milliliter per kilogram
MO	=	morphine sulphate
mm	=	millimeter
m/sec	=	meter per second
N	=	sample size
NAL	=	naloxone
NF-κB	=	nuclear factor kappa B
NMDA	=	N-methyl-D-aspartate
NO	=	nitric oxide
NOS	=	nitric oxide synthase
NRM	=	nucleus raphe magnus
NSAIDs	=	non-steroidal anti-inflammatory drugs
NSS	=	normal saline
OI	=	*Oroxylum indicum* root extract
PAG	=	periaqueductal gray

PGE_2	=	prostaglandin E_2
p.o.	=	per os
PTZ	=	pentylenetetrazole
REMA	=	resazurin microplate assay
sec	=	second
STATs	=	signal transducer and activator of transcription
TBARS	=	thiobarbituric acid-reactive substances
TNF-α	=	tumor necrosis factor-alpha
TPC	=	total phenolic content
vs	=	versus
WT	=	*Walsura trichostemon* root extract
w/w	=	weight by weight

CHAPTER I

INTRODUCTION

Background and Rationale

Pain is a subjective experience, hard to define exactly. Pain can affect all areas of a person's life including sleep, thought, emotion and activities of daily living. Additionally, pain is the most common symptom prompting patients to seek medical attention and is reported by more than 80% of individuals who visit their primary care provider. Despite the frequency of pain symptoms, individuals often do not obtain satisfactory pain relief. This has led to recent initiatives in health care to make pain the fifth vital sign (O'Neil, 2010).

Inflammation is an important protective response, designed to get rid of the organism of both the initial cause of cell injury (e.g., microbes, toxins) and the consequences of such injury (e.g. necrotic cells and tissues). Without inflammation infection would go unchecked, and injured tissues might remain permanent festering sores. Sometimes mechanisms designed to destroy foreign invaders and necrotic tissues have an intrinsic ability to injure normal tissues. When it is inappropriately directed against self tissues or is not adequately controlled, it becomes the cause of injury and disease such as rheumatoid arthritis, atherosclerosis and fibrosis, as well as life-threatening hypersensitivity reactions to insect bites, drugs and toxins. Therefore, great attention is given to the damaging consequences of inflammation (Kumar, Abbas and Fausto, 2005).

Nonopioid analgesics, including acetaminophen and nonsteroidal anti-inflammatory drugs (NSAIDs), are generally used for mild to moderate pain. Nonopioid analgesics relieve various types of acute and chronic pain (e.g., trauma, postoperative, cancer and arthritis pain) and are especially effective for certain types of somatic pain (e.g., muscle and joint pain, inflammatory pain, postoperative pain). Opioid analgesics, such as morphine, fentanyl and meperidine, are recommended for moderate to severe pain that does not respond to nonopioid analgesics alone. Opioid analgesics play a

major role in the treatment of acute pain (e.g., trauma, postoperative pain), breakthrough pain, cancer pain, and some types of chronic noncancer pain (Berry et al, 2006).

Although nonopioid and opioid analgesics are the mainstay of treatment in various types of pain, these drugs are associated with several adverse effects. For example, opioid analgesics frequently cause sedation, confusion, respiratory depression, nausea, vomiting, constipation, pruritus, and urinary retention whereas adverse effects of NSAIDs including gastrointestinal problem, bleeding, kidney dysfunction and hypersensitivity reactions. Therefore, many researchers are searching for new analgesics and anti-inflammatory drugs with higher efficacy and lower side effects from natural products including herbal plants.

The root extract of Ben-Cha-Moon-Yai remedy (BMY) consists of five herbal roots in an equal part by weight, including roots of *Aegle marmelos* Corr. (Ma-Tum), *Oroxylum indicum* Vent. (Phe-Kaa), *Dimocarpus longan* Lour. (Lam-Yai), *Dolichandrone serrulata* (DC) Seem. (Chare-Tare) and *Walsura trichostemon* Miq. (Khad-Linn). This herbal formula has been used as an antipyretic and anti-inflammatory agent in Thai traditional medicine for many centuries, nevertheless there is scarce in scientific evidence involved its biological activity. Recently, Bansuttee et al. (2010) reported an antipyretic effect of BMY remedy using lipopolysaccharide (LPS)-induced fever assay in rats, however there is no scientific evidence to support BMY remedy as an anti-inflammatory agent. Furthermore, many investigators have demonstrated the biological activity of various components of these five plants and some evidence for the extracts from *Aegle marmelos* (Rao et al., 2003; Arul, Miyazaki and Dhananjayan, 2005; Shankarananth et al., 2007), *Oroxylum indicum* (Golikov and Brekhman, 1967; Siriwatanametanon et al., 2009; Zaveri and Jain, 2010) and *Dimocarpus longan* (Okuyama et al., 1999; Ho, Hwang, Yi-Jane Shen and Lin et la., 2007). The present study was aimed to systematically investigate the antinociceptive and anti-inflammatory effects of the root extract of Ben-Cha-Moon-Yai remedy and five herbal root extracts of Ben-Cha-Moon-Yai remedy.

Purpose of the study

To investigate the antinociceptive and anti-inflammatory effects of the root extract of Ben-Cha-Moon-Yai remedy and all five root extracts compared with reference drugs. In addition, the possible mechanisms involved were also investigated.

Hypothesis

The root extract of Ben-Cha-Moon-Yai remedy and all five root extracts have antinociceptive and anti-inflammatory properties in various animal models.

Research design

Experimental Research

Expected benefit and application

The findings obtained from the present study may eventually lead to the development of the novel therapy with minor adverse effects in treating pain and inflammation. Furthermore, this study may provide scientific evidence to support the use of Ben-Cha-Moon-Yai remedy as an analgesic and anti-inflammatory agent.

Key words

Aegle marmelos

Oroxylum indicum

Dimocarpus longan

Dolichandrone serrulata

Walsura trichostemon

Ben-Cha-Moon-Yai remedy

Hot-plate

Writhing Test

Formalin test

Carrageenan-induced paw edema

Prostaglandin E_2-induced paw edema

CHAPTER II
LITERATURE REVIEWS

PAIN

In the 19th century, Mueller, Van Frey and Goldscheider hypothesized the concepts of neuroreceptors, nociceptors and sensory input. These theories developed into the current definition of pain by the International Association for the Study of Pain (IASP) as "an unpleasant sensory and emotional experience associated with actual or potential tissue damage, or described in terms of such damage." (Baumann and Strickland, 2008)

Pain can be categorized base on pain duration (i.e., acute vs. chronic pain) and underlying pathophysiology (i.e., nociceptive vs. neuropathic pain) which are used most often.

Nociceptive pain

Nociceptive pain typically is classified as either somatic (arising from skin, bone, joint muscle or connective tissue) or visceral (arising from internal organs such as the large intestine or pancreas). Whereas somatic pain most often presents as throbbing and well localize, visceral pain manifest as pain feeling as if it is coming from other structure (referred) (Baumann and Strickland, 2008; Green and Harris, 2008).

Neuropathic pain

Neuropathic pain is distinctly different from nociceptive pain in that it becomes disengaged from noxious stimuli or healing and often is described in terms of chronic pain. Neuropathic pain is a result of nerve damage. The examples of neuropathic pain syndromes are postherpetic neuralgia and diabetic neuropathy. They often are under recognized and difficult to treat. The mechanism responsible for neuropathic pain may be the nervous system's endogenous dynamic nature. Nerve damage or certain disease states may evoke changes seen in inflammatory pain, ectopic excitability, enhanced sensory transmission, nerve structure reorganization and loss of modulatory pain

inhibition. This produces spontaneous nerve stimulation, autonomic neuronal pain stimulation and a progressive increase in the discharge of dorsal horn neurons. Clinically, patients present with spontaneous pain transmission (often described as burning, tingling, shock-like or shooting), exaggerated painful response to normally noxious stimuli (hyperalgesia) and/or painful response to normally nonnoxious stimuli (allodynia) (Baumann and Strickland, 2008; Green and Harris, 2008).

Classification of pain

Acute pain

Acute pain usually has a readily definable cause. Its biological function is protective, acting as a warning that an external threat is noxious or signaling organ malfunction. It has a well-defined time of onset, often associated with signs of hyperactivity of the autonomic nervous system, e.g. tachycardia, hypertension and pallor, depending on the severity of the symptoms. Acute pain usually is nociceptive, although it can be neuropathic in nature, with a relatively strong relationship to levels of pathology. Common causes of acute pain include surgery, acute illness, trauma, labor and medical procedures. The best way of managing acute pain is to diagnose and treat the cause (Berry et al., 2006; Baumann and Strickland, 2008; Green and Harris, 2008).

Chronic pain

Chronic pain usually considered to be pain that has lasted for longer than 6 months. It does not signify a danger that requires immediate avoidance and a patient may not interpret such pain as indicating serious disease. This type of pain can be nociceptive, neuropathic or both. Further, adaptation by the autonomic nervous system overtime may lead to the absence of objective physical signs. However, there is often progressive physical deterioration, with sleep disturbance and weight loss. In severe cases, patients undergo serious affective and behavioral changes, e.g. major depression. Subtypes include: pain that persists beyond the normal healing time for an acute injury (e.g., complex regional pain syndrome), pain related to a chronic disease (e.g., pain secondary to osteoarthritis), pain without an identifiable organic cause (e.g.,

fibromyalgia) and the fourth type that many experts believe warrants a discrete classification, pain associated with cancer (Berry et al., 2006; Baumann and Strickland, 2008; Green and Harris, 2008).

Cancer pain

Pain associated with potentially life-threatening conditions is often called malignant pain or cancer pain. This type of pain includes both chronic and acute components and often has multiple etiologies. It is caused by the disease itself (e.g., tumor invasion, organ obstruction), treatment (e.g., chemotherapy, radiation, surgical incisions), or diagnostic procedures (e.g., biopsy, postoperative pain) (Berry et al., 2006; Baumann and Strickland, 2008; Green and Harris, 2008).

Chronic noncancer pain (CNCP)

This type of pain refers to persistent pain not associated with cancer. In contrast to patients with chronic cancer pain, patients with CNCP often report pain levels that only weakly correspond to identifiable levels of tissue pathology and/or respond poorly to standard treatments. Causes of CNCP include acute injury that has proceeded to chronic pain and various chronic conditions (e.g., low back pain, phantom limb pain and sickle cell disease). In some cases, there is no discernible cause, and the pain is considered the disease. CNCP can affect virtually any body system or region, and pain severity ranges from mild to severe (Berry et al., 2006; Baumann and Strickland, 2008; Green and Harris, 2008).

Nociceptors

Nociception is the process by which intense thermal, mechanical or chemical stimuli are detected by a subpopulation of peripheral nerve fibers, called nociceptors. Nociceptors, or pain receptors, are sensory receptors that are activated by noxious insults to peripheral tissues. Structurally, the receptive endings of peripheral pain fibers are free nerve endings. These receptive endings, which are widely distributed in the skin, dental pulp, periosteum, meninges, and some internal organs, translate the

noxious stimuli into action potentials that are transmitted by a dorsal root ganglion to the dorsal horn of spinal cord. The cell bodies of nociceptors are located in dorsal root ganglia (DRG) for the body and the trigeminal ganglia for the face and have both a peripheral and central axonal branch that innervates their target organ and the spinal cord, respectively. Nociceptors are activated by stimuli that have the potential to cause tissue damage.

Nociceptive action potential is transmitted through two types of afferent nerve fibers: myelinated Aδ fibers and unmyelinated C fibers (Figure 1). The larger Aδ fibers have considerably greater conduction velocities, transmitting impulses at rate of 10 to 30 m/sec. The C fibers are the smallest of all peripheral nerve fibers; they transmit impulses at the rate of 0.5 to 2.5 m/sec. Pain conducted by Aδ fibers traditionally is called *fast pain* or first pain (sharp or bright pain). C fiber pain often is described as *slow-wave pain* or second pain because it is slower in onset and longer in duration.

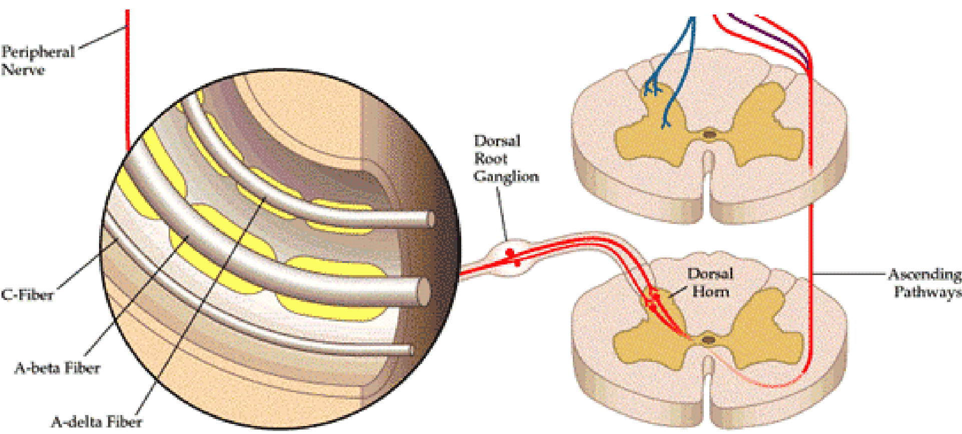


Figure1 Primary afferents with cell bodies in the dorsal root ganglion, include those with large-diameter myelinated (Aβ), small-diameter myelinated (Aδ) and unmyelinated (C) axons (Medscape education, 2007: online).

Unlike other sensory receptors, nociceptors respond to several forms of stimulation, including mechanical, thermal, and chemical. Some receptor respond to a

single type of stimuli (mechanical or thermal) and others, called *polymodal receptors*, respond to all three types of stimuli (mechanical, thermal, and chemical). Therefore, the membranes of nociceptors contain ion channels that are activated by these types of stimuli. Mechanical stimuli can arise from intense pressure applied to skin or from the violent contraction or extreme stretch of muscle. Both extremes of heat and cold can stimulate nociceptors. Chemical stimuli arise from a number of sources, including tissue trauma, ischemia, and inflammation.

A wide range of chemical mediators are released from injured and inflamed tissues, including hydrogen and potassium ions, prostaglandins, leukotrienes, histamine, bradykinin, acetylcholine, and serotonin (Figure 2). These chemical mediators produce their effects by directly stimulating nociceptors or sensitizing them to the effects of nociceptive stimuli: perpetuating the inflammatory responses that lead to the release of chemical agent that act as nociceptive stimuli or increase the response to nociceptive stimuli. For example, bradykinin, histamine, serotonin, and potassium activate and also sensitize nociceptors (Nestler, Hyman and Malenka, 2001; Bear, Connors and Paradiso, 2007; Porth and Matfin, 2009).

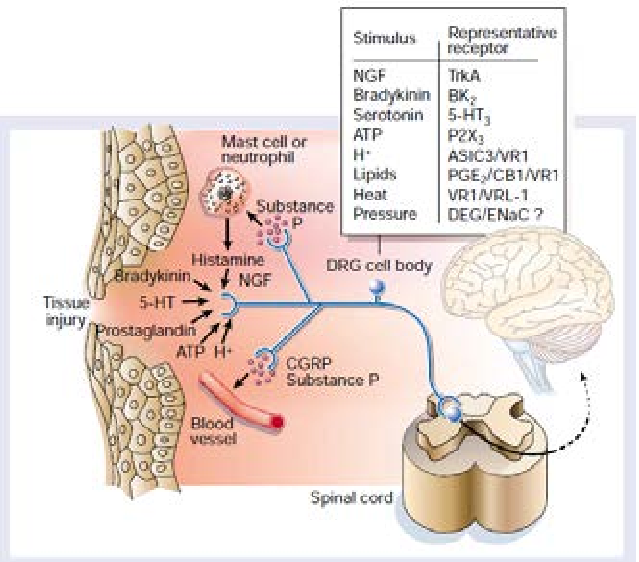


Figure 2 Chemical mediators of injured and inflamed tissues (Julius and Basbaum, 2001)

Mechanisms of pain

The mechanisms of pain are many and complex. As with other forms of somatosensation, the pathways are composed of first-, second- and third-order neurons. The first order neurons and their receptive endings detect stimuli that threaten the integrity of innervated tissues. Second-order neurons are located in the spinal cord and process nociceptive information. Third-order neurons project pain information to the brain. The thalamus and somatosensory cortex integrate and modulate pain as well as the person's subjective reaction to the pain experience (Nestler et al., 2001; Porth and Matfin, 2009). The process of pain pathway consists of 4 steps, including transduction, transmission, perception and modulation.

Transduction

The first step leading to the sensation of pain is stimulation of free nerve endings known as nociceptors. These receptors are found in both somatic and visceral structures. They distinguish between noxious and innocuous stimuli, and they are activated and sensitized by mechanical, thermal and chemical impulse. The underlying mechanism of these noxious stimuli to stimulate or sensitize the nociceptors may be the release of bradykinins, hydrogen and potassium ions, prostaglandins, histamine, interleukins, tumor necrosis factor-α, serotonin and substance P. Receptor activation leads to action potentials that are transmitted along afferent nerve fibers to the spinal cord (Berry et al. 2006; Baumann and Strickland, 2008).

Transmission

Nociceptive transmission takes place in Aδ and C-afferent nerve fibers. Stimulation of small myelinated Aδ fiber evokes sharp, well-localized pain, whereas stimulation of unmyelinated, small-diameter C fiber produces dull, aching, poorly localized pain. These afferent, nociceptive pain fibers synapse in various layers of the dorsal horn in spinal cord, releasing a variety of neurotransmitters, including glutamate, substance P and aspartate. The complex array of events that influence pain can be explained in part by the interactions between neuroreceptors and neurotransmitters that

take place in this synapse. For example, by stimulating sensory myelinated fibers that intraconnect in the dorsal horn with pain fibers, nonnoxious stimuli can have an inhibitory effect on pain transmission. Functionally, the importance of the interplay between these different fibers and various neurotransmitters and neuroreceptors are evident in the analgesic response produced by topical irritants or transcutaneous electrical nerve stimulation. These pain-initiated processes reach the brain through a complex array of a number of ascending spinal cord pathways, which include spinothalamic tract. It is postulated that the thalamus acts as a relay station, as these pathways ascend and pass the impulses to central structure where pain can be processed further (Berry et al. 2006; Baumann and Strickland, 2008).

Pain perception

At this point pain is thought to become the appreciation of signals arriving in higher structures. The brain may accommodate only a limited number of pain signals, and cognitive and behavioral functions can modify pain. Relaxation, distraction, meditation and guided mental imagery may decrease pain by limiting the number of processed pain signals. In contrast, a change in neurobiochemical can affect in state such as depression or anxiety may worsen pain (Berry et al. 2006; Baumann and Strickland, 2008).

Modulation

The body modulates pain through a number of complex processes. One, known as the endogenous opiate system, consists of neurotransmitters (e.g., enkephalins, dynorphins and β-endorphins) and receptors (e.g., mu, delta and kappa) that are found throughout the central nervous system (CNS). Endogenous opioids bind to opioid receptor sites and modulate the transmission of pain impulses. Other receptor types also can influence this system. Activation of N-methyl-D-aspartate (NMDA) receptors, found in the dorsal horn, can decrease the mu-receptor responsiveness to opiates. The CNS also contains a highly organized descending system for control of pain transmission. This system can inhibit synaptic pain transmission at the dorsal horn and

originates in the brain. Important neurotransmitters here include endogenous opioids, serotonin, norepinephrine, γ-aminobutyric acid (GABA) and neurotensin (Nestleret al., 2001; Baumann and Strickland, 2008).

Ascending Pain Pathways

Information about pain as well as temperature in the body is conveyed from the spinal cord to the brain via the spinothalamic pathway (Figure 3). The spinothalamic fibers project up the spinal cord and through the medulla, pons and midbrain without synapsing, until they reach the thalamus. Pain information from the face and head takes a path to the thalamus that is analogous to the spinal path. The small-diameter fibers in the trigeminal nerve synapse first on second-order sensory neurons in the spinal trigeminal nucleus of the brain stem. The axons of these cells cross and ascend to the thalamus in the trigeminal lemniscus (Nestler et al., 2001; Hunter, 2003; Bear, Connors and Paradiso, 2007).

Descending inhibition system

The system begins in an area of the midbrain called the periaqueductal gray (PAG) region. Through research it was found that electrical stimulation of the midbrain PAG regions produced a state of analgesia that lasted for many hours. Subsequently, opioid receptors were found to be highly concentrated in this and other regions of the CNS where electrical stimulation produced analgesia. Because of these findings, the PAG area of the midbrain often is referred to as the endogenous analgesia center (Figure 3).

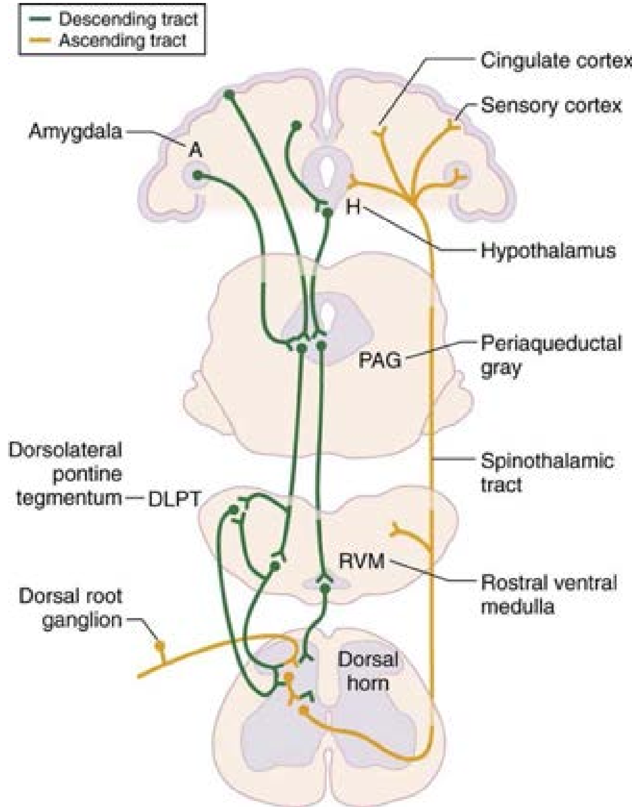


Figure 3 Modulation of pain at several different central nervous system levels (Fields and Basbaum, 1999).

The PAG area receives input from widespread areas of the CNS, including the cerebral cortex, limbic, hypothalamus, brainstem, reticular formation and spinal cord. The neurons of the PAG have axons that descend into an area in the rostral medulla called the nucleus raphe magnus (NRM). The axons of these NRM neurons project to the dorsal horn of the spinal cord, where they terminate in the same layers as the entering primary pain fibers. In the spinal cord these descending pathways inhibit pain transmission by dorsal horn projection neurons. Serotonin has been identified as a neurotransmitter in the NRM medullary nuclei.

Therefore, tricyclic antidepressant drugs, which enhance the effects of serotonin by blocking its synaptic uptake, have been found to be effective in the management of certain types of chronic pain. Additional inhibitory spinal projections arise from noradrenergic neurons in the pons and medulla, which also receive input

from the PAG. The discovery that norepinephrine can block pain transmission led to studies directed at combined administration of opioids and clonidine for pain relief (Hunter, 2003; Bear et al., 2007; Porth and Matfin, 2009).

The gate theory of pain

In the 1960s, Ronald Melzack and Patrick Wall proposed gate control theory (Figure 4). They proposed that certain neurons of the dorsal horns, which project an axon up the spinothalamic tract, are excited by both large-diameter sensory axons and unmyelinated pain axons. The projection neuron is also inhibited by an interneuron and the interneuron is both excited by the large sensory axon and inhibited by the pain axon. By this arrangement, activity in the pain axon alone maximally excites the projection neuron, allowing nociceptive signals to rise to the brain. However, if the large mechanoreceptive axon fires concurrently, it activates the interneuron and suppresses nociceptive signals (Bear et al., 2007).

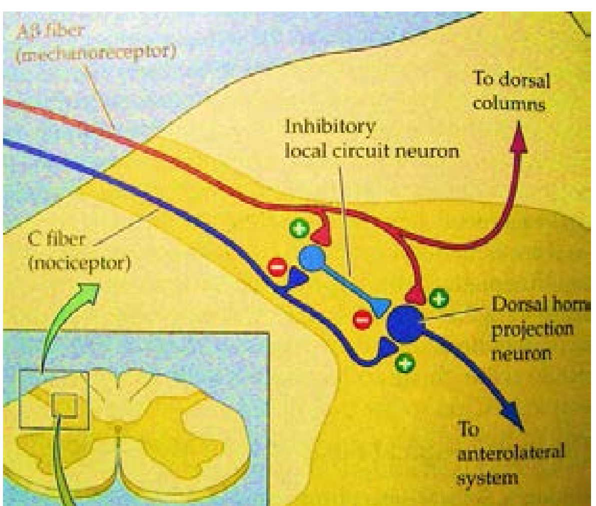


Figure 4 The gate control theory of pain (Bear et al., 2007)

Pharmacologic Therapy

Nonopioid analgesics (Acetaminophen and nonsteroidal anti-inflammatory drugs (NSAIDs))

Acetaminophen, an analgesic and antipyretic, is often selected as initial therapy for mild-to-moderate pain and is considered first-line in several pain situations such as low back pain and osteoarthritis.

NSAIDs have analgesic, antipyretic and anti-inflammatory actions. They appear to act peripherally at pain receptor level. They are particularly useful in treating patients with chronic disease accompanied by both pain and inflammation and for short-term treatment of mild to moderate acute pain, including musculoskeletal injuries and bone pain. Particular indications include the relief of pain accompanying dysmenorrhea, and that associated with neoplastic bone metastases, but they are of minimal use in neuropathic pain (Griffin and Woolf, 2005; O'Neil, 2010; Greene and Harris, 2008).

Mode of action

Acetaminophen preferentially reduces central prostaglandin synthesis by unknown mechanism and blocks pain impulses in the periphery, as a result produces analgesia and antipyresis but has relatively little anti-inflammatory efficacy.

The activity of NSAIDs is ascribed to their inhibition of cyclooxygenase (COX) activity and thus of prostaglandin (PG) synthesis which results in reduced nociceptors sensitization and the recruitment of leukocytes, which produce other inflammatory mediators, and increased pain threshold (Griffin and Woolf, 2005; O'Neil, 2010). COX exists in two isoforms. COX-1 is constitutive in the stomach, kidney, intestines and platelets, while COX-2 is inducible by inflammation in joints, brain, kidney, vascular endothelium and reproductive tract. Activation of COX-1 leads to the formation of autacoids, e.g. protective prostacyclins, in the gastric mucosa and vascular endothelium, prostaglandin E_2 (PGE_2) in the kidney and thromboxane (TXA_2) in the platelets. COX-2 is involved in fever, the central modulation of pain and the initiation of uterine contractions and fetal expulsion in childbirth, but its physiological roles are not

fully defined. COX-2 is mostly inducible by cytokines and other pro-inflammatory stimuli that cause an inflammatory response (Greene and Harris, 2008).

Side effects

Acetaminophen is well tolerated at usual dose and has few clinically significant drug interactions except causing increased hypoprothrombinemic response to warfarin in patients receiving acetaminophen dose of more than 2,000 mg per day. Hepatotoxicity has been reported with excessive use and overdose, and risk of this adverse effect increases in those with hepatitis or chronic alcohol use, as well as those who binge drink or are in fasting state. Regular chronic use of acetaminophen has been associated with chronic renal failure, but reports are conflicting. For these reasons, the maximum dose should be reduced by 50% to 75% in patients with renal dysfunction or hepatic disease and in those who engage in excessive alcohol use (O'Neil, 2010).

NSAIDs demonstrated a flat-dose response curve, with higher doses producing no greater efficacy than moderate dose but resulting in increased incidence adverse effects (e.g. gastrointestinal (GI) irritation, hepatic dysfunction, renal insufficiency, platelet inhibition, sodium retention, and CNS dysfunction). NSAIDs are classified as nonselective (inhibit COX-1 and COX-2) or selective (inhibit only COX-2) based on degree of COX inhibition. COX-2 inhibition is responsible for the anti-inflammatory effects, while COX-1 inhibition contributes increased GI and renal toxicity. The cardiovascular safety of the COX-2 inhibitor (coxibs) has been questioned due to the increased risk of myocardial infarction (MI) and stroke noted in several trials. This increased cardiovascular risk has been confirmed by case-control studies. Consequently, rofecoxib, valdecoxib and parecoxib have been withdrawn by manufacturer. Other coxibs have also produced an increased cardiovascular risk. It has been suggested that the basis for these observations is that selective COX-2 inhibitors significantly reduce levels of prostacyclin (PGI_2), an inhibitor of platelet aggregation, and do not affect thromboxane (TXA_2, a potent vasoconstrictor) formation by COX-1. There is therefore an increased tendency for vascular obstruction (Greene and Harris, 2008; O'Neil, 2010).

Opioid analgesics

Opioids are considered the agents of choice for the treatment of severe acute pain and moderate to severe pain associated with cancer. For chronic pain, their use was once highly controversial, however, use of opioids in chronic pain is now gaining acceptance. Opioids are classified by their activity at site, usual pain intensity treated and duration of action (O'Neil, 2010).

Opioid receptors

Direct evidence that opioids are recognized by specific receptors came from binding studies by Snyder and his colleagues in 1973, though the existence of specific antagonists had earlier suggested that such receptors must exist. Various pharmacological observations implied that more than one type of receptor was involved, the origin suggestion of multiple receptor types arising from *in vivo* studies of the spectrum of actions (e.g. analgesia, sedation, pupillary constriction and bradycardia) produced by different drugs. It was also found that some opioids, but not all, were able to relieve withdrawal symptoms in morphine dependent animals, and this was interpreted in terms of distinct receptor subtypes. The conclusion from these and many subsequent pharmacological studies, now confirmed by receptor cloning, is that three types of opioid receptor, termed mu, delta and kappa (all of them are typical G-protein-coupled receptors; Hunter, 2003).

Opioid agonists and antagonists

Pure agonists (e.g. morphine, pethidine, methadone and fentanyl) include most of the typical morphine-like drugs. They all have affinity for mu receptors and generally lower affinity for delta and kappa sites.

Partial agonist (e.g. buprenorphine) can produce a partial response irrespective of the concentration, and there may even be a decreased response if the optimum concentration is exceeded. They bind with the mu receptor and compete with the agonists, both naturally occurring and exogenous. If they are used in combination with a

complete agonist, they may act as competitive antagonists and level of analgesia may be reduced so that they show only limited activity.

Mixed agonists-antagonists (e.g. pentazocine, nalbuphine and butorphanol) are antagonists at the mu receptor but are still effective as analgesics through agonist effects at the kappa receptor, the agonist effect being either complete or partial.

Pure antagonists are used to reverse respiratory depression post-operatively, to treat opioid poisoning (e.g. naloxone) and to prevent relapse in detoxified opioid addicts (e.g. naltrexone; Hunter, 2003; Greene and Harris, 2008).

Mode of action

Opioid receptors belong to the family of G-protein-coupled receptors and inhibit adenylate cyclase, so reducing the intracellular cAMP. All three receptor subtypes exert this effect and they also exert effects on ion channels through a direct G-protein coupling to the channel. By these means, opioids promote the opening of potassium channels and inhibit the opening of voltage-gated calcium channels, which are the main effects seen at the membrane level. These membrane effects reduce both neuronal excitability (since the increased K^+ conductance causes hyperpolarization of the membrane) and transmitter release (owing to inhibition of Ca^{2+} entry; Hunter, 2003).

Side effects

Side effects common to all opioids include sedation, hallucinations, constipation, nausea, vomiting, urinary retention, myoclonus, and respiratory depression. In terms of medication management, the most frequent are sedation, nausea, and constipation (Table 1). Sedation and nausea are common when initiating therapy and when increasing doses. Nausea can be prevented with centrally acting antiemetic. Sedation usually improves with continued therapy but might become intractable at high doses, and stimulants such as methylphenidate might be needed. Respiratory depression is a serious adverse effect, and usually occurs after acute administration in opioid-naïve patients. Tolerance to respiratory depression develops rapidly with repeated doses, and respiratory depression is rarely a clinically significant problem in pain patients even

those with respiratory impairment. Constipation is a significant adverse effect which tolerance to develop and prophylaxis with stimulant laxatives and stool softeners is recommended (O'Neil, 2010).

Table 1 Functional effects associated with the main types of opioids receptor (Hunter, 2003)

	mu	delta	kappa
Analgesia			
Supraspinal	+++	-	-
Spinal	++	++	+
Peripheral	++	-	++
Respiratory depression	+++	++	-
Pupil constriction	++	-	+
Reduced GI motility	++	++	+
Euphoria	+++	-	-
Dysphoria	-	-	+++
Sedation	++	-	++
Physical dependence	+++	-	+

Inflammation

Inflammation is a protective response that intends to eliminate the initial course of cell injury, remove the damaged tissue and generate new tissue (Port and Sommer, 2009). Excessive inflammation may lead to tissue injury and cause physiologic decompensation, organ dysfunction and death. Inflammation can be divided into two types, including acute and chronic inflammation. The division of inflammation is based according to the time course and cellular components involved. These categories are not mutually exclusive, and some overlap exists.

Acute inflammation is typically of relatively short duration (hours to days) and is characterized by vasodilation, the exudation of protein-rich fluid (plasma) and migration of cells (primarily neutrophils) into the site of injury. Chronic inflammatory diseases include rheumatoid arthritis, systemic lupus erythematosus, silicosis and atherosclerosis. These disorders are characterized by prolonged duration (weeks to months to years) in which active inflammation, tissue destruction and attempts at tissue repair are occurring simultaneously. Infiltration of mononuclear cells and fibrosis are typical histological features of chronic inflammation (Sherwood and Toliver-Kinsky, 2004; Horton-Szar, 2007).

Causes of acute inflammation

- Physical agents, e.g. trauma, heat, cold, ultraviolet light, radiation.
- Irritant and corrosive chemical substances, e.g. acids, alkalis.
- Microbial infections, e.g. pyrogenic bacteria.
- Immune-mediated hypersensitivity reactions, e.g. immune-mediated vasculitis, seasonal allergic rhinitis.
- Tissue necrosis, e.g. ischemia resulting in a myocardial infarction.

Causes of chronic inflammation

Chronic inflammation usually develops as a primary response to:

- Microorganisms resistant to phagocytosis or intracellular killing mechanisms, e.g. tuberculosis, leprosy.

- Foreign bodies, which can be endogenous (e.g. bone, adipose tissue, uric acid crystals) or exogenous (e.g. silica, suture materials, implanted prostheses).
- Some autoimmune diseases, e.g. Hashimoto's thyroiditis, rheumatoid arthritis, contact hypersensitivity reactions.
- Primary granulomatous diseases, e.g. Crohn's disease, sarcoidosis.

Classical signs of acute inflammation

The classic signs of acute inflammation are redness, heat, swelling, pain and loss of function. These classic signs are produced by rapid vascular response and cellular events that are characteristic of acute inflammation. The main function of these events is to bring elements of the immune system to the site of injury and prevent further tissue damage (Kumar, Abbas and Fausto, 2005; Horton-Szar, 2007).

Vascular response

The vascular reactions of acute inflammation consist of changes in the flow of blood and the permeability of vessels.

Widespread vasodilation (hyperemia)

Vasodilation is a classic feature of acute inflammation and is clinically characterized by redness and warmth at site of injury. The purpose of the vasodilatory response is to facilitate the local delivery of soluble mediators and inflammatory cells. Inflammation-induced vasodilation is mediated primarily by nitric oxide (NO) and vasodilatory prostaglandins. Blood flow to the capillary bed is normally limited by the precapillary sphincters. In acute inflammation, a phase of vasodilation occurs when the arterioles and precapillary sphincter relax. This results in increased blood flow to the injured area and increased hydrostatic pressure.

Increased vascular permeability

Endothelial intracellular proteins, such as actin, contract under the influence of chemical inflammatory mediators, such as histamine, bradykinin, nitric oxide and leukotriene B4. Endothelial contraction results in increased fenestrations between

endothelial cells and increased permeability of vessels to plasma proteins. Proteins leak out of the plasma into the interstitial spaces, leading to a decrease in the plasma oncotic pressure. This protein-rich leaking fluid is an exudate. It includes circulating components such as immunoglobulins and coagulation factors. The combined increase in hydrostatic pressure and the decreased oncotic pressure causes net fluid movement from plasma into tissue space, producing swelling, pain, and impaired function. As a result, blood viscosity is increased and blood flow rate is decreased (Kumar, Abbas and Fausto, 2005; Horton-Szar, 2007; Porth and Sommer, 2009).

Cellular events

A critical function of inflammation is to deliver leukocytes to site of injury and to activate the leukocytes to eliminate the offending agents. The most important leukocyte in acute inflammation is neutrophils. These leukocytes ingest and kill bacteria and other microbes and eliminate necrotic tissue and foreign substances. Leukocytes also produce growth factors that aid in repair. The cellular response to inflammation includes recruitment of leukocytes to site of infection or injury, and phagocytosis and intracellular killing.

Recruitment of leukocytes to site of infection or injury

Neutrophils pass between endothelial cell junctions and invade damaged tissue to combat the effects of injury. The movement of leukocytes out of the vessel lumen is termed extravasation, and is achieved in five stages.

1. Margination to the plasmatic zone. This is assisted by the slowing of the blood leukocytes flow nearer to the vessel wall in the plasmatic zone than the axial stream.
2. Rolling of leukocytes due to the repeated formation and destruction of transient adhesions with the endothelium.
3. Adhesions (pavementing)—leukocytes eventually firmly adhere to the vascular endothelium, due to the interaction of paired molecules on the leukocyte and endothelial cell surface, e.g. B_2-integrin and ICAM-1.

4. Transmigration (also called diapesis)—leukocytes pass between the endothelial cell junctions by amoeboid movement through the vessel wall into tissue spaces.
5. Chemotaxis—neutrophils migrate towards and are possibly activated by, chemical substances (chemotaxins) released at sites of tissue injury.

These chemotaxins are thought to be leukotrienes, complement components and bacterial products.

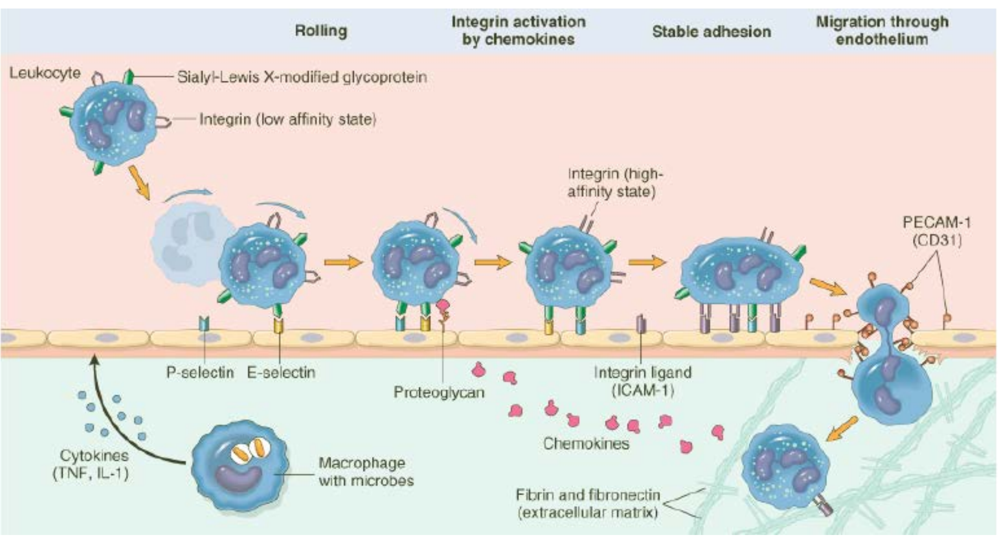


Figure 5 The multistep process of leukocyte migration through blood vessels (Kumar et al., 2005)

Phagocytosis and intracellular killing

Neutrophils and monocytes ingest debris and foreign particles at the site of injury. Cellular pseudopodia engulf the foreign particle and fuse to produce a phagocytic vacuole or phagosome. Phagocytosis is assisted by opsonisation with immunoglobulins and complement components.

Following phagocytosis, leukocytes attempt to destroy phagocytosed material by:

- Discharge of lysosomal enzymes into the phagosome.
- Oxygen-dependent mechanisms, such as H_2O_2, O_2^- , •OH.

- Oxygen-independent mechanisms, such as lactoferrin, lysozyme and hydrolases.

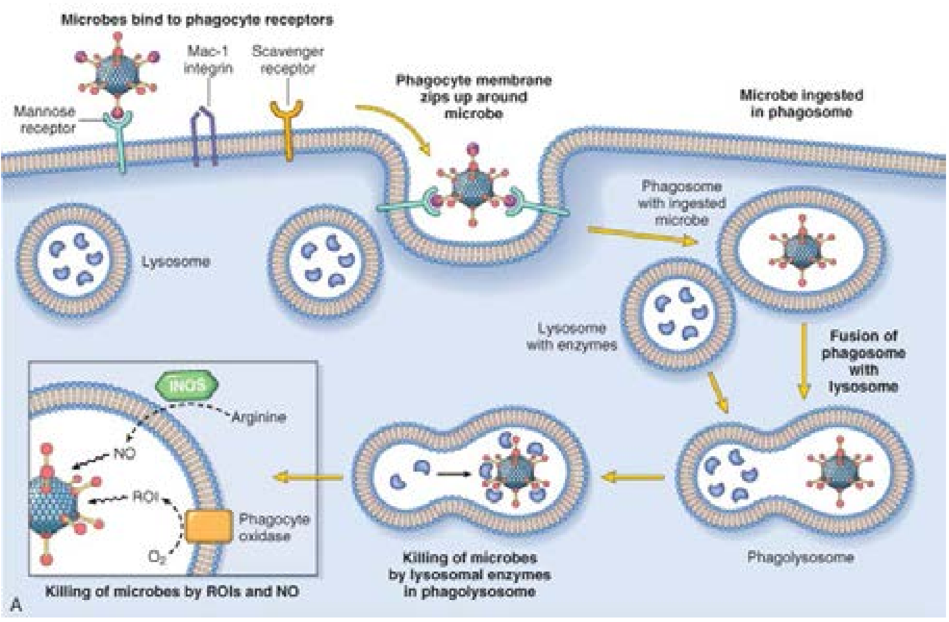


Figure 6 The process of phagocytosis and production of reactive oxygen intermediates within phagocytic vesicles (Kumar et al., 2005).

Chemical mediators of inflammation

Injury causes the release of chemicals that stimulate a vascular response that force fluid and leukocytes to flow to the site of the injury. Stimulated nerve endings signal the brain that there is an injury. The production of active mediators is triggered by microbes or host proteins, such as the complement, kinins, or coagulation systems; those are themselves activated by microbes or damaged tissues. Mediators can act on one target cell or have diverse targets. Once activated and released from the cell, most mediators are short-lived. They may be transformed into inactive metabolites, inactive by enzymes or otherwise scavenged or degraded (Sherwood and Toliver-Kinsky, 2004; Kumar et al., 2005; Horton-Szar, 2007; Porth and Sommer, 2009).

Vasoactive amines

These are preformed inflammatory mediators and so can be rapidly released by inflammatory cells. The most notable example is histamine. Preformed histamine is widely distributed in tissues, the highest concentrations being found in the connective tissues adjacent to blood vessels. It is also found in circulating blood platelets and basophils. Preformed histamine is found in mast cell granules and is released in response to a variety of stimuli. Histamine produces the arteriolar vasodilation and increased vascular permeability of venules seen in early inflammation.

Serotonin is also a preformed vasoactive mediator with actions similar to histamine. It is present in platelets. Release of serotonin (and histamine) from platelets is stimulated when platelets aggregate after contact with collagen, thrombin, adenosine diphosphate, and antigen-antibody complexes.

The complement system

This cascading sequence of serum proteins is made up of more than 20 components; the activated product of one protein activates another. The complement proteins have numerous functions in the body's response to infection.

Kinin system

The kinin system generates vasoactive peptide from plasma proteins called kininogens, by the action of proteases called kallikreins. The kinin system is stimulated by activated coagulation factor XII (The Hageman factor). Activation of kinin system results in release of bradykinin. It exerts its effects by increasing vascular permeability and producing pain. Both effects are cardinal features of acute inflammation. Activation of bradykinin is short lived because it is quickly inactivated by an enzyme called kininase.

Arachidonic acid metabolites

Arachidonic acid is a 20-carbon unsaturated fatty acid found in phospholipids of cell membranes. Release of arachidonic acid by phospholipases initiates a series of

complex reactions that lead to the production of the eicosanoid family inflammatory mediators (prostaglandins, leukotrienes and related metabolites). Eicosanoid synthesis follows one of two pathways: the cyclooxygenase pathway which culminates in the synthesis of prostaglandins and lipooxygenase pathway which culminates in the synthesis of leukotrienes (Figure 7). The prostaglandins, especially vasodilatory prostaglandins, including prostacyclin (PGI_2), PGD_2, PGE_2 and $PGF_{2\alpha}$, induce inflammation and potential the effects of histamine and other mediators.

Like the prostaglandins, the leukotrienes are formed from arachidonic acid, but through the lipooxygenase pathway. The leukotrienes also have been reported to affect the permeability of the postcapillary venules, the adhesion properties of endothelial cells and the extravasation and chemotaxis of neutrophil, eosinophils and monocytes.

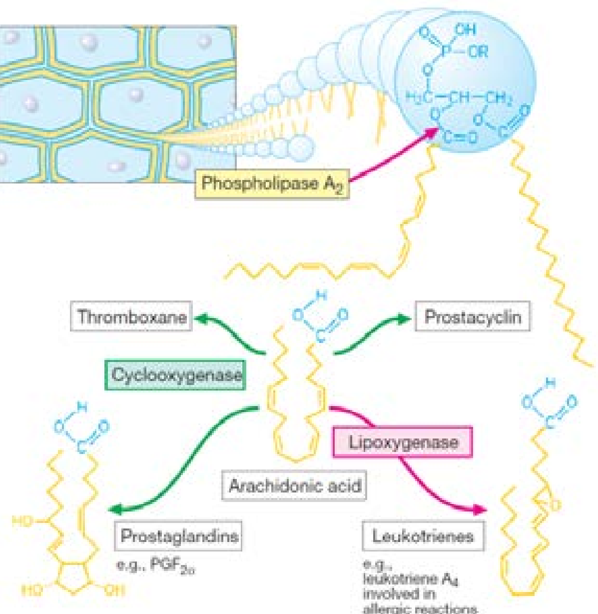


Figure 7 Biosynthesis of the prostaglandins, thromboxanes and leukotrienes by the cyclooxygenase and lipoxygenase pathway (Lüllmann et al., 2000)

Platelet activation factors

Platelet activation factors are released from mast cells and neutrophils during degranulation. They have the following effects: induction of platelet aggregation and

degranulation, increase vascular permeability, induction of leukocyte adhesion to the endothelium and stimulation synthesis of arachidonic acid derivatives.

Cytokines

Cytokines are a family of chemical messengers that act over short distances (autocrine and/or paracrine) by binding specific receptors on target cell surfaces. Cytokines are proteins produced by many cell types, principally activated macrophages and lymphocytes that modulate function of other cells. Tumor necrosis factor alpha (TNF-α) and interleukin1 (IL-1) are key cytokines that mediate acute inflammation. The major cellular source of TNF-α and IL-1 is activated macrophages. The secretion of TNF-α and IL-1 can be stimulated by endotoxin and other microbial products, immune cells, injury, and a variety of inflammation stimuli. TNF-α and IL-1 induce endothelial cells to express adhesion molecules and release cytokines, chemokines, and reactive oxygen species. Furthermore, TNF-α induces priming and aggregation of neutrophils, leading to augmented responses of these cells to other mediators. Chemotactic cytokines, or chemokines, are a family of small proteins that act primarily as chemoattractants to recruit and direct the migration of immune and inflammatory cells. Two classes of chemokines have been identified, including inflammatory chemokines and homing chemokines. Inflammatory chemokines are produced in response to bacterial toxins and inflammatory cytokines. Moreover, these chemokines recruit leukocytes during inflammatory response.

Nitric oxide

Nitric oxide (NO) is a potent vasodilator that is released from endothelial cells and macrophages. NO causes vascular smooth muscle relaxation via cyclic GMP-mediated mechanism resulting in vasodilation at the site of inflammation. It is produced by the action of nitric oxide synthase on L-arginine. NO plays an important role in the inflammatory response, including smooth muscle relaxation and antagonism of platelet adhesion and it serves as an endogenous regulator of leukocyte recruitment. Blocking

NO production under normal conditions, promotes leukocyte rolling and adhesion to postcapillary venules and delivery of exogenous NO reduces leukocyte recruitment. Thus, production of NO appears to be a compensatory mechanism that reduces cellular phase of inflammation.

Acute-phase proteins

Proteins whose serum level dramatically increases during inflammation are called acute-phase proteins. These proteins are produced by the liver and induced by circulating levels of IL-1, e.g. the C-reactive protein.

Pharmacological treatment of inflammation

Although inflammation is a natural response to injury, this process can be uncomfortable for patient, especially when there is fever, pain, and swelling. Anti-inflammatory medication can be given to reduce the inflammatory process and bring comfort to the patient. Anti-inflammatory medication stops the production of prostaglandins resulting in a decrease in the inflammatory process (Kamienski and Keogh, 2006).

Glucocorticoids are frequently used to suppress inflammation, allergy and immune response. Anti-inflammatory therapy is used in many diseases (e.g. rheumatoid arthritis, ulcerative colitis, bronchial asthma, severe inflammatory conditions of the eye and skin). The powerful anti-inflammatory effects of glucocorticoids rest on their capacity to suppress the action of many proinflammatory mediators. Glucocorticoids bind to a corticosteroid receptor (CR) and the complex translocates to the nucleus where it bind to a glucocorticoid response element (GRE). This complex increases the transcription of a number of anti-inflammatory genes and interferes with the binding of transcription factors activating protein and NF-κB to their response element, this action inhibits the production of a range of proinflammatory mediators, including IL-1β and TNF-α. Moreover, glucocorticoids also increase synthesis of the polypeptide lipocortin-1, which inhibits phospholipase A_2 and the synthesis of eicosanoids and PAF (Berry et

al., 2006; Kamienski and Keogh, 2006; Bennett and Brown, 2008). Serious unwanted effects of glucocorticoids are unlikely if the daily dose is below the equivalent of hydrocortisone 50 mg or prednisolone 10 mg. Unwanted effects generally follow prolonged administration of glucocorticoids. The principle adverse effects of chronic glucocorticoids administration are Cushing's syndrome, tendon rupture, immunosuppression, peptic ulcer and glaucoma (Bennett and Brown, 2008).

There are other anti-inflammatory medications that are not chemically the same as corticosteroids medication. These are referred to as nonsteroidal anti-inflammatory drugs (NSAIDs). However, inhibition of prostaglandin synthesis by NSAIDs attenuates rather than abolishes inflammation. Nevertheless, the relatively modest anti-inflammatory actions of the NSAIDs give, to most patients with rheumatoid arthritis, some relief from pain, stiffness, but they do not alter the course of the disease (Kamienski and Keogh, 2006; Neal, 2002).

ANIMAL MODELS

Nociceptive Activity Testing

There are limitations in the use of human subjects for experimentation on pain mechanisms and pathways. Therefore, to study pain transmission, identify new pain targets, and characterize the potential analgesic profile of novel compounds for pain relief, an array of experimental animal pain models has been developed mainly in rat and mouse, reflecting all types of pain. Depending on the model, pain measurements can encompass spontaneous pain behaviors as well as pain evoked by various modalities (Walker, Fox and Urban, 1999; Hogan, 2002; Joshi and Honore, 2006). A few of the most commonly used methods will be described, which may illustrate important aspect of investigations of pain in animals.

Hot-Plate Test

The hot-plate test was described in 1944, and it is one of the most commonly used models of nociception and analgesia in rodents. Originally the test measured nociceptive responses of mice placed on the hot-plate at temperatures varying from 55

to 70°C. Later the test was modified and a constant temperature of about 55°C was used. Subsequently most researchers have used this temperature (Tj∅lsen et al., 1994).

This test consists of introducing a rat or mouse into an open-ended cylindrical space with a floor consisting of a metallic plate that is heated by a thermode or a boiling liquid. A plate heated to a constant temperature produces two behavioral components that can be measured in terms of their reaction times including paw licking and jumping. Both are considered to be supraspinally integrated responses. As far as analgesic substances are concerned, the paw licking behavior is affected only by opioids. On the other hand, the jumping reaction time is increased equally by less powerful analgesics such as acetylsalicylic acid or paracetamol, especially when the temperature of the plate is 50°C or less. The specificity and sensitivity of the test can be increased by measuring the reaction time of the first evoked behavior regardless of whether it is paw-licking or jumping. The behavior is relatively stereotyped in the mouse but is more complex in the rat, which sniffs, licks its forepaws, licks its hind paws, straightens up, stamps its feet, starts and stops washing itself, among other things. These behaviors have been labeled "chaotic defensive movements". Furthermore, this test is very susceptible to learning phenomena, which result in a progressive shortening of the jumping reaction time accompanied by the disappearance of the licking behavior. Thus, the animal may lick the paws and then jump during the first test but will jump almost immediately during subsequent tests. Similarly, even putting the animals on an unheated plate just once to watch the test leads in subsequent tests to a diminution in the reaction time under standard conditions with a constant noxious temperature. Finally, reiteration of the test once a day or once a week inevitably leads to a progressive decrease in the reaction time. In general, these measures are very variable, even within a single laboratory. All these factors make this test a very delicate one to use (Bars et al., 2001).

Writhing test

This test is widely used to evaluate the peripheral analgesic activity and sometimes called the abdominal contortion test, the abdominal constriction response, or

the stretching test, but more commonly it is known as the “writhing test”. Writhing test involves intraperitoneal administration of irritating agent such as phenylbenzoquinone, acetylcholine, dilute hydrochloric, acetic acid, bradykinin, adrenaline, adenosine triphosphate, potassium chloride, tryptamine, and oxytocin.

These irritating agents evoke stereotyped of stretching behavior in the mouse and rat which is variously called, including writhing, stretching, cramping and squirming but the most common known term is writhing. Writhing is characterized by a wave of constriction and elongation passing caudally along the abdominal wall, sometimes accompanied by twisting of the trunk and followed by extension of the hind limbs. These behaviors are considered to be reflexes and involved with visceral pain. The mechanism of the irritating agent is unknown, however several mediators have been proposed. Deraedt et al. (1980) demonstrated the considerable increase of PGE_2 level in the peritoneal fluid of rats which had been injected with acetic acid and disappeared in 90 min. After 90 min the PGE_2 level was less than $PGF_{2\alpha}$. This balance between PGEs, which are hyperalgesic, and $PGF_{2\alpha}$, which has often been shown to be a PGE antagonist, could regulate defense mechanisms. Moreover an examination of cells collected by washing the peritoneum revealed a large decrease between 15 and 30 min after injection of the acetic acid suggested that the prostaglandins could be produced by neutrophil polynuclear cells but also by destruction of macrophages. Various types of prostaglandin biosynthesis inhibitors (non-steroid anti-inflammatory agents, non-narcotic analgesics and some monoamine-oxidase inhibitors and antioxidants) prevented prostaglandin release. Furthermore, Deraedt et al. (1988) also demonstrated that sympathetic component participates in the writhing response induced by intraperitoneal injection of acetic acid in the mouse. Ribeiro et al. (2000) suggested that the release of TNF-α, interleukin-1β and interleukin-8 by resident peritoneal macrophages and mast cells contribute to the nociceptive activity of zymozan and acetic acid in the writhing model. This suggestion is supported by the following observations, including pretreatment of the peritoneal cavities with antisera against these cytokines reduced the nociceptive responses induced by zymosan or acetic acid, peritoneal cells harvested

from cavities injected with zymosan or acetic acid released both interleukin-1β and TNF-α and although individual injection of TNF-α, interleukin-1β or interleukin-8 did not induce the nociceptive effect, intraperitoneal injection of a mixture of these three recombinant cytokines underlied a significant nociceptive writhing response. Analgesic activity of the test compound is determined utilizing the number of writhes which are counted over an interval of time. Unfortunately, the number of writhes decrease spontaneously with time therefore it is impossible to evaluate the duration of action of an analgesic on a single animal. Furthermore, the number of cramps is subject to a great deal of variability. This test enables to detect both central and peripheral analgesic activity. The advantage of this method is that it allows for the evidence to be obtained for effects produced by weak analgesics. On the other hand, it lacks of specificity because positive results are also produced by numerous other substances, including some that have no analgesic action, e.g., adrenergic blockers, antihistamines, muscle relaxants, monoamine oxidase inhibitors, and neuroleptics. Thus, a positive result with this test does not necessarily mean there is analgesic activity.

The specificity can be improved by undertaking a preliminary Rotorod test to detect and eliminate molecules that alter the motor performance of the animal (Pearl et al., 1969b). Although the writhing test has a poor specificity, it is sensitive and predictive, as shown by the correlation between ED_{50} values obtained in rats using this test and analgesic doses in humans (Bars et al., 2001).

Formalin test

The formalin test is considered as a model for chronic pain (Dubuisson and Dennis, 1977). This procedure involves subcutaneous injection of dilute formalin into the plantar surface of the rodent. After injection of the formalin the animals presented behavioral responses such as elevation or favoring of the paw and excessive licking or biting of the injected paw. This test may allow the researchers to dissociate between inflammatory and non-inflammatory pain which is a rough classification of analgesics as well as to identify mechanism of analgesics between central and peripheral component

(Hunskaar and Hole, 1987; Shibata et al, 1989; Tj∅lsen et al., 1992). In the rat and mouse, intraplantar injections of formalin produce a biphasic response. Two distinct periods of high licking activity can be discriminated, an early phase lasting from 0-5 min and a late phase lasting from 20 to 30 min after the injection of formalin (Hunskaar and Hole, 1987; Rosland et al, 1990). Hunskaar and Hole (1987) demonstrated that centrally acting analgesics inhibited the responses in both phases. On the other hand, the nonsteroidal anti-inflammatory drugs and the steroids inhibited the responses only the late phase. The results suggested that the early phase is due to a direct effect on nociceptors and that prostaglandins do not play an important role during this phase in contrast to late phase that seems to be an inflammatory response with inflammatory pain that can be inhibited by anti-inflammatory drugs (Hunskaar and Hole, 1987). In consistent with the previous studies (Hunskaar and Hole,1987; Shibata et al., 1989) demonstrated that centrally acting drugs such as narcotics inhibited both phases equally whereas peripherally acting drugs such as aspirin, oxyphenbutazone, hydrocortisone and dexamethasone only inhibited the second phase. Furthermore, pain response in the first phase was inhibited by capsaicin and Des-Arg9-(Leu8)-bradykinin (bradykinin inhibitor). The second phase was inhibited by compound 48/80 pretreatment, indomethacin and bradykinin inhibitor. Therefore, it is suggested that substance P and bradykinin participate in the manifestation of the first phase response, and histamine, serotonin, prostaglandin and bradykinin are involved in the second phase. These results indicated that the first and second phase responses induced by formalin have distinct characteristic properties. Tj∅lsen et al (1992) proposed that the early phase resulted from C-fiber activation, while the late phase appeared 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.

Anti-inflammatory activity testing

Inflammatory process involves a series of events that can be elicited by numerous stimuli, e.g. infectious agent, ischemia, antigen-antibody interactions, chemical, thermal or mechanical injury. The response is accompanied by the clinical

signs of erythema, edema, hyperalgesia and pain. Pharmacological methods for testing acute and subacute inflammation include UV-erythema in guinea pigs, vascular permeability, oxazolone-induced ear edema in rats and mice, croton-oil edema in rats and mice, paw edema in rats, pleurisy tests and granuloma pouch technique (Vogel, 2008).

Paw edema

One of the cardinal signs of inflammation is the presence of edema. It is not surprising, that edema tests are among the most prominent models used to assess the efficacy of drugs for treating inflammatory disease such as arthritis (Lombardino, 1985).

Among many methods used for screening the anti-inflammatory drugs, one of the most commonly employed techniques is based on the ability of agents to inhibit the edema produced in the hind paw of the rat after injection of a phlogistic agent. Many phlogistic agents (irritants) have been used, such as brewer's yeast, formaldehyde, dextran, egg albumin, kaolin or carrageenan.

The effect can be measured in several ways. The hind limb can be dissected at the talocrural joint and weighted. Usually, the volume of the injected paw is measured before and after application of the irritant and the paw volume after 3 or 6 hr is calculated compared with the volume measured immediately after injection of the irritant for each animal. Effectively treated animals show much less edema. Maintenance of a constant temperature in the laboratory is important since the amount of paw swelling and the degree of inhibition achieved by NSAIDs decrease with decreasing temperature (Lombardino, 1985). The difference of average values between treated animals and control groups is calculated for each time interval and statistically evaluated. The difference at the various time intervals gives some hints for the duration of the anti-inflammatory effect (Vogel, 2008).

Carrageenan-induced rat paw edema model has a biphasic response (Vinegar et al., 1969). The early phase (1–2 hr) is mainly mediated by histamine, serotonin and kinins, while prostaglandins appear to be the most important mediators in the late phase (3–5 hr) after intraplantar injection of carrageenan (Di Rosa and Sorrentino, 1968; Di

Rosa, 1972). The oedema at 3 hr after the application of carrageenan was considered to reach the highest response (Andrade et al., 2007; Kale et al., 2007). The rats have been the animal of choice for the study of carrageenan-induced paw edema response for many years because it has been fully established. Previous study demonstrated the time course pattern in carrageenan-induced mouse paw edema resembles that observed in the rat (Levy, 1969; Posadas et al., 2004). The mouse paw edema responds to anti-inflammatory drugs that causes a reduction in inflammatory responses. Therefore an advantage to using mice rather than rats is the amount of compound needed because the difference between the usual rat and mouse weights is between five and ten fold (Levy, 1969). Thus, mouse paw oedema has been widely used to test new anti-inflammatory drug candidates and to study the mechanisms involved in inflammation (Posadas et al., 2004).

Ben-Cha-Moon-Yai Remedy

Ben-Cha-Moon-Yai remedy is widely used by traditional doctors in Thailand to treat many types of fever and inflammation. Ben-Cha-Moon-Yai remedy is composed of five herbal roots in an equal part by weight, including roots of *Aegle marmelos* (Ma-tum), *Oroxylum indicum* (Phe-kaa), *Dimocarpus longan* (Lam-yai), *Dolichandrone serrulata* (Chare-tare) and *Walsura trichostemon* (Kad-lin). Many studies have shown that all those plants contain many alkaloids, sterols and other compounds. The only scientific evidence that may support its use as an antipyretic drug came from a work of Bansuttee et al. (2010) which reported an antipyretic effect of Ben-Cha-Moon-Yai remedy using the lipopolysaccharide (LPS)-induced fever in rats. However, there is no scientific evidence to support BMY remedy as an anti-inflammatory agent. Some scientific evidences that may support its use as an anti-inflammatory and analgesic agent came from previous studies which demonstrated the anti-inflammatory and the analgesic activities of various parts of *Aegle marmelos* (Rao et al., 2003; Arul, Miyazaki and Dhananjayan, 2005; Shankharananth et al., 2007; Ghangale et al., 2008), *Oroxylum indicum* (Golikov and Brekhman, 1967; Ali et al., 1998; Siriwatanametanon et al., 2009; Zaveri and Jain, 2010) and *Dimocarpus longan* (Okuyama et al., 1999; Ho et al., 2007), three herbal plants in Ben-Cha-Moon-Yai remedy.

Aegle marmelos (Linn.) Corr.

Figure 8 Fruits and roots of *Aegle marmelos* Corr.

Family Rutaceae

Vernacular names

Thailand: matum, tum (Pattani), ma pin (north). Bael or bel fruit (En). Bel Indient (Fr). Indonesia: maja, maja batu. Malaysia: bilak, bila, bel. Philippines: bael. Burma: opesheet, okshit. Cambodia: bnau. Laos: toum. Vietnam: trai mam.

Distribution

Bael grows wide in dry forest in the Indian Peninsula, Sri Lanka, Pakistan and Bangladesh. It is an old cultivated tree in that region, particularly found in temple gardens in India. It has spread to Indo-China, South-East Asia (in particular Thailand, northern Malaysia, eastern Java and north Luzon) and other parts of the tropics (Sunarto, 1991).

Uses

The ripe bael fruit is usually eaten fresh. The pulp is often combined with sugar and tamarind to prepare a refreshing drink in Indian homes, as well as squash, jam and nectar. The green fruits have digestive, stomachic and astringent properties. Ripe fruits are used in chronic diarrhea and dysentery and act as a tonic for the heart and brain. They are also useful in adjuvant treatment of bacillary dysentery, assisting the healing of the ulcerated mucosa of the intestines. The roots, in the form of a decoction, are used to

treat melancholia, intermittent fevers and heart palpitations and also form a component of a popular Ayurvedic medicine dashmool. The bitter-tasting leaves are used as a febrifuge and a poultice made of them is used for ophthalmic disorders and ulcers. Fresh leaves have been used to treat weakness of the heart, dropsy and beri-beri. Bengal quince has also been used for stomach ache, snakebite, cholera, convulsions, dyspepsia, malaria, nausea, spasms, thirst, tumors, sores, itches and proctitis and as an abortifacient. It also finds use as an anodyne, astringent, dentifrice, digestive, piscicide, refrigerant, restorative and laxative (Williamson, 2002).

Description

A deciduous tree, 20 to 25 feet in height and 3 to 4 feet in grinth, with straight, sharp, axillary thorns and trifoliate aromatic leaves. The flowers are greenish white. The fruits are globose, 2 to 4 inch in diameter, gray or yellowish and with smooth, hard, aromatic rind. Seeds are numerous, oblong and compressed, and the pulp is mucilaginous, thick, orange red in color. The root bark is 3.5 mm thick, curved, with its surface cream yellow or grayish in color. The surface is rough, irregular, and shallow with ridges along the line of lenticels and ruptured all over. The stem bark is externally gray and internally cream in color. The outer surface is rough and warty. It is 4 to 8 mm thick, firm in texture and occurs as flat or channeled pieces. The fracture is tough and gritty in the outer region and fibrous in the inner; taste is sweet. The root in transection shows a pentarch to heptarch stele, the cork cambium arising in the pericycle. The cork is lignified and stratified, the phelloderm is composed of a wide zone of parenchymal cells with strands of stone cells in the mature bark. The medullary group in the inner region is uni-to triseriate, while in the outer region it is bi-to pentaseriate (Kapoor, 2001).

Properties

The pulp is soft, yellow or orange, very fragrant and pleasantly flavored. The edible portion (pulp) amounts to 56-77% of the fruit and contains per 100 g: water 61.5 g, protein 1.8 g, fat 0.39 g, carbohydrates 31.8 g, ash 1.7 g, carotene 55 mg, thiamine 0.13 mg, riboflavin 1.19 mg, niacin 1.1 mg, and vitamin C 8 mg. The fruit is rich in tannin

(up to 20% in the rind). Marmelosine ($C_{13}H_{12}O_3$), volatile oil, limonene, alkaloids, coumarines and steroids are also present in different parts of tree (Sunarto, 1991).

Phytochemicals

The leaves contain alkaloids including aegelenine, aegeline and condensed tannins. The roots and aerial parts contain skimmianine, 7, 8-Dimemethoxy-1-hydroxy-2-methyl anthraquinone and 6-hydroxy-1-methoxy-3-methyl anthraquinone. The fruit contains marmelosin, alloimperatorin, marmelide psoralen and tannic acid. The roots contain umbelliferone, psoralen, xanthotoxin, dimethoxy coumarin and scopoletin. The heartwood yields α-xanthotoxol-8-O-β-D-glucoside and the seeds contain luvangetin (Williamson, 2002).

Pharmacological activities

Analgesic, Anti-inflammatory and Antipyretic Activity

Rao et al. (2003) demonstrated analgesic and anti-inflammatory activity of the extract of unripe fruit of *Aegle marmelos*. Analgesic activity was assessed utilizing Randall-selitto test in mice. Anti-inflammatory activity was assessed utilizing carrageenan-induced paw edema in rats. Arul, Miyazaki and Dhananjayan (2005) demonstrated anti-inflammatory, antipyretic and analgesic properties of the serial extracts of the leaves of *A. marmelos*. Anti-inflammatory property was assessed utilizing carrageenan-induced paw edema and cotton-pellet granuloma in rats. Antinociceptive activity was assessed utilizing acetic acid-induced writhing in mice and antipyretic activity was assessed utilizing yeast-induced hyperpyrexia in mice. Similarly, Ghangale et al. (2008) also evaluated anti-inflammatory activity of the aqueous extract of *A. marmelos* using rat paw oedema model and proposed that *A. marmelos* possesses anti-inflammatory activity. Shankharananth et al. (2007) demonstrated that the methanolic extract of the leaves of *A. marmelos* at a dose level of 200 and 300 mg/ kg possesses significant analgesic activity on acetic acid-induced writhing and tail flick test in mice.

Antiulcerogenic Activity

Rao et al. (2003) reported that pretreatment of rats with the extract of unripe fruit of *A. marmelos* produce a significant reduction in severity and incidence of indomethacin, acetic acid and cysteamine-induced ulcer.

Anticonvulsant activity

Anticonvulsant activity was studied by Sankari et al. (2010) utilizing maximal electroshock (MES) or pentylenetetrazole (PTZ) and found that oral administration of ethanolic extract of the leaves of *A. marmelos* resulted in a significant suppression of hind limb tonic extensions in mice.

Antifertility Activity

Sathiyaraj et al. (2010) studied antifertility activity of aqueous extract of the leaves of *A. marmelos* (250, 350 mg/kg for 45 days) and found a significant decrease in the weights of testis, epididymes and seminal vesicle. In addition, they also found a reduction in sperm count and motility of male albino rats.

Hepatoprotective Activity

Singanan et al. (2007) worked on *A. marmelos* leaf extract on alcohol-induced liver injury in albino rats and reported data of the excellent hepatoprotective effects. Similarly, Khan and Sultana (2009) studied on hepatoprotective effect of methanolic extract of *A. marmelos* on CCl_4^- mediated hepatic oxidative stress and the findings indicated that *A. marmelos* attenuates CCl_4^-mediated hepatic oxidative stress in rats.

Antidiarrhoeal Activity

Mazumder et al. (2006) reported antidiarrhoeal potential of chloroform extract of *A. Marmelos* root and the extract was found to be mostly active against the strains of *Vibrio cholera*, followed by *Escherichia coli* and *Shigella spp*. Furthermore, animals pretreated with *A. Marmelos* root extract showed significant inhibitory activity against castor oil-induced diarrhea. Brijesh et al. (2009) studied on the antidiarrhoeal activity of

A. marmelos unripe fruit extract and the extract showed cidal activity against *Giardia* and rotavirus.

Antithyroid Activity

Panda and Kar (2006) isolated scopoletin (7-hydroxy-6-methoxy coumarin) from *A. marmelos* leaves and evaluated for its potential to regulate hyperthyroidism. It was observed that oral administration of scopoletin (1 mg/kg for 7 days) to levo-thyroxine treated animals decreased serum thyroid hormones level. It was proved that scopoletin has superior therapeutic activity than the standard antithyroid drug, propylthiouracil.

Antidiabetic Activity

The aqueous extract of *A. marmelos* leaves was evaluated for hypoglycemic and antioxidant effects by Upadhya et al. (2004) using alloxan-induced diabetes in male albino rats and proposed that the aqueous extract may be useful in the long-term management of diabetes. Similarly, the antihyperlipidemic activity of the aqueous extract of *A. marmelos* fruits was demonstrated by Marzine and Gilbart (2005) using the streptozotocin-induced diabetic Wistar rats. Sundaram et al. (2009) worked on the alcoholic extract of *A. marmelos*, *Momordica charantia* and *Eugenia jambolana* against streptozotocin-induced diabetic rats and confirmed their protective activity against laboratory induced cell necrosis. Whereas, Kuttan and Sabu (2004) studied on the leaves extract of *A. marmelos* on alloxan-induced diabetes and reported that the extract was capable of reducing oxidative stress by scavenging lipid peroxidation and enhancing certain antioxidant levels which causes lowering of elevated blood glucose level. Beside of all above cited work, Hema and Lalithakumari (1999) had presented a tremendous result of *A. marmelos* and documented its hypoglycemic action along with other pharmacological actions on molecular level.

Anticancer Activity

Latica and Costa (2005) evaluated the anticancer potential of folk medicine used in Bangladeshi and tested the extracts of *A. marmelos* for cytotoxic action using brine

shrimp lethality assay, sea urchin eggs assay and MTT assay using tumor cell lines. The extract of *A. marmelos* was found to exhibit toxicity on all used assays. Jagetia, Venkatesh, and Baliga (2005) reported the anticancer effect of the hydroalcoholic extract of bael leaves in the animal model of Ehrlich ascites carcinoma and proposed that the induction of apoptosis may be due to the presence of skimmianine in the extract. Similarly, Subramaniam et al. (2008) isolated 1-hydroxy-5, 7-dimethoxy-2-naphthalene carboxaldehyde (HDNC, marmelin) from *A. marmelos* and found that HDNC inhibits proliferation of HCT-116 colon and HEp-2 alveolar epithelial carcinoma cells.

Radioprotective Activity

Radioprotective effect of *A. marmelos* extract was studied by Jagetia and Venkatesh (2005) by exposing mice to different doses of gamma-radiation and found that oral administration of the extract results in an increase in radiation tolerance by 1.6 Gy. Again, Jagetia et al. (2006) studied effects of this plant extract on radiation-induced changes in the peripheral blood and small intestine of Swiss albino mice. They reported that *A. marmelos* extract significantly reduces the deleterious effect of radiation in intestine and bone marrow of mouse.

Antifungal Activity

Pitre and Srivastava (1987) demonstrated the antifungal activity of the ethanolic root extract against *Aspergillus fumiganus* and *Trichphyton mentagrophytes*. Rana, Singh and Taneja (1997) evaluated the antifungal activity of essential oils isolated from the leaves of Bael using spore germination assay. The oil exhibited variable efficacy against different fungal isolates and 100% inhibition of spore germination of all the fungi tested was observed at 500 ppm. They proposed that the essential oils from bael leaves may interfere with the Ca^{2+}-dipicolonic acid metabolism pathway and possibly inhibit the spore formation. Patil, Chaudhary and Settipalli (2009) reported the antifungal, antidiarrheal and antimicrobial activities of the ethanolic extract *of A. marmelos* leaves.

Antimicrobial Activity

Maheshwari et al. (2009) studied on the ethanolic extract of dried fruit pulp of *A. marmelos* against various intestinal pathogens i.e. *Shigella boydii*, *S. sonnei* and *S. flexneri* and proposed that certain phytochemicals including phenols, tannins and flavonoids were effective against all pathogens. It was also confirmed by Kaur et al. (2009) by treating *E. coli* with *A. marmelos* fruit extract. In consonance, Citarasu et al. (2003) also performed some experiments with *A. marmelos* on certain pathogenic bacteria like *Salmonella typhi*, *Pseudomonas aeruginosa*, *Aeromonas hydrophyla* and *Vibrio sp*., and concluded its positive bactericidal effects.

Anti-asthmatic Activity

Arul, Miyazaki and Dhananjayan (2004) investigated anti-asthmatic activity of the alcoholic extract of the leaves of *A. marmelos* and the revealed the positive relaxant effect in isolated guinea pig ileum and tracheal chain of the extract.

Toxicity Studies

Total alcoholic, total aqueous, whole aqueous and methanolic extracts were collected from the leaves of *A. marmelos* by Veerappan et al. (2007) and studied in experimental rats for their toxicity. No histopathological changes were found when the extracts of *A. marmelos* were administered intraperitoneally for 14 days at the dose of 50 mg/kg body weight. The collected data demonstrated that the extracts of the leaves of *A. marmelos* have a high margin of drug safety.

Oroxylum indicum Vent.

Figure 9 Fruit, flowers and roots of *Oroxylum indicum* Vent.

Family Bignoniaceae

Synonyms

Bignonia indica L. var. 'ALFA'a (1753), *Bignonia pentandra* Lour. (1790), *Calosanthes indica* (L.) Blume (1826).

Vernacular names

Midnight horror (En). Indonesia: pongporang (Sundanese), kayu lanang, mungli (Javanese). Malaysia: beka, bonglai, kulai. Philippines: pingka-pingkahan (Tagalog), abong-abong (Bisaya), Kamkampilan (Iloko). Cambodia: pi ka. Laos: lin may, ung ka. Thailand: phe kaa (central), litmai (northern), lin faa (north-eastern).

Distribution

Oroxylum indicum is found from India eastward to southern China and the Philippines, and throughout South-East Asia; in Indonesia eastward to Sulawesi and the Lesser Sunda Islands. Locally cultivated near human settlements.

Uses

Throughout its distribution area, the bitter bark is employed for intestinal complaints. It is credited with astringent and tonic properties and widely used for diarrhea and dysentery.

In Malaysia, a decoction of the leaves is drunk for stomach-ache. Externally it is employed in cholera, fever, childbirth and rheumatic swellings. The boiled leaves are employed as a poultice during and after childbirth and in dysentery as well as for an enlarged spleen. Leaf poultices may be further applied for toothache and headache. In Java, the pounded bark mixed with water is taken in gastritis and to purify the blood. In northern Sulawesi, the inner bark is used to arrest bleeding.

In the Philippines, a decoction of the root is credited with antirheumatic, antidysenteric and diuretic properties; the leaves are used in antirheumatic baths. In Thailand, the root and root bark are used for diarrhea and dysentery, while the stem bark is applied for ulcers and abscesses.

In Vietnamese folk medicine, a decoction of the seeds is used for cough, bronchitis and gastritis. Externally the seeds are applied to ulcers. A decoction of the dried root bark or stem bark is used in the treatment of allergic diseases, urticarial, jaundice, asthma, sore throat, laryngitis, hoarseness, gastralgia, diarrhea and dysentery. An alcoholic maceration of the fresh bark is externally applied on allergic dermatitis. In Thai folk medicine, the root is employed as a tonic and antidiarrheal, whereas the seed is used as a laxative and expectorant.

Throughout South-East Asia, cooked flowers, buds and young pods are highly esteemed as a vegetable. In Java, flowers, young shoots and the stem bark are consumed fresh as a side dish. The wood can be used as firewood although it has poor quality.

Description

A semi-deciduous, sparingly branched tree up to 27 m tall; trunk up to 40 cm in diameter, bark grey, with prominent leaf scars, twigs thick, pithy, later hollow, lenticellate. Leaves crowded, imparipinnate, 3-4 times pinnate, 0.5-2 m long; petiole long, rachis swollen at points of insertion; stipules absent; leaflets ovate to oblong, 4-11 (-15) cm x 3-9 cm, base cuneate or mostly oblique, apex acuminate, entire, with scattered glands on the lower surface. Inflorescence an erect raceme, terminal, 25-150 cm long, peduncle and rachis partitioned. Flowers bisexual, pedicel 2-4 cm long,

bracteolate; calyx coriaceous, campanulate, containing water in bud, 2-4 cm long, 1.5-2 cm in diameter, brown or dirty violet, becoming almost woody in fruit; corolla funnel-shaped, about 10 cm long, lobes 5, subequal, margin wrinkled, reddish outside, yellowish to pinkish inside; stamens 5, inserted in the throat, hairy at the base; ovary superior, 2-celled, many-ovuled. Fruit a pendent capsule, sword-shaped, 45-120 cm x 6-10 cm, valves flat, almost woody, finally black, Seed 5-9 cm x 2.5-4 cm, including the membranous and transparent wing. Seedling with epigeal germination; hypocotyl elongated; cotyledons leafy.

Properties

The various parts of *Oroxylum indicum* are rich in flavonoids. The leaves contain the flavonoids baicalein (5, 6, 7-trihydroxyflavone), scutellarein (4', 5, 6, 7-tetrahydroxyflavone), and their glycosides baicalin (baicalein-7-glucuronide) and scutellarin (scutellarein-7-glucuronide). The stem and root bark contain e.g. baicalein, scutellarein, oroxylin A (5, 7-dihydroxy-6-methoxyflavone), chrysin (5, 7-dihydroxyflavone) and p-coumaric acid. Baicalein and oroxidin (wogonin-7-O-'BETA'-D-glucuronide) have been isolated from the seeds. Other compounds mentioned in the literature include the prenylated naphthoquinone lapachol, and the anthraquinone derivative aloe-emodin. Dichloromethane extracts of the stem bark and root of *Oroxylum indicum* were found to have antimicrobial activities against Gram-positive bacteria (*Bacillus subtilis* and *Staphylococcus aureus*), Gram-negative bacteria (*Escherichia coli* and *Pseudomonas aeruginosa*) and yeast (*Candida albicans*). Bioassay-guided chromatographic fractionation led to the isolation of flavonoids (e.g. baicalein, chrysin and oroxylin A) and lapachol as active constituents. Lapachol was found to be active against the Gram-positive bacteria; 5 µg gave a zone of inhibition equivalent to that shown by 5 µg of streptomycin, whereas 5 µg of chrysin gave inhibition zones of equal size to that of 5 µg of streptomycin against *Pseudomonas aeruginosa*. The inhibitory activity of lapachol from *Oroxylum indicum* root against soya bean 5-lipoxygenase (IC_{50} 0.79 µg/ml) was equivalent to that of the positive control (the flavonoid fisetin; IC_{50} 0.97 µg/ml), whereas 50 µg/ml) of the dichloromethane extract of the root bark gave 100%

inhibition of leukocyte lipooxygenase. These activities might indicate an anti-inflammatory effect for the dichloromethane extract, mainly due to its lapachol content. The isolated flavonoid baicalin also showed inhibitory effects against the human T cell leukemia virus type 1 and the human immunodeficiency virus (HIV-1). Baicalein furthermore showed antiproliferative activity in cultured rabbit vascular muscle cells and lipooxygenase activity in vitro. Other Pharmacological activities of baicalin and baicalein include anti-inflammatory activity of baicalin in the rat adjuvant arthritis model, inhibition of LPS-induced IL-1 production by both flavonoids and inhibition by baicalein of leukotriene C-4 biosynthesis by rat resident peritoneal macrophages. The methanol extract of the young perianth exhibited strong antitumor-promoting activity when tested against 12-O-tetradecanoylphorbol-13-acetate (TPA) induced Epstein-barr virus early antigen activation (Rasadah, 2001).

Pharmacological Activities

Anti-inflammatory Analgesic and Antiallergic activities

A liquid extract of bark lowered the vascular permeability and suppressed the inflammatory edema of rats sensitized with egg protein, formalin or histamine. This extract had no effect on vascular permeability of rats sensitized with horse serum or xylene (dimethylbenzene). The anti-inflammatory effect of this preparation was more pronounced in the sensitized animal than in the normal ones (Golikov and Brekhman, 1967). A lipophilic extract of *O. indicum* stem bark gave 100% inhibition of leukocyte lipoxygenase at a concentration of 50 g/ml which was mainly due to lapachol. The inhibitory activity of lapachol from *O. indicum* root against soya bean 5-lipoxygenase (IC_{50} 0.79 µg/ml) was equivalent to that of the positive control (the flavonoid fisetin; IC_{50} 0.97 µg /ml), whereas 50 µg/ml of the dichloromethane extract of the root bark gave 100% inhibition of leukocyte lipoxygenase. These activities might indicate an anti-inflammatory effect for the dichloromethane extract, mainly due to its lapachol content (Ali et al., 1998). Similarly, the anti-inflammatory activity of the extract of *O. indicum* was evaluated by Siriwatanametanon et al. (2009). They reported NF-κB inhibitory effect (IC_{50} 47.45 µg/ml) by luciferase assay and inhibitory effect on the release of PGE_2 (IC_{50}

26.98 μg/ml) by ELISA of *O. indicum*. The study was conducted to analyze anti-inflammatory and analgesic activities of the different extracts from root bark of *O. indicum*. Anti-inflammatory activity was assessed utilizing carrageenan-induced paw edema and cotton pellet-induced granuloma formation in rats. Whereas, analgesic activity was assessed using tail-flick method in rats and acetic acid-induced writhing in mice (Zaveri and Jain, 2010).

Antiproliferative Activity

A study was conducted to analyze the antiproliferative activity of several Bangladeshi medicinal plant extracts on different human cell lines including erythroleukemic K562 cells, B lymphoid Raji and T lymphoid Jurkat human tumor cell lines. The data obtained indicated that the ethanolic extract of the stem bark of *O. indicum* showed an antiproliferative activity on all analyzed human tumor cell lines: erythroleukemic K562 cells (IC_{50}=3.77±0.32 mg/mL), B lymphoid Raji (IC_{50}= 23.20±9.6 mg/mL) and T lymphoid Jurkat (IC_{50}=4.11±0.1 mg/mL). The same plant extracts were screened for their activity in inhibiting the interactions between nuclear factors and double stranded target oligonucleotides mimicking the transcription factors such as nuclear factor-kappa B (NF-κB), activator protein (AP-1), signal transducer and activator of transcription (STATs), cAMP response element binding protein (CREB) and GATA-1 factors. The results showed that high concentration of *O. indicum* extract was unable to inhibit almost all TFs/DNA interactions, while it is active on the other AP-1/DNA interactions only when added at 50 mg/mL (Lampronti et al., 2008).

Anticancer activity

A study revealed that eight species of plants used in Bangladeshi folk medicine exhibited some cytotoxic activities in the brine shrimp lethality assay, sea urchin eggs assay, hemolysis assay and MTT assay using tumor cell lines. The extract of *O. indicum* showed the highest toxicity on all tumor cell lines tested, with an IC_{50} of 19.6 mg/mL for CEM, 14.2 mg/mL for HL-60, 17.2 mg/mL for B-16 and 32.5 mg/mL for HCT-8 (Costa-Lotufo et al., 2005). The study was performed to investigate the *in vitro* effects of

baicalein form methanolic extract of the fruits of *O. indicum* on induction of apoptosis in the HL-60 cell line. The result exhibited the inhibition of proliferation of HL-60 cells due to 36-48 hr exposure to 10 or 20 µM of baicalein and they proposed that the effect was associated with the accumulation of cells at S or G2M phases. However, proliferation inhibition at a higher dose may be associated with induction by apoptosis (Roy et al. 2007).

Gastroprotective Activity

The presented data was reported the isolation of two new flavonoids from the stem bark of *O. indicum* possesses ulcer protective effects against various gastric ulceritis inducing models in rats (Babu et al. 2010).

Immunomodulatory Activity

The n-butanol extract (100 mg/kg) of *O. indicum* root bark had the great immunomodulatory activity was against different experimental animal models. This observation suggested that *O. indicum* possessed immunomodulatory activity by enhancing specific immune response (humoral immunity) and non-specific immune response (phagocytosis) of the body as well as exhibiting antioxidant potential. They proposed that the n-butanol extract of *O. indicum* root bark produced a significant immunostimulant activity (Gohil, Zaveri and Jain, 2008).

Antioxidant Activity

Free radical scavenging potential of the different extracts of leaves of *O. indicum* was assessed *in vitro* using diphenyl-picryl-hydrazyl (DPPH) assay. The presented data showed significant antioxidant activity of ethyl acetate, methanolic and water extracts of leaves of *O. indicum* (IC_{50} 49.0, 55.0 and 42.5, respectively) (Gupta et al. 2008). Similarly, the serial extracts of *O. indicum* stem and root bark were evaluated the free radical scavenging property by using 2, 2-azinobis-3-ethylbenzothiazoline-6-sulphonate (ABTS) radical cation decolorization assay, scavenging of nitric oxide radical and reduction of ferric ions by ortho- phenanthroline color method and inhibition of lipid

peroxide formation. The n-butanol extracts of stem and root bark of *O. indicum* was reported to be potent scavenging activity (Zaveri and Dhru, 2009). In addition, the different extracts of *O. indicum* stem bark were investigated for the free radical scavenging activity via total antioxidant assay and β-carotene bleaching assay. The ethanol extract showed maximum antioxidant potential in β-carotene bleaching assay while chloroform extract showed maximum reducing power in total antioxidant activity (Kalaivani and Mathew, 2009). The study proposed that maximum antioxidant potential of ethanol extract may be due to its free radical scavenging activity, while the chloroform extract may be due to its reducing potential.

Antimicrobial Activity

Islam, Eti and Chowdhury (2010) fractionated the extract of *O. indicum* stem bark with petroleum ether, chloroform, hexane and carbon tetrachloride using modified Kupchan partitioning method and tested for antimicrobial activity through standard disc diffusion method. They reported the antimicrobial activity against the gram-positive and gram-negative bacteria, and some fungi of the extracts.

Toxicity Studies

An ethanol-water (1:1) extract of bark and root had a maximum tolerated dose at 1.0 g/kg i.p. in mice (Dhar et al., 1968). Three fraction of 70% ethanol extract of bark were tested for acute and subchronic toxicities in mice. The results revealed that when orally administered at doses up to 800 mg/kg, every fraction had no acute or subchronic toxicity. But intraperitoneal injection of either the first or the second fraction caused mortality (Glinsukon, 1987).

Dimocarpus longan Lour.

Figure 10 Fruits and roots of *Dimocarpus longan* Lour.

Family Sapindaceae

Synonyms

- ssp. *Longan* var. *longan*: *Dimocarpus longan* Lour. (1790), *Euphoria longana* Lamk (1792) nom. Illeg., *Nephelium longana* Cambess. (1829).

- ssp. *Longan* var. *longepetiolulatus* Leenh.: *Euphoria morigera* Gagnep. (1950) nom. inval.

- ssp. *Longan* var. *obtusus* (Pierre) Leenh.: *Euphoria scandens* Winit & Kerr.

- ssp. *malesianus* Leenh. var. *malesianus*: *Nephelium malaiense* Griff. (1854), *Euphoria cinerea* Radlk. (1878) nom. illeg., *Euphoria malaiensis* Radlk. (1879) nom. illeq., *Euphoria gracilis* Radlk. (1913) nom. illeg.

- ssp. *malesianus* Leenh. var. *echinatus* Leenh.: *Euphoria nephelioides* Radlk. (1914) nom. illeg.

Vernacular names

- ssp. *longan* var. *longan*: longan (En). Longanier, oeil de dragon (Fr). Indonesia, Malaysia: lengkeng. Burma: kyet mouk. Cambodia: mien. Laos: lam nhai, nam nhai. Thailand: lamyai pa. Vietnam: nhan.

- ssp. *longan* var. *obtusus*: Thailand: lamyai khruer, lamyai tao.

- ssp. *malesianus* var. *malesianus*: Malaysia: mata kucing (Peninsular Malaysia and Sabah), isau, sau, kakus (Sarawak). Indonesia: buku, ihau (Kalimantan), medaru (Sumatra)

Distribution

Ssp. *longan* var. *longan*: Whereas some authors limit the area of origin to the mountain chain from Burma through southern China, others extend it to south-west India and Sri Lanka, including the lowlands. The crop is mainly grown in south China, Taiwan and north Thailand with small acreages elsewhere in Indo-China as well as Queensland (Australia) and Florida (United States) and scattered trees at higher elevations in South-East Asia.

Ssp. *longan* var. longepetiolulatus: southern Vietnam.

Ssp. *longan* var. *obtusus*: Indo-China, cultivated in Thailand.

Ssp. *malesianus* var. *malesianus*: all over Indo-China and Malaysia, greatest variation found in Borneo.

Ssp. *malesianus* var. *echinatus*: Borneo and the Philippines.

Observation

Tree, up to 40 m tall and 1 m trunk diameter, sometimes buttressed, exceptionally a scandent shrub; branches terete with 5 faint grooves, sometimes warty lenticellate, rather densely ferruginous tomentose.

Leaves 2-4(-6)-jugate, axial parts mostly densely hairy; petiole 1-20 cm, petiolules 0.5-35 mm long; leaflets elliptical, 3-45 cm x 1.5-20 cm, 1-5 times longer than wide, chartaceous to coriaceous, above often tomentose in basal part of midrib, beneath thinly tufted-tomentose mainly on midrib and nerves. Inflorescences usually terminal, 8-40 cm long, densely tufted-tomentose; cymules (1-)3-5-flowered; pedicels 1-4 mm; bracts patent, 1.5-5 mm long; flowers yellow-brown; calyx lobes 2-5 mm x 1-3 mm; petals 5, 1.5-6 mm x 0.6-2 mm, densely woolly to glabrous; stamens (6-)8(-10), filament 1-6 mm.

Fruit drupaceous, 1-3 cm in diameter, lobe(s) broad-ellipsoid to globular, smooth to warty or sometimes up to 1 cm aculeate, sometimes granular, glabrescent, yellowbrown. Seed globular with shining blackish-brown testa; seed enveloped by a thin fleshy, translucent white arilloid.

Uses

Longans as well as the minor fruits of the species are mainly eaten fresh. There are substantial canning industries for longan in Thailand, China and Taiwan. Large fruits are used, preferably those with small seeds. Fruit can be canned in its own juice with little or no sugar, due to the high level of soluble solids. Canned longans retain their individual flavor better than do rambutan or lychee. Longans can be preserved dry, either intact or after removal of the pericarp. The dried flesh is black, leathery and smoky in flavor and is used mainly to prepare a refreshing drink. A liqueur is made by macerating the longan flesh in alcohol.

The seeds are used as a shampoo, like soap berries (*Sapindus saponaria* L.), because of their saponin content. Both the seed and the fruit flesh of longan have several medicinal uses.

The leaves, which contain quercetin and quercitrin, and flowers are sold in Chinese herb markets. The red, hard longan timber and the fairly hard, light brown to yellow 'mata kucing' timber are useful, but rarely available. In eastern Thailand ssp. *longan* var. *obtusus* is grown as an ornamental climber (Choo and Ketsa, 1991).

Phytochemicals

Significant amounts of phenolics, fatty acids and proteins exist in longan fruit. Phenolic compounds including gallic acid, corilagin, ellagic acid and their conjugates, (-) epicatechin, 4-O-methylgallic acid, flavone glycosides, glycosides of quercetin and kaempferol from longan fruit pericarp (Jaitrong et al., 2006; Rangkadilok et al., 2005; Shi et al., 2008; Sun et al., 2007), and ethylgallate1-β -O-galloyl-D-glucopyranose, methyl brevifolin carboxylate, grevifolin and 4-O-α-L-rhamnopyranosyl-ellagicacid, gallic acid, corilagin and ellagic acid from longan seed (Zheng et al., 2009) have been identified.

In addition, longan pericarp contains significant amounts of polysaccharides (Jiang et al., 2009). The polysaccharides are mainly composed of L-arabinofuranose (32.8%), D-glucopyranose (17.6%), Dgalactopyranose (33.7%) and D-galacturonicacid (15.9%) (Yang et al., 2009).

Pharmacological Activities

Anti-inflammatory

The high levels of phenolic compounds of water and ethanolic extract prepared from dried longan flowers including flavonoids, condensed tannins, and proanthocyanidins were identified by Ho et al. (2007). Moreover, the extracts showed prominent inhibitory effects on prostaglandin E_2 and nitric oxide production through lipopolysaccharide-stimulated RAW 264.7 cell model. They concluded that proanthocyanidins involved with the anti-inflammatory activity.

Antianxiety and Analgesic Activities

The extract of *D. longan* arillus was assessed for anxiolytic-like effect using the Vogel-type anti-conflict method in mice. The extract exhibited significant anxiolytic-like effect at 2 g/kg. The results from the bioassay-oriented isolation revealed adenosine to be the active principle and adenosine presented significant anti-conflict effect at a dose of 30 mg/kg. Furthermore, adenosine also produced the analgesic effect in writhing method (Okuyama et al., 1999).

Antioxidant Activity

The antioxidative effect of water and ethanolic extract prepared from dried *D. longan* flowers were reported the radical scavenging activity similar to pure compounds, including gallic acid, myricetin, and epigallocatechin gallate (Ho et al., 2007). Rangkadilok et al. (2007) identified high levels of polyphenolic compounds such as corilagin, gallic acid and ellagic acid. They also reported the high radical scavenging activity of dried longan seed extracts when comparing fresh seed and dried pulp extracts. However, the results demonstrated that major contributors of the high

antioxidant activity may be due to other phenolic or flavonoid glycosides and ellagitannins present in longan fruit. Previous study identified three phenolic compounds in ethanolic extracts of *D. longan* fruit pericarp, namely gallic acid, corilagin, and ellagic acid and reported that the ethanolic extracts of *D. longan* fruit pericarp prepared from high pressure extraction method presented higher level of extraction yield, phenolic content, and antioxidant activity than conventional extraction methods. Antioxidant activity was assessed in various antioxidant models, including 1, 1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging activity, superoxide anion radical scavenging activity, total antioxidant capacity, and lipid peroxidation inhibitory activity (Prasad et al., 2009). In addition, Ripa, Haque and Bulbul, 2010 indicated that ethyl acetate and chloroform fraction of ethanol extract prepared from leaves and stems of Nephelium longan possess potently antioxidant activity through DPPH radical scavenging assay. Furthermore, longan polysaccharides showed a good antioxidant were id activity *in vitro* (Jiang et al., 2009). However, methylation of polysaccharides from longan pericarp reduced radical scavenging activity (Yang et al., 2010). Pan et al. (2008) demonstrated antioxidant activity of the ethanolic extract of *D. longan* peel using 2, 2'-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay, hydroxyl radical scavenging assay, reducing power and total antioxidant capacity. The extract showed excellent antioxidant in all test systems. Furthermore, they also evaluated the decrease of lipid oxidation utilizing thiobarbituric acid-reactive substances (TBARS) assay and reported a significant reduction in lipid oxidation of the extract. Similarly, the extract of *D. longan* fruit pericarp was analyzed the antioxidant activity using various antioxidant models including, DPPH radical scavenging activity, total antioxidant capacity and superoxide anion radical scavenging activity. The result showed high antioxidant activity in all the tested models. Furthermore, three phenolic acids in the extract, namely gallic acid, ellagic acid, and corilagin were identified and quantified by HPLC. Corilagin content was the highest compared to other phenolic acids identified (Prasad et al., 2010).

Anticancer Activity

Polyphenol-rich longan seed extract is a free-radical scavenger that possesses known pharmacological properties and is used by humans for therapeutic purposes. The anticancer activity of ethanolic extract of *D. longan* fruit pericarp was investigated using HepG2, A549, and SGC 7901 cancer cell lines. The result indicated significant anticancer activity of its extract (Prasad et al., 2009). Furthermore, polyphenol-rich longan seed extract also inhibited the proliferation of Colo320DM, SW480 and HT-29 by blocking cell cycle progression during the DNA synthesis phase and inducing apoptotic death, reduced the expression of cyclin A and cyclin D1, activated caspase3 and increased the Bax/Bcl-2 ratio (Chung et al., 2010). It was suggested that a polyphenol-rich longan extract can be employed as a potential novel treatment agent for cancer.

Antityrosinase Activity

Tyrosinase is an enzyme involved in melanin production. Alterations in melanin production might be responsible for a part of the histopathological features unique to malignant melanoma. Therefore, tyrosinase inhibitors may be clinically useful for the treatment of skin cancer. Recently more attention is being paid to the use of natural plant extracts in the cosmetic industry as tyrosine inhibitors which are used as skin-whitening agents (Roh et al., 2004). The extracts of seed and fruit pericarp of *D. longan* were reported to have antityrosinase activity (Rangkadilok et al., 2007; Prasad et al., 2010).

Anti-glycated Activity

Glycation has been confirmed to have a significant role in diabetic complications and normal aging (Yamaguchi et al., 2000). Polysaccharides from longan pericarp showed a good and stable anti-glycated activity (Yang, Zhao and Jiang, 2009). Ultrasonic-assisted extraction technique can increase the anti-glycated activity of polysaccharides obtained from longan pericarp. The role of longan potential polysaccharides in preventing diabetics and normal aging is worth further evaluation.

Memory-enhancing Activity

Park et al. (2010) reported that subchronic administration of the aqueous extract of longan fruit could enhance learning and memory, and its beneficial effects are mediated, in part, by BDNF expression and immature neuronal survival.

Dolichandrone serrulata DC. Seem.

Figure 11 Flower, fruit and leaves of *Dolichandrone serrulata* DC. Seem.

Family Bignoniaceae

Synonyms *Stereospermum serrulata* DC.

Observation

Deciduous tree to 25 m with narrow cylindrical crown and slender branches.

Bark is pale brown, smooth or slightly flaking.

Leaf is to 43 cm, once-pinnate, 3-5 pairs of leaflets, 5-14 × 3-6 cm, elliptic with tapering tip and strongly asymmetric base, usually with scattered teeth.

Flower is 12-21 cm, pure white, opening at night, in short unbranched clusters of 3-7 flowers at end of twigs, 2-3 cm.

Fruit is up to 85×1.8 cm, pointed, spirally twisted.

Seed 2.2-2.8 × 0.5-0.8 cm, rectangular, thin with transparent wing.

Uses

The flower of this plant has a bitter taste and has been used as a vegetable. The bark is used in Thai traditional medicine as an antifever and antiinflammatory agent (Sinaphet et al., 2006).

Phytochemicals

A phenolic triglycoside, dolichandroside, was isolated from the branches of *D. serrulata* together with decaffeoyl-verbascoside, verbascoside, isoverbascoside,

markhamioside A, 2-O-apiosylver-bascoside, luteoside bandixoside (Sinaphet et al., 2006).

Pharmacological activity

Phomkaivon and Areekul, 2009 reported that ethanolic extract from D. serrulata flowers had antioxidant activity in the determination of total phenolic content (TPC) and ferric-reducing antioxidant power assay (FRAP).

Walsura trichostemon Miq.

Figure 12 Fruits and flowers of *Walsura trchostemon* Miq.

Family Meliaceae

Description

Botany evergreen of briefly deciduous trees, very rarely with latex of sap.

Leaves odd-pinnate, stalks swollen and jointed. Alternate, spirally arranged, leaflets usually opposite, no stipules.

Flowers mostly white or yellow, regular, bisexual, in branched clusters at upper leaf axils, 4-5 free spreading petals, stamens longer than petals, style short, disc ring-like.

Fruits fleshy or leathery, not splitting, 1-2 seeds with aril.

Distribution

Walsura trichostemon Miq is a plant of family Meliaceae that has been found in evergreen forest drought throughout Southeast Asia such as Myanmar, Cambodia. Thailand found in North, Northeast and southeastern, which know the local name of Musk Mallow tree (Polyium, Ta-Ngam and Thongnoi, 2009).

Uses

Apart from *W. trichostemon* are using in Thai traditional medicine, as an tendon disabilities, staunch, wash the wound, hemorrhoids and eating to reduce irritation (Polyium et al., 2009) and are used for paresis (Chuakul and Boonpeng, 2004).

Pharmacological Activities

Antimycobacterial and Cytotoxic activity

Antibacterial screening showed the crude methanol extract had a good activity against isolated bacterial strains (MIC between 62.5 and 125 µg/ml). *S. milleri*, *S. aureus* MRSA 2036 21083, *S. aureus* ATCC 25923 and *S. Mutans* ATCC 27175 were the most sensitive bacteria (MIC = 62.5 µg/ml). *S. pneumonia*, *S. aureus* (MRSA), *B. subtilis* ATCC 26633 and *B. pertussis* showed MIC value of 125 µg/ml (Chotsang, Aroonrerk, and Charoenying, 2006).

Sequential extracts of *W. trichostemon* stem bark were tested for their antimycobacterial activity against *Mycobacterium tuberculosis* H37Ra strain (Anti-TB) using the green fluorescent protein microplate assay (GFPMA) and cytotoxic activity against human mouth carcinoma (KB), human small cell lung cancer (NCI-H187) and breast cancer (MCF-7) cancer cell lines, tested using the resazurin microplate assay (REMA). Crude extract showed inhibitory effect against *M. tuberculosis* with MIC_{50} of 50 g/ml and showed inhibitory effect against KB, NCI-H187 and MCF-7 cancer cell lines with IC_{50} values of 1.35- 36.38 g/ml (Polyium et al., 2009).

Sequential extracts of *W. trichostemon* leaf were tested for their antimycobacterial activity against *M. tuberculosis* H37Ra strain (Anti-TB) using the green fluorescent protein microplate assay (GFPMA) and anti-proliferative assay for cancer cell lines against human mouth carcinoma (KB), human small cell lung cancer (NCI-H187) and breast cancer (MCF-7) cancer cell lines, tested using the resazurin microplate assay (REMA). Crude ethyl acetate extract showed inhibitory effect against *M. tuberculosis* with MIC_{50} of 50 µg/ml. Crude hexane, ethyl acetate and methanol extract showed inhibitory effect against KB with IC_{50} values of 10.42- 40.14 µg/ml., NCI-H187 with IC_{50} values of 17.02-25.76 µg/ml and MCF-7 with IC_{50} values of 26.07-36.37 µg/ml. The results showed that the extracts from the leaves of *W. trichostemon* have a significant antimycobacterial and cytotoxic activity (Polyium and Malaphan, 2010).

CHAPTER III
MATERIALS AND METHODS

EXPERIMENTAL ANIMALS

Male ICR mice weighing 18-25 g were obtained from the National Laboratory Animal Centre, Mahidol University, Salaya, Nakornpathom. Animals were maintained in the animal facility of the Faculty of Pharmaceutical Sciences, Chulalongkorn University under the standard condition of temperature (25±2°C), 50-60% of humidity, 12 hr/12 hr light/dark cycles and provided with food and water *ad libitum*. The animals were kept under laboratory conditions for one week prior to the start of the experiments. At the end of each experiment, the animals were sacrificed with carbon dioxide asphyxiation. This study protocol was approved by the Institutional Animal Care and Use Committee of the Faculty of Pharmaceutical Sciences, Chulalongkorn University, Bangkok, Thailand (Appendix A).

DRUGS

The following drugs were used: normal saline solution (NSS; General Hospital Products Public Co., Thailand), 2% Tween 80 (Srichansahaosoth Co., Thailand), morphine sulfate (MO; Thai FDA), indomethacin (IND; Sigma Chemical Co., USA), acetic acid (Merck, Germany), formalin (Merck, Germany), λ-carrageenan (Sigma Chemical Co., USA), prostaglandin E_2 (PGE_2; Sigma Chemical Co., USA), the root extract of Ben-Cha-Moon-Yai remedy (BMY; 125-500 mg/kg) and five herbal root extracts of Ben-Cha-Moon-Yai remedy: *Aegle marmelos* root extract (AM; 25-400 mg/kg), *Oroxylum indicum* root extract (OI; 25-400 mg/kg), *Dimocarpus longan* root extract (DL; 25-400 mg/kg), *Dolichandrone serrulata* root extract (DS; 25-400 mg/kg) and *Walsura trichostemon* root extract (WT; 25-400 mg/kg).

Morphine sulfate, formalin, acetic acid, λ-carrageenan and prostaglandin E_2 were dissolved in normal saline solution.

Indomethacin, the root extract of Ben-Cha-Moon-Yai remedy and five herbal root extracts of Ben-Cha-Moon-Yai remedy were suspended in 2% Tween 80 solution. Morphine sulfate and indomethacin were used as standard analgesic drugs.

Indomethacin was also used as a standard anti-inflammatory drug. The control animals were given with an equivalent volume of vehicle via the same route.

EXPERIMENTAL METHODS

Antinociceptive Activity Testing

Hot-plate Analgesic Testing

Hot-plate test was carried out according to the method previously described by Woolfe and MacDonald in 1948. Male ICR mice weighing 18-25 g were used (N=10 per group). In these experiments, the hot-plate (Harvard apparatus, USA) measuring 28×28 cm was maintained at 55±0.5°C and surrounded by a clear Plexiglas wall cylinder, 20 cm in diameter and 30 cm in height to confine the animal to the heated surface during testing.

On the day of testing, animals were randomly assigned to one of eight treatment groups and underwent 3 pre-drug baseline trials on the hot-plate spaced 5-10 min apart. Only those animals which had a pretreatment hot-plate latency time of less than 45 sec were utilized in these studies. After pre-drug baseline trials, mice were administered various treatments and retested. Each mouse was placed on the hot-plate from an elevation of 5 cm and the latency to the licking of a hind paw or vigorous jumping up from the surface of the metal plate was used as the end point and recorded with a stopwatch. If this behavior was not observed within 45 sec the animal was removed from the hot-plate, given a score of 45 sec for its hot-plate latency and returned to its home cage. The average of the last two trials served as the baseline pre-drug latency.

Immediately, after the third baseline trial on the hot-plate, the animals were administered NSS (10 ml/kg) and MO (10 mg/kg) intraperitoneally or 2% Tween 80 (10 ml/kg), various doses of the root extract of Ben-Cha-Moon-Yai remedy (BMY; 125, 250 and 500 mg/kg) and five herbal root extracts of Ben-Cha-Moon-Yai remedy (25, 50, 100, 200 and 400 mg/kg) orally. The post-drug latency was measured for 7 subsequent trials at 15, 30, 45, 60, 90, 120 and 240 min after drug administration. To avoid tissue damage

the cut-off time was set at 45 sec. The time-course of hot-plate latency was expressed as the mean percent maximum possible effect (%MPE) according to the following formula:

$$\%MPE = \frac{(\text{post-drug latency}) - (\text{pre-drug latency})}{(\text{cut-off time}) - (\text{pre-drug latency})} \times 100$$

with cut-off time for hot-plate test = 45 sec.

The area of analgesia for the hot-plate assays was derived by computing the area under the corresponding 0-240 min time-course-%MPE curves; areas were calculated using the trapezoidal rule (Tallarida and Murray, 1987).

Figure 13 Hot-Plate Analgesiometer

Analysis of the mechanism of antinociceptive action of herbal root extracts

The possible participation of the opioid system in the antinociceptive effect of three herbal root extracts was investigated using the model of mouse hot-plate test. Animal were pretreated with naloxone (NAL; 5 mg/kg, i.p.) 10 min before oral administration of *Aegle marmelos* root extract (AM; 400 mg/kg), *Dolichandrone serrulata* root extract (DS; 200 mg/kg) or *Walsura trichostemon* root extract (WT; 400 mg/kg).

Formalin test

The test was based on the method described by Hunskaar and Hole in 1987. Male ICR mice weighing 18-25 g were used (N=8 per group). Analgesic activity testing was determined using formalin-induced paw licking method. On the day of testing, animals were randomly assigned to one of nine treatment groups. Twenty microliter of 2.5% formalin solution was injected subcutaneously into the left hind paw of each mouse 30 min after intraperitoneal administration of NSS (10 ml/kg) and MO (10 mg/kg) or 1 hr after oral administration of 2% Tween 80 (10 mg/kg), indomethacin (IND; 10 mg/kg), various doses of the root extract of Ben-Cha-Moon-Yai remedy (BMY; 125, 250 and 500 mg/kg) and five herbal root extracts of Ben-Cha-Moon-Yai remedy (25, 50, 100, 200 and 400 mg/kg). Following the formalin injection, animals were immediately placed in an observation cylinder. The time that animal spent licking the injected paw in the early phase (0–5 min) and the late phase (15–30 min) after formalin injection was recorded with a stopwatch. The percentage of inhibition of early and late phases was analyzed using the following formula:

$$\text{\% Inhibition of paw licking} = \frac{\text{Time (control) - Time (test)}}{\text{Time (control)}} \times 100$$

with Time = mean time spent in paw licking (sec).

Figure 14 Formalin-induced paw licking in mouse

Acetic acid-induced writhing test

These studies employed the acetic acid-induced writhing method described by Koster et al. in 1959. Male ICR mice weighing 18-25 g were used (N=8 per group). On the day of testing, animals were randomly assigned to one of seven treatment groups. Mice were then administered 2% Tween 80 (10 mg/kg), IND (10 mg/kg), various doses of the root extract of Ben-Cha-Moon-Yai remedy (125, 250 and 500 mg/kg) or five herbal root extracts of Ben-Cha-Moon-Yai remedy (25, 50, 100, 200 and 400 mg/kg) orally 1 hr before intraperitoneal injection of 0.6% acetic acid (10 ml/kg). Each animal was then placed in a transparent observation cylinder. The number of writhing events (contraction of abdominal muscle together with hind limb extension) were observed and counted at 5 min intervals for a period of 30 min after the acetic acid administration (Nguemfo et al., 2007). Antinociceptive activity was reported as percentage of inhibition of the writhing response compared with the vehicle control group. The percentage of inhibition of the writhing response was calculated using the following formula:

$$\%\ \text{Inhibition of writhing response} = \frac{\text{Wr (control)} - \text{Wr (test)}}{\text{Wr (control)}} \times 100$$

with Wr = mean number of writhes.

Figure 15 Writhing response in mouse

Rota-rod test

Male ICR mice weighing 18-25 g were tested on the rota-rod (N = 8 per group) as previously described by Dunham and Miya in 1957. The animals were placed on a horizontal rod (3.5 cm diameter) rotating at 16 rpm (Ugo Basile, Italy). Mice capable of remaining on the rotating rod for 60 sec or more in three successive trials were selected for the study. Each mouse was treated with 2% Tween 80 (10 ml/kg) or the root extract of Ben-Cha-Moon-Yai remedy (BMY; 500 mg/kg) and five herbal root extracts of Ben-Cha-Moon-Yai remedy (400 mg/kg) orally and placed on the rotating rod at 30, 60, 90, 120 and 240 min after drug administration. The results were expressed as the time in second which the animal enables to remain on the rota-rod during 60 sec (Pearl et al., 1969)

.

Figure 16 Rota-rod test in mice

Anti-inflammatory Activity Testing

Carrageenan-induced paw edema in mice

The anti-inflammatory activity was determined using carrageenan-induced edema in the hind paw according to the method of Winter et al. in 1962 with some modifications. Male ICR mice, 18-25 g (N = 8 per group) were used. On the day of testing, animals were randomly assigned to one of seven treatment groups. Each mouse was pretreated with 2% Tween 80 (10 ml/kg), indomethacin (IND; 10 mg/kg), various doses of the root extract of Ben-Cha-Moon-Yai remedy (BMY; 125, 250 and 500 mg/kg) and five herbal root extracts of Ben-Cha-Moon-Yai remedy (25, 50, 100, 200 and 400 mg/kg) orally. One hour later, 1% carrageenan solution (50 μl) were injected subcutaneously into the plantar surface of the left hind paw of each mouse (Chao et al., 2009). The mouse's paw was marked with black ink at the level of the lateral malleolus and immersed in saline up to this mark. The paw volume was measured before and after injection of carrageenan at 1, 2, 3, 4, 5 and 6 hr using plethysmometer (Ugo Basile, Italy). Edema was expressed as the mean increase in paw volume relative to control animals. The percentage of inhibition of edema was analyzed using the following formula:

$$\% \text{ Inhibition of edema} = \frac{(Vc - Vt)}{Vc} \times 100$$

with Vc = edema volume in control group; Vt = edema volume in tested group.

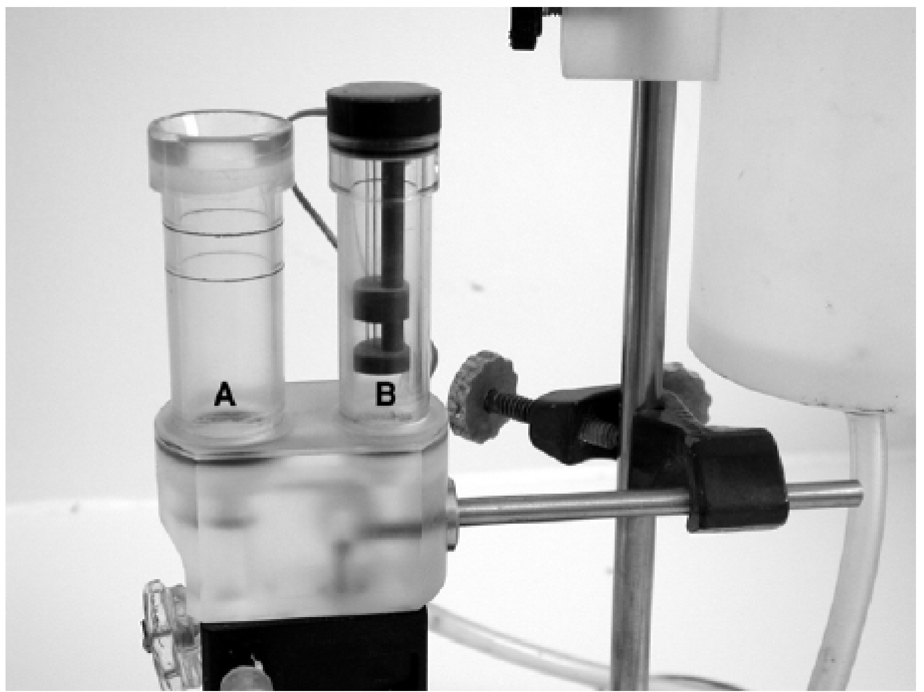


Figure 17 Plethysmometer. The larger one (A) is used to measure fluid displaced by the paw, a volume change that is precisely mirrored in the smaller tube (B) containing a transducer which is linked to a decoder capable of digitally displaying volumes.

Analysis of the mechanism of anti-inflammatory activity of five herbal root extracts

The mechanism of anti-inflammatory activity of five herbal root extracts of Ben-Cha-Moon-Yai remedy (AM, OI, DL, DS and WT) were investigated using the model PGE_2-induced acute inflammation in mice. Male ICR mice 18-25 g (N = 8 per group) were used. On the day of testing, animals were treated orally with 2% Tween 80 (10 ml/kg), indomethacin (IND; 10 mg/kg) or AM, OI, DL, DS and WT (400 mg/kg). One hour later, animals were challenged by subcutaneous injection of 0.01% PGE_2 solution (50 µl) into the plantar surface of the left hind paw (Akkol et al., 2008; Castardo et al., 2008). The paw volume was measured before and after injection of PGE_2 at 0.5, 1, 1.5, 2, 3 and

4 hr using plethysmometer (Ugo Basile). Edema volume was expressed as the mean increase in paw volume in relation to control. The percentage of inhibition of edema was analyzed using the following formula as described above.

DATA TERATMENT AND STATISTICAL ANALYSE

The results were expressed as mean±S.E.M. Differences in mean values between groups were analyzed by a one-way analysis of variance (ANOVA) and Student's *t*-test followed by a post-hoc Tukey test for multiple comparisons. Statistical significance was assessed as $p<0.05$.

CHAPTER IV
RESULTS

MOUSE HOT-PLATE TEST

To demonstrate the validity of the hot-plate analgesic testing following drug administration, mice received morphine sulphate (MO; 10 mg/kg) intraperitoneally (i.p.) and were tested during the subsequent 240 min period. As expected MO significantly ($p<0.001$) increased the hot-plate latency producing an area of analgesia of 12,835.65±1,909.33 %MPE-min compared with that of normal saline solution (NSS) (-10,873.10±4,166.42 %MPE-min; Figure 18).

Initial studies utilizing the hot-plate test in mice to examine the efficacy of the root extract of Ben-Cha-Moon-Yai remedy and five herbal root extracts of Ben-Cha-Moon-Yai remedy in producing analgesia. Mice were administered orally 2% Tween 80 or various doses of the root extract of Ben-Cha-Moon-Yai remedy (BMY; 125, 250 and 500 mg/kg) and five herbal root extracts of Ben-Cha-Moon-Yai remedy (25, 50, 100, 200 and 400 mg/kg).

All doses of BMY (125, 250 and 500 mg/kg) significantly ($p<0.05$, $p<0.001$ and $p<0.01$, respectively) increased the hot-plate latency when compared to the vehicle group. BMY 250 mg/kg significantly ($p<0.05$) increased the hot-plate latency when compared to BMY 125 mg/kg (Figure 19). The analgesic peak effects of BMY 125, 250 and 500 mg/kg were reached within 90, 240, 120 min after oral administration, respectively. Individual time courses of the responses are shown in Figure 20.

AM 400 mg/kg significantly ($p<0.05$) increased the hot-plate latency when compared to the vehicle group (Figure 21). The analgesic peak effect of AM 400 mg/kg was reached within 120 min after oral administration. Individual time courses of the responses are shown in Figure 22.

All doses of OI and DL (25-400 mg/kg) did not produce analgesic response when compared to the vehicle group (Figure 23 and 25). Individual time courses of the responses are shown in Figure 24 and 26.

DS at the doses of 200 and 400 mg/kg significantly (p<0.01, p<0.05, respectively) increased the hot-plate latency when compared to the vehicle group (Figure 27). The analgesic peak effects of DS 200 and 400 mg/kg were reached within 120 and 90 min after oral administration, respectively. Individual time courses of the responses are shown in Figure 28.

WT at the doses of 100, 200 and 400 mg/kg significantly (p<0.05, p<0.01 and p<0.01, respectively) increased the hot-plate latency when compared to the vehicle group (Figure 29). The analgesic peak effects of WT 100, 200 and 400 mg/kg were reached within 120, 30 and 90 min after oral administration, respectively. Individual time courses of the responses are shown in Figure 30.

In order to investigate any role of the opioid receptor in AM, DS and WT actions, mice were then administered NSS (10 ml/kg, i.p.), naloxone (NAL; 5 mg/kg, i.p.), a short-acting opioid receptor antagonist, 2% Tween 80 (10 ml/kg, p.o.), AM (400 mg/kg, p.o.), DS (200 mg/kg, p.o.), WT (400 mg/kg, p.o.) or the combination of NAL and AM (5/400 mg/kg), the combination of NAL and DS (5/200 mg/kg) and the combination of NAL and WT (5/400 mg/kg). NAL alone failed to produce significant response when compared to vehicle group. AM, DS, WT at the dose tested produced significant (p<0.001) response when compared to vehicle group. The inclusion of naloxone with AM, DS and WT significantly (p<0.001) attenuated the analgesic response due to AM, DS and WT indicating that opioid receptors are involved in the analgesic response produced by AM, DS and WT (Figure 31, 33, 35, respectively). Individual time courses of the responses are shown in Figure 32, 34, 36, respectively.

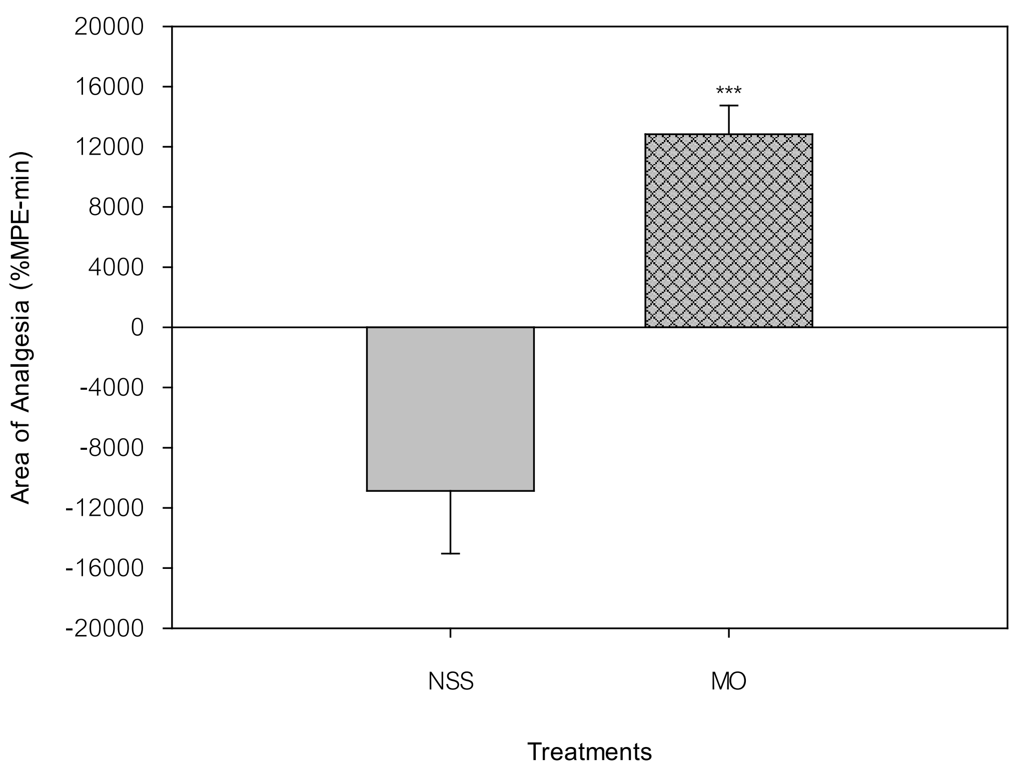


Figure 18 Area of analgesia (%MPE-min) from 0-240 minutes after intraperitoneal administration of normal saline solution (NSS; 10 ml/kg) and morphine sulphate (MO; 10 mg/kg). N=10 for all groups. ***p<0.001 significantly different compared to NSS.

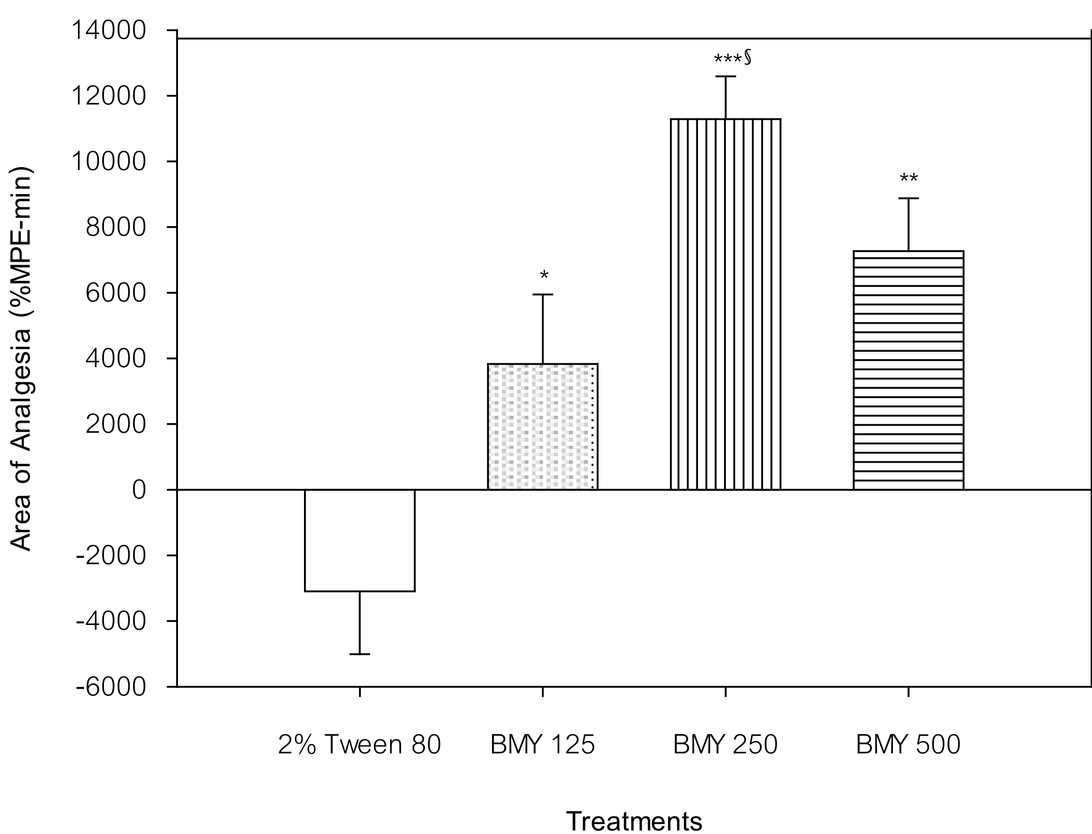


Figure 19 Area of analgesia (%MPE-min) from 0-240 minutes after oral administration of 2% Tween 80 (10 ml/kg) and various doses of the root extract of Ben-Cha-Moon-Yai remedy (BMY; 125-500 mg/kg). N=10 for all groups.

$^{*}p<0.05$, $^{**}p<0.01$, $^{***}p<0.001$ significantly different compared to 2% Tween 80.

$^{\S}p<0.05$ significantly different compared to BMY 125 mg/kg.

Mouse Hot-plate Test

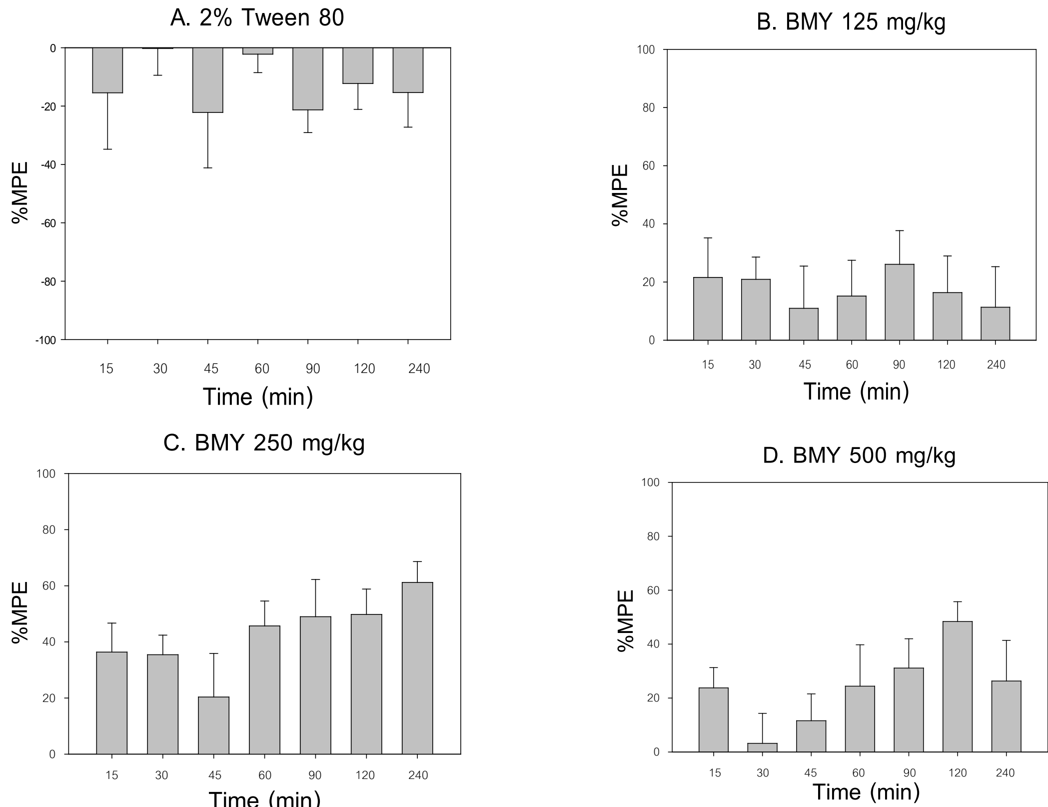


Figure 20 Individual time courses of the response (%MPE versus time (min)) after oral administration of various doses of the root extract of Ben-Cha-Moon-Yai remedy (BMY; 125-500 mg/kg).

A. 2% Tween 80 10 ml/kg, B. BMY 125 mg/kg, C. BMY 250 mg/kg, D. BMY 500 mg/kg

N=10 for all groups.

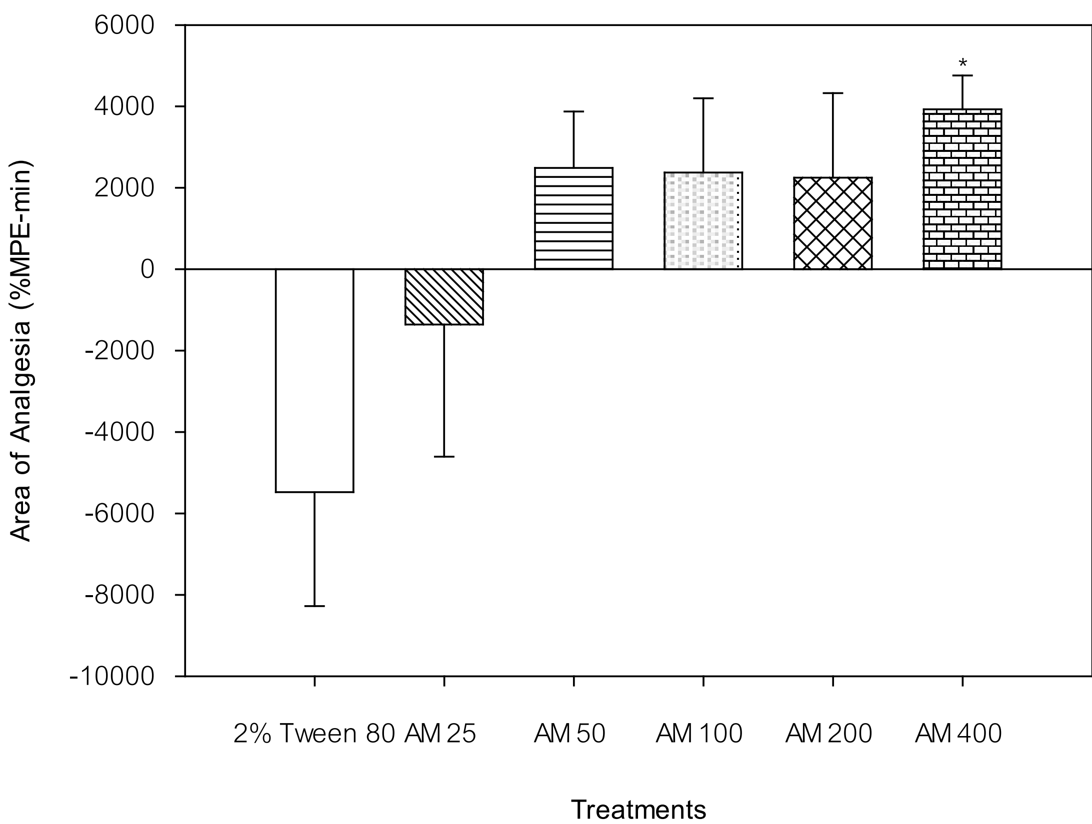


Figure 21 Area of analgesia (%MPE-min) from 0-240 minutes after oral administration of 2% Tween 80 and various doses of *Aegle marmelos* root extract (AM; 25-400 mg/kg). N=10 for all groups. $^{*}p<0.05$ significantly different compared to 2% Tween 80.

Mouse Hot-plate Test

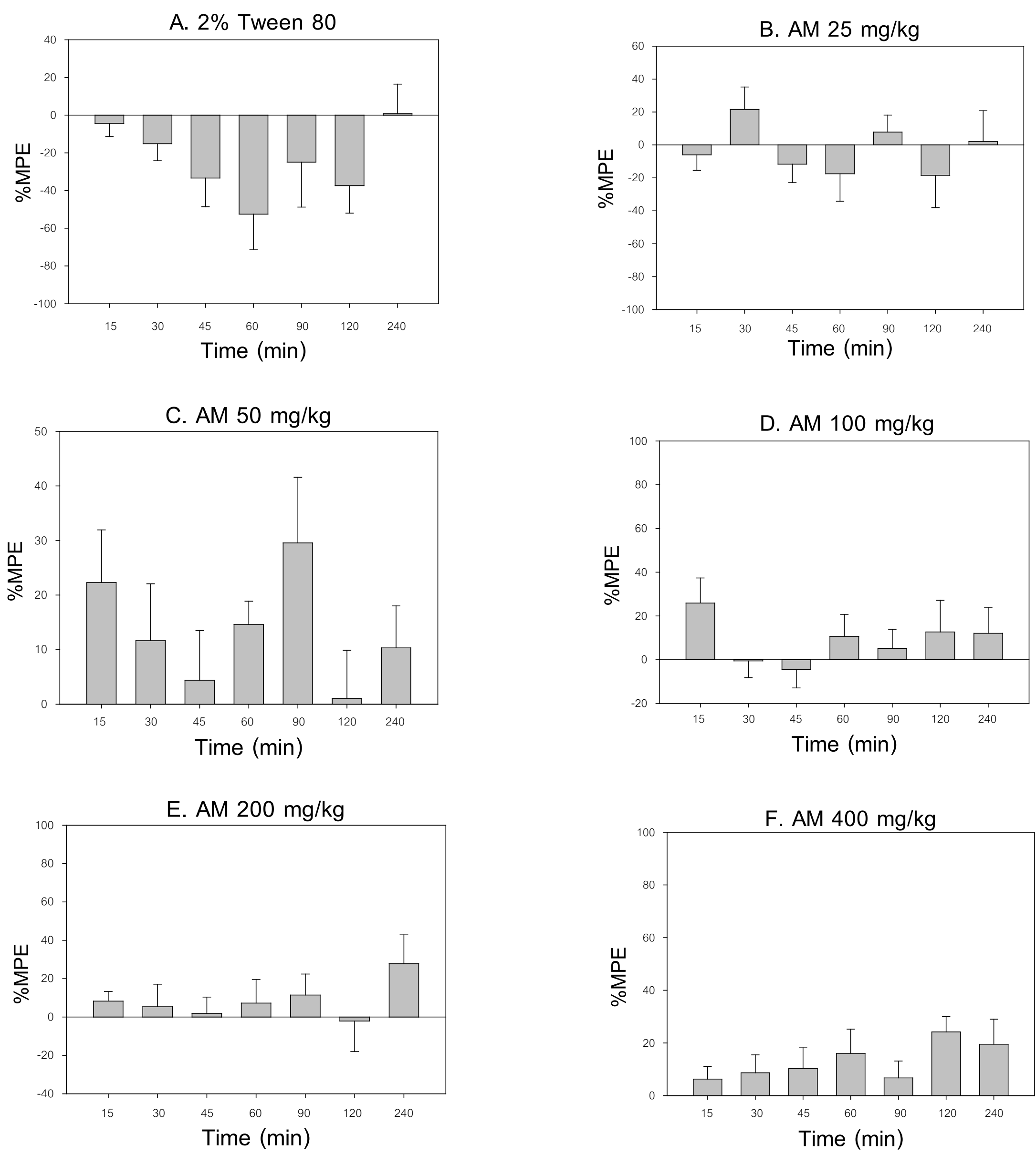


Figure 22 Individual time courses of the response (%MPE versus time (min)) after oral administration of various doses of *Aegle marmelos* root extract (AM; 25-400 mg/kg). A. 2% Tween 80 10 ml/kg, B. AM 25 mg/kg, C. AM 50 mg/kg, D. AM 100 mg/kg, E. AM 200 mg/kg, F. AM 400 mg/kg. N=10 for all groups.

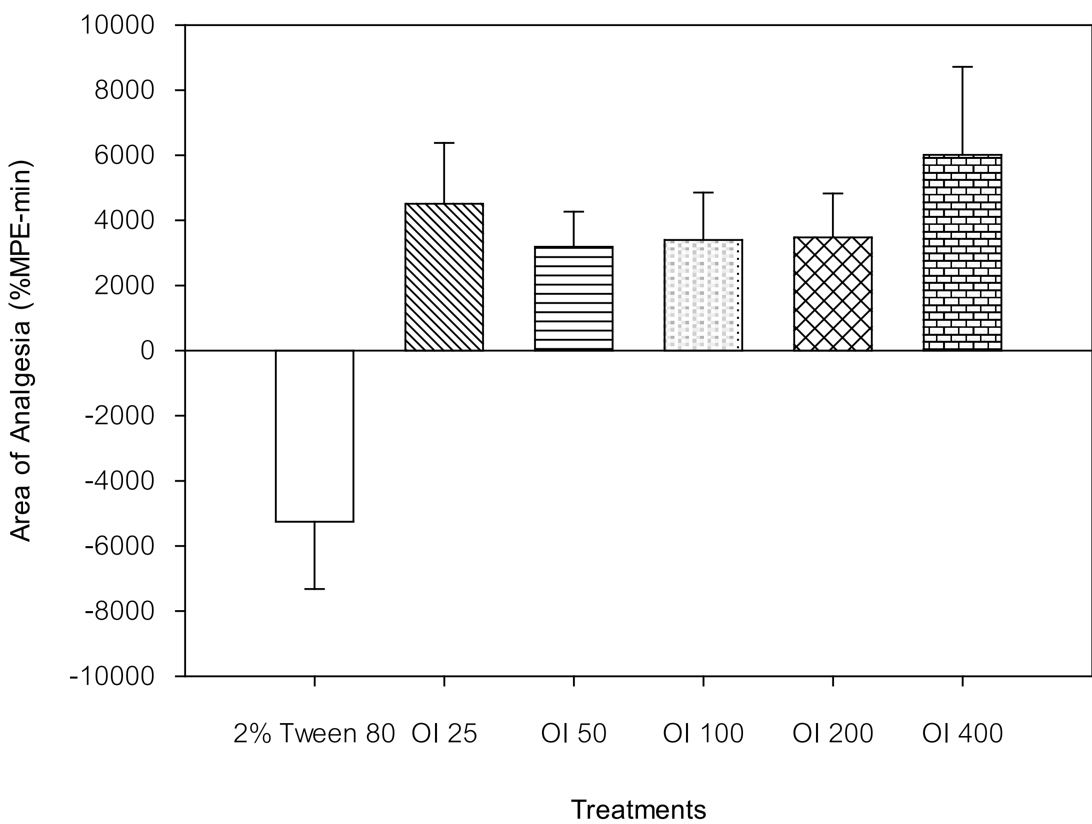


Figure 23 Area of analgesia (%MPE-min) from 0-240 minutes after oral administration of 2% Tween 80 and various doses of *Oroxylum indicum* root extract (OI; 25-400 mg/kg). N=10 for all groups.

Mouse Hot-plate Test

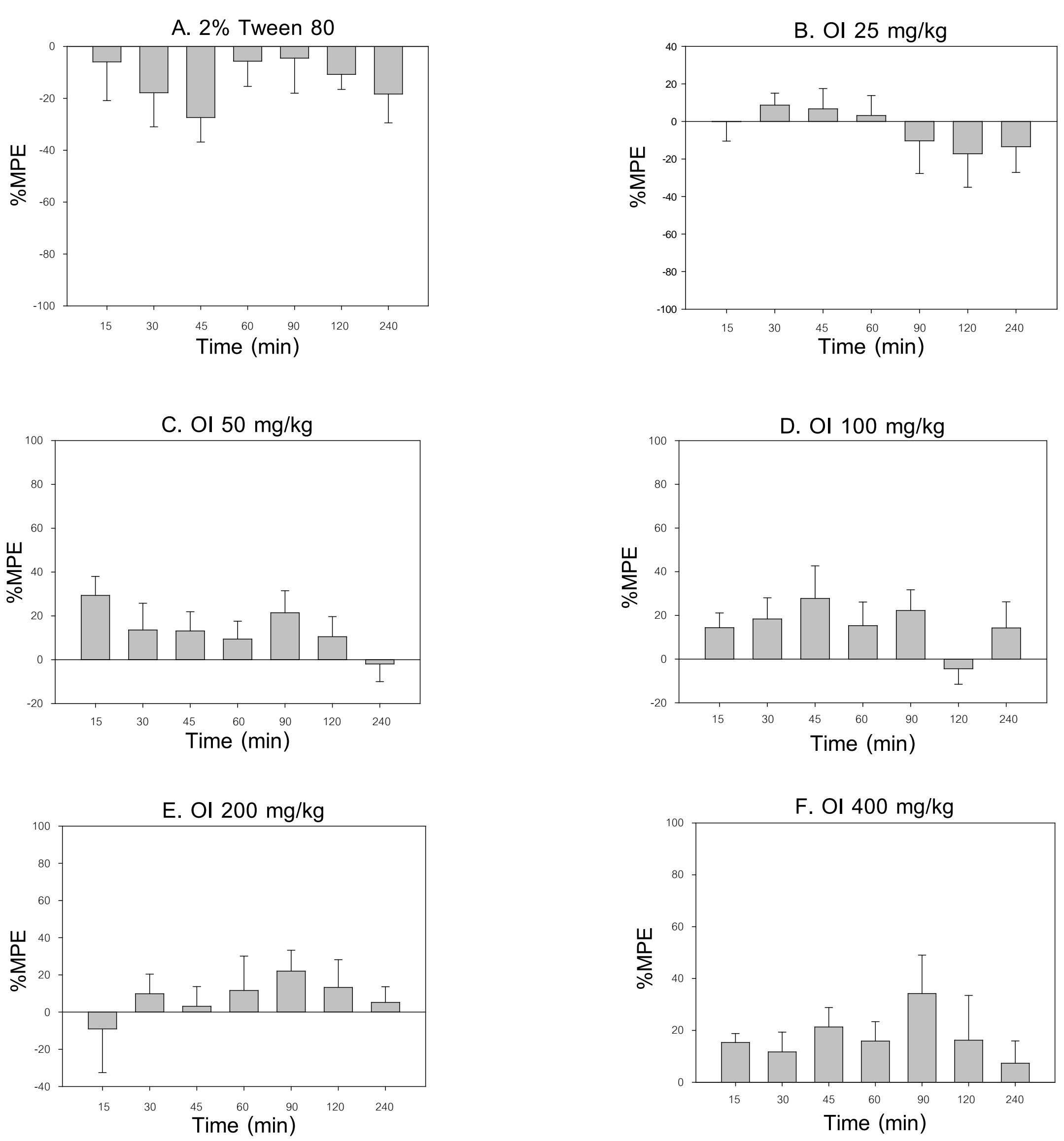


Figure 24 Individual time courses of the response (%MPE versus time (min)) after oral administration of various doses of *Oroxylum indicum* root extract (OI; 25-400 mg/kg). A. 2% Tween 80 10 ml/kg, B. OI 25 mg/kg, C. OI 50 mg/kg, D. OI 100 mg/kg, E. OI 200 mg/kg, F. OI 400 mg/kg. N=10 for all groups.

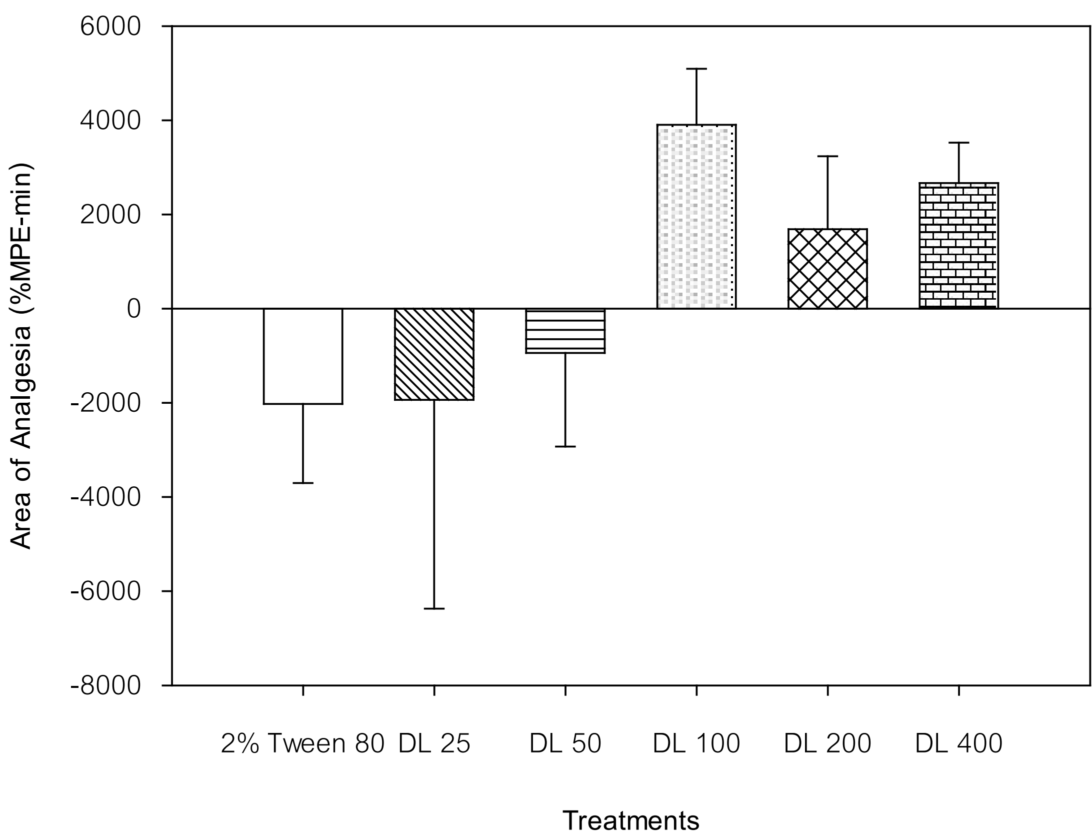


Figure 25 Area of analgesia (%MPE-min) from 0-240 minutes after oral administration of 2% Tween 80 and various doses of *Dimocarpus longan* root extract (DL; 25-400 mg/kg). N=10 for all groups.

Mouse Hot-plate Test

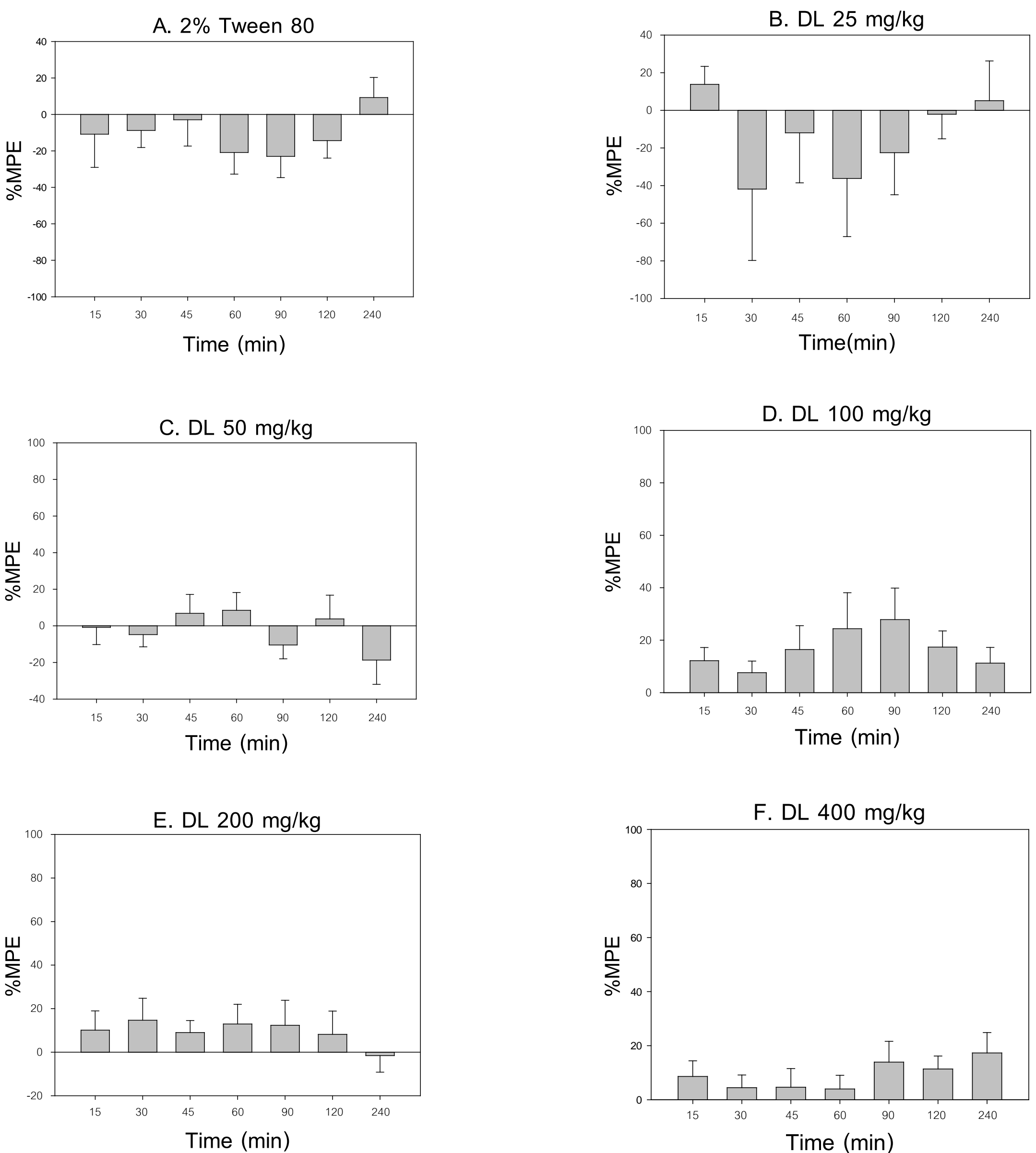


Figure 26 Individual time courses of the response (%MPE versus time (min)) after oral administration of various doses of *Dimocarpus longan* root extract (DL; 25-400 mg/kg). A. 2% Tween 80 10 ml/kg, B. DL 25 mg/kg, C. DL 50 mg/kg, D. DL 100 mg/kg, E. DL 200 mg/kg, F. DL 400 mg/kg. N=10 for all groups.

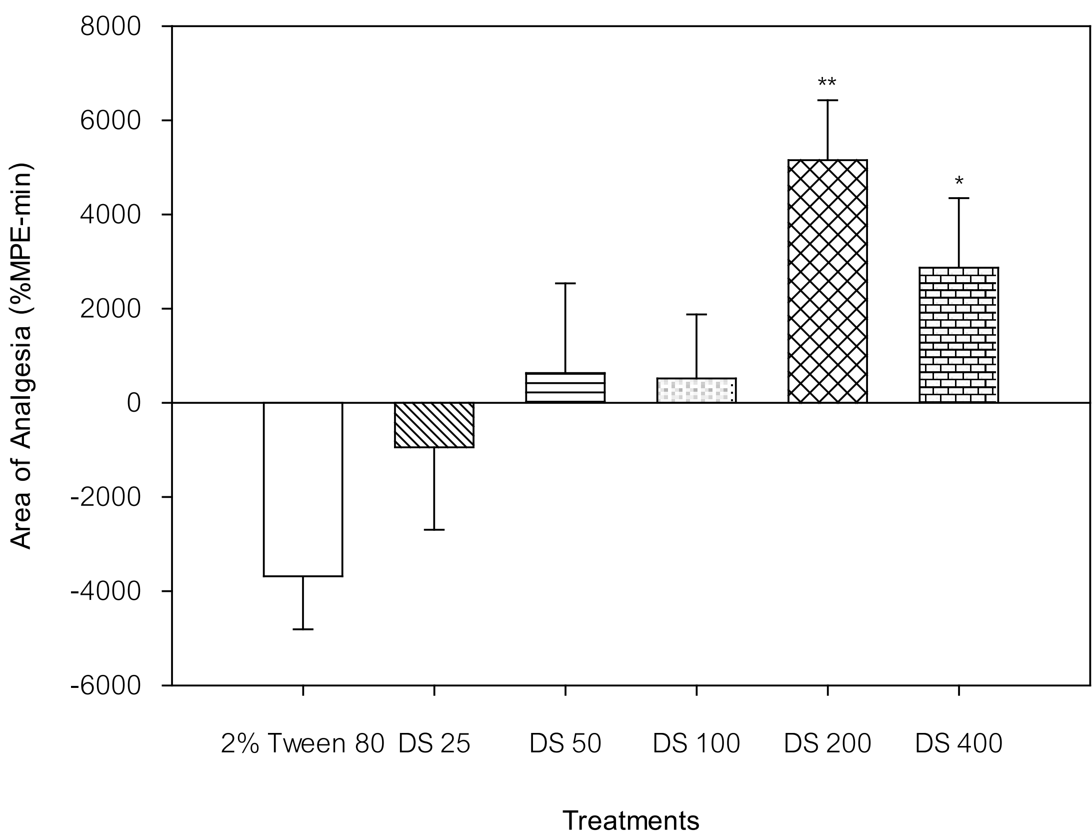


Figure 27 Area of analgesia (%MPE-min) from 0-240 minutes after oral administration of 2% Tween 80 and various doses of *Dolichandrone serrulata* root extract (DS; 25-400 mg/kg). N=10 for all groups. $^{*}p$<0.05, $^{**}p$<0.01 significantly different compared to 2% Tween 80.

Mouse Hot-plate Test

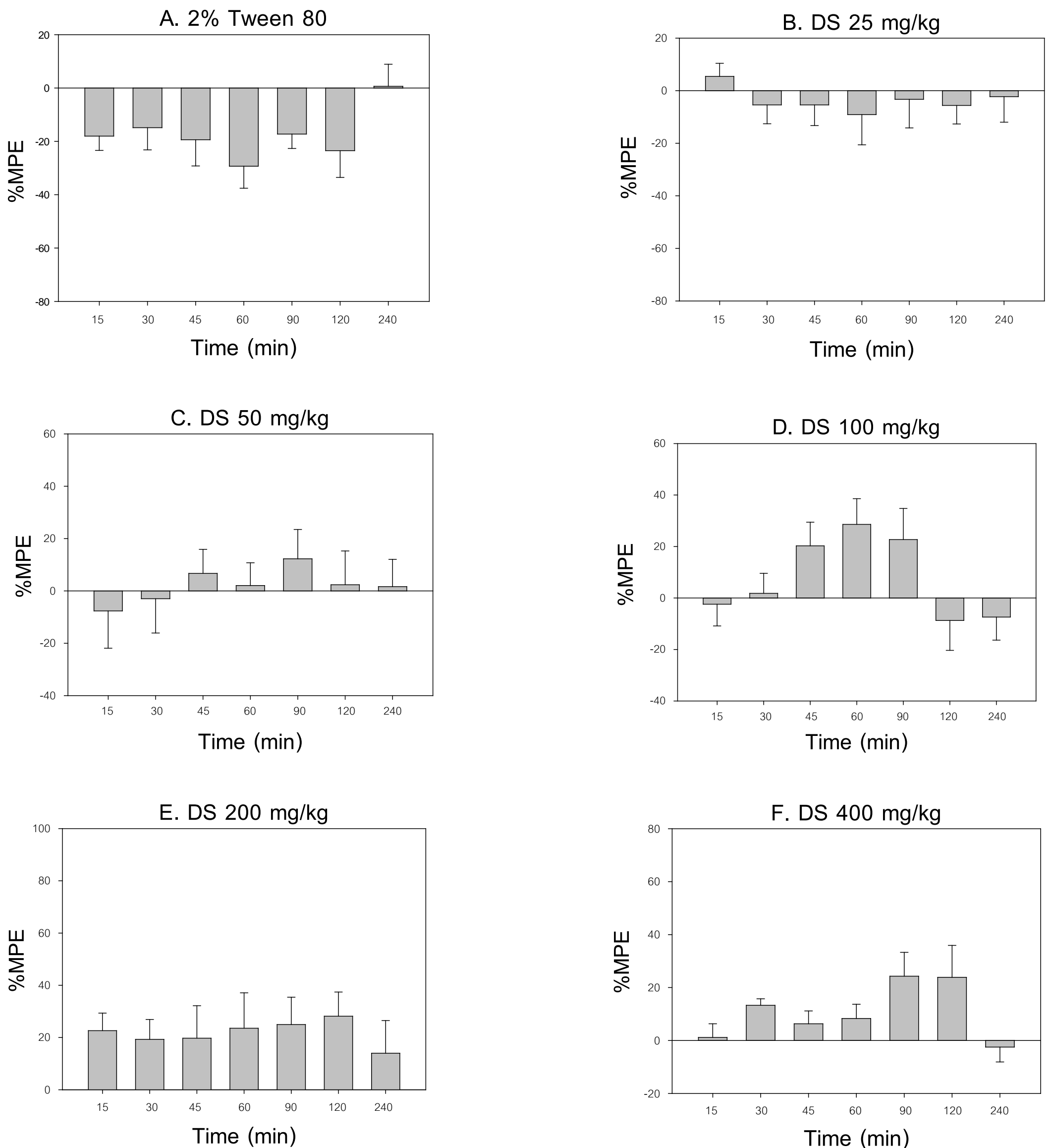


Figure 28 Individual time courses of the response (%MPE versus time (min)) after oral administration of various doses of *Dolichandrone serrulata* root extract (DS; 25-400 mg/kg).

A. 2% Tween 80 10 ml/kg, B. DS 25 mg/kg, C. DS 50 mg/kg, D. DS 100 mg/kg, E. DS 200 mg/kg, F. DS 400 mg/kg. N=10 for all groups.

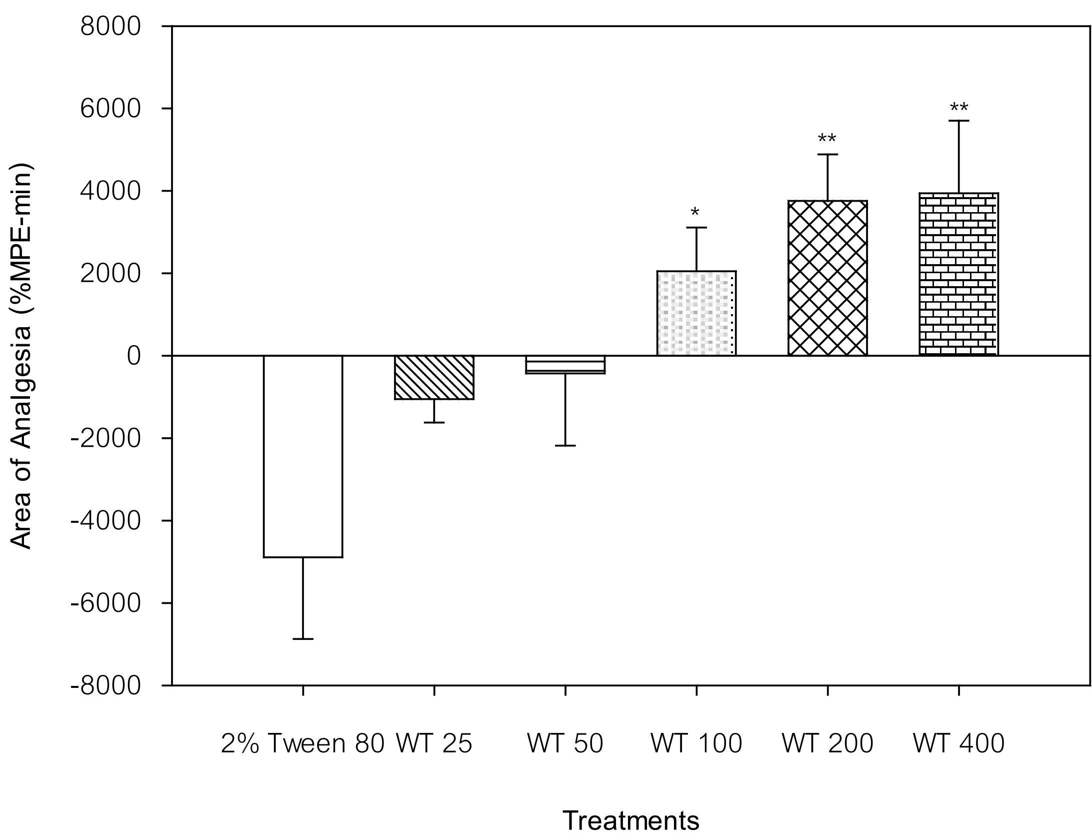


Figure 29 Area of analgesia (%MPE-min) from 0-240 minutes after oral administration of 2% Tween 80 and various doses of *Walsura trichostemon* root extract (WT; 25-400 mg/kg). N=10 for all groups. $^{*}p$<0.05, $^{**}p$<0.01 significantly different compared to 2% Tween 80.

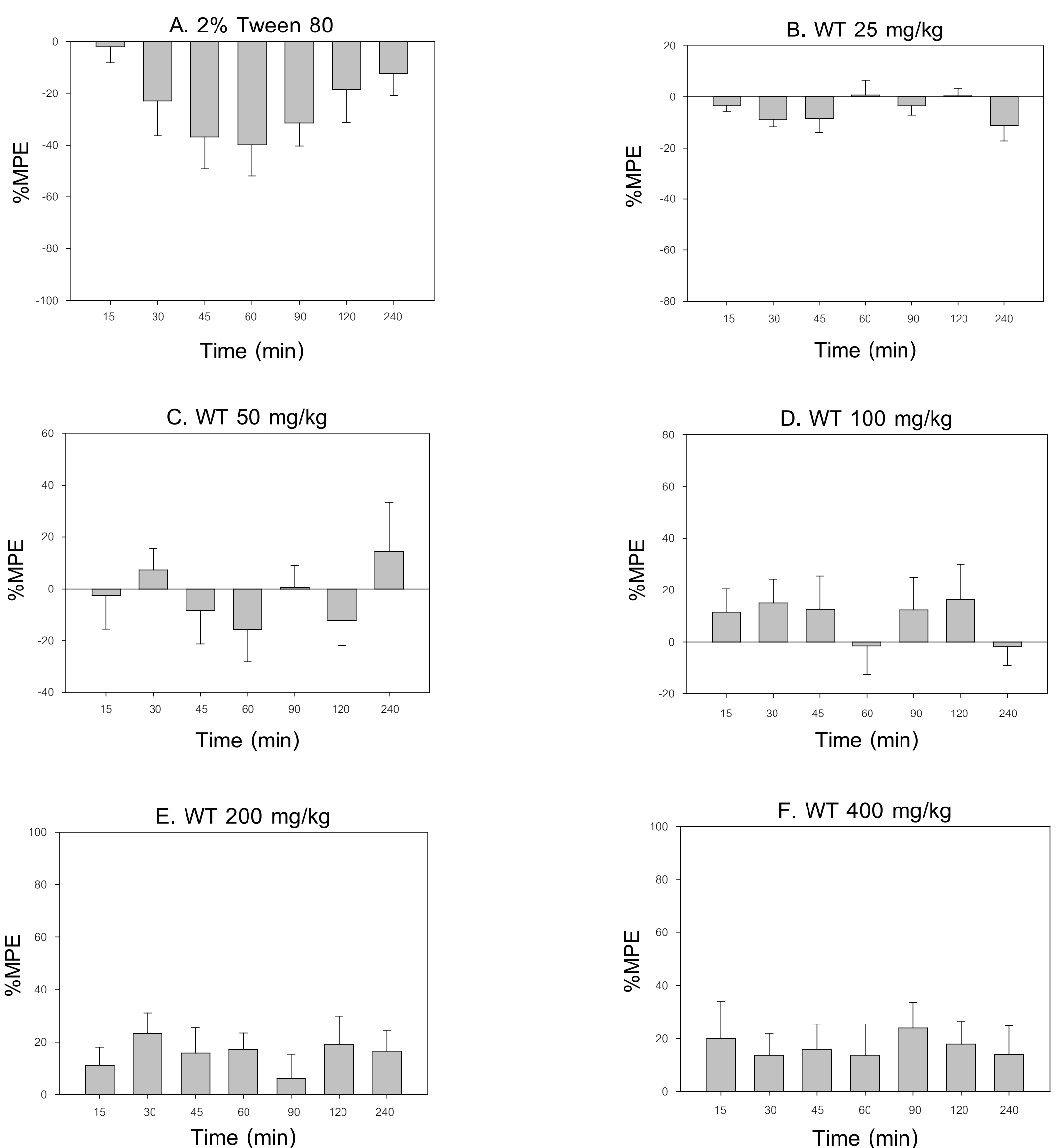


Figure 30 Individual time courses of the response (%MPE versus time (min)) after oral administration of various doses of *Walsura trichostemon* root extract (WT; 25-400 mg/kg).

A. 2% Tween 80 10 ml/kg, B. WT 25 mg/kg, C. WT 50 mg/kg, D. WT 100 mg/kg, E. WT 200 mg/kg, F. WT 400 mg/kg. N=10 for all groups.

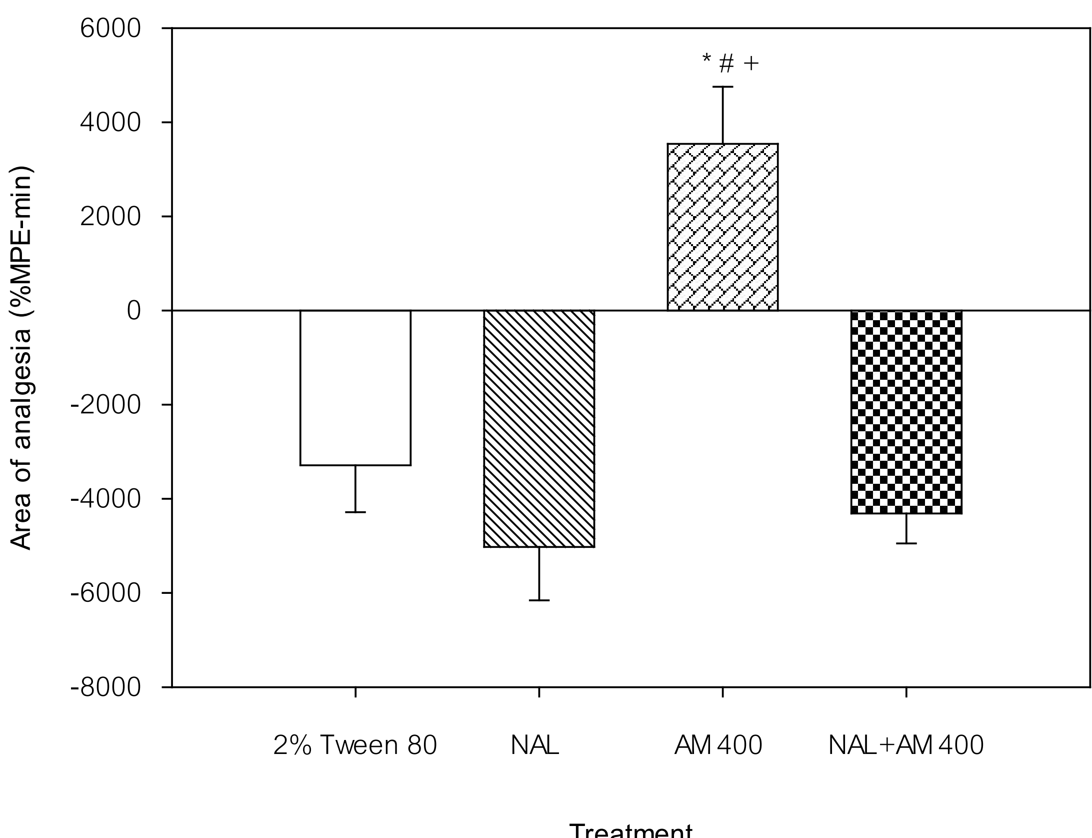


Figure 31 Area of analgesia (%MPE-min) from 0-240 minutes after administration of 2% Tween 80 (10 ml/kg, p.o.), naloxone (NAL; 5 mg/kg, i.p.), *Aegle marmelos* root extract (AM; 400 mg/kg, p.o.) and the combination of naloxone and AM (5/400 mg/kg). N=10 for all groups.

$^{*}p<0.001$ significantly different compared to 2% Tween 80.

$^{\#}p<0.001$ significantly different compared to NAL.

$^{+}p<0.001$ significantly different compared to NAL+AM 400.

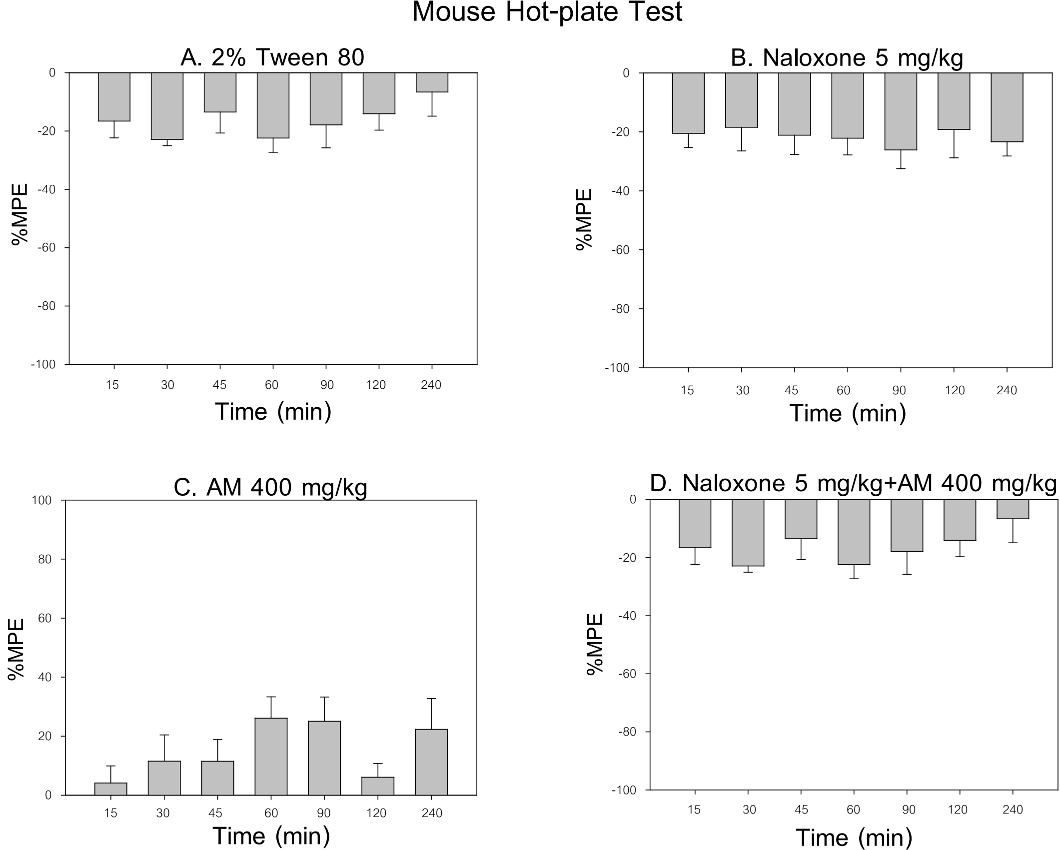


Figure 32 Individual time courses of the response (%MPE versus time (min)) after administration of 2% Tween 80 (p.o.), naloxone 5 mg/kg (i.p.), *Aegle marmelos* root extract 400 mg/kg (AM, p.o.) and the combination of naloxone and AM (5/400 mg/kg).
A. 2% Tween 80, B. Naloxone 5 mg/kg, C. AM 400 mg/kg, D. Naloxone 5 mg/kg + AM 400 mg/kg. N=10 for all groups.

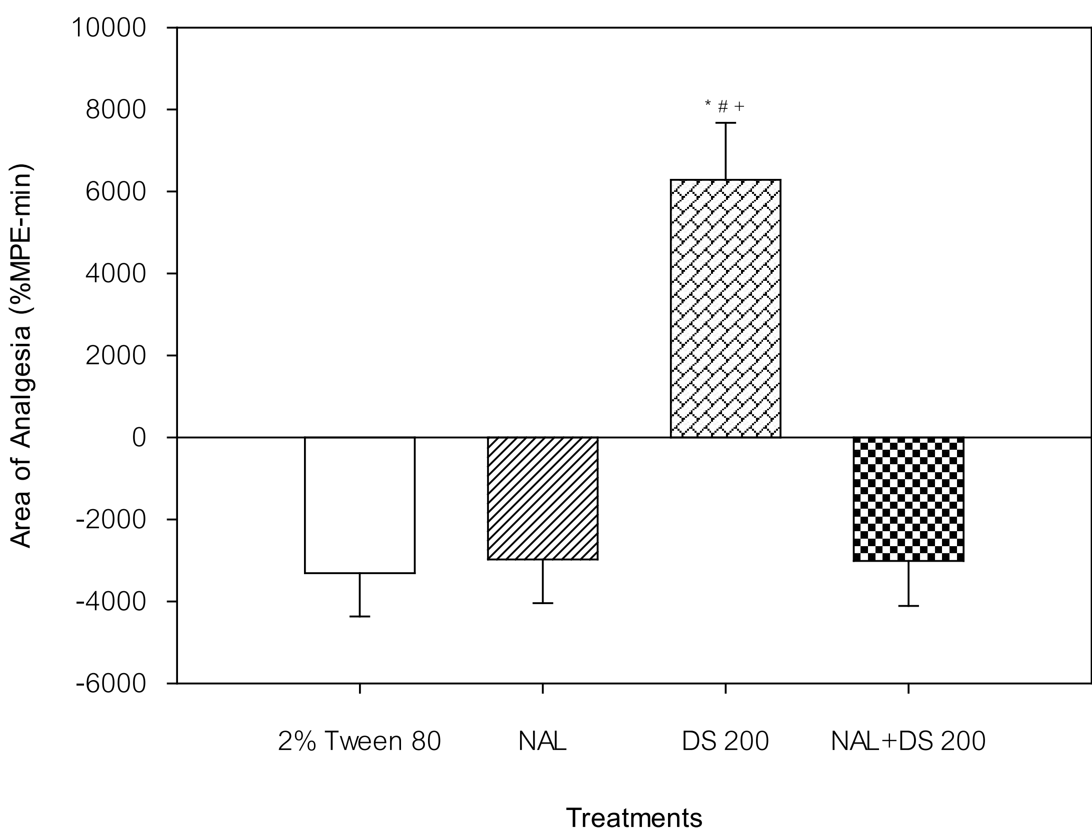


Figure 33 Area of analgesia (%MPE-min) from 0-240 minutes after administration of 2% Tween 80 (10 ml/kg, p.o.), naloxone (NAL; 5 mg/kg, i.p.), *Dolichandrone serrulata* root extract (DS; 200 mg/kg, p.o.) and the combination of naloxone and DS (5/200 mg/kg). N=10 for all groups.

$^{*}p<0.001$ significantly different compared to 2% Tween 80.

$^{\#}p<0.001$ significantly different compared to NAL.

$^{+}p<0.001$ significantly different compared to NAL+DS 200.

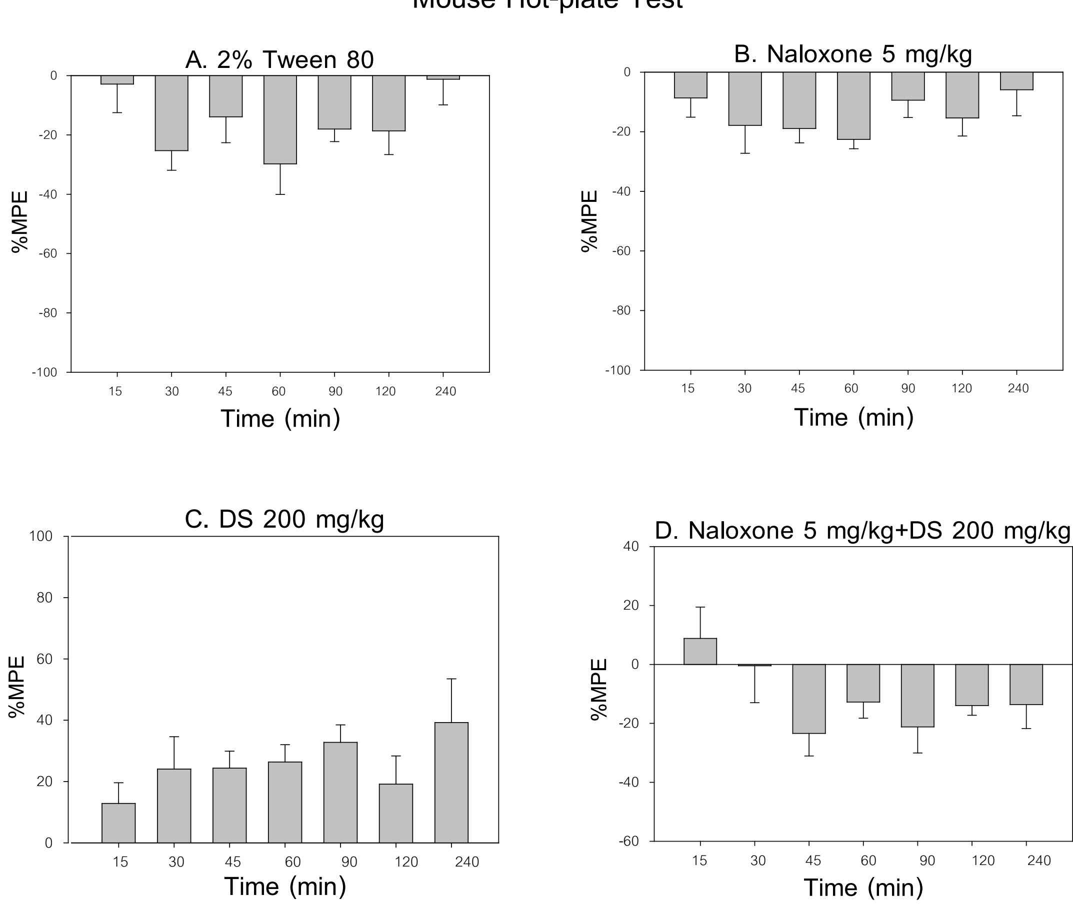


Figure 34 Individual time courses of the response (%MPE versus time (min)) after administration of 2% Tween80 (p.o.), naloxone 5 mg/kg (i.p.), *Dolichandrone serrulata* root extract 200 mg/kg (DS, p.o.) and the combination of naloxone and DS (5/200 mg/kg).

A. 2% Tween 80, B. Naloxone 5 mg/kg, C. DS 200 mg/kg, D. Naloxone 5 mg/kg + DS 200 mg/kg. N=10 for all groups.

Mouse Hot-plate Test

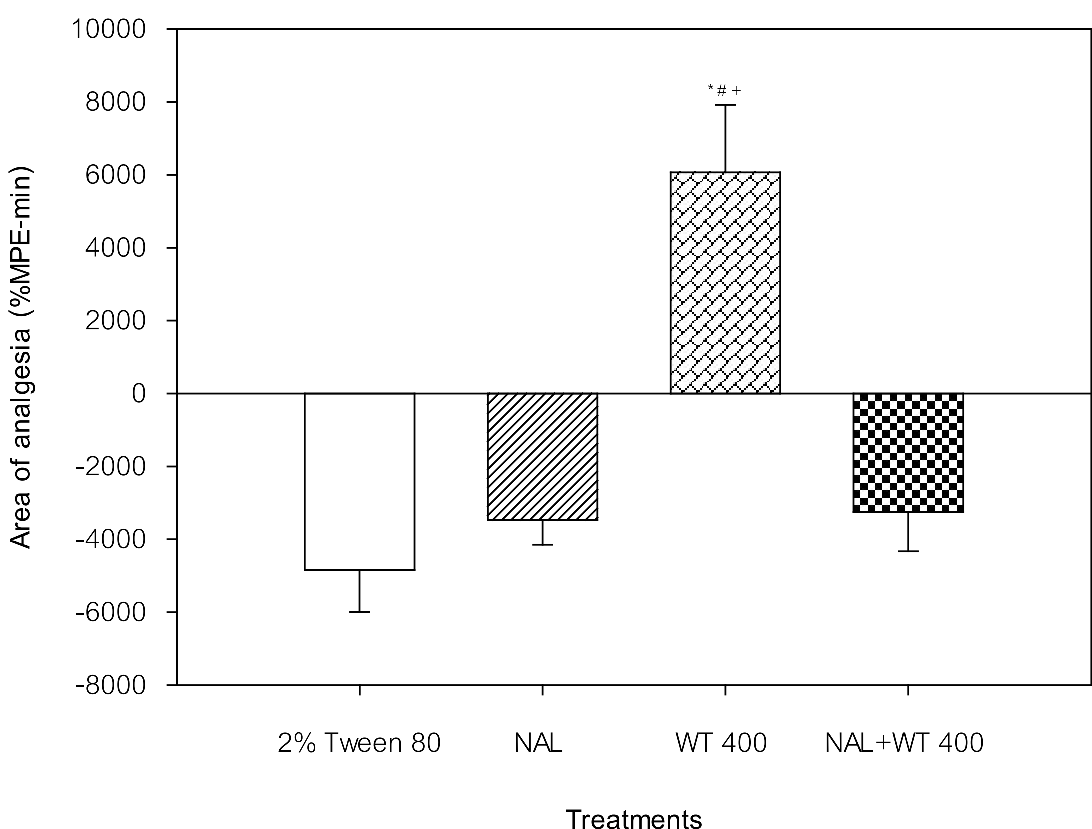


Figure 35 Area of analgesia (%MPE-min) from 0-240 minutes after administration of 2% Tween 80 (10 ml/kg, p.o.), naloxone (NAL; 5 mg/kg, i.p.), *Walsura trichostemon* root extract (WT; 400 mg/kg, p.o.) and the combination of naloxone and WT (5/400 mg/kg). N=10 for all groups.

$^{*}p<0.001$ significantly different compared to 2% Tween 80.

$^{\#}p<0.001$ significantly different compared to NAL.

$^{+}p<0.001$ significantly different compared to NAL+WT 400.

Mouse Hot-plate Test

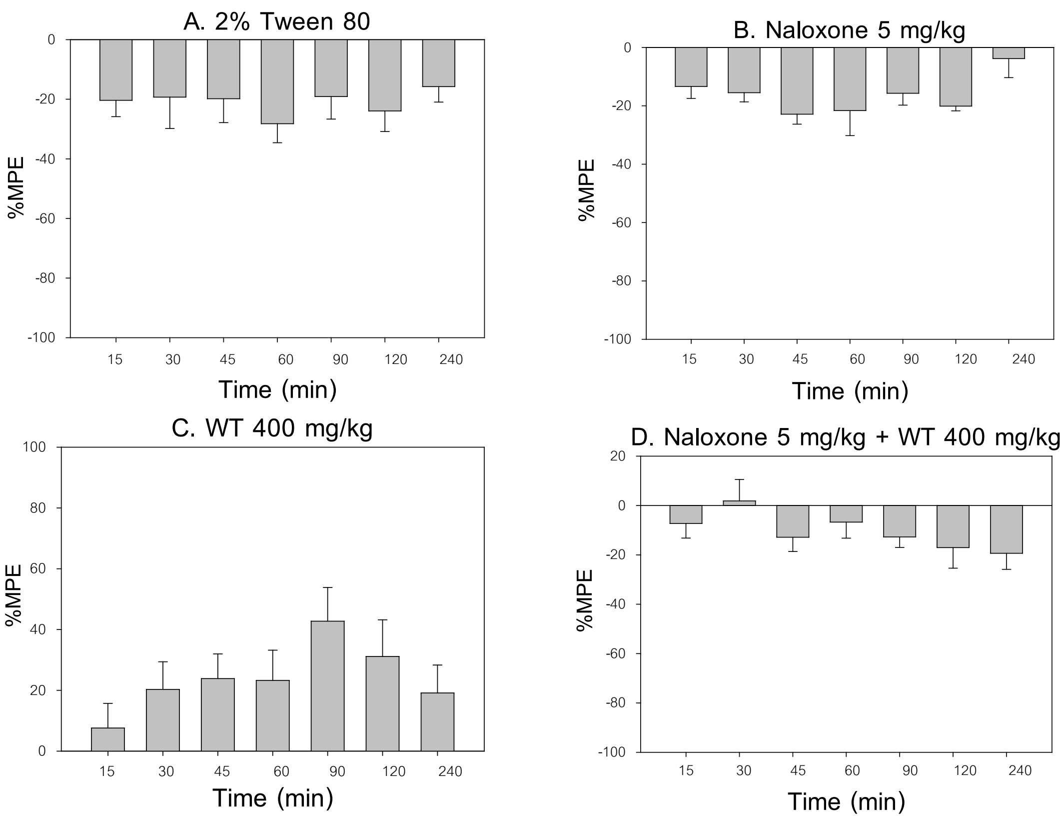


Figure 36 Individual time courses of the response (%MPE versus time (min)) after administration of 2% Tween 80 (p.o.), naloxone 5 mg/kg (i.p.), *Walsura trichostemon* root extract 400 mg/kg (WT, p.o.) and the combination of naloxone and WT (5/400 mg/kg). A. 2% Tween 80, B. Naloxone 5 mg/kg, C. WT 400 mg/kg, D. Naloxone 5 mg/kg + WT 400 mg/kg. N=10 for all groups.

FORMALIN TEST IN MICE

To demonstrate the validity of formalin test following drug administration, mice received morphine sulfate (MO; 10 mg/kg) intraperitoneally or indomethacin (IND; 10 mg/kg) and were observed for paw licking at early phase (0-5 min) and late phase (15-30 min). As expected MO significantly (p<0.001 and p<0.001, respectively) decreased the licking time of both early and late phases by 90.87% and 97.69%, respectively producing mean time spent on paw licking of 7.48±1.84 and 3.67±1.08 sec compared with that of NSS (81.92±8.60 and 158.80±6.08 sec, respectively; Figure 37, Table 3). IND significantly decreased the licking time in the late phase compared with that of vehicle group (Figure 38-43; Table 4-9).

Study then utilized the formalin test in mice to examine the efficacy of BMY and five herbal root extracts (AM, OI, DL, DS and WT) in producing analgesia. Mice were administered orally 2% Tween 80, IND (10 mg/kg), various doses of BMY (125, 250 and 500 mg/kg) or AM, OI, DL, DS and WT (25, 50, 100, 200 and 400 mg/kg).

Only BMY 250 mg/kg significantly (p<0.05) decreased the licking time by 20.03% producing mean time spent on paw licking of 90.82±6.93 sec compared with that of vehicle group (113.56±4.83 sec) in the early phase. For the late phase, All doses of BMY (125, 250 and 500 mg/kg) significantly (p<0.01) decreased the licking time by 48.96%, 56.85% and 51.30%, respectively producing mean time spent on paw licking of 80.97±18.20, 68.47±14.99 and 77.27±18.43 sec, respectively when compared with that of vehicle group (158.66±13.07 sec). The reference drug, IND (10 mg/kg) also caused significant (p<0.01) inhibition of the late phase of formalin-induced nociception, producing 56.58% inhibition when compared to the vehicle group (Figure 38, Table 3).

AM at dose of 400 mg/kg significantly (p<0.05) decreased the licking time in the early phase by 34.55% producing mean time spent on paw licking of 64.19±4.08 sec compared with that of vehicle group (98.06±5.45 sec). Furthermore, AM 400 mg/kg also significantly (p<0.01 and p<0.05, respectively) decreased the licking time in the early phase compared with that of AM 25 and 100 mg/kg (103.42±6.64 and 98.60±4.03 sec, respectively). IND (10 mg/kg), the reference drug, caused significant (p<0.001)

inhibition of the late phase of formalin-induced nociception, producing 61.71% inhibition when compared to the vehicle group. AM 400 mg/kg significantly ($p<0.001$) decreased the licking time by and 59.85% producing the mean time spent on paw licking of 58.44±5.50 sec compared with that of vehicle group (145.57±17.16 sec). The antinociceptive efficacy of AM 400 mg/kg is comparable to IND. Additionally, AM 400 mg/kg significantly ($p<0.001$, $p<0.001$, $p<0.001$ and $p<0.01$, respectively) decreased the licking time in the late phase compared with that of AM 25, 50, 100 and 200 mg/kg (Figure 39, Table 4).

All doses of OI failed to decrease the licking time in the early phase induced by formalin. However, OI at the doses of 100, 200 and 400 mg/kg significantly ($p<0.01$, $p<0.001$, $p<0.001$, respectively) decreased the licking time by 49.40%, 61.78% and 68.28%, respectively producing mean time spent on paw licking of 73.07±9.98, 55.18±9.03 and 45.80±17.08 sec, respectively compared with that of vehicle group (144.41±10.68 sec) during the late phase. IND (10 mg/kg) caused significant ($p<0.01$) inhibition of the late phase of formalin-induced nociception, producing 48.96% inhibition when compared to the vehicle group. In addition, OI 100 mg/kg significantly ($p<0.05$) decreased the licking time in the late phase compared with that of OI 25 mg/kg. OI 200 mg/kg significantly ($p<0.001$ and $p<0.01$, respectively) decreased the licking time in the late phase compared with that of OI 25 and 50 mg/kg. OI 400 mg/kg significantly ($p<0.001$) decreased the licking time in the late phase compared with that of OI 25 and 50 mg/kg. The antinociceptive efficacy during the late phase of OI 50-400 mg/kg is comparable to IND (Figure 40, Table 5).

All doses of DL failed to decrease the licking time in the early phase induced by formalin. However, DL 200 and 400 mg/kg significantly ($p<0.01$ and $p<0.001$, respectively) decreased the licking time during the late phase by 53.57% and 88.14%, respectively producing mean time spent on paw licking of 56.90±12.12 and 14.52±6.54 sec, respectively compared with that of vehicle group (122.54±15.26 sec). IND (10 mg/kg) caused significant ($p<0.01$) inhibition only in the late phase of formalin-induced nociception, producing 56.39% inhibition when compared to the vehicle group.

Furthermore, in the late phase, DL 200 mg/kg significantly (*p*<0.01 and *p*<0.05, respectively) decreased the licking time compared with that of DL 25 and 50 mg/kg. DL 400 mg/kg also significantly (*p*<0.001) decreased the licking time in the late phase compared with that of DL 25-100 mg/kg. The antinociceptive efficacy during the late phase of DL 200 and 400 mg/kg are comparable to IND (Figure 41, Table 6).

DS 400 mg/kg significantly (*p*<0.001) decreased the licking time in the early phase by 38.17% producing mean time spent on paw licking of 64.53±3.99 sec compared with that of vehicle group (104.38±5.50 sec). DS 400 mg also significantly (*p*<0.01, *p*<0.05, *p*<0.01 and *p*<0.05, respectively) decreased mean time spent on paw licking in the early phase compared with that of IND and DS at the doses of 25, 50 and 100 mg/kg, respectively. For the late phase, DS 200 and 400 mg/kg significantly (*p*<0.01, *p*<0.001, respectively) decreased the licking time in the late phase by 45.22% and 55.31%, respectively producing mean time spent on paw licking of 73.99±7.81 and 60.36±5.32 sec, respectively compared with that of vehicle group (135.07±10.01 sec). DS 200 mg/kg significantly (*p*<0.01) decreased the licking time in the late phase compared with that of DS 25 mg/kg. DS 400 mg/kg significantly (*p*<0.001) decreased the licking time compared with that of DS 25 mg/kg. IND (10 mg/kg) caused significant (*p*<0.001) inhibition of the late phase of formalin-induced nociception, producing 61.15% inhibition when compared to the vehicle group. The antinociceptive efficacy during the late phase of DS 200 and 400 mg/kg are comparable to IND (Figure 42, Table 7).

In the early phase, WT 400 mg kg significantly (*p*<0.05) decreased the licking time by 31.66% producing mean time spent on paw licking of 72.32±4.15 sec compared with that of vehicle group (105.03±6.11 sec). WT 400 mg/kg significantly (*p*<0.01 and *p*<0.05, respectively) decreased the licking time compared with that of WT 50 and 100 mg/kg. For the late phase, WT 200 and 400 mg/kg significantly (*p*<0.01 and *p*<0.001, respectively) decreased the licking time by 44.49% and 53% producing mean time spent on paw licking of 78.59±7.71 and 66.55±5.82 sec, respectively compared with that of vehicle group (141.59±13.19 sec). WT 400 mg/kg also significantly (*p*<0.01)

decreased the licking time compared with that of WT 25 mg/kg. IND (10 mg/kg) caused significant ($p<0.001$) inhibition of the late phase of formalin-induced nociception, producing 61.74% inhibition when compared to the vehicle group. The antinociceptive efficacy during the late phase of WT 200 and 400 mg/kg are comparable to IND (Figure 43, Table 8).

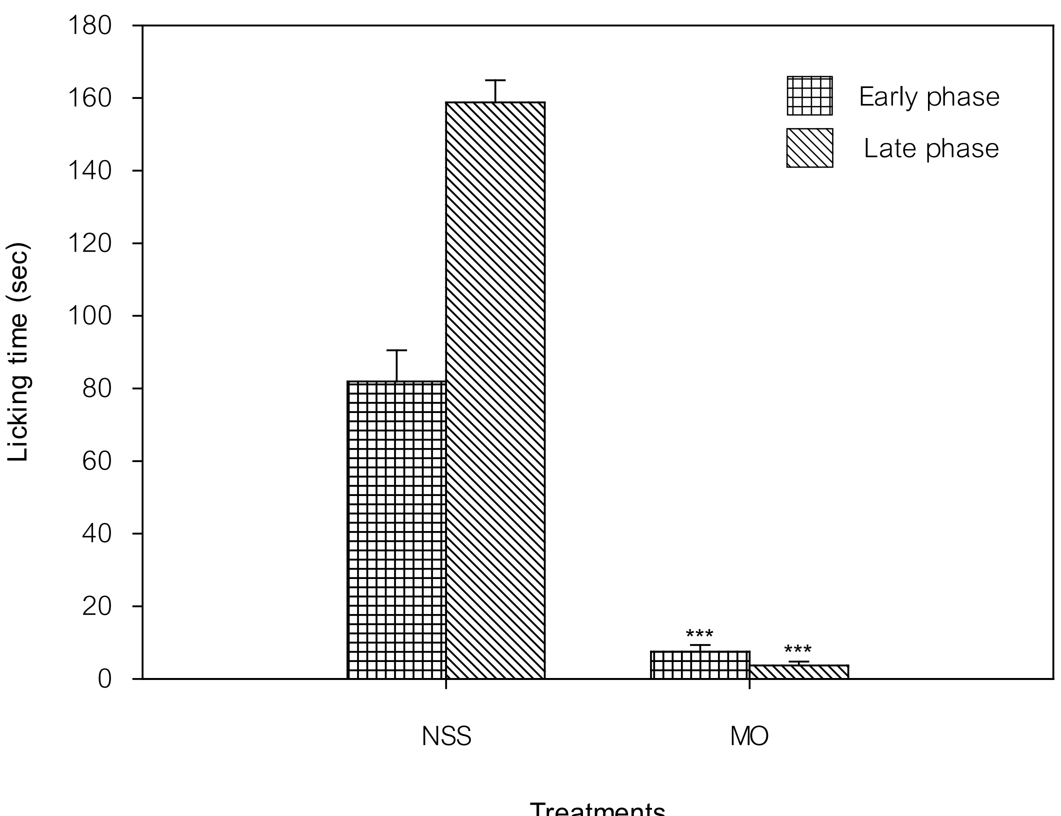


Figure 37 Time spent on paw licking after intraperitoneal administration of 0.9% normal saline solution (NSS; 10 ml/kg) and morphine sulphate (MO; 10 mg/kg). N=8 for all groups. $^{***}p<0.001$ significantly different compared to NSS.

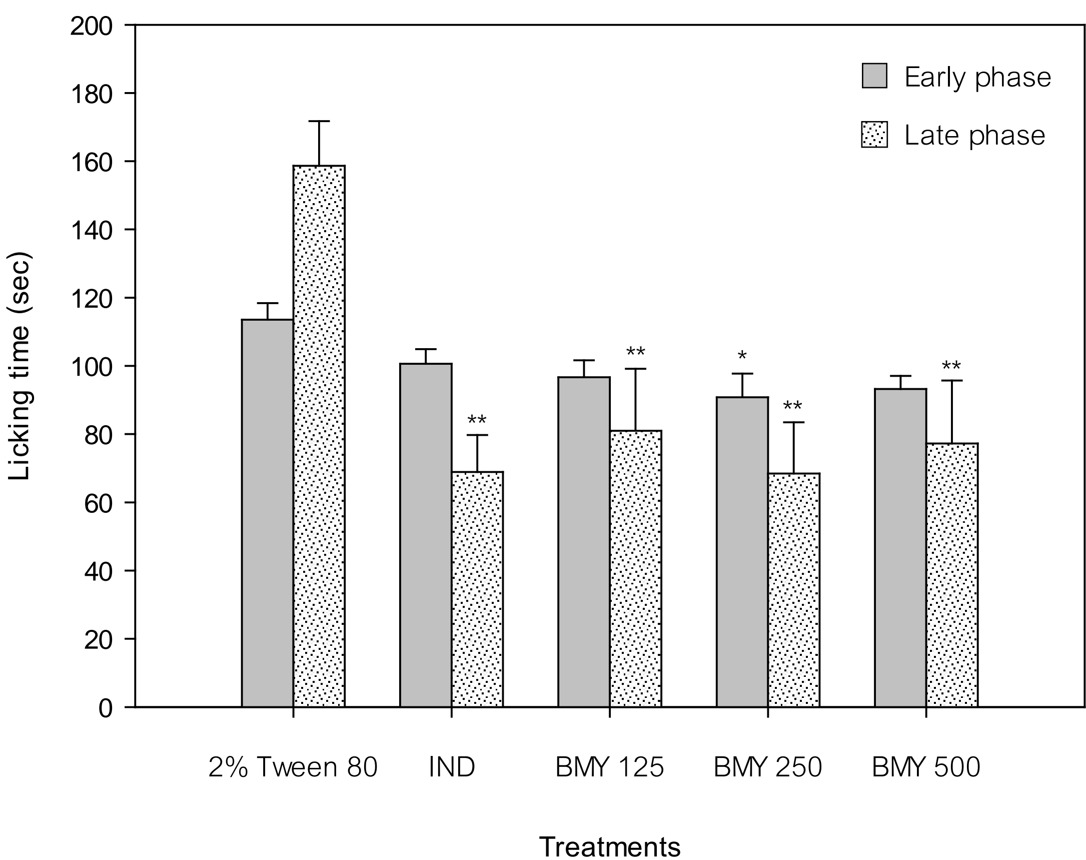


Figure 38 Time spent on paw licking after oral administration of 2% Tween 80 (10 ml/kg), indomethacin (IND; 10 mg/kg) and various doses of the root extract of Ben-Cha-Moon-Yai remedy (BMY; 125-500 mg/kg). N=8 for all groups. $^{*}p<0.05$, $^{**}p<0.01$ significantly different compared to 2% Tween 80.

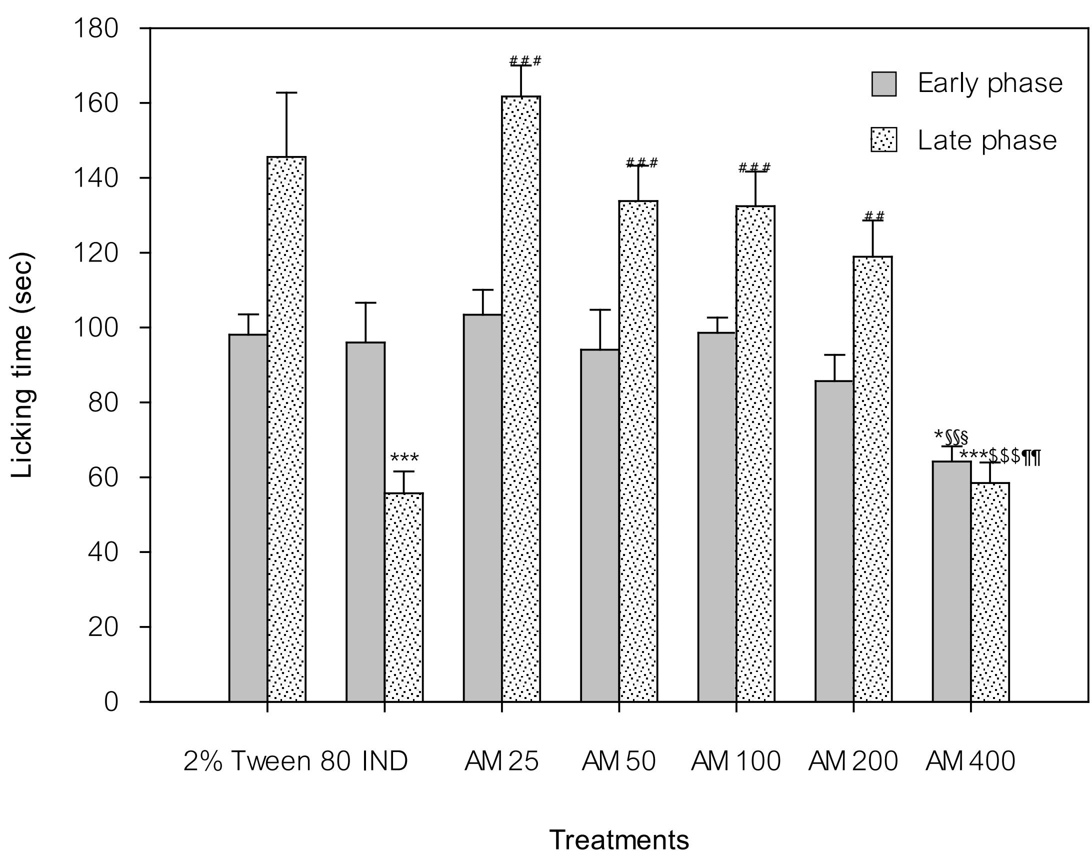


Figure 39 Time spent on paw licking after oral administration of 2% Tween 80 (10 ml/kg), indomethacin (IND; 10 mg/kg) and various doses of *Aegle marmelos* root extract (AM; 25-400 mg/kg). N=8 for all groups.

$^{*}p<0.05$, $^{***}p<0.001$ significantly different compared to 2% Tween 80.

$^{§}p<0.05$ significantly different compared to AM 100 mg/kg.

$^{¶¶}p<0.01$ significantly different compared to AM 200 mg/kg.

$^{§§}p<0.01$ significantly different compared to AM 25 mg/kg.

$^{\$\$\$}p<0.001$ significantly different compared to AM 25-100 mg/kg.

$^{\#\#}p<0.01$, $^{\#\#\#}p<0.001$ significantly different compared to IND 10 mg/kg.

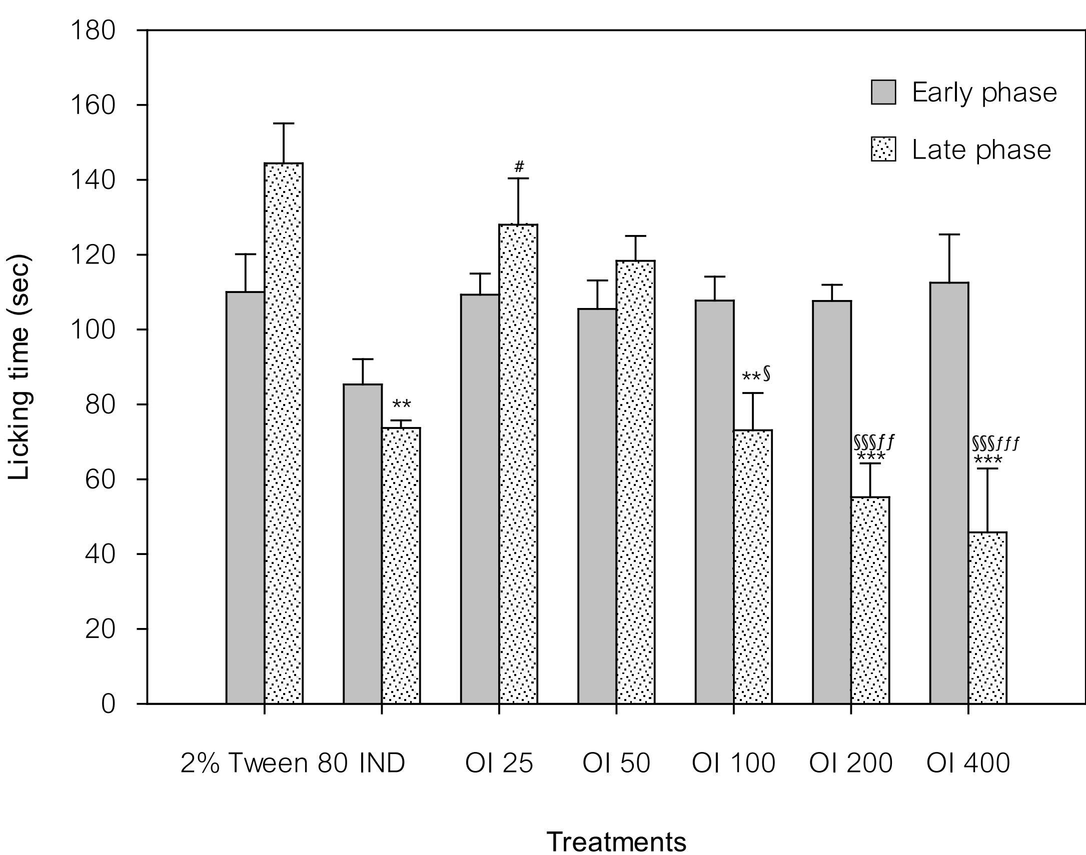


Figure 40 Time spent on paw licking after oral administration of 2% Tween 80 (10 ml/kg), indomethacin (IND; 10 mg/kg) and various doses of *Oroxylum indicum* root extract (OI; 25-400 mg/kg). N=8 for all groups.

$^{**}p$<0.01, $^{***}p$<0.001 significantly different compared to 2% Tween 80.

$^{§}p$<0.05, $^{§§§}p$<0.001 significantly different compared to OI 25 mg/kg.

^{ff}p<0.01, ^{fff}p<0.001 significantly different compared to OI 50 mg/kg.

$^{\#}p$<0.05 significantly different compared to IND 10 mg/kg.

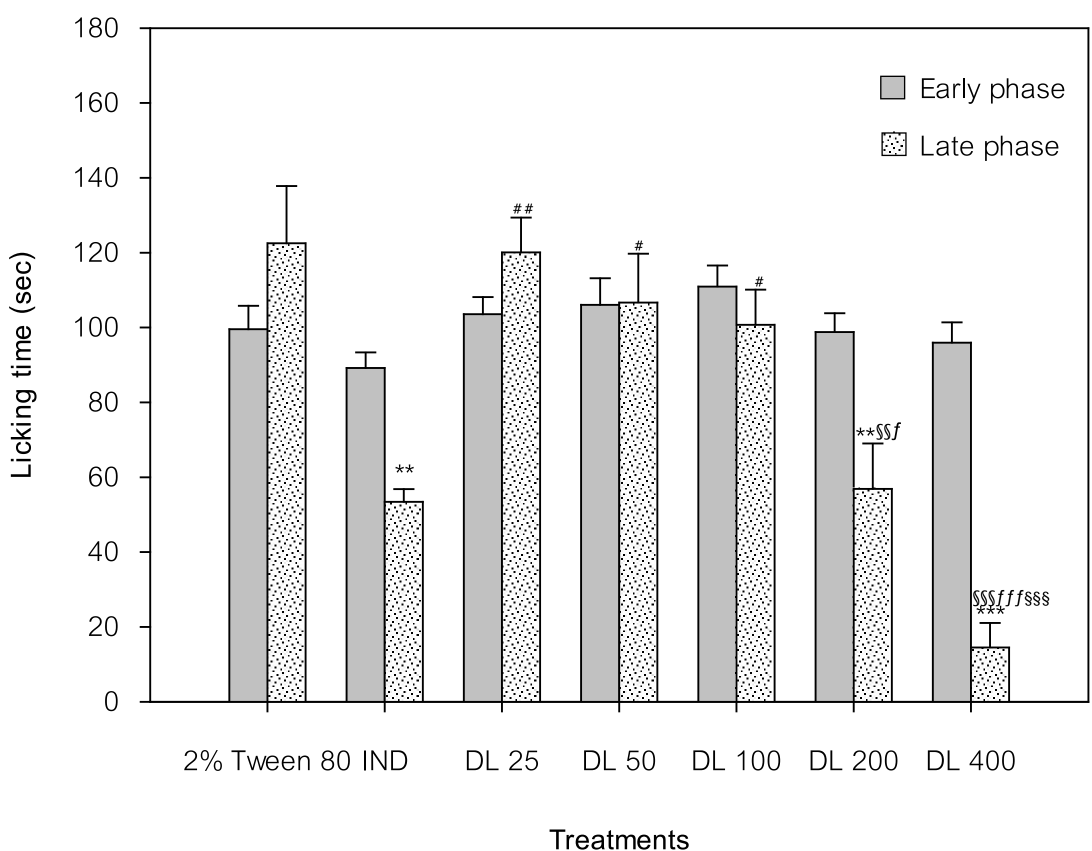


Figure 41 Time spent on paw licking after oral administration of 2% Tween 80 (10 ml/kg), indomethacin (IND; 10 mg/kg) and various doses of *Dimocarpus longan* root extract (DL; 25-400 mg/kg). N=8 for all groups.

$^{**}p<0.01$, $^{***}p<0.001$ significantly different compared to 2% Tween 80.

$^{f}p<0.05$, $^{fff}p<0.001$ significantly different compared to DL 50 mg/kg.

$^{§§}p<0.01$, $^{§§§}p<0.001$ significantly different compared to DL 25 mg/kg.

$^{§§§}p<0.001$ significantly different compared to DL 100 mg/kg.

$^{\#}p<0.05$, $^{\#\#}p<0.01$ significantly different compared to IND 10 mg/kg.

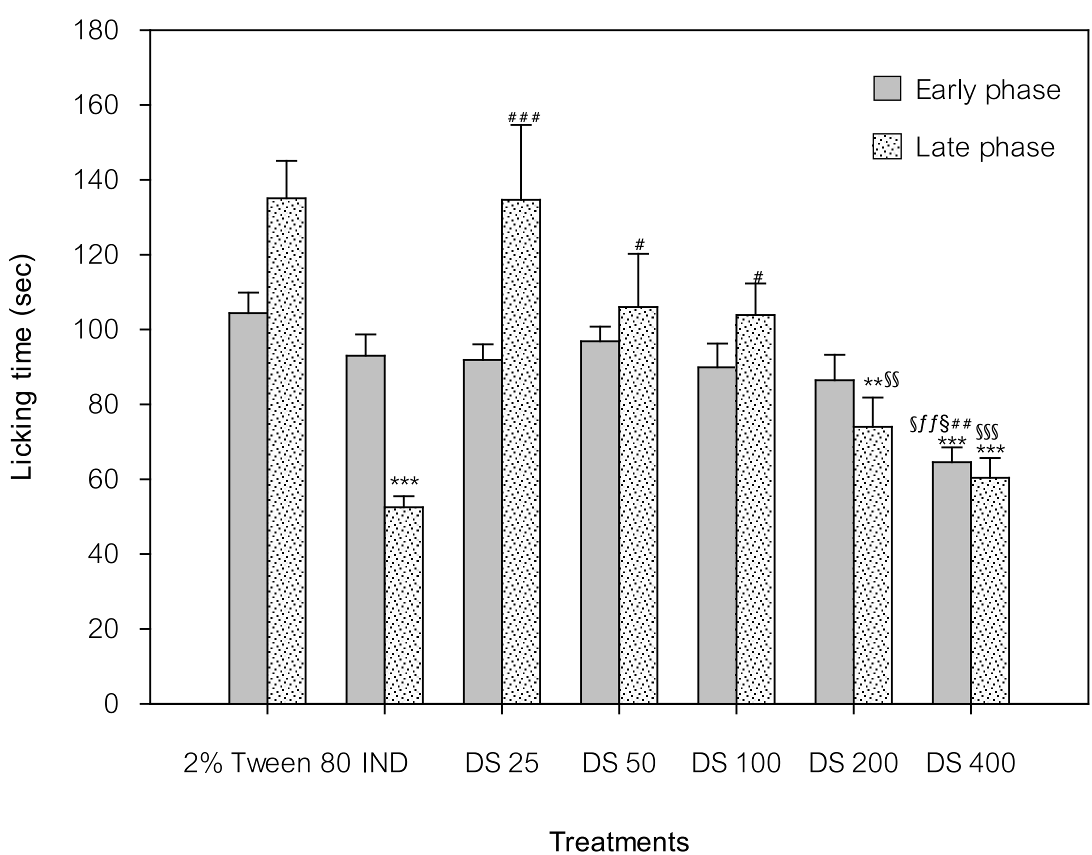


Figure 42 Time spent on hind paw licking after oral administration of 2% Tween 80 (10 ml/kg), indomethacin (IND; 10 mg/kg) and various doses of *Dolichandrone serrulata* root extract (DS; 25-400 mg/kg). N=8 for all groups.

$^{**}p<0.01$, $^{***}p<0.001$ significantly different compared to 2% Tween 80.

$^{§}p<0.05$ significantly different compared to DS 100 mg/kg.

$^{§}p<0.05$, $^{§§}p<0.01$, $^{§§§}p<0.001$ significantly different compared to DS 25 mg/kg.

$^{ff}p<0.01$ significantly different compared to DS 50 mg/kg.

$^{\#}p<0.05$, $^{\#\#}p<0.01$, $^{\#\#\#}p<0.001$ significantly different compared to IND 10 mg/kg.

Formalin test in mice

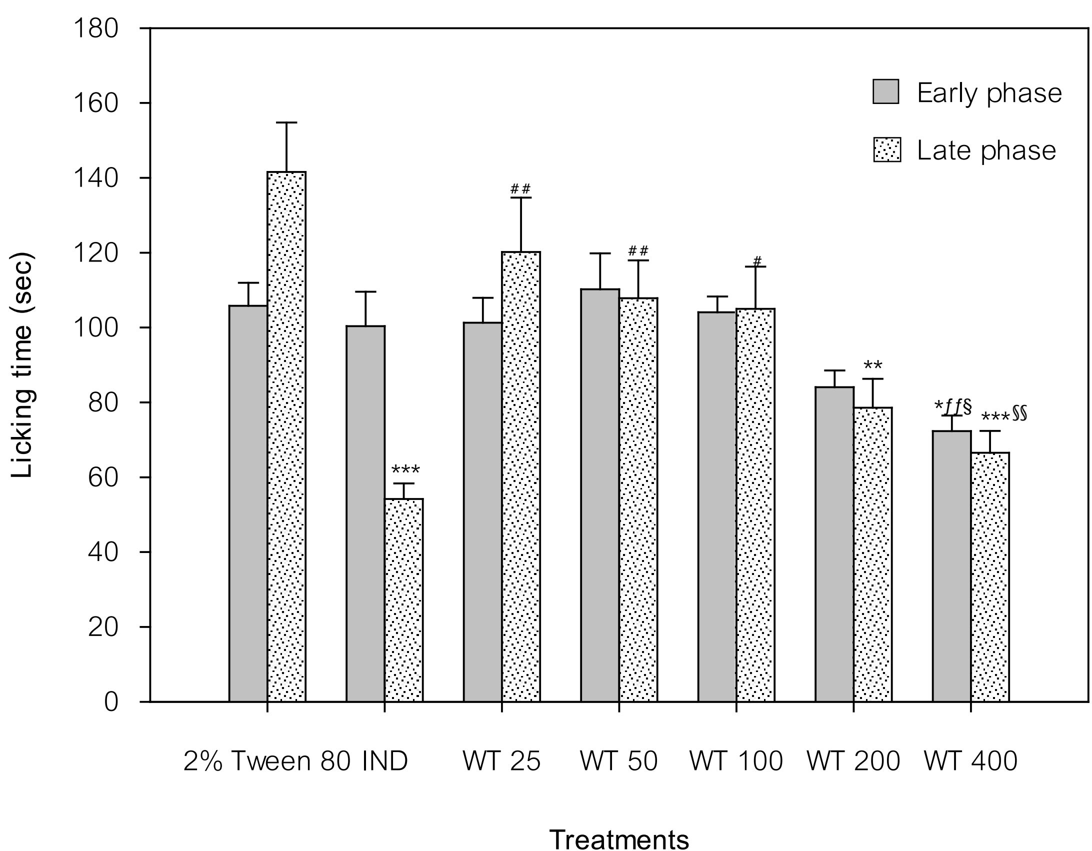


Figure 43 Time spent on paw licking after oral administration of 2% Tween 80 (10 ml/kg), indomethacin (IND; 10 mg/kg) and various doses of *Walsura trichostemon* root extract (WT; 25-400 mg/kg). N=8 for all groups.

$^{*}p<0.05$, $^{**}p<0.01$, $^{***}p<0.001$ significantly different compared to 2% Tween 80.

$^{\S}p<0.05$ significantly different compared to WT 100 mg/kg.

$^{\S\S}p<0.01$ significantly different compared to WT 25 mg/kg.

$^{ff}p<0.01$ significantly different compared to WT 50 mg/kg.

$^{\#}p<0.05$, $^{\#\#}p<0.01$ significantly different compared to IND 10 mg/kg.

Table 2 Time spent on paw licking after intraperitoneal administration of 0.9% normal saline solution (NSS; 10 ml/kg) and morphine sulfate (MO; 10 mg/kg). Each value represents mean±S.E.M. N=8 for all groups. Inhibition is reported as percentage compared to vehicle control.

Treatment	Dose (mg/kg)	Licking time (sec) (% inhibition)	
		Early phase	Late phase
NSS		81.92±8.60	158.80±6.08
MO	10	7.48±1.84*** (90.87%)	3.67±1.08*** (97.69%)

*** $p<0.001$ significantly different compared to NSS.

Table 3 Time spent on paw licking after oral administration of 2% Tween 80 (10 ml/kg), indomethacin (IND; 10 mg/kg) and various doses of Ben-Cha-Moon-Yai remedy (BMY; 125-500 mg/kg). Each value represents mean±S.E.M. N=8 for all groups. Inhibition is reported as percentage compared to vehicle control.

Treatments	Dose (mg/kg)	Licking time (sec) (% inhibition)	
		Early phase	Late phase
2% Tween 80		113.56±4.83	158.66±13.07
IND	10	100.61±4.33 (-11.40%)	68.89±10.85** (-56.58%)
BMY	125	96.67±4.97 (-14.87%)	80.97±18.20** (-48.96%)
	250	90.82±6.93* (-20.03%)	68.47±14.99** (-56.85%)
	500	93.20±3.89 (-17.93%)	77.27±18.43** (-51.30%)

*$p<0.05$, **$p<0.01$ significantly different compared to 2% Tween 80.

Table 4 Time spent on paw licking after oral administration of 2% Tween 80 (10 ml/kg), indomethacin (IND; 10 mg/kg) and various doses of *Aegle marmelos* root extract (AM; 25-400 mg/kg). Each value represents mean±S.E.M. N=8 for all groups. Inhibition is reported as percentage compared to vehicle control.

Treatments	Dose (mg/kg)	Licking time (sec) (% inhibition)	
		Early phase	Late phase
2% Tween 80		98.06±5.45	145.57±17.16
IND	10	95.98±10.65 (-2.12%)	55.73±5.83*** (-61.71%)
AM	25	103.42±6.64 (5.45%)	161.76±8.25$^{\#\#\#}$ (11.12%)
	50	94.09±10.62 (-4.05%)	133.80±9.44$^{\#\#\#}$ (-8.08%)
	100	98.60±4.03 (0.55%)	132.43±9.26$^{\#\#\#}$ (-9.02%)
	200	85.68±7.03 (-12.62%)	118.91±9.70$^{\#\#}$ (-18.31%)
	400	64.19±4.08$^{*\ \S\S\ \S}$ (-34.55%)	58.44±5.50$^{***\$\$\$\ \P\P}$ (-59.85%)

$^{*}p$<0.05, $^{**}p$<0.01 significantly different compared to 2% Tween 80.

$^{\S}p$<0.05 significantly different compared to AM 100 mg/kg.

$^{\S\S}p$<0.01 significantly different compared to AM 25 mg/kg.

$^{\P\P}p$<0.01 significantly different compared to AM 200 mg/kg.

$^{\#\#}p$<0.01, $^{\#\#\#}p$<0.001 significantly different compared to IND 10 mg/kg.

$^{\$\$\$}p$<0.001 significantly different compared to AM 25-100 mg/kg.

Table 5 Time spent on paw licking after oral administration of 2% Tween 80 (10 ml/kg), indomethacin (IND; 10 mg/kg) and various doses of *Oroxylum indicum* root extract (OI; 25-400 mg/kg). Each value represents mean±S.E.M. N=8 for all groups. Inhibition is reported as percentage compared to vehicle control.

Treatments	Dose (mg/kg)	Licking time (sec) (% inhibition)	
		Early phase	Late phase
2% Tween 80		110.03±10.08	144.41±10.68
IND	10	85.31±6.77 (-22.47%)	73.70±2.00** (-48.96%)
OI	25	109.31±5.63 (-0.65%)	128.02±12.38$^{\#}$ (-11.35%)
	50	105.48±7.62 (-4.13%)	118.32±6.67 (-18.06%)
	100	107.74±6.40 (-2.08%)	73.07± 9.98**§ (-49.40%)
	200	107.65±4.31 (-2.16%)	55.18±9.03$^{***\S\S\S\ ff}$ (-61.78%)
	400	112.52±12.87 (2.26%)	45.80±17.08$^{***\S\S\S\ fff}$ (-68.28%)

$^{**}p$<0.01, $^{***}p$<0.001 significantly different compared to 2% Tween 80.

$^{\S}p$<0.05, $^{\S\S\S}p$<0.001 significantly different compared to OI 25 mg/kg.

^{ff}p<0.01, ^{fff}p<0.001 significantly different compared to OI 50 mg/kg.

$^{\#}p$<0.05 significantly different compared to IND 10 mg/kg.

Table 6 Time spent on paw licking after oral administration of 2% Tween 80 (10 ml/kg), indomethacin (IND; 10 mg/kg) and various doses of *Dimocarpus longan* root extract (DL; 25-400 mg/kg). Each value represents mean±S.E.M., N=8 for all groups. Inhibition is reported as percentage compared to vehicle control.

Treatments	Dose (mg/kg)	Licking time (sec) (% inhibition)	
		Early phase	Late phase
2% Tween 80		99.55±6.25	122.54±15.26
IND	10	89.21±4.14 (-10.39%)	$53.44±3.40^{**}$ (-56.39%)
DL	25	103.56±4.56 (4.02%)	$120.08±9.32^{\#\#}$ (-2.01%)
	50	106.04±7.11 (6.52%)	$106.66±13.05^{\#}$ (-12.96%)
	100	110.94±5.62 (11.44%)	$100.72±9.42^{\#}$ (-17.81%)
	200	98.77±5.04 (-0.78%)	$56.90±12.12^{**\S\S f}$ (-53.57%)
	400	95.92±5.47 (-3.64%)	$14.52±6.54^{***\S\S\S fff\S\S\S}$ (-88.14%)

$^{**}p<0.01$, $^{***}p<0.001$ significantly different compared to 2% Tween 80.

$^{\S\S}p<0.01$, $^{\S\S\S}p<0.001$ significantly different compared to DL 25 mg/kg.

$^{f}p<0.05$, $^{fff}p<0.001$ significantly different compared to DL 50 mg/kg.

$^{\S\S\S}p<0.001$ significantly different compared to DL 100 mg/kg.

$^{\#}p<0.05$, $^{\#\#}p<0.01$ significantly different compared to IND 10 mg/kg.

Table 7 Time spent on paw licking after oral administration of 2% Tween 80 (10 ml/kg), indomethacin (IND; 10 mg/kg) and various doses of *Dolichandrone serrulata* root extract (DS; 25-400 mg/kg). Each value represents mean±S.E.M. N=8 for all groups. Inhibition is reported as percentage compared to vehicle control.

Treatments	Dose (mg/kg)	Licking time (sec) (% inhibition)	
		Early phase	Late phase
2% Tween 80		104.38±5.50	135.07±10.01
IND	10	93.00±5.66 (-10.89%)	$52.48\pm2.98^{***}$ (-61.15%)
DS	25	91.88±4.18 (-11.97%)	$134.68\pm19.98^{\#\#\#}$ (-0.29%)
	50	96.84±3.94 (-7.22%)	$105.98\pm14.25^{\#}$ (-21.53%)
	100	89.92±6.32 (-13.84%)	$103.88\pm8.44^{\#}$ (-23.10%)
	200	86.42±6.84 (-17.20%)	$73.99\pm7.81^{**\S\S}$ (-45.22%)
	400	$64.53\pm3.99^{***\S ff\S\#\#}$ (-38.17%)	$60.36\pm5.32^{***\S\S\S}$ (-55.31%)

$^{**}p<0.01$, $^{***}p<0.001$ significantly different compared to 2% Tween 80.

$^{\S}p<0.05$ significantly different compared to DL 100 mg/kg.

$^{\S}p<0.05$, $^{\S\S}p<0.01$, $^{\S\S\S}p<0.001$ significantly different compared to DL 25 mg/kg.

$^{ff}p<0.01$ significantly different compared to DL 50 mg/kg.

$^{\#}p<0.05$, $^{\#\#}p<0.01$, $^{\#\#\#}p<0.001$ significantly different compared to IND 10 mg/kg.

Table 8 Time spent on paw licking after oral administration of 2% Tween 80 (10 ml/kg), indomethacin (IND; 10 mg/kg) and various doses of *Walsura trichostemon* root extract (WT; 25-400 mg/kg). Each value represents mean±S.E.M. N=8 for all groups. Inhibition is reported as percentage compared to vehicle control.

Treatments	Dose (mg/kg)	Licking time (sec) (% inhibition)	
		Early phase	Late phase
2% Tween 80		105.03±6.11	141.59±13.19
IND	10	100.38±9.15 (-5.13%)	54.16±4.17*** (-61.74%)
WT	25	101.28±6.64 (-4.28%)	120.20±14.52$^{\#\#}$ (-15.10%)
	50	110.20±9.60 (4.14%)	107.85±10.11$^{\#\#}$ (-23.82%)
	100	104.08±4.19 (-1.64%)	104.98±11.27$^{\#}$ (-25.85%)
	200	84.05±4.46 (-20.57%)	78.59±7.71** (-44.49%)
	400	72.32±4.15^{*ff§} (-31.66%)	66.55±5.82***§§ (-53.00%)

$^{*}p<0.05$, $^{**}p<0.01$, $^{***}p<0.001$ significantly different compared to 2% Tween 80.

$^{§}p<0.05$ significantly different compared to DL 100 mg/kg.

$^{§§}p<0.01$ significantly different compared to DL 25 mg/kg.

$^{ff}p<0.01$ significantly different compared to DL 50 mg/kg.

$^{\#}p<0.05$, $^{\#\#}p<0.01$ significantly different compared to IND 10 mg/kg.

ACETIC ACID-INDUCED WRITHING IN MICE

Studies then utilized the acetic acid-induced writhing method to examine the analgesic efficacy of BMY and five herbal root extracts (AM, OI, DL, DS and WT). Each mouse was administered orally 2% Tween 80, indomethacin (IND; 10 mg/kg), various doses of BMY (125, 250, 500 mg/kg) or AM, OI, DL, DS and WT (25, 50, 100, 200, 400 mg/kg).

To demonstrate the validity of acetic acid-induced writhing method, IND 10 mg/kg was used as a positive control. As expected IND significantly ($p<0.001$) decreased writhing response by 85.48% compared with 2%Tween 80. All doses of BMY (125, 250 and 500 mg/kg) significantly ($p<0.05$, $p<0.01$, $p<0.001$, respectively) decreased the number of writhes induced by acetic acid by 32.78%, 43.57% and 59.34%, respectively when compared to vehicle control. The antinociceptive efficacy of BMY 500 mg/kg is comparable to IND (Figure 44).

AM at doses of 200, 400 mg/kg significantly ($p<0.001$) decreased the number of writhes induced by acetic acid by 69.36% and 87.54%, respectively when compared to vehicle control. AM at doses of 200, 400 mg/kg significantly ($p<0.001$) decreased the number of writhes when compared to AM (25, 50 and 100 mg/kg). IND significantly ($p<0.001$) decreased writhing response by 83.16% compared with 2% Tween 80. The antinociceptive efficacy of AM 200 and 400 mg/kg are comparable to IND (Figure 45).

OI at doses of 100, 200, 400 mg/kg significantly ($p<0.05$, $p<0.05$ and $p<0.001$, respectively) decreased the number of writhes induced by acetic acid by 33.84%, 37.16% and 58.31%, respectively when compared to vehicle control. IND significantly ($p<0.001$) decreased the number of writhes by 78.55% when compared to vehicle control. The antinociceptive efficacy of OI 400 mg/kg is comparable to IND (Figure 46).

DL at doses of 100, 200, 400 mg/kg significantly ($p<0.05$, $p<0.01$, $p<0.001$, respectively) decreased the number of writhes by 33.55%, 38.98% and 67.73%, respectively when compared to vehicle control. DL 400 mg/kg significantly ($p<0.01$, $p<0.01$ and $p<0.05$, respectively) decreased the number of writhes when compared to

DL at doses of 25, 50 and 100 mg/kg. IND significantly decreased the number of writhes by 75.40% when compared to vehicle control. The antinociceptive efficacy of DL 400 mg/kg is comparable to IND (Figure 47).

DS at doses of 200, 400 mg/kg significantly ($p<0.05$ and $p<0.01$, respectively) decreased the number of writhes by 37.18% and 45.49%, respectively when compared to vehicle control. DS 400 significantly ($p<0.05$) decreased the number of writhes when compared to DS 25 mg/kg. IND significantly ($p<0.001$) decreased the number of writhes by 85.92% when compared to vehicle control. IND has higher antinociceptive efficacy than DS 25-400 mg/kg (Figure 48).

WT at doses of 200, 400 mg/kg significantly ($p<0.001$) decreased the number of writhes by 49.98 % and 61.50%, respectively when compared to vehicle control. WT 200 mg/kg significantly decreased the number of writhes ($p<0.001$, $p<0.01$, respectively) when compared to WT 25 and 50 mg/kg. WT 400 mg/kg significantly ($p<0.001$ and $p<0.01$, respectively) decreased the number of writhes when compared to WT 50 and 100 mg/kg. IND significantly ($p<0.001$) decreased the number of writhes by 93.52% when compared to vehicle control. IND has higher antinociceptive efficacy than WT 25-400 mg/kg (Figure 49).

Acetic acid-induced writhing in mice

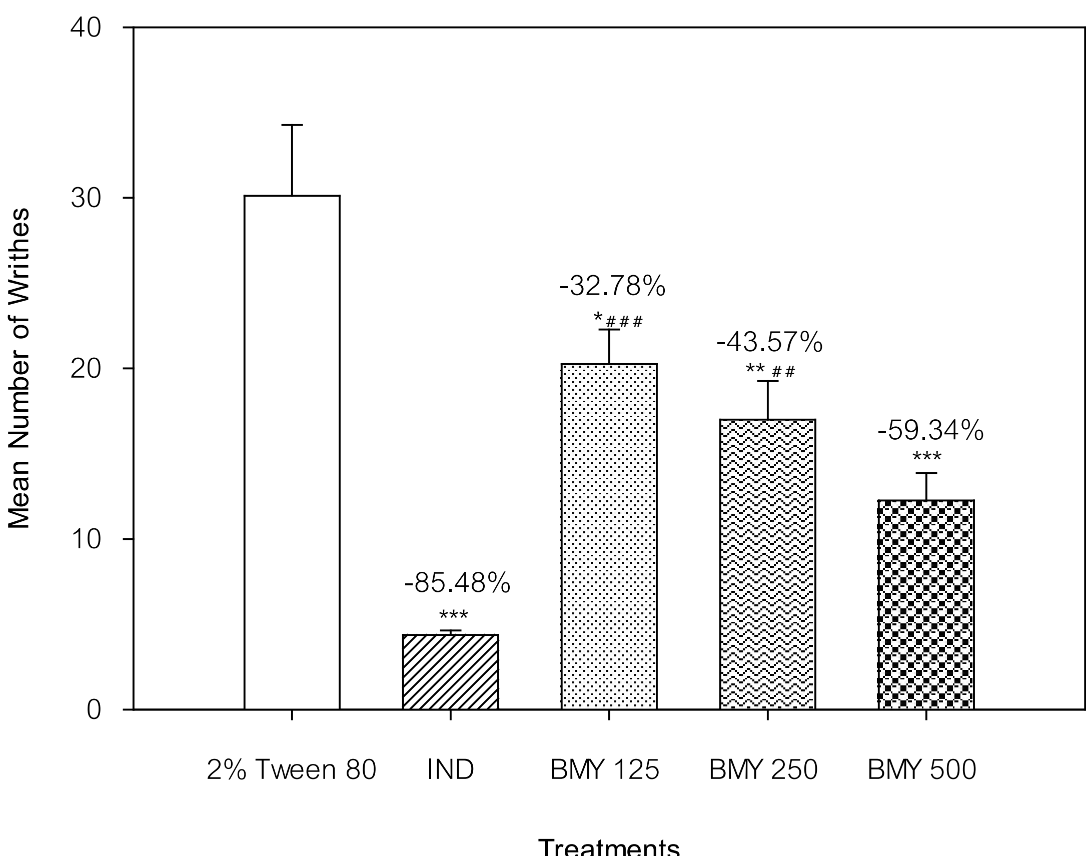


Figure 44 Mean number of writhes after oral administration of 2% Tween 80 (10 ml/kg), indomethacin (IND; 10 mg/kg) and various doses of the root extract of Ben-Cha-Moon-Yai remedy (BMY; 125-500 mg/kg). N=8 for all groups.

$^{*}p<0.05$, $^{**}p<0.01$, $^{***}p<0.001$ significantly different compared to 2% Tween 80.

$^{\#\#}p<0.01$, $^{\#\#\#}p<0.001$ significantly different compared to IND 10 mg/kg.

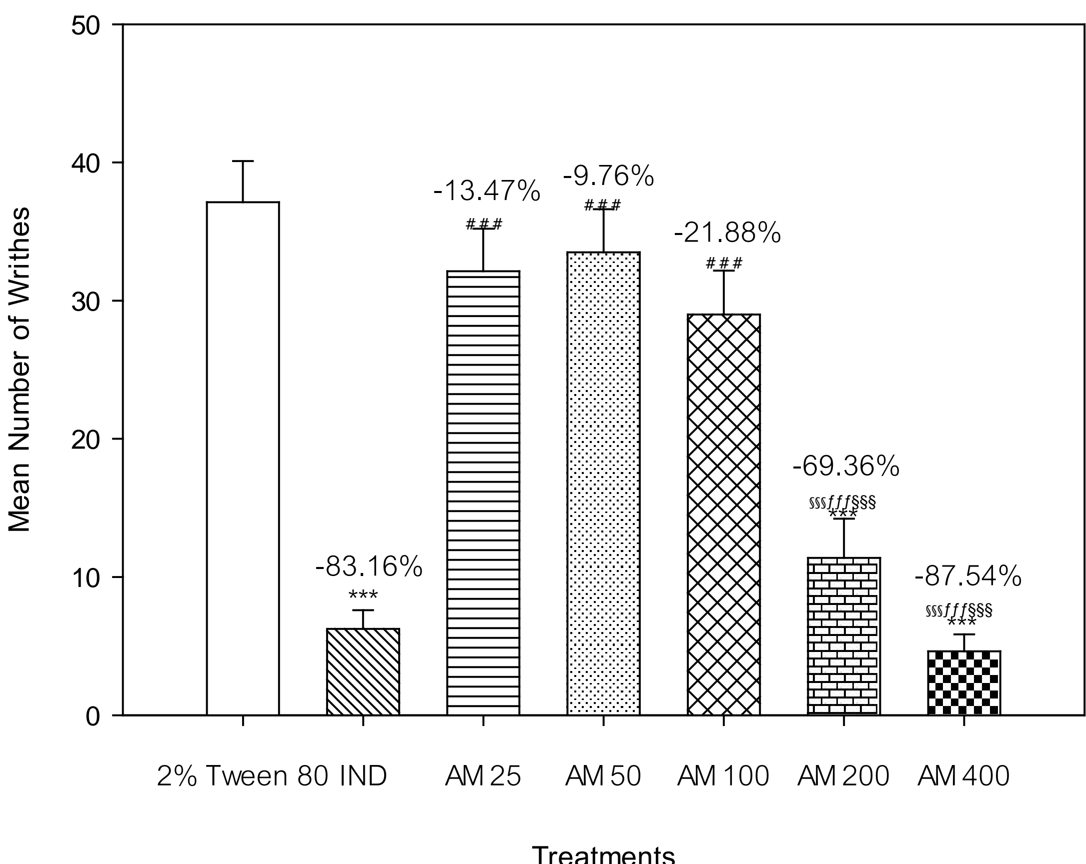


Figure 45 Mean number of writhes after oral administration of 2% Tween 80 (10 ml/kg), indomethacin (IND; 10 mg/kg) and various doses of *Aegle marmelos* root extract (AM; 25-400 mg/kg). N=8 for all groups.

$^{***}p<0.001$ significantly different compared to 2% Tween 80.

$^{\S\S\S}p<0.001$ significantly different compared to AM 25 mg/kg.

$^{fff}p<0.001$ significantly different compared to AM 50 mg/kg.

$^{\S\S\S}p<0.001$ significantly different compared to AM 100 mg/kg.

$^{\#\#\#}p<0.001$ significantly different compared to IND 10 mg/kg.

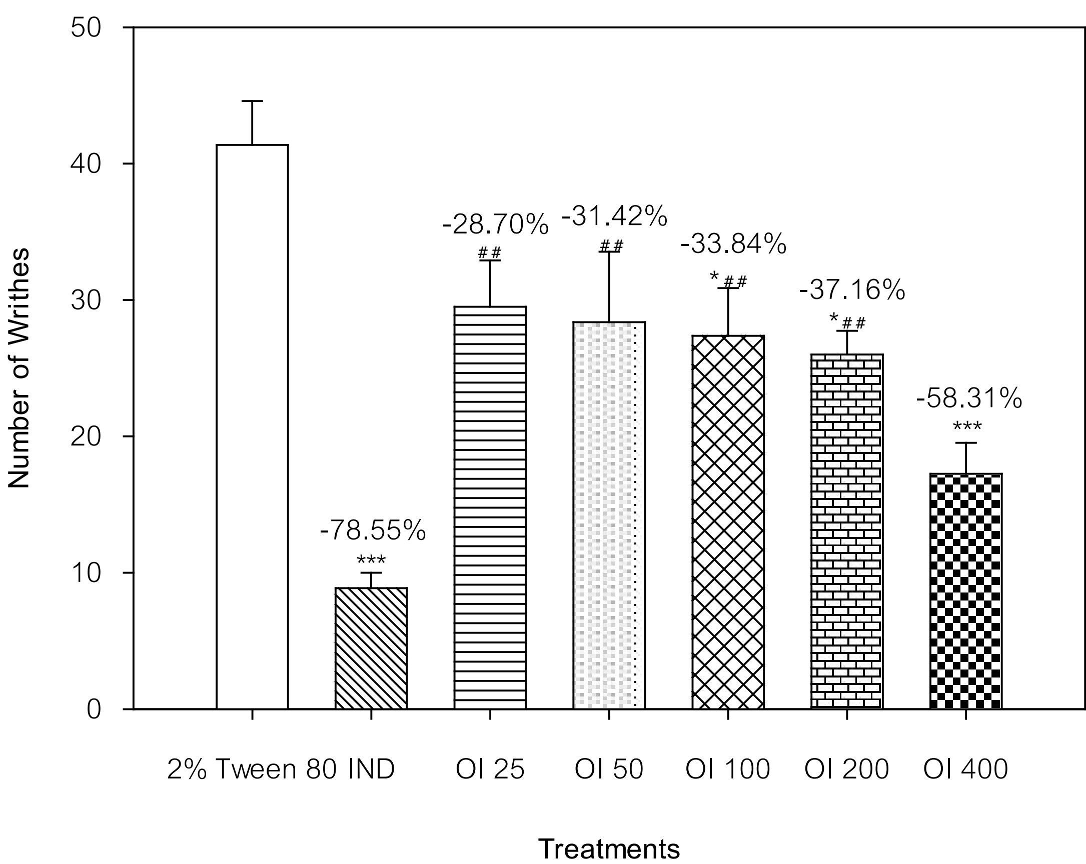


Figure 46 Mean number of writhes after oral administration of 2% Tween 80 (10 ml/kg), indomethacin (IND; 10 mg/kg) and various doses of *Oroxylum indicum* root extract (OI; 25-400 mg/kg). N=8 for all groups.

$^{*}p<0.05$, $^{***}p<0.001$ significantly different compared to 2% Tween 80.

$^{\#\#}p<0.01$ significantly different compared to IND 10 mg/kg.

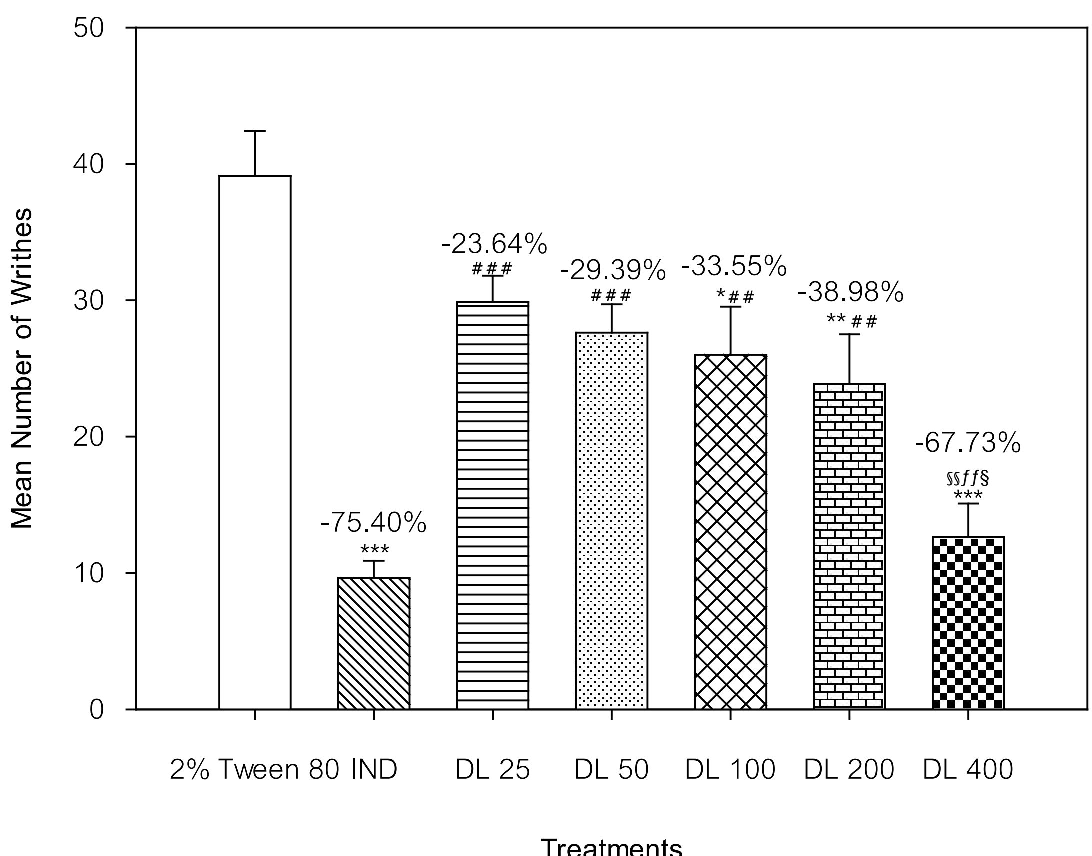


Figure 47 Mean number of writhes after oral administration of 2% Tween 80 (10 ml/kg), indomethacin (IND; 10 mg/kg) and various doses of *Dimocarpus longan* root extract (DL; 25-400 mg/kg). N=8 for all groups.

$^{*}p$<0.05, $^{**}p$<0.01, $^{***}p$<0.001 significantly different compared to 2% Tween 80.

$^{§}p$<0.05 significantly different compared to DL 100 mg/kg.

$^{§§}p$<0.01 significantly different compared to DL 25 mg/kg.

^{ff}p<0.01 significantly different compared to DL 50 mg/kg.

$^{\#\#}p$<0.01, $^{\#\#\#}p$<0.001 significantly different compared to IND 10 mg/kg.

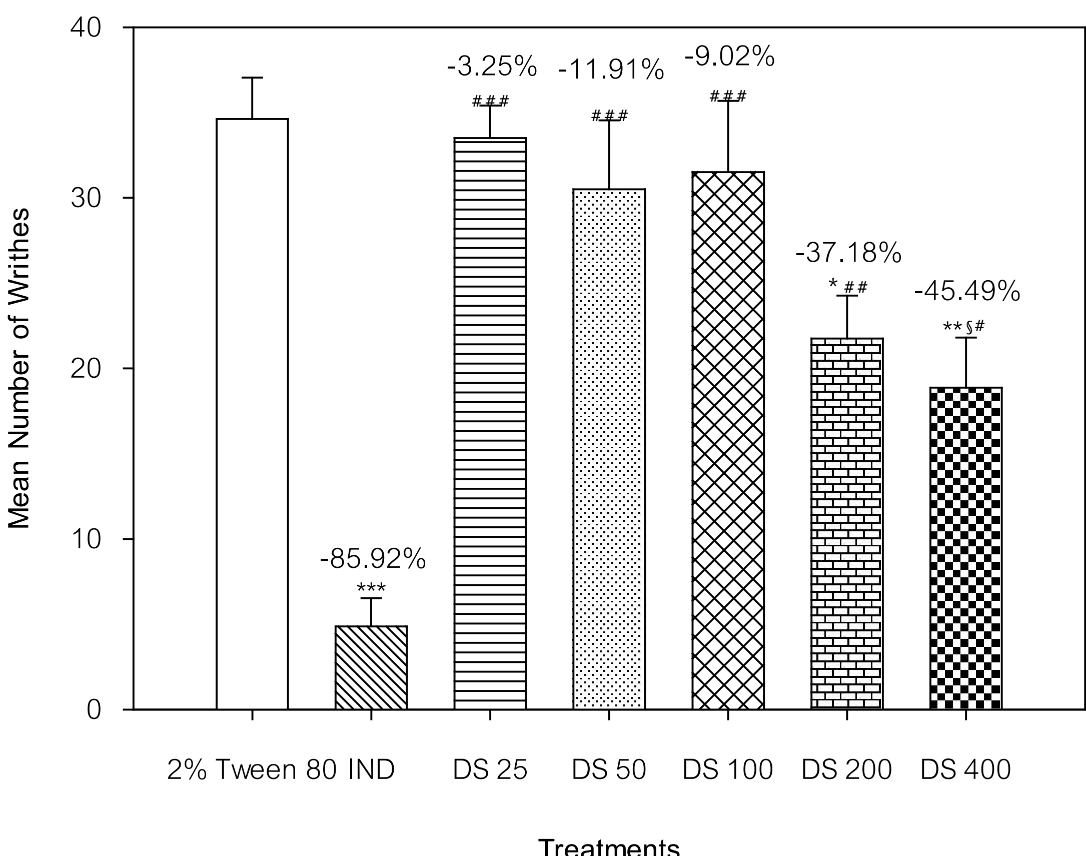


Figure 48 Mean number of writhes after oral administration of 2% Tween 80 (10 ml/kg), indomethacin (IND; 10 mg/kg) and various doses of *Dolichandrone serrulata* root extract (DS; 25-400 mg/kg). N=8 for all groups.

$^{*}p<0.05$, $^{**}p<0.01$ significantly different compared to 2% Tween 80.

$^{\S}p<0.05$ significantly different compared to DS 25 mg/kg.

$^{\#}p<0.05$, $^{\#\#}p<0.01$, $^{\#\#\#}p<0.001$ significantly different compared to IND 10 mg/kg.

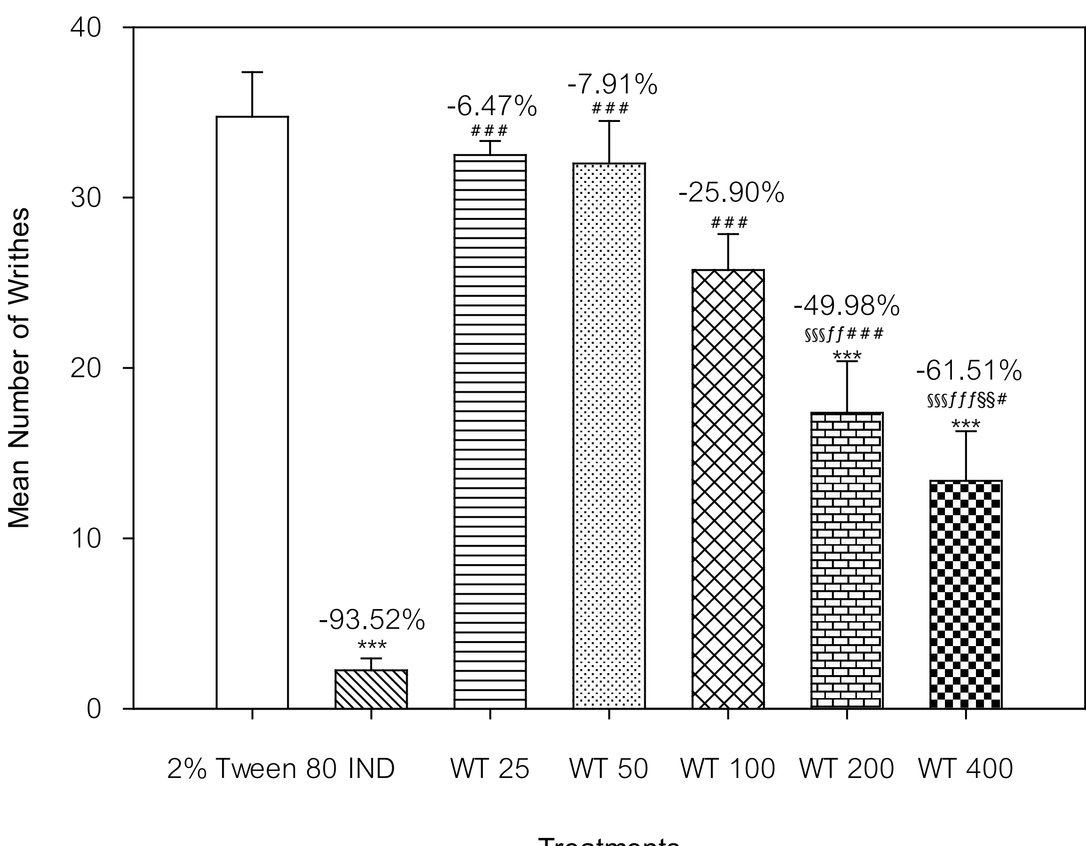


Figure 49 Mean number of writhes after oral administration of 2% Tween 80 (10 ml/kg), indomethacin (IND; 10 mg/kg) and various doses of *Walsura trichostemon* root extract (WT; 25-400 mg/kg). N=8 for all groups.

$^{***}p<0.001$ significantly different compared to 2% Tween 80.

$^{§§}p<0.01$ significantly different compared to WT 100 mg/kg.

$^{ff}p<0.01$, $^{fff}p<0.001$ significantly different compared to WT 50 mg/kg.

$^{§§§}p<0.001$ significantly different compared to WT 25 mg/kg.

$^{\#}p<0.05$, $^{\#\#\#}p<0.001$ significantly different compared to IND 10 mg/kg.

Effects of the root extract of Ben-Cha-Moon-Yai remedy and five root extracts of Ben-Cha-Moon-Yai remedy on the hot-plate test

The area of analgesia (%MPE-min) of BMY at doses of 125, 250 and 500 mg/kg were compared with each herbal root extract at doses of 25, 50 and 100 mg/kg, respectively. BMY 125 mg/kg and all five root extracts at the dose of 25 mg/kg have comparable analgesic effect (Figure 50A). BMY 250 mg/kg showed significant (p<0.01) analgesic effect when compared to AM and OI at the dose of 50 mg/kg. BMY 250 mg/kg also showed significant (p<0.001) analgesic effect when compared to DL, DS and WT at the dose of 50 mg/kg (Figure 50B). BMY (500 mg/kg) displayed significant (p<0.05) analgesic effect when compared to DS 100 mg/kg (Figure 50C).

Effects of the root extract of Ben-Cha-Moon-Yai remedy and

five root extracts of Ben-Cha-Moon-Yai remedy on the hot-plate test

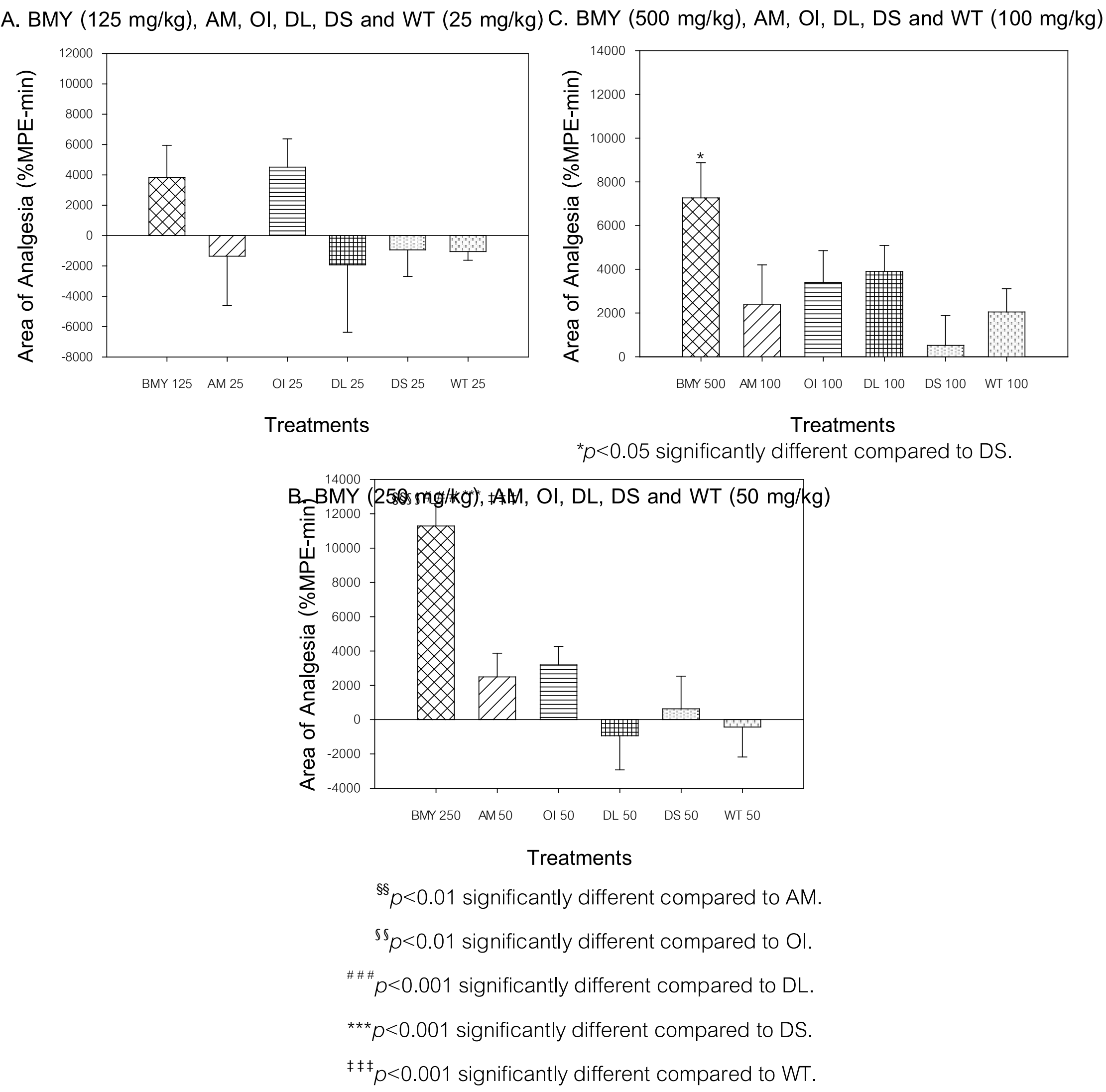


Figure 50 Area of analgesia (%MPE-min) from 0-240 minutes of the root extract of Ben-Cha-Moon-Yai remedy (BMY), *Aegle marmelos* root extract (AM), *Oroxylum indicum* root extract (OI), *Dimocarpus longan* root extract (DL), *Dolichandrone serrulata* root extract (DS) and *Walsura trichostemon* root extract (WT).

A. BMY (125 mg/kg), AM, OI, DL, DS and WT (25 mg/kg), B. BMY (250 mg/kg), AM, OI, DL, DS and WT (50 mg/kg), C. BMY (500 mg/kg), AM, OI, DL, DS and WT (100 mg/kg).

Effects of the root extract of Ben-Cha-Moon-Yai remedy and five root extracts of Ben-Cha-Moon-Yai remedy on the formalin test

Time spent on paw licking of BMY at doses of 125, 250 and 500 mg/kg were compared with each herbal root extract at doses of 25, 50 and 100 mg/kg respectively. During the early phase BMY 125 mg/kg and all five root extracts at the dose of 25 mg/kg have comparable analgesic effect, while BMY 125 mg/kg showed significant ($p<0.01$) analgesic effect when compared to AM 25 mg/kg in the late phase (Figure 51A). Similarly, BMY 250 mg/kg and all five root extracts at the dose of 50 mg/kg have comparable analgesic effect during the early phase, however BMY (250 mg/kg) showed significant ($p<0.01$ and $p<0.05$, respectively) analgesic effect when compared to AM and OI at the dose of 50 mg/kg during the late phase (Figure 51B). BMY 500 mg/kg and OI 100 mg/kg produced significant ($p<0.05$) analgesic effect when compared to AM 100 mg/kg during the late phase, whereas BMY 500 mg/kg and all five root extracts at the dose of 100 mg/kg produced comparable analgesic effect in the early phase (Figure 51C).

Effects of the root extract of Ben-Cha-Moon-Yai remedy and
five root extracts of Ben-Cha-Moon-Yai remedy on the formalin test

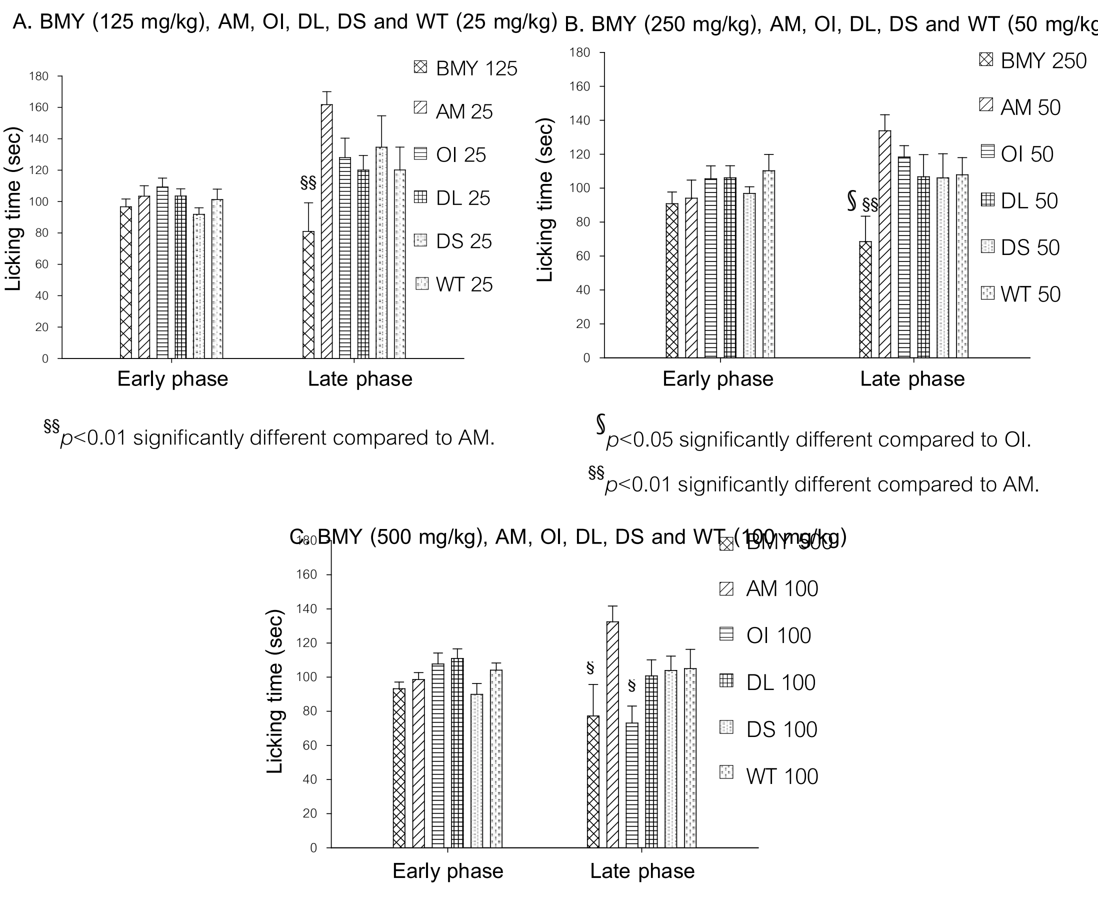


Figure 51 Time spent on paw licking of the root extract of Ben-Cha-Moon-Yai remedy (BMY), *Aegle marmelos* root extract (AM), *Oroxylum indicum* root extract (OI), *Dimocarpus longan* root extract (DL), *Dolichandrone serrulata* root extract (DS) and *Walsura trichostemon* root extract (WT).

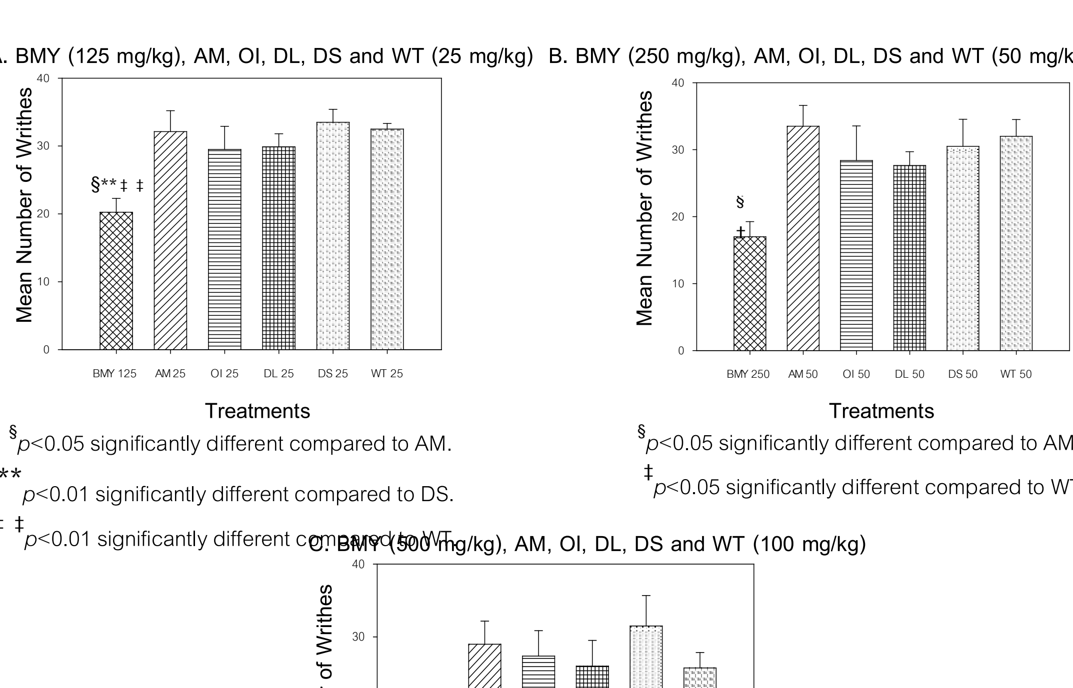
A. BMY (125 mg/kg), AM, OI, DL, DS and WT (25 mg/kg), B. BMY (250 mg/kg), AM, OI, DL, DS and WT (50 mg/kg), C. BMY (500 mg/kg), AM, OI, DL, DS and WT (100 mg/kg).

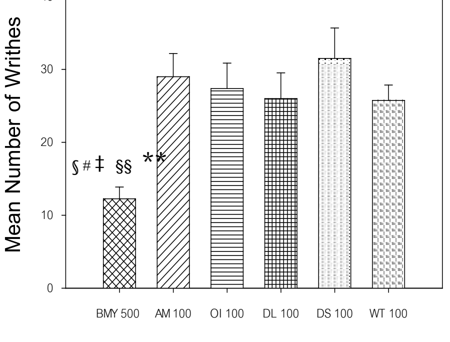
Effects of the root extract of Ben-Cha-Moon-Yai remedy and five root extracts of Ben-Cha-Moon-Yai remedy on the acetic acid-induced writhing test

Mean number of writhes of BMY at doses of 125, 250 and 500 mg/kg were compared with each herbal root extract at doses of 25, 50 and 100 mg/kg, respectively. The result demonstrated that BMY 125 mg/kg showed significant ($p<0.05$, $p<0.01$ and $p<0.01$, respectively) analgesic effect when compared to AM, DS and WT at the dose of 25 mg/kg (Figure 52A). Similarly, BMY 250 mg/kg showed significant ($p<0.05$) analgesic effect when compared with AM and WT at the dose of 50 mg/kg (Figure 52B). BMY 500 mg/kg showed significant ($p<0.01$, $p<0.05$, $p<0.05$, $p<0.01$ and $p<0.05$, respectively) analgesic effect when compared to AM, OI, DL, DS and WT at the dose of 100 mg/kg (Figure 52C).

Effects of the root extract of Ben-Cha-Moon-Yai remedy and

five root extracts of Ben-Cha-Moon-Yai remedy on the acetic acid-induced writhing test





§$p<0.05$ significantly different compared to OI.

#$p<0.05$ significantly different compared to DL.

‡$p<0.05$ significantly different compared to WT.

§§$p<0.01$ significantly different compared to AM.

**$p<0.01$ significantly different compared to DS.

Figure 52 Mean number of writhes of the root extract of Ben-Cha-Moon-Yai remedy (BMY), *Aegle marmelos* root extract (AM), *Oroxylum indicum* root extract (OI), *Dimocarpus longan* root extract (DL), *Dolichandrone serrulata* root extract (DS) and *Walsura trichostemon* root extract (WT).

A. BMY (125 mg/kg), AM, OI, DL, DS and WT (25 mg/kg), B. BMY (250 mg/kg), AM, OI, DL, DS and WT (50 mg/kg), C. BMY (500 mg/kg), AM, OI, DL, DS and WT (100 mg/kg).

ROTA-ROD TEST

In order to determine the effect of BMY and five herbal root extracts (AM, OI, DL, DS and WT) on motor response, mice were administered 2% Tween 80, BMY (500 mg/kg), AM, OI, DL, DS or WT (400 mg/kg) orally and tested on the rota-rod apparatus for 5 subsequent trials at 30, 60, 90, 120 and 240 min after drug administration. The results showed that BMY, AM, OI, DL, DS and WT at doses tested did not affect the motor response of the animals (Figure 53).

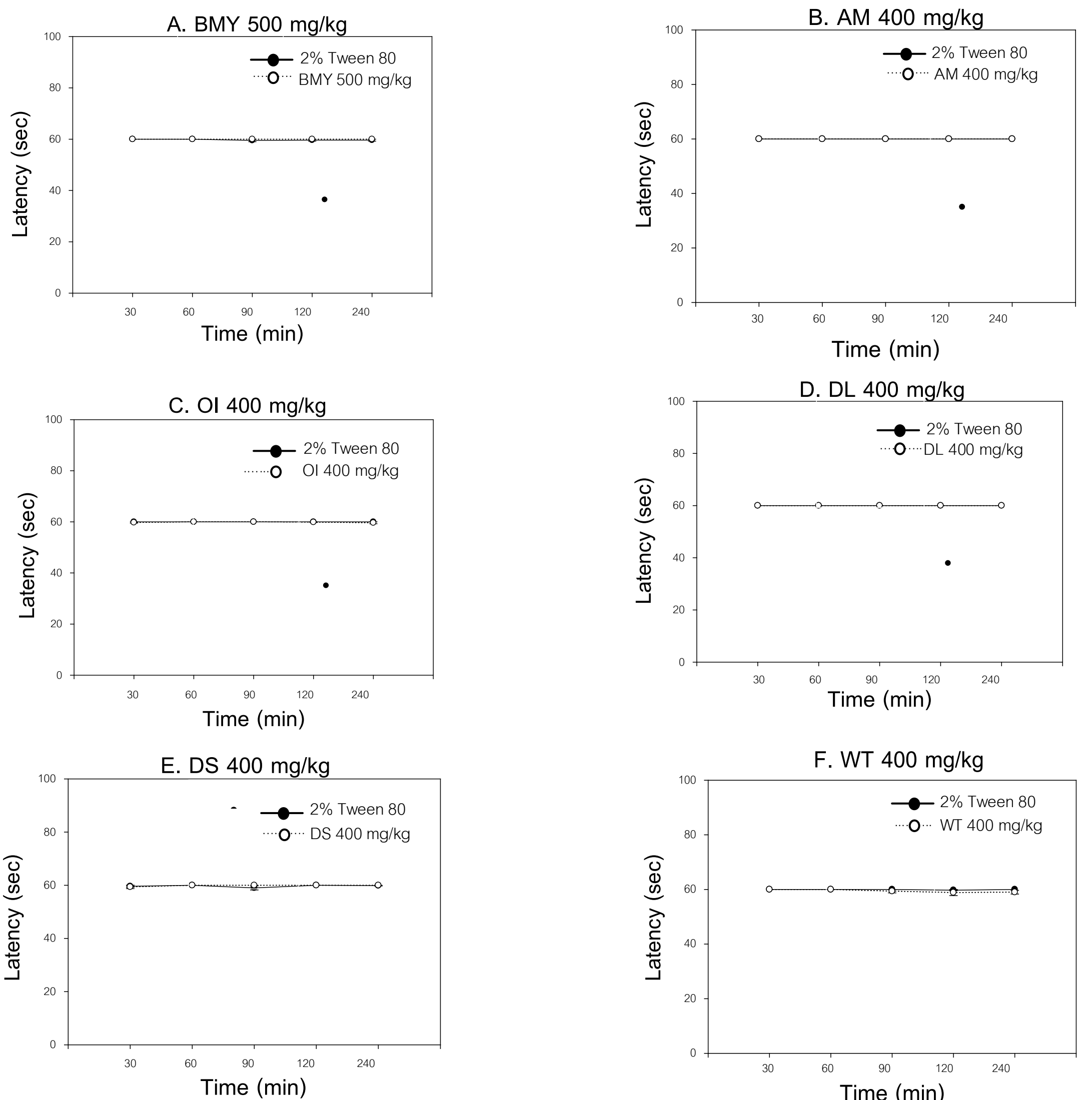


Figure 53 Rota-rod latency of each extract after oral administration compared to 2% Tween 80 (10 ml/kg). N=8 for all groups.

A. The root extract of Ben-Cha-Moon-Yai remedy (BMY; 500 mg/kg). B. *Aegle marmelos* root extract (AM; 400 mg/kg). C.*Oroxylum indicum* root extract (OI; 400 mg/kg). D *Dimocarpus longan* root extract (DL; 400 mg/kg). E. *Dolichandrone serrulata* root extract (DS; 400 mg/kg). F. *Walsura trichostemon* root extract (WT; 400 mg/kg).

CARRAGEENAN-INDUCED MOUSE PAW EDEMA

Studies then utilized the carrageenan-induced mouse paw edema test to examine the anti-inflammatory efficacy of BMY and five herbal root extracts (AM, OI, DL, DS and WT). Each mouse was administered orally 2% Tween 80, indomethacin (IND; 10 mg/kg), various doses of BMY (125, 250, 500 mg/kg) or AM, OI, DL, DS, WT (25, 50, 100, 200, 400 mg/kg).

To demonstrate the validity of carrageenan-induced mouse paw edema test, IND 10 mg/kg was used as a positive control. As expected IND significantly decreased paw edema at 2, 3, 4 and 5 hr after carrageenan administration compared with that of 2% Tween 80 and showed a maximum inhibition of paw edema of 72.22% at 5 hr. BMY 125 mg/kg significantly (p<0.05) decreased paw edema and produced an inhibition of paw edema of 32.91% at 3 hr after carrageenan administration compared with that of 2% Tween 80. BMY at the dose of 250 mg/kg significantly (p<0.001) decreased paw edema at 3 and 4 hr compared with that of 2% Tween 80 and produced a maximum inhibition of paw edema of 51.61% at 4 hr. BMY 250 mg/kg also significantly (p<0.01 and p<0.05, respectively) decreased paw edema at 4 and 5 hr compared to BMY 125 mg/kg. The highest dose of BMY (500 mg/kg) significantly (p<0.001, p<0.001 and p<0.05, respectively) decreased paw edema at 3, 4 and 5 hr compared to 2% Tween 80 and produced a maximum inhibition of paw edema of 65.77% at 3 hr. BMY 500 mg/kg also significantly (p<0.05, p<0.01 and p<0.01, respectively) decreased paw edema at 3, 4 and 5 hr compared to BMY 125 mg/kg. The anti-inflammatory effect of BMY 500 mg/kg was comparable to IND (Table 9).

AM at the dose of 400 mg/kg significantly (p<0.05) decreased paw edema at 3 and 4 hr after carrageenan administration compared to 2% Tween 80 and produced an inhibition of paw edema of 23.93% at 4 hr. AM 400 mg/kg also produced a significant decrease (p<0.05) in paw edema at 4 hr compared to AM 25 mg/kg. IND 10 mg/kg significantly (p<0.001) decreased paw edema at 2, 3, 4, 5 and 6 hr after carrageenan administration compared to 2% Tween 80 and produced a maximum inhibition of paw

edema of 67.18% at 4 hr. All doses of AM (25-400 mg/kg) produced less anti-inflammatory effect than IND (Table 10).

OI at the dose of 200 mg/kg significantly (p<0.05) decreased paw edema at 3 and 4 hr after carrageenan administration compared to 2% Tween 80 and produced a maximum inhibition of paw edema of 26.18% at 4 hr. OI at the dose of 400 mg/kg significantly (p<0.05, p<0.01 and p<0.05, respectively) decreased paw edema at 3, 4 and 5 hr compared to 2% Tween 80 and showed a maximum inhibition of paw edema of 32.14% at 3 hr after carrageenan administration. OI 400 mg/kg also produced a significant decrease (p<0.05) in paw edema at 3 and 4 hr compared to OI 25 and 50 mg/kg. IND 10 mg/kg significantly (p<0.001) decreased paw edema at 2, 3, 4, 5 and 6 hr after carrageenan administration compared to 2% Tween 80 and showed a maximum inhibition of paw edema of 68.83% at 4 hr. All doses of OI (25-400 mg/kg) produced less anti-inflammatory effect than IND (Table 11).

DL at the dose of 200 mg/kg significantly (p<0.05) decreased paw edema at 3, 4 and 5 hr after carrageenan administration compared to 2% Tween 80 and produced a maximum inhibition of paw edema of 34.85% at 4 hr. DL at the dose of 400 mg/kg significantly (p<0.05, p<0.01 and p<0.05, respectively) decreased paw edema at 3, 4 and 5 hr compared to 2% Tween 80 and produced a maximum inhibition of paw edema of 39.39% at 4 hr. DL 400 mg/kg also significantly (p<0.05) decreased paw edema at 3, 4 and 5 hr compared to DL 25 mg/kg. IND 10 mg/kg significantly decreased paw edema at 2, 3, 4 and 5 hr after carrageenan injection compared to 2% Tween 80 and showed a maximum inhibition of paw edema of 71.21% at 4 hr. All doses of DL (25-400 mg/kg) produced less anti-inflammatory effect than IND (Table 12).

DS at the dose of 200 mg/kg significantly (p<0.05) decreased paw edema at 4 hr after carrageenan administration compared to 2% Tween 80 and produced an inhibition of paw edema of 29.69%. DS at the dose of 400 mg/kg significantly (p<0.05) decreased paw edema at 3 and 4 hr after carrageenan administration compared to 2% Tween 80 and produced a maximum inhibition of paw edema of 34.38% at 4 hr. IND 10 mg/kg significantly decreased paw edema at 2, 3, 4, 5 and 6 hr after carrageenan

administration compared to 2% Tween 80 and showed a maximum inhibition of paw edema of 64.06% at 4 hr. All doses of DS (25-400 mg/kg) produced less anti-inflammatory effect than IND (Table 13).

WT at the dose of 25 mg/kg significantly (p<0.01 and p<0.05, respectively) decreased paw edema at 3 and 4 hr after carrageenan administration compared to 2% Tween 80 and produced a maximum inhibition of paw edema of 36.76% at 3 hr. WT at doses of 50 and 100 mg/kg significantly (p<0.01) decreased paw edema at 3 and 4 hr compared to 2% Tween 80 and produced a similar maximum inhibition of paw edema of 42.65% at 3 hr. WT at the dose of 200 mg/kg significantly (p<0.001 and p<0.01, respectively) decreased paw edema at 3 and 4 hr compared to 2% Tween 80 and produced a maximum inhibition of paw edema of 47.06% at 3 hr. WT at the dose of 400 mg/kg significantly (p<0.001, p<0.001 and p<0.05, respectively) decreased paw edema at 3, 4 and 5 hr compared to 2% Tween 80 administration and produced a maximum inhibition of paw edema of 48.53% at 3 hr. IND 10 mg/kg significantly decreased paw edema at 2, 3, 4, 5 and 6 hr after carrageenan administration compared to 2% Tween 80 and showed a maximum inhibition of paw edema of 73.53% at 3 hr. All doses of DL (25-400 mg/kg) produced less anti-inflammatory effect than IND (Table 14).

Table 9 Change of edema volume (ml) of oral administration of 2% Tween 80, indomethacin (IND; 10 mg/kg) and the root extract of Ben-Cha-Moon-Yai remedy (BMY; 125-500 mg/kg). N=8. $^{*}p<0.05$, $^{**}p<0.01$, $^{***}p<0.001$ significantly different compared to 2% Tween 80. $^{\dagger}p<0.05$, $^{\dagger\dagger}p<0.01$ significantly different compared to BMY 125 mg/kg. $^{\#\#}p<0.01$, $^{\#\#\#}p<0.001$ significantly different compared to IND 10 mg/kg.

Treatments (mg/kg)	Paw edema (ml)±S.E.M. (% Inhibition)					
	1 hr	2 hr	3 hr	4 hr	5 hr	6 hr
2%Tween 80	0.029±0.0051	0.0525±0.0031	0.0913±0.0058	0.0775±0.0082	0.0675±0.0118	0.0400±0.0091
IND 10 mg/kg	0.0250±0.0019 (-13.79%)	0.0313±0.0029* (-40.48%)	0.0400±0.0060*** (-56.19%)	0.0238±0.0037*** (-69.35%)	0.0188±0.0023** (-72.22%)	0.0338±0.0053 (-15.63%)
BMY 125 mg/kg	0.0338±0.0046 (16.38%)	0.0500±0.0063$^{\#\#}$ (-4.76%)	0.0613±0.0069* (-32.91%)	0.0675±0.0053$^{\#\#\#}$ (-12.90%)	0.0750±0.0082$^{\#\#\#}$ (11.11%)	0.0550±0.0057 (37.50%)
BMY 250 mg/kg	0.0338±0.0053 (16.38%)	0.0575±0.0070 (9.52%)	0.0463±0.0080*** (-49.34%)	0.0375±0.0049***†† (-51.61%)	0.0400±0.0073† (-40.74%)	0.0463±0.0073 (15.63%)
BMY 500 mg/kg	0.0375±0.0031 (29.31%)	0.0513±0.0040 (-2.38%)	0.0313±0.0040***† (-65.77%)	0.0338±0.0056***†† (-56.45%)	0.0338±0.0068*†† (-50%)	0.0325±0.0041 (-18.75%)

Table 10 Change of edema volume (ml) of oral administration of 2% Tween 80, indomethacin (IND; 10 mg/kg) and *Aegle marmelos* root extract (AM; 25-400 mg/kg). N=8. $^{*}p<0.05$, $^{***}p<0.001$ significantly different compared to 2% Tween 80. $^{\S}p<0.05$ significantly different compared to AM 25 mg/kg. $^{\#}p<0.05$, $^{\#\#}p<0.01$, $^{\#\#\#}p<0.001$ significantly different compared to IND 10 mg/kg.

Treatments (mg/kg)	Paw edema±S.E.M. (% Inhibition)					
	1 hr	2 hr	3 hr	4 hr	5 hr	6 hr
2%Tween 80	0.0513±0.0048	0.0625±0.0041	0.0750±0.0046	0.0838±0.0037	0.0850±0.0073	0.0788±0.0058
IND 10 mg/kg	0.0400±0.0019 (-22.03%)	0.0350±0.0019*** (-44%)	0.0275±0.0025*** (-63.33%)	0.0275±0.0025*** (-67.18%)	0.0313±0.0035*** (-63.24%)	0.0375±0.0037*** (-52.41%)
AM 25 mg/kg	0.0463±0.0032 (-9.84%)	0.0625±0.0031$^{\#\#\#}$ (0%)	0.0738±0.0026$^{\#\#\#}$ (-1.67%)	0.0825±0.0049$^{\#\#\#}$ (-1.55%)	0.0800±0.0066$^{\#\#\#}$ (-5.88%)	0.0788±0.0061$^{\#\#\#}$ (-0.06%)
AM 50 mg/kg	0.0438±0.0018 (-14.72%)	0.0600±0.0033$^{\#\#\#}$ (-4.00%)	0.0725±0.0059$^{\#\#\#}$ (-3.33%)	0.0800±0.0053$^{\#\#\#}$ (-4.53%)	0.0775±0.0037$^{\#\#\#}$ (-8.82%)	0.0763±0.0042$^{\#\#}$ (-3.24%)
AM 100 mg/kg	0.0413±0.0029 (-19.59%)	0.0575±0.0041$^{\#\#}$ (-8.00%)	0.0700±0.0038$^{\#\#\#}$ (-6.67%)	0.0763±0.0050$^{\#\#\#}$ (-9.01%)	0.0738±0.0073$^{\#\#\#}$ (-13.24%)	0.0750±0.0089$^{\#\#}$ (-4.82%)
AM 200 mg/kg	0.0413±0.0035 (-19.59%)	0.0550±0.0042$^{\#\#}$ (-12.00%)	0.0650±0.0038$^{\#\#\#}$ (-13.33%)	0.0713±0.0035$^{\#\#\#}$ (-14.98%)	0.0700±0.0033$^{\#\#\#}$ (-17.65%)	0.0663±0.0046$^{\#}$ (-15.93%)
AM 400 mg/kg	0.0413±0.0023 (-19.59%)	0.0525±0.0031$^{\#\#}$ (-16.00%)	0.0575±0.0016$^{*\#\#\#}$ (-23.33%)	0.0638±0.0026$^{*\S\#\#\#}$ (-23.93%)	0.0625±0.0059$^{\#\#}$ (-26.47%)	0.0625±0.0067$^{\#}$ (-20.69%)

Table 11 Change of edema volume (ml) of oral administration of 2% Tween 80, indomethacin (IND; 10 mg/kg) and *Oroxylum indicum* root extract (OI; 25-400 mg/kg). N=8. $^{*}p<0.05$, $^{**}p<0.01$, $^{***}p<0.001$ significantly different compared to 2% Tween 80. $^{†}p<0.05$ significantly different compared to OI 25 and 50 mg/kg. $^{\#}p<0.05$, $^{\#\#}p<0.01$, $^{\#\#\#}p<0.001$ significantly different compared to IND 10 mg/kg.

Treatments (mg/kg)	Paw edema±S.E.M. (% Inhibition)					
	1 hr	2 hr	3 hr	4 hr	5 hr	6 hr
2% Tween 80	0.0488±0.0035	0.0600±0.0033	0.0700±0.0027	0.0763±0.0026	0.0738±0.0053	0.0700±0.0065
IND 10 mg/kg	0.0375±0.0025 (-23%)	$0.0275±0.0016^{***}$ (-54.17%)	$0.0225±0.0016^{***}$ (-67.86%)	$0.0238±0.0018^{***}$ (-68.83%)	$0.0300±0.0033^{***}$ (-59.29%)	$0.0338±0.0026^{***}$ (-51.79%)
OI 25 mg/kg	0.0488±0.0035 (0.10%)	$0.0613±0.0044^{\#\#\#}$ (2.08%)	$0.0700±0.0042^{\#\#\#}$ (0%)	$0.0738±0.0026^{\#\#\#}$ (-3.22%)	$0.0738±0.0042^{\#\#\#}$ (0.07%)	$0.0725±0.0059^{\#\#\#}$ (3.57%)
OI 50 mg/kg	0.0475±0.0053 (-2.46%)	$0.0600±0.0063^{\#\#\#}$ (0%)	$0.0675±0.0062^{\#\#\#}$ (-3.57%)	$0.0725±0.0070^{\#\#\#}$ (-4.86%)	$0.0713±0.0058^{\#\#\#}$ (-3.32%)	$0.0700±0.0053^{\#\#\#}$ (0%)
OI 100 mg/kg	0.0475±0.0041 (-2.46%)	$0.0575±0.0025^{\#\#\#}$ (-4.17%)	$0.0650±0.0053^{\#\#\#}$ (-7.14%)	$0.0713±0.0044^{\#\#\#}$ (-6.50%)	$0.0688±0.0061^{\#\#\#}$ (-6.72%)	$0.0688±0.0051^{\#\#}$ (-1.79%)
OI 200 mg/kg	0.0450±0.0033 (-7.60%)	$0.0500±0.0042^{\#\#}$ (-16.67%)	$0.0525±0.0045^{*\#\#\#}$ (-25%)	$0.0563±0.0037^{*\#\#\#}$ (-26.18%)	$0.0588±0.0055^{\#\#}$ (-20.28%)	$0.0613±0.0061^{\#}$ (-12.50%)
OI 400 mg/kg	0.0425±0.0025 (-12.73%)	$0.0463±0.0032^{\#}$ (-22.92%)	$0.0475±0.0041^{*†\#\#}$ (-32.14%)	$0.0525±0.0056^{**†\#\#}$ (-31.10%)	$0.0538±0.0050^{*\#}$ (-27.07%)	$0.0575±0.0056^{\#}$ (-17.86%)

Table 12 Change of edema (ml) volume of oral administration of 2% Tween 80, indomethacin (IND; 10 mg/kg) and *Dimocarpus longan* root extract (DL; 25-400 mg/kg). N=8. $^{*}p<0.05$, $^{**}p<0.01$, $^{***}p<0.001$ significantly different compared to 2% Tween 80. $^{\S}p<0.05$ significantly different compared to DL 25 mg/kg. $^{\#}p<0.05$, $^{\#\#}p<0.01$, $^{\#\#\#}p<0.001$ significantly different compared to IND 10 mg/kg.

Treatments (mg/kg)	Paw edema±S.E.M. (% Inhibition)					
	1 hr	2 hr	3 hr	4 hr	5 hr	6 hr
2% Tween 80	0.0425±0.0049	0.0725±0.0077	0.0850±0.0089	0.0825±0.0065	0.0725±0.0092	0.0613±0.0079
IND	0.0313±0.0012	0.0300±0.0038**	0.0263±0.0026***	0.0238±0.0026***	0.0300±0.0042***	0.0363±0.0046
10 mg/kg	(-26.47%)	(-58.62%)	(-69.12%)	(-71.21%)	(-58.62%)	(-40.86%)
DL	0.0438±0.0026	0.0725±0.0053$^{\#\#}$	0.0825±0.0065$^{\#\#\#}$	0.0788±0.0072$^{\#\#\#}$	0.0713±0.0051$^{\#\#\#}$	0.0600±0.0087
25 mg/kg	(2.94%)	(0%)	(-2.94%)	(-4.55%)	(-1.72%)	(-2.12%)
DL	0.0438±0.0026	0.0713±0.00581$^{\#\#}$	0.0738±0.0073$^{\#\#\#}$	0.0713±0.0064$^{\#\#\#}$	0.0650±0.0053$^{\#\#}$	0.0575±0.0075
50 mg/kg	(2.94%)	(-1.72%)	(-13.24%)	(-13.64%)	(-10.34%)	(-6.20%)
DL	0.0413±0.0035	0.0688±0.0029	0.0700±0.0046$^{\#\#\#}$	0.0688±0.0058$^{\#\#\#}$	0.0575±0.0041$^{\#}$	0.0500±0.0087
100 mg/kg	(-2.94%)	(-5.17%)	(-17.65%)	(-16.67%)	(-20.69%)	(-18.43%)
DL	0.0400±0.0042	0.0638±0.0125$^{\#}$	0.0575±0.0073$^{*\#}$	0.0538±0.0068$^{*\#}$	0.0488±0.0040*	0.0475±0.0025
200 mg/kg	(-5.88%)	(-12.07%)	(-32.35%)	(-34.85%)	(-32.76%)	(-22.51%)
DL	0.0388±0.0029	0.0625±0.0037$^{\#}$	0.0538±0.0037$^{*\S\#}$	0.0500±0.0033$^{**\S\#}$	0.0463±0.0037*§	0.0425±0.0077
400 mg/kg	(-8.82%)	(-13.79%)	(-36.76%)	(-39.39%)	(-36.21%)	(-30.67%)

Table 13 Change of edema volume (ml) of oral administration of 2% Tween 80, indomethacin (IND 10 mg/kg) and *Dolichandrone serrulata* root extract (DS; 25-400 mg/kg). N=8. $^{*}p$<0.05, $^{**}p$<0.01, $^{***}p$<0.001 significantly different compared to 2% Tween 80. $^{\#}p$<0.05, $^{\#\#}p$<0.01, $^{\#\#\#}p$<0.001 significantly different compared to IND 10 mg/kg.

Treatments (mg/kg)	Paw edema±S.E.M. (% Inhibition)					
	1 hr	2 hr	3 hr	4 hr	5 hr	6 hr
2% Tween 80	0.0463±0.0026	0.0575±0.0036	0.0725±0.0049	0.0800±0.0057	0.0738±0.0053	0.0688±0.0048
IND 10 mg/kg	0.0388±0.0029 (-16.13%)	0.0388±0.0035** (-32.61%)	0.0300±0.0038*** (-58.62%)	0.0288±0.0029*** (-64.06%)	0.0288±0.0029*** (-60.99%)	0.0325±0.0031** (-52.69%)
DS 25 mg/kg	0.0450±0.0019 (-2.60%)	0.0550±0.0027$^{\#\#}$ (-4.35%)	0.0700±0.0046$^{\#\#\#}$ (-3.45%)	0.0688±0.0079$^{\#\#\#}$ (-14.06%)	0.0688±0.0079$^{\#\#\#}$ (-6.72%)	0.0688±0.0087$^{\#\#}$ (0.07%)
DS 50 mg/kg	0.0438±0.0026 (-5.30%)	0.0538±0.0026$^{\#}$ (-6.52%)	0.0688±0.0035$^{\#\#\#}$ (-5.17%)	0.0675±0.0036$^{\#\#\#}$ (-15.63%)	0.0675±0.0036$^{\#\#\#}$ (-8.41%)	0.0663±0.0050$^{\#\#}$ (-3.57%)
DS 100 mg/kg	0.0438±0.0026 (-5.30%)	0.0525±0.0025$^{\#}$ (-8.70%)	0.0650±0.0038$^{\#\#\#}$ (-10.34%)	0.0638±0.0042$^{\#\#}$ (-20.31%)	0.0638±0.0042$^{\#\#}$ (-13.50%)	0.0625±0.0045$^{\#\#}$ (-9.02%)
DS 200 mg/kg	0.0425±0.0016 (-8.01%)	0.0525±0.0016$^{\#}$ (-8.70%)	0.0588±0.0029$^{\#\#\#}$ (-18.97%)	0.0563±0.0062$^{*\#}$ (-29.69%)	0.0575±0.0065$^{\#\#}$ (-21.98%)	0.0575±0.0059$^{\#}$ (-16.30%)
DS 400 mg/kg	0.0413±0.0012 (-10.71%)	0.0513±0.0035 (-10.87%)	0.0538±0.0046$^{*\#\#}$ (-25.86%)	0.0525±0.0053$^{*\#}$ (-34.38%)	0.0538±0.0056$^{\#}$ (-27.07%)	0.0538±0.0059 (-21.76%)

Table 14 Change of edema volume (ml) of oral administration of 2%Tween 80 (10 ml/kg), indomethacin (IND; 10 mg/kg) and *Walsura trichostemon* root extract (WT; 25-400 mg/kg). N=8. $^{*}p$<0.05, $^{**}p$<0.01, $^{***}p$<0.001 significantly different compared to 2%Tween 80. $^{\#}p$<0.05, $^{\#\#}p$<0.01, $^{\#\#\#}p$<0.001 significantly different compared to IND 10 mg/kg.

Treatments (mg/kg)	Paw edema±S.E.M. (% Inhibition)					
	1 hr	2 hr	3 hr	4 hr	5 hr	6 hr
2%Tween 80	0.055±0.0042	0.0675±0.0036	0.085±0.0060	0.0863±0.0050	0.0763±0.0042	0.0675±0.0036
IND 10 mg/kg	0.0438±0.0032 (-20.45%)	0.0325±0.0025*** (-51.85%)	0.0225±0.0016*** (-73.53%)	0.0238±0.0018*** (-72.45%)	0.0288±0.0029*** (-62.27%)	0.0325±0.0041** (-51.85%)
WT 25 mg/kg	0.0500±0.0075 (-9.09%)	0.0525±0.0075 (-22.22%)	0.0538±0.0086$^{**\#\#}$ (-36.76%)	0.0588±0.0089$^{*\#\#}$ (-31.84%)	0.0688±0.0074$^{\#\#\#}$ (-9.78%)	0.0700±0.0084$^{\#\#}$ (-3.70%)
WT 50 mg/kg	0.0513±0.0055 (-6.82%)	0.0525±0.0049 (-22.22%)	0.0488±0.0051$^{**\#}$ (-42.65%)	0.0563±0.0046$^{**\#\#}$ (-34.74%)	0.0663±0.0046$^{\#\#\#}$ (-13.06%)	0.0688±0.0048$^{\#\#}$ (1.85%)
WT 100 mg/kg	0.0488±0.0040 (-11.36%)	0.0500±0.0038 (-25.93%)	0.0488±0.0074$^{**\#}$ (-42.65%)	0.0563±0.0075$^{**\#\#}$ (-34.74%)	0.0638±0.0080$^{\#\#\#}$ (-16.34%)	0.0675±0.0084$^{\#\#}$ (0%)
WT 200 mg/kg	0.0475±0.0041 (-13.64%)	0.0500±0.0033 (-25.93%)	0.0450±0.0033*** (-47.06%)	0.0500±0.0042$^{**\#}$ (-42%)	0.0575±0.0041$^{\#\#}$ (-24.54%)	0.0613±0.0051$^{\#}$ (-9.26%)
WT 400 mg/kg	0.0475±0.0031 (-13.64%)	0.0475±0.0049 (-29.63%)	0.0438±0.0046*** (-48.53%)	0.0463±0.0042*** (-46.35%)	0.0525±0.0025$^{*\#}$ (-31.10%)	0.0563±0.0032 (-16.67%)

Effects of the root extract of Ben-Cha-Moon-Yai remedy and five root extracts of Ben-Cha-Moon-Yai remedy on carrageenan-induced paw edema

The anti-inflammatory effect of BMY at doses of 125, 250 and 500 mg/kg was compared with all five root extracts at doses of 25, 50 and 100 mg/kg, respectively. AM at the dose of 25 mg/kg showed significant (p<0.05) anti-inflammatory effect when compared to DL 25 mg/kg. BMY at the dose of 250 mg/kg produced significant (p<0.001, p<0.001 and p<0.01, respectively) anti-inflammatory effect when compared to OI, DL and DS (50 mg/kg). AM at the dose of 50 mg/kg demonstrated significant (p<0.01, p<0.01 and p<0.05, respectively) anti-inflammatory effect when compared to OI, DL and DS (50 mg/kg). Similarly, BMY at the dose of 500 mg/kg demonstrated significant (p<0.01) anti-inflammatory effect when compared to OI, DL and DS (50 mg/kg). AM at the dose of 100 mg/kg demonstrated significant (p<0.05) anti-inflammatory effect when compared to OI and DS (100 mg/kg; Table 15).

Table 15 Change of edema volume (ml) at the maximum percentage of inhibition of Ben-Cha-Moon-Yai remedy (BMY; 125, 250 and 500 mg/kg), *Aegle marmelos* root extract (AM; 25, 50 and 100 mg/kg), *Oroxylum indicum* root extract (OI; 25, 50 and 100 mg/kg), *Dimocarpus longan* root extract (DL; 25, 50 and 100 mg/kg), *Dolichandrone serrulata* root extract (DS; 25, 50 and 100 mg/kg) and *Walsura trichostemon* root extract (WT; 25, 50 and 100 mg/kg).

Treatment (mg/kg)	Paw volume (ml)	Treatment (mg/kg)	Paw volume (ml)	Treatment (mg/kg)	Paw volume (ml)
BMY (125 mg/kg)	0.0613±0.0069	BMY(250 mg/kg)	0.0375±0.0049$^{\S\S\S\dagger\dagger\dagger\#\#}$	BMY (500 mg/kg)	0.0313±0.0040$^{\S\S\dagger\dagger\#\#}$
AM (25 mg/kg)	0.0463±0.0032†	AM (50 mg/kg)	0.0438±0.0018$^{\S\S\dagger\dagger\#}$	AM (100 mg/kg)	0.0413±0.0029$^{\S\#}$
OI (25 mg/kg)	0.0725±0.0059	OI (50 mg/kg)	0.0725±0.0070	OI (100 mg/kg)	0.0650±0.0053
DL (25 mg/kg)	0.0788±0.0072	DL (50 mg/kg)	0.0713±0.0064	DL (100 mg/kg)	0.0575±0.0041
DS (25 mg/kg)	0.0688±0.0079	DS (50 mg/kg)	0.0675±0.0036	DS (100 mg/kg)	0.0638±0.0042
WT (25 mg/kg)	0.0538±0.0086	WT (50 mg/kg)	0.0488±0.0051	WT (100 mg/kg)	0.0488±0.0074

$^{\S}p<0.05$, $^{\S\S}p<0.01$, $^{\S\S\S}p<0.001$ significantly different compared to OI.

$^{\dagger}p<0.05$, $^{\dagger\dagger}p<0.01$, $^{\dagger\dagger\dagger}p<0.001$ significantly different compared to DL.

$^{\#}p<0.05$, $^{\#\#}p<0.01$ significantly different compared to DS.

Prostaglandin E_2-induced mouse paw edema

The mechanism of five herbal root extracts of Ben-Cha-Moon-Yai remedy (AM, OI, DL, DS and WT) in producing anti-inflammation were examined utilizing the PGE_2-induced paw edema test. Mice were administered orally 2% Tween 80, IND (10 mg/kg) or AM, OI, DL, DS, WT (400 mg/kg) 1 hour before injection of PGE_2 into the plantar surface of the left hind paw.

To demonstrate the validity of the PGE_2-induced paw edema test, IND (a cyclooxygenase inhibitor) was used as a positive control. As expected IND 10 mg/kg significantly decreased paw edema at 0.5, 1, 1.5, 2 hr after PGE_2 administration compared to 2% Tween 80 ($p<0.01$, $p<0.001$, $p<0.001$ and $p<0.01$, respectively) and showed a maximum inhibition of paw edema of 55.44% at 1.5 hr. AM 400 mg/kg significantly decreased paw edema at 0.5, 1 and 1.5 hr after PGE_2 administration compared to 2% Tween 80 ($p<0.05$) and showed a maximum inhibition of paw edema of 31.77% at 1.5 hr. AM 400 mg/kg significantly displayed less anti-inflammatory effect when compared to IND at 1 hr after PGE_2 administration ($p<0.01$; Table 16).

OI 400 mg/kg significantly decreased paw edema at 0.5, 1 and 1.5 hr after PGE_2 administration compared to 2% Tween 80 ($p<0.05$) and showed a maximum inhibition of paw edema of 29.34% at 1.5 hr. IND 10 mg/kg significantly decreased paw edema at 0.5, 1, 1.5 and 2 hr compared to 2% Tween 80 ($p<0.01$, $p<0.001$, $p<0.001$ and $p<0.01$, respectively) and showed a maximum inhibition of paw edema of 53.70% at 1.5 hr. The anti-inflammatory effect of OI was comparable to IND (Table 17).

DL 400 mg/kg significantly decreased paw edema at 0.5, 1 and 1.5 hr after PGE_2 administration compared to 2% Tween 80 ($p<0.05$) and showed a maximum inhibition of paw edema of 20.83% at 1 hr. IND 10 mg/kg significantly decreased paw edema at 0.5, 1, 1.5 and 2 hr compared to 2% Tween 80 ($p<0.001$, $p<0.001$, $p<0.001$ and $p<0.01$, respectively) and showed a maximum inhibition of paw edema of 56.14% at 1.5 hr. DL 400 mg/kg significantly displayed less anti-inflammatory effect than IND at 1, 1.5 and 2 hr after PGE_2 administration ($p<0.01$, $p<0.001$ and $p<0.05$, respectively; Table 18).

DS 400 mg/kg significantly decreased paw edema at 1 and 1.5 hr after PGE_2 administration compared to 2% Tween 80 ($p<0.05$) and showed a maximum inhibition of paw edema of 27.50% at 1.5 hr. IND 10 mg/kg significantly decreased paw edema at 0.5, 1 1.5 and 2 hr compared to 2% Tween 80 ($p<0.01$, $p<0.001$, $p<0.001$ and $p<0.01$, respectively) and showed a maximum inhibition of paw edema of 62.50% at 1.5 hr. DS 400 mg/kg significantly showed less anti-inflammatory effect than IND at 0.5, 1 and 1.5 hr after PGE_2 administration ($p<0.05$, $p<0.05$ and $p<0.01$; Table 19).

WT 400 mg/kg significantly decreased paw edema at 1 and 1.5 hr after prostaglandin E_2 administration compared to 2% Tween 80 ($p<0.01$ and $p<0.05$, respectively) and showed a maximum inhibition of paw edema of 29.34% at 1.5 hr. IND 10 mg/kg significantly decreased paw edema at 0.5, 1, 1.5, 2 and 3 hr compared to 2% Tween 80 ($p<0.01$, $p<0.001$, $p<0.001$, $p<0.01$ and $p<0.05$, respectively) and showed a maximum inhibition of paw edema of 53.70% at 1.5 hr. WT 400 mg/kg significantly showed less anti-inflammatory effect than IND at 1 and 1.5 hr after PGE_2 administration ($p<0.05$; Table 20).

Table 16 Change of edema volume (ml) of oral administration of 2% Tween 80 (10 ml/kg), indomethacin (IND; 10 mg/kg) and *Aegle marmelos* root extract (AM; 400 mg/kg) after prostaglandin E_2 administration. N=8 for all groups. $^{*}p<0.05$, $^{**}p<0.01$, $^{***}p<0.001$ significantly different compared to 2% Tween 80. $^{\#\#}p<0.01$ significantly different compared to IND 10 mg/kg.

Treatments (mg/kg)	Paw edema±S.E.M. (%Inhibition)					
	0.5 hr	**1 hr**	**1.5 hr**	**2 hr**	**3 hr**	**4 hr**
2%Tween 80	0.0700±0.0033	0.0613±0.0029	0.0513±0.0044	0.0350±0.0033	0.0238±0.0037	0.0163±0.0026
IND 10 mg/kg	0.0557±0.0557** (-20.41%)	0.0371±0.0018*** (-39.41%)	0.0229±0.0028*** (-55.44%)	0.0186±0.0034** (-46.94%)	0.0171±0.0028 (-27.97%)	0.0114±0.0014 (-29.89%)
AM 400 mg/kg	0.0600±0.0027* (-14.29%)	0.0500±0.0027$^{*\#\#}$ (-18.43%)	0.0350±0.0038* (-31.77%)	0.0250±0.0033 (-28.57%)	0.0238±0.0026 (-0.21%)	0.01500±0.0019 (-7.98%)

Table 17 Change of edema volume (ml) of oral administration of 2% Tween 80 (10 ml/kg), indomethacin (10 mg/kg) and *Oroxylum indicum* root extract (OI; 400 mg/kg) after prostaglandin E_2 administration. N=8 for all groups. $^{*}p<0.05$, $^{**}p<0.01$, $^{***}p<0.001$ significantly different compared to 2% Tween 80.

Treatments (mg/kg)	Paw edema±S.E.M. (%Inhibition)					
	0.5 hr	**1 hr**	**1.5 hr**	**2 hr**	**3 hr**	**4 hr**
2%Tween 80	0.0725± 0.0045	0.0600±0.0038	0.0513± 0.0051	0.0363±0.0037	0.0225±0.0031	0.0188±0.0023
Indomethacin 10 mg/kg	0.0525±0.0036** (-27.59%)	0.035±0.0039*** (-41.67%)	0.0238±0.0026*** (-53.70%)	0.0188±0.0029** (-48.35%)	0.0163±0.0018 (-27.78%)	0.0125±0.0016 (-33.16%)
OI 400 mg/kg	0.0575±0.0036* (-20.69%)	0.0450±0.0033* (-25.00%)	0.0363±0.0026* (-29.34%)	0.0250±0.0019 (-31.13%)	0.0238±0.0018 (5.56%)	0.0175±0.0025 (-6.42%)

Table 18 Change of edema volume (ml) of oral administration of 2% Tween 80 (10 ml/kg), indomethacin (IND; 10 mg/kg) and *Dimocarpus longan* root extract (DL; 400 mg/kg) after prostaglandin E_2 administration. N=8 for all groups. $^{*}p$<0.05, $^{**}p$<0.01, $^{***}p$<0.001 significantly different compared to 2% Tween 80. $^{\#}p$<0.05, $^{\#\#}p$<0.01, $^{\#\#\#}p$<0.001 significantly different compared to IND 10 mg/kg.

Treatments (mg/kg)	Paw edema±S.E.M. (%Inhibition)					
	0.5 hr	1 hr	1.5 hr	2 hr	3 hr	4 hr
2%Tween 80	0.0738±0.0026	0.0600±0.0033	0.0513±0.0035	0.0350±0.0027	0.0250±0.0038	0.0188±0.0029
IND 10 mg/kg	0.0575±0.0016*** (-22.09%)	0.0325±0.0031*** (-45.83%)	0.0225±0.00164*** (-56.14%)	0.0213±0.0023** (-39.29%)	0.0150±0.0019 (-40.00%)	0.0138±0.0018 (-26.86%)
DL 400 mg/kg	0.0650±0.0027* (-11.92%)	0.0475±0.0031$^{*\#\#}$ (-20.83%)	0.0413±0.0023$^{*\#\#\#}$ (-19.59%)	0.0313±0.0023$^{\#}$ (-10.71%)	0.0238±0.0019 (-5.00%)	0.0188±0.0023 (-0.27%)

Table 19 Change of edema volume (ml) of oral administration of 2% Tween 80 (10 ml/kg), indomethacin (10 mg/kg) and *Dolichandrone serrulata* root extract (DS; 400 mg/kg) after prostaglandin E_2 administration. N=8 for all groups. $^{*}p<0.05$, $^{**}p<0.01$, $^{***}p<0.001$ significantly different compared to 2%Tween 80. $^{\#}p<0.05$, $^{\#\#}p<0.01$ significantly different compared to IND 10 mg/kg.

Treatments (mg/kg)	Paw edema±S.E.M. (%Inhibition)					
	0.5 hr	1 hr	1.5 hr	2 hr	3 hr	4 hr
2% Tween 80	0.0725±0.0031	0.0625±0.0025	0.0500±0.0033	0.0363±0.0050	0.0238±0.0037	0.0200±0.0019
IND 10 mg/kg	$0.0538±0.0026^{**}$ (-25.86%)	$0.0325±0.0031^{***}$ (-48.00%)	$0.0188±0.0023^{***}$ (-62.50%)	$0.0188±0.0023^{**}$ (-48.35%)	0.0175±0.0016 (-26.47%)	0.0163±0.0018 (-18.75%)
DS 400 mg/kg	$0.0650±0.0033^{\#}$ (-10.34%)	$0.0488±0.0048^{*\#}$ (-22.00%)	$0.0363±0.0046^{*\#\#}$ (-27.50%)	0.0288±0.0023 (-20.80%)	0.0200±0.0019 (-15.97%)	0.0175±0.0016 (-12.50%)

Table 20 Change of edema volume (ml) of oral administration of 2% Tween 80 (10 ml/kg), indomethacin (10 mg/kg) and *Walsura trichostemon* root extract (WT; 400 mg/kg) from 0.5-4 hr after prostaglandin E_2 administration. N=8 for all groups. $^{*}p<0.05$, $^{**}p<0.01$, $^{***}p<0.001$ significantly different compared to 2% Tween 80. $^{\#}p<0.05$ significantly different compared to IND 10 mg/kg.

Treatments (mg/kg)	Paw edema±S.E.M. (%Inhibition)					
	0.5 hr	1 hr	1.5 hr	2 hr	3 hr	4 hr
2%Tween 80	0.0738±0.0026	0.0638±0.0026	0.0513±0.0029	0.0363±0.0026	0.0300±0.0027	0.0200±0.0027
IND 10 mg/kg	0.0575±0.0025** (-22.09%)	0.0363±0.0032*** (-43.18%)	0.0238±0.0037*** (-53.70%)	0.0200±0.0033** (-44.90%)	0.0175±0.0025* (-41.67%)	0.0125±0.0016 (-37.50%)
WT 400 mg/kg	0.0625±0.0041 (-15.31%)	0.0475±0.0036$^{**\#}$ (-25.55%)	0.0363±0.0038$^{*\#}$ (-29.34%)	0.0275±0.0025 (-24.24%)	0.0214±0.0034 (-28.57%)	0.0171±0.0028 (-14.29%)

Effective doses of the root extract of Ben-Cha-Moon-Yai remedy (BMY), *Aegle marmelos* root extract (AM), *Oroxylum indicum* root extract (OI), *Dimocarpus longan* root extract (DL), *Dolichandrone serrulata* root extract (DS) and *Walsura trichostemon* root extract (WT) in various antinociceptive and anti-inflammatory activity testing models

The effective doses of BMY, AM, OI, DL, DS and WT in various antinociceptive and anti-inflammatory activity testing models were summarized in Table 21.

Table 21 Summary of the effective doses of the root extract of Ben-Cha-Moon-Yai remedy (BMY), *Aegle marmelos* root extract (AM), *Oroxylum indicum* root extract (OI), *Dimocarpus longan* root extract (DL), *Dolichandrone serrulata* root extract (DS) and *Walsura trichostemon* root extract (WT) in various antinociceptive and anti-inflammatory activity testing models.

Root Extracts (mg/kg)	Antinociceptive Activity Testing Models				Anti-inflammatory Activity Testing Models
	Hot-plate test	Formalin test		Writhing test	Carrageenan-induced paw edema test
		Early phase	Late phase		
BMY	125, 250, 500	250	125, 250, 500	125, 250, 500	125, 250, 500
AM	400	400	400	200, 400	400
OI	NS	NS	100, 200, 400	100, 200, 400	200, 400
DL	NS	NS	200, 400	100, 200, 400	200, 400
DS	200, 400	400	200, 400	200, 400	200, 400
WT	100, 200, 400	400	200, 400	200, 400	25, 50, 100, 200, 400

NS = no significance was observed

144

CHAPTER V
DISCUSSION AND CONCLUSION

These studies have demonstrated the antinociceptive and anti-inflammatory effects of the root extract of Ben-Cha-Moon-Yai remedy (BMY) and five herbal root extracts of Ben-Cha-Moon-Yai remedy (AM, OI, DL, DS and WT) in various animal models. Antinociceptive property was assessed utilizing thermally-induced (hot-plate) and chemically-induced (formalin and writhing tests) pain models in mice. The involvement of opioid receptors in the analgesic effects of each herbal root extracts of Ben-Cha-Moon-Yai remedy was also investigated. Anti-inflammatory activity was assessed utilizing carrageenan-induced mouse paw edema, an acute inflammation model. The mechanism of anti-inflammatory actions of all five herbal root extracts was further investigated utilizing prostaglandin E_2-induced mouse paw edema model.

Firstly, analgesic effect of BMY and all five herbal root extracts (AM, OI, DL, DS, WT) was evaluated utilizing the standard mouse hot-plate test (Woolfe and MacDonald, 1944), a central analgesic activity testing model. The hot-plate test produces two behavioral components including paw licking and jumping with all four feet in rats and mice. Both behaviors are considered to be supraspinally integrated responses (Bars et al., 2001). This model usually employs morphine (MO) as a reference drug. In this study, MO showed potent analgesic effect on the response indicating the sensitivity of this test. BMY and AM, OI, DL, DS, WT were administered orally to the animals by suspending in 2% Tween 80. The oral administration was chosen in order to imitate the normal consumption of Ben-Cha-Moon-Yai remedy, the Thai traditional antipyretic and anti-inflammatory medicine.

Results from the present study indicated that all doses of BMY (125-250 mg/kg), *Aegle marmelos* root extract (AM; 400 mg/kg), *Dolichandrone serrulata* root extract (DS; 200 and 400 mg/kg) and *Walsura trichostemon* root extract (WT; 100-400 mg/kg) have significant analgesic action in the hot-plate test. The antinociceptive peak response of BMY (125-500 mg/kg) and AM (400 mg/kg), DS (200 and 400 mg/kg) and WT (100, 200 and 400 mg/kg) was observed at different time points starting from 90-240 min after

orally administration. This may partly due to variable absorption of the herbal root extracts from the gastrointestinal tract of rodents causing a delay effect. The results supported that BMY, AM, DS and WT at specified doses have central analgesic effect. The rest of the extracts including *Oroxylum indicum* root extract (OI) and *Dimocarpus longan* root extract (DL) showed negligible analgesic action in the hot-plate model. These results are consistent with the previous studies. Shankarananth et al. (2007) demonstrated the analgesic activity of *Aegle marmelos* leaves extract in a thermal-induced nociception model, tail-flick test. Zaveri and Jain (2009) reported the analgesic activity of *Oroxylum indicum* root bark extract in the same model.

Naloxone, a short acting opioid antagonist, was utilized to investigate the involvement of opioid receptors in the analgesic effects of the effective root extracts including AM (400 mg/kg), DS (200 mg/kg) and WT (400 mg/kg) utilizing hot-plate test. The results demonstrated the attenuation of the analgesic response of AM, DS and WT by naloxone suggesting the involvement of opioid receptors in analgesia produced by these three herbal root extracts.

In order to measure the analgesic effect of BMY, AM, OI, DL, DS and WT against chemical stimuli, formalin test was chosen. In This test, animals displayed nociceptive behaviors consisting of shaking, licking and biting the affected paw in two distinct phases. The early phase (acute pain) initiates immediately after formalin injection and lasts for five minutes as a result of chemical stimulation of primary afferent nociceptors. The late phase (inflammatory pain) initiates 20 min after formalin injection and lasts for 10 min arising from peripheral inflammation and functional changes in the dorsal horn of the spinal cord (Hunskaar and Hole, 1987; Shibata et al., 1989; Tjølsen et al., 1992). Previous studies reported that substance P and bradykinin participate in the early phase, whereas histamine, serotonin, prostaglandin, nitric oxide and bradykinin are involved in late phase (Hunskaar and Hole, 1987; Tjølsen et al., 1992). Each phase of formalin test reflects different mechanisms; drugs that act predominantly on central nervous system inhibit both phases equally while peripherally acting drugs inhibit only the late phase (Hunskaar and Hole, 1987; Shibata et al., 1989; Rosland et al., 1990). In

addition, the late phase is selectively attenuated by cyclooxygenase inhibitors (Yaksh et al., 2001).

In this study, we employed MO and IND as reference drugs. MO, a central analgesic drug, demonstrated potent analgesic effects in both phases while IND, a peripheral acting drug, demonstrated analgesic response only in the late phase. BMY at doses of 125 and 500 mg/kg produced significant analgesic action only in the late phase, while BMY at the dose of 250 mg/kg produced significant analgesic action in both phases. BMY at the dose of 250 mg/kg is likely to be the most effective dose in this model. The highest dose of AM (400 mg/kg) demonstrated significant analgesic responses in both phases of formalin-induced nociception test indicated that AM possess analgesic property in both acute and inflammatory pain. OI at doses of 100-400 mg/kg and DL at doses of 200 and 400 mg/kg produced significant analgesic responses only during the late phase suggesting antinociceptive activity of OI and DL in inflammatory pain. DS at the dose of 200 mg/kg produced significant analgesic action only in the late phase, while the highest dose of DS (400 mg/kg) produced significant analgesic action in both phases suggesting analgesic property in both acute and inflammatory pain. WT at the dose of 200 mg/kg produced significant analgesic action only in the late phase, while the highest dose of WT (400 mg/kg) produced significant analgesic action in both phases. The results suggested the analgesic property of WT in both acute and inflammatory pain. All the results from the formalin test indicated that BMY, AM, DS and WT displayed central analgesic action, while OI and DL exhibited peripheral analgesic action.

Studies were then undertaken to investigate the peripheral analgesic effect of BMY, AM, OI, DL, DS and WT utilizing the acetic acid-induced writhing test. The writhing test is based on the postulation that acetic acid acts by releasing endogenous mediators that stimulate the nociceptive neurons as a result of prostanoids mediators and is used as a screening tool for the assessment of analgesic properties of a test compound (Collier et al., 1968). The writhing response is presumed to be induced by local peritoneal receptor activation (Bentley et al., 1983). The nociceptive properties of

acetic acid may be due to the release of cytokines, including TNF-α, interleukin-1β, and interleukin-8 by resident peritoneal macrophages and mast cells (Ribeiro et al., 2000). In mice acetic acid was reported to cause an increase in the peritoneal fluid levels of PGE_2 and PGF_2, as well as lipooxygenase products (Deraedt et al., 1980), and the release of sympathetic nervous system mediators (Duaret, Nakamura and Ferreira, 1988). This response can be prevented by various inhibitors of prostaglandin biosynthesis including nonsteroidal anti-inflammatory agents, non-narcotic analgesics, some monoamine oxidase inhibitors and antioxidants prevented prostaglandin release (Deraedt et al., 1980).

In the present study, indomethacin (IND), a nonsteroidal anti-inflammatory drug, produced significant analgesic response in the acetic acid-induced writhing test. All dose of BMY, AM (200 and 400 mg/kg), OI (100-400 mg/kg), DL (100-400 mg/kg), DS (200 and 400 mg/kg) and WT (200 and 400 mg/kg) showed significant analgesic responses in this model indicating the peripheral antinociceptive property of these extracts. These results were consistent with previous studies that reported analgesic activity of *Aegle marmelos* leaves extract (Arul et al., 2005, Shankarananth et al., 2007), *Oroxylum indicum* root bark extract (Zaveri and Jain, 2009) and the active ingredient of *Dimocarpus longan* extract (Okuyama et al., 1999) in the acetic acid-induced writhing method. The proposed mechanism of BMY and all five root extracts may be due to the reduction on the liberation of those inflammatory mediators or by direct blockade of receptors resulting in a peripheral analgesic action.

Results from hot-plate, formalin and writhing tests indicated both central and peripheral antinociceptive properties of BMY. AM, DS and WT also displayed both central and peripheral analgesic activities, while OI and DL exhibited only peripheral analgesic effects. This is the first study that demonstrated the analgesic properties of BMY, DS and WT. These studies also provide additional scientific support to the use of *Aegle marmelos* (Ma-tum) and *Walsura trichostemon* (Kad-lin) roots as analgesic drugs in Thai traditional medicine.

Furthermore, to exclude the possible cause of non-specific disturbances of motor coordination caused by BMY, AM, OI, DL, DS and WT, the rota-rod test was performed. This test has been used to determine a compound's ability to produce skeletal muscle relaxation, convulsions and depression of the CNS. Results from this study indicated neither detectable relaxant nor sedative effects of the highest doses of BMY, AM, OI, DL, DS and WT. Therefore, the behavioral responses observed in the hot-plate, writhing and formalin tests were likely not the motor dysfunction but rather than a true antinociceptive effect.

Carrageenan-induced paw edema in rats was originally described by Winter et al. in 1962. Modification of the carrageenan-induced paw edema test in mice was first established by Levy in 1969. Levy demonstrated that carrageenan causes an oedema in the mouse paw similar, as time course, to that shown by Winter in the rats. Since then, the mouse paw edema test has been increasingly used to evaluate the anti-inflammatory drug candidates (Posadas et al., 2004). Inflammation induced by carrageenan is acute, nonimmune, well-researched and highly reproducible. Cardinal signs of inflammation (edema, hyperalgesia and erythema) develop immediately following subcutaneous carrageenan injection, resulting from actions of proinflammatory agents. The oedema at 3 hr after the application of carrageenan was considered to reach the highest response (Andrade et al., 2007; Kale et al., 2007). The inflammatory response resulted from carrageenan can be modulated by inhibitors of specific molecules within the inflammatory cascade (Morris, 2003). Carrageenan-induced paw edema test, a standard experimental model of acute inflammation is characterized by a biphasic response. The first phase (1-2 hr after carrageenan injection) is due to liberation of serotonin and histamine in paw tissues, whereas the second phase is sustained by the liberation of prostaglandins especially those of the E series (Di Rosa, 1972; Morris, 2003). Continuity between two phases is believed to be mediated by kinins (Vinegar et al., 1969; Perianayagum et al., 2006).

The results demonstrated that IND significantly reduced paw edema at 2 hr or more after carrageenan administration (during second phase). The effect of IND in

decreasing paw edema only at the second phase could be explained by the fact that IND is a cyclooxygenase inhibitor and contributes to the reduction of prostaglandins synthesis. These results are consistent with the previous study which showed that IND caused strong inhibition of the second phase without affecting the development of the first phase (Vinegar et al., 1969).

All doses of BMY showed significant reduction of paw edema at 3 hr or more, suggesting that BMY produces an anti-edematous effect at the second phase. The highest dose of AM (400 mg/kg), OI (200-400 mg/kg), DL (200-400 mg/kg), DS (200-400 mg/kg) and all doses of WT (25-400 mg/kg) significantly reduced paw edema at 3 hr or more, suggesting that these five root extracts produce anti-inflammatory effect during the second phase which involves prostaglandin synthesis. This effect may be due to the interference by BMY and all five root extracts on the liberation of prostaglandins, or the blockade of the prostaglandin receptors. Results of AM and OI were consistent with previous studies that showed anti-edematogenic effect of the extract of *Aegle marmelos* fruits and leaves and the extract of *Oroxylum indicum* root bark at the second phase of carrageenan-induced paw edema in rats (Rao et al., 2003; Arul et al., 2005; Zaveri and Jain, 2009). This is the first study that demonstrated the anti-inflammatory properties of BMY, DS and WT. These studies also provide additional scientific support to the use of *Aegle marmelos* (Ma-tum), *Dolichandrone serrulata* (Chare-tare), *Oroxylum indicum* (Phe-kaa) and *Walsura trichostemon* (Kad-lin) roots as anti-inflammatory drugs in Thai traditional medicine.

In order to clarify the anti-inflammatory action of all five root extracts during the second phase of inflammation-induced by carrageenan, PGE_2-induced paw edema model was utilized according to the method of Akkol et al. (2008) and Castardo et al. (2008). PGE_2 is generally considered as a key proinflammatory mediator, and its role has been extensively studied in several inflammatory events. High levels of prostaglandin E_2 have been found in inflammatory exudates, and the injection of prostaglandin E_2 directly into tissue have been shown to induce a number of classical signs of inflammation (Cluadino et al., 2006). IND, a positive control, significantly

inhibited the edematogenic effect of PGE_2 starting at 0.5 hr and last for 2 hr or more. The highest dose of all five root extracts significantly inhibited the edematogenic effect induced by PGE_2 at 0.5 hr or more after PGE_2 administration. Results from these studies indicated the involvement of inhibition of PGE_2 effects in the anti-inflammatory effect of all five root extracts. Therefore, it was confirmed that all five root extracts exert their anti-inflammatory actions by blocking the effects of prostaglandins especially PGE_2. Further studies are needed to determine the specific mechanism (s) of action of BMY and all five herbal root extracts.

In order to investigate the root extract that contribute to the analgesic or anti-inflammatory effects of BMY, each root extract at the same dose was compared to BMY. The analgesic and anti-inflammatory effects of BMY at all doses tested (125-500 mg/kg) were more potent than each individual root extract at doses of 25-100 mg/kg. The analgesic property of all doses of BMY was most likely resulted from all five root extracts. The anti-inflammatory property of the lowest dose of BMY (125 mg/kg) was most likely resulted from all five root extracts, while for the higher doses of BMY (250 and 500 mg/kg) was mainly due to AM and WT. This could be due to additive and/or synergistic effects of some herbal roots in the remedy. It is believed that some herbal roots in the remedy may reduce the toxicity of other roots and exert other pharmacological effects that are beneficial. This might be a reason why Thai traditional doctors prefer to use Ben-Cha-Moon-Yai remedy instead of single root as an anti-inflammatory or antipyretic agent.

In conclusion, the present study demonstrated that BMY, AM, DS and WT possess both central and peripheral antinociceptive properties, while OI and DL possess only peripheral analgesic property. The analgesic mechanisms of AM, DS and WT are most likely involved with the opioid pathway. Additionally, BMY and all five herbal root extracts also demonstrated anti-inflammatory property. The anti-inflammatory mechanism of all five herbal root extracts may involve the interference on the liberation of prostaglandins or inhibition of prostaglandin E_2 effects. The mechanism (s) of action of BMY and all five herbal root extracts requires further investigation. These findings may

eventually lead to the development of novel therapies with minor adverse effects in treating pain and inflammatory conditions.

FUTURE RESEARCH

The future research could comprise of several objectives as listed below

(1) To better understand the mechanism of the root extract of Ben-Cha-Moon-Yai remedy and five herbal root extracts that involved in producing analgesic and anti-inflammatory effects.

(2) To better characterize the mechanism of analgesic effects of five herbal root extracts using other opioid antagonists.

(3) To investigate the anti-inflammatory effect of Ben-Cha-Moon-Yai remedy and five herbal root extracts in the standard experimental animal model of chronic inflammation.

(4) To investigate the potential use of Ben-Cha-Moon-Yai remedy and five herbal roots extracts in combination with other analgesics or anti-inflammatory drugs.

(5) To investigate other routes of administration that might be more appropriate and enhance the analgesic or anti-inflammatory effects of Ben-Cha-Moon-Yai remedy and five herbal root extracts.

(6) To elucidate side effects and toxic effects of Ben-Cha-Moon-Yai remedy and five herbal root extracts at high dosage or after chronic use.

These studies may provide important clues to help understand the mechanism underlying the analgesic and anti-inflammatory effects of Ben-Cha-Moon-Yai remedy and all five herbal root extracts and further support the use of such compounds in clinical setting.

REFERENCES

Andrade, S. F., Cardoso, L. G. V., Carvalho, J.C.T., and Bastos, J. K. Antiinflammatory and antinociceptive activities of extract, fractions and populnoic acid from bark wood of *Austroplenckia populnea*. Journal of Ethnopharmacology 109 (2007): 464–471.

Arul, V., Miyazaki, S., and Dhananjayan, R. Mechanisms of the Contractile Effect of the Alcoholic extract of *Aegle marmelos* Corr. on Isolated Guinea pig ileum and tracheal chain. International Journal of Phytotherapy and Phytopharmacology 11 (2004): 679-683.

Arul, V., Miyazaki, S., and Dhananjayan, R. Studies on the Anti-inflammatory, Antipyretic and Analgesic properties of Leaves of *Aegle marmelos* Corr. Journal of Ethnopharmacology 96 (2005): 159-163.

Babu, T. H., et al. Gastroprotective Flavonoid constituents from *Oroxylum indicum* Vent. Bioorganic & Medicinal Chemistry Letters 20 (2010): 117-120.

Bansuttee, S., Manohan, R., Palanuvej, C., Ruangrungsi, N., and Towiwat, P. Antipyretic effect of Ben-Cha-Moon-Yai Remedy. Journal of Health Research 24 (2010): 181-185.

Bars, D. L., Gozariu, M., and Cadden, S. W. Animal Models of Nociception. Pharmacological Reviews 53 (2001): 597-652.

Baumann, T. J. and Strickland, J. Pain Management. In Dipiro, J. T., Talbert, R. L., Yee, G. C., Matzke, G. R., Wells, B. G., and Posey, L. M. (eds.). Pharmacotherapy: A Pathophysiologic Approach, pp. 1089-1104. United States of America: MCGRAW-HILL Medical Publishing Division, 2008.

Bear, M. F., Connors, B. W., and Paradiso, M. A. Pain. Neuroscience Exploring the Brain, pp. 408-420. United States of America: Lippincott Williams & Wilkins, 2007.

Bentley, G. A., Newton, S. H., and Starr, J. Studies on Antinociceptive Action of α-agonist Drugs and Their Interactions with Opioid Mechanisms. British Journal of Pharmacology 79 (1983): 125-134.

Bennett, P. N., and Brown, M. J. Drug for inflammation and rheumatological disease. Clinical pharmacology, pp. 255-260. Spain: Churchill Livingstone Elseveir, 2008.

Berry, P. H., Covington, E. C., Dahl, J. L., Katz, J. A., and Miaskowski, C. Pain: Current Understanding of Assessment, Management, and Treatments, pp. 1-39. National Pharmaceutical Council: American Pain Society, 2006.

Brijesh, S., Daswani, P., Tetali, P., Antia, N., and Birdi, T. Studies on the Antidiarrhoeal activity of *Aegle marmelos* unripe fruit: Validating Its Traditional usage. BMC Complementary and Alternative Medicine 9 (2009): 1-12.

Choo, W. K., and Ketsa, S. *Dimocarpus longan* Lour. [Online]. 1991. Record from Proseabase. Verheij, E.W.M. and Coronel, R.E. (Editors). PROSEA (Plant Resources of South-East Asia) Foundation, Bogor, Indonesia. Available from: http://www.proseanet.org. [2011, April 30]

Chotsang, P., Aroonrek, N., and Charoenying, P. Allelopathic and Antibacterial Activities of Organic Solvent Extracts from the Stem bark of *Walsura trichostemon* Miq. Proceedings 32nd Congress on Science and Technology of Thailand (2006), Bangkok, Thailand.

Chung, Y. C., Lin, C. C., Chou, C. C., and Hsu, C. P. The Effect of Longan Seed Polyphenols on Colorectal Carcinoma Cells. European Journal of Clinical Investigation 40 (2010): 713-721.

Citarasu, T., Rajajeyasekar, R., Venkatmalingam K., Dhandapani, P. S., and Peter, M. M. Effect of Wood apple *Aegle marmelos* (Diacotyledons, Sapindales, Rutaceae) extract as an antibacterial agent on pathogens infecting prawn (*Penaeus indicus*) larviculture. Indian Journal of Marine Sciences 32 (2003): 156-161.

Costa-Lotufo, L. V., et al. Studies of the Anticancer Potential of Plants used in Bangladeshi folk medicine. Journal of Ethnopharmacology 99 (2005): 21–30.

Deraedt, R., Jouquey, S., Delevallee, F., and Flahaut, M. Release of Prostaglandins E and F in an Algogenic Reaction and Its Inhibition. European Journal of Pharmacology 61 (1980): 17-24.

Dhar, M. M., Dhawan, B. N., Mehrotra, B. N., and Ray, C. Screening of Indian plants for Biological Activity. Indian Journal of Experimental Biology 6 (1968): 232-247.

Duaret, I. D., Nakamura, M., and Ferreira, S. H. Participation of the Sympathetic System in Acetic Acid Induced Writhing in Mice. Brazilian Journal of Medical and Biological Research 21 (1988): 341–43.

Di Rosa, M., and Sorrentino, L. The Mechanism of the Inflammatory Effect of Carrageenan. European Journal of Pharmacology 4 (1968): 340–342.

Di Rosa, M. Biological Properties of Carrageenan. Journal of Pharmacy and Pharmacology 24 (1972): 89–102.

Fields, H. L., and Basbaum, A. I. Textbook of Pain. pp. 309-329. Edinburgh: Churchill Livingstone, 1999.

Ghangale, G. R., Surve, V. S., Anbarasan, K., and Gatne, M. M. Evaluation of *Aegle marmelos* (Bael) for Anti-inflammatory activity in Rats. The Journal of Bombay Veterinary College 16 (2008): 15-16.

Glinsukon, T. Toxicological report. Symposium on development of Medical Plant for Tropical Diseases (1987) Bangkok, Thailand. Feb 26-27.

Gohil, P., Zaveri, M., and Jain, S. Immunomodulatory Activity of n-Butanol Extract of *Oroxylum indicum*. Pharmaceutical Biology 46 (2008): 914-919.

Golikov, P. P., and Brekhman, I. I. Pharmacological study of a Liquid extract from the Bark of *Oroxylum indicum*. Rastitel'nye Resursy 3 (1967): 446.

Greene, R. J., and Harris, N. D. Pathology and Therapeutics for Pharmacists, pp. 455-467. Great Britain: Pharmaceutical Press, 2008.

Griffin, R. S. and Woolf, C. Pharmacology of Analgesia. In Golan, D. E., Tashjian, A. H., Armstrong, E. J., Galanter, J. M., Armstrong, A. W., Arnaout, R. A. and Rose, H. S. (eds.). Principles of Pharmacology: The Patholophysiologic Basis of Drug Therapy, pp. 229-242. United States of America: Lippincott Williams & Wilkins, 2005.

Gupta, R. C., Sharma, V., Sharma, N., Kumar, N. and Singhet, B. *In Vitro* Antioxidant Activity from Leaves of *Oroxylum indicum* (L.) Vent. -A North Indian Highly Threatened and Vulnerable Medicinal Plant. Journal of Pharmacy Research 1 (2008): 65-72.

Hema, C. G., and Lalithakumari, K. Screening of Pharmacological actions of *Aegle marmelos*. Indian Journal of Pharmacology 20 (1999): 80-85.

HO, S. C., HWANG, L. S., YI-JANE SHEN, Y., and LIN, C. Suppressive Effect of a Proanthocyanidin-rich Extract from Longan (*Dimocarpus longan* Lour.) Flowers on Nitric Oxide Production in LPS-Stimulated Macrophage Cells. Journal of Agricultural and Food Chemistry 55 (2007): 10664-10670.

Horton-Szar, D. Inflammation, Repair and cell death. Pathology. pp. 5-11. Chaina. Mosby Elsevier. 2007.

Hunskaar, S., and Hole, K. The formalin test in mice: dissociation between inflammatory and non-inflammatory pain. Pain 30 (1987): 103-14.

Hunter, L. Analgesic drugs. Pharmacology, pp. 562-583. Scotland: Churchill Livingstone, 2003.

Isalm, M. K., Eti, I. Z., and Chowdhury, J. A. Phytochemical and Antimicrobial Analysis on the Extract of *Oroxylum indicum* Linn. Stem-bark. Iranian Journal of Pharmacology & Therapeutics 9 (2010): 25-28.

Jagetia, G. C., Venkatesh, P., and Baliga, M. S. *Aegle marmelos* (L.) CORREA Inhibits the Proliferation of Transplanted Ehrlich Ascites Carcinoma in Mice. Biological and Pharmaceutical Bulletin 28 (2005): 58-64.

Jagetia, G. C., and Venkatesh, P. Radioprotection by oral administration of *Aegle marmelos in vivo*. Journal of Environmental Pathology, Toxicology and Oncology 24 (2005): 315-332.

Jagetia, G. C., Venkatesh, P., Archana, P., Krishnanand, B. R., and Baliga, M. S. Effects of *Aegle marmelos* on the peripheral blood and small intestine of mice exposed to gamma radiation. Journal of Environmental Pathology, Toxicology and Oncology 25 (2006): 611-624.

Jaitrong, S., Rattanapanone, N., and Manthey, J. A. Analysis of the Phenolic compounds in Longan (*Dimocarpus longan* Lour.) peel. Proceedings of the Florida State Horticultural Society 119 (2006): 371-375.

Jiang, G. X., et al. Extraction and Structural Identification of alkali-soluble polysaccharides of Longan (*Dimocarpus longan* Lour.) fruit Pericarp. Innivative Food Science and Emerging Technologies 10 (2009): 638-642.

Joshi, S. K. and Honore, P. Animal Models of Pain for Drug Discovery. Expert Opinion and Drug Discovery 1 (2006): 341-352.

Julius, D. and Basbaum, A. I. Molecular Mechanisms of Nociception. Nature 413 (2001): 203-209.

Hogan, Q. Animal Pain Models. Regional Anesthesia and Pain Medicine. 27 (2002): 385–401.

Kalaivani, T., and Mathew, L. Phytochemistry and Free Radical Scavenging Activities of *Oroxylum indicum*. Environment & We are International Journal of Science & Technology 4 (2009): 45-52.

Kale, M., Misar, A. V., Dave, V., Joshi, M., and Mujumdar, A. M. Antiinflammatory Activity of *Dalbergia lanceolaria* Bark Ethanol Extract in Mice and Rats. Journal of Ethnopharmacology 112 (2007): 300–304.

Kamienski, M., and Keogh, J. Pharmacology Demystified. United State of America: McGraw-Hill, 2006.

Kapoor, L. D. *Aegle marmelos*. Hand book of Ayurvedic Medicinal Plants, pp. 21. United States of America: CRC Press LLC, 2001.

Kapoor, L. D. *Oroxylum indicum*. Hand book of Ayurvedic Medicinal Plants, pp. 252. United States of America: CRC Press LLC, 2001.

Khare, C. P. *Aegle marmelos*. Indian Herbal Remedies, pp. 27-28. Germany: Springer-Verlag Berlin Heildelberg, 2004.

Koster, R., Anderson, M., and De-Beer, E. J. Acetic Acid for Analgesic Screening. Federation Proceedings 18 (1959): 412-418.

Kaur, S., Kaur, P., Walia, A., and Kumar, S. Antigenotoxic Activity of Polyphenolic Rich Extracts from *Aegle marmelos* in Human Blood Lymphocytes and *E.coli* PQ 37. Records of Natural Products 3 (2009): 68-75.

Khan, T. H., and Sultana, S. Antioxidant and Hepatoprotective Potential of *Aegle marmelos* Correa. Against CCl_4^- Induced Oxidative Stress and Early Tumor Events. Journal of Enzyme Inhibition and Medicinal Chemistry 24 (2009): 320-327.

Kumar, V., Abbas, A. K. and Fausto, N. Pathologic Basis of Disease. pp. 48-80. Chaina: Elsevier Saunders, 2005.

Kuttan, R., and Sabu, M. C. Antidiabetic Activity of *Aegle marmelos* and Its Relationship with Its Antioxidant Properties. Indian Journal of Physiology and Pharmacology 48 (2004): 81–88.

Lampronti, I., Khan, M. T., Borgatti, M., Bianchi, N., and Gambari, R. Inhibitory Effects of Bangladeshi Medicinal Plant Extracts on Interactions Between Transcription Factors and Target DNA Sequences. Evidence-based Complementary and Alternative Medicine 5 (2008): 303–312.

Latica, V., and Costa, L. Evaluation of anticancer potential used in Bangladeshi folk medicine. Journal of Ethnopharmacology 99 (2005): 21-38.

Levy, L. Carrageenan Induced Paw Edema in the Mouse. Life Sciences 8 (1989): 801-808.

Litwack, K. Somatosensory function, pain and headache. In Porth, C.M., and Matfin, G. (eds.). Pathophysiology concepts of altered health states, pp. 1233-1239. Chaina: Lippincott Williams & Wilkins, 2009.

Lombardino, J. G. Nonsteroidal anti-inflammatory drugs. The United State of America: John Wiley Sons, Inc., 1985.

Lüllmann, H., Ziegler, A., Mohr, K. and Bieger, D. Antipyretic Analgesics and Antiinflammatory Drugs. Color Atlas of Pharmacology. pp.198-201. Germany: Staudigl Donauwörth, 2000.

Maheshwari, V. L., Joshi, P. V., and Patil, R. H. *In Vitro* Antidiarrheal Activity and Toxicity Profile of *Aegle marmelos*. Dried Fruit Pulp. Natural Product Radiance 8 (2009): 498-502.

Marzine, P. S., and Gilbart, R. The Effect of an Aqueous Extract of *A. marmelos* fruits on Serum and Tissue Lipids in Experimental Diabetes. Journal of the Science of Food and Agriculture 85 (2005): 569-573.

Mazumder, R., et al. Antidiarhoeal Evaluation of *Aegle marmelos* (Correa) Linn. Root Extract. Phytotherpy Research 20 (2006): 82-84.

Morris, C. J., Carrageenan-Induced Paw Edema in Rat and Mouse. In Winyard, P. G. and Wiloughby, D. A. (eds.). Inflammation Protocols, pp. 115-121. United States of America: Human Press Inc., 2003.

Neal, M. J. Medical Pharmacology at Glance. Great Britain: MPG Books Ltd, Bodmin Cornwall, 2002.

Nestler, E. J., Hyman, S. E. and Malenka, R. C. Pain. Molecular Neuropharmacology A Foundation for Clinical Neuroscience, pp. 434-451. United States of America: McGraw-Hill Companies, 2001.

Okuyama, E., Ebihara, H., Takeuchi, H., and Yamazaki, M. Adenosine, The Anxiolytic-Like Principle of the Arillus of *Euphoria longana*. Planta Medica 65 (1999): 115-119.

O'Neil, C. Pain Management. In Chisholm-Burns, M. A., Schwinghammer, T. L., Wells, B. G., Malone, P. M., Kolesar, J. M. and Dipiro, J. T. (eds.). Pharmacotherapy Principle & Practice, pp. 567-579. United States of America: McGraw-Hill Medical, 2010.

Pan, Y., et al. Antioxidant Activity of Microwave-Assisted Extract of Longan (*Dimocarpus Longan* Lour.) Peel. Food Chemistry 106 (2008): 1264-1270.

Panda, S., and Kar, A. Evaluation of the Antithyroid, Antioxidative and Antihyperglycemic Activity of Scopoletin from *Aegle marmelos* leaves in Hyperthyroid Rats. Phytotherapy Research 20 (2006): 1103-1105.

Park, S. J., et al. The Memory-Enhancing Effects of *Euphoria longan* Fruit Extract in Mice. Journal of Ethnopharmacology 128 (2010): 160-165.

Patil, R. H., Chaudhary, B., and Settipalli, S. Antifungal and Antiaflatoxigenic Activity of *Aegle marmelos*. Pharmacognosy Journal 1(2009): 298-301.

Pearl, J., Stander, H., and McKean, D. B. Effects of Analgesics and Other Drugs on Mice in Phenylquinone and Rota-rod Test. Journal of Pharmacology and Experimental Therapeutics 167 (1969): 9-13.

Phomkaivon, N., and Areekul, V. Screening for Antioxidant Activity in Selected Thai Wild Plants. Asian Journal of Food & Agro-Industry 2 (2009): 433-440.

Pitre, S., and Srivastava, S. K. Pharmacological, Microbiological and Phytochemical Studies on the Root of *Aegle marmelos*. Fitoterapia 58 (1987): 194.

Polyium, U., Ta-Ngam, P., and Thongnoi, A. Antimycrobial and Cytotoxic Activity of Crude Extract from the Stem bark of *Walsura trichostemon* Miq. International Conference on the Role of Universities in Hands-On Education Rajamangala University of Technology Lanna, Chiang-Mai, Thailand 23-29 Aug 2009.

Polyium, U., and Malaphan, T. Antimycrobial and Cytotoxic Activity of Crude Extract From the Leaf of *Walsura trichostemon* Miq. The 2nd Annual International Conference of Northeast Pharmacy Research, Mahasarakharm, Thailand, 13-14 Feb 2010.

Porth, C. M., and Sommer, C. Inflammation, tissue repair, and wound healing. In Porth, C. M., and Matfin, G. (eds.). Pathophysiology concepts of altered health states, pp. 377-390. Chaina: Lippincott Williams & Wilkins, 2009.

Posadas, I., et al. Carrageenan-induced Mouse Paw Oedema is Biphasic, Age-Weight Dependent and Displays Differential Nitric Oxide Cyclooxygenase-2 Expression. British Journal of Pharmacology 142 (2004): 331–338.

Prasad, K., et al. Antioxidant and Anticancer Activities of High Pressure-Assisted Extract of Longan (*Dimocarpus longan* Lour.) Fruit Pericarp. Innovative Food Science and Emerging Technologies 10 (2009): 413-419.

Prasad, K., et al. Enhanced Antioxidant and Antityrosinase Activities of Longan Fruit Pericarp by Ultra-High-Pressure-Assisted Extraction. Journal of Pharmaceutical and Biomedical Analysis 51 (2010): 471–477.

Rana, B. K., Singh, U. P., and Taneja, V. Antifungal Activity and Kinetics of Inhibition by Essential Oil Isolated From Leaves of *Aegle marmelos*. Journal of Ethnopharmacology 57 (1997): 29-34.

Rangkadilok, N., Worasuttayangkurn, L., Bennett, R. N., and Satayavivad, J. Identification and Quantification of Polyphenolic Compounds in Longan (*Euphoria longana* Lam.) Fruit. Journal of Agricultural and Food Chemistry 53 (2005): 1387-1392.

Rangkadilok, N., et al. Evaluation of Free Radical Scavenging and Antityrosinase Activities of Standardized Longan Fruit Extract. Food and Chemical Toxicology 45 (2007): 328-336.

Rasadah, M.A. *Oroxylum indicum* (L.) Kurz. [Online]. 2001. Record from Proseabase. Van Valkenburg, J.L.C.H.; and Bunyapraphatsara, N. (eds.). PROSEA (Plant Resources of South-East Asia) Foundation, Bogor, Indonesia. Available from: http://www.proseanet.org. [2011,April 8]

Rao, C. V., Amresh, S. K. O., Mehrotra, S., and Pushpangadan, P. Analgesic, Anti-inflammatory and Antiulcerogenic Activity of the Unripe fruits of *Aegle marmelos*. Acta Pharmaceutica Turcica 45 (2003): 85-91.

Ribeiro, R. A., et al. Involvement of Resident Macrophages and Mast Cells in the Writhing Nociceptive Response Induced by Zymosan and Acetic Acid in Mice. European Journal of Pharmacology 387 (2000): 111–18.

Ripa, F. A., Haque, M., and Bulbul, I. J. *In Vitro* Antibacterial, Cytotoxic and Antioxidant Activities of plant *Nephelium longan*. Pakistan Journal of Bilogical Sceinces 13 (2010): 22-27.

Roh, J. S., Han, J. Y., Kim, J. H., and Hwang, J. K. Inhibitory Effects of Active Compounds Isolated from Safflower (*Carthamus tinctorius* L.) Seeds for Melanogenesis. Biological and Pharmaceutical Bulletin 27 (2004), 1976–1978.

Roy, M. K., et al. Baicalein, a Flavonoid Extracted From a Methanolic Extract of *Oroxylum indicum* Inhibits Proliferation of a Cancer Cell Line *In vitro* Via Induction of Apoptosis. Pharmazie. 62 (2007):149-53.

Sankari, M., Chitra, V., Silambujanaki, P., and Raju, D. Anticonvulsant Activity of Ethanolic Extract of *Aegle marmelos* (Leaves) in Mice. International Journal of PharmTech Research 2 (2010): 640-643.

Sathiyaraj, K., et al. Antifertility Effect of Aqueous Leaf Extract of *Aegle marmelos* on Male Albino Rats. International Journal of Current Pharmaceutical Research 2 (2010): 26-29.

Shankarananth, V., et al. Analgesic Activity of Methanol Extract of *Aegle marmelos* Leaves. Fitoterapia 78 (2007): 258-259.

Sherwood, E. R. and Toliver-Kinsky, T. Mechanisms of the Inflammatory Response. Best Practice & Research Clinical Anaesthesiology 18 (2004): 385-405.

Shi, J. Y., et al. Identification of (-)-Epicathechin as the Direct Substrate for Polyphenoloxidase from Longan Fruit Pericarp. Food Science and Technology 41 (2008): 1742-1747.

Shibata, M., Ohkubo, T., Takahashi, H., and Inoki, R. Modified Formalin Test: Characteristic Biphasic Pain Response. Pain 38 (1989): 347-52.

Silbernagl, S. and Lang, F. Color Atlas of Pathophysiology, pp. 194-197. Germany: Staudigl Druck, Donauwörth, 2000.

Sinaphet, B., Noiarsa, P., Ruchirawat, S., Otsuka, H., and Kanchanapoom, T. Dolichandroside, a New Phenolic Triglycosides from *Dolichandrone serrulata* (DC.) Seem. Journal of Natural Medicines 60 (2006): 251-254.

Singanan, V., Singanan, M., and Begum, H. The Hepatoprotective Effect of Bael Leaves (*Aegle Marmelos*) in Alcohol Induced Liver Injury in Albino Rats. International Journal of Science & Technology 2 (2007): 83-92.

Siriwatanametanon, N., Fiebich, B., Prieto, J.M., Efferth, T., and Heinrich, M. Thai Medicinal Plants and the Search for New Anti-inflammatory and Anticancer Agents. Planta Med (2009): 75.

Subramaniam, D., et al. Activation of Apoptosis by 1-Hydroxy-5, 7-Dimethoxy-2-Naphthalene-Carboxaldehyde, a Novel Compound from *Aegle marmelos*. Cancer Research 68 (2008): 8573-8581.

Sunarto, A. T. *Aegle marmelos* (L.) Correa [Online]. 1991. Record from Proseabase. Verheij, E. W. M. and Coronel, R. E. (eds.). Prosea (Plant Resources of South-East Asia) Foundation, Bogor, Indonesia. Available from: http://www.proseanet.org. [2011, May 10]

Sundaram, E. N., Raddy, U., Maheswara, P., and Singh K. P. Effect of Alcoholic Extracts of Indian Medicinal Plants on the Altered Enzymatic Activities of Diabetic Rats. Indian Journal of Pharmaceutical Sciences 71(2009): 594-598.

Sun, J., Shi, J., Jiang, Y. M., Xue, S. J., and Wei, X. Y. Identification of Two Polyphenolic Compounds with Antioxidant Activities in Longan Pericarp Tissues. Journal of Agricultural and Food Chemistry 55 (2007): 5864-5868.

Tallarida, R. J., and Murray, R. B. Manual of Pharmacologic Calculation with Computer Programs, 2nd ed. New York: Springer-Verlag, 1987.

Tjølsen, A., Berge, O. G., Hunskaar, S., Rosland, J. H., and Hole, K. The Formalin Test: an Evaluation of the Method. Pain 51 (1992): 5-17.

Tjølsen, A., Hole, K., and Hunskaar, S. Animal Models in Pain Research. In Svendsen, P. and Hua, J. (eds.). Handbook of Labaratory Animal Science. United States of America: CRC Press, 1994.

Upadhya, S., Shanbhag, K. K., Suneetha, G. and Naidu, B. M. A Study of Hypoglycemic and Antioxidant Activity of *Aegle marmelos* in Alloxan Induced Diabetic Rats. Indian Journal of Physiology and Pharmacology 48 (2004): 476-480.

Veerappan, A., Miyazaki, S., Kadarkaraisamy, M., and Ranganathan, D. Acute and Subacute Toxicity Studies of *Aegle marmelos* an Indian Medicinal Plant. Phytomedicine 14 (2007): 209-215.

Vinegar, R., Schreiber, W., and Hugo, R. Biphasic Development of Carrageenan Oedema in Rats. Journal of Pharmacology and Experimental Therapeutics 166 (1969): 96-103.

Vogel, H. G. Drug Discovery and Evaluation: Pharmacological Assay, pp. 983-115. 3rd ed. Germany: Springer-Verlag, 2008.

Walker, K., Fox, A. J. and Urban, L. A. Animal Models for Pain Research. Molecular Medicine Today 5 (1999): 319-321.

Williamson, E. M. *Aegle marmelos*. Major Herbs of Ayurveda, pp. 25-28. China: Churchill Livingstone, 2002.

Winter, C. A., Risley, E. A., and Nuss, C. W. Carrageenan-Induced Edema in Hind Paw of the Rats as an Assay for Anti-inflammatory Drugs. Proceedings of the Society of Experimental Biology and Medicine 111 (1962): 544-547.

Woolfe, G., and MacDonald, A. D. The Evaluation of the Analgesic Action of Pethidine Hydrochloride (Demerol). The Journal of Pharmacology and Experimental Therapeutics 80 (1944): 300.

Wecker, L., Crespo, L. M., Dunaway, G., Faingold, C., and Watts, S. Brody's Human Pharmacology Molecular to Clinical, pp.163-169. Canada: Mosby Elsevier, 2010.

Yamaguchi, F., Ariga, T., Yoshimura, Y., and Nakazawa, H. Antioxidative and Anti-glycation Activity of Garcinol from *Garcinia indica* Fruit Rind. Journal of Agricultural and Food Chemistry 48 (2000): 180-185.

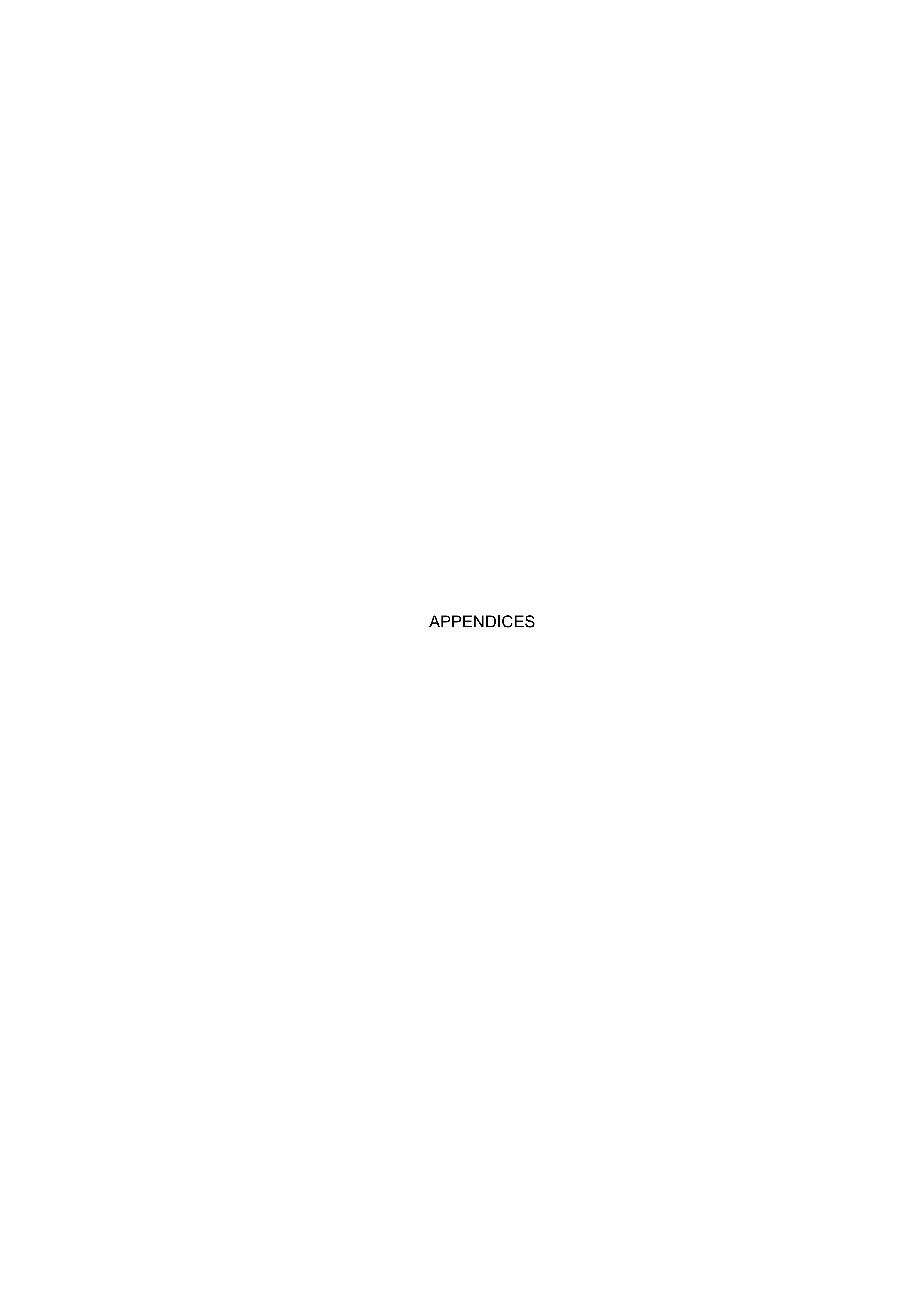
Yang, B., Zhao, M., and Jiang, Y. Anti-Glycated Activity of Polysaccharides of Longan (*Dimocarpus longan* Lour.) Fruit Pericarp Treated by Ultrasonic Wave. Food Chemistry 114 (2009): 629–633.

Yang, B., Zhao, M. K., Prasad, K. N., Jiang, G., and Jiang, Y. Effect of Methylation on the Structure and Radical Scavenging Activity of Polysaccharides from Longan (*Dimocarpus longan* Lour.) fruit pericarp. Food Chemistry 118 (2010): 364-368.

Zaveri, M., and Dhru, B. *In Vitro* Antioxidant Potential of Stem and Root Bark of *Oroxylum indicum*. Journal of Global Pharma Technology 3 (2009): 42-48.

Zaveri, M., and Jain, S. Anti-inflammatory and Analgesic Activity of Root bark of *Oroxylum indicum*, Vent. Journal of Global Pharma Technology 2 (2010): 79-87.

Zheng, G. M., et al. Polyphenols from Longan Seeds and Their Radical-Scavenging Activity. Food Chemistry 116 (2009): 433-436.



APPENDICES

Appendix A

Certificate of Project Approval by the Institutional Animal Care and Use Committee,
Faculty of Pharmaceutical Sciences, Chulalongkorn University,
Bangkok, Thailand

Chulalongkorn University Animal Care and Use Committee

Certificate of Project Approval	☐ Original ☐ Renew
Animal Use Protocol No. 12-33-007	**Approval No. 12-33-007**

Protocol Title
Antinociceptive and anti-inflammatory effects of five root extracts of ben-cha-moon yai remedy

Principal Investigator
Pasarapa Towiwat, Ph.D.

Certification of Institutional Animal Care and Use Committee (IACUC)
This project has been reviewed and approved by the IACUC in accordance with university regulations and policies governing the care and use of laboratory animals. The review has followed guidelines documented in Ethical Principles and Guidelines for the Use of Animals for Scientific Purposes edited by the National Research Council of Thailand.

Date of Approval	**Date of Expiration**
January 30, 2012	January 30, 2015

Applicant Faculty/Institution
Faculty of Pharmaceutical Sciences, Chulalongkorn University, Phyathai Rd., Pathumwan BKK-THAILAND. 10330

Signature of Chairperson	**Signature of Authorized Official**
[signature]	[signature]
Name and Title	**Name and Title**
THONGCHAI SOOKSAWATE, Ph.D. Chairman	PARKPOOM TENGAMNUAY, Ph.D. Associate Dean (Research and Academic Service)

The official signing above certifies that the information provided on this form is correct. The institution assumes that investigators will take responsibility, and follow university regulations and policies for the care and use of animals.

This approval is subjected to assurance given in the animal use protocol and may be required for future investigations and reviews.

Appendix B

Preparation of five herbal root extracts and thin-layer chromatograms of the methanolic extract of five herbal roots of Ben-Cha-Moon-Yai remedy

PREPARATION OF FIVE HERBAL ROOT EXTRACTS

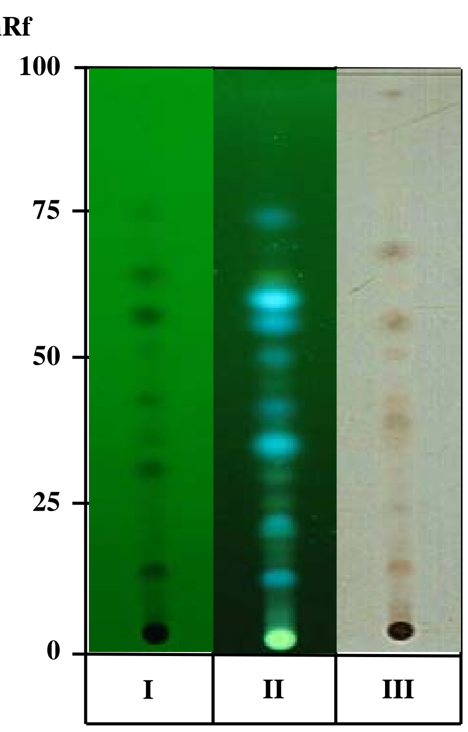
Ben-Cha-Moon-Yai remedy consists of five herbal roots of *Aegle marmelos* Corr. (Ma-Tum; AM), *Oroxylum indicum* Vent. (Phe-Kaa; OI), *Dimocarpus longan* Lour. (Lam-Yai; DL), *Dolichandrone serrulata* (DC) Seem. (Khare-tare; DS) and *Walsura trichostemon* Miq. (Khad-Lin; WT). They were collected from Chiangrai, Tak, Nakhon Ratchasima and Surin Province and authenticated by Associate Professor Dr. Nijsiri Ruangrungsi. The voucher specimens were deposited at the College of Public Health Sciences, Chulalongkorn University, Thailand. All five roots were washed, air-dried under shade and ground to coarse powders. Each dried-root powder was exhaustively macerated with absolute ethanol in a closed conical flask at room temperature, and filtered. The filtrate was evaporated to dryness under vacuum. Maceration was continued with water until exhaustion, and the filtrate was lyophilized to dryness. The yield of the extracts were weighed, recorded and stored at -20°C. The root extract of Ben-Cha-Moon-Yai remedy (BMY) was prepared by mixing each extract in the quantity equivalent to the traditional remedy preparation. The extract was prepared by Ms. Rawiwan Manohan, College of Public Health Sciences, Chulalongkorn University, Bangkok, Thailand.

Thin-layer chromatographic identification

One gram of the root powder of *Aegle marmelos* Corr., *Oroxylum indicum* Vent., *Dimocarpus longan* Lour., *Dolichandrone serrulata* (DC) Seem. and *Walsura trichostemon* Miq. was macerated with 20 ml of methanol for 12 hr, filtered and evaporated to dryness. The residue was dissolved in 0.5 ml of methanol. Apply 10 µl to the thin-layer chromatographic plate, using silica gel 60 F_{254} as the coating substance. The plate was removed and allowed to dry and observed for the produced spots under short-wave (254 nm) and long-wave (366 nm) ultraviolet light. The plate was sprayed with the mixture solution of 10% sulfuric acid reagent (conc. sulfuric acid 10 ml in methanol 90 ml). The plate was then placed in the hot air oven at 105°C for 5 min. The thin-layer chromatograms of all five root extracts were prepared by Ms. Rawiwan

Manohan, College of Public Health Sciences, Chulalongkorn University, Bangkok, Thailand.

Figure 54 Thin-layer chromatogram of the methanolic extract of *Aegle marmelos* root



Solvent system

Toluene: Ethyl acetate 75:25

Detection

I = detection under UV light 254 nm

II = detection under UV light 366 nm

III = detection with 10% sulfuric acid *,**

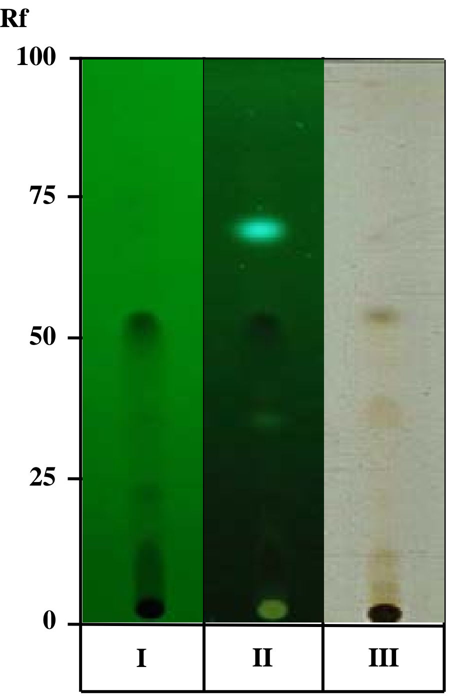
*10% sulfuric acid reagent

Preparation: conc. sulfuric acid 10 ml in methanol 90 ml

**Spot color Development

Heat the plate at 120 °C for 10 minutes after sprayed.

Figure 55 Thin-layer chromatogram of the methanolic extract of *Oroxylum indicum* root



Solvent system

Toluene: Ethyl acetate 75:25

Detection

I = detection under UV light 254 nm

II = detection under UV light 366 nm

III = detection with 10% sulfuric acid*,**

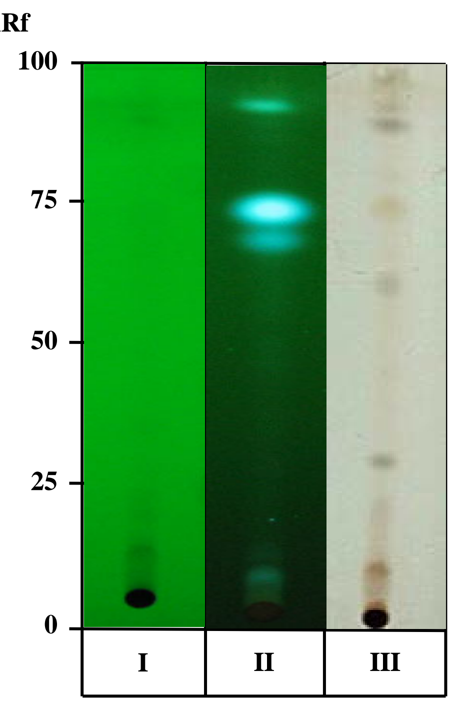
*10% sulfuric acid reagent

Preparation: conc. sulfuric acid 10 ml in methanol 90 ml

**Spot color Development

Heat the plate at 120 ^{o}C for 10 minutes after sprayed.

Figure 56 Thin-layer chromatogram of the methanolic extract of *Dimocarpus longan* root



Solvent system

Chloroform: Methanol 9: 1

Detection

I = detection under UV light 254 nm

II = detection under UV light 366 nm

III = detection with 10% sulfuric acid*,**

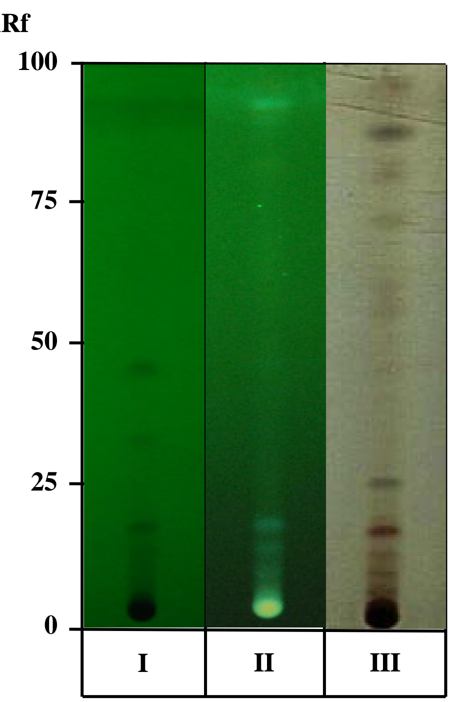
*10% sulfuric acid reagent

Preparation: conc. sulfuric acid 10 ml in methanol 90 ml

**Spot color Development

Heat the plate at 120 °C for 10 minutes after sprayed.

Figure 57 Thin-layer chromatogram of the methanolic extract of *Dolichandrone serrulata* root



Solvent system

Chloroform: Methanol 9: 1

Detection

I = detection under UV light 254 nm

II = detection under UV light 366 nm

III = detection with 10% sulfuric acid*,**

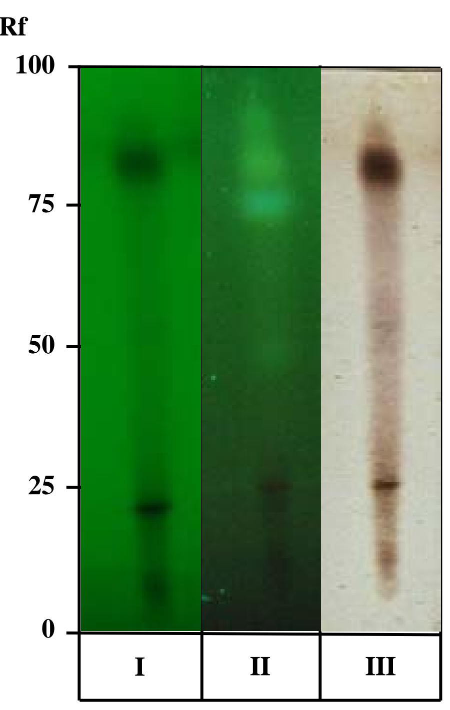
*10% sulfuric acid reagent

Preparation: conc. sulfuric acid 10 ml in methanol 90 ml

**Spot color Development

Heat the plate at 120 °C for 10 minutes after sprayed.

Figure 58 Thin-layer chromatogram of the methanolic extract of *Walsura trichostemon* root



Solvent system

n-butanol: acetic acid : water 4 : 1 : 5

Detection

I = detection under UV light 254 nm

II = detection under UV light 366 nm

III = detection with 10% sulfuric acid*,**

*10% sulfuric acid reagent

Preparation: conc. sulfuric acid 10 ml in methanol 90 ml

**Spot color Development

Heat the plate at 120 °C for 10 minutes after sprayed.

Appendix C

Data of the mouse hot-plate test

Table 22 Latency (sec) in the mouse hot-plate test from 0-240 min after oral administration of the root extract of Ben-Cha-Moon-Yai remedy (BMY; 125-500 mg/kg). N=10 for all groups. Data presented as mean±S.E.M.

Treatments (mg/kg)	Baseline (sec)	15 min	30 min	45 min	60 min	90 min	120 min	240 min
2% Tween 80	23.57±1.88	22.14±2.98	23.80±2.41	20.48±2.97	23.50±1.73	19.69±1.90	22.03±1.07	21.59±1.57
BMY 125	22.30±1.26	28.10±2.35	27.08±1.76	25.93±2.50	26.75±2.47	28.68±2.26	26.05±2.65	25.74±2.35
BMY 250	19.92±1.17	29.50±2.56	28.77±1.88	25.91±3.56	31.18±2.18	31.84±3.51	33.15±1.95	35.69±1.66
BMY 500	22.14±2.98	27.15±1.61	22.55±1.92	24.52±1.76	27.34±3.13	29.39±2.15	33.15±1.53	27.03±3.58

Table 23 Latency (sec) in the mouse hot-plate test from 0-240 min after oral administration of *Aegle marmelos* root extract (AM; 25-400 mg/kg). N=10 for all groups. Data presented as mean±S.E.M.

Treatments (mg/kg)	Baseline (sec)	15 min	30 min	45 min	60 min	90 min	120 min	240 min
2% Tween 80	23.33±1.89	22.23±2.60	20.95±1.83	17.75±2.12	14.84±1.22	19.61±3.45	17.51±1.01	24.54±3.16
AM 25	22.62±1.62	22.15±1.43	27.99±2.81	20.48±2.62	19.96±3.00	24.91±2.31	20.20±2.90	25.01±2.81
AM 50	19.51±1.42	24.39±2.68	23.29±2.09	21.63±0.94	23.29±1.57	27.31±2.99	20.47±1.68	22.29±2.19
AM 100	22.06±1.46	28.91±2.07	22.73±1.08	21.99±0.82	24.21±2.58	23.97±1.30	25.72±2.57	25.82±2.22
AM 200	19.44±1.41	21.66±1.56	21.39±2.46	20.64±1.67	21.66±2.62	22.75±2.44	19.73±3.26	27.50±3.23
AM 400	20.15±1.03	22.01±0.86	22.48±1.62	23.02±1.72	24.69±1.98	21.99±1.54	26.20±1.56	25.30±2.17

Table 24 Latency (sec) in the mouse hot-plate test from 0-240 min after oral administration of *Oroxylum indicum* root extract (OI; 25-400 mg/kg). N=10 for all groups. Data presented as mean±S.E.M.

Treatments (mg/kg)	Baseline (sec)	15 min	30 min	45 min	60 min	90 min	120 min	240 min
2% Tween 80	20.08±1.28	19.75±2.92	16.93±2.04	14.26±1.01	19.64±1.41	19.57±3.24	17.49±1.89	16.72±1.11
OI 25	19.66±2.03	21.19±1.92	21.63±2.64	22.36±2.79	21.23±2.98	19.71±2.28	17.55±2.29	18.53±1.37
OI 50	18.79±1.53	25.64±2.89	23.32±2.47	22.91±1.83	21.08±2.77	23.65±3.19	21.04±2.72	18.79±1.94
OI 100	19.85±1.22	23.59±1.77	24.48±2.68	26.65±3.86	23.39±2.89	24.69±3.03	18.71±2.06	22.79±3.34
OI 200	21.74±2.12	23.41±2.52	24.74±2.38	23.53±1.92	26.07±2.71	25.77±3.36	23.99±3.29	23.61±2.46
OI 400	18.78±1.04	22.70±1.42	21.66±2.40	24.72±1.66	23.33±1.71	28.66±3.51	23.99±4.01	21.27±1.75

Table 25 Latency (sec) in the mouse hot-plate test from 0-240 min after oral administration of *Dimocarpus longan* root extract (DL; 25-400 mg/kg). N=10 for all groups. Data presented as mean±S.E.M.

Treatments (mg/kg)	Baseline (sec)	15 min	30 min	45 min	60 min	90 min	120 min	240 min
2% Tween 80	20.31±1.37	19.21±2.78	17.66±2.83	20.84±2.15	16.33±1.80	15.96±1.19	17.641±1.52	22.62±2.95
DL 25	21.29±2.75	26.66±1.59	19.47±2.28	22.93±2.81	19.62±1.85	21.09±2.05	23.76±1.95	26.83±2.34
DL 50	20.20±1.69	20.54±2.09	19.37±2.01	22.32±2.59	22.70±2.46	18.43±1.63	22.39±2.87	17.33±1.36
DL 100	16.46±1.34	20.18±1.54	18.66±1.70	20.56±3.06	23.18±3.76	24.98±3.29	21.58±1.93	19.95±1.76
DL 200	18.59±1.58	21.91±1.97	22.60±2.75	21.45±1.36	23.02±1.63	22.91±2.29	21±2.60	18.92±1.63
DL400	16.89±0.85	19.36±1.76	18.20±1.47	18.48±1.80	18.11±1.44	20.67±2.47	20.30±1.15	21.67±2.38

Table 26 Latency (sec) in the mouse hot-plate test from 0-240 min after oral administration of *Dolichandrone serrulata* root extract (DS; 25-400 mg/kg). N=10 for all groups. Data presented as mean±S.E.M.

Treatments (mg/kg)	Baseline (sec)	15 min	30 min	45 min	60 min	90 min	120 min	240 min
2% Tween 80	22.38±1.24	18.53±1.46	19.07±2.24	17.79±2.53	16.48±0.96	18.73±1.51	17.98±1.22	22.39±2.29
DS 25	19.03±1.93	20.37±2.13	18.64±1.67	18.67±1.71	18.27±1.52	19.68±1.88	18.41±1.89	19.74±1.86
DS 50	20±1.84	19.60±1.87	20.71±1.60	22.46±1.74	21.31±1.90	24.24±2.17	21.26±2.79	21.61±1.85
DS 100	20.78±1.55	20.94±1.42	21.70±1.67	25.10±2.62	26.89±2.76	25.67±3.28	20.03±1.73	19.53±2.31
DS 200	19.24±1.47	24.91±2.28	24.40±2.05	25.36±2.83	25.65±3.20	26.23±2.22	25.88±3.03	23.75±2.86
DS 400	16.47±1	17.11±0.99	20.34±0.92	18.39±1.35	18.96±1.57	23.08±2.71	23.19±3.40	16.03±1.33

Table 27 Latency (sec) in the mouse hot-plate test from 0-240 min after oral administration of *Walsura trichostemon* root extract (WT; 25-400 mg/kg). N=10 for all groups. Data presented as mean±S.E.M.

Treatments (mg/kg)	Baseline (sec)	15 min	30 min	45 min	60 min	90 min	120 min	240 min
2% Tween 80	22.41±1.43	21.90±2.06	18.28±2.04	15.40±1.00	14.69±1.09	16.16±1.49	19.43±1.93	20.20±1.91
WT 25	17.91±1.04	16.98±1.31	15.58±1.17	16.00±1.06	18.01±1.79	17.09±1.56	18.03±1.28	15.30±0.93
WT 50	20.94±1.58	21.67±2.25	23.06±2.02	19.52±2.80	18.28±2.06	21.79±1.62	18.62±2.04	25.80±3.56
WT 100	18.64±1.43	21.96±2.10	22.53±2.63	22.10±3.16	18.54±2.66	23.23±2.30	22.50±3.60	18.89±1.28
WT 200	15.91±1.20	19.67±1.45	22.78±2.33	21.09±2.24	20.92±2.09	18.27±2.16	21.20±2.92	20.76±2.35
WT 400	16.79±1.06	23.00±3.73	21.18±1.78	22.07±2.04	21.56±2.90	24.18±2.24	22.07±2.22	21.49±2.86

Table 28 %MPE-Time in the mouse hot-plate test from 0-240 min after oral administration of various doses of the root extract of Ben- Cha-Moon-Yai remedy (BMY; 125-500 mg/kg). N=10 for all groups. Data presented as mean±S.E.M.

Treatments (mg/kg)	15 min	30 min	45 min	60 min	90 min	120 min	240 min	Area of analgesia
2% Tween 80	-15.45±19.33	-0.27±9.18	-22.18±18.98	-2.22±6.32	-21.28±7.79	-12.25±8.88	-15.36±11.86	-3,097.97±1,909.93
BMY 125	21.57±13.60	20.93±7.65	10.96±14.51	15.17±12.32	26.09±11.59	16.35±12.62	11.33±13.93	3,832.50±2,117.28
BMY 250	36.36±10.33	35.42±6.97	20.35±15.54	45.71±8.86	48.98±13.27	49.80±9.03	61.20±7.43	11,287.41±1,306.67
BMY 500	23.79±7.50	3.22±11.12	11.59±9.96	24.39±15.37	31.12±10.87	48.40±7.32	26.31±15.07	7,269.91±1,607.88

Table 29 %MPE-Time in the mouse hot-plate test from 0-240 min after oral administration of various doses of *Aegle marmelos* root extract (AM; 25-400 mg/kg). N=10 for all groups. Data presented as mean±S.E.M.

Treatments (mg/kg)	15 min	30 min	45 min	60 min	90 min	120 min	240 min	Area of analgesia
2% Tween 80	-4.39±7.04	-15.16±8.99	-33.36±15.21	-52.53±18.61	-24.95±23.80	-31.37±14.60	0.83±15.59	-5,477.36±2,798.69
AM 25	-6.05±9.40	21.57±13.60	-11.69±11.18	-17.52±16.66	7.82±10.28	-18.45±19.65	2.09±18.70	-1,360.65±3,246.89
AM 50	22.30±9.63	11.65±10.41	4.39±9.12	14.63±4.24	29.56±12.01	1.03±8.85	10.32±7.69	2,487.85±1,386.15
AM 100	25.87±11.50	-0.58±7.69	-4.51±8.44	10.64±10.06	5.11±8.77	12.66±14.49	12.04±11.69	2,376.00±1,825.23
AM 200	8.32±4.98	5.39±11.72	1.90±8.52	7.28±12.24	11.45±10.98	-2.08±15.92	27.77±15.06	2,251.36±2,077.67
AM 400	6.28±4.78	8.68±6.80	10.36±7.80	16.04±9.21	6.74±6.39	24.20±5.83	19.53±9.51	3,930.13±828.09

Table 30 %MPE-Time in the mouse hot-plate test from 0-240 min after oral administration of various doses of *Oroxylum indicum* root extract (OI; 25-400 mg/kg). N=10 for all groups. Data presented as mean±S.E.M.

Treatments (mg/kg)	15 min	30 min	45 min	60 min	90 min	120 min	240 min	Area of analgesia
2% Tween 80	-5.94±14.93	-17.86±13.12	-27.39±9.44	-5.70±9.71	-4.50±13.53	-10.78±5.77	-18.34±11.12	-2,940.06 ± 1,612.55
OI 25	-0.06±10.43	8.67±6.37	6.73±10.80	3.17±10.62	-10.33±17.43	-17.24±17.80	-13.44±13.70	-2,108.61 ± 2,880.54
OI 50	29.36±8.65	13.58±12.17	13.12±8.77	9.41±8.18	21.43±10.03	10.49±9.19	-1.93±8.07	2,367.03 ± 994.35
OI 100	14.35±6.74	18.35±9.70	27.76±14.91	15.29±10.80	22.20±9.48	-4.44±7.06	14.25±11.96	2,439.20 ± 1,180.23
OI 200	-9.06±23.51	9.83±10.56	3.11±10.61	11.64±18.49	22.02±11.27	13.24±14.92	5.22±8.43	2,287.47 ± 1,128.74
OI 400	15.35±3.44	11.69±7.62	21.33±7.47	15.87±7.50	34.22±14.83	16.22±17.26	7.31±8.61	3,764.78 ± 2,279.81

Table 31 %MPE-Time in the mouse hot-plate test from 0-240 min after oral administration of various doses of *Dimocarpus longan* root extract (DL; 25-400 mg/kg). N=10 for all groups. Data presented as mean±S.E.M.

Treatments (mg/kg)	15 min	30 min	45 min	60 min	90 min	120 min	240 min	Area of analgesia
2% Tween 80	-10.84±18.18	-8.77±9.36	-2.94±14.40	-20.87±11.83	-22.99±11.67	-14.38±9.56	9.25±10.99	-2,021.55±1,681.64
DL 25	13.84±9.51	-41.85±37.91	-11.94±26.58	-36.19±30.90	-22.51±22.33	-2.06±13.10	5.12±21.12	-1,936.09±4,432.66
DL 50	-0.84±9.38	-4.76±6.70	6.85±10.33	8.50±9.74	-10.46±7.54	3.80±12.97	-18.69±13.28	-940.47±1,987.57
DL 100	12.17±5.01	7.61±4.42	16.43±9.09	24.37±13.72	27.85±12.03	17.38±6.16	11.25±5.98	3,905.23±1,187.40
DL 200	10.11±8.85	14.66±10.11	9.01±5.52	12.93±9.04	12.37±11.47	8.19±10.66	-1.58±7.58	1,688.16±1,548.59
DL 400	8.61±5.80	4.48±4.66	4.64±6.89	3.99±5.05	13.93±7.69	11.38±4.80	17.33±7.53	2,666.82±856.36

Table 32 %MPE-Time in the mouse hot-plate test from 0-240 min after oral administration of various doses of *Dolichandrone serrulata* root extract (DS; 25-400 mg/kg). N=10 for all groups. Data presented as mean±S.E.M.

Treatments (mg/kg)	15 min	30 min	45 min	60 min	90 min	120 min	240 min	Area of analgesia
2% Tween 80	-18.03±5.35	-14.87±8.32	-19.37±9.84	-29.32±8.26	-17.27±5.40	-23.47±10.03	0.66±8.26	-3,682.57 ± 1,126.55
DS 25	5.43±4.98	-5.42±7.17	-5.40±7.88	-9.10±11.50	-3.30±10.85	-5.60±7.07	-2.29±9.70	-942.56 ± 1,750.79
DS 50	-7.64±14.24	-2.99±13.09	6.70±9.19	2.07±8.68	12.28±11.20	2.36±12.91	1.64±10.43	631.92 ± 1,904.79
DS 100	-2.42±8.43	1.81±7.76	20.27±9.16	28.60±9.99	22.70±12.08	-8.73±11.60	-7.42±8.94	519.79 ± 1,358.46
DS 200	22.63±6.72	19.30±7.61	19.73±12.42	23.56±13.53	24.98±10.47	28.15±9.27	13.98±12.52	5,154.56 ± 1,269.98
DS 400	1.16±5.16	13.30±2.45	6.31±4.87	8.34±5.34	24.31±8.99	23.88±12.04	-2.49±5.61	2,870.22 ± 1,476.94

Table 33 %MPE-Time in the mouse hot-plate test from 0-240 min after oral administration of various doses of *Walsura trichostemon* root extract (WT; 25-400 mg/kg). N=10 for all groups. Data presented as mean±S.E.M.

Treatments (mg/kg)	15 min	30 min	45 min	60 min	90 min	120 min	240 min	Area of analgesia
2% Tween 80	-2.00±6.27	-22.97±13.42	-36.84±12.32	-39.85±12.01	-31.34±8.95	-18.45±12.66	-12.37±8.46	-4,699.61±1,982.49
WT 25	-3.29±2.48	-8.85±2.94	-8.45±5.53	0.65±5.89	-3.49±3.58	0.35±3.10	-11.32±5.94	-1,051.71±571.31
WT 50	-2.62±12.98	7.26±8.41	-8.35±12.91	-15.72±12.49	0.62±8.33	-12.13±9.72	14.49±18.86	-430.94±1,748.23
WT 100	11.55±9.01	15.05±9.21	12.61±12.86	-1.49±11.11	12.39±12.55	16.38±13.52	-1.78±7.31	2,048.29±1,063.64
WT 200	11.11±7.00	23.17±7.90	15.91±9.64	17.19±6.23	6.13±9.35	19.18±10.75	16.62±7.84	3,759.23±1,125.86
WT 400	20.00±13.96	13.57±8.17	15.96±9.43	13.39±12.04	23.88±9.62	17.90±8.49	14.02±10.80	3,944.24±1,758.08

Table 34 Latency (sec) in the mouse hot-plate test from 0-240 min after administration of naloxone (5 mg/kg; i.p.), 2% Tween 80 (10 ml/kg; p.o.), *Aegle marmelos* root extract (AM; 400 mg/kg; p.o.) and the combination of naloxone and *Aegle marmelos* root extract (5/400 mg/kg). N=10 for all groups. Data presented as mean±S.E.M.

Treatments (mg/kg)	Baseline (sec)	15 min	30 min	45 min	60 min	90 min	120 min	240 min
Naloxone 5 mg/kg	23.61±0.47	19.20±1.19	19.74±1.65	19.23±1.24	18.98±1.09	18.20±1.09	19.72±1.94	18.65±1.04
2% Tween 80 10 ml/kg	23.20±0.55	19.78±0.99	18.29±0.49	20.54±1.15	18.48±0.79	19.49±1.50	20.24±1.08	21.69±1.90
AM 400 mg/kg	22.41±0.75	23.48±1.23	25.09±2.07	25.34±1.11	28.34±1.63	28.42±1.48	24.02±0.63	27.85±1.75
Naloxone + AM	22.44±0.61	18.97±0.76	17.97±1.33	18.29±0.56	16.01±0.36	18.63±1.00	18.43±0.86	19.02±1.04

Table 35 Latency (sec) in the mouse hot-plate test from 0-240 min after administration of naloxone (5 mg/kg; i.p.), 2% Tween 80 (10 ml/kg; p.o.), *Dolichandrone serrulata* root extract (DS; 200 mg/kg; p.o.) and the combination of naloxone and *Dolichandrone serrulata* root extract (5/200 mg/kg). N=10 for all groups. Data presented as mean±S.E.M.

Treatments (mg/kg)	Baseline (sec)	15 min	30 min	45 min	60 min	90 min	120 min	240 min
Naloxone 5 mg/kg	21.78±1.02	20.04±1.35	18.18±1.60	17.73±0.71	16.74±0.86	19.82±1.24	18.61±0.99	20.88±1.56
2% Tween 80 10 ml/kg	24.34±0.49	23.74±2.04	19.22±1.29	21.27±2.12	18.50±1.84	20.58±1.13	20.50±1.63	24.01±1.92
DS 200 mg/kg	22.44±0.72	25.64±1.13	27.90±2.18	27.90±1.32	28.13±1.68	29.85±1.32	26.54±2.28	31.48±3.02
Naloxone + DS	21.72±1.31	23.82±2.63	22.41±2.40	16.94±1.16	19.00±1.46	17.64±0.88	18.66±1.25	19.16±1.38

Table 36 Latency (sec) in the mouse hot-plate test from 0-240 min after administration of NSS (10 ml/kg, i.p.), naloxone (5 mg/kg; i.p.), 2% Tween 80 (10 ml/kg; p.o.), *Walsura trichostemon* root extract (WT; 400 mg/kg, p.o.) and the combination of naloxone and *Walsura trichostemon* root extract (5/400 mg/kg). N=10 for all groups. Data presented as mean±S.E.M.

Treatments (mg/kg)	Baseline (sec)	15 min	30 min	45 min	60 min	90 min	120 min	240 min
NSS	22.41±0.93	20.74±1.01	17.35±1.84	18.39±1.05	15.67±0.80	16.18±0.78	17.89±1.52	19.55±1.56
Naloxone 5 mg/kg	21.50±0.62	18.41±1.04	17.93±0.75	16.23±0.74	16.55±1.93	17.83±1.13	16.75±0.91	20.62±1.62
2% Tween 80 10 ml/kg	22.58±0.70	18.20±0.95	18.39±2.09	18.46±1.41	16.48±1.13	18.62±1.18	17.52±1.05	19.19±1.08
WT 400 mg/kg	21.83±0.64	23.73±1.75	26.37±2.18	27.26±2.05	27.20±2.20	31.65±2.50	29.26±2.71	26.34±2.13
Naloxone + WT	22.02±0.85	20.32±1.54	22.20±2.24	19.11±1.46	20.49±1.62	19.17±1.20	18.47±1.46	17.88±1.16

Table 37 %MPE-Time in the mouse hot-plate test from 0-240 min after administration of naloxone (5 mg/kg; i.p.), 2% Tween 80 (10 ml/kg; p.o.), *Aegle marmelos* root extract (AM; 400 mg/kg, p.o.) and the combination of naloxone and *Aegle marmelos* root extract (5/400 mg/kg). N=10 for all groups. Data presented as mean±S.E.M.

Treatments (mg/kg)	15 min	30 min	45 min	60 min	90 min	120 min	240 min	Area of analgesia
Naloxone 5 mg/kg	-20.50±4.85	-18.43±8.04	-21.12±6.53	-22.14±5.67	-26.13±6.34	-19.18±9.61	-23.34±4.83	-5,022.12±1,132.62
2% Tween 80 10 ml/kg	-16.57±5.77	-22.88±2.13	-13.48±7.20	-22.40±4.88	-17.88±7.88	-14.08±5.62	-6.63±8.25	-3,288.21±996.50
AM 400 mg/kg	4.14±5.77	11.52±8.89	11.50±7.33	26.10±7.21	25.06±8.21	6.09±4.62	22.32±10.45	3,542.10±1,211.8
Naloxone + AM	-15.52±2.48	-20.42±6.50	-18.79±2.53	-29.32±3.74	-17.44±4.70	-18.00±3.32	-15.94±5.52	-4,310.20±637.97

Table 38 %MPE-Time in the mouse hot-plate test from 0-240 min after administration of naloxone (5 mg/kg; i.p.), 2% Tween 80 (10 ml/kg; p.o.), *Dolichandrone serrulata* root extract (DS; 200 mg/kg; p.o.) and the combination of naloxone and *Dolichandrone serrulata* root extract (5/200 mg/kg). N=10 for all groups. Data presented as mean±S.E.M.

Treatments (mg/kg)	15 min	30 min	45 min	60 min	90 min	120 min	240 min	Area of analgesia
Naloxone 5 mg/kg	-8.65±6.48	-17.84±9.39	-18.92±4.80	-22.56±3.13	-9.40±5.81	-15.37±6.08	-5.90±8.73	-2,977.61±1,062.50
2% Tween 80 10 ml/kg	-2.86±9.64	-25.31±6.59	-13.90±8.72	-29.75±10.30	-18.03±4.21	-18.64±7.99	-1.22±8.65	-3,312.66±1,057.05
DS 200 mg/kg	12.86±6.74	24.07±10.54	24.37±5.55	26.36±5.66	32.78±5.71	19.15±9.20	39.25±14.24	6,287.52±1,390.54
Naloxone + DS	8.82±10.64	-0.46±12.53	-23.40±7.65	-12.76±5.48	-21.21±8.82	-13.96±3.28	-13.62±8.12	-3,013.01±1,095.76

Table 39 %MPE-Time in mouse hot-plate test from 0-240 min after administration of NSS (10 ml/kg, i.p.), naloxone (5 mg/kg; i.p.), 2% Tween 80 (10 ml/kg; p.o.), *Walsura trichostemon* root extract (WT; 400 mg/kg; p.o.) and the combination of naloxone and *Walsura trichostemon* root extract (5/400 mg/kg). N=10 for all groups. Data presented as mean±S.E.M.

Treatments (mg/kg)	15 min	30 min	45 min	60 min	90 min	120 min	240 min	Area of analgesia
NSS	-9.13±6.80	-23.79±9.71	-20.56±8.63	-31.48±5.53	-29.39±5.98	-20.87±7.06	-12.65±4.95	-4,716.28±1,027.29
Naloxone 5 mg/kg	-13.37±4.10	-15.51±3.12	-22.88±3.41	-21.62±8.6	-15.70±4.08	-20.12±1.61	-3.83±6.51	-3,472.54±675.06
2% Tween 80 10 ml/kg	-20.36±5.48	-19.28±10.5	-19.82±8.03	-28.21±6.37	-19.11±7.54	-23.91±6.91	-15.75±5.21	-4,838.38±1,152.64
WT 400 mg/kg	7.60±8.11	20.30±9.09	23.87±8.12	23.27±9.94	42.75±11.08	31.14±12.06	19.14±9.18	6,066.40±1,850.51
Naloxone + WT	-7.29±5.92	1.88±8.64	-12.86±5.78	-6.75±6.49	-12.74±4.25	-17.08±8.31	-19.36±6.51	-3,251.08±1,075.98

Appendix D

Data of the acetic acid-induced writhing in mice

Table 40 Dose-response and time-course effect of 2%Tween 80, indomethacin (IND; 10 mg/kg) and various doses of the root extract of Ben-Cha-Moon-Yai remedy (BMY) on acetic acid-induced writhing in mice. N=8 per group. Data presented as mean±S.E.M.

Treatments (mg/kg)	Mean Number of Writhes						Total
	0-5 min	6-10 min	11-15 min	16-20 min	21-25 min	26-30 min	
2%Tween 80	1.88±0.58	5.38±0.73	7.38±0.96	6.50±0.91	5.50±0.80	3.50±0.94	30.12±4.15
IND 10	0.38±0.18	0.38±0.18	0.88±0.30	1.12±0.35	1.12±0.23	0.50±0.27	4.38±0.26
BMY 125	1.38±0.53	4.25±0.53	5.25±0.70	4.75±0.82	2.25±0.45	2.25±0.45	20.12±2.03
BMY 250	1.88±0.48	3.25±0.31	4.25±0.62	3.75±0.70	2.12±0.48	1.75±0.36	17.00±2.26
BMY 500	1.00±0.33	2.00±0.60	2.88±0.58	2.88±0.23	2.12±0.30	1.38±0.18	12.25±1.62

Table 41 Dose-response and time-course of 2%Tween 80, indomethacin (IND; 10 mg/kg) and various doses of *Aegle marmelos* root extract (AM) on acetic acid-induced writhing in mice. N=8 per group. Data presented as mean±S.E.M.

Treatments (mg/kg)	Mean Number of Writhes						Total
	0-5 min	6-10 min	11-15 min	16-20 min	21-25 min	26-30 min	
2%Tween 80	2.00±1.36	7.62±2.01	9.88±1.08	9.00±1.25	5.12±0.69	3.5±0.60	37.12±2.97
IND 10	0	0.88±0.40	2.00±0.50	1.25±0.49	1.75±0.65	0.38±0.26	6.25±1.35
AM 25	1.38±0.38	6.12±1.23	6.00±0.84	7.88±0.88	6.00±0.80	4.75±0.56	32.12±3.08
AM 50	2.50±0.87	7.50±1.44	8.5±0.82	6.25±0.98	5.12±0.64	3.62±1.13	33.5±3.11
AM 100	1.88±0.93	7.12±1.86	7.38±0.75	6.62±0.94	4.12±0.79	1.88±0.64	29±3.17
AM 200	0.12±1.12	2.62±1.27	3.50±0.96	2.38±0.38	2.00±0.50	0.75±0.41	11.38±2.84
AM 400	0	1.62±0.53	1.25±0.49	1.00±0.38	0.38±0.26	0.38±0.26	4.62±1.22

Table 42 Dose-response and time-course effect of 2%Tween 80, indomethacin (IND; 10 mg/kg) and various doses of *Oroxylum indicum* root extract (OI) on acetic acid-induced writhing in mice. N=8 per group. Data presented as mean±S.E.M.

Treatments (mg/kg)	Mean Numbers of Writhes						Total
	0-5 min	6-10 min	11-15 min	16-20 min	21-25 min	26-30 min	
2%Tween 80	4.00±0.92	8.62±1.18	8.12±0.48	6.62±0.42	7.00±1.04	7.00±1.07	41.38 ± 3.22
IND 10	0.38±0.18	2.25±0.36	3.00±0.60	1.88±0.67	1.12±0.23	0.25±0.16	8.88 ± 1.12
OI 25	2.38±0.68	6.88±1.14	7.25±1.05	6.12±0.97	3.38±0.78	3.50±0.94	29.5 ± 3.4
OI 50	3.25±0.80	8.12±1.96	6.75±1.22	5.00±1.21	3.75±0.90	1.50±0.63	28.38 ± 5.17
OI 100	2.00±0.38	4.12±0.67	8.38±1.19	5.75±1.19	4.88±1.06	2.25±0.53	27.38 ± 3.49
OI 200	2.75±0.36	7.38±1.21	6.12±0.40	5.00±0.84	3.00±0.53	1.75±0.41	26 ± 1.73
OI 400	0.62±0.38	4.25±0.84	4.00±0.84	4.25±0.56	2.25±0.56	1.88±0.67	17.25 ± 2.27

Table 43 Dose-response and time-course effect of 2%Tween 80, indomethacin (IND; 10 mg/kg) and various doses of *Dimocarpus longan* root extract (DL) on acetic acid-induced writhing in mice. N=8 per group. Data presented as mean±S.E.M.

Treatments (mg/kg)	Mean Number of Writhes						Total
	0-5 min	6-10 min	11-15 min	16-20 min	21-25 min	26-30 min	
2%Tween 80	2.12±0.83	7.38±1.28	9.25±0.90	8.00±0.76	6.88±0.97	5.50±0.76	39.12±3.29
IND 10	0.25±0.16	2.75±0.59	2.62±0.62	2.62±0.60	0.88±0.30	0.50±0.27	9.62±1.28
DL 25	1.75±0.41	7.00±1.27	8.5±0.78	6.38±0.62	3.00±0.46	3.25±0.88	29.88±1.93
DL 50	1.62±0.65	6.25±0.84	6.25±0.80	5.62±0.88	4.00±0.57	3.88±0.64	27.62±2.07
DL 100	1.5±0.38	6.38±1.08	6.75±1.13	4.75±0.53	4.38±0.80	2.25±0.65	26.01±3.53
DL 200	2.00±0.50	7.12±1.47	5.88±0.83	4.38±0.50	2.62±0.62	1.88±0.44	23.88±3.62
DL 400	1.00±0.42	4.12±1.02	3.75±0.70	1.62±0.46	1.12±0.40	1.00±0.42	12.62±2.47

Table 44 Dose-response and time-course effect of 2%Tween 80, indomethacin (IND; 10 mg/kg) and various doses of *Dolichandrone serrulata* root extract (DS) on acetic acid-induced writhing in mice. N=8 per group. Data presented as mean±S.E.M.

Treatments (mg/kg)	Mean Number of Writhes						Total
	0-5 min	6-10 min	11-15 min	16-20 min	21-25 min	26-30 min	
2%Tween 80	2.01±0.89	6.12±0.77	9.88±0.93	5.88±0.90	5.75±0.59	5.00±0.42	34.62±2.43
IND 10	0.12±0.12	0.75±0.41	2.25±0.92	0.50±0.27	0.88±0.30	0.38±0.26	4.88±1.65
DS 25	2.25±0.45	7.38±1.19	8.50±0.92	6.38±0.90	5.50±0.91	3.50±0.78	33.5±1.91
DS 50	1.38±0.56	7.00±2.18	8.25±1.16	4.38±0.65	6.12±0.91	3.38±1.18	30.5±4.04
DS 100	2.25±0.80	6.50±1.36	9.00±1.18	5.62±1.21	5.38±1.29	2.75±1.13	31.5±4.18
DS 200	1.50±0.42	6.00±1.48	5.00±0.82	5.50±0.68	2.50±0.60	1.25±0.45	21.75±2.52
DS 400	1.50±0.57	3.62±1.24	4.00±0.78	4.12±1.09	3.38±0.86	2.25±0.75	18.88±2.94

Table 45 Dose-response and time-course effect of 2%Tween 80, indomethacin (IND; 10 mg/kg) and various doses of *Walsura trichostemon* root extract (WT) on acetic acid-induced writhing in mice. N=8 per group. Data presented as mean±S.E.M.

Treatments (mg/kg)	Mean Number of Writhes						Total
	0-5 min	6-10 min	11-15 min	16-20 min	21-25 min	26-30 min	
2%Tween 80	0.75±0.31	4.50±1.28	7.12±1.01	8.00±1.12	8.38±0.80	6.00±0.80	34.75±2.62
IND 10	0	0.25±0.16	0.38±0.26	1.25±0.67	0.12±0.12	0.25±0.16	2.25±0.70
WT 25	1.50±0.50	8.00±0.78	8.38±0.86	7.62±0.62	4.12±0.58	2.88±0.48	32.5±0.82
WT 50	0.62±0.26	5.62±0.80	8.50±0.76	8.00±0.84	5.62±0.68	3.62±0.80	32.00±2.5
WT 100	1.62±0.46	6.38±1.25	7.75±0.65	4.88±0.58	2.75±0.45	2.38±0.62	25.75±2.10
WT 200	0.62±0.32	3.88±1.01	4.25±1.11	4.50±1.05	2.50±0.63	1.62±0.53	17.38±3.02
WT 400	0.50±0.27	2.88±1.09	2.88±0.67	3.75±0.96	1.88±0.55	1.50±0.42	13.38±2.91

Appendix E

Data of the rota-rod test in mice

Table 46 Effect of the root extract of Ben-Cha-Moon-Yai remedy (BMY; 500 mg/kg; p.o.) in the rota-rod test. N=8 per group. Data presented as mean±S.E.M.

Treatments (mg/kg)	Time (mean±S.E.M.)				
	30 min	**60 min**	**90 min**	**120 min**	**240 min**
2%Tween 80	60±0	60±0	59.5±0.5	59.62±0.38	59.62±0.38
BMY 500	60±0	60±0	60±0	60±0	60±0

Table 47 Effect of *Aegle marmelos* root extract (AM; 400 mg/kg; p.o.) in the rota-rod test. N=8 per group. Data presented as mean±S.E.M.

Treatments (mg/kg)	Time (mean±S.E.M.)				
	30 min	**60 min**	**90 min**	**120 min**	**240 min**
2%Tween 80	60±0	60±0	60±0	60±0	60±0
AM 400	60±0	60±0	60±0	60±0	60±0

Table 48 Effect of *Oroxylum indicum* root extract (OI; 400 mg/kg; p.o.) in the rota-rod test. N=8 per group. Data presented as mean±S.E.M.

Treatments (mg/kg)	Time (mean±S.E.M.)				
	30 min	**60 min**	**90 min**	**120 min**	**240 min**
2%Tween 80	60±0	60±0	60±0	60±0	60±0
OI 400	59.75±0.25	60±0	60±0	59.87±0.12	59.62±0.37

Table 49 Effect of *Dimocarpus longan* root extract (DL; 400 mg/kg; p.o.) in the rota-rod test. N=8 per group. Data presented as mean±S.E.M.

Treatments	Time (mean±S.E.M.)				
(mg/kg)	30 min	60 min	90 min	120 min	240 min
2%Tween 80	60±0	60±0	60±0	60±0	60±0
DL 400	60±0	60±0	60±0	60±0	60±0

Table 50 Effect of *Dolichandrone serrulata* root extract (DS; 400 mg/kg; p.o.) in the rota-rod test. N=8 per group. Data presented as mean±S.E.M.

Treatments	Time (mean±S.E.M.)				
(mg/kg)	30 min	60 min	90 min	120 min	240 min
2%Tween 80	59.62±0.37	60±0	59±0.75	60±0	59.87±0.12
DS 400	59.37±0.62	60±0	60±0	60±0	60±0

Table 51 Effect of *Walsura trichostemon* root extract (WT; 400 mg/kg; p.o.) in the rota-rod test. N=8 per group. Data presented as mean±S.E.M.

Treatments	Time (mean±S.E.M.)				
(mg/kg)	30 min	60 min	90 min	120 min	240 min
2%Tween 80	60±0	60±0	60±0	59.75±0.25	60±0
WT 400	60±0	60±0	59.37±0.62	58.87±1.12	59±0.68

VITAE

Miss Chayanin Kiratipaiboon was born on February 12, 1983 in Bangkok, Thailand. She graduated with Doctor of Pharmacy from Srinakharinwirot University in 2007. After graduation, she had worked as a clinical pharmacist at Bangkok Hospital Pattaya, Chonburi, Thailand for 2 years.