ORIGINAL ARTICLE

Investigation of the anti-inflammatory, analgesic and antipyretic activities of the extracts from the rhizome of *Dioscorea membranacea* Pierre in experimental animals

Wantana Reanmongkol[1], Arunporn Itharat[2] and Pisit Bouking[3]

Abstract

Reanmongkol, W., Itharat, A. and Bouking, P.

Investigation of the anti-inflammatory, analgesic and antipyretic activities of the extracts from the rhizome of *Dioscorea membranacea* Pierre in experimental animals

Songklanakarin J. Sci. Technol., March 2007, 29(Suppl. 1) : 49-57

The effects of the ethanol and aqueous extracts from the rhizome of *Dioscorea membranacea* Pierre (*D. membranacea*) on inflammatory response using carrageenin-induced paw edema in rats, were examined. Antinociceptive activity using writhing, hot plate and formalin test in mice and the antipyretic activity in yeast-induced fever in rats, were also examined. Oral administration of the ethanol extract at the dose of 1600 mg/kg significantly decreased the paw edema induced by carrageenin in rats. The aqueous extract (1600 mg/kg) also significantly suppressed the carrageenin-induced paw edema in rats. The ethanol extract had no significant effects on antinociceptive response in writhing, formalin and hot plate tests and antipyretic

---

[1]Ph.D.(Pharmacology), Assoc. Prof., [3]Dip.(Science), Technician, Department of Clinical Pharmacy, Faculty of Pharmaceutical Sciences, Prince of Songkla University, Hat Yai, Songkhla 90112 Thailand. [2]Ph.D. (Pharmacognosy), Assoc. Prof., Applied Thai Traditional Medicine Centre, Faculty of Medicine, Thammasart University, Klong Luang, Pathum Thani, 10120 Thailand.

Corresponding e-mail: wantana.r@psu.ac.th

activities in yeast-induced fever in rats. No significant effects on writhing test and yeast-induced fever were observed after oral administration of the aqueous extract in experimental animals. These results suggest that the ethanol and aqueous extracts of *D. membranacea* rhizome possess anti-inflammatory action but no analgesic or antipyretic actions and their anti-inflammatory action may act at some site(s) of action or inhibit of some inflammatory mediators, which is different from the action of aspirin.

Key words : *Dioscorea membranacea*, rhizome, ethanol extract, aqueous extract, anti-inflammatory activity

บทคัดย่อ

วันทนา เหรียญมงคล[1] อรุณพร อิฐรัตน์[2] และ พิศิษฐ์ บัวกิ่ง[1]

ตรวจหาฤทธิ์แก้อักเสบ ระงับปวด และลดไข้ ของสารสกัดจากเหง้าหัวข้าวเย็น (*Dioscorea membranacea* Pierre) ในหนูทดลอง

ว. สงขลานครินทร์ วทท. มีนาคม 2550 29(ฉบับพิเศษ 1) : 49-57

ทำการทดสอบผลของสารสกัดด้วยเอทานอลและน้ำจากเหง้าของหัวข้าวเย็น โดยสังเกตผลต่อการตอบสนองต่อการอักเสบซึ่งเกิดจากการเหนี่ยวนำด้วยคาร์ราจีนินในหนูขาว ผลการระงับปวด โดยการเหนี่ยวนำให้เกิดความปวดด้วยกรดอะเซติก ความร้อน และฟอร์มาลินในหนูถีบจักร และผลต่อการลดไข้ โดยการเหนี่ยวนำให้เกิดไข้ด้วยยีสต์ในหนูขาว เมื่อป้อนสารสกัดด้วยเอทานอลที่ขนาด 1600 มก./กก. เข้าทางปากในหนูขาว พบว่าสามารถลดการบวมอย่างมีนัยสำคัญที่อุ้งเท้าซึ่งเกิดจากการเหนี่ยวนำด้วยคาร์ราจีนิน สารสกัดด้วยน้ำ (1600 มก./กก.) สามารถลดการบวมอย่างมีนัยสำคัญที่อุ้งเท้าซึ่งเกิดจากการเหนี่ยวนำด้วยคาร์ราจีนินได้เช่นกัน ไม่มีสารสกัดใดจากเหง้าของหัวข้าวเย็น ที่สามารถลดอาการปวดในการทดสอบฤทธิ์แก้ปวดในหนูถีบจักร และลดไข้ซึ่งเกิดจากการเหนี่ยวนำด้วยยีสต์ในหนูขาว จากผลการทดลองนี้ชี้แนะว่า สารสกัดด้วยเอทานอลและน้ำจากเหง้าของหัวข้าวเย็น มีฤทธิ์แก้อักเสบ แต่ไม่มีฤทธิ์ระงับปวด หรือลดไข้ และฤทธิ์ต้านการอักเสบอาจออกฤทธิ์ที่ตำแหน่งหรือยับยั้งตัวสื่อการอักเสบซึ่งแตกต่างจากการออกฤทธิ์ของแอสไพริน

[1]ภาควิชาเภสัชกรรมคลินิก คณะเภสัชศาสตร์ มหาวิทยาลัยสงขลานครินทร์ อำเภอหาดใหญ่ จังหวัดสงขลา 90112 [2]ศูนย์การแพทย์แผนไทยประยุกต์ คณะแพทยศาสตร์ มหาวิทยาลัยธรรมศาสตร์ อำเภอคลองหลวง จังหวัดปทุมธานี 10120

Dioscorea membranacea Pierre (*D. membranacea*), family Dioscoreaceae, is known in Thai as Hua-Khao-Yen. It has been long used as common ingredients in several preparations, including those used in the treatments of inflammatory diseases e.g., arthritis, lymphopathy, dermopathy, venereal diseases, leprosy and cancers. Hua-Khao-Yen is available as drugs in traditional drug stores throughout Thailand (Boonyaratanakornkit and Chantarateptavan, 1993).

Several compounds have been isolated and identified from *Dioscorea* spp. rhizome. For examples, glycosides and steroidal saponins were isolated from *D. futschauensis*, *D. spongiosa* and *Dioscorea panthaica* (Liu *et al*., 2003; Yin *et al*., 2003; Dong *et al*., 2004).

In addition, many pharmacological activities of *Dioscorea* spp. Rhizome have been studied. The antiosteoporotic, antifungal, anti-hypercholesterolemia and anticancer activities from *Dioscorea* spp. rhizome have been reported (Yin *et al*., 2004a; Yin *et al*., 2004b; Sautour *et al*., 2004; Kwon *et al*., 2003; Ma *et al*., 2002; Wang *et al*., 2004). The extract of *Dioscorea* rhizome protected against acute kidney and liver injuries in rats induced by acetaminophen and ethanol (Lee *et al*., 2002a; Lee *et al*., 2002b). The methanol extract of *D. tokoro* Makino root reduced the proliferation

of human fibroblast-like synovial cells stimulated by interleukin-1 beta and tumor necrosis factor-alpha (Kim *et al*., 2004).

However, only few studies of *D. membranacea* rhizomes have been reported. Dioscorealides A and B and 1,4-phenanthraquinone, dioscoreanone, compounds from *D. membranacea* rhizome, were isolated and identified (Itharat *et al*., 2003). Recently, it has been reported that the ethanol extract from *D. membranacea* rhizome was potently cytotoxic against cancer cell lines (Itharat *et al*., 2004).

In the present study, in order to evaluate the potential anti-inflammatory effect of the extract of *D. membranacea* rhizomes, we investigated the anti-inflammatory activity in experimental animal model using carrageenin-induced paw edema in rats. The analgesic and antipyretic activities were also examined using the writhing, hot plate and formalin tests in mice and yeast-induced fever in rats, respectively.

Materials and methods

Plant material

The rhizomes (Hua-Khao-Yen) of *D. membranacea* Pierre (Dioscoreaceae) were collected from Amphor Pa-tue, Chumporn Province. Authentication of plant material was carried out at the herbarium of the Department of Forestry, Bangkok, Thailand, where the herbarium voucher has been kept. A duplicate set has been deposited in the herbarium of Southern Center of Thai Medicinal Plants at Faculty of Pharmaceutical Sciences, Prince of Songkla University, Songkhla, Thailand. The voucher number is SKP A062041305.

Preparation of the extract from the rhizomes of *D. membranacea*

The small pieces of the rhizome of *D. membranacea* were dried at 50°C, powdered and extracted by methods corresponding to those practised by Thai traditional doctors. For the ethanolic extracts, dried ground rhizome of *D. membranacea* (100 g) was percolated with 95% ethanol, then concentrated to dryness under reduced pressure. The water extract was obtained by boiling dried ground rhizome of *D. membranacea* (100 g) for 30 min in distilled water (300 ml), all extracts being filtered and freeze dried. The percentage yields of the ethanol and aqueous extracts were 3.4% and 21.9%, respectively. The ethanol and aqueous extracts were dissolved in cosolvent solution (propylene glycol : tween 80 : water = 4:1:4) and used as the test extract. All doses are expressed in terms of crude extract (mg/kg body weight).

Animals

All animals were obtained from the Animal House, Faculty of Sciences, Prince of Songkla University, Hat Yai, Songkhla, Thailand. Animals used in this study were male Swiss albino mice, weighing 30-38 g and Wistar rats with the weight ranging from 150-210 g. The animals were housed for at least one week in the laboratory animal room prior to testing. Food and water were given *ad libitum* unless otherwise specified. All experimental protocols were approved by the animal ethics committee of Prince of Songkla University.

Carrageenin-induced paw edema

According to the method described by Winter *et al*. (1962), the initial right hind paw volume of the rats was measured using a plethysmometer (Ugo Basile) and then 0.1 ml of 1% (w/v) carrageenin was subcutaneously injected into the subplantar region of the right hind paw. The volume of the right hind paw was measured at 1, 2, 3, 4 and 5 hr after carrageenin injection and the edema volume was determined. Cosolvent, the extract or aspirin was orally administered 30 min before carrageenin injection.

Antinociceptive Activity

1. Writhing test

Writhing behavior was tested, in which 0.6% acetic acid solution (10 ml/kg body weight) was injected intraperitoneally and the number of writhings and stretchings was counted over a 20 min period as previously reported (Koster *et al*.,

1959; Hendershot & Forsaith, 1959). The plant extract (400, 800 and 1600 mg/kg), a reference analgesic drug, aspirin (200 mg/kg), or cosolvent was orally administered 30 min before intraperitoneal injection of acetic acid.

2. Hot plate test

The hot plate test was carried out according to the method described by Woolfe & Mac Donald (1944). Mice were placed on a hot plate maintained at 55°C ± 1°C. Latency of nociceptive response such as licking of a hind limb or jumping was measured. Starting thirty minutes after oral administration of the test agents except morphine (15 min after administration), the nociceptive response was measured every 15 min over a 60 min period. Morphine sulfate was injected subcutaneously. The cut-off time was 45 sec. Only the mice that showed nociceptive responses within 15 sec were used for the experiments.

3. Formalin test

Thirty minutes after oral administration of the extract (400, 800 and 1600 mg/kg), aspirin (200 mg/kg) or cosolvent except morphine (15 min after administration), 20 µl of 2.5% formalin in saline was injected subcutaneously to a hind paw of the mice. Morphine sulfate was injected subcutaneously. The time spent licking the injected paw was recorded and the data were expressed as total licking time in the early phase (0-5 min) and the late phase (15-30 min) after formalin injection (Hunskaar *et al*., 1985)

Antipyretic activity

Antipyretic activity of drug was measured by slightly modifying the method described by Adams *et al*. (1968). Male Wister rats were fasted overnight with water *ad lib* before the experiments. Pyrexia was induced by subcutaneously injecting 20% (W/V) brewer's yeast suspension (10 ml/kg) into the animal's dorsum region. Seventeen hours after the injection, the rectal temperature of each rat was measured using a digital thermometer (SK-1250 MC, Sato Keiryoki Mfg. Co., Ltd., Japan). Only rats that showed an increase in temperature of at least 0.7°C were used for experiments. Test agent or cosolvent was administered orally and the temperature was measured at 1, 2, 3, 4, and 5 hr after drug administration.

Statistical analysis

Data are expressed as mean ± SEM and were analyzed statistically by one-way ANOVA procedures, followed by using Dunnett's test. A difference was considered significant at $p<0.05$.

Results

Effect of the ethanol and aqueous extract of *D. membranacea* and aspirin on carrageenin-induced paw edema in rats.

Oral administration of the ethanol extract at the dose of 1600 mg/kg significantly suppressed the paw edema at 2 and 3 hr after carrageenin injection in rats. The aqueous extract (1600 mg/kg) also significantly decreased the carrageenin-induced paw edema at 2, 3, 4 and 5 hr after carrageenin injection in rats. Aspirin (200 mg/kg), the standard control, also reduced paw edema in this test (Table 1).

Effect of the ethanol and aqueous extracts of *D. membranacea* and aspirin on acetic acid-induced writhing in mice.

Neither the ethanol nor aqueous extract of *D. membranacea* rhizome affected the numbers of writhings and stretchings induced by intraperitoneal 0.6% acetic acid after oral administration (400, 800, 1600 mg/kg) in mice. However, the reference drug aspirin (200 mg/kg) produced significant protective effects towards the acetic acid induced pain (Table 2).

Effect of the ethanol extract of *D. membranacea*, aspirin and morphine on hindpaw licking in the formalin test in mice.

The ethanol extract of *D. membranacea* rhizome (400, 800 and 1600 mg/kg, p.o.) had no significant suppression of the licking activity in both phases of the formalin-induced pain in mice. Aspirin (200 mg/kg) reduced the licking activity only in the late phase. In contrast, the reference antinociceptive drug morphine sulfate (10 mg/kg,

Table 1. Effect of the ethanol and aqueous extract of *D. membranacea* and aspirin on carrageenin-induced paw edema in rats.

Drug	Dose (mg/kg, p.o.)	Paw volume (ml)					
		0 hr	1 hr	2 hr	3 hr	4 hr	5 hr
Cosolvent	-	3.93±0.16	4.81±0.17	5.26±0.16	6.06±0.15	6.67±0.14	6.82±0.15
Aspirin	200	3.55±0.07	3.92±0.12*	3.99±0.12*	4.10±0.14*	4.32±0.10*	4.59±0.08*
D. membranacea	400	3.77±0.14	4.45±0.27	5.01±0.25	5.98±0.25	6.29±0.27	6.34±0.28
(ethanol)	800	4.03±0.12	4.70±0.30	5.49±0.27	5.91±0.33	6.43±0.18	6.55±0.19
Cosolvent	-	4.38±0.17	5.51±0.24	6.89±0.24	7.59±0.13	8.27±0.15	8.19±0.17
Aspirin	200	4.57±0.10	5.04±0.18	5.29±0.22*	5.36±0.28*	5.64±0.28*	5.81±0.25*
D. membranacea (ethanol)	1600	4.60±0.07	5.35±0.17	6.10±0.25*	6.53±0.35*	7.24±0.42	7.50±0.51
Cosolvent	-	4.49±0.13	5.57±0.18	6.22±0.14	6.94±0.26	7.52±0.25	7.77±0.20
Aspirin	200	4.60±0.07	4.96±0.13*	5.01±0.13*	5.21±0.15*	5.63±0.08*	5.76±0.08*
D. membranacea	400	4.46±0.10	5.43±0.25	6.12±0.29	6.52±0.21	7.27±0.32	7.50±0.40
(aqueous)	800	4.33±0.06	5.31±0.19	6.05±0.18	6.77±0.21	7.28±0.21	7.59±0.20
	1600	4.49±0.19	5.12±0.17	5.53±0.19*	5.89±0.30*	6.20±0.37*	6.29±0.47*

The initial hind paw volume of the rat was determined volumetrically. Thirty min after test agent administration (p.o.), 1% carrageenin in saline was subcutaneously injected in a volume of 0.1 ml into the right hind paw at time 0 and the paw volume was measured at 1 hr intervals for 5 hr. Each point represents the mean±S.E.M of 6 rats. *p<0.05, compared with the control group (Dunnett's test).

Table 2. Effect of the ethanol and aqueous extracts of *D. membranacea* and aspirin on acetic acid-induced writhing in mice.

Drug	Dose (mg/kg p.o.)	No. of writhing (counts/20 min)
Cosolvent	-	57.8±4.0
Aspirin	200	15.3±3.7*
D. membranacea	400	61.3±4.5
(ethanol)	800	58.3±3.6
	1600	57.5±5.0
Cosolvent	-	59.5±5.5
Aspirin	200	28.4±4.2*
D. membranacea	400	60.6±6.8
(aqueous)	800	59.2±4.5
	1600	72.7±5.4

The ethanol extract and aqueous extract of *D. membranacea* were orally administered. After 30 min, 0.6% acetic acid solution (10 ml/kg) was intraperitoneally injected in mice. Immediately after injection, the number of writhings was counted over a 20-min period. Each datum represents the mean ± S.E.M. of 10 mice. * p< 0.05 compared with the control group (Dunnett's test)

s.c.) significantly inhibited the licking activity in both phases of formalin-induced nociception (Table 3).

Effect of the ethanol extract of *D. membranacea*, aspirin and morphine on nociceptive response induced by heat in mice.

The mean latency of nociceptive responses to thermal stimuli is summarized in Table 4. Neither the ethanol extract of *D. membranacea* rhizome (400, 800 and 1600 mg/kg, p.o.) nor aspirin (200 mg/kg, p.o.) significantly exerted protective effects on heat-induced pain in mice. By contrast, a centrally acting analgesic drug, morphine sulfate (10 mg/kg, s.c.) markedly increased pain latency.

Effect of the ethanol and aqueous extracts of *D. membranacea* and aspirin on brewer's yeast-induced fever in rats.

The ethanol and aqueous extracts of *D. membranacea* rhizome (400, 800 and 1600 mg/kg, p.o.) did not show any significantly effect on

Table 3. Effect of the ethanol extract of *D. membranacea*, aspirin and morphine on hindpaw licking in the formalin test in mice.

Drug	Dose (mg/kg, p.o.)	Early Phase (sec)	Late Phase (sec)
Cosolvent	-	71.6±5.7	89.5±9.5
Aspirin	200	57.6±4.8	34.7±8.6*
Morphine	10	11.5±2.2**	0.0±0.0**
D. membranacea	400	63.9±6.2	82.3±11.0
(ethanol)	800	73.1±6.9	67.3±7.5
	1600	68.4±8.0	66.5±9.6

Thirty min after test drug administration (p.o.), 2.5% formalin was subcutaneously injected to a hindpaw in a volume of 20 µl. Each datum represents the mean licking time ± S.E.M. from 10 mice in the early phase (0-5 min) and the late phase (15-30 min) after formalin injection. *p<0.05, **p< 0.01 compared with the control group (Dunnett's test)

Table 4. Effect of the ethanol extract of *D. membranacea*, aspirin and morphine on nociceptive response induced by heat in mice.

Drug	Dose (mg/kg, p.o.)	Latency of nociceptive response (sec)			
		15	30	45	60 min
Cosolvent	-	15.7±1.2	13.9±0.7	12.7±0.8	12.0±0.7
Aspirin	200	14.9±2.1	16.5±1.7	14.1±1.3	12.6±0.7
Morphine	10	28.5±1.7*	29.0±2.8*	29.8±1.4*	28.5±1.5*
D. membranacea	400	16.2±1.7	14.3±1.6	12.5±0.6	12.7±0.9
(ethanol)	800	15.4±2.0	16.2±1.5	12.7±1.5	10.6±0.8
	1600	15.3±1.2	13.3±1.6	12.6±1.2	11.4±0.5

Beginning 30 min after oral administration of test agents (or 15 min after morphine injection, s.c.), the nociceptive response was measured every 15 min over a 60-min period. Each datum represents the latency of nociceptive responses (sec) ± S.E.M. (n=10) *p< 0.05 compared with the control group (Dunnett's test)

yeast-induced fever in rats while the reference drug aspirin suppressed fever induced by yeast in rats (Table 5).

Discussion

The results demonstrate that the ethanol and aqueous extracts obtained from *D. membranacea* rhizome at the dose of 1600 mg/kg exhibited anti-inflammatory activity by significant suppression of the paw edema induced by carrageenin in rats but they were less potent than aspirin (200 mg/kg). It has been reported that the methanol extract of *Dioscorea tokoro* rhizome reduced the pro-inflammatory cytokines and mediators in the synoviocytes of rheumatoid arthritis (Kim *et al*., 2004). As the carrageenin-induced paw edema model is used for evaluation of anti-inflammatory activity of the compounds involving several chemical mediators such as prostaglandins, serotonin, histamine and bradykinin (Vinegar *et al*., 1987), it is possible that some active compounds e.g., dioscorealides or dioscoreanone, isolated from *D. membranacea* rhizome (Itharat *et al*., 2003) and an inhibition of some inflammatory mediators may be involved in this activity. Nevertheless, further experiments are needed to elucidate its anti-inflammatory action.

The writhing test is generally used for screening of antinociceptive effects (Koster *et al*., 1959; Hendershot and Forsaith, 1959). Unfortunately, the ethanol and aqueous extracts of *D. membranacea* rhizome did not show any significant inhibition on acetic acid-induced writhing response while the reference drug aspirin (200 mg/kg) produced significant protective effects towards the acetic acid-induced pain in mice.

The formalin test is another pain model, which assesses the way an animal responds to moderate continuous pain generated by injured tissue (Tjolsen *et al*., 1992). The effects of drugs on licking responses in the early and late phases reportedly represent antinociceptive action on sensory receptor stimulation and anti-inflammatory action, respectively (Dubuisson and Dennis, 1977; Hunskaar and Hole, 1987). In the present study, the ethanol extract of *D. membranacea* rhizome had no significantly suppression of the licking activity in either phase of the formalin-induced pain in mice. Morphine significantly

Table 5. Effect of the ethanol and aqueous extracts of *D. membranacea* and aspirin on brewer's yeast-induced fever in rats.

Drug	Dose (mg/kg, p.o.)	Average rectal temperature (ºC)					
		0 hr	1 hr	2 hr	3 hr	4 hr	5 hr
Cosolvent	-	38.1±0.2	37.6±0.1	37.6±0.2	37.4±0.2	37.4±0.2	37.3±0.2
Aspirin	200	38.2±0.1	37.3±0.2	37.1±0.1	36.8 +0.2*	36.7±0.3*	36.5±0.3*
D. membranacea	400	38.5±0.2	37.9±0.2	37.8±0.2	37.4±0.1	37.3±0.2	37.1±0.2
(ethanol)	800	38.3±0.2	37.8±0.2	37.5±0.2	37.5±0.1	37.4±0.2	37.2±0.2
	1600	38.0±0.1	37.6±0.2	37.3±0.2	37.3±0.2	37.0±0.1	37.0±0.2
Cosolvent	-	37.6±0.1	37.5±0.1	37.2±0.1	37.0±0.1	37.0±0.2	37.1±0.2
Aspirin	200	37.6±0.1	36.8±0.1*	36.2±0.1*	36.3±0.1*	36.3±0.1	36.4±0.1*
D. membranacea	400	37.6±0.1	37.2±0.2	36.7±0.1	36.8±0.1	36.6±0.1	36.8±0.1
(aqueous)	800	37.8±0.1	37.5±0.1	37.0±0.2	37.2±0.2	37.1±0.3	37.3±0.2
	1600	37.5±0.1	37.2±0.1	36.8±0.2	36.9±0.2	36.7±0.2	36.9±0.1

Twenty percent of yeast suspension was subcutaneously injected into the dorsum region of rats. Seventeen hours after injection, rectal temperature was measured (time 0) and then drugs were orally administered. The temperature was again measured at 1, 2, 3, 4 and 5 hr after drug administration. Each datum represents the mean rectal temperature (ºC) ± S.E.M. (n = 6) *p<0.05, compared with the control group (Dunnett'ss test).

reduced the licking activity in both phases while aspirin decreased the licking activity only in the late phase.

Thermic painful stimuli are known to be selective to centrally but not peripherally acting analgesic drugs (Chau, 1989). In the present study, morphine, a centrally acting analgesic drug, produced an inhibitory effect on the nociceptive response in this test, while the ethanol extract of *D. membranacea* rhizome and aspirin failed to affect the responses.

Neither the ethanol nor aqueous extract of *D. membranacea* rhizome showed any significant effect on yeast-induced fever in rats while the reference drug aspirin suppressed fever induced by yeast in rats by inhibiting the synthesis of prostaglandin E2 (Dascombe, 1985; Vane, 1987).

The ethanol and aqueous extract of *D. membranacea* rhizome suppressed the paw edema induced by carrageenin in rats but had no significant effect on nociceptive response induced by chemicals or heat in mice and fever induced by yeast in rats, compared with aspirin, a nonsteroidal anti-inflammatory drug, which possesses analgesic, antipyretic and anti-inflammatory activities by inhibition of prostaglandin synthesis via cyclo-oxygenase activity (Vane, 1987). Thus, the anti-inflammatory action of the extracts from *D. membranacea* rhizome may act at some site(s) of action or inhibit of some inflammatory mediators that are different from those of aspirin.

Based on these results, we conclude that the ethanol and aqueous extracts of *D. membranacea* rhizome result in an anti-inflammatory activity, and that its actions on inflammation may be have a different mechanism from that of aspirin. However, further studies are needed to clarify the anti-inflammatory mechanisms of the extracts of *D. membranacea* rhizome.

Acknowledgments

The authors are very grateful to Faculty of Pharmaceutical Sciences, Prince of Songkla University, for financial support of this work.

References

Adams, S.S., Hebborn, P. and Nicholson, J.S. 1968. Some aspects of the pharmacology of ibufenac, a non-steroidal anti-inflammatory agent. J. Pharm. Pharmac., 20: 305-312.

Boonyaratanakornkit , L. and Chantarateptavan, V. 1993. Identification and specification of Khao-Yen-Neua and Khao-Yen-Tai. Thai. J. Pharm. Sci., 1: 79-90.

Chau, T. 1989. Pharmacology methods in the control of inflammation. **In**: Modern Methods in Pharmacology, Vol. V, Alan. R. Liss., Inc., New York, pp. 195-212.

Dascombe, M.J. 1985. The pharmacology of fever. Prog. Neurobiol., 25: 327-373.

Dong, M., Feng, X.Z., Wang, B.X., Ikejima, T. and Wu, L.J. 2004. Steroidal saponins from *Dioscorea panthaica* and their cytotoxic activity. Pharmazie., 59: 294-296.

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

Hendershot, L.C. and Forsaith, J. 1959. Antagonism of the frequency of phenylquinone-induced writhing in the mouse by weak analgesics and non-analgesics. J. Pharmacol. Exp. Ther., 125: 237-240.

Hunskaar, S., Fasmer, O.B. and Hole, K. 1985. Formalin test in mice, a useful technique for evaluating mild analgesics. J. Neurosci. Meth., 14: 69-76.

Hunskaar, S. and Hole, K. 1987. The formalin test in mice: dissociation between inflammatory and non-inflammatory pain. Pain 30: 103-114.

Itharat, A., Plubrukarn, A., Kongsaeree, P., Bui, T., Kaewpradub, N. and Houghton, P.J. 2003. Dioscorealides and dioscoreanone, novel cytotoxic naphthofuranoxepins, and 1,4-phenanthraquinone from *Dioscorea membranacea* Pierre. Org. Lett., 5: 2879-2882.

Itharat, A., Houghton, P.J., Eno-Amooquaye, E., Burke, P.J., Sampson, J.H. and Raman, A. 2004. In vitro cytotoxic activity of Thai medicinal plants used traditionally to treat cancer. J. Ethnopharmacol., 90: 33-38.

Kim, M.J., Kim, H.N., Kang, K.S., Baek, N.I., Kim, D.K., Kim, Y.S., Kim, S.H. and Jean, B.H. 2004. Methanol extract of Dioscoreae Rhizoma inhibits pro-inflammatory cytokines and mediators in the synoviocytes of rheumatoid arthritis. Int. Immunopharmacol., 4: 1489-1497.

Koster, R., Anderson, M. and de Beer, E.J. 1959. Acetic acid for analgesic screening. Fed. Proc., 18: 412.

Kwon, C.S., Sohn, H.Y., Kim, S.H., Kim, J.H., Son, K.H., Lee, J.S., Lim, J.K. and Kim, J.S. 2003. Anti-obesity effect of *Dioscorea nipponica* Makino with lipase-inhibitory activity in rodents. Biosci. Biotechnol. Biochem., 67: 1451-1456.

Lee, S.C., Tsai, C.C., Chen, J.C., Lin, J.G., Lin, C.C., Hu, M.L. and Lu, S. 2002a. Effects of "Chinese yam" on hepato-nephrotoxicity of acetaminophen in rats. Acta Pharmacol. Sin., 23: 503-508.

Lee, S.C., Tsai, C.C., Chen, J.C., Lin, C.C., Hu, M.L. and Lu, S. 2002b. The evaluation of reno- and hepatoprotective effects of huai-shan-yao (Rhizome Dioscoreae). Am. J. Chin. Med., 30: 609-616.

Liu, H., Kiong, Z., Li, F., Qu, G., Kobayashi, H. and Yao, X. 2003. Two new pregnane glycosides from *Dioscorea futschauensis* R. KUNTH. Chem. Pharm. Bull. (Tokyo), 51: 1089-1091.

Ma, H.Y., Zhao, Z.T., Wang, L.J., Wang, Y., Zhou, Q.L. and Wang, B.X. 2002. Comparative study on anti-hypercholesterolemia activity of diosgenin and total saponin of *Dioscorea panthaica*. Zhongguo Zhong Yao Za Zhi, 27: 528-531.

Sautour, M., Mitaine-Offer, A.C., Miyamoto, T., Dongmo, A. and Lacaille-Dubois, M.A. 2004. Antifungal steroid saponins from *Dioscorea cayenensis*. Planta Med., 70: 90-92.

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

Vane, J. 1987. The evolution of non-steroidal anti-inflammatory drugs and their mechanisms of action. Drugs, 33 Suppl 1: 18-27.

Vinegar, R., Truax, J.F., Selph, J.L., Johnston, P.R., Venable, A.L. and McKenzie, K.K. 1987. Pathway to carrageenan-induced inflammation in the hind limb of the rat. Fed. Proc., 46: 118-126.

Wang, S.L., Cai, B., Cui, C.B., Liu, H.W., Wu, C.F. and Yao, X.S. 2004. Diosgenin-3-O-alpha-L-rhamnopyranosyl-(1-->4)-beta-D-glucopyranoside obtained as a new anticancer agent from *Dioscorea futschauensis* induces apoptosis on human colon carcinoma HCT-15 cells via mitochondria-controlled apoptotic pathway. J. Asian Nat. Prod. Res., 6: 115-125.

Winter, C.A. Risley, E.A. and Nuss, G.W. 1962. Carrageenin-induced edema in hind paw of the rat as an assay for anti-inflammatory drugs. Proc. Soc. Exp. Biol. Med., 111: 544-547.

Woolfe, G. and MacDonald, A.D. 1944. The evaluation of the analgesic action of pethidine hydrochloride (Demerol). J. Pharmacol. Exp. Ther., 80: 300-330.

Yin, J., Kouda. K., Tezuka, Y., Tran, Q.L., Miyahara, T., Chen, Y. and Kadota, S. 2003. Steroidal glycosides from the rhizomes of *Dioscorea spongiosa*. J. Nat. Prod., 66: 646-650.

Yin, J., Kouda. K., Tezuka, Y., Le Tran, Q.L., Miyahara, T., Chen, Y. and Kadota, S. 2004a. New diarylheptanoids from the rhizomes of *Dioscorea spongiosa* and their antiosteoporotic activity. Planta Med., 70: 54-58.

Yin, J., Tezuka, Y., Kouda. K., Tran, Q.L., Miyahara, T., Chen, Y. and Kadota, S. 2004b. Antiosteoporotic activity of the water extract of *Dioscorea spongiosa*. Biol. Pharm. Bull., 27: 583-586.