ORIGINAL ARTICLE

Antinociceptive and antipyretic activities of extracts and fractions from *Dracaena loureiri* in experimental animals

Wantana Reanmongkol[1], Sanan Subhadhirasakul[2] and Pisit Bouking[3]

Abstract

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Antinociceptive and antipyretic activities of extracts and fractions from *Dracaena loureiri* in experimental animals
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Dried coarsely powdered material from the stem woods of *Dracaena loureiri* Gagnep (*D loureiri*) has extracted with hexane and methanol to give hexane and methanol extracts, respectively. The methanol extract was roughly separated into four fractions. They were methanol, methanol + water, chloroform and ethyl acetate fractions. The effects of the methanol extract, hexane extract, methanol fraction, methanol + water fraction, ethyl acetate fraction and chloroform fraction on nociceptive response using writhing, hot plate and formalin tests in mice and the antipyretic activity in yeast-induced fever in rats, were examined. General behavior was also examined using pentobarbital-induced sleep in mice. The LD_{50} value of intraperitoneally injected the methanol extract, hexane extract, methanol fraction, ethyl acetate fraction and chloroform fraction in mice was 1.67 g/kg, >7 g/kg, 739.73 mg/kg, 489.77 mg/kg and 1.67 g/kg, respectively. Oral administration of the methanol extract and methanol fraction of *D. loureiri* (100-400 mg/kg) dose de-

[1]Ph.D.(Pharmacology), Assoc. Prof., Department of Clinical Pharmacy, [2]Ph.D.(Chemistry of Natural Products), Assoc. Prof., Department of Pharmacognosy and Pharmaceutical Botany, [3]Technician, Department of Clinical Pharmacy, Faculty of Pharmaceutical Sciences, Prince of Songkla University, Hat Yai, Songkhla 90112 Thailand.
Corresponding e-mail: rwantana@ratree.psu.ac.th
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pendently decreased the number of writhings and stretchings induced by acetic acid and licking activity of the late phase in the formalin test. All extracts or fractions of *D. loureiri* had no effects on heat-induced pain in mice. Only the methanol fraction of *D. loureiri* suppressed yeast-induced fever in rats. Neither extracts nor fractions affected paw edema induced by carrageenin in rats. The methanol extract of *D. loureiri* (100-400 mg/kg, p.o.) prolonged the duration of pentobarbital-induced sleep in mice. These results suggest that the methanol extract and the methanol fraction of *D. loureiri* possess analgesic effect. Only the methanol fraction of the extract exhibited antipyretic effect.

Key words : *Dracaena loureiri*, extract, antinociceptive, antipyretic

บทคัดย่อ

วันทนา เหรียญมงคล[1] สนั่น ศุภธีรสกุล[2] และ พิศิษฐ์ บัวกิ่ง[1]

ผลของสารสกัดจากแก่นจันทน์แดงต่อการแก้ปวดและแก้ไข้ในหนูทดลอง

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ผงบดหยาบจากจันทน์แดงนำมาสกัดด้วยเฮกเซนและเมทานอลได้สารสกัดด้วยเฮกเซนและสารสกัดด้วยเมทานอล สารสกัดด้วยเมทานอลนำมาแยกอย่างหยาบออกเป็นสี่ส่วนคือ ส่วนที่แยกด้วยเมทานอล ส่วนที่แยกด้วยเมทานอลกับน้ำ ส่วนที่แยกด้วยเอทิลอะซีเตท และส่วนที่แยกด้วยคลอโรฟอร์ม ทำการทดสอบผลทางเภสัชวิทยาของสารสกัดด้วยเมทานอล สารสกัดด้วยเฮกเซน ส่วนที่แยกด้วยเมทานอล ส่วนที่แยกด้วยเมทานอลกับน้ำ ส่วนที่แยกด้วยเอทิลอะซีเตท และส่วนที่แยกด้วยคลอโรฟอร์มในหนูทดลอง โดยสังเกตผลของสารสกัดต่อการระงับปวดซึ่งเกิดจากกรดอะเซติก ความร้อน และฟอร์มาลินในหนูถีบจักร และผลต่อการลดไข้ซึ่งเกิดจากการเหนี่ยวนำโดยยีสต์ในหนูขาว ยังได้สังเกตผลต่อพฤติกรรมทั่วไปโดยใช้การเหนี่ยวนำให้หลับด้วยเพ็นโทบาร์บิตาลในหนูถีบจักร ขนาดของสารสกัดด้วยเมทานอล สารสกัดด้วยเฮกเซน ส่วนที่แยกด้วยเมทานอล ส่วนที่แยกด้วยเอทิลอะซีเตท และส่วนที่แยกด้วยคลอโรฟอร์ม ที่ทำให้หนูถีบจักรตาย 50% มีค่า 1.67 ก./กก., มากกว่า 7 ก./กก., 739.73 มก./กก., 489.77 มก./กก. และ 1.67 ก./กก. ตามลำดับ เมื่อฉีดเข้าทางหน้าท้องในหนูถีบจักร เมื่อป้อนสารสกัดด้วยเมทานอลและส่วนที่แยกด้วยเมทานอลจากจันทน์แดง (100-400 มก./กก.) เข้าทางปากในหนูทดลอง พบว่า สามารถลดจำนวนของการบิดและยืดของลำตัวเมื่อถูกกระตุ้นโดยกรดอะเซติก และลดการเลียในช่วงเฟสหลังของการทดสอบด้วยฟอร์มาลินในหนูถีบจักร ผลของสารสกัดด้วยเมทานอล และส่วนที่แยกด้วยเมทานอล จะเพิ่มขึ้นตามขนาดของสารสกัดที่ใช้ ไม่มีชั้นใดของสารสกัดจากจันทน์แดงที่มีผลต่อการทดสอบด้วยความร้อนในหนูถีบจักร เฉพาะส่วนที่แยกด้วยเมทานอลเท่านั้นที่มีฤทธิ์ลดไข้ซึ่งเกิดจากการเหนี่ยวนำด้วยยีสต์ในหนูขาว ไม่มีชั้นใดของสารสกัดจากจันทน์แดงที่มีผลลดการบวมที่อุ้งเท้าซึ่งเกิดจากการเหนี่ยวนำด้วยคาร์ราจีนินในหนูขาว สารสกัดด้วยเมทานอล (100-400 มก./กก.) เสริมฤทธิ์การหลับของเพ็นโทบาร์บิตาลให้ยาวนานขึ้น จากผลการทดลองนี้เสนอว่า สารสกัดด้วยเมทานอล และส่วนที่แยกด้วยเมทานอลจากจันทน์แดง มีฤทธิ์แก้ปวด เฉพาะส่วนที่แยกด้วยเมทานอลที่มีฤทธิ์ลดไข้

[1]ภาควิชาเภสัชกรรมคลินิก [2]ภาควิชาเภสัชเวทและเภสัชพฤกษศาสตร์ คณะเภสัชศาสตร์ มหาวิทยาลัยสงขลานครินทร์ อำเภอหาดใหญ่ จังหวัดสงขลา 90112

Dracaena loureiri Gagnep (*D. loureiri*) is known in Thai as Chan-Daeng or Chan-Pha or Lakka chan in the family Agavaceae. It is a shrub or slender much-branched tree (Garder *et al.*, 2000). When this plant becomes old, it has a red core in the stem and then the stem gradually decays until all cores become red, this core wood is called Chan-Daeng. Most of the *D. loureiri* plant grows in the high moutains. It can be found in any parts of Thailand (Thai Traditional Medicine Association, 1964; Pongbunrod, 1979). *D. loureiri* has been used as a folk medicine e.g., antipyretic, anti-inflamma-

tory and for pain relief (Thai Traditional Medicine Association, 1964; Pongbunrod, 1979). The root of *D. loureiri* has been used as antidiarrhea (Perry, 1980). Some chemical constituents and biological activities from the stems of *D. loureiri* have been reported (Meksuriyen & Cordell, 1988a). Fifteen flavonoid derivatives have been isolated from the chloroform fraction, they were (7S, 12b*R*)-10-hydroxy-11-methoxy-dracaenone; (7*R*, 12b*R*)-7, 10-dihydroxy-11-methoxy-dracaenone; (3*S*)-7,4'-dihydroxy-3-(4-hydroxybenzyl)-chromane; loureirin A, B, C and D; 7,4'-dihydroxyflavone; (2*S*)-7-hydroxyflavanone; (2*S*)-pinocembrin; (2*S*)-7,4'-dihydroxy-5-methoxyflavone; 4,4'-dihydroxy-2'-methoxychalcone; (2*R*)-7,4'-dihydroxyflavan; (2*R*)-4'-hydroxy-7-methoxyflavan and (3*R*)-eucomol. Among the fifteen isolated compounds, (3*S*)-7,4'-dihydroxy-3-(4-hydroxybenzyl)-chromane, loureirin D and (2*S*)-pinocembrin showed antibacterial activity against *S. aureus* and *B. subtilis*. The cytotoxic activity of all these compounds was also tested and only 4,4'-dihydroxy-2'-methoxychalcone could be considered active. Racemic and natural laevorotatory 7,4'-dihydroxyflavan showed fungitoxicity activity in TLC bioassay against *Botrytis cinerea* and *Cladosporum herbarum*. Retrodihydrochalcones from the leaves of *D. loureiri*, have also been isolated (Meksuriyen & Cordell, 1988b). Furthermore it has been reported that retrodihydrochalcones and homoisoflavones, isolated from the stem wood of *D. loureiri* extract, possessed estrogen agonist activity (Meksuriyen & Cordell, 1988b; Ichikawa, *et al*., 1997). Recently, stilbenoids, isolated from the stem wood of *D. loureiri*, were reported to have potent inhibitory activity against COX-1 and COX-2 enzymes but non-selective activity (Likhitwitayawuid, *et al*., 2002).

Although *D. loureiri* has been used for a long time as herbal medicine in Thailand, no pharmacological studies *in vivo* have previously been conducted on analgesic and antiinflammatory actions of this plant. In the present study, in order to evaluate the potential existence of analgesic and antiinflammatory activities of the extract obtained from *D. loureiri*, we investigated the antinociceptive effects of the extract using the writhing, hot plate and formalin tests in mice, and its anti-inflammatory activity in carrageenin-induced paw edema in rats. Furthermore, we also investigated the antipyretic activity of *D. loureiri* in yeast-induced fever in rats, although the antipyretic action of *D. loureiri* has been reported (Wasuwat, 1967). In addition, we also studied the general behavior using pentobarbital-induced sleep in mice.

Material and Methods

Plant material

The stem wood (Chan-Daeng) or crude drugs of *Dracaena loureiri* Gagnep. (Agavaceae) were collected and purchased from herbal drugstores in Songkhla and Satun Provinces, Thailand. The crude drugs were identified by direct comparison with authentic specimens in the Department of Pharmacognosy and Pharmaceutical Botany, Faculty of Pharmaceutical Sciences, Prince of Songkla University. Voucher specimens of crude drugs have been deposited in the same place.

Preparation of the extract from the stems of *Dracaena loureiri*

The dried coarsely powdered wood of *D. loureiri* (1.98 kg) were macerated with 10.0 L of n-hexane for five days and then filtered and evaporated to give a syrupy mass. The marc was remacerated with n-hexane (10.0 L) four times, filtered and evaporated. All syrupy masses were combined to give 21.96 g n-hexane extract. The marc was dried in open air and then was macerated with methanol using the same procedure as described above to give 417.87 g methanolic extract.

Fractionation of crude extract

A 10.0 g portion of crude methanolic extract was fractionated using silica gel (Merck, Germany; SiO_2 230-400 mesh ASTM) column chromatography. The column was eluted with chloroform until the eluate was pale or no clear major spot was detected on TLC. The eluting solvent was changed to ethyl acetate and the column eluted until no clear major spot was detected on TLC,

then, the eluting solvent changed to methanol and 30% water in methanol. By repeating the above procedure, the crude methanolic extract was roughly separated into four main fractions, chloroform, ethyl acetate, methanol and 30% water in methanol fractions. The methanolic crude extract, 102.86 g, gave chloroform, ethyl acetate, methanol and 30% water in methanol fractions of 15.94, 36.55, 25.96 and 1.88 g, respectively. All doses were expressed in terms of each fraction of dried crude extract (mg/kg body weight).

Animals

All animals used in this study were obtained from the Animal House, Faculty of Sciences, Prince of Songkla University, Hat Yai, Songkhla, Thailand. Male Swiss mice with the weight ranging from 28-38 g were used for all experiments except for yeast-induced fever test, in which male Wistar rats with the weight ranging from 140-220 g were used. The rats were handled for 5-10 min daily for several days before experiments. 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 procedures described were reviewed and approved by the Institutional Committee for Ethical Use of Animals.

Acute toxicity

The 50% lethal dose of each fraction of the *D. loureiri* extract was estimated by the up-and-down method in mice (Bruce, 1985). Doses were adjusted by a constant multiplicative factor; viz. 1.5, for this experiment. The dose for each successive animal was adjusted up or down depending on the previous outcome.

Antinociceptive Activity

1. Writhing test

Writhing behaviour 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 of each fraction (100, 200 and 400 mg/kg), a reference analgesic drug, aspirin (200 mg/kg), or cosolvent vehicle was orally administered 30 min before acetic acid.

2. Hot plate test

The hot plate test was carried out according to the method described by Woolfe & MacDonald (1944). Mice were placed on a hot plate maintained at 55ºC ± 1ºC. Latency of nociceptive response such as licking, flicking of a hind limb or jumping was measured. Starting thirty minutes after p.o. 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 administration of the *D. loureiri* extract of each fraction (100, 200 and 400 mg/kg, p.o.), aspirin (200 mg/kg, p.o.) or cosolvent except morphine (15 min after administration), 20 µl of 2.5% formalin in saline was injected subcutaneously to a hindpaw 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 actvity of drug was measured by slightly modifying the method described by Adams *et al*. (1968). Male Wistar 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 animals' dorsum region. Seventeen hours after the injection, the rectal temperature of each rat was measured using a digital thermometer (SK-1250MC, Sato Keiryoki Mfg. Co., Ltd., Japan). Only rats that showed an increase in temperature

of at least 0.7ºC were used for the experiments. Test agent or cosolvent vehicle was administered orally and the temperature was measured at 1, 2, 3, 4 and 5 hr after drug administration.

Carrageenin-induced paw edema

According to the method described by Winnter *et al*. (1962), the initial right hindpaw 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 right hind paw was measured at 0.5, 1, 2, 3, 4, and 5 hr after carrageenin injection, and the edema volume was determined. The data were expressed as percentage swelling, compared with the initial hindpaw volume of each rat. Co-solvent, each fraction of *D. loureiri* extract or aspirin was orally administered 30 min before carrageenin injection.

Pentobarbital-induced sleep

Pentobarbital (50 mg/kg) was injected intraperitoneally to mice. The duration of sleep was measured as the period between the loss and the recovery of the righting reflex. Each fraction of the extract of *D. loureiri* (100, 200 and 400 mg/kg), and cosolvent vehicle were administered orally 30 min before pentobarbital (Ferrini *et al*., 1974).

Chemicals

The following drugs were used: morphine sulfate, brewer's yeast, carrageenin lambda (AR grade, Sigma Chem. Co., St. Louis, U.S.A.), aspirin (AR grade, Srichand United Dispensary Co., Ltd., Bangkok, Thailand), sodium chloride (AR grade, Carlo Erba, Germanny), acetic acid (AR grade, J.T, Baker Inc., Phillipsburg, U.S.A.), silica gel (SiO_2 230-400 mesh, ASTM, Merck KGaA, Germany), n-hexane, chloroform, methanol and ethyl acetate (AR grade, Merck KGaA, Germany). *D. loureiri* extract of each fraction and aspirin were dissolved in cosolvent solution (propylene glycol : ethanol : tween 80 : water = 4:1:1:4), and administered orally in a constant volume (10 ml/kg for mice and 5 ml/kg for rats) 30 min before the experiments. Morphine sulfate was dissolved in 0.9% sodium chloride solution and administered subcutaneously. All drug solutions were prepared immediately before starting the experiments.

Statistical Analysis

Data are expressed as means ± SEM and were analyzed statistically using unpaired Student's t-test. A difference was considered significant at $p<0.05$.

Results

Acute toxicity

In the acute toxicity test, signs of toxicity included muscle weakness, lethargy, loss of righting reflex and death. The LD_{50} value of intraperitoneally injected *D. loureiri* extract of each fraction in mice was as follows:

Methanol extract = 1.67 g/kg

n-Hexane extract: > 7 g/kg, no lethal within 7 days

Methanol fraction = 739.73 mg/kg

Methanol + water fraction: not enough to test

Ethyl acetate fraction = 489.77 mg/kg

Chloroform fraction = 1.67 g/kg

Effects of *D. loureiri* on nociceptive responses

Writhing test

Oral administration of the methanol extract (Figure 1A) and the methanol fraction (Figure 1B) of *D. loureiri* (100-400 mg/kg) dose dependently attenuated the number of writhings and stretchings induced by intraperitoneal 0.6% acetic acid. There were no significant effects in the n-hexane extract, methanol + water fraction, ethyl acetate fraction and chloroform fraction of *D. loureiri* (data not shown). The reference drug aspirin (200 mg/kg) also produced significant protective effects towards the acetic acid-induced pain.

Hot plate test

Neither the *D. loureiri* extract of each extract or fraction (100, 200 and 400 mg/kg, p.o.)

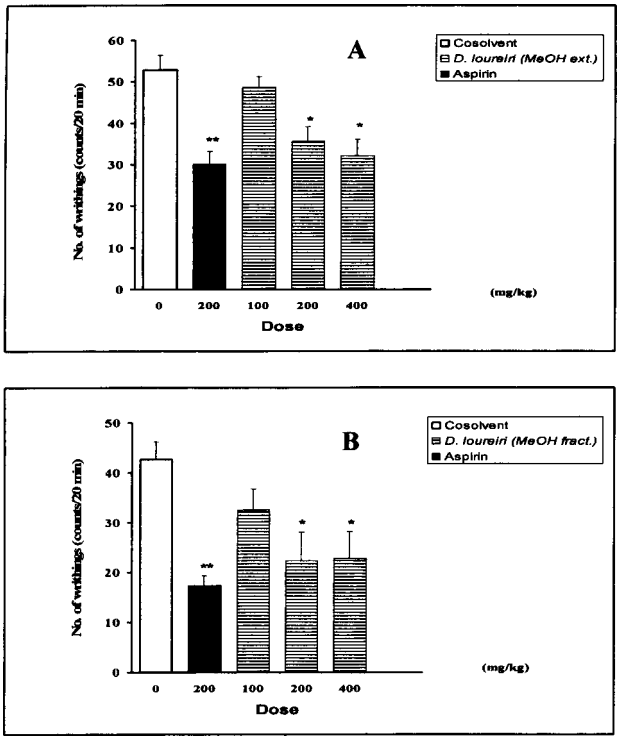

Figure 1. Effect of *D. loureiri* (methanol extract, A; methanol fraction, B) and aspirin on acetic acid-induced writhing in mice.
Each column represents the mean ± S.E.M. (n=10)
*** p<0.05, ** p<0.01 compared to the control group (Student's t-test)**

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 (data not shown).

Formalin test

The methanol extract, methanol fraction and ethyl acetate fraction of *D. loureiri* reduced the licking activity only in the late phase but not in the early phase (Table 1). Aspirin (200 mg/kg) also produced similar effects on formalin-induced pain. The n-hexane extract, methanol + water fraction and chloroform fraction had no effects on both phases in this test (data not shown). In contrast, the reference antinociceptive drug morphine sulfate (10 mg/kg, s.c.) significantly reduced the licking activity against both phases of formalin-induced nociception.

Effect of *D. loureiri* on yeast-induced fever in rats

Only the methanol fraction (Table 2) of *D. loureiri* suppressed yeast-induced fever. A reference drug aspirin also reversed yeast-induced fever. However, the other fractions (data not shown) of the extract had no significant effects on pyrexia induced by yeast .

Effect of *D. loureiri* on carrageenin-induced paw edema in rats

Neither extracts nor fractions (data not shown) of *D. loureiri* affected paw edema induced by carrageenin in rats while aspirin (200 mg/kg) significantly reduced the carrageenin-induced paw edema.

Table 1. Effect of the methanol extract, methanol fraction and ethyl acetate fraction of *D. loureiri*, aspirin and morphine on hind-paw licking in the formalin test in mice.

Drug	Dose (mg/kg, p.o.)	Early Phase (sec)	Late Phase (sec)
Cosolvent	-	75.9±7.5	164.2±22.4
Aspirin	200	70.2±9.1	99.9±28.7*
Morphine	10	23.2±5.1***	0.0±0.0***
D. loureiri	100	64.3±10.7	78.5±20.0*
(methanol extract)	200	77.0±10.8	72.0±19.5*
	400	85.2±9.3	55.1±18.5**
Cosolvent	-	65.7±11.3	80.9±15.3
Aspirin	200	49.4±4.7	27.4±9.9*
Morphine	10	2.8±1.1**	0.0±0.0**
D. loureiri	100	66.7±14.4	58.4±8.9
(methanol fraction)	200	69.0±10.8	42.7±7.8
	400	60.4±9.2	29.1±7.0*
Cosolvent	-	52.5±8.0	129.7±19.8
Aspirin	200	35.0±4.7	29.9±12.0*
Morphine	10	2.8±1.1**	0.0±0.0**
D. loureiri	100	52.1±7.1	111.7±11.5
(ethyl acetate fraction)	200	63.3±8.7	68.3±8.0*
	400	40.2±4.8	45.8±13.7*

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, ***p<0.001 compared with the control group (Student's t-test).

Table 2. Effect of the methanol fraction of *D. loureiri* extract and aspirin on brewer's yeast-induced fever in rats.

Drug	Dose (mg/kg, p.o.)	Average rectal temperature (ºC)					
		0	1hr	2hr	3hr	4hr	5hr
Cosolvent	-	38.0±0.2	37.4±0.2	37.7±0.2	37.6±0.3	37.4±0.3	37.3±0.3
D. loureiri	100	38.0±0.2	37.1±0.1	36.9±0.1*	36.9±0.2	37.1±0.2	37.1±0.1
(methanol fraction)	200	37.4±0.3	36.7±0.2*	36.7±0.2*	36.5±0.2*	36.6±0.2	36.9±0.3
	400	37.6±0.2	36.9±0.2	36.4±0.3*	36.5±0.3*	36.5±0.3	36.6±0.3
Aspirin	200	38.0±0.1	36.6±0.2*	36.3±0.4*	36.8±0.2*	36.9±0.2	37.1±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 (Student's t-test).

Effect of *D. loureiri* on pentobarbital-induced sleep in mice

Only the methanol extract (Table 3) of *D. loureiri* dose-dependently (100-400 mg/kg, p.o.) prolonged the duration of pentobarbital-induced sleep while the others (data not shown) of the extract had no significant effects on sleep induced by pentobarbital in mice.

Discussion

The results demonstrate that the methanol extract and the methanol fraction obtained from the stem wood of *D. loureiri* attenuated nociceptive responses to chemical stimuli in the acetic acid-induced writhing and in the formalin test in mice. The methanol fraction of *D. loureiri* suppressed yeast-induced fever while the methanol extract had no effect on yeast-induced hyperthermia in rats. It is possible that methanol fraction which was partially purified, possesses a higher amount of active substance(s) than does the methanol extract.

The methanol extract and the methanol fraction of *D. loureiri* exerted protective action in the writhing test similar to the reference peripheral analgesic compound, aspirin. This test is generally used for screening of antinociceptive effect (Koster *et al*., 1959; Hendershot & Forsaith, 1959). Thus the active compound (s) in the methanol extract and the methanol fraction of *D. loureiri* may possess analgesic action.

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 all extracts and fractions of *D. loureiri* failed to affect the response. These findings, therefore, suggest that the apparent antinociceptive action of the active compound(s) in the methanol extract and the methanol fraction of *D. loureiri* may be mediated through peripheral but not central mechanism(s).

The formalin test is another pain model, which assesses the way an animal responds to moderate, continuous pain generated by injured tissue (Tjolsen, 1992). The effects of drugs on the 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). The methanol extract, the methanol fraction and the ethyl acetate fraction of *D. loureiri* produced a dose-related reduction of licking activity only in the late phase but did not affect the responses in the early phase, suggesting the anti-inflammatory action of this extract. However, the ethyl acetate fraction of *D. loureiri* had the effect only on the formalin test but

Table 3. Effect of the methanol extract of *D. loureiri* on pentobarbital-induced sleep in mice.

Drug	Dose (mg/kg, p.o.)	Duration of pentobarbital-induced sleep (min)
Cosolvent	-	64.2±3.8
D. loureiri	100	77.1±3.4**
(methanol extract)	200	80.0±3.1*
	400	101.8±7.1**

The methanol extract of *D. loureiri* was orally administered. After 30 min, pentobarbital (50 mg/kg, i.p.) was injected, and sleeping time was measured. Each datum represents the mean ± S.E.M. (n=10). Each datum represents the mean ± S.E.M. from 10 mice. *p<0.05, **p<0.01 compared with the control group (Student's t-test).

not on writhing model. It is possible that some active compound(s) contained in the ethyl acetate fraction, are different from those of the chemicals obtained in the methanol extract and the methanol fraction of *D. loureiri*.

Only the methanol fraction of *D. loureiri* decreased yeast-induced fever. It is possible that most of the active compound(s) affecting the fever are in the methanol fraction. It is interesting to determine the active constituents in this fraction.

Unfortunately, no extracts or fractions of *D. loureiri* affected paw edema induced by carrageenin in rats. Thus it does not support the indication for use of *D. loureiri* to relieve inflammation in folk medicine.

It seems that a sedative effect of the methanol extract of *D. loureiri* could apparently account for the antinociceptive responses in the tests used in this study. Thus, the sedative effect of the methanol extract of *D. loureiri* on analgesic responses cannot be excluded. However, it is possible that the metabolism or excretion of pentobarbital may be inhibited by the methanol extract of *D. loureiri*.

Our preparation of *D. loureiri* differed somewhat from that of Likhitwitayawuid *et al*. (2002). They used hexane, ethyl acetate and methanol extract from the stem wood of *D. loureiri* for investigation and found that only the ethyl acetate fraction possessed inhibitory activity against COX-1 and COX-2 enzymes. In our method, we obtained the n-hexane and methanol extracts and then the methanol extract was fractionated to chlorofrom, ethyl acetate, methanol and methanol + water fractions. These fractions were tested for activities. In our results, the methanol extract and the methanol fraction of *D. loureiri* showed analgesic activities while the ethyl acetate fraction exerted only a weak effect. These results are not reconcilable; it is possible that there are some processes of extraction that differ from those of Likhitwitayawuid *et al*. (2002) and/or the active compound(s) contained in the ethyl acetate fraction tested *in vivo* is not high enough to show the activity and/ or some compound may counteract that of activity and some active compound(s) preferably contained in the methanol fraction. The variability of sources of crude drugs may also produce variable constituents in the extracts. In addition, there are some differences in *in vivo* and *in vitro* models.

In conclusion, these results suggest that the methanol extract and the methanol fraction of *D. loureiri* possess analgesic effect. Only the methanol fraction of the extract exhibited antipyretic effect.

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