ORIGINAL ARTICLE

Antipyretic activity of the extracts of *Hibiscus sabdariffa* calyces L. in experimental animals

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Abstract

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The effects of the extracts from *Hibiscus sabdariffa* calyces L. (*H. sabdariffa*) on nociceptive response using writhing, hot plate and formalin test in mice and the antipyretic activity in yeast-induced fever in rats, were examined. Anti-inflammatory activity was also investigated on carrageenin-induced paw edema in rats. No acute toxicity was observed in mice after oral administration of the ethanol and aqueous extract of *H. sabdariffa* calyces at the dose of 15 g/kg. Oral administration of the ethanol extract at the dose of 800 mg/kg significantly decreased the number of contortions and stretchings induced by acetic acid in mice. The aqueous extracts had no effect on this test. Neither the ethanol nor aqueous extract had an effect in the formalin and hot plate tests in mice. The ethanol and the vacuum dried extract of *H. sabdariffa* calyces (200-800 mg/kg, p.o.) decreased the yeast-induced fever in rats. The *H. sabdariffa* extract had no effect on carrageenin-induced paw edema in rats. These results suggest that the ethanol and aqueous extract (vacuum dry) of *H. sabdariffa* calyces possess antipyretic action through mechanisms that are different from that of aspirin.

Key words : *Hibiscus sabdariffa* calyces, extract, antipyretic, experimental animal

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บทคัดย่อ

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ฤทธิ์แก้ไข้ของสารสกัดจากดอกกระเจี๊ยบในสัตว์ทดลอง

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ทดสอบผลของสารสกัดจากดอกกระเจี๊ยบในหนูถีบจักรต่อการระงับปวด ซึ่งเกิดจากกรดอะเซติก ความร้อน และฟอร์มาลิน และผลต่อการลดไข้ซึ่งเกิดจากการเหนี่ยวนำโดยยีสต์ นอกจากนี้ยังได้ทดสอบผลต้านการอักเสบซึ่งเกิดจากการกระตุ้นให้เกิดการบวมที่อุ้งเท้าด้วยคาร์ราจินินในหนูขาว ไม่พบความเป็นพิษเฉียบพลันจากการป้อนสารสกัดเอทธานอลและสารสกัดน้ำของดอกกระเจี๊ยบขนาด 15 มก./กก. เมื่อป้อนสารสกัดเอทธานอลขนาด 800 มก./กก. เข้าทางปากในหนูถีบจักร พบว่าสามารถลดจำนวนของการบิดและยืดของลำตัวเมื่อถูกกระตุ้นโดยกรดอะเซติกในหนูถีบจักร ส่วนสารสกัดน้ำไม่มีผลต่อการทดสอบนี้ ทั้งสารสกัดเอทธานอลและสารสกัดน้ำไม่มีผลต่อการทดสอบด้วยฟอร์มาลินและความร้อนในหนูถีบจักร สารสกัดเอทธานอลและสารสกัดน้ำที่ทำให้แห้งด้วยความดันของดอกกระเจี๊ยบ (200-800 มก./กก) มีฤทธิ์ลดไข้ซึ่งเกิดจากการเหนี่ยวนำโดยยีสต์ในหนูขาว สารสกัดของดอกกระเจี๊ยบไม่มีผลต้านการอักเสบซึ่งเกิดจากการกระตุ้นให้เกิดการบวมที่อุ้งเท้าด้วยคาร์ราจินินในหนูขาว จากการทดลองนี้เสนอว่าสารสกัดเอทธานอลและสารสกัดน้ำที่ทำให้แห้งด้วยความดันของดอกกระเจี๊ยบมีฤทธิ์ลดไข้ ซึ่งมีกลไกการออกฤทธิ์ที่ต่างจากการแก้ไข้ของแอสไพริน

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Hibiscus sabdariffa (*H. sabdariffa*) L., family Malvaceae, is known in Thai as Kra-Jeab. It is an annual shrub and commonly used to make beverages. The calyces are used to make beverages and have been used in folk medicines and claimed effective as diuretics, stomachic, aphrodisiac, antiseptic, astringent, cholagogue, digestive, sedative, laxative, antimicrobial or as remedy for pyrexia, abscesses, heart ailments and hypertension (Perry, 1980).

Pharmacological activities of *H. sabdariffa* calyces extract have been studied. It inhibited serum lipids and showed an antiatherosclerotic activity in cholesterol-fed rabbits and rats (el-Saadany *et al*., 1991; Chen *et al*., 2003). In addition, *H. sabdariffa* extract protected human erythrocytes against lipid peroxidation (Suboh *et al*., 2004). It decreased blood pressure in spontaneously hypertensive rats (Onyenekwe *et al*., 1999; Odigie *et al*., 2003) which was mediated through acetylcholine and histamine-like mechanisms and via direct vasorelaxant effects (Adegunloye *et al*., 1996) and in patients with essential hypertension (Faraji and Tarkhani, 1999; Herrera-Arellano *et al*., 2004). Furthermore, it possessed radical scavenging activity, anticytotoxicity induced by tert-butyl hydroperoxide in rat primary hepatocytes and antimutagenic activity against methylazoxymethanol acetate and heterocyclic amines in F344 rats (Duh and Yen, 1997; Tseng *et al*., 1997; Chewonarin *et al*., 1999).

Some active compounds isolated from *H. sabdariffa* calyces and their pharmacological activities have been reported. Protocatechuic acid, a phenolic compound, protected against tert-butyl hydroperoxide and lipopolysaccharide-induced hepatic damage in rats (Tseng *et al*., 1996; Tseng *et al*., 1997; Liu *et al*., 2002; Lin *et al*., 2003). It also inhibited tumor promotion in mouse skin and possessed an apoptosis inducer in human leukemia cells (Tseng *et al*., 1998; Tseng *et al*., 2000). Anthocyanin, red color in roselle possessed antioxidant capacity and reduced liver lesions induced by tert-butyl hydroperoxide and paracetamol in rats (Wang *et al*., 2000; Tsai *et al*., 2002; Ali *et al*., 2003).

Although *H. sabdariffa* has been popularly used as beverage in Thailand and many pharma-

cological activities are reported, only few pharmacological studies on the antipyretic action of this plant have been undertaken despite the claims of its effectiveness in relief of pyrexia in folkloric medicines (Perry, 1980). In the present study, in order to evaluate the potential antipyretic of the extract of *H. sabdariffa* calyces, we investigated the antipyretic activity in an experimental animal model using yeast-induced fever in rats. The analgesic and anti-inflammatory effects of this calyces were also examined using the writhing, hot plate and formalin tests in mice, and in carrageenin-induced paw edema in rats, respectively.

Materials and methods

Plant material

Red calyces of *H. sabdariffa* L. were collected at Khounmeet District in Songkhla Province, Thailand. The plant was identified by direct comparison with herbarium specimens in the PSU Herbarium, Department of Biology, Faculty of Sciences, Prince of Songkla University. A voucher specimen of plant material has been deposited in the Faculty of Pharmaceutical Sciences, Prince of Songkla University, Thailand.

Preparation of the extract from the calyces of *H. sabdariffa*

The dried calyces of *H. sabdariffa* were extracted in hot water (70°C) and then filtered. The filtrates were evaporated by vaccum drying and spray drying under reduced pressure to give the aqueous extracts. In the same way, the dried calyces of *H. sabdariffa* were also extracted with 95% ethanol and then filtrated and evaporated under reduced pressure to give ethanol extract. The aqueous and ethanol extract were used as the test extracts. All doses are expressed in terms of crude extract (mg/kg body weight).

Animals

All animals were obtained from the Animal House Facility Unit, 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 weight ranging from 150 to 210 g. The animals were housed for at least one week in the laboratory animal room prior to study. 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 (Ref no 0521.05/459).

Acute toxicity

The 50% lethal dose (LD50) of the extract of *H. sabdariffa* calyces in mice was estimated by the up and down method (Bruce, 1985). Doses were adjusted up or down by a constant multiplicative factor (1.5) depending on the previous outcome.

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 and Forsaith, 1959). The plant extract (200, 400 and 800 mg/kg), a reference analgesic drug, aspirin (200 mg/kg), or cosolvent 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 & 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 *H. sabdariffa* extract (200, 400 and 800 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 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 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^{o}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.

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, *H. sabdariffa* extract or aspirin was orally administered 30 min before carrageenin injection.

Chemicals

The following drugs were used: morphine sulfate, brewer's yeast, carrageenin lambda, aspirin (AR grade, Sigma Chem. Co., St. Louis, U.S.A.), sodium chloride (AR grade Carlo Erba., Germany), acetic acid (AR grade, J.T, Baker Inc., Phillipsburg, U.S.A.), formalin (Labscan Asia Co., Ltd., Bangkok, Thailand), propylene glycol (Vidhyasom, Bangkok, Thailand), tween 80 (Srichand United Dispensary Co., Ltd., Bangkok, Thailand) and ethanol (AR grade, Merck KGaA, Geramany). The ethanol extract of *H. sabdariffa* and aspirin were dissolved in cosolvent solution (propylene glycol : tween 80 : water = 4:1:4) and the aqueous extract (spray dry, vacuum dry) of *H. sabdariffa* was dissolved in distilled water 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 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

Acute toxicity

In the acute toxicity test, no any toxicity was observed within 7 days after oral administration at the high dose of 15 g/kg ethanol and aqueous extract of *H. sabdariffa* calyces in mice.

Effects of the *H. sabdariffa* extract on nociceptive responses

Writhing test

Oral administration (800 mg/kg) of the ethanol extract of *H. sabdariffa* significantly inhibited the numbers of writhings and stretchings induced by intraperitoneal 0.6% acetic acid. The reference drug aspirin (200 mg/kg) also produced significant protective effects against the acetic acid induced pain. The aqueous extract (spray dried and vacuum dried) of *H. sabdariffa* had no significant effect on the writhing test (Table 1).

Table 1. Effect of extract of *H. sabdariffa* and aspirin on acetic acid induced writhing in mice.

Drug	Dose (mg/kg p.o.)	No. of writhing (counts/20 min)	% inhibition
Cosolvent	-	63.0±4.1	
Aspirin	200	14.7±2.7*	76.7
H. sabdariffa	200	66.5±3.2	-5.6
(ethanol)	400	58.1±6.9	7.8
	800	20.6±2.7*	67.3
Water	-	52.3±5.7	
Aspirin	200	17.2±2.1*	67.1
H. sabdariffa	200	38.7±7.5	26.0
(spray dry)	400	46.3±6.2	11.5
	800	35.0±4.1	33.1
Water	-	49.5±8.4	
Aspirin	200	15.2±2.6*	69.3
H. sabdariffa	200	49.8±3.7	-0.6
(vacuum dry)	400	41.1±4.8	17.0
	800	48.1±4.3	2.8

The extract of *H. sabdariffa* was 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. Values represent mean ± S.E.M., each from 10 mice. * p< 0.05 compared with the control group (Dunnett's test)

Hot plate Test

The mean latency time of nociceptive responses to thermal stimuli is summarized in Table 2. Neither the extract of *H. sabdariffa* (200, 400 and 800 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 time.

Formalin test

Neither the ethanol nor aqueous extract of *H. sabdariffa* calyces (200, 400 and 800 mg/kg, p.o.) significantly suppressed the licking activity in either phase 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, s.c.) significantly inhibited the licking activity against both phases of formalin-induced nociception (Table 3).

Effect of *H. sabdariffa* extract on yeast-induced fever in rats

The ethanol extract (400, 800 mg/kg) of *H. sabdariffa* significantly reversed yeast induced fever at 2, 4 and 5 hr and vacuum dried extract (800 mg/kg) also significantly reduced yeast induced fever at 3, 4 and 5 hr after drug administration in rats while the spray dried aqueous extract had no effect on yeast-induced fever. The reference drug aspirin also suppressed fever induced by yeast in rats (Table 4).

Effect of *H. sabdariffa* extract on carrageenin-induced paw edema in rats

The *H. sabdariffa* extracts (ethanol, spray dry and vacuum dry) had no effect on carrageenin-induced paw edema whereas aspirin (200 mg/kg) significantly reduced paw edema induced by carrageenin in rats (Table 5).

Table 2. Effects of the extract of H. sabdariffa, 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	-	13.7±1.1	13.5±1.4	14.6±1.0	13.7±1.1
Aspirin	200	14.5±1.8	13.1±1.4	13.2±1.3	13.8±0.9
Morphine	10	32.0±2.6*	33.3±2.2*	33.1±2.8*	31.6±2.1*
H. sabdariffa	200	17.2±1.4	18.9±1.7	14.9±2.1	13.6±1.2
(ethanol)	400	16.1±1.0	17.9±2.1	12.7±1.5	12.7±1.1
	800	17.6±2.1	15.2±1.8	16.3±1.6	15.4±1.0
Water	-	20.6±1.6	18.2±1.8	19.5±2.9	17.9±1.6
Aspirin	200	20.5±2.9	16.5±1.6	18.7±1.7	18.5±1.0
Morphine	10	40.9±1.2*	41.4±0.8*	39.8±1.7*	41.0±0.6*
H. sabdariffa	200	19.2±2.0	22.6±2.3	23.3±2.5	19.2±1.7
(spray dry)	400	18.2±1.3	22.2±3.0	21.4±2.9	23.1±2.4
	800	21.8±2.2	18.2±2.3	18.9±2.2	21.0±1.7
Water	-	17.4±1.8	18.0±2.0	16.4±1.4	14.5±1.4
Aspirin	200	18.9±1.6	14.9±1.1	18.7±1.1	15.4±0.9
Morphine	10	38.0±1.1*	35.5±2.4*	33.6±2.0*	32.2±1.5*
H. sabdariffa	200	17.9±1.6	20.0±1.5	18.2±1.9	15.9±1.3
(vacuum dry)	400	17.6±1.5	18.5±1.3	15.2±1.5	14.4±0.5
	800	14.6±1.3	14.4±1.6	17.9±1.7	14.5±1.0

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. Values represent the latency time of nociceptive responses (sec) ± S.E.M. (n=10) * p< 0.05 compared with the control group (Dunnett's test).

Table 3. Effect of the extract of *H. sabdariffa*, 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	-	60.4±4.0	81.7±14.6
Aspirin	200	62.0±4.0	40.4±6.2*
Morphine	10	2.8±1.1**	0.0±0.0**
H. sabdariffa	200	51.1±5.5	90.5±6.4
(ethanol)	400	54.6±5.8	62.7±10.1
	800	48.3±4.9	54.1±12.2
Water	-	63.3±4.4	69.5±7.2
H. sabdariffa	200	48.0±4.2	75.7±9.2
(spray dry)	400	69.6±5.7	66.5±7.0
	800	52.7±5.3	64.5±7.5
H. sabdariffa	200	54.6±3.8	77.8±12.1
(vacuum dry)	400	58.9±6.6	76.5±9.6
	800	50.8±6.4	72.5±6.8

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

Table 4. Effect of the extract of *H. sabdariffa* 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	-	37.8±0.1	37.7±0.2	37.5±0.2	37.3±0.2	37.3±0.2	37.2±0.1
Aspirin	200	37.8±0.2	36.9±0.1*	36.6±0.1*	36.4±0.1*	36.3±0.1*	36.2±0.1*
H. sabdariffa	200	37.9±0.2	37.6±0.1	37.4±0.1	37.2±0.1	36.9±0.2	36.9±0.2
(ethanol)	400	37.8±0.1	37.2±0.2	37.0±0.2*	36.8±0.2	36.7±0.2*	36.6±0.2*
	800	37.7±0.1	37.2±0.1	37.0±0.1*	36.7±0.1	36.6±0.1*	36.6±0.1*
Water	-	38.5±0.1	38.6±0.1	38.7±0.1	38.4±0.1	38.3±0.1	38.3±0.1
Aspirin	200	38.8±0.1	37.9±0.1*	37.6±0.1*	37.4±0.2*	37.4±0.2*	37.3±0.2*
H. sabdariffa	200	38.6±0.1	38.6±0.1	38.3±0.1	38.2±0.1	38.1±0.1	38.1±0.1
(spray dry)	400	38.7±0.1	38.7±0.1	38.5±0.1	38.3±0.2	38.3±0.1	38.1±0.1
	800	38.7±0.2	38.7±0.2	38.4±0.2	38.1±0.2	38.1±0.1	38.1±0.1
Water	-	38.5±0.1	38.6±0.1	38.5±0.2	38.3±0.1	38.1±0.2	38.1±0.2
Aspirin	200	38.3±0.2	37.6±0.2*	37.3±0.2*	37.3±0.3*	37.2±0.3*	37.1±0.2*
H. sabdariffa	200	38.1±0.3	38.4±0.2	37.7±0.4	37.7±0.2	37.8±0.2	37.7±0.2
(vacuum dry)	400	38.2±0.3	38.5±0.2	38.1±0.3	38.0±0.3	37.7±0.3	37.9±0.3
	800	38.1±0.2	38.0±0.4	37.6±0.3	37.4±0.3*	37.4±0.3*	37.4±0.3*

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's test).

Table 5. Effect of the extract of *H. sabdariffa* and aspirin on carrageenin-induced paw edema in rats.

Drug	Dose (mg/kg, p.o.)	Paw volume (ml)					
		0	1hr	2hr	3hr	4hr	5hr
Cosolvent	-	3.89±0.12	4.77±0.08	5.65±0.24	6.81±0.20	7.25±0.21	7.30±0.19
Aspirin	200	4.19±0.15	4.50±0.14	4.74±0.17*	5.12±0.32*	5.30±0.40*	5.38±0.51*
H. sabdariffa	200	4.02±0.09	4.91±0.15	6.11±0.25	7.31±0.36	7.89±0.43	7.94±0.49
(ethanol)	400	4.32±0.05	5.20±0.11	6.36±0.22	7.51±0.20	7.95±0.24	8.08±0.27
	800	4.27±0.08	5.23±0.12	6.71±0.08	7.54±0.11	7.99±0.22	7.73±0.27
Water	-	3.90±0.16	4.99±0.22	6.44±0.17	7.25±0.19	7.49±0.15	7.00±0.25
Aspirin	200	4.04±0.16	4.33±0.19*	4.42±0.14*	5.12±0.31*	4.97±0.23*	4.65±0.13*
H. sabdariffa	200	3.94±0.10	4.86±0.10	6.21±0.16	7.32±0.28	6.92±0.19	6.79±0.17
(spray dry)	400	3.99±0.10	4.89±0.07	6.04±0.26	7.27±0.28	7.04±0.41	6.68±0.32
	800	4.04±0.09	4.70±0.05	5.91±0.29	7.01±0.45	6.80±0.27	6.47±0.22
Water	-	4.48±0.09	5.40±0.13	6.72±0.29	7.60±0.40	7.74±0.32	7.90±0.45
Aspirin	200	4.60±0.08	4.85±0.08*	5.14±0.13*	5.37±0.16*	5.33±0.16*	5.94±0.23*
H. sabdariffa	200	4.42±0.04	5.61±0.15	6.97±0.14	7.55±0.15	7.92±0.17	7.84±0.15
(vacuum dry)	400	4.63±0.09	5.60±0.12	6.24±0.18	6.91±0.46	7.03±0.47	6.91±0.39
	800	4.53±0.11	5.68±0.22	6.25±0.41	6.89±0.35	7.15±0.40	6.99±0.41

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 value represents mean ± S.E.M., each from 6 rats. *p<0.05, compared with the control group (Dunnett's test).

Discussion

In the present study, no acute toxicity was observed after oral administration even at the high dose of the extract of *H. sabdariffa* calyces, so it has potential safety for consumption. However, *H. sabdariffa* calyces extract could cause liver injury and induce testicular toxicity after prolonged usage of high dose in rats (Akindahunsi and Olaleye, 2003; Orisakwe *et al*., 2004).

The ethanol extract of *H. sabdariffa* significantly inhibited the acetic acid-induced writhing response at the dose of 800 mg/kg while the aqueous extract (spray dried and vacuum dried) of *H. sabdariffa* at the same dose range had no significant effect in this test. As the writhing test is generally used for screening of antinociceptive effects (Koster *et al*., 1959; Hendershot&Forsaith, 1959), the ethanol extract of *H. sabdariffa* may possess analgesic action. However, this interpretation should be made with care, as some non-analgesic agents, e.g., sympathomimetics, central nervous system stimulants, serotonin antagonists and monoamine oxidase inhibitors have been reported to decrease the writhing response (Hendershot and Forsaith, 1959; Brittain *et al*., 1963; Vogel and Vogel, 1997).

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, whereas all the extracts of *H. sabdariffa* calyces and aspirin failed to suppress the responses. These findings, therefore, suggest that the apparent antinociceptive action of the active compound(s) in the ethanol extract of *H. sabdariffa* may be mediated through peripheral but not central mechanism(s). However, Dafallah and al-Mustafa (1996) found that the aqueous extract of H. sabdariffa showed a significant effect on the hot plate reaction time. Different sources of plant materials may contribute to difference in chemical compositions and account for the discrepancy of the findings.

The formalin test is another model to assess the 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 & Dennis, 1977; Hunskaar and Hole, 1987). In the present study, neither the ethanol nor aqueous extract of *H. sabdariffa* calyces significantly suppressed the licking activity in either the early or late phase in mice. Morphine significantly reduced the licking activity in both phases whereas aspirin decreased the licking activity only in the late phase.

Both the ethanol and aqueous extract (vacuum dry) of *H. sabdariffa* calyces decreased the fever induced by yeast in rats. From the result, the ethanol extract showed stronger fever lowering effect than that of the aqueous extract (vacuum dried). Thus, it is possible that more active compound(s) for antipyretic action may be included in the ethanol extract than in the aqueous extract. The fever condition entails enhanced formation of cytokines such as interleukins, interferons and tumor necrosis factor, and the cytokines increase the synthesis of prostaglandin E2. Aspirin suppresses this response by inhibiting the synthesis of prostaglandin E2 (Dascombe, 1985; Vane, 1987). The *H. sabdariffa* calyces extract may be involved in the inhibition of some of these substances inducing fever.

Unfortunately, none of the extracts of *H. sabdariffa* calyces had any significant effect on carrageeninñinduced hind paw edema in rats. In contrast, aspirin, a nonsteroidal anti-inflammatory drug, decreased the paw edema by inhibition of prostaglandin synthesis via cyclooxygenase activity (Vane, 1987).

Since the extract of *H. sabdariffa* calyces had no significant effect on either formalin-induced pain in mice or carrageenin-induced paw edema in rats but suppressed fever induced by yeast, the antipyretic action of *H. sabdariffa* calyces may act at different mechanism from that of aspirin.

Based on these results, we conclude that the ethanol and aqueous extract (vacuum dried) of *H. sabdariffa* calyces have antipyretic effects, and

that the actions on pyrexia may be different from that of the aspirin.

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