การศึกษาคุณสมบัติทางเภสัชวิทยาบางประการ ของสารสกัดด้วยน้ำจากสมุนไพรโคกกระออม

Some Pharmacological Activities of an Aqueous Extract from Cardiospermum Halicacabum

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บทคัดย่อ

การศึกษาฤทธิ์ของสารสกัดด้วยน้ำจากสมุนไพรโคกกระออมในการระงับปวดด้วยการกระตุ้นด้วยกรดน้ำส้มและการใช้ความร้อนพบว่า สารสกัดด้วยน้ำมีความแรงในการระงับอาการปวดเป็นครึ่งหนึ่งของมอร์ฟีนซัลเฟตที่ขนาด 25 มก/กก เมื่อทดสอบฤทธิ์ต้านการอักเสบโดยการกระตุ้นการบวมที่อุ้งเท้าหนูด้วยคาราจีแนน พบว่าสารสกัดโคกกระออมที่ความเข้มข้น 0.7 ก/กก มีผลต่อการต้านการอักเสบได้ดีกว่าน้ำเกลือแต่น้อยกว่า indomethacin (45 มก/กก) อย่างไรก็ตาม สารสกัดสามารถลดอาการบวมใกล้เคียงกับ indomethacin ที่เวลา 5 ชม. หลังได้รับยา เมื่อทำการศึกษาฤทธิ์ในการลดไข้ที่เกิดจากการกระตุ้นด้วยยีสต์ในหนู พบว่ามีฤทธิ์ลดไข้โดยเริ่มต้นออกฤทธิ์ที่เวลา 30 นาทีและ มีระยะเวลานาน 6 ชม. สารสกัดโคกกระออมไม่มีฤทธิ์ในการฆ่าเชื้อแบคทีเรีย แต่มีฤทธิ์ในการยับยั้งเชื้อรา Mentagophyte และ Tricophyton เล็กน้อยที่ความเข้มข้นถึง 10000 ไมโครกรัมต่อมล และการออกฤทธิ์ในด้านเภสัชวิทยาจะขึ้นกับขนาดและความเข้มข้นของสารสกัด

Abstract

Some pharmacological activities of an aqueous extract from Kok-kra-orn (Cardiospermum halicacabum) were investigated. Its analgesic property using writhing test and modified hotplate method was approximately half potency of morphine sulfate (25 mg/kg). Its anti-inflammatory effect, using carragenan-induced paw edema model, was found to be better than normal saline but less than indomethacin (45 mg/kg). However, the extract at 0.7 g/kg appears to have a relatively similar intensity to indomethacin after 5 hours of administration. When antipyretic action using yeast-induced hyperthermia test was carried out, it was found that the antipyretic effect in rat was also found with onset of 30 min and 6 hours duration. The aqueous extract had no antibacterial activity but sh.owed mild antifugal activity against *Mentagophyte* spp. and *Trichophyton* spp. All studied pharmacological activities of the extract (0.175-0.7 g/kg) appear to be a dose dependent.

คำสำคัญ: โคกกระออม, ฤทธิ์ต้านจุลชีพ, การระงับปวด, การต้านการอักเสบ, การลดไข้, การฆ่าเชื้อแบคทีเรีย, และเชื้อรา

Keywords: Cardiospermum halicacabum, Pharmacological activities, Antimirobial activities

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Introduction

Cardiospermum halicacabum (Family Spindaceae), known in Thailand as "kok-kra-orm" is an annual herbaceous creeper which flower all year round. It has a bitter and acrid taste (Chinese Medicinal Herbs of Hong Kong, 1988). The plant is widely distributed in the North-eastern part of Thailand and has been used as a source of folk medicine for the treatment of several diseases such as rheumatism, cold and fever as well as for traumatic injury and some skin diseases (Jayaweera, 1982). In the present study, some pharmacological activities of aqueous extract *of C. halicacabum* were investigated. The analgesic property of the extract was carried out using writhing test (Lanhers *et al.*, 1991) and modified hot plate method (Chitcharoenthum and Khunkitti, 1997). The antipyretic effect was assessed by yeast-induced hyperthermia test (Lanhers *et al.*, 1991) and anti-inflammatory effect was investigated by carragenan-induced paw edema test (Lanhers *et al.*, 1991) as well as its antimicrobial acivities were investigated.

Materials and Methods

Chemicals

Diclofinac, lambda carrageenan, (type IV) and chlorhexidine diacetate were purchased from Sigma, USA. Morphine sulfate was from T.P. Drug, Bangkok. Paracetamol were from LBS, Bangkok, Thailand. Indomethacin was from Fluka, Germany. Sodium chloride was purchase from Merck, Germany. All culture media were from Difco Laboratory, USA

Preparation of *C. halicacabum* plant extract

Fresh *C. halicacabum* (100 g) were boiled in purified water (5000ml) until the volume was reduced to approximately 2500 ml. After the aqueous extract was cooled, the filtrate collected by Freezed-drying and kept in the dessicator.

Analgesic activity

Acetic acid-induced abdominal writhing test (Lanhers, *et al.* 1991)

Groups of 8 male mice weighing 18-22 g were fed either the extract (0.175-1.5 g/kg) or a reference drugs: indomethacin (45 mg/kg), Paracetamol (200 mk/kg), diclofenac (40 mg/kg), morphine sulfate (25 mg/kg) or 0.9% NaCl (10 ml/kg) solution at 60 min prior to i.p injection of 0.1 ml/10 g body weight of 1% (v/v) acetic acid. Each mouse was observed in an individual box for the number of writhes they elicited at every 5 min and the observation was taken for 1 hour. The data was calculated and presented as the percentage of protection.

$$\% \text{ Protection} = \frac{100 \text{ X (mean pain response of the control-mean pain response of the treated mice)}}{\text{(mean pain response of the control)}}$$

Modified Hot Plate Method (Chitchareonthum and Khunkitti, 1997) Five glass cylinders of 12.5 x 24 cm (diameter and height) with a known rate of temperature changed within 3 min (0.2 °C per sec) were used. Female albino mice of 23-25 gm were acclimatized in the glass cylinder for 4 hours before the experiment began. Control threshold of each mouse was taken twice in hourly interval before and after drug administration. The elapse time to behavioral changes such as paw padding (shaking), paw licking and jumping were recorded. The results were expressed as % antinociceptic effect.

$$\% \text{ Antinociceptic effect} = \frac{100 \text{ X (mean treated time- mean control time)}}{\text{(mean control time)}}$$

Anti-inflammatory activity using carrageenan-induced paw edema test (Lanhers, *et al.* 1991)

Male rats weighing 200-250 g were used in this study. Before treatment, the average volume of the back paw of each rat (V_o) was determined using Plethysmometer 7150, Ugo Basile, Apelex, Fr. Group of 6 rats were fed per oral either the plant extract (0.175-1.5 g/kg), or indomethacin (45 mg/kg), or 0.9% NaCl at 60 min prior to intradermal

inject with 0.1 ml of 1%w/v carragenan in the left paw and 0.1 ml of normal saline in the right paw. The volume of treated paws (V_t) were determined at 1, 2, 3, 4, 5, 6 and 24 hours. The results were expressed as the percentage of inhibition.

$$\% \text{ Inhibition} = \frac{100 \text{ X } [(V_t - V_o)_{control} - (V_t - V_o)_{treatment}]}{(V_t - V_o)_{control}}$$

Antipyretic activity using yeast-induced hyperthermia test (Lanhers, *et al.* 1991)

Female albino mice weighing 25.6-31.0 g were injected subcutaneously with 0.3 ml/mouse of either the suspension of brewer yeast extract (0.5g/kg), or 0.9% NaCl. The rectal temperature was recorded using a Thermister probe (yellow spring) connected to Harvard Temperature recorder (Takara Instrument, Japan) every hour. Five hours after yeast injection, the rectal temperature was recorded to select hyperthermic mice which have the rectal temperature $\geq$ 0.5°C higher than their normal temperature (T_5). Then the selected mice were randomly divided into groups of 10 mice and being fed either the plant extract (0.75-3 g/kg) or normal saline (1.0 ml). The rectal temperature of each mice were recorded hourly (T_t). The results were expressed as the percentage of temperature change

$$\% \text{ Temperature Change} = \frac{100 \text{ X } (T_t - T_5)}{T_5}$$

Antibacterial activity

Microorganisms used for the assay were *Pseudomonas aeruginosa* ATCC 9027, *Staphylococcus aureus* ATCC 6538, *Escherischia coli* ATCC 25922 and *Bacillus subtillis* ATCC 6633. A 0.2 ml of an overnight culture of microorganisms in tryptic soy broth at 37°C was seeded in 20 ml of molten tryptic soy agar and in a petri dish. The seeded plates were allowed to dry for 30 min. Then, the holes with 3 mm diameter were aseptically made. Forty µl of the test compounds at the concentrations of 2000, 4000, 8000, 10000, 12000 µg/ml were then added to each hole, respectively and allowed diffusion to take place for one hour and then incubated at 37°C for 24 hours. After 24 hours of incubation, the sensitivity of the organisms to the test compound was recorded by measuring the inhibition zones. These were performed in 6 replicates. Chlorhexdine diacetate (0.05%) was used as control. The effect of pH on its activity were performed by adjusting pH of medium to pH 4, 7.4, 9 with either 0.1 N HCL or 0.1N NaOH

Antifungal activity study

Human pathogenic fungal strains were obtained from clinical isolates of Siriraj Hospital, Bangkok. There were *Trichophyton* spp. e.g. *T. mentagophytes simi*-LMO-023 ยุทธชัย *T. mentagophytes* ธารทิพย์, *T. rubrum*-LMO-003, *T. rubrum* CDC, *T. rubrum* ชีวบุตร, *T. schoenleinii*, *T. tonsurans* CDC, *T. violaceum*, *T. violaceum* CDC, *T. concentricum*; *Microsporum* spp. e.g. *M. gypseumsimi*-LMO 007 ลัดดา, *M. gypseum* CDC, *M. canis*, *M. canis* CDC, *M. ferrugeneum* น้อง, *M. ferrugeneum* มินท์, *M. gallinae*; *Epidermophyton* spp. e.g. *E. floccosum*, *E. floccosum*-LMO-002 สุชาติ. Geometric dilutions of the test compounds (4000-10000 µg/ml) were freshly prepared in Yeast Nitrogen Base (NYB) in 0.01 M phosphate buffer pH 7.0. Consequently, 1-5 x105 cells/ml of the fungi were added to the test and incubated at 25°C for 14 days. 2.5 µg/ml of Amphotericin B was used as a control. The effect of pH on its activity was performed by adjusting pH of medium to pH 4, 7.4, 9 with either 0.1 N HCl or 0.1N NaOH. Tubes were inspected at day 14 for the presence of fungal growth as evidenced by turbidity. The minimum inhibitory concentration (MIC) was defined as the lowest concentration that inhibited visible growth. The results were confirmed by plate agar diffusion method.

Results and Discussion

Analgesic activity

Intraperitoneal injection of acetic acid produced an abdominal writing response by activating the chemosensitive nociceptor in animal. (Chen *et al.*, 1995). Table 1 demonstrated that after 1 and 2 hour, the inhibition of the writing responses in mice was increased as the concentrations of the extract increased up to 0.7 g/kg. The aqueous extract at 0.7 g/kg inhibited the writing responses in mice at 40.7% and 41.4 respectively, which was less than all reference drugs. However, as the concentration of the extract increased to 1.4 g/kg, the percent protection reduced to 14.7%. This might be due to the solubility limitation of active compounds which is not yet known. Generally, acetic acid-induced writing responses indicate analgesic action but not specify that whether the analgesic effect is due to the central or peripheral inhibitions. The thermal painful stimuli, therefore, indicated central analgesic properties (Martnez-Vazquez *et al*, 1996). The extract exhibited a 10-30% antinociceptive effect within 1 to 2 hour. The modified hot plate test indicated that after 2 hours, the antinociceptic effect of the extract showed dose dependent whereas at 1 hour maximum effect was found at 0.75 g/kg whereas at 1.5 g/kg the effect was about 10%. (Fig. 1) It might be due to the solubility limitation of the bioactive compounds which could affect on its absorption. However, after 2 hours, the extract at concentration of 3.0 g/kg had about 50% antinociceptic effect of 25mg/kg morphine sulfate which was higher than paracetamol 200 mg/kg and diclofinac (40 mg/kg) (Fig 1). These results revealed that like indomethacin, paracethamol and diclofinac, the extract exhibited the peripheral analgesic action and like morphine sulfate, the extract was had centrally analgesic action. The centrally analgesic effect was greater than that of diclofenac but lesser than morphine. Taken at 3 g/kg dose, the extract exerted greater effect than diclofenac by 1.5 fold but lesser than that of morphine by 2.2 folds. The analgesic activity of the extract was in between morphine and diclofenac. In this test also showed the weak and brief analgesic effect of paracetamol.

Anti-Inflammatory Activity

Fig 2 showed that the extract inhibited the paw edema at the early and late phases in rats. In general, it has been known that carrageenan-induced paw edema had a pronounce biphasic reaction in rats with different inflammatory mediators. In the early phase, it was related to the release of histamine and like substances. The late phase was associated with the activation of plasma kinins and tissue prostaglandins (Chen *et al.*, 1995). As a result, the antiinflammatory action of the extract as well as indomethacin migth be due to inhibition of histamine and histamine like substances and of kinins and prostaglandins. As shown in table 2, a pretreatment by *C. halicacabum* extract reduced carrageenan-induced paw edema in a dose dependent way from 0.175 to 0.7 g/kg. Again at 1.4 g/kg, the inhibition of edema decreased. Apparently, the maximal inhibition was found at 30 min after pretreatment before gradually decreased or remained relatively steady and increased again after 24 hours. Indomethacin reached a maximum intensity at 3 hours and gradually decreased until 6 hour and reach the second peak at 24 hours. The extract at all concentrations had less antiinflammatory action than indomethacin.

Antipyretic Effect

Normal mice had rectal temperature of 37.3°C which can be increased to 42°C by the Brewer yeast injection (0.5g/kg). The hyperthermia in mice was induced within 4 hours and reach the peak at about 7 hours (Fig 3). The plant extract (0.75-3 g/kg) had the antipyretic effect within one hour and at peak about 3 hours after administration (Fig 4).

Antimicrobial activity

The aqueous extracts at very high concentrations (4000-10000 µg/ml) exhibited no

antibacterial activity at the pH studied. The inhibition zone diameter of 0.05% chlorhexidine was 12-14 mm. Of 19 dermatophytes species, 5 strains were inhibited by 10000 µg/ml of the extract which were *T. metagophytes* ยุทธชัย, *M. canis*, *M. ferrugincum* น้อง and *E. floccosum*, *E. floccosum* LMO-002 สุชาติ. When the pH of *C. halicacabum* extracts was adjusted. The inhibition were found at pH 4.0 and pH 9.0. In this study, Of 19 strains, the extract had antifungal activity against 10.52% of *Epidermopyhton*, 0.52% of *Microsporum* and 5.26% of *Trichophyton*. Some species were confirmed by plate agar diffusion methods (Table 3). It was found that *E. floccosum simi*-LMO 002 สุชาติ was sensitive to the extract at as low as 4000 µg/ml and *T mentagophytes* ยุทธชัย was at 6000 µg/ml. The extract (10000 µg/ml) at pH 4 and 7.4 inhibited *E. floccosum simi*-LMO 002 สุชาติ and *T mentagophytes* ยุทธชัย but no activity was found at pH 9.0 (table 4). As a result, the extract exhibited quite less activity at high concentrations and effective at pH 4 to 7.4. At higher pH, the extract was inactive. This might be due to the active was inactivated or dissociated and could not penetrate through the cell of dermatophytes.

Conclusion

Among all tests, the extract seems to possess strong antipyretic and anti-inflammatory as well as analgesic effect. The analgesic effect of *C. halicacabum* was in between peripheral acting analgesic and central acting analgesic as morphine. In an antimicrobial studies, the extract was effective against few *Epidermophyton* spp. and very little on *Trichophyton* spp. at very high concentrations. No antibacterial activity was found. This finding confirmed a study of Sadique *et al* (1987) and supported the traditional application of *C. halicacabum* for treatment of swelling and inflammation as well as rhumatism (Jayaweera, 1982; Chinese Medicinal Herbs of Hong Kong, 1988) More information was needed to understand effects of the plant.

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Table 1 Analgesic Activity of Test Compounds: Writhing Test (n=8)

Extract and drug used	Dose	Pain response		%Protection	
		1h	2h	1h	2h
Control (0.9% NSS)	10 ml/kg	68.1 ± 9.3	66.1 ± 6.0	-	-
Indometacin	45 mg/kg	34.6 ± 7.3	35.0 ± 7.9	49.1	47.0
Paracetamol	200 mg/kg	37.7 ± 10.2	30.6 ± 7.1	44.6	53.7
Diclofinac	40 mg/kg	34.9 ± 8.0	37.4 ± 5.8	48.7	43.4
Morphine sulfate	25 mg/kg	1.4 ± 1.0	20.4 ± 11.1	97.9	69.1
C. halicacabum	0.175 g/kg	49.0 ± 5.6	52.0 ± 6.2	27.9	21.3
C. halicacabum	0.35 g/kg	47.4 ± 6.9	57.0 ± 5.8	30.3	13.8
C. halicacabum	0.7 g/kg	40.3 ± 10.3	38.7 ± 8.4	40.7	41.4
C. halicacabum	1.4 g/kg	58.0 ± 6.5	58.3 ± 7.4	14.7	11.8

Table 2 Inhibitory effect of *C. halicacabum* extract and indomethacin on carrageenan-induced edema (n=6)

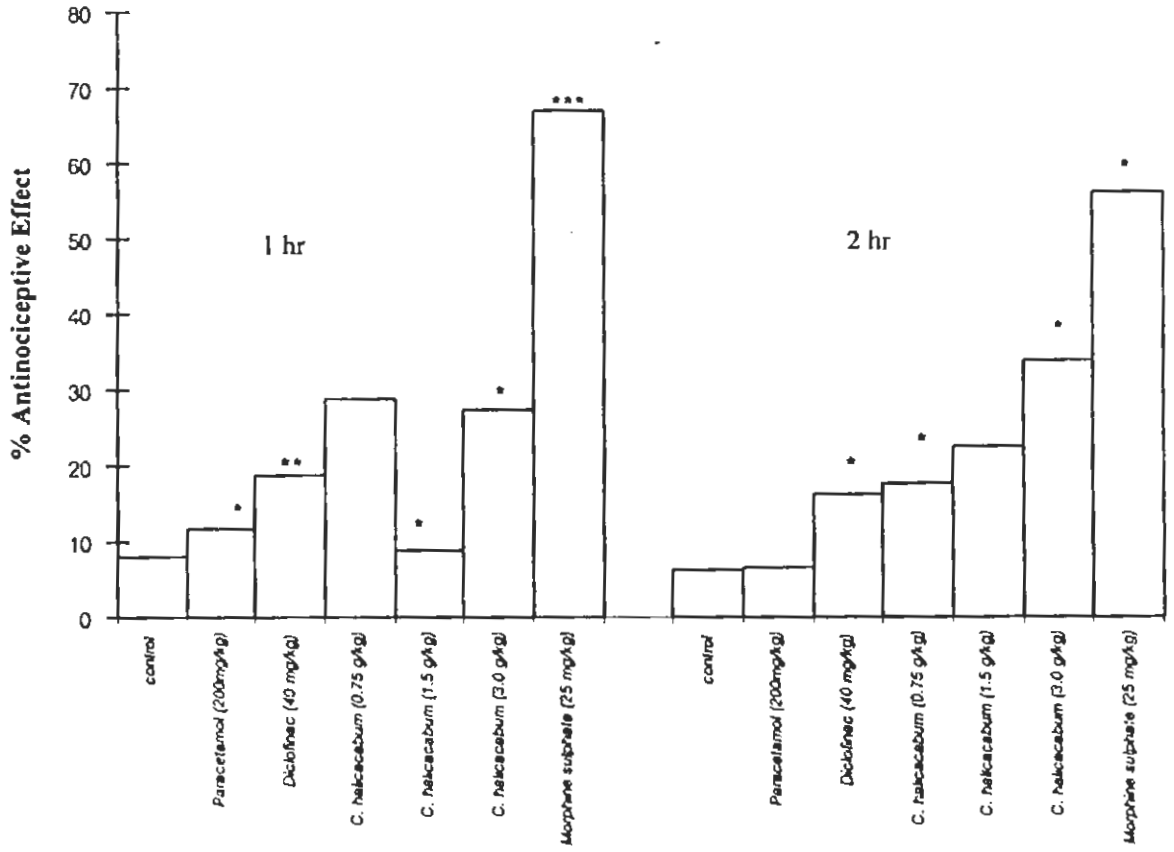
Group	Percentage of inhibition							
	0.5h	1h	2h	3h	4h	5h	6h	24h
Indomethacin (45 mk/kg)	48.93	52.37	54.27	56.67	51.18	41.72	38.11	70.84
C. halicacabum extract (0.175 /kg)	26.24	20.68	24.04	21.31	18.49	18.22	12.15	39.54
C. halicacabum extract (0.35 g/kg)	44.64	35.12	26.60	26.95	25.14	25.39	18.52	55.62
C. halicacabum extract (0.7 g/kg)	57.37	45.20	24.27	31.04	40.60	42.01	35.56	63.36
C. halicacabum extract (1.4g/kg)	45.29	25.97	25.05	27.76	27.93	31.30	23.67	51.10

Table 3 Inhibition zone diameters (mm) of *C. halicacabum* extract against dermatophytes by agar diffusion method.

Dermatophytes	Aqueous extract (µg/ml)				Amphotericin B
	10000	8000	6000	4000	2.5 µg/ml
E. floccum simi-LMO-002 สุชาติ	18-20	15-17	15-17	14-15	10-12
M. Gypseum simi-LMO-007 ลัดดา	0	0	0	0	10
T. mentagophytes ยุทธชัย	10-12	11-14	10-14	0	15
T. mentagophytes ธารทิพย์	0	0	0	0	12-15
T. rubum	13	0	0	0	11-12

Table 4 Inhibition zone (mm) of *C. halicacabum* extract (10000 µg/ml) at various pHs against dermatophytes by agar diffusion method.

Dermatophytes	pH			Amphotericin B
	4.0	7.4	9.0	2.5 µg/ml
E. floccum simi-LMO-002 สุชาติ	15-17	15-20	0	11
M. Gypseum simi-LMO-007 ลัดดา	0	0	0	10
T. mentagophytes ยุทธชัย	20	12-20	0	10
T. mentagophytes ธารทิพย์	0	0	0	12-15
T. rubum	0	0	0	12



*p < 0.05, **p<0.01 and ***p<0.01 significant level by paired t-test

Figure 1 Analgesic activity of the extract of *C. halicacabum* and reference drugs using modified hot plate method.

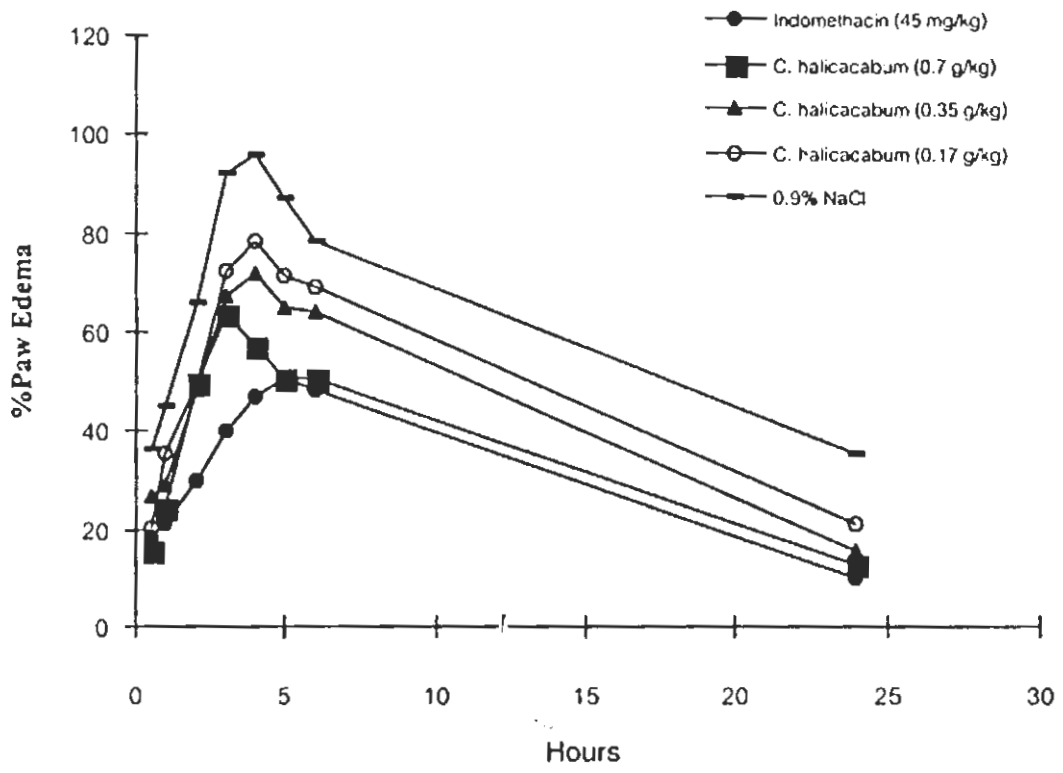


Figure 2 Anti-inflammatory activity of the extract of *C. halicacabum* and indomethacin using carrageenan-induced paw edema

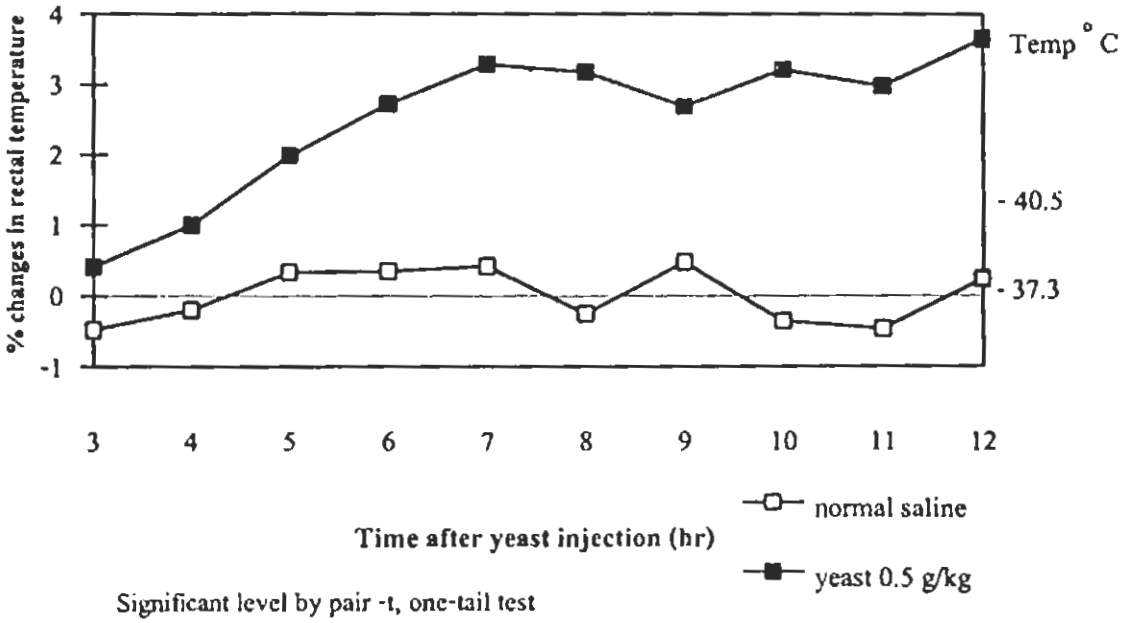


Figure 3 The profile of yeast-induced fever

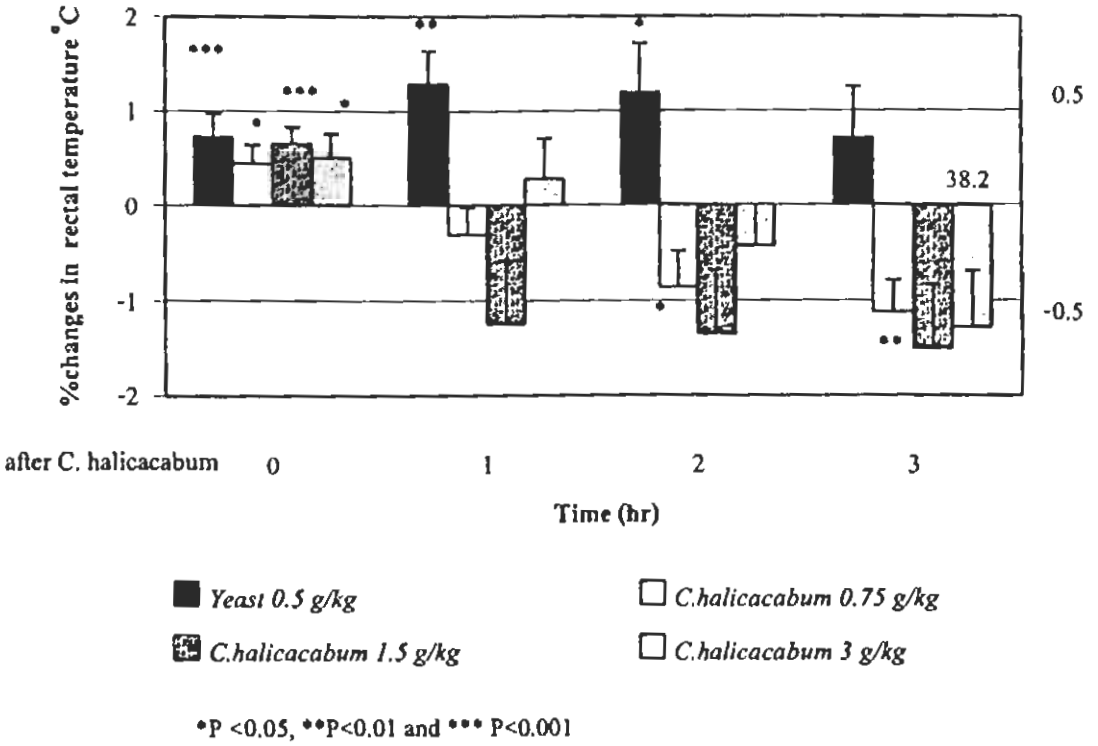


Figure 4 Antipyretic activity of an aqueous of *C. halicacabum*