ORIGINAL ARTICLE

ANTI-INFLAMMATORY AND ANALGESIC EFFECTS OF *HEDYCHIUM CORONARIUM* KOEN

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ABSTRACT

Successive hexane, chloroform and methanol extracts of the rhizome of *Hedychium coronarium Koen.* (HC) were subjected to evaluate analgesic and anti-inflammatory activities in animal model. In acetic acid-induced writhing test, the chloroform and methanol extract at doses of 400 mg/kg body weight elicited 27.23 and 40.59% inhibition of writhing reflex respectively. Both the chloroform and methanol extracts showed significant elongation of tail flick time (41.15 and 61.32% elongation respectively) at 400 mg/kg body weight. In carrageenan induced rat paw edema test, the chloroform and methanol extracts at a dose of 400 mg/kg body weight showed statistically significant ($P<0.01$) inhibition of paw edema by 27.46 and 32.48%, respectively at the third hour after carrageenan injection.

Keywords: *Hedychium coronarium,* analgesic activity, anti-inflammatory activity, writhing, radiant heat tail-flick, carrageenan.

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INTRODUCTION

Inflammation is a pathophysiological response of living tissue to injuries that leads to the local accumulation of plasmatic fluid and blood cells. Although it is a defense mechanism that helps body to protect itself against infection, burn, toxic chemicals, allergens or other noxious stimuli, the complex events and mediators involved in the inflammatory reaction can be induced, maintain or aggravate many diseases (Sosa *et al*, 2002). However, studies have been continuing on inflammatory diseases and the side effects of the currently available anti-inflammatory drugs pose a major problem during their clinical use. Therefore, development of newer and more powerful anti-inflammatory drugs with lesser side effects is necessary.

Hedychium coronarium Koen (Bengali name: Dolon Champa) is an erect herb belonging to the family Zingiberaceae. The plant is available in all tropical countries. The rhizome of the plant is used in the treatment of diabetes (Bhandary *et al.*, 1995). It is also used as antirheumatic, excitant, febrifuge and tonic (Jain *et al.*, 1995). Previous phytochemical investigations showed that the plant contains the diterpenes-coronarin A, coronarin B, coronarin C, coronarin D and isocoronarin D (Nakatani *et al.*, 1994). Though the plant is traditionally used in many parts of Bangladesh, no scientific report is available to validate the folkloric use. As a part of our continuing studies on the medicinal plants of Bangladesh, we investigated the analgesic and anti-inflammatory activities of different extracts of *H. coronarium.*

MATERIALS AND METHODS

Plant material

The rhizome of *H. coronarium* was collected from the botanical garden, University of Dhaka, Bangladesh in May, 2002 and was identified (voucher specimen No. DUH-02) by the taxonomist of the Department of Botany, University of Dhaka. Collected plants, after cutting into small pieces, were dried and pulverized into a coarse powder and stored into an air-tight container.

Extraction and *sample preparation*

The pulverized coarse powder of the rhizome of HC (455 gm) was extracted with hexane, chloroform and methanol by successive cold extraction. All the extracts obtained, were filtered off and evaporated to dryness *in vacuum* at low temperature and reduced pressure by rotary evaporator. The hexane extract was designated as HCH, chloroform extract as HCC and methanol extract as HCM. All the extracts (HCH, HCC and HCM) were dissolved in normal saline by using 0.1% tween-80.

Experimental animals

Swiss albino mice (20-25 g) and *Long Evans* rats (140-160 g) of either sex were obtained from the animal house of the International Center for Diarrhoeal Disease and Research, Bangladesh (ICDDR, B). The animals were given standard feed developed by ICDDR, B and water *ad libitum* and kept in the laboratory environment (12 h dark/12 h light cycle) for seven days for acclimatization. Animals were kept under fasting for overnight and weighed before the experiment.

Drugs and chemicals

The following chemicals and drugs were used: aminopyrine (Sigma-Aldrich), acetic acid (Merck, Germany), morphine (Jayson Pharmaceuticals Ltd., Bangladesh), carrageenan (Sigma-Aldrich) and phenylbutazone (Sigma-Aldrich).

Acetic acid induced writhing test

The peripheral analgesic activity of different extracts of HC was determined by the acetic acid induced writhing inhibition method (Whittle, 1964). The pre-screened Swiss albino mice employed for this experiment were divided into eight groups as shown in Table I. The inhibition of writhing in mice by the plant extracts (HCH, HCC and HCM) at doses of 200 and 400 mg/kg, p.o. were compared against inhibition of writhing by a standard analgesic agent, aminopyrine given p.o. at a dose of 50 mg/kg body weight. The control group received the saline containing 0.1% tween-80. Acetic acid (0.7%) at a dose of 0.1 ml/10g was administered intraperitoneally 40 min after the administration of the test materials. After an interval of 10 min, numbers of writhing were counted for 10 min. The percent inhibition of writhing was measured using the formula,

$$\text{Percent inhibition of writhing} = (1-W_t/W_c) \times 100$$

where, W_C and W_t represent the average number of writhing produced by the control and test group, respectively.

Radiant heat tail-flick method

The analgesic activity was determined by radiant heat tail-flick model in mice (D'Amour and Smith, 1941). The animal groups are treated as described in the writhing methods. Morphine (2 mg/kg) administered sub-cutaneously was used as the standard analgesic agent. Tail-flick latency was assessed by the analgesiometer (Inco, India). The strength of the current passing through the naked nicrome wire was kept constant at 5 ampere. The distance between heat source and the tail was 1.5 cm and the application site of the heat on the tail was maintained within 2 cm, measured from the root of the tail. Cut-off reaction time was 10 sec to avoid any tissue injury during the process. Tail-flick latency was measured after 1 h of the drug administration.

Anti-inflammatory study

In this experiment, carrageenan induced rat hind paw

Table 1: Analgesic activity of different extracts of *Hedychium coronarium* on acetic acid induced writhing response and radiant heat tail-flick model in mice.

Group	Acetic acid induced writhing response in mice				Radiant heat tail-flick	
	Dose[a]	Writhings[b]	% Inhibition		Reaction time (sec)[b]	% of elongation
Control	-	33.7±1.65	-		4.05±0.21	-
HCH	200	30.8±1.67	8.66		4.78±0.23	18.11
	400	28.0±0.58*	16.83		4.97±0.29	22.63
HCC	200	29.0±1.57	13.86		5.47±0.80	34.98
	400	24.5±1.34**	27.23		5.72±0.62*	41.15
HCM	200	27.9±1.39*	17.08		5.67±0.27*	39.92
	400	20.0±1.53**	40.59		6.53±0.26**	61.32
Aminopyrine	50	16.5±1.38**	50.99		-	-
Morphine	2[c]	-	-		7.97±0.63**	96.7
One-way ANOVA	F	15.9			7.94	
	df	7, 40			7, 40	
	P	<0.001			<0.001	

[a]40 min after drug treatment, mice were injected i.p. with 0.7%(v/v) acetic acid (0.1ml/10g); 10 min after the injection, the number of writhing was counted for 10 min.
[b]Values are Mean ± SEM (n = 6); One-way ANOVA; ***P*<0.01, **P*<0.05 compared to control.
[c]Morphine was administered subcutaneously.

edema was used as the animal model of acute inflammation (Winter, 1962). The animals were divided into groups as shown in table 2. 1 hour after the oral administration of test materials (200 and 400 mg/kg, p.o.), standard drug (80 mg/kg, p.o.) or saline to respective treatment groups, 1% carrageenan solution was injected to the sub-planter region of right hind paw of each rat. The volume of paw edema was measured at different time intervals for a period of 24 h by mercury displacement method. Traveling microscope (ELFO Scientific Apparatus, India) was used to record the volume of mercury displaced after immersion of the inflamed paw. Prior to immersion into mercury, the inflamed right hind paw was labeled with permanent ink to confirm uniform immersion during each episode of measuring paw volume. The average percent increase in paw volume was calculated and compared against the control group. Percent inhibition was calculated using the following formula.

$$\% \text{ inhibition of paw edema} = (1\text{-}V_t/V_c) \times 100$$

V_c and V_t represent average paw volume of control and treated animals, respectively.

Statistical analysis

The results were analyzed for statistical significance using one-way ANOVA followed by Dunnett's test. A *P* value <0.05 was considered significant.

RESULTS

As shown in table 1, methanol extract of HC (200 and 400 mg/kg, p.o.) showed a significant (*P*<0.01) reduction in the number of writhing with 17.08 and 40.59% of inhibition, respectively. The chloroform extract showed 27.23% reduction at a dose of 400 mg/kg. The standard drug aminopyrine produced 50.99% inhibition at a dose of 50 mg/kg body weight.

In radiant heat tail-flick model, percentage of tail flick elongation was 39.92 and 61.32 in methanolic extract of HC at doses of 200 and 400 mg/kg body weight, respectively. The chloroform extract showed 41.15% elongation of tail flick latency at higher dose. The result was found to be statistically significant (*P*<0.01) in comparison to the control.

In carrageenan induced rat paw edema test for acute inflammation, HCC and HCM exhibited statistically significant (*P* <0.01) inhibition of paw volume by 27.46 and 32.39%, respectively at a dose of 400 mg/kg body weight, which was comparable to that of standard drug phenylbutazone (42.54% inhibition, *P*<0.001) given p.o. at a dose of 80 mg/kg body weight after 3 h of carrageenan administration (table 2)

DISCUSSION

The abdominal constriction response induced by acetic acid is a sensitive procedure to establish peripherally acting analgesics. The response is thought to be mediated by peritoneal mast cells (Ronaldo *et al*, 2000), acid sensing ion channels (Voilley, 2004) and the prostaglandin pathways (Vogel and Vogel, 1997). The significant antinociceptive activity of HCC and HCM might be due to the presence of analgesic principles acting with the prostaglandin pathways. However, true analgesic activity can only be ensured by the combination

Table 2: Anti-inflammatory activity of different extracts of *Hedychium coronarium* on carrageenan induced rat paw edema.

Group	Dose[a] (mg/kg)	Carrageenan induced rat paw edema[b] Mean ± SEM (% inhibition of paw volume)				
		1 h	2 h	3 h	4 h	24 h
Control	-	81.0±2.31	117.7±5.04	111.7±2.47	83.3±3.35	58.1±1.42
HCH	200	74.3±2.42 (8.23)	107.3±3.99 (8.78)	100.0±1.26** (10.45)	81.5±2.86 (2.20)	57.5±1.91 (1.15)
	400	71.0±2.02* (12.35)	104.8±4.59 (10.90)	98.3±1.98** (11.94)	75.0±2.54 (10.00)	56.8±1.17 (2.29)
HCC	200	72.5±3.64 (10.49)	101.7±3.43* (13.60)	94.5±3.27** (15.37)	74.3±3.00 (10.80)	57.0±2.42 (2.00)
	400	69.0±2.13** (14.81)	92.8±2.89** (21.10)	81.0±0.73** (27.46)	59.5±0.67** (28.60)	56.6±1.38 (2.58)
HCM	200	69.7±2.59** (13.99)	98.5±3.27** (26.29)	92.0±2.22** (17.61)	73.8±2.70 (11.4)	56.6±1.89 (4.30)
	400	64.3±1.15** (20.58)	88.0±3.08** (25.21)	70.45±1.41** (32.39)	55.0±1.00** (34.00)	55.0±1.00 (5.44)
PBZ	80	58.2±1.27** (28.13)	77.0±1.91** (34.56)	64.2±1.80** (42.54)	54.3±2.03** (34.80)	54.5±1.12 (6.30)
One-way ANOVA	F	8.44	12.3	56.1	20.1	0.61
	df	7, 40	7, 40	7, 40	7, 40	7, 40
	P	<0.0001	<0.0001	<0.0001	<0.0001	>0.05

[a]1hr after treatment of test material, p.o., carrageenan was administered in rat hind paw

[b]Values are Mean ± SEM (n = 6); paw volume is expressed in change of height (in mm) of Hg bath (in parentheses, % inhibition of edema). One-way ANOVA; ***P*<0.01, **P*<0.05 compared to control. PBZ = Phenylbutazone.

of at least two methods as the acetic acid induced abdominal constriction test can provide false positive results (Le Bars, 2001). To investigate whether HC has true analgesic potential, radiant heat tail-flick method was also used. In the tail-flick method both the chloroform and methanolic fractions increased the stress tolerance capacity of the animals and hence also indicate the possible involvement of a higher center ((Whittle, 1964).

The carrageenan-induced paw edema model in rats is known to be sensitive to cyclooxygenase inhibitors and has been used to evaluate the effect of non-steroidal anti-inflammatory agents, which primarily inhibit the cyclooxygenase involved in prostaglandin synthesis (Seibert *et al.*, 1994). The time course of edema development in carrageenan-induced paw edema model in rats is generally represented by a biphasic curve (Vinegar *et al*, 1969). The first phase of inflammation occurs within an hour of carrageenan injection and is partly due to the trauma of injection and also to histamine and serotonin component (Crunkhorn and Meacock, 1971). Prostaglandins (PGs) play a major role in the development of the second phase of inflammatory reaction which is measured at 3 h (Di Rosa and Willoughby, 1971). The presence of PGE_2 in the inflammatory exudates from the injected foot can be demonstrated at 3 h and period thereafter ((Vinegar *et al*, 1969). Therefore, it can be inferred that the inhibitory effect of different extracts of HC on carrageenan-induced inflammation could be due to inhibition of the enzyme cyclooxygenase leading to inhibition of prostaglandin synthesis. Significant inhibition of paw edema in the early hours of study by HC could be attributed to the inhibition of histamine (Hirasawa *et al.*, 1991) and/or serotonin.

Although different extracts of *H. coronarium* exhibited significant analgesic and anti-inflammatory activities, the exact mechanisms underlying the observed pharmacological effects can only be elucidated after isolation of active constituents using a wide range of experimental models.

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