Research Paper

Antinociceptive and antiinflammatory effects of *Centella asiatica*

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Received: 24.3.2004
Revised: 23.7.2004
Accepted: 25.7.2004

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ABSTRACT

Objective: To evaluate the effects of *Centella asiatica* (CA) upon pain (antinociception) and inflammation in rodent models.

Material and Methods: The antinociceptive activity of the water extract of CA (10, 30, 100 and 300 mg/kg) was studied using acetic acid-induced writhing and hot-plate method in mice. The antiinflammatory activity of CA was studied in rats by prostaglandin E_2-induced paw edema.

Results: Water extract of CA revealed significant antinociceptive activity with both the models. The activity was statistically similar to aspirin but less potent than morphine. The CA extract also revealed significant antiinflammatory activity. This effect was statistically similar to the non-steroidal antiinflammatory drug, mefenamic acid.

Conclusion: These results suggest that the water extract of CA possesses antinociceptive and antiinflammatory activities.

KEY WORDS: Analgesic, inflammation, PGE_2

Introduction

Centella asiatica (CA) of the family Umbelliferae is commonly found in parts of India, Asia and the Middle East. It is known as 'Pegaga' in Malaysia, 'Luei Gong Gen' or 'Tung Chain' in China, 'Vallarai' in Tamil Nadu (India) and 'Daun Kaki Kuda' in Indonesia.[1] It is a perennial, herbaceous creeper growing up to 30 cm in height with fan-shaped leaves. CA has been used in traditional medicine in Asia for hundreds of years.[2] The major constituents are triterpene saponins, mainly asiaticoside, sapogenin asiatic acid, madecassoside and madecassic acid.[3] In Malaysia, this herb is commonly eaten fresh as a vegetable, especially by Malay communities. It is also believed to have beneficial effects in improving memory, and treating mental fatigue, anxiety and eczema.[4] Ayurvedic medicine has effectively used CA in the treatment of inflammation, anemia, asthma, blood disorders, bronchitis, fever, urinary discharge and splenomegaly.[5]

The aqueous extract of CA possesses antioxidant, cognitive-enhancing and antiepileptic properties.[6] Although this herb has many useful claims, the mechanism of its medicinal effects are not understood. The objectives of this study were to evaluate the antinociceptive and antiinflammatory activities of CA extract in mice and rats.

Material and Methods

Chemicals

Acetic acid, naloxone hydrochloride, prostaglandin E_2 (PGE_2), aspirin (ASA), mefenamic acid (MA) and other standard laboratory chemicals were obtained from Sigma Chemicals, Dorset, England. All drugs and extracts were dissolved in normal saline prior to use. Morphine sulfate was generously given by the Department of Biomedical Sciences, Faculty of Allied Health Sciences, National University of Malaysia, Kuala Lumpur, Malaysia.

Plant material and extraction

Centella asiatica whole plant was purchased from a local producer in Selangor, Malaysia and was authenticated by the

curator of the phytomedical herbarium, Institute of Bioscience, Universiti Putra Malaysia. Voucher specimen (SK 533/03) was deposited in the herbarium. One kg of wet CA was oven-dried at 50° C for 24 h and ground to powder. The powder was extracted using distilled water in a Soxlet apparatus according to the previously published method.[7] The resultant extract was freeze-dried and kept at –20° C prior to use.

Animals

Adult male ICR Balb/c mice (20-25 g) were used for all the antinociceptive experiments. Adult male Sprague-Dawley rats (200-250 g) were used to study the antiinflammatory activity. The animals (five per cage) were maintained under standard laboratory conditions (light period of 12 h/day and temperature 27° C ± 2° C), with access to food and water *ad libitum*. The experimental procedures were carried out in strict compliance with the Institutional Animal Ethics Committee regulations. All experiments were performed in the morning according to the guidelines for the care of laboratory animals.[8]

Acetic acid-induced writhing

The antinociceptive activity of CA was assessed using writhing test (abdominal constriction test).[9] Acetic acid solution (10 ml/kg, 0.6%) was injected intraperitoneally, and the contraction of abdominal muscles together with stretching of the hind limbs was cumulatively counted over a period of 0.5 h beginning 5 min after acetic acid injection. The extract was administered (0, 10, 30, 100 and 300 mg/kg, i.p.) 0.5 h before the acetic acid injection. Antinociceptive activity was expressed as the percentage inhibition of abdominal constrictions between control animals and mice pre-treated (n=6) with the extract using the ratio:

(Control mean – Treated mean) x 100 / Control mean

In an attempt to investigate the participation of the opioid system in the antinociceptive effect of this plant extract, separate groups of mice (n=6) were pretreated with non-specific opioid receptor antagonist, naloxone (5 mg/kg, i.p.), injected 15 min before the administration of the extract.

Hot-plate test

The hot-plate test was performed to measure response latencies according to the method previously described.[10] The hot-plate (Model 7280, Ugo Basile, Italy) was maintained at 55.0 ± 0.2° C and the animals were placed into the perspex cylinder on the heated surface and the time (sec) to discomfort reaction (licking paws or jumping) was recorded as response latency, prior to and 30, 60, 120 and 150 min after administration of the extract (0, 10, 30, 100 and 300 mg/kg, i.p.). A latency period of 20 sec was defined as complete analgesia and the measurement was terminated if it exceeded the latency period in order to avoid injury.

PGE_2-induced inflammation

Sprague Dawley rats were divided into 5 groups (n=6/group). Groups 1 and 2 were given saline i.p. Thirty min after saline injection the control group (Group 1) received 0.1 ml of saline at the hind paw and Group 2 received PGE_2 (100 IU) through intraplantar route. Groups 3 and 4 received 2 and 4 mg/kg CA extract (i.p.) respectively. This was followed by the administration of PGE_2 through the intraplantar route. Group 5 received 10 mg/kg mefenamic acid i.p. prior to PGE_2 administration. The paw volume was measured every 0.5 h for 4 h using the method published previously.[11]

Statistical analysis

Numerical results are expressed as mean±SD, unless otherwise stated. One-way analysis of variance (ANOVA) was used for statistical comparison; $P<0.05$ being the criterion for statistical significance. The significant treatment means were further subjected to Duncan multiple post test.

Results

Effect of CA extract on the acetic acid-induced writhing

As shown in Table 1, water extract of CA (10, 30, 100 and 300 mg/kg, i.p.) showed a significant dose-dependent reduction in the number of writhing with approximately 13%, 45%, 64% and 85% of inhibition respectively. Maximum inhibition was observed at the dose of 300 mg/kg, which was statistically similar to the control drug aspirin (100 mg/kg). Morphine (10 mg/kg) showed the most potent inhibition.

The mechanism of the CA-induced antinociception was investigated using the opioid receptor antagonist, naloxone. The antinociceptive effect induced by the aqueous extract of CA (300 mg/kg) was significantly antagonized by pretreatment with naloxone in the writhing test (Table 1). Naloxone also inhibited the other doses of CA in this test **(data not shown)**.

Effect of CA extract on hot-plate test

Table 2 shows the time course of the antinociception produced by the aqueous extract of CA (0, 10, 30, 100 and 300 mg/kg). Intraperitoneal administration of the extract resulted in significant and dose-dependent prolongation of the response latency in the hot-plate test. The effect reached a peak at approximately 60 min after administration and then gradually decreased.

Table 1

Effect of *Centella asiatica* extract on acetic acid-induced writhing in mice

Group (mg/kg)	*No. of writhing*	*Percentage inhibition*[1]
Control - normal saline	33.6 ± 3.9[a]	-
CA (10)	29.3 ± 3.4[a]	12.8
CA (30)	18.3 ± 3.0[b]	45.5
CA (100)	12.1 ± 3.5[c]	64.0
CA (300)	4.9 ± 2.6[d]	85.4
ASA (100)	6.25 ± 1.3[d]	81.4
Morphine (10)	0	100
CA (300) + Naloxone (5)	15.4 ± 1.5[c]	54.2
One-way ANOVA F	114.08	
df	7, 40	
P	*< 0.05*	

CA: *Centella asiatica*

Values are mean±SD; n=6 in each group

[1]Percentage inhibition when compared to control

[a-d]Significantly different at $P<0.05$

Table 2

Effect of *Centella asiatica* extract on hot-plate reaction time in mice

Group (mg/kg)		*Time (min)*					*One-way ANOVA*		
		Pre-	*30*	*60*	*120*	*150*	*df*	*F*	*P*
Control - saline		5.60 ± 0.38^{ax}	5.48 ± 0.55^{ax}	5.45 ± 0.52^{ax}	6.20 ± 0.61^{ax}	5.30 ± 0.67^{ax}	4, 25	2.373	ns
CA (10)		5.60 ± 0.36^{ax}	6.02 ± 0.35^{ax}	5.90 ± 0.49^{ax}	6.08 ± 0.43^{ax}	6.25 ± 0.41^{ax}	4, 25	2.084	ns
CA (30)		6.18 ± 0.68^{ax}	6.82 ± 0.32^{bx}	7.50 ± 0.58^{by}	7.08 ± 0.27^{bx}	7.38 ± 0.71^{by}	4, 25	5.608	<0.05
CA (100)		5.73 ± 0.28^{ax}	7.75 ± 0.33^{cy}	8.13 ± 0.55^{by}	8.00 ± 0.32^{cy}	7.42 ± 0.92^{by}	4, 25	19.843	<0.05
CA (300)		5.72 ± 0.18^{ax}	8.83 ± 0.28^{dy}	9.42 ± 0.76^{cy}	8.85 ± 0.32^{dy}	8.97 ± 0.60^{cy}	4, 25	58.174	<0.05
CA (300) + Naloxone (5)		5.93 ± 0.58^{ax}	7.50 ± 0.57^{cy}	7.18 ± 0.65^{by}	7.50 ± 0.20^{bbcy}	7.12 ± 0.41^{by}	4, 25	9.760	<0.05
One-way ANOVA	F	1.535	51.513	35.547	46.737	22.031			
	df	5, 30	5, 30	5, 30	5, 30	5, 30			
	P	*ns*	*< 0.05*	*< 0.05*	*< 0.05*	*< 0.05*			

CA=*Centella asiatica.* Data expressed as mean±SD (sec); n=6 in each group
[a-d]Significantly different at *P*<0.05, in the same column
[x-y]Significantly different at *P*<0.05, in the same row

Table 3

Effect of *Centella asiatica* on prostaglandin E_2-induced hind paw edema in rats

Time (min)	*Control (Saline)*	*Control (MA 10 mg/kg)*	*CA (mg/kg)*			*One-way ANOVA*		
			2	*4*	*10*	*df*	*F*	*P*
0	0.22 ± 0.03^{ax}	0.20 ± 0.03^{ax}	0.27 ± 0.04^{ax}	0.27 ± 0.04^{ax}	0.26 ± 0.03^{ax}	4,25	2.186	ns
30	0.63 ± 0.03^{bx}	0.62 ± 0.01^{ax}	0.59 ± 0.06^{bx}	0.59 ± 0.07^{bx}	0.55 ± 0.08^{bx}	4,25	1.849	ns
60	0.62 ± 0.03^{bx}	0.54 ± 0.11^{ax}	0.54 ± 0.07^{bx}	0.56 ± 0.06^{bcx}	0.50 ± 0.04^{bcx}	4,25	2.494	ns
90	0.63 ± 0.03^{bx}	0.50 ± 0.89^{ax}	0.55 ± 0.06^{bx}	0.52 ± 0.07^{bcdx}	0.48 ± 0.03^{bcdx}	4,25	0.1282	ns
120	0.62 ± 0.03^{bx}	0.57 ± 0.07^{ax}	0.53 ± 0.06^{bx}	0.49 ± 0.06^{cdey}	0.44 ± 0.04^{cdey}	4,25	9.966	<0.05
150	0.63 ± 0.02^{bx}	0.55 ± 0.05^{ax}	0.56 ± 0.08^{bx}	0.44 ± 0.05^{defy}	0.39 ± 0.04^{dey}	4,25	21.112	<0.05
180	0.62 ± 0.04^{bx}	0.51 ± 0.07^{ay}	0.51 ± 0.07^{by}	0.41 ± 0.05^{defyz}	0.37 + 0.08^{ez}	4,25	14.158	<0.05
210	0.62 ± 0.03^{bx}	0.48 ± 0.06^{axy}	0.49 ± 0.18^{bxy}	0.39 ± 0.06^{efy}	0.33 ± 0.06^{aey}	4,25	8.279	<0.05
240	0.63 ± 0.04^{bx}	0.47 ± 0.06^{ay}	0.49 ± 0.17^{bxy}	0.37 ± 0.06^{efyz}	0.31 ± 0.02^{aez}	4,25	11.158	<0.05
One-way ANOVA F	109.5	0.9363	5.241	18.034	21.346			
df	8, 45	8,45	8,45	8,45	8,45			
P	*< 0.05*	*ns*	*< 0.05*	*< 0.05*	*< 0.05*			

MA=mefenamic acid; CA=*Centella asiatica.* Data expressed as mean±SD (cm^3)
[a-f]Significantly different at *P*<0.05, in the same column
[x-z]Significantly different at *P*<0.05, in the same row

Effect of CA extract on PGE_2-induced inflammation

The extracts of CA elicited dose-dependent antiinflammatory activity (Table 3). At 2 mg/kg concentration, the extract had mild antiinflammatory property. However, at 4 and 10 mg/kg, the antiinflammatory activity was significantly different from the control ($P<0.05$). The antiinflammatory activity of mefenamic acid was found to be similar to that of the CA (4 mg/kg). Interestingly, the 10 mg/kg CA showed a significantly higher effect when compared to mefenamic acid.

Discussion

Administration of CA extracts showed significant antinociceptive activity in the hot-plate and acetic acid-induced writhing tests (Table 1 and 2). These results indicate that the plant extract possesses centrally and peripherally mediated antinociceptive properties.[12]

The hot-plate method is one of the most common tests for evaluating the analgesic efficacy of drugs/compounds in rodents.[13] However, care must be taken for drugs/compounds that produce false positive results by modifying the behavior of the rodents.[14] The inhibition of antinociception by naloxone, an opioid receptor antagonist, in both the hot-plate and abdominal writhing test revealed these results were positive and the mechanism of CA antinociception might involve opioid receptors. The potency of antinociception was less than morphine and aspirin at similar doses.

Intraperitoneal administration of CA reduced the PGE_2-induced paw edema significantly. The extract showed significant antiinflammatory activity even at 2 mg/kg when compared to control and larger dose was found to be more effective then mefenamic acid. The antiinflammatory activities of various herbs have been closely related to the high content of triterpenes.[15]

Inamdar *et al* [3] reported bioactive terpene acids such as asiatic acid and madecassic acid from the water-methanol extraction of CA. These phytocompounds may be present in the crude extract of CA that may account for the antinociceptive and antiinflammatory activities. The results from this present study strongly indicate that the water extract of CA possesses antinociceptive and antiinflammatory activities. These findings justify the traditional use of this plant in the treatment of inflammatory conditions or rheumatism. Further biological studies using major phytochemicals from CA, asiaticoside, sapogenin asiatic asid, madecassoside and madecassic acid, are suggested to determine the active chemical(s) responsible for these activities of the extract.

Acknowledgements

This study was supported by the State government of Selangor, Malaysia and Universiti Putra Malaysia.

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