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Water Extract of Leaves and Stems of Preflowering but not Flowering Plants of *Anisomeles indica* Possesses Analgesic and Antihyperalgesic Activities in Rats

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Abstract

According to Sri Lankan traditional medicine, a decoction made from stems and leaves of *Anisomeles indica* Kuntze (Lamiaceae) possesses analgesic activity. However, the validity of this claim has not been scientifically tested. The aim of this study was to investigate analgesic and antihyperalgesic activities of this plant using a water extract made from the leaves and stems. The water extracts were made from leaves and stems of both preflowering (E1) and flowering plants (E2). E1 showed a dose-dependent analgesic effect up to 6 h of treatment when tested in rats using the hot plate and the tail flick techniques. Further, the analgesic effect of E1 was not accompanied by toxic effects. This effect was neither gender dependent nor dependent on the stage of the estrous cycle. E1 also showed a dose-dependent antihyperalgesic activity in the hot plate test. In contrast, E2 did not show any analgesic effect (500 mg/kg). The analgesic effect produced by E1 was not abolished by naloxone. E1 dose-dependently retarded the amplitude of the spontaneous contractions of isolated dioestrous rat uterus. Further, E1 induced a dose-dependent plasma membrane stabilisation effect on rat erythrocytes. Collectively, these observations suggest that the analgesic and antihyperalgesic effects of E1 are mediated from inhibition of COX-1, thus impairing the synthesis of prostaglandins. A change in chemical contents that accompanies flowering could be one possible reason for the inability of E2 to demonstrate analgesic effect.

Keywords: *Anisomeles indica* Kuntze, analgesia, antihyperalgesia, COX-1 inhibition, Sri Lanka.

Introduction

Anisomeles indica Kuntze (Lamiaceae), Yakwanassa in Sinhala and Peyameratti in Tamil, is a large perennial herb which commonly grows in the wild in Sri Lanka (Jayaweera, 1981). In Sri Lankan traditional medicine, a decoction made from aerial parts of this plant, irrespective of its growth stage, has been used for many years as an analgesic. However, so far, no attempts have been made to scientifically validate the claimed analgesic activity of *A. indica*.

The main aim of this study was therefore to assess the analgesic and antihyperalgesic potential of decoctions made from leaves and stems of preflowering and flowering plants of *A. indica* using rats. The other aim was to investigate its sub-chronic toxicological effects in view of recent publications on toxic effects of herbal extracts (Hsu et al., 1998; Penn, 1988).

Materials and methods

Chemicals

Aspirin (State Pharmaceutical Corporation, Colombo, Sri Lanka), carrageenan (Sigma Chemical Company St. Louis MO, USA), charcoal and formalin (BDH Chemicals, Poole, England), ether and naloxone hydrochloride (Fluka, Buchs, Switzerland), methyl cellulose (Griffin and George Ltd., London, UK), AST and ALT assay kits (Randox Laboratories Ltd., Co. Antrim, UK) were used.

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Collection of plants and preparation of plant extracts

Fresh *A. indica* plants at the preflowering and flowering stages were collected separately from an abandoned field area around Colombo, Sri Lanka between September and November 1998 and were authenticated by Professor R.N. de Fonseka, Department of Botany, University of Colombo, Sri Lanka. A voucher specimen (No. 20 – AI) has been deposited in the museum of the Department of Zoology, University of Colombo. The two categories of plants were cut into small pieces and boiled separately in distilled water (200 g of plant material per litre of water) under reflux conditions, for 3 h. After 3 h, the boiled extracts were filtered through cotton wool and each filtrate was further reduced to 100 ml by boiling under reduced pressure. The concentrated extracts were then freeze-dried. The freeze-dried extracts were dark brown solid masses (yield: 5%) which were made into small pieces using a glass rod. The masses obtained from the preflowering and the flowering plants were designated E1 and E2, respectively.

Phytochemical tests and pH of the extract

The presence or the absence of alkaloids, phenols, coumarins, triterpenoids, amino acids and peptides, saponins, steroids and flavonoids in E1 and E2 were investigated using the standard tests described by Farnsworth (1966). The pH of the extracts was determined using a pH meter (TOA Electronics, Tokyo, Japan).

Animals

Cross-bred albino rats from a colony maintained at the Department of Zoology, University of Colombo were used: males and females weighing 150–200 g (for testing analgesic activity), and males weighing 250–300 g (for toxicological studies). The rats were housed under standardised animal house conditions (temperature; 28–31 °C, photoperiod: approximately 12 h natural light and relative humidity: 55–60%) and had access to food (Vet House Ltd., Colombo, Sri Lanka) and water *ad libitum*.

Treatment of animals with the plant extracts

Appropriate weights of E1 and E2 were dissolved in distilled water (DW) to obtain 125, 250 and 500 mg/kg of body weight in 1 ml solution (both E1 and E2 showed saturation at 500 mg/kg in 1 ml). After oral treatment of either E1 or E2 the animals were continuously observed for 1 h for overt signs of acute clinical toxicity (lachrymation, salivation, diarrhoea) or stress (exopthalmia, fur erection).

Evaluation of analgesic effects of E1 and E2

Eighty-four male rats were randomly divided into seven equal groups (n = 12/group). The rats in group 1 were orally treated with 1 ml of DW, group 2 with 500 mg/kg of E2 and groups 3, 4, and 5, respectively, with 125, 250 and 500 mg/kg of E1 in 1 ml DW. The rats in group 6 were orally treated with 133.5 mg/kg aspirin suspended in 1 ml of 1% methyl cellulose while those in group 7 were treated with 1 ml 1% methyl cellulose.

Certain analgesics show a difference in effectiveness between the sexes and among different stages of the estrous cycle in rats (Kavaliers & Innes, 1993). To determine whether there is such a difference in the analgesic effect of *A. indica*, 60 female rats in three different stages of their estrous cycle were selected by microscopic examination of their vaginal smears (diestrus: n = 24, pro-estrus: n = 12, estrus: n = 24) and treated as follows. Rats in each stage of the oestrous cycle were randomly divided into two equal groups. Half the number of rats in each group were treated orally with 500 mg/kg E1 (in 1 ml). The balance in each group were used as controls and treated orally with 1 ml DW/rat.

The time taken to experience pain in seconds (reaction time) of rats in each of the above groups was determined three hours before and 1, 3 and 6 h post-treatment using hot plate and tail flick techniques as described by Langerman et al. (1995). In the hot plate technique, the rat was placed on an enclosed hot plate (Model MK 35 A, Muromachi Kikai Co. Ltd., Tokyo, Japan) at 50 °C and the time taken to lick the hind paw or to jump up (the reaction time) was noted. In the tail flick technique, the tail of a rat was immersed 3–4 cm from its tip, in a water bath at 55 °C and the time taken to flick the tail (the reaction time) was noted. A cutoff time for the hot plate reaction time was set at 20 s while for the tail flick it was set at 10 s. The percentage maximum possible effect (% MPE) of the analgesic effect was calculated using the following equation:

$$\%\ \text{MPE} = \frac{(\text{post-treatment latency}) - (\text{per-treatment latency})}{(\text{cutoff latency}) - (\text{pre-treatment latency})} \times 100$$

Evaluation of antihyperalgesic activity of E1

Forty-eight male rats were randomly divided into four equal groups. The rats in groups 1–3 were orally treated with 125, 250 and 500 mg/kg of E1 respectively and those in the group 4 with 1 ml of DW. Immediately afterwards, the rats in all the groups were injected with 0.05 ml of 1% carrageenan suspension in normal saline into the plantar surface of the left hind paw (Richardson et al., 1998). One hour later, the reaction time of these rats was assessed using the hot plate technique as described previously.

Testing of opioid receptor mediation in inducing analgesia

Naloxone abolishes the analgesic effects mediated through opioid receptors (Ganong, 1995). Male rats (n = 24) were

randomly divided into two equal groups. The rats in one group were subcutaneously injected with 5 mg/kg naloxone hydrochloride in 0.1 ml normal saline while those in the other group were treated with 0.1 ml of normal saline (Ratnasooriya & Dharmasiri, 1999). Forty-five minutes later, the rats in both groups were orally treated with 500 mg/kg E1 in 1 ml water. After 1 h, the reaction times of these rats were assessed using the hot plate and tail flick techniques as described previously.

Prostaglandin synthesis inhibition activity

This test was based on the claim that the cyclooxygenase I (COX-I) inhibitor indomethacin inhibits the spontaneous contractions of the rat uterus *in vitro* (Lindsey et al., 1999; Uguru et al., 1998). Therefore, COX-I inhibitory effect of E1 was tested as follows.

Nine female rats in diestrus (Perusquia et al.,1993) were selected by microscopic examination of vaginal smears. They were sacrificed with an overdose of ether, their uterine horns removed, and cut into approximately 1 cm pieces. These uteri were individually placed in a 50 ml organ bath containing Kreb's Henseleit solution having the following composition (mmol/l): Na^+ 143, K^+ 5.8, Ca^{2+} 2.6, Mg^{2+} 1.2, Cl^- 128, $H_2PO_4^-$ 1.2, HCO_3^- 25, SO_4^{2-} 1.2 and glucose 11.1 at a pH of 7.4. The organ bath was maintained at 37 °C and aerated with a gas mixture of 95% O_2 and 5% CO_2. The spontaneous activity of the uteri was recorded isometrically under a resting tension of 1 g until the contractions became regular (usually within a 10 min) using an isometric sensor (Star Medicals, Tokyo, Japan). After the contractions became regular, the normal activity of the uteri was recorded for a further 10 min. Following this, E1 was added sequentially into the organ bath so that the final concentrations became 10, 20, 30, and 40 µg/ml in the organ bath (n = 3/dose). Ten µg/ml of aspirin (n = 3) was used as the reference drug to inhibit COX-I (Rang et al., 1995). After each treatment the spontaneous activity of the uteri was further recorded for 20 min. The amplitude and frequency of contractions, the lag period for the cessation of contractions and the EC_{50} for the inhibition of the contractions were calculated.

Plasma membrane stabilization action of E1

Indomethacin, a COX-1 inhibitor stabilises the plasma membrane of rat erythrocytes *in vitro* (Perez et al., 1995). This property of E1 was tested using heat-induced haemolysis as modified by Ratnasooriya and Dharmasiri (1999). Twenty µl of uncoagulated fresh rat blood was added into vials containing 1.00 ml of 0.15 M phosphate buffered saline (PBS) (pH = 7.4). These vials were treated with either aspirin (n = 3) or E2 in PBS (n = 3), so that the final concentrations of the vials were 50, 100, 150 µg/ml E1 or 50 µg/ml of aspirin and for controls (n = 3) 15 µl of PBS. Thereafter, the vials were mixed well and incubated at 37 °C for 15 min followed by 25 min at 54 °C. The vials were centrifuged at 3200 g for 2 min. The absorbance of the supernatant was measured at 540 nm using a spectrophotometer (JASCO V500, Jasco Corporation, Tokyo, Japan). The percentage inhibition of haemolysis with respect to the controls was calculated.

Effects on intestinal motility

Certain non-steroidal anti-inflammatory drugs (NSAID) used as analgesics cause diarrhoea by increasing intestinal motility (Laurence & Bennett, 1992). To investigate whether E1 has this property 12 male rats were starved for 24 h but water was provided *ad libitum*. These rats were randomly divided into two equal groups. The groups were orally treated either with 500 mg/kg E1 or with 1 ml DW. One hour later, the rats in both groups were orally treated with 5% charcoal suspension. After 20 min these rats were sacrificed with ether, the small intestines removed gently, stretched and total length of the small intestines and the distance travelled by the charcoal from the pyloric end towards the caecal end was measured. The percentage distance traveled was calculated as described by Akah et al. (1998).

Induction of gastric lesions

To investigate whether E1 causes gastric lesions like NSAID used as analgesics (Rang et al., 1995), male rats (n = 12) were starved for 24 h but provided with water *ad libitum*. These rats were randomly divided into two equal groups. These groups were orally treated either with 500 mg/kg E1 or with 1 ml DW. Five hours later these rats were sacrificed with ether, the stomachs removed, fixed with 10% formalin, opened along the greater curvature and examined for haemorrhagic lesions using a magnifying lens (Puntero et al., 1997).

Sedative activity

Twenty-four male rats were randomly divided into two equal groups. The animals in one group were orally treated with 1 ml of 500 mg/kg E1 and the other group was treated orally with 1 ml DW. After 1 h, the rats were individually placed in the centre of the rat hole board and observed for 7.5 min. During this period, the number of rears, number of head dips, locomotory activity, number of faecal boluses produced and the time spent for head dips were noted and the time spent per head dip was then calculated according to File and Wardill (1975).

Effects on muscle strength and coordination

Twenty-four male rats were randomly divided into two equal groups. The rats of one group were orally treated with 1 ml of 500 mg/kg E1, while those in the other group were orally treated with 1 ml DW. After 1 h the rats were individually subjected to a bar-holding test to evaluate muscle strength and followed by the Bridge test to evaluate muscle coordination

and the latency to fall in the bar-holding test and the latency to slide off in the Bridge test (in s) was recorded up to 1 min, respectively (Plaznic et al., 1993).

Haemotoxicity, hepatotoxicity and effect on body weights

Twelve male rats were randomly divided into two equal groups. The rats in one group were orally treated with 1 ml of 500 mg/kg E1 daily for seven consecutive days (between 9 and 12 h). Those in the other group were treated with 1 ml DW in the same manner. These rats were observed during the treatment period for overt clinical signs of toxicity and stress. In addition, their food and water intake was noted. On day 8, blood was collected from the tails of these rats and their red blood cell counts, white blood cell counts (total and differential), haemoglobin content and packed cell volume were assessed as described by Ghai (1993). Another 12 rats were randomly divided into two equal groups and were treated as described above to investigate the hepatotoxic activity of E1. On day 1 post-treatment, the body weights of these rats were measured using an electronic balance (MP 6000, Chyo Balance Corporation, Tokyo, Japan). The rats were then lightly anaesthetized with ether, blood collected by cardiac puncture and allowed to clot for 15–20 min. The blood was then centrifuged at 3200 g for 15 min, serum separated and the activities of Aspartate Transaminase (AST) and Alanine Transaminase (ALT) in each serum sample assessed using standard enzyme assay kits.

Statistics

The results are expressed as means ± SEM. The statistical analyses were made using Student's *t*-test, Mann-Whiney *U*-test, one-way ANOVA and linear regression analysis. A P value ≤0.05 was considered as significant.

Results

Phytochemicals and pH of E1 and E2

Qualitative chemical test revealed the presence of alkaloids, phenols, saponins, triterpenoids and coumarins in both E1 and E2. Aqueous solutions of E1 and E2 had a pH of 5.8. However, E1 was more hygroscopic than E2.

Analgesic effect

A comparison of the reaction time of rats treated with E2 and the controls, as assessed by the hot plate and the tail flick tests and % MPE showed that E2 did not exert a significant analgesic effect. In contrast, E1 exerted a significant and dose-dependent analgesic effect (for the 1st h: $r^2 = 1$; $P < 0.01$, for the 3rd: $r^2 = 0.99$; $P < 0.01$, for the 6th $r^2 = 0.94$; $P < 0.01$) in both the above tests and in the % MPE (Tables 1 and 2). EC_{50} for the analgesic effect was 176.4 mg/kg at 1 h, 489.6 mg/kg for 3 h and 1532.7 mg/kg for 6 h post-treatment in the hot plate test.

The analgesic effect of E1 is higher than aspirin at 1 h (250 mg/kg by 35% and 500 mg/kg by 45%).

Table 1. Effects of oral treatment of different doses of pre-flowering (E1) and flowering (E2) plant extracts of *A. indica* on the reaction times of rats in hot plate and tail flick tests (means ± SEM, n = 12 per group).

	Reaction time(s)							
	Hot plate				Tail flick			
	Pretreatment	1 h	3 h	6 h	Pretreatment	1 h	3 h	6 h
Control	13.4 ± 1.3	11.7 ± 1.3	12.1 ± 1.0	11.5 ± 1.0	2.9 ± 0.3	2.3 ± 0.2	2.3 ± 0.1	2.5 ± 0.2
125 mg/kg E1	16.3 ± 0.7	16.3 ± 0.7	16.0 ± 1.1	13.2 ± 1.0	3.5 ± 0.3	3.0 ± 0.3	3.0 ± 0.1	3.0 ± 0.2
250 mg/kg E1	14.2 ± 1.0	19.3 ± 2.3**	16.8 ± 2.0*	13.8 ± 0.7*	2.8 ± 0.2	4.3 ± 0.3**	3.1 ± 0.2**	0.9 ± 0.2*
500 mg/kg E1	13.4 ± 1.1	25.4 ± 3.9**	18.2 ± 1.1**	14.3 ± 1.0*	3.2 ± 0.4	3.8 ± 0.6**	3.3 ± 0.4**	3.3 ± 0.4
500 mg/kg E2	12.5 ± 0.7	12.6 ± 1.1	13.6 ± 0.9	12.1 ± 1.4	2.3 ± 0.2	2.6 ± 0.2	2.4 ± 0.1	2.4 ± 0.2
1% methyl cellulose	12.53 ± 0.8	11.47 ± 0.68	11.5 ± 0.9	11.30 ± 1.0	2.8 ± 0.3	3.03 ± 0.3	3.3 ± 0.3	2.7 ± 0.1
133.5 mg/kg Aspirin	11.09 ± 1.1	17.5 ± 2.3*	16.0 ± 2.3	11.7 ± 1.1	2.6 ± 0.2	3.2 ± 0.3	3.3 ± 0.4	2.7 ± 0.4

As compared with controls: *$P < 0.05$, **$P < 0.01$ (Student's *t*-test).

Table 2. Effect of oral treatment of different doses of pre-flowering (E1) and flowering (E2) plant extarcts of *A. indica* on the % maximum possible effect (MPE) of hot plate and tail flick test on rats (means ± SEM, n = 12 per group).

	% MPE					
	Hot plate			Tail flick		
	1 h	3 h	6 h	1 h	3 h	6 h
Control	–133.1 ± 92.4	–107.2 ± 63.1	–157.4 ± 93.9	–9.8 ± 4.2	–10.0 ± 5.1	–9.7 ± 5.0
125 mg/kg E1	–62.0 ± 193.0	–283.0 ± 376.0	–455.0 ± 268.0	–11.9 ± 9.5	11.4 ± 6.3	–11.5 ± 5.8
250 mg/kg E1	0.0 ± 105.0*	–15.6 ± 84.2	–64.7 ± 55.0	20.2 ± 4.0**	2.8 ± 3.7**	0.2 ± 3.0
500 mg/kg E1	153.1 ± 90.6*	–53.0 ± 126.0*	–124.0 ± 132.0	10.0 ± 9.0**	0.7 ± 4.8*	0.5 ± 6.0
500 mg/kg E2	–5.6 ± 15.8	9.2 ± 14.2	–15.7 ± 21.4	1.9 ± 2.7	–1.9 ± 2.4	1.8 ± 2.6

As compared with controls: *$P < 0.05$, **$P < 0.01$ (Mann-Whitney *U*-test).

Table 3. Effects of oral treatment of 500 mg/kg of pre-flowering (E1) plant extracts of *A. indica* on the reaction times of female rats in three different stages of their estrous cycle in the hot plate and tail-flick tests (means ± SEM, C – Control; T – treated).

			Reaction time(s)							
			Hot plate				Tail flick			
Stage of the oestrous cycle		n	Pre-treatment	1 h	3 h	6 h	Pre-treatment	1 h	3 h	6 h
Pro-estrous	C	6	11.5 ± 1.2	11.3 ± 0.9	10.9 ± 0.7	11.2 ± 0.8	2.4 ± 0.2	2.3 ± 0.2	2.2 ± 0.2	2.4 ± 0.3
	T	6	12.1 ± 0.9	23.0 ± 4.7*	15.1 ± 2.7	11.1 ± 0.9	2.4 ± 0.2	4.3 ± 0.8*	2.9 ± 0.4	2.2 ± 0.2
Estrous	C	12	11.5 ± 1.0	11.4 ± 0.7	10.9 ± 1.0	11.5 ± 0.9	2.2 ± 0.1	2.0 ± 0.1	1.9 ± 0.1	1.9 ± 0.1
	T	12	10.7 ± 0.8	22.3 ± 1.5**	12.9 ± 1.5	8.5 ± 0.9	2.1 ± 0.1	3.2 ± 0.3**	2.0 ± 0.1	2.0 ± 0.1
Diestrous	C	12	14.8 ± 0.8	14.0 ± 0.8	14.6 ± 0.7	14.7 ± 0.8	3.7 ± 0.4	3.4 ± 0.3	3.8 ± 0.4	3.4 ± 0.3
	T	12	15.9 ± 0.7	23.7 ± 1.5**	13.8 ± 0.7	15.0 ± 0.8	3.4 ± 0.3	4.5 ± 0.5*	3.4 ± 0.3	3.1 ± 0.2

As compared with controls: *$P < 0.05$, **$P < 0.01$ (Student's *t*-test).

In the females, the analgesic effect produced by 500 mg/kg of E1 was not significantly different among the three different stages of their oestrous cycle. However, the reaction time was significantly ($P < 0.05 - 0.01$) increased in all three groups compared to their respective controls after 1 h of post-treatment in the hot plate and tail flick tests (Table 3). However, in the females the analgesic effect was not significant at both 3 h and 6 h post-treatment.

Antihyperalgesic effect

Oral administration of E1 showed a significant ($P < 0.01$) and dose-dependent ($r^2 = 0.99$, $P < 0.05$) increase in the reaction time of injured rats at 1 h post-treatment in the hot plate test demonstrating its antihyperalgesic activity (reaction time: control vs. 125 mg/kg vs. 250 mg/kg vs. 500 mg/kg; 11.8 ± 0.8 vs. 13.1 ± 2.0 vs. 15.7 ± 1.9 vs. 22.7 ± 3.0 s). The EC_{50} for the antihyperalgesic activity was 314.29 mg/kg. This EC_{50} is higher (by 44%) than that of E1 for the analgesic effect at 1 h in the hot plate test.

Opioid receptor mediation

In the naloxone experiment, naloxone hydrochloride did not significantly impair the analgesia produced by E1 (naloxone + E1 vs. saline + E1; reaction time in hot plate: 25.2 ± 4.5 vs. 22.9 ± 1.6 s, tail flick: 3.5 ± 0.2 vs. 3.3 ± 0.1 s).

Prostaglandin synthesis inhibition activity

E1 reduced the amplitude of spontaneous contractions of the isolated diestrous rat uterus in a dose-dependent manner ($r^2 = 0.99$, $P < 0.01$). The EC_{50} for the inhibition of the contractions was 24.2 µg/ml of E1. Ten µg/ml aspirin also inhibited the amplitude of contractions of the uterus but not the frequency. On the other hand, E2 had no significant inhibitory effect on the spontaneous contractions of the uterus (Table 4). The lag periods for the initiation of the inhibition of contractions were 190.0 ± 50.1 s for E1 and 240.0 ± 30.0 s for aspirin.

Table 4. Effects of different concentrations of pre-flowering (E1) plant extracts of *A indica* and aspirin on the spontaneous contractions of the isolated dioestrous uterus of rat (means ± SEM, n = 3 per dose).

	Amplitude of contractions (g)		Frequency of contractions/min	
	E1	Aspirin	E1	Aspirin
Normal uterus	0.89 ± 0.12	0.86 ± 0.16	4.17 ± 0.42	1.59 ± 0.42
Concentration of E1/Aspirin				
10 µg/ml	0.66 ± 0.08	0.28 ± 0.01	3.90 ± 0.61	1.67 ± 0.31
20 µg/ml	0.57 ± 0.05		3.10 ± 0.81	
30 µg/ml	0.48 ± 0.09		3.07 ± 0.43	
40 µg/ml	0.34 ± 0.02		3.10 ± 0.53	

For E1, amplitude of the contractions dose-dependently decreased ($r^2 = 0.99$, $P < 0.01$).

Table 5. Effects of different concentrations of pre-flowering (E1) plant extracts of *A. indica* and aspirin on the haemolysis induced by heat *in vitro.*

	% inhibition of haemolysis	
Concentration (µg/ml)	E1	Aspirin
50	48.7 ± 0.6	13.7 ± 0.7
100	53.1 ± 0.7	18.3 ± 1.3
150	55.1 ± 0.6	23.9 ± 1.3
200	58.5 ± 0.6	30.5 ± 0.9
250	59.2 ± 0.7	40.1 ± 1.4
300	62.8 ± 0.7	47.5 ± 1.2

Percentage inhibition was dose dependent ($r^2 = 0.97$, $P < 0.01$).

Plasma membrane stabilising activity

E1 caused a dose-dependent inhibition of haemolysis induced by heat as seen with aspirin *in vitro* ($r^2 = 0.97$, $P < 0.01$) (Table 5). The EC_{50} for the inhibition by E1 was 57.0 µg/ml while that of by aspirin was 326.7 µg/ml.

Intestinal motility

500 mg/kg E1 did not produce a significant effect on the motility of the small intestine as indicated by the % distance the charcoal had travelled in the small intestine (control vs. treatment: 53.9 ± 2.3 vs. 51.8 ± 2.0%).

Gastric lesions

This treatment also failed to cause any macroscopic haemorrhagic lesions in the mucosa of the stomach at 5 h of post-treatment.

Sedative effect

The analgesia induced by the single oral dose of E1 was not accompanied by a significant sedative effect in terms of the observed parameters in the rat hole board test (number of rears: control vs. E1 22.7 ± 2.6 vs. 21.8 ± 2.6; number of head dips: control vs. E1 6.6 ± 1.6 vs. 8.8 ± 1.3; locomotory activity: control vs. E1 19.0 ± 1.6 vs. 17.7 ± 2.5; number of faecal boluses: control vs. E1 2.8 ± 0.6 vs. 2.9 ± 0.3; time/head dip; control vs. E1 0.9 ± 0.1 vs. 0.9 ± 0.1 s).

Effects on muscle strength and coordination

A single dose of 500 mg/kg E1 failed to induce a significant impairment in the muscle strength (latency to fall in the bar holding test; control vs. treatment: 56.8 ± 2.1 vs. 56.2 ± 2.1 s) or muscle coordination (latency to slide off in the Bridge test; control vs. treatment 56.6 ± 1.6 vs. 56.6 ± 1.7 s).

Haemotoxicity, hepatotoxicity, effect on body weights and other toxic effects

Seven-day oral treatment of 500 mg/kg E1 did not bring about significant haemotoxic effects in rats according to the blood parameters investigated (data not shown) and in the body weights (control vs. treatment 282.2 ± 5.8 vs. 268.8 ± 6.8). This treatment also had not caused a significant elevation in the activities of serum AST and ALT (AST control vs. treatment: 41.3 ± 5.4 vs. 38.7 ± 5.8 U/l, ALT control vs. treatment 16.8 ± 0.8 vs. 17.0 ± 1.2 U/l). Treatment with E1 did not bring about overt signs of acute clinical toxicity stress or suppression of food and water intake according to the general observations.

Discussion

The results show that E1 but not E2 possesses a powerful analgesic and antihyperalgesic activity. The analgesic effect had a rapid onset (1 h of treatment) and a fairly long duration of action (up to 6 h of post-treatment) and likely to be mediated predominantly through supraspinal mechanisms (Hough et al., 1999). The results obtained with the bar-

holding test and the Bridge test show that the highest dose of E1 does not impair the muscle strength and coordination along with the analgesic effect produced by it. Therefore, the increase in the reaction time in the hot plate is due to a true analgesic effect because drugs causing the relaxation of muscles produce false positive results in the hot plate test (Grasioso et al., 1998). This analgesic effect was neither associated with overt signs of clinical toxicity nor stress nor gastric lesions nor altered intestinal motility, nor depressed body weight. Further, even a seven-day treatment of E1 was not haemotoxic or hepatotoxic or renotoxic (Dharmasiri, 2001). The water and food intake of treated rats appeared to be comparable with that of control. The non-induction of gastric lesions and the unalteration of intestinal motility by E1 make it superior to NSAIDs which, usually produces gastric lesions and diarrhoea (Laurence & Bennett, 1992).

The analgesic effect of E1 was neither gender dependent nor dependent on the stage of the estrous cycle, indicating its equipotency in both sexes. However, its duration of action was shorter in females than in males. This may be due to rapid metabolism of the active component in females. The powerful antihyperalgesic activity of E1 shows its ability to suppress chronic pain. The analgesic potency of E1 is higher than that reported for some other plants such as *Psychotria sarmentosa* (Ratnasooriya & Dharmasiri, 1999) and *Mucuna prurita* (Ratnasooriya et al., 1999). E1 also showed a higher analgesic activity than 133.5 mg/kg aspirin, a dose equivalent to normal therapeutic doses administered to humans for pain-relieving purposes (Laurence & Bennett, 1992).

Antinociceptive effects can be brought about by sedatives (Rang et al., 1995). However, E1 had no sedative effect when tested in the rat hole board. The negative results obtained in the naloxone hydrochloride test excludes the possibility of opioid receptor mediation in the induction of analgesia either through a direct involvement of E1 with the receptors or indirectly via the E1-induced release of endogenous opioid peptides due to stress (Ganong, 1995). Further, the H_1-receptor agonists produce analgesic effects (Hough et al., 1999). H_1 agonists usually increase the uterine contractions (Rang et al., 1995). Since E1 caused a decrease in the contractility of rat uterus, an analgesic effect through H_1-receptor mediation is also unlikely.

Cyclooxygenase-I (COX-I) inhibitors such as aspirin and indomethacin are potent analgesics which impair the synthesis of prostaglandins involved in the normal sensation of pain (Rang et al., 1995). The antinociceptive effect caused by E1 could most probably be due to the inhibition of COX-I since E1 dose-dependently impaired the amplitude of spontaneous contractions of the isolated dioestrous rat uterus as seen with aspirin. The spontaneous activity of the isolated dioestrous rat uterus is claimed to be initiated by prostaglandins (PG) synthesised via the COX-I pathway (Lindsey et al., 1999). The long delay to inhibit the contractions of the dioestrous rat uterus both by E1 and aspirin excludes the possibility of direct receptor mediation in the inhibition process: direct receptor mediated effects on the spontaneous activity of rat uterus are usually visible within 30–60 s of treatment *in vitro* (Perusquia et al., 1993). E1 dose-dependently stabilised the plasma membrane of rat erythrocytes (as judged by the inhibition of heat induced haemolysis of rat blood) providing further support to the notion that E1 has COX-I inhibitory activity: both aspirin (in this study) and indomethacin (Perez et al., 1995), which are COX-I inhibitors, stabilise the plasma membrane of rat erythrocytes *in vitro*. COX-1 inhibitors, like aspirin and indomethacin, have analgesic and antihyperalgesic activities (Rang et al., 1995). Thus, the analgesic and the antihyperalgesic activities of E1 can be related to its COX-1 inhibitory activity. However, the antihyperalgesic activity of E1 is less potent than its analgesic activity as indicated by the EC_{50} s of E1 for the two activities.

Phytochemical tests revealed that the classes of phytochemicals present were common to E1 and E2. However, a difference in phytochemical composition between E1 and E2 has been reported (Dharmasiri et al., 2000). This chemical difference may account for the lack of analgesic activity in E2. However, this study shows for the first time a change of a bioactivity of a plant with flowering. Further, this study reveals the importance of the consideration of the growth stage of plants if anticipated therapeutic effects are to be obtained.

In conclusion, the results of this study provide scientific support for the use of *A. indica* as an analgesic in the traditional medicine of Sri Lanka. However, it is essential to use pre-flowering plants to induce analgesic or antihyperalgesic effects. It may be possible to develop a potent analgesic and antihyperalgesic drug with minimal side effects from *A. indica*.

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