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Tender Leaf Extract of *Terminalia catappa* Antinociceptive Activity in Rats

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Abstract

In Sri Lankan folklore, the juice of tender leaves of *Terminalia catappa* L. (Combretaceae) is recommended for pains, including headaches. However, the validity of this claim is not fully scientifically proven. The aim of this study was to investigate analgesic, antihyperalgesic and anti-inflammatory activities of this juice/extract in rats. Tender leaves were macerated in a mortar to obtain juice/extract (40% v/w; 2.5 g of leaves produced 1 mL extract). Different doses of the extract (5, 10, or 15 mL kg^{-1}) or water were orally administered to male rats and the 10 mL kg^{-1} dose to female rats of different stages of estrous cycle. 1, 3 and 5 h later, analgesic potential was determined. Different sets of rats, were orally treated with 10 mL kg^{-1} of extract and these rats were subjected to carrageenan induced paw edema, inflammatory and formalin induced pain tests. All the 3 doses were well tolerated. The 10 and 15 mL kg^{-1} doses significantly ($P < 0.05$) increased the reaction time and changed the % maximum possible effect at 3 h post treatment in the hot plate test. In contrast, none of the doses exhibited analgesic activity in the tail flick test. Further, in the females, the 10 mL kg^{-1} dose induced a significant analgesia as in males, and this effect was not affected by the stage of the estrous cycle. The antinociceptive action of the extract was not blocked by naloxone nor by metachlopramide. Further, the extract was devoid of sedative activity. In the carrageenan study, the extract showed neither anti-inflammatory nor antihyperalgesic activities. In the formalin pain test, the extract significantly reduced the pain in the early phase but not in the late phase. It was concluded that *T. catappa* leaf extract is useful as an analgesic but not as an antihyperalgesic in mild to moderate pain, supporting its folklore use in Sri Lanka.

Keywords: Analgesia, anti-inflammatory, antinociception, hyperalgesia, Sri Lanka, *Terminalia catappa.*

Introduction

Trees of *Terminalia catappa* L. (Combretaceae), Indian-almond in English, Kottamba in Sinhala and Amandi in Tamil, are very common along the coast of Sri Lanka. According to the Sri Lankan Ayurvedic pharmacopoeia different parts of this plant are recommended for diseases such as diarrhoea, gonorrhoea and several skin ailments including scabies (Jayasinghe, 1979; Jayaweera, 1982). According to Sri Lankan folklore, the juice of tender leaves of this plant is recommended for severe pains including headaches. If this claim is correct then the juice of tender leaves of *T. catappa* should possess analgesic and/or antihyperalgesic activity and possibly anti-inflammatory activity too. However, this has not been scientifically tested although a methanol extract of dried leaves and stems of this plant has been shown to have analgesic activity in rats when tested by Hippocratic screening procedures (Esposito-Avella et al., 1985).

The aim of this study was to investigate the analgesic, antihyperalgesic and anti-inflammatory potential of the fresh juice of tender leaves of *T. catappa.* Such an investigation is useful as many people in Sri Lanka still rely on herbal medicine for their primary health care. If the folklore claims are found to be scientifically valid then the people can be encouraged to use the juice to treat mild to moderate pain states and if not, its use could be discouraged. Also if potent activity is found, then it may be possible to isolate lead compounds for future development of new pain killing drugs.

Materials and methods

Fresh tender leaves of *T. catappa* were plucked from trees found in the campus garden of the University of Colombo between January and July, 1999. The identity of these leaves were authenticated by Professor A.S. Seneviratna,

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Department of Botany, University of Colombo. A voucher specimen has been deposited at the Department of Zoology Museum, University of Colombo (specimen number 18 TC). The leaves were thoroughly macerated in a porcelain mortar and the resulting juice/extract was squeezed through a muslin cloth. The yield was 40% (v/w); 2.5 g of fresh leaves produced 1 mL of extract.

The pH of the extract was determined using an electronic pH meter (TOA Electronic Ltd., Tokyo, Japan). The presence or absence of alkaloids, flavonoids, phenols, coumarins and steroids were examined using standard chemical tests (Farnsworth, 1966).

Adult cross-bred male albino rats (weighing 200–225 g) and females (weighing 175–200 g) from our own colony were used as experimental animals. These animals were kept in standardised animal house conditions (temperature: 28–31 °C; photoperiod approximately 12 h natural light per day; relative humidity: 50–55%) with free access to pelleted food (Vet House Ltd., Colombo, Sri Lanka) and tap water.

102 male rats were randomly divided into 8 groups. These rats were orally treated (11.00–12.00 h) either with distilled water (DW), or extract in the following manner. Group 1 (n = 12, 5 mL kg^{-1}), 2 (n = 12, 10 mL kg^{-1}), 3 (n = 12, 15 mL kg^{-1}), 4 (n = 10, 5 mL kg^{-1} DW), 5 (n = 11, 10 mL kg^{-1} DW), 6 (n = 10, 15 mL kg^{-1} DW), 7 (n = 9, 2 mg kg^{-1} indomethacin in 1 mL of % methyl cellulose), 8 (n = 12, 4 mg kg^{-1} indomethacin in 1 mL of 1% methyl cellulose), 9 (n = 8, 8 mg kg^{-1} indomethacin in 1 mL of 1% methyl cellulose) and 10 (n = 6, 1 mL 1% methyl cellulose). The IC_{50} values of the extract and indomethacin was determined using linear regression analysis.

Pro-estrus (n = 14), estrus (n = 15) and diestrus (n = 24) females were selected by vaginal smearing using normal saline (0.9% NaCl: w/v). The rats in each stage of the estrus cycle were randomly divided into two groups. One group was orally treated (11.00–12.00) with 10 mL kg^{-1} of DW) [pro-estrus (n = 6), estrus (n = 7) and diestrus (n = 12)] and the other with 10 mL kg^{-1} of extract [pro-estrus (n = 8), estrus (n = 8) and diestrus (n = 12)]. Following treatment, all these rats were continuously observed for overt clinical signs of toxicity, stress and gross behavioural abnormalities.

13 male rats were randomly assigned into two groups. One group (n = 7) was given subcutaneously 5 mg kg^{-1} of naloxone hydrochloride (Fluka Chemicals, Buchs, Swizerland), an opioid antagonist, in 0.1 mL saline, and the other group (n = 6) with 0.1 mL normal saline. 45 min later, all these rats were orally treated with 10 mL kg^{-1} of extract.

15 male rats were randomly selected and divided into two groups. The first group (n = 8) was orally administered with 1.5 mg kg^{-1} of metochlopramide (Ipca Laboratories Ltd., Mumbai, India), dopamine (D_2) antagonist, in 1 mL of 1% methyl cellulose (Griffin and George Ltd., Wembley, UK). The second group (n = 7) was orally treated with 1 mL of 1% methylcellulose. 1 h later, both groups of rats were orally treated with 10 mL kg^{-1} of extract.

Nociception was determined in all rats used in the above experiments (except those in groups 7, 8, and 9) 5–6 h before treatment and 1, 3 and 5 h post-treatment using the tail flick method (Langerman et al., 1995). Briefly, the time taken (in s) to flick the tail (the reaction time) when the tail is immersed (5–6 cm from its tip) in a water bath at 55 °C (the reaction time) was determined. A cut-off time of 15 sec was used to avoid tissue damage. The reaction time was also converted to percent of maximal possible effect, % (MPE) according to the following formula:

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

Nociception of these rats was also evaluated 5–6 h before treatment and 1, 3 and 5 h post-treatment (indomethacin only at 3 h post-treatment) using the hot plate technique (Langerman et al., 1995). Briefly, a rat was placed on a hot plate (Model MK 35 A, Muromachi Kikai Co. Ltd., Tokyo, Japan) maintained at 50 °C, and the time taken (in sec) either to lick the hind paw or to jump from the surface of the hot plate (the reaction time) was determined. A cut off time of 20 sec was used to avoid tissue damage. The reaction time was also converted to % MPE as described earlier.

In a separate set of experiments, 12 male rats were randomly divided into 2 equal groups. The first group (n = 6) was orally treated with 10 mL kg^{-1} of extract, and the second group (n = 6) with 10 mL kg^{-1} of DW. 1 h later, the volume of the left hind paw of these rats were determined by water displacement technique. The paw was dipped (up to a pre-marked circle on the tibio-tarsal joint) in a 10 mL glass vial filled with water (diameter: 17 mm and height: 51 mm). The displaced water was then collected in a small non-stick plastic container and the volume of the displaced water was measured. Immediately following this, 0.05 mL of 1% carrageenan (Sigma Chemical Company, St. Louis, MO, USA) suspension in normal saline was injected subcutaneously into the subplantar surface of the left hind paw (Winter et al., 1962). The paw volume was monitored once again at 3, and 5 h post-treatment using the above method. The change in paw volume at different times was calculated by subtracting the initial paw volume from that at 3, and 5 h after carrageenan treatment.

In another experiment, 18 male rats were randomly divided into 2 equal groups and one group was orally treated with 10 mL kg^{-1} of extract and the other group with 10 mL kg^{-1} of DW. Immediately after, all these rats were injected with 0.05 mL of 1% carrageenan into the subplantar surface, subcutaneously. The reaction time of these rats was determined using the hot plate techniques as described earlier, at 3 h post-treatment to investigate whether the leaves has anti-hyperalgesic activity.

20 male rats were randomly assigned into two groups of 10 each. One group was orally treated with 10 mL kg^{-1} of

extract and the other with 10 mL kg^{-1} of DW. 3 h later, each of these rats was injected subcutaneously with 50 µL of 2.5% formalin at subplantar surface of the left hind paw. These rats were individually placed in an observation cage and the severity of the pain was recorded using a scoring system [0, if the rat walked or stood firmly on the injected paw; 1, partially elevated or favoured the paw; 2, elevated the paw without contact with the floor; or 3, locked, bit or shook the paw] (Dubbinson & Dennis, 1977) for 10 min (early phase). Following 5 min rest, scoring was again made for 45 min (late phase).

12 male rats were randomly divided into 2 equal groups. One group was treated with 10 mL kg^{-1} of extract and the other with 10 mL kg^{-1} of DW. After 1 h, these rats were individually placed in the centre of the rat hole-board apparatus and observed for 7.5 min period. During this period the number of rears, number of head dips, locomotory activity and the number of faecal boluses produced were recorded (File & Wardill, 1975). The time spent per head dip was then computed.

Another 12 male rats were randomly divided into 2 equal groups. The first group was treated with 10 mL kg^{-1} of extract and the other group with 10 mL kg^{-1} of DW. Then, 1 h later, these rats were subjected to a bar holding test [to evaluate the muscle strength (Plaznic et al., 1993)] and the Bridge test [to evaluate the muscle co-ordination (Plaznic et al., 1993)] and the latency to fall and slide off (in sec) was recorded, respectively.

To investigate the nerve plasma membrane stabilising activity of extract, the effect of the extract on the heat-induced haemolysis of rat erythrocytes was assessed (Perez et al., 1995). Briefly, the extract was prepared in 0.15 M phosphate buffered saline (PBS, pH 7.4). 20 µL of fresh rat blood was added to 1 mL PBS and to this either 30 µL of extract (n = 6) or PBS (n = 6) was added. The contents were mixed and incubated at 37 °C for 15 min followed by 25 min at 54 °C. The contents were spun for 5 min at 3200 g using a lab centrifuge (Eltex of Sweden Ltd., Bradford, UK). The supernatant was removed and the absorbance of the supernatant was measured at 540 nm using a spectrophotometer (Jasco V 500, Jasco Corporation, Tokyo, Japan).

The results are expressed as means ± SEM. Statistical analysis was done using Mann-Whitney U-test except where pooling of results were made using Kruskal-Wallis test. P values equal or less than 0.05 were considered as significant.

Results

All the three doses were well tolerated with no overt signs of clinical toxicity, stress or behavioural abnormalities.

The extract was brownish in colour and had a pH of 3.8. Chemical testing showed the presence of alkaloids, flavonoids, phenols and coumarins.

Tables 1 and 2, summarise the results obtained with the tail flick and hot plate techniques. Among the males, since there was no significant difference ($P > 0.05$; Kruskal-Wallis test) in the reaction times of different DW treated groups (n = 31) these results were pooled together. None of the doses significantly ($P < 0.05$) prolonged the reaction time or altered the % MPE in the tail flick technique compared to DW control and their respective pre-treatment values. However, the mid-dose induced a significant ($P < 0.01$) and marked prolongation in the reaction time and significant ($P < 0.00001$); change in % MPE in the hot plate technique at 3 h post-treatment. The high dose too produced significant ($P < 0.05$) increases in the reaction time and % MPE but was less potent. However, both the mid and high doses failed to significantly ($P > 0.05$) alter the reaction time and % MPE at 1 and 5 h post-treatment. In contrast, the low dose had no significant ($P > 0.05$) effect on both the reaction time and % MPE at 1, 3 and 5 h post-treatment. All 3 doses of the reference analgesic drug, indomethacin produced a significant ($P < 0.05$) prolongation of the reaction time at 1 and 3 h post-treatment in hot plate test. The IC_{50} values for the extract and indomethacin were 7.8 mL kg^{-1} and 3.37 mg kg^{-1}, respectively.

Among the females (both in the control and treatment groups), there was no significant difference ($P > 0.05$); between the reaction time and % MPE in different stages of the estrous cycle. Therefore, these results were pooled. Compared to pooled DW controls or with their respective pre-treatment values, the mid dose significantly ($P < 0.05$) increased both the reaction time and % MPE.

In the naloxone study, with hot plate technique, subcutaneous administration of naloxone did not significantly ($P > 0.05$) impair the reaction time induced by the mid-dose (saline + extract vs. naloxone + extract: 14.9 ± 2.6 vs. 15.7 ± 1.5 sec).

In the metochlopramide experiment with the hot plate technique, the oral administration of this drug did not significantly ($P > 0.05$); change the reaction time provoked by the 10 mL kg^{-1} dose of extract (1% methyl cellulose + extract vs. metochlopramide + extract: 16.2 ± 2.4 vs. 17.7 ± 2.7 sec).

In the carrageenan study, the mid dose failed to significantly ($P > 0.05$) impair the increase in paw volume both at 3 h (control vs. treatment: 0.73 ± 0.06 vs. 0.77 ± 0.06 mL) and 5 h post-treatment (control vs. treatment: 0.60 ± 0.74 vs. 0.74 ± 0.06 mL). Further, this dose of extract also failed to prolong the reaction time significantly ($P > 0.05$) at 3 h following carrageenan treatment when evaluated in the hot plate (control vs. treatment: 17.25 ± 2.25 vs. 17.71 ± 0.96 sec).

The results obtained in the formalin test are summerised in Table 3. The mid-dose of extract significantly ($P < 0.05$) reduced the pain during early phase but had no significant ($P > 0.05$) effect during the late phase of the test.

In the rat hole-board test, none of the parameters investigated was significantly ($P > 0.05$) altered by the mid-dose (control vs. treatment: number of rears 21.6 ± 1.7 vs. 23.7 ± 1.6, number of head dips 8.2 ± 0.6 vs. 9.4 ± 0.8, time per head dip 1.4 ± 0.2 vs. 3.1 ± 1.1 sec, locomotory activity 22.4 ± 1.9 vs. 22.1 ± 1.2 and number of fecal boluses 3.6 ± 0.6 vs. 3.8 ± 0.7).

Table 1. Analgesic effect of fresh tender leaf extract (E) of *T. catappa* in male and female rats of different stages of estrous cycle measured in terms of reaction time in the hot plate and tail flick algesimetric tests (means ± SEM; ranges in parentheses).

		Hot Plate Reaction Time(s)				Tail Flick Reaction Time(s)			
Treatment	n	Pre-treatment	1 h	3 h	5 h	Pre-treatment	1 h	3 h	5 h
Males	31	11.1 ± 0.8 (5–19)	10.8 ± 0.8 (4–20)	10.4 ± 0.8 (4–21)	11.0 ± 0.9 (2–23)	2.9 ± 0.1 (1–5)	2.9 ± 0.2 (1–5)	2.9 ± 0.2 (2–5)	2.6 ± 0.1 (2–4)
water (pooled)									
5 mL kg^{-1} E	12	13.8 ± 1.2 (7–19)	13.5 ± 1.0 (9–21)	13.6 ± 1.8 (5–22)	14.0 ± 1.8 (7–22)	2.6 ± 0.3 (1–5)	3.4 ± 0.5 (1–8)	3.5 ± 0.4 (2–5)	3.5 ± 0.3 (2–5)
10 mL kg^{-1} E	12	11.1 ± 1.1 (7–19)	11.7 ± 0.9 (9–21)	17.5 ± 1.7** (10–27)	12.4 ± 1.0 (9–17)	2.9 ± 0.3 (2–4)	3.0 ± 0.2 (2–4)	4.3 ± 0.5 (3–8)	3.3 ± 0.2 (2–4)
15 mL kg^{-1} E	12	10.5 ± 1.1 (6–19)	11.4 ± 1.2 (6–22)	13.7 ± 1.5* (8–24)	12.1 ± 3.1 (4–45)	2.5 ± 0.2 (2–5)	2.7 ± 0.2 (1–4)	2.4 ± 0.2 (2–4)	2.4 ± 0.2 (2–3)
Females 10 mL kg^{-1} E or water									
Pro-estrus	6	13.7 ± 1.6	11.6 ± 2.0	11.6 ± 1.9	11.6 ± 1.3	4.0 ± 0.2	2.4 ± 0.2	25 ± 0.3	2.8 ± 0.4
Water		(7–18)	(6–21)	(6–17)	(8–16)	(4–5)	(2–3)	(2–4)	(2–4)
E	8	11.4 ± 1.1 (8–16)	12.4 ± 1.2 (8–19)	13.8 ± 3.1 (6–33)	14.2 ± 0.4 (8–22)	2.5 ± 0.3 (2–4)	3.0 ± 0.3 (2–5)	2.4 ± 0.2 (2–4)	2.3 ± 0.2 (2–3)
Estrus	7	12.6 ± 1.4	10.5 ± 0.7	11.5 ± 1.7	9.8 ± 1.2	3.3 ± 0.4	2.8 ± 0.3	2.6 ± 0.3	1.7 ± 0.1
Water		(7–17)	(8–14)	(7–18)	(5–15)	(2–5)	(2–4)	(2–4)	(1–2)
E	8	10.0 ± 1.4 (6–17)	14.9 ± 2.3 (7–27)	12.7 ± 1.4 (9–19)	10.2 ± 1.4 (6–16)	3.4 ± 0.2 (3–5)	2.7 ± 0.2 (2–4)	2.6 ± 0.2 (2–4)	2.2 ± 0.1 (2–3)
Diestrus	12	12.7 ± 1.0	10.5 ± 1.0	12.1 ± 1.1	14.1 ± 1.2	2.8 ± 0.3	2.8 ± 0.4	2.4 ± 0.3	2.5 ± 0.2
Water		(8–19)	(6–17)	(6–20)	(7–19)	(1–5)	(2–7)	(2–6)	(2–4)
E	12	12.2 ± 1.3 (4–8)	14.8 ± 2.1 (7–29)	15.5 ± 1.5 (10–26)	14.0 ± 1.5 (9–23)	2.5 ± 0.2 (2–4)	2.8 ± 0.2 (2–4)	2.6 ± 0.2 (2–4)	2.2 ± 0.1 (2–3)
Water pooled	25	13.0 ± 0.2 (7–19)	10.7 ± 0.7 (6–21)	12.1 ± 0.8 (6–20)	12.6 ± 0.8 (5–19)	3.2 ± 0.2 (1–5)	2.7 ± 0.2 (1–7)	2.5 ± 0.2 (2–6)	2.3 ± 0.1 (1–4)
E pooled	28	11.3 ± 0.8 (4–19)	14.2 ± 1.2 (7–29)	14.8 ± 1.2* (6–33)	12.9 ± 0.9 (6–24)	2.8 ± 0.1 (2–5)	2.8 ± 0.1 (2–5)	2.6 ± 0.1 (2–4)	2.2 ± 0.1 (2–3)

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

Likewise, the mid-dose did not significantly ($P > 0.05$) change the latency to fall in the bar holding test (control vs. treatment: 56.4 ± 0.4 vs. 57.5 ± 1.9 sec) or the latency to slide off in the Bridge test (control vs. treatment: 56.1 ± 1.9 vs. 55.9 ± 2.8 sec). The extract also failed to significantly ($P > 0.05$) change the absorbance in the heat induced hemolysis test of rat red blood cells (control vs. treatment: 0.740 ± 0.02 vs. 0.728 ± 0.04).

Discussion

The results demonstrate that the extract of tender leaves of *T. catappa* has analgesic activity as evaluated in the hot plate test (in terms of prolonged reaction time and % MPE) and not in the tail flick test. This suggests that the antinociceptive activity of the extract is mediated supraspinally, possibly at the thalamus (Porter, 1991), rather than spinally at spinothalamic and/or reticulothalamic pathways (Porter, 1991): the hot plate test predominately measures supraspinally organised responses while tail flick test predominately measures spinal reflexes (Wong et al., 1994). Some plant extracts [such as *Mucuna pruriens* (L.) DC. (Fabaceae) (Ratnasooriya et al., 1999)] and synthetic drugs [such as nifedipine (Wong et al., 1994)] produce analgesia by acting supraspinally as evident with this extract. The analgesic action had a slow onset (only at 3 h) short duration of action (reversible by 5 h), and was neither gender-dependent [morphine (Kavaliers & Innes, 1993)] nor dependent on the stage of the estrous cycle [some herbal analgesics such as *Murraya koenigii* (L.) Sprengel (Rutaceae) (Ratnasooriya et al., 1994b)] and not accompanied with undesirable side effects (in terms of overt clinical signs, abnormal behavioural patterns, change in the reaction times of bar holding and Bridge tests or impairment of locomotory activity in the rat hole-board).

The dose response curve of the analgesic effect of *T. catappa* extract was bell-shaped. Bell-shaped dose-response

Table 2. Analgesic effect of fresh tender leaf extract (E) of *T. catappa* as % maximum possible effect (% MPE) (means ± SEM; ranges in parentheses).

	% MPE in Hot Plate		
Treatment	1 h	3 h	5 h
Males			
Water (pooled)	−128.5 ± 75.1 (−2050–96)	−55.6 ± 39.7 (−1000–211)	−154.8 ± 85.6 (−2033–127)
5 mL kg^{-1} E	−56.5 ± 44.9 (−493–115)	−24.2 ± 50.0 (−507–150)	−35.4 ± 71.0 (−593–445)
10 mL kg^{-1} E	3.0 ± 21.4 (−156–153)	116.9 ± 44.9** (14–587)	2.9 ± 12.1 (−93–305)
15 mL kg^{-1} E	−49.2 ± 57.1 (−700–122)	14.3 ± 43.5* (−433–275)	−19.5 ± 51.3 (−546–305)
Females			
10 mL kg^{-1} water (pooled)	−131.0 ± 75.2 (−1929–108)	−117.7 ± 68.8 (−1571–99)	−48.2 ± 21.5 (−323–86)
10 mL kg^{-1} E	−24.4 ± 49.4 (−1300–186)	−21.3 ± 64.4* (−1650–447)	−61.2 ± 58.6 (−1500–136)

As compared with controls: * P < 0.05, ** P < 0.00001 (Mann-Whitney U-test).

Table 3. Effect of fresh tender leaf extract (E) of *T. catappa* on the formalin-induced pain model in rats (means ± SEM; ranges in parentheses).

Treatment	n	Early phase	Late phase
10 mL kg^{-1} Water	10	1.76 ± 0.14 (1–2)	2.21 ± 0.04 (1–2)
10 mL kg^{-1} E	12	1.42 ± 0.14* (1–2)	2.10 ± 0.10 (1–2)

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

curves have been reported with other synthetic (Li et al., 1996) and herbal analgesics (Ratnasooriya et al., 1999). Such an action can be attributed to desensitisation (Scuka & Muzrzymas, 1991) or down-regulation of receptors (Stewart & Badiani, 1993) if receptors mediation is involved in the precipitation of antinociceptive action in this extract. Alternatively, the damping of the analgesic effect with the high dose of the extract may result from the coexistence of component(s) with different extract$_{max}$, which blocks pain inhibition pathways of the brain. Such a mode of action is proposed for analgesics such as morphine (Roumy & Jean-Marie, 1998) and sedatives such as midazolam (Lau et al., 1998).

The analgesia induced by the extract was not attenuated by naloxone, an opioid receptor antagonist, suggesting that the antinociception is not mediated via opioid mechanisms. This observation also indicates the noninvolvement of endogenous opioids (Roumy & Jean-Marie, 1998) and cholecystokininergic mechanisms (Benedetti, 1997) in extract induced analgesia.

Analgesia can also be produced by a variety of stressors via opiod mechanisms (Badio et al., 1995). But such a mode of action is unlikely in this study as none of the extract-treated animals exhibited signs of stress (in terms of overt clinical signs, behaviour and number of fecal boluses produced in the rat hole-board).

Analgesia can be induced via a dopaminergic mechanism (Jensen & Yaksh, 1986). However, such a mode of action is unlikely in this study as metochlopramide, dopamine (D_2) receptor antagonist, failed to block analgesia induced by the extract.

Some sedatives posses analgesic activity (Rang et al., 1995), but, the analgesic action of the extract is unlikely to be mediated via sedation as none of the parameters monitored in the rat hole-board technique changed: this is a sensitive and reliable test used widely to test potential sedatives (File & Wardill, 1975).

In the formalin test, the mid-dose of the extract inhibited the early phase of pain and had no effect on the late phase. Further, this dose of the extract failed to reduce the carrageenan induced paw edema. Collectively, these observations suggest that the extract does not act via the inhibition of peripheral prostaglandin synthesis (Rang et al., 1995; Ahmadiani et al., 1998; Puntero et al., 1997). However, a possibility exists that the extract exerts its analgesic actions through a paracetamol (acetaminophen) type of action as is claimed for fruit extract of *Mormodica dioica* Roxb. ex Willd. (Cucurbitaceae) (Ratnasooriya et al., 1994a) and seed extract of *Mucuna pruriens* Blume (Rubiaceae) (Ratnasooriya et al., 1999) or leaves and stem extract of *Psychotria sarmentosa* Blume (Rubiaceae) (Ratnasooriya & Dharmasiri, 1999). In the formalin test, the early phase is

claimed to be a direct result of stimulation of nociceptors in the paw (Schwarz & Puil, 1998). Thus, the impairment of this phase by the extract suggests that the extract induces analgesia by inhibiting the transduction of the nociceptive message (Ahmadiani et al., 1998) as with local anaesthetics (Schwarz & Puil, 1998). However, this afferent transmission blockade is unlikely be due to a nerve membrane stabilizing action since the extract failed to inhibit the heat induced haemolysis of erythrocytes. Nevertheless, if the active component precipitating analgesia is a metabolite of the extract, then it is unlikely to observe an affect in the above test as is evident in the study.

The mid-dose of the extract failed to prolong the reaction time in the hot plate 3 h following carrageenan treatment: this inflammatory pain test has been proved to be a very useful test to assess antihyperalgesic activity. Thus, the extract is devoid of any antihyperalgesic activity and may also not be useful as a therapeutic agent for moderate pain.

The extract contained alkaloids, flavonoids, phenols and coumarines. Further, four new hydrolizable tannins, Terflavins A and B, Tergallagin and Tercatain have been isolated from the leaves of *T. catappa* (Tanaka et al., 1986).

In conclusion, this study scientifically demonstrates that tender leaf extract of *T. catappa* possesses marked analgesic activity without any antihyperalgesic or anti-inflammatory activity. Our results could justify the use of *T. catappa* in Sri Lankan folk medicine as an analgesic. Its use must be encouraged at least in mild to moderate pain as these leaves can be obtained easily free of charge.

References

Ahmadiani A, Fereidoni M, Semnanian S, Kamalinejad M, Saremi S (1998): Antinociceptive and anti-inflammatory effects of *Sambucus ebulus* rhizome extract in rats. *Ethnopharmacol 6*: 229–235.

Badio B, Shi D, Garraffo HM, Daly JW (1995): Antinociceptive effects of the alkaloid epibatidine: Further studies on involvements of nicotinic receptors. *Drug Develop Res 36*: 45–59.

Benedetti F (1997): Cholecystokinin type A and B receptors and their modulation of opioid analgesia. *NIPS 12*: 263–268.

Dubbinson D, Dennis SG (1977): The formalin test: A quantitative study of the anlgesic effect of morphine, meperidin and brain stem stimulation in rats and cats. *Pain 4*: 161–174.

Esposito-Avella M, Brown P, Tejeira I, Buitrago R, Barrois L, Sanches C, Gupta MP, Cedeno J (1985): Pharmacological screening of Panamanian medicinal plants Part I. *Int J Crude Drug Res 23*: 17–25.

Farnsworth NR (1966): Biological and phytochemical screening of plants. *J Pharm Sci 55*: 225–276.

File SE, Wardill A (1975): Validity of head-dipping as a measure of exploration in modified hole-board. *Psychopharmacology 44*: 53–57.

Jayasinghe DM (1979): *Ayurveda Pharmacopoeia*. Department of Ayurveda, Colombo, Sri Lanka.

Jayaweera DMA (1982): *Medicinal Plants used in Ceylon*. Part II, National Science Council of Sri Lanka, Colombo, Sri Lanka.

Jensen TS, Yaksh TL (1986): Effects of an intrthecal dopamine agonist, apomorphine on thermal and chemical evoked noxious responses in rats. *Brain Res 296*: 285–293.

Kavaliers M, Innes DGL (1993): Sex differences in naloxone and Try-MIF-1-induced hypoalgesia. *Pharmacol Biochem Behav 46*: 777–780.

Langerman L, Zakouski MI, Piskoun B, Grant GJ (1995): Hot plate versus tail flick: Evalution of acute tolerance to continuous morphine infusion in the rat model. *J Pharmacol Toxicol Meth 34*: 23–28.

Lau CE, Wang Y, Ma F (1998): Pharmacokinetic-pharmacodynamic modeling of the coexistance of stimulatory and sedative components for midazolam. *Eur J Pharmacol 346*: 131–144.

Li BY, Nalwalk JW, Barker LA, Cumming P, Parsons MF, Hough LB (1996): Characterization of the antinociceptive properties of cimetidine and a structural analog. *J Pharmacol Exp Ther 276*: 500–508.

Perez RM, Perez S, Zavala HA, Salazar M (1995): Anti-inflammatory activity of the bark of *Hippocratea excelsa. J Ethnopharmacol 47*: 85–90.

Plaznic A, Stefanski R, Palejko W, Kotawski W (1993): The role of accumbense GABA-B receptors in the regulation of rat behaviour. *Neurosci Res Comm 12*: 23–30.

Porter F (1991): *Encyclopedia of Human Biology Vol: 5*, Academic Inc., Press New York.

Puntero BF, Peinado II, Villardel Fresno AM (1997): Anti-inflammatory and anti-ulcer activity of *Teucrium buxifolium. J Ethnopharmacol 55*: 93–98.

Ramesh M, Rao YN, Rao AVNA, Prabhakar MC, Rao CS, Muralidhar N, Reddy BM (1998): Antinociceptive and anti-inflammatory activity of a flavonoid isolated from *Caralluma attennuata. J Ethnopharmacol 62*: 63–66.

Rang HP, Dale MM, Ritter JM (1995): *Pharmacology*. Churchil Livingstone: London.

Ratnasooriya WD, Fernandopulle BMR, Manickavasger S (1994a): Analgesic activity of *Mormodica dioica* fruit extract in rats. *Med Sci Res 22*: 307–308.

Ratnasooriya WD, Peiris LDC, Amarasekara AS (1994b): Analgesic activity of *Murraya koenigii. Med Sci Res 22*: 837–840.

Ratnasooriya WD, Lokupitiya EYK, Dharmasiri MG (1999): Analgesia and sedation induced by *Mucuna prurita* in rats. *Med Sci Res 27*: 403–406.

Ratnasooriya WD, Dharmasiri MG (1999): Water extract of *Psychotria sarmentosa* has analgesic and antihyperalgesic activity in rats. *Med Sci Res 27*: 715–718.

Roumy M, Jean-Marie Z (1998): Neruopeptide FF, pain and analgesia. *Eur J Pharmacol 345*: 1–11.

Schwarz SKW, Puil E (1998): Analgesic and sedative concentrations of lignocaine shunt tonic and burst firing in thalamocortical neurones. *Br J Pharmacol 124*: 1633–1642.

Scuka M, Mazrzymas JW (1991): Post synaptic potentiation and desensitization at the vertebrate end-plate receptors *Progress Neurobiol 38*: 19–33.

Stewart J, Badiani A (1993): Tolerance and sensitization to the behavioural effects of drugs. *Behav Pharmacol 4*: 289–312.

Tanaka T, Nonaka G, Nishioka I (1986): Tannins and related compounds. XLII. Isolation and characterisation of four new hydrolizable tannins. terflavins A and B, tergallagin and tercatain from the leaves of *Terminalia catappa* L. *Chem Pharm Bull 34*: 1039–1049.

Winter CA, Risley EA, Nuss GW (1962): Carrageenan-induced oedema in hind paw of the rat as an assay for anti-inflammatory drugs. *Proceed Soc Exper Biol Med 111*: 544–547.

Wong CH, Dey P, Yarmush J, Wu W, Zbuzek UK (1994): Nifedipine-induced analgesia after epidural injections in rats. *Anesth Analg 79*: 303–306.