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ANTINOCICEPTIVE ACTION OF AQUEOUS EXTRACT OF THE LEAVES OF *IXORA COCCINEA*

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The aim of this study was to examine the antinociceptive potential of leaves of *Ixora coccinea* (family: Rubeaceae). One of four doses (500, 750, 1000 or 1500 mg/kg, n = 8/dose) of aqueous leaf extract (ALE) or 1 ml of distilled water was orally administered to male rats and antinociceptive activity was ascertained using three models of nociception (tail flick, hot plate and formalin tests). The results showed that ALE possesses considerable antinociceptive activity (when evaluated in hot plate and formalin test but not in tail flick test). The antinociceptive activity of the ALE had a rapid onset (within 1 h) and a fairly long duration of action (up to 5 h) with a peak effect at 3 h. Further, the antinociceptive activity was dose-dependent and was not associated with harmful side-effects or toxicity even following subchronic administration. The antinociceptive action was mediated centrally at the supraspinal level mainly via dopaminergic mechanism. In addition, it is likely that antioxidant activity of the ALE could have played an auxiliary role in inducing antinociception. Dopaminergic and antioxidative activities of ALE could arise, respectively, from its quaternary base alkaloid and flavonoid constituents.

Keywords: Ixora coccinea – antinociception – pain – toxicology

INTRODUCTION

The search for new pharmacologically active agents obtained by screening natural sources such as microbial fermentations and plant extracts has led to the discovery of many clinically useful drugs that play a major role on the treatment of human diseases. Sri Lanka with its great diversity of flora possesses many plant species of medicinal value. The plant kingdom represents a virtually untapped reservoir of new and exciting chemical compounds, many of them extraordinary biodynamic.

Ixora coccinea Linn (family – Rubiaceae) (Sinhala: Ratmal, Tamil: Vedchi) is a shrub [13] with small, obovate to oval-oblong, rounded to subcordate base leaves on branched hard heavy twings. In Sri Lanka, it is distributed in the monsoon and intermediate rain forest areas [13]. The hexane fraction of the flowers has shown signifi-

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cant antigenotoxic properties, and triterpenoid ursolic acid has been isolated as the active constituent [15].

The antitumour activity of *Ixora coccinea* flowers has been studied in comparison to interperitoneal transplanted Dalton's lymphoma (ascitic and solid tumours) and Ehrlich ascites carcinoma (ECA) tumors in mice, and shown to be effective in increasing the life span of DLA and ECA ascitic tumor bearing mice [16]. It is also less active against solid tumors (DLA) as compared to asutic tumors. It was not toxic to normal lymphocytes, whereas it was toxic to transformed lymphocytes from leukaemic patients, acute lymphoblastic leukaemia (ALL) and chronic myelogenous leukaemia (CML) and K-562 suspension cultures. The active fraction inhibited tritiated thymidine incorporation in cellular DNA [16].

Ursolic acid in the active fraction of *I. coccinea* flowers is shown to be chemoprotective when tested both against cisplantin [17] and cyclophosphamide [18] induced toxicity in mice as well as hepatoprotective. It also significantly prolonged the life span of cisplatin treated mice and maintained their blood urea nitrogen levels in the near normal range [17]. Aqueous and ethanolic extracts of *Ixora coccinea* plant shows high and specific antimicrobial activity on prokaryotic systems [27]. Lugeol isolated from the petroleum ether fraction of the ethanol extract of the leaves of *Ixora coccinea* showed anti-inflammatory activity in the carrageenan-induced paw edema test in rats and anti-mitotic activity in a preliminary cytotoxic study using the "Alium test" [25].

In Sri Lanka the ayurvedic system of medicine utilizes the roots of *Ixora coccinea* for treatment of a variety of unrelated ailments/disease such as hiccoughs, nausea, loss of appetite, fever and gonorrhoea. The flowers and bark are used on reddened eyes and eruptions. A decoction of the flowers is given for haemoptysis, catarrhal bronchitis and dysmenorrhoea [13]. The use of flowers and roots for the treatment of wide range of ailment prompted us to include *Ixora coccinea* in our research program in search of analgesics from natural sources. The flowers are seasonal and the roots are not available in abundance, whilst the leaves are available throughout the year even in large quantities. Therefore our study was carried out on aqueous extract of the leave of *Ixora coccinea.*

MATERIALS AND METHODS

Collection of herbs

Fresh leaves of *Ixora coccinea* was collected from in Kaleliya and Mirigama in the Gampaha district of Sri Lanka in August 2001 and was identified and authentiated by Professor B. A. Abeywickrama of the Botany Department of the University of Colombo. A voucher specimen (wdr/sad 1003) was deposited at the museum of the Department of Zoology.

Preparation of the aqueous leaf extract (ALE)

The pieces of leaves were washed under running water, air-dried and cut into small pieces. The pieces (234 g) were mascerated with water and were then refluxed with 3 L water for two days in a round bottom flask fitted to a Leibig condenser. The brownish red solution was filtered and freeze-dried (12 g, yield 4.3%) and stored air tight at room temperature (30–32 °C). The freeze-dried powder was dissolved in distilled water (DW) to obtain the required dosages in 1 ml solution (500, 750, 1000 and 1500 mg/kg).

Animals

Healthy adult cross-breed albino male rats (weight: 200–250 g) were used in the study. The animals were kept in plastic cages under standardized animal house conditions with continuous access to pelleted food (Master Feed Ltd., Colombo, Sri Lanka) and tap water.

Hot plate and tail flick tests

Fourty-eight rats were selected and divided randomly into six groups. Food was withheld from these rats for 16 h and different concentrations of aqueous leaf extract (ALE) or vehicle (control) was administered orally in the following manner: Group 1 (n = 16) with 1 ml of DW; group 2 (n = 8) with 1 ml 500 mg/kg of ALE; group 3 (n = 8) with 1 ml 1000 mg/kg of ALE; group 4 (n = 8) with 1 ml 1500 mg/kg of ALE and group 5 (n = 8) were intramuscularly injected with 25 mg/kg meparadine hydrochloride as a positive control. Three to four hour before treatment (pre-treatment) and then at hourly intervals for 6 h post-treatment (either with ALE or vehicle or reference drug), these rats were subjected to hot plate and tail flick test [4].

Formalin test

Thirty-two rats were divided randomly into 4 groups and were orally administered the ALE or vehicle as follows: group 1 (n = 6) with 1 ml 500 mg/kg of ALE; group 2 (n = 9) with 1 ml 1000 mg/kg of ALE; group 3 (n = 8) with 1 ml 1500 mg/kg of ALE; group 4 (n = 10) with 1 ml vehicle. Three hours after administration, each rat was subcutaneously injected with 0.05 ml 2.5% formalin solution (BDH Chemicals, Poole, UK) into the subplantar surface of the left hind paw. Rats were then observed for 60 min and the number of lickings of the injected hind paw and the amount of time spent licking the injected paw were recorded in two phases: 1st phase, 1–5 min and 2nd phase, 15–60 min [7].

Investigation for opioid receptor mediation

Twelve rats were fasted overnight (but with free access to water) and randomly divided into two equal groups. Those in group 1 were subcutaneously injected with 5 mg/kg of nalaxone (an opioid receptor antagonist) and those in group 2 with isotonic saline. After 45 min, rats in both groups were orally administered with 1 ml of 1500 mg/kg ALE. These rats were subjected to the hot plate test before treatment (nalaxon or saline) and 3 h after extract treatment [22].

Investigation for dopamine receptor mediation

Fifteen male rats were randomly divided into two groups. Group 1 (n = 9) was orally treated with 1000 mg/kg of metachlopramide, dopamine antagonist in 1 ml of 1% methylcellulose. Group 2 (n = 6) was orally treated with 1 ml of methylcellulose. One hour later, both groups of rats were orally treated with 1 ml of 1500 mg/kg dose of ALE and nociception was determined 5–6 h before treatment and 1 h post-treatment, using the hotplate technique [22].

Evaluation of sedative activity

Sixteen rats were randomly divided into two equal groups and were fasted for 16 h (but with free access to water). Rats in group 1 were orally administered with 1 ml of DW and those in group 2 with 1 ml of 1500 mg/kg of ALE. Each of these rats was then placed on a rat hole-board apparatus and was given 7.5 min trial [9]. During this period, the number of head dips, rears and crossings were scored.

Evaluation of effects on muscle coordination and strength

Twelve rats were treated either with 1500 mg/kg of ALE (n = 6) or vehicle (n = 6) thrice a day for seven consecutive days. On the day 7 post-treatment, the rats were subjected to the Bridge test, bar holding test and righting reflex test [4] and the respective latencies were recorded.

Evaluation of subchronic toxicity

The rats used in the above investigation were observed each day of treatment (6–8 h) and on day 1, post-treatment for presence of Straub's tail reaction, overt signs of toxicity, stress and aversive behaviours as described by Deraniyagala et al. [4]. The rectal temperature of these rats was also determined using a clinical thermometer (Oson Duopris Company Ltd., Germany).

Effects on fertility, gestation length and neonatal developmental parameters of pups

Sixteen day 1 pregnant rats were randomly divided into two equal groups. One group was orally administered with 1000 mg/kg of ALE and the other group with 1 ml of DW daily for 7 consecutive days. On day 14 of pregnancy, the rats were laporatomized under mild ether anesthesia and numbers of uterine implants, viable implants, dead implants and corpora lutea were recorded. The size and the distribution of the uterine implants were also noted. The animals were sutured, treated locally and subcutaneously with tetracycline® and allowed to recover and deliver. The number of pups born was recorded and these were examined for any obvious external gross malformations. Their weights were determined on postnatal days 1 and 5. In addition, on day 5 of parturition the cranial length and the cervicosacral length were determined. The pups were monitored daily for general health, eye opening and appearance of fur.

Based on these laporatomy and neonatal data the following were computed as described by Deraniyagala et al. [4]: quantal pregnancy, implantation index, gestation index, pre-implantation loss, post-implantation loss, live birth index, fetal survival ratio, litter index and viability index.

Evaluation of effect on haematological parameters, serum SGPT, SGOT creatinine and urea levels

Twelve rats were treated either with 1500 mg/kg of ALE (n = 6) or vehicle (n = 6) thrice a day for seven consecutive days. On day 7 post-treatment, blood (1.5–2.0 ml) was collected from the tails of these rats under mild ether anesthesia using aseptic precautions. The WBC, RBC, counts and haemoglobin content of fresh blood was determined using standard techniques [10]. Another aliquot of blood was allowed to clot at room temperature (28–30 °C) and centrifuged at 3200 rpm for 5 min. The serum was collected and the SGOT (EC 2.6.1.1), SGPT (EC 2.6.1.2) creatinine and urea levels were determined using Randox kits (Randox Laboratories Ltd., Co., Antrium, U.K.) and a spectrophotometer (Jasco V560, Jasco Corporation, Tokyo, Japan).

Evaluation of prostaglandin synthesis inhibition activity

The experiment was carried out according to Dharmasiri et al. [5]. One centimeter portions of isolated dioestrus rat uteri were suspended in a 50 ml organ bath containing Kreb's Henseleit solution. The spontaneous contractions of the uteri were recorded using an isometric sensor (Medicals, Tokyo, Japan) for 10 min. The organ bath was treated in triplicate with the ALE so that the final concentrations of extract in the organ bath become 1, 5, 10 and 30 μg/ml and the contractions were recorded

for further 10–15 min. The mean amplitude and frequency of contractions were calculated.

Evaluation of plasma membrane stabilising activity

This activity was evaluated using heat-induced haemolysis of rat erythrocytes in vitro as described by Dharmasiri et al. [5]. The concentrations of the ALE usd were 3125, 1062.5, 31.25, 15.62 and 7.81 μg/ml. Saline was used as the control and aspirin was used as the positive reference drug. The absorbance of the supernatant was measured at 540 nm spectrophotometrically. The percentage of haemolysis with respect to control was calculated.

Evaluation of antioxidant activity

Antioxidant activity was assessed using thiobarbituric acid reactive substances assay as described by Dorman et al. [6]. The concentrations of the ALE used were 0.25, 2.5, 12.5, 18.8, 25, 50 and 125 μg/ml. Hundred microgram per milliliter of butylated hydroxy toluene (BHT) was used as the positive reference and DW was used as the control. The absorbance was measured at 532 nm and the antioxidant index was calculated.

Chemical analysis

Phytochemical screening of the ALE was carried to according to Farnsworth [7]. The ALE was subjected to column chromatography (30 cm length and 3.7 cm diameter) on reverse phase C-18 silica gel (Fluka Chemie G). The column was eluted with water, mixtures of methanol and water, methanol, mixtures of methanol and ethyl acetate, ethyl acetate, mixtures of ethyl acetate and dichloromethane, dichloromethane, mixtures of dichloromethane, hexane and finally with hexane. The fractions with similar thin layer chromatography (t.l.c) spots were combined after inspecting under U.V. light. The combined fractions were subjected to t.l.c (Aldrich -silica gel precoated on plastic plates and Fluka Chemie G reverse phase C-18 precoated glass plates). The mobile phases were 60% dichloromethane in hexane, 10% methanol in dichloromethane for normal phase chromatography and methanol and 50% methanol in water for reverse phase chromatography. The t.l.c plates were sprayed with colour reagents specific for various classes of compounds [4].

Statistical analysis

The data are expressed as the mean ± SEM. Statistical analysis was performed using Mann-Whitney U-test. Significant values were set at $P \leq 0.05$. Linear regression analysis was performed to assess dose-dependencies.

RESULTS

Hot plate and tail flick tests

Table 1 shows the results of hot plate test. The highest dose of ALE caused a significant ($P \leq 0.05$) prolongation of the reaction time in the hot plate test from 1st hour to 6th hour, both compared to the control and to its pretreatment values, the maximum effect has been observed at 3rd hour (66% compared to pretreatment and 63% compared to control). With the mid-dose, significant prolongation of reaction times were evident from 2nd to 5th hour. On the other hand, the lower dose (500 mg/kg) induced a significant prolongation of reaction time between 2nd to 4th hours. In contrast, the reference drug meparadine prolonged the reaction time in the 1st and 2nd hour only. The analgesic effects at the 2nd ($r^2 = 0.91$, $P \leq 0.05$) and 3rd hour ($r^2 = 0.94$, $P \leq 0.05$) were dose-dependent.

In the tail flick test there was no significant alteration ($P > 0.05$) in the tail flick reaction time in any of the ALE treated rats as compared to control rats (data not shown).

Formalin test

Table 2 shows the effect of ALE on the formalin test. All the three doses of the ALE significantly ($P < 0.05$) impaired the number of lickings and the time spent on licking both in the early and late phases. However, both these effects in the early phase were

Table 1
The effect of oral administration of different doses of aqueous leaf extract of *Ixora coccinae* on the hot plate reaction time of rats

Treatment	Hot plate reaction time(s) (mean ±SEM)						
	Pretreatment	1st hour	2nd hour	3rd hour	4th hour	5th hour	6th hour
500 mg/kg n=8	8.57±0.52	9.44±0.63	10.75±1.44*	11.27±0.94*	10.33±0.92*	9.38±0.72	9.81±0.77
1000 mg/kg n=8	8.16±0.98	10.76±1.19	11.96±0.98*	14.17±1.11*	12.71±0.97*	12.12±1.53*	10.38±1.11
1500 mg/kg n=8	8.85±0.68*	11.35±0.41*	12.35*±1.63	14.68±1.49*	12.61±1.6*	11.77±1.38*	11.14±1.37*
Control n=16	8.82±0.45	8.86±0.39	8.71±0.42	9.00±0.51	8.66±0.508	7.9±0.43	8.2±0.43
Meparadine 25 mg/kg n=8	7.87±0.22	17.67±1.30*	14.45±0.70*	7.97±1.00	NI	NI	NI

*Values are significant at $P \leq 0.05$; NI: not investigated.

Table 2
The effect of oral administration of different doses of water extract of *Ixora coccinae* leaves on formalin test

Treatment extract, mg/kg	Early phase		Late phase	
	Time spent on licking(s)	No. of licking	Time spent on licking(s)	No. of licking
Control n = 10	87.6 ± 13.6	17.8 ± 1.7	345.1 ± 36.6	74.2 ± 7.1
500 n = 6	56.2 ± 3.4*	12.8 ± 1.2*	124.8 ± 1.8**	23.8 ± 1.1**
1000 n = 9	51.8 ± 3.7**	12.8 ± 1.3	56.8 ± 5.4**	23.3 ± 1.6**
1500 n = 8	49.6 ± 11.6*	9.2 ± 2.1**	108.4 ± 20.5**	19.8 ± 4.4
Morphene n=6	17.3 ± 3.9**	4.5 ± 0.4**	102.2 ± 2.2**	10.5 ± 0.8**

Values are significant * at $P < 0.05$, ** at $P < 0.01$.

significantly ($P < 0.05$) higher than in the late phase. The reference drug morphene also significantly ($P < 0.05$) reduced the number of lickings and the duration of lickings in both phases.

Opioid receptor mediation

As shown in Table 3, with the hot plate technique, subcutaneous administration of nalaxone did not significantly ($P \geq 0.05$) impair the prolongation of reaction time induced by 1500 mg/kg of ALE.

Table 3
The effect of nalaxon injection on the hot plate reaction time of *Ixora coccinae* leaves water extract (1500 mg/kg)

Treatment	Pretreatment(s)	Posttreatment(s)
Nalaxone + extract n = 7	11.4 ± 1.1	13.43 ± 1.58
Saline + extract n = 7	10.8 ± 1.0	15.4 ± 1.5*

*Values are significant at $P \leq 0.05$.

Dopamine receptor mediation

The results of metachlopramide study is depicted in Table 4. As shown, metachlopramide significantly ($P < 0.05$) curtailed the prolongation of reaction time (by 52%) induced by 1500 mg/kg of ALE.

Sedative activity

In the rat hole-board test, 1500 mg/kg of ALE failed ($P > 0.05$) to significantly alter any of the three parameters investigated (number of dips: 9.2 ± 1.3 vs 5.6 ± 0.9, number of rears: control vs. treatment; 15.4 ± 2.3 vs 9.5 ± 1.3, number of crossings: 16.4 ± 2.8 vs 10.0 ± 2.4).

Effects on muscle coordination and muscle strength

None of the latencies in the Bridge, bar and righting reflex tests was significantly ($P > 0.05$) altered by 1500 mg/kg of ALE.

Subchronic toxicity

Subchronic treatment of the ALE did not elicit any overt signs of toxicity, stress or aversive behavior. There was also no change in the rectal temperature of rats treated with ALE (data not shown).

Effects on fertility, gestation length and neonatal developmental parameters of pups

None of the investigated fertility parameters, gestational length and neonatal developmental parameters of pups born was significantly ($P > 0.05$) altered by 1000 mg/kg

Table 4
The effect of metachlopramide injection on the hot plate reaction time of *Ixora coccinae* leaves water extract

Treatment	Pretreatment(s)	Post-treatment(s)
1500 mg/kg n = 9	10.0	8.0 ± 0.9*
Control n = 6	9.0 ± 0.6	12.2 ± 0.5

*Values are significant at $P \leq 0.05$.

dose of ALE, compared to control. Further, no obvious external gross morphological deformities were detected in pups treated both with the ALE and control.

Haematological parameters, serum SGOT, SGPT creatinine and urea levels

None of the enzyme levels (SGOT: control vs treatment; 34.1 ± 5.6 U/l vs 35.1 ± 5.5 U/l and SGPT: control vs treatment; 16.9 ± 3.7 U/l vs 14.4 ± 1.8 U/l), creatinine (0.6 ± 0.1 mg/dl vs 0.2 ± 0.02 mg/dl), urea (18.3 ± 2.5 mg/dl vs 15.4 ± 1.8 mg/dl) or haemotological parameters (haemoglobin: control vs treatment; 19.4 ± 0.9 vs 19.3 ± 0.8 g/dL; RBC: $6.5 \pm 0.2 \times 10^6$ vs $5.5 \pm 0.2 \times 10^6$ cells/mm^3; WBC $6.0 \pm 8.8 \times 10^3$ vs $11.1 \pm 13.4 \times 10^3$ cells/mm^3) were altered significantly ($P > 0.05$) by the ALE.

Prostaglandin synthesis inhibition activity

In the isolated rat uterine preparation, all the concentration of ALE tested failed to significantly ($P > 0.05$) alter either the frequency or amplitude of spontaneous contractions (data not shown).

Plasma membrane stabilization activity

All of the concentration of ALE tested failed to induce a significant ($P > 0.05$) change in the absorbance in the heat induced-haemolysis test of rat blood cells. In contrast, aspirin the reference drug ($P < 0.05$) significantly impaired the absorbance at all concentrations tested (data not shown).

Table 5
Antioxidant activity of aqueous leaf extract of *Ixora coccinae*

Sample	Concentration mg/ml	Antioxidant index
BHT (Butylated hydroxyl toluene)	0.1	91.20 ± 5.68
Extract	0.01	1.60 ± 8.33
	0.1	12.27 ± 2.08
	0.5	12.41 ± 2.29
	0.75	16.74 ± 5.55
	1.0	38.67 ± 8.58
	2.0	44.26 ± 13.82
	5.0	74.10 ± 9.54

Antioxidant activity

As shown in Table 5, the ALE had a mild (compared to BTH control) but a dose-dependent ($r^2 = 0.9$, $P < 0.05$) antioxidant activity.

Chemical analysis

Phytochemical screening of the ALE showed the presence of quaternary base alkaloids, flavonoids, tannins/polyphenols, steroids and/or terpenoids and saponins. Thin layer chromatography of the methylene chloride, methylene chloride/hexane & Hexane column fractions showed the presence of phenols (R_f 0.76, 0.12, 0.08), flavonoids (R_f 0.83, 0.7), steroids (R_f 0.09) and triterpenoid glycosides (R_f 0.64, 0.17, 0.73) and a quaternary base (R_f 0.15) on spraying with characteristic reagents.

DISCUSSION

We examined the antinociception potential of an ALE of *Ixora coccinea* using normal rats and three widely used, reliable and sensitive analgesic tests. The results show, for the first time, that the ALE when given orally possesses marked and dose-dependent antinociceptive activity as evaluated from hot plate (in terms of prolonged reaction time) and formalin tests (in both early and late phases) but not in tail flick test. Lack of motor deficiencies (in terms of Bridge and bar holding tests) and presence of dose-dependent antinociceptive activity suggest that the ALE-induced antinociception was genuine and possibly receptor mediated. The positive results in the hot plate test suggest that the ALE-induced antinociception is mediated centrally at the supraspinal level [29] possibly acting on a descending inhibitory pain pathway [26] and that the ALE is effective against acute phasic nociceptive pain [29]. The ALE-induced inhibition of the early phase of formalin test provides additional support for this inference [8]. On the other hand, impairment of the late phase of formalin test indicate that the ALE is effective against continuous inflammatory pain, possibly by inhibiting relase and/or activity of inflammatory mediators such as histamine, serotonin, bradykinin [28]. In fact, we have previously shown presence of antihistamine activity in the ALE [23]. Furthermore, the suppression of both phases of the formalin test indicates that the ALE has peripherally mediated antinociceptive actions as well [8]. The negative results obtained in the tail flick test [29] taken together with the positive results in the other two tests rules out that a spinally mediation action in ALE-induced antinociception. However, with the test models used it is not possible to judge whether the extract is effective against neuropathic pain.

The antinociceptive activity of the ALE had a rapid onset (within 1 h) and fairly long duration of action (about 5 h). The maximum pain relief was evident at 3 h. Such a mode of action does not necessitates frequent administration of this ALE in pain relief, which is a desirable feature. However, the antinociceptive activity of the

extract was slightly lower than meparadine in hot plate test and morphine in formalin test. Clinically, these two drugs are usually used in the management of moderate to severe pain particularly of visceral origin [1].

In rats, food deprivation induces antinociception [see 4] but such a mode of action is unlikely in this study as food was available *ad libidum* and there was no suppression of food intake. Stress can provoke analgesia [21]. However, ALE was not stressogenic (in terms of fur erection, exopthalmia and aversive behaviors). Therefore, antinociception due to stress can be ruled out. Some sedatives induce analgesia [1], but the antinociception activity of the ALE is unlikely to be mediated via sedation as none of the parameters in the hole-board test was altered. The ALE also failed to inhibit heat-indiced haemolysis of rat erythrocytes *in vitro.* Thus, the antinociception is unlikely to be due to membrane stabilizing effect and/or raising of nociception threshold as reported with some herbal drugs [5, 24] and local anaesthetics [21]. The ALE did not induce Straub's tail reaction, CNS stimulating behaviors (such as excitation, spontaneous agitation or enhanced locomotion in the hole-board) or marked breathing depression. Further, the aninociception action of the ALE was not blocked by nalaxone, an opioid receptor antagonist. Collectively, these data indicates that antinociception is mediated independent of opioid mechanisms. However, the impairment of the number of paw licking and the time spent on paw licking in the formalin test at both initial and late phases could arise not only from the presence of opioids and/or opiodiomimetics but could also arise from presence of phenolic constituents [3] and steroidal constituents [20] as was present in this ALE. Prostaglandins induce pain and prostaglandin synthesis blockers are potent analgesics [1, 21]. However, antinociception in this study is unlikely to be mediated via prostaglandin synthesis inhibition as the ALE failed to suppress contractions of rat isolated uterine preparation.

In contrast, dopamine receptor antagonist, metachloropromide, markedly attenuated the antinociception induced by the ALE. This suggests that the antonociception is mediated via dopaminergic mechanisms; dopamine is a transmitter in the descending pain inhibiting pathway in brain and dopamine agonists are shown to act as analgesics [1]. Some dopamine agonists such as apomorphine, selegiline, entacaporn possess tertiary nitrogen [14], which could exist as quaternary salts in combination with plant acids. Some of the dopamine agonists also have phenolic groups as was present in the ALE. Interestingly, recently, dopaminergic activity has been shown in two plants belongs to the family Rubeaceae [19]. In this respect, it is noteworthy that decoction made from Sri Lankan *Scoparia dulcis* plants also possess similar mode of antinociceptive action [24]. Clinically, excessive day time sleepiness is reported with dopamine receptor agonists [1]. However, such an action was not evident in our study. Oxygen free radicals are now implicated with pain [11]. The ALE had a mild antioxidant activity which could play an auxiliary role in the induction of antinociception.

Subchronic treatment of this ALE did not provoke unacceptable side-effects and was well tolerated: neither produced overt signs of clinical toxicity nor hepatotoxicity, nor nephrotoxicity, nor haematotoxicity, nor fertility, nor gross changes in pre-

and neo-natal development of pups. Further, the ALE appears to be non-terotogenic. It is now recognized that administration of several dopamine agonists and dopamine precursor levodopa is associated with increased male libido [2, 12]. Our ALE may have a similar effect but further experimentations are required for definitive conclusions.

Although, we did not come across repeats of the use of leaves of *Ixora coccinea* in folkfloric medicine our study clearly shows that the ALE of *Ixora coccinea* has antinociception activity when tested in rats. Thus, this ALE as it is may have the potential as cost effective therapeutic for pain. Further, these findings suggest that isolation of the active constituents could be potentially useful for the development of antinociceptives useful in the management of pain.

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