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Antinociceptive activity and toxicological study of aqueous leaf extract of *Justicia gendarussa* Burm. F. in rats

W.D. Ratnasooriya[1], S.A. Deraniyagala[2], D.C Dehigaspitiya.[2]

[1] *Department of Zoology, University of Colombo, Colombo 03, Sri Lanka*
[2] *Department of Chemistry, University of Colombo, Colombo 03, Sri Lanka.*
Correspondence : e-mail : wdr@zoology.cmb.ac.lk

ABSTRACT - According to Sri Lankan traditional medicine, leaves of *Justicia gendarussa* Burm. F. possesses analgesic action. But, validity of this claim has not been scientifically assessed. This study examined the oral antinociceptive potential of aqueous leaf extract (ALE) of *J. gendarussa* in rats using different concentrations (1500, 2000 and 3000 mg/kg). The results showed moderate and significant ($P<0.05$) antinociceptive action when tested in hot plate but not in tail flick test. This antinociceptive action had a rapid onset (2 h), moderate duration of action (2-4 h) and was dose-related. However, its antinociception effect was 2-5 folds weaker than morphine. In addition, ALE significantly impaired both phases of formalin test of nociception. Naloxone blocked the antinociceptive activity of ALE. Collectively, these observations suggest that ALE mediates its antinociception centrally at supraspinal and peripheral sites *via* opioid receptor mechanisms, and is effective against neurogenic and continuous inflammatory pain. In addition, ALE showed strong antioxidant activity which could also contributes to its antinociception. ALE was well tolerated (in terms of overt clinical signs, hepatic, renal and haemotoxicities). Phytochemical screening revealed the presence of alkaloids, flavonoids, saturated steroidal saponins or triterpinoidal saponins and amino acids and/or amines in ALE. It is concluded that ALE of *J. gendarussa* possesses safe, moderate oral antinociceptive activity.

KEY WORDS - Antinociception, *Justicia gendarussa,* Opioid mechanisms, Pain impairment.

INTRODUCION

Justicia gendarussa Burm. F. (Family: Acanthaceae), (Sinhala: Kalu-weraniya, Tamil: Karunochchi) is a shade loving, quick growing, evergreen scented shrub (0.6 – 1.2 m tall) with simple, opposite, lanceolate or linear- lanceolate, acute at base, tapering into rounded apex and glabrous and shining leaves (8- 12.5 cm long, 1.2-2cm broad) with purple veins prominent at beneath. Its stem is quadrangular, thickened at and above the nodes and internodes 2-7 cm long. The flowers are in terminal or axillary spikes and are irregular, bisexual, sessile, white with pink or purple spots inside and red in the throat and lip. It is considered to be a native of China and distributed in Sri Lanka, India and Malaya, wild or cultivated. It is rather common in low country (below 300 m) in Sri Lanka (1, 2). The leaves of *J. gendarussa* are rich in potassium salts and contain alkaloids and aromatic amines (1, 3). The water and 80% ethanol extracts of aerial parts of this plant has shown to possess HIV type 1 reverse transcriptase inhibitory activity *in vitro* (4).

In the traditional medicine the leaves of the plant is recommended to treat number of ailments such as fever, hemiplegia, rheumatism, arthritis, muscle pain, lumbago, headache and earache (1, 5, 6).The wide medicinal use of the leaves of this plant indicate that it may have antinociceptive potential. However, to our knowledge, there are no published scientific studies on the antinociceptive activity on the leaves of *J. gendarussa* and its potential toxicity. This study examines the antinociceptive potential and toxicity of an aqueous leaf extract (ALE) of this plant.

MATERIALS AND METHODS

Plant selection and identification

Mature leaves of *J. gendarussa* were collected from Mirigama in the Gampaha district of Sri Lanka in August 2005 and was identified and authenticated by Dr. H. Kathriarachchi of Department of Plant Science, University of Colombo. A voucher specimen (WDR/ Kalu-weraniya 1) is deposited at the museum of the Department of Zoology, University of Colombo.

Preparation of the extract

The leaves were shade dried, crushed into small pieces and 800 g of these were refluxed with distilled water (DW), (7 L) for two days in a flask fitted with a Leibig condenser. The brownish solution was filtered and was boiled further (1 hour) to get a concentrated solution. It was freeze-dried (54 g, yield 6.75%) and stored air tight in the refrigerator (4°C). The freeze-dried powder

was dissolved in DW to obtain the required concentrations of ALE in 1 mL solution (1500, 2000 or 3000 mg/kg). Taking into consideration the metabolic rate of rat, the low dose tested was 7.5 times higher than that is usually recommended by the traditional practitioners of Sri Lanka in prescribing herbal decoctions (7) which is within the accepted range for the rat model (8).

Pharmacological evaluation

Experimental animals

Adult albino male rats (weight: 175-225 g) were used in the study. These animals were kept under standardized animal house conditions (temperature: 28-31°C, photoperiod: approximately 12 hours natural light per day, relative humidity: 50-55 %) with free access to pelleted food (Master feed Ltd., Colombo, Sri Lanka) and water. The research was conducted in accordance with the internationally accepted principles for animal use and care and guidelines and rules of the Faculty of Science, University of Colombo for animal experimentations. All surgical interventions were done under ether anaesthesia using aseptic precautions.

Evaluation of antinociceptive activity using hot plate and tail flick tests

Forty three rats were selected and assigned into 5 groups. The rats were orally administered with the ALE or DW (control) in the following manner. Group 1 (n = 12) with 1 mL of DW, 2 (n = 9) with 1 mL of 1500 mg/kg of ALE, 3 (n = 9) with 2000 mg/kg of ALE, 4 (n = 9) with of 3000 mg/kg of ALE and 5 (n = 4) with 1 mL of 15 mg/kg of morphine sulphate (Pharmachemie B. V., Harlem, Netherlands) (an opioid receptor agonist) (9) as the positive control. Before treatment and up to 6 h post treatment these rats were subjected to hot plate and tail flick tests (10) at hourly intervals.

Evaluation of effects on muscle coordination and strength

Twelve male rats were treated either with 3000 mg/kg of ALE (n = 6) or DW (n = 6) and 2 h post-treatment, the rats were subjected to the bar holding test (9) (to evaluate muscle strength), Bridge (11) and righting reflex tests (12) (to evaluate muscle coordination) and the respective latencies (in seconds) were recorded.

Investigation of opioid receptor mediation in inducing antinociception

Twelve male rats were selected and randomly divided into two equal groups (n = 6/group). Those in group 1 were intraperitoneally injected with 1.5 mg/kg of naloxone hydrochloride (Troikaa Pharmaceuticals Ltd, Gujarat, India), an opioid receptor antagonist (9), and those in group 2 with isotonic saline (0.9% NaCl, w/v). After 45 min, the rats in both groups were orally administered with 3000 mg/kg of ALE. These rats were subjected to the hot plate test 1 h before and 2 h after *the treatment (13).*

Investigation for dopamine receptor mediation

Twelve rats were randomly divided into two equal groups (n = 6/group). The animals in group 1 were orally treated with 1.5 mg/kg of metoclopramide (GlaxoSmithKline, Pakistain Limited), a dopamine antagonist (9), in 1 mL of 1% methylcellulose (Griffin and George Ltd., London, UK). Those in group 2 were orally treated with 1mL of 1% methylcellulose. One hour later, both groups of rats were orally treated with 3000 mg/kg of ALE and nociception was determined before treatment (dopamine antagonist) and 2 h post-treatment, using the hot plate technique (13).

Investigation for muscarinic receptor mediation

Twelve male rats were randomly divided into two equal groups (n = 6/group). Those in groups 1 were intraperitoneally injected with 2 mg/kg of atropine sulphate (Laboratorre Renaudin, Paris, France), a muscarinic receptor antagonist (9), and those in group 2 with isotonic saline. After 10 min, the rats in both groups were orally administered with 3000 mg/kg of ALE. These rats were subjected to the hot plate test before treatment and 2 h after ALE treatment (13).

Evaluation of sedative activity

Twelve rats were randomly divided into two equal groups (n = 6/group) and the rats in group 1 were orally administered with 1mL of DW and those in group 2 with 3000 mg/kg of ALE. After 2 h, these rats were individually placed in the center of the rat hole-board apparatus and were observed for 7.5 min. During this period, the numbers of rears and head dips, locomotory activity and the number of faecal boluses were recorded. The time spent on head dips and time per dip was then calculated (14).

Formalin test

Sixteen rats were divided into 2 groups and were orally administered the ALE or vehicle as follows; group 1 (n = 10) with 1mL of DW, group 2 (n = 6) with 3000 mg/kg of ALE. Two hours after administration, each of these rats was subcutaneously injected with 0.05 mL 2.5% formalin solution (BDH Chemicals, Poole, UK) into the sub plantar surface of the left hind paw. The rats were then observed for 30 min and the numbers of licking, flinching, lifting and time spend on licking of the injected paw were recorded in two phases,1-5 min (1st phase) and 20–30 min (2nd phase) (15).

Evaluation of prostaglandin synthesis inhibition activity

Female rats in dioestrous stage were selected by vaginal smearing. They were sacrificed with an overdose of ether, their uterine horns were removed and cut into approximately 1 cm pieces. These uteri were individually placed in a 50 mL organ bath containing Kreb's Henseleit solution (pH 7.4) (8).The organ bath was maintained at 37 °C and aerated continuously with a gas mixture of 95% O_2 and 5% CO_2. The spontaneous contractions of the uteri were recorded isometrically using a transducer (Star Medicals, Tokyo, Japan) under a resting tension of 1 g. After the contractions became regular, the normal activity of the uteri was recorded for a further 10 min. Following this, ALE was added sequentially into the organ bath so that the final concentration became 8, 16 or 24 µg/mL in the organ bath. Aspirin (10 µg/mL) was used as the reference drug. After each treatment the spontaneous contractions of the uteri was recorded for 10 min. Then the mean amplitude and frequency of contractions were calculated (16).

Evaluation of membrane stabilization

The neuronal membrane stabilizing activity of ALE was assessed using the heat-induced haemolysis of rat erythrocyte model (13). Briefly, fresh rat blood (20 µL) was added to vials containing 1 mL of 0.15 M phosphate buffered saline (PBS), (pH 7.4). To this, either different concentrations of 5 µL of ALE [0.75, 0.5, 0.25 or 0.125 mg/mL (n = 4)] or PBS (n = 4) or 1 mg/mL aspirin, the positive reference drug (n = 4) was added. Thereafter, the vials were mixed and incubated at 37 °C for 15 min followed by 25 min at 54 °C. The vials were then centrifuged (Wifuge Lab Centrifuge, Eltex Swedwn Ltd., Bradfod, U.K.) at 3200 g for 5 min and the absorbance was measured at 540 nm using a spectrophotometer (Jasco V 500, Jasco Corporation, Tokyo, Japan). Heat-induced haemolysis in terms of the absorbance values of control and different treated were compared.

Evaluation of antioxidant activity

Antioxidant activity was assessed using thiobarbituric acid (TBA) reactive substances assay based on fowl egg yolk (17). Briefly, into snap capped vials 10 µL of different concentrations of ALE (50, 100, 500 or 750 µg/mL) and egg yolk 50 µL were added. DW (10µL) was used as the control. Acetic acid (20% solution, 150 µL) and TBA (0.8%, 150 µL) were added to each snap capped vial. Total volume was adjusted to 400 µL by adding DW. These mixtures were vortex for 5 s and kept in a water bath (LCH-110 Lab Thermo Cool, Tokyo, Japan) at 95°C for 60 min. Butanol (1 mL) was added to each tube and vortex for 5 s. After centrifuging at 1500 g for 5 min, butanol layer was separated. Butylated hydroxy toluene (BHT), ascorbic acid and vitamin E (100 µg/mL) were used as positive controls. The absorbance was measured at 532 nm and the antioxidant index was calculated as follows: Anti oxidant index = (1-*T*/C) × 100 (where *T* – absorbance of treated and *C*- absorbance of control)

Toxicological studies

Evaluation of sub chronic toxicity

Twelve rats were randomly divided into two groups (n = 6/group) and their body weights were measured using an electronic balance (MP6000, Chyo Balance Corporation, Tokyo, Japan).Those in the first group were orally treated with 3000 mg/ kg of ALE and those in group two with 1 mL of vehicle (DW) once a day (12.00-13.00 h) for 21 consecutive days. Throughout the treatment period these rats were observed once a day (13.00-14.00 h) for survival, any overt signs of toxicity (salivation, diarrhoea, yellowing of fur, postural abnormalities, behavioural changes, marked impairments of food and water intake and body weight), stress (fur erection and exopthalmia) and aversive behaviours (biting and scratching behaviour, licking at tail, paw and penis and intense grooming behaviour). The rectal temperature of these rats was also determined using a clinical thermometer (TM-II, normal glass, Focal Corporation, Tokyo, Japan). On the last day of treatment, the body weight of each rat was recorded.

Evaluation of effect on haemotological parameters, serum glutamic-pyruvate transaminase (SGPT), serum glutamic-oxaloacetic transaminase (SGOT) and serum urea and creatinine levels

The blood from the rats used for toxicological study was collected from tails into two eppendorff tubes. One with EDTA and the other without EDTA under ether anesthesia using aseptic precautions. White blood cell (WBC) count, Red blood cell (RBC) count, and differential count (DC) were determined using standard techniques (18). Using serum, SGOT (EC 2.61.1) and SGPT (EC 2.6.1.2), urea and creatinine levels were determined with enzyme kits (Randox Laboratories Ltd., Atrium, U.K.) and a spectrophotometer as described by the manufacturer.

Phytochemical analysis

Phytochemical screening of the ALE was carried out according to Farnsworth (19). The ALE was also subjected to column chromatography. The column was packed with silica gel C-18 reverse phase material

(Fluka Chemie G, Buchs, Switzerland) using DW. It was eluted with solvents of decreasing polarity starting with DW, mixtures of methanol and DW, methanol, mixtures of methanol and ethyl acetate, ethyl acetate, mixtures of ethyl acetate and dichloromethane, dichloromethane, mixtures of dichloromethane and hexane and finally with hexane. The fractions with similar thin layer chromatography (TLC) spots under UV light were combined. The combined fractions were again subjected to TLC (Aldrich silica gel precoated on plastic plates and Fluka chemie G reverse-phase C-18 precoated glass plates). The mobile phases were methanol for reverse phase chromatography and 30% dichloromethane in hexane and 15% dichloromethane in hexane for normal phase chromatography. The TLC plates were sprayed with colour reagents specific for various classes of compounds (20). ($AlCl_3$ test for flavonoids; vanillin-phosphoric acid for steroids; p-toluene sulphonic acid for steroid and flavonoids; Liebermann-Burchard test for triterpenoid glycoside and cholesterol; Dragendoff's reagent for alkaloids and/or organic bases; Iodoplatinate for alkaloids and/or various nitrogenous heterocyclic compounds; diazotized p-nitro-aniline for phenols and ninhydrin test for amino acids and amines).

Statistical analysis

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

RESULTS

Evaluation of nociception using hot plate and tail flick tests

As shown in Table 1 all the 3 doses of ALE failed to significantly ($P>0.05$) alter the reaction time at 1st h post-treatment. On the other hand, all the 3 doses of ALE significantly ($P<0.05$) and dose dependently (2nd h $r^2 = 0.97$; $P<0.05$, 3rd h $r^2 = 0.96$; $P<0.05$) increased the reaction time both at 2nd h (low by 35.4%; mid by 36.7% and high by 43.0%) and 3rd h (low by 31.8%; mid by 17.6% and high by 22.3%) as compared to control. In addition, the highest dose also significantly ($P<0.05$) prolonged the reaction time at 4th h post-treatment (by 28.4%). In contrast, morphine induced a marked prolongation of reaction time from 1st h to 4th h post-treatment (1st h by 59.0%; 2nd h by 103.8%; 3rd h by 60.0% and 4th h by 45.7%). At each time point, the prolongation of reaction time by morphine was markedly higher than that produced by the highest dose ALE (1st h by 48.3%; 2nd h by 42.5%; 3rd h by 30.8 % and 4th h by 13.5%).

In contrast, in the tail flick test, there was no significant ($P>0.05$) alteration in the reaction time induced by any of the ALE doses as compared to control (Table 2).

Effect on muscle coordination and strength

None of the latencies in the Bridge, bar holding and righting reflex tests were significantly ($P>0.05$) altered by 3000 mg/kg of ALE (Table 3).

Opioid receptor mediation

As shown in the Table 4, intraperitoneal administration of naloxone significantly ($P<0.05$) impaired the prolongation of reaction time induced by 3000 mg/kg of ALE.

Dopamine receptor mediation

Oral administration of metoclopramide did not significantly ($P>0.05$) alter the reaction time in hot plate test induced by 3000 mg/kg of ALE. The results are shown in Table 5.

Muscarinic receptor mediation

Intraperitoneal administration of atropine did not produce a significant ($P>0.05$) alteration in the reaction time induced by 3000 mg/kg of ALE (Table 6).

Sedative activity

The results obtained in the rat-hole board test are summarized in Table 7. As shown, the locomotory activity (by 53.0%) and number of rearings (by 46.8%) were significantly ($P<0.05$) increased by the highest dose of ALE.

Formalin test

As shown in Table 8, number of liftings (1st phase by 55.1% and 2nd phase by 70.2%), number of lickings (1st phase by 51.4% and 2nd phase by 48.1%) and cumulative time spent on licking (1st phase by 47.9% and 2nd phase by 38.0%) were significantly ($P<0.05$) impaired by the highest dose of ALE.

Evaluation of prostaglandin synthesis inhibition activity

In the isolated rat uterine preparations, all the concentrations of ALE tested failed to significantly ($P>0.05$) alter the frequency or the amplitude of the spontaneous contractions (Table 9).

Evaluation of membrane stabilization

All the concentrations 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. On the other hand, aspirin, positive reference drug significantly ($P<0.05$) reduced the absorbance as compared with the control (PBS) (Table 10).

Evaluation of antioxidant activity

As shown in Table 11, ALE had a comparable antioxidant activity to BHT, ascorbic acid and vitamin E. Further, the antioxidant activity of ALE was dose depended (r^2 = 0.8239; P< 0.05).

Toxicological studies

Subchronic treatment of the ALE did not elicit any overt signs of toxicity stress or aversive behaviours. Further, as compared with the control, ALE did not induce any significant (P>0.05) change in the haemotological parameters and serum enzyme levels of SGOT, SGPT, creatinine and urea (Tables 12 and 13). Further, ALE also did not cause significant (P>0.05) changes in the rectal temperature and body weight (Table 14).

Phytohemical analysis

Phytochemical screening (19) of the ALE showed the presence of alkaloids, flavonoids and saturated steroidal saponins or triterpenoidal saponins. The reverse phase TLC's obtained from the fractions collected from the reverse phase C-18 column chromatogram (eluting solvents - DW, mixtures of methanol and DW, methanol, mixtures of methanol and ethyl acetate, ethyl acetate, mixtures of ethyl acetate and dichloromethane, dichloromethane, mixtures of dichloromethane and hexane and hexane), on spraying with characteristic reagents showed the presence of alkaloids (mobile phase -methanol, R_f 0.44, 0.46 and 0.59), flavonoids (mobile phase - methanol, R_f 0.28 and 0.65), steroids (mobile phase - methanol, R_f 0.51, 0.50 and 0.81) triterpenoid glycoside (mobile phase -methanol R_f 0.12, 0.3, 0.68 and 0.78) and amino acids and/or amines (mobile phase - methanol, R_f 0.08, 0.1 and 0.21). The normal phase TLC's of column fractions gave positive colour reactions for alkaloids (mobile phase - 30% CH_2Cl_2: 70% hexane R_f 0.37), flavonoids (mobile phase - 30% CH_2Cl_2: 70% hexane R_f 0.33 and mobile phase -15% CH_2Cl_2: 85% hexane R_f 0.76 and 0.23), steroids (mobile phase - 30% CH_2Cl_2: 70% hexane R_f 0.17 and mobile phase 15% CH_2Cl_2: 85% hexane R_f 0.62 and 0.87) and triterpenoid glycosides (mobile phase - 30% CH_2Cl_2: 70% hexane R_f 0.26 and mobile phase - 15% CH_2Cl_2: 85% hexane R_f 0.72).

DISCUSSION

This study examined the antinociceptive potential of ALE of *J. gendarussa* in rats using thermal and chemical models of nociception. These models are accurate, reliable and widely used in the evaluation of potential antinociceptive agents (10, 13, 15, 16). The results show, for the first time, that the ALE possesses moderate, dose-dependent oral antinociception action of rapid onset (2^{nd} h) and a modest duration of action (2-4 h) when evaluated in the hot plate test but not in the tail-flick test. In addition, the highest dose of ALE (only dose tested) also suppressed both phases of the formalin test. The antinociception activity of ALE was not a false positive result as there were no ALE-induced global motor impairments (as judged by bar, Bridge or righting reflex tests) or hypothermia. Further, even with subchronic administration of high dose of ALE there were no overt signs of clinical toxicity, mortality, hepatotoxicity (in terms of SGOT and SGPT levels), renotoxicity (in terms of serum creatinine and urea levels) or haemotoxicity (determined by RBC and WBC counts and DC of blood smears). This indicates the ALE is relatively non-toxic and tolerable. However, the antinociceptive action of ALE was 2-5 fold less effective than morphine, one of the most potent analgesics of current use. Nevertheless, the onset and duration of the antinociception of ALE and morphine were comparable. These observations collectively suggest that ALE of *J. gendarussa* has the potential to be used, as a safe orally active and cheap therapeutic agent for mild to moderate pains in developing countries where still about 80% of people use traditional medicine for primary health care (21) and accesses to western drugs are limited. It also shows the potential of this plant species as a natural source of chemical scaffold for synthesis of novel, effective, safe and cheap antinociceptives. In this regard, it is interesting to note that, almost half the some 850 small molecules introduced as drugs were derived from plant sources (21).

The positive results obtained with ALE in the hot plate indicate that ALE provoked antinociception is mediated centrally at supraspinal level (22) and is effective against phasic transient pain (22). The ability of ALE to suppress both phases of formalin test suggests that it is effective against both neurogenic and continuous inflammatory pain even of peripheral origin (23). On the other hand, lack of prolongation of the reaction time in tail flick test suggests that the ALE-induced antinociception is not mediated spinally: tail flick test predominantly measures spinal reflexes (22).

In rats, food deprivation induces antinocieption (24), but such a mode of action is unlikely here as food and water was available *ad libidum*. Stress provokes antinociception (9). However, ALE was not stressogenic (in terms of fur erection and exopthalmia) and this mode of action is unlikely. Antinociception can be

Table 1. The effect of oral administration of aqueous extract of Justicia gendarussa leaves on the hot plate reaction time.

Treatment (mg/kg)		Hot plate reaction time (s) (mean±SEM)						
		Pre-treatment	1 h	2 h	3 h	4 h	5 h	6 h
Control	(n = 12)	7.7 ± 0.3	8.3 ± 0.6	7.9 ± 0.4	8.5 ± 0.5	8.1 ±0.5	7.9 ± 0.5	8.3 ± 0.6
1500	(n = 9)	8.1 ± 0.3	8.6 ± 0.3	10.7 ± 0.6*	11.2 ± 1.0*	9.0 ± 0.3	8.3 ± 0.2	9.2 ± 0.4
2000	(n = 9)	7.7 ± 0.4	8.8 ± 0.3	10.8 ± 0.3*	10.0 ± 0.2*	9.1 ± 0.2	8.6 ± 0.4	8.0 ± 0.4
3000	(n = 9)	7.9 ± 0.3	8.9 ± 0.9	11.3 ± 0.7*	10.4 ± 0.8*	10.4 ± 0.6*	8.9 ± 0.5	8.7 ± 0.6
morphine	(n = 4)	8.8 ± 0.7	13.2 ± 0.8*	16.1 ± 1.3*	13.6 ± 1.6*	11.8 ± 1.9*	9.7 ± 1.7	7.6 ± 0.2

**Values are significant at P≤0.05 vs. control.*

Table 2. The effect of oral administration of aqueous extract of Justicia gendarussa leaves on the tail flick reaction time.

Treatment (mg/kg)		Tail flick reaction time (s) (mean±SEM)						
		Pre-treatment	1 h	2 h	3 h	4 h	5 h	6 h
Control	(n = 12)	1.65 ± 0.13	1.52 ± 0.07	1.55 ± 0.08	1.48 ± 0.06	1.47 ±0.04	1.54 ± 0.05	1.62 ± 0.04
1500	(n = 9)	1.97± 0.23	1.73 ± 0.07	1.76 ± 0.08	1.96 ± 0.06	1.89 ± 0.04	1.93 ± 0.05	1.66 ± 0.04
2000	(n = 9)	1.57 ± 0.11	1.76 ± 0.12	1.59 ± 0.09	1.62 ± 0.19	1.62 ± 0.11	1.54 ± 0.12	1.74 ± 0.15
3000	(n = 9)	1.61 ± 0.09	1.73 ± 0.15	2.13 ± 0.19	1.71 ± 0.14	1.65 ± 0.16	1.74 ± 0.12	1.66 ± 0.14

Values are not significant, P>0.05 vs. control

Table 3. The effect of the aqueous leaf extract of Justicia gendarussa on sedative parameters (mean±SEM)

Treatment		Locomotory activity	Number of rears	Number of head dips	Total time spent on head dips (s)	Time/head dip (s)	Faecal boluses
Control	(n = 9)	18.1±3.4	14.3±2.1	6.8±0.8	9.23±2.30	1.23±0.16	1.6±0.4
3000 mg/kg	(n = 6)	27.7±1.5*	21.0±2.0*	10.0±1.4	14.4±2.70	1.41±0.22	0.7±0.5

**Values are significant at P≤0.05 vs. control*

Table 4. The effect of the aqueous leaf extract of Justicia gendarussa on formalin test (mean±SEM)

Treatment		1st phase (0 – 5 min)				2nd phase (20 – 30 min)			
		Number of flinchings	Number of liftings	Number of lickings	Licking time (s)	Number of flinchings	Number of liftings	Number of lickings	Licking time (s)
Control	(n = 10)	5.6±1.0	8.9±1.0	14.4±1.4	80.7±7.6	0.7±0.5	9.4±2.5	13.5±2.5	56.8±6.9
3000 mg/kg	(n = 6)	4.17±1.1	4.0±0.8*	7.0±1.8*	42.0±8.8*	3.3±0.7	2.8±0.3*	7.0±1.2*	35.2±8.1*

** As compared with the control value significant at P < 0.05*

Table 5. The effect of the aqueous leaf extract of Justicia gendarussa on toxicological parameters (mean±SEM)

Treatment		Toxicological parameters					
		RBC $\times 10^4$ (cells/mm^{-3})	WBC $\times 10^3$ (cells/mm^{-3})	SGPT (U/L)	SGOT (U/L)	Serum creatinine (mg/dL)	Serum urea (mg/dL)
Control	(n = 6)	559.2±15	8.3±0.4	22.7±0.8	39.5±2.2	0.77±0.11	39.70±7.56
3000 mg/kg	(n = 6)	526.2±21	9.1±0.5	21.1±2.4	36.0±6.6	0.70±0.13	32.99±1.82

SGPT - serum glutamic-pyruvate transaminase SGOT- serum glutamic-oxaloacetic transaminase
Values are not significant, P>0.05 vs. control

Table 6. The effect of the aqueous leaf extract of Justicia gendarussa on differential count of blood (mean±SEM)

Treatment		Neutrophils (%)	Lymphocytes (%)	Monocytes (%)	Eosinophils (%)	Basophils (%)
Control	(n = 6)	30.3±0.7	67.8±0.8	1.2±0.4	0.8±0.3	0.0±0.0
3000 mg/kg	(n = 6)	29.8±1.1	67.8±1.5	1.5±0.6	0.7±0.3	0.0±0.0

Values are not significant, P>0.05 vs. control

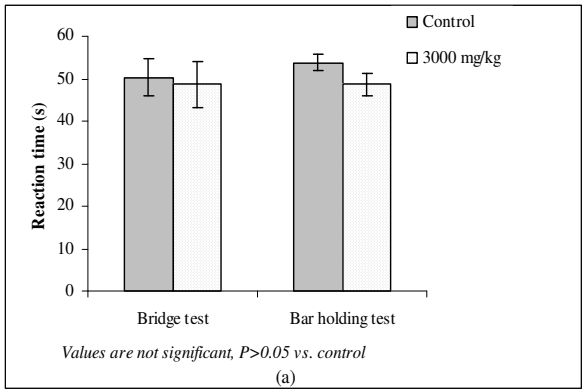
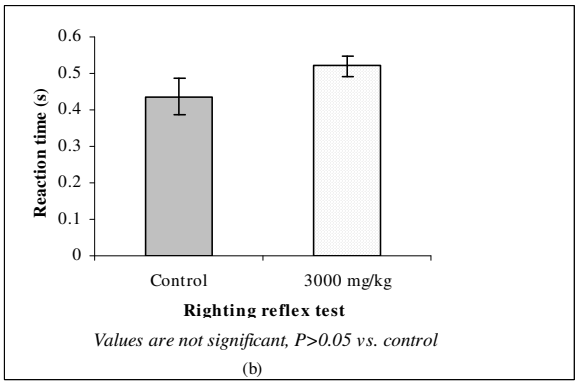


Figure 1. The effect of the aqueous leaf extract of Justicia gendarussa on (a) reaction time of Bridge, barholding tests (mean±SEM).



(b) righting reflex tests

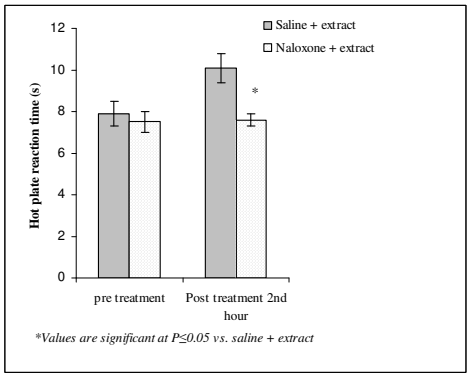
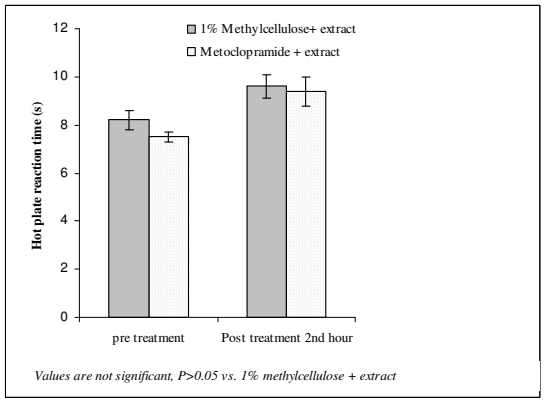


Figure 2. The effect of naloxone on the hot plate reaction time of the aqueous leaf extract of Justicia gendarussa (mean±SEM).



igure 3. The effect of metoclopramide on the hot plate reaction time of the aqueous leaf extract of Justicia gendarussa (mean±SEM).

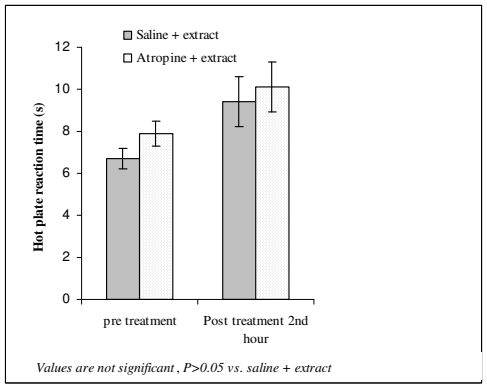


Figure 4. The effect of atropine on the hot plate reaction time of the aqueous leaf extract of Justicia gendarussa (mean±SEM).

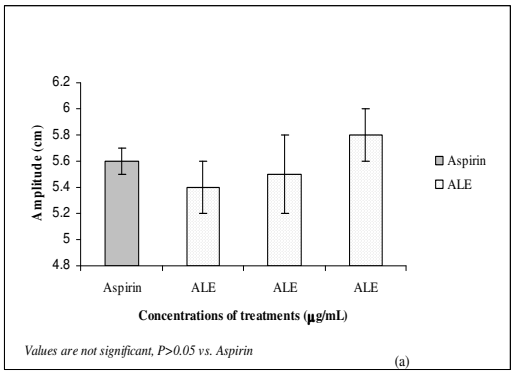


Figure 5. The effect of the aqueous leaf extract (ALE) of Justicia gendarussa on contractions of rat isolated uterus (a) Effect on amplitude (mean±SEM).

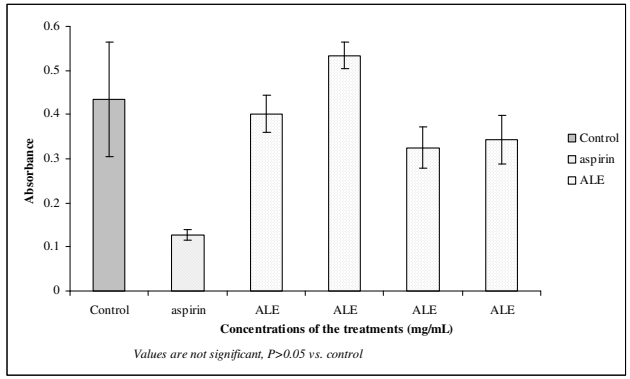


Figure 6. The effect of the aqueous leaf extract (ALE) of Justicia gendarussa on membrane stabilization (mean±SEM).

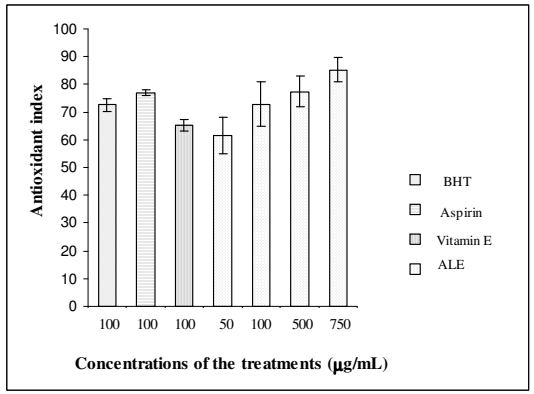
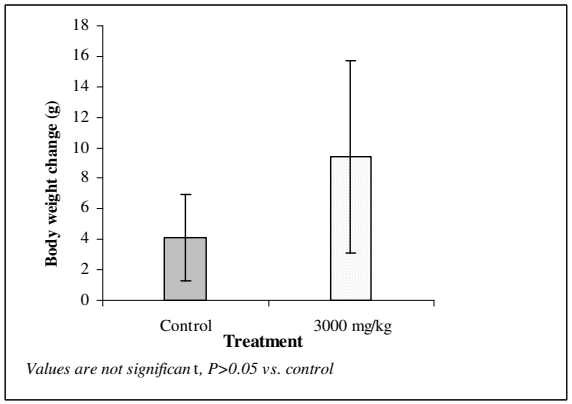


Figure 7. In vitro antioxidant index of the aqueous leaf extract (ALE) of Justicia gendarussa (mean±SEM).



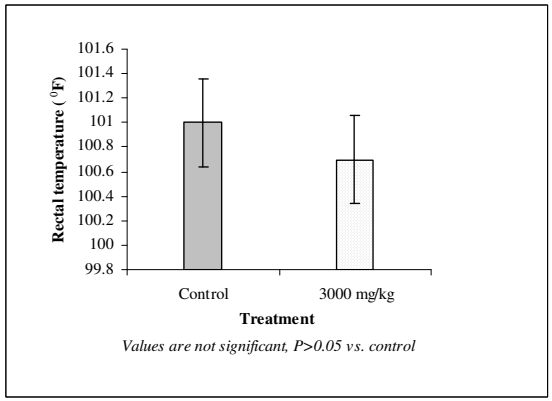


Figure 8. The effect of the aqueous leaf extract of Justicia gendarussa on (a) body weight change of rats (mean±SEM)

(b) rectal temperature (mean±SEM)

precipitated *via* cholinergic mechanisms (25). However, atropine, a muscarinic receptor antagonist (9) failed to curtail ALE induced antinociception. This suggests the ALE does not mediate antinociception *via* cholinergic mechanisms. Further, the absence of overt signs characteristic of cholinergic stimulation (such as salivation) following ALE administration lends further evidence in support of this notion. Dopamine receptor agonist have been shown to induce antinociception (26) but involvement of dopaminergic mechanisms in inhibiting pain is also unlikely in this study as metocloropromide, a dopamine receptor antagonist (9) failed to suppress ALE-induced anticiception. Neuronal membrane stabilizers and agents raising nociception threshold in neurons provoke antinociception (9). However, this mode of action appears to be non operative as ALE failed to inhibit heat-induced heamolysis of rat erythrocytes *in vitro* (9). Prostaglandins are linked with pain and prostaglandin synthesis inhibiting drugs are potent analgesics (9). However, antinociception with ALE is unlikely to be due impairment of prostaglandin synthesis as it failed to suppress the amplitude and the frequency of spontaneous contractions of rat isolated dioestrous uterine preparations. Sedatives can also have antinociception action (9). Since ALE promoted rather than suppressed sedatives parameters in the rat hole-board test, antinociception *via* sedation is unlikely in this study.

In contrast, ALE-induced nociception was blocked by naloxone, a potent opioids receptor antagonist (27) indicating the involvement of opioid mechanisms. Opioids suppress both phases of formalin test (28) and such an action was evident with ALE. This fact provides further support in favour of opioid mediation with ALE. Such an opioid mediated action may result from the presence of opioidiomimetric constituents such as alkaloids present in ALE and/or due to enhance release of endogenus opioid peptides. Several plant alkaloids are shown to be powerful antinociceptives operative *via* opioid mechanism (29, 30). Moreover, phenolic and steroidal constituents were present in the ALE and these are also known to impair both phases of the formalin test (31, 32). It is possible that these two chemical constituents could also have played a crucial role in inducing antinociception in this study. Oxygen free radicals are now implicated with pain (33). ALE possessed marked antioxidant activity when tested with thiobarbituric acid reactive substrate assay. It is thus possible that the antioxidant activity of ALE could also have contributed to its antinociception cation. Antioxidant activity of ALE could result from its flavonoids (34, 35). In conclusion, this study scientifically show, for the first time, that ALE of *J. gendarussa* possesses moderate and safe oral antinociceptive action justifying it's use as a pain alleviator in traditional medicine of Sri Lanka. It may be possible to develop a safe, potent and cost-effective novel antinociceptive pharmaceutical from this medicinal plant.

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