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Evaluation of *in vivo* anti-inflammatory and analgesic activity of *Dillenia indica* f. *elongata* (Miq.) Miq. and *Shorea robusta* stem bark extracts

Preet Amol Singh¹, Narsihma Baba Brindavanam², Gaya Prasad Kimothi², Vidhu Aeri^{1*}¹Department of Pharmacognosy and Phytochemistry, Jamia Hamdard University, Hamdard Nagar, New Delhi, 110062, India²Dabur Bio-resource Development Department, DRDC, Ghaziabad, India

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ABSTRACT

Objective: To evaluate the *in vivo* anti-inflammatory and analgesic potential of stem bark extract of *Dillenia indica* f. *elongata* (Miq.) Miq. (*D. indica* f. *elongata*) and its comparison with *Shorea robusta* Gaertn. (*S. robusta*) and respective standard drugs in experimental animals.

Methods: Analgesic models (hot plate, tail flick and formalin induced paw licking) along with acute (carrageenan-induced) and chronic (formalin-induced) models of inflammation were evaluated for analgesic and anti-inflammatory potential of the plant extracts.

Results: The results of the study showed that the ethyl acetate extracts of *D. indica* f. *elongata* (100 and 300 mg/kg) and *S. robusta* (100 and 300 mg/kg) possessed good central as well as peripheral analgesic activity as compared with pentazocine and indomethacin (10 mg/kg) respectively. The extracts showed significant ($P < 0.01$) activity in carrageenan- and formalin-induced chronic inflammation models by using indomethacin (8 mg/kg) and diclofenac (13.5 mg/kg) as standard drugs respectively.

Conclusions: It can be concluded that the presence of major constituents like flavonoids, tannins and phenols in the ethyl acetate extracts of stem bark of *D. indica* f. *elongata* (100 and 300 mg/kg) and *S. robusta* (100 and 300 mg/kg) may be responsible for its analgesic and anti-inflammatory activity.

1. Introduction

Inflammation is a complex physiological process associated with pain as a secondary process and its hallmarks include swelling, redness, pain and fever (tumor, rubor, dolor and calor)[1]. Inflammation is associated with the release of chemicals, basically leukotrienes, prostaglandins, bradykinin, histamine, interleukin-1 and platelet-activating factor, from tissues and migrating cells[2]. Steroidal and non-steroidal anti-inflammatory drugs are the most clinically accepted chemical therapeutics for the treatment of inflammation-related diseases, such as rheumatism, arthritis, cardiovascular diseases and asthma[3]. The greatest disadvantage of the presently available potent synthetic drugs for the treatment of inflammation-related diseases is their serious adverse effects and the chances of reoccurrence of symptoms after discontinuation[4]. Therefore, naturally occurring agents which have

lesser side effects with good analgesic and anti-inflammatory activities are required as a substitute to chemical therapeutics[5].

Dillenia indica f. *elongata* (Miq.) Miq. (*D. indica* f. *elongata*) is an evergreen forest plant rich in lupeol group of triterpenoids like betulin, betulinic acid and flavonoids which possess wide spectrum of pharmacological activities[6]. Bark (possesses astringent properties), fruit (possesses laxative properties, treats fever and cough)[7], alcoholic extract (has central nervous system depressant activity) and the mixed juice of leaf, bark and fruits are given orally for the treatment of cancer and diarrhoea[8].

Shorea robusta Gaertn. (*S. robusta*) (Dipterocarpaceae) is a plant used traditionally in India for treatment of many ailments and the plant contains chemical constituents such as polyphenols, shoreaphenol, chalcone glycoside ($C_{21}H_{22}O_7$), etc. The bark is used for the treatment of piles, ulcers and wounds[9,10]. The resin of *S. robusta* possesses astringent properties for which it is given for the treatment of diarrhoea, dysentery, skin, ear troubles and it is well documented in literature for its good analgesic and anti-inflammatory properties[10,11].

There is little information about the analgesic and anti-inflammatory

*Corresponding author: Dr. Vidhu Aeri, PhD, Department of Pharmacognosy and Phytochemistry, Jamia Hamdard University, Hamdard Nagar, New Delhi, 110062, India.

Tel: 09873496942

E-mail: vidhuaeri@yahoo.com

properties of *D. indica* f. *elongata* in literature. However, oral reports from a herbal medical practitioner in Pinjore, Haryana, India, indicate that extract of the plant is consumed orally for the treatment of pain and inflammation. Furthermore, the claim to investigate this plant was due to the activities of its bark, leaves and fruits used indigenously[12]. The bruised bark is applied as cataplasm for the arthritis patients[13]. So, the present study was undertaken to scientifically evaluate the anti-inflammatory and analgesic potential of ethyl acetate extracts of stem bark of *D. indica* f. *elongata* and its comparison with standard drugs and the stem bark extract of *S. robusta*.

2. Materials and methods

2.1. Plant materials

Bark of *D. indica* f. *elongata* was collected from Pinjore garden in Haryana, India. Likewise, bark of *S. robusta* was collected from Dehradun in Uttarakhand, India. The barks of *D. indica* f. *elongata* and *S. robusta* were authenticated with the help of taxonomist, Department of Botany, Punjabi University, Patiala, India with voucher No. PUN-59202 and PUN-59458, respectively.

2.2. Preparation of extracts

The barks were cut into small pieces, air dried and powdered. The powdered plant materials (500 g each) were extracted with ethyl acetate at 35 °C by using reflux for 12 h. The filtrates were concentrated separately under reduced pressure at 40 °C and stored in refrigerator. The reddish brown coloured extract of *S. robusta* [yield 2.124% (w/w) with respect to the dry material] and the dark brown coloured extract was obtained from *D. indica* f. *elongata* [yield 1.065% (w/w) with respect to the dry material]. For the administration to animals, the doses of ethyl acetate extracts of *D. indica* f. *elongata* (100 and 300 mg/kg) and *S. robusta* (100 and 300 mg/kg) were suspended in 0.5% (w/v) aqueous carboxy methyl cellulose.

2.3. Quantification of polyphenols and alkaloids in extracts

After qualitative analysis, screening was done to ascertain the quantitative presence of polyphenols and alkaloids in *D. indica* f. *elongata* and *S. robusta* plant extracts. The concentrations of alkaloids and flavonoids in these extracts were determined by using gravimetric method[14]. The determination of the total phenolic content was carried out by using spectroscopic method[15], while tannins were determined by using titrimetric method[16].

2.4. Drugs and chemicals

Betulin standard was purchased from Sigma-Aldrich Chemical Corporation (USA). Carrageenan was obtained from (S.D. Fine chemicals Limited, Mumbai, India), pentazocine (Ranbaxy laboratories,

Ahmedabad, India), indomethacin (IPGA, Mumbai, India), diclofenac sodium (Novartis, India), formalin (S.D. Fine chemicals Limited, Bombay, India).

2.5. High-performance liquid chromatography (HPLC) analysis of betulin in extracts

The HPLC analysis of betulin was performed in *D. indica* f. *elongata* and *S. robusta* extracts by using HPLC (waters, alliance) which consisted of a 100- μ L injector, waters reciprocating pump, 4 line in-line degasser and 2998 Photodiode Array Detector detector with Empower 2 integration software for the analysis. The chromatographic separation was achieved by using reverse phase Hypersil C18 column (250 mm) under isocratic elution of acetonitrile: water 85:15 (v/v) with a flow rate of 1.0 mL/min and the run time was set at 30 min. The detection wavelength was set at 210 nm and the injection volume was set at 20 μ L. All chromatographic operations were carried out at ambient temperature. Column temperature was maintained at 25 °C. The reference standard betulin was obtained from Sigma-Aldrich Chemical Corporation (USA). Distilled water was prepared with a Milli-Q academic water purification system (Millipore, Bedford, MA, USA). HPLC grade acetonitrile (Merk Ltd, Mumbai, India) was used for the HPLC analysis. All the solvents were membrane filtered through 0.45 mm pore size (Millipore). The chromatographic peak of betulin in plant extracts was confirmed by comparing their retention time and UV spectra with betulin standard.

2.6. Animals

Male Wistar Albino rats weighing 100–120 g were procured from Jamia Hamdard University, New Delhi. All animals were housed in polypropylene cages at (25 \pm 2) °C and relative humidity (30%–70%) with 12 h of light: 12 h of dark cycles. The animals were acclimatised to the laboratory conditions at least one week before starting the experiment. In all experimental models of inflammation and analgesic, six rats were used in each group. They were provided free access to food (M/s. Hindustan Lever Ltd., Mumbai, India) and water *ad libitum*. The experimental protocol No. 1081 got clearance from the Institutional Animal Ethics Committee and the care of the animals was taken according to the Committee for the Purpose of Control and Supervision on Experiments on Animals guidelines.

2.7. Analgesic activity

2.7.1. Hot plate method in rats

The hot plate method was selected for estimating centrally mediated analgesic effects of ethyl acetate extract of *D. indica* f. *elongata* and *S. robusta*[2]. The central analgesic drug, pentazocine, was used as a reference drug. For the experiments, six groups ($n = 6$) of Wistar Albino rats (100–120 g) were placed on a hot plate maintained at room temperature for 15 min for environmental adaptation. Food was

withdrawn on the previous night of the experiment. Group 1 (control group) received the vehicle [0.5% carboxy methyl cellulose (CMC), *p.o.*] and Group 2 (standard group) was administered with pentazocine (30 mg/kg, *i.p.*). Groups 3 and 4 received ethyl acetate extract of *D. indica f. elongata* (100 and 300 mg/kg, *p.o.* respectively), whereas Groups 5 and 6 were administered with ethyl acetate extract of *S. robusta* (100 and 300 mg/kg, *p.o.* respectively). Each animal was then individually placed on the hot plate maintained at 55 °C. Assessment of anti-nociceptive responses, such as licking paws or jumping off the hot plate, was determined at 15, 30, 45, 60 and 90 min after administration of the samples.

2.7.2. Tail flick method in rats

The tail flick test was employed for the purpose of assessing central analgesic activities of *D. indica f. elongata* and *S. robusta* by using pentazocine (25 mg/kg, *p.o.*) as a standard drug[17]. This method involved immersing extreme 3 cm of the rat's tail in a water bath containing water at temperature of (55.0 ± 0.5) °C. Animals reacted by withdrawing the tail within few seconds and the reaction time was noted on withdrawal of the tail by using stop-watch. Each animal served as its own control and two readings were obtained for the control at 0 and 10 min interval. The average of the two values was the initial reaction time. Group 1 received the vehicle (0.5% CMC, *p.o.*), and Group 2 was treated with pentazocine (25 mg/kg, *p.o.*). Groups 3 and 4 were administered with ethyl acetate extract of *D. indica f. elongata* (100 and 300 mg/kg, *p.o.* respectively), whereas Groups 5 and 6 received ethyl acetate extract of *S. robusta* (100 and 300 mg/kg, *p.o.* respectively). The reaction time of the tested groups were observed at 0.5, 1.0, 2.0, 3.0, 4.0 h after the latency period of 30 min after the administration of the tested samples. Time of no response was set at 2 min.

2.7.3. Formalin-induced pain

Formalin-induced paw licking test was used to assess to the peripheral analgesic activity of the extracts by using peripheral analgesic drug, *i.e.* indomethacin, as reference[18]. Pain was induced by injecting 0.05 mL of 2.5% formalin in distilled water in the subplantar of the right hind paw of rats. The control group received the vehicle (0.5% of CMC, *p.o.*) while indomethacin (10 mg/kg, *p.o.*) was given to the standard group. Groups 3 and 4 were administered by the extract of *D. indica f. elongata* (100 and 300 mg/kg, *p.o.* respectively), whereas Groups 5 and 6 were administered by *S. robusta* (100 and 300 mg/kg, *p.o.* respectively) 30 minutes prior to injecting formalin. The number of licks on the injected paw was indicative of pain. The number of licks in the early phase (0–5 min) and late phase (15–30 min) were counted after injecting formalin. The early phase represented neurogenic while the late phase represented inflammatory pain response.

2.8. Anti-inflammatory activity

2.8.1. Carrageenan-induced acute inflammation

The male Wistar Albino rats (100–120 g) were divided into six

groups (*n* = 6). The first group received CMC (0.5% 10 ml/kg, *p.o.*). The second group was treated with indomethacin (8 mg/kg, *p.o.*). Groups 3 and 4 received ethyl acetate extract of *D. indica f. elongata* (100 and 300 mg/kg, *p.o.* respectively), whereas Groups 5 and 6 were administered by ethyl acetate extract of *S. robusta* (100 and 300 mg/kg, *p.o.* respectively). Acute inflammation was induced by subplantar injection of 0.1 mL of 1% carrageenan (in 1% CMC w/v) in the right hind paw of the rats[2,19]. The animals were put on fast overnight prior to the start of experiment. The rats were pre-treated with the samples an h before the administration of carrageenan. The paw thickness (cm) of the rats was measured by vernier calliper at 0, 30, 60, 120 and 240 min interval after the carrageenan injection.

2.8.2. Formalin-induced chronic inflammation

The chronic inflammation study was carried out by using formalin to induce chronic inflammation model in which male Wistar Albino rats weighing between 100–120 g were randomly selected[18]. Rats were divided into six groups (*n* = 6). Group 1 served as the toxic control (induced by formalin), Group 2 was treated with diclofenac sodium (13.5 mg/kg, *p.o.*). Groups 3 and 4 was treated with *D. indica f. elongata* (100 and 300 mg/kg, *p.o.* respectively) whereas Groups 5 and 6 received *S. robusta* (100 and 300 mg/kg, *p.o.* respectively). The paw thickness of the left hind paw of each animal was measured with vernier calliper on the 0th day. On day 1 and day 3, they were injected with 0.1 mL of 2% v/v formaldehyde in normal saline into the subplantar region of the left hind paw for induction of chronic inflammation. Dosing with standard drug, *i.e.* diclofenac sodium, and extracts was started on the same day and continued for 10 days.

2.9. Statistical analysis

The results expressed in this study were evaluated by ANOVA between the control and test groups, followed by Dunnet's test. Significant levels were at *P* < 0.05 and *P* < 0.01 (95% confident limits).

3. Results

3.1. Quantification of polyphenols and alkaloids in extracts

The ethyl acetate extracts of *D. indica f. elongata* and *S. robusta* had shown positive results of polyphenols and alkaloids. It was investigated that both *D. indica f. elongata* and *S. robusta* possessed higher amount of flavonoids than other polyphenols and alkaloids as shown in Table 1.

Table 1

Quantification of polyphenols and alkaloids in plant extracts.

Plants	Polyphenols (% w/v)	Tannins (% w/v)	Alkaloids (% w/w)	Flavonoids (% w/w)
<i>D. indica f. elongata</i> (bark)	1.400	4.800	0.610	5.836
<i>S. robusta</i> (bark)	1.900	1.000	1.670	6.120

The data represent the higher amount of flavonoids in ethyl acetate stem bark extracts of *D. indica f. elongata* and *S. robusta*.

3.2. HPLC analysis of betulin in extracts

Betulin, a reported anti-inflammatory compound, was analysed in ethyl acetate extracts of *D. indica* f. *elongata* and *S. robusta* by using HPLC. Extract of *D. indica* f. *elongata* showed 2.510% w/w of betulin having least retention time, i.e. 10.603 min, by using HPLC while betulin was not detected in *S. robusta*. Graph of standard betulin and betulin in the extract of *D. indica* f. *elongata* is represented in Figure 1A, B respectively.

3.3. Analgesic activity

3.3.1. Hot plate method in rats

The analgesic activity possessed the ethyl acetate extract of *D. indica* f. *elongata* and *S. robusta* using hot plate test in Wistar Albino rats is illustrated in Figure 2. Pentazocine showed significant ($P < 0.01$) increase in reaction time over 90 min as compared with control group. Ethyl acetate extract of *D. indica* f. *elongata* and *S. robusta* (300 mg/kg) showed significant ($P < 0.05$) analgesic activity at 60 and 30 min respectively, whereas *D. indica* f. *elongata* and *S. robusta* (100 mg/kg) did not show significant analgesic activity. The results are shown in Figure 2.

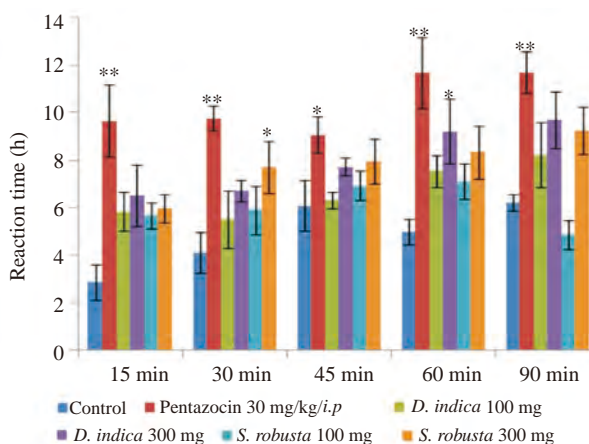


Figure 2. Analgesic activity (central) of *D. indica* f. *elongata* and *S. robusta* by using the hot plate method.

The data represent mean \pm SEM ($n = 6$). *: $P < 0.05$, **: $P < 0.01$, compared to corresponding control.

3.3.2. Tail flick test

Pentazocine (25 mg/kg, *p.o*) showed significant ($P < 0.01$) increase in reaction time at 1 h which got significantly ($P < 0.01$) reduced at 4 h as compared to control group. Ethyl acetate extract of *S. robusta* (100 and 300 mg/kg) showed significant ($P < 0.01$) analgesic activity which was more potent than standard pentazocine from 0.5 h to 1 h. It

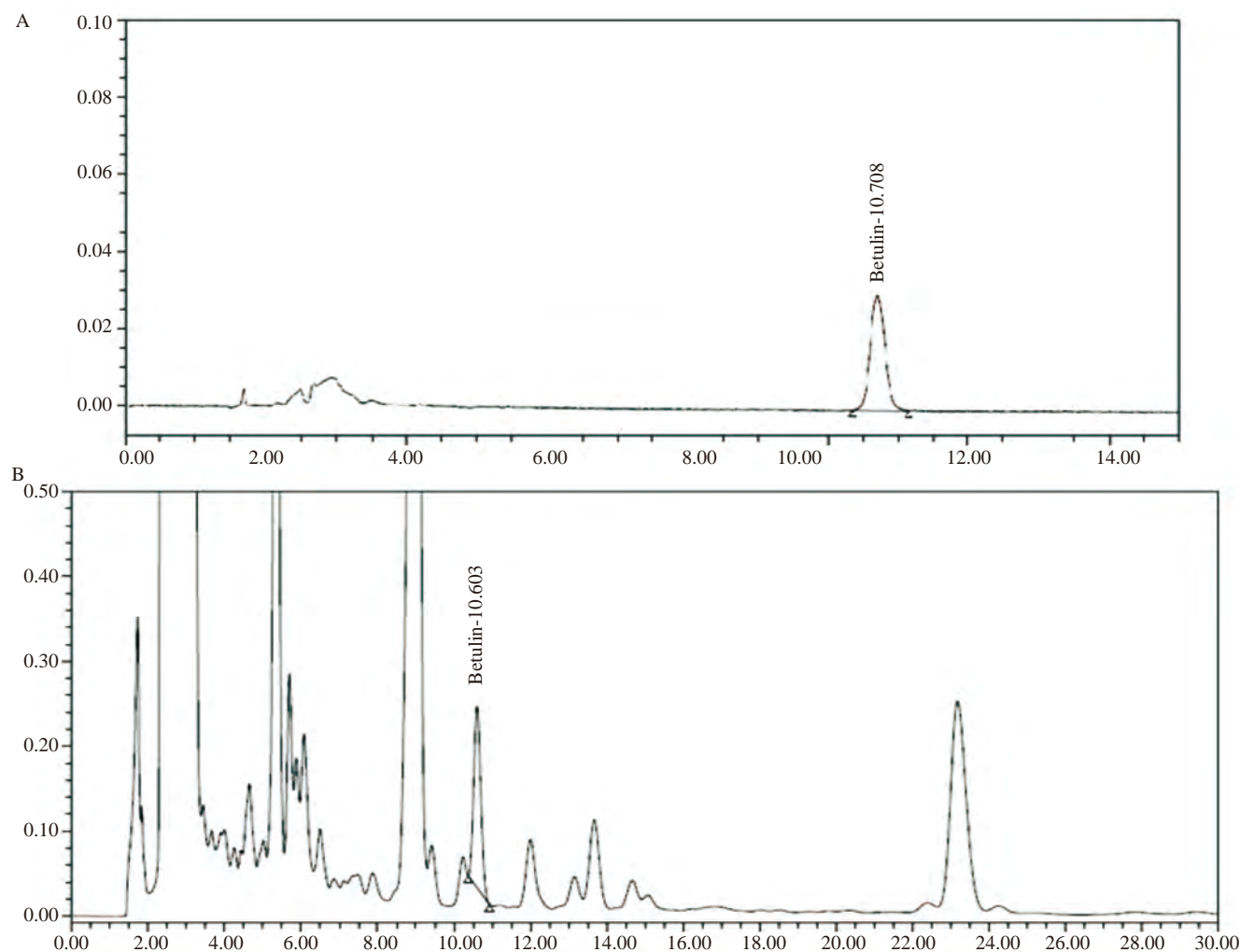


Figure 1. (A) HPLC chromatogram of standard betulin. (B) HPLC chromatogram of betulin in ethyl acetate extract of *D. indica* f. *elongata*.

showed significant ($P < 0.01$) decrease in activity at 4 h as compared with control group. *D. indica* f. *elongata* (100 mg/kg) possessed significant ($P < 0.01$) analgesic activity more than that of standard drug pentazocine at 1 h by increasing the reaction time which got significantly ($P < 0.01$) decreased at 4 h as compared with control group as represented in Figure 3. On the other hand, *D. indica* f. *elongata* (300 mg/kg) did not show significant activity. The response was dose-dependent (Figure 3).

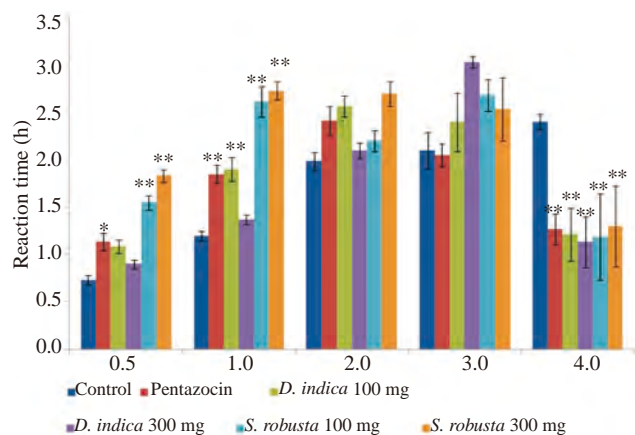


Figure 3. Analgesic activity (central) of *D. indica* f. *elongata* and *S. robusta* by using the tail flick method. The data represent mean \pm SEM ($n = 6$). *: $P < 0.05$, **: $P < 0.01$, compared to corresponding control.

3.3.3. Formalin-induced pain

Indomethacin, a nonsteroidal anti-inflammatory drug, showed a significant ($P < 0.01$) decrease in the number of paw lickings at the second phase (15–30 min). *D. indica* f. *elongata* and *S. robusta* (100 and 300 mg/kg) extracts were potent than indomethacin and significantly ($P < 0.01$) decreased the number of paw lickings at the second phase as represented in Figure 4. *D. indica* f. *elongata* (300 mg/kg) at the second phase (15–30 min) was the most protected group with very less number of paw lickings as represented in Figure 4. Moreover, *S. robusta* and *D. indica* f. *elongata* (100 mg/kg) in the first phase (0–15 min) significantly ($P < 0.01$) increased the number of paw lickings as compared with the control.

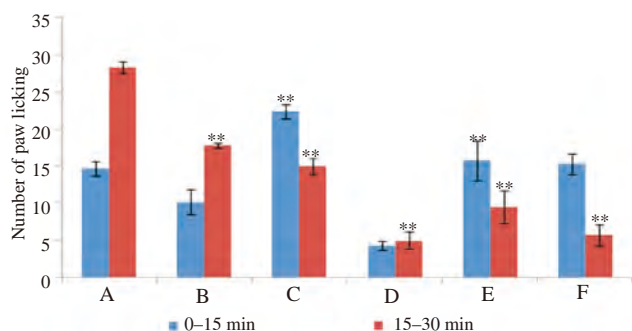


Figure 4. Analgesic activity (peripheral) of *D. indica* f. *elongata* and *S. robusta* by using formalin induced paw licking test. The data represent mean \pm SEM ($n = 6$). *: $P < 0.05$, **: $P < 0.01$, compared to corresponding control. A: Control; B: Indomethacin; C: *D. indica* 100 mg/kg; D: *D. indica* 300 mg/kg; E: *S. robusta* 100 mg/kg; F: *S. robusta* 300 mg/kg.

3.4. Anti-inflammatory activity

3.4.1. Carrageenan-induced acute inflammation

Carrageenan-induced paw edema remained even 4 h after its injection into the subplantar region of rat paw. Indomethacin, a standard drug, inhibited the edema formation due to carrageenan over 240 min at the dose of 8 mg/kg. The ethyl acetate extract of *D. indica* f. *elongata* (100 and 300 mg/kg) significantly inhibited edema formation over 240 min. Moreover, groups of *D. indica* f. *elongata* (100 and 300 mg/kg) remained more protected than indomethacin group as shown in Figure 5. On the other hand, ethyl acetate extract of *S. robusta* had shown less anti-inflammatory activity than *D. indica* f. *elongata* had down. Extract of *S. robusta* (100 mg/kg) remained more potent than *S. robusta* (300 mg/kg) and possessed good anti-inflammatory activity. In the present study, the ethyl acetate extract of *D. indica* f. *elongata* and *S. robusta* exhibited weak inhibitory effect at the early phase but inhibited the increase of paw volume during the late phase (3 h after carrageenan injection) of inflammation (Figure 5).

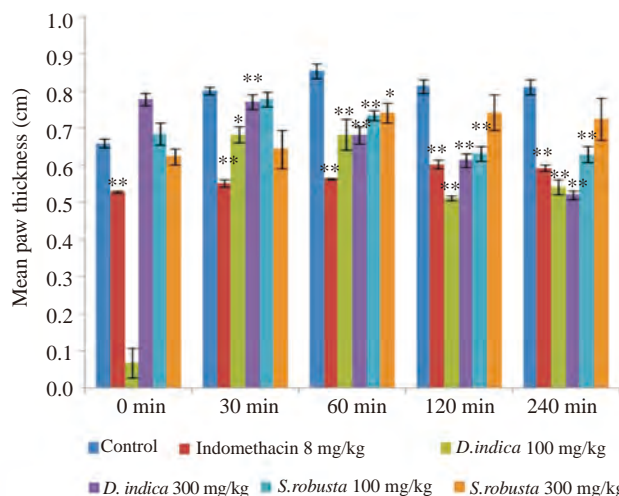


Figure 5. Anti-inflammatory (acute) activity of *D. indica* f. *elongata* and *S. robusta* by using carrageenan-induced model. The data represent mean \pm SEM ($n = 6$). *: $P < 0.05$, **: $P < 0.01$, compared to corresponding control.

3.4.2. Formalin-induced chronic inflammation

The paw thickness was measured daily for 10 days after the induction of inflammation, but on the 1st, 4th, 6th, 8th, 10th days the extracts had shown visible results in rats. The inflammation was significantly ($P < 0.05$) reduced from day 4 in *D. indica* f. *elongata* and *S. robusta* (300 mg/kg) administered groups by 35.42% and 39.22% respectively. The treatment with ethyl acetate extract of *S. robusta* (300 mg/kg) had shown the maximum inhibition (52.04%) of paw edema on the 8th day as compared to toxic control and it was also more protected than the standard drug diclofenac. On the other side, *D. indica* f. *elongata* (300 mg/kg) was more protected than diclofenac on the 10th day showing the maximum inhibition (54.41%) of paw edema as represented in Figure 6. Both *D. indica*

f. elongata and *S. robusta* (300 mg/kg) possessed good anti-inflammatory activity but *D. indica f. elongata* was more potent than *S. robusta* and diclofenac sodium on the 10th day (Table 2).

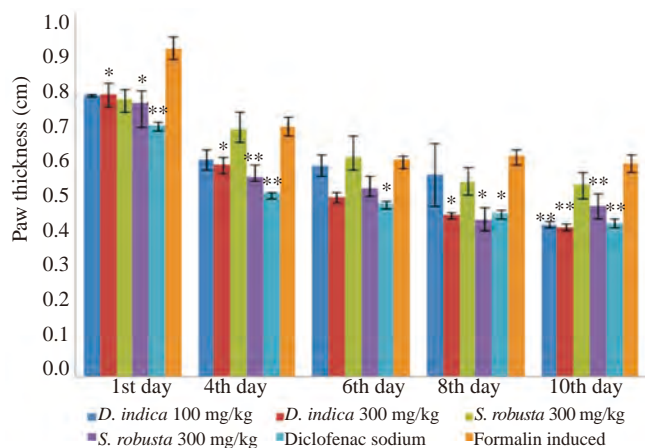


Figure 6. Anti-inflammatory (chronic) activity of *D. indica f. elongata* and *S. robusta* by using formalin-induced chronic model.

The data represent mean \pm SEM ($n = 6$). *: $P < 0.05$, **: $P < 0.01$, compared to corresponding control.

Table 2

The percentage of inhibition of ethyl acetate extracts of *D. indica f. elongata*, *S. robusta* and diclofenac sodium on formalin-induced chronic inflammation.

Days	<i>D. indica</i> 100 mg/kg	<i>D. indica</i> 300 mg/kg	<i>S. robusta</i> 100 mg/kg	<i>S. robusta</i> 300 mg/kg	Diclofenac sodium 13.5 mg/kg	Formalin induced
1st	14.04	13.87	15.06	16.52	23.33	0.00
4th	33.70	35.42	24.55	39.22	44.08	23.70
6th	35.57	45.29	33.01	42.58	47.33	33.53
8th	38.30	50.74	40.50	52.04	50.24	32.53
10th	53.58	54.41	41.58	47.89	53.19	34.79

The data represent the percentage of inhibition of inflammation induced by formalin as compared to toxic control. Toxic control: Formalin induced; Standard drug: Diclofenac sodium; *D. indica*: Ethyl acetate extract of *D. indica f. elongata* (*p.o.*); *S. robusta*: Ethyl acetate extract of *S. robusta* (*p.o.*).

4. Discussion

Pain is categorised into two types: peripheral and central. The peripheral pain involves peripheral pain afferent neurons activated by disagreeable stimulus while central pain is activated by afferent inputs pain sensation[17]. The hot plate test was used to find central analgesic activity and is regarded to be a supraspinally organized response involving higher brain functions[10]. The results obtained from the hot plate method indicated that *S. robusta* and *D. indica f. elongata* (300 mg/kg) possessed supraspinally mediated analgesic action by increasing the reaction time of Wistar Albino rats as compared to control group. The tail flick test was further studied to corroborate central analgesic activity of the extract. Since the extracts had shown analgesic activity in tail flick test, it is indicated that the extracts were acting centrally on opioid receptors, *i.e.* μ , κ_3 , κ_1 , d_2 and d [10,17]. Ethyl acetate extracts of *S. robusta* (100 and 300 mg/kg) and *D. indica f. elongata* (100 mg/kg) had shown central analgesia by increasing the reaction time of Wistar Albino

rats in tail flick test. *D. indica f. elongata* (300 mg/kg) remained less active, which may be due to dose dependent response of the extract or its higher dose stimulating messengers antagonising, its analgesic response. The formalin-induced paw licking test was carried out to investigate peripheral analgesic activity and to fortify the evidence of the anti-nociceptive activity of the extracts seen in hot plate and tail flick tests. The test includes two phases. The early phase (0–5 min) related to acute neurogenic pain activates nociceptors and primary afferent fibres by formalin to release bradykinin and tachykinins[18,20]. It is followed by an inflammatory pain which is accompanied by the release of inflammatory mediators such as serotonin, excitatory amino acids, histamines and prostaglandins[10]. *D. indica f. elongata* and *S. robusta* (100 and 300 mg/kg) had shown significant decrease in the number of paw lickings in rats at the second phase, thus suggesting that the extracts inhibited inflammatory mediators active during the second phase.

The results obtained from the inflammatory studies suggest that the extracts markedly inhibited paw oedema by oral administration in both carrageenan- and formalin-induced chronic inflammation. Carrageenan-induced paw edema in rats is biphasic in nature. The early phase involves the release of kinins, histamines and serotonin while the late phase involves the release of prostaglandins[21]. In the present study, the ethyl acetate extract of *D. indica f. elongata* and *S. robusta* had shown a weak inhibitory effect at the early phase but inhibited the increase of paw volume during the late phase (3 h after carrageenan injection) of inflammation. Based on this biphasic nature of carrageenan and significant activity observed during the last phase of carrageenan inflammation, it may be suggested that the extracts inhibited prostaglandin or the release of late mediators involved in carrageenan-induced paw edema[2,20]. Formalin-induced chronic inflammation is one of the most desirable model for the evaluation of chronic inflammation studies. Inflammation caused by formalin is again biphasic in nature. The early phase is neurogenic mediated by substance P and bradykinin followed by a tissue mediated response involving the release of histamines and prostaglandins[22]. The study confirms that the extracts were more potent during the late phase, *i.e.* on the 4th and 10th day, thus inhibiting tissue mediated response involving the release of histamines and prostaglandins.

The phytochemical screening of the extracts possessed high amount of flavonoids which has been attributed to inhibit pain perception and inflammation[23]. Betulin, a pentacyclic-triterpenoid, known to possess a good anti-inflammatory activity by inhibiting enzyme phospholipase A2, cyclooxygenase-2, cytokines, chemokines[24,25], was quantified (2.510% w/w) in *D. indica f. elongata* better anti-inflammatory activity of *D. indica f. elongata* than the extract of *S. robusta* (devoid of betulin), which can be linked to the synergistic action of betulin in promoting anti-inflammatory activity. It is concluded in the present study that the

ethyl acetate extracts of *D. indica* f. *elongata* and *S. robusta* have both central and peripheral anti-nociceptive activity which may be due to the blockade of opioid receptors (κ , μ , δ), prostaglandins and histamines. The presence of flavonoids, phenols and tannins might have played a major role in promoting anti-nociceptive and anti-inflammatory activity.

Conflict of interest statement

We declare that we have no conflict of interest.

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