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Research Article

Pharmacological Evaluation of Traditional Medicinal Plant of *Dyschoriste Littoralis* Nees

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ABSTRACT

To evaluate the analgesic, anti-inflammatory and anti-pyretic activity of aerial parts of plant extracts and isolated compounds of *Dyschoriste littoralis* Nees (Fam: Acanthaceae). The drug materials were prepared by successive soxhlation of petroleum ether, chloroform, ethyl acetate and methanol. Analgesic activity was performed by tail-flick technique in mice. The inflammatory reaction is readily produced in rats in the form of paw edema with the help of carrageenan (0.1 ml of 1% w/v) which was injected into the right hind paw sub-plantar region of rat to determine the anti-inflammatory effect. Pyrexia was induced by injecting subcutaneously 15% w/v 10 ml/kg yeast suspended in 0.5% w/v methyl cellulose solution into the animal's dorsum region. Bioactive compounds were isolated by running column chromatography, characterized and percentage of activity of bioactive principle was calculated. The methanol extract at a dose of 600 mg/kg body (P<0.05) and isolated compound B (P<0.001) shown the more increase in percentage of activity than a reference diclofenac sodium (P<0.001). The isolated compounds A & B shows the reduction in body temperature within 30 min as that one and the same to standard drug paracetamol. DLME (methanolic extract) at a dose of 600 mg /kg exhibited a maximum percentage of activity in the dose dependent inhibition than DLEAE (ethyl acetate extract) on carrageenan-induced rat paw edema. These results suggest that the antipyretic, analgesic, and anti-inflammatory activity were may be due to its bioactive principle luteolin and quercetin present in the extract and reveals that *Dyschoriste littoralis* could be used as potential drug for the treatment of pain, fever and inflammation.

Keywords: *Dyschoriste littoralis;* anti-pyretic; analgesic; anti-inflammatory; luteoline; quercetin

INTRODUCTION

Inflammation is a homeostatic phenomenon and is usually associated with pain as a secondary process resulting from the release of analgesic mediators (Hunskaar et al, 1987; Osadebe et al, 2003). When inflammation is left to itself, it displays a short course of reaction (acute inflammation), but under certain conditions, it becomes a more sustained event (chronic inflammation) such as that prevailing in human rheumatoid arthritis. A vast array of substances, the so-called mediators of inflammation, are formed or released either concurrently or successively at the site of injury from various plasma or cell sources in response to an etiological factor (Hollander 1982). Pyrexia/fever may be a result of secondary impact of infection or one of the consequences of tissue damage, inflammation, graft rejection and malignancy or other diseased status. Pyrexia was enhanced by formation of cytokines (IL 1β, IL-6, Interferon α, β and TNFα). This pro-inflammatory mediator increases the synthesis of PGE_2 in many tissues near to the pre-optic hypothalamic area via an increase in cyclic AMP and triggers the hypothalamus to elevate the normal body temperature by promoting an increase in heat generation and decrease in heat loss (Doscombe, 1985).

During the course of reviewing the traditional medicinal plants in various states of India, the investigation of the efficacy of plant-based drugs used in the traditional medicine have been paid great attention because they are cheap, have little side effects and according to WHO still about 80 % of the world population rely mainly on plant-based drugs (Kumara, 2001). It was seen that many plants have remarkable effects as on pyrexia, pain, infections, and liver ailments. During an appraisal of medicinal plants, it was found that plants belong to acanthaceae family was potential for its antipyretic, anti-inflammatory and analgesic activity (Kiritikar and Basu, 1987), and they were traditionally used as, antipyretic, anti-inflammatory and analgesic activity in various parts of India. From the above background, we plan to evaluate the plant used by

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most of the traditional practitioners in Tamilnadu and ethno medical information given in various literatures named as *Dyschoriste littoralis* Nees belongs to the family Acanthaceae for its antipyretic, anti-inflammatory and analgesic activity.

MATERIALS AND METHODS

Plant collection, drying, pulverizing and preparation of extract

The *Dyschoriste littoralis* Nees herb was collected from the moist places near Tirunelveli, identified and authenticated by Mr.V.Chelladurai, Botanist, Central Council for Ayurveda and Siddha, Government of India. The voucher specimen was preserved in our laboratory for future reference. After collection of the plant, the root was removed; the aerial part was washed thoroughly in tap water and dried in shade for about 10 days under controlled temperature (25± 2 ºC), powdered and passed through a 40 mesh sieve and stored in a well closed container for further use. Coarsely powdered dried aerial plant (1.3 kg) was successively soxhlated using petroleum ether, chloroform, ethyl acetate and methanol for 72 h at room temperature respectively. The extracts were filtered and the solvents evaporated to dryness under reduced pressure in an Eyela rotary evaporator at 40 to 45°C. The percentage yield was noted as 2.91 for petroleum ether, 4.58 for chloroform, 3.50 for ethyl acetate and 7.91 for methanol. Preliminary phytochemical screening was performed (Harborne, 1987).

Chemicals and instruments

Solvents for extraction were purchased from Qualigens fine chemical (P) limited Mumbai. TLC was carried out using Merck aluminium sheet coated with silica gel GF_{254} (0.2 mm). The melting points were recorded in a Technico melting point apparatus. The UV Spectrum was measured with a Shimatzu UV-1700 double beam Spectrophotometer. The IR spectra of isolated compounds were recorded on a Jasco FTIR-4100 in potassium bromide discs. ^{1}H NMR spectra were recorded on a Bruker 300 FT NMR Spectrometer using $CDCl_3$ as solvent and tetramethylsilane (TMS) as internal standard. Mass studies were done by JEOL-SX-102 instrument.

Pharmacological studies

Experimental animals

Wistar albino rats and Swiss albino mice were used for pharmacological evaluations. The animals were maintained in colony cages at 25±2°C, relative humidity of 45-55%, under a 12 h light and dark cycle; they were fed standard animal feed. All the animals were acclimatized for a week before use. The Institutional Animal Ethics committee approved the protocol adopted for the experimentation of animals.

Acute toxicological studies

Acute oral toxicity was performed as per the OECD 423 guidelines (Ecobichon, 1997). Three female Albino mice weighing 22–25 g was used in the study. The animals were fasted overnight before the treatment. Each animal was administered orally a dose level of 5 mg/kg body weight by gastric intubation. The mice were housed individually in polypropylene cages and were observed for clinical signs of toxicity at 30 min, 1 h, 2 h, 4 h and for 48 h post dosing, with special attention during the first 4 h. The administered dose was assigned as toxic if mortality was observed in three animals. If no mortality was observed, the procedure was repeated with further higher doses such as 50, 300, 1000, 2000 and 3000 mg/kg body weight.

Antipyretic activity

Antipyretic activity was determined on male Wistar albino rats, weighing about 200-250 g. The rats showing 37.5 $\pm$ 0.5°C were selected and then they were fasted for 24 hours before inducing pyrexia. Their normal body temperature was recorded. The normal body temperature of each rat was measured rectally at predetermined intervals. Fever was induced as per the method by injecting subcutaneously 15% w/v 10 ml/kg yeast suspended in 0.5% w/v sodium carboxy methyl cellulose (CMC) solution into the animal's dorsum region (Zhao et al, 2005) and injected site is massaged in order to spread and they were allowed to food. After advocating this presumed dose uniformly, the rats were returned to their housing cages. After 19 h of yeast injection, the rectal temperature of each rat was measured again using a digital thermometer. Only rats that showed a net increase in temperature of at least 0.7°C were used for the antipyretic study. The animals were divided into 8 groups of 6 each and numbered. The control, standard and test substances were given to the animals by gastric tube. After the drug was administered, the temperature of all the rats in each group was recorded at an interval of 30 min, 1 h, 2 h, 3 h and 4 h. The mean temperature was found out for each group and compared with the standard drug values. The animals marked as group -I received orally 5 ml/kg of body weight of 0.5 % CMC. The animals marked group-II, group III, group, IV group V, group VI group VII and group VIII received orally 150 mg/kg of paracetamol in 0.5 % CMC, 300 mg/kg, 600 mg/kg of DLEAE, 300 mg/kg, 600 mg/kg of DLME, and isolated compounds (A) & (B)10 mg/kg respectively.

Analgesic activity

The test extracts and the standard drugs were administered in the form of a suspension (0.5% CMC) by oral route. Test for analgesic activity was performed by tail-flick technique (Miranda et al, 2003) on Swiss albino mice (25-35 g) of either sex selected by random sampling technique. Each group consisted of six animals. Diclofenac sodium at a dose level of 10 mg/kg was ad-

ministered orally as reference drug for comparison. The test extracts at two dose levels (300, 600 mg/kg) and isolated compound A & B at a dose level of 10 mg/kg were administered orally. The reaction time was recorded at 30 min, 1, 2 and 3 h after the treatment, and cut-off time was 10 sec. The percent analgesic activity (PAA) was calculated by the following formula,

$$PAA = \left[\frac{T_2 - T_1}{10 - T_1} \right] X\ 100$$

Where T_1 is the reaction time (s) before treatment and T_2 is the reaction time (s) after treatment.

Anti-inflammatory activity

The inflammatory reaction is readily produced in rats in the form of paw edema using carrageenan. The rats were divided into eight groups, each consisting of six animals. DLEAE and DLME of (300 and 600 mg/kg in 0.5 % w/v CMC was administered orally. The first and second groups received 5 ml/kg of 0.5 % w/v CMC as vehicle control and 10 mg/kg (p.o) of diclofenac sodium as standard respectively, for comparative pharmacological assessment. Group-III, IV, V, VI, VII, and VIII treated with 300 and 600 mg/kg of DLEAE and DLME and isolated compound A& B at a dose level of 10 mg/kg respectively. After 30 min of extracts administration, 0.1 ml of 1% w/v carrageenan was injected into the right hind paw sub-plantar region of each rat. The left paw served as reference (non-inflammatory paw) for comparison. The paw volumes of both paws of control and extract treated rats were measured at 1 h, 2 h, 3 h, 4 h and 5 h after carrageenan administration (Winter et al, 1962). The percentage inhibition for each rat and each group was obtained by using the formula C−T/C×100 where C is the edema rate of control group and T for treated group.

RESULTS

Acute toxicity

The animals were observed for toxic symptoms of behavioural changes, locomotion, convulsions and mortality. The extracts found to nontoxic up to the dose of 3000 mg/kg**.**

Anti pyretic activity

Ethyl acetate and methanol extracts of the *Dyschoriste littoralis* Nees were screened for antipyretic activity (Table 1). Methanol extract at the dose of 300 mg/kg & 600 mg/kg body weight showed maximum antipyretic activity response ($P < 0.001$) equivalent to that of paracetamol after 1 h of administration, but isolated compounds A & B shows the reduction in body temperature within 30 min as that one and the same to standard drug paracetamol. The results indicated that the major component responsible for antipyretic activity may be present in the methanol extract and compounds isolated from the same extract has been identical in activity in terms of time to that of standard drug.

Analgesic activity

The results of analgesic testing indicate that the test extracts and isolated compounds exhibited an increase in percentage of analgesic activity at 30 min of reaction time when compare to standard drug and an increase in activity at 1 h which reached a peak level at 2 h. Decline in activity was observed at 3 h Table 1. Among the extracts, methanol extract at a dose of 600 mg/kg body weight ($P < 0.05$) and isolated compound B ($P < 0.001$) shown the more increase in percentage of activity than a reference diclofenac sodium ($P < 0.001$).

Anti-inflammatory activity

The results for the anti-inflammatory activity of DLEAE tested by carrageenan induced rat paw edema were recorded in Table 2. DLEAE and DLME produces maximum activity at a dose of 300 mg/kg showed 37.61% and 46.51% protective effect respectively after 2 h of administration. DLEAE and DLME at a dose of 600 mg/kg showed a significant protective effect (38.28 % and 50.938%) of paw edema. The standard drug diclofenac sodium (10 mg/kg) produced the maximum protective effects of 43.78%. Thus, DLME at a dose of 600 mg/kg exhibited a maximum percentage of activity in the dose dependent inhibition than DLEAE on carrageenan-induced rat paw edema (Table 2). Similarly, the isolated compound A & B ($P < 0.001$) exhibited more than 10 % increase in activity than standard drug (Table 1).

Confirmation of bioactive isolated active principle

Fractionation in column chromatography sorted out the following bioactive molecules known as luteolin and quercetin. **Luteolin:** The ^{1}H-NMR investigation has given the following chemical shifts (δ in ppm). 5.12 (s, 1H, OH), 5.45 (s, 1H, OH), 5.70 (s, 1H, OH), 5.99 (s, 1H, OH), 6.31-7.59 (m, 6H, Ar-H). The M^+ peak appeared at 286 confirmed as luteoline by the molecular mass of the compound in mass spectrum and IR (KBr) cm-1: 3513 (OH), 3096 (Ar-CH), 1734 (C=O), 1032 (C-O-C). The molecular formula was found to be $C_{15}H_{10}O_6$.

Quercetin: The ^{1}H-NMR investigation has given the following chemical shifts (δ in ppm). 4.72 (s, 1H, OH), 5.08 (s, 1H, OH), 5.41 (s, 1H, OH), 5.87 (s, 1H, OH), 6.20 (s, 1H, OH), 6.80-7.82 (m, 6H, Ar-H). The M^+ peak appeared at 302 confirmed as luteoline by the molecular mass of the compound in mass spectrum and IR (KBr) cm-1: 3538, (OH), 3074 (Ar-CH), 1739 (C=O),1023 (C-O-C). The molecular formula was found to be $C_{15}H_{10}O_7$.

DISCUSSION

Pyrexia may be due to the infection or one of the sequence of the tissue Damage, inflammation, graft rejection or other diseased states (Mahesh et al, 2009). The enhanced formation of pro inflammatory mediators like cytokines, interleukin 1β, α, and TNF – α) in-

Table 1: Antipyretic activity of the extracts of *Dyschoriste littoralis Nees*

Groups	-18 h	0 h	30 min	1 h	2 h	3h
Group I-Control	35.00±0.44[a]	37.00±0.36[a]	37.33±0.33[a]	38.00±0.25[a]	38.50±0.34[a]	39.58±0.20[a]
Group II: Paracetamol (150mg/kg)	33.83±0.54[a]	38.50±0.25[a]	37.50±0.40[a]	36.75±0.35[a]	36.33±0.65[a]	35.67±0.44[a]
Group III: DLEAE 300 mg/kg	35.50±0.22[a]	38.42±0.20 [a]	38.08±0.27[a]	37.08±0.20 [a]	36.67±0.21[a]	36.33±0.16 [a]
Group IV: DLEAE 600 mg/kg	35.50±0.22 [a]	38.75±0.17 [a]	38.25±0.17 [a]	37.17±0.16 [a]	37.25±0.25 [a]	36.08±0.08 [a]
Group V: DLME 300 mg/kg	35.50±0.22 [a]	38.75±0.17 [a]	38.08±0.23 [a]	37.67±0.21[a]	36.83±0.16 [a]	36.25±0.17 [a]
Group VI: DLME 600 mg/kg	35.50±0.22 [a]	38.75±0.25 [a]	37.58±0.27 [a]	37.25±0.25 [a]	36.92±0.37 [a]	35.83±0.27 [a]
Group VII: Luteolin 10 mg/kg	34.50±0.34[a]	38.50±0.22[a]	37.33±0.33 [a]	36.33±0.21 [a]	35.83±0.30 [a]	35.17±0.30 [a]
Group VIII: Quercetin 10 mg/kg	35.50±0.22 [a]	38.75±0.17 [a]	37.92±0.20 [a]	37.17±0.16 [a]	36.58±0.20 [a]	36.00±0.12 [a]

Data expressed as mean ± SEM from six different experiments. Significance levels [a]p<0.001 as compared with the respective control.

creases the synthesis of prostaglandin E_2 (PGE_2) near the pre-optic hypothalamus area, and leads to triggering the hypothalamus to elevate the body temperature due to the infected or damaged tissue (Sanjib et al, 2010). Paracetamol a non-steroidal anti-inflammatory drug (NSAID), is commonly prescribed to treat fever inhibits cyclooxygenase -2 (COX-2) to decrease the body temperature by inhibiting PGE2 biosynthesis. It causes toxic effects to the liver cells, glomeruli, cortex of brain and heart muscles due to inhibition of COX-2, but natural COX-2 inhibitors have lower selectivity with fewer side effects (Chattopadhyay et al, 2005).

The antipyretic activity demonstrated that the extracts possess a significant effect in maintaining normal body temperature and reducing Brewer's yeast induced pyrexia in rats, and their effects, are comparable with that of standard antipyretic drug paracetamol. Such reduction in rectal temperature of tested animals by both the extracts at 300 mg/kg and 600 mg/kg may be due to the presence of flavonoids. The presence of flavonoids in DLEAE, DLME and the presence of isolated luteolin, may be responsible for antipyretic activity. The antipyretic activity of luteolin isolated from other medicinal plants was already established and the activity was attributed due to the inhibition of synthesis of TNF-α and anti-oxidant activity associated with amelioration of inflammatory actions of cytokines (Shah et al, 2011). It was also evident from the study that the antipyretic activity of DLME at 600 mg/kg is almost similar to that of paracetamol. Thus, the antipyretic activity supported the claims of traditional practitioners of *Dyschoriste littoralis* Nees as an antipyretic remedy.

The present study indicates that the methanol extract has shown significant analgesic action in mice, by increasing the latency period in the tail flick test. Preliminary phytochemical screening revealed the presence of flavonoids compound in *Dyschoriste littoralis* Nees and they are recognized as powerful antioxidants. In the analgesic activity, flavonoids primarily target prostaglandins, which are involved in controlling pain perceptions (Rajnarayana et al, 2001). Therefore, it can be assumed that *Dyschoriste littoralis* Nees extracts might suppress the formation of prostaglandins by inhibiting or antagonizing the enzyme cyclooxygenase (Das et al, 2010) and also makes the nociceptors more sensitive to pain producing agents such as bradykinin and ultimately relieve the sensation of pain. Carrageenan induced paw oedema method is the most commonly used method for screening and evaluation of anti-inflammatory activity of natural and synthetic compounds. The initial phase of study observed at 1 h may be attributed due to the release of histamine and serotonin (Crunkhon and Meacock, 1971). The oedema maintained during the plateau phase is presumed to be due to the release of kinin like substances (Van Arman et al, 1965; Di Rosa et al, 1971). The second accelerating phase of swelling may be due to the release of prostaglandins, i.e., PGE_2 and nitric oxide. During the inflammatory phase, from the macrophage, the reactive free radical nitric oxide (NO) is synthesized by inducible NO synthase (NOS), where the excessive production of NO plays a pathogenic role both in acute and chronic inflammations (Clancy et al, 1998). NO is reported for vasodilatations, increase in vascular permeability, edema formation and inducing the synthesis of prostaglandins at the site of inflammation (Moncada et al, 1991; Grisham et al, 1999). DLEAE and DLME showed significant anti-inflammatory activity at 2 h against carrageenan injection, suggesting that it predominantly inhibited the release of inflammatory mediators from phlogistic stimuli. The anti-inflammatory activity may be due to presence of flavonoids in DLEAE

Table 2: Anti-inflammatory and analgesic activity of the extracts of *Dyschoriste littoralis Nees*

Group/Treatment	Percent Anti-inflammatory activity				Percent Analgesic activity			
	30 min	1 h	2 h	3h	30 min	1 h	2 h	3h
Group I-Control	---	----	---	---	1.85± 1.85^{Ns}	5.78 ± 2.59 ^{Ns}	7.87± 2.50 ^{Ns}	3.93± 2.49 ^{Ns}
Group II: Diclofenac sodium (10mg/kg)	32.45± 9.83^{b}	36.50± 4.33^{c}	43.78± 3.69^{c}	33.04± 2.97^{b}	35.18± 3.89^{c}	42.82 ± 5.20^{c}	48.61 ± 5.83^{c}	29.17± 3.42^{c}
Group III: DLEAE 300 mg/kg	20.17± 5.33^{b}	33.73± 5.33^{c}	37.61± 2.75^{c}	19.70± 2.46^{b}	29.41± 3.03^{a}	35.30± 2.63 ^{a}	35.30± 2.63^{a}	19.60± 1.96 ^{a}
Group IV: DLEAE 600 mg/kg	29.82± 4.22^{c}	38.50± 4.57^{c}	38.28± 2.01^{c}	26.40± 3.10 ^{c}	33.33± 2.48 ^{a}	39.22± 1.96 ^{a}	43.14± 3.61 ^{a}	25.49± 2.48 ^{a}
Group V: DLME 300 mg/kg	20.17± 6.71^{a}	36.10± 4.97^{c}	46.51± 4.02^{c}	23.17± 2.63 ^{b}	37.26± 2.48^{a}	41.18± 3.03 ^{a}	45.10± 2.47^{a}	21.56± 2.48 ^{a}
Group VI: DLME 600 mg/kg	28.94± 5.88^{c}	45.63± 5.33^{c}	50.93± 2.48^{c}	27.13± 1.40^{c}	45.10± 2.47^{a}	50.98± 1.96 ^{a}	52.84± 4.23 ^{a}	31.37± 3.61 ^{a}
Group VII: Luteolin 10 mg/kg	35.08± 4.43^{c}	46.82± 5.45^{c}	53.82± 5.48^{c}	27.42± 2.37^{c}	45.10± 3.92 ^{c}	52.94± 4.29^{c}	54.90± 3.61 ^{c}	35.30± 2.63^{c}
Group VIII: Quercetin 10 mg/kg	25.77± 3.41^{b}	42.46± 8.68^{c}	53.37± 4.78^{c}	29.62± 1.97^{b}	47.06± 2.63 ^{a}	56.76± 2.41 ^{a}	58.67± 2.56^{a}	33.33± 2.48 ^{a}

Data expressed as mean ± SEM from six different experiments. Significance levels $^{a}p<0.05$, $^{b}p<0.01$and $^{c}p<0.001$ as compared with the respective control

and DLME, confirmed by phytochemical investigations and the compound luteoline isolated from the column fractions. In the present study, flavonoids of *Dyschoriste littoralis* Nees was found to suppress carrageenan induced edema significantly (*P*<0.01) but its anti-inflammatory action was less effective than diclofenac sodium. The analgesic activity is thought to be due to the presence of flavonoids and its primarily target towards the prostaglandins which are involved in controlling pain perceptions. Therefore, it can be assumed that *Dyschoriste littoralis* Nees extracts might suppress the formation of prostaglandins by inhibiting or antagonizing the enzyme cyclooxygenase and also makes the nociceptors more sensitive to pain producing agents such as bradykinin and ultimately relieve the sensation of pain. These findings suggest that the *Dyschoriste littoralis* could be used as a potential drug for the treatment of pain, fever and inflammation. However further studies in the aspects of COX enzyme inhibition is required to rule out the molecular level interaction for the pain, perception and inflammation pathway.

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