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Antinociceptive and Antipyretic Effects of *Plectranthus amboinicus* (Lour) Spreng Leaves

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ABSTRACT

The leaves of *Plectranthus amboinicus* (Lour) Spreng were traditionally used for the treatment of pyrexia and acute pains. The present study was carried out using acetic acid-induced writhing and tail immersion tests in mice while, yeast-induced pyrexia in rats. The leaves were subjected to successive extraction using the various solvents (petroleum ether, chloroform, ethanol and water) in the increasing order of polarity. It was found that the leaves revealed the presence of alkaloids, carbohydrates, glycosides, proteins, amino acids, flavonoids, quinine, tannins, phenolic compounds and terpenoids. Both extracts (500 mg/kg, p.o.) produced the significant ($P<0.01$) Antinociceptive and antipyretic effects. The observed pharmacological activities provide the scientific basis to support traditional claims as well as, exploring some new and promising leads.

KEYWORDS: Pyrexia, Acute pains, Ethanolic extract, Aqueous extracts, *Plectranthus amboinicus*.

INTRODUCTION

Analgesia (pain) is an ill defined unpleasant, sensation usually evoked by an external or internal noxious stimulus. Pain is a warning signal and primarily protective in nature, but causes discomfort. Analgesics are the drug that selectively relieves pain by acting in the CNS or on peripheral pain mechanisms, without significantly altering consciousness. The plant *Plectranthus amboinicus* (Lour.) Spreng belongs to family Lamiaceae, known as country borage in English.[1] It is large succulent aromatic perennial herb, shrubby below, hispidly villous or tomentose.[2] It is found or cultivated

throughout India, Ceylon and Moluccas.[3] earlier claims showed that the leaves are being traditionally used as a diuretic.[2] Upon literature review it was found that the plant contains butylanisode, β-caryophyllene, quercetin, ursolic acids, triterpenic acids, α-pinene, β-pinene, thymol, eugenol, carvacrol, 1,8-cineole, β-phellandrene, p-cymene, salvigenin, crisimaritin and chrysoeriol.[4,5,6,7,8,9] Many pharmacological properties have reported including urolithiasis,[10,11] antiepileptic,[12] antitumour and antimutagenic,[13] neuropharmacoligical,[14] radioprotective effect,[15] antioxidant,[16] anti-microbial,[17,18] anti-bacterial, anti-fungal properties.[19,20] However there are no reports to our knowledge on its antinociceptive and antipyretic activities. Hence, the present study was undertaken to investigate antinociceptive and antipyretic potential of the ethanolic and aqueous leaf extract of *Plectranthus amboinicus* (Lour) Spreng in experimental animal models.

MATERIALS AND METHODS

Plant material

The leaves of *Plectranthus amboinicus* (Lour) Spreng were collected from the fields of Kanchipuram, Tamil Nadu. It was authenticated by Director, Plant Anatomy Research Centre (PARC), Chennai. A voucher specimen no. PARC/2007/89 has been deposited in the institute.

Extraction of plant material

The 500 gms of air-dried leaves were made into coarse powder. The powdered material was successively extracted with petroleum ether, chloroform, ethanol and water by cold maceration in increasing order of their polarity.[21] In addition the fresh powder was defatted with petroleum ether and extracted with 95% ethanol (72 hours) and water (24 hours) separately. The extracts were filtered with muslin cloth and solvent was distilled off.

Phytochemical tests

The petroleum ether (PEPA), chloroform (CEPA), ethanolic (EEPA) and aqueous (AEPA) extracts of *Plectranthus amboinicus* were subjected to preliminary, qualitative phytochemical investigation.[22] The percentage yield of PEPA, CEPA, EEPA, AEPA were found to be 2.47, 3.69, 12.2 and 18.1 respectively.

Animals

Healthy, adult Wistar rats (180-260 gms) and Swiss albino mice (18-25 gms) of either sex were obtained from the animal house, Vel's College of Pharmacy, Pallavaram, Chennai (CPCSEA/12-12-00/PH-07-14). The animals were maintained in well-ventilated room temperature with natural 12hrs + 12hrs day night cycle in the propylene cages. The animals were fed with balanced pellet and water *ad libitum*. The animals were housed for one week prior to the experiments to acclimatize to the laboratory conditions.

Acute toxicity study

Six Wistar rats (180-260 gms) and six Swiss albino mice (18-25 gms) of either sex were dosed with extracts in different concentrations and were observed for any symptoms of toxicity for 48 hrs as per guidelines no. 425 (OECD 2001) and LD_{50} was estimated > 5000mg/kg. Based on the results obtained from this study the doses of further pharmacological studies were fixed to be 500 mg/kg.[23]

Antinociceptive activities

Acetic acid-induced writhing

Mice were divided in to four groups each consisting of 6 mice. They were starved for 18 hrs. The treatment regimen is as follows:

Group 1 (Control) :Vehicle (3ml/kg, p.o.), 1% suspension of Tween-80

Group 2 (Standard) :Aspirin (150mg/kg, p.o.)

Group 3 (Test-1) :Ethanolic leaf extract (500mg/kg, p.o.)

Group 4 (Test-2) :Aqueous leaf extract (500mg/kg, p.o.)

After half an hour all mice received a 0.7% aqueous solution of acetic acid 10 mg/kg, i.p. and writhings were counted for 10 minutes after the acid injection.[24]

Acetic acid induced writhing test.

$$\% \text{ inhibition} = \frac{\text{Mean no. of writhing in control} - \text{Mean no. of writhing in test group}}{\text{Mean no. of writhing in control group}}$$

Tail immersion method

Mice were divided in to four groups each consisting of 6 mice. The treatment regimen is as follows:

Group 1 (Control) :Vehicle (3ml/kg, p.o.), 1% suspension of Tween-80

Group 2 (Standard) :Pentazocine (30mg/kg, p.o.)

Group 3 (Test-1) :Ethanolic leaf extract (500mg/kg, p.o.)

Group 4 (Test-2) :Aqueous leaf extract (500mg/kg, p.o.)

The distal part of the tails of the animals was immersed in hot water maintained at 55.0±1.0 ^{o}C. The time taken to withdraw the tail was noted as reaction time. A cut off time of 10 sec was maintained at 55 ^{o}C to prevent tissue damage. The reaction time was measured at 0, 15, 30, 45 and 60 min after treatment, respectively.[24]

Antipyretic activity

The test was performed in rats by injecting 10 ml/kg s.c. of 15% aqueous solution of Brewer's yeast to induce pyrexia. Rectal temperature of each animal was taken before and 24h after the yeast injection using digital clinical thermometer. Animal that did not show a minimum increase of 0.7 ^{o}C in temperature 24h after yeast injection were discarded. The selected animals were divided in to 4 groups and treated as follows:

Group 1 (Control) :Vehicle (3ml/kg, p.o.), 1% suspension of Tween-80

Group 2 (Standard) :Paracetamol (20mg/kg, i.p.)

Group 3 (Test-1) :Ethanolic leaf extract (500mg/kg, p.o.)

Group 4 (Test-2) :Aqueous leaf extract (500mg/kg, p.o.)

The rectal temperature of each animal was again recorded at 0.5, 1, 1.5 and 2 h after treatment.[25]

Data analysis

SPSS (version 13.0) statistical program was used to carry out a one-way analysis of variance was used to carry out a one-way analysis of variance (ANOVA) on data followed by Dunnett's t test. $P<0.01$ was considered as significant, when compare to standard drug.

RESULTS

Phytochemical tests

The crude extract was found to be positive for the presence of alkaloids, carbohydrates, glycosides, proteins, amino acids, flavonoids, quinine, tannins, phenolic compounds and terpenoids. However the crude extract was found negative for the presence of steroids and anthocyanins.

Effect on acetic acid writhing

The ethanolic and aqueous extract (500 mg/kg, p.o.) significantly reduced the acetic acid-induced writhing by 60.02 and 51.39% respectively (Table 1).

Effect on tail-immersion tests

Both the extracts induced significant protection (Table 2) in mice in tail-immersion tests being the ethanolic extracts the most active comparable with the standard drug pentazocine (30mg/kg, p.o.).

Antipyretic effect

Both the extracts showed a marked antipyretic effect (Fig 1) by causing a reduction in yeast-induced fever. Ethanolic extract showed the effect to the same degree as paracetamol (20 mg/kg, i.p.).

Table 1: Effect of ethanolic and aqueous extracts of *Plectranthus amboinicus* leaf in acetic acid induced writhing in mice

Treatment	**Dose**	**Writhing count**	**Inhibition (%)**
Control	3ml/kg, p.o.	37.89±1.09	--
Aspirin	150mg/kg, p.o.	11.24±0.06*	70.34
Ethanolic extract	500mg/kg, p.o.	15.15±0.04*	60.02
Aqueous extract	500mg/kg, p.o.	18.42±0.11*	51.39

Values are expressed as mean ± SEM of six samples, * represents $P<0.01$ when compared to control,
Data were analysed by One-way ANOVA followed by Dunnett's test

Table 2: Effect of ethanolic and aqueous extracts of *Plectranthus amboinicus* leaf on tail-immersion tests in mice

Treatment	**Dose**	**Reaction time (Sec)**	**Latency (%)**
Control	3ml/kg, p.o.	2.8±0.49	--
Pentazocine	30mg/kg, p.o.	4.64±0.73*	65.71
Ethanolic extract	500mg/kg, p.o.	4.06±0.82*	45.00
Aqueous extract	500mg/kg, p.o.	3.87±0.38*	38.21

Values are expressed as mean ± SEM of six samples, * represents $P<0.01$ when compared to control,
Data were analysed by One-way ANOVA followed by Dunnett's test

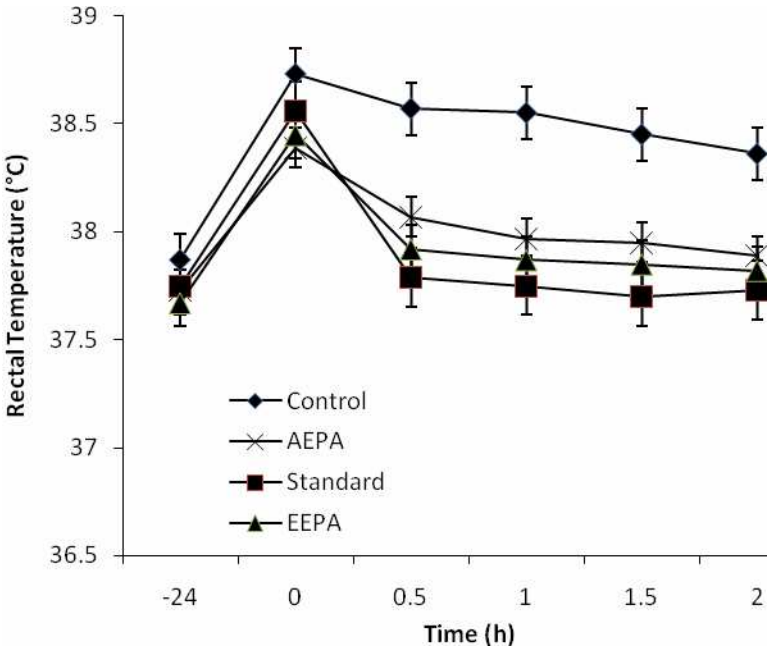

Fig 1: Effect of ethanolic and aqueous extracts of *Plectranthus amboinicus* leaf on yeast induced pyrexia in rats. Values are expressed as mean ± SEM of six samples. Data were analysed by One-way ANOVA followed by Dunnett's test.

DISCUSSION

Several experimentally induced laboratory models were employed in evaluating the antinociceptive and antipyretic effect of ethanolic and aqueous extracts of *Plectranthus amboinicus*. It is necessary to apply tests which differ with respect to stimulus quality, intensity and duration, to obtain as complete a picture as possible of the analgesic properties of a substance using behavioural nociceptive tests. The results obtained showed that the both extracts possess significant analgesic effect on the various pain models used. A significant inhibitory effect was shown by both the extracts on writhing test (a test useful for evaluating mild analgesic non-steroidal anti-inflammatory agents). This suggests that the analgesic effect of the extract may be peripherally mediated. The extracts were also showed a significant effect in the tail-immersion tests (Centrally acting analgesic drugs elevate pain threshold of animals towards heat and pressure). The effect of the extracts on this pain models indicates that it might be centrally acting.[25]

The extract caused a better hypothermal activity against yeast-induced pyrexia in rats. Subcutaneous injection of yeast induces pyrexia by increasing synthesis of prostaglandin and is used to screen agents for antipyretic effect.[24]

The Antinociceptive and antipyretic activity of *Plectranthus amboinicus* Lour. may be due to the individual or combined action of bio-active constituents present in it. The findings will be helpful for further phytochemical and pharmacodynamic investigations to find the active constituents responsible for the activity, which may explore some new and promising leads.

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