Indian J Physiol Pharmacol 2005; 49 (3) : 289–296

PHARMACOLOGICAL STUDIES OF *CLERODENDRON COLEBROOKIANUM* WALP, A POTENT HYPOTENSIVE PLANT

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(Received on January 12, 2005)

Abstract : *Clerodendron colebrookianum* Walp., (Fam: Verbenaceae) locally known is "NEFAFU" is widely used for curing various diseases. Here some pharmacological properties of this plant were studied using rat & mice of either sex. Methanol extract (MLE) of various concentrations (50, 100, 200 mg/kg of body wt) were tested on animals. Carrageenin induced rat paw oedema model with three hours for oedema formation was used to test anti-inflammatory activity. It was observed that the plant extract significantly inhibits the Carrageenin induced rat paw oedema. The acetic acid induced writhing test by injecting 0.6% acetic acid (i.p.) followed by injecting MLE & tail immersion test, both in hot & cold water was used to test the analgesic effect of the plant In all the four experiments MLE (200 mg/kg, of body wt.) has been found mostly effective in inhibiting Carrageenin induced rat paw oedema, the number of writhings induced by acetic acid & elevated pain threshold in hot & cold-water test. It reduced the number of abdominal writhing induced by acetic acid and elevated pain threshold in hot tail flick test. The effect of methanol extract (MLE) on phenobarbitone induced sleeping time was also tested, here again MLE (200 mg/kg of body wt) showed remarkable prolongation in sleeping time. Seasonal variation on the activities of the plant extract was also investigated in the study. The plant samples were collected in the months of January and July of the year. It has been observed that the January collection of the plant showed higher activities in most of the parameters in these experiments and also showed significantly higher values in the proximate analysis. The leaves of the *C. colebrookianum* were practically found to be non-toxic.

Key words : *clerodendron colebrookianum* walp anti-inflammatory writhing analgesic phenobarbitone sleeping time proximate analysis

INTRODUCTION

Medicinal plants play a key role in the human healthcare. About 80% of the world population relies to treat a plethora of diseases on traditional remedies, which is predominantly based on plant materials. The traditional medicine refers to a broad range of ancient, natural healthcare practices including folk/tribal practices as

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well as Ayurveda, Siddha, Amchi, Unani. These medical practices originated since time immemorial and developed gradually to a large extent, by relying or based on practical experiences without significant references to modern scientific principles. Medicinal herbs are an indispensable part of the traditional medicine practiced all over the world due to low costs, easy access and ancestral experience (1). These practices incorporated ancient beliefs were passed from generation to generation. Though effective but herbal medicines are unscientifically exploited and/or improperly used, therefore this field needs proper study in the light of modern science (2).

Various species of the genus Clerodendron have been reported to possess medicinal properties (3, 4, 5). *Clerodendron colebrookianum* Walp (Verbenaceae), an evergreen shrub, commonly called as 'Nefafu' by the local people, grows generally in the moist and waste places of North-Eastern regions at higher altitudes (5). The plant reported to contain triacontane, amyrin, clerodin, (24s) ethyl cholesta 5, 22, 25 trien 3-ol, clerodolone, clerodendoside, B-sitosterol, clerosterol, daucosterol, colebrin A-E (4, 5, 6). The leaves and leaf twigs of this plant are used as home remedy for high blood pressure by the people of North-Eastern regions of India (4, 7). The roots *of Clerodendron colebrookianum* Walp have anthelmintic, antibacterial, anti-fungal properties (8) and are reported to cure bronchial asthma, gastrointestinal tract disorders, syphilis and gonorrhea and several hematological disorders (3). The present study aims to investigate some pharmacological activities of the plant extract and seasonal variation of the activities.

METHODS

Extract preparation :

The plants were collected from near by areas of Guwahati, Assam in January and July months of 2002. A botanist of Gauhati University identified the plants. A voucher specimen of the plant has been preserved in the Division of Life Sciences of Institute of Advanced Study in Science and Technology for future record. After proper identification, the plants were shade dried, powdered separately, collected in the two different seasons. The powdered plant material was exhaustively extracted with methanol in soxhlet apparatus. The extract was completely dried in rotary vacuum evaporator below 40°C. The extract was now ready for use. All the experiments were done in two sets using these extracts processed and prepared separately of the two seasons.

Air-dried and powdered plant materials were subjected to preliminary phyto-chemical screening (9) and proximate analysis (10) of the plant was determined following the official procedure given in standard books. Moisture contents (11) (on fresh sample), ash values, extractive values (10) of the leaves of the plant were determined Metal contents are determined using Atomic Absorption Spectrophotometer (Model AAS – 680, Shimazdu, Japan).

The extract was administered to animals after making a suspension of the extract with proper amount of 2.5% Tween-80.

Animals

Male Wistar rats (160–210 gm) and Swiss albino mice (20–35 gm) of either sex were used. They were maintained on a standard normal diet and provided with water *ad libitum*. Approval of the Institutional Animal Ethics Committee has been obtained for this entire work.

Carrageenin induced paw oedema in rat:

Pedal inflammation in rats was produced according to the method of Awe et al (12). An injection was made of 1% carrageenin (Sigma, USA) suspension (prepared in normal saline) into the right hind foot of each rat in the planter region. Test groups of rats were given MLE at a dose of 50, 100, 200 mg/kg (i.p.) 30 minutes before carrageenin injection. At the same time control group received 0.2 ml of Tween-80 and reference group received 10 mg/kg *indomethacin* (i.p.) (M/S. Jagsonpal Pharmaceuticals Ltd., India). Increase of linear paw circumference was measured by tying a piece of cotton thread round the rat's paw and noting the point of intersection of two ends on a meter scale (12, 13). This was taken as an index of paw volume, which is a measure of oedema. Measurements were taken immediately before and at 3 hours after carrageenin injection. The inhibitory activity was calculated according to the following formula (12):–

$$\text{Percent inhibition} = \frac{[(Ct - Co)\text{ of control} - (Ct - Co)\text{ of treated}]}{(Ct - Co)\text{ of control}} \times 100$$

Ct=Linear paw circumference 3 hours after carrageenin injection
Co=Linear paw circumference 3 hours before carrageenin injection

Analgesic activity:

Acetic acid induced writhing test (Chemical stimulation):

Male Swiss albino mice were divided into four groups. Group I was used as a control group, received 0.2 ml of Tween-80, an hour before (i.p.) injection of 0.6% v/v acetic acid (10 ml/kg, prepared in normal saline). Test groups (Group 2 and 3, Test 1 and 2) received MLE at a dose of 100 and 200 mg/kg an hour before acetic acid injection and reference group received 400 mg/kg of Dispirin (M/S. Rickitt Benckiser Ltd., India). The number of abdominal constrictions (writhing) and stretching with a jerk of the hind limb was counted between 5 and 15 minutes after administering acetic acid.

Percent protection against writhing movement was taken as index of analgesia and it was calculated with the help of the following formula (12):–

$$\%\,\text{Protection} = \frac{(\text{Number of writing in control} - \text{Number of writhing in treated})}{\text{Number of writhing in control}} \times 100$$

Tail immersion method:–

Hot tail flick test:

Albino mice of either sex were used. All the mice were screened by exposure to the thermal stimulus. Those showing positive response were divided into four groups, e.g. control, Test 1, Test 2 and reference. Before experiment animals of all the groups were fasted for 24 hours with water given *ad libitum* maintained at room temperature. Group wise animals received i.p. injection

of Tween-80 (control), 100 and 200 mg/kg of MLE (test 1 and 2) and 1 mg/kg of Pentazocine as reference (M/S. Ranbaxy Lab. Ltd., India). About 5 cm of the tail of mice was dipped in warm water kept constant at 50 ± 0.7°C. The time taken to withdraw the tail clearly out of water was considered as the reaction time with the cutoff time being 60 sec. First reading was discarded and the reaction time was taken as a mean of next two readings. The latent period of the tail flick response was taken as the index on antinociception and was determined immediately after injection, second reading was taken 30 minutes after injection and third reading was taken 60 minutes after injection. The maximum reaction time was fixed at 4 min. The maximum possible analgesia (MPA) was calculated as: (14).

$$MPA = \frac{[\text{Test reaction time} - \text{Saline reaction time}]}{[240 - \text{Saline reaction time}]}.$$

Cold tail flick test:

Albino mice of either sex was taken and screened by exposure to the cold stimulus. Those showing positive response were divided into four groups e.g. control, Test 1, Test 2 and reference. Before experiment animals of all the groups were fasted for 24 hours with water given *ad libitum* maintained at room temperature. Group wise animals received i.p. injection of Tween-80 (control), 100 and 200 mg/kg of MLE (Test 1 and 2) 1 mg/kg of Pentazocine. About 5 cm of the tail of mice was dipped in a cold 1:1 mixture of water and ethylene glycol kept constant at –10 ± 0.7°C. The time taken to withdraw the tail clearly out of water was considered as the reaction time with the cutoff time being 60 sec. First reading was discarded and the reaction time was taken as a mean of next two readings, The latent period of the tail flick response was taken as the index on antinociception and was determined immediately after injection, second reading was taken 30 minutes after injection & third reading was taken 60 minutes after injection. The maximum reaction time was fixed at 4.2 min. The maximum possible analgesia (MPA) was calculated as: (14, 15).

$$MPA = \frac{[\text{Test reaction time} - \text{Saline reaction time}]}{[252 - \text{Saline reaction time}]}.$$

Effect on phenobarbitone sleeping time:

Albino mice of either sex were divided into four groups e.g. control, Test-1, Test-2 and Reference. Control group received 0.2 ml of 2.5% Tween-80, i.p. and test groups (1 and 2) received (100 and 200 mg/kg) of MLE respectively i.p. and reference group received Chlorpromazine (10 mg/kg, i.p.) (M/S. Medopharm Lab. Pvt Ltd., India) 30 minutes later animals of all the groups were injected with 20 mg/kg of phenobarbitone, i.p. (M/S. Biodeal Lab. Pvt.. Ltd., India). The duration of righting reflex was taken as a measure of sleeping time (12).

Statistical analysis:

Results of biochemical estimates are expressed as Mean ± SEM. Total variation present in a set of data was estimated through one-way analysis of variance (ANOVA).

RESULTS

The values of successive solvent extraction, proximate analysis and inorganic metal ion determination of the plant are recorded in Table I. The values of eleven metals have been determined separately in two seasons of the year (Table I). The plant samples collected in the January month show higher concentration of metal ions than that of the June collection of the plant In contrary, it has been observed that the Cobalt ion content in the July collection of the plant is 200.00 ppm, whereas in January collection it is 145.00 ppm only. The values of Magnesium and Iron ion contents are significantly high in the plant in both the seasons than that of the other metal ion contents. The plant contains no Pb ion in both the seasons.

TABLE I: Preliminary Phytochemical Screening and Proximate analysis of *C. colebrookianum* leaves.

Parameters	*Results*	
	January collection	*July collection*
Extractive values (% W/V)		
Pet Ether (60-80°C)	1.23	1.70
Chloroform	0.46	0.54
Ethyl acetate	3.65	3.32
Methanol	14.47	12.67
Water	15.21	16.11
Proximate analysis (% w/v)		
Foreign organic matter	0.90	1.20
Moisture content	3.24	3.84
Total ash	12.10	11.50
Acid soluble ash	2.70	2.50
Water soluble ash	4.23	4.68
Sulfated ash	21.64	20.24
Alcohol soluble extractive	3.60	3.40
Water soluble extractive	11.70	12.20
Inorganic metal ion content (ppm)		
Sodium	1783.22	1663.32
Potassium	2453.40	2242.50
Calcium	896.60	875.80
Cobalt	145.50	200.20
Copper	462.90	458.00
Iron	4520.20	3612.30
Magnesium	6631.20	5332.30
Zinc	1104.60	1034.40
Lead	–	–
Nickel	103.20	112.50
Manganese	432.40	395.20

Methanol extract (MLE) of the leaves of the C *colebrookianum* was administered in the dose of 50, 100 and 200 mg/kg, i.p. and 200 mg/kg dose was found to inhibit 87.5% carrageenin induced rat paw oedema (Table II). 200 mg/kg dose showed higher activities than the lower doses ($P<0.001$). The January collection plants are found to be more potent in respect of paw oedema reduction.

TABLE II: Effect of MLE on carrageenin induced rat paw oedema at 3 hours.

Group	*Dose (mg/kg)*	*Mean increase in paw circumference (c.m.) ± S.E.E.*	*Percent inhibition (%)*
Control	Tween-80	0.80±0.009	–
MLE	50	0.45±0.008	43.75
MLE	100	0.25±0.006	68.75
MLE	200	0.10±0.010***	87.50
Reference (Indomethacin)	10	0.20±0.012***	75.00

***$P<0.001$, compared with control, n = 6.

$$\text{Percent inhibition} = \frac{[(Ct-Co)_{\text{of control}} - (Ct-Co)_{\text{of treated}}]}{(Ct-Co)_{\text{of control}}} \times 100$$

Ct=Linear paw circumference 3 hours after carrageenin injection
Co=Linear paw circumference 3 hours before carrageenin injection

In the acetic acid writhing experiment MLE of the plant was administered i.p. in the dose of 100 and 200 mg/kg b.w. and 200 mg/kg dose showed significant effects on

TABLE III: Effect of MLE an Acetic Acid induced writhing in mice.

Group	*Dose (mg/kg)*	*Mean number of writhings ± S.E.M. (15 min)*	*Percent inhibition of writhings (%)*
Control	Tween-80	21.17±0.48	–
MLE	100	9.30±0.42	56.07
MLE	200	7.30±0.42***	65.52
Reference (Dispirin)	400	7.00±0.52***	66.93

***P<0.001, compared with control, n = 6.

rats (P<0.001) in a dose dependent manner. 200 mg/kg dose exhibited a 65.51% inhibition in writhing in rats. Dispirin was used as the reference drug in this experiment (Table III).

The analgesic effect of the plant extract was investigated in two different doses (100 and 200 mg/kg) following the hot and cold-tail flick methods (14, 15). The leaf extract, in contrary to an earlier report (5) showed significant analgesic effects, which is evident from the results obtained from: acetic acid writhing, the hot and cold water experiments (Table IV and V). In this case also, the plant collected in the month of January exhibited significant effects than that of the July collection.

TABLE IV: Effect of MLE on pain threshold in thermal induced pain.

Group	*Dose (mg/kg)*	*MPA value (Mean ± S.E.M.) (%)*
Control	Tween-80	7.92±0.73
MLE	100	12.30±0.60
MLE	200	23.60±0.44***
Reference (Pentazocine)	1	59.30±0.12***

***P<0.001, compared with control, n = 6.

TABLE V: Effect of MLE on pain threshold in cold induced pain.

Group	*Dose (mg/kg)*	*MPA value (Mean ± S.E.M.) (%)*
Control	Tween-80	16.53±0.23
MLE	100	41.83±0.48
MLE	200	65.93±0.70***
Reference (Pentazocine)	1	80.10±0.40***

***P<0.001, compared with control, n = 6.

TABLE VI: Effect of MLE on Phenobarbitone induced sleeping time.

Group	*Dose (mg/kg)*	*MPA value (Mean ± S.E.M.) (%)*
Control	Tween-80	48.83±1.08
MLE	100	85.50±2.69
MLE	200	108.00±1.06
Reference (Chlorpomazine)	1	252.83±1.94

***P<0.001, compared with control, n = 6.

From Table II, III and IV it is clear that MLE 200 mg/kg is the most effective dose in these three cases, moreover this dose also have shown its effect on phenobarbitone induced sleeping time, where it has prolonged the sleeping time in a dose dependent pattern (Table VI).

The leaves of the C *colebrookianum* were practically found to be non-toxic. LD_{50} of the MLE in mice was determined following the methodology of Handa et al (16) and found to be 18 g/kg, i.p. in mice.

DISCUSSION

The results obtained from proximate analysis and inorganic metal ion content determinations along with the extractive values provide certain parameters. These

can serve as markers in fingerprint analysis in identification of the drugs in whole or when incorporated in poly-herbal formulations. It has also been observed that the plant samples collected in two different seasons vary in their phytocontents, which is evident from the results (Table I). The Methanol extract of the plant collected in the month of January as well as June was studied separately for preliminary evaluation of pharmacological properties. The plant extract of January collection shows more significant results than that of the extract of the plant collected in the June month of the year. Results of methanol extract of the plant collected in the month of January have been reported here in this paper.

Most of the metal ions in the January collection of the plant samples are found to be higher in comparison to mat of the plants collected in the June month of the year. The presence of higher concentration of Magnesium and Iron is significant and requires further work on this aspect of the plant to explore the correlation with the use of this plant in various diseases related to blood pressure and heart by the ethnic people of this region.

This study establishes the anti-inflammatory, analgesic effects of the extract of C *colebrookianum* and its ability to prolong Phenobarbitone induced sleeping time. The carrageenin induced inflammatory process is believed to be biphasic (17). The initial phase seen at the first hr is attributed to the release of histamine and serotonin (18). The second accelerating phase of swelling is due to the release of prostaglandin, bradykinin and lysozyme. It has been reported that second phase of oedema is sensitive to both clinically useful steroidal and non-steroidal anti-inflammatory agent (19). The anti-inflammatory activity exerted by the methanol extract (MLE) of C *colebrookianum* suggests that they could have acted by affecting kinnin, prostaglandin, bradykinin and lysozyme synthesis. It is reported that the presence of terpenes, glycosides and sterols in plants has been found to exert active anti-inflammatory effect (20).

Other important results obtained in the present work were the central and peripheral analgesic activities demonstrated by the inhibitory action on the acetic acid induced writhing, hot and cold water test Where, acetic acid induced writhing and hot water test is used to study the action on peripheral nervous system (21) and cold-water test is used to study the action on central nervous system. Other investigators have shown that the cold tail flick is the selective method able to screen centrally acting opiate like analgesic agents, and is not sensitive to analgesic acting peripherally (14). The experiment confirms the analgesic activity of the MLE of the leaves of this plant. The strong anti-inflammatory and analgesic activity of the methanol extract of the plant prove that the presence of terpenes, sterols, glycosides and other polar bioactive components may be responsible for these activities (20).

The LD_{50} of the leaves of the plant was studied and found to be 18 g/kg, i.p. in mice. Further studies shall aim at characterizing and purifying compounds in this plant with potential bioactive properties. In addition, studies into the mechanism of action of

compounds so isolated will be necessary, with the ultimate objective of developing pharmacologically active *and* potential therapeutic agents from the natural product.

ACKNOWLEDGEMENTS

The authors are thankful to the Director, Institute of Advanced Study in Science and Technology, Guwahati (IASST) for giving the facilities to carry out the work. Thanks are also due to Dr. G. Sarma, Curator, Gauhati University, and Dr. P. Gogoi, Botany Dept., D.R. College, Golaghat for identification of the plant and to Mr. Sabin Kalita, Lab Attendant for helping in performing the experiments. We are thankful to the DST, Govt. of India for financial support to perform the work.

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