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Evaluation of Analgesic and Anti-inflammatory activity of aqueous extract of *Clerodendrum colebrookianum* in experimental animals

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Abstract

The present study was carried out to establish the analgesic and anti-inflammatory activity of aqueous extract of *Clerodendrum colebrookianum* leaves in experimental animals. The anti- nociceptive activity was measured by chemically inducing algesia with acetic acid and thermally induced algesia with hot plate in mice while the anti-inflammatory activity was measured by using caragennan induced rat paw edema. The aqueous extract of *C. colebrookianum* was prepared accordingly and was subjected to the experimental animals at a dose rate of 100 and 300 mg/kg body weight and administered subcutaneously 30 min prior to subjection of the above mentioned assays. The extract at all concentration, was found to exhibit significant anti-nociceptive ($P<0.01$) and anti-inflammatory activities. It is concluded that the AECC may probably exert its analgesic effect by either inhibiting the enzymatic synthesis of prostanoids or by inhibiting the non-enzymatic free radical based synthesis of isoprostanes. However, the anti-inflammatory activity of meloxicam and AECC may be attributed to their involvement in suppressing the cascade of acute vascular response with suppression of all 3 phases such as histamine and serotonin; kinins; and prostaglandins.

Keywords: Analgesic; Abdominal constriction; Eddy's hot plate; Anti-inflammatory; Carageenan; Clerodendrum colebrokianum.

Introduction

Herbal medicines are the oldest remedies known to mankind. Several herbs known for their medicinal properties have been in use since time immemorial. The growing interest in herbs is a part of the movement towards change in lifestyle based on the belief that the plants have a vast potential for their use as a curative medicine. In the present scenario, the demand for herbal products is growing exponentially throughout the world for their potential medicinal value.

Clerodendrum colebrookianum (Verbenaceae) locally known as "Phuinam" is a common shrub, used by the Mizo people as vegetables, has several medicinal property. Traditionally, the plant is used in the treatment of rheumatism, asthma and other inflammatory diseases. The plant is also reported to have antidiabetic, anti-hypertensive and sedative properties. The pharmacological studies revealed that the extract from *Clerodendrum colebrookianum* Walp protects rat heart against oxidative stress induced by ischemic-reperfusion injury (IRI) [1]. Fresh juice treatment significantly reduced the SGOT level while ethylacetate extract increased the SGPT level without damaging the liver cells in rats [2], extract of *C. colebrookianum* increased the antioxidant capacity of blood and had an inhibitory effect on the basal level of lipid peroxidation of liver and kidney [3]. Although, some literatures above cited are scientifically proven but there was no scientific validation against anti-nociceptive and anti-inflammatory activities of the plant, therefore, there is a necessity to explore the plant and to conduct pharmacological studies to ascertain the anti-nociceptive and anti-inflammatory activities accordingly.

Materials and Methods

Plant material

Shrubs of *C. colebrookianum* were carefully uprooted and collected from the College campus and were washed gently till the whole plant material looked clean and was mopped by blotting paper and weighed. The plant material was air dried in shade for a period of two weeks. On complete drying, whole plant material was ground to powder with a grinder and sieved through sieve number 22.

Preparation of plant extract

For preparing cold aqueous extract, the method used by Manjunatha *et al.,* (2005) [4] was followed with slight modification. One hundred and twenty five (125) grams of powder of *C. colebrookianum* was soaked in 1 liter of double distilled water for a period of 4 days with intermittent stirring and at the end of 4^{th} day the content was filtered with muslin cloth followed by Whatman filter paper number 1. The extract obtained was further subjected to vacuum evaporation at (60-70)°C for 24 hours and lyophilized for successive 24 hours. Lyophilization was stopped when the extract appeared sufficiently dry. Further the material was stored at -40°C in deep freezer in air tight containers until use.

Animals

Adult healthy Swiss albino mice (25-30 grams) and Wistar rats (100-120 grams) of either sex were procured from Ghosh Enterprises, Kolkata. Animals were housed in polypropylene cages, had free access to standard balanced ration, clean drinking water and were maintained in standard laboratory conditions (12:12 hour light/dark cycle at ambient temperature ranging between 12-25°C).

Chemicals and Drugs

Acetic acid and carrageenan were purchased from Merck India Pvt. Ltd. Mumbai while Meloxicam and Pentazocin were purchased from Jagsonpal Pvt. Ltd, Amar Nagar, Faridabad, India. All the chemicals and solvents were of analytical grade.

Phytochemical screening

Phytochemical tests were conducted on the

aqueous extract of *Clerodendrum colebrookianum* as per standard procedures (Edeoga *et al.*, 2005) [5].

Acute oral toxicity

The acute oral toxicity studies of both the extracts were undertaken in mice (30 10 grams body weight). The animals were divided into 4 groups with 5 numbers of animals in each group. Group I received the extract of *C. colebrookianum* @ 1000 mg/kg body weight, group II received the extract @ 3000 mg/kg, Group III received the same @ 6000 mg/kg and Group IV received the extract @ 9000 mg/kg. The study was done for 72 hours and during this period the animals were observed for mortality, any sign of abnormality, feeding-watering as well as body weight change.

Evaluation of anti-nociceptive activity

Acetic acid induced writhing model

Twenty apparently healthy male mice (30 ± 10 grams body weight) were randomly divided into 4 groups having 5 animals in each group. The control (10 ml/kg), standard (Meloxicam at 10 $mg.kg^{-1}$ PO) and test drugs (aqueous extracts at 100 and 300 $mg.kg^{-1}$ PO, respectively) were administered in the respective groups. After 1 hour of the drug administration, 1% acetic acid was administered @ 0.5 ml per 100 grams of body weight intra-peritoneally in all the groups to assess the protective index of the drug against the anagogic stimuli [6].

Number of writhes in 15 minutes from the time of injection of acetic acid were counted and considered for calculation of protective index as described by Somchit *et al.* (2004). [7]

Protective index = [(control mean- treatment mean) x 100/ control mean]

Hot plate induced writhing model

Before the commencement of the study, the mice (30 ± 10 grams body weight) were placed on the Eddy's hot plate at a temperature of 55 ± 1°C to cull the animals showing erratic reaction. The best responding 20 animals (7.75 ± 1.25 seconds) of either sex were selected and randomly divided into 4 groups of 5 animals each. Zero, 30 and 60 minutes after the administration of test and reference compounds, animals of all the groups were individually placed on the hot plate maintained at 55 ± 1°C. The time taken in seconds for discomfort reaction (fore paw licking or

jumping) was observed and compared with pre-treatment readings [8]. Cut off latency period of 15 seconds was defined as complete analgesia. Further, per cent maximum possible effect (% MPE) was calculated from pre- and post-treatment readings [9].

$$\% MPE = R_t - R / Co - R \times 100$$

R_t : reaction time after treatment

R : control reaction time

Co: cutoff time

Evaluation for anti- inflammatory activity

Carrageenan-induced paw oedema

Before the commencement of the experiment, the animals were fasted for 12 hours. Paw oedema was induced by injecting flogistic agent viz. carrageenan (1% in 0.9% NaCl) @ 0.1 ml at the right hind paw in subplantar region. The standard and test drugs were administered simultaneously with carrageenan injection [10]. The paw volumes were measured at time 0 (V_o- pre-treatment) and then measured again at 1, 2, 3, and 4 hour (V_t where, t is 1, 2, 3and 4 hour reading, respectively) after the carrageenan administration by the water displacement method with the help of the plethysmometer. Percentage of oedema inhibition (% PI) was calculated with the help of above recordings [11].

$$P I = (V_t-V_o) \text{ Control} - (V_t-V_o) \text{ Treated} / (V_t-V_o) \text{ Control} \times 100$$

V_t: volume of paw at time't'

V_o: pretreatment volume of paw

Results

Phytochemical screening

The phytochemical screening of AECC showed the presence of tannins by ferric chloride

and gelatin test; terpenoides and cardiac glycosides by Salkowski test and Keller – Killani test; saponins by foam test and flavonoides by lead acetate and ferric chloride test.

Acute toxicity studies

Aqueous extract of *C. colebrookianum* @ 1000 $mg.kg^{-1}$, 3000 $mg.kg^{-1}$, 6000 $mg.kg^{-1}$ and 9000 $mg.kg^{-1}$ did not produce any mortality or apparent sign of overt toxicity during the period of observation for 3 days. Therefore, the extract was found to have LD_{50} above 9000 $mg.kg^{-1}$. However at 9000 $mg.kg^{-1}$, some behavioral changes like depression and reduced alertness were observed.

Acetic acid induced writhing model

The effect of AECC on acetic acid-induced writhing test in mice is shown in Table 1 and Figure 1. Administration of AECC at 100 and 300 $mg.kg^{-1}$ PO, significantly reduced (P<0.01) the acetic acid induced writhing to 18.751.88 and 8.751.43 writhes/15 min, respectively as compared to the vehicle control (30.752.92 writhes/15 min).

S.No.	Treatment (mg/kg body weight, PO)	Number of Writhes/15min	% Protection Index
A	Vehicle	30.75±2.92 [c]	-
B	Meloxicam(10)	5.75±0.85 [b]	81.30
C	AECC(100)	18.75±1.88 [a]	39.02
D	AECC(300)	8.75±1.43 [a]	71.54
F Value		34.22**	
P Value		0.000	

Table 1: Effect of AECC on acetic acid induced pain in mice

Values are mean ± SEM, n=6 in each groups. **: P<0.01, compared to vehicle control. In the column bearing a common superscript do not differ significantly, but different superscript differ significantly

Eddy's hot plate model

The effect of AECC on Eddy's hot plate model is shown in Table 2 and Figure 2. In this model of thermal pain, the AECC @ 100 and 300 $mg.kg^{-1}$ possessed significant (P<0.05-P<0.01) analgesic property as reflected by delayed post-treatment pain latency (54.06 ± 4.7) and 45.50 ± 11.35 seconds, respectively) compared to their respective pre-treatment latency (5.62 ± 1.24 and 13.98 ± 2.35 seconds, respectively) which was higher than pentazocin @ 10 $mg.kg^{-1}$ (42.83 ± 10.10 seconds).

Carragennan induced rat paw edema model

The anti-inflammatory effect of AECC monitored up to 4 hours post-treatment in carrageenan–induced rat paw edema model is shown in Table 3 and Figure 3. At 100 and 300 $mg.kg^{-1}$, PO, AECC exerted significant anti-inflammatory effect (P<0.05) at 120 minutes of post-treatment. Both the doses of aqueous extract, during this period inhibited the rat paw oedema by 24.13 and 6.89 %, respectively while that of meloxicam (@ 4 $mg.kg^{-1}$, PO), inhibited the paw edema by 89.65 %. Thus meloxicam @ 4 $mg.kg^{-1}$, PO, had greater efficacy in inhibiting the rat paw edema compared to the AECC @ 100 and 300 $mg.kg^{-1}$, PO, respectively.

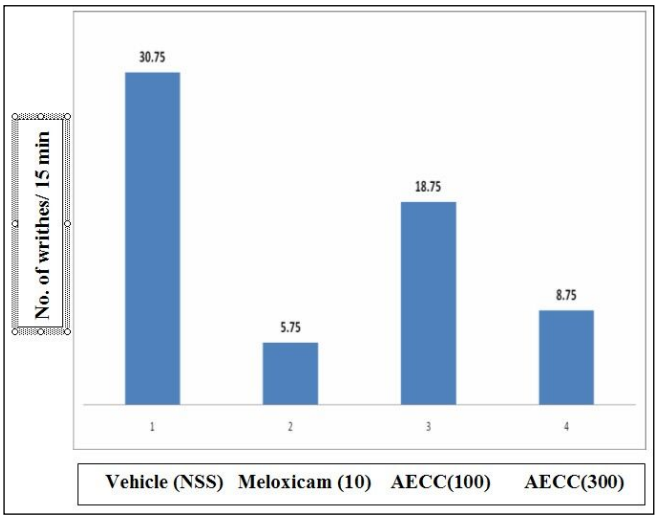

Fig.1. Effect of AECC on acetic acid induced pain in mice

Group	Treatment (mg/kg body weight, PO)	Latency (Seconds)			
		Pre-treatment	0 min post-treatment	30 min Post-treatment	1 hour Post-treatment
A	Vehicle	21.5±6.89	17.25±2.89	17.62±4.42	32.11±6.3
B	Pentazocin (10sc)	14.73±1.99	16.79±6.95	43.14±9.39	42.83±10.10
C	AECC (100)	15.62±1.24	19.17±3.84	28.97±6.88	54.06±4.7
D	AECC (300)	13.98±2.35	13.14±1.79	37.54±7.99	45.50±11.35
t – Value		0.802		3.301**	5.097**
P – Value		0.435		0.005	0.000

Table 2: Effect of AECC on Eddy's hot plate induced pain in mice

Values are mean ± SEM, n=6 in each groups. **: P<0.01, compared to vehicle control. In the column bearing a common superscript do not differ significantly, but different superscript differ significantly

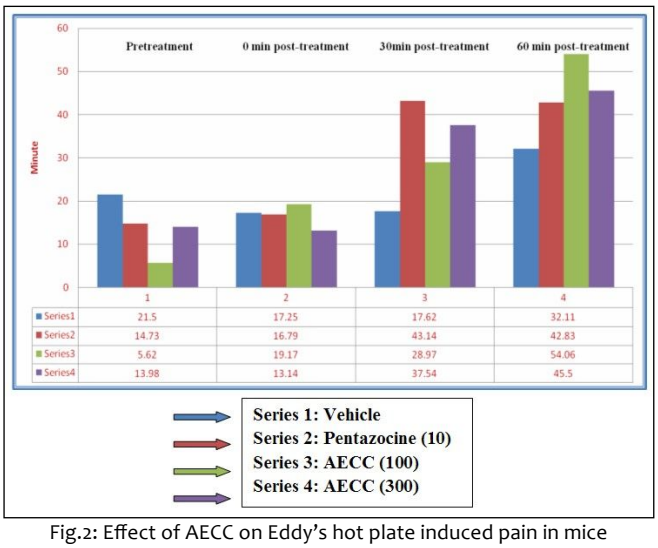

Fig.2: Effect of AECC on Eddy's hot plate induced pain in mice

Group	Treatment (mg/kg body weight, PO)	Post-treatment paw volume (CC)				
		0 min	1 hour	2 hour	3 hour	4 hour
A	Vehicle	0.88±0.018	1.29±0.06	1.17±0.02[b]	1.24±0.02	1.23±0.03
B	Meloxicam(4mg/kg)	0.86±0.047	1.00±0.08 (65.85%)	0.89±0.06 (89.65%) [b]	0.98±0.07 (66.66%)	0.97±0.08 (68.57%)
C	AECC(100)	0.71±0.044	1.14±0.01 (-4.87%)	1.07±0.05 (24.13%) [ab]	1.08±0.08 (-2.77%)	1.00±0.09 (17.14%)
D	AECC (300)	0.74±0.079	1.08±0.04 (17.07%)	1.05±0.05 (6.89%) [a]	1.05±0.05 (13.88%)	1.10±0.04 (-2.85%)
F- Value			2.334	4.602*	2.879	2.807
P-Value			0.113	0.017	0.069	0.073

Table.3: Effect of AECC on carrageenan-induced rat paw oedema

Values are mean ± SEM, n=6 in each groups. *: P<0.05, compared to vehicle control. In the column bearing a common superscript do not differ significantly, but different superscript differ significantly

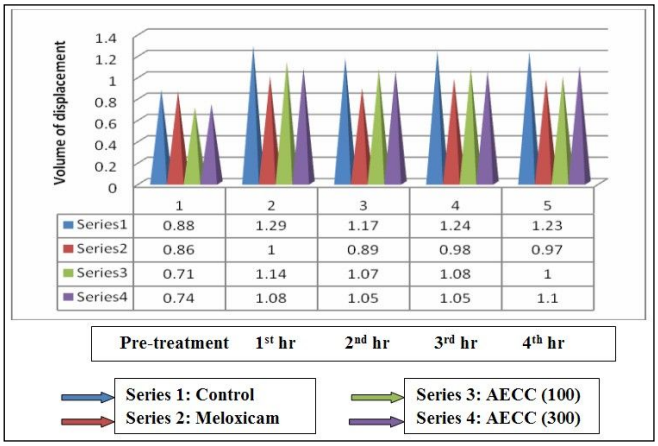

Fig.4.13. Effect of AECC on carrageenan-induced rat paw oedema

Discussion

The abdominal constriction produced after administration of acetia acid is related to sensitization of nociceptors to prostaglandins. Chemical and physical stimuli activate the Ca^{2+} dependent translocation of group IV cytosolic PLA_2 (c PLA_2), which has high affinity to arachidonic acid, to the membrane where it hydrolyzes the sn- 2 ester bond of membrane phospholipids (particularly phosphatidylcholine and phosphatidylethanolamine), releasing arachidonate. Arachidonate is metabolized to the cyclic endoperoxide prostaglandin G (PGG) and H (PGH) by the cyclo-oxygenase (COX) and hydroperoxidase (HOX) activities of the prostaglandin G/H synthases.

Isomerases and sythases effect the terminal transformation of arachidonate into terminal prostanoids.

Further isoeicosanoids, a family of eicosanoids isomers, are formed non-enzymatically by direct free radical based attack on arachidonic acid and related substrates [12]. Unlike eicosanoids, these compounds are generated on the esterified lipid in cell membrane, from which they are cleaved, presumably by phospholipases and the free isoeicosanoids circulate in the blood. Since several isoeicosanoids (isoprostanes) are formed which can activate the protanoid receptor, it may be speculated that they may contribute to the pathophysiology of pain and inflammatory responses in a manner insensitive to COX inhibition [13].

It is, therefore, postulated that AECC may probably exert its analgesic effect by either inhibiting the enzymatic synthesis of prostanoids or by inhibiting the non-enzymatic free radical based synthesis of isoprostanes.

Thermal stimuli causes pain by stimulation of nociceptive receptors and transmitted over intact neural pathways. Both tail flick and hot plate tests are selectively used to evaluate centrally acting analgesics [14]. The thermal stimulus is also described as an acute, non-inflammatory nociceptive stimulus as it causes direct stimulation of the nociceptors without causing any inflammatory-mediated nociception. In this present study, mice treated with AECC were more tolerant to the thermal stimulus than those receiving normal saline alone. The analgesic activity was comparable to pentazocine, an opioid analgesic whose analgesic activity is mediated through central route. The analgesic effect of opioids arise from their ability to directly inhibit the ascending transmission of nociceptive information from the spinal cord dorsal horn and to activate pain control circuits that descends from the mid brain via the rostral ventromedial medulla to the spinal cord dorsal horn [15]. Since, AECC could increase the latency period in mice which was comparable to those mice receiving pentazocine, it may explain the possibility of involvement of a centrally mediated analgesic activity.

Carrageenan-induced acute vascular response occurs in 3 phases with involvement of histamine and serotonin; kinins; and prostaglandins in phase 1, 2 and 3, respectively [16]. Anti-inflammatory activity of meloxicam and aqueous extracts may be attributed to their involvement in suppressing the cascade of acute vascular response with suppression of all 3 phases.

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