



ANTI- INFLAMMATORY AND ANTI-NOCICEPTIVE EVALUATION OF VARIOUS EXTRACTS OF *ENECOSTEMA* *AXILLARE*

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ABSTARCT

Anti-inflammatory and anti-nociceptive effects of the whole plant extracts of *Enecostema Axillare* was evaluated by carrageenan induced paw oedema and Tail-flick method, respectively. The percentage reduction in the rat paw-volume and increase in the time of withdrawl response of tail from the heat source was observed in the mice, treated with chloroform and methanolic extract of *Enecostema Axillare*. The anti-inflammatory and anti-nociceptive activity produced by the whole plant extract of *Enecostema Axillare* was evaluated statistically by student's "t" test.

Key words : Anti-inflammatory, Anti-nociceptive, *Enecostema Axillare*.

INTRODUCTION

Enecostema Axillare (Family : Gentianaceae) is a perennial herb widely distributed in India, Srilanka, Malaya, Tropical Africa and West Indies. It is erect herb and the leaves are lanceolate and has apex acute¹. In Indian ethno medicine, it is locally known as Chota chirayata in Hindi, Mamijava in Gujarati and Vellaragu in Tamil^{2, 3}. The literature survey and phyto chemical investigation reveals the presence of amino acids, flavanoids and sugars⁴. The entire plant has been used as anti-inflammatory and in the treatment of rheumatitis, diabetes, fungistatic, skin diseases and snake bite.⁵⁻⁸ Based on these traditional uses, the present investigation was undertaken to study the anti-inflammatory and anti-nociceptive activity of the whole plant extracts of *Enecostema Axillare* in comparison with diclofenac sodium and paracetamol, well known anti-inflammatory and anti-nociceptive agents, respectively.

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EXPERIMENTAL

Preparation of the extract

Fresh plant materials of *Enecostema Axillare* were collected in and around Virudhunagar district, Tamil Nadu and authenticated at the Taxonomical division of ANJA College of Arts and Science, Sivakasi by Dr.V.Ganesh. The uprooted plant materials were washed with water and shade dried at room temperature for 20 days. The dried plant materials were powdered using a pulverizer and passed through sieve No. 10. 500 g of the powdered and sieved plant material was extracted with 1.0 L. of chloroform (Ranbaxy Ltd, India) and 1 L. of methanol (s.d. fine) for 24 h using a Soxhlet extractor in a successive manner. The extracts thus obtained was concentrated by distillation and the concentrated extract was stored in the dessicator (yield 2.93 % and 12.6 %, respectively). The preliminary phytochemical investigations^{9, 10} of the chloroform and methanolic extract show the presence of carbohydrates, glycosides, flavones and anthrocyanins. The results were tabulated in Table 1.

Table 1. Phytochemical analysis of extracts of *Enecostema Axillare*

Constituents	Extracts	
	Chloroform	Methanol
Alkaloids	—	—
Carbohydrates and Glycosides	+	+
Phytosterols	—	—
Fixed oils and Fats	—	—
Saponins	—	—
Tannins	—	—
Proteins	—	—
Gums and Mucilage	—	—
Flavones Anthrocyanins	—	+
Anthrocyanins	—	+
+ Indicates Presence; — Indicates Absence		

Anti-inflammatory activity

Albino rats of either sex weighing 150–200 g were used in this study. They were provided with standard diet and water *ad libitum*. The rats were divided into six groups, each consists of six animals. Acute inflammation was induced by injecting 0.1 mL of 1%

w/v solution of carrageenan as a phlogistic agent into the sub-plantar aponeurosis of the hind paw of rats.¹¹⁻¹⁵ First group received 1.0 mL of 5% carboxy methyl cellulose solution orally (control), the second group received diclofenac sodium (10 mg / kg, orally), and the third and fourth group received chloroform extract (200 and 400 mg / kg, orally) respectively. The fifth and sixth group received methanolic extract (200 and 400 mg /kg, orally), respectively, 30 minutes before carrageenan injection. Paw volume was measured with a plethysmometer before and 3 h after the carrageenan injection. Results are expressed as percentage inhibition of inflammation in the treated groups compared to control groups and are tabulated in Table 2.

Table 2. Anti-inflammatory evaluation of extracts of *Enecostema Axillare*

Treatment	Dose (mg / kg)	Mean Paw volume (mL)	% inhibition
Control	1 mL	0.65 ± 0.03	—
Diclofenac sodium	10	0.20 ± 0.04*	69.24
Chloroform extract	200	0.50 ± 0.04*	21.54
Chloroform extract	400	0.48 ± 0.03*	26.20
Methanolic extract	200	0.29 ± 0.04*	55.40
Methanolic extract	400	0.22 ± 0.04*	66.20

Anti-nociceptive activity

Albino mice of either sex weighing between 25 – 30 g were used in this study. The animals were provided with water *ad libitum*. The anti-nociceptive activity was studied in INCO analgesiometer by tail flick method^{16, 17}. The animals were divided into six groups, each consists of six animals. First group served as control, received 1.0 mL of 5% carboxy methyl cellulose solution, orally. The second group served as standard, received paracetamol (50 mg/ kg, orally). The third and fourth group received chloroform extract (200 and 400 mg/ kg, orally) respectively. The fifth and sixth group received methanolic extract (200 and 400 mg/ kg, orally) respectively. The observations were made at 15, 30, 45, and 60 minutes of time intervals after the drug administration and are tabulated in Table 3.

Table 3. Anti-nociceptive evaluation of extracts of *Enecostema Axillare*

Treatment (mg/ kg p.o)	Dose (mg / kg)	Time of withdrawal from heat source (sec) mean \pm SEM					
		15 min	30 min	45 min	60 min	90 min	120 min
Control	1 mL	3.8 \pm 0.20	3.8 \pm 0.20	3.6 \pm 0.21	3.8 \pm 0.20	3.4 \pm 0.20	3.4 \pm 0.20
Paracetamol	50	5.4 \pm 0.50*	6.2 \pm 0.37*	8.0 \pm 0.31*	9.0 \pm 0.44*	7.7 \pm 0.58*	7.4 \pm 0.47*
Chloroform extract	200	4.0 \pm 0.31	4.0 \pm 0.31	4.2 \pm 0.30*	4.1 \pm 0.32	3.8 \pm 0.21*	4.0 \pm 0.33
Chloroform extract	400	4.2 \pm 0.34	4.3 \pm 0.28	4.1 \pm 0.29	4.8 \pm 0.33*	4.5 \pm 0.35*	4.6 \pm 0.37
Methanolic extract	200	5.0 \pm 0.48	5.4 \pm 0.50	6.3 \pm 0.38*	7.8 \pm 0.47	6.8 \pm 0.51	5.1 \pm 0.49*
Methanolic extract	400	5.2 \pm 0.51	7.0 \pm 0.55*	7.4 \pm 0.38*	8.1 \pm 0.43	6.8 \pm 0.49*	5.7 \pm 0.53

*p < 0.001 ; Values are expressed as mean ; n = 6.

Statistical analysis

Results were expressed as mean \pm SEM and student's "t" test were used to assess statistical significance.

RESULTS AND DISCUSSION

Present study showed that the reduction of carrageenan induced paw oedema in rats was observed in fifth and sixth groups animals following oral administration of the methanolic extract than in third and fourth groups animals received chloroform extract of *Enecostema Axillare*, but it was less than standard diclofenac sodium. The data in Table 2, suggests that the methanolic extract of *Enecostema Axillare* possess a moderate anti-inflammatory activity. This may be due to the presence of active constituents such as flavones and flavonoids¹⁸. Flavonoids have been reported to inhibit arachidonic acid metabolism and prostaglandin synthetase activity. Mainly carrageenan induced oedema is mediated by histamine, 5-HT, bradykinin, polymorphonuclear leukocytes, prostanoids, nitric oxide, neuropeptides and cytokines^{19,20}.

It also shows that increase in the time of withdrawal of tail from the heat source was observed following oral administration of the methanolic extract of *Enecostema Axillare*, but it was less than those treated with standard drug paracetamol. The data Table 3, suggests that the methanolic extract of *Enecostema Axillare* possess a moderate anti-nociceptive activity and the chloroform extract is devoid of anti-nociceptive activity.

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