December 2009 Volume 5 Issue 4

Trade Science Inc.

Natural Products

An Indian Journal

Full Paper

NPAIJ, 5(4), 2009 [198-201]

Analgesic and anti-inflammatory activities of *Clausena dentata* in experimental animal models

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Received: 22nd July, 2009 ; Accepted: 1st August, 2009

ABSTRACT

The anti-inflammatory and analgesic effect of hexane, chloroform and methanol extracts of the root bark of *Clausena dentata* were evaluated in experimental animals of either sex. The extracts were vacuum dried and tested for analgesic activity in Wister mice and anti-inflammatory activity in Wister rats at oral dose levels of 50,100 and 150 mg/kg body weight. The analgesic activity was studied by tail-flick method using Paracetamol 100mg/kg oral dose as standard. The basal reaction time to radiant heat was recorded by pacing the tip (last 1-2 cm) of the tail on the radiant heat source. A cut – off period of 10 seconds was observed to prevent damage to the tail. The reaction time was recorded at 15, 30, 45 and 60 min after treatment. The percentage protection against tail-flicking was calculated, allowing maximum tolerability time of 10 sec. The anti-inflammatory activity was studied by carrageenan induced paw-edema using Diclofenac Sodium at a oral dose of 20mg/kg as standard. The initial paw volume was measured plethysmographically within 30 sec of the injection. The relative increase in the paw volume was measured in control, standard and extract treated groups 1 hr and 4 hr after carrageenan injection. The hexane, chloroform and methonolic extracts exhibited significant analgesic activity ($p<0.01$) at a dose of 150mg/kg. Anti-inflammatory activity of the extracts manifested only after 4 hours of carrageenan administration. The percentage reduction of edema was found to be 73% with hexane, followed by 71% with methanol and 55.2% with chloroform at 150mg/kg oral dose ($p<0.01$). The results indicated that, the hexane extract of *Clausena dentata* exhibited more significant activity at a dose of 150mg/kg than methanol and chloroform extracts in the treatment of pain and inflammation.

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KEYWORDS

Analgesic;
Anti inflammatory;
Clausena dentata;
Paw edema.

INTRODUCTION

Medicinal plants play a significant role in various ancient traditional system of medicine such as Ayurveda, Siddha, Unani and Chinese traditional medicines and modern medicine. They play an important role in preventive and curative treatment, despite advances in modern medicine. The high cost of modern medicine, their unavailability an most importantly, the serious side effects of certain drugs have resulted in a significant re-

turn to traditional medicine.[1]

Clausena dentata (Willd.) M.Roem is a small tree plant, belonging to the family of Rutaceae and found in India, Sri Lanka and China.[2] It is popularly known as Anai chedi in Tamil. *Clausena dentata* is used by locals of Yercaud and Boda Hills for its medicinal and nutritive value. The stem bark of *Clausena dentata is* used in veterinary medicine for the treatment of wounds and sprains.[1] In Cambodia the stem is considered bitter tonic and astringent; the infusion is given for colic pain with diarrhoea.[3] The root bark of Clausena dentata was found to contain coumarins. The dried powdered root stock is used by the tribes in Chotanagpur region of India for decayed teeth.[4] In the present study, vacuum dried hexane, chloroform, and methanol extracts of *Clausena dentata* stem bark were evaluated for their analgesic activity by tail-flick method and acute anti inflammatory activity by carrageenan induced paw edema method.

MATERIALS AND METHODS

Plant materials and preparation of extract

The root bark of *Clausena dentata* was collected from Kadagaman, Tiruvannamalai Dist, Tamilnadu, India. The root bark were dried in the shade and pulverized to a coarse powder and was extracted by maceration successively with hexane, chloroform, methanol for 72 h and concentrated at 40^0 c under reduced pressure using Buchi R-153 Rotavapor. The extracts were named HE, CE, and ME for hexane, chloroform and methanol respectively.[5]

Animal

Wister albino mice (25-35g body weight) and Wister albino rats (180-200g body weight) of either sex were procured from the animal house of Sri Ramachandra University, Tamil Nadu, India after obtaining approval from the Institutional animal ethical committee. The animals were maintained in colony cages at a temperature of 25 ± 2^0 c, relative humidity of 45-55%, maintanted under 12 hrs light and dark cycle (0600 to 1800h-light : 1800to 0600 h- dark). The animals were fed with standard animal feed (Hindustan lever ltd) and water ad libitum. All the animals were acclimatized for a week before use .HE, CE and ME were dissolved in 1% tween 80 and administered orally by using feeding canula.

Analgesic activity by tail-flick method

The analgesic activity was determined by tail- flick method using Wister albino mice of either sex selected by random sampling technique.[6] The basal reaction time to radiant heat was recorded by pacing the tip (last 1-2 cm) of the tail on the radiant heat source. A cut – off period of 10 seconds was observed to prevent damage to the tail. Paracetamol at a dose level of 100 mg/kg (SC) was administered as standard drug for comparison. The extracts at 3 dose levels (50,100,150 mg/kg) were administered per oral. The reaction time was

TABLE 1 : Analgesic activity of *Clausena dentata* root bark extracts:

Sr. No	Treatment (n=6)/group	Dose (mg/kg)	Mean time(in sec) ±SD				% Protection
			15min	30min	45min	60min	
1.	Control 1% Tween80	--	3.50±0.080	3.50±0.080	3.40±0.080	3.40±0.080	---------
2.	Paracetamol	100	6.15±0.105	6.15±0.105	8.20±0.08	9.2*±0.08	92
3.	Hexane (HE)	50	3.50±0.116	3.50±0.116	4.01±0.147	4.16±0.103	41.6
		100	4.05±0.151	4.35±0.137	4.63±0.121	5.11*±0.147	51
		150	5.10*±0.080	5.40*±0.080	7.0*±0.080	7.60*±0.080	76
4.	Chloroform (CE)	50	3.50±0.080	3.50±0.051	3.51±0.041	3.71±0.07	37
		100	3.73±0.051	3.93±0.103	4.18±0.187	4.65*±0.147	46.5
		150	4.50±0.080	4.80±0.080	5.10*±0.080	6.70*±0.080	67
5.	Methanol (ME)	50	3.51±0.11	3.70±0.080	3.50±0.07	3.88±0.07	38
		100	3.93±0.050	4.11±0.750	4.40±0.080	4.71±0.200	47
		150	4.80±0.080	5.05*±0.100	6.80*±0.080	7.10*±0.08	71

(n=6) Values are expressed as Mean± S.D, *p <0.01 when compared with control group.

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recorded at 15, 30, 45 and 60 min after treatment. The percentage protection against tail-flicking was calculated, allowing maximum tolerability time of 10 sec. The analgesic activity data were presented in TABLE 1.

Acute anti inflammatory activity

The acute anti inflammatory activity was evaluvated by carrageenan –induced paw edema methodin Wister albino rats of either sex by using a plethysmograph. Diclofenac sodium (20 mg/kg) was administered as a standard drug.[7] The animals were divided into groups as shown in TABLE 1. Acute inflammation was produced by subplantar injection of 0.1 ml of 1%W/V suspension of carrageenan with 2% gum acacia in normal saline, in the right hind paw of the rats, one hour after oral administration of the extracts at 3 dose levels (50,100,150 mg/μg) per oral by feeding cannula. The initial paw volume was measured plethysmographically within 30 sec of the injection. The relative increase in the paw volume was measured in control, standard and extract treated groups 1 hr and 4 hr after carrageenan injection. The percentage increase in the paw-volume in animals treated with standard drug and the extracts of *Clausena dentata* were compared with the increase in paw-volume of untreated control animals.[8,9] The percentage inhibition of edema was calculated by using the formula

$$\% \text{ inhibition} = \frac{(C - S)}{C} \times 100$$

Where C = mean relative changes in the paw volume of the control, S = mean relative changes in the paw volume of the test

Statistical analysis

The values were expressed as mean ±S.D. Statistical significance was calculated by using student's T-test.[10]

RESULTS

Carrageenan-induced paw edema method

In the present study, analgesic activity by tail flick method shows the results given in TABLE 1. It was observed that HE, CE and ME exhibited analgesic activity at a graded dose response. Among the extracts, HE (76%) had greater activity than ME (71%) and CE (67%) at the dose level of 150mg/kg . The results of anti-inflammatory activity were expressed in TABLE 2. In acute anti-inflammatory model, significant activity of the extracts manifested only after 4 hours of carrageenan administration. The percentage reduction of edema was found to be greater with HE (73%), followed by ME (71%) and CE (55.2%) at a dose of 150 mg/kg body weight.

TABLE 2 : Acute anti-inflammatory activity of *Clausena dentata* root bark extracts

Sr. No	TREATMENT	DOSE (mg/kg)	Mean paw volume ± SD. 1 hr	4 hr	% Reduction of edema
1.	Control (1% Tween 80)	-----	0.169 ±0.001	0.170±0.001	--------
2.	Diclofenac Sodium	20	0.231*±0.001	0.045*±0.001	73.5
3.	Hexane (HE)	50	0.182±0.005	0.138±0.001	18
		100	0.182±0.005	0.107±0.001	37
		150	0.180*±0.004	0.044*±0.002	73
4.	Chloroform (CE)	50	0.181±0.001	0.140±0.001	14
		100	0.178±0.004	0.122±0.001	21
		150	0.175*±0.004	0.081*±0.001	52
5.	Methanol (ME)	50	0.170±0.001	0.137±0.004	17
		100	0.169±0.011	0.109±0.007	35
		150	0.167*±0.011	0.049*±0.082	71

(n=6) Values are expressed as Mean± S.D, *p <0.01when compared with control group.

DISCUSSION

Inflammation has different phases; the first phase is caused by an increase in vascular permeability, the second one by infiltrate of leucocytes and the third one by granuloma formation. We determined anti-inflammatory activity by using inhibition of carrageenan-induced inflammation which is one of the most feasible methods to screen anti-inflammatory agents. The development of carrageenan-induced edema is bi-phasic; the first phase is attributed to the release of histamine, serotonin and kinins and the second phase is related to the release of prostaglandins and bradykinins.[11-15] We observed that hexane, methanol and chloroform extracts showed significant inhibition against carrageenan-induced paw edema in the dose dependent manner.[16] This response tendency of the extract in carrageenan-induced paw edema revealed good peripheral anti-in-

flammatory properties of the hexane extract. This anti-inflammatory effect of may be due to the presence of flavonoids and coumarins namely impertanin, dentain and nordentain in the root bark. It has been reported that a number of flavonoids possess anti-inflammatory[17] and analgesic[18] activities. Flavonoids are known to inhibit the enzyme prostaglandin synthetase, more specifically the endoperoxidase and reported to produce anti-inflammatory effects.[19] Since, prostaglandins are also involved in the pain perception; inhibition of their synthesis might be the possible reason for the analgesic activity of the ethanolic extract. Literature reveals the presence of coumarins namely; 3-(1,1-dimethylallyl) xanthyletin in the root and stem bark of Clausena dentate.[4,21] Studies done by O-Kennedy and Thones[20] have established the analgesic activity of coumarin, The presence of coumarins in *Clausena dentata* may be responsible for its analgesic and anti-inflammatory activity. Thus, it is concluded that the hexane, chloroform and methanol extracts of root bark of *Clausena dentata* produces significant analgesic and anti-inflammatory activities in dose dependent manner.

ACKNOWLEDGEMENT

The authors wish to thank the management of Sri Ramachandra University, Chennai, for providing facilities to carryout the work.

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