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Analgesic and anthelmintic activity of a protease extracted from the latex of the plant *Calotropis gigantea* Linn

Smriti Rekha Chanda[1]*, Indranil Chanda[1], Sadhan Kr.Dutta[2], Subrat Kar[3]

[1]Department of Pharmacy, Girijananda Chowdhury Institute of Pharmaceutical Science, Hathkhowapara, Azara, Guwahati-781017, Assam, (INDIA)

[2]Department of Pharmacy, Gupta College of Technological Sciences, Asansol-713301 West Bengal, (INDIA)

[3]Department of Pharmachemistry, Seemanta Institute of Pharmaceutical Sciences, Jharpokharia-757086, Orissa, (INDIA)

E-mail : das_smritirekha@rediffmail.com

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ABSTRACT

The analgesic and anthelmintic activities of the new protease fraction Calotropain-G extracted from the latex of the plant *Calotropis gigantea Linn*. was evaluated using acetic acid induced writhing test and hot plate test in mice and in earthworm respectively. Calotropain-G showed significant analgesic and anthelmintic activity. The analgesic effect may be due to the inhibition of synthesis or action of prostaglandins. The potency of the compound in anthelmintic activity was found to be inversely proportional to the time taken for paralysis or death of the worms.

KEYWORDS

Protease;
Calotropain-G;
Analgesic;
Writhing;
Anthelmintic.

INTRODUCTION

Calotropis gigantea Linn. (Asclepiadaceae) is a much branched, hardy, wolly shrub, 1-5 m in height. It is also known as 'madar' in English and 'ark' in Hindi. Stems are woody, round and tender ones covered with soft, loosely appressed, whitish, waxy or sometimes powdery pubescence. Flowers are lilac or pale rose or purple and some times light greenish yellow or white in colour[1]. This plant is native to India, Sri Lanka, China and Malesia. Traditionaly the latex of the plant is used to treat leukoderma, tumors, ascites, and diseases of the abdomen, painful joints and swellings. The latex is also used as expectorant, depilatory and anthelmintic[2]. The reported medicinal properties are antipyretic activity[3], procoagulant activity[4], anti-diarrhoeal activity[5], histamine action[6], cardiotonic action[7]. The plant has been reported to contain oxypregnane-oligogly cosides[8], calotropin, uscharin, calotoxin, calactin, uscharidin and gigantin[1]. The latex contains the proteases, calotropain D_I and D_{II}[9] and calotropain F_I and F_{II}[10]. In this study, we investigated the analgesic and anthelmintic activities of the isolated protease fraction 'Calotropain-G'.

MATERIAL AND METHODS

Plant material

The Latex of *Calotropis gigantea* was collected from Mayurbhanj district (Orissa, India) in February 2006. The plant was authenticated at Botanical Survey of India, Howrah, West Bengal (India). A voucher specimen was deposited at the Central National Herbarium of Botanical Survey of India.

Preparation of extracts

Latex was collected from the stem of the plant by incision into 0.01% EDTA solution in a flask. The col-

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lected crude latex was kept overnight in cold condition in refrigerator. The latex was then filtered through ordinary filter paper to remove the white gummy material. To the clear crude extract chilled acetone was added slowly with constant shaking. The solution was kept overnight in a refrigerator and then centrifuged. The precipitate was dried[11,12,13]. Chemical test for protein[14] and protease assay were carried out using standard procedures[15].

Test animals

Swiss albino mice of both sexes weighing between 20–25 gm were used for the test of LD_{50} value and analgesic activities. They were obtained from the animal house, S.I.P.S., Jharpokharia, Orissa. They were maintained under standard environmental conditions and were fed with standard pellet diet supplied by Hindustan Lever Ltd., Kolkata, India and water *ad libitum*.

Pheritima posthuma (earthworm obtained from Horticulture Department) were selected to study anthelmintic activity due to its anatomical and physiological resemblance with the intestinal roundworm parasites of human beings.

Toxicity study

The extracted compound (named as 'Calotropain-G') was dissolved in glass distilled water and used for the toxicity study. The experiment was carried out taking 10 groups of animals each group containing 10 mice. The LD_{50} value was determined by the method Litchfield and Wilcoxon[16]. The animals were administered with Calotropain-G intraperitoneally, starting from the dose of 20 mg/kg up to 110 mg/kg bodyweight. From the observed value three dose levels of 5, 7 and 15 mg/kg were selected for the estimation of analgesic activity.

Analgesic activity

Acetic acid induced writhing response in mice

The method described by Turner[17] was used. Thirty mice were divided into 5 groups (n= 6) Group 1 received sterile water for injection (SWFI, 10 ml/kg i.p.), group 2, 3 and 4 received Calotropain-G (5,7,15 mg/kg i.p. respectively), while group 5 received standard drug Acetylsalicylic acid, (ASA, 100 mg/kg i.p.)

10 ml/kg of 0.6 % aqueous solution of acetic acid was given (injected intraperitoneally) to each mouse thirty minutes after treatment. Each mouse was then placed in a transparent plastic observation cage and the number of writhes was counted for 10 minutes after 5 minutes after intraperitoneal injection of acetic acid. A significant reduction in the number of writhes by drug treatment as compared to vehicle treated animals was considered as a positive analgesic response. The percentage inhibition of writhing was then calculated.

Hot-plate test

This test was carried out according to the method of Turner[17]. Only mice which reacted within 15 sec when placed on a hot- plate maintained at 55 ± 1^0C, were selected. The mice were then grouped into 5 (n=6), and treated either with sterile water for injection (10 ml/kg, i.p.), Calotropain-G (5, 7, 15 mg/kg, i.p.) or Morphine (5mg/kg, i.p.). Each mice was screened by placing them on the hot plate and reaction time recorded in seconds for fore paw licking or jumping.

Anthelmintic activity

The anthelmintic activity was observed according to method described by Kailashraj and Kurup[18]. Earthworms (*Pheritima posthuma*) of nearly equal size (8±1 cm) were selected for its anatomical and physiological resemblance with the intestinal roundworm parasites of human beings[19,20].

Each earthworm was placed in petridishes containing 15 ml of normal saline (9 mg/ml) as control, Calotropain-G in different dilutions (2.5, 5, 10, 25 and 50 mg/ml) and standard compound Piperazine Citrate also in normal saline (15 mg/ml). Observations were made for the time taken to paralyse and/or death of individual worms up to four hours of test period. Paralysis was said to occur when the worms did not revive even in normal saline. Death was concluded when the worms lost their motility following with fading away of their body colour[21].

Statistical analysis

The experimental results were expressed as the mean ± S.E.M. data were assessed by the method of analysis of ANOVA followed by student's t-test. P value of < 0.05 was considered as statistically significant.

RESULT

The compound Calotropain-G gave positive test

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TABLE 1: Effects of calotropain-G and acetyl salicylic acid (ASA) on writhing induced by acetic acid in mice

Treatment	Dose (i.p.)	No. of writhings (per 10 min)	Inhibition (%)
Control (SWFI)	10 ml/kg,	30.68±2.72	-
Calotropain-G	5 mg/kg	24.57±2.83*	19.92
Calotropain -G	7 mg/kg	21.39±1.49*	30.28
Calotropain -G	15 mg/kg	15.82±1.37*	48.44
ASA	100 mg/kg	9.81±1.51*	68.02

Values expressed as mean ± SEM; (n=6); * (p<0.05) control vs. treated groups

TABLE 2: Effect of calotropain-G and morphine on hot plate reaction time in mice

Time (min)	Control (SWFI) 10 ml/kg	Calotropain -G 5 mg/kg	Calotropain -G 7 mg/kg	Calotropain -G 15 mg/kg	Morphine 5 mg/kg
0	8.5±0.65	7.8±0.80	8.02±0.42	9.1±0.03	8.7±0.35
30	9.5±0.69	9.92±0.44	10.8±0.88	11.63±0.80*	22.66±2.08*
60	9.7±0.73	10.78±0.29	10.07±0.70	12.87±0.56*	21.16±1.32*
90	10.7±0.66	11.04±0.30	11.37±0.42*	15.37±0.46*	18.07±1.04*
120	11.16±1.67	11.31±0.60	12.67±1.06*	18.07±0.46*	17.03±1.05*

Values expressed as mean ± SEM; (n = 6); * (p<0.05) control vs. treated groups

TABLE 3: Anthelmintic activity of Calotropain-G

Compound	Concentration (mg/ml)	Time (min)	
		For paralysis	For death
Control (Normal saline)	9	-	240 #
Piperazine citrate	15	19.43±0.47*	32.28±0.63*
Calotropain-G	2.5	146.78±2.86*	-
	5	118.48±1.23*	151.18±2.71*
	10	74.21±1.14*	116.42±1.82*
	25	27.23±0.62*	49.25±0.42*
	50	16.31±0.31*	28.29±0.23*

Values are expressed as mean ± SEM from Six observations, * (p<0.05) control vs. treated group, # Total test period where no paralysis or death occurred to the worms

for protein. Calotropain-G also showed significant activity in protease assay. The LD_{50} of Calotropain-G when given through intraperitoneal route in mice was found to be 56 mg/kg body weight.

The results presented in TABLE 1 shows that Calotropain-G (at the doses of 5, 7 and 15 mg/kg) and the standard drug ASA (at the dose of 100 mg/kg) exhibited significant and dose dependant inhibition of acetic acid induced writhing when compared to that of control. As shown in TABLE 2, Calotropain-G (at the doses of 7 and 15 mg/kg) and the standard drug Morphine (at the dose of 5 mg/kg) increased the reaction time significantly and dose dependently when compared to that of control.

The anthelmintic activity of the compound Calotropain-G on *P.posthuma* is exhibited in TABLE 3. The perusal of the data reveals that Calotropain-G showed only paralysis but no mortality (up to four hours or 240 minutes) at the concentration of 2.5 mg/ml. The other test concentrations of Calotropain-G (5, 10, 25 and 50 mg/ml) and the standard drug Piperazine Citrate (at the concentration of 15 mg/ml) showed significant anthelmintic activity when compared with total test period (240 minutes) of control group where no paralysis or death occurred to the worms.

DISCUSSION AND CONCLUSION

The analgesic activity of Calotropain-G was studied for peripheral and central activities. Acetic acid induced writhing method is not only simple and reliable but also affords rapid evaluation of peripheral type of analgesic action. In this test, the animals react with characteristic stretching behavior, which is called writhing. The abdominal constriction is related to the sensitization of nociceptive receptors to prostaglandins. It is therefore possible that Calotropain-G produced analgesic effect may be probably due to the inhibition of synthesis or action of prostaglandins. The hot plate method originally described by Woolfe and Mac Donald[22]. This test has been found to be suitable for evaluation of centrally but not of peripherally acting analgesic. The validity of this test has been shown even in the presence of substantial impairment of motor performance[23]. The centrally acting analgesics generally elevate the pain threshold of mice towards heat. The present findings of the study indicate that the Calotropain-G may be centrally acting.

The anthelmintic activity reveals concentration dependent nature. The potency of the compound is found to be inversely proportional to the time taken for paralysis or death of the worms.

In conclusion, the present study demonstrates that Calotropain-G has marked analgesic and anthelmintic activities. Further studies may reveal the exact mechanisms of action responsible for the analgesic and anthelmintic activities of Calotropain-G.

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