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A study on Analgesic,CNS Depressant ,Muscle Relaxant Activity of Marine Gastropode Species of *Conus betulinus*

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Abstract

The *conus betulinus* is collected from portnovo of Chidambaram. The Venom of *conus betulinus* was extracted with 1.1 % (v/v) of acetic and was centrifuged at 12,000 RPM at 4^0c. Then supernatant sample was collected and freeze dried then stored in sealed ampoules. The present study is evaluated for analgesic (50,100 and 200 mcg kg^{-1}, i.p.), central nervous system (CNS) depressant (50, 100 and 200 mcg kg^{-1} i.p.) and muscle relaxant activity (50,100 and 200 mcg kg^{1}, i.p) from wistar rat. The analgesic activity was assayed by tail flick test (thermally induced pain). The CNS Depressant and muscle relaxant activity was assayed by determining with using actophotometer and rotarod tests. In the test 200 mcg kg^{-1} of all activities is nearly equal to the standard.

Key Words: *conus betulinus*, CNS-Depressant, Analgesic, Muscle Relaxant

Introduction

Nature offers wide scope as plants and microbes have been the source of medications from ancient days. In this context, the rich diversity of marine organisms, due to their unique physiological adaptations to the harsh marine environment, produce natural products which offer a good source of pharmacologically active agents with the potential to produce valuable therapeutic entities ***Thakur et al.,* 2005**;.The number of natural products isolated from marine organisms exceeded 18,000 in 2007 ***MarinLit, 2007***. Though a wide range of useful drugs including antibiotics, analgesic, anti-inflammatory, anticoagulants, CNS depressants, antipyretic agents etc., have been isolated from marine organisms, only a few marine derived products are in the market and several of them are in clinical trials (Thakur *et al*., 2005).Marine organisms encompass roughly a half of the total biodiversity, thus offering a vast source to discover useful therapeutics. Academic researchers began to collaborate with pharmacologists in 1989 and the potential of the oceans became clear with many unique bioactive substances being extracted from marine plants and invertebrates ***Fenical, 1997***.The number of conotoxins were characterized, the vermivorous species of *conus betulinus*, which was found to be highly toxic to vertebrates, the purified *conus betulinus* was characterized as a new family of conotoxins. ***chen et al,1998.***

Pain is an unpleasant sensation localized to a part of the body. It is both sensation and emotion. Pain usually occurs when peripheral nociceptors are stimulated in response to tissue injury, visceral distension, or other factors. In such situation, pain perception is a normal physiologic response mediated by healthy nervous system ***Fields and Martin, 2008***.

Those substances depressing the CNS have Variety of Manifesting ranging Mildest of ataractic [transqvillzation] is severe specific such as barbiturate. Since CNS depressant activity varies according to part of the nervous system affected and the degree of depression the screens is unspecific ***Glaser and Mayer, 2009***

The Skeletal Relaxant with calming effect reduces anxiety and tension and out. This effect can be easily studied in animal using various methods, which should be adjusted in such a way that a normal rat can stay on the rod for an appreciable period of time which is reduced upon administration of drug which relaxes skeletal muscle method calming effect degree can be studied.

Material and methods

Collection of molluscs

Fresh and live *conus betulinu* were collected from the port novo coastal area (Lat. 09º17´11.3˝ N and Long. 79º 09´17.1˝ E), Cuddalore District, Tamil Nadu, India, during the morning hours (8.30 am - 9.30 am) with support from local fishermen. Collected samples were transported with the use of plastic containers and identified at the marine biology department of Annamalai University Chidambaram and then transported to the pharmacology Laboratory of Department of Pharmacy. The samples were then washed with tap water until the removal of sand and mud from the shells. Animal portion were cracked using hammer, tissue portion were removed from their shells, the venom duct and venom bulb of each animal was dissected out and homogenized in distilled water in a tissue homogenizer. The Venom was extracted with 1.1 % (v/v) of acetic and was centrifuged at 12,000 RPM at 4^0c. Then supernatant sample was collected and freeze dried then stored in sealed ampoules. The freeze-dried and refrigerated crude venom *conus betulinus* was dissolved in distilled water freshly, during the experiment studies

Analgesic activity: The assessment of analgesic activity was carried out by measuring the sensitivity of the tip of the tail (last 1-2 cm) of adult albino rats placed gently in warm water maintained at 55±2°C and the active rats flicking the tail within 5 seconds were selected for the study. The active rats were divided into five groups of six animals each. The Group I was the control and received normal saline. The Group II was the standard reference group and received Pentazocine (100 mg kg^{-1}). The Group III Group IV and group V animals received *conus betulinus* extracts at50mcg, 100 mcg and 200 mcg kg^{-1}, respectively. The basal reaction time of all groups of animals after treatment was recorded at different time intervals of 15, 30, 60 mins ***Turner, 1965; Kulkarni, 1999***

Central Nervous System (CNS) Depresant Activity: The spontaneous locomotors activity and equipped with photosenser ***Asakura et al., 1993***. The rats were individually placed in a transparent cage (25x48x18 cm^3) and the locomotors activity and rearing were recorded for 10 min. The animals were divided into five groups with Group I serving as a control. The Group II was treated with standard diazepam (4 mg kg^{-1}.) and group III, IV, and V were treated with *conus betulinus* extracts at a dosage level of 50, 100 and 200 mcg kg^{-1}. The locomotors activity was again observed after 30 min of drug administration. The experiment were repeated at an interval of 30 and 60 mins the percentage of changes in the activity was recorded.

Motor coordination: Five groups of mice (n=6) were fed orally with *conus betulinus* (50, 100 and 200 mcg/kg) or vehicle and the effect on motor coordination was assessed using rotarod apparatus ***Dunham MW and, Miya TS 1957***. The animals rat were trained to remain for 3 min on the rod rotating at a speed of 25 rpm. On the next day either vehicle or *conus betulinus* (50, 100 and 200 mcg/kg) was administered orally and their ability to remain on the rotating rod was assessed before and 30 min after the oral administration. The fall-off time from the rod was noted for each animal.

Result and Discussion

Table - 1

EFFECT OF *CONUS BETULINUS* CRUDE EXTRACT ON TAIL FLICK RESPONSE ON RAT (ANALGESIOMETER)

Drug	Dose (mg/Kg)	Mean reaction time	Mean reaction time after administration of Drug		
			15 (min)	30 (min)	60 (min)
Control (saline)	0.2ml	3.62 ± 0.22	3.82 ± 0.34	3.68 ± 0.26	3.59 ± 0.19
CB extract	0.05	3.71 ± 0.31	4.76 ± 0.33	5.41 ± 0.42	5.67 ± 0.31
CB extract	0.10	3.92 ± 0.31	5.42 ± 0.33	5.86 ± 0.37	5.98 ± 0.42
CB extract	0.20	3.87 ± 0.26	6.17 ± 0.47	7.58 ± 0.40	8.23 ± 0.22
Pentazocin (std)	100.0	3.78 ± 0.33	6.93 ± 0.31	8.52 ± 0.21	9.32 ± 0.31

Values are mean ± SME; n=6 in each group. Percentage inhibition is significantly different at, $P < 0.05$, As compared to control.

Table - 2

EFFECT OF *CONUS BETULINUS* CRUDE EXTRACT ON SPONTANEOUS MOTOR ACTIVIT ON RAT (ACTOPHOTOMETER)

Drug	Dose (mg/Kg)	Mean reaction time	Mean reaction time after administration of Drug	
			30 (min)	60 (min)
Control (saline)	0.2ml	412.67 ± 5.04	404.50 ± 6.34	392.39 ± 4.26
CB extract	0.05	421.22 ± 5.09	155.70 ± 1.06	149.40 ± 0.79
CB extract	0.10	414.13±5.29	135.60 ±0 .67	108.04 ± 0.81
CB extract	0.20	408.82±3.88	86.97 ± 0.77	69.93 ± 0.99
Diazepam (std)	4.00	419.64±6.65	3.6.57 ±1.15	29.74 ± 0.96

Values are mean ± SME; n=6 in each group. Percentage inhibition is significantly different at, $P < 0.05$, As compared to control.

Table-3

EFFECT OF *CONUS BETULINUS* CRUDE EXTRACT ON MOTOR COORDINATION ON RAT (ROTA ROD EXPERIMENT)

Drug	Dose (mg/Kg)	Mean reaction time	Mean reaction time after administration of Drug	
			30 (min)	60 (min)
Control (saline)	0.2ml	207.00±5.17	216.50±4.33	231.39±4.09
CB extract	0.05	216.67±4.75	154.67±4.69	146.83±7.39
CB extract	0.10	214.50±6.69	128.33±3.21	99.17±3.56
CB extract	0.20	221.50±5.50	84.10±2.56	43.95±1.06
Diazepam (std)	4.00	419.99±5.14	41.8±1.91	21.07±0.57

Values are mean ± SME; n=6 in each group. Percentage inhibition is significantly different at, $P < 0.05$, As compared to control.

The analgesic activity of CB extract was evaluated by tail flick method in rat to assess central (narcotic) analgesic activity. ***Vogel HG. Berlin, and Heidelberg 2002***. The results of tail flick study clearly indicated that the CB had significant action revealing the involvement of the CNS

in analgesic. This implies that the CB exerted analgesic activity interfering the central mechanisms for the transmission of painful messages in mice.

The tail flick test is thermally induced model where radiant heat is used as a source of pain. Here, radiant heat (through a hot nichrome wire) is applied to the tail of mice and the withdrawal of tail from the radiant heat source (hot nichrome wire) is considered as flicking response to thermally induced pain. The flicking reaction which is the end point of this test may be mediated as a spinal reflex. Analgesics of only narcotic (central) type, e.g., morphine, pethidine, pentazocine, etc., can increase the tail flick latency period indicating analgesia. ***Seth UK et al, 1972***. The difference in tail flick latency in (seconds) of saline (control) treated groups and CB venom extract (test) are presented in (table-I). CB pretreatment induced related changes in tail-with drawal latencies when compared to control group. The maximum analgesic effect reached at 60 min after administration. The effect was dose dependent. A cut off time of 10 seconds has taken as maximum analgesic response to avoid damage to the tail due to heat. The maximum analgesic response was observed at a dose of 200 mcg/kg of CB was found to nearly similar as standard.
Most of the centrally acting analgesics have certain CNS depressant effects. The locomotors activity was evaluated to assess the CNS-depressant property of CB on the motor activity in rat. Most of the centrally active analgesic agents influence the locomotors activities in human beings and rodents mainly by reducing the motor activity because of their CNS depressant property ***Muthal AV and Chopde CT, 1993***. Locomotor activity is considered as an index of wakefulness or alertness of mental activity and a decrease may lead to calming and sedation as a result of reduced excitability of the CNS ***Singh N et al, 2011***. The results of the present study showed significant influence in locomotor activity of rat by CB treatment demonstrating decrease in locomotor activity and hence indicating its CNS depressant property in rat. This effect was dose dependent and the effect was observed after 30 minutes of drug administration and persisted for 60 min (table-2). The effect CB at the dose of 200 mcg/kg was found to be nearly similar as compared to standard response.

In muscle relaxant evaluation, the CB extract induced decrease in fall off time was due to the loss of muscle grip implying skeletal muscle relaxation Demonstration of marked muscle relaxant effect by the rotarod study indicated that CB extract induced neurological deficit accompanied with taming or calming effect in rat, thereby further supporting its CNS-depressant effect.the results of motor co-ordination test are presented in (table-3). It was found that the CB exhibited a marked reduction in motor co-ordination in rat found to be dose dependent and rat were unable to hold on the rotating rod. The effect CB at the dose of 200mcg/kg was found to be nearly as compared to standard response.

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