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

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Abstract

The *conus araneosus* is collected from portnovo of Chidambaram. The Venom of *conus baraneosus* 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 araneosus*, 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; Glaser and Mayer, 2009***).The number of natural products isolated from marine organisms exceeded 18,000 in 2007 (***MarinLit,2007***). 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 marine gastropods known as cone snails(conus) constitute an unusually species rich group of venomous predators,one of the largest single genera (>500 species) of living marine invertebrates. There are about 300 species in the genus *conus*. The shells of these animals are very beautiful, especially when the periostracum (outer covering) is removed. It is mainly distributed in Indian Ocean and indo-west pacific region into the following area (***hylleberg, J. & Kilburn, R.N. 2002***.) the physiology and pharmacological characterization of the venoms of a few cone snails was carried out. A first comprehensive study of the effect of different *conus* venom (***Kohn, et al,1960***.) demonstrated that there are striking differences in potency, in particular venoms of fish-hunting *conus* are much more lethal than those of the other groups when tested in vertebrates.the first biochemical attempt characterizing the biologically active components of cone snail venoms was reported. The number of conotoxins was characterized; the vermivorous species of *conus araneosus*, which was found to be highly toxic to vertebrates, the purified *conus araneosus* was characterized as a new family of conotoxins. (***Chen et al, 1998***). The present studies investigate analgesic ,CNS depressant and musle relaxant activity of *conus araneosus*

Material and methods

Collection of molluscs

Fresh and live *conus araneosus* 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 araneosus* 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 V was the standard reference group and received Pentazocine (100 mg kg^{-1}). The Group II,III and IV animals received *conus araneosus* extracts at 50mcg, 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 V was treated with standard diazepam (4 mg kg^{-1}.) and groupII, III, IV, were treated with *conus araneosus* 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 araneousus* (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 araneosus* (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 araneosus* CRUDE EXTRACT ON TAIL FLICK RESPONSE ON RAT (ANALGESIOMETER)

Drug	**Dose (mcg/Kg)**	**Mean reaction time**	**Mean reaction time after administration of Drug**		
			15 (min)	**30 (min)**	**60 (min)**
Control (saline)	0.2ml	3.92 ± 0.29	3.78 ± 0.38	3.58 ± 0.26	3.42 ± 0.22
CA extract	0.05	3.76 ± 0.33	4.46 ± 0.27	5.11 ± 0.48	5.37 ± 0.39
CA extract	0.10	3.82 ± 0.31	5.12 ± 0.38	5.82 ± 0.31	6.18 ± 0.32
CA extract	0.20	3.67 ± 0.26	6.47 ± 0.57	7.18 ± 0.44	8.13 ± 0.23
Pentazocin	100	3.79 ± 0.33	6.74 ± 0.35	8.12 ± 0.29	9.42 ± 0.41

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 araneosus* CRUDE EXTRACT ON SPONTANEOUS MOTOR ACTIVITY 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	414.27 ± 6.04	396.50 ± 5.14	392.39 ± 4.26
CA extract	0.05	428.42 ± 5.79	145.45 ± 2.06	139.40 ± 0.49
CA extract	0.10	434.23±4.24	138.23 ±0 .27	118.34 ± 1.11
CA extract	0.20	424.62±4.28	82.91 ± 0.77	71.53 ± 0.91
Diazepam	4.00	410.72±6.15	41.27 ±1.23	32.34 ± 1.06

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 araneosus* 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	217.27±6.17	206.32±4.23	261.32±4.89
CA extract	0.05	218.77±5.45	163.47±4.29	146.23±6.42
CA extract	0.10	242.21±6.34	122.13±4.41	102.37±3.66
CA extract	0.20	231.32±6.55	79.18±3.52	54.63±2.06
Diazepam	4.00	410.25±5.64	51.08±1.91	32.37±0.78

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

The tail flick test was considered to be selective to examine compounds acting through opioid receptor the extracts increased pain threshold which means basal latency, which indicates that it may act via centrally mediated analgesic mechanism.

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 CA was found to nearly similar as standard. Overall, the analgesic action of extract of *conus araneosus* is assumed to be due to inhibition of prostaglandin synthesis and its role on both central and peripheral analgesic mechanism. The extract may act as more significant analgesic activity.

The spontaneous locomotor activity is a test to appraise the level of excitability of the CNS (**Mansur, R.M,et al 1980**) and any decrease of this activity may be narrowly related to sedation resulting from depression of the central nervous system.(**Ozturk, Y et al 1996**) Gamma-amino-butyric acid (GABA) is the major inhibitory neurotransmitter in the central nervous system. Different anxiolytic, muscle relaxant, sedative-hypnotic drugs are elucidation their action through GABA, therefore it is possible that extracts of *conus araneosus* may acts by potentiating GABAergic inhibition in the CNS via membrane hyperpolarization which leads to a decrease in the firing rate of critical neurons in the brain or may be due to direct activation of GABA receptor by the extracts (**Kolawole, O.T et al,2007**) The locomotor activity is a measure of the level of excitability of the CNS and decrease of this activity may be closely related to sedation resulting from depression of the central nervous system. 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, 201)1***. The results of the present study showed significant influence in locomotor activity of rat by CA 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 CA at the dose of 200 mcg/kg was found to be nearly similar as compared to standard response.

The observed muscle relaxant effect of the extract may be due to the agonistic effect on GABA/benzodiazepine receptor complex.(**Vikas G & Payal,2010**) In muscle relaxant evaluation, the CA 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 CA 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 CA 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 CA at the dose of 200mcg/kg was found to be nearly as compared to standard response

Conclusion

Based on the results of the present study, it can be concluded that the extract of *conus araneosus* possesses potent analgesic, CNS depressant and muscle relaxants properties, which support its use in recent medicine. However, further studies are needed to understand the exact mechanisms of action and to isolate the compound (s) responsible for such activity.

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