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# Some Neuropharamacological Effects of the Crude Extract of *Conus parvatus* in Mice

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**Abstract:** The present study was aimed to characterize the type of conotoxin present in *Conus parvatus*. (CP) belongs to family conidae, through neuro-pharmacological activities of the crude venom extract on some of the CNS animal experiment using mice as animal model. The effects of CP on CNS were studied, by using spontaneous motor activity, gross behavior, rota-rod performance, analgesic activity and potentiation of pentobarbitone sleeping time in mice. Preliminary evaluation of acute toxicity was also carried out; The $LD_{50}$ value was found to be 425.20 mcg $kg^{-1}$ by i.p. route. The extract (200 mcg $kg^{-1}$ i.p.) was found to produce, reduction in spontaneous motor activity, potent analgesic activity, reduction in motor coordination and prolonged pentobarbitone-sleeping time. From the above all pharmacological activities, it may concluded that, the conotoxin present in CP is most likely to be a α-conotoxin and also it will be more suitable to continue the studies in the areas of analgesic and other CNS depressant therapeutic areas after isolation of the pure cono-peptide from CP.

**Key words:** *Conus parvatus*, sedation, spontaneous motor activity, gross behavior, motor coordination, pentobarbitone sleeping time

## INTRODUCTION

Predatory marine snails of the genus Conus (family conidae) with over 500 species may comprise the largest single genus of marine animals living today. These species inhabit in tropical reef environments throughout the world. According to their prey preference, cones snails can be classified into three major groups: the piscivorous preying upon fish (*e.g.*, *Conus striatus*, *C. geographus*), the molluscivours eating mollusk (e.g., *C. textile*, *C. pennaceus*) and the vermivorous feeding upon *polychaete annelids* (e.g., *C. imperialis*, *C. vexillum*). All cone snails are venomous predators and have developed a sophisticated biochemical arsenal to rapidly immobilize their prey. Their venoms are complex mixtures of small, disulfide-bridged polypeptide toxins (conotoxins) that inhibit the function of ion channels and neurotransmitter receptors. In addition to the vital role in prey capture and defense against predators, conotoxins are useful tools in neuroscience to characterize receptors and receptor subtypes due to their high binding affinity and specificity. In many cases the determination of ion channel has depended on finding a specific biotoxin (Olivera and Cruz, 2001; Miller, 1995).

Conotoxins also offer great potential as leads in drug development and indeed the N-type calcium channel blocker from *Conus magus*, ω-conotoxin is currently in clinical trial for the treatment of stroke and chronic pain. It is anticipated that the discovery of new toxins displaying characteristically high specificities will increase our understanding of the physiology, pharmacology, biochemistry and structure of their receptors and may provide leads, to new pharmaceuticals.

## MATERIALS AND METHODS

**Preparation of crude venom toxin extract:** Specimens of *Conus parvatus* were collected from Portonova, Chidambaram, Tamil Nadu, Southern India, dissected and a crude extract prepared from the venom duct material as previously described. Briefly, ground dried ducts were extracted with 30% acetonitrile/water acidified with 0.1% trifluoroacetic acid, centrifuged and the supernatants retained. Crude venom extract was lyophilized and stored at -20°C.

**Animals:** The protocol was approved by, The Institutional Animal Ethics Committee of Annamalai University Faculty of Medicine. The pharmacological experiments were conducted using Swiss albino mice weighing 20-25 g Animals were maintained under standard nutritional and environmental conditions of 50±10% RH and 12 h light and 12 h dark cycle throughout the experiment. The animals were used after

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an acclimatization period of at least 5 days to the laboratory environment and provided with standard food pellets and water *ad libitum*. The animals were deprived of food 24 h before experimentation. The animal ethical committee clearance was obtained from the institution for the present study.

**Acute toxicity test:** Mice were divided into groups of six each and CP was injected i.p. In doses from 50 to 500 mcg $kg^{-1}$ death within 24 h was recorded. The $LD_{50}$ was estimated from the graph of percent mortality against log-dose of the extracts using the (Miller and Tainter, 1944) method.

**Analgesic activity:** To evaluate the central analgesic effects of crude venom extract of CP. Tail flick test was performed by time taken for mouse to withdraw the tail when immersed in hot water maintained at 55±0.5°C 1st group was treated with saline, while group 2nd, 3rd, 4th received the extract (50, 100, 200 mcg $kg^{-1}$ i.p.).The group 5th treated with Pentazocine (10 mg $kg^{-1}$, i.p.) as standard drug.

**Spontaneous motor activity (SMA):** Spontaneous motor activity was performed using Actophotometer (Techno LE3806, India). Mice were grouped of six of each. The 1st group was treated with normal saline, while group 2nd, 3rd, 4th received the extract (50, 100 and 200 mg $kg^{-1}$ i.p.) and group 5th received diazepam 4 mg $kg^{-1}$ i.p. Activity was automatically recorded 30 min after treatment and at every 10 min. The experiments were repeated at an interval of 30 min, for a total of 120 min. Results of the treated groups were compared with those of control group at each time interval (Amos *et al*., 2001). SMA measurements started 30 min after the administration of the extract and the results were compared with those of control.

**Gross behavioral activity:** After an intra-peritoneal administration of the same test doses (acute toxicity concentration) of venom extract to a group of 6 mice, each animal was observed for gross behavioral extracts. The behaviors of the animals were continuously observed for 3 h after administration of the venom extract, then at the end of every 30 min for next 3 h. The study was done for 6, 12 and 24 h.

**Motor coordination:** Rota-rod (Techno, India) biological research apparatus was used for the test. The instrument (a horizontal rotation device) was set at a rate of 16 revolutions per minute(Fujimori and Cobb, 1965). Mice were placed on the rod and those that were able to remain on the rod longer than 3 min were selected for the study. 1st group was treated with saline, while group 2nd, 3rd and 4th received the (50, 100, 200 mg $kg^{-1}$ i.p.) and group 5th received diazepam 4 mg $kg^{-1}$ i.p. Mouse unable to remain on the rod at least for three min was considered as a positive test and the time of its fall was recorded.

**Pentobarbital-sleeping time:** Albino mice were grouped of six each. They were treated as follows; 1st group was treated with saline, while group 2nd, 3rd and 4th received the extract (50, 100, 200 mg $kg^{-1}$ i.p.) and group 5th received diazepam 4 mg $kg^{-1}$ i.p. The effects were recorded as follows: Time elapsed between the administrations of pentobarbital until loss of righting reflex was recorded of as the onset of sleep, while the time from the loss to its recovery was considered as the duration of sleep (Ming-Chin, 1998).

**Statistical analysis:** All the data obtained were expressed as mean±standard error. Differences in means were estimated by means of ANOVA followed by Dunnet's post hoc test. Results were considered significant at $p<0.05$.

## RESULTS

**Acute toxicity and general behavioral studies:** The LD50 of CP by i.p. route in mice was found to be 425.20 mcg $kg^{-1}$. While conducting the toxicity studies animals were observed continuously for any general behavioral changes and significant reduction of spontaneous loco-motor motility, drowsiness and remarkably quiet were observed.

**Analgesic activity:** Analgesic activity was investigated by tail flick test in mice. The difference in tail flick latency (sec) before and after treatment, in saline treated group was 3.50±0.22 (Table 1). CP pretreatment induced related changes in tail-withdrawal latencies when compared to control group. The maximum analgesic effect reached

Table 1: Effect of *C. parvatus* crude extract on tail flick response in mice

| | | | Mean reaction time after administration of drug | | |
|---|---|---|---|---|---|
| Drug | Dose (mg $kg^{-1}$) | Mean reaction time (min) | 15 (min) | 30 (min) | 60 (min) |
| Control (saline) | 0.2 mL | 3.50±0.22 | 3.82±0.34 | 3.65±0.26 | 3.29±0.19 |
| CP extract | 0.05 | 3.83±0.31 | 4.67±0.33 | 5.33±0.42 | 5.83±0.31 |
| CP extract | 0.10 | 4.17±0.31 | 5.33±0.33 | 6.00±0.37 | 6.33±0.42 |
| CP extract | 0.20 | 4.00±0.26 | 6.17±0.47 | 7.83±0.40 | 8.50±0.22 |
| Pentazocin (std) | 10.0 | 3.67±0.33 | 6.83±0.31 | 8.67±0.21 | 9.17±0.31 |

Values are mean±SME; n = 6 in each group. Percentage inhibition when compared to control. Significantly different at, $p<0.05$

Table 2: Effect of *C. parvatus* crude extract on spontaneous motor activity in mice

| Drug | Dose (mg kg$^{-1}$) | Mean reaction time (min) | Mean reaction time after administration of drug | |
|---|---|---|---|---|
| | | | 30 min | 60 min |
| Control (saline) | 0.2 mL | 420.67±5.04 | 413.50±6.34 | 398.39±4.26 |
| CP extract | 0.05 | 404.22±5.09 | 95.70±1.05 | 74.09±0.79 |
| CP extract | 0.10 | 417.13±25.29 | 82.50±0.67 | 44.04±0.81 |
| CP extract | 0.20 | 418.82±3.88 | 29.97±0.77 | 18.93±0.99 |
| Diazepam (std) | 4.00 | 407.64±6.65 | 36.57±1.15 | 19.74±0.96 |

Values are mean±SME; n = 6 in each group. Percentage inhibition when compared to control. Significantly different at $p<0.05$

Table 3: Effect of *C. parvatus* crude extract on motor coordination in mice

| Drug | Dose (mg kg$^{-1}$) | Mean reaction time (min) | Mean reaction time after administration of drug | |
|---|---|---|---|---|
| | | | 30 min | 60 min |
| Control (saline) | 0.2 mL | 207.00±5.17 | 216.50±4.33 | 231.39±4.09 |
| CP extract | 0.05 | 206.67±4.75 | 104.67±4.69 | 106.83±7.39 |
| CP extract | 0.10 | 214.50±6.69 | 50.33±3.21 | 54.17±3.56 |
| CP extract | 0.20 | 221.50±5.50 | 36.10±2.56 | 43.50±1.06 |
| Diazepam (std) | 4.00 | 419.99±5.14 | 33.80±1.91 | 19.07±0.57 |

Values are mean±SME; n = 6 in each group. Percentage inhibition when compared to control. Significantly different at $p<0.05$

Table 4: Effect of *C. parvatus* crude extract on Pentobaritone induced sleeping time in mice

| Treatments | Drug (mg kg$^{-1}$) | Onset of sleep (min) | Duration of sleep (min) |
|---|---|---|---|
| Control (Pentobaritone) | 40.0 | 3.68±0.15 | 42.92±1.88 |
| Test (*C. parvatus* ) | 0.05 | 3.32±0.23 | 47.47±1.91 |
| Test (*C. parvatus* ) | 0.10 | 3.12±0.24 | 53.39±0.97 |
| Test (*C. parvatus* ) | 0.20 | 2.27±0.22 | 71.49±1.33 |
| Standard (Diazepam) | 4.00 | 2.16±0.14 | 93.15±2.15 |

Values are mean±SME; n = 6 in each group. Percentage inhibition when compared to control. Significantly different at $p<0.05$

at 60 min after administration. The effect was dose dependent. A cut off time of 15 sec was taken as maximum analgesic response to avoid damage to the tail due to heat.

**Spontaneous motor activity:** CP produced significant decrease in the spontaneous motor activity in mice. This effect was dose dependent and the effect was observed within 30 min of drug administration and persisted for 60 min (Table 2).

**Motor coordination:** Results of motor coordination test are presented in (Table 3). It was found that, the CP exhibited a marked reduction in motor coordination in mice was found to be dose dependent and mice were unable to hold on the rotating rod.

**Pentobaritone induced sleeping time:** Prior administration of CP significantly potentiated pentobarbitone-induced sleeping time in mice. Various sleep time of mice treated with Pentobaritone with or without extract are shown in (Table 4). The normal sleeping time was found to be 42.92 min in mice treated with pentobarbitone alone. Prior administration of CP significantly potentiated onset of action and duration of action of pentobarbitone-induced sleeping time in mice. The maximum duration of sleeping was observed at a dose of 200 mcg kg$^{-1}$ of CP and was approximately 76.75% as standard.

**Gross behavior pattern:** After an intra-peritoneal administration of the same test doses (acute toxicity concentration) of venom extract to a group of 6 mice, each animal was observed for gross behavioral extracts, there was a significant reduction in responses when compared with control (Table 5).

## DISCUSSION

The present research reports some neuro-pharmacological activities of venom toxin extract of *Conus parvatus* in mice. Results indicated that the CP significantly increased in tail flick withdrawal response. Tail flick analgesic testing is usually considered suitable for centrally acting analgesic in a dose dependent fashion.

The extract was found to produce alteration in general behavior pattern, significant reduction of spontaneous motor motility, gross behavior pattern, reduced motor coordination and potentiation of pentobarbitone induced sleeping time. The present findings suggest that CP possesses CNS-depressant action.

The extract significantly reduced spontaneous motor activity. The activity is a measure of the level of excitability of the CNS and this decrease may be closely related to sedation resulting from depression of the central nervous system (Ozturk *et al*., 1996).The CP crude venom extract possessed central nervous system depressant activity as indicated by the decrease in gross behavior (Ming-Chin, 1998) in mice. It also showed a marked sedative effect as indicated by the reduction in gross behavior and potentiation of pentobarbitone induced sleeping time.

Earlier studies have related prolongation of barbital hypnosis to pentobarbital metabolic inhibition or action on the CNS involved in the regulation of sleep (Dubois *et al*., 1986). It is generally accepted that the sedative effect of drugs can be evaluated by measurement of spontaneous motor activity and pentobarbitone induced sleeping time in laboratory animal model (Adzu *et al*., 2002). These results corroborate those of (Fujimori and Cobb, 1965), who proposed that the enhancement of barbital hypnosis is a good index of CNS depressant activity. Results of the

Table 5: Effect of *C. parvatus* crude extract on gross behavioral studies in mice

| Observation | Effects | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| Gross activity | Up to 3 h | 3 ½ h | 4 h | 4 ½ h | 5 h | 5 ½ h | 6 h | 12 h | 24 h |
| Respiration | ↓ | ↓ | ↓ | ↓↓ | ↓↓ | ↓↓ | ↓↓ | ↓↓ | ↓↓ |
| Writhing | ++ | ++ | + | + | + | - | - | - | - |
| Tremor | ++ | ++ | + | + | + | + | - | - | - |
| Convulsions | ++ | ++ | + | - | - | - | - | - | - |
| Hind limb paralysis | + | + | ++ | ++ | ++ | ++ | ++ | ++ | ++ |
| Sense of touch and sound | ↑↑ | ↑↑ | ↑ | ↑ | - | - | - | - | - |
| Salivation | ↑ | ↑ | - | - | - | - | - | - | - |
| Diarrhea | - | - | - | - | - | - | - | - | - |
| Mortality | - | - | - | - | - | - | - | - | - |

+: Mild effect, ↓: Mild depression, ↑: Mild stimulation, - : No effect, ++: Strong effect, ↓↓: Strong depression, ↑↑: Strong stimulation, *: Observation of 3 animals death after 36 h

gross behavior test (Table 5) further support the neuro-sedative activity and its possible application in anxiety condition (Amos *et al*., 2001).

## CONCLUSIONS

From the previous work done on crude venom of *C. geographus* has shown to consist of α-GID is the sixth α-conotoxin and the second neuronally active α-conotoxin (McIntosh *et al*., 1999; Wishart *et al*., 1995), showing the importance of α-conotoxins targeting both muscle and neuronal nAChRs, It was understood that α-Conotoxins are competitive antagonists of acetylcholine (ACh) binding to the nAChR Neuronal nicotinic acetylcholine receptors (nAChR) represent important targets for the development of novel drugs for the treatment of pain and various disorders of the central nervous system (Lloyd and Williams, 2000).

Present findings indicate that the CP has got more potent analgesic activity as pentazocin at the concentration of (200 mcg), it was also found to posses more CNS depressant activity as it show a moderate to strong sedative activity as the dose increased which results in significant reduction of spontaneous motor motility, gross behavior pattern, reduced motor coordination and potentiation of pentobarbitone induced sleeping time.

Therefore, CP crude extract may consist of content of alpha-conotoxins, might be contributing in part to the experimental pharmacological effects. Further studies are planned to establish mechanism of CNS-depressant action of CP by using various agonists and antagonists. From the above all pharmacological activities, as the activities of the crude extract has more resemblances α-conotoxin, it may concluded that, the conotoxin present in CP is most likely to be a α-conotoxin and also it will be more suitable to continue further studies on the areas of analgesic and other CNS depressant therapeutic areas after isolation of the pure cono-peptide from CP.

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