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Bioactive potential of crude venom from marine snail, *Conus zeylanicus*

Pawan Kumar[1,*], P. P. Srivastava[1], S.K. Chakraborty[1] & S. M. Shivaprakash[2]

[1]ICAR-Central Institute of Fisheries Education, Off Yari Road, Panch Marg, Versova, Mumbai – 400 061, Maharashtra, India.

[2]Director of Extension, Karnataka Veterinary, Animal and Fisheries Sciences University (KVAFSU), Nandinagar, Bidar-585 401 Karnataka, India.

*[E-mail : kpawann8@gmail.com]

Abstract

The crude toxin was fractionated stepwise using diethyl-amino-ethyl (DEAE) cellulose anion exchange chromatography. A total amount of 2.5 g of the crude venom was extracted from 40-55 specimens. The protein content of crude toxic venom was found to be maximum 281.6 ± 1.36 µg/mL and minimum 52.8 ± 0.88 µg/mL. The mice bioassay for lethality was performed on male albino mice weighing 20 ± 2 found to be lethal at 1hr: 40minutes:15 second at 1.0 ml and 2hr: 20minutes: 10 seconds at 0.75 mL. Toxicity of crude extracts of the coniids injected intra-peritoneally with different doses in male albino mice are shown the symptoms of toxicity. The crude venom exhibited hemolytic activity on chicken erythrocytes, which was estimated as 4 HU. Analgesic activity test was carried out on albino mice by tail-flick method showed an analgesic effect. The crude venom exhibited neurostimulatory response on mice brain and AChE activity ranging between 81% and 175% was caused by the venom of C. zeylanicus. The fractionated toxin was characterized by performing SDS-PAGE on 12.0% gel system using standard protein markers yielded four clear bands 20 kDa, 40 kDa, 58 kDa, and 100 kDa. The present study reveals the pharmacological potential of the crude venom of Conus zeylanicus could be utilized for a better assessment of the clinical manifestations produced by the venomous marine animals.

[**Keywords:** *Conus zeylanicus;* hemolytic activity; SDS-PAGE; venom; AChE; bioassay]

1. Introduction

Cone snails (genus *Conus*) are invertebrate venomous predators comprising approximately 700 species[1], with each *conus* species producing a distinctive repertoire of 100-200 venom peptides[2, 3]. Cone snails (genus *Conus*) are predatory gastropods that are found in tropical marine habitats around the world. Cone snails are a large genus of venomous snails that use a complex mixture of physiologically active peptide for prey capture[4]. The venom peptides exhibits excitotoxic shock as well as alters neurotransmission compress effect a highly evolved uses of combination drug strategy in natural systems[5, 6]. The venoms of cone snails are a natural resource of peptides with a promising pharmaceutical potential for developing drugs that target ion channels, cell-surface neurotransmitter receptors and transporters. During 50 mllion years of evolution, cone snails have turned to expertize in neuropharmacology. Some instructive lessons could be learned from cone snails for our modern drug design[5]. Several postulates have been presented to explain the generation of conotoxin diversity[7, 8, 9, 10]. In future, extensive analysis of the cone snail's genes may provide a fundamental basis to elucidate the mechanisms of *conus* peptide biosynthesis and diversity. About 5070 species of Mollusca have been recorded from India of which, a total of 3370 species are from the marine environment while resting from the freshwater and terrestrial environment[11]. Kohn[12] reported 48 species of *Conus* from India but increased the number to seventy-seven[13, 14]. Although 48 different species of cone snails prevail in India, no systematic study of the painkilling properties of their toxins has been carried out in the past. In India, studies were carried out on the biomedicinal aspect of species available viz *Conus figulinus, Conus lorosii, Conus inscriptus. Conus amadis, Conus mutabilis* and *Conus lentiginosus*. The studies have been shows that the venom from the cone snails had pronounced pain killing effect on laboratory mice. The cone snails from Indian waters have been poorly studied especially with regards to their toxicity. Therefore, the present study was undertaken to gain a better understanding of the toxicity and biological properties of the crude venom extracted from the *C. zeylanicus* collected from the coast of Mumbai for their pharmaceutical potential.

2. Materials and Methods

2.1 Preparation of crude extract

The samples of *Conus zeylanicus* were collected along the of Khardanda beach, Mumbai. The specimens were kept alive in saltwater till their sacrificed. A total amount of 2.5 g of crude venom was extracted from about 40-50 specimens. Venom was extracted from freshly sacrifice animals as described by Cruz et al[15]. The soft body of the animal was removed by opening the shell. The venom duct and bulb of each animal was dissected and taken out. The venom duct was ground and mixed with double-distilled water (DDW). The homogenate was centrifuged at 10,000 rpm for 15 minutes at room temperature. The supernatant was collected for lyophilisation and stored immediately at 4^0C and lyophilized in Labconco freezone Lyophilizer (6L capacity). The lyophilized powder was resuspended in double distilled water (DDW) and it was considered as crude venom. After centrifugation, the supernatant was separated, lyophilized and stored at -20 ^{0}C for further analysis.

2.2 Experimental Animals

The pharmacological experiments were conducted using male albino mice of Kausauli strain weighing 20±2 g were procured from the animal house of M/S Haffkine Bio-pharma, Mumbai, and were maintained in the laboratory, following the basic codal formalities of the Ethical Committee of the Institute's. The animals were housed in the animal house maintained under standard hygienic conditions with a 12-hour light and dark cycle throughout the experiment.

2.3 Partial Purification

Partial purification of the crude extract was carried out through DEAE cellulose anion exchange chromatography[16]. Ten fractions were collected in a stepwise gradient with 0.1 to 1.0 M NaCl in phosphate buffer saline (PBS). The collected fractions were stored at -20°C for further use.

2.4 Protein Estimation

The protein estimation was carried out by the method Lowry et al[17], using bovine serum albumin (BSA) as a standard.

2.5 Mice Bioassay

The mice bioassay for lethality was done by the method of Gouiffes et al[18]. The bioassay of lethality was done by using clinically healthy male albino mice (20±2 g). The mice were maintained in a healthy condition in the laboratory. The crude venom dissolved @ 5 mg/mL in PBS (pH 7.4) was injected intraperitoneally (i.p) to the mice in doses of 0.25, 0.50, 0.75 and 1.0 mL of each fraction was injected i.p to the mice. Mice in triplicate sets were maintained for each dose. The i.p. injected mice were kept under observation in rearing cages. The time of injection and the time of death were recorded, besides recording the behavioural changes before death were recorded for 24 hrs.

2.6 Hemolytic Study

The hemolytic activity of crude venom on the chicken was tested by micro hemolytic method[19]. The blood was centrifuged at 5,000 rpm for 5 min. and the supernatant was discarded, subsequently the pellet suspended in normal saline (pH 7.4). The procedure was repeated thrice and 1 % erythrocyte suspension was prepared by adding 99 mL normal saline to 1 mL of packed RBC. The hemolytic assay was performed in 96 well 'U' bottom microtitre plates. A row was selected for chicken erythrocyte suspensions. Serial two-fold dilutions of the crude extract of venom (100 µL; 1 mg crude in 1 mL PBS) were made in PBS (pH 7.2) starting from 1:2 ratios. An equal volume of 1% erythrocyte was added to each well. The plates were thoroughly mixed the RBC and venom extract. The plates were kept at room temperature for two hours before reading the results. Appropriate control was included in the tests. Erythrocyte suspension to which double distilled water was added with 100µL served

as blanks for negative control. Button formation at the bottom of the wells was taken as negative control. The reciprocal of the highest dilution of the venom extract showing the hemolysis was defined as one hemolytic unit.

2.7 Neuromodulatory Activity

AChE Activity

The method of Ellman et al[20] was followed to see the Acetylcholine activity (AChE) activity by preparing enzyme source obtained from male albino mouse. Three mL phosphate buffer (pH 8.0) was taken in each tube to which 0.1 ml of enzyme source (2% w/v homogenate) was added and stirred. Then 100 µL of 0.01 M DTNB (5, 5-dithiobis-2-nitrobenzoic acid) was added and the initial color was measured spectrophotometrically at wave length of 412 nm. The test solution of toxin (100 µL) in different concentrations such as 100, 200, 400, 800 and 1000 µg were added. Control experiment was also run simultaneously with 100 µL of triple distilled water containing no toxins. To start the reaction, 20 µL of acetyl thiocholine iodide (ATCI) (0.075 M) was added to each tube as substrate and then the reaction was allowed to continue for 15 minutes at room temperature. The colour developed was measured as final reading at 412 nm in UV-VIS Spectrophotometer. All experiments were conducted in duplicate.

2.8 Immunomodulatory Activity

Immunomodulatory activity was analysed through *in vitro* phagocytosis of *Candida albicans* by polymorphonuclear cells (PMN) (slide method) following the procedure given by Kulkarni and Karande[21].

2.9 Evaluation of Analgesic Activity

Tail-Flick Method

Analgesic activity was measured according to the D`amour and Smith[22] using a tail-flick analgesia meter (Harvard, USA 50-9495, 230 V and 50 Hz) with a variable 150 W, 25 V quartz lamp as the heat source. During the testing period, the mice were restrained in a plastic tube, to which they had been previously adapted for 10 minutes twice a day for three days. The tail-flick latency was recorded as the time of onset of stimulation to the withdrawal of the tail from a light beam. The beam of light was focused on some spot, at about 6 cm from the tip of the tail of each animal. The intensity of the radiant heat was identical in all the experiments. The reaction time of the animal was then displayed and noted down. Mice without administration of any toxin or known pain killer were used as controls while those injected intra-peritoneally with Paracetamol (Crocin® @ 0.25 mL/ 20 ± 2 g mice) served as reference standards. Experimental mice in triplicates received 0.25 mL of toxin i.p. and subjected to a light intensity of 4 different current strengths viz. 3.0, 3.5, 4.0 amps. Analgesic activity was determined as a ratio between the difference in reaction time of envenomated mice and control, since analgesic potential would be proportional to the difference in tail-flick latency between the toxin and control. The mice were tested after 30 minutes of injection. The cut-off time was taken as 10 s to avoid damage to skin due to prolonged exposure to heat and the animals not showing any response even at the end of 10 s were assumed to have complete analgesia.

2.10 Molecular weight determination (SDS-PAGE) Analysis

Sodium dodecylsulphate-polyacrylamide gel electrophoresis (SDS-PAGE) which utilized 5% stacking gel and 10% resolving polyacrylamide gels was carried out to estimate the molecular weight of the hemolytic toxin according to the method of Laemmli[23]. This is the most convenient way of determining the molecular weight of proteins. In this technique, five molecular weight markers (9, 29, 40, 72 and 150 kDa) were used. 10 µl of the marker was loaded in the right well as marker and the crude proteins were loaded subsequently wells. Upon completion of electrophoresis gel run, the gel was washed gently with distilled water to remove excess SDS, stained in Coomassie Brilliant Blue R-250 (Coomassie brilliant blue R-250, 1.25 g methanol, 227 mL; glacial acetic acid, 46 mL; distilled water to complete a volume of 500 mL) for two hours at room temperature and de-stained (methanol, 7 mL; glacial acetic acid, 7 mL; and distilled water to reach 100 mL) for 48 hours. Protein bands were visualized as dark blue bands on a light blue background. Samples were denatured by boiling in buffer containing SDS and β-mercaptoethanol prior to loading onto the gel. Following electrophoresis at 30 mA

for four hours, gels were stained by Coomassie blue staining. Hence, migration of proteins in the gel was only according to their molecular weight.

2.11 Statistical Analysis

The observations were tabulated and the significance of difference was calculated by the Student t-test. Differences between different groups were analyzed by statistically compared with the controls by one-way ANOVA, an all cases statistical significance is indicated by $p<0.05$ considered as significant. All the experiments, mean ± standard error calculated and are included in the figure. The present prolongation of reaction time was also calculated for graphical presentation.

3. Results

Protein Estimation

The protein content in the lyophilized crude extract of *C. zeylanicus* was found to be 281.6 ± 1.36 µg/mL. In the protein purified fractions was found to vary between a minimum of 52.8 µg/mL (F10) and a maximum of 192.4 µg/mL (F1) (Table I).

3.1 Mice Bioassay for Lethality

Non-lethal dose was found to be 0.25 mL containing 52.8 and 0.50 mL containing 150.0 µg/mL. The minimum lethal dose of venom extract was found to be 0.75 mL containing 220.0 µg/mL of protein for 20 ± 2 g mice, wherein death occurred in 2hr: 20minutes: 10 second i.p. injection. When injected with the highest dose of 1.0 mL, containing 281.6 µg/mL of protein death occurred in 01 hr: 40 minutes: 15second. Toxicity of crude extracts of the coniids injected intraperitoneally with different doses in male albino mice are shown the symptoms of toxicity observed (Table II).

3.2 Hemolytic Assay

In-vivo effect of hemolytic assay conducted on chicken erythrocytes revealed that the crude venom of column fractionated venom of *C. zeylanicus* induced spontaneous hemolysis of chicken blood found to be 4 Hemolytic Units (HU) from the protein content in the crude extract of *C. zeylanicus* was 281.6 µg/mL.

3.3 Neuromodulatory Activity

In - vitro effect on AChE activity

Crude venom of *C. zeylanicus* showed a neuromodulatory response on the mice brain AChE activity at the minimum concentration 175% at concentration of 100 µg but decrease the same, up to 81 % at higher concentration of 1000 µg. (Table III).

3.4 Immunomodulatory Activity

Phagocytosis is considered as first line of defense mechanism against microbial infections. In our present study the venom of *C. zeylanicus* showed a neurostimulatory response on the mice brain. Stimulating activity up to 10.25% was shown by lower concentration 100 µg/ mL but had a suppressive activity between -4.10% and -6.18% was exhibited by higher concentrations 800 and 1000 µg/ mL (Table IV).

3.4 Analgesic Activity

Tail-Flick Method

The crude protein of *C. zeylanicus* showed an analgesic ratio (AR) of after injection 30, 60 and 90 min. The crude proteins of the *C. zeylanicus* were tested, exhibited pronounced analgesic activity. The analgesic ratio increased with increase in time. Analgesic activity in terms of tail-flick response observed response time before injection in control was 3.65 sec and response time after injection 6.83, 7.28, 7.82 was observed at 0.25 µg/mL dose; 0.50 µg/ mL dose was 7.23, 7.46, 8.01 and 0.75 µg/mL dose was 7.55, 7.66, 8.35 after 30, 60 and 90 min compared with control and paracetamol respectively (Table V).

3.5 SDS-PAGE Activity

In SDS-PAGE on 12.0% gel, crude protein of *C. zeylanicus* afforded four bands ranging from 20, 40, 58 and 100 kDa have been identified indicating that these samples possess some protein bands in common. The molecular weight of protein was found to be approximately 20 kDa (Fig 1). From the above results it is clearly indicated that these samples possess some protein bands in common.

4. Discussion

4.1 Protein Estimation

The present investigation found the respective crude protein toxin contents to be 281.6 µg/mL fraction one 192.4 µg/mL and fraction ten 52.8 µg/ml. The recorded symptoms of toxic effects were compared well with those reported for other coniids snails *viz*; *C. striatus*, *C. amadis* and *C. mutabilis*[24, 25, 26, 27, 28]. Nayak[29] (2011) estimated crude venom protein of *C. inscriptus* was 330 µg/mL fraction one 210 µg/mL and fraction ten 50 µg/mL. In case of *C. betulinus* crude venom protein 290 µg/mL, F1 containing 180 µg/mL and F10 containing 40 µg/ml. *C. monile* crude venom 420 µg/mL, F1- 270 µg/mL, and F10 containing 70 µg/mL. Kumar et al[30, 31] have showed protein content in the crude toxin was 342.0 µg/ml in *C. inscriptus* and 432.5 µg/mL in *C. lentiginosus* and *C. zeylanicus* 281.6 µg/ml respectively.

4.2 Mouse Bioassay

In the present investigation, instances of toxic effects of various *Coniids* have been well proven and established. The death time varied between 01 hr: 40 min: 15 sec and 02hr: 20min: 10sec in *C. zeylanicus* from the protein at dose of 1.0 and 0.75 mL. Upon envenomation, the common symptoms that were exhibited by the test mice in the present study were palpitation, scratching, violent jumping, dragging of hind limbs and jumping**.** Lewis and Garcia[32] have recorded that a number of Coniids showed toxicity on insects and fishes. Bingham et al[33, 34]. showed that the composition of Coniids venom varies among different species. Nallathambi[26] reported that only the fractions, III and V were able to elicit any symptoms in mice by intracerebral injection (i.c.) with most of the activity concentrated in I and V which produced shaking, convulsions, sleep like activity etc. Purified conotoxin GIV from *C. geographus* by subjecting the venom to a Sephadex G-50 column chromatography followed by DEAE cellulose chromatography, *C. lentigenosus* and *C. mutabilis* venom extracts were partially purified by DEAE-cellulose chromatographic procedure, but out of all the fractions, only fraction 8 in case of *C. mutabilis* showed lethal effects, whereas all other fractions proved as non-lethal, although they elicited symptoms of toxicity in various degrees[28].

4.3 Hemolytic Assay

The crude extract of column fractionated venom of *C. zeylanicus* induced spontaneous hemolysis on chicken blood. The present study observed from the hemolytic unit (HU) recorded for *C. zeylanicus* is four, less than those reported earlier for *C. lentiginosus* and equivalent to *C. mutabilis* by Sakthivel[28]. Hemolytic activity is indicative of cytolytic activity and most cytotoxins have considerable potential as anticancer and antiviral agents. Lattore[35] postulated that the lethality of *C. textile* venom arose not from its neurotoxic properties but from the hemotoxic activities but Kobayashi et al[36] presented evidence to the contrary. Hashimoto[37] reported that the hemolytic factor was not adsorbed on DEAE cellulose from 0.1% NaCl solution. Potent hemolytic activity was discernible in the venom of *C. inscriptus.* Results of the hemolytic property of these venoms were studied confirm to those reported in earlier studies such as those of Nallathambi[26], Ramu[27], and Sakthivel[28].

Shanmuganandam[38] reported that the venom of the vermivorous cone snail *C. figulinus* does not contain any hemolytic properties besides paralytic peptides.

4.4 Nauromodulatory activity

A dose-dependent neuromodulatory response on mouse brain AChE activity was also evinced by the venom of *Conus zeylanicus.* Venom showed an inhibitory effect on AChE activity ranging between 81% and 175% was caused by venom of *C. zeylanicus* at higher concentrations are in conformity with earlier findings of Elancheran[39], who reported elevation of AChE activity to be caused at higher doses of tetrodotoxin, while results from these species are in conformity with the findings of Wankhede[40] who reported crude ovarian extracts of the horseshoe crab to enhance mouse brain AChE activity at lower doses and suppressing the same at higher doses (800 and 1000 µg). Reports on effects of other fish poisons include those of ciguatoxin and jellyfish poison[41]. Kuriaki and Nagano[42] reported that the Acetyl Choline Esterase (AChE) has been the most sensitive enzyme to the puffer poison.

4.5 Immunomodulatory Activity

The present study revealed that the venom *C. zeylanicus* had an immunostimulating effect at lower concentrations but at higher concentrations, they exhibited immunosuppressive effects. lmmunostimulation was found to decrease with the increasing concentration of the venom in all the cases. Al-Hassan *et al*[43] had shown that the wound healing activity exhibited by the epidermal secretion of the Gulf catfish was associated with immunomodulation as well as the prostaglandin pathway. lmmunomodulation by marine toxins is a poorly studied subject[44]. The present results thus open up new vistas for research on the effects of these *Conus* venom on wound healing, tissue regeneration, and related activities.

4.6 Analgesic Activity

The crude proteins of the *C. zeylanicus* were tested, exhibited pronounced analgesic activity. The analgesic ratio decreased with an increase in time. Analgesic activity in terms of tail-flick response observed response time before injection 3.65 sec and after injection 3.88, 3.84 and 3.83 sec after 30, 60 and 90 min was observed respectively. The crude protein of *C. zeylanicus* showed an analgesic ratio (AR) of 0.75 µg/ml dose before injection was 4.67 and after injection was 7.55, 7.66 and 8.35 after 30, 60 and 90 min respectively, when compared with the standard (Paracetamol), *C. zeylanicus* showed the more prominent effect. The present result indicates the potent analgesic activity of the venom studied about almost similar to that of paracetamol. Gouiffes et al[18] reported that no local anesthetic activity or analgesic effect was observed after administration of Bistramide 'A'- toxin. Intra-cisternal injection of the substance at dose of 1.5 mg/kg of the b.w. did not cause death in mice but immobility with loss of muscle tone was rapidly apparent after 5 min of injection. Shanmuganandam[38] demonstrated the effectiveness of *Conus fugulinus* venom on the skin of guinea pig as an infiltration anesthetic agent whereas, Marwick[45] reported *Conus magus* venom had an analgesic effect 1,000 times stronger than morphine. In the present study, the analgesic activity was measured only with the crude venom and therefore, the analgesic activity is likely to increase much folds if the purified fraction or fractions are tested for this activity. The present study exhibited a pronounced analgesic activity. The analgesic ratio decreased with increase in time.

4.7 SDS-PAGE

In the present study SDS-PAGE on 12.0% gel of crude protein of *C. zeylanicus*, yielded 3 prominent bands could be observed at 20-100 kDa ranging from 9 to 150 kDa. In this context, it may be noticed that the SDS-PAGE profiles of CZ1, CZ2 and CZ3 are almost identical. The concentration of protein in the crude extract was found to be 281.6 µg/mL. One lower peptide below 30 kDa have been identified in SDS-PAGE. This finding is in similarity with the results of Shiomi et al[46] that the partially purified echotoxins extracted from *Monoplex echo*, has molecular mass 7 kDa by gel filtration on Sephadex G-75 column. Saravanan et al[6] isolated 14 kDa proteins from the *Conus figulinus*. Periyasamy et al[47] different molecular weight marker proteins were used for *C. inscriptus* 97, 63, 61, 42 kDa and *C. betulinus* 93, 61, 42, 40 kDa band was appeared and was detected on the gel that represented protein of 97-2 kDa. Alam[48] reported that molecular weight calculated for *Conus catus* 13.50 kDa by gel filtration marker on Sephadex G-50 column. Nearly equal to the molecular weight of conotoxin GIV (13.0 kDa) from *C. geographus* calculated using SDS-PAGE[24]. Two factors, one lethal to fish and other to mice

have been purified from *Conus striatus* having a molecular weight between 10.0 kDa to 14.0 kDa and 10.0 kDa respectively[49, 50]. From the present investigation on the venoms of this *C. lentigenosus*, it can be concluded that the venoms of these *C. lentigenosus* are having higher potential to be evaluated as compounds with clinical significance, as these venoms are thermostable up to 70 ^{0}C, viable even after long storage up to 18 months, beyond which there was reduction in the potency. The present results are in accordance with those of Kobayashi *et al*[36, 51, 52] who reported that the venom of *C. geographus*, *C. textile* and *C. imperialis* were stable up to 100°C when heated for 15 min. The venom of *C. amadis* was stable upto 60°C[27], and that of *C. betulinus* upto 63 °C[26]. At higher temperatures, the venom lost its activity. Preliminary investigations on *C. mutabilis* and *C. lentiginosus* revealed a potent analgesic activity to be exhibited by their venoms[28]. Similar instances of stability have been encountered by other researchers.

5. Conclusion

The study exhibited the effects of crude venom extract on column chromatography, SDS PAGE, characterization of the protein responsible for the bioactivity. Further, the purification and structural elucidation of compounds are required to confirm the designation of venoms in the proposed groups. This will greatly help utilize these compounds for the prosperity and well-being of humankind. There is an urgent need for further purification of conotoxins, using ultra-modern techniques such as 2D-gel electrophoresis HPLC, FPLC, which may throw light on the individual characterization of peptides for any further medical use for human being. The study suggests that technology may lead to the development of wonderful new peptide drug in future from *Conus* species. Thus, the results of the present study indicate a very strong hemolytic activity of *C. zeylanicus*. The study strongly suggests that these conotoxins could be utilized as a probing tool to investigate the pharmacological potential. These characteristics emphasize the need for isolation and molecular characterization of new active toxins in *C. zeylanicus* in the near future.

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TABLE I. Protein content of crude and partially purified fractions of *C. lentigenosus*

Samples	Protein (µg/ml)
Crude	281.6 ±1.36
F1	192.4 ± 0.68
F2	178.6 ± 0.61
F3	166.3 ± 0.58
F4	159.3 ± 1.66
F5	152.3 ± 0.68
F6	135.7 ± 0.38
F7	121.6 ± 0.48
F8	99.8 ± 0.88
F9	72.3 ± 0.34
F10	52.8 ± 0.88

TABLE II. Toxicity of crude extracts of the *C. lzeylanicus* injected intraperitoneally in male albino mice 20 ± 2 g with different doses

Injected volume (ml)	Amount of protein (µg/mL)	Death time (H:Min:Sec)	Symptoms of toxicity	Remarks
0.25	52.8	-	-	Non - lethal
0.50	150.0	00:00:00	Palpitation and Scratching	Non - lethal
0.75	220.0	02:20:10	Violent jumping and Dragging of hind limbs	**Lethal**
1.0	281.6.5	01:40:15	Convulsions and Jumping	**Lethal**

TABLE III. *In vitro* effect of the venom of *C. zeylanicus* on the mouse brain AChE activity

Sample (µg)	µ moles of Ach hydrolysed/ mg protein/hr	Activity (%)
Control	0.08868	-----
100	0.15605	175
200	0.14888	165
400	0.12275	138
800	0.11125	125
1000	0.07145	81

TABLE IV. Phagocytosis of *Canadida albicans* by Polymorphonuclear cells (PMN) when treated with venom

Concentration (µg/mL)	Phagocytic Index	Level of modulation (%)
100	2.321	10.25
200	2.245	7.83
400	2.240	7.45
800	2.000	-4.10
1000	1.852	-6.18

TABLE V: Analgesic activity by tail-flick response time (min) in terms of analgesic ratio (AR) in mice injected with the crude venom *C. zeylanicus*.

Drugs	Dose (mg/ml)	Before injection (min)	After administration of drug			% increase in reaction time after 90 min
			30 min	60 min	90 min	
Control	0.2 ml	3.65±0.34	3.88±0.38	3.84±0.43	3.83±0.25	4.93
Paracetamol	5	3.69±0.36	6.63±0.33	7.27±0.26	7.46±0.22	120.0
Extract	0.25	4.26±0.42	6.83±0.51	7.28±0.36	7.82±0.37	104.17
Extract	0.50	4.46±0.26	7.23±0.24	7.46±0.35	8.01±0.26	109.13
Extract	0.75	4.67±0.36	7.55±0.23	7.66±0.31	8.35±0.26	118.01

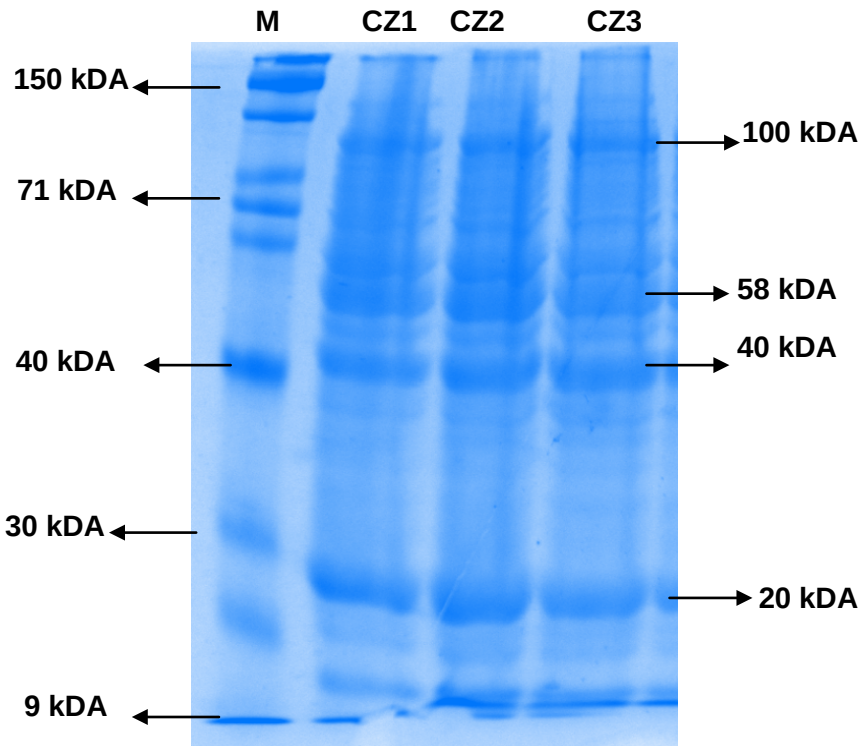

Fig.1: SDS-PAGE analysis of crude protein extracts of the *C. zeylanicus*. Lane M: protein molecular weight marker

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