International Journal of Fisheries and Aquaculture Vol. 4(4), pp. 71-76, 9 March, 2012
Available online at http://www.academicjournals.org/IJFA
DOI: 10.5897/IJFA11.019
ISSN 2006-9839 ©2012 Academic Journals

Full Length Research Paper

Bioactivity of venom extracted from the sea anemone *Anthopleura asiatica* (Cnidaria: Anthozoa): Toxicity and Histopathological studies

Ramkumar S.[1]*, Arun Sudhagar S.[2] and Venkateshvaran K.[1]

[1]Fisheries Resources, Harvest and Post Harvest Division, Central Institute of Fisheries Education, Mumbai, India.
[2]Aquatic Environment and Health Management Division, Central Institute of Fisheries Education, Mumbai, India.

Accepted 21 October, 2011

The bioactivity of the venom from a locally available sea anemone, *Anthopleura asiatica* collected from the Mumbai coast was studied. The crude venom from sixty sea anemones (Sixty numbers) was extracted in aqueous medium. The protein content of the crude venom was 4.3459±0.027 mg/ml. The crude venom was found to be lethal at 1ml when injected intra-peritoneal to kasuali strain male albino mice (20±2 g). The crude venom was partially purified by anion exchange chromatography using a step-wise gradient of 0.1-1.0M NaCl and 10 fractions each of 15 ml, F1 – F10 were collected. Fractions F8, F9 and F10 exhibited lethality to mice, upon envenomation. The symptoms of toxicity observed in the mice indicated that the venom affected the central nervous, cardiovascular and urinary systems. Histopathological study revealed accumulation of polymorphic nuclear cells with necrosis in brain, hemolysis in heart, occlusion with hemolysed blood in kidney and necrosis, vacuolation with pleomorphic nuclear material and hemolysis in liver.

Key words: Sea anemone, venom, toxicity, histopathology.

INTRODUCTION

The sea anemones which are exclusively marine anthozoan invertebrates possess specialized stinging cells, the nematocysts in their body surface (Alsen et al., 1982; Bosmans and Tytgat., 2007). These cells contain venom which is used to paralyze the prey, to defense against predators and to fight territorial disputes (Lotan et al., 1996; Yanagihara et al., 2002). This venom contains variety of active compounds, including potential toxins affecting voltage-gated Na^+ and K^+ channels, acid-sensing ion channels, pore-forming toxins (actinoporins) and protease inhibitors (Belmonte et al., 1994; Beress et al., 1975; Diochot et al., 2003). Of 9000 species of cnidarins, some 70 species have been reported to be toxic to man (Picken and Skaer., 1996). A few species of sea anemones possess highly toxic venoms hazardous for humans, among which *Phyllodiscus semoni* venom caused unexplained acute renal failure in the victim (Mizuno et al., 2000) and in most of the cases venom causes local inflammations, pain and in few cases edema. Through ages studies have proved that sea anemone toxins have hemolytic activity, cardiac toxicity, neurotoxicity and nephrotoxicity (Bunc et al., 1999; Wang et al., 2004; Kawai et al., 2004; Mizuno et al., 2007).The sea anemone from Indian waters have been poorly studied especially with regard to their toxicity. The present study was therefore undertaken to gain a better understanding of the toxicity of the venom extracted from the intertidal sea anemone *Anthopleura asiatica* collected from the coast of Mumbai.

Corresponding author. E-mail: ramfrm@gmail.com. Tel: +91 9167134881.

Abbreviations: PBS, Phosphate buffer saline; **BSA,** bovine serum albumin.

MATERIALS AND METHODS

Sample collection and storage

The sea anemone *A. asiatica* (Uchida, 1958) was collected from the

Table 1. Protein content of crude venom and partially purified fractions of *Anthopleura asiatica* (values are mean ± SE of triplicate sets).

S/N	Sample	Protein (mg/ml)
1.	Crude venom	4.3459±0.027
2.	F1	0.6106±0.004
3.	F2	0.5531±0.024
4.	F3	0.8291±0.004
5.	F4	0.6220±0.003
6.	F5	0.3222±0.005
7.	F6	0.5886±0.002
8.	F7	0.3626±0.004
9.	F8	1.2388±0.058
10.	F9	0.9303±0.036
11.	F10	0.8573±0.006

Khardhanda Beach (Mumbai) during low tide (0.52 m). They were transported live to the laboratory and stored immediately at -20°C for further analysis.

Preparation of crude venom

Sixty anemones weighing 25 g each were homogenized in three volumes of distilled water. The homogenate was filtered and centrifuged at 10,000 rpm at 4°C for 15 min. The supernatant collected was dialyzed against D-glucose. The dialyzed solution was further centrifuged at 16,000 rpm for 20 min at 4°C. The supernatant was collected and stored at -20°C for further use.

Partial purification of crude extract

Partial purification of the crude extract was carried out using DEAE cellulose Anion Exchange Chromatography (Shiomi et al., 1987). 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.

Protein estimation

The protein content of Crude venom extract and partially purified fractions were obtained by Lowery's method (Lowery et al., 1951) using bovine serum albumin (BSA) as a standard.

Mice bioassay for lethality

Clinically healthy Kausauli strain male albino mice of mean weight 20±2 g procured from M/s. Haffkine Biopharma, Mumbai, were maintained in healthy condition in the laboratory, following the codal formalities of the Institute's Ethical Committee. Mice in triplicate sets were challenged intraperitoneally with 0.25, 0.50, 0.75 and 1.0 ml of the crude venom. In the case of partially purified fractions, 1ml of the each fraction was injected. A control was maintained in each case by injecting an equal volume of PBS (pH 7.4). The time of injection and lethality, and behavioral changes were also recorded. Autopsy of the dead mice was performed to study the anatomical and histopathological changes.

Histopathology

Brain, heart, liver and kidney were dissected out from the mice that died upon envenomation. The dissected organs were fixed in 10% formalin for a minimum period of 24 h and further processed according to Lefkowitch (1987). Prepared sections were examined and photographed under a microscope (LAMBOMED, CX II).

RESULTS AND DISSCUSSION

In the present study, toxic proteins have been isolated from the body of *A. asiatica* using distilled water as the extraction medium (Sokotun et al., 2007). The protein content of the crude venom of *A. asiatica* was found to be 4.3459±0.027 mg/ml which is higher than the previous work in anemones from Indian waters (Ravindran et al., 2010). The results are presented in the Table 1.

Lethality was observed in mice injected intraperitoneally with crude venome, F8, F9 and F10, similar to proteins isolated from the sea anemone *Actineria villosa* (Oshiro et al., 2004). This reveals the instance of toxicity in sea anemone. The dose and time at which the crude and fractionated proteins were toxic has been tabulated (Table 2). The potency of the presently extracted toxins is comparable with other toxins extracted from various other tropical and temperate sea anemones (Bruhn et al., 2001; Honma, et al., 2003).

Immediately upon being injected with the venom, the mice exhibited the following symptoms: palpitation, restlessness, exploring around the cage, standing on hind legs, flexing of the muscles, stretching of body, wiping the mouth with forelimbs, excess defecation and micturition, and hairs were found to stand nape. As time advanced and the degree of illness worsened, the symptoms observed were tonic convulsions, paralysis of forelimbs, opaqueness of eyes, dragging of hind limbs, violent jumping, lying on back, gasping for the breath and foaming from the mouth. Finally there was bleeding from the mouth, violent convulsions and then death. This may be due to the action of anemone toxin on the organ systems. Similar observations have also been reported by Gondran et al. (2002). However, the mice envenomated with other fractions did not show any of the above symptoms and found to be normal.

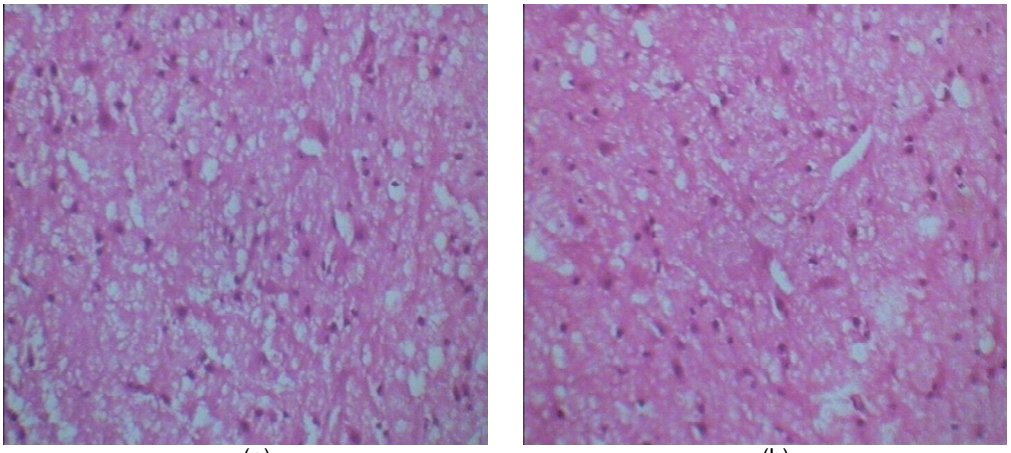
Histopathological observations revealed that, the toxic extract had similar effects on the organs of mice viz, brain, heart, kidney and liver irrespective of the nature of its purity.

Brain: Hemolysis in the cerebral vessels was not common. Accumulation of polymorphic nucleus cells with necrotic changes was common to observe. Area of sponges was discernable at certain regions (Figure 1). This can be attributed to the fact that the sea anemones have been reported to possess neurotoxin of polypeptide nature (Bruhn et al., 2001; Zaharenko et al., 2008).

Heart: Ventricle was totally blocked with hemolysed blood. Blood vessels were also enlarged with hemolysed

Table 2. Toxicity of crude venom and partially purified extracts of *Anthopleura asiatica* injected intraperitoneal to male albino mice of 20 ± 2 g weight (values are mean ± SE of triplicate sets).

S/N	Sample	Dose (ml)	Death time (Min)	Remark
1	Crude Venom	0.25	-	Non lethal
		0.50	-	Non lethal
		0.75	-	Non lethal
		1.0	645±17	Lethal
2	F1	1.0	-	Non lethal
3	F2	1.0	-	Non lethal
4	F3	1.0	-	Non lethal
5	F4	1.0	-	Non lethal
6	F5	1.0	-	Non lethal
7	F6	1.0	-	Non lethal
8	F7	1.0	-	Non lethal
9	F8	1.0	725±22	Lethal
10	F9	1.0	685±12	Lethal
11	F10	1.0	684±19	Lethal
12	FU	1.0	-	Non lethal

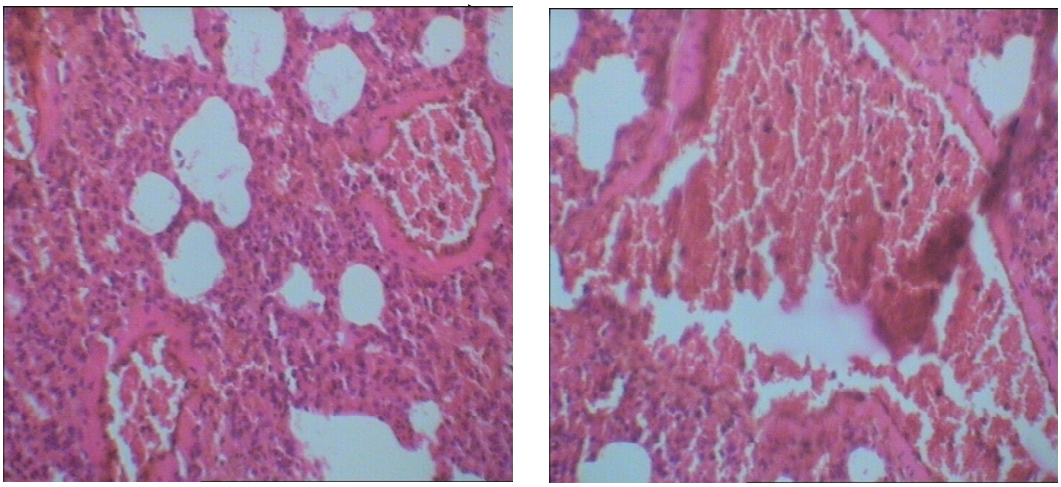


(a) (b)

Figure 1. Effect of toxins from Sea Anemone, *A. Asiaticaon;* the brain of male albino mice, arrows indicating a) spongiosis in brain b) necrotic cells with polymorphic nuclei (H&E, ×400).

blood (Figure 2). It implies acute cardiotoxicity of the sea anemone toxins. Instances of the cardiotoxicity by anemone toxins have been reported earlier from *Anthopleura sp* (Ouyang et al., 2005) and *Anthopleura elegantissima* (Bruhn et al., 2001).

Kidney: The changes observed in kidney included, tubules filled with homogenous mucus like substance; larger blood vessels showed occlusion with hemolysed blood; no changes were observed in glomeruli (Figure 3). According to the report of Luciano et al. (2004), venom toxins were localized in the damaged kidney tissues, this might be due to glomerular filtration which leads to high degree of localization of the venom in the kidneys. The kidneys may also be a secondary site of damage due to hemolysis in heart. The hemoglobin thus released reaches the tubular filtrate of the kidney. If the amount of hemoglobin in blood is excess to the reabsorbing capacity of the proximal tubules, it would lead to precipitation of unabsorbed hemoglobin. This would further block the tubules, eventually causing renal damage (Ravindran et al., 2010; Chen et al., 1976). Recent study in *P. semoni* revealed that, envenomation caused acute renal damage in rats as a result of



(a) (b)

Figure 2. Effect of toxins from sea anemone, *A. asiatica* on the heart of male albino mice, arrows indicating a) enlarged blood vessels (H&E, X 100) b) hemolysed blood in ventricle (H&E, ×400).

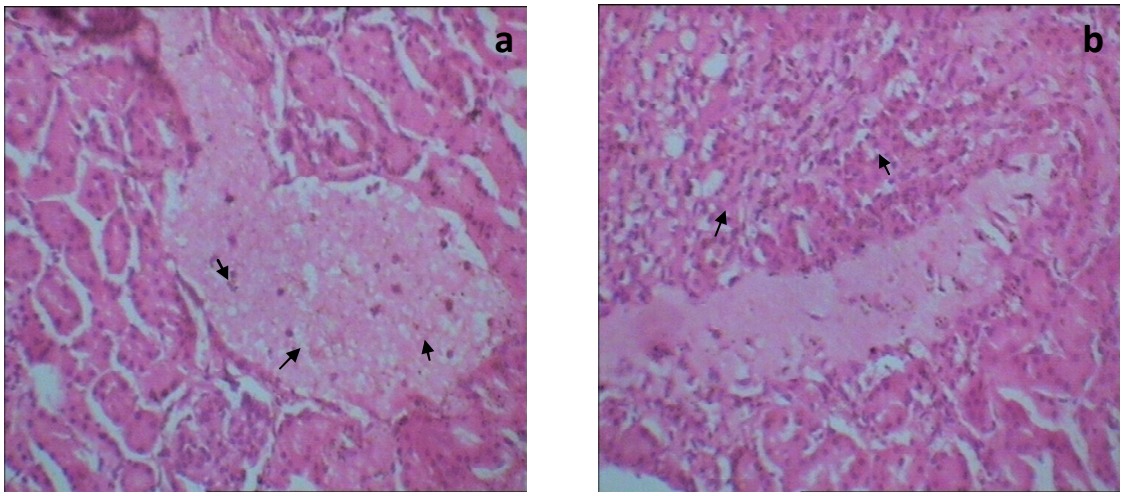


Figure 3. Effect of toxins from sea anemone, *A. asiatica*on the kidney of male albino mice, arrows indicating a) homogenous mucus like substance (H&E, ×400) b) occlusions with hemolysed blood (H&E, ×100).

induction of complement activation (Mizuno et al., 2007).

Liver: In the present study distinctive changes were noticed in the liver. They were occlusion with hemolysed blood in the central vein; engorgement of blood vessels with hemolysed blood; necrosis with fragmented nuclei in the hepatocytes around the central veins; vacuolation with pleomorphic nuclear material in liver tissue. In few cases nuclear chromatin was markedly dissolved to show heterochromatisation (Figure 4). These changes might have been due to detoxification of the toxin in liver. As no recent studies are available in sea anemone in this context, some representative groups from marine invertebrates are used to discuss the findings of the present study. Plancitoxin obtained from *Acanthaster planci* (Crown-of-thorns, an echinoderm) induced apoptosis in hepatocytes of envenomated mice (Ota et al., 2006).

Conclusion

This study demonstrates that the sea anemone, *A. asiatica* produces one or more toxins that may have direct effects on cardiac system, nervous system, hepatic system and excretory system in mammalian model. Further studies could explore and characterize the active agents responsible for toxicity, in order to reveal the bio-pharmacological properties of the toxin.

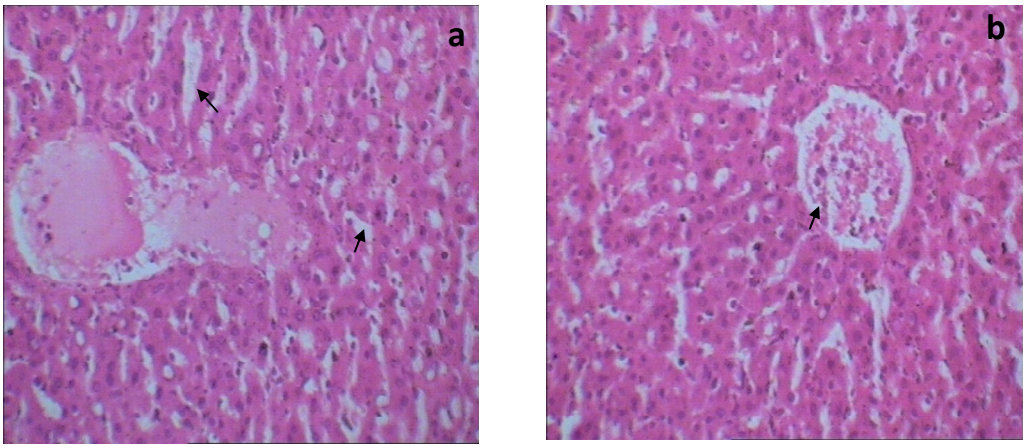


Figure 4. Effect of toxins from sea anemone, *A. asiatica* on the liver of male albino mice, arrows indicating a) vacuolation with pleomorphic nuclear material in hepatocytes b) hemolysed blood in the central vein (H&E, ×400).

ACKNOWLEDGEMENTS

This paper is a tribute to the co-author (Late) Dr K. Venkateshvaran, Principal Scientist, CIFE, Mumbai. The authors thank Central Institute of Fisheries Education, Mumbai for facilities provided. The authors also thank Kanagarajan and Baskara Doss for their technical assistance.

REFERENCES

Alsen C, Peters T, Scheufler E (1982). Studies on the mechanism of the positive inotropic effect of ATX II (*Anemonia sulcata*) on isolated guinea pig atria. J. Cardiovasc. Pharmacol., 4: 63–69.

Belmonte G, Menestrina G, Pederzolli C, Krizaj I, Gubensek F, Turk T, Macek P (1994). Primary and secondary structure of a pore-forming toxin from the sea anemone, *Actina equina*, and its association with lipid vesicles. Biochimica et Biophysica Acta., 1192: 197-204.

Beress L, Beress R, Wunderer G (1975). Purification of three polypeptides with neuro and cardiotoxic activity from the sea anemone *Anemonia sulcata*. Toxiconomy, 13: 359.

Bosmans F, Tytgatb J (2007). Sea anemone venom as a source of insecticidal peptides acting on voltage-gated Na^+ channels. Toxiconomy, 49: 550–560.

Bruhn T, Schaller C, Schulze C, Sanchez-Rodriguez J, Dannmeier C, Ravens U, Heubach JF, Eckhardt K, Schmidtmayer J, Schmidt HI (2001). Isolation and characterisation of five neurotoxic and cardiotoxic polypeptides from the sea anemone *Anthopleura elegantissima*. Toxiconomy, 39: 693-702.

Bunc M, Drevensek G, Budihna M, Suput D (1999). Effects of equinatoxin II from *Actinia equina* (L.) on isolated rat heart: The role of direct cardiotoxic effects in equinatoxin II lethality. Toxiconomy, 37: 109–123.

Chen WY, Yen TS, Chen JT, Hsieh BS, Hsu HC (1976). Acute renal failure due to ingestion of raw bile of grass carp (*Ctenopharyngodon idellus*). J. Formosan Med. Assoc., 75: 149–157.

Diochot S, Loret E, Bruhn T, Beress L, Lazdunski, M (2003). APETx1, a new toxin from the sea anemone *Anthopleura elegantissima*, blocks voltage-gated human ether-a-go-go-related gene potassium channels. Mol. Pharmacol., 64: 59–69.

Gondran M, Eckeli AL, Miges PV, Gabilan NH, Rodrigues ALS (2002). The crude extract from the sea anemone, *Bundosoma caissarum* elicits convulsions in mice: Possible involvement of the glutamatergic systems. Toxiconomy, 40: 1667.

Honma T, Iso T, Ishida M, Nagashima Y, Shiomi K (2003). Occurrence of type III sodium channel peptide toxins in two species of sea anemones. Toxiconomy, 41: 637.

Kawai N, Konno K (2004). Molecular determinants of two neurotoxins that regulate sodium current inactivation in rat hippocampal neurons. Neurosci. Lett., 361: 44–46.

Lefkowitch HJ (1987). Histopathology of disease. Churchill Living Stone publisher, New York, p. 233.

Lotan A, Fishman L, Zlotkin E (1996). Toxin compartmentation and delivery in the cnidaria: The nematocyst's tubule as a multiheaded poisonous arrow. J. Exp. Zool., 275: 444–451.

AL, Randall RJ (1951). Protein measurement with folin phenol reagent. J. Biol. Chem., 193: 265.

Luciano MN, da Silva PH, Chaim OM, dos Santos VL, Franco CR, Soares MF (2004). Experimental evidence for a direct cytotoxicity of *Loxosceles intermedia* (brown spider) venom in renal tissue. J. Histochem. Cytochem., *52*: 455–467.

Mizuno M, Nishikawa K, Yuzawa Y, Kanie T, Mori H, Araki Y, Hotta N, Matsuo S (2000). A case report of acute renal failure following a sting presumably by a sea anemone. Am. J. Kidney Dis., 36: 10.

Mizuno M, Nozaki M, Morine N, Suzuki N, Nishikawa BK, Morgan P, Matsuo S (2007). A Protein Toxin from the Sea Anemone *Phyllodiscus semoni* Targets the Kidney and Causes a Severe Renal Injury with Predominant Glomerular Endothelial Damage. Am. J. Pathol., 171(2): 402-414.

Oshiro N, Kobayashi C**,** Iwanaga S, Nozaki M, Namikoshi M, Spring J, Nagai J(2004). A new membrane-attack complex/perforin (MACPF) domain lethal next term toxin from the nematocyst venom of the Okinawan previous term sea anemone next term *Actineria villosa*. Toxiconomy, 43(2): 225-228.

Ota E, Nagashima Y, Shiomi K (2006). Caspase-independent apotosis induced in rat liver cells by plancitoxin I, the major lethal factor from the crown-of-thorns starfish *Acathaster planci* venom. Toxiconomy, 48: 1002–1010.

Ouyang P, Xie JG, Liu J, Xiao WX, Liu YB, Wang L, Liang D, Wang YH, Xu DL, Liu YL, Xu AL (2005). Effects of a novel recombinant polypeptide from the sea anemone *Anthopleura* sp. on left ventricular function in dogs with acute cardiac insufficiency. Di Yi Jun Yi Da Xue Xue Bao., 25(1): 37–39.

Picken LER, Skaer RJ (1996). A review of researches on nematocysts. In: W.J. Rees (ed.), The cnidaria and Their Evolution. Symp. Zool. Soc. London, Acad., New York, 16: 19-50.

Ravindran VS, Kannan L, Venkateshvaran K (2010). Biological activity of sea anemone proteins: I. Toxicity and Histopathology. Indian J. Exp. Biol., 47: 1225-1232.

Shiomi K, Takamiya M, Yamanaka H, kikuchi T (1987). Purification of a lethal factor in the skin secretion from the oriental catfish *Plotosus*

lineatus. Nippon suisan gakkaishi, 53: 1275.
Sokotun IN, Il'ina AP, Monastyrnaya MM, Leychenko EV, Es'kov AA, Anastuk SD, Kozlovskaya EP (2007). Proteinase Inhibitors from the Tropical Sea Anemone *Radianthus macrodactylus*: Isolation and Characteristic. Biokhimiya, 72(3): 368-374.
Wang L, Ou J, Peng L, Zhong X, Du J, Liu Y (2004). Functional expression and characterization of four novel neurotoxins from sea anemone *Anthopleura sp*. Biochem. Biophys. Res. Commun., 313: 163–170.
Yanagihara AA, Kuroiwa JMY, Oliver LM, Chung JJ, Kunkel DD (2002). Ultrastructure of a novel eurytele nematocyst of *Carybdea alata* Reynolds (Cubozoa Cnidaria). Cell Tiss. Res., 308: 307–318.
Zaharenko AJ, Ferreira WA, Oliveira JS, Richardson M, Pimenta DC, Konno K, Portaro FCV, de Freitas JC (2008). Proteomics of the neurotoxic fraction from the sea anemone *Bunodosoma cangicum* venom: Novel peptides belonging to new classes of toxins. Comp. Biochem. Physiol. D., 3(3): 219-225.