DIVERSITY OF CONUS SPECIES AND IMPORTANCE OF CONOTOXINS

1Ravi Gugulothu, 2Balaji Guguloth 3Srinu Rathlavath & 4Suresh Kummari
1,3,4Teaching Faculty, College of Fisheries Science, Pebbair, Wanaparthy, Telangana - 509104
2Fisheries Scientist, Krishi Vigyan Kendra, Mamnoor, Warangal Urban
3P. V. Narsimha Rao Telangana Veterinary University, Telangana
Corresponded Mail: gbalu002@gmail.com

ABSTRACT
The genus Conus belongs to the group Conidae which is one of the largest groups of gastropods. These species are widely distributed all over the world but scantily reported. Species specific identification is mainly based upon the radular structure. Predacious gastropod cone shells have a diverse mechanism of prey capture and divided into three major feeding types namely; vermivorous, piscivorous and molluscivorous. Piscivorous species are very dangerous, can kill and swallow the prey of similar size. Toxins from this genus are referred as conotoxins. There are 5 (α-conotoxins, δ-conotoxins, κ-conotoxins, μ-conotoxins and ω-conotoxins) different conotoxins based upon the binding site. Conotoxins have a valuable probe in physiological and pharmacological studies. The studies on the conotoxin is still at infancy stage, so far only 100 out of a potentially identified 50,000 toxins have been extracted and analyzed from Conus. A single species of Conus can yield 100-200 peptides with potential therapeutic uses but attention on this conotoxin research has to be initiated. Since India is blessed with hundreds of Conus species, the potential bioactive property of these toxins has to be established for their effective utilization.

KEYWORDS: Conus, Molluscan diversity, radular structure, conotoxin, bioactive properties.

INTRODUCTION
Molluscs are highly diversified group differ in size, shape, as well as in habit and habitats. The history of Malacological study is immense and interesting in India. Studies on Indian molluscs were initiated by the Asiatic Society of Bengal (1784). Benson (1830) was the first to publish a systematic paper on Mollusca. During the recent years, the contribution from Zoological Survey of India, Central Marine Fisheries Research Institute and other several maritime universities enhanced the knowledge of the mollusc and fauna of India. Nearly 5,070 species have been recorded in India till today, of which 3,370 species have been reported to be from marine habitats (Subba Rao, 1991), belonging to 220 families and 591 genera. Bivalves are considered to be the most diverse (1100 species) group followed by cephalopods (210 species), gastropods (190 species), polyplacophore (41 species) and scaphopods (20 species). India has extensive molluscan resources along the coast. These resources have been exploited since time immemorial in the numerous bays, brackish waters, estuaries and seas around the sub-continent for the food, pearls and shells. Rich molluscan diversity has been described from Andaman & Nicobar Islands, which includes over 1000 species from the marine region (Subba Rao, 1991), while Gulf of Mannar and Lakshadweep were found to be represented with 428 and 424 species respectively (Venkatraman et al., 2004). Gastropods occupy significant place in the commercial shell-craft manufacturing industry. The marine gastropod resources in India comprise a variety of species and are exploited regularly for various purposes (Ramadoss, 2003). Predacious gastropod cone shells have a diverse mechanism of prey capture and divided into three major feeding types namely; vermivorous, piscivorous and molluscivorous (Kohn, 1978). Among the various feeding types, piscivorous species are very dangerous. They can kill and swallow the prey of similar size (Kohn, 1956). When in contact with the prey, few Conus spp. use single radular teeth for injection of the venom and others use more than one radular teeth. The venom of each species of Conus has estimated to comprise between 100-200 peptide components (Myers et al., 1993). Olivera and Teichert (2007) indicated that the conotoxins have proved to be a valuable probe in physiological and pharmacological studies of ion channels and other receptors. So far only 100 out of a potential 50,000 toxins have been extracted and analyzed from Conus. Since a species of Conus can yield 100-200 peptides with potential therapeutic uses, it is hoped that discovery of new species of cone snails would add to the knowledge of extant species as well as to the chemical diversity of pharmacologically active peptides. A unique feature of conotoxins is due to their high degree of post-translational modification which is up to 75% of the amino acids in a single conotoxin found to be modified (Jimenez et al., 1997 and Olivera, 1997). McIntosh et al., (2001) have reported the presence of serotonin, a smooth muscle relaxation compound in the venom of the Cone snail, C. imperialis. Studies on conotoxin are very much limited in India, especially in the south east coast of India. The present review discusses about the diversity of the Conus species in India and World with main emphasis on the toxin types and their importance.

Taxonomy and diversity of Conus species
Conidae is one of the largest groups of gastropods, belongs to the super-family Toxoglossa, which are
characterized by possession of venom apparatus. This group comprises about 500 species in the family Conidae, most of them are members of the single genus Conus, with a few exceptions. Nearly, all taxonomists agree that large and worldwide extensive dispersal of this genus should be split into smaller groups, but scheme has been generally accepted for dividing the several hundred Conus species into compact generic groups (Olivera et al., 1990). Cone snails are the inhabitants of shallow water and are found in variety of microhabitats (Kohn, 1978). These species are known to attach to the alga of coral reefs, crawl under the coral heads, and prefer sandy or coral rubble substrate (Halstead, 1965).

Conus species are highly diversified group of organisms documented by different authors from different localities. Kohn (2001) revealed the geographical variation of species diversity of Conus species along the North-East coast of Papua New Guinea. Kohn (1978) reported 70 Conus species from Sri Lanka and 64 species whereas in Maldives and Chagos. Richmond (1999) documented 198 species of Conidae from Western Indian Ocean. The Conidae documented from the Philippines was 287 species (Poppe, 2008) while in Vietnamese waters, 122 species have been recognized (Nguyen, 2005). Few studies indicated limited number of Conus species in the Krusadai Island (Satyamurti, 1952). Rockel et al. (1995) gave a detailed note on world living Conus and documented 316 valid species along with several subspecies and forms from the tropical Indo-Pacific region. In recent years, their taxonomic identification was felt to be very important due to the use of Conus venom in neurological research and drug discovery (Kohn et al., 1999).

SubbaRao (1991) reviewed the Conids of Andaman and Nicobar Islands, recorded about 45 Conus species. Conus are common in Gulf of Mannar, Andaman and Nicobar Islands and Gulf of Kutch and Lakshadweep where coral rocks are found. The study on Indian Conus species started way back 1860's. The taxonomy and distribution of Indian Conidae was studied by Winckworth (1949). A total of 14 Conus species was reported from Krasadai Island of Gulf of Mannar (Satyamurti, 1952). Among the various Conus species, C. milneedwadsi is the only species protected in India (WPA, 1972). A recent study on Conus diversity of Tamil Nadu indicated the existence of 60 species (Franklin et al., 2009), indicated that the richness of Conus in Gulf of Mannar is far better than other parts of Tamil Nadu.

**Structure of poisonous gland**

Toxoglossan gastropods are among the most highly populous groups of marine invertebrates, due to use of their venom while capturing their prey. These include cone snails (Conidae), auger snails (Terebridae), and augur snails (Turridae) (Taylor et al., 1993). They are most diverse and rich group of predatory snails in number of species of Toxoglossate molluscs (Kohn, 1998). In most of the molluscan taxonomy, cone snails are generally brought under the single large genus Conus. While other groups such as augers are assigned by the most taxonomists to several different genera like Terebra, Hastula, Duplicaria in the family Terebridae (Bouchet and Rocroi, 2005). A venom apparatus made up of a muscular venom bulb and a tubular venom gland generally characterizes these marine molluscs.

In general, the anatomical studies of venom apparatus are limited (Hinegardner, 1958, Halstead, 1988). There are only few reports on the venom apparatus like the anatomical structure of venom apparatus of C. imperialis.
Venom obstruct the potassium channel and have 

- conotoxins such as conotoxin SVIA from 

et al., revealed radular 

Toxins are injected into target animals 

Conus 

conotoxins and nicotinic 

2229 C. geographus C. strength of contraction of the 

C. magus 

conotoxin 

To avoid these imprecision in species identification, reliable non-shell characters are needed. The molluscan radular teeth are often unique to species and genus corresponding to shape, structure and the most widely 

Research mainly concentrate on purifying and 

characterizing the toxins from the venom gland of Conus and demonstrating its multiple toxin property, mostly small peptides that targets specific receptors or channels (Craig, 2000, Terlau and Olivera, 2004 and Olivera, 1997).

Types of conotoxins

Conotoxins of Conus species contain tremendously diverse, natural pharmacology compounds consisting of about 10 to 30 amino acid residues with one or more disulfide bonds (Joseph et al., 2010). Conotoxin family comprises around thousands different peptides, most have a corresponding ion channel family target such as ω-conotoxins and Ca channels, α-conotoxins and nicotinic receptors and different conotoxins have different binding sites on the same ion channel target i.e., μ-conotoxin and δ-conotoxin to sites (Myers et al., 1993 and Olivera, 2002). Only a small fraction of the entire conopeptide diversity has been analysed and reviewed (Rojas, et al., 2008). Currently, conotoxins are a valuable tool of scientific research, due to the intense pharmacological activity presented by the peptides. Five conotoxin activities have been determined so far like α- (alpha) conotoxins, δ- (delta) conotoxins, κ- (kappa) conotoxins, μ- (mu) conotoxins and ω- (omega) conotoxins

α-conotoxins

α-conotoxins completely blocks the post synaptic acetylcholine receptor of vertebrate skeletal muscle, which results in paralysis and death (Grayet al., 1981). It contains two cysteine bonds and thirteen to fifteen residues. Majority of the α-conotoxins were extracted from the fish eating species (Joseph et al., 2010). These are reported from C. striatus (Jones and McIntosh, 2001). It has been observed that alterations in sodium channel gating produced by the venom of the marine snail C. striatus (Hahn et al., 1991). The action was studied using the voltage-clamp technique on medullated nerve and produced repetitive firing of action potential when the Ranvier node was depolarized under current clamp conditions.

δ-conotoxins

It is relatively less toxic towards vertebrates. The delta conotoxins such as conotoxin SVIA from C. striatus bind with high affinity to voltage independent sodium channels. The venom of C. purpurascens contains a delta conotoxin (PVIA) termed the 'lock jaw' peptide, which specifically targets the voltage sensitive sodium channels.

κ-conotoxins

These toxins can obstruct the potassium channel and have a knotting or inhibitor cysteine knot scaffold. The knotting scaffold is a very special disulfide through disulfide knot, peptide toxins. The small size, multiple disulfide bonding and ingenious variations make conotoxins to have tremendous natural pharmacological value.

μ-conotoxins

It blocks muscle voltage sensitive sodium channel with only minimal binding to neuronal sodium channel. This is unlike tetrodotoxin and saxitoxin which bind to both muscle and nerve sodium channels with equal affinity and independent of voltage. These are conotoxin GIII isoforms from C. geographus venom (Jones and McIntosh, 2001). These toxins are similar to tetrodotoxin in the selective blocking of skeletal muscle sodium channels, as well as competitively inhibiting saxitoxin binding to receptor site 1 of voltage sensitive sodium channels.
**ω-conotoxins**

These toxins are no less restrained in activity or diverse in sequence than the other classes of conotoxins. The effect of ω conotoxin M VII A is 100 to 1000 times that of morphine. Therefore a synthetic version of ω conotoxin M VII A has found application as an analgesic drug Ziconotide (Prialt).

**Conotoxin Research in India**

Oceans are the potential source of bio-medically important substances, while considering the pharmacologically active compounds from marine resources in relation to drugs and medical agents, Conus toxins (conotoxin) have a great attention because of their thousands of different pharmacologically active peptides (Olivera et al., 1997). The conotoxins are a group of neuroactive polypeptides found in the venom of all marine gastropod snail of the genus Conus. These potent bioactive products play an important role in the explosive growth of biomedical science and its highly active neurotoxin substances act as molecular probes in neurophysiological research and offer fascinating possibilities in elucidating the nature of both nervous and muscular functions. It modulates the activity of ion channels like potassium, sodium and others (Terlau and Olivera, 2004). Gowd et al. (2005) indicated that the works of Baldomero Oliver on Preliminary studies on conotoxin from Indian waters were initiated as early as 1960 (Kohn et al., 1959). Limited studies on the toxic property of C. amadis and C. Aulicus revealed that it is necessary to strengthen the studies on effect of conotoxin on invertebrates and vertebrates (Kasinathan et al., 1989a, b). Pharmaceutical property was partially studied in C. amadis (Ramu, 1994) and in C. lorisquisi (Saminathan, 1997). The identification and characterization of peptides from Conus snails were taken by Gowd et al. (2005) in Indian region. Sarma et al. (2005) elucidated the structure of conotoxin C. amadis Joseph et al. (2010) reviewed the potential of conotoxins for relieving the pain. Several Conus peptides are widely used as research tools in neuroscience, such as ω-conotoxin and some are potential therapeutic agents, such as Ziconotide (Gowd et al., 2005). Sudarsalal et al. (2003) investigated the modulatory activity of sodium channels in a δ-conotoxin from an Indian marine snail (C. amadis). The isolated novel conotoxins from Indian marine cone snails revealed different novel peptides targeting on Na and Ca channels (Gowd et al., 2005). The research on conotoxin is still in an infancy stage in India and much remains to be found. It is high time that concentrated and organized research on marine bioactive compounds has to be taken up. However, till date, there has not been a demonstrable growth in Conus toxin research. Since India is blessed with hundreds of Conus species, the potential bioactive property of these toxins has to be established for their effective utilization.

**Conotoxin research in the World:**

Kohn (1956) was the pioneer in the study of hunter or prey relationship of cone snails and they recognized that the venom of cone snails may possess therapeutic components. Salivary gland secretion of Conus species is one of the important venoms possessing analgesic property and also the most extensively studied (Sakthivel, 1999). Endean et al. (1963, 1974 and 1977) studied the venom of different species of cone snails in Australia and reported that venom contains a diversity of biologically active components. Their pharmacological studies could help to establish the venom caused paralysis of the skeletal musculature of representatives in all vertebrate classes but in invertebrates, their investigation also revealed that crude venoms of several conids had a complex pharmacological picture which suggested the presence of more than one active compound (Endean and Rudkin, 1963). Olivera (1990) was the primary innovator of the successful laboratory techniques in the study of venom components extracted from cone snails. They developed a new intracranial bioassay technique to study the property toxin in mice. The first Conus peptide extracted is α13 amino acid peptide with two disulphide bonds from C. geographus confirmed by chemical synthesis of α-conotoxin (Cruz et al., 1978; Gray et al., 1981). Clark et al. (2001) injected the venom fraction directly into the central nervous system of mammals, instead of using the intraperitoneal injection which resulted in better observation. However, when the same fractions were injected intracranially or intra-cerebrally, the true pharmacological diversity of Conus venoms was revealed by the amazing array of different behavioural phenotypes elicited in the mice (Olivera et al., 1990). The conotoxin inhibit calcium currents and synaptic transmission at the frog neuromuscular junction (Kerr and Yoshikami, 1984). Sleeper peptide a sleep inducing peptide, γ-carboxyglutamate has been isolated from fish-hunting cone snail, C. geographus (McIntosh et al., 1984).

Now-a-days, shaker peptides are known as ω-conotoxins (GVIA and MVIIA), ω-conotoxin GVIA is the most widely used toxin in neuroscience followed by tetraodotoxin (Olivera et al., 1990). In Brazil, three Conus species of large diameter are potentially dangerous, all are piscivores viz; C. regius, C. centurio and C. ermineus were identified from Fernando de Noronha Islands. Alonso et al. (2003) reported that conotoxin leads to the neuropathic pain and other neurological conditions. Ziconotide, a derivative of conotoxin helps to manage the severe chronic pain (Oren Bogin, 2005; Joseph et al., 2010). Terlau and Olivera (2004) reviewed the conotoxin and its impact on ion channels. Recently, efforts have been made to obtain the pharmaceutically valuable extracts from venom ducts of Toxoglossate gastropod genus Conus in the Gulf of Mexico.

**CONCLUSION**

The conotoxins have proved to be a valuable probe in physiological and pharmacological, and it has significant application in neurobiology and other biomedical sciences. Since a species of Conus can yield 100-200 peptides with potential therapeutic uses but research on conotoxin is still in an infancy stage in India and more and more research has needed to be done in future days to make use of bioactive properties of conotoxins. In view of the fact that India is blessed with hundreds of Conus species, the potential bioactive property of these toxins has to be established for their effective utilization.

**REFERENCES**


Conus species and importance of conotoxins


Saminathan, R. (1997) Biology and Pharmacology of the venomous cone snail Conusloroisii (Kiener) from the south east coast of India. Centre of Advanced study in marine biology. Annamalai University, Tamil Nadu, India.


