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Ciguatoxin – an Emerging Biological Hazard among Reef Fishes of India

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Abstract

Ciguatera Fish Poisoning (CFP) is an emerging food safety hazard which has been reported in southern peninsular India in recent times. Ciguateric fishes mostly associated with coral reef ecosystem are implicated in food poisoning outbreaks. USFDA guidance for fish and fishery products classifies CFP as "reasonably likely to occur" in fishes harvested from coral reef regions. Ciguatera Fish Poisoning has been reported from tropical or subtropical areas around the world between latitudes 35°N and 35°S, particularly in the Caribbean, Pacific and Indian Ocean and in the Flower Garden Banks area in the northern Gulf of Mexico. Action levels for CFP limits are now listed as 0.01 ppb for Pacific and 0.1 ppb for Caribbean ciguatoxin. CFP is considered as a natural toxin and USFDA has listed out 12 group of fishes under ciguatera hazard category. With recent EU import rejections of some of the seafood consignments originated from India, CFP has emerged as an important food safety concern. Although, no fatality has been reported so far, morbidity symptoms observed from cases of hospitalization is a definite concern to the export trade. This review deliberates on the significance of CFP, its distribution and hazard control measures.

Keywords: Ciguatoxin, CFP, mouse bioassay, reef fish, mass spectrometry, food safety

Harmful Algal Blooms and Biotoxins

Phytoplankton is the most important constituent of the marine food web and comprises 40% of the total fixed global primary productivity (Falkowski, 1984; D'Silva et al., 2012). In a typical algal bloom scenario, algal cells multiply upto 10^5 to 10^6 cells l^{-1} of seawater (Smith et al., 1993). Around 60-80 phytoplankton species are known to be harmful or toxic, in which 75% are contributed by dinoflagellates (Van Dolah, 2000; Wells et al., 2015; Roberts et al., 2004) and mostly responsible for the production of Harmful Algal Bloom (HAB). Reports of HABs are increasing in frequency, intensity and geographic distribution due to climate change and increased rates of coastal eutrophication (Paerl, 1988; Smayda, 1992; Hallegraeff, 1993; Nixon, 1995; Richardson & Jorgensen, 1996; Daranas et al., 2001). Seafoodborne intoxications, caused by marine biotoxins like Ciguatoxin (CTX), Saxitoxin (STX), Okadaic Acid (OA), Brevetoxin (PbTx), Domoic Acid (DA), Palytoxin (PLTX), Pectenotoxin (PTX), Tetrodotoxin (TTX) and Yessotoxin (YTX) result from the ingestion of contaminated fish and shellfish with the marine algal toxins (MATs) (Garthwaite, 2000; Botana, 2008; Lawrence et al., 2011; Shi, 2012). Marine biotoxins or the seafood toxin comes under the category of naturally occurring chemical hazards (FDA, 2011).

Incidents of biotoxin related hazards are reported globally from Europe, Africa, North America, Central and South America, Asia, Oceania etc. (Yasumoto et al., 1978; Underdal et al., 1985; Perl et al., 1990; Rodrigue et al., 1990; Morris et al., 1990; McMahon & Silke, 1998; DeSchrijver et al., 2002; FAO, 2004; Aune et al., 2007). Hence, producers of shell fishes and fin fishes have to ensure that the product must not contain the marine biotoxins in quantities that exceed 80 μg kg^{-1} for PSP, 20 mg kg^{-1} of DA for ASP, 160 μg kg^{-1} of OA equivalents (Dinophysis toxins and Pectenotoxins in combination), one milligram of Yessotoxin equivalents per kilogram for YTX, 160 micrograms of Azaspiracid equivalents per kilogram for AZP and any detectable level 100 g of fish for Ciguatoxin (FDA, 2011). Table 1 describes the major seafood poisoning syndromes, sources, mechanism of action, clinical

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symptoms and treatments. The extent of threat on human health from HABs in Indian waters remains unreported and unregulated. Until 1980s, the phenomenon of Paralytic Shellfish Poisoning (PSP) was virtually unknown in Indian waters. Indian waters are regularly seen with algal bloom occurrences and a report stated that a total of 101 bloom incidents and 39 causative species responsible for blooms during the period from 1908 to 2009, of which *Noctiluca scintillans* and *Trichodesmium erythraeum* were the most common species contributing to HAB events (D'Silva et al., 2012).

Ciguatera toxin

Existence of ciguatoxicity cannot be indicated by any highly visible surface phenomenon such as red tide as seen in the case of Paralytic Shellfish Poisoning (DeFouw et al., 2001). Hence an early warning to alert incidence of CFP is not possible. Until 2016, the occurrence of Ciguatera Fish poisoning was virtually unknown to fishes from Indian Coast. This region specific biotoxin has been reported very recently from Mangalore and Kerala coast (Rajeish et al., 2016; Rajisha et al., 2017a;b). In reports of CFP cases *Lutjanus bohar* commonly known *Chempalli* fish species was detected as ciguatoxic fish from south west coast of India and caused intoxication in local population. Incidence of ciguatoxin from Indian Coast led to the fact that prevalence of ciguatera toxin in fishes from our reef ecosystem is mainly due to bioaccumulation or biotransformation of toxic dinoflagellate *Gambierdiscus toxicus* through the food web.

As per the observation of UNESCO (2016), there has been a shift in the distribution and occurrence of biotoxins around the world because of climate change. This phenomenon can be attributed to tthe consecutive import rejections from the European Union for Indian seafood consignments for presence of Ciguatoxin. Intoxication due to CTX was reported from fish samples collected from South West Coast of India (Rajeish et al., 2016; Rajisha et al., 2017a;b). CFP is emerging as an important food safety concern which has to be addressed. There is no fatality reported so far, but the symptoms of ciguatera exist as a primary concern to the fisheries sector and export trade.

History of Ciguatoxin

Ciguatera fish poisoning (CFP) is a seafood-borne illness associated with a wide variety of gastrointestinal, neurological and cardiovascular symptoms in humans. The term "cigua" refers to intoxication caused by the ingestion of coral reef fishes (Juranovic & Park, 1991; Scheuer, 1994; DeFouw et al., 2001), which was first used by Don Antonio Para in Cuba in 1787 as a trivial name in Spanish to represent a univalve mollusk *Turbo livona pica* (Dickey, 2008). The incidence of ciguatera has been depicted from centuries back, since the time of Alexander the Great (356-323 B.C) (Scheuer, 1994; Pearn, 2001; Wong et al., 2014) and Homer's Odyssey (800 B.C) (Ragelis, 1984).

Bioaccumulation of ciguatoxin in fish

Ciguatoxin (CTX) is an important biotoxin resulting from the consumption of coral associated fishes. Ciguateric fish orally accumulates complex, more polar and sodium channel activating ciguatoxin (CTX) through the food web (Lewis, 2001). A benthic dinoflagellate known as *Gambierdiscus toxicus*, is responsible for the production of Gambiertoxin. *G. toxicus* is responsible for the production of less polar toxin precursors known as Gambiertoxins. It is transferred and metabolized into the more polar Ciguatoxin (CTX) by the fish itself through the food web (Holmes et al., 1991; DeFouw et al., 2001; Friedman et al., 2017). CTXs are bio accumulated and concentrated in the food chain and both herbivorous and carnivorous fish can become toxic. Small fish ingest the toxin and then are being devoured by larger fish, so that the fish in the higher trophic level of the food web contains high CTX concentrations which are in turn consumed by humans (Banner et al., 1960; Gillespie et al., 1986; Crump et al., 1999; Lehane & Lewis 2000; Dickey & Plakas 2010). Yasumoto et al. (1977) was first to consider *G. toxicus* as the responsible species for CTX accumulation based on the hypothesis of Randall (1958).

Worldwide distribution of ciguatera

Estimated number of people affected from this intoxication has been reported to be ranging from 10,000 to 50,000 on annual basis as per earlier reports (Baden et al., 1995, Lewis, 2001). Recent reports indicate the range has increased to 50,000 to 5,00,000 individuals annually (Lehane & Lewis, 2000; Caillaud et al., 2010), which signifies the intensity of occurrence even though it is difficult to ascertain the under reporting of cases (Tester et al., 2010, Skinner et al., 2011, Friedman et al., 2017). Fig. 1 shows the current global distribution of CFP according to FAO (2017).

Table 1. Marine Algal Toxins, source, clinical symptoms and treatments of poisoning syndromes (Caillaud et al., 2010; Shi, 2012; Friedman et al., 2017)

	Seafood Poisoning Syndromes					
			Fishes		Shell fishes	
Toxin Group	Ciguatera fish Poisoning (CFP)	Paralytic Shellfish Poisoning (PSP)	Diarrhetic Shellfish Poisoning (DSP)	Amnesic Shellfish Poisoning (ASP)	Neurotoxic Shellfish Poisoning (NSP)	Azaspiracid Shellfish Poisoning (AZP)
	Ciguatoxin (CTX)	Saxitoxin (STX)	Okadaic Acid (OA)	Domoic Acid (DA)	Brevetoxin (BTX)	Azaspiracid (AZA)
Source	*Gambierdiscus toxicus*	*Alexandrium catenella A. minutum A. tamarense; Gymnodinium catenatum; Pyrodinum bahamense Alteromonas tetraodonis; Moraxella sp.*	*Dinophysis acuminata; D. acuta; D. fortii; D. norvegica; Prorocentrum lima*	*Pseudo-nitzschia multiseries* and *P. autralis*	*Karenia brevis*	*Protoperidinium crassipes; Azadinium spinosum*
Action on	Nerve, Muscle, Heart, Brain VGSC* openers	Nerve, Brain VGSC* blockers	Enzymes Protein phosphatases inhibitors	Brain Glutamate receptors stimulators	Nerve, Muscle, Lungs, Brain VGSC* openers	
Symptoms	*Mild case* After 3-5 h - Diarrhea, nausea, vomiting, and abdominal pain. After 12-18 h - hot-cold inversion, muscular aches, tingling and numbness of lips, tongue, and perioral region Metallic taste, dryness of mouth, anxiety, prostration dizziness, chills, sweating, dilated eyes, blurred vision, and temporary blindness. *Extreme Case* Paralysis and death may occur in a few extreme cases.	*Mild case* Within 30 min - Tingling sensation or numbness around lips, gradually spreading to face and neck, prickly sensation infingertips and toes, headache, Dizziness, nausea, vomiting, diarrhea. *Extreme Case* Muscular paralysis, pronounced respiratory difficulty, choking sensation, death through respiratory paralysis may occur within 2-24 h after ingestion.	*Mild case* After 30 min to a few hours (seldom more than 12 h): diarrhea, nausea, vomiting, abdominal pain. *Extreme Case* Chronic exposure may promote tumor formation in the digestive system.	*Mild case* After 3-5 hours - nausea, vomiting, diarrhea, abdominal cramps. *Extreme Case* Decreased reaction to deep pain, dizziness, hallucinations, confusion, short-term memory loss, seizures.	*Mild case* After 3-6 h - chills, headache, diarrhea, muscle weakness, muscle and joint pain, nausea, vomiting *Extreme Case* Paresthesia, altered perception of hot and cold, difficulty in breathing, double vision, trouble in talking and swallowing	*Mild case* Nausea, vomiting, severe diarrhea, and stomach cramps, similar to DSP and NSP *Extreme Case* Suspected carcinogen
Treatment	Symptomatic, Gut emptying and decontamination with charcoal is recommended.	Gastric lavage, artificial respiration. No lasting effects.	Recovery after 3 days, irrespective of medical treatment.	Supportive care	Supportive care	Supportive care

* Voltage Gated Sodium Channel

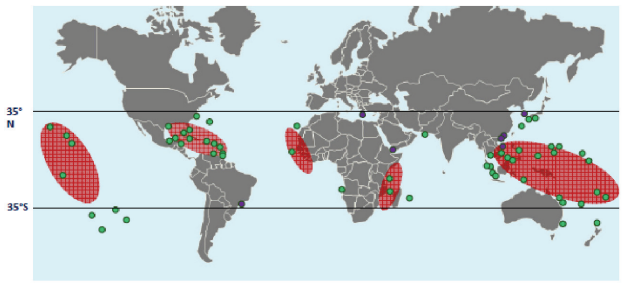


Fig. 1. Current distribution of toxins (Source FAO, 2017)

CFP recognized worldwide as CTX group toxins, is classified into three major categories according to their occurrence as Caribbean (C- CTXs), Pacific (P-CTXs) and Indian Ocean (I-CTXs) origin. It was identified for the first time in fish caught from Europe (EFSA, 2010) and hence the geographical distribution of CFP affects globally in the tropical and subtropical regions. Around 400 reef associated fin fish species (Halstead, 1978, Caillaud et al., 2010) are found to be ciguatoxic due to the process of biotransformation in the food web, but comparatively small number of species are regularly implicated in ciguatera poisoning (Lehane & Lewis, 2000). Dinoflagellates responsible for the production of ciguatoxin exist within coastal waters between 35ºN and 35ºS of the equator (Lewis, 2001; FDA, 2011). Spread of ciguatera has been ascribed to many factors that include increased number of fishing community, consumption of contaminated fishes associated with oceanic oil rings habitats, increased number of travel and trade, increase in ocean temperature, importation of contaminated fish in to new areas where it is not reported earlier (Frenette et al., 1988; Morton et al., 1992; Glaziou & LeGrand, 1994; Moulignier et al., 1995; Bruneau et al., 1997; Lewis, 2001; Pottier et al., 2001; DeHaro et al., 2003; Sheppard & Rioja, 2005; Villareal et al., 2007).

Ciguateric fish

Most of these ciguateric fishes comes under the category of top selling, good tasting and highly demanded food fishes in the world market (Friedman et al., 2017). FDA (2011) has listed common reef associated fin fishes implicated in CFP (Table 2).

Other different ciguatoxic fish species are also reported around the world from various researchers (Blythe et al., 1992; Vernoux & Lejeune, 1994; Hokama et al., 1998; Lewis et al., 1999; Hsieh et al., 2009; Azziz et al., 2012; Chan, 2013). Gillespie et al. (1986) reported narrow-barred Spanish mackerel, *Scomberomorus commersoni* ciguatoxic from Australian coastline. Chinain et al. (2010a) reported Scarids (Parrotfish) and Acanthurids (Unicorn fish) as high-risk ciguatoxic fish species from French Polynesia.

Table 2. Common reef associated fin fishes with Ciguatera toxicity (FDA, 2011; Friedman et al., 2017)

Species	Family
Barracuda	Sphyraenidae
Amberjack	Seriola
Grouper	Serranidae
Snapper	Lutjanidae
Po'ou (*Cheilinus* spp.)	Labridae
Jack	Carangidae
Trevally (*Caranx* spp.)	Carangidae
Wrasse	Labridae
Surgeon fish	Acanthuridae
Moray eel	Muraenidae
Roi (Cephalopholis spp.)	Serranidae
Parrot fish	Scaridae

Clinical diagnosis of ciguatera symptoms

Clinical criteria include a wide array of symptoms characterized into three major groups: neurological, gastrointestinal and cardiac. Preliminary symptoms start with gastrointestinal (e.g. nausea, diarrhea and vomiting, abdominal pain) problems which begin within 6-12 h of fish consumption and resolve spontaneously within 1-4 days. Secondarily, the neurological symptoms which affects the central and peripheral nervous systems (e.g. paresthesia in the extremity and circumoral regions, pruritis, dysuria, myalgia, hallucinations, depression, cold allodynia, giddiness, vertigo, visual, balance and behavioral disturbance, loss of consciousness) set in, but in some cases they may starts simultaneously with the initial symptoms (DeMotta & Noceda 1985; Pearn, 2001; Arena et al., 2004; Friedman et al., 2007; Stewart et al., 2010). Third category includes the cardiac symptoms (e.g. hypotension, bradycardia) at the early stage of toxicity and proceed in combination with the initial two categories of symptoms (Chateau-Degat et al., 2007a; Katz et al., 1993). All these symptoms start within 2-30 h after toxic fish consumption (Caillaud et al., 2010) and may persist from weeks to months and years. According to Chan (2016), CFP is rarely fatal (<0.1% fatality) and Lewis

(2000) reported that it may be higher in the Indian Ocean. Certain foods and behaviours potentiate the ciguatoxic symptoms like nuts, caffeine, pork, chicken, alcohol consumption, tobacco smoking, fish consumption etc. (Gillespie et al., 1986; Glaziou & Martin, 1993; Lewis, 2000; Lewis, 2001; Chateau-Degat et al., 2007b). Clinical diagnosis of ciguatera fish poisoning is considered as a challenge to emergency physicians because of the patients exhibit or present with a mixture of gastrointestinal, neuro-cutaneous and constitutional symptoms (Cheng & Chung, 2004). There is no effective treatment for this poisoning syndrome and the available remedy is based on acute symptomatic and supportive care for the patients (Friedman et al., 2017). Intravenous mannitol (one gram/kilogram body weight over a 30 to 45 min period) and atropine (0.5 mg every 3-5 min) was administered as dosage (Pearn et al., 1989; Baden et al., 1995; Lewis, 2001). CFP diagnosis is done in suffered individuals based upon the visible symptoms, time of onset and previous history of fish consumption (whether reef associated or its toxic history). There are no reliable biomarkers or documentation symptoms yet discovered to confirm the exposure of this toxicity. Distinct differences are present in case of CFP symptoms according to various geographical distributions. In the Pacific CTX, neurological symptoms are dominated, whereas in the Caribbean, gastrointestinal problems are highly dominated (Lewis, 2001). Indian Ocean CTX exhibits a group of symptoms like hallucinations, mental depression, lack of coordination, etc. along with typical ciguatera symptoms (Lewis, 2001). Table 3 describes common CFP associated symptoms in humans.

Detection methods for Ciguatoxin

Ciguatoxin emerging as a new toxin from our coast and the absence of purified standards and complex nature of CTX in fish tissue will be a major concern for the development of a laboratory analytical method. USFDA and NOAA laboratories in Japan and Australia have been developed *in-vitro* assay protocol for determination of ciguatoxin in fish (Dickey, 2008). Traditional methods are practiced among local population, which include animal

Table 3. Clinical diagnosis of ciguatera symptoms (Morris et al., 1982; Coleman, 1990; Lewis, 2001; Arena et al., 2004; Chateau-Degat et al., 2007a; Baumann et al., 2010; Friedman et al., 2017)

Category	Symptoms
Gastrointestinal	Abdominal pain, Vomiting, Diarrhoea, Nausea
Neurological	Generalized weakness, Vertigo
	Lingual paraesthesia, Extremities Paraesthesia, Circumoral Paraesthesia
	Arthralgia and Myalgia
	Dental pain, Ataxia
	Paradoxical Temperature sensation
	Respiratory paralysis
	Coma, Weakness in the extremities, Headache
	Myalgia and Arthralgia
Cardiovascular	Dizziness
	Hypotension (systolic BP <100 mmHg)
	Bradycardia (pulse rate <60 beats/min)
	Chest pain
Others	Chills
	Sweating
	Shortness of breath
	Itching (two to three days)
	Nightmares, mental depression, hallucinations
	Lack of coordination and loss of equilibrium

testing, observing the bleeding at the tail of the fish fillet, observing silver coins turning black on a hypothetical cooked fish, feeling a sensation on tingling when rubbing the liver on gums etc. (Banner et al., 1963; Chinain et al., 2010b). These methods are practically not suitable for the determination of CTX toxicity. An ideal or recognized official method for CTX detection in fish is not yet established (Caillaud et al., 2010; Friedman et al., 2017). Ciguatoxin is a lipid soluble compound and most of the sample preparation methods are based on acetone or methanol extraction (Caillaud et al., 2010).

Mouse Bioassay and other *in vivo* and *in vitro* methods

Mouse bioassay (MBA) has been widely used for the selective determination of ciguatoxicity in fishes introduced by Banner et al. (1960) and further refined by Yasumoto et al. (1984). In this method the lethality is estimated in terms of Mouse Units (MU). After intra peritoneal injection of crude fish ether extract into mice, signs of toxicity is observed up to 24 h. The toxicity and relationship between dose and time to death is used to quantify toxicity (Lewis, 1995). Other *in vivo* assays for the detection of CTX include chicken assay (Kosaki et al., 1968), Brine Shrimp assay (Granade et al., 1976; Bienfang et al., 2008), mosquito larvae assay (Bagnis et al., 1985), Diptera Larvae assay (Labrousse & Matile, 1996), etc. These assays are not recommended for CTX quantification, hence not widely used in laboratories for screening of ciguatoxin (Caillaud et al., 2010). MBA is followed as an official testing method for Paralytic and Diarrheic shellfish toxins as per European Union and FDA guidelines (FDA, 2011; AOAC, 2012). In case of CFP, MBA is used for the screening of ciguatera implicated reef fish samples. Yasumoto et al. (1984) and Caillaud et al. (2010) suggested that any fish containing above 2.5 Mouse Unit (MU) 100 g^{-1} should be avoided as food, since it has long term neurological effects. Sub lethal doses were in the range between 0.18 and 0.45 MU 20 mg^{-1} of ether extract (Wong et al., 2005). The utility of MBA method is limited by the requirement of dose response curve because of the lapse of purified CTXs for accurate quantification; hence the curve is not linear (Hoffman et al., 1983; Lehane & Lewis, 2000; Lewis, 2003). Lewis (1995; 2003) revised the MBA extraction protocol and Wong et al. (2005; 2009) developed a solid phase extraction (SPE) clean up method for CTX fish extract for MBA. Routine analysis of samples by mouse bioassay cannot be recommended since it is non-specific and ethically objectionable (Abraham et al., 2012). Sodium channel specific cytotoxicity (Manger et al., 1993; 1995) and sodium channel receptor binding in rat brain synaptosomal preparations (Lombet et al., 1987; Lewis et al., 1991; Poli et al., 1997) were developed as an alternative to *in vivo* assay. *In vitro* mouse neuroblastoma assay was used as a screening procedure, using an ouabain-veratridine dependent method by Dickey et al. (1999) and Manger et al. (1995). EFSA (2009) recommended *in vitro* assay as an alternative to *in vivo* animal assays for monitoring and investigation of Marine Algal Toxins (MATs).

Physico-chemical detection of CTX in fish

USFDA applied a two- tiered protocol for monitoring of CFP which includes *in vitro* assay and Mass Spectrometry analysis (FDA, 2011; Friedman et al., 2017).

Mass Spectrometry

The main physico-chemical methods used in toxin analysis are chromatographic methods with optical (UV and flourescent detectors) or mass spectrometric detectors (Quilliam, 2003). A typical LC-MS system comprises HPLC for analyte separation, an atmospheric pressure ionization interface to produce ionized molecules and mass spectrometer (MS) in which ions are separated and detected in a high vacuum environment. Various ciguatoxin congeners were quantified using mass spectrometry method for Pacific, Caribbean and Indian Ocean forms. For Pacific ciguatoxin, P-CTX-1, P-CTX-2 and P-CTX-3 congeners with molecular masses identified as 1111.6 & 1095.5 Da were isolated from carnivorous fishes (Lewis et al., 1991: 1993; Lewis & Jones, 1997). Another two congeners for P-CTX are CTX-3B (49-epi-CTX-3C) and CTX-3C with molecular ions 1023.6 Da and M-seco-CTX-3C with m/z 1041.6 Da were isolated from *Gambierdiscus toxicus* (Satake et al., 1993; Chinain et al., 2010b; Roeder et al., 2010). Molecular mass of m/z 1061.6 Da identified for CTX-4B (GT-4B) isolated from *Gambierdiscus* sp. and herbivorous fish as source organisms and also for 52-epi-ciguatoxin-4B (CTX-4A; GT-4A) isolated from *G. toxicus* (Murata et al., 1990; Satake et al., 1996; Yasumoto et al., 2000; Roeder et al., 2010). $[M+H]^+$ ions 1057.6 Da for CTX-2A1 congener were isolated from both *G. discus* and carnivorous fish and 1039.5 Da for CTX-2C1 was determined from *G. toxicus* as

source organism (Satake et al., 1998; Roeder et al., 2010). Almost 10 congeners are identified for Pacific ciguatoxin as fish and algae as causative species and six congeners for Caribbean ciguatoxin (C-CTX) were isolated from carnivorous fishes as source organisms. C-CTX having a m/z 1141.6 Da for C-CTX-1 and C-CTX-2, 1127.6 Da for C -CTX-1127, 1143.6 Da for C-CTX-1143, 1157.6 Da for C-CTX-1157 and 1159.6 for C-CTX-1159 have been identified as congeners by various researchers (Vernoux & Lewis, 1997; Lewis et al., 1998; Lewis et al., 1999; Pottier et al., 2002a;b; Pottier et al., 2003). Indian Ocean ciguatoxin (I-CTX) was isolated from carnivorous fishes with molecular masses m/z 1141.6 Da for I-CTX-1 & I-CTX-2 and 1157.6 Da for I-CTX-3 & I-CTX-4 congeners (Hamilton et al., 2002a;b). Mass Spectrometry (MS) is an excellent tool for the identification and characterization of ciguatoxin congeners, since it is provided enhanced sensitivity and selectivity by measuring accurate masses or a series of fragment ions. Lewis et al. (1994) introduced Ion Spray (IS) as ion source for MS analysis. Yogi et al. (2014) carried out an LC-MS/MS analysis using Triple Quadrupole Mass Spectrometry, in which 14 reference toxins were used and pure CTX-1B and CTX3C were prepared from fish samples collected from Japan.

Nuclear Magnetic Resonance (NMR)

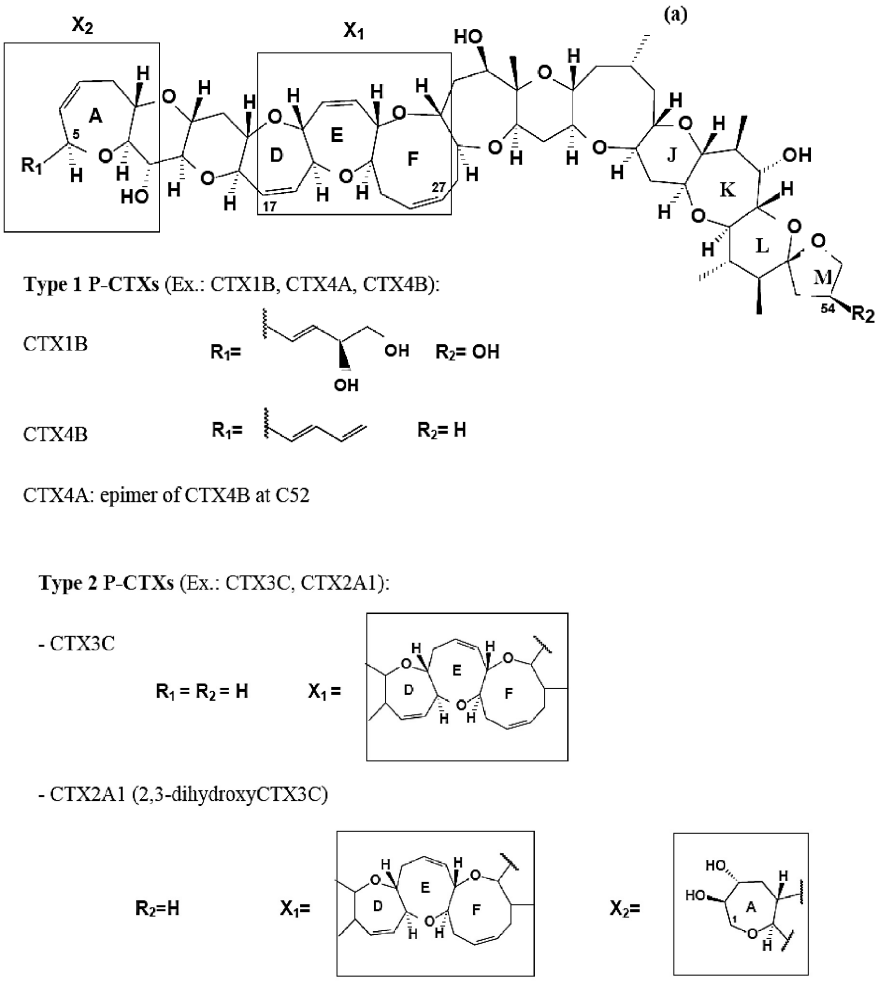
Modern NMR has proved as a vital technique for the full elucidation of the chemical structures of novel biotoxins (Shi et al., 2012). Proton (^{1}H) NMR and Carbon-13 (^{13}C) enables determination of the proton environment (number and configuration of neighboring protons) and identification of the number and type of carbon atoms in an organic molecule respectively. The combination of ^{13}C and ^{1}H NMR in 2D experiments along with FT IR and UV Visible NIR allows the elucidation of the carbon connectivity and 3-dimensional chemical structure of complex organic molecules (Shi et al., 2012). So far only Pacific and Caribbean CTXs are structurally elucidated and NMR is the key technique used for this purpose. CTX is a group of highly oxygenated and cyclic polyether molecules and structurally related with the Brevetoxin (PbTx) group (Lewis, 2001). Murata et al. (1989) started the pioneer work in the structural confirmation of Pacific CTX and its precursor from *G. toxicu*s using NMR methods. From the Pacific P-CTX-1(m/z 1111 Da), P-CTX-2 and P-CTX-3 (both has m/z 1095 Da) were structurally isolated from carnivorous fish and P-CTX-3C (m/z 1045) was isolated from *G. toxicus* (Lewis et al., 1991; 1993; Satake et al., 1993; 1996; 1998). Caribbean C-CTX-1 and C-CTX-2 with molecular mass 1141 Da were structurally elucidated from carnivorous fish (Vernoux & Lewis, 1997; Lewis et al., 1998). P-CTX-4A and P-CTX-4B with molecular mass 1061 Da has been structurally elucidated from *G. toxicus* and herbivorous fishes (Murata et al., 1990). CTX are structurally distinct from other biotoxins (Yasumoto & Murata, 1993) and using Mass Spectrometry and NMR techniques, several minor toxins are also detected (Lewis & Jones, 1997; Vernoux & Lewis, 1997). Around 20 ciguatoxin congeners are structurally elucidated by Yasumoto et al. (2000) using high energy Mass Spectrometry and NMR techniques.

Chemical and Structural Properties of CTX based on NMR analysis

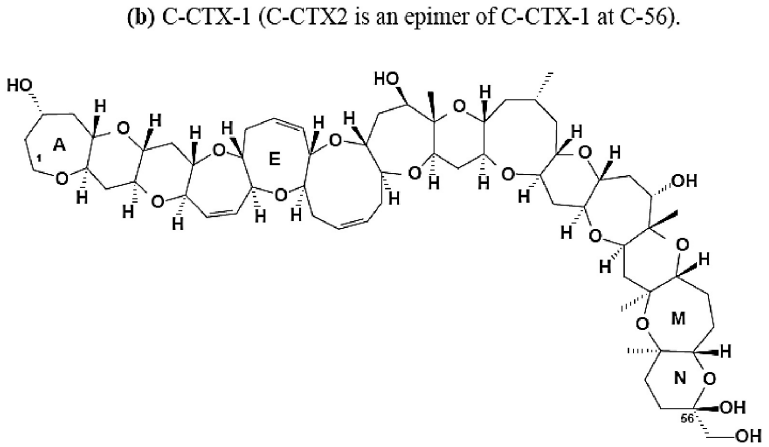
Pacific CTX was divided in to Type I and Type II based on number of carbon atoms (60 and 57) respectively and the structure of the ether ring (Murata et al., 1990; Legrand et al., 1998). Caribbean CTXs contains 62 number of carbon and 14 numbers of E rings. Vernoux & Lewis (1997) first isolated and structurally identified two C-CTXs and later Pottier et al. (2002a; 2002b) identified additional congeners. Hamilton et al. (2002b) isolated four I-CTXs, but their structural characteristics were unidentified. Structure of P-CTX (Type I and Type II) and C-CTX elucidated by different researchers were given in Fig. 2. (Murata et al., 1990; Lewis et al., 1991; Satake et al., 1996; Lewis & Jones, 1997; Vernoux & Lewis, 1997; Lewis et al., 1998; Yasumoto et al., 2000; Lewis, 2001; FAO, 2004; Caillaud et al., 2010; FDA, 2011)

Risk assessment of CFP for food safety

EFSA, 2010 panel on contaminants in the food chain assessed, Ciguatoxin as an emerging biotoxin for which widely screened toxicity assay MBA has found limitations due to insufficient detection and ethical concerns. *In vitro* assay and receptor binding assay have been developed as alternatives; but they need further development and only few laboratories have the needed facility for cell based assays (Caillaud et al., 2010). Hence, LC-MS/MS Tandem Mass Spectrometry is only considered as valued method, in which reference standards need to be developed taking into account the distinctive nature of CTX sourced from various geographical regions (Friedman et al., 2017). The major preventive measures for ciguatera include, avoiding ciguateric



a) P-CTX Type I with 13 number of E rings and 60 number of carbons & Type II with 57 number of carbons



b) Caribbean CTX 1 & CTX 2 with 14 number of E rings and 62 number of carbons

Fig. 2 a) Structure of Pacific Ciguatoxin (Type I & II) and b) structure of Caribbean Ciguatoxin (Lewis et al., 1998; Lewis, 2001; FAO, 2004; Caillaud et al., 2010)

fish, proper surveillance and reporting of incidence based on clinical data, community outreach and education to avoid misdiagnosis and under reporting of cases (Friedman et al., 2017). The ciguatera transmission from person to person which include effects on the embryo/ fetus via placenta and breast feed infant via mothers milk showed the risk of ciguatera toxicity in humans (Bagnis & Legrand, 1987; Blythe & De Silva, 1990; Ruff & Lewis, 1994; Karalis et al., 2000). The amount of toxins is directly correlated to the size of the fish and results indicated that large sized fishes had more ciguatoxin in comparison to small fishes (Pottier et al., 2001). Hence, it is advisable for the consumers to take only fishes of small size. Ban or size restrictions on certain reef fish species can be taken as an initial safety measure to protect the consumers from the lethal effects of this toxicity. European Union regulation states that "Fishery products containing biotoxins such as ciguatoxin or muscle-paralyzing toxins must not be placed on the market" but there is no reference analytical method suggested for CFP samples, which restricts the implementation of regulatory safety limits (Caillaud et al., 2010).

Indian seafood trade is heavily dependent upon the export of the highly prized coral reef fishes. In one such case the remnant head waste of *Lutjanus* species deemed for export was implicated in CFP toxicity in Mangalore coast during September 2016 (MTNN, 2016; Times of India, 2016). The workers of nearby exporting fish firm were also hospitalized due to consumption of fish heads, which was considered as a waste in the fish export factory. This incident showed the failure of hazard identification from the industry and most of the industries handling the export of reef fishes have the responsibility for the proper checking and diagnosis of their consignments, so as to ensure the export safety of our products. Coral reef fisheries mainly contributed by major species such as snappers, reef cods, croakers, trevally and barracuda are highly demanded species in the foreign markets. Pre-export testing is an important hazard control measure for coral reef fishes. Although reports on the existence of ciguatera from our coast is rare, ICAR-CIFT has initiated monitoring of coral reef fishes for presence of ciguatoxin (Rajeish et al., 2016; Rajisha et al., 2017a; b). Climate change and globalization of trade has led to an increase in the spread of ciguatera, hence guidance is needed for those countries where CFP risk management programmes has not yet been implemented (FAO, 2017).

Future Directions

Ciguatoxin has not been reported from Indian coastal regions before 2016. Now there is a prevalence of ciguatoxin from Indian Coast. That indicates that there may be climate change induced shift in the habitat of G. *toxicus*, which is the causative organisms responsible for producing CTX. Hence, risk management approach is required for protection of our aquatic habitats against demographic expansion of *G. toxicus* and further bioaccumulation in coral reef fishes. Now, there is an urgent need to periodically monitor all coral reef species that hugely contributes to the export basket as well as for domestic consumption There is a distinct possibility of bioaccumulation of ciguatoxin in our ecosystem and efforts should be initiated for complete characterization of Indian Ocean Ciguatoxin and development of validated analytical methods.

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