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ASSESSING THE CONTENT AND TOXIC LEVEL OF CIGUATOXINS OF MARINE FISHES IN THE MARKETS IN NHA TRANG (KHANH HOA)

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ABSTRACT The quality of seafood is a subject, which is concerned in many countries. **Ciguatoxin** is one of the most dangerous toxins accumulated in fish and the poisonously potential source to consumers.

Based upon the previously studied results of accumulation of **ciguatoxin** in coral reef fishes in Nha Trang bay in 1999, 13 species of fish in the market in Nha Trang - Khanh Hoa were sampled for studying **ciguatoxin** to assess the quality of fish from April to August 2000. The result of bioassay on mice showed that the quality of almost species of fish was safe in terms of **ciguatoxin**. The extracts from viscera of species such as Tomato hind (Cephalopholis sonnerati), White edged lyretail (Variola albimarginata), Golden threadfin bream (Nemipterus virgatus), Golden rabbitfish (Siganus guttatus) caused death in mice within 6 – 19 h, but the accumulated level was low.

ÑANH GIA HAM LÖÔNG VA ÑOC TÍNH CIGUATOXIN CUA CA BIEN ÑÖÔC BAN TREN THÒ TRÖÔNG NHA TRANG (KHANH HOA)

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TOM TAT Chat löông hai san la van ñe ñöôc quan tam ô nhieu quoc gia. **Ciguatoxin** la mot trong nhöng loai ñoc to nguy hiem ñöôc tích luy ô ca va co the la nguon gay ngo ñoc tiem tang cho ngöôi sö dung.

Döa tren ket qua ñieu tra **ciguatoxin** ô mot so loai ca ran vung bien Nha Trang - Khanh Hoa nam 1999, 13 loai ca tren thò tröông Nha Trang – Khanh Hoa ña ñöôc thu mau nghien cöu **ciguatoxin** ñe ñanh gia chat löông ca tö thang 4 – 8/2000. Ket qua thöû nghiem tren chuot cho thay, hau het cac loai ca la ñam bao an toan ve mat **ciguatoxin**. Chat chiet tö noi quan cua mot so loai nhö ca Mu ca chua (Cephalopholis sonnerati), Mu ñuoi diem trang (Variola albimarginata), Löông dai ñuoi (Nemipterus virgatus), Dìa cong (Siganus guttatus) mac du gay chet cho chuot trong vong 6 – 19 giô nhöng bieu hien möc ño nhiem **ciguatoxin** thap.

I. INTRODUCTION

Ciguatoxin is a type of toxin produced by some microalgae species and accumulated in fish via the food chain. It can cause poisoning to consumers with the high content. More than 300 species of marine fishes have been incriminated, to date (Halstead B.M., 1959).

Ciguatoxin can cause symptoms: neurological (muscle pains, myalgia, headache, inability to sleep, paraesthesiae, blurred eyes, itching...), cardiovascular (cyanosis, dizziness, pallor...), gastrointestinal (abdominal pain, nausea, actual vomiting, diarrhoea...)

For the poison of ciguatera, although the amount of intoxicated consumers has been reported not completely and the rate of mortality has been low, the affect to the sea economics has been remarkable. The poisoning of ciguatera caused by consuming the marine fish occurred in some places in the world such as the group of islands in Kauai (Katz A. R., et al., 1993), Hawaii (Abbott I. A. and G. P. Wilder, 1994), California (America) (Fish imported from Samoa Island; Zlotnick B. A., et al., 1995), the Island of Madagascar (Habermehl G. G. M. et al., 1994; Boiser P., et al., 1995), the Island of Queensland and Tonga (Australia) (Lewis R. J. and Geoffrey K. King, 1996), Hongkong (about 76 per cent was the imported fish; Lusonghui and I. J. Hodgkiss, 1999), Romblon (Philippine) (Rhodora V. Azana and Lilibeth N., 1999).

Controlling the numerous amount of fishes accumulating high content of c**iguatoxin** was carried out in some countries (Hokama Y. et al., 1994). The quality and the safety to consumers were controlled strictly. With the poisonous limit of **ciguatoxin** level, it has been difficult to give out the accurate data so far. However, at the concentration of 0.1ppb CTX (10^{-10} mole/kg – 1M CTX = 1120), it can cause poisoning to human. In the case of mouse bioassay, the death time of mice is not less than six hours.

In Vietnam, during the last few years, some studies of Institute of Oceanography were concerned in the toxins of marine organisms. The results showed that some harmful algae species existed in the coastal waters and some species of crabs, fishes and mollusks accumulated toxins from microalgaes. However, controlling toxins in the seafoods was still limited.

II. MATERIALS AND METHODS

- 13 species of marine fishes collected at the markets: Chut, Xom Moi, Cua Be port in Nha Trang City from April to August 2000 (Table 1).

- Methods for extraction and bioassays:

+ According to Lewis 1995.

+ Using the table of levels described by Hokama Y. et al., 1994.

+ Estimating the toxin level and content (Mouse Unit-MU): According to Hokama Y., 1994; Lewis, 1995 (Table 2).

1 MU: death within 24 hr. of 20 gr. mouse; contain 7 – 9 ng CTXs/100 mg of crude extract/mouse injected. If the death time is less than 6 hours, the sample is considered as the high toxic level.

Table 1: The species of fish collected in Nha Trang market

Vietnamese name	English name	Latin name
Trac mat ño	Red bigeye	Priacanthus macracanthus, Cuvier, 1829
Chuon ñat mot gai	Stairy flying gurnard	Dactyloptena peterseni (Nystrom, 1887)
Hao mình cao	While-linned crevalle	Kaiwarinus equula (Temmink & Schelegel,1844)
Mu ca chua	Tomato hind	Cephalopholis sonnerati (Valenciennes,1828)
Mu ñuoi diem trang	White edged lyretail	Variola albimarginata, Baissac, 1953
Dìa cong	Golden rabbitfish	Siganus guttatus (Bloch, 1787)
Trao	Yellowtail scad	Atule mate (Cuvier, 1833)
Hong cham bac	Ehrenberg's snapper	Lutjanus ehrenbergi (Peters, 1869)
Mu cham ño	Red- spotted grouper	Epinephelus akaara (Temmink & Schelegel, 1842)
Sôn ña vay rang cöa	Pinecone soldierfish	Myripristis murdjan (Forscal, 1775)
Trao mat to	Bigeye scad	Serla crumenopthalmus (Bloch, 1793)
Löông ñuoi dai	Golden threadfin bream	Nemipterus virgatus (Houttyn, 1782).
Hong bung cong	Hunched snapper	Lutjanus gibbus (Forscal, 1775)

Table 2: Mouse toxicity sssay scoring

Level of toxicity	Description of visible clinical symptoms in mouse after extract injection
0	No ill effect
1	15-60 min.: muscle contraction in lower back area (flexion), increased respiration, immobile (inactive), recovery.
2	Similar to 1, but recover in 2 – 3 h, pilo-erection.
3	Recover in 12 –24h: similar to 2, muscle contraction, paralysis in the extremities (usually hind legs), rapid and irregular breathing, immobile, closed eyes, pilo-erection, light cyanosis (tail).
4	Symptoms as in 3, but death within 24 –48h.
5	Symptoms as in 3 and 4, death in less than 6 h.

III. RESULTS AND DISCUSSION

1. The crude ciguatoxin content

According to the above result, the ciguatoxin content of viscera was almost higher than that of muscle. That means, in term of amount, as a whole the ability of accumulation of some compounds, in particular ciguatoxin was high in viscera (Table 3).

Table 3: The crude ciguatoxin content of fish species in Nha Trang markets from April to August 2000 (% wet weight)

Species	April - May		June - July		August	
	Muscle	Viscera	Muscle	Viscea	Muscle	Viscera
Red bigeye (Priacanthus macracanthus)	1.63	0.94	0.41	0.53		
Stairy flying gurnard (Dactyloptena peterseni)	2.1	0.5				
White-linned crevalle (Kaiwarinus equula)	1.18	0.65	0.74	0.75	0.61	1.53
Golden threadfin bream (Nemipterus virgatus)	0.53	0.69			0.75	0.58
Hunched snapper (Lutjanus gibbus)	0.52	1.1			0.5	0.76
Tomato hind (Cephalopholis sonnerati)	0.58	1.93			0.5	2.1
White edged lyretail (Variola albimarginata)	0.65	0.84	0.66	0.92	0.88	0.73
Golden rabbitfish (Siganus guttatus)	0.62	1.14	0.70	1.20	0.60	1.15
Yellowtail scad (Atule mate)	0.51	0.65	0.48	2.67		
Ehrenberg's snapper (Lutjanus ehrenbergi)			0.52	1.10	0.58	1.65
Red-spotted grouper (Epinephelus akaara)			0.69	1.28		
Pinecone soldier fish (Myripristis murdjan)					1.29	1.33
Bigeye scad (Serla crumenopthalmus)			0.69	0.8		

2. The result of mouse bioassay and quality of fish.

All the results of bioassay are presented from tables: 4 – 6.

According to the presented results in tables: 4 – 6, the extract of almost muscles caused the light symptoms of intoxication on mice such as pilo-erection, irregular breathing, immobile... The mice recovered gradually after injection 2-3 h and the weight decreased tendentiously after 3 –4 days. The range of toxic levels was from 0 – 3 and the toxin content was approximate to 0.5 MU or 17.5 – 22.5 ng **ciguatoxin**/100mg crude extract. The extracts of the muscle of Yellowtail scad (Atule mate), White-linned crevalle (Kaiwarinus equula), Red bigeye (Priacanthus macracanthus), Golden threadfin bream (Nemipterus virgatus) were negative in terms of **ciguatoxin**.

Table 4: The result of bioassay on mice of samples collected in April and May 2000

Species	Times	Symptoms		Level of toxicity	
		Muscle	Viscera	Muscle	Viscera
Red bigeye	April 20th 2000	Normal	Normal	0	1
Stairy flying gurnard	April 20th 2000	Light	Light	2	2
White-linned crevalle	April 20th 2000	Normal	Normal	0	0
Golden threadfin bream	April 21st 2000	Normal	Light.	1	2 0.5 MU
Hunched snapper	April 21st 2000	Light	Light	2 0.5 MU	3 0.5 MU
Tomato hind	May 22nd 2000	Light	Pilo-erection, mild to rapid breathing, wobbly, paralysis in hind legs, diarrhoea. Death < 8 h.	2 0.5 MU	4
While edged lyretail	May 22nd 2000	Light	Light	2	2 0.5 MU
Golden rabbitfish	May 22nd 2000	Light	Inactive, pilo-erection, rapid breathing. Death within 6h.	2 0.5 MU	5
Yellowtail scad	May 22nd 2000	Normal	Normal	0	0

Table 5: The result of bioassay on mice of samples collected in June and July 2000

Species	Times	Symptoms		Level of toxicity	
		Muscle	Viscera	Muscle	Viscera
White-linned crevalle	June 20th 2000	Normal	Normal	1	2
Golden rabbitfish	June 20th 2000	Normal	Normal	1	2
Golden threadfin bream	June 21st 2000	Normal.	Immobile, pilo-erection, abnormal cramps. Death within 13 h45.	0	4
Ehrenberg's snapper	June 21st 2000	Light	Light	2	2
Red-spotted grouper	July 16th 2000	Light	Light	2	2
Yellowtail scad	July 16th 2000	Normal	Light	0	1
While edged lyretail	July 16th 2000	Light	Pilo-erection, diarrhoea. Death within 14 h.	2 0.5 MU	4
Red bigeye	July 16th 2000	Light	Light	1	2

Table 6: The result of bioassay on mice of samples collected in August 2000

Species	Times	Symptoms		Level of toxicity	
		Muscle	Viscera	Muscle	Viscera
While-linned crevalle	Aug. 18th 2000	Light	Light	2	3 0.5 MU
Golden threadfin bream	Aug. 18th 2000	Light	Light	2	3 0.5 MU
Hunched snapper	Aug. 18th 2000	Light	Light	2 0.5 MU	3 0.5 MU
Golden rabbitfish	Aug. 20th 2000	Light	Light	2	3 0.5MU
While edged lyretail	Aug. 20th 2000	Light	Pilo-erection, closed eyes, erected tail. Death within 14 h.	2 0.5MU	4
Ehrenberg's snapper	Aug. 20th 2000	Light	Light	2	2
Tomato hind	Aug. 20th 2000	Light	Light	3 0.5 MU	3 0.5 MU
Pinecone soldierfish	Aug. 20th 2000	Light	Light	3 0.5 MU	3 0.5 MU

The extract of Hunched snapper (Lutjanus gibbus) caused the further diarrhoea (0.5 MU) (May) and for the extracts of White edged lyretail (Variola albimarginata) (June - July and August), Pinecone soldierfish (Myripristis murdjan), the effect was relatively strong, including the abnormal cramps, convulsions, but the mice recovered after 24 h. For Hunched snapper (Lutjanus gibbus), Pinecone soldierfish (Myripristis murdjan) Tomato hind (Cephalopholis sonnerati) (August), the extract made the mean weight of mice decreased. The content of CTXs was about 0.5 MU.

Although the extracts from the muscles of some species caused the limited effect on the mice, the content of **ciguatoxin** was low and didn't affect to the quality of fish for consumption.

For the extracts of viscera, toxic level and effect to the mice was commonly higher and stronger than that of muscle. However, the toxin content was also low, the range of toxic levels was from 1-5, and the content was approximate to 0 - 5 MU.

The extract of viscera of Tomato hind (Cephalopholis sonnerati), Golden rabbitfish (Siganus guttatus) (April - May) caused the almost symptoms of **ciguatoxin** on mice and made mice die less than 8 h. With the clear, enough strong symptoms and the ability of making mice die, this confirmed that the **ciguatoxin** content of viscera of some species may be high.

From the viscera of Golden threadfin bream (Nemipterus virgatus) (June - July), the extract caused death in mice within 13h. The extract from viscera of White edged lyretail (Variola albimarginata) caused the strong and longer symptoms on mice, including the diarrhoea, mice died within 14 h (June - July) and 19 h (August).

Although the effect to mice was at the different levels, generally the quality of fish is good for consumption. However, the viscera of some species should be discarded.

Thus, for 13 species of fish collected to assess the quality in terms of **ciguatoxin** during April to August 2000, almost extracts of muscle caused the mild symptoms on mice. The results showed that the quality of muscle was good. The extract of viscera of some species was more toxic than that of muscle. This was confirmed by the studied results (Hokama Y. et al., 1994). Among the extracts from viscera, Siganus guttatus should particularly be paid attention to because the death time of mice was short. This species has the large food chain, **ciguatoxin** from viscera may be extracted from the foods in stomach partly.

With two species of Tomato hind (C. sonnerati) and White edged lyretail (V. albimarginata) belonging to carnivorous group, the viscera should be carefully used because of their high ability of accumulating **ciguatoxin** via food chain. Particularly, only the viscera of Golden threadfin bream (N. virgatus) (the herbivorous fish) was determined to have **ciguatoxin** in this study (caused death in mice). Although the viscera are not greatly valid in use, this reflected the trend of accumulation of **ciguatoxin** in some fish species in Nha Trang markets during the collecting time.

3. The rate of the contaminated samples and died mice

Assessing the toxic level, the symptoms on the mice of the samples which had the most toxic level (caused death in mice) did not still include all the specific types of c**iguatoxin**. Among 20.51% died mice caused by extracts from viscera (16/78 mice), only 3/16 (18.75%) had the hind limb paralysis, the rest 13/16 (81.25%) did not have this symptom. According to Hokama Y. et al., 1994, among the mice had the hind limb paralysis, the rate of mortality was up to 93%, the death time was within 6 h. While only 51% **ciguatoxin** intoxicated mice which did not include the hind limb paralysis died, the death time was within 24 h. In this study, the extract from viscera of Tomato hind (Cephalopholis sonnerati) should be studied further.

Table 7: The rate of died mice by bioassay of samples

Amount of bioassays		Total mice for bioassay		Total died mice (%)		Total survival mice (%)	
Muscle	Viscera	Muscle	Viscera	Muscle	Viscera	Muscle	Viscera
26	26	71	78	0 (0%)	16 (3*) (20.51%)	71 (100%)	62 (79.49%)

Among 52 studied samples (26 muscles, 26 visceras), only 5/16 of viscera (19.23%) caused death in mice less than 24 h (1-5 MU), 21/26 of the rest (80.77%) had not **ciguatoxin**. All muscles (26/26 = 100%) were negative and safe to consume.

Comparing to the studies of ciguatoxin of some different fish species in other areas such as Hawaii Islands for Caranx sp. and Seriola dumeriili (in the total of 292 examined fishes, 86 (29%) were negative while 206 (71%) were considered non-edible or rejected. Caranx sp. alone (146 specimens) showed 72% in the high level of **ciguatoxin** while 28% were in the edible group (Hokama et al., 1990)); and in the result of Hong T. W. P. et al., 1994 (from 1991 –1994, 107 (13.8%) of total 777 Caranx sp. were found to be edible while 86.2% (670) were considered to be non-edible), the rate of non-edible fish in Nha Trang was low.

IV. CONCLUSION

During the time (from April to August 2000) for collecting the samples of fish to control the quality in terms of **ciguatoxin** at the Nha Trang markets by using the mouse bioassay, the results showed that:

- All muscles of fish were not poisonous in terms of **ciguatoxin**, manifested by the light intoxication or no effect to mice. Some samples such as Tomato hind (Cephalopholis sonnerati), Pinecone soldier fish (Myripristis murdjan), the toxin concentration was at the 3rd level (0.5 MU) but it didn't affect to the quality of fish.

- The extracts of viscera of almost species had the effect stronger than that of muscle, manifested by the clear and longer symptoms. Particularly, the extract of Tomato hind (Cephalopholis sonnerati), White edged lyretail (Variola albimarginata), Golden threadfin bream (Nemipterus virgatus), Golden rabbitfish (Siganus guttatus) caused death in mice with the short time (within 6 – 19 h) should be paid attention to.

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