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Toxicological studies on puffer fishes, *Lagocephalus sceleratus* and *Amblyrhynchotes hypeslogenion* in Suez Gulf, Red Sea, Egypt.

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Received 1st December 2010, Accepted 5th May 2011

Abstract

Thirty five samples of puffer fish *Lagocephalus sceleratus* (Gemelin, 1789) and twenty two samples of *Amblyrhynchotes hypeslogenion* (Blecker, 1852) were collected from El Attka Port, Suez Gulf, Red Sea from September 2007 to May 2008, using mouse bioassay technique. The toxicity of *L. sceleratus* showed the highest value for ovaries (666.9 MU/g), intestine (555.7 MU/g) and liver (573.3 MU/g). The muscle and skin had the lowest toxicity (170 and 153.4 MU/g respectively). The second species *A. hypeslogenion* showed toxicity values: 270, 230.4, and 220 MU/g for skin, liver and muscles respectively.

Keywords: Tetrodotoxin, puffer fish, toxicity of fishes, Red Sea fishes, *Lagocephalus sceleratus*.

1. Introduction

Puffer fish toxins (TTX) have drawn scientists' attention due to their involvement in human intoxication and socioeconomic impacts brought by those incidents. TTX has frequent involvement in fatal food poisoning all over the world (Zaki, 2004 and Sasaki *et al*., 2007). It is naturally occurring in a wide variety of animals as well as puffer fish (Noguchi *et al.*, 2006).

The toxicity of puffer fish has individual, anatomical, geographical and seasonal variations (Hashimoto and Kamiya, 1970). The majority of reported cases of human poisoning have occurred in Southern Asia including Malaysia, Taiwan, Hong Kong and Korea (Kan *et al.*, 1978, Yang *et al.*, 1996 and Kanchanapongku, 2001). Also many poisoning cases by puffer fish were recorded in Egypt (Ali, 1996 and Zaki, 2004).

The origin of TTX in puffer fish is still controversial whether it is exogenous (Ali *et al.,* 1990; Ali, 1991; Yasumoto and Yotsu-Yamashita, 1996), or endogenous (Matsumura, 1998), or it may have bacterial origin (Yang *et al.*, 2007).

Thirteen tetraodontid species of puffer fish inhabit the Egyptian Red Sea, Suez Canal, River Nile and Mediterranean Sea. Those represent 13 % of the total number of species reported all over the world (Mohamed, 2003). There were some studies on toxicity of some species of puffer fish in our area, these studies showed that some species have toxic liver, gonad, intestine, skin and muscles with anatomical and seasonal variation in toxicity (Sherif *et al.,* 1994; Ali *et al.*, 1995; Kotb, 1998 and Sabrah *et al.*, 2006).

Due to many poisoning cases recorded in Egypt particularly at last months, the present study comes to throw light on toxicity of two species of puffer fish *L. sceleratus* and *A. hypeslogenion.*

2. Materials and Methods

2.1. Sampling

Samples of puffer fish *L. sceleratus* were monthly collected from local fishermen from Al Attka Port, Suez Gulf (map for study area figure 1). *A. hypeslogenion* were seasonally obtained from local fishermen at the area near the National Institute of Oceanography and Fisheries, Suez.

The total length and weight for fresh samples were directly measured and kept in plastic bags at -20 C°. *L. sceleratus* (35 samples) ranged between 14 and 53 cm in length and in weight between 120 to 2230 gm. The 35 samples were 16 males and 19 females. *A. hypeslogenion* (5 males, 9 females and 8 non differentiated sex samples) with length ranged between 7.5 to 13.5 cm and weight ranged between 6.1 to 42 g.

2.2. Mouse Bioassay Method

Toxicity of different organs (muscle, skin, liver, gonad and digestive tract) was measured according to Japanese Official Mouse Bioassay Method for Tetrodotoxin in puffer fish (Kawabata, 1978). One gram from each organ was individually extracted with

5 ml of glacial acetic acid (1%) and heating for 10 min in water bath at 100 C°. The mixture was cooled down to room temperature and filtrated. The muscle, skin and liver were also tested for *A. hypeslogenion*.

One ml of the filtrate solution was intraperitoneal (IP) administrated into each of three Albino Swiss male mice weighing 20±29 were obtained from mice was observed for symptoms and time of death was recorded. The median death time (the period between injection and death) was used to calculate the mouse units (MU). The toxicity level of samples, expressed in MU/g, was calculated from the dose-death time relationship table of Kawabata (1978) where 1 MU was defined as the amount of toxin required to kill 20 g male mouse in 30 minutes after IP injection.

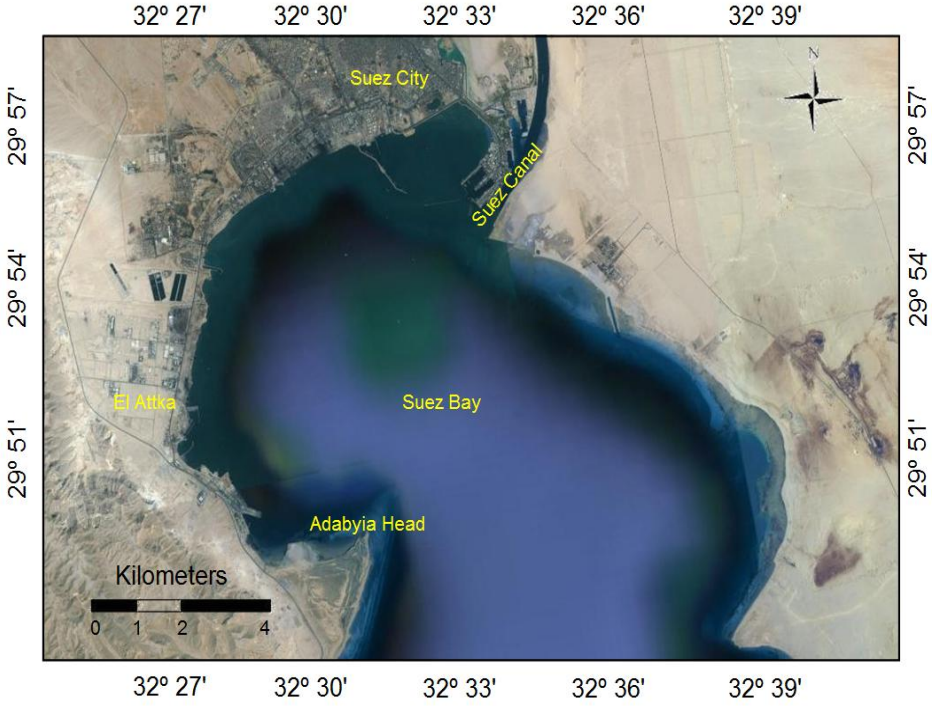

Figure 1. Map for the study area.

3. Results

3.1. *Lagocephalus sceleratus*:

Toxcicity of samples examined in this study were reported in Table (1). About fifty seven percent of samples were toxic. It means that out of nineteen investigated females, thirteen were toxic (about 68.4%) and out of sixteen investigated males seven were toxic (about 43.7%).

Anatomical distribution of toxicity.

Eight samples (22.8%) of muscles from 35 examined samples were toxic. The toxicity level ranged between 5 and 170.05 MU/g. Three females were strongly toxic and five samples were weakly toxic (three females and two males). The highest toxicity level 170.05 MU/g was recorded in March, 2008.

Nine samples of skin (25.7 %) from the 35 samples were toxic (5 to 153.4 MU/g). Two females were strongly toxic, four females and three males were weakly toxic. The highest toxicity level was 153.4 MU/g which was recorded in December, 2007.

Eleven samples of liver from 34 examined were toxic. About 32.3% of samples have toxic liver. The toxin content was ranged between 5 and 573.7 Mu/g. Three females were strongly toxic, six females and two males were weakly toxic. The highest toxicity level was 573.7 Mu/g, was recorded in February.

Eleven samples of ovaries (61.1 %) from eighteen examined were toxic. The toxicity range was between 5.7 and 666.9 MU/g. Eight of samples were strongly toxic and three were weakly toxic. The highest toxicity level 666.9 MU/g was recorded in March, 2008.

Four samples of examined testes were toxic. About 50 % of them had toxic testes. The toxicity range was

between 5 to 49.5 MU/g. The four toxic samples were weakly toxic. The highest toxicity level 49.5 MU/g was recorded in April, 2008.

Thirteen samples of digestive tract (D.T) examined were toxic. 37.15 of them had toxic D.T. the toxicity range was between 5.7 to 555.7 MU/g. Four females were strongly toxic, five females and four males were weakly toxic. The highest toxicity level 555.7 MU/g was recorded in March, 2008.

3.2. *Amblyrhynchotes hypeslogenion*

The number of samples of this puffer fish examined in this study were twenty two. Twenty samples were toxic and two were non toxic with 90.9% toxicity percentage (Table, 2).

Anatomical distribution of toxicity:

Eighteen samples of examined muscles were toxic. About 81.8 %of samples have toxic muscles. The toxicity range was between 9.7- 220 MU/g. There were eight strongly toxic samples (4 females, 2 males and 2 undifferentiated sex), 10 samples were weakly toxic (four females, two males and four undifferentiated sex), and four samples were non toxic. The highest toxicity level 220 MU/g was recorded in March.

Nineteen specimens of examined samples for skin were toxic. About 86.3% of samples have toxic skin. The toxicity range was between 7.11- 283 MU/g. There were five strongly toxic samples (three females and two males), and 14 were weakly toxic samples (6 females, 2 males and 6 undifferentiated sex). The highest toxicity level (283 MU/g was recorded in November, 2007.

Samples of liver have ten toxic ones. The percent of toxic samples was 71.4%. The toxicity range was between 6.54- 230.4MU/g. There were four strongly toxic samples (two males and two females), and six weakly toxic samples (one male, three females and two undifferentiated sex samples). The highest toxicity level (230.4 MU/g was recorded in March, 2008.

Table 1. The toxicity of different organs of *L. sceleratus* using mouse bioassay technique.

Date	No	Length (cm)	Weight (gm)	Sex	Toxicity MU/g				
					Muscle	Skin	Liver	Gonad	D.T
9-2007	1	21	215	M	<5	<5	<5	*	<5
	2	18	168	M	<5	<5	<5	*	<5
10-2007	3	26	266.2	F	<5	5.4	5.25	5.7	5.7
	4	24	220	F	<5	5.7	9.3	6.27	5.8
	5	20	132	M	<5	<5	<5	9.9	6.27
11-2007	6	31	315	F	<5	5.7	6.27	54.02	6.27
	7	37	450	M	<5	<5	8.2	20.73	13.14
	8	34	470	F	10.23	7.5	7.5	*	5.7
	9	34	437	F	<5	<5	19.25	109.25	125.4
	10	33.5	400	M	5.7	11.2	57	*	6.8
12 -2007	11	38	540.3	F	136.25	153.4	188	173.3	146.47
	12	36	567	M	<5	<5	6.2	6.8	4.8
	13	32	347	M	<5	<5	<5	*	<5
1-2008	14	39	810	F	<5	<5	7.64	<5	<5
	15	40	700	M	< 5	< 5	26.7	< 5	76.5
	16	37	620	F	7.4	49.7	10.23	58.5	7.64
2 - 2008	17	39	698.5	F	108.2	112.6	573.7	109.2	120.3
	18	36	530	F	7.6	< 5	10.75	4.7	10.23
	19	36	485	M	< 5	< 5	< 5	< 5	< 5
	20	17	120.5	M	< 5	< 5	*	*	< 5
	21	22	137	M	< 5	< 5	< 5	*	< 5
3 – 2008	22	53	1300	M	14.8	5.2	5.7	19.9	13.7
	23	50	1820	F	170.05	82.8	98	666.9	555.7
	24	52	2230	F	73.5	57.6	48.4	196.9	88.3
	25	49	2200	F	6.8	12.13	8.05	281	11.3
4 – 2008	26	40	630	F	< 5	< 5	< 5	< 5	< 5
	27	36	400	F	< 5	< 5	112.7	168.02	36.4
	28	36	410	M	< 5	17.5	5.7	49.5	5.7
	29	48	815	F	< 5	< 5	< 5	< 5	< 5
	30	29	312	M	< 5	< 5	< 5	16.8	< 5
	31	17	178	M	< 5	< 5	< 5	*	< 5
	32	28	305	F	< 5	< 5	19.5	58.7	27.2
5 – 2008	33	18	153	F	< 5	< 5	< 5	< 5	< 5
	34	14	120	M	36.8	21.7	7.5	*	49.7
	35	49	730	F	19.7	5.7	< 5	158.2	< 5

(F = Female, M = Male, * not tested sample. D.T. digestive tract)

Table 2. The toxicity of different organs *in Amblyrhnchotes hypselogenion*.

Date	No	Length(cm)	Weight(g)	Sex	Toxicity MU/g		
					Muscle	Skin	Liver
8 - 2007	1	8.3	15.1	F	20.7	21.9	*
	2	8	10.4	M	97.9	23.5	13.14
	3	7.8	10.6	Th	30.9	30.9	*
	4	8.5	10.2	Th	23.5	20.7	*
	5	9	10.7	Th	129.06	93.5	*
	6	7.5	8.8	Th	175	27.5	19.69
	7	9	13.6	F	23.5	30.9	*
	8	8	7.9	F	21.34	27.5	*
	9	8.5	9.7	M	109.1	28.2	*
	10	7.5	6.1	Th	25.63	12.21	*
11 – 2007	11	13.5	46.2	F	9.7	26.02	28.02
	12	8.2	11.2	F	10.07	283.5	14.35
	13	8	10	Th	< 5	7.11	6.54
	14	8	9.9	M	< 5	< 5	< 5
	15	8.5	10.1	Th	9.7	25.1	8.2
1 – 2008	16	12	35	F	147.5	114.17	28.1
	17	11.5	32	F	185	78.7	6.27
	18	10.2	21.5	Th	22.05	8.05	13.2
	19	9.8	13.6	M	73	119.2	220
3 – 2008	20	13	40.3	F	220	270	136.7
	21	13.5	42	F	179.3	81.9	108.7
	22	12.5	40	M	113.2	226.7	230.4

M= male, F=female, Th= thread like., *= non tested sample

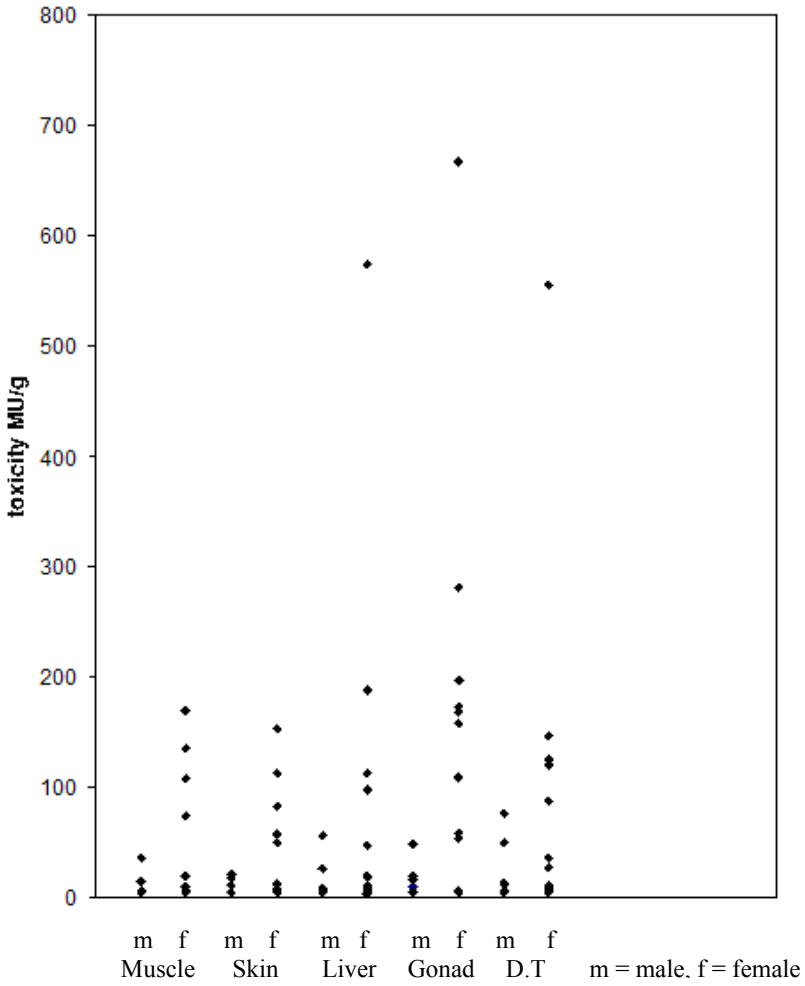

Figure 1. Anatomical variation in *L. sceleratus* toxicity.

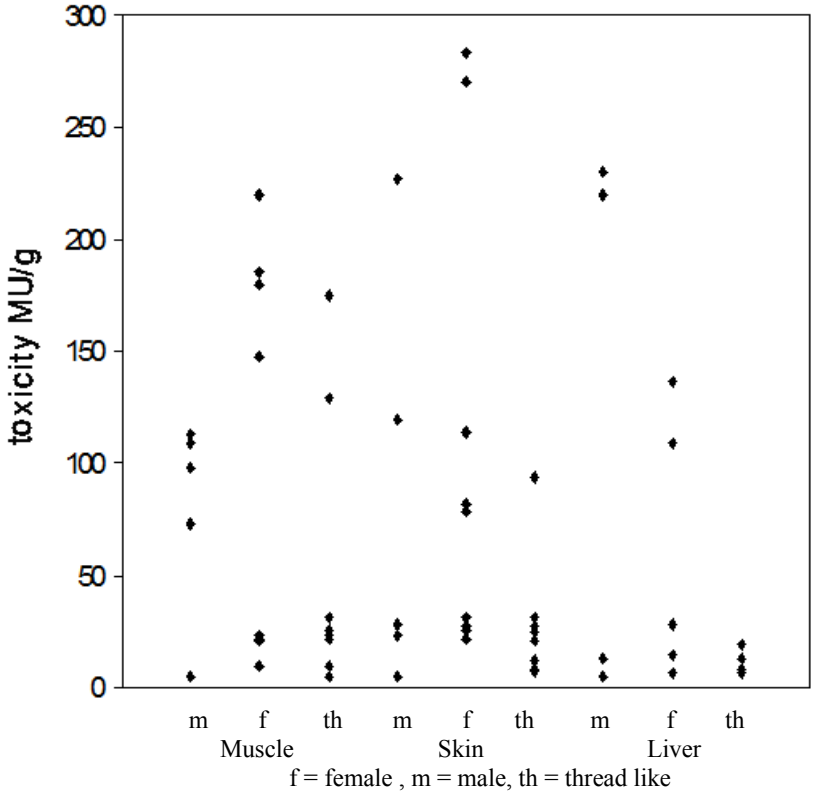

Figure 2. Anatomical variation in *A. hypselogenion* toxicity.

4. Discussion

A lot of studies all over the world have carried out on many species of puffer fish and other organisms containing TTX and confirmed that the toxicity of these species is due to marine neurotoxin TTX, (Halstead, 1978, Mosher *et al.*, 1984 and Noguchi *et al.*, 2006).

The present study tested the toxicity of two species *L. sceleratus* and *A. hypeslogenion*. The most toxic organ was the ovary (666.9 MU/g). These results agreed with Kanoh *et al*. (1984); Fuchi *et al*. (1988); Ali *et al.* (1995); Kotb (1998); Sabrah *et al*. (2006) and Ikeda *et al.* (2010).

There were variations in toxicity of individuals at the same season and also in toxicity of organs in the same individuals. The seasonal and anatomical variations of toxicity were recorded in *L. sceleratus*. The highest toxicity levels for ovaries, muscles, and digestive tract were recorded during March (spring). This result agreed with Kodama *et al*. (1983); Kotb (1998) and Sabrah *et al*. (2006) who found that the toxicity generally begins to increase just before the spawning activity and then sharply decreased activity. The highest toxicity score for liver (573.7) and skin (153.4) were recorded during February (2008) and December (2007) respectively (Table 1).

A. hypeslogenion samples showed the highest toxicity score at all organs; muscles, skin, and liver respectively at spring season. The highest toxicity value, which recorded for skin, agreed with that reported by Shiomi *et al*. (1985) and Hwang *et al*. (1988), for *L. lunaries* and *A. hypeslogenion* respectively. The toxicity of skin of puffer fish and other animals containing TTX may be due to presence of parasitic bacteria which is responsible for producing TTX or may be due to presence of TTX secretory glands on the skin (Tanu *et al.*, 2002).

L. sceleratus is used as food in many countries especially in Egypt, Red Sea sector. This species shouldn't be eaten especially the large sample which reachs the maturity stage (more than 40 cm, Sabrah *et al.,* 2006) because it sometimes has moderately toxic muscles (more than 100 MU/g)especially during spring and summer. This agreed with El Sayed *et al.* (2003) as they also recorded the highest toxicity value for muscles during March (127 MU/g). Other organs like gonads and liver can be used as a source of TTX which have many pharmaceutical uses as it has analgesic activity as it is highly selective sodium channel blocker (Neil *et al.*, 2007).

According to many cases of poisoning which take place recently in Egypt at Suez, Marsa matrouh, Alexandria, and Port Said (reported by the Egyptian

news papers through 2008, 2009, and 2010) the monitoring for fishing of these species should be regularly taken place.

Acknowledgment

I am deeply grateful to Ass. Prof. Dr. Abdallah Elsayed Ali assistant professor of marine biotoxins, Marine science departement, faculty of science, Suez Canal University. For his valuable suggestion and for his keen supervision. Iam also deeply greatful to Prof. Dr. Mohamed Naser El-din Gomaa, Professor of marine toxins food toxicology and contaminants departement, national research center. For his kind supervision.

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دراسات على سمية اسماك القراض لاجوسيفالس سكليراتس و امبليرنكوتس هيبسلوجنيون عائلة التترادونتيدى فى خليج السويس البحر الاحمر

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قمنا خلال هذه الدراسة باختبار سمية نوعين من أسماك القراض لاجوسيفالس سكليراتس و امبليرنكوتس هيبوسلوجنون حيث تم تجميع العينات من الصيادين العاملين فى ميناء الأتكة فى خليج السويس والبحر الأحمر الإختبار باستخدام الفئران 20جرام طبقاً لـ kawabata سنة 1978 وكانت النتائج كالتالى أعلى سمية فى النوع الأول كانت فى المبيض 666.9 وحدة فأر ثم الكبد 573.3 ثم المعدة 555.6وحدة فأر أما عن العضلات والجلد فكانت أقل سمية وهى 170 و 153.4 وحدة فأر على التوالى أما النوع الثانى أعلى سمية كانت كالتالى 270 و 230.4 و 220 وحدة فأر للجلد ، الكبد ثم العضلات على التوالى.