Toxicity and Toxin Properties Study of Puffer Fish Collected from Sabah Waters

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ABSTRACT: Fatal food poisonings associated with the consumption of puffer fish have occurred for decades in Malaysia, but the causative species or toxins have never been documented. Herein, we investigated the toxicity on *Lagocephalus lunaris*, *Lagocephalus spadiceus* and *Lagocephalus sceleratus* collected from the coastal waters of Sabah. Toxicity assessment by mouse bioassay revealed that *L. spadiceus* was non-toxic. The toxicity scores of toxin extracted from muscle, liver and skin of *L. spadiceus* were all less than 2 MU/g and the toxin is considered too mild to be detected since the mice do not die in 30 minutes after injection. However, the mice showed the symptoms of having neurotoxin infection. The toxicity level of two other species remains unknown and the research is in progress. Further study about the toxicity of puffer fish is carried out using High Performance Liquid Chromatography (HPLC) and Liquid Chromatography/ Mass Spectrometry (LC/MS) in order to verify the toxicity level in different part of puffer fish tissues.

Keywords: Mouse Bioassay, Puffer fish, Tetrodotoxin (TTX)

Introduction

Puffer fish sometimes posses a strong toxin which is responsible for the puffer poisoning and most of the cases of poisoning have been reported to be caused by marine species of the family Tetraodontidae (Noguchi and Ebesu, 2001). There are 185 species and 28 genera of puffer fish in the family Tetraodontidae (Oliveira et al., 2006). Among the 185 species, *Lagocephalus lunaris*, *Lagocephalus spadiceus* and *Lagocephalus sceleratus* are the three most common puffer fish species in Malaysia and the former are eaten by some locals leading to several poisoning cases including a few fatalities being reported (Kan et al., 1987).

At least 16 species of puffer fishes have been recorded in Malaysia (Cantor, 1849; Scott, 1959) and several studies related to the toxicology on puffer fish were carried out in this country. The first documented poisoning case in Malaysia was reported to be occurred in Sabah with four intoxications reported in 1985 (Lyn, 1985) and nine fatal cases in 1987 (Kan et al,. 1987). Both cases are resulting from onsumption of puffer fish.

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In early 1970s, three species of puffer fishes, *Lagocephalus lunaris lunaris*, *L. lunaris spadiceus* and *Arothron stellatus* collected from west Malaysia waters were found to be toxic and caused mouse lethality (Berry and Hassan 1973), however, detail toxin properties verification was not carried out during these study.

In this study, we investigate the toxicity and toxin properties of puffer fish collected from Sabah waters with the aim to verify the toxicity level of the fish.

Materials and Methods

Study area

Sampling was carried out at Mandi Darah Island, Sabah. Mandi Darah Island is a small island which is located in Kudat Division. A total of 96 samples of *Lagocephalus lunaris*, *Lagocephalus spadiceus* and *Lagocephalus sceleratus* were caught from the site with the help of laboratory assistant and fishermen. The geographical co-ordinate of the sampling site from station 1 is N 06'56.342' and E 117° 19.241', station 2 is N 06' 55.901' and E 117° 19.942' and station 3 is N 06' 55.402' and E 117° 18. 130'.

Field Sampling

A total of 96 puffer fish comprising *Lagocephalus lunaris* (39), *Lagocephalus spadiceus* (30) and *Lagocephalus sceleratus* (27) were collected in July 2009 at Pulau Mandi Darah, Sabah using a bottom trawl net. After hauling, the catch was removed and

sorted into species groups. The puffer fish specimens were kept frozen at -20°C during field trip and transferred to the laboratory for fish identification and toxin extraction analyses.

Fish identification

Puffer fish were identified as *Lagocephalus lunaris*, *Lagocephalus spadiceus* and *Lagocephalus sceleratus* based on FRI (2004), Atack (2006) and Ngy et al. (2008). Different species were identified according to their morphological characteristics including the distribution patterns of small spines on dorsal. The body weights (BW), standard length (SL) and total length (TL) were measured with the total length (TL) being measured to the nearest 0.1 cm and body weight (BW) to the 0.1 g, respectively.

Toxicity analysis

All samples were partially thawed and dissected separately into muscle, skin, liver, intestine and gonads. Each tissue was examined for its toxicity by mouse bioassay and the lethal potency by the Japanese Standard Method for TTX (Kawabata, 1978) or the AOAC for PST (Williams, 1984). The total weight of each tissue was recorded before being minced into small portion at about 5.0 g. The sample was then extracted with an equal volume of 0.1% acetic acid by heating in a boiling water bath for 5 minutes. The slurry was centrifuged at 10 000 *g* for 15 min to obtain the supernatant. The collected supernatant was later used for TTX detection. The mice used for the bioassay were male of the ddY strain each weighing around 20 g. The time and condition of the mice after injection were recorded. One milliliter of the puffer fish extracts were injected intraperitoneally. Symptoms were noted and the time of death was recorded at the last gasping breath of the mouse. The toxicity was expressed as mouse unit (MU). For both TTX and PST, one MU is defined as the amount of toxin required to kill a 20 g mouse in 30 min and 15 min, respectively.

Result and Discussion

The puffer fish samples collected in this study were identified as three different species namely *Lagocephalus lunaris, Lagocephalus spadiceus* and *Lagocephalus sceleratus*, which are common species in East Malaysia Waters. Note that both *Lagocephalus lunaris* and *Lagocephalus spadiceus* can de differentiated based on their morphological characteristics such as the distribution patterns of small spines in their dorsal bodies (Ngy et al., 2008). For *L. lunaris*, the distribution patterns of small spines are elliptical shape that extends to the base of the dorsal fin ray. *L. spadiceus* differs from *L. lunaris* based on the tadpole like shapes pattern whose tail reaches the base of the dorsal fin ray (**FIG. 1**). Basically *L. lunaris* and *L. spadiceus* are the same in morphological characteristics except spine distribution pattern. The shape of the body is relatively elongate with a broad head which is a little box shaped and the eyes are large rounded with an orbital edge at the lower part. The body is metallic gold colour, darker on the back and sides, and almost silvery gold colour underneath. A broad silvery band runs longitudinally along the mid lateral line from the mouth to the base of the caudal fin. Due to the weak condition of fish, we could not determine if they were male or female. Note that it is difficult to determine the gender at a glance since the sex of *Lagocephalus* genus can only be differentiated based on colour and body size. However, puffers often have tendency to have sexual dimorphism so the male tends to be brighter than female (Thresher, 1984). In this study, a few individuals were confirmed as female with the presence of eggs.

A total of 27 individuals of *Lagocephalus sceleratus* were collected. In general, the body is elongated, slightly compressed laterally and inflatable. Scales are absent, but the body is covered with small spines on the belly. The body is brownish in colour with black, regularly distributed spots of equal size dorsally and a clear wide silver band being present on the lower parts of the side, from the mouth to the caudal fin. Also, a silvery ring is clearly present in front of the eyes and the belly is white.

Toxicity assessment by mouse bioassay revealed that *L. spadiceus* was non-toxic (less than 2 MU/g; data not shown). The toxicity scores of toxin extracted from muscle, liver and skin of *L. spadiceus* were all less than 2 MU/g and the toxin is considered too mild to be detected. The mice survived in 30 min after injection but showed some symptoms of having neurotoxin infection including suffocation and cramp. At the first 10 min after injection, the mice appeared weak and started to have cramps on their hind legs and continued with the slight suffocation after that as evident by their attempt to jump to get more oxygen from outside. However, this condition did not stay for long and the animals appeared to recover slowly and active again, probably due to insufficient toxin concentration in *L. spadiceus* sample to cause death to the mice. All the results show that *L. spadiceus* is considered non toxin and this finding is in agreement with that of Japan Food Hygiene Association and Tani classification of puffer fish toxicity in Japan (Othman et al., 2006).

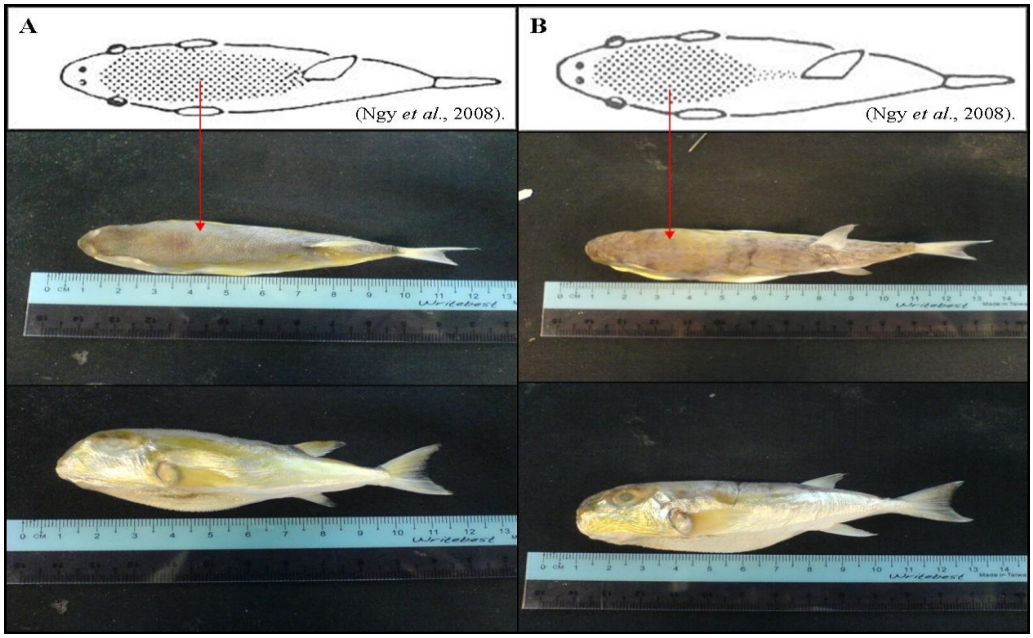

FIG. 1- Puffer fish of the genus *Lagocephalus* collected from the coastal water of Mandi Darah Island, Sabah. (A) Species identification for *L. lunaris* and (B) *L. spadiceus* are based on the morphological characteristics, i.e., distribution patterns of small spines in their dorsal bodies.

Conclusion

Samples collected from Mandi Darah Island, Sabah was identified as *Lagocephalus lunaris*, *Lagocephalus spadiceus* and *Lagocephalus sceleratus*. Tissues of *Lagocephalus spadiceus* are considered non-toxic as their toxic level is too low to cause fatal to mice. However, the level toxicity of two other species remains unknown and the research is in progress. Further study about toxicity of puffer fish will be carried out by using High Performance Liquid Chromatography (HPLC) and Liquid Chromatography/ Mass Spectrometry (LC/MS) by our research group in order to verify the toxicity level in different tissues of puffer fish that commonly consumed, especially in Sabah and Sarawak.

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