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Toxicity and toxin identification in *Colomesus asellus*, an Amazonian (Brazil) freshwater puffer fish

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Abstract

Toxicity and toxin identification in *Colomesus asellus*, an Amazonian (Brazil) freshwater puffer fish. By using four different techniques—mouse bioassay, ELISA, HPLC and mass spectrometry—we evaluated the toxicity in the extracts of *C. asellus*, a freshwater puffer fish from the rivers of the Amazon, and identified for the first time the components responsible for its toxicity. The T20G10 monoclonal antibody raised against TTX, and employed in an indirect competitive enzyme immunoassay, showed very low affinity for the *C. asellus* extracts, indicating that TTX and its analogs are not the main toxic components of the extracts. This antibody was efficient in detecting presence of TTX in a total extract of *Sphoeroides spengleri,* which is one of the most toxic puffer fish found in the Atlantic coast. Extracts of *C. asellus* were toxic when administered intraperitonially into mice with an average toxicity of 38.6 ± 12 mouse unit (MU)/g, while HPLC analysis indicated a lower toxin content ($7.6 \pm 0\ 5$ MU/g). The HPLC profile showed no traces of TTX, but only the presence of PSPs (STX, GTX 2 and GTX 3). These toxins were also confirmed by electrospray ionization mass spectrometry.

Keywords: *Colomesus asellus*; *Sphoeroides spengleri*; ELISA; Freshwater puffer fish; Saxitoxin; Gonyautoxin; HPLC; Mass spectrometry

1. Introduction

Puffer fishes are poisonous and may cause a characteristic clinical poisoning with a high mortality rate, but in Japan and China, have long been a delicacy (Lange, 1990). In Occidental countries, puffer fishes are only sporadically consumed and have been involved in several human poisonings, mainly by the species belonging to the genus *Sphoeroides* (Almeida and Rocha, 1989; Ochoa et al., 1997; MMWR, 2002). Toxinological studies of some *Sphoeroides* species have been reported (Correa et al., 1990; Freitas et al., 2003; Oliveira et al., 2003).

The poison of freshwater puffers may be composed by tetrodotoxin (TTX) or saxitoxin (STX) and its analogs, the predominant toxin being

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dependent on the species. These guanidinium toxins inhibit electrical signaling in many excitable cells (nerves and muscles) by binding to the site 1, blocking the pore of the voltage-gated sodium channels (VGSC) (Cestèle and Catterall, 2000).

The correct identification whether which toxin, TTX or STX, is the main toxic component of a determined puffer poison is not always a simple task. For example, *Tetraodon fangi*, the Thailand freshwater puffer involved in some food poisonings in humans (Saitanu et al., 1991), was first reported to posses mainly TTX as its poison (Laobhripatr et al., 1990). Later, however, the major toxins of this fish were identified as STXs (Sato et al., 1997). Furthermore, other freshwater puffers such as *Tetraodon leiurus complex* and *Tetraodon suvatii* from Thailand and *Tetraodon cutcutia* and *Chelonodon patoca* from Bangladesh may posses many paralytic shellfish poisoning (PSP) toxins (such as STX, neo-STX, decarbamoyl-STX, GTX 2, GTX 3, and decarbamoyl-GTX 2 and 3) (Kungsuwan et al., 1997; Zaman et al., 1997).

Nowadays, at least 185 species of puffer fishes in the family Tetraodontidae, distributed in 28 genera are known. In the genus *Colomesus* there are only two species described (Fishbase, 2005). Both species occur in Brazil: *Colomesus asellus* (Amazon puffer) is an exclusively freshwater puffer living in the Amazon basin, and *Colomesus psittacus* (banded puffer) inhabits brackish waters at the Brazilian northeast coast estuaries. *C. psittacus* was reported to possess an edible flesh and a very toxic liver (Sawaya, 1966), but the toxins and toxicity of *C. asellus* are as yet completely unknown.

In this work, by using four different techniques (mouse bioassay, ELISA, HPLC and mass spectrometry), we report for the first time the high toxicity found in *C. asellus* and the identification of PSPs (STX and gonyautoxins) as the main components of its poison.

2. Material and methods

2.1. Extraction

Twenty-four specimens of *C. asellus* (Müller and Troschel, 1848) were collected from the River Tocantins, in the town harbor of Cametá, Pará State, Brazil, in March of 2003. The total length of animals (in cm) and body weight (in grams) varied from 9 to 15.6 and from 18 to 96, respectively. The extraction procedure was according to Oliveira et al. (2003) with slight modifications. Briefly, six separated extracts were prepared from total body of *C. asellus* as described below (for the number of specimens used and weights, see Table 1).

Extracts were prepared by double extraction with 1% acid acetic in 70% ethanol (2 ml/g), filtered,

Table 1
Toxicity of *Colomesus asellus* extracts (MU means mouse unit) evaluated by different techniques

Sample	Number of specimens	Average body weight (g)	Toxicity				ELISA[a]
			Mouse bioassay		HPLC		
			MU/g	MU/indiv	MU/g	MU/indiv	
1	4	172.00	53.2	2287.5	17.74	764.5	0.0045
2	4	162.60	31.2	1268.3	4.99	202.8	0.0057
3	3	82.60	46.0	1267.5	10.18	280.3	0.0022
4	6	176.80	44.0	1296.7	5.35	157.6	0.0032
5	3	212.20	37.8	2675.0	4.36	308.4	0.0027
6	4	121.50	19.4	590.20	3.23	98.1	0.0064
			38.60 ± 12.01	1564.20 ± 768.49	7.64 ± 5.49	301.95 ± 239.45	0.0041 ± 0.0017
Positive control[b]	285	10.92	45.53	nd	nd	nd	93.77

Six independent total body extracts of *C. asellus* were analyzed by mouse bioassay, high-performance liquid chromatography (HPLC) and enzyme-linked immunosorbent assay (ELISA), and the toxicity expressed as mouse unit per gram of fresh tissue (MU/g) or per mean of body weight from one specimen individually (MU/indiv). A total body extract of several specimens of *Sphoeroides spengleri* was employed as positive control.

[a]Except to the *S. spengleri* extract that was based on it's IC_{50} (converted to MU/g of fresh tissue), the values were obtained from the highest concentration of each extract.

[b]Extract of *Sphoeroides spengleri* (positive control); nd, not determined.