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Short communication

Toxicities and distribution of tetrodotoxin in the tissues of puffer fish found in the coast of the Baja California Peninsula, Mexico

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Abstract

Toxicities and tetrodotoxin distribution in tissues of five puffer fish species commonly found in the littoral of Baja California Peninsula, Mexico (*Sphoeroides annulatus, S. lobatus, S. lispus, Arothron meleagris* and *Canthigaster punctatissima*) were evaluated by bioassay and HPLC. The toxicities estimated as tetrodotoxin-equivalents of all species were more than 0.42 μg/g in at least one of the tissues tested, and the highest was found in S. lispus liver (130 μg/g). © 1999 Elsevier Science Ltd. All rights reserved.

The puffer fish species found in Mexican coastline have been considered edible and non-toxic, thus, special regulation for its consumption or preparation as food to prevent poisoning does not exist at present. Although there is a lack of systematic documentation of fish poisoning cases in Mexico, 18 human poisoning cases have been officially registered during the last 30 years in the state of Baja California Sur alone. Mexico is the second largest puffer fish exporter in the world

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now. The puffer fish fishery in the Peninsula of California increased from 33 tons in 1993, to 198 tons in 1997, bringing a higher risk for human poisoning in the area considering that people are not advised about its consumption. Goe and Halstead (1953) carried out a qualitative study about *Sphoeroides annulatus* toxicity in the Gulf of California describing its tissue distribution in liver, muscle and intestine. In this study, we aim to provide the first information with regard to toxicity level and tetrodotoxin (TTX) tissue distribution in the most common puffer fish found in northwest coastline of Mexico.

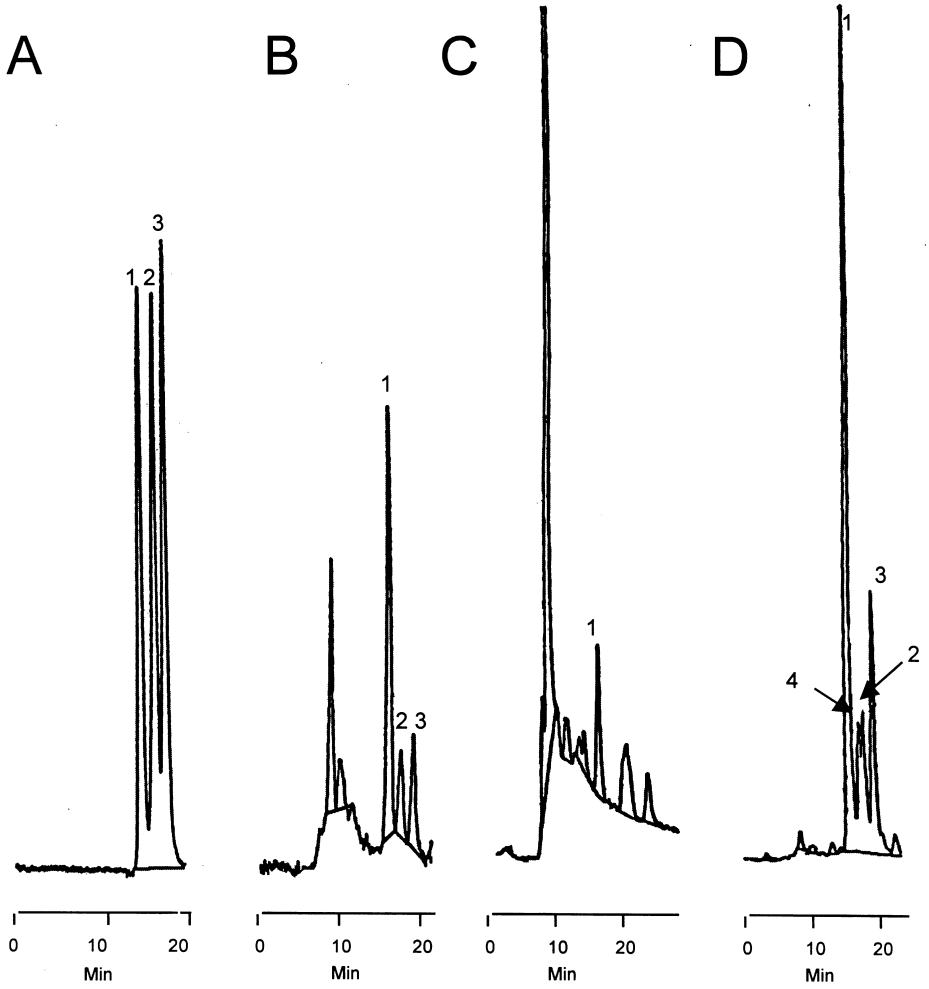

Fig. 1. Chromatograms of standard TTX mixture and sample of puffer fish. (A) Mixture of TTX (0.64 μg), 4-*epi*TTX (1.0 μg) and 4,9-anhydroTTX (1.5 μg), (B) muscle of *A. meleagris* (black phase), (C) liver of *S. annulatus*, (D) intestine of *A. meleagris* (black phase). (10 μl each). TTX (1), 4-*epi*TTX (2), 4,9-anhydroTTX (3) and 11-norTTX-6(S)-ol (4) are indicated.

The puffer fish were collected along the littoral of the Baja California. One specimen of *Arothron meleagris* in the black phase, and one in the golden phase, were captured at Punta Pericos (the reason for the difference in color in the same species remains unexplained and is not known whether this behavior serves an ecological purpose; Goodson, 1988). Five specimens of *S. lispus*, three specimens of *S. lobatus*, twelve specimens of *S. annulatus*, and ten specimens of *Canthigaster punctatissima* were captured at Ojo de Liebre lagoon, El Pardito island, S. Juan de la Costa, and Gaviota island, respectively. All fishes were frozen and transported to the laboratory for toxin extraction and analysis. Defrosted fish were dissected, and the different tissues collected (liver, intestine, gonads, muscle, and of mucus of the skin). The same tissues from one species were mixed, and 100 g of each (or

Table 1
Distribution of TTX and TTX-like compounds in tissues of puffer fish found in the littoral of the Baja California peninsula, Mexico

		Bioassay Equiv. (μg/g)	HPLC (μg/g)		
Species	Tissue	TTX	TTX	4-*epi*TTX	4,9-anhydroTTX
Arothron meleagris (black phase)	Mucus	47	34	10	75
	Muscle	4.3	5.0	–	3.3
	Liver	2.7	4.3	–	3.3
	Intestine	67	52	13	38
A. meleagris (goldhen phase)	Mucus	20	2.5	2.0	5.5
	Muscle	2.9	1.0	0.65	–
	Liver	0.85	1.2	–	–
	Intestine	2.1	0.90	1.5	–
	Gonad	1.2	–	–	–
Sphoeroides annulatus	Mucus	–	–	0.5	–
	Muscle	–	–	–	1.7
	Liver	22	6.9	–	2.3
	Intestine	0.42	0.55	3.0	1.0
	Gonad	0.46	–	0.5	–
S. lispus	Mucus	5.0	1.8	2.5	–
	Muscle	2.3	2.5	–	1.0
	Liver	130	160	7.0	55
	Intestine	10	11	5.5	2.5
	Gonad	12	13	–	1.5
S. lobatus	Mucus	0.42	–	–	–
	Muscle	–	–	–	–
	Liver	–	–	–	–
	Intestine	–	–	–	–
	Gonad	–	–	–	–
Canthigaster punctatissima	Mucus	1.1	–	1.4	–
	Muscle	–	–	–	–
	Liver	0.68	–	0.49	–
	Intestine	0.51	0.49	1.0	–
	Gonad	3.9	2.5	–	–

just the amount obtained) separately disrupted and homogenized with a blender machine with 100 ml of 0.1 N HCl, boiled for 5 min, and adjusted to pH 4 with 1 N HCl. The supernatant containing the toxin was obtained by centrifugation at 1100 ***g*** for 5 min and stored in refrigerator until use.

Bioassay was performed according to AOAC method (1995) for Paralytic Shellfish Poison. Swiss Webster male mice weighing 18–23 g each, in groups of 3 animals, were injected intraperitoneally with aliquots of toxin extract to be killed within an interval of 7–15 min. The toxicity was determined by the average surviving time, according to the standard dose–lethal time plot prepared by using the commercial TTX (Sigma Chemical Co., St Louis, MO, USA). One mouse unit estimated by this method was defined to be equivalent to 0.22 μg of TTX, and the toxicity was expressed as the concentration of TTX-equivalents (μg/g fish tissue). For HPLC analysis, each pooled toxin extract (2 ml) was lyophilized, resuspended in 1 ml of 0.05 M acetic acid, and centrifuged at 10,000 ***g*** for 15 min. Aliquots (10 μl) of the supernatant were applied to a fluorometric HPLC for TTX (Yasumoto and Michishita, 1985). Briefly, TTX and its analogs were separated by HPLC with a Develosil ODS-5 (4.6 × 250 mm) column (Nomura Chemical, Seto, Japan) and an aqueous solution containing 3% acetonitrile, 0.045 M ammonium heptafluorobutyrate and 0.05 M ammonium acetate buffer (pH 5.0) as mobile phase. The eluted compounds were sequentially derived to fluorophores by post-column reaction with 4 N NaOH at 105°C in stainless tube, and detected by a fluoromonitor (Ex 365 nm and Em 510 nm). The quantitative determination of TTX, and its chemically equivalents, 4-*epi*TTX, and 4,9-anhydroTTX (Nakamura and Yasumoto, 1985), was carried out by comparison of the authentic mixture of the same toxins under the similar conditions [Fig. 1(A)]. The detection limits of TTX, 4-*epi*TTX, and 4,9-anhydroTTX were 0.2, 0.3, and 0.4 μg/g, respectively.

The toxicities estimated by bioassay and by HPLC are shown in Table 1. The HPLC chromatogram of *A. meleagris* (black phase) muscle is shown in Fig. 1(B) as the representative. The toxicities of all species were estimated more than 0.42 μg/g as TTX by bioassay in at least one of the tissues tested. The highest toxicity level was found in *S. lispus* liver (130 μg/g), while *S. lobatus* showed toxicity only in mucus at low level (0.42 μg/g). By HPLC analysis, TTX was generally detected in the tissues which were indicated to be toxic by bioassay. The major toxic compounds in these were identified as TTX, 4-*epi*TTX and 4,9-anhydroTTX, which were also detected in most tissues. The TTX concentrations of mucus in all species, except *S. annulatus*, muscle of *A. meleagris* (golden phase), and liver of *S. annulatus*, were estimated higher by bioassay than by HPLC. On the chromatogram of the liver of *S. annulatus* [Fig. 1(C)], no peaks corresponding to other known TTX analogues were shown. Thus, low sensitive TTX analogues on HPLC, or saxitoxin related compounds (Nakamura et al., 1984; Zaman et al., 1998), are suspected as the toxic compounds in these samples because the symptoms of the mice were similar to those of TTX. Presence of 11-norTTX-6(S)-ol (Yotsu-Yamashita et al., 1992) in mucus and intestine of *A. meleagris* (black phase), and muscle and liver of *S. lispus*, were suggested by the peaks that appeared between those of TTX and 4-*epi*TTX in Fig. 1(D) (Yasumoto and

Yotsu-Yamashita, 1996). 11-OxoTTX (Khora and Yasumoto, 1989), and 11-norTTX-6(R)-ol (Endo et al., 1988), previously isolated from Japanese *A. nigropunctatus*, were also detected in the present samples. The two different color phase of *A. meleagris* showed a similar TTX tissue distribution pattern when comparing mucus and liver (mucus > liver), while *S. annulatus* and *S. lispus* showed an opposed pattern, the liver being more toxic than the mucus. According to this result, the currently considered innocuous puffer fish reported here appear toxic in at least one tissue.

It is noteworthy to emphasize the difference in toxicity and toxin tissue distribution between the two color phases of *A. meleagris*. The black phase appears more toxic and the toxin tissue distribution decreases in the following order: intestine > mucus > muscle > liver, while the yellow phase, being more attractive, shows less toxicity but is the mucus the most toxic tissue followed by muscle > intestine > gonads > and liver. One may wonder if this helps as an effective defense strategy at this particular stage.

The international marketing of puffer fish seems to be increasing and fish toxicity should be monitored. Our observations may help official authorities to regulate the capture and consumption of toxic fish species in Mexico, such as the puffer fish included in this study, in order to avoid the risk of lethal poisoning.

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