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‘Diarrhetic’ Type Shellfish Poisoning in Nigeria

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Abstract The safety of shellfish found and consumed in Nigeria is doubtful because no investigations have been carried out on their toxicity. The occurrence and toxicity of toxins in the commonly consumed Nigerian shellfish from Lagos, Warri, Oron, and Port Harcourt (PH) were investigated. Albino Wistar mice treated with chloroform extract of hepatopancreas (HP) from PH shellfish died 7–8 min post treatment. They convulsed for 30–60 s prior to death. The aqueous phase obtained from the diethyl ether extraction of the same HP resulted in the death of one out of two mice injected with it, 39 min post treatment.

Keywords Shellfish toxins · Acute toxicity · Mouse bioassay · Nigeria

Diarrhetic shellfish poisoning (DSP) is a toxic episode caused by the consumption of shellfish contaminated by certain species of marine microorganisms, such as dinoflagellates. Benthic dinoflagellates of the genus *Prorocentrum* are usually found in tropical and subtropical waters and some of them produce phycotoxins that are potentially toxic to man (Bomber and Aikman 1991). Shellfish are filter feeders and accumulate algal toxins in their hepatopancreas following a harmful algal bloom (Madigan et al. 2006). Two main causes of DSP are okadaic acid (OA) and dinophysistoxin I (DTX-1) (Vale and Sampayo 2002; Yasumoto et al. 1985; Hu et al. 1992). Although DSP is unlikely to cause death, it induces debilitating diarrhea and vomiting with no known medical treatments. Other symptoms include nausea and abdominal pain which usually start 30 min to 12 h following ingestion and may persist for 3–4 days (van Egmond et al. 1993). In the course of the sample collection, some of the shellfish consumers revealed that when they eat shellfish at certain periods of the year they develop nausea, diarrhea, and vomiting. Shellfish is one of the sources of protein in the diet of the people of the Niger Delta and Southwest regions of Nigeria. It is found and consumed in the following Nigerian cities: Oron, Warri, Lagos, Calabar, Port Harcourt (PH), and Yenegoa. It is usually bought from the open market live or semi-processed and dried. In other parts of Nigeria, it is consumed as a delicacy. In many countries, the level of toxins in shellfish is constantly monitored to ensure the safety of the shellfish consumed by the public (van Egmond et al. 1993; Nuñez and Scoging 1997) and the mouse bioassay (Yasumoto et al. 1978) is the commonest method of monitoring. Despite high consumption rate of shellfish in Southern Nigeria, their safety is doubtful, as no investigation has been carried out on their toxicity. The present study investigates the occurrence of DSP toxins in Nigerian shellfish.

Materials and Methods

The chemicals used for this study were of analytical grade and purchased from Sigma–Aldrich, St. Louis, MO. Albino Wistar mice (16–20 g) of both sexes were obtained from the Laboratory Animal House of the Faculty of Veterinary

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Medicine, University of Nigeria, Nsukka and were handled as humanely as possible according to the Faculty's guide on laboratory animal care and use. They were kept in metal cages and given water ad libitum before the commencement of the study. They were fed on grower's ration with the following composition: 14.5% crude protein, 4.8% crude fat, 7.2% crude fiber, 8% crude ash, 0.8% calcium, 0.62% phosphorus, 0.33% available phosphorus, 0.6% lysine, 0.29% methionine, 0.52% methionine/cystine, 8000 i.u. Vitamin A, 3400 i.u. Vitamin D3, 25 mg Vitamin E, 4 mg Vitamin B2, 50 mg Vitamin C, 30 mg Manganese, 30 mg Zinc, 0.15% Sodium, and 2300 Kcal/Kg metabolisable energy.

Shellfish samples were collected in February 2005 from the following locations in Southern Nigeria- Lagos, Warri, Oron, and PH (Fig. 1). All the samples were taken to the laboratory alive. Shellfish samples from Warri and Oron were identified as *Iphigenia laevigata* (smooth false donax) and the samples from Lagos and PH were identified as *Senilia senilis* (heavy africanask).

Toxin extraction was done by the method described by Stabell et al. (1991a) and Ramstad et al. (2001). The shellfish samples were vapor-boiled and the shells dislodged from the flesh. The hepatopancreas (HP), which consists of 15% of the entire mussel, was dissected out. Ten grams of the hepatopancreas was weighed out from each sample for the mouse bioassay, homogenized, and stored at –20°C prior to the mouse bioassay. For Lagos, and PH samples, 20 g instead of 10 g of hepatopancreas was used because an extra 10 g was needed for further extraction with diethyl ether at the final step. Using diethyl ether or chloroform in the final step of extraction usually facilitates extraction of the truly diarrhetic toxins or a wider range of toxins (Stabell et al. 1991a; Ramstad et al. 2001), respectively. The homogenized hepatopancreas was extracted with 200 mL acetone and filtered through Whatman No.1 filter paper. The filtrate was dried on ice and the residue was re-dissolved in 5 mL of petroleum ether and 5 mL of 80% methanol. The mixture was transferred to a tube, capped, shook for 1 min and centrifuged at 3000 rpm for 1 min. The upper petroleum ether layer containing lipid components was discarded. Another 5 mL of petroleum ether was added into the tube, shook for 1 min and centrifuged at 3000 rpm for 1 min. Again the upper petroleum ether layer was discarded and the 80% methanol fraction was retained for further extraction.

Chloroform was used in the final step of extraction of the samples from Oron and Warri, while for the samples from Lagos and PH the 80% methanol fraction was divided into two equal parts; chloroform was used in the final step

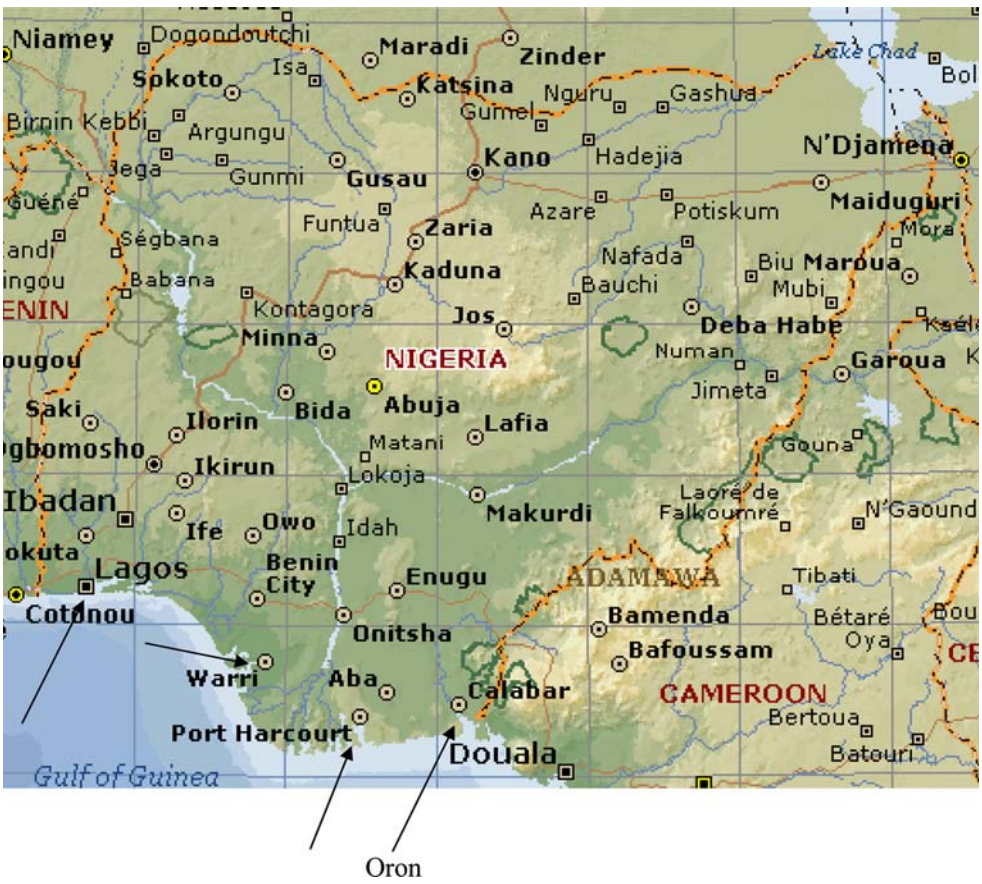

Fig. 1 Map of Nigeria; *arrows* are pointing at cities, namely Lagos, Warri, Port Harcourt, and Oron (along the Gulf of Guinea) where shellfish samples were collected. Stock R (2006) Nigeria. Microsoft® Student 2007 [DVD]. Redmond, WA: Microsoft Corporation

of extraction for one half while diethyl ether was used for the other half. Two milliliter (2 mL) of distilled water and 5 mL of chloroform were added to the 80% methanol fraction in a tube, shaken and centrifuged as before. The upper water–methanol layer was discarded and the chloroform fraction was dried on ice. The residue was stored at –20°C. The other half of the 80% methanol fraction was dried on ice and re-dissolved with 3 mL of distilled water to make an aqueous suspension. The aqueous suspension was extracted with 4 mL of diethyl ether twice to extract the truly diarrhetic toxins. The two ether fractions were combined and backwashed with 1 mL of distilled water. The ether was evaporated to dryness on ice and the residue stored at –20°C. The residue from the aqueous phase was also stored at –20°C.

The mouse bioassay was done as described by Yasumoto et al. (1978). Each residue (chloroform, diethyl ether, and aqueous phase) was suspended in 2 mL of 1% Tween 65 and 0.5 mL of the suspension was injected intraperitoneally (i.p.) to duplicate mice weighing between 16 and 20 g. One milliliter was given i.p to another mouse of the same weight range. Control mice were treated i.p. with 0.5 mL of 1% Tween 65. Toxicity of each extract was determined as time of injection to death of the mice. Mortality confirmed positive result for DSP toxins and one mouse unit (MU; 4 μg OA) was defined as the amount of toxin needed to kill a 16–20 g mouse (Yasumoto et al. 1989). Changes in mice behavior were also recorded. Post mortem was carried out on all the mice used in this study to check for gross pathological lesions.

Results and Discussion

All mice treated with HP-chloroform extract of shellfish collected from PH (Group B) in February, died within 7–8 min after the injection (Table 1). They convulsed for 30–60 s prior to death. None of the mice treated with HP-chloroform extract of shellfish collected from Lagos (Group A) died within 24 h of administering the extract. No death was recorded in the mice treated with HP-chloroform extract of shellfish collected from Oron (Group C) and Warri (Group D). Post mortem of mice in Group A revealed congestion of the intestines and accumulation of blood in the abdominal cavity. No post mortem lesions were found in Groups C and D mice. No deaths were recorded 24 h post administration of the diethyl ether extract (Table 2). With the exception of the control mice, all the others showed severe abdominal discomfort seen as flattening of the abdomen against the floor and regular licking of the ventral abdomen.

The aqueous phase obtained from the diethyl ether extraction of the HP of shellfish collected from PH resulted in the death of one out of two mice (Group G) that were injected with it, 39 min post administration (Table 2). Prior to death the mouse showed rapid backward (head to tail) wavelike contractions lasting about 10 s. Both mice showed serious difficulty in breathing and staggering movements. Post mortem of both mice revealed slight congestion of the intestine and accumulation of fluid in the abdomen.

The deaths recorded in the mouse bioassay test conducted with HP-chloroform extract of shellfish collected in February from PH, were very rapid occurring 6–8 min post administration of the extract. According to Amzil et al. (1999), when such neurological symptoms and rapid deaths (5–10 min) are observed, great caution must be exercised to find out whether these symptoms are caused by paralytic shellfish poisoning (PSP) toxins or other polar toxins. Given the rapid death and neurological symptoms observed and the fact that the mouse bioassay is sensitive to other algal toxins than those causing

Table 1 Mouse bioassay of chloroform extract

Group (Location)	Mouse	Volume of extract injected (i.p) (mL)	Time (min) between injection and death
A (Lagos)	I	0.5	Alive
	II	0.5	Alive
	III	1.0	Alive
B (PH)	I	0.5	8
	II	0.5	7
	III	1.0	6
C (Oron)	I	0.5	Alive
	II	0.5	Alive
	III	1.0	Alive
D (Warri)	I	0.5	Alive
	II	0.5	Alive
	III	1.0	Alive
Control	I	0.5 (Tween 65)	Alive

Table 2 Mouse bioassay of diethyl ether extract and aqueous phase

Group (Location) Extract	Mouse	Volume of extract injected (i.p) (mL)	Time (min) between injection and death
E (Lagos)	I	0.5	Alive
Diethyl ether	II	0.5	Alive
	III	1.0	Alive
F (PH)	I	0.5	Alive
Diethyl ether	II	0.5	Alive
	III	1.0	Alive
G (PH)	I	0.5	Alive
Aqueous phase	II	1.0	39
Control	I	0.5 (Tween 65)	Alive

diarrhea e.g., pectenotoxins (PTX) and yessotoxins (YTX), it was suspected that the mice did not die due to the classical DSP toxins (OA and DTX-1) but rather from the other co-extractable toxins. Extraction with chloroform includes a wider range of toxins such as PTX, YTX, and other unknown toxin(s) that are neurotoxic (Stabell et al. 1991a; Ramstad et al. 2001).

Using diethyl ether in the final step of extraction usually facilitates extraction of the truly diarrhetic toxins. However, unlike the mice treated with chloroform extract, death was not recorded with the ether extract i.p. administration indicating that most of the toxin could have remained in the water phase during the extraction process or are absent in the sample (Table 2). The death of one out of the two mice, 39 min after injection with the aqueous phase extract further strengthens suspicion of the former. Since YTX and PTX (Stabell et al. 1991b) will remain in the water phase following diethyl ether extraction, there is a strong probability that the death recorded from the injection of the aqueous phase extract was caused by YTX and/or PTX. Post mortem of both mice injected with the aqueous phase revealed fluid accumulation in the abdomen and moderately congested intestine. The fluid accumulation seen in the abdomen could be responsible for the difficulty in breathing observed in both mice. The abdominal discomfort and jerky movements noticed in the mice injected with the diethyl ether extract of PH shellfish HP is indicative of mild poisoning. Perhaps the amount and/or type of toxin could not result in death of any of the injected mice.

Death was not recorded in the mice injected with HP-chloroform extracts of shellfish from Lagos (Group A), Oron (Group C), and Warri (Group D). However, post mortem examination of mice treated with extract of the HP from Lagos revealed congestion of the intestines and accumulation of blood in the abdominal cavity. No post mortem lesion was found in the mice treated with HP samples from Oron and Warri. It is possible that the toxin responsible for the death and neurological symptoms observed in Group B is the same toxin responsible for the signs of toxicity found in Group A since both are the same species of shellfish i.e., *Senilia senilis.* The difference in the degree of toxicity could be due to different concentrations of the toxin. It is also possible that the shellfish from PH were exposed to a more harmful type of algae compared to the Lagos mussels.

The deaths and pathologic changes recorded in this work, confirm the fear that the shellfish found and consumed in some parts of Nigeria may be toxic**.** The most effective means of ensuring a nontoxic supply of shellfish is by instituting a shellfish toxicity monitoring program by the Federal Government of Nigeria as in other countries. Further work should be done to identify the toxins found in the Nigerian shellfish and at different periods of the year.

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