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Short Communication

Detection of tetrodotoxin and saxitoxin in dried salted yellow puffer fish (*Xenopterus naritus*) eggs from Satok Market, Kuching, Sarawak

Mohd Nor Azman, A. and *Wan Norhana, M.N.

Fisheries Research Institute, 11960 Batu Maung, Penang

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Abstract

The detection of tetrodotoxin (TTX) and saxitoxin (STX) in dried salted yellow puffer fish (*Xenopterus naritus*) eggs bought from Satok Market, Kuching, Sarawak was carried out by mouse bioassay method. The amount of TTX and STX detected in the samples ranged from 95.6-195.5 Mouse Unit (MU)/g and 1.72-3.58 MU/g respectively. The results indicate that the dried salted eggs samples were found to contain TTX 9-20 times above the regulatory limit for human consumption (10 MU/g). Although detected, the amount of STX in salted eggs extract was slightly below the accepted threshold limit (4 MU/g). The local public in Sarawak should be educated on the potential danger of consuming dried salted puffer fish eggs in addition to the current warnings on puffer fish.

Introduction

Yellow pufferfish (*Xenopterus naritus*) (Figure 1) or locally known as 'ikan buntal kuning' is a common fresh water puffer fish found in Sarawak. It has become a tourist attraction is Sarawak through the 'yellow puffer fish festival' celebrated every year. The Yellow puffer fish belongs to the family Tetraodontidae and can be easily identified by the prominent yellowish or golden coloration especially at the lower part of the body. The fish is found in the coastal waters especially in areas fringing the mangroves along the Batang (River) Sarabas in Betong, Sarawak. Most of the puffer fishes in Tetraodontidae family, including Yellow puffer fish contain neurotoxins especially Tetrodotoxin (TTX) in their body parts including skin, muscle, reproductive tissue (gonads) and digestive tissue (liver) (Noguchi *et al.*, 2006). Saxitoxin (STX), a toxin belonging to paralytic shellfish toxin poisoning group, can also be found in puffer fish (Deeds *et al.*, 2008). The co-occurrence of TTX and STX has been reported within the same species of puffer fish (Ngy *et al.*, 2009).

Yellow puffer fish has been regularly consumed in Sarawak. However, only two mortality cases due to this species have been reported so far. This is associated with the consumption of salted yellow puffer fish roes in Saratok, a district next to Betong, Sarawak (Razak *et al.*, 2009). Although the locals are aware of the poisonous effect of yellow puffer fish, this fish and its products (salted eggs/roes) (Figure 2) are easily available and much sought after by the locals. Salted puffer fish eggs are delicacies in Sarawak and can reach up to RM 30-40 per kilogram (Muliadi and Muhammad Raduan, 2008). The presence of toxin in salted puffer fish eggs could be more harmful because they are eaten whole and the toxin could not be removed by special preparation as in whole fresh puffer fish.

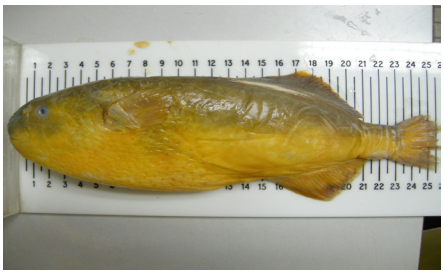

Figure 1.Yellow puffer fish, *X. naritus*

Figure 2. Dried salted yellow puffer fish (*X. naritus*)eggs

There is very limited information on the toxicity of yellow puffer fish including its product. Toxins from yellow puffer fish (*X. naritus*)of Sg Saribas, Sarawak (Othman Bojo *et al.*, 2006; Mohamed *et al.*, 2008) and Andaman Sea (Kungsuwan, 1993) has been documented. Hence, this short study was initiated to determine the prevalence of toxin (TTX and STX) in dried salted yellow puffer fish eggs bought from a market in Kuching, Sarawak.

*Corresponding author.
Email: *wannorhana@yahoo.com*
Tel: 604-6263925/26; Fax: 604-6262210

Materials and Methods

Specimen collection

A total of seven samples (about 150 g each) of dried salted yellow puffer fish eggs were bought from the famous Satok market, Kuching, Sarawak in December 2010.The eggs referred here are actually the gonad or the ovary of the pufferfish. The salted eggs were placed in a polyethylene bag and transported by air in a cooler box to the Fisheries Research Institute, Penang, Malaysia for toxicity determination.

Toxin extraction and mouse-bioassay

The presence of PSP and TTX in the samples was determined by a mouse bioassay method (Kawabata, 1978; AOAC, 1990). Briefly, the salted eggs were cut into small pieces and homogenized with an ultrasonic homogenizer (OMNI-Ruptor 4000, Georgia, USA). For TTX, about 5 g of homogenized tissue was extracted with an equal volume of acetic acid (0.1% v/v). The mixture was boiled (BUCHI B-480, Germany) for 5 min with occasional stirring and was cooled down before being centrifuged (Eppendorf 5430, Hamburg, Germany) at 10,000× *g*, 25°C for 15 min. The clear supernatant was collected and tested for toxicity. Similarly, 5 g of puffer fish eggs tissue was prepared for STX extraction. The homogenized puffer fish eggs were extracted with 0.1N hydrochloric acid (0.1% v/v) by heating in a boiling water bath for 5 min and centrifuged at 10,000 g for 15 min.

The clear supernatant was collected and 1.0 ml of the supernatant was collected and tested for toxicity by injecting intraperitoneally into white mice. A total of 7-9 (18-22 g) healthy and active mice were used in testing each puffer fish eggs extract. Toxicity symptoms were observed after injection and the time of death was recorded at the last gasping breath of the mice. A group of mice were injected with 1.0 ml of 0.1N acetic acid or 0.1N hydrochloric acid to serve as control.

The toxicity of each extract was expressed as mouse units (MU). One MU is theamount of TTX required to kill a male mouse (Balb C strain, reared at the Fisheries Research Institute) weighing 18 - 20 g within 30 min after injection or the amount of STX toxin required to the mouse of the same body weight in 15 min after injection. The average death time was referred to the Sommers table and the corresponding toxicity, was calculated.The scores of the toxicity were classified as: Non toxic < 10 MU/g, weakly toxic ≥ 10-100 MU/g, moderately toxic ≥ 100-1000 MU/g and strongly toxic ≥ 1000 MU/g (Tani, 1985).

Table 1. Detection of TTX in dried salted eggs of yellow puffer fish (*X. naritus*)

Samples	Range of death time (min)	Average death time (min)	Average MU/g	Toxicity score
1	5.44-24.08	13.9	95.6	Moderate
2	6.44-16.18	7.41	119.9	Moderate
3	3.31-5.47	4.44	163.6	Moderate
4	4.06-12.15	6.49	195.5	Moderate
5	4.67-29.06	13.55	140.3	Moderate
6	4.53-9.52	6.51	154.8	Moderate
7	4.45-8.30	5.40	166.2	Moderate

Table 2. Detection of STX in dried salted eggs of yellow puffer fish (*X. naritus*)

Samples	Range of death time (min)	Average death time (min)	Average MU/g	Toxicity score
1	4.06-6.51	5.33	1.72	Non-toxic
2	5.25-6.01	5.63	2.52	Non-toxic
3	5.16-5.32	5.24	3.03	Non-toxic
4	5.11-5.50	5.30	2.94	Non-toxic
5	4.36-5.01	4.69	3.58	Non-toxic
6	6.26-6.34	6.31	2.72	Non-toxic
7	5.14-7.34	6.25	2.56	Non-toxic

Results and Discussions

In this study, the extraction of TTX from puffer tissue was carried out with 0.1N acetic acid because TTX is extremely soluble in weak acid (Che Nin *et al.*, 2010). Although it is considered as non specific and lacking in sensitivity at low concentration, we still use the common mouse bioassay method for toxicity determination as others (Noguchi *et al*., 2006; Sabrah *et al*., 2006; Huang *et al*., 2008; Che Nin *et al*., 2010) because of the simplicity and 'real time' effect of the toxin.

Tables 1 and 2 give the details of toxicity testing of dried salted yellow puffer fisheggs as determined by the mouse bioassay method. To our knowledge, this is the first report on the occurrence of both TTX and STX in dried salted yellow puffer fish eggs from Sarawak. Almost all puffer fish egg extracts were lethal to the mice. The average death time is shown in the respective tables. The signs preceding death were almostidentical. Themicedisplayedamarkedlethargic condition immediately after injection. Following the initial lethargic state, signs of respiratory developed and these were accompanied by restlessness and agitation. The mice started to have cramps on their hind legs. Shortly after this stage, convulsion set in as could be seen from the jumping, thrashing and gasping to get more oxygen from outside, followed by death.The controlled mice (injected with 1.0 ml 0.1N acetic acid or 0.1N hydrochloric) displayed uneasiness and stopped moving after injection. After a few minutes they were back to normal.

The TTX in the yellow puffer fish salted egg samples ranged from 95.6-195.5 MU/g and could

be considered as moderately toxic based on Tani classification. This is also about 9-20 times higher than the regulatory limit set for human consumption at 10 MU/g (Japan Food Hygiene Association, 2005). The amount of TTX recorded in this study is much higher than the muscle, skin, liver and gonad of forty-eight yellow puffer fish samples from Saribas River (0.7-4.5 MU/g) as reported by Mohamed *et al.* (2008).On the other hand, Che Nin *et al.* (2010) did not detect TTX in yellow puffer fish (*X. naritus*) muscle samples even by using the highly sensitive Gas Chromatography-Mass Spectrometry. This is not surprising as pufferfish in general has been reported to show large individual, regional and seasonal variations in toxicity (Noguchi *et al.*, 2006). Meanwhile the high concentrations of toxins in puffer fish eggs are common. According to Alcock (2010), the ovary is the organ with the highest concentration of toxin in an adult pufferfish. The liver too, usually demonstrates high toxicity, except in the spawning season, when the ovary becomes more toxic by accumulating TTX transferred from the liver. Ovary toxicity is the highest when the ova are maturing. This is why the puffer fish eggs are rejected by predators. This property of puffer fish may have a selective advantage that accrues them from a decrease in predation of eggs and juveniles (Alcock, 2010).

On the other hand, the amount of STX in all yellow puffer fish egg extracts ranges from 1.72-3.58 MU/g. These concentrations are slightly below the regulatory limit of 4 MU/g (Japan Food Hygiene Association, 2005). In this study, the symptoms observed for STX and TTX toxicity to mice were similar. The only factor in discriminating STX and TTX toxicity is only based on the shorter death time. Thus the STX reported in this study could also be a more concentrated TTX (because of a shorter death time). Further study will be carried out to confirm the presence of TTX and STX and quantify them with HPLC and LC-MS/MS.

As mentioned in the introduction, there is scarce information on the toxicity of puffer fish especially its products. In Taiwan, the incident of TTX poisoning due to ingestion seasoned dried puffer fish fillet showed a high toxicity of 525 MU/g (Du *et al.*, 1999). In Malaysia, two deaths resulting from the consumption of salted yellow puffer fish (X. naritus) roes have been recorded (Razak *et al*., 2009), however the amount of TTX detected in the roes was not mentioned.

Conclusion

Based on the results of this short study, we could say TTX is the main toxin that is associated with the toxicity of dried salted yellow puffer fish eggs. Further studies should be carried out to determine whether the process of salting and drying would maintain, enhance or reduce the amount of TTX or STX in yellow puffer fish eggs. Lastly, local people of Sarawak should be advised on the potential danger of dried salted yellow pufferfish eggs in addition to the current warnings on puffer fish.

References

Alcock, J. 2010. Ciguatoxin and tetradotoxin in fish eggs. Implication for evolution and public health. Journal of Medical Toxicology 6: 234-236.

AOAC, 1990. Paralytic Shellfish Poison. Biological method. Final action. In: Hellrich, K. (ed.), Official Methods of Analysis. 15th Edition, pp. 881-882, sec 959.08. Association of Official Analytical Chemists, Arlingthon, Virginia, USA.

Che Nin, M., Norjuliana, M.N., Gan, L.H., Razak, L. and Mohamad, S. 2010. Screening of tetradotoxin in puffers using gas chromatography and mass spectrometry. Journal of Chromatography A. 1217: 7455-7459.

Du, S.S., Fu, Y.M., Shih, Y.C., Chang, P.C., Lue, Y.H. and Hwang, D.F. 1999. First report on suspected food poisoning with ingestion of dried seasoned fish fillet. Journal of Food and Drug Analysis 7: 163-167

Huang, H.N., Lin, J. and Lin, H.L. 2008. Identification and quantification of tetradotoxin in the marine gastropod Nassarius by LC-MS. Toxicon 51(5):774-779.

Japan Food Hygiene Association. 2005. Puffer toxin. In: Environmental Health Bureau. Ministry of Health and Welfare. Tokyo (eds). Shokuhin Eisei Kensa Shishin (Manual for methods for food sanitation testing). Tokyo: Japan. Japan Food Hygiene Association, pp 661-666.

Kawabata, T. 1978. Assay method for tetrodotoxin. In: Food Hygiene Examination Manual, Vol. II, pp. 232-240. Japan Food Hygiene Association, Tokyo.

Kungsuwan, A. 1993.Survey on Poisonous Puffer fish in Andaman Seas. In:Proceeding of the Seminar on Fisheries 1993, Department of Fisheries, Bangkok, Thailand, 15–17 September 1993

Othman Bojo, K.A., Rahim, A., Mohamad, S., Long, S. M., Nyanti, L., Ismail, N. and Teen, L. P. 2006. Toxicity of the Yellow Puffer Fish *Xenopterus naritus* from Sungai Saribas Sarawak. Research Bulletin, FRST. 1: 5-6.

Mohamad, S., Lim, P. T., Bojo, O., Nyanti, L., Ismail, N., Rahim, K. A., M. Long, S. 2008. Toxicity of freshwater puffer fish *Xenopterus naritus* (Tetraodontidae) collected from East Malaysia. IOC-WESTPAC Scientific Symposium. 21st-25th May 2008.

Muliadi Y. and Mohammad Raduan, M.A. 2008. Ikan Buntal Kuning (*Lagocephalus Lunaris*) as a Tourism Product in Betong, Sarawak. Journal of Southeast Asean Studies 13(1): 245-263.

Ngy, L., Yu, C.F., Taniyama, S., Takatani, T. and Arakawa, O. 2009. Co-occurrence of tetradotoxin and saxitoxin in Cambodian marine puffer fish *Takifugu oblongos*. African Journal of Marine Science 31(3): 349-354.

Noguchi, T., Arakawa, O. and Takatani, T. 2006. TTX accumulation in puffer fish. Comparative Biochemistry and Physiology Part D 1: 145– 152.

Razak, L., Che Nin, M., Norjuliana, M.N. and Sazaroni, M.R. 2009.Case Study – Puffer fish Poisonings in Malaysia. Asia Pacific Association of Medical Toxicology Conference, 19-23 October 2009. Beijing, China. Available at: *http://www.asiatox.org./8APAMT20%20PP?PP60-Puffer fish%20 Poisoning%20.*

Sabrah, M.M., El-Ganainy, A.A. and Zaky, M.A. 2006. Biology and toxicity of the pufferfish, *Lagocephalus sceleratus* (Gmelin, 1789) from the Gulf of Suez. Egyptian Journal of Aquatic Research 31(1): 283-297.

Tani, I. 1985. Toxicological Studies of Puffers in Japan. Teikoku. Tosho.Co. Tokyo