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# Frequent occurrence of tetrodotoxin in the marine gastropod *Nassarius glans* causing a food poisoning in Khanh Hoa province, Vietnam in 2020

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**ABSTRACT**

A fatal neurotoxic poisoning case happened in Khanh Hoa province in 2020 after eating a certain number of marine gastropods, later identified as *Nassarius glans*, one of the common marine gastropods in Vietnam. As the remaining causative food in the incident, 62 specimens were collected to examine tetrodotoxin toxicity individual variation and frequency of toxic specimens by using HILIC/MS-MS analysis. 100% of studied specimens exhibited toxicity (556 ± 821 MU/g) beyond the regulatory level of consumption (10 MU/g) for puffer(fish) recommended in Japan and extensive variation (18–4,046 MU/g). The result pointed out that only 5 g of soft tissue from *N. glans* (equivalent to 2–3 specimens) containing maximum toxicity detected in the present study may cause human death if consumed. Fhe first time, this study identified TTXs in the gastropods as a causative toxin in the poisoning in Vietnam. Moreover, 65.5% of studied specimens with high toxicity higher than 100 MU/g, including 16.1%, showing extremely high toxicity (> 1,000 MU/g). The results suggested that this gastropod is quite dangerous for human consumption and should be alerted to public awareness.

**Keywords:** HILIC/MS-MS, *Nassarius glans*, poisoning, tetrodotoxin, Vietnam.

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## INTRODUCTION

Food poisoning due to marine gastropod consumptions has been reported sporadically in Asian countries [1], including Vietnam [2]. The causative gastropods were identified belong to Nassariidae, Naticidae, Olividae, Turbinidae, Trochidae, Charonidae, Babylonidae, Tutufadae, and Niothadae [1]. The poisonings with fatal symptoms by consumption of *Nassarius* gastropods had been reported in Khanh Hoa province, Vietnam, where victims showed neurotoxic symptoms [1, 2]. Nevertheless, causative toxins could not be confirmed due to lacking specimens. In our previous screening of the toxins in some common gastropods collected on the Khanh Hoa coast, *Nassarius glans* was reported as one of the TTX-bearing species [3]. However, for the evidence that TTXs are the causative agent in the poisoning cases, it is requested to have scientific data on toxicity individual variation as well as the frequency of toxic specimens. However, this subject has not yet detail investigated due to insufficient poisonous specimens. On 11 September 2020, three fishermen caught some marine gastropods by their snorkeling diving in Van Ninh district coastal water, Khanh Hoa province. About half of the self-caught gastropod was shared by a friend's family in Khai Luong island (Van Thanh commune, Van Ninh district, Khanh Hoa province) as a gift, and another half was boiled and eaten by them at 16:00 in the same day. Symptons occurred about 30 minutes after eating, including tingling in lips and tongue, limbs, dizziness, nausea, and headache. At 19:00, symptoms became severe, all victims were sent to the Tu Bong district General clinic; unfortunately, one died on the way. At about 1:00 am the next day, two victims were transferred to the General Hospital of Khanh Hoa province for intensive care and recovered later, luckily. Another haft gastropod numbers, which gave the family in Khai Luong island, was recognized as strange food; therefore, they were not consumed. They were identified as *Nassarius glans* (Fig. 1) and collected for toxin analysis. This paper describes the result of the study on individual toxicity of tetrodotoxins (TTXs) and the frequency of toxic specimens in the poisonous gastropod specimens. This data provides critical information to confirm that TTXs in *N. glans* caused the poisoning in Vietnam.


*Figure 1.* Photos of *Nassarius glans* in this study

## MATERIALS AND METHODS

### Specimen collection

As the remaining causative food in the poisoning case in Khanh Hoa province, 2020; 62 specimens of marine gastropod (later identified as *Nassarius glans*), which originated from Van Ninh district coastal water, Khanh Hoa province was collected and transferred into the VAST-Keylab on Seafood and Environment Safety, Institute of Oceanography under cold condition. After taking photos and identifying the scientific name (by the gastropod taxonomist, Mr. Bui Quang Nghi), they were cleaned outside, weight, measured in length and width, then deshelled to collect whole soft tissues individually (Table 1).

*Table 1.* Information on marine gastropod collected from the poisoning case in Khanh Hoa province, 2020

| Poisoning date | Poisoning place | Species | n | Length (cm) | Width (cm) | Whole body weight (g) | Weight of soft tissue (g) |
|---|---|---|---|---|---|---|---|
| 11st, September 2020 | Khanh Hoa | *Nassarisus glans* | 62 | 3.9 ± 0.3 | 2.0 ± 0.2 | 5.8 ± 1.0 | 2.7 ± 0.6 |

### Chemicals and instruments

Formic acid and acetic acid were purchased from Wako Pure Chemicals (Osaka, Japan). Ammonium hydroxide of 25% for LC-MS additive was purchased from Sigma-Aldrich (Tokyo, Japan). Acetonitrile was purchased from Kanto Chemicals (Tokyo, Japan). Tetrodotoxin (1 mg) ($C_{11}H_{17}N_3O_8$, 319.27 ug/g) was purchased from Tocris Bioscience (Bristol, UK); *4-epi*TTX ($C_{11}H_{17}N_3O_8$, 319.27 ug/g) and anh-TTX ($C_{11}H_{15}N_3O_7$, 301.25 ug/g) were granted by Prof. Shigeru Sato, Kitasato University, Japan. Hydrophilic interaction liquid chromatography-mass spectrometer (HILIC/MS-MS) was applied on a Shimadzu triple-quadrupole mass spectrometer system (LCMS−8040; Shimadzu Corporation, Kyoto, Japan).

### Extract and analysis of tetrodotoxin

Tetrodotoxin was extracted according to Supapun et al., [4]. The extracts were treated using an ENVI-Carb SPE cartridge 250 mg/3 mL (Sigma Aldrich Japan, Tokyo, Japan), eluted by diluted with a four-fold volume of acetonitrile. The soft tissue of each snail individual was homogenized with acetic acid 1% (1 g/4 mL), boiled for 5 mins, cooled down at room temperature, and centrifugated at 10,000 rpm in 10 mins to collect the supernatant.

TTXs in the eluates were analyzed by the HILIC/MS-MS method according to Boundy et al. [5] with some modifications. The LC separation was carried out on a Waters Xbrige (HILIC) Amide column (4.6 mm I.D ×150 mm, 3.5 µm) at 60 $^oC$ with a 5 µL sample volume injected. Mobile phases were water/formic acid/ammonium hydroxide (500/0.075/0.3 v/v/v) (A) and acetonitrile/water/formic acid (700/300/0.1 v/v/v) (B) with a flow rate of 0.6 mL/min. The chromatographic conditions were as follows: initial conditions 100% B, held for 20 min; then a linear gradient 50:50 A and B within 15 min, held for 9.90 min.

The ion source parameters of the MS spectrometer were as follows: entrance potential (EP), 10 V; curtain gas (CUR), 30 psi; ion spray voltage (IS); 4,500 V; source desolvation temperature (TEM), 250 $^oC$; source ion block temperature, 400 $^oC$; desolvation gas flow; 1,000 L/h; nebulizer gas flow, 2 L/min; and collision gas flow rate, 0.15 mL/min. Multiple reaction monitoring (MRM) was performed in positive electrospray ionization ($ESI^+$).

Our study did not conduct recovery tests with spiked samples because of the limited availability of toxin references. Single-point calibration was applied in this analysis. However, the limit of detection (LOD) (S/N = 3) and the limit of quantitation (LOQ) (S/N = 10) obtained on our HILIC/MS-MS were 38 and 126 nM of TTX, respectively.

The TTX toxicities were calculated from HILIC/MS-MS data and expressed in mouse units (MU/g) according to Nakamura and Yasumoto [6], in which 1 mg TTX corresponds to 4,500 MU, 1 mg *4epi*-TTX corresponds to 709 MU and 1 mg 4,9-anhydroTTX corresponds to 92 MU. One MU is the dose of toxin that will kill a 20-g male mouse (ddY) in 30 min.

## RESULTS AND DISCUSSION

In the HILIC/MRM chromatogram, the retention time (Rt) of Anh-TTX, *4epi*-TTX, and TTX was 27.58, 28.41, and 29.02 mins, respectively (Figure 2a). The peaks with the same retention times of those were observed in all extracts of *N. glans* specimens, respectively (Figure 2b) indicated the presence of TTX, *4epi*-TTX, and AnhTTX in the extracts of *N. glans*. The result, again, confirmed that TTXs were toxins in *N. glans* [3]. The toxin profile was similar to previous studies, in which TTX, *4epi*-TTX, and AnhTTX were toxic compounds in marine gastropods in Vietnam [2, 3].

As shown in Table 2, TTX was found in the highest level (113.8 ± 168.7 µg/g), followed was AnhTTX (75.0 ± 121.8 µg/g) and then *4epi*-TTX (51.9 ± 72.1 µg/g). TTX was known as the most toxic, which was almost more than 6-fold than *4epi*-TTX and 49-fold than AnhTTX toxicities [6, 7]; therefore, more than 90% of total toxicity in the *N. glans* was contributed by TTX, estimated.

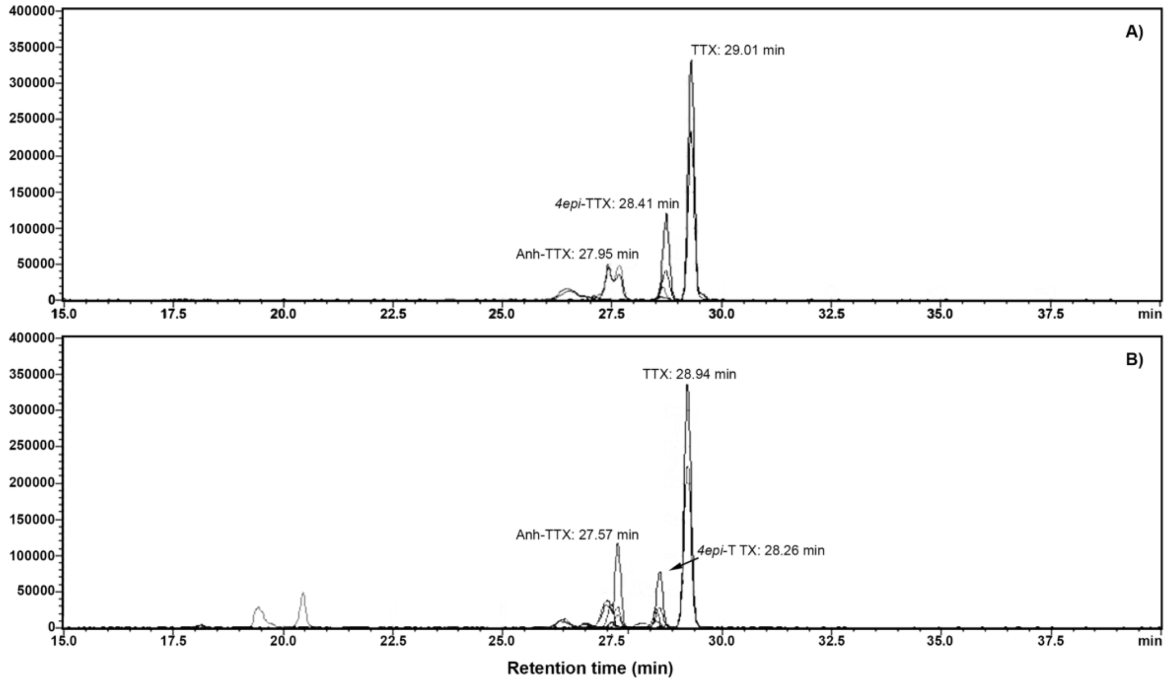


*Figure 2.* HILIC/MS-MS chromatographs of A) TTXs standard; B) TTXs in the extract of *Nassarius glans* collected in the poisoning case in Khanh Hoa province, 2020

All specimens showed toxicity beyond the regulatory level of consumption (10 MU/g) for puffer(fish) recommended in Japan. Moreover, the toxicity level (556±821MU/g) was higher than our previous study (412.7 ± 107.3 MU/g) [3]. The toxicity was also very varied among the specimens (18–4,046 MU/g) (Table 2), which was on a broader range than that in our previous study (187–787 MU/g) [3]. The difference could be due to the larger investigated specimen numbers in the present study compared to the previous.

*Table 2.* Level of TTXs and toxicity in Nassarius glans ($n$ = 62) collected from the poisoning case in Khanh Hoa province, 2020

| Statistic value | Level of TTXs (µg/g) | | | Total toxicity* (MU/g) | Frequency (%) of toxicity (MU/g) in range | | |
|---|---|---|---|---|---|---|---|
| | *4epi*-TTX | TTX | AnhTTX | | 10–100 | 100–1,000 | > 1,000 |
| Range | 0–338.5 | 2.6–831.2 | 0–709.8 | 18–4,046 | 35.5 | 48.4 | 16.1 |
| Mean ± SD | 51.9 ± 72.1 | 113.8 ± 168.7 | 75.0 ± 121.8 | 556 ± 821 | | | |

*Notes:* *: 1 mg TTX corresponding to 4,500 MU; 1 mg 4-*epi*TTX corresponding to 709 MU and 4,9-anhydroTTX corresponding to 92 MU [5].

The highest TTX toxicity observed in *N. glans* was higher than that reported in Taiwan (2,992 MU/g) [8] or in Vietnam [3] and comparable in Japan (4,290 MU/g) [9]. The minimum lethal dose (MLD) is estimated to be approximately 10,000 MU [1]; therefore, only 5 g of soft tissue from *N. glans* (equivalent to 2–3 specimens) containing maximum toxicity detected in the present study may cause death to humans if consumed. Forty out of 62 specimens (occupied 64.5%) exhibited toxicity higher than 100 MU/g, including 10 specimens (occupied 16.1%) that showed extremely high toxicity (> 1,000 MU/g). The result was reasonable to the poisoning information that about 60 specimens (half of the caught gastropod amount, estimated) caused fatal poisoning symptoms for 3 victims. The data, for the first time, indicated that TTXs in *N. glans* were a causative toxin in the poisoning in Vietnam.

*N. glans* has been reported as being a TTX-bearing species from other Asian countries such as Japan and Taiwan [9, 10] and Vietnam [3]. According to Noguchi et al., [1]; TTX in small necrophagous gastropods may come from their food, such as dead toxic puffers. However, the origin of TTX in marine gastropods is still being determined because some other species collected at the same location and simultaneously had no toxins [6, 7]. The present result, together with previous results, indicated that *N. glans* in Vietnam is quite dangerous for human consumption, and needed to have stronger alert public awareness. TTX in *N. glans*, together with their food sources, would be an exciting subject for understanding the mechanism of toxin contamination in this gastropod. On the other hand, TTX occurrence and toxicity in gastropods may show seasonal variation [1]. The occurrence of poisoning in the present study in autumn (September) was similar to that reported in our previous study in Vietnam [2]. However, the poisonings by consumption of *Nassarius* gastropods in China and Taiwan were often reported in summer [1]. This difference should be an interesting subject for further investigation.

## CONCLUSION

HILIC/MS-MS analysis detected a certain level of TTX and its derivatives (AnhTTX and *4epi*-TTX) was detected in all of 62 specimens of *Nassarius glans* collected in the poisoning case in Khanh Hoa province, 2020. For the first time, this study identified TTXs in the gastropods as a causative toxin in the poisoning in Vietnam. The results of high toxicity, wide individual variation, and high frequency of high toxicity specimens indicated that the gastropod *Nassarius glans* in Vietnam is quite dangerous for human consumption and should be in stronger public awareness.

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## REFERENCES

[1] Noguchi, T., Onuki, K., and Arakawa, O., 2011. Tetrodotoxin poisoning due to

pufferfish and gastropods, and their intoxication mechanism. *ISRN toxicology*, *2011*, 276939. doi: 10.5402/2011/276939

[2] Ha, D. V., and Sato, S., 2010. Toxicity of some marine snails responsible for recent food poisonings in Vietnam. *Vietnam Journal of Marine Science and Technology*, *10*(3), 89–95.

[3] Dao, H. V., Pham, K. X., Hoang, B. X., Tanioka, M., Watanabe, R., and Suzuki, T., 2020. Occurrence of tetrodotoxin in three *Nassarius* gastropod species in Khanh Hoa province, Vietnam. *Fisheries science*, *86*, 181–186. https://doi.org/10.1007/s12562-019-01375-3

[4] Brillantes, S., Samosorn, W., Faknoi, S., and Oshima, Y., 2003. Toxicity of puffers landed and marketed in Thailand. *Fisheries science*, *69*(6), 1224–1230. doi: 10.1111/j.0919-9268.2003.00749.x

[5] Boundy, M. J., Selwood, A. I., Harwood, D. T., McNabb, P. S., and Turner, A. D., 2015. Development of a sensitive and selective liquid chromatography–mass spectrometry method for high throughput analysis of paralytic shellfish toxins using graphitised carbon solid phase extraction. *Journal of Chromatography A*, *1387*, 1–12. doi: 10.1016/ j.chroma.2015.01.086

[6] Nakamura, M., and Yasumoto, T., 1985. Tetrodotoxin derivatives in puffer fish. *Toxicon*, *23*(2), 271–276. https://doi.org/10.1016/0041-0101(85)90149-7

[7] Dang, Q. M., Pham, X. K., Dao, V. H., Le, H. K. H., Nguyen, T. H., Phan, B. V., and Doan, T. T., 2015. Tetrodotoxin and saxitoxin in some *Nassarius* species (*Nassarius* Duméril, 1806) collected in Khanh Hoa waters. *Coll. Mar. Res. Works*, *21*, 70–79. (in Vietnamese with English abstract).

[8] Hwang, D. F., Shiu, Y. C., Hwang, P. A., and Lu, Y. H., 2002. Tetrodotoxin in gastropods (snails) implicated in food poisoning in Northern Taiwan. *Journal of food Protection*, *65*(8), 1341–1344. doi: 10.4315/0362-028X-65.8.1341

[9] Taniyama, S., Isami, Y., Matsumoto, T., Nagashima, Y., Takatani, T., and Arakawa, O., 2009. Toxicity and Toxin Profile of Tetrodotoxin Detected in the Scavenging Gastropod *Nassarius* (*Alectrion*) *glans* 'Kinshibai'. *Journal of the Food Hygienic Society of Japan*, *50*(1), 22–28. doi: 10.3358/shokueishi.50.22

[10] Narita, H., Noguchi, T., Maruyama, J., Nara, M., and Hashimoto, K., 1984. Gastropod, "Hanamushirogai" *Zeuxis siquijorensis*. *Bulletin of the Japanese Society of Scientific Fisheries*, *50*(1), 85–88. https://doi.org/10.2331/suisan.50.85