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

Dao Viet Ha*, Le Ho Khanh Hy, Pham Xuan Ky, Bui Quang Nghi, Nguyen Phuong Anh, Phan Bao Vy, Doan Thi Thiet

*Institute of Oceanography, VAST, Vietnam*

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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. The 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.

*Corresponding author at: Institute of Oceanography, 01 Cau Da St., Nha Trang city 650000, Khanh Hoa, Vietnam. E-mail addresses: daovietha69@gmail.com*
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. Symptoms 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 half 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](image)
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

<table>
<thead>
<tr>
<th>Poisoning date</th>
<th>Poisoning place</th>
<th>Species</th>
<th>n</th>
<th>Length (cm)</th>
<th>Width (cm)</th>
<th>Whole body weight (g)</th>
<th>Weight of soft tissue (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>11st, September 2020</td>
<td>Khanh Hoa</td>
<td><em>Nassarius glans</em></td>
<td>62</td>
<td>3.9 ± 0.3</td>
<td>2.0 ± 0.2</td>
<td>5.8 ± 1.0</td>
<td>2.7 ± 0.6</td>
</tr>
</tbody>
</table>

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\textsubscript{11}H\textsubscript{17}N\textsubscript{3}O\textsubscript{8}, 319.27 µg/g) was purchased from Tocris Bioscience (Bristol, UK); 4-epi-TTX (C\textsubscript{11}H\textsubscript{15}N\textsubscript{3}O\textsubscript{7}, 301.25 µg/g) and anh-TTX (C\textsubscript{11}H\textsubscript{15}N\textsubscript{3}O\textsubscript{7}, 301.25 µg/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.
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.

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

<table>
<thead>
<tr>
<th>Statistic value</th>
<th>Level of TTXs (µg/g)</th>
<th>Total toxicity* (MU/g)</th>
<th>Frequency (%) of toxicity (MU/g) in range</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>4epi-TTX</td>
<td>TTX</td>
<td>AnhTTX</td>
</tr>
<tr>
<td>Range</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0–338.5</td>
<td>2.6–831.2</td>
<td>0–709.8</td>
<td>18–4,046</td>
</tr>
<tr>
<td>Mean ± SD</td>
<td>51.9 ± 72.1</td>
<td>113.8 ± 168.7</td>
<td>75.0 ± 121.8</td>
</tr>
</tbody>
</table>

Notes: *: 1 mg TTX corresponding to 4,500 MU; 1 mg 4-epiTTX 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**


