A NOTO ON CIGUATERA POISONING IN OKINAWA AND THE TOXIN
OF A GROUPER, EPINEPHELUS FUSCOGUTTATUS FORSKÅL*

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Ciguatera, widely known fish poisoning in the southern area, has attracted many investigator’s attention not only by its peculiar symptoms but also by its economical problems in utilizing the fish resource. Although a bulk of research has been devoted to species identification and geographic distribution of toxic fishes, chemical studies on the natures of toxin have started only in recent years.

Hashimoto demonstrated that the toxic principle from a poisonous barracuda, Sphyraena picuda, is soluble in fat solvents and the cat-feeding test is preferable to mouse intraperitoneal injection which had been commonly used for routine examination on toxicity of puffer and paralytic shellfish. Later, mongoose-feeding test, mouse injection test with partially purified toxin and frog sciatic nerve test have been developed by Banner et al. and Hess et al. By using these methods, an ether-soluble toxin was isolated from the muscle of Lutjanus bohar and designated ciguatera toxin, independently.

Among the fishermen in Okinawa, frequent outbreaks of ciguatera poisoning have been known from old, and some of the poisonous species were described by Kudaka and by Watanabe. No laboratory work, however, has been conducted. It is very interesting that the toxicity of fish is believed by people to disappear when fishes are kept in ice for about a week, and in fact, lutjanids are used as materials for “kamaboko” and consumed widely without any hazards.

A survey on the toxic species in Okinawa and preliminary experiments on the extraction of toxin from a grouper, Epinephelus fuscoguttatus, were carried out. The validity of story on the loss of toxicity during storage was also examined. The present paper deals with the results of these experiments.

Experimental and Results

1. Materials

Twelve species of fish caught in the vicinity of Okinawa Island and believed to cause occasional poisoning were available for the experiments. Eleven specimens among them were tested at Okinawa within a few days after catch, while the others...
were sent to our laboratory in frozen state and stored at $-20^\circ\text{C}$.

2. **Bioassay**

Cats were used as test animals. In feeding tests, a portion of cooked muscle was fed to cats at under 10% of body weight. When the extracts were to be tested, they were dried and packed into a few pieces of capsule to be administered orally, or dissolved in water to be injected subcutaneously.

**Table 1. Incidence of toxic fish**

<table>
<thead>
<tr>
<th>Species</th>
<th>Range of body weight (kg)</th>
<th>Tested in Okinawa</th>
<th>Tested in Tokyo*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>No. of specimen</td>
<td>No. of toxic specimen</td>
</tr>
<tr>
<td><em>Lutjanus vaigiensis</em></td>
<td>1.7-8.1</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>(QUOY &amp; GAIMARD)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>L. bohar</em></td>
<td>0.7-5.4</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>(FORSKål)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>L. fulviflamma</em></td>
<td>0.6-2.0</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>(FORSKål)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>L. monostigma</em></td>
<td>0.5-0.54</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>(CUVIER &amp; VALENCIENNES)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Variola louti</em></td>
<td>0.6-3.5</td>
<td>3</td>
<td>1</td>
</tr>
<tr>
<td>(FORSKål)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Epinephelus fuscoguttatus</em></td>
<td>2.5-13.4</td>
<td>4</td>
<td>2</td>
</tr>
<tr>
<td>(FORSKål)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Epinephelus sp.</em></td>
<td>1.0-1.1</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td><em>Pogonopera punctata</em></td>
<td>0.4</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>(CUVIER &amp; VALENCIENNES)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Gymnothorax meleagris</em></td>
<td>1.1</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>(SHAW)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>G. pictus</em></td>
<td>1.0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>(AHL)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Gymnothorax sp.</em></td>
<td>1.0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td><em>Gymnothorax sp.</em></td>
<td>0.9</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

* Tested from 10 to 40 days after catch

Cats fed poisonous specimens or injected toxic extracts usually developed the following symptoms: the animals first became sluggish, sitting in arched posture. Vomiting and in some cases diarrhea frequently appeared in a few hours. Weakness in limbs, especially in hind limbs, was observed and the cats became unable to stand or walk in right position. This state lasted fairly for a long time. In the most severe case, prostration, hypersalivation, and lacrimation followed by death were observed. Generally, recovery of the intoxicated animals was very slow and they frequently died in a few weeks from loss of appetite.

As shown in Table 1, nine specimens were found to be toxic among thirty-eight individuals tested. Details of the test in which the specimens were judged to be
toxic are presented in Table 2.

According to the method employed by HALSTEAD, aqueous extracts of the flesh

<table>
<thead>
<tr>
<th>Species</th>
<th>Body weight of specimens (kg)</th>
<th>Body weight of cats (kg)</th>
<th>Test materials</th>
<th>Dose (g)</th>
<th>Response</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>L. vaigiensis</em> (No. 2)</td>
<td>5.57</td>
<td>1.5</td>
<td>Liver</td>
<td>40</td>
<td>Vomiting, prostration</td>
</tr>
<tr>
<td><em>L. bohar</em> (No. 21)</td>
<td>5.40</td>
<td>0.9</td>
<td>Muscle</td>
<td>30</td>
<td>Vomiting, prostration</td>
</tr>
<tr>
<td><em>V. louti</em> (No. 27)</td>
<td>2.53</td>
<td>0.8</td>
<td>*</td>
<td>60</td>
<td>*</td>
</tr>
<tr>
<td><em>Epinephelus sp.</em> (No. 6)</td>
<td>0.97</td>
<td>0.6</td>
<td>Viscera</td>
<td>46</td>
<td>*</td>
</tr>
<tr>
<td><em>E. fuscoguttatus</em> (No. 12)</td>
<td>13.42</td>
<td>0.6</td>
<td>Muscle</td>
<td>30</td>
<td>*</td>
</tr>
<tr>
<td><em>E. fuscoguttatus</em> (No. 18)</td>
<td>10.84</td>
<td>0.6</td>
<td>*</td>
<td>30</td>
<td>*</td>
</tr>
<tr>
<td><em>E. fuscoguttatus</em> (No. 47)</td>
<td>11.0 **</td>
<td>1.1</td>
<td>*</td>
<td>107</td>
<td>Vomiting, weakness of limbs, lacrymation, hypersalivation, death</td>
</tr>
<tr>
<td><em>E. fuscoguttatus</em> (No. 48)</td>
<td>10.0 **</td>
<td>0.8</td>
<td>*</td>
<td>61</td>
<td>Vomiting, weakness of limbs</td>
</tr>
<tr>
<td><em>E. fuscoguttatus</em> (No. 49)</td>
<td>10.0 **</td>
<td>0.8</td>
<td>*</td>
<td>85</td>
<td>Vomiting, prostration</td>
</tr>
</tbody>
</table>

* Tested in Okinawa
** Head and viscera had been removed from the specimens

<table>
<thead>
<tr>
<th>Specimen</th>
<th>Fraction</th>
<th>Body weight of mice (g)</th>
<th>Dose (mg)</th>
<th>Equivalent to flesh (g)</th>
<th>Response</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>L. vaigiensis</em> (No. 2)</td>
<td>Petr. ether fr.</td>
<td>18~22</td>
<td>42</td>
<td>13</td>
<td>None</td>
</tr>
<tr>
<td></td>
<td>Ethereal fr.</td>
<td>18~22</td>
<td>32</td>
<td>28</td>
<td>None</td>
</tr>
<tr>
<td></td>
<td>Aq. EtOH fr.</td>
<td>18~22</td>
<td>47</td>
<td>20</td>
<td>None</td>
</tr>
<tr>
<td><em>E. fuscoguttatus</em> (No. 47)</td>
<td>Petr. ether fr.</td>
<td>15</td>
<td>28</td>
<td>6</td>
<td>None</td>
</tr>
<tr>
<td></td>
<td>Ethereal fr.</td>
<td>21</td>
<td>8</td>
<td>17</td>
<td>None</td>
</tr>
<tr>
<td></td>
<td>Aq. EtOH fr.</td>
<td>13</td>
<td>50</td>
<td>8</td>
<td>None</td>
</tr>
</tbody>
</table>
of three toxic specimens of *E. fuscoguttatus* (No. 47, 48, 49) were prepared by homogenizing the materials with twice volumes of water for 20 minutes at room temperature. After centrifugation, each supernatant was injected intraperitoneally to each three individuals of mouse weighing 11 to 13 g. No symptoms of intoxication were observed in succeeding 48 hours. In another experiment, test fractions prepared from toxic specimen of *L. vaigiensis* (No. 2) and *E. fuscoguttatus* (No. 18) were injected into mice intraperitoneally by following the procedures developed by BANNER and his colleagues*. The mice developed no symptoms as indicated in Table 3.

Each 21.5 and 10.0 g portion of flesh of toxic specimen of *E. fuscoguttatus* (No. 47) was fed to two individuals of rat weighing 99 and 103 g. No symptom appeared in 48 hours. As these tests on mice or rats were carried out in parallel

<table>
<thead>
<tr>
<th>Specimen</th>
<th>Storage period in days</th>
<th>Body weight of cats (kg)</th>
<th>Test materials</th>
<th>Dose (grams of flesh)</th>
<th>Response</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Lutjanus bohar</em> (No. 21)</td>
<td>5</td>
<td>0.6</td>
<td>Muscle</td>
<td>60</td>
<td>Vomiting, prostration</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>0.9</td>
<td></td>
<td>40</td>
<td></td>
</tr>
<tr>
<td></td>
<td>51</td>
<td>0.5</td>
<td></td>
<td>15</td>
<td>None</td>
</tr>
<tr>
<td></td>
<td>52</td>
<td>0.5</td>
<td></td>
<td>17</td>
<td></td>
</tr>
<tr>
<td></td>
<td>52</td>
<td>0.8</td>
<td></td>
<td>19</td>
<td></td>
</tr>
<tr>
<td></td>
<td>52</td>
<td>1.5</td>
<td></td>
<td>51</td>
<td></td>
</tr>
<tr>
<td><em>Variola louti</em> (No. 27)</td>
<td>1</td>
<td>0.8</td>
<td></td>
<td>40</td>
<td>Vomiting, prostration</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>0.5</td>
<td></td>
<td>10</td>
<td></td>
</tr>
<tr>
<td></td>
<td>44</td>
<td>0.6</td>
<td></td>
<td>26</td>
<td>None</td>
</tr>
<tr>
<td></td>
<td>44</td>
<td>0.8</td>
<td>Aq. extracts</td>
<td>50</td>
<td></td>
</tr>
<tr>
<td></td>
<td>45</td>
<td>0.6</td>
<td></td>
<td>50</td>
<td></td>
</tr>
<tr>
<td><em>Epinephelus fuscoguttatus</em> (No. 18)</td>
<td>0</td>
<td>0.6</td>
<td>Muscle</td>
<td>40</td>
<td>Vomiting, prostration</td>
</tr>
<tr>
<td></td>
<td>45</td>
<td>0.5</td>
<td></td>
<td>13</td>
<td></td>
</tr>
<tr>
<td></td>
<td>50</td>
<td>0.8</td>
<td></td>
<td>60</td>
<td></td>
</tr>
<tr>
<td></td>
<td>194</td>
<td>0.7</td>
<td>Aq. extracts</td>
<td>100</td>
<td>None</td>
</tr>
<tr>
<td></td>
<td>200</td>
<td>0.6</td>
<td></td>
<td>100</td>
<td></td>
</tr>
<tr>
<td><em>E. fuscoguttatus</em> (No. 47)</td>
<td>0</td>
<td>1.1</td>
<td>Muscle</td>
<td>107</td>
<td>Vomiting, ataxia, hypersalivation, death</td>
</tr>
<tr>
<td></td>
<td>0</td>
<td>1.2</td>
<td></td>
<td>95</td>
<td>Vomiting, prostration, ataxia</td>
</tr>
<tr>
<td></td>
<td>47</td>
<td>1.0</td>
<td></td>
<td>40</td>
<td>None</td>
</tr>
<tr>
<td></td>
<td>56</td>
<td>1.6</td>
<td></td>
<td>100</td>
<td></td>
</tr>
<tr>
<td></td>
<td>57*</td>
<td>0.8</td>
<td></td>
<td>100</td>
<td></td>
</tr>
<tr>
<td></td>
<td>62*</td>
<td>0.4</td>
<td>Aq. EtOH extracts</td>
<td>50**</td>
<td>Vomiting, prostration</td>
</tr>
<tr>
<td></td>
<td>62*</td>
<td>0.6</td>
<td></td>
<td>50**</td>
<td></td>
</tr>
</tbody>
</table>

* The muscle was cooked immediately after catch and stored in frozen state.

** Administered by subcutaneous injection.
with cat feeding tests which proved the toxicity of test materials, the rodents were found not suitable for bioassay.

3. Loss of toxicity during storage

As shown in Table 4, the toxicity of three species of fish, *L. bohar*, *V. louti*, and *E. fuscoguttatus* disappears during storage at $-20^\circ$C in about 50 days. Cooking prior to freezing is found to be effective in maintaining toxicity as seen on a specimen of *E. fuscoguttatus* (No. 47).

4. Extraction of the toxin

The muscle of toxic *E. fuscoguttatus* was used for the extraction of toxin. The chopped flesh (100 g, No. 12) was extracted three times with each 500 ml of boiling water for 30 minutes. After cooling, the residue was separated from the extracts by filtration and the combined extracts were concentrated to about 80 ml. Insoluble materials were removed by centrifugation, and the supernatant was messed up to 100 ml. Oral administration of each 30 ml portion of this solution caused intoxication on cats weighing 0.7 kg and 0.6 kg, respectively, while the cat weighing 0.9 kg fed 95 g of the residue developed no symptoms.

Fractionation of the toxin was attempted following the method of BANNER and his colleagues. The chopped flesh (250 g, No. 12) was extracted three times with each 500 ml portions of ethanol by boiling the slurry for 30 minutes. The combined filtrate were concentrated in vacuum to 50 ml and diluted with 150 ml of distilled water. The aqueous ethanolic solution was extracted first with petroleum ether and next with diethyl ether in a liquid-liquid extractor. Each fractions obtained were examined on toxicity after removal of solvent by cat-feeding test as shown in Table 5. The results indicate that the toxin is extracted with neither petroleum ether nor diethyl ether from aqueous ethanolic layer, but remains in aqueous layer. Direct extraction with ether from dehydrated muscle was attempted. Fifty grams of flesh (No. 12) was smashed thoroughly with anhydrous sodium sulfate and extracted

<table>
<thead>
<tr>
<th>Sample</th>
<th>Body weight of cats (kg)</th>
<th>Dose (mg)</th>
<th>Equivalent to raw flesh (g)</th>
<th>Response</th>
</tr>
</thead>
<tbody>
<tr>
<td>Muscle</td>
<td>1.0</td>
<td>107</td>
<td></td>
<td>Vomiting, ataxia, hypersalivation, prostration</td>
</tr>
<tr>
<td>Petr. ether</td>
<td>0.8</td>
<td>430</td>
<td>100</td>
<td>None</td>
</tr>
<tr>
<td>extracts</td>
<td>0.8</td>
<td>441</td>
<td>100</td>
<td></td>
</tr>
<tr>
<td>Ether extracts</td>
<td>0.9</td>
<td>41</td>
<td>100</td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.8</td>
<td>41</td>
<td>100</td>
<td></td>
</tr>
<tr>
<td>Aq. EtOH solution</td>
<td>0.8</td>
<td>1.650</td>
<td>100</td>
<td>Vomiting, prostration ataxia</td>
</tr>
<tr>
<td></td>
<td>0.7</td>
<td>1.650</td>
<td>100</td>
<td>Prostration, ataxia</td>
</tr>
</tbody>
</table>
with diethyl ether in a Soxhlet extractor for 8 hours. The ethereal extract was evaporated to dryness. Oily substance thus obtained (0.365 g) showed no toxicity when fed to a cat weighing 0.6 kg.

Chopped flesh (35 g, No. 12) was extracted three times with each 70 ml portions of 85% ethanol by boiling for 30 minutes. The combined extracts were dried up in vacuum, and the residue was further extracted twice with each 25 ml portions of hot acetone for ten minutes. Insoluble material was filtered off after cooling and the combined filtrate was dried up. Oily substance thus obtained (0.25 g) showed no toxicity when fed to a cat weighing 0.6 kg. While acetone-insoluble material (0.87 g) caused intoxication when fed to a cat weighing 0.6 kg.

Toxic solution was prepared from specimen No. 47 by slightly modifying the BANNER method where 70% ethanol was used as initial extractant and extraction with petroleum ether was bypassed. After extraction with ether, the aqueous solution was concentrated to 125 ml. Fifty ml of this solution was dialyzed through cellophane membrane against 1 l of distilled water for 5 hours. The outer solution was changed every hour. As shown in Table 6 administration of the diffusates to cats either orally or subcutaneously caused intoxication, while the dialyzates showed no toxicity.

<table>
<thead>
<tr>
<th>Table 6. Results of dialysis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fraction</td>
</tr>
<tr>
<td>-----------</td>
</tr>
<tr>
<td>Dialyzates</td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td>Diffusates</td>
</tr>
<tr>
<td></td>
</tr>
</tbody>
</table>

Discussion

Informations given by many persons strongly suggest that the species referred to be negative in Table 1 might be toxic sometimes together with following species which were not available in this study: Lethrinus sp., Plectropomus truncatus, Sphyraena picuda, Aleuteres criptus, Cheilinus sp., and Siganus sp. The results given in Table 4 support the fishermen’s saying that toxicity of fishes disappears during storage. In the light of this phenomenon further examination would be necessary for the interpretation of the incidence of toxicity in fishes listed in Table 1.

High incidence of toxicity was found on E. fuscoguttatus. In Okinawa this species is believed to be more poisonous than other ciguateric fishes such as red snappers,
and sometimes fatal. Symptoms on cats markedly differed from those observed on cats intoxicated by a barracuda, in which the transient paralysis of limbs and rapid recovery were far more apparent. The difference between the toxin of *E. fuscoguttatus* and the ciguatera toxin obtained from *L. bohar* is evidenced by fractionation test. According to Banner and his colleagues, although the toxin of the species of *Epinephelus, Gymnothorax, Caranx, and Sphyraena* can be extracted exactly in the same way as that of *L. bohar*, the toxin of *Ctenochaetus striatus* is not removed by extraction with ether. The same author reported that barracuda may carry a toxin of different pharmacological action than that causing the usual ciguatera. These findings strongly suggest that ciguatera comprises several different types of poisoning. In order to draw a conclusion, however, further chemical and pharmacological investigation of the toxin should be necessary.

**Acknowledgment**

Grateful acknowledgment is made to Dr. F. Yasuda for species identification of the fishes. Thanks are also due to Mr. K. Kudaka for his assistance in collecting the samples.

**References**

1) Hashimoto, Y.: *This Bull.* 21 (11), 1153 (1956).
8) Watanabe, M.: *Contribution from the Zoological Department, Research Institute for Natural Resources*, No. 55, 1 (1946).