Lethality Study on the Toxin from Xanthid Crab in Dalipuga, Iligan City

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ABSTRACT

Aqueous extracts were prepared from various parts of the crab Pilumnus vespertillo *at three different dose levels: 2.5, 5, and 10 mg/g. The extracts were tested for toxicity using the standard mouse assay. Cheliped muscle extracts were observed to be toxic to mice at dose levels 5 and 10 mg/g, with an average death time of 27 and three minutes, respectively. Extracts from abdominal muscles showed lethal activity only at 10 mg/g, with*

INTRODUCTION

Crabs belong to the crawling forms of decapod crustaceans distinct from their allied forms, the shrimps and the lobsters. Dominated mostly by marine species, they, however, are represented by both freshwater and semi-terrestrial forms. In the Philippines, marine crabs support a fishery industry and contribute to the diet of many Filipinos.

True crabs of the suborder Brachyura are a highly successful group, showing a variety of different types and habits. There are shore crabs, spider crabs, swimming crabs, pea crabs, and sponge crabs (1). These crabs conceal themselves under rock or coral reefs during the day and crawl at night. They are often caught through torch fishing and by gill nets.

While there are crabs popular to man, there are also known species that have caused deaths due to ingestion of their meat or even the broth (2,3,4, 5,6,7). These allegedly poisonous crabs are widely distributed and are found in coral reefs and many coastal seas in the Indo-Pacific region and also in the Philippines. There are eight families of brachyuran crabs having poisonous representatives: *Xanthidae*, *Majidae*, *Parthenopidae*, *Portunidae*, *Raninidae*, *Grapsidae*, *Gegarcinidae*, and *Domiidae*. Majority of the toxic species belongs to the family *Xanthidae*.

Rumphius (8) gave an interesting observation about poisonous crabs, saying that they have black "fingers", as if they are marked as poisonous by nature. These black- or brown-fingered crabs are especially numerous among *Xanthidae* and are rarely found among other brachyuran families (9).

Ingestion even of cooked crab meat may cause the following disorders in man: paresthesia, muscular paralysis, aphasia, nausea, vomiting, collapse, hypersalivation, muscular exhaustion, convulsion, and respiratory distress

(2,10). Within a few minutes or a few hours after ingestion, death can occur.

Serious investigations have been conducted to characterize the toxin, determine its source, as well as its pharmacologic activity. Regional variations in crab toxicity have also been studied (11,12).

The toxic principle has been found to vary chemically with species and geographical location. Dr. Hashimoto and his group at the University of Tokyo (2,13) discovered that the toxin from coral reef crabs (*Atergatis floridus* and *Zosimus aeneus*) of Japan is identified chemically with saxitoxin (STX), which is elaborated by the planktonic dinoflagellate *Gonyaulax catenella*. Partially purified toxin was shown to be indistinguishable from saxitoxin by paper chromatography (10,11, 14). Several other dinoflagellates have been found to produce saxitoxin and other chemically related compounds (15). An exogenous source for the toxin in the crab has therefore been suggested. However, the major toxin present in Australian specimens of *Atergatis floridus* does not appear to be saxitoxin (10,14).

The similarity of the crab poison with tetrodotoxin (TTX) in certain pharmacologic actions has also been reported. Screening tests on both *Zosimus aeneus* and *Platypodia granulosa* from Ryukyu and Amami Islands, Onoa Atoll, and Gilbert Islands revealed that they contained paralytic poison with a dose-death time curve similar to TTX (2,11).

Other kinds of toxins have also been identified from *Zosimus aeneus* and *Atergatis floridus* in Okinawa, Japan (16,17). These are the gonyautoxins I, II, and III. It was suggested that these toxins come from certain calcareous red algae, *Jania* sp., as evidenced by its presence inside the stomach of the crabs. Yasumoto et al. (18) have claimed that neosaxitoxin and gonyautoxin II were the minor toxins of *Atergatis floridus* and *Zosimus aeneus* from Ishigaki, Okinawa, Japan. Trace amounts of gonyautoxin I was also detected in the latter species. Platypodia granulosa contained only saxitoxin.

Several authors (19,2,11,20,21,14, 22,23,10,24,25,26,27) have studied the properties of crab toxin. According to Santos (25), the molecule is small (less than 24 Angstroms), heat stable, protease resistant, and hydrophilic. It exists as a cation under physiological conditions and apparently depends upon electrostatic interactions for its biological activity. According to Hashimoto et al. (2), adjustment of pH to 3.0 did not affect the toxin, but its toxicity was reduced by half when the pH was increased to 10. The toxin was readily dialyzed, completely extracted with methanol, only slightly with ethanol, and not at all with diethyl ether, petroleum ether, hexane, chloroform, and 1-butanol.

There is little information on Philippine crabs and their toxins, although there are a good number of poisonous species in the country. *Pilumnus vespertilio*, which belongs to the family *Xanthidae*, is found in the Philippines and the rest of the Indo-Pacific Region. Its presence in Dalipuga Bay, Iligan City, has been one of the reasons for its choice as subject in this study, which aims to provide baseline information on toxin from this locally available species. Crude extracts of the toxic principle from different parts of the body of the crab were prepared and relative toxicities determined. Any information gathered here will definitely be of interest to toxicologists, as well as the general public.

MATERIALS AND METHODS

Sample collection. Specimens of *Pilumnus vespertilio* were collected at the littoral reef area of Dalipuga, Iligan City, during the day, and also at night, and their habitat noted. The crabs were frozen and transported in dry ice to the laboratories of the University of the Philippines at Dil-

iman, Quezon City. They were kept in the freezer until the experiment.

Toxin extraction. The procedure of Endean et al. (26) was followed. The abdominal muscles and intestinal parts were separated from the chelipeds. The tissues were macerated separately using a mortar and pestle; contamination was avoided by washing the apparatus thoroughly with distilled water between successive macerations. A suspension of 250 grams macerated tissue in 250 mL distilled water was boiled in a water bath for 20 minutes and centrifuged at 3000 rpm for 15 minutes. The supernate was filtered through Whatman Filter Paper No. 1, acidified with 2 N HCl to pH 3, and centrifuged again at the same speed for 15 minutes. The supernatant liquid was neutralized to pH 7 with 2 N NaOH, then dialyzed against two changes of distilled water, each of 1500 mL volume. The dialysis was done for 18 hours each time. The 300 mL dialyzate was concentrated in a Rotavap and then lyophilized. A portion of the dialyzate was tested for toxicity.

Toxicity determination. The lyophilized residue was reconstituted into aqueous solutions with different concentrations: 2.5, 5, and 10 mg/g. A standard mouse assay was employed by intraperitoneal administration of 1 mL of solution for every 20 grams of mouse. Four mice were used per dose level, and death time in minutes recorded.

A control group was treated similarly as the experimental mice, except that extracts of edible crab (locally named "lambay") were used.

RESULTS AND DISCUSSION

Table 1 shows the lethality of *Pilumnus vespertilio* using the abdominal and gastrointestinal tissue extracts. It is seen that only the third dose level (10 mg/g) demonstrated lethal effects on mice with death time ranging from 7 to 36 minutes, or with an average death time of 16.75 minutes. According to the standard mouse assay, a 10-minute death time indicates a toxin that is very potent. After injection, and before its death, the mouse exhibited various symptoms, like paralysis of the limbs, rapid muscular contraction, and aphasia.

The cheliped muscles, at dose levels 2 and 3, showed lethal effects on the mice (Table 2), which exhibited the same reactions to intraperitoneal injection of the extract. Dose level 2 gave the shortest death time, with an average of three minutes.

The control group exhibited no lethal effects.

The cheliped muscle extract had greater toxic effects than abdominal and intestinal muscle extracts. The observed death time of about three minutes indicates that further purification of the crude extracts would produce a more potent toxin. The difference in activity may be attributed to the calcareous algae which were observed to be growing on or associated with the crab's appendages/chelipeds. According to Yasumoto et al. (18), this calcareous algae is a source of gonyautoxin. An exogenous source of the crab toxin is therefore possible, but its metabolic synthesis by the

Table 1. Lethality of abdominal and intestinal muscle extract of the crab *Pilumnus vespertilio*.

Dose Levels	Concentration (mg/g)	Mouse Weight (g)	Volume Extract (mL)	Death time (min)	No. of mice that died
1	2.5	15.8	0.79	-	0
		13.6	0.68	-	
		17.1	0.68	-	
		16.0	0.80	-	
2	5.0	21.5	1.07	-	0
		19.5	0.92	-	
		19.4	0.93	-	
		19.4	0.93	-	
3	10	20.3	1.01	36	4
		19.3	0.96	17	
		17.1	0.86	7	
		18.3	0.91	7	

Table 2. Lethality of cheliped muscle extract of the crab *Pilumnus vespertilio*.

Dose Levels	Concentration (mg/g)	Mouse Weight (g)	Volume Extract (mL)	Death time (min)	No. of mice that died
1	2.5	17.2	0.86	-	0
		17.6	0.88	-	
		12.7	0.64	-	
		15.1	0.76	-	
2	5.0	17.2	0.86	10	4
		17.6	0.88	11	
		17.2	0.86	35	
		17.2	0.86	38	
3	10	18.7	0.94	2	4
		17.2	0.86	3	
		18.5	0.92	4	
		19.3	0.97	3	

animal cannot yet be ruled out. Another aspect of interest which this study has brought out is the effect of the toxin on the mice's neuromuscular system, as indicated by the rapid muscle spasms and limb paralysis.

The varied toxicity of parts of the crab *Pilumnus vespertilio* has been shown in this study, but deeper and more thorough investigations of all aspects of its activity still has to be done. ■

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