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## Palliative Measures to Paralytic Shellfish Poisoning (PSP)

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## Abstract

The Philippines has been monitoring for disastrous harmful algal blooms (HABs), locally known as a toxic red tide for several years. HABs can be a cause of the proliferation of dinoflagellate *Pyrodinium bahamense var. compressum* (Pbc). Pbc blooms are said to affect tropical bivalves such as the green mussel *Perna viridis* or locally known as tahong and the thorny oyster *Spondylus squamosus* [14]. When the shellfish consumed the Pbc and eaten by the people, it causes paralytic shellfish poisoning (PSP). These Pbc are highly potent due to its capability to block the sodium channels of the membranes of the neuron cell and lead to sudden death and paralysis [8]. The test subjects in this study were the white mice, *Mus musculus*. The mouse bioassay method was from the "Practical Guide on Paralytic Shellfish Poisoning Monitoring in the Philippines, 2002", which was provided by the Bureau of Fisheries and Aquatic Resources (BFAR). Ingesting the toxin to the mice's mouth was through the 1-mL tuberculin syringe. In this study, the palliative measures after ingesting the toxin are the ingestion of coconut milk (gata) and virgin coconut oil (VCO). The experimental method of research explicitly used the factorial design in determining the effects of coconut milk (gata) and VCO as an antidote to PSP. The findings of the study showed that coconut milk (gata) is much more useful than the virgin coconut oil (VCO) as a palliative measure to PSP. Furthermore, it is best to introduce the treatment right after the ingestion of the toxin.

Keywords: Palliative measure; poisoning; red tide; shellfish.

## 1. Introduction

The Philippines, surrounded by coastal waters, has been monitoring for disastrous harmful algal blooms (HABs) or locally known as a toxic red tide for several years. The increase of *Pyrodinium bahamense* var. *compressum* (Pbc) dinoflagellate have been associated with the paralytic shellfish poisoning (PSP) cases in the country. Pbc blooms are said to affect tropical bivalves such as the green mussel, *Perna viridis* or locally known as tahong and the thorny oyster *Spondylus squamosus* [14].

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Red tide is also potentially toxic to human health. Humans can become seriously ill from eating shellfish contaminated with red tide toxin and sometimes lead to death. HAB is predominant with Pbc dinoflagellateproducing saxitoxin that causes PSP in the Philippines, one of the tropical regions. The neurotoxin is watersoluble, acid stable, and relatively heat stable even in high temperature [10]. These Pbc are highly potent due to its capability to block the sodium channels of the membrane of the neuron cell and lead to sudden death and paralysis [13].

The first reported outbreak of toxic red tide in the Philippines occurred in June 1983 in Maqueda Bay, in Samar [3]. A second major outbreak occurred in mid-1987 still in the same place. In Samar Sea, toxic red tide once again reported in September and remain present in parts of the Central Philippines until March 1989 [15].

In July 2013, a PSP report was received by the Philippines' Event-Based Surveillance & Response Unit from Tarangnan, Western Samar. A team of expert from the Department of Health conducted an outbreak investigation to identify the implicated source and risk factors on coastal villages known for green mussel production and exportation [22]. Obtained PSP is by consumption of green mussels, precisely as a mussel broth, harvested in Cambatutay Bay in Tarangnan. The high toxicity of saxitoxin is involved both in green mussel and seawater samples. The cases were primarily presented with neurological symptoms consistent with other PSP outbreaks [11].

As stated by the Manila Bulletin news as of 18<sup>th</sup> of January 2017, the Bureau of Fisheries and Aquatic Resources-Region 8 (BFAR-8) had issued a warning that red tide toxins continue to persist in the bays of Eastern Visayas. There was one reported death in the town of Daram, Samar and twenty other victims hospitalized in the same municipality for eating contaminated shellfish. With this, some people resort to preventive measures such as avoiding the consumption of any seafood, rinsing the shellfish before cooking or throwing away the open shells [5]. Some people adequately considered palliative measures such as drinking coconut milk (gata) after ingesting contaminated shellfish [1]. However, using coconut milk (gata) as an antidote to PSP is not yet established. The effectiveness of coconut milk (gata) as an antidote to PSP is not yet established. Moreover, people using this palliative measure to treat PSP might be at risk for other side effects of the coconut milk (gata).

This study is limited to the effect of coconut milk (gata) and virgin coconut oil (VCO), to the red tide toxin (saxitoxin) found in *tahong* and oysters.

The extracted toxin from the microalgae is the dinoflagellate species of *Pyrodinium bahamense* var. *compressum*. The toxin produced by the dinoflagellates specifically the *Pyrodinium bahamense* var. *compressum* that is accumulated by mussels and oysters were extracted and injected into the mice. The *Mus musculus* (white mice) were used and ingested with the different coconut milk concentrations and VCO and were observed for 24 hours. The result of this study served as an immediate first-aid response to the intoxication of the toxin from *Pyrodinium bahamense* var. *compressum* to the residents who are consumers of shellfish. When people consumed contaminated shellfish and felt the symptoms, it would lessen the number of deaths from paralytic shellfish poisoning because of this awareness.

#### 2. Literature Review

Red tides had always been a concern in the Philippines because of the large numbers of consumers who are at risk of poisoning and its potential economic impact in the fishing industry. There were forty-two (42) outbreaks caused by toxins out of the 2,107 paralytic shellfish poisoning cases and 117 deaths from 1983 to 2001. Before, only a few coastal areas of the country were affected in scattered locations, but today, this has grown to a total of twenty (20) coastal regions. For Manila Bay, during the 1992, Pyrodinium red-tide outbreak, around 38,500 fisher folks were displaced from their livelihood due to the red tide scare. Estimated economic losses for displaced fisher folks was Php3.4 billion (in 2002 prices) [21].

Dr. Rafael D. Guerrero III, former director of the Philippine Council for Aquatic and Marine Resources Development (PCAMRD), said red tide is a natural phenomenon brought about by the bloom or predominance of a floating microscopic organism known as dinoflagellates. These "single-celled organisms can swim at the maximum rate of one meter per hour, by means of two whip-like flagella."

The University of the Philippines at Los Baños (UPLB) said the name red tide was coined due to the sea water discoloration which ranges from amber, red, brown, yellow orange to purple caused by the highly-densed population of dinoflagellates. To make it short, its present name was given. In science, it is called harmful algal blooms (HABs).

## • Red Tide

A red tide is a common term used for a harmful algal bloom. Harmful algal blooms, or HABs, occur when colonies of algae, simple plants that live in the sea and freshwater, grow out of control while producing toxic or harmful effects on people, fish, shellfish, marine mammals, and birds. The human illnesses caused by HABs, though rare, can be debilitating or even fatal. While many people call these blooms 'red tides,' scientists prefer the term harmful algal bloom. This bloom, like many HABs, is caused by microscopic algae that produce toxins that kill fish and make shellfish dangerous to eat. The toxins may also make the surrounding air difficult to breathe. As the name suggests, the bloom of algae often turns the water red [19].

• Paralytic Shellfish Poisoning (PSP)

The first recorded outbreak of paralytic shellfish poisoning (PSP) in the Philippines occurred in June-September 198 in the Samar Sea [9] the causative organism was *Pyrodinium bahamense* var. *compressum* and about 20 deaths resulted, mostly from eating bivalves, with a few from planktivorous fishes [9, 2]. Many persons were said to have felt relief from PSP symptoms by drinking coconut milk with brown sugar, a traditional palliative [7].

Paralytic shellfish poisoning is a marine toxin with both gastrointestinal and neurologic symptoms reported worldwide. It is caused predominantly by the consumption of contaminated shellfish. *Pyrodinium bahamense* var. *compressum* is the main causative algae that cause paralytic shellfish poisoning. These marine bio-toxins are highly potent due to its capability to block the sodium-channel of the neuron cell membrane and lead to

sudden death and paralysis [13]. PSP toxins are heat-stable and water-soluble non-proteinaceous toxins. The PSP are typically comprised of saxitoxin and its derivatives that are subdivided into three categories which are carbamate toxin (highly potent), dacarbamoyl toxin (intermediate toxin) and *N*-sulfocarbamoyl toxin (least potent) [22, 12]. This Saxitoxin is the most toxic and also the most well studied among the other associated PSP toxins.

The major transvector for PSP are the bivalve molluscs (mussels, clams, oysters, with the Alaskan butterclam having the highest concentrations) [23]. PSP toxins are also found in certain crabs and snails which feed on coral reef seaweed. The transvectors accumulate the toxins via feeding in their digestive organs and soft tissues, apparently without harm to the transvectors.

Humans, birds and fish can all be affected by PSP toxins. Herbivorous zooplankton is the primary transvector that can in turn transmit the toxin to fish and possibly other marine creatures that consume zooplankton (Baden 1983). The usual route for humans is the consumption of raw or cooked contaminated shellfish.

• Science-Based Health Benefits from Coconut Herbal Medicine

Coconut has many claims of health benefits in traditional medicine. Only recently that laboratory studies and research has been made to verify its effectivity and science has uphold some its claims as cure for many health problems [17].

There is a misconception about coconut oil that has been propagated long since being high in saturated fats that can cause heart diseases. In the contrary, recent research have shown that the saturated fats found in coconut oil is a type of unique fat molecule known as medium-chain fatty acids (MCFA) that prevents heart diseases. The medium-chain fatty acids found in coconut oil increases the HDL level (good cholesterol) while lowering the LDL (bad cholesterol) in the blood thus improving the ratio of HDL to LDL which is the basis for heart disease risks. The p-Coumaric acid in coconut oil prevents the formation of arterial plaque by preventing the stickiness of the blood platelet, lowering the blood pressure and reducing the risk of damaging the arteries and preventing the development of atherosclerosis [17].

According to [18], during the first toxic dinoflagellate bloom in 1983, the use of coconut milk as an antidote for red tide toxin was tried by the locale through oral route. Based on the records of the Catbalogan Integrated Provincial Hospital (CIPH) in Samar, there were more than 80% of the patients that was given an oral dose of the locale antidote have recovered. The levels of toxicity of the contaminated shellfish ingested by the victims could not be determined to make a more meaningful evaluation of the efficacy of coconut milk as an antidote.

The chemical composition of coconut milk shows difference in factors such as geographical location of the nuts, the method of extraction and the degree of dilution if water or liquid endosperm is added during extraction. The coconut milk is furtherly composed of carbohydrates, which is about 10% in coconut endosperm. Some of them are, D-galactose, galacturonic acid, galactomannan, and L-rhamnose. Proteins that are approximately about 3%. The dominating proteins are albumins and globulins, and the protein content of the undiluted milk ranges from 5-10% on dry basis. It is composed also of vitamins mainly, vitamin A, thiamin (or vitamin B), riboflavin (or

vitamin B<sub>2</sub>), niacin and vitamin C. And other active principles such as organic acids (malic acid, citric acid), phytosterols and minerals (calcium, potassium, sodium, magnesium, phosphorus).

In this study, it was proven that coconut milk is much more effective palliative measure to paralytic shellfish poisoning than virgin coconut oil (VCO).

## 3. Methodology

The method of research utilized in this study is the experimental method. The design is used to determine the effects of coconut milk (gata) and virgin coconut oil (VCO) as a palliative measure to paralytic shellfish poisoning (PSP). The test subjects were white mice (Mus musculus), approximately four-month-old. The researchers followed the Regulations and Ethical Considerations in Animal Experiments: International Laws and Islamic Perspectives (Mohammad 2012) while handling the test subjects. The researchers used thirty-six (36) mice and prepared three (3) experimental set-ups. Each set-up has twelve (12) mice treated with: (1) water, (2) coconut milk (gata), and (3) virgin coconut oil (VCO), respectively. Of the twelve (12) mice in each set-up, six (6) were immediately given the treatment right after the ingestion of the toxin, and the other six (6) were treated later if there is a manifestation of the symptoms for PSP after the intake of the toxin as shown in Table 1. When the mouse appeared distressed, leaped high into the air, thrashed, limped, lied down or slowly gasped for air, were the manifestations of PSP symptoms. The exact death time was on the last gasping breath of the mice.

	Treatment	Number of Mice/Set – up		
Setup		Number of mice treated after ingestion of PSP	Number of mice treated after symptoms of PSP showed	Number of Test Subjects
1	Water	6	6	12
2	Coconut Milk (Gata)	6	6	12
3	Virgin Coconut Oil (VCO)	6	6	12
Total				36

 Table 1: Prepared Experimental Set – up.

#### 3.1 Sample Site

The collection of the green mussel, *P. viridis* that is locally known as tahong, was on the coastal waters of Irong-Irong Bay, Brgy. Lucerdoni, Tarangnan, Western Samar. Based on the laboratory results posted by BFAR on the Shellfish Bulletin No. 35 Series of 2017, the green mussels were positive to paralytic shellfish poison beyond the regulatory limit before the conduct of this study.

#### 3.2 Sample Collection

All samples were collected on a random basis as possible concerning likely influencing environmental factors, e.g., tidal state, rainfall, wind, etc., to avoid introducing any bias to the results.

The sampling method of shellfish for commercial harvesting was used as this can influence the degree of contamination. The barangay chairman and fisherfolks of the said barangay assisted in harvesting the sample. Shellfish were made free from adhering mud and sediment by rinsing and scrubbing with fresh water of potable quality, were placed inside a food-grade plastic bag, and labeled. The ice packs used must not come into direct contact with the sample bags with the green mussels. The sample bags were in the cooler before transporting it. Samples were delivered to the laboratory within 24 hours after the collection with temperatures between  $1^{\circ}C - 8^{\circ}C$ .

The researchers adopted the Protocol for sampling and transport of shellfish for the purpose of Official Control Monitoring of classified shellfish production areas under Regulation (EC) No. 854/2004, [5].

## 3.3. Extraction of Toxin from Shellfish Samples

The extraction of the toxin from the shellfish was at the Chemistry Laboratory, Leyte Normal University, Tacloban City, Leyte. A blender was used to homogenize the drained fresh shellfish tissue until no more lumps were present. The 100g of homogenate and 200 mL of distilled water was mixed in a 500-mL beaker with the addition of 100 mL of 0.1N HCl.

The ideal pH range of the homogenate is between three and four, where all PSP components are in optimal stability. The mixture was heated at  $85^{\circ}$ C for five minutes with constant stirring to distribute the heat evenly.

The sample was allowed to cool and centrifuged at 4000 rpm for 10 minutes. Through proper decantation, the extraction of supernatant liquid was done.

#### 3.4 Mouse Bioassay Method

The mouse bioassay method was adopted from the "Practical Guide on Paralytic Shellfish Poisoning Monitoring in the Philippines, 2002," which was provided by the Bureau of Fisheries and Aquatic Resources (BFAR). The standard weight of the mice ranges from 17.0 to 22.0 g.

#### 3.5 Ingestion of the Extracted Toxin to the White Mice

The 1-mL toxic extract in the tuberculin syringe was ingested into the mouth of the white mice, and the researchers observed and recorded the reaction of the mice.

The mice were marked on its tail with a felt-tip marker to indicate the trial number for each mouse and were put back into its cage for observation of PSP symptoms.

#### 3.5 Ingestion of the Coconut Milk (Gata) And Virgin Coconut Oil (VCO) As An Antidote To The Toxin

The coconut milk (gata), virgin coconut oil (VCO), and water (control) were taken into the mice's mouth using 1-mL tuberculin syringe after the ingestion of PSP and after the symptoms of PSP showed.

#### 4. Results and Discussion

## 4.1 Results

Table 2 shows the number of alive white mice (*Mus musculus*) after the ingestion of the treatments in each setup: water, coconut milk (gata) and virgin coconut oil (VCO).

	Treatment	Number of Mice/Set – up $(n = 12)$		Total Number of
Setup		Number of alive mice treated after ingestion of PSP	Number of alive mice treated after symptoms of PSP showed	Alive Test Subjects
1	Water	0	0	0
2	Coconut Milk (Gata)	6	5	11
3	Virgin Coconut Oil (VCO)	5	4	9

Table 2: Alive White Mice (Mus musculus) ingested with PSP after 16 hours.

As shown in Table 2, when the test subjects were treated with water (Setup 1) right after they were ingested with toxin and likewise, treated them after the symptoms of PSP showed, none of them survived.

Meanwhile, in Setup 2, all the six mice survived when ingested with coconut milk after the intake of the toxin whereas, five out of six or 83% survived when treated after the manifestation of PSP.

The third setup uses the virgin coconut oil (VCO) as the treatment. There were five out of six or 83% survived when treated after the ingestion of the toxin while four out of six or 67% survived when treated after the PSP manifested.

## 4.2 Discussion

All the test subjects died using water as the treatment after ingested with PSP, and when treated after the PSP symptoms took place. Whereas all the mice survived when treated with coconut milk (gata), and only one test subject died when treated with virgin coconut oil (VCO) after the immediate ingestion of PSP. On the other hand, one of the white mice died when ingested with the coconut milk (gata) after the manifestation of the PSP, while two subjects died when treated with virgin coconut oil (VCO) as well. All the surviving white mice can stand, move, and displayed no signs of PSP symptoms after 16 hours of both treatments.

## 5. Conclusion

The results of this study showed that coconut milk (gata) and virgin coconut oil (VCO) were both effective palliative measures to paralytic shellfish poisoning. However, coconut milk (gata) is much more effective than virgin coconut oil (VCO). Furthermore, the result shows that it is better to introduce the treatment after the ingestion of the toxin.

#### 6. Policy Implications of the Study

Based on the latest laboratory results of the Department of Agriculture through the Bureau of Fisheries and Aquatic Resources and Local Government Units (LGUs), the Shellfish Bulletins are issued. These series of Shellfish Bulletins informed the consumers that shellfishes collected on identified places that are still positive for paralytic shellfish poisoning (PSP) that is beyond the regulatory limit are not safe for human consumption. The Shellfish Bulletins likewise warn the consuming public to refrain from eating such contaminated shellfish to refrain from untoward incidents.

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