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Identification and hemolytic activity of jellyfish (*Rhopilema* sp., Scyphozoa: Rhizostomeae) venom from the Persian Gulf and Oman Sea

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Abstract. *Jafari H, Honari H, Zargan J, Jahromi ST. 2019. Identification and hemolytic activity of jellyfish (*Rhopilema *sp., Scyphozoa: Rhizostomeae) venom from the Persian Gulf and Oman Sea. Biodiversitas 20: 1228-1232.* The present study investigated the hemolytic capacity of the crude venom extracted from isolated nematocysts of *Rhopilema* sp. Scyphozoa: Rhizostomeae. Nematocyst was used at various concentrations to evaluate the hemolytic activity by using the nematocysts of human, mice, and sheep. Mean concentration-dependent hemolysis could be observed from 200 µg/mL of protein equivalents or higher with variable potencies in different species. The crude venom was analyzed using sodium dodecyl sulfate-polyacrylamide gel electrophoresis SDS-PAGE. Molecular weight with 3 clear bands including 45, 65 and 95 kDa appeared to be the major protein components of the venom. The results of our experiments indicated that venom of *Rhopilema* sp. induces hemolysis in the studied species and determined that the increase in the amount of toxin has a positive correlation with the increase of cell lysis. This study showed that the venom of *Rhopilema* sp. may have many biologically active principles, which need further studies in the future.

Keywords: Venom, Nematocysts, Hemolytic assay, *Rhopilema*

INTRODUCTION

Jellyfish belong to the largest known Medusa in the Cnidaria phylum. Some of this species belonging to this phylum such as *Rhopilema* sp. are reported in the Persian Gulf (Qeshm island) and the Oman Sea (Bandar E- Jask). Their populations become very large in many seasons of the year (Jouiaei et al. 2015). Jellyfish blooming causes change in fisheries stock due to food competition. In current years, there have been many reports of jellyfish blooms in different parts of the world such as Italy, Turkey, and China. Such changes in the population and distribution have also been reported in the Persian Gulf and Oman Sea. (Daryanabard and Dawson 2008). Jellyfish blooms of various species have always interested the researchers since they often cause damage to swimmers. Most injured people do not need supplementary therapies and hospitals, but some show systemic complications and very few cases end in mortality. A change in the number of jellyfishes can lead to changes in marine ecosystems or destruction of the marine installations (Purcell et al. 2005, 2007). On the other hand, jellyfishes contain important compounds, especially collagen (Addad et al. 2011; Zhuang et al. 2009). In addition, at present, some invasive species such as *Phyllorhiza* sp. in the coastal waters have also severely affected the tourism industry in many coastal tourist spots (Johnson et al. 2005). The bloom of a giant jellyfish, *Nemopilema nomurai*, has caused fishery damages and danger to sea bathers in the waters of China, Korea, and Japan.

The objective of this research is to investigate the cytotoxic and hemolytic activities of crude venom extract of *Rhopilema* sp. using a number of *in-vitro* assays. Toxins resulting from some of the jellyfish species typically for the development of new drugs also have promising applications in cardiovascular medicine and target medicine of nerve molecular biology. Therefore, it is useful to study jellyfish venom which is beneficial in human health using animal studies. Such experiments in rat confirmed that jellyfish can be used to cure arthritis (Billingham et al. 1973). Other researchers have described that the various venoms from different jellyfish species have biological functions (Deshane et al. 2003). A few publications have dealt with the nematocyst toxins of *Rhopilema* sp. on the basis of a newly developed method for isolating undercharged nematocysts of these jellyfishes. The present study was performed to examine the biological properties of crude extracts from *Rhopilema* sp**.** from the Persian Gulf and Oman Sea.

MATERIALS AND METHODS

Sampling

Jellyfish medusae of the *Rhopilema* sp. were collected by diving from Bandar- E Jask in the southwest of Iran (25◦31'51.81"N and 47◦49'11.86" E) throughout the coastal waters of the Oman Sea during September to November 2017 (Figure 1). These specimens were identified as *Rhopilema* sp. according to Kramp (1961) and Kitamura and Omori (2010).

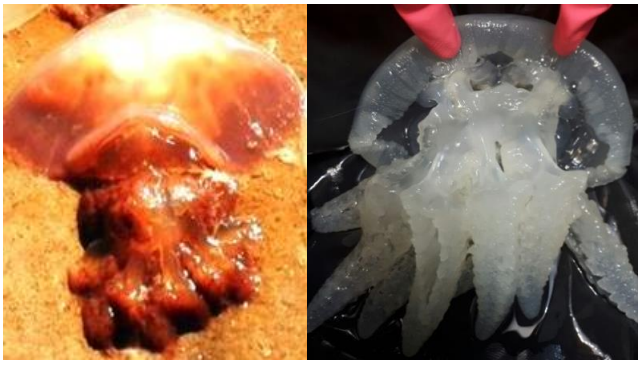

Figure 1. *Rhopilema* sp. morphotypes from the Oman Sea

Isolation the venom

Purification of isolated nematocyst preparation

The procedure made by Bloom et al. (1998) has been used to isolate the venom existing in nematocysts cells. For this purpose, tentacles were removed from live jellyfish. Then, tentacles were refrigerated in bottles with two volumes of seawater for one to four day. Once a day the containers were vigorously shaken and an aliquot filtered through a fine sieve. The decision to continue digestion of individual samples was based on microscopic examination of the filtered sediment and tentacles. The final sediments were then lyophilized and stored at −70 °C until use.

Preparation of nematocyst venom

The powder resulting from the lyophilized nematocysts was dissolved (1: 6) in twice distilled water. Then the solution was examined by microscope to determine the amount of nematocysts cells. The Mechanical homogenization and sonication (Hielscher, UP100H (was performed in three periods of 20 seconds in dry ice. Next, the solution was centrifuged for 1 hour at 20,000 rpm. The solution was stored in 1 mL tubes in -20 °C.

Determination of the concentration of extracted venom

To determine the concentration of toxin, 10 microliters (µL) of the solution was added to 1.5 mL tubes, and then the volume was brought to the final volume of 100 mL. In another tube, only 100 µL of distilled water was poured. Then, one mL of Bradford solution was added to each tube and kept at room temperature for 5 minutes. The absorbance was measured at 595 nm by a spectrophotometer (Shimadzu, UV-1280). The numbers obtained were compared with the standard curve and the protein concentration was calculated (Bradford 1976).

Purification of venom using gel chromatography

For partial purification of toxin, 1 mL of the raw venom was applied to a 3 x 6 cm column of Sephadex G200 chromatography column using 5.05 molar buffer at pH = 0.7 and then the absorption of the fractions was determined at 280 nm.

Investigating the poisoning rate

The bioassay of lethality was conducted using clinically healthy mice (25 ± 2 g). The mice were maintained in the laboratory in a healthy condition. Mice in triplicate were tested subcutaneously with different concentrations at 10, 100, 200, and 300 micrograms of toxin which were dissolved at 5 mg/mL in phosphate buffer saline (PBS) in a final injection volume of 0.5 mL. Control was maintained in each case by injecting an equal volume of PBS (pH 7.4). The time of injection and death, as well as behavioral changes before death were recorded for 24 hours. The results of the main test were calculated using Pearson-Carver statistical method.

Estimating the lytic activity

Human, mouse, and sheep red blood cells were used to evaluate the lytic activity of the venom. Blood samples were transferred to the tubes containing anti-clot material. Then, 20 mL of phosphate buffer saline (PBS) was added (pH 7.4) to 1 mL of blood, and centrifuged at 5000 rpm for 5 minutes at 4°C. The supernatant was drained and re-centrifuged at 5000 rpm for 5 minutes. Finally, the erythrocytes were diluted up to 2%. A 96-well plate was used to determine the optical density. For each well, 300 µL of three types of blood cells (human, mice, and sheep) were inoculated with 2% dilution, and then 60 µL of the sample extracted with concentrations of 50, 100, 200 and 400 µg/mL were added to each well. The mixture of poison and blood cells was placed at room temperature for 4 hours. Then, 150 µL of the supernatant was removed from the wells and 150 µL of the Bradford Reagent was added to each well. Finally, the absorbance was measured at 595 nm. The wells containing blood cell suspension and wells with phosphate buffer saline were considered as negative and positive controls respectively (Bloom et al. 1998).

SDS-PAGE (sodium dodecyl sulfate-polyacrylamide gel electrophoresis) was used for the separation of proteins based on their molecular weight and their differential rates of migration through a sieving matrix (a gel) under the influence of an applied electrical field.

In this study total protein from protein compositions of studied jellyfish poison were isolated by Coomassie-stained 12% SDS-PAGE gel (Figure 2).

RESULTS AND DISCUSSION

SDS-PAGE

SDS-PAGE isolated protein compositions of studied jellyfish poison. Figure 1 shows that the venom contains proteins with different molecular weights, but most proteins are positioned in three molecular weights of 45, 65 and 95 kDa (Figure 1).

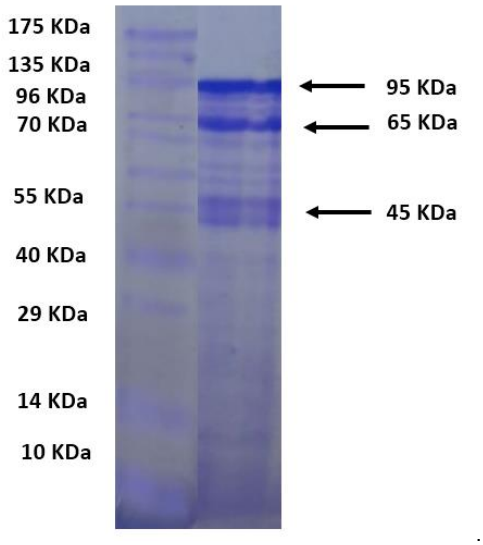

Figure 2. SDS-PAGE Gel (12% polyacrylamide gel with Coomassie blue analysis showing *Rhopilema* sp. crude venom (*right*) and standard marker (*left*)

Determination of the LD50 value

The dilution series was used in the main test and subcutaneous injection was performed in 4 mice (25 ± 2 g). Then the results were calculated using Pearson-Carver statistical method according to the equation below. Logarithm of the 100% death rate ± (logarithmic coefficient)/(dose per sample number) (Σ dead number - n/2) = α & β. Eventually, the LD50 infusion rate for Balb/c was 0.45 µg/kg.

Hemolytic activity

To evaluate the effect of the hemolytic effect, 50 to 800 µg of the venom was used to influence the human, mouse, and sheep blood cells. As shown in Figure 3, the amount of lysis of blood cells increased with the venom concentration. Concentrations of 250, 300 and 400 micrograms per mL caused a 50% lysis of blood cells of mice, humans, and sheep.

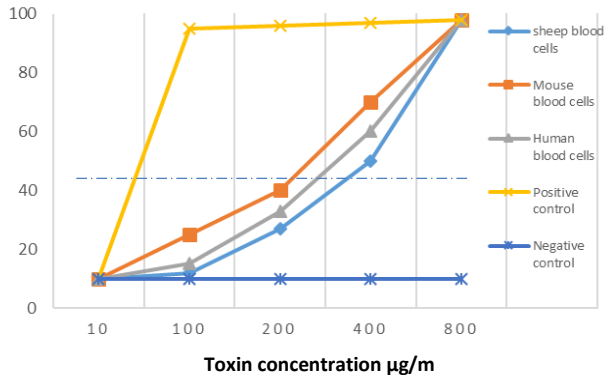

Figure 3. Hemolytic activity of *Rhopilema* sp. venom in mice, humans and sheep blood cells

Extraction of total genomic DNA

DNA was purified from each specimen (gonad tissue) using NEX™Diagnostics kit following the manufacturer's protocol. Amplification of partial COI gene was conducted based on universal published primers by Folmer (1994). Forward, HCO 1490: (5' GGT CAA CAA ATC ATA AAG ATT TGG -3') and reverse HCO 2198: (5' TAA ACT TCA GGG TGA CCA AAA AAT CA -3').

The Neighbour-Joining tree is presented in Figure 3.All Iranian *Rhopilema* sp. in group sequences clustered into two distinct clusters and as sister groups with GenBank *Rhopilema* sp. This was in terms of the close relationships of *Rhopilema* sp. with *R. esculentum* with each pair forming a highly supported monophyletic group of 100% support.

Discussion

Pharmacology and toxicology of jellyfish venom have been investigated since 1960s and most of them have tried to extract its active components as a new natural source of medicine (Sugantahi et al 2001). In this research, an optimized method for isolating the venom has been presented. In order to evaluate the hemolytic activity of the venom, it was necessary to design a method that does not reduce the activity of the toxin. In this study, the properties of the hemolysis activity and poison detection of studied species have been investigated based on the technique using enzyme and lytic activities. According to lytic cell activity and cytotoxicity, the venom has been investigated for its efficacy as a possible anticancer drug (Maisano et al. 2013; Lee et al. 2011). The moon jellyfish has a strong fibrinolytic effect (Rastogi et al. 2102). The Persian Gulf and Oman Sea are areas where there are many varieties of marine jellyfishes. Different biodiversity in the Persian Gulf and Oman Sea has led to the creation of sea-bloom in some seasons, especially in spring and summer (Gharibi et al. 2016). In this study, the genus *Rhopilema* sp. was isolated and investigated. There are several methods for extracting jellyfish toxins, but modified method reported from Bloom et al. (1998) were used in this research. In this appropriate method, we obtained a suitable concentration that maintains the activity and active ingredients of the poison.

Marine jellyfish venom is sensitive to heat and its activity is greatly reduced by high temperature. But in this study, nematocysts isolated from sea jellyfish were lyophilized (Gamma model 1-16 LSCplus, Germany) by dry temperature. Performing proper sonication using acetate buffer containing NaCl and frozen tentacles in a phosphate buffer solution were performed based on the method of Nagai et al. (2002) in order to release the poison from nematocysts is also an important step for increasing the concentration of extracted poison. In this research, we introduced a modified method and proposed optimal conditions for venom extraction. This method has better efficacy than other methods, such as dialysis dehydration and ultrasound diffusion. In dialysis method, large amounts of poison proteins are usually lost, but its hemolytic activity is maintained. Conversely, in ultrasound diffusion method a good concentration of protein would be obtained,

but its hemolytic activity is reduced (Li et al. 2009). Molecular weight of venom protein was determined by SDS PAGE using standard protein markers and calculated. Three distinct bands with molecular weights of 45, 65 and 95 kDa were recorded (Figure 1). In support, previous studies by Nagai et al. (2002), have reported 44 kDa for *Chiropsalmus quadrumanus*. Also hemolytic components were studied by Keen and Crown (1969) who confirmed it to have molecular weight of 70 kDa which compares to our present study showing 65 KDa.

In this study, the lytic effect of the venom on blood cells was evaluated in different species. Also, the effects of different concentrations of poison against human, sheep and mice blood cells were investigated. The results showed that increase in the amount of toxin has a direct relationship with the increase of hemolytic activity, while the increase in concentration does not increase uniformly with the amount of cell lysis (Figure 2). Walker et al (1977) determined the intravenous LD50 in mice (weight unspecified) to be 0.3 mg/kg and estimated the main toxin, which was a basic protein to have a molecular weight of approximately 70 KDa Also, the type of blood cells is also a contributing factor in lysis effect (Ponce et al. 2013). Rottini et al. (1995) showed that sheep red blood cells were more sensitive than mouse and human red blood cells, while Torres and Aguilar (2001) showed that the jellyfish *Cassiopeia xamachana* species has more lethargic effect on human red blood cells than on sheep red blood cells. Kang et al. (2009) investigated the lytic activity of *N. nomuria* on dogs, cats, guinea pigs, humans, and rabbits. They showed that the red blood cells of dogs were most sensitive. In the present study, we showed that the rate of lysis of blood cells in mice, sheep, and humans differs. Therefore, it can be summarized that the level of poison increases with the concentration level of venom and also in the same concentrations, it takes less time for the blood cells of the mouse to lyse compared to human and sheep blood cells. It can be suggested that the rate of blood cell lysis in studied jellyfish is unpredictable. This effect can be altered by changing the poison concentration of the venom (Kang et al. 2009). This study showed that the venom of *Rhopilema* sp. may have many biologically active principles, which need further studies in future. Thus, the biologically active compounds present in the venom of jellyfish are found to be useful tools for pharmacological research.

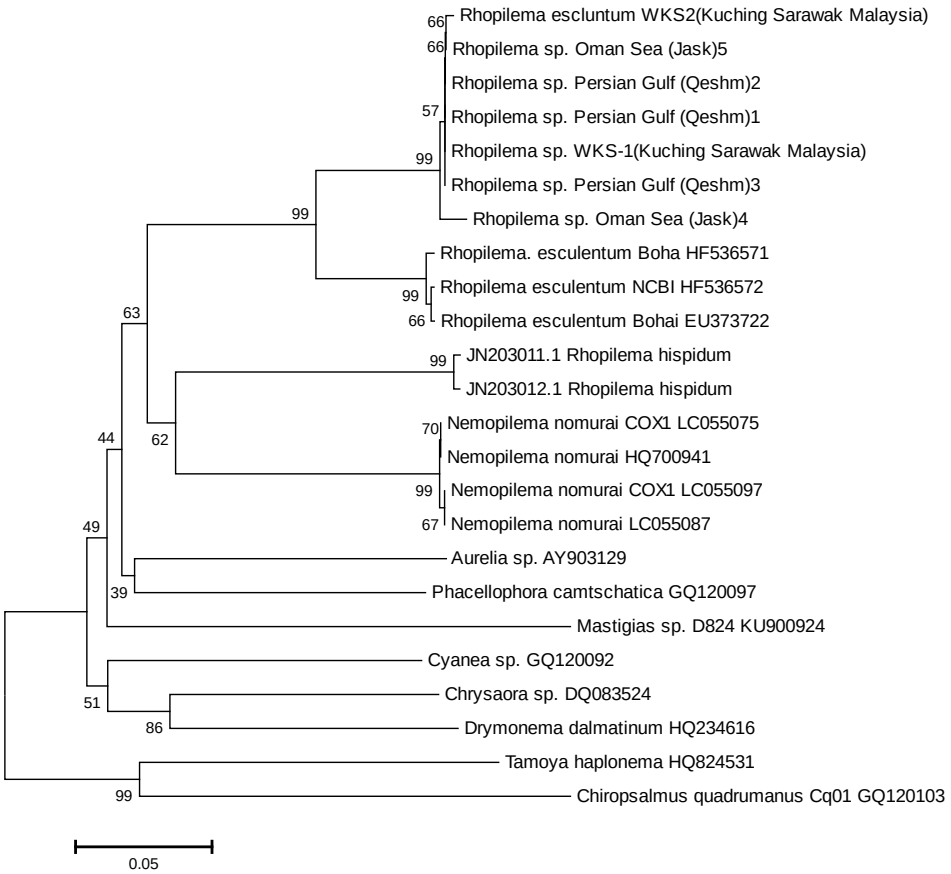

Figure 3. Neighbor-joining tree based on genetic distance analysis of COI sequences showing the genetic relationships of *Rhopilema* sp. and *Rhopilema esculatum* with similar genetic distances to the samples caught from Oman Sea area. *Tamoya haplonema* and *Chiropsalmus quadrumanus* species were used as outgroups. Scale shown refers to genetic distance based on nucleotide substitutions. Numbers at branching points are bootstrap support

In this study, a suitable method for isolating the venom was introduced and it was shown that proteins of 45, 65 and 95 kDa constitute the main venom proteins. Due to the presence of active compounds in the poison of studied species, and especially the hemolytic compounds, it can be considered for further investigations to produce recombinant proteins for some specific purposes. It was also found that the amount of cell lysis depends on the concentration of venom and the type of blood cells and varies in different species.

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