Original Article

Characterization and Pharmacological Activities of Jellyfish, *Chrysaora hysoscella* Captured in Bushehr Port, Iran

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ABSTRACT

Background: Cutaneous reactions like pruritus and erythema are common in warm months of the year in Bushehr Port, Persian Gulf, Iran due to jellyfish envenomation. This study reports isolation of the Chrysaora hysoscella nematocysts and evaluating its pharmacological activities during a bloom in 2013.

Methods: The venom of C. hysoscella captured in Jofre area in Bushehr port was analyzed. The electrophoretic profile was assessed by SDS-PAGE (12.5%) and the crude sample was analyzed using reverse phase HPLC. Caseinase activity was also determined.

Results: After separation of tentacles and isolation of their nematocysts, three different major protein components were revealed at 72-250 kDa with SDS-PAGE, signifying the presence of peptides in its venom**.** Two major peaks at 8.62 and 11.23 min were observed in reverse phase HPLC of the crude venom denoting protease peptide structural identities. Caseinase activity of C. hysoscella's venom was extremely low as compared with other jellyfish venoms.

Conclusion: This was the first report on the structural examination of jellyfish in Persian Gulf and may pave the way for determination and separation of destructive enzymes inducing cutaneous reactions in fishermen and swimmers.

Keywords: Caseinase, Cnidarian Venoms, Nematocyst, Scyphozoa, Sea Nettle.

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INTRODUCTION

Fishing over the past half century has resulted in an increase in previously suppressed gelatinous zooplanktons (jellyfish) feeding on marine food which is not much consumed by the fish anymore [1, 2]. Jellyfish species are one of the oldest living creatures and are shaped by a gelatinous bell and trailing tentacles. *Chrysaora hysoscela* and four other jellyfish species belonging to Schyphomedusae have bloomed in several parts of the world, including Adriatic Sea, over the last 200 years [3]. *Crambionella orsini* has become abundant in warm waters including the coasts of southern provinces of Iran boundary to Persian Gulf [4].

The presence of these creatures has become dangerous for swimmers and fishermen causing local and systemic reactions including redness, pain, rash, itching and other cutaneous manifestations especially in warm months of the year.

The aim of this study was extracting the jellyfish nematocyst to study venom protein components and caseinase content by SDS-PAGE analysis and RP-HPLC.

MATERIALS AND METHODS

Nematocyst Preparation

Specimens of *C. hysoscella* were collected from Jofre area in Bushehr strait of Persian Gulf during its bloom as shown in Fig. 1. The captured jellyfish were held in ice and immediately transferred to our laboratory for further experiments (Fig 2). Nematocysts were isolated from *C. hysoscella* [5] with minor modifications. In brief, tentacles were passed through four layers of medical gauze for separation of detached nematocysts. This procedure was repeated three

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more times to completely separate them from tentacles. The final filtrates were centrifuged (700g) at 4 °C for 20 min and the nematocysts were lyophilized and stored at -20 °C.

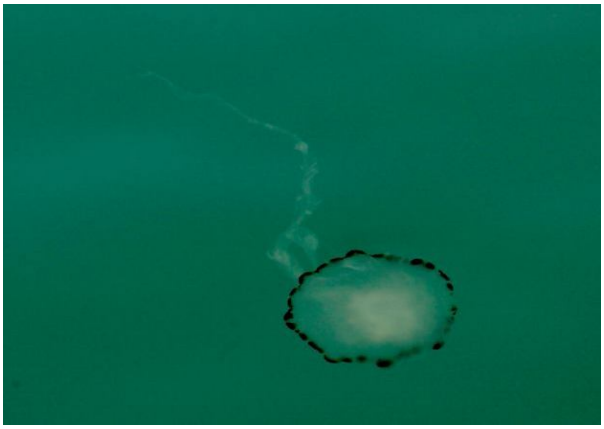

Figure1. *Chrysaora hysoscella* species in the Bushehr coastal part.

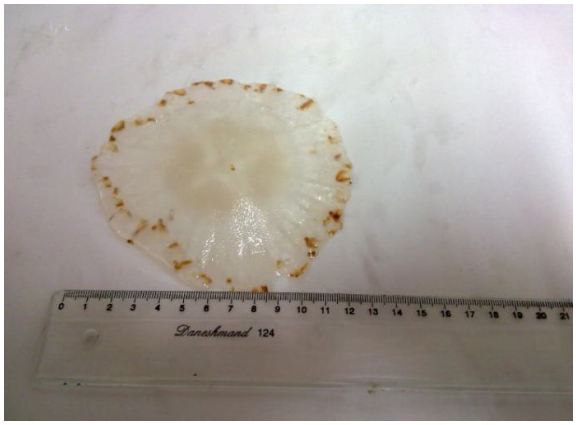

Figure2. Aboral surface of the *Chrysaora hysoscella* jellyfish in our laboratory.

Venom Extraction

Lyophilized venom was placed into screw top vials with distilled water and glass beads (8000 beads; 0.5 mm) and shaken 5 times in a minibead mill at 5000 rpm for 30 minutes [6, 7]. The jellyfish venom was separated with a pipette and transferred to an Eppendorf tube and was centrifuged (3,000 g) at 4 °C for 1 min. The supernatant was used as *C. hysoscella* venom and its protein concentration was determined [8].

SDS-PAGE

Electrophoresis was performed according to Laemmli UK. Method [9] using 12 % resolving gel with 4% stacking gel. Jellyfish samples were suspended in SDS-PAGE buffer (62.5 mM Tris-Hcl, pH 6.8, 10% glycerol, 2% SDS and 0.01% Bromophenol Blue) and were incubated at 95 °C for 5 min, then stored at -20 °C. Electrophoresis was carried out at 100V at room temperature using Tris-Glycine Buffer. The molecular-weight marker of 6.5-200 kDa (Sigma marker wide range, USA) was run parallel with the venom for molecular weight determination. For Coomassie staining, gels were placed for 30 min in Coomassie blue G (0.1% in 40% methanol and 10% acetic acid) and then destained in water for 30 min

Caseinase Activity

To assess caseinase activity of venom, colorimetric experiment was performed [10] with minor modifications: one milliliter of 0.5% casein was incubated for 2 h at room temperature with 400 µl of test solutions containing 30 µg of venom in 0.0008 M calcium chloride at pH 8.8. To stop the reaction, trichloroacetic acid (5%) was added to the reaction mixture. The supernatants were quantified by Bradford method [8] to evaluate hydrolyzed peptides.

HPLC Analysis

The jellyfish crude venom (100 µg) was dissolved in distilled water (50 µL) and the insoluble residue was discarded by centrifugation at 13000×rpm for 10 min at 4 °C. For the separation of venom fragments by HPLC instrument (Knauer- Germany) with UV detector, 50 µl of the prepared venom (5 µg/µl) was injecte -
250 × 4.6 mm) and eluted in a linear gradient of acetonitrile containing 0.05% TFA (solution C) and 0.05% TFA in water (solution D) at 1ml/min flow rate. Protein fractions were detected at 214 nm and 280 nm, and collected manually. HPLC was carried out at room temperature.

Ethical Statement

We have to confirm that all animal procedures of this study were in accordance with the guidelines for animal care prepared by Committee on Care and Use of Laboratory Animal Resources, National Research Council, USA. The Ethics Committee of the university approved the study.

RESULTS

SDS-PAGE Analysis of Jellyfish Venom

The electrophoretic profile, with 12.5% acrylamide gel of jellyfish venom showed at least 3 different major protein components with a range

of molecular mass between 72kDa and 250 kDa (Fig. 3). Resolution of separate bands below 36 kDa was not possible.

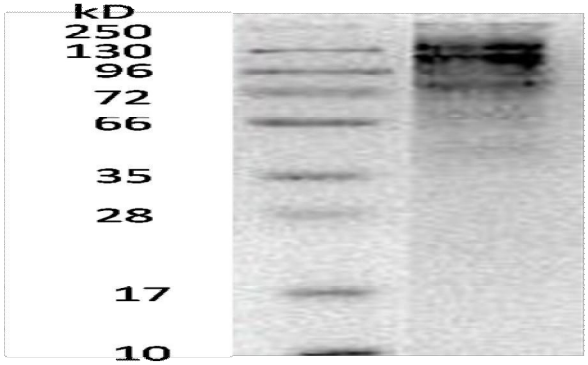

Figure 3. *Chrysaora hysoscella* venom was loaded in lane 1.The protein components were separated by SDS-PAGE (12% acrylamide) and stained with coomassie blue.

Proteolytic Activity

Caseinase activity of the venom was assayed by colorimetric experiments. Caseinase (1%) absorbance increased from 0.07 to 0.082 percent at the end of our experiment and 0.03 g of protein wa

Purification by RP-HPLC

HPLC chromatography of *Chrysaora* venom in C18 column resulted in 15 fractions. Absorbance was recorded at 280 and 214 nm. Maximum optical density of the fractions was 30 mAU. Isolation details are depicted in Fig. 4. Two major peaks at 8.62 and 11.23 min could be observed from reverse phase HPLC of the crude venom.

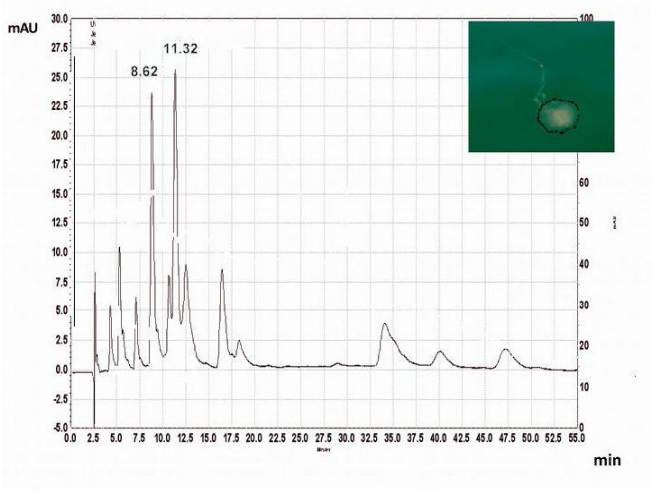

Figure 4. HPLC profile of *Chrysaora hysoscella*.

DISCUSSION

Jellyfish are ancient creature and have thrived in the oceans from 500 million years ago [11] . In recent years, there were several reports of jellyfish blooms, and their population size and distribution area have increased in many countries like Italy and Turkey causing cutaneous injuries to fishermen [12, 13]. Persian Gulf has numerous venomous animals, including some species of jellyfish specially in warm months of the year causing immediate and delayed immune reactions (pain, burning sensation, itching, papulonodular reaction and urticaria) [14, 15]. *C. hysoscella* was the main species of gellyfish collected from Jofre fishing port along the coastal part of Bushehr, southern Iran. Protein content of its nematocyst extraction showed three protein bands in the range of 75-250 kDa by SDS-PAGE, signifying the presence of peptides in its venom that are different from other jellyfishes [16, 17].

Jellyfish venoms are complex mixtures of toxins including, caseinase, hyaluronidase, phospholipase A_2 and other destructive enzymes, responsible for its pathological effects on humans by direct toxic or antigenic properties [18, 19]. Caseinase activity of *C. hysoscella*'s venom was extremely low as compared with other jellyfish venoms (*Nemopilema nomurai, Rhopilema esculenta, Cyanea nozakii,* and *Aurelia aurita*) showing its minimal role in this creature envenomation [20].

The crude sample analyzed in reverse phase HPLC demonstrated two major peaks at the retention times of 8.62 and 11.23 min, denoting the presence of protease peptides. The other minor peaks possibly accounted for lipids, biogenic amines and nucleosides.

CONCLUSION

This study was the first preliminiary report on biochemical properties of *C. hysoscella* as one of the poisonous jelly fish causing blooms in Iran, Namibia and Benguela ecosystems [21, 22] necessitating more studies for isolation of different peaks carried out by HPLC responsible for its detrimental effects in humans to pave a suitable treatment in envenomed patients.

ACKNOWLEGEMENTS

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