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**RESEARCH ARTICLE**

# Hemolytic Assay of Jellyfish Venom (*Chiropsalmus Quadrigatus)* Haeckel, 1880 against Chicken Blood

**S.Kumaralingam[1] and K.Venkateswaran[2]**

**1.** Department of Marine Science, Bharathidasan University, Trichy.
**2.** Central Institute of Fisheries Education, Versova Mumbai-400 061.

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## *Abstract*

The present study was carried out of Hemolytic activity of jellyfish against chicken blood. In this paper outcome results is *In vitro* hemolytic model using a crude extract, the observed hemolytic concentration was found to be highest with 7.05 ± 0.07 hemolytic units (HU)/Protein for the 10$^{th}$ fraction and lowest with 2.26 ± 0.16 hemolytic units (HU)/Protein for the 1$^{st}$ fraction in chicken erythrocytes. Estimation of cytotoxic study in the chicken blood was elaborately discussed in this paper.

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## Introduction

Jellyfish are abundant in the Arabian seas along with Mumbai coast waters. It is reported that jellyfish have been creating problems at times for coastal fisheries and nuclear power plants situated in coastal areas. There are various species of jellyfish, which are hazardous to human beings. There are several instances of jellyfish stings leading to death of individuals (Burnett *et al.* 1989).

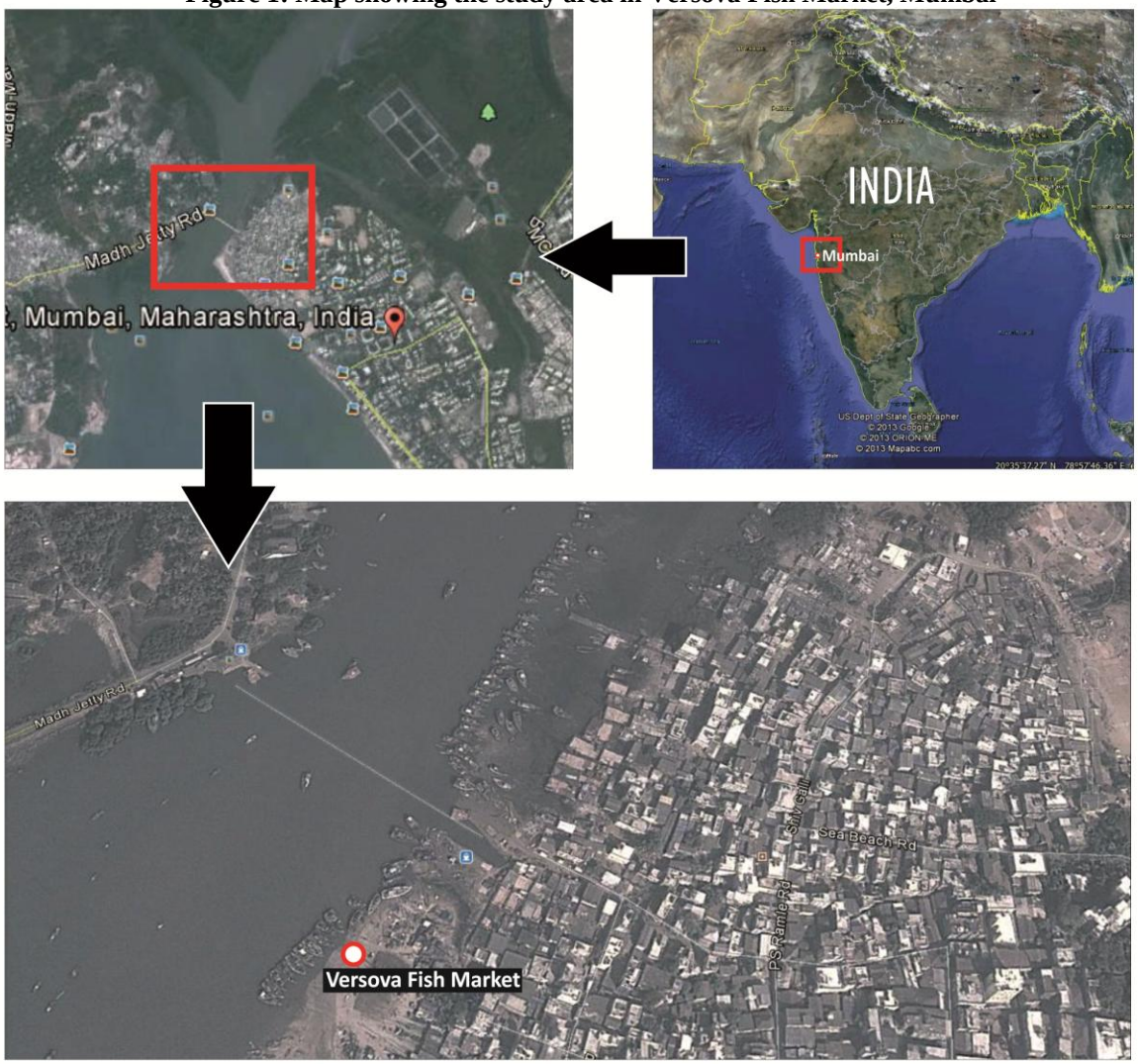
The most common jellyfish genera responsible for human envenomations include *Chiropsalmus, Chironex, Carybdea, Chrysaora, Pelagia, Aurelia,* etc. Their venom varies from mildly dangerous to being capable of inflicting death to humans. In addition to this, jellyfish cause hindrance in fishing operation while haul is collected on the deck and also creates hindrance in recreational activities such as swimming, SCUBA diving, etc. but so far few studies only conducted on biopharmaceuticals potentials venom of jellyfish. The first chirodropid described was *Chiropsalmus quadrumanus* (Agassiz 1862). Haeckel (1880) was identified *Chiropsalmus quadrigatus* an indo-pacific region for the first major work on jellyfish classification. This classification was extened by Mayer (1910), Kramp (1961) and Southcott (1956 and 1967). Daniel Praveen paul and Debanjan sengupta. (2008) studied Isolation, Characterization and comparison of the Venom of Jellyfishes *Pelagia notiluca* and *Chrysaor quinquecirrha.* In recent studies on Biopharmaceutical potential of nematocyst venom of *Chriropsalmus quadrigatus* (Kumaralingam 2012). The jellyfish *Chiropsalmus quadrigatus* is found in Indian waters and very common along the Mumbai coast, but so far no studies have been conducted on their toxicity In present study are focused Hemolytic activities of the particular species against with chicken blood.

### Materials and Methods

***Study site***

The study was conducted in two stations in Versova Fish Market Mumbai, (Fig. 1)
Station-I: 19º08.321'N, 72º48.099'E,
Station-II: 19º08.378'N, 72º48.138'E

**Figure 1: Map showing the study area in Versova Fish Market, Mumbai**




Jellyfish (*Chiropsalmus quadrigatus)* was collected from Mumbai coast on March 2008, by commercial fish trawlers operating off Versova and brought to the laboratory in seawater. In the laboratory, the tentacles was dissected out and frozen at -20$^0$C. Alternate freezing and thawing for 3 times yielded undischarged nematocysts. The solution (Phosphate buffer saline) containing these undischarged nematocysts was centrifuged thrice, each time for 10 minutes at 18,000 rpm in a refrigerated centrifuge break the nematocysts. The supernatant was used as crude venom. Crude venom was partially purified using ion-exchange chromatography with DEAE-cellulose and 10 fractions were obtained (each 15 ml) against a step-wise (0.1 to 1.0M) gradient of NaCl as the eluent.

***Hemolytic Assay***

The assay was carried out according to Pani Prasad and Venkateshvaran (1997) in `` v `` shaped Laxbro microtitre plates. (Fig-2) The lyophilized toxin and the lethal fractions were assayed. The concentration of the toxin was 5 mg/ ml. One row of well was used for only one toxin fraction. Initially 100 µl of normal saline was added to each well. Then 100 µl of the 1$^{st}$ toxin fraction was added to the first well and was thoroughly mixed. From this 100 µl was transferred to the next well and this process was repeated up to the last well from which 100 µl of the dilution was discarded. Then 100 µl of the prepared erythrocyte suspension was added to each well. A negative control was kept by mixing 100 µl of normal saline and 100 µl of 1% RBC suspension and positive control by mixing 100 µl of distilled water and 100 µl of 1% RBC suspension. Formation of a fine `` Button cell`` with regular margin indicates the negative reaction. A uniform red colored suspension of the lysed RBC indicates the positive result. The plates

were incubated for 2 hours at room temperature and the results were read. Hemolytic activity was expressed as hemolytic Unit (HU), 1 HU being defined as the amount of protein required to cause 50% hemolysis or the reciprocal of the highest dilution of the toxin in which a hemolytic pattern was obtained.

## Result

***Taxonomy of Jellyfish under Study***

*Chiropsalmus quadrigatus* Haeckel, 1880 (Fig-3)
Kingdom: Animalia
Phylum : Cnidaria
Class : Cubozoa
Order : Chirodropidae
Genus : *Chiropsalmus*
Species : *quadrigatus*

***Hemolytic Assay***

Crude extract at different fractions from 1-10 of *C. quadrigatus* was checked for hemolytic activity against chicken blood. (Table-1) The hemolytic activity increased with the concentration of the venom. The lowest hemolytic activity was found with $1^{th}$ fraction whereas the highest hemolytic activity was found with the $10^{th}$ fraction (Fig-4).

**Table 1. Hemolytic activity of crude/fraction extracts from the jellyfish species (*Chiropsalmus quadrigatus)* against chicken blood (Values mean of triplicate sets)**

| Crude/Fraction | Hemolytic unit |
|---|---|
| 1 | 2.26 ± 0.16 |
| 2 | 2.50 ± 0.19 |
| 3 | 3.00 ± 0.21 |
| 4 | 3.50 ±0.08 |
| 5 | 3.90 ± 0.06 |
| 6 | 4.17 ± 0.05 |
| 7 | 4.60 ± 0.25 |
| 8 | 5.30 ± 0.14 |
| 9 | 6.15 ± 0.20 |
| 10 | 7.05 ± 0.07 |

**Fig-2: Showing Hemolytic activity of *Chiropsalmus quadrigatus***

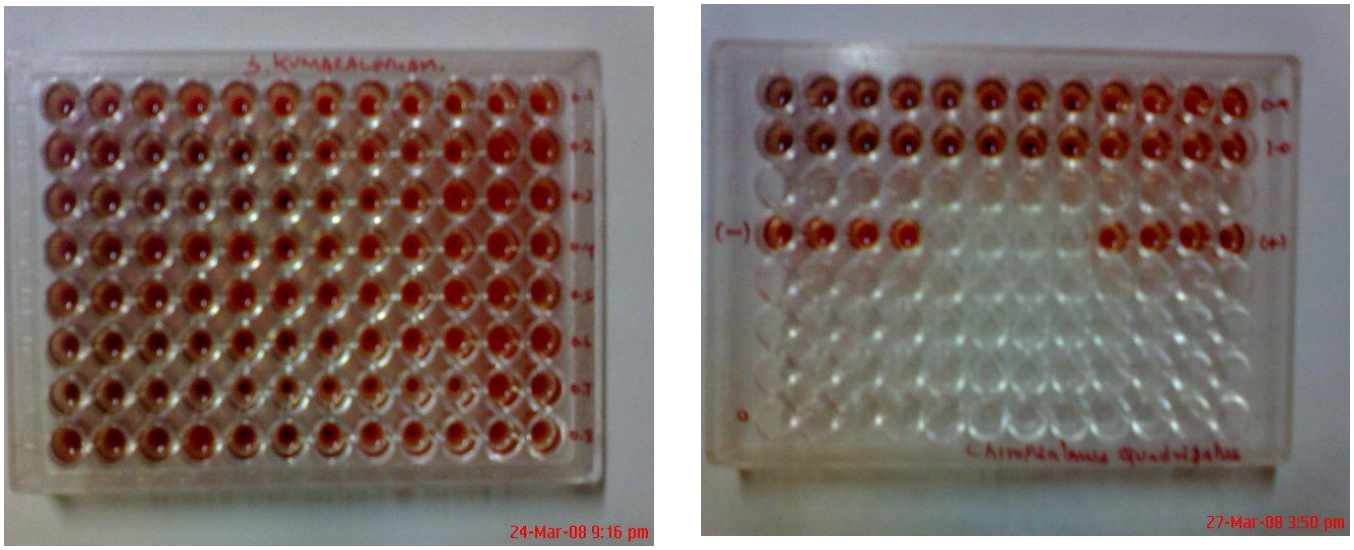



**Fig-3: Taxonomy of Jellyfish under Study**

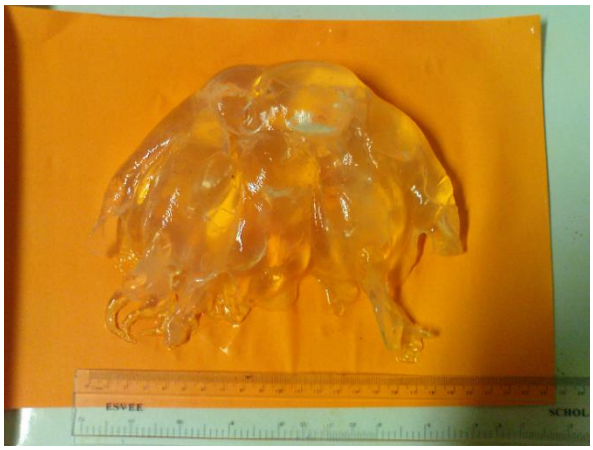
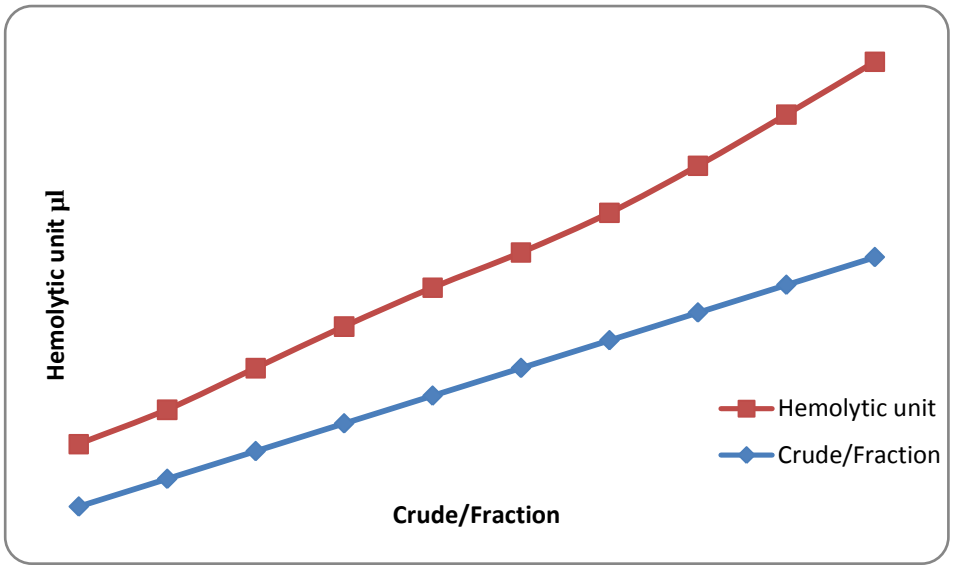


**Figure: 4 Hemotoxicity test for crude venom extract of *Chiropsalmus Quadrigatus* (Crude/Fraction vs. Hemolytic Unit µl)**



## Discussion

***Hemolytic Assay***

Jellyfishes contain a range of active biological compounds including some potent toxins. It has been reported that the venoms present in different species of jellyfishes are known to possess potent hemolytic properties.

It is noteworthy to mention that most of the earlier reports claim that *In vitro* hemolysis can serve as a sensitive test to express the degree of cytotoxicity. Hence, I tried to evaluate the *In vitro* hemolytic activity of this nematocyst extract in addition to conducting a toxicity and analgesic study. In my present study with the *In vitro* hemolytic model using a crude extract, the observed hemolytic concentration was found to be highest with 7.05 ± 0.07 hemolytic units (HU)/Protein in chicken erythrocytes. All the previous hemolytic studies workers - Helmholz *et al*., (2007) on *Cyanea capillata* and *Ctanea lamarckii*; Huahua Yu *et al*., (2007) on *Rhopilema esculentum*; indicated a positive result on hemolysis test. In my study paper the crude venom sample Indicate same value of the results.

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