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Toxicity and Larvicidal Activity of Jellyfish, Crambionella Stuhalmanni (Chun, 1896)and Chrysaora Quinquecirrha(Desor 1848) Extract Against Dengue Vector Aedes aegypti

KEYWORDS: Jellyfish, larvicidal activity, toxicity, Ades aegypti etc.

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ABSTRACT *Mosquitoes are the most critical group of insects in the context of public health, because they transmit numerous diseases, which in turn resulting in millions of deaths annually. The frequent use of systemic insecticides to manage insect pests leads to the destabilization of ecosystems and an enhanced resistance to insecticides by pests, and hence the clear need for alternatives. To evaluate the toxicity and larvicidal activity against dengue vector, Aedes aegypti (Ae. Aegypti) using jellyfish extract. We determined the jellyfish nematocysts extracts effectiveness by using ice-freezing method, for which the animals were collected from Vellar estuary, Parangipettai during post monsoon season. The probit analysis of each tested concentration and control were observed by using software SPSS 16 version package. The each tested concentration variable was assessed by DMRT method. Venom extracts were obtained from C. stuhalmanni and C. quinquecirrha, showed protein content 394 µgmL□1and 252 µgmL- 1 respectively. In SDSPAGE, molecular weight with 9 clear bands of 14, 25, 35, 31, 40, 65, 69, 75 and 85 kDa in C. stuhalmanni and 8 clear bands of 14, 26, 35, 30, 45, 55 70 and 80 kDa in C. quinquecirrha crude extracts. In toxicity assay minimum lethal doses of both crude samples showed 0.5 ml per 10 ± 2gm in Ocypoda crab. A significant larvicidal potential was recorded after 42h post treatment against the dengue vector, A. aegypti. The highest larval mortality was observed in the jellyfishes extracts. The LC50 and LC90 values of C. stuhalmanni and C.quinquecirrha extract were 166.102 and 286.550mg/l and 140.20 and 341.06 mg/l respectively. Our results suggest that this an ideal eco-friendly approach for the control larvae of the dengue vector A. aegypti. The present study could be speculated that the venom of C. quinquecirrha and C. Stuhalmanni have many biological active principles, and it needs further studies.*

Introduction

Dengue is transmitted by mosquitoes of the genus Aedes which are widely distributed in subtropical and tropical areas of the world and is classified as a major global health threat by the World Health Organization (1). Dengue virus is primarily transmitted by Aedes mosquitoes, particularly Aedes aegypti(Ae. aegypti) and bite during the daytime. Mosquitoes are a serious threat to public health transmitting several dangerous diseases for over 2 billion people in the tropics(2). Ae. aegypti, the primary carrier for viruses that cause dengue fever, dengue hemorrhagic fever and yellow fever are widespread over large areas of the tropics and subtropics and is reported to infect more than 100 million people every year in more than 110 countries in the tropics(3).

Mosquitoes are the most important group of insects in terms of public health importance as they transmit a number of causative agents of diseases, such as malaria, filariasis, dengue fever, etc. causing millions of deaths every year(4). Mosquito bites may also cause allergic responses including local skin reactions and systemic reactions such as urticaria and angioedema (5). Mosquito borne diseases have an economic impact, including loss in commercial and labour outputs, particularly in countries with tropical and subtropical climates; however, no part of the world is free from vector-borne diseases(6). The yellow fever mosquito, Aedes aegypti (L., 1762), is responsible for dengue fever in India, where the number of dengue fever cases has increased significantly in recent years. Some 2.5 billion people two-fifths of the world's population are now at risk from dengue. The WHO has estimated that there may be 50 million dengue infections worldwide every year.

The disease is now endemic in more than 100 countries in Africa, America, Eastern Mediterranean, South-east Asia and the Western Pacific, the last two being the most seriously affected (7). WHO (2009), in the absence of effective vaccine and drugs, dengue prevention and control programs have depended on vector control. Management of this disease vector using synthetic organic chemical insecticides has failed because Aedes mosquitoes have developed insecticide resistance (8). For the control of mosquito vectors, dichlorodiphenyl trichloroethane, organophosphates and pyrethroids are being used as adulticides/larvicides for the latest several decades in the National Vector Borne Disease Control programme in India, depending on the requirements of the given disease control programme (9). Although effective repeated use of these controlling agents has fostered several environmental and health concerns, including disruption of natural biological control systems, outbreaks of other insect species, widespread development of resistance and undesirable effects on non-target organisms (10).

The biodiversity of the marine ecosystem provides an important source of chemical compounds which, due to antiviral, antibacterial, antifungal and anticancer activities, have many therapeutic applications (11-14). Many of the marine macro algae produce a variety of secondary metabolites, such as polyphenols, terpenes, sterols, lectins, inhibitors of proteinases, fatty acids, enzymes, and polysaccharides (12) which act as potential bioactive compounds of interest for pharmaceutical applications. Natural products have been regarded as an important sources which could produces potential chemotherapeutic agents. Toxins/nematocysts extracts derived from some of the jellyfishes as a model for the development of new drug has promising applications in pharmaceuticals. To the best of our knowledge, there is no documented literature on larvicidal activity and toxicity

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on Ocypod crab using jellyfish nematocyst extracts. In the present study, an attempt has been made to evaluate the crab toxicity and mosquito larvicidal effect of jellyfish, C. quinquecirrha and C. Stuhalmanni extract against A. aegypti.

Materials and methods

Preparation of C. Stuhalmanni and C.quinquecirrha extract

The samples of jellyfishes were collected from Vellar estuary, Parangipettai, along the southeast coast of India during the summer season (April & May, 2014). The collected live specimens were kept in the glass bowl along with ice for 15 minutes. Due to stress condition, the tentacles released the nematocysts, which were filtered using 0.5 mm mesh sieve and filtered by Whatman No.1 filter paper. The nematocysts were centrifuged at 5000 rpm for 15 min. The supernatant was collected in separate cleaned beakers for lyophilization and stored at4° C until further used. The crude extract was filtered and dialyzed by using Sigma (USA) dialysis membrane - 500 (average flat width: 24.26 mm; average diameter: 14.3 mm; approximate capacity: 1.61 mL/cm) against D-glucose to remove excess water. Then, the supernatant obtained was lyophilized (Free ZoneR Freeze Dry Systems, Labconco, USA) and stored at 4° C in labelled 25-mL vials kept in containers until their analysis(15). The nematocyst extract yield and aperture were estimated by counting at 0.2 mL under a light microscope.

Toxicity assay in Crab (LD50)

The lethal and paralytic activities were studied in the Ocypod crab(Ocypoda macrocera) followed by the method of (16), the crabs weighing about 10 ± 2 gms were collected from the seashore of Parangipettai. Extracts of C. stuhalmanni and C.quinquecirrha were injected to the crabs at the junction of body and chelate leg. A group of five animals were challenged with various doses (0.25, 0.50, 0.75 and 1.0 ml) of both crudes. Stock solution 5mgml-1 prepared. The effect of these concentrations on the crabs leads to colour change, foaming, paralytic effect, restlessness and mortality.

Fig. 1: Showing the crab O. macrocera **Fig. 2: Injection of venom**

Protein estimation

Protein estimation was carried by the method of (17) .The standard protein sample was prepared at 2 mg/ml of BSA. The assay relies on the binding of the dye Commassie Blue G 250 to the protein molecule measured calorimetrically at 595 nm in HITACHI-220S, dilution of protein standards concentration 20, 40, 60, 80 and100 µg/100 µL.

Molecular weight determination SDS PAGE

The proteinaceous nature of C. stuhalmanni and C. quinquecirrha crude extracts were subjected to electrophoresis following the method of (18) 10 in 12% polyacrylamide slab gels. This is the most convenient way for determining the molecular weight of proteins. In this technique, SDS detergent was being used to make uniform charge all over the protein samples and --Mercaptoethanol was used to break the disulphide linkage, which makes all proteins in the same shape. Hence, migration of proteins in the gel was only according to their molecular weight. The CPMI was used as the moleculermarker11.

Larval collection and Larvicidal activity

The larvae of Ades aegypti were collected from overhead and ground level water tanks, house hold water reservoirs of different sites of Salem, Tamilnadu, India. Larvae of Ades aegypti were collected through dipping method from the rice fields of surrounding areas. In laboratory they were kept in trays with sufficient water in stress free, pathogen free, hygienic condition. Larvae were provided with finely ground dog biscuit periodically.

The larval mortality bioassays were carried out according to the test method of larval susceptibility as suggested by the World Health Organization (19).Twenty five early fourth instar larvae were introduced in a glass beaker with 100 ml of C. stuhalmanni and C. quinquecirrha extract at various concentrations (from 100–400 mg/L). For each concentration, four replicates were performed, for a total of 100 larvae, and a control treatment with tap water was included. The larval mortality was calculated after a 42 h exposure period, during which no food was given to the larvae. The lethal concentrations (LC50 and LC90) were calculated by probit analysis (20).In records of the effect of concentration, moribund and dead larvae were considered as affected. LC50 and LC90 value indicated 50% and 90% mortality, respectively; 95% confidence limit of upper and lower confidence levels were calculated from a series of "exposure" concentration by comparing the percentage of mortality in the treated group to control by probit analysis (SPSS, version 16).

Results and Discussion

Cnidarian constitute the taxonomically most extensive group of venomous animals. Scientists have studied biochemistry, pharmacology and toxicology of jellyfish venom since 1960s and most of them have tried to extract its active components as a new natural source of medicine. A large number of marine organisms are known to possess bioactive substances that have tremendous pharmaceutical potential for the future (21). Although considerable progress has been made to isolate and characterise the toxic components of marine cnidarians (22). In the present study the jellyfish C. stuhalmanni and C. quinquecirrha were identified by the standard literatures (23-24). From the jellyfish 5.50.mg and 4.95 mg of straw yellow gummy mass lyophilised crude venom was obtained. The amount of protein content in crudes showed 394 and 312µg/mL respectively. In toxicity assay minimum lethal doses of both crude samples showed 0.5 ml per 10 ± 2gm in Ocypoda crab. The lethal and paralytic activities were studied in the Ocypod crab (Ocypoda macrocera) is showed in figures 1 & 2. The result of the further observation made on the colour change of crab, foaming paralytic effect and restlessness of the crabs are duly presented in table1&2.

Molecular weight of these proteins were determined by SDS PAGE on polyacrylamide slab gel using standard protein marker and calculated to be 97KDa,85KDa,70 KDa,55KDa,30KDa, and 15KDa in the same order. The crude venoms of C. stuhalmanni and C. quinquecirrha showed the following distinct bands recording low to high molecular weight (Fig.3). Nine distinct clear bands has molecular weight of 14, 25, 35, 31, 40, 65, 69, 75 and 85 kDa in C. stuhalmanni and 8 clear bands of 14, 26, 35, 30, 45, 55 70 and 80 kDa in C. quinquecirrha crude extracts. Similar results have also been reported by Macek &Andrulah(25). The above results supports the previous studies i.e.

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Subsequent results were found from the cnidarian Lebrunia danae with molecular weights of 62.5 and 58 kDa(26). Jellyfish venom from different species vary in activity and composition. It was reported that a major component in C.quinquecirrha venom had a 19kDa molecular weight, whereas major protein component in Chrysaora achlyos venom was 55 kDa(27) and Chironex fleckeri venom contained a 20 kDa protein(28). Toxic fractions of C. fleckeri venom have been reported to contain components of molecular sizes from 10 to 600 kDa, although a larger one may represent macromolecular aggregates (29). However, further purification and characterization of compounds are required to confirm the type of toxins in these jellyfishes. The presently obtained toxic proteins from jellyfish extracts could be categorized as (i) Medium sized Cytolytic Actinoporins (~ 20kDa), (ii) Cardio stimulatory proteins (~ 28kDa) and (iii) Cytolysin with or without Phospholipase in both crude and fractionated proteins (~ 40kDa).

Table 1: Toxicity of C. stuhalmanni crude (5 mgkg-1 of body wt.) i.p., injection to Ocypod crab (10 ± 2 gms)

S. No	Concentration	No. of animals	Death	Observation			
5mg/ml)crude (ml)		10		Colour change	Foaming	Paralytic effect	Restlessness
1.	Control	10	Nil	-	-	-	-
2.	0.25	10	Nil	+	-	-	+
3.	0.50	10	4	+	+	-	+
4.	0.75	10	6	+	+	+	-
5.	1.00	10	7	+	+	+	-

Table 2: Toxicity of C. quinquecirrha crude venom (5 mgkg1 of body wt.) i.p., injection to Ocypod crab (10 ± 2 gms)

S. No	Concentration	No. of animals	Death	**Observation**			
5mg/ml) crude (ml)		10		Colour change	Foaming	Paralytic effect	Restlessness
1.	Control	10	Nil	-	-	-	-
2.	0.25	10	Nil	-	-	-	+
3.	0.50	10	4	-	+	-	+
4.	0.75	10	5	+	+	+	+
5.	1.00	10	8	+	+	+	+

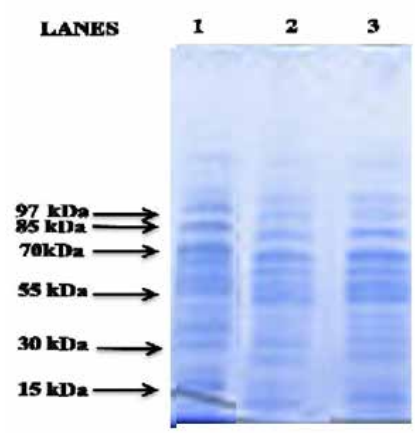

Fig.3: SDS PAGE (12% polyacrylamide gel with Commassie Blue) Analysis of C. stuhalmanni and C. quinquecirrha showing (1) Protein standard marker (2) C. stuhalmanni Crude venom (3) C. quinquecirrha crude venom

The use of bioactive compounds of marine origin is an alternative pest control method, which can help to minimize the usage of toxic pesticides and their deleterious effects on non-target insect species in the environment. Various commercially available chemically synthesized insecticides and repellants like N,N-diethyl-3- methylbenzamide, d-trans allethrin, transfluthrin, prallethrin are functional against these vectors, but have secondary impacts on environment and human health. In this context, insecticides from marine origin have gained palpable attention due to their numerous beneficial prospects of being eco-friendly, non-toxic to the nontarget organisms, cost effective, as well as natural availability. In general, A. aegypti is used in insecticide screening trials because it is usually less susceptible and easy to colonize in the laboratory (30).

During the last decade, various studies on natural marine products against mosquito vectors indicated them as possible alternatives to synthetic insecticides (31-32) .Bond et al., have also been reported(33) the naturally derived insecticide spinosad is highly toxic to Aedes and Anopheles mosquito larvae. In the present study, Larvicidal activity of the jellyfish extract increased the mortality of mosquito larvae in a concentration-dependent manner are depicted in Table3&4.

Table3. Larvicidal activity of C. stuhalmanni extract against fourth instar A. aegypti larvae.

Conc (mg/l)	Mortality 24 h (%)	LC50 (mg/l) (95% FL)	LC90 (mg/l) (95% FL)	Chi-square	Regression
100	12.12±1.3	**166.102 286.550 12.462** (181.490-196.712) (316.987-361.266)			Y=10.817±5.29
200	50.66.±1.5				
300	86.66±2.5				
400	100±0.0				

Each value ($\bar{X}$±SE) represents mean of four values. Significant at P<0.5

Table4. Larvicidal activity of C.quinquecirrhaextract against fourth instar A. aegypti larvae

Conc (mg/l)	Mortality 24 h (%)	LC50 (mg/l) (95% FL)	LC90 (mg/l) (95% FL)	Chi-square	Regression
100	37.33±1.5	140.2341.6 14.942 (121.4–160.1)(322.3–360.2)			Y=9.144±4.58
200	70.66.±2.0				
300	93.33. ±2.8				
400	100±0				
Control	1.5±0.3				

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Each value ($X \pm SE$) represents mean of four values.- Significant at $P<0.5$

Among the four tested concentrations, 200 µg/mL of concentration was found to exhibit relatively high larvicidal activity against A. aegypti. The LC50 and LC90 values of C. stuhalmanni and C.quinquecirrha extract were 166.102 and 286.550mg/l, and, 140.20 and 341.06 mg/l (Table.3) respectively. Previous literature also indicated that the dichloromethane: methanolic crude extract of C. antennina, showed larvicidal activity against Culex quinquefasiatus Say, 1823 second instar larvae with a LC50 value of 100 µg ml−1.In vitro and in vivo antiplasmodial activity of polyherbal extracts of C. antennina have been reported against Plasmodium falciparum (34-35) observed LC50 value 116.8 mg/L against An. stephensiwhile working with methanol extract of Clitoria ternatea L. seed. In a recent work with methanol extract of Moringa obeifera seed on 3rd instar larvae of the same mosquito species(36) found LC50 and LC90 values 72.45 mg/L and 139.82 mg/L respectively. In the present study, however, C. stuhalmanni and C.quinquecirrha extract exhibited moderate larvicidal activity with LC50 value of 200 mg/L against dengue vector, A. aegypti, which could be useful tool for probing biological, pharmacological activities. The marine peptides are thus currently considered to be promising components for the development of drugs for the treatment of malaria. Molecular studies in order to determine the structure of proteins of nematocyst and pharmacological efficacy of the extract are in progress.

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