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Research Article

BIOLOGICAL AND PHARMACOLOGICAL ACTIVITIES OF JELLY FISH *CRAMBIONELLA STUHALMANNI* (CHUN, 1896) AND *CHRYSAORA QUINQUECIRRHA* (DESOR, 1848)

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ABSTRACT

Venom extracts were obtained from *C. stuhalmanni* and *C. quinquicirrha* showed protein content 394 µgmL^{-1}and 252 µgmL^{-1} respectively. In SDS-PAGE, 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. quinquicirrha* crude extracts. Hemolytic assay from two nematocysts venom on human blood, goat blood showed 32 HU, 16 HU and 64 HU, 32 HU in *C. stuhalmanni* and *C. quinquicirrha* respectively. In toxicity assay minimum lethal doses of both crude samples showed 0.5 ml per 10 ± 2gm in Ocypoda crab and 1.0ml, at 1.10 ± 0.03 and 1.56 ± 0.03 min & sec in mice respectively. Analgesic ratio of both extracts in hot plate and tail flick method were found to be significant. Inhibition of writhing response and CNS depressant activity percentage were 35%, 55% and 90%, 95% in *C. Stuhalmanni* and *C. quinquecirrha* extracts. Decrease in licking time and licking frequency by the mice in formalin test and also showed prompt anti-inflammatory activity. Thus the venom of two jelly fishes is found to be useful tools for probing biological or pharmacological activity.

Key words: Paralysis, Venom, Nematocysts, Hemolytic assay, Analgesic ratio and anti-inflammatory.

INTRODUCTION

Toxicity is a common feature of cnidarians[1], and a large amount of toxins have been identified in cnidarian nematocysts[2]. A variety of toxins were produced and used for prey capture and defense by the cnidarians, which include Anthozoa, Scyphozoa, Cubozoa and Hydrozoa[3]. Cnidae are specialized sub cellular organelles of diverse structure and function that are plentiful amongest Cnidarians[4]. They can be classified into three main types, nematocyst, spirocyst and ptychocyst, based on structural and functional features. The major function of nematocyst is the delivery of venom through the skin, whereas spirocysts are adhesive and ptychocysts are involved in protection[5]. The later two structures are found in Anthozoans, whereas the jellyfish (Scyphozoans and Cubozoan) contain only nematocyst. The biological roles of toxins delivered by nematocysts include the capture and killing of prey, digestion, repelling of predators and interspecies spatial competition[2].

Toxins derived from some of the jelly fishes as a model for the development of new drug also has promising applications in cardiovascular medicine and target medicine of nerve molecular biology. Hence, it is useful to study jellyfish venom which is benefited to human health by using mice study. Evidence from rat experiments showed that jellyfish can be used to cure arthritis[6]. Other investigators have reported that the various venoms from different jellyfish species have biological functions[7]. Only few publications have deal with the nematocyst toxins of *C. stuhalmanni* and *C. quinquicirrha* on the basis of a newly developed method for isolating undercharged nematocysts of these jelly fishes. The present study was undertaken to investigate the biological properties of crude extracts from *C. stuhalmanni* and *C. quinquicirrha*.

MATERIALS AND METHODS

Extraction of nematocyst

The extraction method was followed by Yanagihara et al. [4]. The live animals were kept inside the glass bowl along with some amount of distilled water in an ice container for15 min. During stress condition the nematocysts were extracted from the tentacles. The same procedure was repeated thrice. The collected nematocysts containing toxins were collected with 0.5 mm mesh sieve and filtered by Whatman No.1 filter paper. The supernatant was kept for lyophilization which was later transformed into crystalline powder and stored at 4^{o}C for further use.

Protein estimation

Protein estimation was carried by the method of Bradford et al., [8]. 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 and 100 µg/100 µL.

Haemolytic study

Crude extracts of *C. stuhalmanni* and *C. quinquicirrha* were assayed on goat and human erythrocytes followed by the method of Pani Prasad and Venkateshwaran, [9]. The chicken, goat, cow and human blood were obtained from the source, 2.7% ethylenediamine-tetraacetic (EDTA) solution as an anticoagulant at 5% of the blood volume and brought to the laboratory. The blood was centrifuged thrice at 5000 rpm for 10 minutes. 1% erythrocyte suspension was prepared for hemolytic study.

Hemolytic assay was performed in 'V' shaped sterile Laxbro microtitre plate. Serial two fold dilutions of the venom extract (100 µl; 1 mg C. *stuhalmanni* and *C. quinquicirrha* toxin in 1 ml PBS) were made in PBS (pH 7.2) starting from 1 : 2. An equal volume of 1% human RBC was added to each well. The plates were shaken for mixing the RBC and venom extract. The plates were incubated at room temperature for 2 hrs before reading the results. Erythrocytes suspension to which distilled water was added (100 µl respectively) served as blanks for negative control. Button formations in the wells were taken as negative. Reciprocal of the highest dilution of the venom extract showing the hemolysis was taken as one hemolytic unit.

Molecular weight determination SDS PAGE

The proteinaceous nature of *C. stuhalmanni* and *C. quinquicirrha* crude extracts were subjected to electrophoresis following the method of Laemmli, [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 β-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 molecular marker[11].

Toxicity assay in Crab (LD_{50})

The lethal and paralytic activities were studied in the Ocypod crab (*Ocypoda macrocera)* (Fig. 2 & 3.) followed by the method of Lane and Dodge, [12]. The crabs weighing about 10 ± 2 gms were collected from the seashore of Parangipettai. Extracts of *C. stuhalmanni* and *C. quinquicirrha* 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 5 $mgml^{-1}$ prepared. The effect of these concentrations on the crabs leads to colour change, foaming, paralytic effect, restlessness and mortility.

Fig. 2: Showing the crab *O. macrocera*

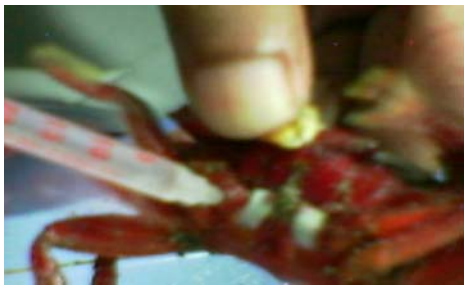

Fig. 3: Injection of venom

Experimental animals

Albino (Wister) rats and Swiss albino mice weighing 170 ± 20 g and 20 ± 2 g of either sex were used for the study. The animals were procured and housed in the animal house maintained under standard hygienic conditions at 20 ± 2°C humidity (60 ± 10%) with 12 hour day and night cycle with food and water *ad libitum*. The set of rules followed for animal experiment were approved by the Institutional Ethical Committee Reg. No: 160/1999/CPCSEA-/11.01.2008, Rajah Muthiah Medical College, Annamalai University.

Drugs

All the standard drugs (Pentazocine, Diazepam, Carrageenan, Aspirin and Indomethacin) were obtained from various chemical units- E. Merck India Ltd. and S. D. Fine Chem. Ltd. (India).

Mice bioassay

The bioassay for lethality was done by the method of Gouiffes *et al.* [13]. The bioassay of lethality was done by using clinically healthy male albino mice (20 ± 2 gms). The mice were maintained in a healthy condition in the laboratory. Mice in triplicate were tested intraperitoneally with different concentration at 0.25, 0.50, 0.75 and 1.0 ml of the *C. stuhalmanni* and *C. quinquicirrha* crude extracts were dissolved at 5 $mgmL^{-1}$ in phosphate buffer saline (PBS). A control was maintained in each case by injecting an equal volume of PBS (pH 7.4). The time of injection and death, besides behavioral changes before death were recorded for 24 hours.

Analgesic activity

Tail - flick method

Analgesic activity was described by the method of D'amour and Smith, [14] using tail flick analgesia meter (Harvard, USA 50-9495, 230 V and 50 Hz) with a variable 150 W, 25 v lamp as the heat source. The both crudes were dissolved in PBS (pH 7.2) at the dose of 5 mg/kg of body weight and then injected *i.p.* to mice. Mice without administration of extracts or known painkiller were used as control (Normal Saline) and Pentazocine (Dose 5 mg/kg s.c) serves as a reference standard. The mice were tested 30 mins after injection.

Hot plate method

Hot plate method was described by Eddy & Leimbach, [15] and Tornos *et al.* [16] The animal was individually placed on a hotplate and maintained for 55°C. Reaction of animal such as paw licking or jump response was taken as an end point. *C. stuhalmanni* and *C. quinquicirrha* crude extracts were injected at dose level of 5 mg/kg of body weight was injected *i.p.* to mice (20 ± 2 gm).

Writhing test

Writhing was induced in mice by intraperitoneal administration of 0.1 ml of 1% acetic acid. The number of writhing movements was counted for 20 min. The writhing test was performed after the administration of the vehicle or drug.

Formalin test

Formalin test method was followed by Shibata *et al.* [17] Twenty micro liters (20µl) of 5% formalin were injected subcutaneously into the right hind paw of mice. The time (in seconds) spent in licking and biting responses of the injected paw was taken as an indicator of pain response. Responses were measured for 5 min. After formalin injection for 30 min. *C. stuhalmanni* and *C. quinquicirrha* crude (5 mg/kg, *s.c*) extracts were administered 60 min before formalin injection. Indomethacin (10 mg/kg, *s.c*) was administered 30 min before formalin injection. Control received the same volume of saline by oral administration.

Central nervous system depressant activity

The CNS depressant activity was followed by Dews, [18] and Kulkarni & Dandiya, [19] using Actophotometer (Medicraft, Model No: 600 M-6 D S. No. PA- 0135). Male albino mice (20 ± 2 gm) were housed with a 12:12 hour dark–light cycle. The concentrations of extracts were 2 mg/kg of body weight. A mouse without administration of any toxin or known painkiller was used as control. The basal reaction time and administration of *C. stuhalmanni* and *C. quinquicirrha* crude extracts after 15, 30, 45, 60 and 120 min were noted and percentage decrease of motor activity represented.

Anti -inflammatory activity (Paw edema formation)

Anti -inflammatory activity was following the method of Winter *et al.* [20] Male rat weighing 100 ± 2 g were divided into groups of six animals. A volume of 0.05 ml of 1% Carrageenan in Normal Saline Solution (NSS) in 0.2 M Carbonate buffer was injected intradermally into the plantar side of the right hind paw of the rats. Test drugs and vehicle were given 1 hour prior to Carrageenan injection. Paw volumes were measured using a plethysmometer (Model 7150, Ugo Basile, Italy) before as well as 1, 3 and 5 hour after carrageenan administration at an interval of 60 minutes.

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 posses 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 jelly fish, *C. Stuhalmanni* (464 gms. in wet wt.) and *C. quinquicirrha* (585 gms. in wet wt.) were collected and identified by following the standard literature of Cairnes *et al,* [23]and Fernando and Fernando[24]. From the jelly fish 5.50 gms and 4.95 gms of straw yellow gummy mass lyophilized crude venom was obtained. The amount of protein content in crudes showed 394 and 312 µgmL^{-1} respectively. Molecular weight of these proteins were determined by SDS PAGE on 12% polyacrylamide slab gel using standard protein marker and calculated to be 97 kDa, 85 kDa, 70 kDa, 55 kDa, 30 kDa and 15 kDa 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. 1). Nine distinct clear bands has molecular weight of 14, 25, 35, 31, 40, 65, 69, 75 and 85 kDa in *C. stuhalmanni* crude and eight distinct clear bands has the molecular weight of 14, 26, 35, 30, 45, 55, 70 and 80 kDa in *C. quinquecirrha* crude.

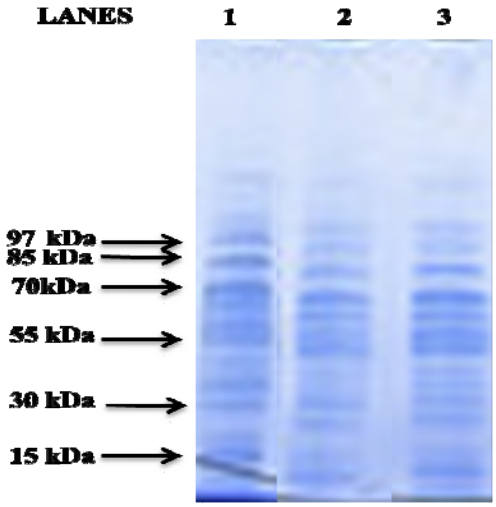

Fig.1: SDS- PAGE (12% polyacrylamide gel with Commasive Blue) Analysis of *C. stuhalmanni* and *C. quinquicirrha* showing (1) Protein standard marker (2) *C. stuhalmanni* Crude venom (3) *C. quinquicirrha* crude venom

It supports the previous studies *i.e.,* Subsequent results were found from the cnidarian *L. danae* with molecular weights of 62.5 and 58 kDa.[25] Jelly fish venoms from different species vary in activity and composition. It was reported that a major component in *C. quinquecirrha* venom had a 19 kDa molecular weight, where as major protein component in *Chrysaora achlyos* venom was 55 kDa.[26] The venom of *Chiropsalmus quadrigatus* contained a hemolytic toxin of 44 kDa[27] and that from *C. fleckeri* nematocysts 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 the larger ones may represent macromolecular aggregates.[29]

Biological characterization was carried out by employing different bioassay methods which revealed that two crude toxins posses were varying degree of hemolytic, lethal, analgesic, edema introducing and neurotoxic activity. The hemolytic activity is reported to be the most common biological activity and the modes of action of these heamolysins have been described by several researches. [30] The hemolytic component (haemolysin) was studied by Keen and Crone, [31] who confirmed it to have a molecular weight of about 70 kDa. In the present study the haemolytic activities were tested from crude of *C. Stuhalmanni* and *C. quinquecirrha* on human and goat blood erythrocytes. Human and goat blood were the vulnerable to lysis provoked. *C. Stuhalmanni* crude sample on human blood was showed maximum 32 HU and minimum 16 HU in goat blood and *C. quinquecirrha* crude samples was showed maximum in 64 HU human blood, minimum in 32 HU goat blood. It supports the previous studies *i.e.*, the specific hemolytic activity have been found in extracts of many cnidarians such as *Chironex fleckeri,*[28, 29] *Cassiopeia xamachana* [32,33] and in *Hydra attenuate.*[34] Further studies of extracts of isolated nematocysts and of tentacles from which nematocysts had been removed confirmed the presence of a hemolytic agent of molecular weight 70 kDa.[35]Although haemolysis may be of some significance in experimental situations, which has not been observed in clinical situations. However, no systematic clinical study of haemolysis has been conducted.

Several jelly fishes are considered among the most hazardous creatures known due to toxicity of their stings to humans.[3, 36] The toxins within the nematocysts of the box jellyfish *C. fleckeri* combine to represent one of the most potent lethal animal venoms in that they can cause human death within minutes.[37]Freeman and Turner, [38] assayed toxin by intravenous injection in 20 g mice to determine a 'mouse unit.' Baxter and Marr, [39] determined accurate intravenous LD50 values in 25 g mice for their toxins. In our study the minimum lethal dose of *C. Stuhalmanni* and *C. quinquecirrha* crude samples were found to be 0.5 ml per 10 ± 2gm crab (*O. macocera*). 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 Table. 1 & 2.

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 (5mgml^{-1}) crude (ml)	No. of animals	Death within 30 min.	Observation			
				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. quinquicirrha* crude venom (5 mgkg^{-1} of body wt.) *i.p.,* injection to Ocypod crab (10 ± 2 gms).

S. No	Concentration (5mgml^{-1}) fractionated (ml)	No. of animals	Death within 30 min.	Observation			
				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	+	+	+	+

Table 3: Toxicity of *C. stuhalmanni* crude venom (5 mgkg^{-1} of body wt.) *i.p.,* injection to male albino mice (20 ± 2 gms).

S. No	Concentration (5mgml^{-1}) Crude (ml)	No. of animals	Death time (Min : Sec) (Mean ±S.E.M)	Symptoms
1.	Control	5	-	-
2.	0.25	5	-	Palpitation, - No Lethality
3.	0.50	5	-	Palpitation, excess defecation - No Lethality
4.	0.75	5	-	Palpitation, excess defecation - No Lethality
5.	1.00	5	1.10 ± 0.03	Palpitation, excess defecation, dragging of hind limbs and paralysis. - **Lethal**

Table 4: Toxicity of *C. quinquicirrha* crude venom (5 mgkg^{-1} of body wt.) *i.p.,* injection to male albino mice (20 ± 2 gms).

S. No	Concentration (5mgml^{-1})Crude (ml)	No. of animals	Death time (Min : Sec) (Mean± S.E.M)	Symptoms
1.	Control	5	-	-
2.	0.25	5	-	No symptoms – No Lethality
3.	0.50	5	-	Palpitation, excess defecation - No Lethality
4.	0.75	5	-	Palpitation, excess defecation - No Lethality
5.	1.00	5	1.56 ± 0.03	Palpitation, excess defecation, dragging of hind limbs and paralysis. - **Lethal**

In mice bioassay the crude extract of *C. Stuhalmanni* and *C. quinquecirrha* showed lethality at a dose of 1.0ml, the death time was recorded at 1.10 ± 0.03 and1.56 ± 0.03 min & sec respectively. Flowing observation were made Palpitation, excess defecation, dragging of hind limbs and paralysis (Table. 3 & 4). Freeman and Turner, [40] found the toxicity in mice (0.2 - 5.0µg/kg), extracted from the Australian Jelly fish *Chiropsalmus quadrigatus* and *Chironex fleckeri.* Venom extracted from *Chironex fleckeri*[41] at high dose 10 µg/kg caused cardiac failure in anaesthetized rabbits. Walker, [42] 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.

Analgesic activity is reported in terms of analgesic ratios. Both crude extracts of jelly fish injected mice showed greater analgesic ratio. In tail flick method 12.24 ± 0.19AR and 11.04 ± 0.31AR recorded as a maximum analgesic ratio in *C. quinquecirrha* and *C. Stuhalmanni* extracts after 2 hrs of extract administration and minimum 6.8 ± 0.18AR and 4.64 ±0.22AR showed by *C. quinquecirrha* and *C. Stuhalmanni* extracts at 5 hrs and 6 hrs respectively (Table. 5). In hot plate method, both extracts showed the maximum paw licking of 6.33 ± 0.32 AR in after 15 min of extract administration and minimum 2.33 ± 0.33AR in 2 hrs of *C. quinquecirrha* extracts administration. Maximum jump response 5.66 ± 0.14 AR was observed in 15 min of *C. Stuhalmanni* extract administration and minimum 3.33 ± 0.33AR observed in 2 hrs. In *C. quinquecirrha* extracts showed maximum 5.33 ± 0.33AR in 30 min and minimum 2.66 ± 0.32AR in 1 hrs respectively (Table. 6). The studies on analgesic activity of *C. quinquecirrha* and *C. Stuhalmanni* are very less but more literatures are available on biological aspects. Analgesic effect of medicinal plants is a widely studied subject.

Table 5: Analgesic activities in terms of tail flick response of *C. stuhalmanni* extracts at 5mgkg^{-1} of 20 ± 2 g male albino mice.

Treatment	Mean reaction time before administration of drug	Mean reaction time after administration of drug							
		15 (min)	30 (min)	60 (min)	2nd hr	3rd hr	4th hr	5th hr	6th hr
Control (Saline)	3.50 ± 0.22	3.82 ± 0.32	3.66 ± 0.31	3.29 ± 0.21	3.40 ±0.24	3.66 ±0.32	3.12 ±0.18	3.52 ±0.24	3.22 ±0.18
Crude C.s extract	3.63 ± 0.21	6.14 ± 0.14	8.12 ± 0.24*	9.16 ±0.28**	11.04 ±0.31***	8.64 ±0.34*	6.15 ±0.14	5.12 ±0.21	4.64 ±0.22
Crude C. q extract	3.45 ± 0.31	6.84 ± 0.18*	9.48 ± 0.24**	10.12 ±0.42**	12.24 ±0.19***	11.12 ±0.24***	9.64 ±0.31**	8.24 ±0.24*	6.87 ±0.22
Pentazocine (std)	3.67 ± 0.32	6.83 ± 0.14*	8.67 ± 0.21*	9.17 ± 0.31**	10.11 ±0.10***	8.24 ±0.32*	5.61 ±0.14	4.11 ±0.13	3.52 ±0.14

Values are mean ± SEM; n=6 in each group. Percentage inhibition when compared to control. *Values are statistically significant at P< 0.05.

**Values are statistically significant at P< 0.01. ***Values are statistically significant at P< 0.001.

Table 6: Analgesic activities in terms of hot plate response of *C. stuhalmanni* extracts at 5mgkg^{-1} of 20 ± 2 g male albino mice.

Treatment	Basal reaction time (Sec)		Response time after extracts injection (Mean ± S.D)									
	Paw licking	Jump response	Paw licking					Jump response				
			15 min	30 min	45 min	1 hrs	2 hrs	15 min	30 min	45 min	1 hrs	2 hrs
Control (Saline)	3.33 ± 0.33	2.33 ± 0.33	4.33 ± 0.33	2.33 ± 0.33	2.66 ± 0.32*	3.33 ± 0.33	3.16 ± 0.33*	4.66 ± 0.33	2.16 ± 0.33	3.66 ± 0.14*	2.66 ± 0.33	2.33 ± 0.33
Crude C. s extract	3.33 ± 0.33	6.16 ± 0.33	6.33 ± 0.32*	4.6 ± 0.33*	4.33 ± 0.14*	4.66 ± 0.33*	3.66 ± 0.32*	5.33 ± 0.33**	5.66 ± 0.14	4.66 ± 0.33	3.66 ± 0.33*	3.33 ± 0.33
Crude C. q extract	4.33 ± 0.33	5.33 ± 0.33	6.33 ± 0.33*	3.33 ± 0.32**	4.6 ± 0.10**	4.33 ± 0.14*	2.33 ± 0.33	5.33 ± 0.32*	5.33 ± 0.33**	4.33 ± 0.33	2.66 ± 0.32*	3.66 ± 0.32*
Pentazocine (std)	3.66 ± 0.33	4.33 ± 0.33	3.33 ± 0.33*	4.66 ± 0.32*	2.66 ± 0.33	2.66 ± 0.33***	3.33 ± 0.14	4.33 ± 0.33*	3.33 ± 0.33*	2.66 ± 0.33*	2.33 ± 0.14*	3.33 ± 0.33**

Values are mean ± SEM; n=6 in each group. Percentage inhibition when compared to control. *Values are statistically significant at P< 0.05. **Values are statistically significant at P< 0.01. ***Values are statistically significant at P< 0.001.

The present result was coincided with another author worked in molluscs and plants. The pain killing effect may be due to the selective modulation the neuronal nicotinic receptors in the spinal cord and brain as found out by Day in Ecudoran frogs, as reported by Marwick.[43] Gouiffes *et al.* [13] reported that no local anaesthetic activity or analgesic effect was observed after administration of Bistramide A – toxin. Intracisternal injection of the substance at a dose level of 1.5 mg/ kg of body weight (a dose equal to the *i.p.* LD_{50}) did not cause mortality in mice; but, immobility with loss of muscle tone was rapidly apparent (5 min after injection). Marwick, [43] observed the venom of *Conus magus* have 1,000 times stronger analgesic activity than morphine. Sakthivel, [44] studied the analgesic property of *Conus lentiginosus* and *C. mutabilis,* which was 128 times more than that of paracetamol. Shanmuganandam, [45] reported the effectiveness of *Conus figulinus* venom on guinea pig skin as an infiltration anaesthetic agent. Salivary gland secretion of the gastropod *Conus* sp. is one of the most important venoms to possess analgesic property. [46, 44]

The both extracts (200 mg/kg), administered intraperitoneally, significantly inhibit acetic acid induced writhing in mice. There writhing are related to increase in the peritoneal level of prostaglandins and leukotrienes.[47] In writhing test *C. Stuhalmanni* and *C. quinquecirrha* extracts showed 30.65±2.10 and 21.95±1.68 writhing and Inhibition of writhing response percentage was 35% and 55% (Table. 7). The results strongly suggest that the mechanism of action of extract may be linked to lipoxygenase and/or cycloxygenase.

The pain in the early phase of formalin test was due to the direct stimulation of the sensory nerve fibres by formalin while the pain in the late phase was due to inflammatory mediators, like histamine, prostaglandins, serotonin and bradykinins. [48, 49, 50] This test is believed to be a more valid analgesic model which is better correlated with clinical pain.[50, 51] In this study, the extract caused a dose-dependent decrease in licking time and licking frequency by the mice injected with formalin signifying the analgesic effect of the extracts (Table. 8.). In this investigation jelly fish compounds showing the decrease of CNS depressant activity 90% and 95% were recorded in *C. Stuhalmanni* and *C. quinquecirrha* extracts (Table. 9.).

Inflammatory (edema) is defined as soft tissue swelling due to expansion of the interstitial volume and can be localised or generalised. Al-Hassan *et al.* [52] have reported that the action of toxin was competitively inhibited by pre-treatment with atropine and indomethacin. The mechanisms of anti-inflammatory activity may be related to the anti-phlogistic action of the tannins. NSAID such as indomethacin used in this study are known to inhibit cyclooxygenase enzymes I and II which are implicated in the production of inflammation-mediating agent prostaglandin E2 (PGE2) from arachidonic acid.[53, 54, 55] The pattern of anti-inflammatory and analgesic activities exhibited by this extract was similar to that of indomethacin which suggests that the jelly fish extracts activity may be mediated by cyclooxygenase I and II inhibition (Table. 10.).

Table 7: Writhing effects of *C. stuhalmanni* and *C. quinquicirrha* crude venom (5 mgkg^{-1} of body wt.) extracts on male albino mice (20 ± 2 gms)

S. No	Treatment	n	Dose mg/kg	Route of administration	No. of writhes	Inhibition of writhing response (%)
1	Control	6	----	*i.p*	48.06±4.04	-
2	Aspirin (std)	6	300	*i.p*	10.05±2.12**	80
3	C. s Crude	6	200	*i.p*	30.65±2.10	35
4	C. q Crude	6	200	*i.p*	21.95±1.68**	55

C. s = *C. stuhalmanni, C. q* = *C. quinquicirrha*, Mean = S.E.M, n= of 6 animals, ** = P≤0.001= highly significant.

Table 8: Analgesic effect of *C. stuhalmanni*, *C. quinquicirrha* crude venom extracts (5 mgkg^{-1} of body wt.) and indomethacin (10mgkg^{-1}) on male albino mice (20 ± 2 gms) using formalin.

	Control	Crude extract of *C. stuhalmanni* (5mgkg^{-1})		Crude extract of *C. quinquicirrha* (5mgkg^{-1})		Indomethacin (10mgkg^{-1})
		0.5	1.0	0.5	1.0	
Duration (sec)	12.8 ±2.2	5.5±0.8*	9.7 ±0.6*	6.7±0.5*	7.2 ±0.3*	5.0±0.5*
Frequency/30min	22.5 ±3.5	8.7±0.3*	13.2±1.6*	9.5±0.6*	14.8±1.5*	14.3±1.8*

*Values are statistically significant at P< 0.05.

Table 9: Central nervous system depressant activity of *C. stuhalmanni* and *C. quinquicirrha* crude venom extracts at 2mgkg^{-1} of 20 ± 2 g male albino mice

Treatment 5mgkg^{-1}	Dose (mgkg^{-1})	Mean reaction time before administration of drug (Sec)	Mean reaction time after administration of drug (Sec)	
			30 min	60 min
Control (Saline)	0.2	208.14 ± 5.24	216.14 ± 3.86	202.64 ± 6.44
C. s Crude	2.0	214.62 ± 6.14	44.24±3.96***	21.14 ± 2.1***
C. q Crude	2.0	212.18 ± 3.84	32.12±3.82***	12.16 ± 3.4***
Diazepam (Std)	4.00	204.63 ± 4.64	33.68±3.84***	18.14 ± 2.86***

Values are mean ± SEM; n=6 in each group. Percentage inhibition when compared to control.

*Values are statistically significant at P< 0.05. **Values are statistically significant at P< 0.01. ***Values are statistically significant at P< 0.001.

Table 10: Anti inflammatory formation effect of *C. stuhalmanni* and *C. quinquicirrha* crude venom extracts on at 2 mgkg^{-1} of 20 ± 2 g male albino mouse.

Group	n	Dose mg/kg	Paw volume increase (mm)			Inhibition (%)		
			1 hrs	3 hrs	5 hrs	1 hrs	3 hrs	5 hrs
Control	6	-	0.38±0.07	0.69±0.05	0.085±0.01	-	-	-
Indomethacin	6	300	0.10±0.02**	0.21±0.02**	0.27±0.03**	72	70	67
C. s Crude	6	50	0.23±0.04*	0.48±0.01*	0.50±0.02*	39	33	39
C. q Crude	6	100	0.19±0.03*	0.40±0.01*	0.42±0.04*	50	44	52

n = 6 animals in each group, * = p≤0.01 (significant), ** = p≤0.01 (highly significant)

The present study could be speculated that the venom of *C. Stuhalmanni* and *C. quinquecirrha* may have many biologically active principals, which need further elaborate to study in future. Thus, the importance of biologically active compounds present in the venom of jelly fish and has become evident and found to be useful tools for probing biological or pharmacological activity.

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REFERENCES

1. Gunthorpe L. Cameron A M. Widespread but variable toxicity in scleractinian corals. Toxicon. 1990; 28: 119 – 1219.
2. Macek P. Polypeptide cytolytic toxins from sea anemones (Actinaria). FEMS Microbiol. Immunol. 1992; 5: 121– 129.
3. Halstead BW. Poisonous and Venomous Marine Animals of the World. Darwin Press, Princeton, NJ. 1988; 243-263.
4. Yanagihara AA, Kuroiwa, JMY, Oliver LM, Chung JJ, Kunkel DD. Ultra structure of a novel euryteles nematocyst of Carybdea alata Reynaud (Cubozoa Cnidaria). Cell Tissue Res. 2002; 308: 307-318.
5. Rifkin J. Jellyfish mechanisms. Chapter 6. In: Williamson J, Fenner P, Burnett, J, Rifkin J. (Eds.), Venomous and Poisonous Marine Animals. University of New South Wales Press, Sydney; 1996; 2: 121–173.
6. Hsieh YHP. Jelly fish as food. Hydrobiologia, 2001; 451: 11–17
7. Rottini G, Gusmani L, Parovel E. Purification and properties of a cytolytic toxin in venom of the jellyfish *Carybdea marsupialis*. Toxicon. 1995; 33: 315-326.
8. Bradford MM. A rapid and sensitive method for the quantization of microgram quantities of protein utilizing the principle of protein-dye binding. Anal. Biochem. 1976; 72-254.
9. Pani Prasad K, Venkateshwaran K. Micro hemolytic assay, International Training Manual on Advance Techniques in Marine Biotoxinology, CIFE, India; 1997. p. 41.
10. Laemmli UK. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature. 1970; 227 (5259): 680-685.
11. Sambrook J, Russell DW. Molecular cloning-Laboratory manuals 3rdedition, vol-3. Cold spring harbor laboratory press, cold spring harbor, New York; 2001. A.8.40 - A.8.49.
12. Lane CE, Dodge E. The toxicity of *Physalia* nematocysts. *Biol.* Bull., Mar. Biol. Lab., Woods Hole. 1958; 115: 219-226.
13. Gouiffes D, Juge M, Grimaud N, Welin L, Sauviat MP, Barbin Y. A new toxin from the Urochordata *Lissoclinum bistratum* Sluiter: isolation and preliminary characterization. Toxicon. 1988; 26(12): 1129- 1136.
14. D'Amour FE, Smith DL. A method for determining loss of pain sensation**.** J Pharmacol Exp Ther**.** 1941; 41: 419 - 424.
15. Eddy NB, Leimbach D. Synthetic analgesics. II. Dithienylbutenyl and dithienylbutylamines. J. Pharmacol. Exp. Ther. Mar. (1953) 107 (3): 385- 393.
16. Tornos MP, Saenz MT, Garcia MD, Fernandez MA. Antinociceptive effects of the tubercles of *Anredera leptostachys.* J. of Ethnophar. 1999; 68(3): 229- 234.
17. Shibata M, Ohkubo T, Takahashi H, Inoki R. Modified formalin test: Characteristic biphasic response. Pain. 1989; 38: 347–352.
18. Dews PB. The measurement of the influence of drugs on voluntary activity in mice. Brit. J. of Pharmacol. 1953; 8: 46- 48.
19. Kulkarni SK, Dandiya PC. Influence of chemical stimulation of central DA system on the "open field" behaviour of rats. Pharmacopsychiatry. 1975; 8: 45- 50.
20. Winter CA, Risley EA, Nuss GW. Carrageenan-induced edema in hind paw of the rat as an assay for anti-inflammatory drugs. Proc Soc Exp Biol Med. 1962; 111: 544-47.
21. Qasim S Z. Pharmaceutical potential of marine organisms. In: Aravindan and S. D. R Kumari (Eds), Advances in Aquatic Biology and Fisheries, University of Kerala, India; 1998. p. 5-10.
22. Russell FE. Marine toxins and venomous and poisonous marine animals In: *Advances in Marine Biology* (Blaxter J.H.S, Russell, F.S. and Yonge, C.M., Eds.) Academic Press, London; 1984. p. 725.
23. Cairns S, Calder DR, Brinckmann-Voss A, Castro CB, Pugh PR, Cutress CE. Common and Scientific Names of Aquatic Invertebrates from the United States and Canada: Cnidaria and Ctenophora (Bethesda, MD: American Fisheries Society); 1991. p. 75.
24. Fernando SA, Fernando OJ. A field guide to the common invertebrates of the east coast of India. CAS in Marine Biology, Annamalai University; 2002. p. 63.
25. Rodrıguez JS, Vazquez KC. Isolation and biological characterization of neurotoxic compounds from the sea anemone *Lebrunia danae* (Duchassaing and Michelotti, 1860) Arch. Toxicol. 2006; 80: 436- 441.
26. Radwan FF, Gershwin LA, Burnett JW. Toxinological studies on the nematocyst venom of *Chrysaora achlyos.* Toxicon. 2000; 38: 1581-1591.

27. Nagai H, Takuwa-Kuroda K, Nakao M, Oshiro N, Iwanga S, Nakajima T. A novel protein toxin from the deadly box jellyfish (sea wasp, Habu-kurage) *Chiropsalmus quadrigatus*. Biosci. Biotechnol. Biochem. 2002; 66: 97–102.
28. Olson CE, Pockl EE, Calton GJ, Burnett JW. Immunochromatographic purification of a nematocyst toxin from the Cnidarian *Chironex fleckeri* (sea wasp). Toxicon. 1984; 22: 733-742.
29. Bloom DA, Radwan FFY, Burnett JW. Toxinological and immunological Studies of capillary electrophoresis fractionated *Chrysaora quinquecirrha* (Doser) fishing tentacle and *Chironex fleckeri* Southcott nematocysts venoms. Com. Biochem and Physiol. 2001;47B: 815.
30. Long KO, Burnett J W. Isolation characterization and comparison of hemolytic peptides in nematocyst venoms of two species of jellyfish (C. *quinquecrrha* and C. *capillata)* Comp. Biochem. Physiol. 1989; 948(4):641-646.
31. Keen TE, Crone HD. The hemolytic properties of extracts of tentacles from the cnidarian *Chironex fleckeri*. Toxicon. 1969; 7: 55-63.
32. Radwan FFY, Burnett JW, Bloom DA, Coliano T, Eldefrawie ME, Erderly H. A comparison of the toxinological characteristics of two *Cassiopea and Aurelia* species. Toxicon. 2001; 39: 245-257.
33. Torres M, Aguliar MB, Falcon A. Electrophysiological and hemolytic activity elicited by the venom of the jellyfish *Cassiopea xamachana*. Toxicon. 2001; 39: 297-1 307
34. Klug M, Weber J, Tardent P. Hemolytic and toxic properties of *Hydra attenuata* Nematocysts. Toxicon. 1989; 27: 325-339.
35. Endean R, Monks SA, Cameron AM. Toxins from the box-jellyfish Chironex fleckeri. Toxicon. 1993; 31: 397–410.
36. Fenner PJ, Williamson JA. Worldwide deaths and severe envenomation from jelly fish sting. Med. J. Aus. 1996; 165: 658-661.
37. Burnett J, Currie B, Fenner P, Rifkin J, Williamson J. Cubozoan (Box Jellyfish) Chapter 9. In: Williamson, J., Fenner, P., Burnett, J., Rifkin, J. (Eds.), Venomous and Poisonous Marine Animals. University of New South Wales Press, Sydney; 1996. p. 236–283.
38. Freeman SE, Turner RJ. A pharmacological study of the toxin in a Cnidarian, Chironex fleckeri Southcott. Br J Pharmacol. 1969; 35(3): 510–520.
39. Baxter, E.H, Marr G. M. Sea wasp (*Chironex fleckeri*) venom: lethal, hemolytic and dermonecrotic properties, Toxicon. 1969; 7: 195.
40. Freeman SE, Turner RJ. Cardiovascular effects of cnidarian toxins: a comparison of toxins extracted from *Chiropsalmus quadrigatus* and *Chironex fleckeri*. Toxicon. 1972; 10(1): 31-37.
41. Koyama T, Noguchi K, Matsuzaki T, Sakanashi M, Nakasone J, Miyagi K, et al. Haemodyamic effects of the crude venom from nematocysts of the box- jelly fish *Chiropsalmus quadrigatus* (Habu-kurage) in anaesthetized rabbits. Toxicon. 2003; 41: 621-631.
42. Walker MJA, Martinez TT, Godin DV. Investigations into the cardiotoxicity of a toxin from the nematocysts of the jellyfish, *Cyanea capillata*. Toxicon. 1977; 15: 339-346.
43. Marwick C. Medical news and perspectives. J. Am. Medical Assoc. 1998; 279(21): 1679- 1681.
44. Sakthivel A. Biomedicinal activity of *Conus lentiginosus* and *Conus mutabilis* from Mumbai coast [M. F. Sc., Dissertation]. C.I.F.E, Mumbai, India. 1999; p. 60.
45. Shanmuganandam P. Studies on the venom *Conus fugulinus* Linnaeus (Mollusca: Gastropoda) from the south east coast of India [Ph.D. thesis]. Annamalai University, India; 1995. p. 214.
46. Spampinato S, Speroni E, Govani P, Pistacchio E, Pomagnoli C, Murani G. Effect of omega conotoxin and verapamil on antinociceptive, behavioural and thermoregulatory responses to opiods in the rat. Eur. J. Pharma. (1994; 254(3): 229- 238.
47. Deraedt R, Jougney S, Benzoni J, Peterfalvi M. Release of prostaglandins E. and F in algogenic reaction and its inhibition. Eur. J. of Pharmacol. 1980; 61: 16-24.
48. Dharmasiri JR, Jayakody AC, Galhena G,. Liyanage SSP, Ratnasooriya, WD. Anti inflammatory and analgesic activities of mature fresh leaves of *Vitex negundo*. J Ethnopharmaco.l 2003; 87: 199-206.
49. Murray CW, Porreca F. Cowan A. Methodological refinements in the mouse paw formalin test an animal model of tonic pain. J Pharmacol Methods. 1988; 20: 175-186.
50. Tjolsen A, Berge DG, Hunskaar S, Rosland JH Hole K. The formalin test: an evaluation of the method. Pain. 1992; 51: 5-17.
51. Ghannadi A, Hajhashemi V.Jafarabadi H. An investigation of the analgesic and anti-inflammatory effects of *Nigella sativa* seed polyphenols. J Med Food. 2005; **8**: 488-493.
52. Al-Hassan JM, Thomson M, Ali M, Fayad S, Elkhaead A, Thulesius O, et al. Vasoconstrictor components in the Arabian Gulf Catfish, *Arius thalassinus* epidermal secretions. Comp. Biochem. Physiol. 1986; 88B: 813- 822.
53. Moody JO, Robert VA, Connolly JD, Houghton PJ. Anti-inflammatory activities of the methanol extracts and an isolated furanoditerpene constituent of *Sphenocentrum jollyanum* Pierre (Menispermaceae). J Ethnopharmacol, 2006; 104: 87-91.
54. Dhara AK, Suba V, Sen T, Pal S, Chaudhuri AKN. Preliminary studies on the anti inflammatory and analgesic activity of the methanol fraction of the root extract of Tragia *involucrate* Linn. J Ethnopharmacol. 2000; 72: 265-268.
55. Wu KK. Aspirin and other cyclooxygenase inhibitors: new therapeutic insights. *Seminar Vascular Me.* 2003; 3: 107-112.