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Pharmacological and biomedical properties of sea anemones *Paracondactylis indicus, Paracondactylis sinensis, Heteractis magnifica* and *Stichodactyla haddoni* from East coast of India

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ABSTRACT

Objective: To explore the biomedical and pharmacological activity of *Paracondactylis indicus* (*P. indicus*), *Paracondactylis sinensis* (*P. sinensis*), *Heteractis magnifica* (*H. magnifica*) and *Stichodactyla haddoni* (*S. haddoni*). **Methods:** The live sea anemones were kept inside the glass bowl along with some amount of distilled water in an ice container for 15 min. During stress condition, nematocysts released from the tentacles were collected and centrifuged at 5 000 rpm for 15 min. The supernatant were collected in separate cleaned beakers for lyophilisation. **Results:** The protein content of crude extracts was 15.2, 28.7, 18.2 and 35.4 μg/mL. In hemolytic assay, the *P. indicus* was sensitive (16.842 HT/mg) on chicken blood but *P. sinensis* was less sensitive (1.114 HT/mg) on chicken and goat blood. Whereas *H. magnifica* and *S. haddoni* showed hemolysis (0.879, 0.903 HT/mg and 56.263, 0.451 HT/mg) in chicken and goat blood. In antimicrobial assay, the methanol extract of *P. indicus* showed maximum inhibition zone of 9.7 mm against *S. typhii* and *P. sinensis* showed 9.8 mm against *K. pneumonia* in methanol and ethanol extracts. Whereas the *H. magnifica* and *S. haddoni* showed maximum of 10 mm against *S. typhii, K. pneumonia* in methanol and ethanol extracts. **Conclusions:** The high toxic sea anemones may also contain some biologically active agents which has haemolytic, analgesic and anti-infilamatory activity.

1. Introduction

The marine environment has proven to be a rich source of extremely potent compounds that have demonstrated significant activities in anti-tumour, anti-inflammatory, analgesic, immunomodulatory, allergy and anti-viral activities. A small number of marine microbes, plants and animals have already yielded more than 16 000 novel compounds with hundred of new compounds still being discovered every year[1]. All sea anemones produce venom which is delivered by specialized stinging organelles, known as nematocysts, located on body surfaces and in high concentration on tentacles. Nematocysts are employed in defense against predators and in the capture of prey[2]. The venom consists of various kinds of polypeptide toxins[3]. Natural products released into the water are rapidly diluted and therefore, need to highly potent to have any effect, for this reason and becomes of the immense biological diversity in the sea as a whole, it is increasingly recognised that a huge number of natural products and chemicals entities exist in the ocean[4,5].

Sea anemone like other cnidarians are produced biologically active polypeptides and protein. Most of the peptide contains 46 – 49 amino acid residues in a single polypeptide chain that is cross linked by three disulfide bridges[6]. Sea anemones produce toxin, which are used for prey acquisition or as chemical signals for repelling predators. According to their mode of action, sea anemone toxin can be divided into neurotoxin and cytotoxins. Sea anemones (Actiniaria) produce four different classes of cytolytic polypeptides[7].

2. Materials and methods

2.1. Sample collection and identification

The four species of sea anemones, *Paracondactylis indicus* (*P. indicus*), *Paracondactylis sinensis* (*P. sinensis*),

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Heteractis magnifica (*H. magnifica*) and *Stichodactyla haddoni* (*S. haddoni*) were collected from Mandapam coast, India. The live specimens were brought to the laboratory with sea water in an air tightened pack and kept in live condition until further extraction. The collected specimens were identified using the standard literature[8].

2.2. Animals

Male albino mice weighing (20 ± 2) g were housed under standard laboratory condition. The animal had free access to food and water. The animal ethical committee of institute approved all the experimental protocols of study.

2.3. Extraction of nematocyst

The crude extract was prepared by alternative thawing method of Anderluh *et al*.[9]. The live animals were kept inside the glass bowl along with some amount of distilled water in an ice container for 15 minutes. During stress condition the nematocysts were released from the tentacles. The same procedure was repeated for thrice. The collected nematocysts containing toxins were filtered by Whatman No.1 filter paper.

2.4. Centrifugation and lyophilisation

In order, the collected nematocysts were centrifuged at 5 000 rpm for 15 min. The supernatant was collected in separate cleaned beakers for lyophilisation. The pure aqueous extracts were kept for lyophilisation which then transformed into crystalline powder for various bioassays.

2.5. Protein estimation

Protein concentration was determined by the method of Rajesh Bhattacharjee and Parames[10]. Bovine serum albumin (BSA) was used as a standard.

2.6. Hemolytic assay

Crude extracts of *P. indicus*, *P. sinensis*, *H. magnifica* and *S. haddoni* were assayed on chicken and goat blood followed by the method of Pani Prasad and Venkateshvaran[11]. Samples of chicken and goat bloods were obtained from the nearby slaughterhouse in Parangipettai using 2.7 % ethylene diamine tetra acetic acid (EDTA) solution as an anticoagulant at 5 % of the blood volume and brought to the laboratory. The blood was centrifuged thrice at 5 000 rpm for five minutes. 1% erythrocyte suspension was prepared for hemolysis study.

2.7. Analgesic activity

2.7.1. Hot plate method

Hot plate method was described by Eddy and Leimbach[12]. The animals were individually placed on a hotplate and maintained for 55 ℃. Reaction of animals such as paw licking or jump response was taken as an end point. Extracts were injected i.p to male albino mice at dose level of 5 mg/kg of body weight (20 ± 2) g.

2.8. Central nervous system depressant activity

The CNS depressant activity was performed according to Kulkarni & Dandiya[13] using actophotometer. Male albino mice were housed with a 12:12 hour dark – light cycle. The concentrations of extract were 5 mg/kg of body weight. Mice without administration of any crude toxin were used as a control.

2.9. Edema formation

The crude toxin from the anemone was assayed for edema formation activity following the modified method of Smith[14]. Group of two mice in each was injected with 0.1 mL of the toxin in the right foot pad and with 0.1 mL of buffered saline in left pad. Two hours after injection, percentage of increase in size was measured with a vernier caliper and the increase in size of the envenomated foot relative to the saline injected foot was taken as the Edema Ratio (ER). The minimum edematic dose was defined as the dose causing 105 % ER.

2.10. Mice bioassay for lethality

This bioassay has done by using clinically healthy male albino mice (20±2) g were maintained under a healthy condition at the laboratory. Mice in triplicate were tested intraperitoneally injection with 0.25, 0.50, 0.75 and 1.00 mL of the crude toxin, dissolved at 5 mg/mL. A control will be maintained in each case by injecting an equal volume of PBS (pH 7.2). The times of injection and death, besides the behavioural changes of the mice before death was recorded.

2.11. Antibacterial test

The antibacterial activity was determined by standard disc diffusion method of Murugan and Santhana Ramasamy[15]. The following microorganisms, *Staphylococcus aureus(S. aureus)*, *Salmonella typhi(S. typhi)*, *Salmonella paratyphi(S. paratyphi)*, *Klebsiella pneumonia(K. pneumonia)*, *Vibrio cholerae(V. cholerae)* and *Pseudomonas aeruginosa(P. aeruginosa)* were used. The extracts were applied to 6 mm sterile discs in aliquots of 30 μL of solvent, allowed to dry at room temperature, and placed on agar plates seeded with microorganisms. The bacteria were maintained on nutrient agar plates and incubated at 37 ℃ for 24 hrs. The zone of growth inhibition was measured.

3. Results

3.1. Protein estimation

The amount of protein content in *P. indicus*, *P. sinensis*, *H. magnifica* and *S. haddoni* were showed 15.2, 28.7, 18.2 and 35.4 μg/mL respectively.

3.2. Hemolytic assay

The hemolytic activity of crude extracts shown moderate activity on chicken and goat erythrocytes. In chicken blood induced pronounced hemolysis with maximum specific hemolytic activity of 16.842 HT/mg in *P. indicus* and minimum of 1.114 HT/mg in *P. sinensis*. Whereas the *H. magnifica* and *S. haddoni* extracts showed hemolysis in both chicken and goat blood, with specific hemolytic unit of 0.879, 0.903 and 56.263, 0.451 HT/mg respectively.

3.3. Analgesic activity

Analgesic activity of *P. indicus*, *P. sinensis*, *H. magnifica*

and *S. haddoni* extracts on hot plate method were tested using male albino mice (20±2) g. The analgesic ratio (AR) was recorded based on paw licking and jumping response after extracts administration.

In hot plate method, mice treated with crude extract of *P. indicus* and *P. sinensis* showed maximum paw licking of 5 times at 120 and 15 second, respectively. In the jumping response test, mice treated with crude extract of *P. indicus* showed 5 AR at 60 sec, which was comparatively higher than that treated with *P. sinensis*. Whereas mice treated with crude extract of *H. magnifica* and *S. haddoni* also showed significantly inhibiting the paw-licking and jumping response in a time dependent manner as shown in Figure 1 & 2.

3.4. Central nervous system depressant activity

The maximum decrease of depressant activity of 34.3 %, 39.19 %, 32.43 % and 22.80 % were recorded in the crude extracts of *P. indicus*, *P. sinensis*, *H. magnifica* and *S. haddoni*. Percentage decrease of motor activity has been calculated from the basal score and after 10 min of injection of extract (Table 1).

3.5. Edema formation

The toxins extracted from four different sea anemone species was taken for the edema forming activity in the mouse foot pad. All the four species were exhibited edema formation in the mice foot pad. The crude extract of *H. magnifica* showed an ER of 200 %, followed by *P. indicus* the (ER) 160 % was observed and (ER) of 120% was observed in both *P. sinensis* and *S. haddoni* (Table 2).

3.6. Mice bioassay for lethality

Crude extract of sea anemones, when intraperitoneally injected into male albino mice at doses of 0.25, 0.50, 0.75 and 1.0 mL, showed symptoms of toxicity. The extract of *P. indicus*, *P. sinensis*, *H. magnifica* and *S. haddoni* at a dose of 1.0 mL (containing 5 mg of crude toxin) protein were lethal to mice upon IP injection; however, the maximum lethality were observed in all the sea anemone extracts at a dose level of 1.0 mL than other doses.

3.6.1. Behavioral changes in mice

Lying on belly with forelimbs spread wide, running around the cage in an excited manner, escape reaction, prolonged palpitation, closed eyes, grooming, shivering of forelimbs, loss of balance, opaque eyes, squeaking, tonic convulsions, gasping for breath, arching of body backwards, paralysis, micturiction, flexing of muscles, prodding (insensitive to stimuli), diarrhea, lethargy, dragging of hind limbs, rolling of tail, foaming from mouth and exophthalmia were the common alterations observed upon envenomation on various samples of sea anemones such as *P. indicus*, *P. sinensis*, *H. magnifica* and *S. haddoni* samples at 5.0 mg/mL

3.7. Antibacterial test

Crude extracts obtained from sea anemones displayed strong antibacterial activity against all strains (Figure 3 and 4). Inhibition zones were showing maximum 9.7 mm against *S. typhii* in methanolic extract of *P. indicus* and minimum 6 mm showed against *S. aureus* in aqueous extract. In *P. sinensis* extract showed 9.8 mm against *K. pneumonia* of methanolic and ethanolic extracts and minimum of 6 mm showed *S. aureus* in aqueous extract. In the case of *H. magnifica* and *S. haddoni* showed maximum 10 mm against *S. typhii*, *K. pneumonia* in methanol and ethanolic extracts and minimum of 6mm showed against *S. aureus* in aqueous extract respectively.

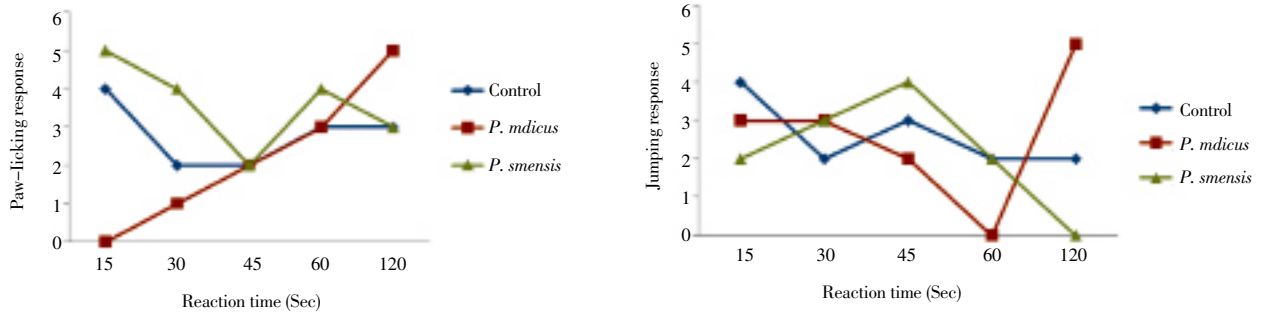

Figure 1. Analgesic activities in terms of hotplate response of *P. indicus* and *P. sinensis* extract at 2 mg /kg of (20±2) g male albino mice.

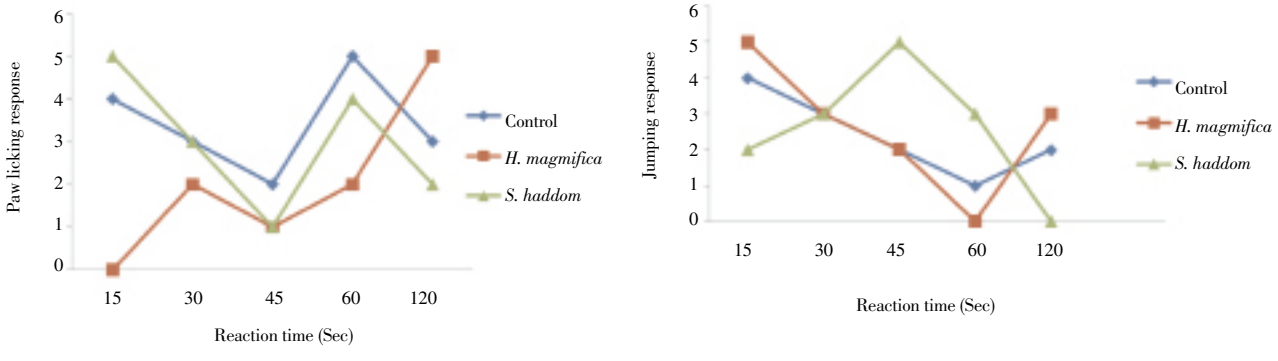

Figure 2. Analgesic activities in terms of hotplate response of *H. magnifica* and *S. haddoni* extract at 2 mg/kg of (20±2) g male albino mice.

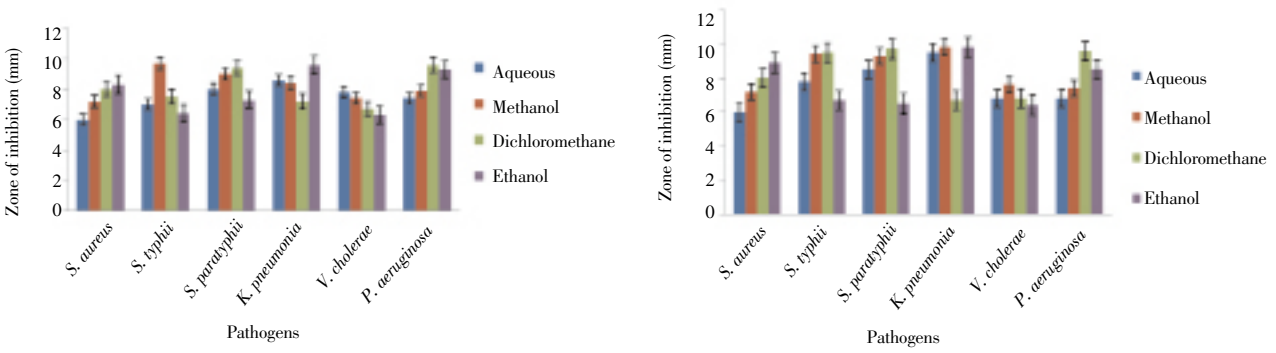

Figure 3. Antibacterial activity of *P. indicus* and *P. sinensis* against human pathogens.

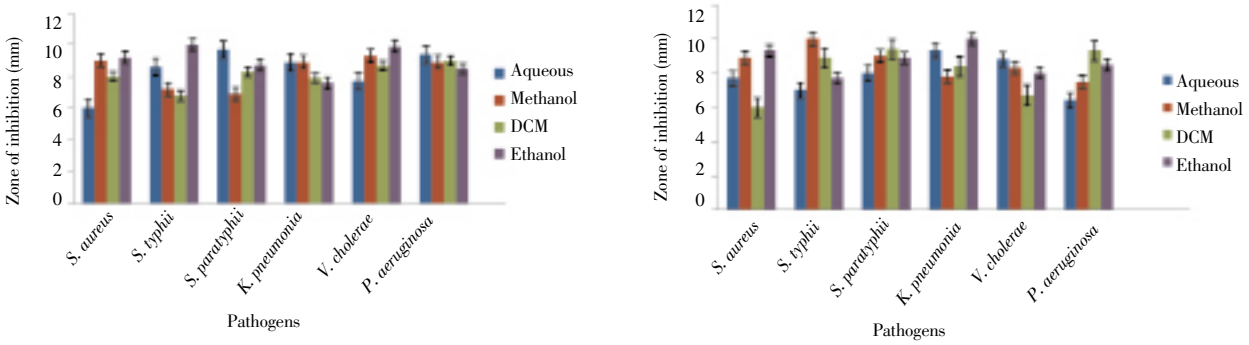

Figure 4. Antibacterial activity of *H. magnifica* and *S. haddoni* against human pathogens.

Table 1
Central nervous system activity of *P. indicus, P. sinensis, H. magnifica* and *S. haddoni* extract at 2 mg/kg of (20±2) g male albino mice.

Treatment (2 mg/kg)	Body weight (g)	Locomotor activity (scores) in 10 min		% Decrease of motor activity
		Before treatment	After treatment	
Control	20.85	534	485	9.17
P. indicus	20.97	431	283	34.33
P. sinensis	20.10	324	197	39.19
H. magnifica	20.97	370	250	32.43
S. haddoni	20.10	285	220	22.80

Table 2
Edema formation effect of *P. indicus, P. sinensis, H. magnifica* and *S. haddoni* extract on at 2mg/kg of (20±2) g male albino mice.

Treatment (2 mg/kg)	Paw edema volume (mm)		
	Before injection	After injection	Edema ratio in %
Control (Normal saline)	0.4	0.5	–
Crude extract of *P. indicus*	0.5	0.8	160
Crude extract of *P. sinensis*	0.4	0.6	120
Crude extract of *H. magnifica*	0.5	1.0	200
Crude extract of *S. haddoni*	0.5	0.6	120

4. Discussion

The importance of the biologically active compounds present in the venom of different marine organisms has become evident. The study of these compounds has permitted an improved understanding in the fields pharmaceutical, neural, and biological sciences, and has allowed for the development of novel drugs. Sea anemones contain a range of active biological compounds including some potent toxins. It has been reported that the venoms present in different species of sea anemones are known to possess potent hemolytic properties[16].

The present study showed hemolytic activity in all sea anemone extracts against chicken and goat blood. Previously, Rottini *et al*[17] showed haemolytic activity from jelly fish *C. marsupialis* nematocyst venom assayed in sheep red blood cells but not in human red blood cells. Torres *et al*[18]. have reported the jelly fish *Cassiopea xamachana* venom showed a higher hemolytic activity in human RBCs than sheep RBCs.

Hemolytic activity has been described on a variety of cnidarian venoms against erythrocytes of many different species. Karthikayalu *et al*.[19] have studied characterization, purification and phylogenetic analysis of a cytolysin from

the sea anemone *Heteractis magnifica* of the Indian Ocean. Venoms isolated from the red sea soft corals *Nephthea* sp., *Dendronephthya* sp. and heteroxenia fuscescens were found to posses hemolytic toxins[20].

Rottini *et al*[17] demonstrated that *Carybdea marsupialis* jellyfish venom exhibits unpredictable hemolytic activities in different species, such as sheep, human, and rabbit. The analgesic activity of *P. indicus, P. sinensis, H. magnifica* and *S. haddoni* extracts on mice exhibited better activity. This results support the earlier findings that crude extracts of some echinoderms[21]. The extraction from spines of *Acanthaster planci* was lethal to mice, and sea anemone nematocylst[22,23] and the toxicity of TTX produced microbes from puffer fish[24].

The symptoms observed in envenomoted mice are indicative of central nervous system (CNS) toxicity and nephrotoxicity. Present study showing the sea anemone *P. indicus*, *P. sinensis, H. magnifica* and *S. haddoni* extracts provides does-dependant lethality to male albino mice, with displaying adverse effect such as sedation, respiratory distress, paralysis, and death. Similar symptoms are also reported in roe extracts two sciaenids[25,26].

The antimicrobial activity of molluscan reported here agrees with the results of previous studies[27,28]. In this present study, aqueous, methanol, dichloromethane and ethanol extracts of *P. indicus, P. sinensis H. magnifica* and *S. haddoni* exhibited zone of inhibition against all human pathogens. Among this, *H. magnifica* and *S. haddoni* showed 10 mm against *S. typhii* and *K. pneumonia*. Previously, Williams *et al*.[29] have reported from the benthic sea anemone *Stichodactyla haddoni* collected from the Indian coast against the fish pathogen *Aeromonas hydrophila*.

Nylund *et al*.[30] reported that the crude organic extract of marine algae *Bonnemaisonia hamifera* inhibited the growth of nine bacteria. Later, the same author demonstrated that polyhalogenated 2-heptanone from this alga, strongly inhibits the colonization and growth of numerous marine bacteria[31]. Villasin and Pomory,[32], the extract from the body wall of the sea cucumber *Parastichopus parvimensis* had antibacterial properties.

Conflict of interest statement

We declare that we have no conflict of interest.

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