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RESEARCH ARTICLE

ANTICOAGULANT PROPERTIES OF THE SEA ANEMONES MUCUS (*HETERACTIS MAGNIFICA* AND *STICHODACTLYLA HADDONI*)

Annadurai, D., Prithiviraj, N., Shanthasubitha, S and Sadeesh Kumar, R

CAS in Marine Biology, Annamalai University, Parangipettai-608 502, India

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ABSTRACT

Mobile In the present study, anticoagulation activity of mucus extracted from sea anemones *Heteractis magnifica* and *Stichodactlyla haddoni* at three different concentrations was tested in blood plasma, using two assay system and the results are presented in Table 1. In APTT assay the activity was a maximum in *Stichodactyla haddoni* this species prolonged clotting time of maximum 700 seconds about 8.7 fold higher than that of control (80 seconds) of all the concentration tested and the clotting time is higher in the *Stichodactyla haddoni* then *Heteractis magnifica*. Thus, the sea anemone mucus extracts time clotting was noted in the ratio 8.7 is the high folds in the species of *Stichodactyla haddoni* in APTT assay. In PT assay a few extract showed higher concentration in the species *Stichodactyla haddoni* 3.5, 2.5 and 2.5 folds higher respectively in 100, 500 and 1000µgmL^{-1} than the control. All other extract shows lower anti coagulant activity in PT assay.

INTRODUCTION

Sea anemones are known to contain several types of toxins, possibly in specialized stinging cells (nematocysts) similar to most members of the phylum Cnidaria and the class Anthozoa. They are distributed in the tropical and subtropical seas of the world. About 9,000 species of Cnidarians, approximately 70 species have been reported to be capable of causing intoxication in human being by means of a stinging cell (Picken and Skaer, 1996). Anemones neurotoxins, which have relatively low molecular weights between 3,000 and 5,000Da, are very important tools in neurophysiological and pharmacological research as blockers and modulators of K^+ and Na^+ channels. Because of their unique effect on sodium channels, several sea anemone polypeptides have been utilized as valuable pharmacological agent (Norton, 1991).

The detailed investigation on the anticoagulant activity of algal and echinoderm marine sulfated furans and sulfated polysaccharides was reported during 1999. Pereira *et al*., (1999) investigated the possible correlation between structure and anticoagulant activity of echinoderm-derived and brown algae-derived fucans, noting that, while the highly branched sulfated fucans from brown algae directly inhibited thrombin, the linear fucans from echinoderms required the presence of antithrombin or heparin cofactor II for inhibition of thrombin, a molecular mechanism also observed with mammalian glycosaminoglycans. These investigators support the use of structural analysis of sulfated polysaccharides from algae and echinoderms and their testing on specific biological assays as an important biochemical approach to investigate molecular mechanisms of anticoagulant activity in mammalian systems.

* Corresponding author: + 91
E-mail address:

Heparin is used to limit the blood clotting during value replacement surgery and thrombus formation in cardio-vascular disorders and surgical procedures. It is also used in myocardial infarction, inflammatory and allergic conditions. However, heparin has a disadvantage as it exhibits side-effects like hemorrhage. Therefore, there is an urgent need of finding alternative anticoagulants. Heparin is being used only as a blood anticoagulant in the clinical operations. It was discovered in 1916 by Mclean Later, *et al* was named as heparin by Howell and Holt, (1918) as it was extracted from dog liver. Heparin is synthesized by the connective tissue type of most cells and is composed of 20-60 disaccharides units are sulfated mostly at c-2 and c-6 of glucosamine and c-2 of the uronic acid moieties. Heparin is used clinically in three major ways: (1) administration of low dosage by subcutaneous injection for the prophylaxis of venous thrombosis (2) administration of standard dosage, intravenously for maintaining the corporeal circulation, such as heart- lung machines, or in real dialysis (Thomas, 1951).Further, heparin and other poly sulphates have been found to be potent and selective inhibitors of HIV -1 replication in cell cultures (Baba *et al*., 1988).

Even though, the investigation of bioactive compounds from these organism, the sea anemones from the Indian waters have been scantily studied especially for their bioactive properties. Hence, the present study was undertaken to investigate the pharmacological potentials (anticoagulant activity of the mucus of the sea anemone) of sea anemone species, *Heteractis magnifica* and *Stichodactlyla haddoni* collected from Gulf of Mannar region, southeast coast of India.

MATERIALS AND METHODS

Sample preparation

Methanol extraction: Sea anemone mucus was extracted following the method as proposed by Bakus *et al.,* (1981). The sea anemone mucus were weighed, 100 g was soaked in 250 ml of methanol for 5 hours. The solvent was then removed and filtered through what man filter paper No.1. The solvent was evaporated at low pressure by using a Buchi Rotavapor at 60 ^{o} C and the extract was stored in refrigerator for further use.

Aqueous extraction**:** The aqueous extract of the samples were prepared by collecting the mucus from the specimens in triple distilled water. The resultant solution was filtered and dialyzed by using Sigma dialysis membrane - 500 (Flat width -24.26 mm, Diameter -14.3 mm and capacity approx. - 1.61 ml/cm) against D-glucose to remove the excess water. The supernatant obtained was lyophilized using the Labcono Freeze Dry System and stored at 4^{o} C in a refrigerator for the further use as mucus aqueous extract.

Plasma preparation**:** Blood samples were collected from healthy volunteers and then anti -coagulated using 3.8% tri-sodium citrate in a polypropylene container (9 Parts of blood to 1 part of tri-sodium citrate solution). It was immediately centrifuged at 4000 x g for 15minutes and the plasma was stored at 4^{0}C until its use.

Blood anticoagulant assay

The activity was tested against the sea anemone mucus samples of two different solvents; the time taken for coagulation was noted. Sea anemone polysaccharide extracts prolonged the time taken for blood clotting, and this was also more pronounced with the assay of activated partial thromboplastine time (APTT), then that of prothrombin time (PT). This activity increased with concentrations of extracts used 100, 500, 1000µg mL^{-1}.

Activated partial thromboplastin time* (*APTT): To a 12 X 75 nm test tube, 0.1mL test plasma and 0.1ml Liquiceilin-E (a phospholipids preparation derived from rabbit brain with ellagic acid as an activator; Tulip Diagnostics Pvt Ltd., India) were added and shaken briefly to mix the reagent and plasma. The tube was placed at 37^{0}C for 20min for incubation. After the incubation, 0.1ml pre-warmed calcium chloride solution was forcibly added into the mixture of plasma and reagent. Simultaneously stop watch started. The test tube was shaken to mix the content sand kept at 37°C for 20 seconds. To this, one ml of aqueous Sea anemone mucus extract was added separately in different concentration (100,500, and 1000µg per ml of 0.9% saline) and kept at 37^{0}C. A stopwatch was stated to record the coagulation time in seconds. The tube was shaken to mix the contents and it was tilted gently back and forth and the stopwatch was stopped as soon as the clot formation began. The steps were repeated for methanol mucus extract (Shanmugam *et al*., 2000, and Muruganandham *et al*., 2002).

Prothrombin time (PT): To a 12x75mm test tube, 0.1 ml of plasma was added and the test tube was placed in water bath for 3-5 mins at 37°C. Then 0.2ml of liquiplastin reagent (Tulip Diagnostics Pvt Ltd, India) pre-warmed at 37°C was added. To this mixture, one ml of aqueous Sea anemone mucus extract was added separately in different concentrations (100, 500 and 1000 µg per ml of 0.9% saline) and kept at 37°C. Further, the similar procedure as mentioned above was followed to record the coagulation time. The same steps were repeated for methanol mucus extract.

RESULTS AND DISCUSSION

Anticoagulation activity of mucus extracted from sea anemones *Heteractis magnifica* and *Stichodactlyla haddoni* at three different concentrations was tested in blood plasma, using two assay system and the results are presented in Table 1.

APTT Assay

The anticoagulation activity of two samples were noted, the plasma was coagulated at 120minutes. The coagulating time of

Table 1 Clotting time (seconds) in sea anemone *(Heteractis magnifica* and *Stichodactlyla haddoni*) mucus extracts assayed by using APTT and PT methods

Name of the species	Solvent used	Clotting time in seconds					
		APTT assay ((µg mL^{-1})			PT assay (µg mL^{-1})		
	Aqueous extract	100µg	500µg	1000µg	100µg	500µg	1000µg
Heteractis		400	400	>400	200	200	200
magnifica	Methanol extract	>400	350	300	150	80	80
Stichodactlyla	Aqueous extract	200	200	250	150	100	150
haddoni	Methanol extract	550	500	>500	120	100	150

sample of *Heteractis magnifica* aqueous extract was noted as 400, 400 and >400 seconds for different concentration of 100, 500 and 1000 respectively and the activity of methanol extract was noted as >400, 350 and 300 seconds. The coagulating time of sample of *Stichodactlyla haddoni* aqueous extract was noted as 200, 200 and 250 seconds for different concentration of 100, 500 and 1000 respectively and the activity of methanol extract was noted as550, 500 and >500 seconds. Control was 80 seconds.

PT Assay

The anticoagulation activity of two samples were noted, the plasma was coagulated at 40seconds.The coagulating time of sample of *Heteractis magnifica* aqueous extract was noted as 200, 200 and 200 seconds for different concentration of 100, 500 and 1000 respectively and the activity of methanol extract was noted as 150, 80 and 80 seconds. The coagulating time of sample of *Stichodactlyla haddoni* aqueous extract was noted as 150, 100 and 150 seconds for different concentration of 100, 500 and 1000 respectively and the activity of methanol extract was noted as 120, 100 and 150 seconds. Control was 40 seconds

In the present study, sea anemone extracts the clotting assay differs according to the concentration and the different

solvent extracts the variation in the anticoagulant activity may be due to the active polysaccharides and sulphate groups attached to them. In APTT assay the activity was a maximum in *Stichodactyla haddoni* this species prolonged clotting time of maximum 700 seconds about 8.7 – fold higher than that of control (80 seconds) of all the concentration tested and the clotting time is higher in the *Stichodactyla haddoni* then *Heteractis magnifica*. But in the case of aqueous extract there is the little bit difference between two species. The species of *Stichodactyla haddoni* in methanol extract shows the anticoagulant activity of 5.0, 4.3 and 3.7 - folds higher respectively in 100, 500 and 1000µgmL^{-1} than the control. In PT assay a few extract showed higher concentration in the species *Stichodactyla haddoni* 3.5, 2.5 and 2.5 folds higher respectively in 100, 500 and 1000µgmL^{-1} than the control. All other extract shows lower anti coagulant activity in PT assay.

Anticoagulant activity of sea anemone extracts (Different solvents) at three different concentrations was tested in blood plasma using two assay systems (Kathiresan *et al*., 2006). The two assays varied in anticoagulant activity in general, time taken for blood clotting was higher in APTT then that in PT assay. For both sea anemones extract treated plasma and untreated control. The similar observation was carried out by Jurd *et al*., 1995 and Kathiresan *et al*., 2006.

The activity is expressed in the clotting time ratio in relation to sulphate groupes attached to them (Shanmugam *et al*., 2000). The anticoagulant activity of the mucus of sea anemone present study coincide with sulphate levels of the extracts *Stichodactyla haddoni* had high sulphate level (3. 94%) compared to Heteractis *magnifica* (3% of dry weight) followed by Terho and Hartila, (1971). The heparin exhibited up to 315-fold higher time. The heparin study in mangrove extracts (Kathiresan *et al*., 2006) proved the 15 folds higher in the species of *Avicenia* species. From the sea anemone mucus extracts time clotting was noted in the ratio 8.7 is the high folds in the species of *Stichodactyla haddoni* in APTT assay.

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