ANTICOAGULANT ACTIVITY OF MARINE GASTROPODS BABYLLONA SPIRATA LIN, 1758 AND PHALIUM GLAUCUM LIN, 1758 COLLECTED FROM CUDDALORE, SOUTHEAST COST OF INDIA

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ABSTRACT

Objectives: Molluscs are highly delicious seafood and they are also very good source for biomedically imported products. Among the molluscs some have pronounced pharmacological activities or other properties which are useful in biomedical area.

Methods: In the present study glycosaminoglycans were isolated from two marine gastropods such as Babyllona spirata and Phalium glaucum.

Results: The isolated glycosaminoglycans were quantified in crude samples and they were estimated as 8.7 gm/kg & 5.3 gm/kg crude in Babyllona spirata and Phalium glaucum respectively. Both the gastropods showed the anticoagulant activity of the crude samples 134 USP units/mg and 78 USP units/mg correspondingly in B. spirata and P. glaucum. In the agarose gel electrophoresis using acetate buffer, the band of the crude samples studied showed the band with similar mobility to standard heparin sulfate.

Conclusion: Among the two gastropods B. spirata showed more anticoagulant activity than that of P. glaucum.

Keywords: B. spirata, P. glaucum Anticoagulant activity, Agarose gel electrophoresis, FTIR

INTRODUCTION

Commonly glycosaminoglycans are classified based on their sulphate position and glycosaminoglycans include heparin, dermatan sulphate, hyaluronan, chondroitin sulphate, keratan sulphate and heparin sulphate. In this group, heparin is a sulphated mucopolysaccharide composed of α-L-iduronic, β-D-glucuronic acid and α-D-galactosamine unit, joined by 1→bonds. The structure of heparin is highly complex and heterogeneous. This compound is involved in a variety of physiological and pathological infections[1].

Anticoagulants have been widely used both clinically and in-vitro medical treatments. In clinical practice, they are drugs of choice for the prevention and treatment of thromboembolic disorders and prophylaxis of thrombotic events both pre and post-surgery. It is estimated that 2-4 patients out of 1,000 receive anticoagulant therapy [23]. In the materials field, anticoagulants are used to improve the hemo-compatibility of medical device and tissue engineering materials [4]. Recently, the concept of "vascular beautifying" has been promoted by the cosmetics industry [5]. Substances with anticoagulant activity are among the first choices as functional components which are being used to open up new areas of application for anticoagulants.

Heparin and heparin like substances appear to be rather ubiquitous natural products which are found in most mammalian tissues including viscera, lung, skin, kidney, liver, muscle, mast cells and basophils [6]. Their strategic location and highly charged nature make them important biological players in cell to cell and cell matrix interactions that take place during normal and pathological events, related to the cell recognition, adhesion, migration and growth [7,8,9,10]. Suggested that GAGs and other important molecules involved in the calcification of carbohydrates in antler of mule deer (Odocoileus hemionus). However heparin and heparin like substances are not only found in higher life forms, but also in lower invertebrates [11][12]lobe[13] [14]. acididans tanduates [15]. A large number of animal species contain GAGs and the marine molluscs are particularly rich of those polysaccharides [16][17]. A compound named mactin had been isolated from Mactrus passula and Cyprina islandica [18]. The chemical analyses and anticoagulant activities of mactin (glycosaminoglycan) were distinguishable from those of heparin. Heparin-like GAGs were isolated from Chiorceus ramous, C. virginus and Hemifusus pugilinus[l9]. The sulphated glycosaminoglycans were also isolated from the bivalves and gastropods [20]. Among several invertebrates, the presence of heparin-like compounds in molluscs were observed by [21-25] studied in-vitro anticoagulant activities of alginate sulphate and its quarterized derivates. Their structure was characterized by elemental analysis such as FTIR, C-NMR and gel permeation chromatography.

[26] Have developed a method for the recovery of heparin and heparin like substances from various marine organisms like flounder, crab, mussel and clams. Since molluscs heparin contains antithrombin-dependant anticoagulant associated with the presence of unique 3-O- sulfated glycosamine residues in all anticoagulant heparin[12]. Many works have been done on anticoagulant potential of different classes of molluscs whereas fewer attempts has made on the class of gastropods. Hence, the present study deals with the isolation of GAGs, anticoagulant activity and characterization of the gastropods, B. spirata and P. glaucum collected from the cuddalore, southeast coast of India.

MATERIALS AND METHOD

Isolation of Glicosaminoglycans

The molluscs B. spirata and P. glaucum were collected from the Cuddalore (Lat. 11°2’4; Long.79°49’49°E) Shells were opened and body tissues were collected. They were blended in 0.4 M sodium sulphate solution (Na2SO4; 3.5 l/kg of the tissue) and kept at 55°C for 1 h 30 min. the pH was adjusted to 11.5 by adding 10% sodium hydroxide (NaOH) solution. Aluminum sulfate (Al2(SO4)3) crystals (80 mg/kg tissues) were added to this solution, and the suspension was heated to 95°C for 1 h. 247 pyridin chloride (PDC) solution (3 g/100 ml of 0.8 M NaCl) was used to precipitate the crude white heparin complex. The precipitate was redissolved in 150 ml of sodium chloride solution (2.0 M) and was incubated at 30°C for 30 min. the precipitate was washed, with ethanol and methanol through centrifugation, and vacuum dried.

Anticoagulant activity

The anticoagulant activities of crude GAG samples were determined by comparing with the concentration necessary to prevent the clotting of sheep plasma using USP (United State Pharmacopeia) method.

Agarose gel electrophoresis

Electrophoresis was carried out in agarose gel plates. The gel plate was prepared with 0.6% agarose (2 to 4 mm thick) containing acetate buffer at pH 3.6 by the following method of [27].
FTIR - (Fourier Transform- Infra Red spectrum analysis)

The lyophilized GAGs samples of B. spirata and P. glaucum (10mg) was mixed with 100mg of dried potassium bromide (Kbr) and compressed to prepare as a salt disc. The disc was then read spectro photometerically (Bio-Rad FTIR-40 model, USA). The frequencies of different components present in each sample were analyzed.

RESULTS

The amount of the crude GAGs sample of B. spirata and P. glaucum was recorded as 8.7 & 5.3 gm/kg respectively. The anticoagulant activity of the B. spirata and P. glaucum crude samples was reported to be 134 USP units/mg and 78 USP units/mg (Table 1).

Agarose gel electrophoresis

Agarose gel electrophoresis of extracted crude glycosaminoglycans from B. spirata and P. glaucum are presented in Fig.1. As it can be observed that the agarose gel electrophoresis using acetate buffer, the band of the heparin like glycosaminoglycans in the crude sample studied showed the band similar mobility to heparin sulfate. The result of agarose gel electrophoresis showed the migration of crude sample as that of standard heparin sulfate. From this it could be inferred that the crude GAGs sample contains complex mixture of anticoagulant compounds.

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Animal</th>
<th>Yield crude GAGs gm/kg</th>
<th>Anticoagulant activity USP units/mg crude sample</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Babylonia spirata</td>
<td>8.7</td>
<td>134</td>
</tr>
<tr>
<td>2</td>
<td>Phalium glaucum</td>
<td>5.3</td>
<td>78</td>
</tr>
</tbody>
</table>

Table 1: Yield and anticoagulant activity of crude samples in B. spirata and P. glaucum

FTIR spectral analysis

Fourier Transform Infra Red spectrum of the lyophilized sample of GAGs showed four peaks at 1411.89, 1292.31, 1213.23, 1157.29, 1041.56, 960.55, 840.96 and 758.02 cm\(^{-1}\) whereas the spectrum of the crude sample of B. spirata showed four peaks at 1215.15, 933.55, 767.67 and 678.94 cm\(^{-1}\). P. glaucum showed the two peaks at 1215.15 and 763.81cm\(^{-1}\).

Fig. 1: A agarose gel Image of GAGs from two gastropods

Fig. 2: FTIR spectrum of Standard heparin sulfate

Fig. 3: FTIR spectrum of crude GAGs of B. spirata
DISCUSSION

Heparin and heparin like compounds, which are present in some invertebrate molluscs, showed high anticoagulant activity and share most of the structural properties with mammalian heparins. In the present study, homogenization of body tissue B. spirata and P. glaucum in acetone and subsequent extraction in acetone/petroleum ether resulted in 50gm of defatted tissue. The heparin obtained from defatted tissue described in the present study has all the features as that of heparin. Similarly, heparin has been prepared from a number of different sources; human [28], clams [21], giant African snail [12] and seaweeds [29]. The use of GC for quantitative separation of sulphated polysaccharides in tissue extracts is preferred. In the present investigation, the yield of crude heparin-like glycosaminoglycans was found as 8.7 & 5.3 gm/kg in B. spirata and P. glaucum. Previously [30] have recorded the yield of heparin and other sulfated mucopolysaccharides from thymus as 274 µg/kg. Similarly, [31] isolated the sulfated mucopolysaccharides by using quaternary ammonium salts and reported the yield as 170, 1740, 8430, 307.0 and 1090.0 µg/kg dry tissues in different molluscan species such as Alolocumbia ater, Perna perna, Mesodesma donacum, Loligo brasilienne and Octopus sp. respectively. [21] isolated heparin with a yield of 2.6g/kg and 3.8g/kg from Anomalocardia brasiliuna and Tivelu macrotroides respectively. [32] obtained that the 7.02gm/kg of heparin like substances in marine molluscs Katelysia opima. [33] reported that the yield of crude heparin from Conus amabdis and Babylonia zeylonica as 2.6 and 8.16 gm/kg respectively. However, the cephalopods such as Sepia aculeata and S. bremamana and Loligo duvaucelli and Doryteuthis cuneatus showed higher net yield of the heparin like sulfated polysaccharides 21.7 gm/kg, 24.0 gm/kg and 16.5 gm/kg, 8.4 gm/kg respectively [34] [35]. [22] had quantified the heparin yield as 2.27g/kg and 2.2g/kg from Tridacna maxima and Perna viridis respectively. [36] reported the total yield of the crude heparin was estimated as 5.4g/kg in Katelysia opima and 4.1g/kg in Donax cuneatus from the defatted tissue. In B. spirata and P. glaucum, generally the glycosaminoglycans content was found to be low as it is high when compared to other molluscs. Among the two gastropods B. spirata showed more anticoagulant compounds than that of P. glaucum.

The heparin isolated from marine clams and mussels has identical structural features and anticoagulant activity of mammalian polysaccharide [37]. Heparin with high anticoagulant activity was isolated from the marine molluscs, Anomalocardia brasiliuna Donax striatus and Tivelu macrotroides [21]. Which showed similar activity like mammalian heparin but differs in molecular weight, the molluscan heparin have a higher molecular weight and high anticoagulant activity [21]. In the present study, the anticoagulant activity of the crude sample of GAG from the whole body tissue of B. spirata and P. glaucum was reported 134 USP units/mg and 78 USP units/mg. [38] showed the activity ranging from 130-150 USP units/mg for extracted products of Spisula solidissima and Cyprina islandica. [21] showed the anticoagulant activity of heparin from two species of molluscs, D. striatus and T. mactriodes as 180 units/mg and 220 units/mg respectively. [34] reported the anticoagulant activity of the crude sample of H. pupillus in crude units/mg. [35] reported the anticoagulant activity of the crude sample of L. duvaucelli was 376.98 USP units/mg and D. sibogae as 376.98 USP units/mg. [36] Vijayabaskar (2008) reported the extraction of GAGs from K. opima and D. cuneatus showed anticoagulant activity of 160USP units/mg and 154USP units/mg respectively. [40] reported the anticoagulant activity of the crude sample of Meretrix custa (sheep blood 22.52 USP units /mg) (chicken blood 20.00 USP units /mg) and (human blood 18.60 USP units /mg). In the present investigated two gastropods B. spirata and P. glaucum tissue is value medicinal due to high quality of anticoagulant compounds. From the above it could be understood that body tissue of B. spirata is a very good potential source of anticoagulant compounds than that of P. glaucum.

Electrophoresis is a selective tool for the identification of different sulfated mucopolysaccharides from different sources. Agarose gel electrophoresis is the only technique to conform the presence of GAGs. The staining solution (toluidine blue) was found to bind only with the sulfated polysaccharide and not bind with other compounds [41]. The electrophoratic migration of the sulfated polysaccharide using acetate buffer (pH 3.6) system depends on the structure of the polysaccharide. In this study, the molecular weight of crude sample of GAGs from gastropods, B. spirata (lane 2) and P. glaucum (lane 3) showed one band which is said to be heparin sulfate. The previous study made by [31] in the invertebrates also reported the same migratory pattern of bands for the sulfated polysaccharides particularly in Octopus sp. which showed polysaccharide (sheep blood 22.52 USP units /mg) (chicken blood 20.00 USP units /mg) and (human blood 18.60 USP units /mg). In the present investigated two gastropods B. spirata and P. glaucum tissue is value medicinal due to high quality of anticoagulant compounds. From the above it could be understood that body tissue of B. spirata is a very good potential source of anticoagulant compounds than that of P. glaucum.

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from the skin of Chimaera sp. Assignment of IR absorption bands and 1240 cm\(^{-1}\)and 1430 cm\(^{-1}\)in the spectrum of fully o-sulphonated HA were based on the reports by [45]. The peak pattern between the standard heparin and the sample was at 1213.2.3 cm\(^{-1}\)in crude sample and 1215.15 cm\(^{-1}\)as standard heparin indicating the presence of GAGs group in the samples analyzed. The FT-IR spectral analysis of the anticoagulant GAGs from marine molluscs B. spirata and P. glaucum showed more or less same peaks lying within the same range of values of the standard heparin. This proves that the extracted anticoagulant GAGs in the body tissue from B. spirata and P. glaucum is also an anticoagulant resembling the heparin and heparan-sulfate substances. The results in this research showed that gastropod, B. spirata tissue having GAGs with high quantity of anticoagulant compounds.

The anticoagulant effect of heparin is closely associated with their molecular weight [46]. Commercially available low molecular weight heparins are less potent than UFH in term of anticoagulation [47]. Crude products isolated from marine organisms have served as a source of drugs and starting materials for synthesis of useful drugs. In addition, because of the differences in the environmental conditions, new biochemical entity having biological activity can be evolved by marine organisms. So, it is believed that the studies of new and unique compounds derived from marine organisms will continue to increase our basic knowledge with respect to pharmacology and medicine.

Thus the result of the present investigation provides information about the isolation, anticoagulant activity and characterization of the crude GAGs from a non-conventional source, the gastropods, B. spirata and P. glaucum. Further the good anticoagulant activity by the anticoagulant compound from this species proves the possibility of its utilization as an additional potent source for the extraction of such anticoagulant compound. Besides the above, the result of the present study is providing baseline data for the future researchers in this line of work and is also throwing more light on the development of marine gastropods, body tissue by the pharmaceutical technologists for the extraction of useful drugs or the active principles or functional units for the synthesis of drugs in future.

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