

EXTRACTION OF HEPARIN LIKE SUBSTANCES FROM THE MURICID GASTROPODS *CHICOREUS RAMOSUS* AND *CHICOREUS VIRGINEUS*

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ABSTRACT

Muscles were screened to isolate heparin-like polysaccharides. The yield of crude heparin in *Chicoreus ramosus* was 10.6 g kg tissue and 10 g kg in *C. virgineus*. Total yield of heparin was 12,369 units/kg tissue in *C. ramosus* and 11,970 units kg in *C. virgineus*.

INTRODUCTION

The sulfated polysaccharide heparin is composed of D-glucouronic acid and D-glucosamine residues and performs several biological functions. It has been widely used as a medicine for more than 40 years, because of its anticoagulant, antithrombotic and antilipaemic activities. It is also used in the treatment of various thrombosis to prevent local extension of venous thrombosis and subsequent pulmonary embolism without causing bleeding (Kakkar 1982). Its efficacy in the treatment of AIDS has been reported by Baba (1988). Heparin binds itself to a consensus peptide in the HIV virus blocking its replication *in vitro*.

Much work has been done on heparin-like mucopolysaccharides from vertebrates. Patel *et al.* (1980) extracted mucopolysaccharides from the skate, *Raja eglanteria*. Commercial heparin is obtained from bovine lung and porcine intestinal mucosa. Though reports on the mucopolysaccharides derived from invertebrates are meagre, there is evidence of heparin and heparin-like substances in their tissues. Tissues of the surf clam, *Spisula solidissima* contain a large amount of heparin-like anti-coagulant material of a sulfated polysaccharide nature (Thomas 1954). Heparin-like compounds were shown in six molluscs species (Cassaro & Dietrich 1977). The presence of heparin in some estuarine bivalves have been reported by Somasundaram *et al.* (1989). Compared with mammalian heparin, molluscan heparin has a higher molecular weight and lower toxicity (Frommhagen *et al.* 1953) Molluscan heparin can be used as an alternative to mammalian heparin. The purpose of the present study was to quantify possible heparin-like mucopolysaccharides

from muscles of the muricid gastropods *Chicoreus ramosus* and *C. virgineus*.

MATERIALS AND METHODS

The gastropods were collected from the Cuddalore coastal waters, south-east coast of India (Lat. 11°42'N; Long. 79°46'E).

The heparin-like substance was extracted according to Holick *et al.* (1985). The soft body was removed from the shell. The gonad, digestive gland, and gut were discarded. The remaining muscles were blended, incubated with 0.4 M Na₂SO₄ (pH 11.5) at 55°C for 90 min, Al₂(SO₄)₃ added, heated for 30 min at 95 °C, cooled to room temperature, suction filtered, cetylpyridinium chloride added until a white precipitate formed at 40 °C, incubated for 24 hrs at 37 °C, centrifuged for 90 min in a refrigerated centrifuge at 3000 rpm, and the heparin complex collected. The complex was redissolved in 2 M NaCl solution at 40 °C to dissociate the pyridinium salt from heparin complex. Two volumes of 95 % ethylalcohol was added to precipitate the crude heparin which was collected by centrifuging and air dried in the oven at room temperature.

Heparin and heparin-like substances were quantified colorimetrically. Azure A (1 g/l distilled water) was stored at 4 °C for two weeks. One ml stock solution was made up to 50 ml with double distilled water. The absorbance of serial dilutions of standard heparin and Azure A was read at 505 nm (Hitachi Double Beam Spectrophotometer). Heparin activity of the unknown sample was determined from the standard curve.

RESULTS AND DISCUSSION

The total yield from *C. ramosus* was 12,369 units/kg, and 11,970 units/kg in *C. virginicus*. The viscera of marine flounder, cod, red crab, mussel and scallop were 10,789; 8,560; 3,418; 1,420, and 9,259 units/kg respectively. Barlow *et al.* (1964) reported the highest values in beef mucosa (21,000 units/kg) and the lowest

in human lung tissue (1,800 units/kg). So the yield of heparin-like substances from the present gastropods are within the expected range. But, the heparin activity depends upon impurities present in the isolated product. The heparin activity can be further enhanced by using High Performance Liquid Chromatography (HPLC).

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