Heparin from seaweeds

Heparin is a life-saving drug. It is used in cardio-vascular surgery. The patients who have undergone valve replacement surgery depend on these drugs for their whole lives. About 5000 patients undergo valve replacement surgery annually in India. Unfortunately, the drug is not available in the market. The reason attributed is non-availability of raw materials. The raw materials are beef lung and porcine intestine. There is a need to investigate other raw materials for heparin. Preliminary investigations were made on flounder, cod, tautog, scup, red crab, mussel, scallop, sea clam[1], back water clam, blood clam and edible oyster[2].

Heparin is a polysaccharide. The seaweeds are rich in polysaccharides. The Indian coastal waters supply a large quantity of seaweeds. The objective of the investigation was to screen common seaweeds for heparin activity and recommend suitable species for extraction of heparin from them.

Sixteen species of seaweeds, *Chaetomorpha media*, *Chaetomorpha linum*, *Ulva lactuca*, *Sargassum myriocystum*, *Gelidiella acerosa*, *Hypnea valentiae*, *Hypnea musciformis*, *Gracilaria edulis*, *Gracilaria arcuata*, *Gracilaria foliifera*, *Gracilaria corticata*, *Champia parvula*, *Laurencia papillosa*, *Acanthophora spicifera*, *Roschera glomerulata* and *Grateloupia filicina* were screened for heparin activity. Heparin was extracted from dry seaweeds by using cetylpyridinium chloride. The cetylpyridinium–heparin complex was dissolved in 2 M NaCl and heparin recovered as sodium salt by ethanol precipitation. Potential species of seaweeds were identified by using a HPLC system. The system consists of a pump (Model 2248 Pharmacia LKB Biotechnology), an injector (Rheodyne model 7125), a column (Ultropac TSK G3000 SW; 600 × 7.5 mm ID, particle size 10 µm LKB No. 2135-360), RI detector (Erma, Japan) and a two-channel recorder (Pharmacia LKB-REC 2). The eluent was glass distilled water at a rate of 1.0 ml/min. Standard heparin (Sigma) solution and the sample solutions were injected at concentration of 1 and 25 mg/ml respectively. The injection volume was 200 µl each. Heparin activity was determined from standard heparin curve prepared by Azure A assay[3].

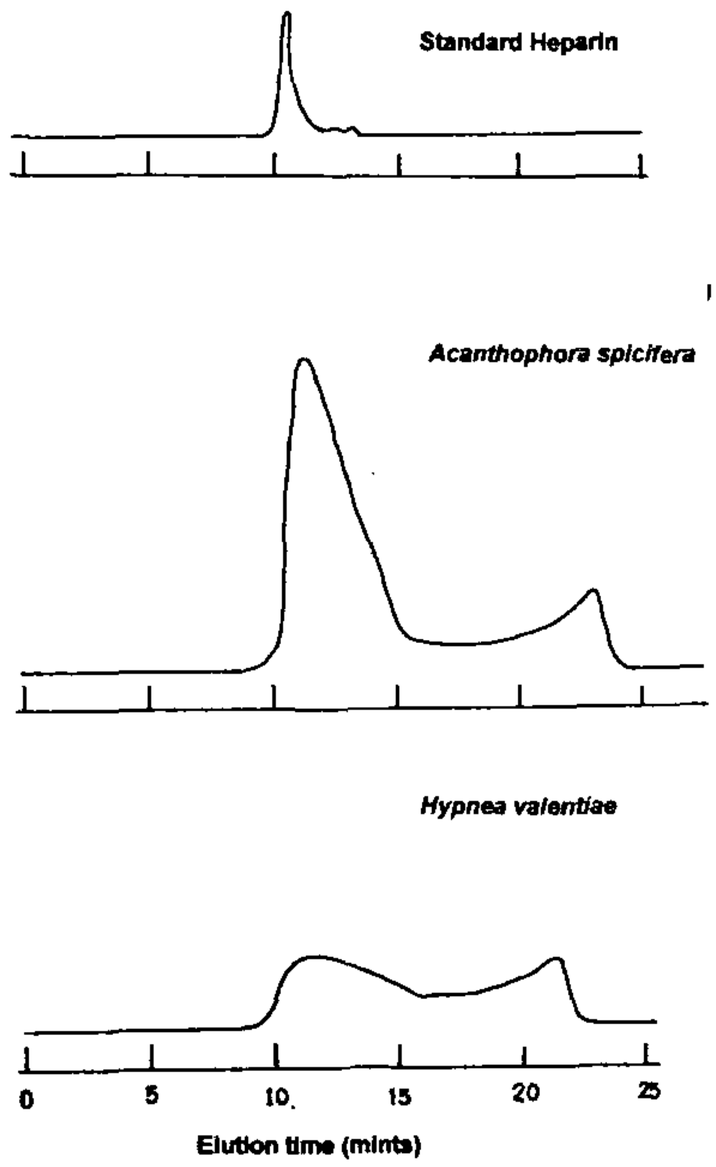

Figure 1. Elution profiles in HPLC of Sigma heparin and extracts of seaweeds, *A. spicifera* and *H. valentiae*.

The rhodophytes, *A. spicifera* and *H. valentiae* showed heparin activity. A second peak of unknown compound was also seen in the extracts (Figure 1). Heparin activity was 28.5 USP units per mg in *A. spicifera* and 11.8 units in *H. valentiae*.

The yield per kg dry seaweed was 4.34×10^5 units in *A. spicifera* and 4.65×10^5 units in *H. valentiae*. Heparin activity ranged from 1.4×10^3 to 2.8×10^5 units per kg tissue in many vertebrates and invertebrates[1,2]. *A. spicifera* and *H. valentiae* showed 10–20 times higher activity than most animal tissues. They are suitable for extraction of heparin.

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A note on the ostracode fauna from the Jurassic beds of Habo Hill, Kachchh

Considerable work has been done on the stratigraphy and palaeontology (dealing chiefly with bivalves, brachiopods, cephalopods, corals, echinoids and foraminifers) of the Jurassic beds of Kachchh for the last one and half century since they were first systematically described[1]. It is, however, surprising to note that ostracodes, which though occur quite commonly in these beds, have not received much attention from the micropalaeontologists. As far as we are aware, only a single work[2] on ostracodes from surface exposures of the Jurassic beds of Kachchh described eight species (all new) from

two localities – Khawda and Lodai. They are: *Cytherella disjuncta*, *C. obscura*, *Cytherelloidea difficila*, *Paracypris contermia*, *Progonocythere grumosa*, *P. implicata*, *P. laeviscula* and *P. prolata*. Besides, there are two papers[3,4] on the ostracodes from the Upper Jurassic–Lower Cretaceous subsurface succession of well 2, Banni, Rann of Kachchh.

With the intention to fill up the above lacuna in our knowledge, we took up the study of ostracodes from the Jurassic beds of Habo Hill, situated in the eastern part of east-westerly trending hill range along southern margin of the Banni, Rann of Kachchh (Figure 1). Five stratigraphic sections from different localities were sampled. They are as follows:

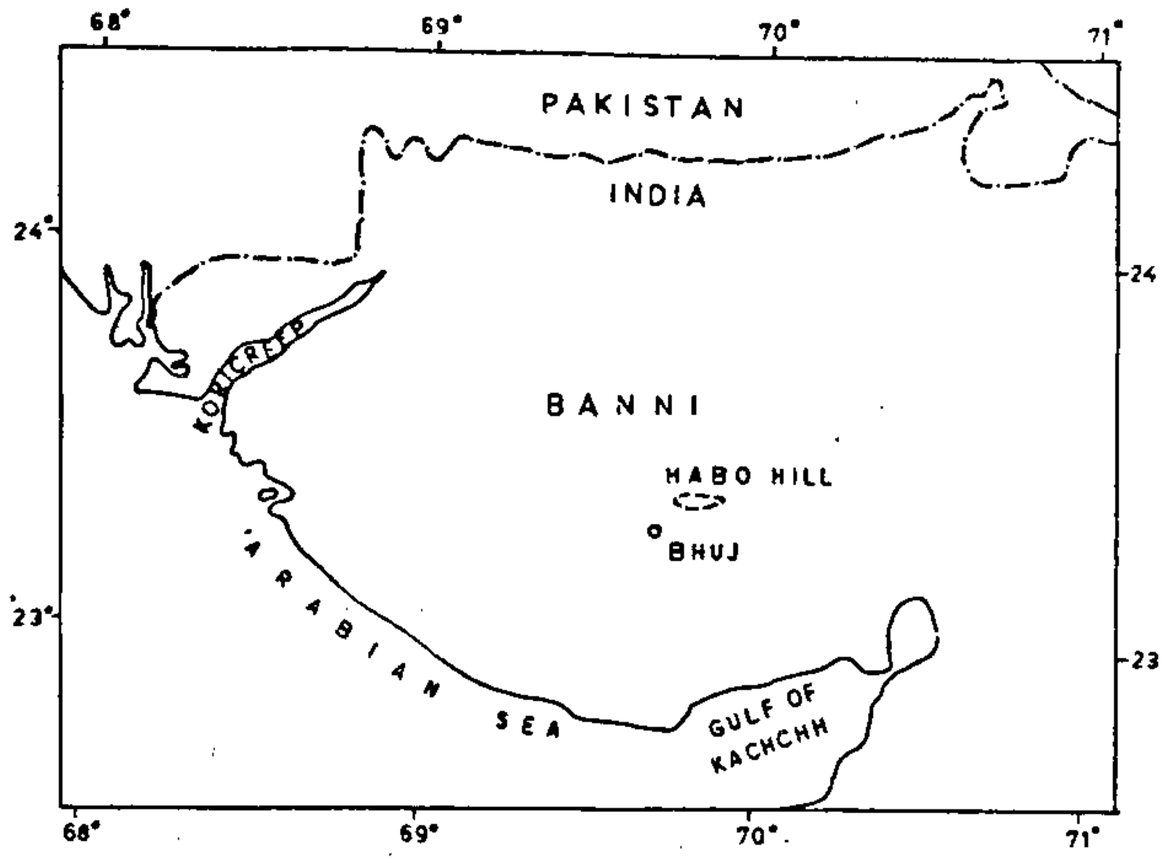

Figure 1. Location of the Habo Hill.

Table 1. Stratigraphic succession of the Jurassic beds of Habo Hill, Kachchh (Modified after Kanjilal, 1978)

AGE	FORMATIONS	MEMBERS	Bed/Lithological units	Proposed ostracode zones
Kimmeridgian	KATROL		Dark brown gypsiferous shales and sandstones	
Callovian–Oxfordian		LODAI	1. Alternate sequence of greenish-brown limestones and shales, top band being oolitic	III *Pirileberis remota*
Callovian	CHARI (= HABO)	RUDRAMATA	2. Greyish-yellow gypsiferous shale 3. Greyish-yellow conglomerate 4. Greyish-yellow gypsiferous shale	
		JHIKADI	5. Highly ferruginous, hard sandstone 6. Yellow-brown gypsiferous shale 7. Reddish-brown hard sandstone with thin bands of shales 8. Reddish-brown friable sandstone 9. Black coral limestone 10. Highly ferruginous, hard sandstone 11. Reddish-brown conglomeratic sandstone	II Poorly fossiliferous
Late Bathonian–Callovian		DHRANG	12. Greyish-yellow gypsiferous shales 13. Alternate sequence of greyish-yellow limestones and gypsiferous shales 14. Yellow gypsiferous shale 15. Greyish-yellow limestone with bands of shales	I *Progonocythere laeviscula*
		BLACK LIMESTONE	16. Black limestone with bands of grey shales	

Table 2. Distribution of ostracodes in the Jurassic beds of Habo Hill

Species \ Proposed ostracode zones / Bed numbers	Chari Formation: I, *Progonocythere laeviscula* 16	15	14	13	12	II, Poorly fossiliferous 11	10	9	8	7	6	5	III, *Pirileberis remota* 4	3	2	1	Katrol Formation
Batella depressa Khosla, Jakhar & Mohammed, n. sp., morphotype B	X																
Cytherelloidea paradifficila Khosla, Jakhar & Mohammed, n. sp.	X																
Mandelstamia sp.	X																
Cytherelloidea sp. cf. *C. atlantolevantiana* Rosenfeld & Honigstein	X	X															
Cytherelloidea sp.	X	X															
Procytheridea kachchhensis Khosla, Jakhar & Mohammed, n. sp.	X	X															
P. ihopyensis Grekoff	X	X	X														
Trichordis (Paratrichordis) parvicarinata Khosla & Jakhar	X		X														
Cytherelloidea ipis Grekoff	X	X		X													
Cytheropterina devai Khosla, Jakhar & Mohammed, n. sp.	X	X	X	X													
Mesocytheridea? mathuri Khosla, Jakhar & Mohammed, n. sp.	X	X	X	X													
Batella befotakaensis (Grekoff)	X	X	X	X	X												
B. clavata Khosla, Jakhar & Mohammed, n. sp.	X	X			X												
Habocythere malgachica (Grekoff)	X	X	X	X	X												
Mandelstamia depecheae Khosla, Jakhar & Mohammed, n. sp.	X	X	X	X	X												
Progonocythere laeviscula Lyubimova & Mohan	X	X	X	X	X												
Trichordis (Paratrichordis) devexa (Grekoff)	X	X	X	X	X												
T. (P.) grumosa (Lyubimova & Mohan)	X	X	X	X	X												
Trichordis (Trichordis) gujaratensis Khosla, Jakhar & Mohammed, n. sp.	X	X	X	X	X												
Amicytheridea triangulata Bate	X	X	X	X									X		X		
Cytherella disjuncta Lyubimova & Mohan	X	X	X	X						X	X		X		X	X	
C. obscura Lyubimova & Mohan	X	X	X	X	X					X	X		X		X	X	
Cytherelloidea bhujensis Khosla, Jakhar & Mohammed, n. sp.	X	X	X	X									X		X	X	
Habocythere diluta Khosla & Jakhar, n. sp.	X	X	X	X	X					X	X		X			X	
Nophrecythere jaisalmerensis (Kulshreshtha, Singh & Tewari)	X	X	X	X	X												X
Amicytheridea sp.		X															
Cytherelloidea dhrangensis Khosla & Jakhar, n. sp.		X	X														
Nophrecythere denticulata (Kulshreshtha, Singh & Tewari)		X	X	X													
Batella depressa Khosla, Jakhar & Mohammed, n. sp., morphotype A		X	X	X	X												
B. falcula (Grekoff)		X		X	X												
Trichordis (Trichordis) praetexta Grekoff		X	X	X	X												
Paracypris contermia (Lyubimova & Mohan)		X	X										X		X		
Acrocythere sp.			X														
Cytheropterina pandeyi Khosla, Jakhar & Mohammed, n. sp.			X														
Cytherella sp.			X	X													
Nophrecythere whatleyi Khosla & Jakhar, n. sp.			X	X	X												
Progonocythere haboensis Khosla & Jakhar, n. sp.			X	X						X							
Cytheropterina sajjaniae Khosla, Jakhar & Mohammed, n. sp.			X		X										X		
Majungaella perforata kachchhensis Khosla, Jakhar & Mohammed, n. subsp.				X	X					X	X		X		X	X	
Cytherella kalajarensis Khosla & Jakhar, n. sp.													X		X		
Habocythere bicruciata (Grekoff)													X		X		
H. ventrisulcata Khosla, Jakhar & Mohammed, n. sp.													X		X		
Habocythere sp.													X		X		
Cytherella masuguluensis Bate													X		X	X	
Pirileberis remota (Grekoff)													X		X	X	
Majungaella rasilis Khosla, Jakhar & Mohammed, n. sp.															X		
Cytherelloidea langijarensis Khosla, Jakhar & Mohammed, n. sp.															X	X	
Schuleridea (Eoschuleridea) soodani Khosla, Jakhar & Mohammed, n. sp.															X		
Majungaella perforata Grekoff																	X
Progonocythere sp.																	X

Section I, exposed along banks of the Kala jar (stream) near Dhrang village (23°23′30″N : 69°50′30″E). *Section II*, exposed along banks of the Langi jar, 2 km east of Dhrang village. *Section III*, exposed along banks of a stream near Habo village (23°21′10″N : 69°51′19″E) and partly along tributary of Kala jar north of the crest of Habo Hill. *Section IV*, exposed along banks of a stream near Kotai village (23°23′24″N : 69°47′12″E). *Section V*, exposed along banks of a stream 1 km east of Fulae village (23°23′29″N : 69°48′43″E).

All the sections yielded rich and excellently well preserved ostracode fauna comprising 49 species (47 from the Chari Formation and three from the basal part of Katrol Formation, one being common to the both). The taxonomy of these ostracodes is being given elsewhere[5]. The present note gives stratigraphic distribution of these ostracodes.

The Jurassic beds of Habo Hill display *quaquaversal* dips, steeper on the north but gentle on the south, east and west. The older beds lie towards the centre, while they are surrounded on all sides by younger ones. The geology of the hill has been previously discussed in detail[6] by Kanjilal and the succession worked out by him is given here in modified form in Table 1. The name Chari Formation has been preferred over the Habo Formation in view of its long usage and familiarity with most of the workers. Further, contrary to general practice, Kanjilal gave number 1 to the youngest beds of this formation and assigned higher numbers to the succeeding older beds. The same practice has been followed in the present work.

The distribution of ostracodes in the Jurassic beds of Habo Hill is given in Table 2. On the basis of these ostracodes it is possible to group beds 1–16 of the Chari Formation into three assemblage zones. These are, in ascending order, as follows: Zone I, *Progonocythere laeviscula* Zone (beds 16–12); Zone II, Poorly fossiliferous Zone (beds 11–5), and Zone III, *Pirileberis remota* Zone (beds 4–1). Late Bathonian–Callovian age is assigned for the beds of Zone I and Callovian age for beds of Zones II and III of the Chari Formation.

The ostracode fauna of the Jurassic beds of Kachchh shows strongest affinity, besides Rajasthan, with those of the Jurassic ostracodes of Majunga Basin, Madagascar[7]. There are as many as 13 species – *Amicytheridea triangulata* Bate, *Cytherelloidea ipis* Grekoff, *Habocythere bicruciata* (Grekoff), *H. malgachica*, *Batella befotakaensis* (Grekoff), *B. falcula* (Grekoff), *Majungaella perforata* Grekoff, *Neurocythere whatleyi* Khosla, Jakhar and Mohammed, *Pirileberis remota* (Grekoff), *Procytheridea ihopyensis* Grekoff, *Progonocythere laeviscula* Lyubimova and Mohan, *Trichordis (Paratrichordis) devexa* (Grekoff) and *Trichordis (Trichordis) praetexta* Grekoff – common to both these areas. Jurassic ostracodes of Kachchh also show a lesser affinity with the ostracodes of Tanzania, Central Saudi Arabia and Israel. Very likely India and Madagascar were close to each other, if not continguous, and connected by an epicontinental sea.

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