SYNOPSIS

“Bioactive Carbohydrates from Marine Source”

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Today research has been focussed on finding pharmaceutical agents with selective pharmaceutical effects and less toxicity. As a consequence of increase in demand, serious research is on its way for seeking therapeutic agents from natural sources. This process has facilitated to produce remarkably a diverse array of natural products containing medicinally useful terpenoid derivatives, alkaloids, glycosides, polyphenolics, steroids and so on. Numerous types of bioactive compounds have been isolated from plant sources, several of them are currently in clinical trials.

Marine organisms are known to have a rich source of structurally diverse bioactive compounds with valuable pharmaceutical potentials. Marine floras include microflora (bacteria, actinobacteria, cyanobacteria and fungi), microalgae, macroalgae (seaweeds), and flowering plants (mangroves and other halophytes). Occupying almost 71% of globe, the ocean is rich in biodiversity and the microflora and microalgae alone constitute more than 90% of oceanic biomass (Kathiresan & Duraisamy, 2005). This vast marine floral resource will offer a great scope for discovery of new drugs. It is increasingly recognized that ocean contains a huge number of natural products and novel chemical entities with unique biological activities that may be useful in finding potential drugs with greater efficacy and specificity for the treatment of human diseases (Haefner, 2003).

Among marine organisms, marine algae are rich sources of structurally diverse bioactive compounds with various biological activities. Recently, their importance as a source of novel bioactive substances is growing rapidly and researchers had revealed that marine algal originated compounds exhibit various biological activities. Seaweed is the term that refers to all types of algae found in ocean. It comes in red, green and brown algae. Seaweeds are important sources of proteins, vitamins and minerals and hence their metabolites are shown promising activity agaist cancer incidence. Seaweed also contains high amount of polyphenol such as catechin, epicatechin, epigallocatechin, gallic acid and gallate (Yoshie *et al.,* 2002).The alcohol extract of red algae exhibit tumorocidal activity on Ehrlich's

Ascites Carcinoma. Algae have recently gained special interest owing to their diverse biological property. There are many reports on immune and anticancerous activity of algae.

Over the last few year medical and pharmacological industry have shown an increase interest in seaweed derived polysaccharides. Due to the wide variations in their molecular weights, structural parameters and physiological characteristics, seaweed polysaccharides show diverse bioactivities, such as antiviral , (Ponce *et al.*, 2003) antiproliferative or antitumor, anticoagulant, antioxidant, anti-inflammatory and anti-complementary effects (Riou *et al*., 1996).

Various anticoagulant-active polysaccharides, especially from marine red and brown algae, have been isolated and characterized. They contain a variety of sulphated galactans and sulphated fucans, and exhibit high anticoagulant activity. However, there are fewer reports of anticoagulant-active polysaccharides from marine green algae than those from brown and red algae. Polysaccharides from marine green algae show potent anticoagulant activity, and represent potential source to be explored (Jurd *et al.*, 1995).

Many anticarcinogens were immunosuppressive agents. They repress tumour growth; meanwhile, they are adverse to immune system of organism. It has become an important aim of research in immunopharmacology and oncotherapy to discover and identify new antitumor drugs which can potentialize the immune function. Sulphated polysaccharides have shown to have effective anti-tumour activities by attacking the cancer cell directly (Sheng *et al*., 2007) or enhancing the host's immune function (Zhou *et al.*, 2004). So keeping in view of above facts, the work has been taken up to study the composition of polysaccharides isolated from green algae (Ulva) after screening a number of algae for polysaccharides. *Ulva* is a green alga which is edible and the biomass availability is more when compared to other algae of Kerala coast and has been selected for the study.

In the present study, a sulphated polysaccharide was isolated from marine algae of Kerala coast. It was purified by gel filtration chromatography. From the three fractions separated major fraction having high carbohydrate content analysed by phenol sulphuric acid test was selected for further study. The chemical composition of the polysaccharide was analysed and the molecular weight was determined by Static light scattering method and we could see that the polysaccharide has a molecular weight of 1.3×10^{6}Da which has not been reported so far. As this particular polysaccharide with the above molecular weight and specific composition has not been reported and studied for its pharmacological activity so

far, the sulphated polysaccharide was characterised by Fourier Transform Infrared (FTIR) Spectroscopy, Matrix Assisted Laser Desorption/Ionization-Time Of Flight (MALDI-TOF) spectroscopy, Scanning Electron Microscope/Energy Dispersive X-ray Spectroscopy (SEM-EDX),Nuclear Magnetic resonance spectrum (^{1}H NMR), Correlation Spectroscopy (COSY) and ^{13}C NMR. It was found that the polysaccharide contained uronic acid, rhamnose, xylose, glucose, mannose and galactose as monosaccharide components.

As the above polysaccharide is a sulphated polysaccharide having high anionic groups the anticoagulant activity of the polysaccharide was studied *in vitro*, *ex vivo* and *in vivo*. The polysaccharide could prevent clotting even at a very low concentration as comparable with heparin. Activated Partial Thromboplastin Time (APTT) and Prothrombin Time (PT) studies invitro clearly established the anticoagulant activity. *Ex vivo* anticoagulant activity study in rat model by APTT assay also indicated the anticoagulant activity of the polysaccharides. The in vivo antithrombotic effect was studied in stasis induced venous thrombosis model in rat also established the antithrombotic activity of polysaccharide. To ensure the feasibility of the above observations the histopathological evaluation of the ligated inferior vena cava was conducted and confirmed the antithrombotic effect of the polysaccharide. Detailed study of the antithrombotic effect of polysaccharide for a longer period was done to rule out the possibility of inducing haemophilia that could be seen with Heparin, Aspirin, Disprins etc. It was interesting to note that the polysaccharide do not induce haemophilia when compared to heparin. So it would be advisable to recommend for the treatment of thrombotic disorder.

In order to check whether the polysaccharide has any toxicity in the living organism the following tests were conducted. (a) 24 hour mortality study was conducted in mice model (b) Sub acute toxicity study was also conducted by evaluating the hepatotoxic marker enzymes, haematological studies, urea and creatine level in mice model. The results clearly indicated that the polysaccharide is non toxic.

Cancer causes an increased risk of venous thromboembolism and the frequency of venous thromboembolism in patients undergoing cancer surgery is roughly twice that seen in patients without malignancies who have comparable operations. As reported earlier, the polysaccharide is having more anionic group and venous thrombosis is the major complication found in patients treated with anticancer drugs, the isolated polysaccharide was tested for its anticancer activity by estimating the superoxide radical scavenging activity, hydroxyl radical scavenging activity, inhibition of lipid peroxidation and nitric oxide

scavenging activity in rat models by following the standard procedures. From the results it could be concluded that the isolated polysaccharide has a very high antioxidant effect.

Anti-inflammatory activity of the polysaccharide was studied in male Swiss albino mice model. Anti-inflammatory activity was determined by carrageenan induced acute and formalin induced chronic mouse Paw edima. There was decrease in the Paw edima in the polysaccharide treated groups. The results showed a significant anti-inflammatory activity for polysaccharide.

The anticancer effect of the polysaccharide was as studied. Two mice cell lines kindly provided from Amala cancer research institute were used for the study. (a) *In vitro* cytotoxicity screening with the isolated polysaccharide for Dalton's Lymphoma Ascites (DLA) and Ehrlich's Ascites Carcinoma (EAC) cell lines were done using trypan blue exclusion method. Very distinct inhibitions of the cancer cell lines were observed. (b) *In vivo* anticancerous activity of the isolated polysaccharide was studied with DLA and EAC cell lines for a period of 30 days in mice. In DLA cell line induced mice, the polysaccharide treatment showed a very good reduction in tumour volume when compared to the controls. In EAC induced and polysaccharide treated mice, the percentage life span was found to be high when compared to the controls.

Cytotoxic screening with the isolated polysaccharide was studied in a panel of human cell lines-OVACAN cell line, PC3 cell lines and HepG2 cell lines using MTT assay. The results showed a decrease in cell viability in a dose dependent manner in the case of OVACAN cell lines.

The above results are discussed in this thesis in eight chapters. From the above results, we could be able to suggest a new bio molecule that could be recommended for the treatment of cancer.