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In vivo Antiviral Activity of Polysaccharide from the Indian Green Alga, *Acrosiphonia orientalis* (J. Agardh): Potential Implication in Shrimp Disease Management

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Abstract: The polysaccharide extracted from the marine green algae, *Acrosiphonia orientalis* was evaluated for *in vivo* antiviral activity against the shrimp pathogen, White Spot Syndrome Virus (WSSV). The algal polysaccharide was rationalized with commercial shrimp feed and orally administrated for a period of 14 days followed by the artificial viral challenge experiment. It was found that shrimp fed medicated diet (4g/kg) exhibited better survival rate (88%) over the control (0%). The immune defence factors such as total haemoycte count, differential haemocyte count and phenoloxidase activity was high for shrimp treated with algal polysaccharide. Therefore, it was concluded that the algal polysaccharide can be utilized as prophylactic drug for the management of WSSV.

Key words: *Acrosiphonia orientalis* % Algal polysaccharide % Antiviral activity % Immunostimulant % Haemocyte % *Penaeus monodon*

INTRODUCTION

Shrimp aquaculture is an economically important industry, with high international trade value. During the past decade, non scientific shrimp farm intensification and improper management strategies have increased the incidence of infectious diseases which in turn declined the growth of this industry. Viral infections are recognized as the principle threatening factor for sustainable shrimp farming. Throughout the world, more than 20 viruses have been identified to cause shrimp diseases [1]. Among these, WSSV is recognized as the most dreadful pathogen causing 100% mortalities within a week of infection in hatcheries and growout conditions, causing tremendous economic loss. The WSSV, belonging to the family *Nimaviridae* (genus *Whispovirus*), has a wide host range. The clinical sign of the infection is characterized by white spots on the exoskeleton and epidermis[1].

The sustainability of shrimp industry is now resort largely on prevention and control of diseases through proper farm management. It is well known that in crustaceans, immunostimulants increase resistance to infectious diseases by enhancing both specific and non-specific defence mechanisms [2]. Application of natural immunostimulants for proactive shrimp disease management is a recently developed ecofriendly approach [3]. Many researchers have demonstrated the use of natural polysaccharide to boost up shrimp immunity [3, 4]. Research pertaining to the immune enhancing mechanism in shrimp using the marine algal polysaccharide against viral diseases is already reported [5].

It has been reported that the seaweeds from the Indian coast are wealthy resource of bioactive compounds [6]. Some of these seaweeds have already been reported to possess antiviral potency [7]. Several Indian seaweeds have been extensively used for the ecofriendly management of shrimp diseases [3]. Therefore, the objective of the current study was to evaluate the *in vivo* antiviral property of *A. orientalis* and to examine the haemolymph parameters to determine the immune status of the shrimp.

MATERIALS AND METHODS

Collection and Extraction of Polysaccharides from *A. orientalis*: *A. orientalis* was collected from the Kollam coast (Southwest coast of India) and polysaccharides

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were extracted from the dried fronds (20 kg) using 0.1 N HCl at 95° C for 12 h and the extract was precipitated by adding ethanol [4]. The polysaccharide was recovered after centrifugation at 5000 rpm (Eppendorf).

Preparation of Medicated Feed: Experimental medicated feed were produced by combining the commercially available feed (Godrej, Mumbai) with polysaccharide of different dose level (2, 4, 6, 8 g/kg).

Treatment Schedule: The WSSV-free juveniles of *Penaeus monodon* Fabricius (Crustacea, Decapoda), weighing 2-5 g/shrimp were purchased from local farm, acclimated to standard conditions: 27±2°C, 15ppt salinity, 12 h of light per day and constant aeration. The stocking densities were maintained at 20 animals per glass aquarium (1000 L). The shrimp were fed with medicated feed in three equal instalments at a rate of 5 % of their body weight for a period of fourteen days. The same diet without polysaccharides was fed to control groups. The water quality parameters including pH, temperature, salinity, dissolved oxygen and ammonia content were monitored using an autoanalyser (Hach).

WSSV Challenge Experiment: The stored/ freshly collected shrimp samples was confirmed for WSSV infection using commercially available PCR based detection kit (Genei, Bangalore). Challenge experiment was performed using the filtrate of the epidermis from the live naturally infected PCR positive shrimps. The epidermis of the infected shrimp was homogenized in brackish water at 4° C at a ratio of 1:9. After centrifugation at 12,000 rpm for 5 min, the supernatant was filtered through 0.45 µm membrane and diluted 500-700 times in brackish water. Fifteen shrimp in each treatment with two replicates, each were challenged. The challenged shrimp were monitored daily for infection/mortality for 2 weeks. The haemolymph was collected from the shrimp after 48 h of pathogenic exposure and subjected to defence factor analysis.

Determination of Host Defence Factors: Haemolymph was collected from the ventral part of haemoceol of the second abdominal segment using 1 ml syringe filled with Alsever's solution as an anticoagulant [8]. The total haemocytes counts (THC) and differential haemocytes counts (DHC) were carried out as described by Jones [9]. proPO assay was measured spectrophotometrically by recording the formation of dopachrome from L dihydrophenylalanine at 490 nm [10].

All the analysis were carried out using pooled haemolymph of 15 shrimp and results were expressed as average of triplicate experiments.

RESULTS

In general, the survival rate of shrimp fed medicated diet was higher (65-88 %) over the controls (0 %) (Fig.1). Out of the four medicated diet, shrimps treated with 4 g/kg exhibited significantly higher survival rate of 88%, whereas in other treatments the survival rate was moderate. During the first seven days of post infection, the survival rate was higher (90%) in all medicated diet treatments. However, at the end of 2nd week the survival rates declined to 65% except in 2 g/kg and 4 g/kg treated groups, whereas in the control, animals were lethargic and moribund after two days of infection and mortality rate was 100% on the 4th day. The clinical signs were identical to those found in shrimp naturally infected with WSSV.

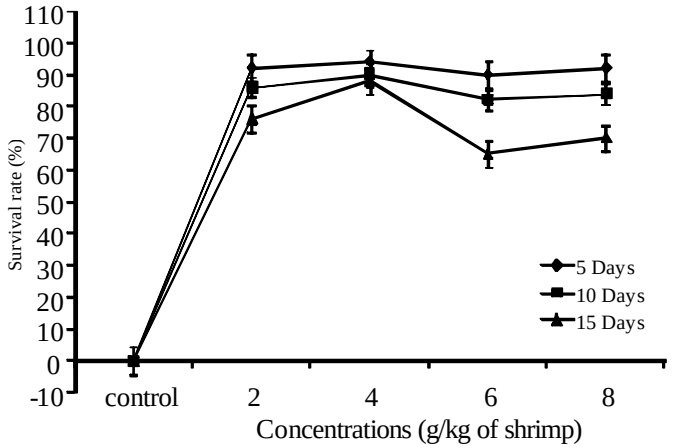

Fig. 1: Percentage of survival rate of shrimp fed with different concentrations of medicated diet (algal polysaccharides) and challenged with WSSV

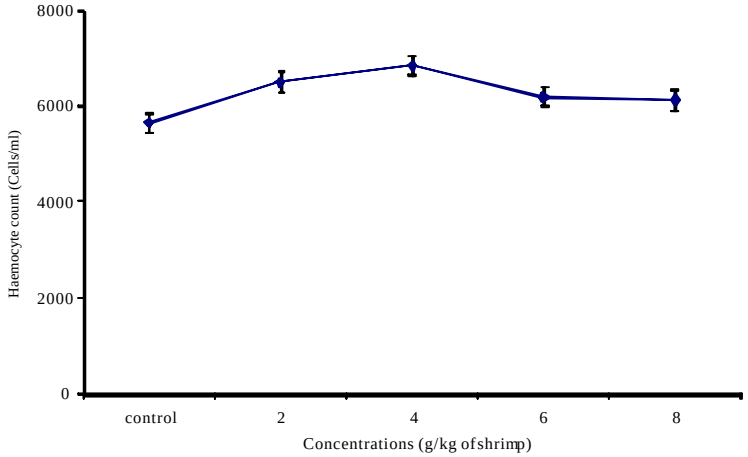

Fig. 2: Total haemocyte count in the haemolymph of shrimp *P. monodon* fed with different concentrations of medicated diet (algal polysaccharides)

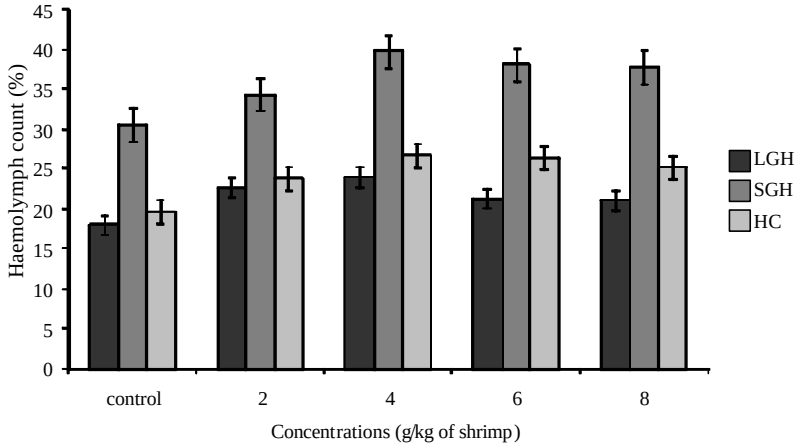

Fig. 3: Differential haemocyte count in haemolymph of shrimp *P. monodon* fed with different concentrations of medicated diet (algal polysaccharides)

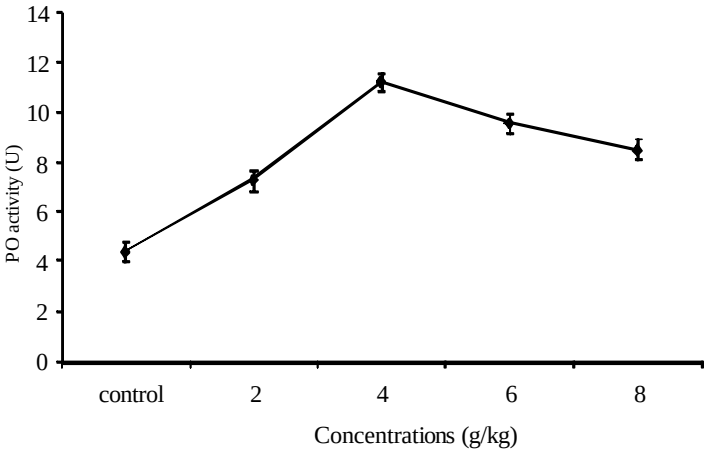

Fig. 4: Phenoloxidase activity in the haemolymph of shrimp, *P. monodon* fed with different concentrations of medicated diet (algal polysaccharides)

The THC was significantly elevated in the shrimp fed 4 g/kg (6.87±0.58 x 10^3 cell/ml), followed by 2 g/kg (6.52±0.23 x 10^3 cell/ml) (Fig. 2). In controls the THC was 5.67±1.2 x 10^3 cells/ml. DHC value of treated and control shrimp varied widely (Fig. 3). The results indicates that the medicated diet enhance the proliferation of all types of haemocytes, whereas in control, haemocyte counts decreased drastically.

Regarding the PO activity, it was higher in 4 g/kg treated shrimp group (15.23 ± 0.28) than that of control (4.38± 0.14). However, the PO activity of the rest of the treatment varied slightly (Fig. 4).

DISCUSSION

The overall result of the present study demonstrated that the oral administration of algal polysaccharide based diet (medicated diet) can produce a reduction in the WSSV infection in *P. monodon*. Chotigeat *et al*. [5] reported that oral administration of fucoidan from brown algae increased the survival rate of WSSV infected *P. monodon.* The polysaccharides from other marine algae of different geographical regions are well known for their antiviral potentials [11, 12]. Data pertaining to the *in vivo* anti WSSV property of certain algae was earlier demonstrated by many authors [7, 13].

Viral and bacterial infections, together with poor water quality, are the main reason for shrimp mortality. Reinforcing resistance against the invading pathogens of shrimp by improving shrimp immunity is an acceptable strategy for defending diseases. Unlike the mammals and fishes, crustaceans order lacks a specific immune system [14]. However, the shrimp immunity is defined as nonspecific internal defence response that included both cellular and humeral components. Haemocytes play an important role in the cellular immune responses, including prevention of blood loss, recognition of non-self, phagocytosis and encapsulation [15]. Usage of natural immunostimulant as a better remedy than administrating vaccines and antibiotics is accepted for the control of pathogen in aquaculture. Immunostimulants has wide range of efficacy [16] and enhance the non-specific defence mechanisms, thereby preventing infectious diseases. The circulating haemocyte number is a stress indicator [17] and haemocyte counts may be a valuable tool in monitoring the health status of crustacean species [18, 19]. In the present study, haemogram profile of the shrimp fed medicated feed varied widely over the control. The proPO-activating system is a vital part of shrimp immune response, which includes recognition of foreign invaders and nonliving entities, activation of a wide range of defence reactions, such as phagocytosis and antibacterial activity, encapsulation and nodule formation. Increase in total haemocytes count is generally accompanied by changes in phenoloxidase activity because these cells are the major store for the proPO system. Our PO activity results were in comparison with those obtained by Huang *et al*. [4] in closely related species, *Fenneropenaeus chinensis*. Therefore, the seaweed based medicated diet at a rate of 4g /kg for two weeks could enhance the immunity of the shrimp.

In conclusion, our study shown the increased resistance to WSSV infection of *P. monodon* treated with medicated diet. This is the first report on the immune stimulant effects of Indian seaweeds in shrimp against WSSV. Considering the large biomass availability and cost effective extraction procedure, this seaweed could be utilized as feed additive/ medication to prevent WSSV in shrimp aquaculture.

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