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Original Article

STUDY ON IMMUNOSTIMULATORY PROPERTY OF FEW SEAWEEDS INJECTED INTRAPERITONEALLY

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ABSTRACT

Objective: Three type of seaweed was chosen for the study namely *Gracilaria corticata, Ulva lactuca* and *Stocheospermum marginatum* and was extracted with ethanol.

Methods: The fishes [Common carp (*Cyprinus carpio)* (weight 10±5g)] were divided into five groups. They were infected with *A. hydrophila* (1.6×10^4 CFU/fish). The infected three groups were injected with seaweed extract (0.20 mg) suspended in saline solution. One group was injected only saline control the other one group was injected with ciprofloxacin (0.20 mg) (standard) suspended in saline. The experiment was carried out for 28 d. Every seven days interval the fishes were injected with seaweed extract and blood parameters of RBC, WBC, HB, Ht, MCV, MCH, and MCHC were recorded.

Results: The MCHC value of Gracilaria *corticata* was recorded in 28th day (29.28±1.15) g/l. In *Stocheospermum marginatum,* the MCHC value was found in (27.19±1.62) g/l on 28th day. The *Ulva lactuca* had a MCHC value of (26.80±2) g/l on 28th day.

Conclusion: From this study, we can understand that the fish injected with seaweed extracts as good Immunostimulants properties.

Keywords: Seaweed, Common carp, Bacterial infection, *Aeromonas hydrophila* Blood parameter, RBC, WBC,HB, Ht, MCV, MCH, MCHC

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INTRODUCTION

Selective utilization of marine algae as a potential source of pharmaceutical agents has been increasing in recent years. Many of the seaweeds possess bio-active components which inhibit the growth of some of the Gram positive and Gram negative bacterial pathogens. The algal extracts were used as a curative and preventive agent for various diseases such as antibiotics, antihelminthics, and cough remedies, antihypertensive, antitumour and anti diarrhoea. Recently we have embarked on the chemical investigation of marine algae with a special accent on their bioactive properties [1]. About 6000 species of seaweeds have been identified and are grouped into different classes like green (Chlorophytes), brown (Phaeophytes) and red (Rhodophytes) algae. Most of the compounds of marine algae show antibacterial activities [2]. Aquaculture has been a growing activity for the last 20 y* worldwide and this impressive development has been attended by some practices potentially damaging to animal health [3]. The bacterial infections are considered the major cause of mortality in aquaculture [4]. Particularly *A. hydrophila and Y. ruckeri as gram-negative, and S. agalactiae, L. garvieae* and *E. faecalis* as gram-positive bacteria cause infectious diseases [5]. The potential hazards of using antibiotics in aquaculture are the development of antibiotic-resistant microorganisms, antibiotic residuals in fish products, contamination of surrounding ecological systems and reduced efficiency of antibiotics against the diseases caused by resistant pathogens [6]. Prevention of fish disease through the stimulation of non-specific immune response by natural compounds is a potential solution for development of sustainable antibiotic-free aquaculture. Numerous compounds with cytostatic, antiviral, antihelmintic, antifungal and antibacterial activities have been detected in green, brown and red algae [7]. Many metabolites isolated from marine algae possess bioactive principles [8]. In the present study seaweed extracts, immunostimulants of non-specific immune response in common carp were studied.

MATERIALS AND METHODS

Collection of fishes

Common carp (weight 10±5g) were collected from a local fish farm in Vellanguli Tirunelveli District. The fish were acclimatised in 100-litre tanks (10 fish/tank) and provided with continuous aeration. During the holding period, the fish were fed a control feed at 5% of their body weight, once a day.

Collection of seaweed

Live samples of the seaweeds were collected by handpicking during low tide from Hare Island in the Gulf of Mannar of Tuticorin coast (08 46' 2.15"N lat; 78 11' 16.05" E long). The seaweeds were identified with the help of Botany expert in our college. After identification, the seaweeds were shade dried and powdered.

Preparation of extracts by soxhlet extraction method

The powdered samples were extracted by using soxhlet apparatus. Ethanol was taken as the solvent for extraction. 25g of the sample and 250 ml of the solvent were taken for extraction. The apparatus was run for 4h and syrupy extracts were collected. The extracts obtained were concentrated using rotary vacuum evaporator. Then the extract was stored in cold storage for further study.

Infection of fishes

Bacterial strain, *A. hydrophila* was obtained from Microbial Type Culture Collection and Gene Bank (MTCC) Institute of Microbial Technology, Chandigarh, India. After obtaining bacteria, it was cultured in tryptone soya broth (Himedia) for 24 h at 37^0 C. After incubation period, the culture was centrifuged at 800g for 15 min at 4^0 C. The packed cells were washed with phosphate-buffered saline (PBS; pH 7.2) twice and then the required dose was prepared in PBS. The bacterial suspension was prepared to 1.6×10^4 CFU/fish as determined using an ineffectual dosage.

Intraperitoneal injection

After infection the fish were divided into five groups and injected intraperitoneally in seven days interval, namely control group injected only saline second one was standard group injected with 0.20 mg ciprofloxacin suspended in saline and the third test group (ethanol extracted seaweed like *Gracilaria corticata*, *Ulva lactuca*, *Stocheospermum marginatum* injected at 0.20 mg of each extract suspended in saline solution). The immunological parameters were analyzed for every 7 d interval. In the experimental period, all group of fishes were fed with control diet.

Haematological studies

a. Blood collection

A blood sample was collected from fish of each group. The fishes were collected and gently wiped with a dry cloth to remove water. Caudal peduncle was cut with a sharp blade and the blood was collected in a watch glass containing EDTA, an anticoagulant (6% Ethylene Diamine Tetra Acetic Acid). The blood was mixed well with the EDTA solution by using a needle and this sample was used for determining Haematological studies.

Immune response in common carp was studied by analysing various parameters like RBC, WBC, HB, Hematocrit values, MCV, MCH, MCHC. All these values were determined using a method originally devised by Yokoyama [9] and later on modified by Christensen *et al.,* [10].

RESULTS

There was a gradual increase in the RBC count (fig. 1) from the initial day to the 28th day the maximum being observed in the fishes injected ciprofloxacin drug (as standard) on 28th day (3±0.06)×10^6 cells/mm^3 followed by *Stocheospermum marginatum* extract on the 28th day (2.66±0.04)×10^6 cells/mm^3 *Gracilaria corticata* (2.63±0.08)×10^6 cells/mm^3 and *Ulva lactuca* (1.46±0.08)×10^6 cells/mm^3 and the control phosphate buffer saline had least amount of RBC was counted (1.22±0.04)×10^6 cells/mm^3. Then WBC level also gradually increased from initial day to 28th day the values being high for the standard ciprofloxacin (23.33±0.22)×10^3cells/mm^3. The red seaweed *Gracilaria corticata* a value of (21.3±0.47) ×10^3cells/mm^3 was noted and the brown seaweed *Stocheospermum marginatum* recorded a value of (20.33±1.11)×10^3cells/mm^3 the green seaweed *Ulva lactuca* value was (19.3±0.06)×10^3 cells/mm^3. The least level was found in control drug (18.78±0.22) ×10^3 cells/mm^3. Haemoglobin count was more at 28th day (6.33±0.04) g % in the fishes injected with ciprofloxacin. There was a steady rise in the value from the initial day to 28th day. In *Gracilaria corticata* the haemoglobin level was (5.66±0.08) g % which was close to the standard group. *Stocheospermum marginatum* and *Ulva lactuca* had the haemoglobin (5.03±0.06) g % and (5±0.05) g % on 28th day. The least haemoglobin was found in the control group on 28th day (3.5±0.08) g %. The haematocrit values also increased from initial day to 28th day. The least haematocrit value was noted in control group (17.45±0.44)% on 28th day. The most value was found in the standard group in 28th day (20.17±0.66)%. The red, brown and green seaweeds had the following haematocrit values (19.33±0.44)%, (18.5±0.08)% and (18.66±0.44)% on 28th day.

The Mean corpuscular volume (MCV) was decreased from initial day to 28th day. The highest value of MCV was found in control group on 28th day (143.03±5.55) fl. And the lowest value was found in a Standard group on 28th day (67.23±3.33) fl. *Gracilaria corticata* had (73.50±1.48) fl on 28th day the brown seaweed *Stocheospermum marginatum* had (69.55±5.18) fl on 28th day and *Ulva lactuca* had (127.81±4.31) fl on 28th day. Similarly, the Mean Corpuscular Haemoglobin (MCH) value also decreases from initial to 28th day. The lowest MCH Value was found in *Stocheospermum marginatum* on 28th day (18.91±0.79) pg and the highest value was noted in *Ulva lactuca* on 28th day (34.25±1.66) pg. the standard group contain (21.10±1.22) pg on 28th day. The red seaweed *Gracilaria corticata* had (21.52±1.48) pg on 28th day the control group was noted (28.69±0.66) pg on 28th day. The Mean corpuscular Haemoglobin Concentration (MCHC) value increased from initial to 28th day. The most value of MCHC was found in 28th day (31.38±1.37) g/l in the standard group and least value of MCHC was found on 28th day (20.06±1.28) g/l in control group. The MCHC value of Gracilaria *corticata* was recorded in 28th day (29.28±1.15) g/l. In *Stocheospermum marginatum* the MCHC value was found in (27.19±1.62) g/l on 28th day. The *Ulva lactuca* had a MCHC value of (26.80±2) g/l on 28th d.

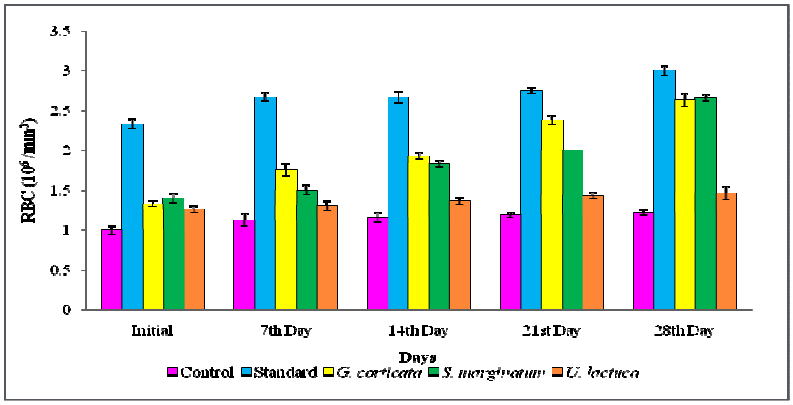

Fig. 1: The RBC value of *Cyprinus carpio* after seaweed extracts treatment

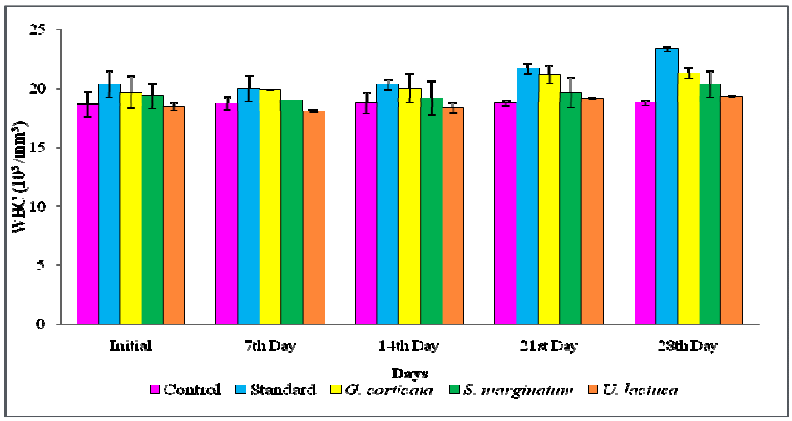

Fig. 2: The WBC value of *Cyprinus carpio* after seaweed extracts treatment

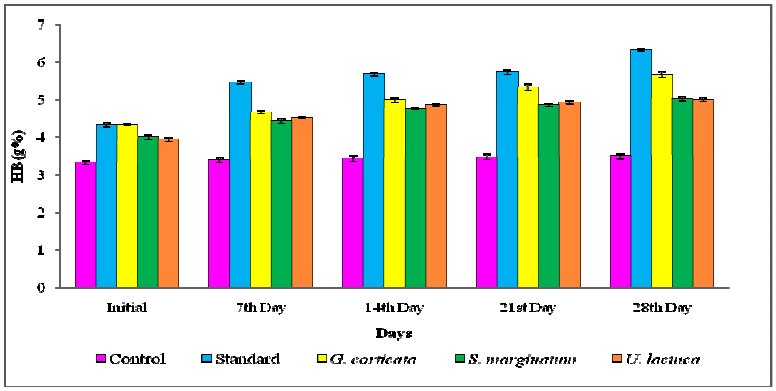

Fig. 3: The Hb value of *Cyprinus carpio* after seaweed extracts treatment

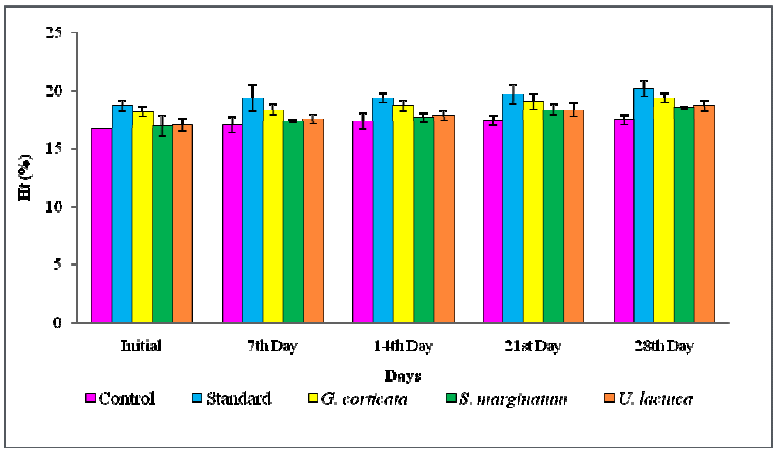

Fig. 4: The Ht value of *Cyprinus carpio* after seaweed extracts treatment

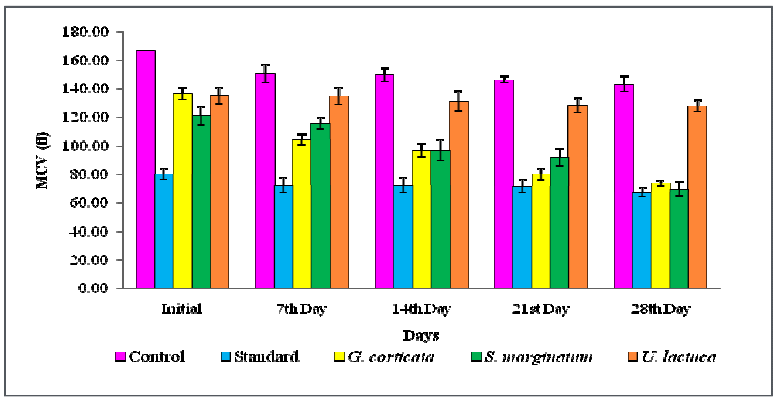

Fig. 5: The MCV value of *Cyprinus carpio* after seaweed extracts treatment

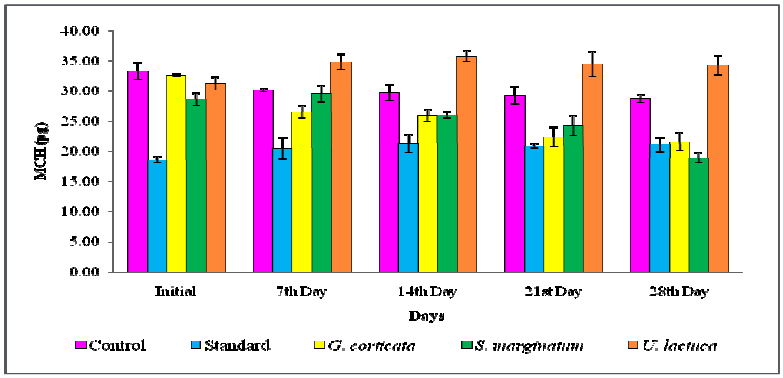

Fig. 6: The MCH value of *Cyprinus carpio* after seaweed extracts treatment

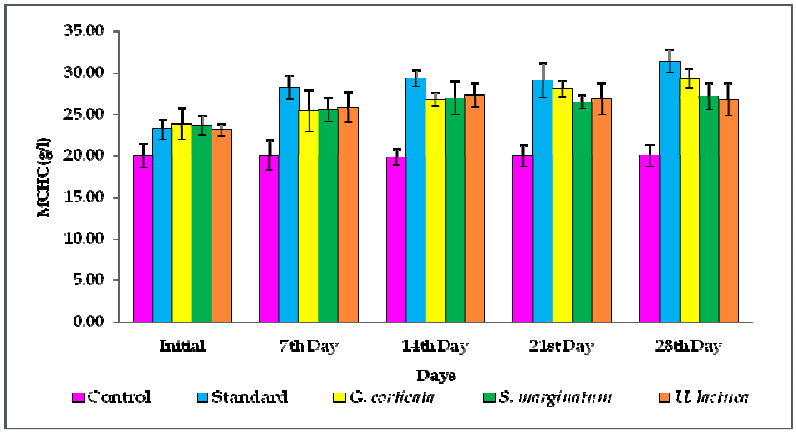

Fig. 7: The MCHC value of *Cyprinus carpio* after seaweed extracts treatment

DISCUSSION

Fish diseases are a great threat to the economic viability of any aquaculture practices. In advanced aquaculture practices, several antibiotics, vaccines and chemotherapeutic agents, as well as some immune-stimulants, have been used to prevent the spread of viral, bacterial, parasitic and fungal diseases [11]. In fishes, immunity can either be the non-specific type which is an innate defensive mechanism, or an acquired specific immunity. Numerous studies have reported a wide range of bioactivities displayed by natural products from plants, fungus and algae, which revealed to be of great interest in the prevention or treatment of pathogens [12-14].

Some recent studies reveal seaweed and algae as a potential source of antimicrobial products [15-18]. Showed that methanol extracts of red horn weed (Ceramium rubrum) (10 mg dry weight/ml) and hexane extracts of oarweed (Laminaria digitata) (31 mg dry weight/ml) evoked strong antibacterial activities against 16 different bacteria tested (marine bacteria and fish pathogenic bacteria). In the present study, the possibility of using seaweeds as immune stimulants was analysed red seaweed (Gracilaria corticata), green seaweed (Ulva lactuca), brown seaweed (Stocheospermum marginatum) one in each group was taken up for the study.

Haematology, based on RBC, WBC, HB, Haematocrit values, MCV, MCH, MCHC has provided valuable information for fishery biologists in the assessment of fish health [19]. The seaweed incorporated diet stimulates the immune system so the haematological parameters like RBC and WBC level are increased after the admission of the immuno-stimulant [20]. In our study fishes were injected the seaweed extract in intraperitoneal and the experiment was carried out in 28 d. Every 7 d interval the blood parameters were tested, the blood parameters have increased from the first d to the last d. In our study seaweed extract act as an immune stimulant. So the extract improved the immune system of fishes.

CONCLUSION

Fish depends more on nonspecific defence mechanism than mammals and Immunostimulant plays a vital role in the health and management strategies of the aquatic organisms. From this study, we have concluded seaweed extract act as an immune stimulant and also it enhances the nonspecific immunity and increases disease resistance of *C. carpio.* We suggest that seaweed as a Good immune stimulatory agent in the aquaculture industry.

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CONFLICT OF INTERESTS

Declare none

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