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Review Article

IMMUNOSTIMULANT EFFECT OF MEDICINAL PLANTS ON FISH

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ABSTRACT
Fishes not only play an important role in the demand of food for humans but they have also emerged as major model organisms for different biomedical researches. A number of experiments with the use of several drugs have been conducted in fish. Diseases in fish caused by bacteria are most widespread. Antibiotics are frequently used to control fish diseases caused by bacteria, but there is an increasing risk of developing antibiotic resistant strains of bacteria. The medicinal plants can act as immunostimulants, conferring early activation to the non-specific defense mechanisms of fish and elevating the specific immune response. The herbs contain many immunologically active components such as polysaccharides, organic acids, alkaloids, glycosides and volatile oils, which can enhance immune functions. Recently, there has been increased interest in the immune stimulating function of some herbs in aquaculture. The non-specific immune functions such as bacteriolytic activity and leukocyte function of fish have been improved by some herbs. Henceforth, this article elucidates certain herbs (medicinal plants) which have been shown experimentally as well as clinically to possess immunostimulant effects in fish, thereby treating different fish diseases.

KEYWORDS: Immunostimulants, herbs (medicinal plants), fish, fish diseases.

INDRODUCTION

Fish are palatable and proteinous food for human beings. India is now at the threshold of blue revolution and it has made a notable progress in the field of inland fisheries. Fishes not only play an important role in the demand of food for humans but they are widely used for various biological experiments[1]. Fishes, particularly *Danio rerio* (zebrafish, a freshwater tropical fish) have emerged as major model organism for biomedical research, such as in developmental genetics, neurophysiology, oncology and biomedicine[1-2]. A number of experiments and the use of drugs have been performed in fish. The antibacterial, antiparasitic and anaesthetic drugs, besides the pharmacokinetic and pharmacodynamic parameters have been well experimented on the fish. Drugs, e.g., tetracyclines, penicillins, macrolides, quinolones, sulfonamides, immunostimulants, anticancer agents, herbal drugs, vaccines, etc. have been successfully experimented on fish. Thus, fish can be used as model organism in the experimental studies[2].

Diseases in fish caused by bacteria, particularly *Aeromonas hydrophila,* are most widespread. Septicaemia caused by motile aeromonads is a ubiquitous problem that affects fishes found in warm, cool and cold fresh water around the world. The bacterium, *A. hydrophila* has been associated with disease in carp, eels, milkfish, channel catfish, tilapia and ayu, and can also be an opportunist in stress-related diseases in salmonids. Antibiotics are frequently used to control disease caused by this bacterium, but there is an increasing risk of developing antibiotic resistant strains of bacteria. Vaccines are being developed against *A. hydrophila* and atypical *A. salmonicida,* but these are not yet commercially available. As *A. hydrophila* is such a heterogeneous species (multiple strains), vaccine development is extremely complex. The medicinal plants can act as immunostimulants, conferring early activation to the non-specific defense mechanisms of fish and elevating the specific immune response. Medicinal plants have been used as medicine and an immune booster for humans for thousands of years. Recently, a growing interest has developed in using herbs in animal feeds by both researchers and feed companies. The herbs contain many immunologically active components such as polysaccharides, organic acids, alkaloids, glycosides and volatile oils, which can enhance immune functions. Therefore, the medicinal plants have been used as medicine to treat different fish diseases and to control of shrimp, especially in the countries like China, Mexico, India, Thailand and Japan[3].

Recently, there has been increased interest in the immune stimulating function of some herbs in aquaculture. The non-specific immune functions such as bacteriolytic activity and leukocyte function were improved by some mixtures of Chinese herbs in shrimp (*Penaeus chinensis*) and tilapia[4]. The non-specific defense mechanisms of fishes include neutrophil activation, production of peroxidase and oxidative radicals, together with initiation of other inflammatory factors[5]. Certain medicinal plants (herbs) possessing immunostimulant effects, as discussed below, have been used experimentally as well as clinically to treat various fish diseases and to control infections in them.

Immunostimulant Effects Of Certain Plants

Lonicera japonica herb has been known as an anti-inflammatory agent and used widely for upper respiratory tract infections, diabetes mellitus and rheumatoid arthritis[6]. It has been reported that *L. japonica* significantly increased the blood neutrophil activity and promoted phagocytosis by the neutrophils in bovine at the correct concentration[7]. Several studies have reported that oral administration of chitin[8] and yeast products (MacroGard, Vitastim and *Saccharomyces cerevisiae*)[9] increased the phagocytic capability of the cells in rainbow trout. It has been further reported[9] that the extracellular activity was very high in fish fed with dietary glucan.

After challenge with *A. hydrophila,* survival of fish fed with the extracts of *Ganoderma lucidum* and *L. japonica* herbs

was improved when compared with the control group. The survival was further enhanced in the group fed the *Ganoderma* extract supplement and when both herbs were used together. It is possible that this is the result of enhancement of some components of non-specific immune system of the fish by *Ganoderma* and a combination of *Ganoderma* and *Lonicera*. There is strong evidence that feeding glucans can modify the activity of the innate immune system of fish and increase the disease resistance in several fish species[10]. *G. lucidium* is a traditional Chinese medicine used for the prevention and treatment of various human diseases in China and other Asian countries[11]. It has been shown that an aqueous extract (AqE) from *G. lucidum* will promote phagocytosis by macrophages in mice immunosuppressed by an anticancer drug, cyclophosphamide. *G. lucidum* will stimulate the proliferation of lymphocytes induced by concanavalin A or lipopolysaccharide and influence gene expression of cytokines[12].

The extracts of four Chinese herbs (*Rheum officinale, Andrographis paniculata, Isatis indigotica* and *L. japonica*) increased the phagocytosis of white blood cells (WBCs) of crucian carp[13]. Immunostimulant effects of the dietary intake of various medicinal plant extracts on fish, rainbow trout (*Oncorhynchus mykiss*), were investigated. The fishes were fed with diets containing aqueous extract (AqEs) of mistletoe (*Viscum album*), nettle (*Urtica dioica*) and ginger (*Zingiber officinale*). Food containing lyophilized extracts of these plants as 0.1% and 1% was used at a rate of 2% of body weight per day for 3 weeks. Plant materials tested for immunostimulatory food additives caused an enhanced extracellular respiratory burst activity ($P<0.001$) compared to the control group. The fishes fed with a diet containing 1% AqE of powdered ginger roots exhibited a significant non-specific immune response. Phagocytosis and extracellular burst activity of blood leukocytes (WBCs) were significantly higher in this group than those in the control group. All plant extracts added to fish diet increased the total protein level in plasma except 0.1% ginger. The highest level of plasma proteins was observed in the group fed with 1% ginger extract containing feed. It was shown that in trout fed with nettle and mistletoe extracts the production of extracellular superoxide anion was of a similar level to that in the control fish[14].

Immunostimulatory effect of the oral administration of the AqE of *Eclipta alba* (Bhangra) leaf was studied in tilapia fish, *Oreochromis mossambicus.* The fishes were fed for 1, 2 or 3 weeks with diets containing the AqE of *E. alba* leaves at 0, 0.01, 0.1 or 1% levels. After each week, non-specific humoral (lysozyme, antiprotease and complement) response, cellular (myeloperoxidase content, production of reactive oxygen and nitrogen species) response and disease resistance against *A. hydrophila* (a bacterial pathogen) were determined. The results indicated that *E. alba* AqE administered as feed supplement significantly enhanced most of the non-specific immune parameters tested. Among the humoral responses, lysozyme activity significantly increased after feeding with AqE for 1, 2 or 3 weeks. No significant modulation was noticed in all the cellular responses tested after 3 weeks of feeding, while reactive oxygen species (ROS) production and myeloperoxidase content showed significant enhancement after 1 week of feeding with AqE. When challenged with *A. hydrophila* after 1, 2 or 3 weeks of feeding, the percentage mortality was significantly reduced in the treated fish. The highest dose of 1% gave better protection than the other doses with the relative percentage survival (RPS) values of 64, 75 and 32 after feeding for 1, 2 and 3 weeks respectively. Finally, the results indicated that dietary intake of AqE of *E. alba* leaves enhances the non-specific immune responses and disease resistance of *O. mossambicus* against *A. hydrophila* bacteria[15].

Three diet variations, in addition to control (without herbal treatment), were used to determine the effects of two Chinese herbs (*L. japonica* and *G. lucidum*) on non-specific immune response of tilapia fish. The herbal diets contained 1.0% of *Lonicera*, 1.0% of *Ganoderma* and a mixture of *Ganoderma* (0.5%) and *Lonicera* (0.5%). The diets were fed for 3 weeks. The respiratory burst activity of WBC, phagocytosis, plasma lysozyme, total protein and total immunoglobulin were monitored. Following 3 weeks after feeding, fish were infected with *A. hydrophila* and mortalities recorded. The study showed that feeding tilapia with *Ganoderma* and *Lonicera* alone or in combination enhanced the phagocytosis by blood phagocytic cells during the whole experimental period and stimulated lysozyme activity after 2 weeks. Respiratory burst activity of phagocytic blood cells, total protein and total immunoglobulin in plasma were not enhanced. Both herbs when used alone or in combination increased the survival of fish after challenge with *A. hydrophila.* The highest mortality (58%) was observed in control fish, followed with fish fed with *Lonicera* extract (43%) and fish fed with *Ganoderma* (30%). The lowest mortality (21%) was observed when fish were fed with a combination of these two medicinal plants. Hence, it can be concluded that the herbal extracts added to diets acted as immunostimulants, and appeared to improve the immune status and disease resistance of fish. It was also shown that *Astragalus* enhanced lysozyme activities in tilapia fish during the whole period of the experiment when fed with low (0.1%) and medium (0.5%) doses. In case of fish fed with *Scutellaria,* there was significant inhibition of extracellular superoxide anion production[3].

Goldfish, *Carassius auratus* were inoculated intramuscularly (50 microl) with *A. hydrophila*. In *A. hydrophila* infected goldfish, fed with diets containing 100 and 200 mg kg(-1) of mixed herbal extracts supplementation feeds, the WBC levels significantly increased ($P<0.05$) throughout the experimental trial compared to the control. The red blood cell (RBC) and haemoglobin (Hb) in goldfish significantly decreased ($P<0.05$) when fed with 100 and 200 mg kg(-1) of mixed herbal extracts supplementation feeds; while it was restored near control when infected fish fed with 400 or 800 mg kg(-1) of herbal extracts supplementation feeds. On the other hand, the haematocrit (Ht) values declined significantly ($P<0.05$) in 100, 200 and 400 mg kg(-1) of mixed herbal supplementation feeding groups on weeks 2 and 4 when compared to control group. The mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC) values almost significantly altered from the control values. The infected goldfish treated with 100 or 200 mg kg(-1) of herbal supplementation feeds exhibited significant ($P<0.05$) decrease in total protein, glucose and cholesterol levels on week 1-4; whereas it was restored when infected fish fed with 400 or 800 mg kg(-1) of herbal supplementation feeds on week 4. In comparison to untreated control goldfish, the respiratory burst activity and phagocytic activity of blood cells was significantly enhanced in infected fish feeding with 200, 400 and 800 mg kg(-1) of herbal supplementation feeds compared to the control. However, the infected fish fed with

all the doses of mixed herbal supplementation feeds, the lysozyme activity was significantly enhanced throughout the experimental period. This study showed that the infected goldfish treated with 400 and 800 mg kg(-1) of herbal supplementation feeds preceding the challenge with live *A. hydrophila* had 30% and 25% mortality. Whilst, 100 and 200 mg kg (-1) of herbal supplementation feeds treated groups were found the mortalities of 50% and 45%, respectively. The results indicated that 400 or 800 mg kg(-1) of mixed herbal supplementation feeds restored the altered hematological parameters and triggered the innate immune system of goldfish against *A. hydrophila*[16].

Efficacy of dietary doses of *Withania somnifera* (Ashwagandha) root powder was evaluated on immunological parameters and disease resistance against *A. hydrophila* infection in *Labeo rohita* (Indian major carp) fingerlings. These fishes were fed with dry diet containing 0 gkg(-1) (control), 1 gkg(-1) (T(1)), 2 gkg(-1) (T(2)) and 3 gkg(-1) (T(3)) of *W. somnifera* root powder for 42 days. Immunological (NBT level, phagocytic activity, total immunoglobulin and lysozyme activity) parameters of fishes were examined at 0, 14, 28 and 42 days of feeding. The fishes were challenged with *A. hydrophila* for 42 days post-feeding and the mortalities (%) were recorded over 14 days post-infection. The results demonstrated that fishes fed with *W. somnifera* root showed enhanced NBT level, phagocytic activity, total immunoglobulin level and lysozyme activity ($p<0.05$) compared with the control group. The survivability was higher in experimental diets than the control group. Dietary *W. somnifera* at the level of 2 gkg(-1) showed significantly ($P<0.05$) higher protection (RPS 42.85+/-0.65%) against *A. hydrophila* infection than control. The results suggested that the *W. somnifera* root powder has a stimulatory effect on immunological parameters and increases disease resistance in *L. rohita* fingerlings against *A. hydrophila* infection[17].

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