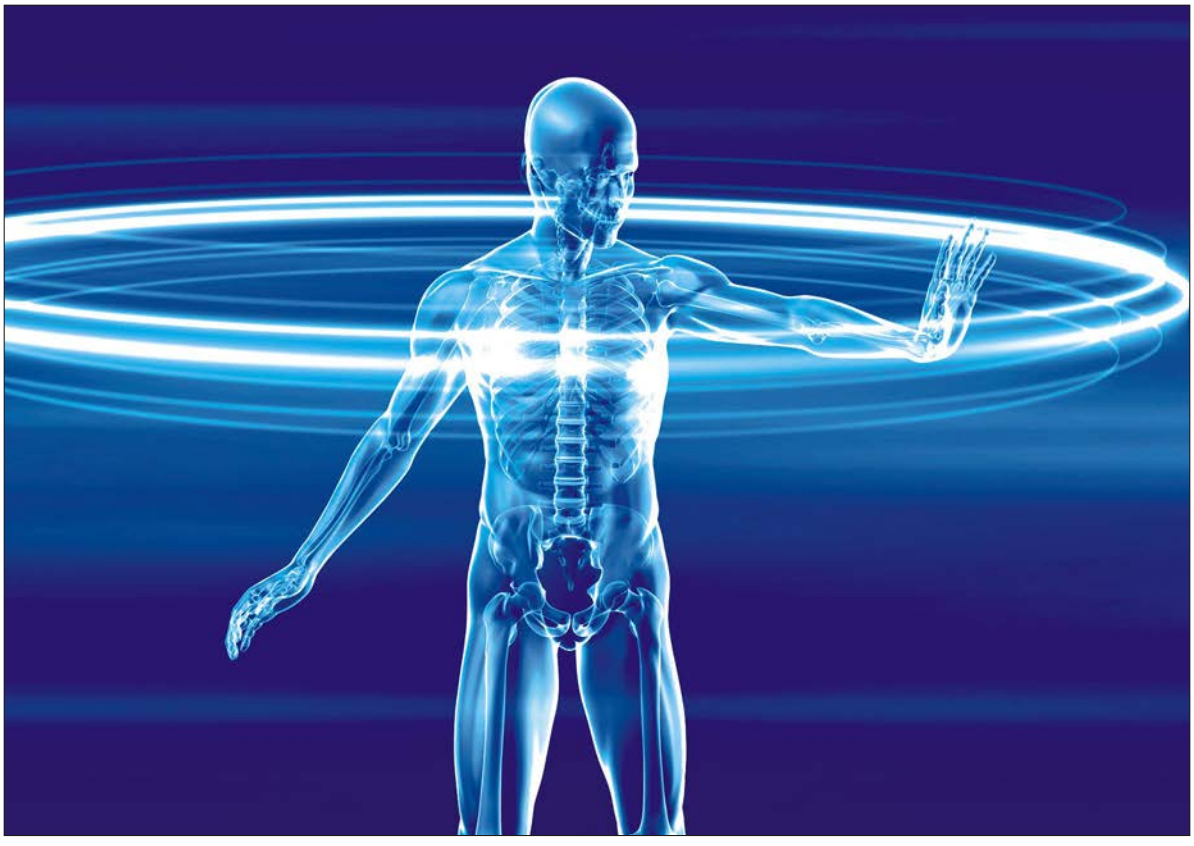

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Development of Immunity by Extract of Medicinal Plant *Ocimum sanctum* on Common Carp *Cyprinus carpio* (L.)

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ABSTRACT

The loss of fish production from infectious diseases accounts about 60% of all diseased fish. One of the major constrains for the development of aquaculture is the loss due to disease. So, we need to modulate the immune system of fish. A study has been carried out to investigate the development of immunity by extract of medicinal plant *Ocimum sanctum* on different concentrations (10, 20 and 30 ppm) against heat killed *A. hydrophila* on the fingerlings of common carp, *Cyprinus carpio*. In the plant extract treated fishes, no mortality was seen. Increase in the weight of the fish observed in plant extract treated groups, (10 ppm) showed more growth than the control and other groups. The low dose (10 ppm) of plant extract showed maximum antibody response and more phagocytic activity than the control and other experimental groups. The 10 ppm was found more efficient. Hepatosomatic index was more in the control group due to the enlargement of liver. However, the plant extract administered group showed decreased HSI.

Key words: *Ocimum sanctum*, *Cyprinus carpio*, *Aeromonas hydrophila*, hepatosomatic index (HSI), antibody titre, phagocytic activity, immunomodulatory effect

INTRODUCTION

Aquaculture productivity constitutes significant portion of national income in many countries of Asia. Large scale mortalities of fish often occur in ponds and loss is due to environmental pollution stress followed by microbial infection (Michael, 1997). The aquaculture and fisheries have a promising role to play in social development by providing nutritional security for the Indian population and contributing to the economic advancement of the farmers and fishery workers as more than 14.66 million fishermen and fish farmers are totally dependent on fisheries for their livelihood in India (Phale *et al*., 2009). The increase the productivity in fish culture has been accompanied by stressful conditions and problems related to fish diseases. Enhancement of the immune system seems to be the most promising method for preventing fish diseases. Some immunostimulants that have been used in aquaculture are: Synthetic substances (Like levamisole), biological substances (bacterial derivatives), nutritional factors, animal and plant components. Unlike chemical substances used in aquaculture, plant immunostimulants have no residuals in the environment and do not cause drug resistance. On the other hand, in traditional medicine plants have been used in order to prevent and treat a lot of diseases in human and animals

(Soltani *et al.*, 2010). Fish which form minor and major links in food webs of ecosystems harbor a wide assay and a time large number of parasites. Fish represent not only the earliest but also the largest class of vertebrates. This triumph of fish has been accomplished despite the fact that they possess both slower and less developed adaptive immune systems than these of higher vertebrates. Due to the aquatic environment fish have unique anatomical and physical characteristics. Fish live in intimate contact with an environment containing both saprophytic and pathogenic microbes capable of digesting and degrading fish tissues (Ravichandran *et al.*, 2010). Common carp is an omnivorous fish, feeds mainly in the benthic region of the pond. They have been serving as a stable food for people (Santhanakumar and Selvaraj, 1995). The ecological spectrum of carp is broad. Best growth is obtained when water temperature ranges between 23 and 30°C. The fish can survive cold winter periods. Salinity up to about 5% is tolerated. The optimal pH range is 6.5-9.0. The species can survive low oxygen concentration (0.3-0.5 mg L^{-1}) as well as super saturation. Carp are omnivorous, with a high tendency towards the consumption of animal food, such as water insects, larvae of insects, worms, molluscs and zooplankton (Milstein *et al.*, 2002). Today domesticated common carp is one of the most important species in freshwater fish culture mostly raised for human consumption, especially in Asia, with an annual production currently exceeding 3.2 million tones (Khalili and Amirkolaie, 2010). One of the major bacterial pathogens in India, *Aeromonas hydrophila* is known to cause a variety of diseases in fish, such as, haemorrhagic septicaemia, infectious dropsy and tropical ulcerative disease and fin rot leading to heavy mortality in aquaculture forms (Karunasagar *et al.*, 1997). *Aeromonas* species are enteropathogens, such strains possess virulence properties, such as the ability to produce enterotoxins, cytotoxins, haemolysins and or ability to invade epithelial cells. The main virulence factors of aeromonas species that can be associated with gastroenteritis (Seethalakshmi *et al.*, 2008). During an outbreak of disease among Indian major carps the causative agent was found to be *A. hydrophila* (Katoch *et al.*, 2003). *Ocimum sanctum* may act at various levels in the immune mechanism, such as, antibody production, release of mediators of hypersensitivity reactions and tissues responses to these mediators in the target organs in modulating the humoral immune response (Mediratta *et al.*, 2002). *Ocimum sanctum* is a medicinal plant in traditional system of medicine, different parts (leaves, stem, flower, root, seeds and even whole plant) of *Ocimum sanctum* Linn. have been recommended for the treatment of bronchitis, malaria, diarrhea, dysentery, skin disease, arthritis, eye diseases, insect bites and so on. The *O. sanctum* L. has also been suggested to possess anti-fertility, anticancer, antidiabetic, antifungal, antimicrobial, cardioprotective, analgesic, antispasmodic and adaptogenic actions (Pattanayak *et al.*, 2010). Plant extracts can act as immunostimulants even at low concentration and hence, its use could be very cost effective. It is bio degradable and environment friendly. Preparation of leaf extract is simple and inexpensive (Logambal *et al.*, 2000). Hence, the present study deals with development of immunity by extract of medicinal plant *Ocimum sanctum* on common carp, *Cyprinus carpio* (L.) injected with heat killed *Aeromonas hydrophila*. The following parameters, such as, mortality and survival, growth, antibody titre, phagocytic activity, hepatosomatic index (HSI) were studied.

MATERIALS AND METHODS

Experimental animal and maintenance: Clinically normal and alive fingerlings were collected from Meenakshi fish farm at Madurai, Tamil Nadu. The collected fishes were transported to the laboratory in polythene bags containing oxygenated water. They were acclimated to laboratory conditions for 15 days in non-chlorinated water in glass tanks. Proper aeration was maintained and fed *ad-libitum*.

Experiment: Methanol extract of *Ocimum sanctum* were prepared and 10, 20 and 30 ppm dilutions were used for the injection. The 10^6 dilution of *Aeromonas hydrophila* (100 µL) was taken and injected into intraperitoneally, seven and three days after primary and secondary administration of plant extract in experimental fish. The control fish received no plant extract.

The present study has been carried out November 2006 to April 2007 (06.11.2006 30.04.2007). The mortality rate and weight gain of *C. carpio* was calculated using standard procedure following the method of Balasubramanian (2006). The antibody titre was recorded and expressed as $\log_2$ antibody titre of serum following bacterial agglutination assay (Michael, 2000). The phagocytic ratio was found out by counting under microscope. The hepatosomatic index is computed by gravimetric method.

RESULTS AND DISCUSSION

The present research was conducted to study the immuno modulatory effect of the plant extract, Tulsi, *Ocimum sanctum* on common corp, *Cyprinus carpio* against heat killed *A. hydropila* as an antigen. The control fish showed 40% mortality on the 15th day. The plant extract treated groups show no mortality (Table 1). Similar results were also obtained by various scientists. The fingerlings injected with 100 µL *Aeromonas hydrophila* releaved 80% survival rate in control group, 100% survival for experimental groups fed with vitamin C (Narmadha *et al*., 2007). Similar results were also observed by Anand (2007). They reported that plant extract treated fishes showed no mortality. The control fishes showed 50% mortality.

The control fish showed less weight as the days are increased. However, the plant extract administered experimental groups showed more weight than the control on all days. When compared to different doses of (10, 20 and 30 ppm) *O. sanctum,* more growth was observed in 10 ppm when compared to the control (Fig. 1). Vadivelmurugan *et al.* (2007) reported that the low dose induced weight gain due to the therapeutic effect on bacterial infection. Since anti ulcerative like vicco turmeric cream has greater influence on the health and immune response of the fish, the body weight increased with increasing dosage of vicco turmeric vanishing cream.

The control group showed peak antibody response on 20th day. In the plant extract administered group, the peak antibody response occured on 15th day and more antibody titre was elicited than the control. Low dose of plant extract treated group showed more antibody response than the high dose and control (Fig. 2). Similar observations were observed by Kaliraj *et al.* (2008) in *C. carpio* treated with *Albizia lebbeck* and *Gymnema sylvestre*. Similar results were observed by Soltani *et al.* (2010) in *C. carpio* treated with essential oil *Zartaria multiflora*. However, the high dose did not produce any effect.

Table 1: Cumulative percent mortality rate of the fingerlings of *C. carpio* IP administered with 0.1 mL of 10^6 cfu mL^{-1} of heat killed *A. hydrophila*. Different dose of *Ocimum sanctum* 10, 20 and 30 ppm were administered 7 and 3 days prior to injection of heat killed *Aeromonas hydrophila*

		Days after administration						
	Dose (ppm)	0	5	10	15	20	25	Total mortality rate (%)
Control	0	0	0	30.0	40.0	40.0	40.0	40.0
Ocimum sanctum	10	0	0	0	0	0	0	0
	20	0	0	0	0	0	0	0
	30	0	0	0	0	0	0	0

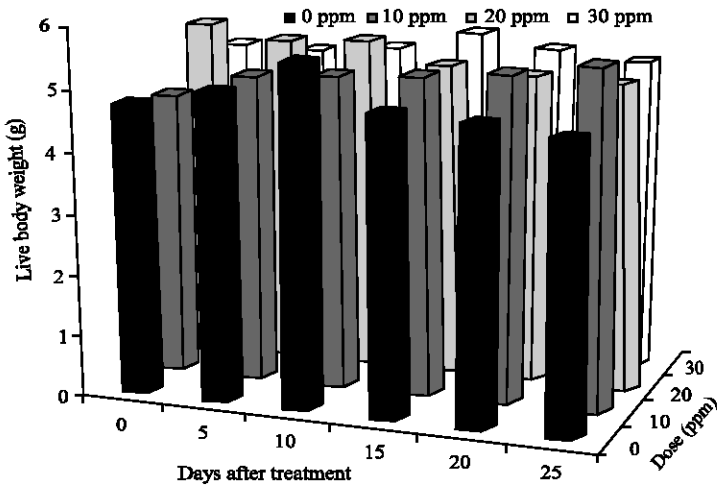

Fig. 1: Live body weight (g) of the heat killed *Aeromonas hydrophila* immunized *C. carpio* treated with different dose of *Ocimum sanctum* 7 and 3 days prior to injection of heat killed *Aeromonas hydrophila*

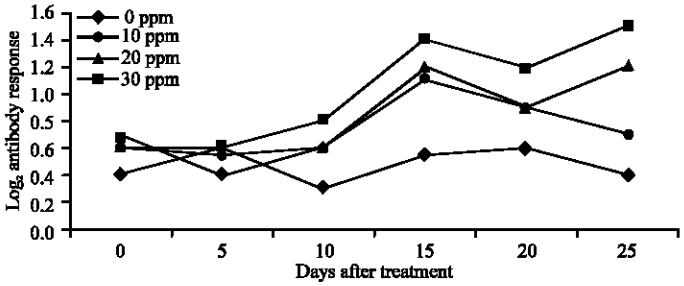

Fig. 2: Antibody titre (log_2 values) of *C. carpio* IP administered with 0.1 mL of 10^6 cfu mL^{-1} of heat killed *Aeromonas hydrophila* cells 100 µL of 10, 20 and 30 ppm of *Ocimum sanctum* were injected 7 and 3 days prior to administration of heat killed *Aeromonas hydrophila*.

The plant extract administered experimental groups showed more phagocytic activity i.e., the plant extracts enhanced the phagocytic activity during the course of 25 days of experiment. The comparison of both control and experimental groups showed more phagocytic activity in the concentration of 10 ppm. *Ocimum sanctum* treated fishes. Therefore, phagocytic activity was found increased in the plant extract treated group than the control (Table 2). Similar results were observed by Durgadevi and Balasubramanian (2009) that the plants extract treated groups showed maximum phagocytic activity than the control groups. Similar results were also observed by Maharajan and Balasubramanian, 2009) in vitamin E treated groups which showed more phagocytic activity when compared to the control groups.

The control group showed maximum Hepatosomatic Index (HSI) on 15th day. The HSI of control group increased upto 15^{th} day and decreased after 20^{th} day (Fig. 3). Similar results were observed by Meenakumari and Jayasreenair, (2000) whose experimental fishes were injected intraperitonially with one ml of *Vibrio anguillarum* culture containing morphological changes and from the analysis it was clear that as the post injection day proceeded, bacteria caused severe damage to the tissue. Slight focal necrosis and vocuolation of hepatocytes were occurred. Congestion

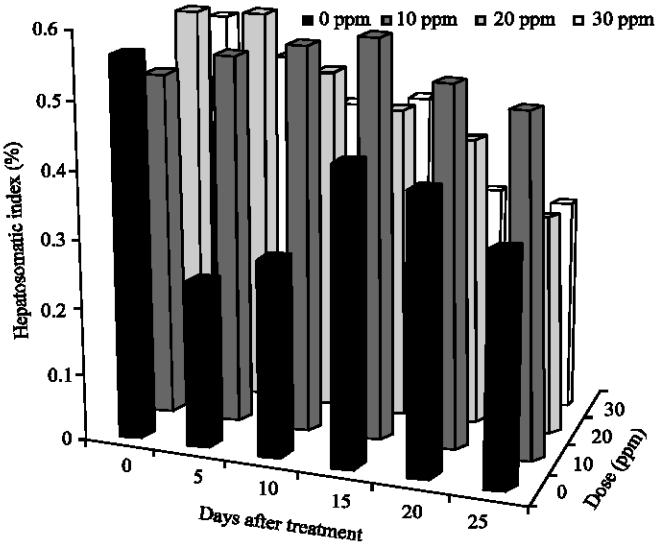

Fig. 3: Hepatosomatic index of C. carpio treated with 100 µL of different concentrations of *Ocimum sanctum*. 0.1 ml of 10^6 cfu mL^{-1} of heat killed *Aeromonas hydrophila* was injected 7 and 3 days after the administration of the plant extracts.

Table 2: Phagocytic activity (%) of blood samples of *C. carpio* treated with 100 µL of different concentrations of plant extract administered prior to the injection of 0.1 mL of 10^6 cfu mL^{-1} of heat killed *Aeromonas hydrophila*

		Days after administration					
	Dose (ppm)	0	5	10	15	20	25
Control	0	0	26.67±20.05	20.00±1.00	46.67±1.53	43.33±1.53	33.33±1.53
Ocimum sanctum	10	0	50.00±30.60	33.33±1.53	70.00±2.00	70.00±4.36	90.00±3.00
	20	0	50.00±1.73	40.00±2.00	56.67±2.08	63.33±3.06	80.00±1.00
	30	0	50.00±2.00	26.67±0.58	56.67±1.53	50.00±1.00	53.33±1.53

and haemorrhages were also found. Degeneration of hepatocytes in the marginal areas and pycnotic nuclei were seen scattered in the neighbouring tissue.

CONCLUSION

The plant extract *O. sancutum* treated fish group showed no mortality and more weight gain. The antibody titre of the plant extract treated fish exhibited earlier and more antibody titre and the phagocytic activity was also more. HSI was found to the normal after plant extract treatment. It is concluded that 10ppm *O. sancutum* is more effective to control *A. hydrophila*. Therefore, *O. sancutum* induces both non-specific and specific immunity.

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