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A Study on the Immunoprotective effect of the Medicinal Plant *Aloe Vera* on the Common Carp *Cyprinus Carpio* (L)

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Abstract

The present study has been carried out to evaluate the immuno stimulatory effect of Aloe vera. A.vera is an active compound and it contains protein, amino acids, and enzymes. Different concentrations (250,500,750mg/kg of food) of extract of Aloe vera were fed to common carp, Cyprinus carpio for 30 days. Fish pathogen, Aeromonas hydrophila with 0.1ml of 10^5CFU/ml was injected intraperitonialy. The cumulative percentage mortality, oxygen consumption and opercular movement were calculated and observed. After the treatment, cumulative percentage mortality, increased opercular movement and oxygen consumption were observed. Thus Aloe vera acts as an immunostimulant to enhance the activity of fish.

Keywords: Cyprinus carpio, Aeromonas hydrophila, Aloe vera, cumulative percentage mortality, Opercular movement, Oxygen consumption.

Introduction

Immunostimulants are valuable for the prevention and control of fish disease in aqua culture as they represent an alternative and supplementary treatment to vaccination. The immunostimulants also have additional effects such as growth enhancement and increase in the survival rates of the fishes under stress[1]. The common carp *C.carpio* is commercially an important fish species in aquaculture[2]. India is rich in rivers, ponds and streams whose major fish fauna consists of *C.carpio.* The aquaculture products were serving as a stable food for people[3]. Riverine fishery is one of the essential economic components of the fishery sector of any state[4]. The fish can eat vegetarian diet of water plants but prefer to scavenge the bottom for insects, crustaceans, crawfish, and benthic worms. The common practice of fish importation for aquaculture was in the form of fingerling (2-3 inches in total length). However, in recent years, eggs, fish larvae (less than 1 inch) and even brood fish are also imported. Such practices contribute to the spread of fish pathogen especially viruses[5].

Aeromonas hydrophila, the most common bacterial pathogen in fresh water fish, *Aeromonas* species are enteropathogens which possess virulence properties, such as, the ability to produce enterotoxins, cytotoxins, haemolysins and ability to invade epithelial cells. The main virulence factors of *A.hydrophila* species that can be associated with gastroenteritis[6].

Immunostimulants seems to be representing a useful alternative to vaccination and chemotherapy in the control of fish disease. They can enhance the non specific immune response[7]. Recently in aqua culture, scores of plant extracts have been tested and used with good results in the control of bacterial and viral disease. *Aloe vera* is an active compound and it includes polysaccharide, mannans, anthraquinones, lectins, salicylic acid, urea, nitrogen, cinnamic acid, phenol, and sulphur. It also contains amino acids, lipids, sterols, tannin and enzymes[8].

The world health organization (WHO) reports that over 70% of the world population needs herbal medicine for primary health care. These medicines are cheaper, non toxic biodegradable and bio compatible. There is a growing interest among researcher and feed companies for the incorporation of herbs in fish feeds as immunostimulants[9]. Fish are an integral component of aquatic ecosystems. In addition to being a desired resource for users of the aquatic habitat, they play important role in energy flow, cycling of nutrients and maintaining community balance in the ecosystem[10].

Aloe vera is a perinial succulent cactus like plant, which grows in hot dry climates. *Aloe vera* supplementation can increase the resistance to *A.hydrophila* and *A.Septicemia*[11]. The relative percent survival was found to be increased in the fish fed with *Aloe vera* so that *Aloe vera* can enhance some of specific and non specific immune responses.ie lysosome activity, serum bacterial activity, total protein and Ig M level. Most of the herbs and herb extracts can be given orally which is the most convenient method of immunostimulation. This study helps to get a wider acceptance for the usage of medical plants in disease prevention and improved health management in aquaculture practices.

Material and Methods

Collection and maintenance of *Cyprinus carpio*: Fingerlings of *C. carpio* were collected from Manju fish farm at Kallidaikurichi, Tirunelveli district, Tamil Nadu, India. The collected fishes (10±1g) were acclimated to laboratory

conditions for 20 days in non chlorinated bore well water. Fishes were reared in the experimental tanks for 30 days in treatment with plant extract supplemented food of different concentrations.

Feed for the fish: The fish feed was prepared from groundnut oil cake, wheat bran and soya bean which were purchased from the local market and were made into the fine granules mechanically. The fine granules were then, made into powder by using a mixie. The powdered groundnut oil cake, soya bean and wheat bran were mixed in the ratio of 5:2:1 and sterilized. A multivitamin tablet was also added. It was made into small pellets[12].

Preparation of experimental feed: The experimental fish were fed with a prepared feed as described earlier with little modification; 250,500,750 mg/kg *A.vera* leaf extract were mixed with sterilized dry diet and fed to healthy normal fish. After 30 days of feeding a challenge test was conducted by injecting the fish pathogen *A. hydrophila,* 0.1 ml of 10^5 CFU/ml of *A. hydrophila* was intraperitonally injected for control and experimental fishes. Every seven days, the Cumulative percentile mortality, oxygen consumption and opercular movement were studied.

Cumulative percentile mortality: It was estimated by observing the number of fish survived throughout the experiment. The survival rate of common carp, *Cyprinus carpio* was obtained by dividing the number of the fish survived to the total number of fishes.

$$\text{Survival rate} = \frac{\text{Number of fishes survived}}{\text{Total number of fishes}}$$

Oxygen consumption in fish: The oxygen consumption of the fingerlings of the control and experimental fish was estimated by Winkler's method.

Opercular movement: The fish is taken in a beaker containing water. The number of opercular movements for a minute was recorded with the help of a stop watch in the control and the experimental fish. The triplicate observation was recorded from each sample for the control and the experimental fishes.

Results and Discussion

The decrease in haemoglobin concentration indicates the fish inability to provide sufficient oxygen to the tissues[13]. It is found that, generally mortality was not observed in 250mg/kg fish group (table-1). The control group showed higher range of (40%) mortality. No Mortality was observed in any group of fish throughout the experimental period[14]. The survival rate challenging the fish, Labeorohita wih A. hydrolium was enhanced when the fishes fed with the diets containing Magnifera kernel[15]. The relative percent survival was found to be increased in fish fed with *A.vera* and is able to enhance specific and non specific immune response in the *C.carpio.*

The opercular movement of fishes in experimental group increased after the treatment (table 2). The length of the fish increased during the period and opercular beats and respiratory rate were also increased. Opercular beat, respiratory rate depend upon size of fish and stress of temperature. Similar finding was observed like a rise in atmospheric temperature due to natural variation would directly influence the water temperature and the ectothermic fishes[16]. The temperature and growth rate are as modulators of the metabolic capacity of fish[17]. The 96 hours LC50 of the household detergents Det-I is 28.5mg/litre and Det-II 41.75mg/litre in Garramullya. They also noticed changes in behavioural responses of Garamullya like opercular movement was 12-15 times more faster than controlled, frequent surfacing, loss of nervous control, try to jump out of media[18].

The experimental group of 250 mg/kg showed increased O_2 consumption of fish (table 3). But the control group showed decrease in oxygen consumption level. The experimental fish treated with 10 mg of turmeric powder showed increase in the O_2 consumption of fish, when compared to 20mg and 30 mg of turmeric powder[19].

Table-1
Cumulative percentage mortality (%) of fingerlings of common carp, *C. carpio* fed with different concentrations of *Aloe vera* and intraperitonially challenged with 0.1ml of 10^5cells of *Aeromonas hydrophila*

Dose (mg/kg) of plant extract	Days after administration					
	0	7	14	21	28	35
Control	0	20	30	40	40	40
250	0	0	0	0	0	0
500	0	0	10	20	20	20
750	0	0	20	30	30	30

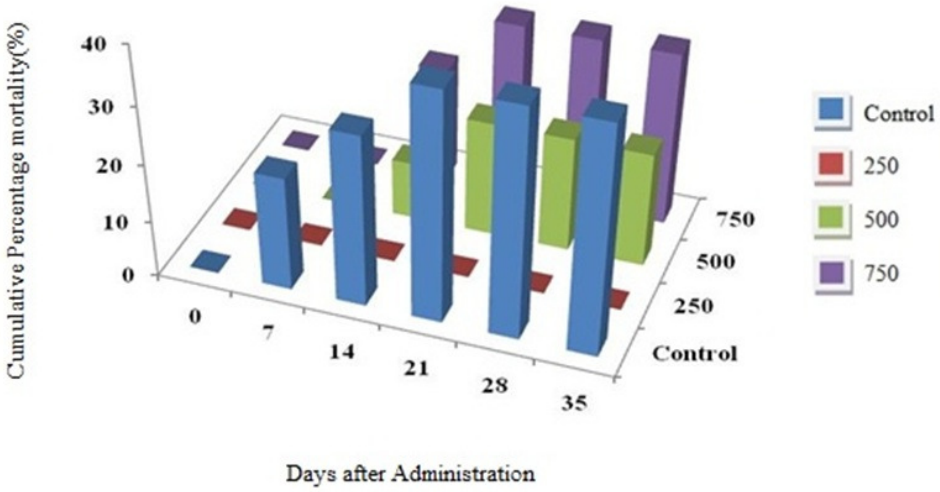

Figure-1
Percentage mortality (%) of fingerlings of common carp, *C. carpio*

Table-2
The opercular movement (No/ minute)of fingerlings of Common carp, *C. carpio* fed with different concentrations of *Aloe vera* and intraperitonially challenged with 0.1ml of 10^5CFU/ml of *A.hydrophila*. Each value (mean ± SD) represents the average performance of five estimations

Dose(mg/kg) of plant extract	Days after administration					
	0	**7**	**14**	**21**	**28**	**35**
Control	88.33 ±1.00	86.66 ±1.52	83.00 ±1.00	81.00 ±1.52	77.33 ±1.00	76.11 ±2.04
250	86.00 ±2.01	91.33 ±1.00	94.00 ±1.52	97.33 ±0.57	99.66 ±1.52	103.00 ±2.00
500	89.30 ±1.00	93.00 ±2.08	98.30 ±1.52	99.60 ±2.00	102.00 ±2.05	105.30 ±1.52
750	90.33 ±2.08	93.66 ±1.00	95.30 ±2.00	98.33 ±2.00	103.00 ±1.08	106.30 ±1.00

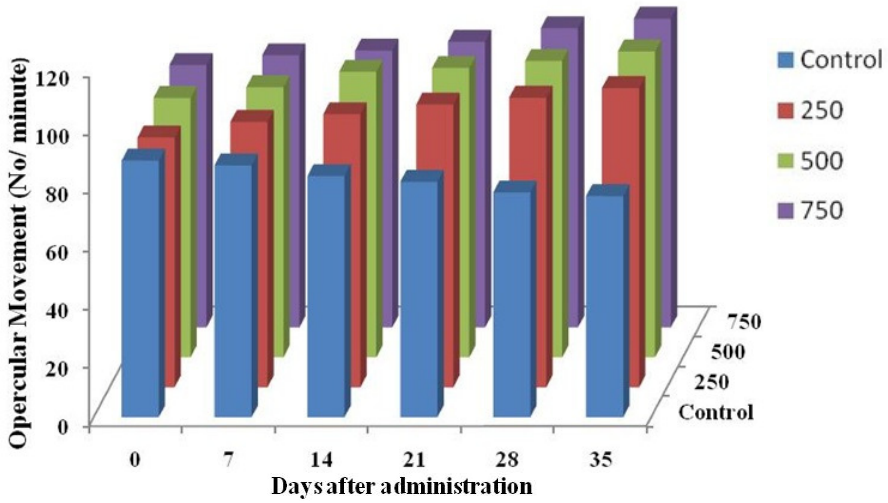

Figure-2
The opercular movement (No/ minute) of fingerlings of Common carp, *C. carpio*

Table-3
Oxygen consumption (mg/g/h) of fingerlings of common *carp, C. carpio* fed with different concentrations of *Aloe vera* and intraperitonially challenged with 0.1ml of 10^5cells of *Aeromonas hydrophila*

Dose(mg/kg) of plant extract	Days after administration					
	0	7	14	21	28	35
Control	0.265 ±0.003	0.231 ±0.003	0.208 ±0.002	0.154 ±0.002	0.142 ±0.002	0.129 ±0.002
250	0.141 ±0.001	0.184 ±0.002	0.203 ±0.002	0.207 ±0.001	0.218 ±0.001	0.236 ±0.001
500	0.105 ±0.001	0.113 ±0.003	0.143 ±0.003	0.155 ±0.001	0.174 ±0.003	0.183 ±0.002
750	0.094 ±0.001	0.065 ±0.002	0.085 ±0.003	0.113 ±0.002	0.123 ±0.002	0.141 ±0.002

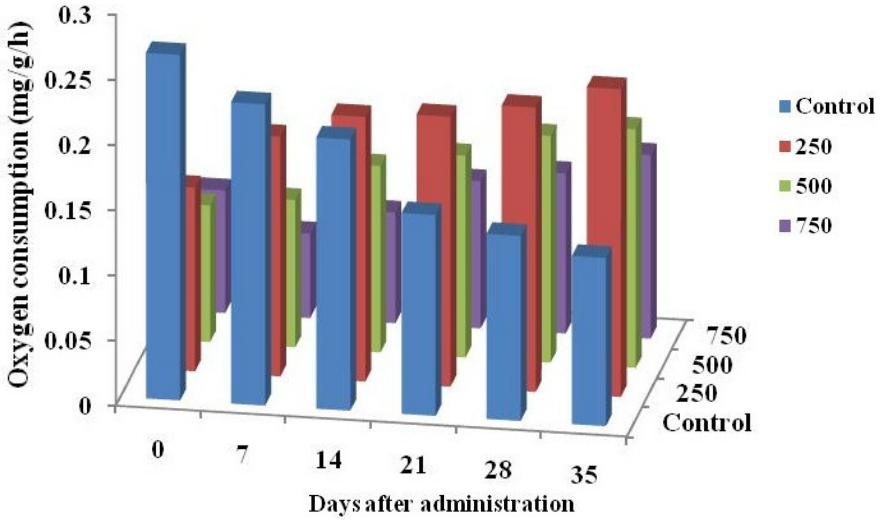

Figure-3
Oxygen consumption (mg/g/h) of fingerlings of common *carp, C. carpio*

Conclusion

According to this study, the plant extract treatment of *Alovera* increased the survival rate of the fish, *C.carpio.* The oxygen consumption and opercular movement of the disease induced fish is low, when compared to the fish treated with the plant extract. Low dose is found to be more effective in curing the disease. Therefore cumulative percentage mortality, the opercular movement and oxygen content of the plant treated fish is correspondingly higher in the experimental fishes. This type of work is useful for further research.

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