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# Therapeutic Effect of *Phyllanthus emblica* on Disease Induced Common Carp *Cyprinus carpio* by *Aeromonas hydrophila*

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## ABSTRACT
The fresh water fish are the important source of animal protein in human population. Fish can adapt themselves to adverse environmental conditions by changing their physiological activities. Immunostimulants like amla (*Phyllanthus emblica*) has the additional effects such as enhancement of growth and increase in the survival rate of the fish under stress. Hematological studies on fish have assumed greater significance due to increasing emphasis on pisciculture and greater awareness on natural freshwater resources in the tropics. The present study focuses on the disease curing effect of the extracts of amla and its role on survival and mortality, opercular movement, oxygen consumption and hematological parameters, such as, leucocytes count and hemoglobin content.

**Key words:** *Cyprinus carpio*, *Aeromonas hydrophila*, *Phyllanthus emblica*, survival, opercular movement , oxygen consumption, leucocyte count, hemoglobin content

## INTRODUCTION
Fish culture is an important industry and its production is increasing worldwide every year. Some countries have sought to improve productivity and profitability by intensification of fish production methods, which can adversely affect fish health and poor environment will lead to an increasing susceptibility to infection (Sakai, 1999). Ancient times, medicinal plants have been used for the treatment of common infectious diseases (Rios and Recio, 2005). The ability of herbs to inhibit bacterial activity has been documented (Dubber and Harder, 2008). Herbs have been used in various countries to control shrimp and fish diseases. The non-specific immune functions, such as, bacteriolytic activity and leucocyte functions were improved by mixtures of chosen Chinese herbs incorporated in shrimps and fish diet (Chang *et al*., 2000). Immunostimulants also have additional effects such as increase in the survival rate under stress (Heo *et al*., 2004). Immunostimulants may be given either alone to activate non-specific defence mechanisms or along with the antigen to enhance the specific immune response.

*Phyllanthus emblica*, also known as amla, has been used in Ayurveda, the ancient Indian system of medicine. It has been used for treatment of several disorders such as common cold, scurvy, cancer and heart diseases. It is believed that the major constituent responsible for these activities is Vitamin C (Ascorbic acid). Ascorbic acid shows antioxidant, anti-inflammatory and anti-mutagenic properties. Blood parameters are considered pathophysiological indicators of the whole body and therefore, are important in diagnosing the structural and functional status of the fish exposed to toxicants (Adhikari and Sarkar, 2004). Hematological studies on fish have assumed greater significance due to increasing emphasis on pisciculture and greater awareness of the pollution of natural freshwater resources in the tropics. Such studies have generally been used as an effect and sensitive index to monitor physiological and pathological changes in fish (Summarwar *et al*., 2012). Hematological analysis can prove valuable knowledge for monitoring the health status of both the wild and culturable fish. Hematological changes depend on the fish species, age, the cycle of sexual maturity and health conditions (Blaxhall, 1972).

*Aeromonas hydrophila* is capable of producing disease and is usually associated with changes in environmental conditions, such as, stress, overcrowding, a sudden change in temperature, transfer of fish, mishandling, poor water quality, high nitrite and carbon dioxide level. A number of immunostimulants include very heterogeneous groups of substances like levamisole, lipo-polysaccharides, glucans, peptidoglycan and muramyl dipeptide on the immune responses have been tested. Immunostimulants seem to be more important as they depend more on non-specific defense mechanisms (Anderson, 1992):

- To evaluate certain physiological and hematological parameters of the fish, *Cyprinus carpio* inoculated with *A. hydrophila*, with the different dose of plant extracts (*Phyllanthus emblica*) added in the diet
- To estimate the proper dose of plant extract for the recovery of the fish

## MATERIALS AND METHODS

Fingerlings of *Cyprinus carpio* were collected from Kallidaikurichi, Manju fish farm at Tirunelveli district, Tamilnadu, India. The collected fish were acclimated to laboratory conditions for 20 days in non-chlorinated bore well water. Fish were reared in the experimental tanks for 30 days in treatment with amla plant extract supplemented food of different concentrations. The fish feed was prepared from groundnut oil cake, wheat bran and soya bean mixed in the ratio of 5:2:1 and was added with multivitamin tablet. It was made into small pellets. The experimental fish were fed with a prepared feed as described earlier with little modification. 250, 500, 750 mg of leaf extract were mixed with 1 kg of sterilized dry diet and fed to healthy normal fish. After 30 days of feeding, the control and experimental fish were intraperitoneally injected with 0.1 mL of $10^5$ CFU $mL^{-1}$ of *Aeromonas hydrophila*.

The mortality and survival rate, oxygen consumption, opercular movement, leucocyte count and hemoglobin content were studied once in every seven days. Survival and mortality was calculated by following the standard procedure. The oxygen consumption of the fingerlings of the control and experimental fish was estimated by Winkler's method. Opercular movement was recorded using the standard procedure. The Total Leucocyte Count (TLC) was made with Neubauer's haemocytometer. Hemoglobin content was analyzed using Shali's-Acid haematin method.

The graduated tube was filled to the 20 mark (on % scale) with 0.1 mL HCl. The blood was drawn by using pipette of Shali's haemoglobinometer to the mark upto 0.02 mL. Wipe the tip of the pipette with cotton, so that no blood was left to stick to its outside. Expel blood into the Shali's tube containing the HCl solution. A small amount of an acid was sucked into the pipette and expel it again into the tube and repeated this twice. The content was mixed quickly but gently with glass-rod for 10 min. The distilled water was drop by drop, mixing between each addition until the colour matched with the standard. The amount of solution in the graduated tube was read and the calibrations gave the hemoglobin concentration in terms of gram/deciliter.

## RESULTS AND DISCUSSION

In the present study, the effect of *Phyllanthus emblica* on disease induced *Cyprinus carpio* was studied (Table 1). The control fish showed 20% mortality on 7th day and 50% of mortality on 35th day but the 250 mg kg$^{-1}$ plant extract treated group showed no mortality. Similar results were observed by Anand (2007). He reported that the plant extract treated fish have no mortality and the control fish showed 50% mortality. Ardo *et al.* (2008) reported that feeding of tilapia with two Chinese medicinal herbs, reduced mortality, when the fish were experimentally infected with fish pathogen, *Aeromonas hydrophila.*

The increasing rate of $O_2$ consumption (mg g$^{-1}$ h$^{-1}$) of fish was observed in response to the decreasing dose of the extract of amla (Table 2). This may be the reason for good survival rate of fish in low concentration of plant extract. Low dose (250 mg kg$^{-1}$) of plant extract treated group showed more oxygen consumption than the higher dose. Tilak *et al.* (2001) reported the damage of gill membrane and change in respiratory pigments, which are the causative factors for the decrease in oxygen consumption. As the days are increased, the fish treated with low dose of plant extract consumed more oxygen as the normal fish. Decreased quantity of $O_2$ consumption in high dose may be due to the fact that the high dose will suppress the normal physiological activity.

The increase in the number of opercular movement was observed with the increase in the plant extract concentration (Table 3). So, also the decreased opercular movement in the control fish is

Table 1: Cumulative percentage mortality of common carp, *C. carpio* fed with different concentrations of *Phyllanthus emblica* and intraperitoneally challenged with 0.1 mL of $10^5$ CFU mL$^{-1}$ of *Aeromonas hydrophila.* Each value represents the average performance of five estimations

| Dose of plant extract (mg kg$^{-1}$) | Days after administration | | | | | |
|---|---|---|---|---|---|---|
| | 0 | 7 | 14 | 21 | 28 | 35 |
| Control | 0.0 | 20.0 | 30.0 | 30.0 | 40.0 | 50.0 |
| Untreated control | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| 250 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| 500 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| 750 | 0.0 | 0.0 | 0.0 | 0.0 | 20.0 | 40.0 |

Values are Mean±SD

Table 2: Oxygen consumption of common carp, *C. carpio* fed with different concentrations of *Phyllanthus emblica* and intraperitoneally challenged with 0.1 mL of $10^5$ CFU mL$^{-1}$ of *Aeromonas hydrophila.* Each value represents the average performance of five estimations

| Dose of plant extract (mg kg$^{-1}$) | Days after administration | | | | | |
|---|---|---|---|---|---|---|
| | 0 | 7 | 14 | 21 | 28 | 35 |
| Control | 0.196±0.03 | 0.209±0.01 | 0.190±0.04 | 0.133±0.02 | 0.128±0.01 | 0.121±0.02 |
| Untreated control | 0.196±0.02 | 0.206±0.02 | 0.225±0.01 | 0.243±0.012 | 0.263±0.012 | 0.266±0.04 |
| 250 | 0.196±0.02 | 0.216±0.02 | 0.226±0.04* | 0.246±0.03* | 0.263±0.01* | 0.266±0.04* |
| 500 | 0.163±0.01 | 0.148±0.02* | 0.196±0.00* | 0.216±0.02* | 0.225±0.01* | 0.263±0.012* |
| 750 | 0.168±0.04 | 0.133±0.01* | 0.149±0.03* | 0.196±0.01* | 0.208±0.01* | 0.216±0.02* |

*Statistically significant, p<0.05, t test, values are Mean±SD

Table 3: Opercular movement of common carp, *C. carpio* fed with different concentrations of *Phyllanthus emblica* and intraperitoneally challenged with 0.1 mL of $10^5$ CFU mL$^{-1}$ of *Aeromonas hydrophila.* Each value represents the average performance of five estimations

| Dose of plant extract (mg kg$^{-1}$) | Days after administration | | | | | |
|---|---|---|---|---|---|---|
| | 0 | 7 | 14 | 21 | 28 | 35 |
| Control | 94.00±2.30 | 92.00±1.00 | 90.00±2.50 | 89.00±1.30 | 84.00±1.20 | 80.00±1.20 |
| Untreated control | 90.00±2.50 | 92.00±1.00 | 93.00±1.30 | 94.00±1.20 | 96.00±1.40 | 100.00±2.80 |
| 250 | 94.00±1.20 | 96.00±1.20* | 98.00±2.20* | 99.00±2.10* | 101.00±2.40* | 103.00±2.40* |
| 500 | 90.00±3.00 | 94.00±2.20 | 96.00±1.40* | 98.00±1.80* | 98.00±2.80* | 100.00±2.80* |
| 750 | 91.00±2.20 | 93.00±1.30 | 94.00±1.10* | 97.00±2.60* | 99.00±2.40* | 102.00±2.60* |

*Statistically significant, p<0.05, t test, values are Mean±SD

Table 4: The total leucocyte count of common carp, *C. carpio* fed with different concentrations of *Phyllanthus emblica* and intraperitoneally challenged with 0.1 mL of $10^5$ CFU mL$^{-1}$ of *Aeromonas hydrophila*. Each value represents the average performance of five estimations

| Dose of plant extract (mg kg$^{-1}$) | Days after administration | | | | | |
|---|---|---|---|---|---|---|
| | 0 | 7 | 14 | 21 | 28 | 35 |
| Control | 3.82±0.03 | 4.37±0.06 | 4.47±0.02 | 3.82±0.03 | 3.81±0.06 | 4.48±0.02 |
| Untreated control | 3.82±0.03 | 3.81±0.06 | 4.16±0.04 | 4.48±0.02 | 5.15±0.03 | 5.45±0.04 |
| 250 | 3.83±0.04 | 4.01±0.06* | 5.45±0.04* | 5.94±0.03* | 5.55±0.04* | 5.71±0.04* |
| 500 | 3.91±0.09 | 4.16±0.04* | 5.15±0.03* | 5.72±0.06* | 5.79±0.02* | 5.82±0.04* |
| 750 | 3.84±0.02 | 4.02±0.03* | 4.48±0.02* | 4.49±0.01* | 4.67±0.03* | 4.85±0.02* |

*Statistically significant, p<0.05, t test, values are Mean±SD

Table 5: Hemoglobin content of common carp, *C. carpio* fed with different concentrations of *Phyllanthus emblica* and intraperitoneally challenged with 0.1 mL of $10^5$ CFU mL$^{-1}$ of *Aeromonas hydrophila*. Each value represents the average performance of five estimations

| Dose of plant extract (mg kg$^{-1}$) | Days after administration | | | | | |
|---|---|---|---|---|---|---|
| | 0 | 7 | 14 | 21 | 28 | 35 |
| Control | 8.51±0.15 | 8.12±0.02 | 8.03±0.03 | 7.85±0.05 | 7.72±0.05 | 7.18±0.08 |
| Untreated control | 7.63±0.05 | 7.90±0.03 | 7.92±0.04 | 8.12±0.03 | 8.21±0.03 | 8.27±0.03 |
| 250 | 8.41±0.05 | 8.54±0.05* | 8.63±0.03* | 8.75±0.04* | 8.54±0.03* | 8.70±0.03* |
| 500 | 7.63±0.05 | 7.77±0.05* | 7.90±0.03* | 8.05±0.045* | 8.21±0.03* | 8.27±0.03* |
| 750 | 7.10±0.05 | 7.36±0.04* | 7.51±0.08* | 7.74±0.04* | 7.92±0.04* | 8.12±0.03* |

*Statistically significant, p<0.05, t test, values are Mean±SD

associated with the diseased condition and also the damaged gills. So, the environmental factors and pathogens alter the physiological activities of the fish. The use of the extracts of amla, controls the bacterial disease in fish culture. Sanauallah and Ahmed (1980) studied that the infestation in the gills of *Lobia rohita* and *Cirrinus mrigala*, differ due to difference in feeding habits of the fish. In the present study, the low dose of plant extract application favours the therapeutic effect of the diseased fish and therefore, the opercular movement in the low dose is in conformity with the oxygen requirement of the fish.

White Blood Cells (WBC) count in experimental groups (250 mg kg$^{-1}$) was significantly higher (p>0.05, statistically significant), when compared with the control group (Table 4). Similar results were observed in Abasali and Mohamad (2010) and Sahu *et al.* (2007), who reported that WBC count was higher in fingerlings of *Labia rohit*a fed with *Mangifera indica* kernel when compared to control. Gopalakannan and Arul (2006) also reported that there was an increase in the WBC count after feeding the carp with immunostimulant like Chitin. The leucocyte count in the low dose treated fish showed the progressive increase in the quantity of cells, when the days are increased as the normal fish, whereas the diseased control fish exhibited the decrease in WBC count.

Hemoglobin content of the plant extract treated groups was found to be increased after the days were increased (Table 5). But in control it was decreased. Similar observations were observed in Abasali and Mohamad (2010), when compared to the control and other experimental groups. Hemoglobin values in all experimental groups also showed an increased trend when compared to control (Prasad and Mukthiraj, 2011). Low dose of plant extract treatment favours the more hemoglobin than the higher dose because at low dose, there was an increased oxygen consumption as in the normal fish. The normal value of hemoglobin in the plant extract treated fish showed the good health status of the fish.

## CONCLUSION

The plant extract treatment of amla increased the survival rate of the fish, *C. carpio*. The oxygen consumption 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, the opercular movement and hemoglobin content of the plant treated fish is correspondingly higher in the experimental fish. This is evidenced by the increase in WBC in the plant treated fish, particularly the low dose treated fish.

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