IMMUNOSTIMULATORY POTENTIAL OF DIETARY AMLA (PHYLLANTHUS EMBLICA) IN GROWTH AND HAEMATOLOGY OF TILAPIA MOSSAMBICUS CHALLENGED WITH PSEUDOMONAS AERUGINOSA

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ABSTRACT
The present study was carried out to evaluate the immunostimulant potential of Amla (Phyllanthus emblica). Formulated diets with different concentrations of ethanolic extract of P.emblica (D1=0%, D2=1% and D3=2%) were fed to T.mossambicus for 40 days, the Specific Growth Rate (SGR), Feed Conversion Ratio (FCR) were calculated and significant increase was observed in SGR & FCR. The experimental fishes were infected with Pseudomonas aeruginosa and after 5 days the haematological parameters like Total Erythrocyte count (TEC), Haemoglobin (Hb), Total leucocyte count (TLC), Differential leucocyte count (DLC), serum total protein, serum albumin and globulin levels were analyzed. The TEC, Hb, TLC, Lymphocytes, Neutrophils and monocyte counts increased significantly in D3 diet fed fishes. Highly significant increase was observed in serum total protein and albumin levels in fishes fed with D2 diet and increase in Globulin levels was insignificant. Basophil and Eosinophil counts decreased significantly. Thus Emblica in fish feed preparations may be included as growth promoter and immunostimulator.

Key words: Phyllanthus emblica, Tilapia mossambicus, Pseudomonas aeruginosa, haematology, Immunostimulants

INTRODUCTION
Aquaculture remains a growing, vibrant and important production sector for high protein food. The growth of intensive aquaculture production has led to a growing interest in treating or preventing fish diseases. Protecting the fishes from disease can be done through two ways. One is by strengthening the Immune power of the, organism to fight the invasion of pathogens, and the second is through medication. Traditionally, antibiotics have been used in aquaculture for the prevention and treatment of bacterial diseases. However, the use of antibiotics in aquaculture poses threats such as development of bacterial strains that are resistant to antibiotic treatment, or the occurrence of antibiotic residues in fish farmed for human consumption. Based on these two considerations, the potential uses of existing antibiotics and approval of new ones for aquaculture are limited. Alternatively, vaccines against specific pathogens have been developed with varying degree of success. The wide range of pathogens in fish farming also limits vaccines’ effectiveness.

Immunostimulants
Immunostimulants seem to represent a useful alternative to vaccination and chemotherapy in the control of fish diseases as they can enhance the non-specific immune response. The Immunostimulants also have additional advantages, such as growth enhancement and increase in the survival rates of the fishes under stress.

Herbs have been used as medicine and an immune booster for humans for thousands of years throughout the world, as many of them are rich sources of volatile oils, saponins, phenolics, tannins, alkaloids, polysaccharides and polyphenolides which are responsible for various activities like anti-stress, appetizer, tonic, anti-microbial and immunostimulant. Recently, in aquaculture the plant extracts from beets and potatoes have been found to be more effective in the control of bacterial and viral diseases. Dietary supplementation of Achyranthes aspera seed stimulated immunity and enhanced resistance to Aeromonas hydrophila infection in Labeo rohita. Feed incorporated with Zingiber officinale and Curcuma longa has enhanced the nonspecific immune response in Cirrhinus mrigala exposed to Pseudomonas aeruginosa.

Amla (Phyllanthus emblica) (Syn: Emblicas officinalis) is one of the foremost plants utilized from antiquity till to date. Amla is regarded as “one of the best rejuvenating herbs” in the Ayurveda, an Indian Tradition medicinal system. Traditionally, the fruit is useful as an astringent, cardiac tonic, diuretic, laxative, liver tonic, diuretic, refrigerant, stomachic, restorative, antipyretic, anti-inflammatory, hair tonic and digestive medicine. The fruits of amla contain a wide variety of phenolic compounds such as tannins, phyllembelic acid, phyllemblin, rutin, curcuminoides and embilco. The fruit is reputed to have a broad range of therapeutic effects including anti-carcinogenesis, and anti-mutagenic, apoptosis, Immunostimulator. Haematology, including erythrocyte count, haemoglobin concentration, haematocrit and leucocyte count has provided valuable information for fishery biologists in the assessment of fish health.

In the present work the ethanolic extract of Amla (Phyllanthus emblica) is incorporated with the formulated diet, fed to Tilapia for 40 days and infected with P.aeruginosa. The immunostimulant potential was assessed by analyzing the haematological parameters.

MATERIALS & METHODS
The experimental fish Tilapia mossambicus (weight 45±5g) were purchased from local fish farm and allowed to acclimate to laboratory conditions for 15 days. During acclimatization they were fed with rice bran and groundnut oil cake ad libitum. During the experimental period the water quality variables: temperature (28±1°C), pH (7.4±0.2), salinity (10±2) and dissolved Oxygen (>5mg/l) were recorded. The water was changed daily in order to maintain the fishes in healthy state. The dry powder of Amla (Phyllanthus emblica) was purchased from the local market (Manufacturers: Zigma Herbal Remedies, Thovalai, Tamilnadu). 10 gram of the
In the present study, the total leucocyte counts (TLC) were counted using the kits. Total erythrocyte counts (TEC) and haemoglobin concentration were also measured. The data were analyzed statistically and students’ t-test was used to test their significance.

**RESULTS**

**Growth Parameters**

The specific growth rate (SGR) and feed conversion ratio (FCR) exhibited an increasing trend in all the three experimental groups; however, it was dose dependent and significantly higher in D3 diet fed fishes (Fig.1 & 2).

**Erythrocytic Parameters**

In the present study, it was observed that the total erythrocyte count (TEC) and haemoglobin concentration levels were higher in both D2 & D3 diet fed fishes over D1 diet fed fishes. However, the increase in the infected fishes was insignificant in D2 diet but highly significant in D3 diet fed fishes (Fig.3 & 4).

**Leucocytic Parameters**

The total leucocyte counts were higher in both Amla extract incorporated diets (D2 & D3) fed fishes than the control diet fed fishes (D1) (Table-1). However, the increase was highly significant only in the D3 diet fed fishes. In differential leucocyte counts, in all the three experimental groups it was observed that the lymphocyte counts were higher followed by neutrophil, monocyte, eosinophil and basophil. The lymphocytes, neutrophils and monocytes exhibited an increasing trend in both D2 & D3 diet fed fishes; however, the lymphocyte and neutrophil increase were significant and highly significant respectively in D3 diet fed fishes only, whereas monocytes exhibited highly significant increase in both D1 & D2 diet fed fishes compared to infected fishes fed with D1 diet. On the other hand, eosinophil and basophil counts exhibited highly significant decrease in both D2 & D3 diet fed fishes as compared to their control counterpart.

**DISCUSSION**

The present study focused to evaluate the immunostimulatory potential of dietary Amla (Phyllanthus emblica) in fish. The fishes fed with Amla extract incorporated diet exhibited an increase in specific growth rate and feed conversion rate. Incorporation of Amla in the diet might have improved palatability, digestion and absorption of nutrients resulting in improved SGR and FCR. Nile Tilapia (Oreochromis niloticus) fed with Echinacea purpurea and Allium sativum fortified diet exhibited significantly higher specific growth rate. Similarly, significant increase in specific growth rate in Nile Tilapia fed with Green Tea (Camellia sinensis) incorporated diet and infected with Aeromonas hydrophila. Thus it is evident that dietary incorporation of Amla plays a significant role as growth promoter. Erythrocytes are a major and reliable indicator of various sources of stress. RBCs transport haemoglobin that in turn transports O₂ and the amount of O₂ received by tissues depends on the maturity of RBCs and amount of Hb. Thus highly significant increase in RBC and Hb levels in D3 diet fed fishes is a response to tolerate stress or on the other hand is a measure to maintain general health. Increase was observed in RBCs in Nile Tilapia fed with Green Tea supplemented diet and infected with Aeromonas hydrophila, similar result were observed in common carp fed with feed incorporated with plants like Inula helenium, Tussilago farfara, Brassica nigra, Echinacea purpurea & Chelidonium majus and infected with Aeromonas hydrophila. Increase in RBCs, Ht and Hb of Nile Tilapia with increasing dietary ginsana levels compared to control fish was also reported. Total and differential leucocyte counts are important indices of non-specific defence activities in fish, as leucocytes are centrally involved in phagocytic and immune responses to parasitic, bacterial, viral and similar challenges. Similarly neutrophilia and monocytosis can be contributed to acute inflammatory response due to infection, as monocytes undergo transformation into macrophages and may be...
involved in phagocytosis and killing of pathogens upon first recognition and subsequent infections. Neutrophil is the first cell to respond to infection within 24 hours, increases during bacterial infections to phagocytose them, but they die after having phagocytosed a few pathogens as they cannot renew their lysosomes used in digesting microbes. Whereas monocytes phagocytose the pathogens not only more efficiently as it is long lived and able to replace lysosomes but also presents the antigens to lymphocytes. Thus significant increase in the TLC, Neutrophil and Monocytes in Amla extract incorporated diet fed fishes can be attributed to the non-specific immune response and increase in Lymphocytes may be a specific pathogen induced Immune response.

An increase in WBC and Lymphocyte counts in Nile Tilapia fed with feed incorporated with Green Tea and infected with Aeromonas hydrophila18, similarly, increase in WBC in common carp fed with feed incorporated with plants like Inula helenium, Tussilago farfara, Brassica nigra, Echinacea purpurea & Helichrysum majus and infected with Aeromonas hydrophila19, the WBC & RBC counts were higher in Lake rohita fingerlings fed with Magnifier indica kernel20, an increase in WBC counts after feeding common carp with ‘Chitin’21, an increase in WBC and lymphocyte counts in Clarias batrachus fed with Musa acuminate peel extract22 and increase in WBC, neutrophil, lymphocyte and monocyte were observed in Cirrhinus mirigala fed with feed supplemented with Ginger and Turmeric and infected with P. aeruginosa9. There is a close relationship between the level of protein synthesis in liver tissue and plasma protein pools, total protein levels in plasma may be elevated due to the increased levels of protein synthesis in liver tissue23. Commonly, increases in the levels of plasma total protein, albumin and globulin in fish are thought to be associated with a stronger innate immune response24. The increase in serum protein content might be in part due to an increase in the WBC, which is a major source of serum protein production such as lysozyme, complement factors and bactericidal peptides25. Serum proteins include various humoral elements of the non-specific immune system and increase in serum total protein, globulin and albumin are likely to be a result of the enhancement of the non-specific immune response of fishes26. Serum albumin not only maintains osmotic pressure but also presents the antigens to lymphocytes. Thus increase in serum Total protein and albumin levels in D3 diet fed fishes in the present experiment may be an indication to increased levels of non-specific immunity and the increased albumin levels may facilitate the transport of more humoral compounds as well as Amla extract in the blood.

Increased levels of serum protein, albumin and globulin levels in Tilapia fed with Green tea incorporated diet and infected with A. hydrophila27. Similar results were also observed in C. carpio fed with feed incorporated with mixed plant extracts (Inula helenium, Tussilago farfara, Brassica nigra, Echinacea purpurea & Helichrysum majus) for 60 days and infected with A. hydrophila27.

CONCLUSION
In the present study it was observed that the Specific Growth Rate and Feed Conversion Ratio of Amla diet fed fishes were significantly higher. Anaemic organisms are prone to infection as the healthy functioning of any cell requires adequate supply of oxygen. As the TEC and Hb values has increased in the Amla diet fed fishes indicating improved health status to combat the pathogens. Increased levels of neutrophils and monocytes in Amla diet fed fishes is a sign of Amla induced improvement of non specific immune response and increase in the lymphocyte counts can be considered as an acute specific immune response. Similarly increased levels of serum total protein can be correlated to the increased levels of TLC both playing a vital role in improving the non specific immunity. Thus from the present study it was observed that incorporation of Amla in fish feed formulations not only acts as Immunostimulator but also as Growth Promoter.

REFERENCES
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Table 2: Comparison of Serum parameters in Tilapia fed with 0% (D1), 1% (D2) and 2% (D3) of Amla incorporated diet and challenged with *P. aeruginosa*.

<table>
<thead>
<tr>
<th>Diet</th>
<th>Total Protein (g/dl)</th>
<th>Albumin (g/dl)</th>
<th>Globulin (g/dl)</th>
<th>A/G ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>D1</td>
<td>3.2±0.3</td>
<td>2.2±0.2</td>
<td>1.0±0.2</td>
<td>2.2±0.3</td>
</tr>
<tr>
<td>D2</td>
<td>3.9±0.4**</td>
<td>3.0±0.4**</td>
<td>0.9±0.1</td>
<td>3.3±0.4**</td>
</tr>
<tr>
<td>D3</td>
<td>3.8±0.3*</td>
<td>2.5±0.3</td>
<td>1.3±0.3</td>
<td>1.9±0.2</td>
</tr>
</tbody>
</table>

* = Significant, ** = Highly Significant

Table 1: Comparison of Total and Differential Leucocytes in Tilapia fed with 0% (D1), 1% (D2) and 2% (D3) of Amla incorporated diet and challenged with *P. aeruginosa*.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>D1</th>
<th>D2</th>
<th>D3</th>
</tr>
</thead>
<tbody>
<tr>
<td>TLC (x 10^9)</td>
<td>4.8±0.7</td>
<td>5.7±0.5</td>
<td>8.2±0.6**</td>
</tr>
<tr>
<td>Lymphocyte (%)</td>
<td>45±2.1</td>
<td>50±3.2*</td>
<td>52±2.7*</td>
</tr>
<tr>
<td>Neutrophil (%)</td>
<td>25±1.8</td>
<td>28±2.3</td>
<td>32±1.5**</td>
</tr>
<tr>
<td>Monocyte (%)</td>
<td>8±1.2</td>
<td>12±0.9**</td>
<td>13±0.7**</td>
</tr>
<tr>
<td>Eosinophil (%)</td>
<td>8±0.6</td>
<td>6±0.3**</td>
<td>2±0.1**</td>
</tr>
<tr>
<td>Basophil (%)</td>
<td>8±0.8</td>
<td>4±0.2**</td>
<td>1±0.1**</td>
</tr>
</tbody>
</table>

* = Significant, ** = Highly Significant