Haematological Studies on Disease Induced Common Carp, *Cyprinus carpio* Fed with Formulated Feed with Plant Extract of *Andrographis paniculata*

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**Abstract:** The present study has been carried out hematological parameters using medicinal plant *Andrographis paniculata* on different concentrations such as, 15mg, 30mg and 45mg formulated diet is studied against 0.1 ml of CFU/ml 10^7 cells *Aeromonas hydrophila* on common carp *Cyprinus carpio*. The 45 mg of plant extract formulated diet showed maximum WBCs content than the control and other experimental groups. Increase in the RBCs count was observed plant extract treated groups, the 45 mg showed more RBCs than the control and other groups. Haemoglobin content was more in plant extract administered group than the control group. The high dose of plant extract (45mg/100g diet) showed more phagocytic activity than the other groups. 45mg was found more efficient.

**Key words:** *Aeromonas hydrophila* • Haemoglobin • RBCs • WBCs • Phagocytes • *Cyprinus carpio*

**INTRODUCTION**

Aquaculture has been a growing activity for the last twenty years worldwide and this impressive development has been attended by some practices potentially damaging to animal health. The continuous use of antimicrobial agents in aquaculture has resulted in more resistant bacterial strains in aquatic environment [1]. Global aquaculture production has been steadily increasing over the last decade. The global production of farmed fish and shell fish (according to FAO categorization) increased from 10.64 million metric tonnes in 1987 to 26.38 million metric tonnes in 1996, indicating an increase of 148%. The corresponding increase in value was 110% from 16.38 billion to US $ 41.55 billion [2].

In aquaculture, infectious diseases are the major problems causing heavy loss of fish farmers. The recent expansion of intensive aquaculture practices has led to a growing interest in understanding fish diseases so that they can be treated or prevented [3]. One of the major bacterial pathogens in India, *Aeromonas hydrophila* is known to cause a variety of diseases in fish, such as, haemorrhagic septicemia, infectious dropsy, tropical ulcerative disease and fin rot leading to heavy mortality in aquaculture forms [4]. During 1994-1995 disease problem struck the booming of aquaculture industry and caused a loss of 10,000-12,000 tones value Rs.250-350 billion [5]. The bacteria *A. hydrophila* were gram negative motile non-sporulating rods occurring singly [6]. During an outbreak of disease among Indian major carps that had more than 75% mortality, the causative agent was found to be *A. hydrophila* [7]. Hence, the present study has been carried out Haematological studies on disease induced common carp, *Cyprinus carpio* (L) fed with formulated feed with plant extract of *Andrographis paniculata*” was planned to evaluate the effect of plant extract the blood parameters of cultured *Cyprinus carpio*. In view of these favourable characteristics the following haematological parameters such as, total RBCs count, total WBCs count, haemoglobin content phagocytic activity were studied.

**MATERIALS AND METHODS**

*Cyprinus carpio* fish (10-15 g) were collected from Ahamed Mohaideen Fish farm Madurai and it’s maintained in the laboratory at 28±1°C in non-chlorinated water. The ground nut oil cake, soy bean and wheat bran were mixed in the ratio of 5:2:1 and sterilized. Add a multivitamin tablet. The above mixed foods were added with different concentrations of plant extract used for experimental fishes and without plant extract food for control fish. The food was made into small pellets. 0.1 ml of 10^7 CFU/ml of
Aeromonas hydrophila was injected intraperitoneally both for plant extract treated fishes and without plant extract treated fishes (Control). After one of the treatment the following haematological studies such as, red and white blood cell counts were determined using neubauer counting chamber. Hemoglobin (Hb) concentrations were determined by Blaxhall and Daisley [8] and Phagocytic cells were counted by under microscope [9]. The statistical significance of difference between control and treated groups were tested by ‘t’ test.

RESULTS AND DISCUSSION

The count of RBCs in the blood of the Cyprinus carpio exposed to be 15mg, 30mg and 45mg of plant extract per 100g of diet. The RBCs count in the control fish was found to be 3.07 ± 1.53. The plant extract treated fishes showed the RBCs 6.10±2.00 ×10⁶ cells /ml (15mg) 6.43 ± 2.00×10⁶ cells /ml (30mg) and 6.67±2.08×10⁶ cells /ml (45mg) in the initial day (0 day) (Table 1). The RBC counts were increased with increasing concentrations of plant extract formulated diet in different days of treatment (7, 14, 21 and 28 days). The results were significant at 0.05 levels, p< 0.05 (t-test). Subeenabegum and Navaraj [10] reported that RBC and haemoglobin content were enhanced in fish fed herbal diets compared to the control group. Harikrishnan et al 2011 [11] also reported that mixed herbal extract supplementation diets restored the altered haematological parameters and triggered the innate immune system of goldfish [12] against A. hydrophila infection.

In the present study the WBC counts were varied from experimental and control fishes. The plant extract formulated diet treated fishes showed more number of WBC cells were observed, when compared to control (diseased) and negative control (normal fish). The WBCs count in the control fish was found to be 2.10 ± 1.00 (Table 1). The plant extract contain formulated diet treated fishes showed maximum number of WBCs were observed in 45mg plant extract mixed diet found to be 3.20 ± 1.00 in the initial day (0 day) and 6.33 ± 0.58 (28 day). These results were supported by Innocent, Fatima and Sivarajani [13] reported that WBCs count was increased with increasing concentration of leaf extract Plumbago rosea formulated diet treated with disease induced Catla catla. Data [14] reported that White blood cell counts were significantly higher in herbal growth promoter feed additive in fish meal on the performance of Nile Tilapia, Oreochromis niloticus (L.). Similar results were also observed by Adewoye [15] observed by increase in the level of White Blood Cell (WBCs) in the group that received the Tephrosia vogelii extract compared with the control in Clarias gariepinus fishes.

Hemoglobin content on disease induced common carp; Cyprinus carpio fed with formulated feed with plant extract of Andrographis paniculata were studied in different days of treatment (0 day to 28 days). In the control fishes showed low level of hemoglobin content (4.83 ± 0.15 g/dl) when compared to negative control fishes (6.20 ± 0.20 g/dl) (Table 1). The different concentrations of plant extract formulated diet treated fishes showed gradually increased the haemoglobin content in different days of treatment. The results were significant at 0.05 level, p< 0.05 (t test). Binukumari and Subisha [16] observed that fingerlings of Oreochromis niloticus exposed to lethal concentration of Moringa oleifera showed an increase in haemoglobin, RBC and WBC count when compared with control fishes. Oral administration of the medical plant, Eclipta alba, on the non-specific immune responses and disease resistance of tilapia (Oreochromis mossambicus) has been investigated [17]. The results indicated that E. Alba administered in the diet significantly enhanced the haematological parameters. Furthermore, when tilapia was challenged with Aeromonas hydrophila mortality was significantly reduced in E. Alba treated fish. Abasali and Sudagar [18] also reported that herbal immunostimulant diet shows significantly increased haemoglobin content in disease induced common carp, Cyprinus carpio.

| Table 1: Haematological changes of C. Carpio intraperitoneally injected with 0.1 ml of 10⁷ CFU / ml of Aeromonas hydrophila and treated with different concentrations of leaf extract of Andrographis paniculata |
|------------------------------------------------------------|------------------------------------------------------------|------------------------------------------------------------|------------------------------------------------------------|
| Days after WBCs CFU/ml                                    | RBCs CFU                                                   | Haemoglobin g/dl                                           | Phagocytes                                                 |
| Treatments                                                | Con 15 30 45                                               | Con 15 30 45                                              | Con 15 30 45                                               | Con 15 30 45                                              |
|-------------------------------------------------------------------------------------------------------------------------------|
| Different concentrations (mg) of plants extracts formulated food                                                              |

<table>
<thead>
<tr>
<th>Treatment</th>
<th>WBCs CFU/ml</th>
<th>RBCs CFU</th>
<th>Haemoglobin g/dl</th>
<th>Phagocytes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>2.10±1.00</td>
<td>2.80±2.08</td>
<td>3.06±1.52</td>
<td>3.20±1.00</td>
</tr>
<tr>
<td>0mg</td>
<td>3.07±1.53</td>
<td>6.10±2.00</td>
<td>6.43±2.08</td>
<td>6.67±2.08</td>
</tr>
<tr>
<td>15mg</td>
<td>4.83±1.05</td>
<td>5.20±2.05</td>
<td>5.47±2.01</td>
<td>5.70±2.05</td>
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<tr>
<td>30mg</td>
<td>6.43±1.05</td>
<td>5.20±2.05</td>
<td>5.47±2.01</td>
<td>5.70±2.05</td>
</tr>
<tr>
<td>45mg</td>
<td>7.10±1.52</td>
<td>5.50±2.08</td>
<td>5.70±2.05</td>
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</tr>
<tr>
<td>7 days</td>
<td>4.30±1.05</td>
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<td>4.50±2.05</td>
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<tr>
<td>14 days</td>
<td>4.30±1.05</td>
<td>5.50±2.05</td>
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<td>5.70±2.05</td>
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<tr>
<td>21 days</td>
<td>4.30±1.05</td>
<td>4.40±2.05</td>
<td>4.50±2.05</td>
<td>4.60±2.05</td>
</tr>
<tr>
<td>28 days</td>
<td>4.30±1.05</td>
<td>4.40±2.05</td>
<td>4.50±2.05</td>
<td>4.60±2.05</td>
</tr>
</tbody>
</table>

*Statistically significant at 0.05 level, p < 0.05 (t-test)
The control group showed more phagocytic activity on 28th day. Plant extracts administered experimental group also showed phagocytic activity on 28th day in all concentrations (Table 1). More phagocytic activity observed in the plant extract treated group than the control. The comparison of both control and experimental groups showed more phagocytic activity in the concentration of 45mg/100g of diet. Therefore, phagocytic activity was found to be increased in the plant extract treated group than the control. The results were significant at 0.05 level, p< 0.05 (t test). Pavaraj et al. [19] reported that Ocimum sanctum leaf extract enhance the phagocytic activity in the experimental fishes C.carpio infected with A. hydrophila. Ardo et al. [20] also reported that feeding Nile tilapia (Oreochromis niloticus) with two herbal extracts (Astragalus membranaceus and Lonicera japonica) alone or in combination significantly enhanced phagocytic cells. Three diet variations, in addition to control (without herbal treatment), were used to determine the effects of Chinese herbs (Lonicera japonica and Ganoderma lucidum) on non specific response of tilapia fish. 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 [21]. From these study, the high concentration of plant extract mixed diet shows maximum number of RBCs, WBCs, Hemoglobin and phagocytic cells were observed compared to lower concentration of plant extract mixed diet.

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REFERENCES


