**Effects of *Helianthus annuus* on Haematological and Biochemical Parameters in *Labeo rohita* (Hamilton, 1822) Fingerlings infected with *Aeromonas hydrophila***

**ABSTRACT**

In the present study, an attempt was made to appraise the curative potential of locally available plant *Helianthus annuus* leaf powder against *Aeromonas hydrophila,* a bacterial pathogen of *Labeo rohita.* The experimental feed was prepared with different concentrations of *H. annuus* leaf powder (0.5g, 1g, 1.5g, 2g and control) and the same were fed to *L. rohita* fingerlings infected with *A. hydrophila,* individual group wisefor 28 days. The effects of different concentrations of feed containing leaf powder of *H. annuus* on Haematology, Biochemistry and Enzyme parameters in the blood of *L. rohita* fingerlings infected with *A. hydrophila* were studied. The results indicated that the parameters of the infected fish gradually increased from abnormal to normal levels at the cessation of the 28th day of experiment in the blood of fish. This change might be attributed to the intake of *H. annuus* leaf powder, containing feed. The results of the present investigation apparently suggested that the selected plant’s leaf powder had curative potential against *A. hydophila* infected *L. rohita* fingerlings. Therefore, it is concluded that the aqua farmers may be encouraged to utilize the leaf powder of *H. annuus* @ 2.0 g concentration in the diet of *L. rohita* fingerlings to cure the bacterial infection, particularly, *A. hydrophila.*

**Keywords:***Helianthus annuus, Labeo rohita, Aeromonas hydrophila,* Haematology.

**Introduction:**

The world's one of the most important aquaculture species is Carps, in terms of production. It is the primary source of animal protein for billions of people worldwide, where capture fishery and aquaculture serve the livelihoods of more than 10% of the global population (Anon. 2020). Fisheries and aquaculture are the fastest-growing industries in the World (Tacon, 2020). India is one of the major fish producing countries in the world employing over seven million persons in fishing and allied industries and contributing 60 crores annual1y to national income. They have been playing an important role in the economic development front on account of their contribution to food and nutritional security, national income, employment opportunities as well as generating livelihood options (Kumar and Shivani, 2014). The advent of aquaculture practices, the production of aqua crops in India comprising of both Indian major carps and exotic carps contribute about 97% of the total aquaculture production (FAO, 2011).

The average annual increase in global consumption of fish has outpaced population growth. Of the global animal protein consumption, 20% is met by fish suggesting the importance of fish in global food security and nutrition. India ranks second in global aquaculture production and Indian major carps (IMCs) contribute to more than 75% of its aquaculture economy (FAO, 2020). *Labeo rohita* (Rohu) is an IMC and among the top eleven finfish species produced in world aquaculture (FAO, 2020). It has high growth potential and very popular regarding consumer preference. Hence, it is considered the most important freshwater species cultured in India. There are several species of aeromonads such as *Aeromonas hydrophila* that can infect fish and other aquatic animals and spread disease to them (Abdella *et al*., 2017; Fernández-Bravo and Figueras 2020).

Aquaculture industries are affected by certain major pathogens such as viruses (Gomez-Casado *et al*., 2011; Vega-Heredia *et al*., 2012), bacteria (Jacobs *et al*., 2009; Frans *et al*., 2011), fungi (Khoo, 2000; Ramaiah, 2006) parasites (Brooker *et al*., 2007; Guo and Woo, 2009) and other undiagnosed and emerging pathogens is now a primary constraint to the culture of many aquatic species, obstructing both economic and social development in many countries and a significant constraint on aquaculture production and trade (Smith, 2006). Disease outbreaks elevated the mortality rate and decrease production efficiency, causing high economic loss to the fish farmers (Madhuri *et al*., 2012; Verma and Gupta, 2015). The most common fish diseases include columnaris, gill disease, ick (ich), dropsy, tail and fin-rot, white spot disease, pop-eye, cloudy eye, swim bladder disease, lice and nematode worms’ infestation (Aquatic community, 2011). In fish, the disease susceptible organs and tissues include skin, gills, fins, liver, kidney, spleen, and intestine (Wassif and Mohammed 2022).

*Aeromonas* species are widely distributed throughout the world and one of the causative pathogens for a variety of fish, animal and human diseases in general and particularly in aquaculture (Martínez-Murcia *et al*., 2005). *A. hydrophila* causes exophthalmia, fin root, darkened and ulcerative lesions on the body and even severe bleeding (Austin and Austin, 2007; Hardi *et al*., 2016). Several *Aeromonas* sp., such as *A. hydrophila, A. sobria,* and *A. salmonicida,* are the causative agents of bacterial septicemia in aquaculture. The mortality rate caused by *Aeromonas* infection could be over 95 % (Zhan *et al*., 2004).

According to Gabriel *et al.* (2004) haematological parameters are directly related to the response of the animal where the fishes live. Hematological, serum biochemical and immunological characteristics are among the important indices of the status of the internal environment of the fish (Edsall, 1999; Luskova *et al*., 2002). Many plant compounds have been found to have non-specific immuno-stimulating effects in humans and animals of which more than a dozen have been evaluated in fish and shrimps (Selvaraj *et al.,* 2005; Kamilya *et al.,* 2008; Pandey and Madhuri, 2010; Ye *et al.,* 2011).

Citarasu *et al*. (2006), Sahu *et al*. (2007), Abasali and Mohamad (2010) and Baba *et al*. (2016) reported reduced glucose levels in different fishes fed with a herbal extract of *Avena sativa* immuno-stimulant diet indicating the capability of plant extract to reduce the effect of stress. Jagruthi *et al*., (2014) found an increase in glucose level in carp fed with herbal extract of *A. sativa* supplementation diet for four weeks; feeding with the plant extracts might have acted like a stress indicator in fish. The herbal drugs act as antistressor and induce immunological parameters such as serum lysozyme activity, SOD, NOS and levels of total protein, globulin and albumin (Wu *et al.,* 2007). Hence, the present study was aimed to evaluate the effects of dietary supplementation of *H. annuus* leaf powder on Haematological parameters of *L. rohita* fingerlings challenged with *A. hydrophila* for the experimental period of 28 days.

**MATERIALS AND METHODS:**

**Collection of Fish Fingerlings**

Infected freshwater fish fingerlings (12 ± 4 g) of *Labeo rohita* (Rohu) were collected from Goldfish Farms at Valamburi Street, Karanthai, Thanjavur District and Tamil Nadu.

**Physiochemical parameters of water**

The methods suggested by APHA (1998) were adopted for the estimation of water quality characteristics and the results thereof are provided.

**Preparation of experimental diets**

The ingredients used for the preparation of experimental feeds were Fish meal, Rice bran, Maize, Soybeans, Groundnut oil cake, Wheat flour, Sunflower seed, Cassava flour, Salt, Vitamin and Minerals procured from the local market. The experimental groups were designated as T1, T2, T3, T4 and T5 and they were fed with prepared fish diet. The respective experimental fish fingerlings’ groups were fed with the feed prepared by incorporating *H. annuus* leaf powder at 0.5g (T2), 1g (T3), 1.5g (T4), 2g (T5) and T1 control (without leaf powder), individually. Diseased *L. rohita* fingerlings were distributed randomly into five groups each with ten fishes in each group. These fishes were fed with the feed prepared at four concentrations and observed at 7, 14, 21 and 28 days for any mortality or clinical signs or signs of recovery from infection. A control group was also maintained to make a comparison with the experimental fish groups.

**Collection of Blood**

Blood was collected from the experimental fishes individually, immediately after their capture from tanks by severing the caudal peduncle as suggested by Michael *et al.,* (1994) once at weekly intervals *i.e.,* 7, 14, 21 and 28 days. A portion of blood collected was used to separate the serum for biochemical estimation of the same and the same was stored in the refrigerator for further analysis.

**Haematological parameters**

RBC and WBC counts were determined by the method of Armour *et al.* (1965). The differential Leucocytes count was enumerated by following the method of Sardar *et al.* (2001). The different leucocytes like neutrophils, basophils, eosinophils, lymphocytes and monocytes were identified, counted and expressed in percentage and they were tabulated. Dethloff *et al*. (1999) method was adopted for the estimation of Heamoglobin (g/dl) and the results were recorded.

**Serum Biochemical Analysis**

Biochemical parameters such as Total serum protein, serum albumin, serum globulin and they were quantified by adopting the Biuret method (Johnson *et al.,* 1999). Trinder (1969) method was adopted for Serum glucose estimation. Serum cholesterol was analyzed by the cholesterol oxidase – peroxidase (CHOD- POD) method suggested by Allian *et al.,* (1974). Serum triglycerides level was estimated by the method of glycerol phosphate oxidase – peroxidase (GPO - POD) as described by Trinder *et al.,* (1969). Serum uric acid of the experimental fishes was diagnosed by the method of urease/POD as prescribed by Newman (1999).

**Estimation of Enzymes**

The amount of Alkaline Phosphatase (ALP), Acid Phosphatase (ACP), Aspartate Aminotransferase (AST) and Alanine Aminotransferase (ALT) was estimated by the modified AMP method of Thomas (1998) and Burtis and Ashwood (1999). All Biochemical estimations were performed in Star Plus Semi Auto Analyser. Haematological changes in the control and extract treated *L. rohita* fish were analyzed by Auto Hematology Analyzer (Mindray BC-2800, Shenzhen Mindray Bio-Medical Electronics Co., Ltd., China).

**Statistical analysis**

The values obtained in the present investigation in triplicates were converted into Mean ± Standard Error values and the same are presented in Tables. The data collected on haematology, biochemistry and enzyme parameters were subjected to one-way Analysis of Variance (ANOVA) followed by SNK: Student -Newman Keuls post hoc test for comparing the impact of different concentrations of leaf powder of *H. annuus* and exposure days on the *L. rohita* fingerlings infected with *A. hydrophila* at 0.05 level. All these analyses were done by using SPSS (Statistical Package for Social Sciences) program version 16.0 for windows.

**RESULTS**

The results of physico-chemical parameters of fish tank water and tap water are provided in Table 1

**Table 1: Physico-chemical parameters of the fish tank water and tap water**

|  |  |  |  |
| --- | --- | --- | --- |
| **S.No** | **Parameters** | **Fish tank water**  **Mean ± S.E. (*n*=3)** | **Tap water**  **Mean ± S.E. (*n*=3)** |
| 1 | Temperature (oC) | 22.43±0.12 | 25.33±0.15 |
| 2 | pH | 6.46±0.07 | 6.77±0.07 |
| 3 | Total alkaloids (mg/l) | 623.33 ±14.53 | 224±2.31 |
| 4 | Total solids (mg/l) | 1953.9±0.76 | 517.6±0.89 |
| 5 | Total dissolved solids (mg/l) | 1277.7±0.49 | 876.33±0.73 |
| 6 | Calcium (mg/l) | 43.13±0.55 | 30.65±0.79 |
| 7 | Magnesium (mg/l) | 556.34±0.98 | 196.38±0.68 |
| 8 | Chloride (mg/l) | 0.02±0.00 | 0.02±0.00 |
| 9 | Carbonate (mg/l) | 16.10±0.06 | 18.20±0.12 |
| 10 | Bicarbonate (mg/l) | 629.94±0.71 | 389.33±0.63 |
| 11 | BOD (mg/l) | 8.14±0.02 | 6.24±0.14 |
| 12 | Dissolved oxygen | 6.5±0.12 | 6.7±0.15 |

The impact of different concentrations of feed consisting of leaf powder of *H. annuus* on total RBC, WBC and Haemoglobin content of *L. rohita* fingerlings infected with *A. hydrophila* is presented in Table 2. It is obvious from the results that the control group (Treatment I) had significantly lowest Total RBC, Total WBC and Haemoglobin during all through the four weeks period when compared to other experimental groups. However, in the treatment groups (II-V) a significant increase was observed in the Total RBC, Total WBC and Haemoglobin count as compared to Control (ANOVA; p<0.05) and this might be due to the intake of *H. annuus* leaf powder containing feed (Table 2). There was a significant difference between all the experimental groups in respect of total RBC, total WBC and Haemoglobin count except Treatment V and Treatment I (SNK Test, p<0.05) (Table 2). The blood extracted from the *L. rohita* fed with the diet consisting of 0.5g of *H. annuus* leaf powder on the 7th day of exposure had the lowest RBC (2.33±0.09106/mm3), WBC (13.76±0.20 103/mm3) and Haemoglobin (5.23±0.12g/dl), respectively. On the other hand, the blood obtained from the fish fed with a diet containing 2.0g of *H. annuus* leaf powder on the 28th day of exposure had the highest number of RBC *i.e.,* 3.27±0.09(106/mm3), WBC *i.e.,* 20.65±0.19 (103/mm3) and Haemoglobin (9.17±0.09g/dl) and it is almost similar to normal total RBC, total WBC and Haemoglobin count (Table 2).

**Table 2: Blood parameters of *L. rohita* (infected with *A.hydrophila* – Treatments I - V) fed with various concentrations of diet containing leaf powder of *H. annuus***

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| **Blood parameters** | **Group/**  **Days** | **Normal** | **Treatment**  **I (Control)** | **Treatment II (0.5 g)** | **Treatment III (1.0 g)** | **Treatment IV (1.5 g)** | **Treatment V (2.0 g)** |
| **Mean ± SE (*n*=3)** | | | | | |
| **RBC 106/mm3** | 7 Days | 3.06±0.01a | 2.20±0.12abcd | 2.33±0.09abc | 2.33±0.07a | 2.37±0.03a | 2.43±0.03ab |
| 14 Days | 3.14±0.02bc | 2.20±0.06abcd | 2.37±0.07abc | 2.43±0.03bc | 2.63±0.09bc | 2.67±0.09ab |
| 21 Days | 3.17±0.01bc | 2.23±0.09abcd | 2.50±0.06abc | 2.57±0.09bc | 2.80±0.06bc | 3.03±0.07cd |
| 28 Days | 3.29±0.04d | 2.50±0.06abcd | 2.70±0.06cd | 2.83±0.07d | 3±0.06cd | 3.27±0.09cd |
| **WBC 103/mm3** | 7 Days | 21.62±0.19a | 12.70±0.13a | 13.76±0.20a | 14.93±0.20a | 15.65±0.18ab | 16.60±0.20a |
| 14 Days | 22.41±0.15bc | 13.94±0.14b | 14.67±0.13b | 15.69±0.17b | 17.67±0.19ab | 18.67±0.15b |
| 21 Days | 23.61±0.18bc | 15.79±0.09c | 16.79±0.11c | 17.76±0.15cd | 18.58±0.19c | 19.64±0.20c |
| 28 Days | 22.62±0.17d | 16.69±0.20d | 17.70±0.14d | 18.66±0.11cd | 19.74±0.13d | 20.65±0.19d |
| **HB g/dl** | 7 Days | 9.04±0.01a | 4.9±0.05abc | 5.23±0.12a | 5.44±0.07a | 5.80±0.06a | 6.07±0.09a |
| 14 Days | 9.11±0.01bc | 5.4±0.15abcd | 5.43±0.67b | 5.87±0.88b | 6.67±0.88b | 7.67±0.88b |
| 21 Days | 9.12±0.02bc | 5.53±0.88abcd | 5.77±0.88c | 6.50±0.58c | 7.63±0.88c | 8.37±0.88c |
| 28 Days | 9.18±0.01d | 5.83±0.03abcd | 7.53±0.88d | 8.03±0.03d | 8.50±0.06d | 9.17±0.09d |

**RBC - Red Blood Cell, WBC- White Blood Cell, HB- Haemoglobin**

**Mean ± SE values are followed by different superscript letter(s) in the same column for each parameter denotes they are statistically significant (ANOVA; SNK Test, p<0.05)**

The effect of different concentrations of feed consisting of leaf powder of *H. annuus* on different leucocytes of *L. rohita* fingerlings infected with *A. hydrophila* is given in Table 3. The various leucocytes count was found to be lower in the blood extracted from the control group *i.e.,* Treatment I throughout the experimental period than the other Treatment groups (II-V).It is evident from the results, statistically, a significant increase was observed in basophils, eosinophils, lymphocytes, monocyte and neutrophils count of *L. rohita* fingerlings collected from Treatment V (ANOVA, p<0.05; vide Table 3). The observed increase in basophils, eosinophils, lymphocytes, monocyte and neutrophils count was statistically different between Treatment I and Treatment IV (SNK Test, p<0.05; vide Table 3). The basophils, eosinophils, lymphocytes, monocyte and neutrophils count ranged from 2.33±0.33%,1.33±0.33%, 17.33±0.33%, 4±0.00% and 2.67±0.58% (on the 7th day of exposure at 0.5g concentration level) to 4.33±0.33%, 4.33±0.33%, 26.33±1.20%, 9.33±0.33% and 6.33±0.33% (on the 28th day of exposure at 2.0g of concentration), respectively*.* The basophils, eosinophils, lymphocytes, monocyte and neutrophils count in the present investigation was gradually increased during different exposure days and at treatment groups and almost reached normal values in the blood of *L. rohita* fingerlings (fed with the diet consisting of 2.0g of *H. annuus* leaf powder) at the end of the 28th day.

**Table 3: Differential leucocytes count of *L. rohita* (infected with *A.hydrophila* – Treatments I - V) fed with various concentrations of diet containing leaf powder of *H. annuus.***

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| **Blood parameters** | **Group/**  **Days** | **Normal** | **Treatment**  **I (Control)** | **Treatment**  **II (0.5 g)** | **Treatment III (1.0 g)** | **Treatment IV (1.5 g)** | **Treatment**  **V (2.0 g)** |
| **Mean ± SE (*n*=3)** | | | | | |
| **Basophils %** | 7 Days | 3.67±0.33ab | 2±0.00abc | 2.33±0.33abcd | 2.67±0.33abcd | 3±0.00abc | 3.33±0.33ab |
| 14 Days | 4±0.00abc | 2.33±0.33abc | 2.67±0.33abcd | 3±0.00abcd | 3.33±0.33abcd | 3.67±0.33abcd |
| 21 Days | 4.67±0.33bcd | 2.67±0.33abc | 3±0.00abcd | 3.33±0.33abcd | 3.67±0.33abc | 4±0.00bcd |
| 28 Days | 5±0.00cd | 3±0.00d | 3.33±0.33abcd | 3.67±0.33abcd | 4±0.00bcd | 4.33±0.33bcd |
| **Eosinophils %** | 7 Days | 4±0.00ab | 1.33±0.67abc | 1.33±0.33abcd | 1.67±0.33abc | 2±0.00a | 2.33±0.33a |
| 14 Days | 4.67±0.33abcd | 1.67±0.33abc | 2±0.00abcd | 2.33±0.33abcd | 2.67±0.33bcd | 3.33±0.33bc |
| 21 Days | 5±0.00bcd | 2±0.00abcd | 2.33±0.33abcd | 2.67±0.33abcd | 3.33±0.33bcd | 3.67±0.33bc |
| 28 Days | 5.33±0.33bcd | 2.33±0.33cd | 2.67±0.33abcd | 3.33±0.33bcd | 3.67±0.33bcd | 4.33±0.33d |
| **Lymphocytes %** | 7 Days | 26.45±0.17a | 15.67±0.33abc | 17.33±0.33abcd | 19.67±0.33a | 22±1.00a | 22.33±0.88 a |
| 14 Days | 27.62±0.15b | 16.33±0.67abc | 18.33±0.88abcd | 21±1.16bcd | 24.67±1.20b | 24.67±1.76bcd |
| 21 Days | 27.44±0.07c | 15.33±0.33abc | 17±0.58abcd | 20.33±0.88bcd | 23±1.16cd | 25±0.58bcd |
| 28 Days | 28.05±0.09d | 15.33±0.88d | 19.33±0.88abcd | 23.33±1.20bcd | 24.67±0.88cd | 26.33±1.20bcd |
| **Monocytes %** | 7 Days | 9.29±0.12a | 3.67±0.33abc | 4±0.00ab | 4.33±0.33a | 4.67±0.33a | 5.67±0.33ab |
| 14 Days | 8.90±0.03bc | 4±0.00abc | 4.33±0.33ab | 5.33±0.33bc | 6±0.00bc | 6.67±0.33ab |
| 21 Days | 9.61±0.10bc | 4.67±0.33abc | 5.33±0.33c | 6±0.00bc | 6.67±0.33bc | 7.67±0.33bc |
| 28 Days | 9.61±0.10cd | 5.67±0.33d | 7.33±0.33d | 7.67±0.33d | 8.33±0.33d | 9.33±0.33d |
| **Neutrophils %** | 7 Days | 6.50±0.06a | 2.33±0.33abcd | 2.67±0.58abcd | 3±0.58acb | 3.33±0.33abcd | 3.67±0.33a |
| 14 Days | 6.53±0.23bc | 2.67±0.33abcd | 3.33±0.33abcd | 3.67±0.33acb | 4.33±0.88abcd | 4.67±0.33bc |
| 21 Days | 6.90±0.06bc | 3.33±0.33abcd | 3.67±0.33abcd | 4±0.58acb | 4.67±0.33abcd | 5.67±0.33bc |
| 28 Days | 7.47±0.07d | 3.67±0.33abcd | 4±0.58abcd | 4.33±0.33cbd | 5.33±0.33abcd | 6.33±0.33cd |

**Mean ± SE values are followed by different superscript letter(s) in the same column for each parameter denotes they are statistically significant (ANOVA; SNK Test, p<0.05).**

The results of the present study revealed that the biochemical parameters (Total Protein, Albumin, Globulin, Glucose, Cholesterol, Triglycerides and Uric Acid) in the Treatment I (Control Group) was comparatively lower than that of the other treatment groups *i.e.,* II-V. A significant increase could be observed in the Total Protein, Albumin, Globulin, Glucose, Cholesterol, Triglycerides and Uric Acid content in the blood of *L. rohita* fingerlings of all other Treatment Groups as compared to the Treatment I (Control Group) (ANOVA, p<0.05; vide Tables 4-5). The difference in Total Protein, Albumin, Globulin, Glucose, Cholesterol, Triglycerides and Uric Acid content between control and experimental groups was found to be statistically significant (at four different concentrations and exposure days) except Treatment III and Treatment IV (SNK Test, p<0.05; vide Tables 4-5). The blood extracted from *L. rohita* fingerlings fed with diet containing 2.0g of *H. annuus* leaf powder (on the 28th day of the experiment) had the highest amount of Total Protein, Albumin, Globulin, Glucose, Cholesterol, Triglycerides and Uric Acid content 2.29±0.06g/dl, 2.12±0.04mg/dl, 2.39±0.02g/dl, 106.81±0.03g/dl, 129.65±0.53mg/dl, 236.73±1.55mg/dl and 0.98±0.01mg/dl, respectively. On the other hand, the blood obtained from *L. rohita* fingerlings fed with a diet containing 0.5g of *H. annuus* leaf powder (on the 7th day of exposure) had the lowest amount of Total Protein, Albumin, Globulin, Glucose, Cholesterol, Triglycerides and Uric Acid content 1.67±0.01g/dl, 1.36±0.03mg/dl, 0.99±0.00g/dl, 79.88±0.01g/dl, 74.58±0.02mg/dl, 198.21±0.92mg/dl and 0.24±0.02mg/dl, respectively (Tables 4-5)*.* It is apparent from the results of the present investigation, at the cessation of 28th day of an experiment, the biochemical parameters almost reached to normal levels where the *L. rohita* fingerlings fed were with diet consisting of 2.0g of *H. annuus* leaf powder and it indicated that the fingerlings recovered from *A. hydrophila* infection.

**Table 4: Biochemical parameters of *L. rohita* fingerlings (infected with *A. hydrophila*) fed with various concentrations of diet containing leaf powder of *H. annuus***

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| **Biochemical**  **Parameters** | **Group/**  **Days** | **Normal** | **Treatment**  **I (Control)** | **Treatment II (0.5 g)** | **Treatment III (1.0 g)** | **Treatment IV (1.5 g)** | **Treatment**  **V (2.0 g)** |
| **Mean ± SE (*n*=3)** | | | | | |
| **Total Protein g/dl** | 7 Days | 2.56±0.05abcd | 1.56±0.01a | 1.67±0.01ab | 1.75±0.02a | 1.86±0.02a | 1.94±0.02abc |
| 14 Days | 2.74±0.03abcd | 1.63±0.03bc | 1.75±0.02ab | 1.85±0.02b | 1.94±0.01b | 1.99±0.01abc |
| 21 Days | 2.64±0.04abcd | 1.67±0.01bc | 1.83±0.02c | 1.97±0.01cd | 1.98±0.01cd | 2.07±0.01abc |
| 28 Days | 2.75±0.06abcd | 1.79±0.00d | 1.93±0.01d | 1.97±0.01cd | 2.08±0.01cd | 2.29±0.06d |
| **Albumin mg/dl** | 7 Days | 2.20±0.04ab | 1.34±0.03abcd | 1.36±0.03a | 1.46±0.01a | 1.55±0.02a | 1.56±0.02a |
| 14 Days | 2.27±0.02ab | 1.37±0.01abcd | 1.47±0.01b | 1.53±0.01b | 1.71±0.01b | 1.77±0.01b |
| 21 Days | 2.37±0.01cd | 1.43±0.00abcd | 1.70±0.01c | 1.77±0.15c | 1.89±0.04dc | 1.96±0.01c |
| 28 Days | 2.42±0.02cd | 1.43±0.02abcd | 1.79±0.04d | 1.88±0.17d | 1.94±0.17dc | 2.12±0.04d |
| **Globulin g/dl** | 7 Days | 2.14±0.01a | 0.88±0.01a | 0.99±0.00a | 1.30±0.04a | 1.68±0.01a | 1.78±0.01a |
| 14 Days | 2.19±0.01b | 0.97±0.02b | 1.46±0.01b | 1.99±0.00c | 1.79±0.00b | 1.97±0.01bc |
| 21 Days | 2.28±0.01c | 1.14±0.03cd | 1.65±0.02c | 1.90±0.01b | 2.11±0.03c | 1.98±0.04bc |
| 28 Days | 2.42±0.01d | 1.18±0.01cd | 1.98±0.01d | 2.10±0.03d | 2.25±0.05d | 2.39±0.02d |
| **Glucose g/dl** | 7 Days | 116.39±0.2a | 77.69±0.00a | 79.88±0.01a | 81.17±0.02c | 83.70±0.03b | 85.36±0.02a |
| 14 Days | 116.46±0.1bcd | 88.97±0.01b | 90.32±0.03b | 93.39±0.08a | 95.26±0.10a | 99.87±0.01b |
| 21 Days | 116.48±0.13bcd | 92.82±0.01c | 95.77±0.01c | 99.31±0.04d | 102.68±0.11c | 106.50±0.24cd |
| 28 Days | 116.93±0.02bcd | 94.96±0.02d | 96.99±0.00d | 99.80±0.03b | 104.29±0.05d | 106.81±0.03cd |

**Mean ± SE values are followed by different superscript letter(s) in the same column for each parameter denotes they are statistically significant (ANOVA; SNK Test, p<0.05)**

**Table 5: Biochemical parameters of *L. rohita* fingerlings (infected with *A. hydrophila*) fed with various concentrations of diet containing leaf powder of *H. annuus***

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| **Biochemical**  **Parameters** | **Group/**  **Days** | **Normal** | **Treatment**  **I (Control)** | **Treatment II (0.5 g)** | **Treatment III (1.0 g)** | **Treatment**  **IV (1.5 g)** | **Treatment**  **V (2.0 g)** |
| **Mean ± SE (*n*=3)** | | | | | |
| **Cholesterol mg/dl** | 7 Days | 133.33±0.13a | 72.27±0.03a | 74.58±0.02a | 78.50±0.16a | 80.40±0.04ba | 85.20±0.04a |
| 14 Days | 133.74±0.04bcd | 75.76±0.12b | 76.76±0.02b | 78.36±0.02b | 86.27±0.01ba | 90.72±0.08cb |
| 21 Days | 133.85±0.02bcd | 86.89±0.02c | 88.59±0.15c | 90.72±0.08c | 95.64±0.09c | 100.46±0.02cb |
| 28 Days | 134.28±0.32bcd | 90.15±0.17d | 96.81±0.22d | 108.32±1.20d | 114.88±2.14d | 129.65±0.53d |
| **Triglycerides mg/dl** | 7 Days | 240.67±0.22cabd | 193.80±0.74ab | 198.21±0.92a | 201.72±1.86a | 219.36±0.94bac | 226.55±2.55cab |
| 14 Days | 242.71±0.20cabd | 197.23±0.99ab | 201.47±0.75b | 210.63±1.19bc | 217.43±1.27bac | 228.47±0.90cab |
| 21 Days | 244.57±0.17cabd | 198.54±1.33bcd | 209.79±0.96c | 214.07±2.82bc | 218.20±1.59bac | 231.67±1.99cab |
| 28 Days | 245.65±0.15cabd | 203.05±1.99bcd | 220.87±1.33d | 225.12±2.81d | 233.47±1.86d | 236.73±1.55bd |
| **Uric acid mg/dl** | 7 Days | 1.05±0.02abcd | 0.23±0.01a | 0.24±0.02ab | 0.28±0.01a | 0.31±0.02a | 0.47±0.01a |
| 14 Days | 1.09±0.02abcd | 0.26±0.01bc | 0.29±0.01ab | 0.37±0.01b | 0.49±0.00b | 0.68±0.01b |
| 21 Days | 1.09±0.02abcd | 0.31±0.02bc | 0.49±0.10c | 0.58±0.00c | 0.85±0.03dc | 0.77±0.01c |
| 28 Days | 1.11±0.02abcd | 0.38±0.01d | 0.78±0.01d | 0.84±0.02d | 0.96±0.02dc | 0.98±0.01d |

**Mean ± SE values are followed by different superscript letter(s) in the same column for each parameter denotes they are statistically significant (ANOVA; SNK Test, p<0.05).**

It is understood from the results that the effect of different concentrations of feed consisting of leaf powder of *H. annuus* on various enzymatic parameters *viz.,* Aspartate Aminotransferase (AST), Alanine Aminotransferase (ALT), Acid Phosphatase (ACP) and Alkaline Phosphatase (ALP) of blood collected from the control group (Treatment I) had comparatively lower amount than the other treatment groups (Treatments II-V). The quantity of AST, ALT, ACP and ALP was found to be significantly higher in the blood extracted from *L. rohita* fingerlings of all treatment groups than the Treatment I (Control group) (ANOVA, p<0.05; vide Table 6). The difference in Alkaline Phosphatase between all experimental groups was found to be statistically significant (at four different concentrations and exposure days) (SNK test, p<0.05; vide Table 6)*.* The blood extracted from the *L. rohita* fed with the diet consisting of 0.5g of *H. annuus* leaf powder on the 7th day of exposure had the lowest amount of AST, ALT, ACP and ALP (19.67±0.11 IU/L, 27.89±0.02 IU/L, 0.38±0.06 IU/L and 18.74±0.04 IU/L, respectively).Contrary to this, the blood obtained from the fish fed with diet containing 2.0g of *H. annuus* leaf powder on the 28th day of exposure had the highest amount of AST, ALT, ACP and ALP (29.98±0.01 IU/L 39.47±0.15 IU/L, 1.07±0.04 IU/L and 33.36±0.12 IU/L, respectively). It is obvious from the results that at the cessation of the 28th day of the experiment, all the four enzyme parameters studied in the blood of *L. rohita* fingerlings infected with *A. hydrophila* gradually increased and attained the normal values and this might be due to the intake of *H. annuus* leaf powder containing feed (Table 6).

**Table 6: Enzymatic parameters of *L. rohita* (infected with *A. hydrophila* – Treatments I - V) fed with various concentrations of diet containing leaf powder of *H. annuus***

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| **Blood parameters** | **Group/**  **Days** | **Normal** | **Treatment**  **I (Control)** | **Treatment II (0.5 g)** | **Treatment III (1.0 g)** | **Treatment IV (1.5 g)** | **Treatment**  **V (2.0 g)** |
| **Mean ± SE (*n*=3)** | | | | | |
| AST (IU/L) | 7 Days | 35.57±0.24 b | 18.43±0.06a | 19.67±0.11a | 20.64±0.18a | 22.59±0.09a | 23.52±0.07a |
| 14 Days | 33.53±0.19c | 19.40±0.11b | 21.27±0.09bc | 23.78±0.01b | 25.63±0.11b | 26.85±0.04cb |
| 21 Days | 34.67±0.10 a | 19.92±0.03c | 21.63±0.04bc | 24.92±0.03c | 26.85±0.05d | 28.53±012cb |
| 28 Days | 36.64±0.24d | 20.17±0.05d | 22.66±0.33d | 26.69±0.29d | 27.83±0.07c | 29.98±0.01d |
| ALT (IU/L) | 7 Days | 47.27±0.58c | 26.41±0.00acb | 27.89±0.02a | 29.33±0.09a | 31.37±0.09a | 32.83±0.05a |
| 14 Days | 46.67±0.16bad | 26.93±0.47acb | 28.84±0.07b | 30.85±0.05b | 32.69±0.15b | 33.66±0.10bc |
| 21 Days | 44.83±0.06bad | 26.92±0.02acb | 29.79±0.16c | 31.85±0.04d | 33.78±0.10c | 34.84±0.03bc |
| 28 Days | 47.57±0.14bad | 27.96±0.01d | 30.68±0.09d | 32.39±0.09c | 35.72±0.11d | 39.47±0.15d |
| ACP (IU/L) | 7 Days | 1.47±0.01abcd | 0.27±0.01a | 0.38±0.06a | 0.47±0.01a | 0.59± 0.01a | 0.65±0.02a |
| 14 Days | 1.47±0.01abcd | 0.35±0.01b | 0.62±0.02b | 0.66±0.02b | 0.75±0.01b | 0.88±0.01b |
| 21 Days | 1.48±0.00abcd | 0.49±0.01c | 0.76±0.01c | 0.80±0.01c | 0.87±0.02cd | 1.01±0.03c |
| 28 Days | 1.5±0.01abcd | 0.56±0.02d | 0.94±0.02d | 0.97±0.01d | 1.04±0.16cd | 1.07±0.04d |
| ALP (IU/L) | 7 Days | 33.89±0.03cb | 17.57±0.17a | 18.74±0.04a | 19.60±0.01a | 23.37±0.28a | 21.62±0.13a |
| 14 Days | 32.74±0.13cb | 18.58±0.34b | 20.85±0.03b | 23.30±0.12b | 25.69±0.0b | 29.78±0.02b |
| 21 Days | 32.71±0.09da | 19.55±0.08c | 24.74±0.06c | 27.52±0.14c | 29.52±0.16c | 30.78±0.04c |
| 28 Days | 33.57±0.16da | 20.54±0.18d | 28.52±0.07d | 29.60±0.22d | 32.37±0.12d | 33.36±0.12d |

**AST - Aspartate Aminotransferase, ALT- Alanine Aminotransferase,** [**ACP- Acid phosphatase, ALP- Alkaline Phosphatase**](https://labtestsonline.org/tests/alkaline-phosphatase-alp)

**Mean ± SE values are followed by different superscript letter(s) in the same column for each parameter denotes they are statistically significant (ANOVA; SNK Test, p<0.05).**

**Discussion**

Water quality is directly related to fish production, and it is essential to a healthy, balanced, and functioning of aquaculture system (DeLong *et al.,* 2009 and Bryan *et al.,* 2011).  Bryan *et al.* (2011) reported that an ideal pH range for freshwater aquaculture should range between 6.5 and 7.0, though a pH range of 6.1 to 8.0 is also considered satisfactory for the survival and reproduction of fish. Ngugi *et al.* (2007) gave a range from 20°C to 35°C as ideal for tilapia culture. According to Riche and Garling (2003), the preferred DO for optimum growth of tilapia is above 5 mg/L. The results of the present work are in conformity with these earlier observations.

Haematological parameters are closely related to the response of animals to the environment. Further, it is stated that the environment where fishes live could exert some influence on the haematological characteristics (Mishra *et al.*, 1977). Over the past few years, infectious diseases caused by *A. hydrophila* have become a major problem in fish culture causing heavy economic losses because of high mortalities. Plant-based extracts have been proven to enhance survival and immunocompetence in cultured fish. Several plant extracts that have active ingredients and various biological activities have been reported as suitable for use as supplements in aquaculture (Citarasu 2010, Madhuri *et al*., 2013, Chakraborty *et al*., 2014, Sivasankar *et al*., 2015, Syahidah *et al*., 2015).

It is apparent from the results of the present investigation that the selected plant had curative potential against *A. hydophila* infected *L. rohita* fingerlings. The present findings agree with the earlier results of Abutbul *et al*., (2004) in Tilapia fish fed with a diet containing ethyl acetate extract of *Rosmarinus officinalis* leaf powder. Rao *et al*., (2006) reported the disease resistance against *A. hydrophila* was enhanced in *L. rohita* fed with 0.5% of *A. aspera*.

The haematological values such as RBC and WBC count and haemoglobin obtained in the present study almost in agreement with earlier workers (Sahu *et al.*, 2007). According to the results, *H. annuus* leaf powder could increase hemoglobin content, WBC and RBC levels in experimental groups compared to control group. In accordance with the present findings, Sahu *et al.,* (2007); Gopalakannan and Arul, (2006)reported that WBC and RBC counts were higher in *L.* *rohita* fingerlings fed with *H. annuus* when compared to control. The hematological parameters in the present investigation such as RBC, WBC, hemoglobin and differential leukocytes counts increased significantly during every weeks after feeding with *H. annuus* leaf powder when compared to control group.

Nya and Austin (2009) observed and reported that increased levels of total erythrocytes, total leucocytes, lymphocytes, monocytes, serum total protein, globulin, phagocytic activity, superoxide anion production (respiratory burst activity) serum lysozyme activity, serum bactericidal activity, serum alternate complement pathway activity in *L.* *rohita* infected with *A. hydrophila* after being fed with dietary *Zingiber officinale* for 14 days. The result of the present study corroborates with the results of Nya and Austin (2009).

Similarly, enhanced serum total protein, albumin and globulin values could be recorded in the experimental groups of *L. rohita* fingerlings fed with *A. aspera* seed and *M. indica* kernel (Rao *et al.,* 2006 and Sahu *et al.,* 2007). The results of the present study obviously demonstrate that the total protein, albumin and total globulin levels increased significantly in fish following feeding of medicinal plant mixed diet. The glucose, triglyceride, ALT, AST and ALP of *L.* *rohita* were not influenced by different levels of *H. annuus* leaf powder. The herbal plants may be used as a potential and promising sources of pharmaceutical agents against fish pathogens in organic aquaculture according to Abdul and Haniffa, (2011) and Turker *et al*., (2009**)**.

**Conclusions**

The results of our findings suggest that the haematological and biochemical parameters of the *L. rohita* fingerlings infected with *A. hydrophila* were significantly recovered after being fed with different concentrations of *H. annuus* leaf powder employed in this study. Plants are undoubtedly considered as important sources of potentially useful chemical compounds for the development of new therapeutic agents against fish pathogens. It is apparent from the results of the present investigation that the leaves of *H. annuus* had curative potential against *A. hydophila* infected *L. rohita* fingerlings. Undoubtedly, this plant’s leaves must possess many phyto-compounds which can act against the target bacteria in the *L. rohita* fingerlings and can recover them from infection when they were fed consistently for 28 days @ 2.0g *H. annuus* leaf powder containing feed. Therefore, it is concluded that the aqua farmers may be encouraged to utilize the leaf powder of *H. annuus* @ 2.0 g concentration in the diet of *L. rohita* fingerlings to cure the bacterial infection, particularly, *A. hydrophila.* Further, this plant’s leaves curative potential against other bacterial and fungal pathogens of fishes may also be studied and reported in future.

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