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Health Promoting Biochemical Effects of Three Medicinal Plants on Normal and *Aeromonas hydrophila* Infected *Labeo rohita*

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ABSTRACT

Present study was carried out to assess the influence of three medicinal plants, *Ocimum bascilicum, Adathoda vasica* and *Calendula officinalis* on the biochemical parameters of normal and *Aeromonas hydrophila*-infected fish-*labeo rohita*. Fish of length, 5-9 cm were selected for the experiment. Five sets each of healthy live specimens and *A. hydrophila*-infected fish were kept in parallel pairs and each pair of fish sets were fed ad libitum for 15 days on one of the following compounded feeds: namely, normal fish feed (control groups), feeds supplemented at 3% concentrations with extracts of three plants. At the culmination of feeding experiment, biochemical analysis of serum for enzymes Serum Glutamate Oxaloacetate transferase (SGOT), Serum Glutamate Phospho Transferase (SGPT), Alkaline phosphatase, triglycerides, total sugars, serum protein were carried out from each set. Highest level of SGOT, SGPT, ACP was recorded in *Ocimum basilicum* supplemented group (60.0, 54.1, 26.3 mg dL^{-1}). When infected maximum level was also observed in *Ocimum basilicum* supplemented group for SGOT, ACP (22.3, 19.3 mg dL^{-1}) and for SGPT in *Calendula officinalis* supplemented group (17.5 mg dL^{-1}). Lipid profile show contrasting result with maximum value recorded in infected animals, triglyceride and cholesterol showed maximum value in *Calendula officinalis* supplemented group (88.2, 96.4 mg dL^{-1}) and for total sugar *Ocimum basilicum* supplemented group recorded maximum (96.3 mg dL^{-1}). Highest serum protein content was in *Calendula officinalis* supplemented group (6.0 mg dL^{-1}) and in infected animals in control with 3.2 mg dL^{-1}. This study revealed that feed supplementation of three medicinal plant extracts alters biochemical parameters to overcome disease induced stress in fishes.

Key words: *Aeromonas hydrophila, Labeo rohita, Calendula officinalis, Adathoda vasica, Ocimum basilicum*

INTRODUCTION

In aquaculture several infectious diseases are reported in twentieth century mainly belongs to species of *Pseudomonas, Aeromonas, Streptococcus* and *Vibrio* species and few parasitic origin like Protozoan, Helminthes and Arthropods. Bacterial species causes high mortality and severe economic loss during its outbreak (Raa, 1996; Wedemeyer, 1996; Udomkusonsri and Noga, 2005; Toranzo *et al*., 2005). Skin ulceration and epidermal damage are the most common symptoms that appear on diseased fishes (Camus *et al*., 1998; Samuelsen *et al*., 2006). *Aeromonas hydrophila* and *Pseudomonas* can survive and multiply in the environment which is rich in organic matter and warm waters.

Aeromonas hydrophila infections normally cause high mortality and loss for a period of time. Infestation of fishes commonly associated with stressed condition of habitat. Common sources of stress are poor water quality, overcrowding or rough handling. Aeromonads are ubiquitous, oxidase-positive, facultatively anaerobic, glucose-fermenting and Gram-negative bacteria that are native to aquatic environments. They have been found in brackish, fresh, estuarine, marine, chlorinated and unchlorinated water supplies worldwide, with the highest numbers obtained in the warmer months (Havelaar *et al*., 1992; Massa *et al*., 2001; Seethalakshmi *et al*., 2008). Aeromonads have been isolated from diseased, cold and warm-blooded animals for over 100 years and from humans since the early 1950s (Mathewson and Dupont, 1992).

Most common disease caused due to *A. hydrophila* infection in fish, are tail rot, fin rot and haemorrhagic septicaemia. Symptoms of *Aeromonas* infection was sloughing off of the scales, hemorrhaging in the gills and anus, ulcers, abscesses, exophthalmia (bulging eyes) and abdominal swelling (dropsy). Internally, there may be the presence of ascitic fluid in the peritoneal cavity, anaemia and swelling of the kidney and liver (Soler *et al*., 2002). Another condition caused by *A. hydrophila*, known as red-fin disease, is characterized by the presence of surface haemorrhages and scale erosion.

Antibacterial therapy will provide only short-term relief if adverse environmental conditions such as high water temperatures, low water flows, low oxygen levels or crowding are not promptly corrected. As use of antibiotic to control microbial pathogens such as *Aeromonas* leads to multidrug resistance, antibiotic residues in environment, transmission of antibiotic in the food chain leads to several problems. So it is inevitable to probe for alternative methods of controlling pathogens. Thus, pharmaceutical industries search bio-active ingredients from the medicinal plants that is traditionally used to cure common ailments (Aboaba *et al*., 2006). One such alternative is the use of herbal medicinal plant-extracts as feed supplement which not only enhance immunity but also increase the size of fishes. Hence in present study we have evaluated the efficiency of three selected medicinal plant extracts *Ocimum basilicum, Calendula officinalis* and *Adathoda vasica* were used against *Aeromonas hydrophila*, in *Labeo rohita* by analyzing the haematological and biochemical parameters.

MATERIALS AND METHODS

Samples: Infected fishes were collected from different sources. The study was carried out from October-2008 to April-2009 in Research Department of Zoology, Periyar E.V.R. College, Tiruchirappalli-620023, Tamilnadu, India. Isolates of *Aeromonas hydrophila* was identified and selected according to different biochemical characterization. Samples were serially diluted and inoculated in nutrient agar medium. Isolated bacterial colonies were streaked on Nutrient agar plates to obtain pure culture. The pure culture were streaked on Nutrient agar slants and stored at 4°C.

Antibacterial screening

Selection of medicinal plant: Selection of medicinal plants is based on the ancient use and its remedial progress observed by people. *Adathoda vasica, Ocimum basilicum* and *Calendula officinalis* leaves were collected from various areas in and around Tiruchirappalli. Collected plants were dried in shade and ground into fine powder and stored in closed containers for further use.

Organic fraction collection: One hundred twenty five grams of powdered plant was successfully extracted with cold butanol. Container containing plant powder was immersed in the solvent and the extract was collected using soxhlet apparatus. Fractions were completely dried by evaporation at room temperature and stored in sterile container.

Experimental set up for *in vitro* studies: Growth experiments were carried out for a period of 15 days in the laboratory. Three replicates were maintained for each treatment. The fishes were weighed accurately in digital electronic balance before the start of the experiment. Healthy live specimens of *Labeo rohita* in the weight of 9-15 g and length 5-9 cm were collected from a fish farm at Thirumanur, Perambalur district. Prior to experimentation the fish were allowed to acclimate to laboratory conditions for ten days. The fish were fed twice daily with combination of rice bran and Bengal gram powder etc., in the form of pellets at the rate of 3% of body weight during acclimation and experimentation. Fish were starved for one day before the initiation of experiments. Animals were introduced into plastic trough of 45 litres capacity. In each plastic trough, 10 animals were maintained. Continuous aeration was given by using compressor air pump to maintain dissolved oxygen at a level of more than 5 ppm in each trough. Water exchange was carried out daily at a rate of 25%.

The fishes were fed with test diets at 3% of their body weight daily which was split into two rations, one feeding in the morning (9.00-9.30 am) and other in the evening (4.30-5.00 pm). The feed was supplied to the fish in the trough by keeping it in a glass plate. The left over feed was removed daily and dried in hot-air oven to assess the food conversion ratio. Faeces were removed daily before the morning feeding. The survival of the fish was observed throughout the experimental period. At the end of growth trial, the final weight of the animal and the dried uneaten feed were taken and used for calculation of growth related parameters.

Inoculum: *Aeromonas hydrophila* was used for challenging the post feed supplemented animals and control fishes. The virulence of the *Aeromonas hydrophila* was tested by determining the LD_{50} by intramuscular administration of various doses in *Labeo rohita*. The cultures were further inoculated in 250 mL nutrient broth and grown for 24 h in order to produce sufficient number of bacteria for the challenge.

Intramuscular challenge: The challenge inoculum was drawn into 1 mL insulin syringe. The fish were netted out individually and the inoculum injected intramuscularly (0.4 mL) in the caudal peduncle. The needle was held at a 45°C angle to the abdomen and the inoculum injected just inside the caudal area. After injection, the fish were released into the tank.

Biochemical analysis

Blood collection: At the end of the experimental period, the animals were deprived of food overnight and then killed by decapitation. Blood was collected by cardiac puncture in tubes containing EDTA solution for the estimation of blood glucose. Serum was separated for the estimation of Sugar, cholesterol, SGOT, SGPT, ALP, Triglycerides and protein (Rehulka, 2000).

Statistical analysis: For all the animals under study, the mean value of sugar, cholesterol, SGOT, SGPT, ALP, Triglycerides and protein content in blood, both in control and experimental fish was estimated out and standard deviation was calculated. Two way ANOVA of the results and post-hoc (SNK) test were carried out using a statistical package (SPSS version.10).

RESULTS

In the present study antimicrobial property of the butanolic extracts of three medicinal plants namely *Calendula officinalis, Adathoda vasica* and *Ocimum basilicum* were tested by using them as feed supplements, against *Aeromonas hydrophilla. Labeo rohita* was procured and fed with supplemented feed for twenty days and infected with median lethal dose of *Aeromonas hydrophila*.

Hepatopancreatic function test: The study evaluated the activity of serum Serum Glutamate Oxaloacetate Transaminase (SGOT), Serum Glutamate Pyruvate Transaminase (SGPT) and Alkaline Phospatase (ALP), in all feed supplemented groups with *Calendula officinalis, Adathoda vasica* and *Ocimum basilicum*.

Serum Glutamate Oxaloacetate Transaminase (SGOT): SGOT level was observed in the feed supplemented groups maximum concentration of 60.0 mg dL^{-1} was observed in *Ocimum basilicum* supplemented animals and lowest concentration in control group with 19.4 mg dL^{-1}. Even though there was gradual decrease in infected animals, the feed supplemented animals showed elevated level when compared with that of control, it ranged between 3.0 mg dL^{-1} in control group to 22.3 mg dL^{-1} in *Ocimum basilicum* group (Table 1).

Serum Glutamate Pyruvate Transaminase (SGPT): Elevated level of SGPT level was observed in the feed supplemented groups maximum concentration of 54.1 mg dL^{-1} was observed in *Ocimum basilicum* supplemented animals and lowest concentration in control group with 16.2 mg dL^{-1}. Even though there was gradual decrease in infected animals, the feed supplemented animals showed elevated level when compared with that of control, it ranged between 3.1 mg dL^{-1} in control group to 17.5 mg dL^{-1} in *Calendula officinalis* supplemented group (Table 2).

Alkaline Phospatase (ALP): Maximum concentration of ALP level of 26.3 mg dL^{-1} was observed in *Ocimum basilicum* supplemented animals and the lowest concentration in *Calendula officinalis* supplemented group with 18.3 mg dL^{-1}. Even though there was gradual decrease in infected

Table 1: SGOT content in the blood of *Labeo rohita*, fed on compounded feed supplemented with phyto-extracts (Mean values±Standard deviation)

Feed supplements	Normal	Infected
Control	19.4±0.5^{d}	3.0±0.1^{d}
Ocimum basilicum	60.0±07^{a}	22.3±0.5^{a}
Calendula officinalis	53.0±0.4^{b}	21.7±0.5^{b}
Adathoda vasica	48.0±0.7^{c}	19.3±0.2^{c}

F = 6021.0 ($p<0.001$). Dissimilar superscripts denote, significantly different values statistically

Table 2: SGPT content in the blood of *Labeo rohita*, fed on compounded feed supplemented with phyto-extracts (Mean values±Standard deviation)

Feed supplements	Normal	Infected
Control	16.2±0.2^{d}	3.1±0.3^{d}
Ocimum basilicum	54.1±0.2^{a}	15.0±0.1^{a}
Calendula officinalis	47.6±0.5^{b}	17.5±0.4^{b}
Adathoda vasica	22.2±0.7^{c}	7.3±0.2^{c}

F = 8954.0 ($p<0.001$). Dissimilar superscripts denote, significantly different values statistically

Table 3: ALP content in the blood of *Labeo rohita*, fed on compounded feed supplemented with phyto-extracts (Mean values±Standard deviation)

Feed supplements	Normal	Infected
Control	23.4±0.5^{d}	3.2±0.2^{d}
Ocimum basilicum	26.3±0.2^{a}	19.3±0.3^{a}
Calendula officinalis	18.3±0.2^{c}	9.5±0.6^{c}
Adathoda vasica	25.0±0.8^{b}	16.0±0.1^{b}

F = 1023.6 ($p<0.001$). Dissimilar superscripts denote, significantly different values statistically

Table 4: Triglyceride content in the blood of *Labeo rohita*, fed on compounded feed supplemented with phyto-extracts (Mean values±Standard deviation)

Feed supplements	Normal	Infected
Control	106.0±0.5^{a}	75.2±0.3^{a}
Ocimum basilicum	52.0±0.2^{c}	81.2±0.4^{c}
Calendula officinalis	58.1±0.1^{b}	88.2±0.2^{b}
Adathoda vasica	40.6±0.4^{d}	82.3±0.4^{d}

F = 11156.4 ($p<0.001$). Dissimilar superscripts denote, significantly different values statistically

animals, the feed supplemented animals showed elevated level when compared with that of control, it ranged between 3.2 mg dL^{-1} in control group to 19.3 mg dL^{-1} in *Ocimum basilicum* group (Table 3). Analysis of Variance revealed that there was significant variation with in feed supplement and also between feed supplemented and infected animals. SNK post hoc analysis showed there was significant variation between all feed supplemented groups.

Lipid profile: Rise in serum lipids, such as total cholesterol and triglycerides are indicators of stress in animals. Hence, present study investigated the level of total cholesterol, total sugars, serum protein and triglycerides in animal groups.

Triglycerides: Reduction in the triglyceride level was observed in the feed supplemented groups. Maximum concentration of 106.0 mg dL^{-1} was observed in control animals and the lowest concentration in *Adathoda vasica* supplemented group with 75.2 mg dL^{-1}. Even though there was gradual decrease in normal feed supplemented animals, there was increase in infected feed supplemented animals showed elevated level when compared with that of control, it ranged between 40.6 mg dL^{-1} in control group to 88.2 mg dL^{-1} in *Calendula officinalis* group (Table 4).

Total sugars: Elevated level of total sugar was observed in the feed supplemented groups maximum concentration of 49.3 mg dL^{-1} was observed in *Ocimum basilicum* supplemented animals and lowest concentration in control group with 40.5 mg dL^{-1}. Infected animals showed elevated level of total sugar in all treatments. Maximum recorded in *Ocimum basilicum* supplemented group with 96.3 mg dL^{-1} and lowest concentration observed in *Calendula officinalis* supplemented group with 79.5 mg dL^{-1} (Table 5).

Cholesterol: Increased cholesterol level was observed in the feed supplemented groups maximum concentration of 76.6 mg dL^{-1} was observed in *Calendula officinalis* supplemented animals and lowest concentration in control group with 39.6 mg dL^{-1}. Infected animals showed elevated level

Table 5: Total sugar content in the blood of *Labeo rohita*, fed on compounded feed supplemented with phyto-extracts (Mean values±Standard deviation)

Feed supplements	Normal	Infected
Control	40.8±0.3^{b}	88.5±0.5^{b}
Ocimum basilicum	49.3±0.3^{a}	96.3±0.3^{a}
Calendula officinalis	40.5±0.3^{c}	79.5±0.4^{c}
Adathoda vasica	42.6±0.9^{b}	88.0±0.8^{b}

F = 338.4 ($p<0.001$). Dissimilar superscripts denote, significantly different values statistically

Table 6: Cholesterol content in the blood of *Labeo rohita*, fed on compounded feed supplemented with phyto-extracts (Mean values±Standard deviation)

Feed supplements	Normal	Infected
Control	39.0±0.7^{d}	61.0±1.4^{d}
Ocimum basilicum	61.0±1.0^{c}	91.0±0.8^{c}
Calendula officinalis	76.6±0.5^{a}	96.4±0.3^{a}
Adathoda vasica	66.0±0.3^{b}	92.8±0.7^{b}

F = 3093.1 ($p<0.001$). Dissimilar superscripts denote, significantly different values statistically

Table 7: Protein content in the blood of *Labeo rohita*, fed on compounded feed supplemented with phyto-extracts (Mean Values±Standard deviation)

Feed supplements	Normal	Infected
Control	4.7±0.2^{b}	3.2±0.3^{b}
Ocimum basilicum	5.0±0.3^{b}	3.4±0.2^{b}
Calendula officinalis	6.0±0.3^{a}	4.0±0.1^{a}
Adathoda vasica	6.0±0.4^{a}	4.0±0.5^{a}

F = 36.79 ($p<0.001$). Dissimilar superscripts denote, significantly different values statistically

of cholesterol in all treatments maximum recorded in *Calendula officinalis* supplemented group with 96.4 mg dL^{-1} and lowest concentration observed in control group with 61.0 mg dL^{-1} (Table 6).

Serum protein: Elevated serum protein level was observed in the feed supplemented groups maximum concentration of 6.0 mg dL^{-1} was observed in *Calendula officinalis* supplemented animals and lowest concentration in control group with 4.0 mg dL^{-1}. Infected animals showed decreased level of serum protein in all treatments maximum recorded in *Calendula officinalis* supplemented group with 4.0 mg dL^{-1} and the lowest concentration observed in control group with 3.2 mg dL^{-1} (Table 7). Analysis of Variation revealed that there was significant variation with in feed supplement and also between feed supplemented and infected animals. SNK post hoc analysis showed there was not much significant variation between supplemented groups.

DISCUSSION

Performing blood chemistry determinations in laboratory, can provide vital information to aid in the diagnosis and management of infected individuals or in health assessment (Pincus, 1996). Hence in this study, various biochemical parameters such as SGOT, SGPT, ALP, total cholesterol, total sugars, serum protein and triglycerides in animal groups of *Labeo rohita* were assessed after 15 day feed trial with herbal extract supplemented diets. Azolla protein concentrate was used as feed supplement for *Labeo rohita* to improve fish meal was done by Sheeno and Sahu (2006).

Similarly in the present study, the commercial compounded feed was supplemented with butanolic extracts of medicinal plants, *Ocimum sanctum* and *Adathoda vasica* to study its impact on several biochemical parameters.

Because of the side effect and the resistance that pathogenic microorganism build against antibiotics much of recent attention has been paid to extract and use biologically active compounds isolated from plant species used traditionally in herbal medicine (Essawi and Scrow, 2000). In accordance with present study, several medicinal plant extracts were used to inhibit *Aeromonas hydrophila* was recorded by Biradar *et al.* (2007). The acetone extract of the roots of *Rumex crispus* and *Acinos rotundifolius* demonstrated significant inhibitory activity against *Aeromonas hydrophila* (Ulukanli *et al.*, 2005). Feed supplement trial using the butanolic extract showed significant increase in the cholesterol, total sugars, triglycerides content in all the infected feed supplemented animal. Among the supplemented groups *Ocimum basilicum* recorded maximum gain. However, when infected with *Aeromonas hydrophila* there was marked decrease in non-feed supplemented control, whereas there was only slight reduction in plant extract supplemented animals. El-Barbary and Mehrim (2009) observed restorative effect against aflatoxin when treated with medicinal herbs in the blood glucose level, total protein and hepatopancreatic function in *Oreochromis niloticus* which was similar to that observed in present study. Tatina *et al.* (2010) also observed increased level of total protein, glucose and triglycerides in *Acipenser ruthenus* when fed on different diets, is evident in the present study. Physiological stress like intramuscular injection of *Aeromonas hydrophila* shows decrease in hepatic glycogen content (Peters *et al.*, 1980; Ejike and Schreck, 1980; Peters and Hong, 1985).

The protein content was maximum in the feed supplemented animals provided by *Ocimum basilicum*. Even though there was reduction in the protein content of infected animals in the supplemented group, they showed higher concentration than that of the normal, control, infected animals. The decrease in the total protein due to *Aeromonas hydrophila* infection is also observed in *Cyprinus carpio* treated with herbal extract of *A. indica*. Reduced level of total protein in Rainbow trout infected with *Aeromonas hydrophila* was recorded by Rehulka (2002). Similarly, the reduction of total protein and alkaline phosphatase was observed when exposed to cadmium by Shalaby (2007). The reduction in protein is significantly due to hypoproteinemia while low level may be the result of losses from the skin lesions, an increase in catabolism in acute inflammation or reduced synthesis due to hepatopathy.

The stress response in fish is generally mediated by neuroendocrine response which includes the release of stress hormones such as cortisol and catecholamines into circulatory system (Barton, 2002). There was significant variation in the serum protein content. Two way ANOVA and SNK post hoc test showed significant variation in the biochemical parameters of serum enzymes between the normal and feed supplemented animals. The activity of AST, ACP and ALT enzymes in blood may also be used as stress indicator (Svoboda, 2001). In the present study, hepatpancreatic enzyme level was restored to normal level in infected fish which is fed with medicinal plant supplemented groups. These observed variations were dependent on the concentration of plant extracts used. Hence, from present study, it could be inferred that the plant extracts of *Ocimum basilicum, Calendula officinalis* and *Adathoda vasica* had beneficial effects of improving the biochemical parameters of the common carp *Labeo rohita*. Adequate concentrations of these plant extracts when incorporated in the regular feed of the fish will definitely improve its nutritive value and there by its growth, as well. The antimicrobial property of these plants will lower the susceptibility of the fish to a variety of bacteria-borne diseases. The present study also accentuates the need for further studies, particularly in the isolation and characterization of active principles to modulate non-specific immune system of fishes and control *Aeromonas* infection.

CONCLUSION

From the present study, it concludes that three medicinal plants can be used to control *Aeromonas hydrophila* infection when appropriate quantity was supplemented with normal feed. Further exploration of bioactive component which is responsible for controlling microbe will make the feed more palatable and easy for large scale field application.

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