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Medicinal plants as immunostimulants for health management in Indian cat fish

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PEER REVIEW

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Comments

This research work is very important to the control of fish disease by medicinal plant. This work brought new insight into fish biology and general immunology. These results are clear and it is concluded that infected groups of fish maintained on herbal plant diets yielded significantly better growth and biochemical parameters than the control. Statistical analysis is good and well correlated. This article is very useful for fish diet against *A. hydrophila*.

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ABSTRACT

Objective: To study the effect of three medicinal plants on growth, survival and immunoprotection in *Mystus montanus* fingerlings.

Methods: The three experimental groups of fish were fed with a diet supplemented of *Ocimum tenuiflorum*, *Zingiber officinale* and *Allium cepa* (0.5 g each), and control group was fed without supplementation of herbal diet. After 45 d of feeding the experimental group fishes were injected with 0.5 mL of *Aeromonas hydrophila* and were supplemented with herbal diet. In the control group fishes were injected with 0.5 mL of physiological saline solution alone.

Results: The blood samples were collected for haemotological analysis after 7 d of post infection. The results showed that the haematological parameters of haemoglobin, serum glutamic-oxaloacetic transaminase, serum glutamic pyruvic transaminase, serum glucose, red blood cell and white blood cell were found to be more in challenged fish. The survival rate of pathogen challenged fish maintained on the herbal diet is better than those in the control group which is supplemented with non-herbal diet.

Conclusions: These results indicated that herbal diet is useful as a growth promoter and for disease resistance of *Mystus montanus* against *Aeromonas hydrophila* infection.

KEYWORDS

Aeromonas hydrophila, Growth performance, Haemotological parameters, *Mystus montanus*, Medicinal plants

1. Introduction

Aquaculture remains a growing, vibrant and important production sector for higher 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 the immune power of the organism to fight the invasion of pathogens, and the second is through medication[1]. The motile *Aeromonas* group, especially *Aeromonas hydrophila (A. hydrophila)*, affects a wide variety of fresh water fish species and occasionally marine fish[2]. This infections normally cause high mortality and loss for a period of time. The commercial vaccines are expensive for fish farming practices and are specific against particular pathogens. The medicinal plants have strong antibacterial effects of active

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compounds known to play an important role in preventing bacterial infections. These compounds have the ability to inhibit the generation of oxygen anions and scavenge free radical, hence reducing stress effects. The dietary supplementation of *Achyranthes aspera* seed stimulated immunity and enhanced resistance to *A. hydrophila* infection of fingerlings (*Labeo rohita*)[3]. The herbs or herbal drugs are used not only against diseases but also as growth promoters, stress resistance boosters and preventatives of infections, and it can also act as immunostimulants. Presently, the research has been initiated to evaluate the feasibility of herbal drugs in fish diseases and additionally, the herbal drugs provide a cheaper source for treatment and greater accuracy than chemotherapeutic agents without causing toxicity[4]. Some plants are rich sources for treatment of cultured fish species and some plants are rich sources of bioactive compounds like volatile oils, saponins, phenolics, tannins, alkaloids, polysaccharides and polypeptides, and these are natural plant products with various activities such as anti-stress, appetizer, anti-microbial, anti-cancer and immune stimulants[5]. Many studies have proved that herbal additives enhanced the growth of fishes and protected them from diseases[6]. Recently in aquaculture, some plant extracts have been tested and used with good results in the control of bacterial and viral diseases. Fourteen herbs have been tested against *A. hydrophila* infection in tilapia (*Oreochromis niloticus*) and among these herbs, the ethanol extract of *Psidium guajava* has been found to exhibit the highest anti-microbial activity[7]. In this present study, authors aimed to evaluate the effect of three medicinal plants as growth promoter and their disease resistance for *Mystus montanus (M. montanus)* fingerlings.

2. Materials and methods

2.1. Fish

A total of 180 *M. montanus* fingerlings [(1.82±0.41) g] were collected from Korampallam fish pond, Tuticorin, Tamilnadu, India. They were acclimated for 15 d in 500 L cement tanks, then distributed into 12 plastic containers at an initial density of 15 fishes per plastic container. During the acclimation period they were fed with the basal diet.

2.2. Preparation of plant powder

The fresh herbal plants, *Ocimum tenuiflorum* (*O. tenuiflorum*), *Zingiber officinalis* (*Z. officinalis*), *Allium sativum* (*A. sativum*), were collected from local market at Tuticorin, Tamil Nadu. They were washed thoroughly 2 to 3 times with running tap water and then with sterile water. Then the plants were dried in shade, powdered and used for diet preparation.

2.3. Diet and experimental design

The experimental protocol was that each treatment (T1, T2, T3 and control) had three replicates, and *M. montanus* in each treatment were fed with the prepared feed for 60 d. Three trials were carried out with the *M. montanus* in 9 plastic container with 20 L of water, three replicates for each treatment and the control were performed. The feed was prepared using common ingredients such as fishmeal, groundnut oil cake, wheat flour, soyflour, tapioca flour, multi-vitamin which were procured from the local markets in Tuticorin. The above ingredients were mixed and grained into powder form. The feed powder is mixed with boiled water to make paste or semi moist dough. The dough was pelletized into pellets and dried. The prepared feed (40% protein) was fed to the *M. montanus* fish at the rate of 5% body weight for twice a day. All fishes were fed daily at 9:00 am, and 4:00 pm each day, and any remaining diets were collected by siphoning before feeding.

The ingredients and percentage composition of the basal diet used in the experiment were given in Table 1. Fishes were treated as follows: T1: feed with addition of 0.5 g of *O. tenuiflorum* in each of three plastic containers; T2: feed with addition of 0.5 g *A. sativum* in each of three plastic containers; T3: feed with addition of 0.5 g of *Z. officinalis* in each of three plastic containers; control group: fed on the basal diet without the addition of herbal diet. The pathogenic bacteria of *A. hydrophila* was obtained from Marine Biological Research Centre, Kamaraj College, Tuticorin. It was maintained on nutrient agar slants and kept in a refrigerator at 4 °C until used. The viable cell count of pathogenic bacteria on the plates was also performed manually. Every seven day each plastic container was cleaned and the water changed for water quality control, temperature, pH and dissolved oxygen were measured daily, the level of dissolved oxygen was observed above 7 mg/L. The water quality parameters were monitored weekly and the mean values recorded were as follows: temperature (28.47 ±0.50) °C and pH (7.01±0.11).

Table 1
Composition of basal diet.

Ingredients (g/100g)	Control	T1	T2	T3
Fish meal	40.00	40.00	40.00	40.00
Ground nut oil cake	25.00	25.00	25.00	25.00
Soybean flour	15.00	15.00	15.00	15.00
Wheat flour	10.00	10.00	10.00	10.00
Tapioca flour	9.00	8.50	8.50	8.50
Vitamin and minerals mix*	1.00	1.00	1.00	1.00
Herbal diets	0.00	0.50	0.50	0.50
Proximate composition (%) moister	10.23	11.02	11.14	10.98
Crude protein	39.40	41.14	40.25	42.32
Carbohydrate	26.00	29.00	23.00	24.00
Fat	5.20	4.80	4.30	4.80

*Vitamin and mineral premix, each kg of premix contained vitamin A (4 000 000 IU), vitamin D (666 666.7 IU), vitamin H (3 333.3 mg,), vitamin K3 (333.3 mg), vitamin B1 (333.3 mg), vitamin B2 (1,666.7 mg), vitamin B6 (500 mg), vitamin B1 (3.33 mg), pantothenic acid (3 333.3 mg), folic acid (333.3 mg), biotin (16.7 mg) niacin (10 000 mg), iron (10 000 mg), manganese (20 000 mg), copper(1 333.3 mg), zinc (166 666.7 mg), iodine (100 mg), cobalt (33.3 mg) and selenium (33.3 mg).

2.4. Estimation of growth parameters

The growth parameters were estimated as follows:
1. Growth weight was estimated weekly throughout the experimental period.

2. Weight gain=Final fish weight (g)−initial fish weight (g).
3. Specific growth rate (SGR) (%)=Net log of final weight−Net log of initial weight/ experimental duration×100.
4. Feed conversion ratio (FCR) (g)=Total feed consumed by fish (g)/Total weight gain by fish (g).
5. Survival=Final No. of fish/initial No. of fish×100.

2.5. Challenging test

After 60 d of feeding, the fish of each group were divided into two subgroups, the first subgroup of each treatment was injected with pathogenic *A. hydrophila* (*i.p.*, 0.5 mL of 10^7 cells/mL). The second subgroup was injected with 0.5 mL of physiological saline as control (*i.p.*). Both subgroups were kept under observation for 7 d to record the survival rate daily.

2.6. Haematological parameters

After the experimental period blood was collected from the caudal vein of three randomly selected fish per tank. All the procedures used were as described previously[8]. The blood samples were used for determining erythrocyte count, white blood count and haemoglobin content[9,10]. The serum mineral Mg^{2+} was determined calorimetrically[8]. The content of cholesterol was also determined. The plasma was obtained by centrifugation of blood at 3000 r/min for 15 min and non-haemolysed plasma was stored in deep freezer for further biochemical analysis. Plasma glucose was determined using glucose kits supplied by Boehringer Mannheim kit and serum protein content was determined calorimetrically[8]. The immunological parameter like serum amylase, serum glutamic oxaloacetic transaminase (SGOT), serum glutamic pyruvic transaminase (SGPT), gamma-glutamyl transpeptidase (GGT) and serum alkaline phosphatase were determined calorimetrically[11,12].

2.7. Statistical analysis

Result was analyzed using SPSS (Version 17.0) software. One way ANOVA and Duncan's multiple range tests were used to determine the significance of difference between groups. All the results were expressed as mean±SD and significant difference were expressed at a significant level of $P<0.05$.

3. Results

3.1. Growth performance

Results (Table 2) showed that the weight gain [(2.76±1.11) g], SGR (0.75%±0.76%) and FCR [(1.15±0.18) g] of *M. montanus* fingerlings increased significantly when fed with a diet containing *A. sativum*. These values decreased significantly in fish group fed with a diet containing *O. tenuiflorum* (T1) [weight gain (1.78±0.81) g, SGR 0.68%±0.09%; FCR (1.49±0.34) g]. The treatment of T3 containing *Z. officinalis* was found to be slightly better compared to T2 with weight gain (2.36 ±1.42) g, SGR 0.85%±0.91% and FCR (0.89±0.42) g. The growth performance was found to be poor in control group [weight gain (1.61±1.31) g, SGR 0.61%±0.20% and FCR (1.56±0.41) g] when compared to T1, T2 and T3.

Table 2
Growth performance of *M. montanus* fed with herbal and non-herbal diets.

Treatment	Initial weight	Final weight	Weight gain (g)	Specific growth rate %	FCR	Survival (%)
Control	1.82±0.41^a	3.43±0.54^a	1.61±1.31^b	0.61±0.20^a	1.56±0.41^a	92%
T1	2.03±0.53^a	4.11±0.20^c	1.78±0.81^{a,b}	0.68±0.09^a	1.49±0.34^c	94%
T2	2.38±0.96^c	5.14±0.36^a	2.76±1.11^b	0.75±0.76^a	1.15±0.18^a	99%
T3	2.99±0.65^a	6.35±1.34^c	2.36±1.42^b	0.85±0.91^a	0.89±0.42^a	96%

^{a,b,c}All values are means of three replicates±SE for triplicate feeding groups and values in the same row with different superscripts are significantly different ($P<0.05$).

3.2. Heamotological parameters

The results for the monitored haematology parameters of *M. montanus* fed with herbal and non-herbal diets after 1 week of infection with *A. hydrophila* are presented in Table 3. Significant ($P<0.05$) differences were observed in the haematology parameters between the infected groups fed with the herbal diet and those fed with non-herbal diet. Overall, the haemaglobin, red blood cell (RBC), white blood cell (WBC), cholesterol, total serum protein and magnesium (Mg^{2+}) values followed a similar pattern; the values were significantly higher in the infected fish fed with the herbal diet than that of those fed with the non-herbal diet.

Table 3
Haematological parameters of *M. montanus* fed with herbal and non-herbal diets.

Parameters	Control	T1	T2	T3
Haemoglobin (g/dL)	8.10±0.41^a	8.60±1.13^a	9.20±0.52^c	10.40±0.84^a
RBC (Cells×10^6 mm^{-3})	2.40±0.82^a	2.90±0.51^a	3.40±0.36^b	4.40±0.60^b
WBC (Cells×10^3 mm^{-3})	22.00±1.53^b	26.00±0.31^a	28.00±1.05^a	31.40±1.06^c
Total serum protein (mg/dL)	1.43±0.26^a	2.01±0.15^b	2.42±0.21^a	3.50±0.15^c
Serum glucose (mg/dL)	74.30±0.52^{a,b}	88.00±0.25^a	92.00±0.43^b	101.20±0.62^a
Cholesterol (mg/dL)	41.20±0.41^a	48.00±0.81^b	51.20±0.92^a	56.20±0.82^a
Mg^{2+} (mg/dL)	0.92±0.32^c	1.61±0.25^a	1.81±0.58^a	2.80±0.24^c

^{a,b,c}All values are means of three replicates±SE for triplicate feeding groups and values in the same row with different superscripts are significantly different ($P<0.05$).

3.3. Immunological parameters

The results for the monitored immunological parameters of *M. montanus* fed with herbal and non-herbal diets after 1 week of infection with *A. hydrophila* are presented in Table 4.

Table 4
Immunological response in *M. montanus* fed with herbal and non-herbal diets.

Parameters	Control	T1	T2	T3
Serum amylase (IU/L)	7.24±0.81[a]	8.15±0.93[a]	9.21±0.48[c]	11.20±1.28[a]
Alkalin phosphatase (IU/L)	3.39±0.17[a]	4.15±1.03[c]	2.83±1.42[a]	6.15±0.96[b]
SGOT (IU/L)	3.49±1.05[a]	4.21±0.49[a]	6.28±0.95[c]	8.31±0.58[a]
SGPT (IU/L)	7.51±1.34[a]	7.69±0.89[c]	8.58±0.53[ab]	10.38±1.41[b]
GGT (IU/L)	7.84±0.8[a]	8.01±1.24[c]	9.28±1.16[a]	12.40±0.81[a]

[a,b,c]All values are means of three replicates±SE for triplicate feeding groups and values in the same row with different superscripts are significantly different (P<0.05).

Significant (P<0.05) difference were observed in the immunological parameters between the fish fed with herbal and non-herbal diet. Over all, the serum amylase, alkaline phosphatase, SGOT, SGPT and GGT values were significantly higher in the infected fish fed with the herbal diet than that of those fed with the non-herbal diet.

3.4. Challenging test

Table 5 showed that the survival rate of T2 was 99%, of which *M. montanus* fed on a diet containing *A. sativum* for three months and injected with pathogenic *A. hydrophila* (0.5 mL of 10^7 cells/mL). The survival rate of T3 was 96%, and T1 94% which fed on a diet containing *Z. officinalis* and *O. tenuiflorum*.

Table 5
Challenge test for herbal and non-herbal diets.

Items	Control	T1	T2	T3
Route of injection	I/P*	I/P*	I/P*	I/P*
Dose	0.5 mL of saline solution	0.5 mL of *A. hydrophila*	0.5 mL of *A. hydrophila*	0.5 mL of *A. hydrophila*
No. of injected fish	10	10	10	10
Mortality	0	5.66±0.57[a]	1.33±0.52[b]	3.66±5.72[a]
Survival	100	94.00±0.57[a]	99.00±0.59[b]	96.00±1.04[b]

*Intraperitoneal.

4. Discussion

As the alternative to chemotherapy, application of natural products, like plant extracts, in aquaculture is new and developing venture which needs further research in fish[13]. The plant extracts used in this study could enhance the serum bactericidal activity in all experimental groups. In agreement with the present findings, Sivaram *et al*. reported that serum bactericidal activity was enhanced in juvenile greasy groupers *Epinphelus tauvina* fed with antibacterial active principles of *Ocimum sanctum* and *Aniasom nifera*[14]. The present study showed that fish fed with same doses of *O. tenuiflorum*, *Z. fficinalis* and *A. sativum* showed significantly increase in SGR, FCR, weight gain and final weight of fishes compared to the control group. Smilarly *Carassius auratus* fed with mixed herbal feed supplements significantly restored the altered haematological and immunological parameters, and triggered the innate immune system against *A. hydrophila*[15]. The present study showed that fish fed with herbs had significantly higher WBC and RBC counts compared to the control; similarly it was reported that WBC and RBC counts were higher in *Labeo rohita* fingerlings fed with *Magnifera indica* kernel when compared to control[16]. There was an increase in the WBC and RBC count after feeding of herbal diets. More over serum protein cholesterol Ca^2 and blood glucose were significantly increased in infected fish fed with herbal diets compared with fish fed with non-herbal diets. In many fish species the blood chemistry parameters were altered throughout the period of severe bacterial infection, as reported that when rainbow trout was infected with a combination of *Aeromonas* and *Streptococcus*, the blood chemistry parameters were altered[17]. The medicinal herbs employed in this study showed statistically significant effects. These effects were demonstrated both *in vitro* and *in vivo*. Herbs evaluated in this study decreased the mortality of fish and also improved mean mass of the surviving fish; while the feed conservation ratio was lower for the treated *M. montanus*, the reduced mortality showed that the herbs can significantly improve product yield. *M. montanus* were infected with *A. hydropilla* by the injection method and diet of herbal supplementation showed survival rate of 100% in T2 and 87% in control. Statistical analysis displayed significant differences (P<0.05) in the mortality of the *M. montanus* between the treatment and control groups. Mortality in herbal treatments and control group were observed to start on the third day of contact with the pathogen but the mortality of all herbal treatments were significantly different from the control when it stabilized to end on the seventh day. In conclusion, the results of the present study observed that the weight gain, SGR and FCR of herbal diets fishes significantly (P<0.05) increased in T2 compared with the control group, Also infection group of fish fed with herbal diets had significantly better hematological and immunological parameters than the fish fed with non-herbal diet.

Conflict of interest statement

We declare that we have no conflict of interest.

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Comments

Background

Protecting the fishes from disease can be done through two ways. One is by the immune power of the organism to fight the invasion of pathogens, and the second is through medication. The present study showed the medicinal plants as immunostimulants for health management in Indian cat fish (*M. montanus*).

Research frontiers

This study aimed to determine the use of medicinal plants against bacterial pathogen. Tested fish were exposed to different concentration of plant powder. Blood samples were collected to determine the heamatological and Immunological parameters.

Related reports

A number of scientists have worked on the protective effect of medicinal plants against fish desease. For example, Ravikumar *et al*. (2010) evaluated the feasibility of using herbal medicines in fish disease management.

Innovations and breakthroughs

Innovative outcome of this research paper is the use of three medicinal plants as feed supplements to control the fish disease.

Applications

This scientific study supports and suggests the use of medicinal plants to control the bacterial fish disease, which is an useful antibacterial agent as a therapeutic agent.

Peer review

This research work is very important for the control of fish disease by medicinal plant. This work brought new insight into fish biology and general immunology. These results are clear and it is concluded that infected groups of fish maintained on herbal plant diets yielded significantly better growth and biochemical parameters than the control. Statistical analysis is good and well correlated. This article is very useful for fish diet against *A. hydrophila*.

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