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EFFECT OF ORAL IMMUNOSTIMULANT *ANDROGRAPHIS PANICULATA* AND RESISTANCE TO *AEROMONAS HYDROPHILA* IN *CATLA CATLA*

Innocent Xavier B.*, Fathima Syed Ali M., Sheeba, Sheeba S.
PG & Research Department of Zoology, St.Xavier's College (Autonomous), Tirunelveli, Tamilnadu, India

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***Corresponding author**
Email: xiyuvaraj1010@gmail.com

ABSTRACT
The present study was designed to evaluate the immunostimulant potential of *Andrographis paniculata* in fish *Catla catla,* post challenged with *Aeromonas hydrophila*. Fishes were divided into 2 groups, one received control diet, and another received *Andrographis paniculata* incorporated diet for 14 days. The heamatological parameters and serum protein level were analyzed between control and experimental. There is a significant increase in TEC ($P<0.05$) and TLC ($P<0.01$) in immune-stimulant incorporated diet (IS). Remarkable increase in Hb content and serum protein was noticed as 2.42 to 3.2g% and 0.52 to 0.98g% respectively. The immunostimulant administered *Cata catla* when challenged with *Aeromonas hydrophila* showed decrease in TEC and drastic increase in TLC ($P < 0.01$) and lymphocyte as 58% when compared to control.

Keywords: *Aeromonas hydrophila, Andrographis paniculata* Immunostimulant.

INTRODUCTION

In aquaculture large scale mortalities of fish occur due to infectious microbial and parasitic diseases caused due to high dense culture or by pollution mediated environmental stress. Protecting the fish from diseases can be done by through two ways. One is by strengthening the self immune power of the organism and the second is through medication[1]. However, recently, use of antibiotics and chemotherapy has been criticized because their use has created problems with resistant bacteria, and toxicity both in fish and environment.

Immunostimulants are substances which enhance the humoral and cellular immune response both in specific and non-specific way[2]. The use of plants as a productive system for immunostimulators facilitates a new and safe method of immunologically active components such as polysaccharides, organic acids, alkaloids, glycosides and volatile oils which can enhance immune function.

Recently there has been an increased interest in the immune stimulating function of some herbs in aquaculture[3]. These natural plant products have various activities like antistress, appetizer, antimicrobials and immunostimulants[4a, 4b,]. Yin (2006)[5] have studied herb extracts have a potential application in fish culture primarily, because they can be easily obtained not expensive and act against a broad spectrum of pathogens.

Andrographis paniculata belong to the family Acanthaceae. The plant extract is known to possess a variety of pharmacological activities. Andrographolide the major constituant of the extract is implicated in its pharmacological activity[6].The herb is well known drug "green chiretta". It has various medicinal propecties like antidiarrhoreal, immunostimulant and anti inflammatory[7].

The present study is aimed to assess the efficacy of *Andrographis paniculata* and to evaluate the immunostimulant activity in the fish *Catla catla,* post infected with *A.hydrophila* by analyzing hematological parameters.

MATERIALS AND METHODS

The experimental fish *Catla catla* were collected from kallidaikurchi fish farm (125±30g) and allowed to acclimatize to laboratory conditions for one week. During acclimatization they were fed with rice bran and groundnut oil cake. Water was renewed daily. During the experimental period the water quality variables temperature (28±1 ^{0}C) pH (7.4±0.2), salinity (10±2) and dissolved oxygen (>5mg^{-1}) were recorded.

Feed Preparation

The basic diet (Control diet) was prepared by mixing rice bran l0g, wheat bran l0g, soyaflour 23g, dry fish meal 24g, groundnut oil cake 23g, and tapioca flour l0g made as a dough, sterilized in pressure cooker for 30 min, cooled and made in the form of noodles by adding a little amount of sunflower oil. They were shade dried and broken into small desirable pieces and stored. Immunostimulant diet was prepared using the same composition of ingredients to which 2g *Andrographis paniculata* powder was added, which was collected from kurchi, local plant garden, and identified in the Herbarium of Centre of Biodiversity and Biotechnology, St.Xavier's College. (Herbarium No: XCH-21181) was shade dried and powdered.

Experimental

Experiment I consists of two groups control and one experimental of 15 fishes each. The control groups received normal diet and the experimental group received feed formulated with, *Andrographis paniculata* powder (Is diet). The fishes were fed with these diets for 14 days, and the heamatological parameters and serum protein level was analyzed after 1st, 3rd, 7th and 14th day respectively.

After 14 days both control and experimental feed fed fishes were infected with the bacteria *Aeromonas hydrophila* previously grown in nutrient broth for 24hrs. 10^{-3} and 10^{-5} cfu/ml bacteria were injected intramuscularly and again hematological parameters and serum proteins were studied after 1st, 3rd, 7th day.

Haematological and Serological analysis

The blood was collected from the fishes by puncturing the heart by using 1ml insulin syringe. For serological analysis the collected blood were centrifuged at 2500 rpm for 14min. Total Erythrocyte count (TEC), Total leucocyte counts (TLC) were enumerated by using Haemocytometer with improved Neubaur ruling chamber (Weber & Sons England), Haemoglobin content (Hb) was estimated by Cyanomethomoglobin method. Blood smears stained with May – Grunewald's Giemsa's stain was used for differential Leucocytes count. The data were analyzed statistically and students't' test was used to test the level of significance. For serological techniques Gornall's method for serum protein estimation was carried out.

RESULTS

The values of TEC, TLC were higher in immunostimulated incorporated diets and the increase was significant (P <0.05) and highly significant (P <0.01) on the 7th day in respectively when compared with standard feed pellets (Table 1 and 2). DLC could not envisage marked differences however over here and there fluctuations were found among the types of cells (Table 3). Higher percentage of haemoglobin were estimated as 6 g % to 6.629% in immunostimulated fishes than control fishes (Figure 1). Serum protein level exhibited an increase from 0.41 to 0.84g% in IS incorporated fishes (Figure 2)

A significant decrease in TEC was noticed in IS fed fishes when infected with *Aeromonas hydrophila* at a dosage of both 10^{-3} and 10^{-5} cfu/ml.whereas there was a drastic increase in the TLC was noted in both 10^{-3} and 10^{-5} cfu/ml. (Table 4 and 5) DLC showed an increase in lymphocytes as 58% in experimentally infected fishes, than in control fishes on the 7th day (Table 6). Hb content also showed an increase in 10^{-5} cfu/ml concentration on experimentally infected fishes on 7th day as 9.49% (Figure 3)

Table 1: TEC (millions/mm^3) in *C.catla* administered with control and IS diet

Duration Days	Diet	RBC million/cells Mean ± SD
1	control	0.648±0.0725
	IS	0.786±6.0545*
3	control	0.648±0.0725
	IS	0.73±0.34848*
7	control	0.648±0.0725
	IS	0.706±0.3726*
14	control	0.648±0.0725
	IS	0.722±0.3604*

P; *significant

Table 2: TLC (million cells/mm^3) in *C.catla* in relation to control and IS diet

Duration Days	Diet	WBC million/cells Mean ± SD
1	control	34210±159.5
	IS	5665.62±78.125
3	control	3321±159.5
	IS	9582.17±213.42*
7	control	3621±159.5
	IS	96667.15±95.35**
14	control	3321±158.5
	IS	5481.24±278.12

P;** more significant *significant

Table 3: DLC in *C.catla* in relation to control and IS diet

Duration Days	Diet	Lymphocyte %	Monocyte %	Neutrophil %	Eosinophil %	Basophil %
1	control	47	24	20	5	4
	IS	46	25	18	6	4
3	control	46	25	14	9	5
	IS	44	23	15	12	6
7	control	45	26	15	8	5
	IS	46	25	16	8	5
14	control	46	24	20	5	4
	IS	47	24	18	6	4

Table 4: TLC (Million cells/mm^3) in *C.catla* pre administered with IS diet in and post challenged with *A. hydrophila*

Duration Days	Dosage cfu/m1	RBC million/cells Mean ± SD
1	control	0.69±0.648
	IS 10^3	0.88±0.894*
	10^5	0.38±0.3531*
3	control	0.63±0.0251
	IS 10^3	0.43±0.0653*
	10^5	0.55±0.0282*
7	control	0.64±0.0725
	IS 10^3	0.47±0.2508*
	10^5	0.37±0.0371*
14	control	0.61±0.0275
	IS 10^3	0.35±0.027*
	10^5	0.33±0.1334*

P;** more significant *significant

Table 5: TLC (cells/mm^3) in *C.catla* pre administered with IS diet and post challenged with *A.hydrophila*

Duration Days	Dosage cfu/m1	WBC million/cells Mean ± SD
1	control	2550±40.82
	IS 10^3	3683.3±62.3*
	10^5	3716.6±47.1**
3	control	2600±108
	IS 10^3	3766.6±62.3**
	10^5	3616.6±300.9**
7	control	2583.3±84.98
	IS 10^3	4000±81.6**
	10^5	4166.6±82.6**
14	control	2550±40.82
	IS 10^3	3900.3±147.2**
	10^5	3713.3±441.1**

P;** more significant *significant

Table 6: DLC (%) in *C.catla* pre administered with IS diet and post challenged with *A.hydrophila*

Duration Days	Dosage cfu/ml	Lymphocyte %	Monocyte %	Neutrophil %	Eosinophil %	Basophil %
1	control	44	31	12	10	3
	IS 10^3	40	32	12	8	8
	10^5	40	35	10	10	5
3	control	43	31	13	9	4
	IS 10^3	45	35	10	5	5
	10^5	50	36	6	4	4
7	control	45	30	12	10	3
	IS 10^3	59	20	12	8	1
	10^5	55	34	8	2	1
14	control	43	32	14	8	3
	IS 10^3	58	25	10	5	2
	10^5	55	30	8	5	2

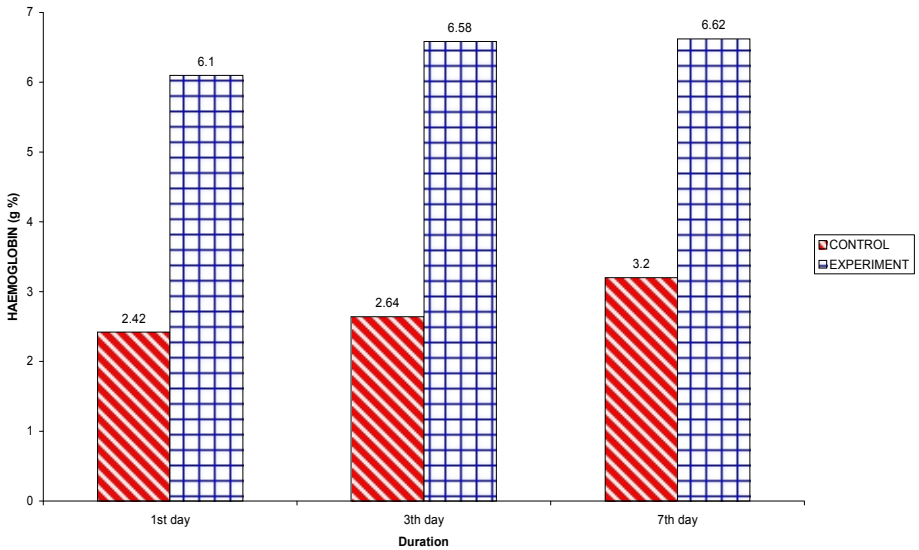

Figure 1: Hb Content (g %) in *C.Catla* administered with control and IS diet

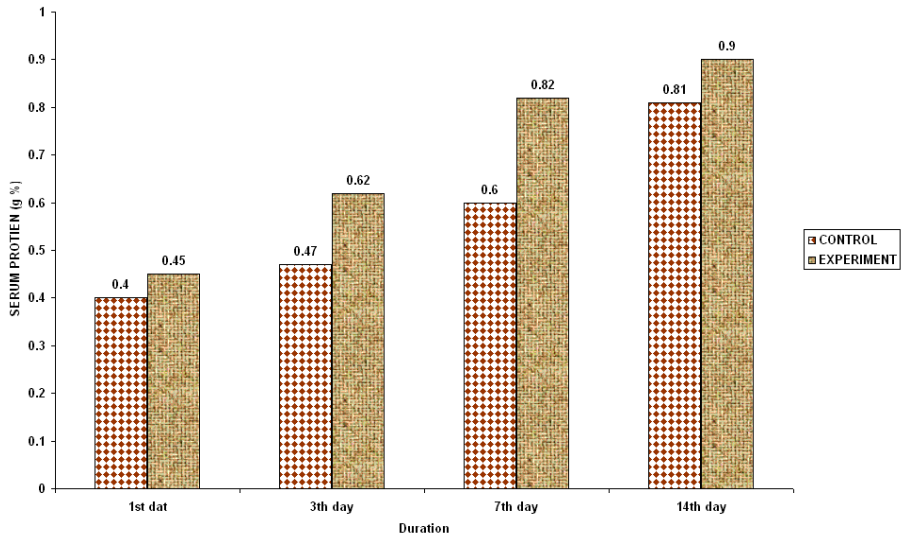

Figure 2: Serum protein content in *Catla catla* administered with control and IS diet

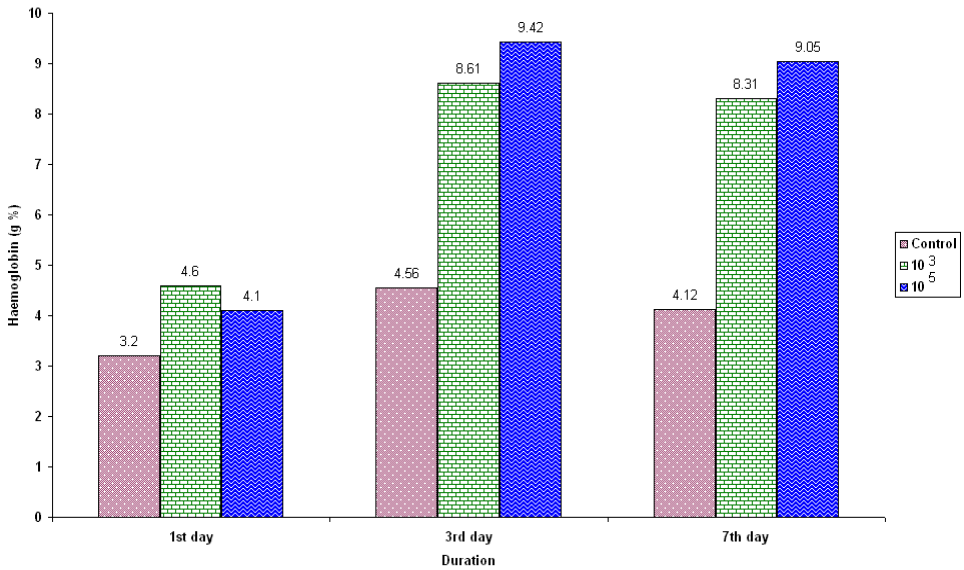

Figure 3: Hb content (g %) in *C.Catla* with IS diet and post challenged wit *A.Hydrophila*

DISCUSSION

In aquaculture many studies report that herbal medicine extracts can be used as immunostimulants for fishes[8],[9,10]. Many recent experiments have shown the immunostimulants can be given alone to induce the *in vitro* and *in vivo* responses[11]. Feed mediated immunization in fish is an effortless and stress free process which can be used for almost any age. So plant derived substances incorporated into standard fish feeds may elicit a specific humoral response and then enhance host defense mechanism[12].

Ethanol extract and purified diterpene andrographoides of *Andrographis paniculata* induces significant stimulation of antibody and delayed type hypersensitivity response in fish[13]

Total and differential leucocyte counts are important indices of nonspecific defense activities of fish[14]. As leucocytes are centrally involved in phagocytic and immune responses to parasitic bacterial, viral and similar challenges[15].

Immune enhancement increase white cell phagocytosis inhibits HIV – I replication and improves CD4 and T Lymphocyte counts[7]. Immunostimulatory activity of *A.paniculata* is evidenced by increased proliferation of lymphocytes and production of interleukins[6]. The TLC, Lymphocytes and neutrophil counts in the present study decreased sharply in the infected fish fed with control diet whereas there is an increase in IS diet fed fishes. These results were was similar to the results of Stephen *et al.*, 2006[1], Ramasamy Harikrishnan *et. al.,* 2010[1],[16].

CONCLUSION

From the above results it is clear that *Andrographis Paniculata* acts as a potent immunostimulant since it induces the blood parameters in the experimental fish *Catla catla*. It can be used as an dietary additive or as an adjuvant to heighten the immune response. The efficacy of *Andrographis paniculata* clearly proved an increase the innate defense mechanim. It is found to be a good choice as a diet supplement to induce some level of disease resistance and enhancement of non specific immunity and act as a potent oral immunostimulant in fishes.

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