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RESEARCH PAPER

Effects of *Verbascum speciosum* on Growth Performance, Intestinal Histology, Immune System and Biochemical Parameters in Rainbow Trout (*Oncorhynchus mykiss*)

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Abstract

In the present study, the efficacy of *Verbascum speciosum* on growth performance, gastrointestinal structure, immune and biochemical parameters in rainbow trout were evaluated. *Verbascum speciosum* was supplemented at 40 (low dose group), 250 (medium dose group), and 500 (high dose group) mg kg^{-1} diet and fed to fish (~9.7 g initially) for 55 days. *Verbascum speciosum* supplementation significantly enhanced the growth performance in low dose group compared to control group**.** Serum complement, total antibody, haemagglutination titer and lysozyme activities were significantly higher in fish administered with medium dose diet in comparison with the basal diet. The low dose group showed an increase in the peroxidase level compared with the control group. In low and medium dose groups significantly higher total protein and globulin contents were also seen. Higher alkaline phosphatase (ALP) and aspartate aminotransferase (AST) activities were also observed in fish that received the high dose of *Verbascum speciosum*. Meanwhile, higher antioxidant activity and lower lipid peroxidation product, indicated by malondialdehyde (MDA), were seen in low dose group. Present study suggests that *Verbascum speciosum,* especially in low and medium doses of administration could affect growth performance, immunological and biochemical parameters in rainbow trout.

Keywords: Rainbow trout, *Verbascum speciosum*, Immune response, Biochemical parameter.

Introduction

Aquaculture fish production has increased significantly over the past few decades, which has led to intensive fish culture practices where stressors like overcrowding, transport, handling, grading and poor water quality are common (Christybapita, Divyagnaneswari and Micheal. 2007). These stress factors can result in immune depression and outbreaks of infections. The use of compounds with immunostimulant and/or antioxidant effects as dietary supplement can improve the innate defense on animals providing resistance to pathogens during periods of high stress (Faggio *et al.,* 2015). Among immunostimulants, depending on their sources, natural ones are preferable because they are biocompatible, biodegradable, cost effective and safe for the environment (Sheikhzadeh, 2013). In recent years, Algae, herbs, plant extracts rich in polyphenols, green tea extracts and essential oils have been extensively used in aquaculture for immune enhancement and improved disease resistance (Guardiola *et al.,* 2016).

Verbascum L., plant of the *Scrophulariaceae* family, is comprised of about 323 species and distributed wordwide (Akdemir *et al.,* 2011). This plant has been known for centuries as a potent medicinal plant according the "folk medicines" for cultures around the world. The leaves and flowers of*Verbascum* are reported to have expectorant, mucolytic and demulcent properties which are used to treat respiratory disorders such as bronchitis, dry coughs, tuberculosis and asthma in traditional Turkish medicine. The species are also used to treat haemorrhoids, rheumatic pain, superficial fungal infections, wounds and diarrhoea, and have inhibitory activities against the murine lymphocytic leukaemia and influenza viruses A2 and B (Akdemir *et al.,* 2011). In recent study, total phenolic content, antioxidant, antifungal and antibacterial activities of *Verbascum speciosum* (*V. speciosum*), were also shown (Nofouzi, 2015).

In spite of *in vitro* studies on beneficial effects of *Verbascum* mainly *V. speciosum*, no information is

available on the possible effect of this herb on fish species. In fact, measuring the immunological and biochemical parameters in fish species treated with herbal derivatives may be a good method to evaluate the possible effects of an herbal drug on fish health (Faggio *et al.*, 2014). Therefore, the aim of this study was to assess the effect of *V.speciosum* on growth performance, gastrointestinal structure, some non-specific immune responses and blood biochemistry in juvenile rainbow trout.

Materials and Methods

Fish

Three hundred and sixty fingerling rainbow trout (9.7 ± 0.3 g mean weight) were taken from a local fish farm in Amol, Iran. Fish were randomly placed in twelve indoor cement tanks (2 × 0.34 × 0.35 m) for eight days for adaptation to the experiment conditions. Each tank was constantly provided with oxygenated free-flowing river water with following characteristics: flow rate 0.9 l s^{-1}, water temperature 13 ± 1ºC, pH 7.8, dissolved oxygen>8 ppm, NH_3 <0.01 mg l^{-1}, NO_2 <0.1 mg l^{-1} and hardness 275 mg l^{-1}. During the experiment, fish were fed with commercial pelleted diet (Faradaneh, Iran) at 3.5% of body weight daily and five times a day. Analysis of basal diet is shown in Table 1.

Experimental Design

The air-dried leaves of *Verbascum speciosum* which were collected from wild nature of Mahabad (West Azerbaijan, Iran) were used. Plant was botanically identified in herbarium of the Pharmacy faculty, Tabriz University of medical sciences (Tabriz, Iran) under code number 16696-tbz fph. Experimental conditions were designed as previously described by previous study (Nootash *et al.*, 2013). In brief, 40, 250, and 500 mg of grounded material were added to one kg of basal pellet. In order to reinforce adhesion of particles to the pelleted diet, 20 ml of fish oil was sprayed on one Kg of feed. The control diet was similarly modified by spraying 20 ml fish oil kg^{-1} on the pellet. The *V. speciosum* supplemented feed was separately preserved in tightly closed plastic bags in cold and dry climate until they were used in the feeding trials. Fish were distributed into four groups in triplicates, with 30 fish per tank and fed with experimental diets for 55 days.

Fish Performance

On day 40 and 55 of trial, all fish in each tank were anesthetized into clove oil bath (50µl l^{-1}). Then, growth parameters including total body weight and length and specific growth rate (SGR) were measured according to the given equations:

$$SGR= 100 (\ln W2 - W1)/T$$

Where W1, W2, T accounted for the initial weight, final weight and number of days in the feeding trial, respectively.

Sample Collection

According to the experimental plan sampling was conducted on day 40 and 55 post-feeding. After 24 h starvation, 10 fish from each tank were anaesthetized in a clove oil bath (50 µl L^{-1}) and blood was collected from the caudal vein. Fish blood was allowed to clot at room temperature for 1 h and was stored in a refrigerator overnight. The clot was then centrifuged at 1500 g for 5 min. The serum was collected and stored in sterile Eppendorf tubes at -20°C until used for assays. It must be noted that all sampling fish for immunological parameters were removed from study after blood collection.

Gastrointestinal Histological Parameters

Intestine and pyloric caeca were collected from four fish in each tank on day 40 and 55 of the trial. It must be mentioned that sampling fish for histology was in addition to the fish used for other parameters. After necropsy, macroscopic observations were performed. Histological samples were prepared according to previous study (Sheikhazdeh *et al.*, 2016).

Serum Immunological Parameters

Lysozyme Activity

The activity of serum lysozyme was determined using turbidometric assay previously published by Andani *et al* (2012) using *Micrococcus lysodiekticus* (Sigma) as lysozyme sensitive bacterium. The serial dilutions of hen egg white lysozyme (Sigma) were prepared in 0.1 M of phosphate-citrate buffer (pH 5.8) and served as the standard. The serum samples (25µl)

Table 1 Composition of the basal diet used during the study

Proximate analysis (%)	SFT_2	SFT_3	FFT_1	FFT_2
Dry matter	89	89	89	89
Crude protein	46	46	40	40
Crude lipid	14	14	16	16
Ash	10	10	10	10
Fiber	3	3	3.5	3.5
Phosphorous	1.2	1.2	1.1	1.1

along with diluted lysozyme (25µl) were transferred into wells of a 96-well plate in triplicate. Then, *M. lysodiekticus* suspension (75µg ml^{-1}) already made in the same buffer was added to all wells. Following repeated shaking, the changes in optical density were monitored every 30 s for 5 min at 450 nm at 20ºC using an ELISA reader. The lysozyme activity of the sample was determined and expressed as µg ml^{-1} serum.

Complement Activity

Alternative complement activity was assessed using Rabbit Red Blood Cells (RaRBC) according to Andani *et al* (2012) procedure. Following washing of the RaRBC three times, the absolute lysis value was prepared by adding 100µl of the RaRBC to 3.4 ml distilled water. The lysate was then exposed to cold centrifugation and the turbidity of the aqueous phase was determined at 414 nm wavelength by a spectrophotometer (Awareness, USA). Subsequently, the serum specimens were diluted in the buffer and 250µl of adjusted volume sera was added to 100µl of RaRBC in test tubes. The prepared solution was kept at room temperature for 90 min with repeated mixing. Then, 3.15 ml of NaCl solution (0.85%) was added to all samples and the tubes were centrifuged for 10 min and the absorbance of the supernatant was quantified again. The level of cell lysis was determined and haemolysis curve was drawn through plotting the haemolysis degree against the volume of serum added on a log/log-scaled graph. The volume producing 50% haemolysis (ACH50) was considered for determining the complement activity of the serum samples and the number of ACH50 was expressed as Units ml^{-1}.

Serum Total Antibody Level

Serum total immunoglobulin was measured according to Siwicki, Anderson and Rumsey. 1994. Following dilution of serum samples with 0.85% NaCl, total protein was estimated by Bradford method. One hundred µl of total serum samples was mixed with an equal volume of polyethylene glycol solution (Sigma) in a 96-well tissue plate. After 2 h incubation at 25º C, plate was spun by cold centrifugation at 5000g. The liquid phase was further diluted and the protein level was determined by Bradford method. This value was deducted from the total protein level and the result was considered as the total immunoglobulin concentration of the serum expressed as mg/ml.

Serum Haemagglutination (HA) Titre

Serum HA titre was measured according to Saurabh *et al* (2010). Briefly, a double serial dilution of the inactivated sera (56°C for 20 min) was made in PBS (with Ca^{2+} and Mg^{2+}), and then 50 µL of 1% Chicken RBC (CRBC) was added to each well of the microtitre plate and incubated for 1h at 37ºC. The HA titre was defined as the last dilution of serum showing minimal positive agglutinin. Values are expressed as reciprocal of the HA titre.

Peroxidase Activity

The peroxidase activity in serum specimens was assayed according to Cuesta *et al* (2005). In brief, 30µl of serum was diluted with 120 µl of Hank's buffer (HBSS) without Ca or Mg in 96-well plates. 50 µl of substrates buffer containing 20 mM TMB and 5 mM H2O2 were added to the above. The enzymatic reaction was stopped after 2 min by adding 50µl of 2 M sulphuric acid and the OD value was measured at 450 nm using a microplate reader. Standard samples without serum were served as blank. The activity of peroxidase enzyme was defined as U mg^{-1} serum proteins

Serum Biochemical Parameters

Serum biochemical metabolites including protein, globulin and cholesterol levels and activity of some liver enzymes including aminotransferase (AST), alkaline phosphatase (ALP), and alanine aminotransferase (ALT) were evaluated by commercial kits and a biochemical automated analyzer (Sheikhzadeh *et al.*, 2012). Total antioxidant activity (Randox Laboratories Ltd.) was determined in the serum specimens using ferric reducing ability of plasma (FRAP) assay. The malondialdehyde (MDA) level in the serum was measured using the thiobarbituric acid test based on previously established procedure (Sheikhzadeh *et al.*, 2012). MDA level was expressed as nmol dl^{-1} of serum.

Statistical Analysis

Analysis of variance (ANOVA) and LSD tests were run to compare different treatments using the SPSS 22. The mean and standard errors were calculated for each treatment. The accepted level of significance was $P \leq 0.05$.

Results

On day 40, fish fed low dose of *V.speciosum* had significantly higher weight than fish in control group. Fish length for fish in different experimental groups had no significant differences on days 40 and 55. Moreover, SGR was significantly enhanced in fish fed low dose of herb in comparison with control group (Table 2).

In post-mortem examination, no relevant gross lesions or microscopic changes were noticed in fish intestine and pyloric caeca in all groups. Light microscopy of fish intestine and pyloric caeca also showed normal appearance in all groups. In fish

intestine and pyloric caeca, the epithelial goblet cell percentage, villus thickness and length in fish intestine did not change during this experiment. In pyloric caeca, fold length and thickness appeared to be in the same condition in all groups (Table 3-4).

Lysozyme activity showed significant elevations in group fed diet supplemented with middle dose of *V.speciosum* in comparison with control group while on day 55; no significant difference (P>0.05) between different groups was noted. On days 40 and 55, complement titer showed a significant elevation in group fed diet supplemented with middle dose of *V. speciosum* in comparison with control group. Meanwhile, dietary intake of *V.speciosum* in middle treatment group increased the serum total antibody content on day 40 of feeding trial whereas no significant changes were noted in total antibody content on day 55 of trial. On day 40, no significant differences in hemagglutination titer was observed among all groups. However, hemagglutination titer on day 55 of trial was significantly higher in group fed diet supplemented with middle dose of *V.speciosum* compared with control group. Fish in group fed diet supplemented with low dose of *V.speciosum* exhibited higher peroxidase content on day 40. On day 55, peroxidase content did not show any significant differences between different groups (Table 5).

On day 40, serum total protein and globulin levels showed significant increase in fish fed diet supplemented with low and medium doses of *V.speciosum* compared with the control group but on day 55, these metabolites were similar between all groups. In the current study, serum cholesterol level in fish fed diet supplemented with middle dose of *V.speciosum* was significantly lower than that of control group on day 40, whereas on day 55, cholesterol level in fish serum did not show any significant differences between different groups (Table 6).

ALP and AST levels of the fish fed diet supplemented with high dose of *V.speciosum* were significantly higher compared with those fed control diet on day 55 of feeding trial. Meanwhile, dietary intake of *V.speciosum* had no significant impact on ALT level in treatment groups compared to control group on days 40 and 55 of trial (Table 7).

Fish fed low dose of herb showed significant increase on antioxidant activity on day 40. Meanwhile, Antioxidant activity in all treatment groups remained unchanged on day 55. Serum lipid peroxidation product of fish fed low dose of *V.speciosum* was considerably lower than that of fish fed control diet on day 55 of feeding trial (Table 8).

Table 2. Growth performance in rainbow trout after feeding different doses of *Verbascum speciosum*

Verbascum speciosum in diet	Final weight (g)		Final length (cm)		Specific growth rate	
	Day 40	Day 55	Day 40	Day 55	Day 40	Day 55
Control	20.44 ± 0.86	36.99 ± 1.16	14.90 ± 0.24	16.01 ± 0.18	5.97 ± 0.21	6.06 ± 0.04
40 mg kg^{-1}	27.52 ± 2.70*	42.18 ± 6.01	15.33 ± 0.22	16.56 ± 0.74	7.25 ± 0.29*	6.12 ± 0.43
250 mg kg^{-1}	20.10 ± 1.31	34.83 ± 2.24	14.14 ± 0.27	15.60 ± 0.30	5.89 ± 0.57	5.94 ± 0.26
500 mg kg^{-1}	21.18 ± 1.10	42.98 ± 2.37	14.63 ± 0.27	16.65 ± 0.34	6.25 ± 0.34	6.39 ± 0.18

Data are mean ± SEM. Those within a column superscripted by different letters are significantly different (P<0.05).

Table 3. Intestinal morphology of rainbow trout after feeding different doses of *Verbascum speciosum*

Intestine	Villus length (×100μ)		Villus thickness (×100μ)		Goblet cell (%)	
Days post challenge	40	55	40	55	40	55
Control	3.65±0.46	4.08±0.58	1.35±0.13	1.16±0.21	30.6±2.8	29.7±3.5
40 mg kg^{-1}	3.04±0.45	4.12±0.7	1.14±0.1	1.2±0.14	25.1±6.3	18.5±3.9
250 mg kg^{-1}	4.2±0.3	3.26±0.52	1.03±0.06	1.05±0.06	39.9±12.4	21.8±3.4
500 mg kg^{-1}	4.46±0.88	4.49±0.61	1.26±0.08	1.21±0.12	27.3±4.8	28.2±5.6

Data are mean ± SEM. Those within a column superscripted by different letters are significantly different (P<0.05).

Table 4. Pyloric caeca morphology of rainbow trout after feeding different doses of *Verbascum speciosum*

Pyloric caeca	Fold length (×100μ)		Fold thickness (×100μ)		Goblet cell (%)	
Days post challenge	40	55	40	55	40	55
control	3.85±0.95	4.15±0.54	1.2±0.073	1.21±0.079	7.37±1.59	6.8±1.75
40 mg kg^{-1}	2.76±0.37	3.43±0.49	1±0.063	1.11±0.047	18.41±6.49	5.99±2.42
250 mg kg^{-1}	3.08±0.48	5.16±1.07	1.16±0.067	1.05±0.042	9.58±1	8.3±1.78
500 mg kg^{-1}	3.84±0.48	4.53±0.81	1.18±0.066	1.28±0.087	8.85±2.71	4.37±1.46

Data are mean ± SEM. Those within a column superscripted by different letters are significantly different (P<0.05).

Discussion

Results of the present study showed that *V.speciosum* in rainbow trout diet augmented the growth performance, biochemical and immunological parameters even though histological examination of fish gastrointestine was not significantly affected by the experimental diets. Improvement in growth parameters was just noted in low dose group on day 40 of trial even though slight improvement was noted on day 55 of sampling. In fish species, improvement in growth performance could be related to better nutrient digestibility and absorption, improved digestive enzymes and maintaining the function and structure of the small intestine, leading to an increased digestive capacity of the gut (Sheikhazdeh *et al.*, 2016). In the current study, no significant changes were seen in gastrointestinal histology in different treatment groups. Therefore, comparing the effects of intestinal morphology with growth performance, it is clear that different mechanisms except enhancement in structure of the small intestine could result in better performance in low dose group.

The non-specific defense mechanisms of fishes include neutrophil activation, production of peroxidase and oxidative radicals, haemagglutination

Table 5 Immunological parameters in rainbow trout serum after feeding different doses of *Verbscum speciosum*

Verbascum speciosum in diet	Complement titer (units ml^{-1})		Total antibody (mg ml^{-1})		Haemagglutination titer		Lysozyme activity (µg ml^{-1})		Peroxidase (450 nm)	
	Day 40	Day 55	Day 40	Day 55	Day 40	Day 55	Day 40	Day 55	Day 40	Day 55
Control	58.75 ± 3.75	77.17 ± 4.86	4.25 ± 0.25	6.26 ± 0.35	3.50 ± 0.5	3.23 ± 0.48	16.34 ± 0.23	27.03 ± 2.37	0.17 ± 0.02	0.19 ± 0.04
40 mg kg^{-1}	69.73 ± 5.62	81.26 ± 4.32	5.06 ± 0.44	6.17 ± 0.41	3.27 ± 0.55	2.36 ± 0.24	19.97 ± 3.07	27.88 ± 2.19	0.21 ± 0.01*	0.23 ± 0.02
250 mg kg^{-1}	79.99 ± 4.88*	91.40 ± 2.88*	6.02 ± 0.33*	6.62 ± 0.31	3.81 ± 0.26	4.90 ± 0.62*	31.47 ± 3.54*	31.89 ± 3.25	0.20 ± 0.07	0.18 ± 0.02
500 mg kg^{-1}	69.21 ± 5.34	77.29 ± 5.25	5.12 ± 0.4	6.26 ± 0.4	2.71 ± 0.75	3.80 ± 0.55	25.18 ± 6.22	21.41 ± 3.93	0.19 ± 0.01	0.19 ± 0.01

Data are mean ± SEM. Those within a column superscripted by different letters are significantly different ($P < 0.05$).

Table 6. Serum metabolites in rainbow trout after feeding different doses of *Verbascum speciosum*

Group	Total protein (g dl^{-1})		Globulin (g dl^{-1})		Cholesterol (mg dl^{-1})	
	Day 40	Day 55	Day 40	Day 55	Day 40	Day 55
Control	2.46 ± 0.05	3.06 ± 0.23	1.43 ± 0.05	1.76 ± 0.1	203 ± 0.54	216.5± 37.6
40 mg kg^{-1}	2.86 ± 0.19*	3.21 ± 0.18	1.60 ± 0.08*	1.86 ± 0.08	196 ± 27.52	200.33 ± 14.98
250 mg kg^{-1}	2.86 ± 0.33*	3.23 ± 0.13	1.63 ± 0.19*	1.82 ± 0.06	163.16 ± 21.67*	203.5 ± 19.85
500 mg kg^{-1}	2.46 ± 0.17	2.96 ± 0.34	1.41 ± 0.07	1.70 ± 0.12	228 ± 29.46	230.33± 42.09

Data are mean ± SEM. Those within a column superscripted by different letters are significantly different ($P < 0.05$).

Table 7 Serum enzyme activity in rainbow trout after feeding different doses of *Verbascum speciosum*

Group	ALP (U lit^{-1})		AST (U lit^{-1})		ALT (U lit^{-1})	
	Day 40	Day 55	Day 40	Day 55	Day 40	Day 55
Control	143.16 ± 0.75	180.83 ± 234.18	265 ± 0	169.66 ± 90.85	15.5 ± 0.54	14.16 ± 4.44
40 mg kg^{-1}	115.66 ± 154.19	149.83 ± 30.98	240.33 ± 51.42	178 ± 36.23	17.83 ± 5.26	12.33 ± 2.58
250 mg kg^{-1}	156 ± 124.9	171.83 ± 147.7	228 ± 59.77	226 ± 75.77	14.5 ± 6.34	15.83 ± 6.46
500 mg kg^{-1}	165.66 ± 130.73	375.16 ± 137.57*	280.16 ± 66.79	272 ± 61.08*	15.5 ± 4.41	15.83 ± 3.81

Data are mean ± SEM. Those within a column superscripted by different letters are significantly different ($P < 0.05$).
ALP: alkaline phosphatase, AST: aspartate aminotransferase; ALT: alanine aminotransferase

Table 8 Antioxidant activity in serum of rainbow trout after feeding different doses of *Verbascum speciosum*

Verbascum speciosum in diet	Total antioxidant activity (mmol L^{-1})		Lipid peroxidation product (nmol mL^{-1})	
	Day 40	Day 55	Day 40	Day 55
Control	0.65 ± 0.00^{a}	0.57 ± 0.03	4.84 ± 0.16	5.81 ± 0.19^{b}
40 mg kg^{-1}	0.99 ± 0.06^{b}	0.61 ± 0.03	4.40 ± 0.28	4.82 ± 0.28^{a}
250 mg kg^{-1}	0.63 ± 0.03^{ab}	0.55 ± 0.03	4.72 ± 0.17	5.64 ± 0.30^{ab}
500 mg kg^{-1}	0.65 ± 0.02^{ab}	0.58 ± 0.03	4.6 ± 0.26	5.22 ± 0.50^{ab}

activity together with initiation of other inflammatory factors (Behara and Swain. 2011). Lysozyme is responsible for bacteriolysis, opsonisation, immune response, and antimicrobial activity (Carbone and Faggio, 2016). In this study, *V.speciosum* in medium dose was able to elevate lysozyme activities in rainbow trout. Similarly, elevated lysozyme level was measured in common carp by feeding Aloe vera, *Mystus keletius* and in rainbow trout after feeding various herbal extracts including Aloe vera, *Solanum triobatum*, *Ocimum sanctum* and *Camellia sinensis* (Alishahi *et al.,* 2010; Sheikhzadeh *et al.,* 2011; Begum and Navaraji. 2012).

The alternative pathway of complement activity is also a powerful non-specific defense mechanism for protecting fish against a wide range of potentially invasive organisms, such as bacteria, fungi, viruses, and parasites (Son *et al.,* 2009). In the present study, serum complement activity in the medium dose group was significantly higher than in the control group. Similarly, elevated level of complement activity upon injection of extracts of three Korean herbs, including *Punica granatum*, *Chrysanthemum cinerariaefolium* and *Zanthoxylum schinifolium* was observed in *Paralichthys olivaceus* (Harikrishnan *et al.,* 2010).

As a natural antibody, IgM is the main immunoglobulin present in teleosts and could provide instantaneous protection of fish against pathogens (Srivastava and Pandey. 2015). Similar to previous immune parameters, supplementations of *V. speciosum* in the medium dose could augment the serum immunoglobulin level in rainbow trout.

Agglutinins are a group of proteins that have different specificities for carbohydrate binding (Saurabh *et al.,* 2010). These agglutinins are Ca^{2+}-dependent and can agglutinate a number of fish pathogens, including parasites. Their ability to bind to terminal sugars on glycoproteins and glycolipids makes them important pattern recognition receptors in innate immunity. In the present study, haemagglutinin level in medium dose group was higher in comparison with the control group. Similarly, higher hemagglutination titer after administration of green tea was observed in rainbow trout (Sheikhzadeh *et al.,* 2011).

Peroxidases play an important role in defense system against extracellular bacterial and parasitic pathogens. Myeloperoxidase and eosinophil peroxidase are important active peroxidases in immune system of fish and found cytoplasmic granules of neutrophils and eosinophil, respectively (Ahmadi *et al.,* 2014). Present study showed that low dose of *V.speciosum* could increase the peroxidase activity in rainbow trout. Similarly, in rainbow trout and common carp higher peroxidase activity following the administration of *Eclipta alba* and *Euphorbia hitra* extracts were observed (Christybapita *et al.,* 2007).

Serum total protein and globulin are considered as indicators for determining immune system activation (Alishahi *et al.,* 2010). Higher protein and globulin levels in the fish fed the low and medium doses of *V.speciosum* were shown in this study. In parallel, activation of innate immune parameters was noted in these two groups. In fact, the activation of immune system was more pronounced in medium dose group in comparison with the low dose group.

V.speciosum could cause a decrease in serum cholesterol value of rainbow trout in middle dose. The influence of some herbs like yarrow in decreasing cholesterol in the blood of rainbow trout confirms this issue (Nafisi Bahabadi *et al.,* 2014). Similarly, decreases in cholesterol level are also reported in rainbow trout and tilapia respectively fed with silymarin extract (Banaee *et al*., 2011) and *Cynodon dactylon*, *Aegle marmelos*, *Withania somnifera*, *Zingber officinale* extracts (Immanuel *et al.*, 2009).

Liver-specific enzymes such as ALP and AST are sensitive measure of hepatotoxicity and histopathological changes. Increase in ALP and AST levels in the high dose group indicates the weakening or damage of normal liver function in this group. Further pathological studies are warranted to prove liver dysfunction in this dose of administration. Conversely, serum activity of ALP, AST, and ALT did not differ between the groups that received low and medium doses of *V.speciosum* in comparison with the control group so we propose that this herb in these doses is a safe supplement.

Present study suggests that the antioxidant activity was better in low dose group than that in control fish on days 40 and 55 even though slight increase in antioxidant activity was noted on day 55 of trial. The present study also indicates that lipid peroxidation product decreased in this treatment group compared to control group. So this herb can protect fish against oxidative stress as a potent antioxidant and free radical scavenger in fish culture. Based on the results of the previous work, the *V. speciosum* methanolic extract has antioxidant activity and remarkable phenolic content which can serve as an excellent natural source of antioxidant agents (Nofouzi, 2015). In polluted environment with oxidative stresses induced by chemicals and contaminants on aquatic organisms (Messina *et al.,* 2014), using a substance with antioxidant activity may be of particular importance.

In conclusion, this preliminary study showed that administration of *V. speciosum* has beneficial effects in rainbow trout, affecting parameters like growth performance, immunity and biochemical parameters. Further investigations are needed to fully understand the interaction between this herb and different fish species even on the molecular level.

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