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ORIGINAL ARTICLE

Dietary Effects of major plantain (*Plantago major* L.) extraction on hematological, biochemical indices-indices and innate immune system in Common carp (*Cyprinus carpio*) fingerlings

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Abstract

Abstract

Major plantain *(Plantago major)* is belonged to Plantaginaceae family, traditionally it was used commonly in folk medicine for skin poultices on wounds, sores, or insect stings. This study was investigated the effect of different levels of major plantain extraction on hematological, biochemical and immunological indices in common carp (*Cyprinus carpio*). Common carp fingerlings (N=240, 18.69±2.09g) were randomly distributed in 12 tanks (3 treatments in 3 replicates). The fingerlings were fed with 0 (as control), 0.1, 0.5 and 1% major plantain extract for 45 days. At the end of the experiment, blood samples were taken from the caudal vein, and some hematological (RBC, HCT, Hb, MCV, MCH, MCHC, WBC, Lymphocyte, Monocyte, Neutrophil) factors, serum biochemical (Glucose, AST, ALT, ALP) and immunological (Total Ig, Globulin, Albumin, Total protein) indices-indices were evaluated. There were no significant differences in RBC, HCT, MCV, ALP, AST, ALT, Total protein, Albumin and total Immunoglobulin concentrations and also Monocytes, lymphocytes and neutrophils between treatments fed different levels of major plantain extract and the control group (P>0.05). Hb, MCH, MCHC, globulin levels in treatment fed with 0.1% major plantain extract was remarkably higher than other groups (P<0.05). Glucose level in treatments fed with 0.1 and 1% major plantain extract were not remarkably different from each other ($P>0.05$), but were significantly higher than two other groups (P>0.05). Finally, due to positive effect of major plantain extract at the level of 0.1% on some hematological indices-indices and the immune responses in common carp, it can be suggested to use major plantain extract to promote immune system in common carp.

Keywords: Major plantain, Innate immune, Biochemical, Extraction, Common carp

INTRODUCTION

Globally, aquaculture has been known as the fastest growing food production technology to meet increasingly sea protein demand (Elbesthi et al. 2020). The aquaculture industry has been reported about 10% growth annually since 1950 (Pauly 2017). According to the FAO, over 91% of global aquaculture production currently being produced in the Asia region (102.9 million tons in 2017). Currently, total global aquaculture production is estimated more than 18.32 million tons (Tacon 2020; Elbesthi et al. 2020).

The Common carp is a widespread freshwater fish of eutrophic waters in lakes and large rivers in Europe and Asia that Dedicated about 4.5 million tons of global aquaculture production in 2016 (Zemheri Navruz et al. 2019). To achieve more output, rapid growth, nutritional efficiency, and increased resistance to disease and stress has been noticed in the aquaculture industry (Tangestani et al. 2011). For long time, Antibiotics, hormones, and chemical drugs have been used as growth and immunity stimulants in aquatic farms (Lee et al. 2012). Many of these chemical treatment in intensive fish culture have been criticized because of their side-effect specially emergence of drug-resistant bacteria, environmental pollution and bioaccumulation of undesired residues as well as weakening the immune systems in culture aquatic species (Tangestani et al. 2011). Some non-

chemical substitutes have been recommended to alternate with chemicals as growth and immune stimulants including: enzymes, organic acids, probiotics, herbs, etc. has been considered in recent year (Zakariaee et al. 2021; Masamha et al. 2010Alagawany et al. 2020).

Herbals medicine and their derivatives have been studied as immune stimulants in humans and animals (Bao et al 2019). Previous studies were approved plant extracts play as growth and immune booster in fish (Paray et al. 2020). Medicinal plants are considered as appropriate alternative of antibiotics and other chemicals to suppress diseases and pollutants in aquaculture farms (Rajabiesterabadi et al. 2020).

Major plantain (*Plantago major*) is belonged to Plantaginaceae family, traditionally were used commonly in folk medic ine for skin poultices on wounds, sores, or insect stings. In traditional Iranian medicine, there are two plantain species including *Plantago major* and *Plantago lanceolate* used as medical treatments. *Plantago major* is in the order Plantaginaceae and the English name plantain is major plantain and grows throughout Iran, USA, Europe, Turkey, Afghanistan, Pakistan, Iraq and the Palestinian Plateau (Ahmad et al. 1980; Behbahani et al. 2017). It also used as an anesthetic, antiviral, anti-inflammatory, astringent, anti-worm, psychoactive, anti-histamine, anti-rheumatic, anti-ulcer, diuretic, laxative and antihypertensive in traditional medicine. In addition, soluble aqueous compounds are isolated from the plantain (major plantain) and increase human lymphocytes and stimulate the immune system. (Kobeasy et al. 2011). Biologically active compounds and components of major plantain include flavonoids, alkaloids, polysaccharides, lipids, phenolic acid derivatives, iridoid glycosides and terpenoids, carbohydrates, caffeic acid derivatives, and also, acetic acid, benzoic acid, cholorogenic acid, citric acid, ferric acid, linoleic acid, salicylic acid and arsenic acid are some other active compounds of this plant (Anushiravani et al. 2020; Reina et al. 2013).

Common carp (*Cyprinus carpio.*) is belonged to Cyprinidae, which is the largest freshwater family (Vilizzi & Walker 1998). Common carp is the most important farmed fish species in the world that constitute 71.9% of freshwater production (Yousefi et al. 2019). High growth rate, affordable food cost, and high tolerance against poor quality and bad farming condition has placed these species among the three most important breeding species in the world (FAO 2006).

Some previous studies are focused on *Plantago lanceolate* effect in other animal physiology such as quail chickens (Temür & Sema 2019), but based on open source data, there is a lack of information about major plantain effects on aquatic animals. So, this study was investigated the effect of major plantain on hematological and innate immune indices in common carp.

MATERIALS AND METHOD

Experimental design: This research was performed in the Shahid Nassir Fazli Barabadi aquaculture center, Gorgan-Iran in February 2020. 240 healthy common carp fingerlings (average weight= 18.2±0.69 g) were purchased from private local farm and adapted with experimental condition for two weeks. The fingerlings were randomly distributed into 12 fiberglass tanks with 50 liters water. Experimental treatments were designed as 3 treatments (0.1, 0.5 and 1% major plantain extract per kilogram of food) and a control group (without major plantain extract) with three replicate (Bilen et al. 2020). 40-50% of the tank water was exchanged daily (Heydarnejad 2012).

Physiochemical indices of water were monitored daily using a portable water quality tester (Model 320-WTW, Germany). Average physicochemical indices of water including temperature, pH, hardness, and dissolved oxygen were adjusted at 26±2°C, 7.2±0.3, 286±1.2mg L^{-1} total calcium carbonate, and 8mg L^{-1} respectively (Heydarnejad 2012).

Major plantain extraction: Extraction of major plantain seeds was performed by Nhu et al. (2019) Method. The first, the seed of major plantain plant from the local market prepared, secondary, the seeds was pulverized by electric mill. Then, 400 g major plantain powder was mixed with 1200 mL of alcohols 96% in a ratio of 1:3 and was shacked for 72 hours,

afterwards, the solution was centrifuged in 5600rpm for 10 minutes at 4°C. Finally, the supernatant was collected and transferred into rotary at 50°C to vapor alcohol from the solution. In order to further concentrate of the extract, an oven with a temperature of 50°C was used.

Diet preparation: A commercial diet (carp feed, Celine company) was prepared for this study. In order to preparation the experimental diets, different levels of extract was certainly mixed with alcohol and after dissolving, sprayed on diets. In order to prevent the effect of alcohol, it was sprayed on basal diet as the control group. To remove the alcohol, experimental diets were placed in a clean, dark and cool place for 24 hours. Fish were fed 2 times in a day at 3% of their body weight (Giri et al. 2019).

Blood sampling, hematological and non-specific immune indices: At the end of the experiment, blood samples were collected from 3 fasted fish (anesthetized with 50mg clove powder in one liter of water) of each tank using a method described by the Feldman (2000). Erythrocytes (red blood cell, RBC) and leukocytes (WBC) numbers were counted using a Neubaur hemocytometer. After fixing (96 % ethanol for 30 min) and staining (Giemsa solution) of the blood smear samples, differential leukocyte counts (monocyte, lymphocyte, and neutrophil) were estimated by optical biology microscope (ECLIPSE E100, Nikon, Tokyo, Japan) (Dorafshan et al. 2008). Hematocrit (HCT) was determined using micro hematocrit capillaries and expressed as percentage of total blood volume (Thompson et al. 1997). Hemoglobin (Hb) concentration was determined by cyanmethemoglobin method (Blaxhall & Daisley 1973). Mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), and mean corpuscular hemoglobin concentration (MCHC) were calculated indirectly using the following formula (Blaxhall & Daisley (1973):

MCV (fL)= (Hct (%) × 10)/RBC (106/mm3)

MCH (pg)= (Hb (g/dL) × 10)/RBC (106/mm3)

MCHC (g/dL/%)= (Hb (g/dL) /Hct (%)) ×100

The non-heparinized samples were centrifuged in 7000rpm for 10 minutes at 4°C and supernatant were accurately collected. Finally, the obtained serum was kept in -80°C until future analysis. Serum alkaline phosphatase (ALP), aspartate aminotransferase (AST), alanine aminotransferase (ALT), total protein (TP), albumin, globulin and glucose were measured using commercial kits (Pars Azmoon®, Iran) according to the protocols suggested by manufacture using spectrophotometry method. Also, total Ig was measured by Enzyme-Linked Immuno-Sorbent Assay (ELISA) (Irma, JAPAN) according to instructions Eastbiopharm® kit (China) (Drabkin & Austin 1932).

Data analysis: All results were reported as the mean ± standard deviation (n=3), and all statistical analyses were performed using SPSS 23.0. (Chicago, IL, USA). All data were compared by one-way analysis of variance (ANOVA), and differences between the means were tested by Duncan's tests. Differences were considered significant when the *P* value was less than 0.05 ($P<0.05$). All graphs were drawn with Microsoft Excel version 2019.

RESULTS

Hematological indices: Blood cells: Hematological indices related to blood cells of common carp (*Cyprinus carpio*) fed with different levels of major plantain extract are presented in Table 1. According to the results, there were no significant differences in RBC, HCT and MCV between treatments and the control group ($P>0.05$). Hemoglobin concentration in treatment fed with 0.1% major plantain extract was remarkably higher than other groups ($P<0.05$); but its level in treatment fed with 0.5 and 1% major plantain extract was not statistically differ from the control group ($P>0.05$). In addition, MCH and MCHC levels in treatment fed with 0.1% major plantain extract was remarkably higher than other groups ($P<0.05$).

Immune cells: The effect of major plantain extract on WBC and differential leukocyte counts are presented in Table 2. WBC in treatment fed with 1% major plantain extract was notably higher than other groups ($P<0.05$). Monocytes, lymphocytes and neutrophils showed no significant difference in all treatments and the control group as well ($P>0.05$).

Table 1. Hematological indices of Common carp (*Cyprinus carpio*) fed with different levels of major plantain extract.

Hematological indices	levels of major plantain extract				
	Control	0.1%	0.5%	1%	Sig
RBC (10^6/mm^3)	2.07±0.18	1.985±0.035	2.06±0.16	1.965±0.115	0.717
HCT (%)	35.00±2.00	32.333±2.021	34.333±2.517	34.00±1.000	0.611
Hb (g dL^{-1})	7.42±0.834^b	9.948±0.787^a	7.311±1.951^b	5.422±0.245^b	0.009
MCV (µm^3)	169.38±5.086	165.52±12.327	166.71±1.687	173.32±8.538	0.653
MCH (pg)	35.81±1.532^b	50.11±3.761^a	35.17±6.684^{bc}	27.69±2.575^c	0.001
MCHC (%)	21.16±1.339^b	30.32±2.092^a	21. 11±4.046^b	15.95±0.741^c	0.001

Values are presented as the mean ± S.D. Different letter denote significant difference between treatments (P<0.05).

Table 2. WBC and differential leukocyte counts of Common carp (*Cyprinus carpio*) fed with different levels of major plantain extract.

WBC and deferential leukocyte indices	levels of major plantain extract				
	Control	0.1%	0.5%	1%	Sig
WBC (10^3/mm^3)	21.00±1.00^b	23.333±1.041^b	22.667±0.577^b	28.50±1.803^a	0.000
Lymphocytes (%)	86.36±5.584	82.56±13.632	91.71±10.794	91.71±3.001	0.579
Monocyte (%)	8.87±4.278	8.69±6.339	3.02±3.01	5.05±3.458	0.362
Neutrophils (%)	4.76±2. 841	8.75±9.214	5.28±7.687	3.24±0.467	0.739

Values are presented as the mean ± S.D. Different letter denote significant difference between treatments (P<0.05).

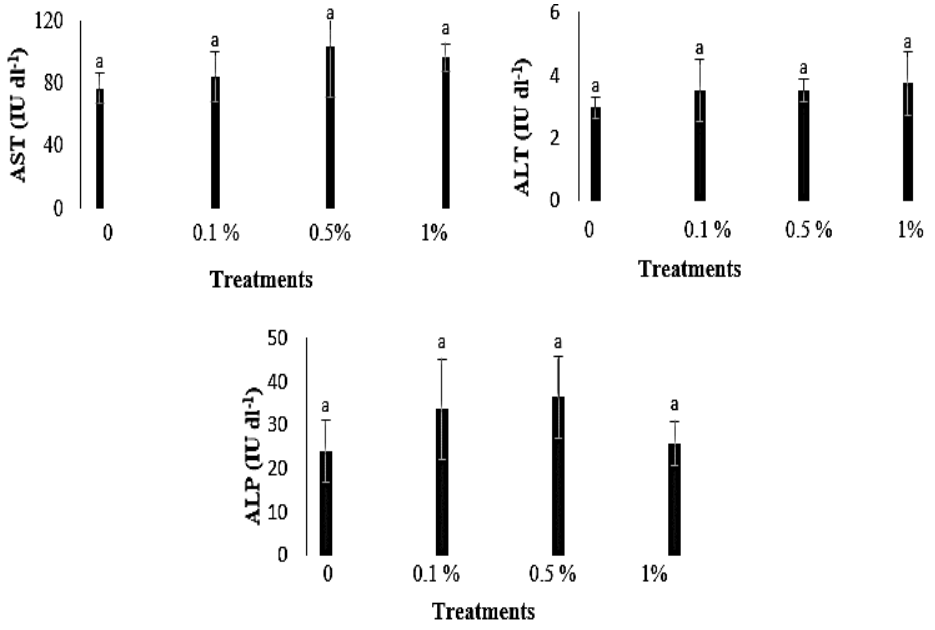

Fig.1. Metabolic enzymes in liver: Alkaline phosphatase (ALP), Aspartate aminotransferase (AST), Alanine aminotransferase (ALT) level in serum of Common carp (*Cyprinus carpio*) fed with different levels of major plantain extract for 45 days. Values are presented as the mean ± S.D. The bars assigned with different letter denote significant difference between treatments (P<0.05).

Non-specific immune: Hepatic metabolic enzymes: The effect of dietary supplemented with different levels of major plantain extract on metabolic enzymes including ALP, AST and ALT in common carp is shown in Figure 1. The results showed no significant differences in ALP, AST and ALT in all groups (P>0.05).

Serum innate immune indices: Albumin and Glucose concentrations in common carp fed with different levels of major plantain extract are mentioned in Figure 2. Albumin concentration was not statistically different between all treatments and the control group (P>0.05). Although glucose level in the control group and treatment fed with 0.5% major plantain extract were not remarkably different from each other (P>0.05), it was significantly higher than other groups (P<0.05). Based on the results, no significant differences were observed in total protein and

Iran. J. Ichthyol. (2024) 11(3): 174-183

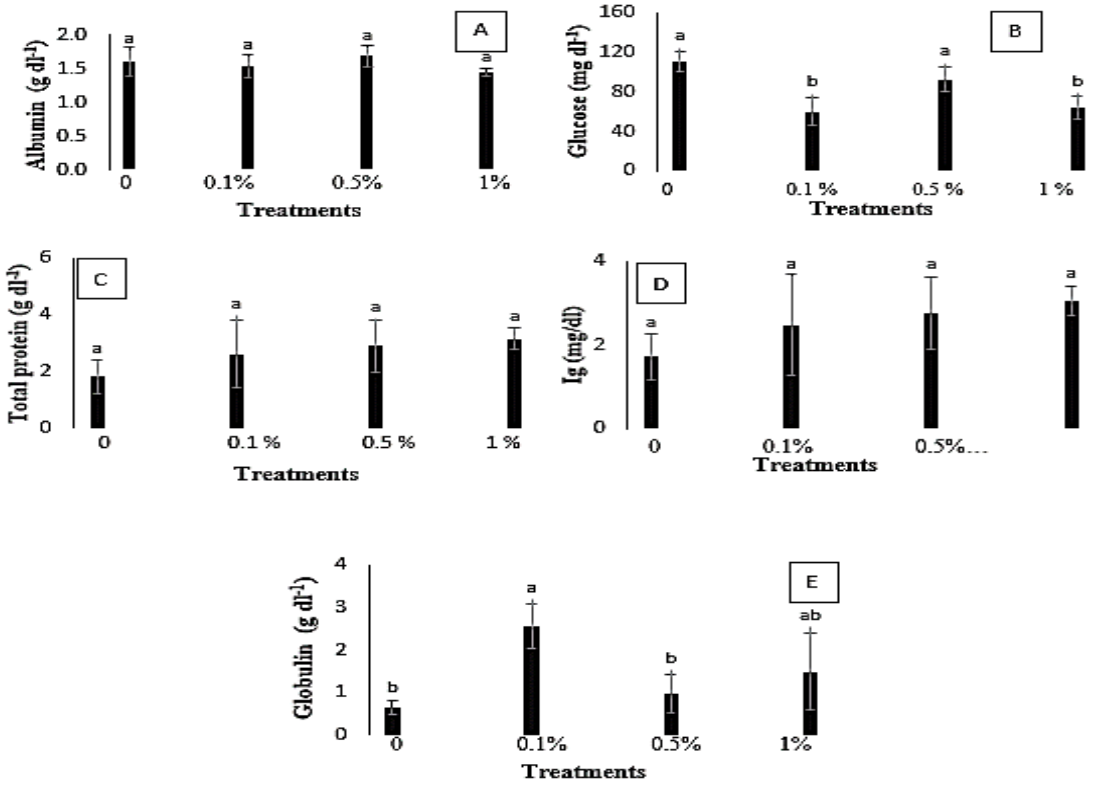

Fig.2. Some serum innate immune indices: A) Albumin, B) Glucose, C) Total protein, D) Immunoglobulin and E) Globulin levels in Common carp (*Cyprinus carpio*) fed with different levels of major plantain extract for 45 days. Values are presented as the mean±S.D. The bars assigned with different letter denote significant difference between treatments (P<0.05).

Immunoglobulin concentrations in treatments fed with different levels of major plantain extract and the control group (P>0.05). However, the amount of globulin in treatment fed with 0.1% the plant extract was noticeably higher than others (P<0.05).

DISCUSSION

Plantaginaceae traditionally was used to treat liver disease, stomach problems and urinary system inflammation. The effect of some species of plantain was investigated in animal feeding (Fu et al. 2019). Despite the unique properties of major plantain, there is limited information about the possible effects of major plantain in fish nutrition. However, there is some positive information about other species of plantain on animals, which caused to improve general performance. For instance, *Asiatica plantain* extract reduced blood pressure in mice (Fu et al. 2019). Also, ribwort plantain *(Plantago lanceolate L.)* is useful to wound healing in mice (Kurt et al. 2018).

Hematological indices are important indices to fish health monitoring (Fazio, 2019). Obtained results showed diets supplemented with major plantain extract had no significant effect on RBC, HCT and MCV. RBC, Hb and HCT levels play a key role to facilitate tissue oxygenation and carbon dioxide elimination. Similar results were reported by Elbesthi et al. (2020). They proved dietary supplemented with *Plantago lanceolate* extract had no notable effect on RBC and HCT in rainbow trout. Some previous studies confirmed that some plants and their derivatives like juniper berry oil (Osman et al. 2019), olive leaf extract (*Olea europea L.*) (Zemheri-Navruz et al. 2019), marjoram extract (*Origanum majorana*) (Yousefi et al, 2020), ginger extract *(Zingiber officinale)* (Mohammadi et al. 2020), and rosemary leaf powder (Yousefi et al. 2019) had no remarkable effect on RBC and HCT levels of common carp. In contrast with our results, it was reported RBC, Hb and HCT levels were remarkably increased by supplementing diet with 200 mg *Origanum majorana*

extract as an herbal supplementation in per kg to common carp diet (Yousefi et al. 2020). Hb level in treatment fed with 0.1% major plantain extract was remarkably higher than other groups. To confirm our result, a published report proved that supplemented diet with *Plantago lanceolate* extract caused a significant increase in Hb concentration in comparison with the control group (Elbesthi et al. 2020). Our findings showed there was no significant difference in MCV between all treatments. Binaii et al. (2014) investigated dietary treated by nettle leaf and stem powder (*Urtica dioica*) on juvenile beluga *(Huso huso)* and then reported the herbal powder had no significant effect on MCV level, but MCHC level was significantly increased. MCH and MCHC level in treatment fed with 0.1% major plantain extract was remarkably higher than other groups. Similar results confirmed our findings reported by Rajabi Esterabadi et al. (2020), Elbesthi et al. (2020), Mohammadi et al. (2020), which they were all found that diet supplemented with plant-treated extracts increased MCH and MCHC in treatments compared to the group fed with non-supplemented diet. This increase might be related to the increase in Hb concentration.

The humeral immunity is an important part of innate immune system includes the production of numerous antibacterial compounds, proteins of inflammation acute phase, complement activated by alternative pathway, cytokines, phagocytosis and inflammation (Bayne & Gerwick 2001). White blood cells (WBC) are a part of the humoral immune system that helps fight infection and defend the body against other foreign materials (Rieger & Barreda 2011). In the present study, WBC in treatment fed with 1% major plantain extract was notably higher than other groups, However, Monocytes, lymphocytes and neutrophils were no significant difference in all treatments. Increasing in WBC due to plant-based supplemented diet causing in innate immune boosting to disease resistance (Yousefi et al, 2020). Increasing in WBC by mixing diets with herbal derivatives have been proved by previous studies (For more information see, Binaii et al. (2014), Adel et al. (2016), Yousefi et al. (2019), Adel et al. (2020) and Yousefi et al. (2020). White blood cells such as neutrophils and macrophages play an important role in defense against pathogens, bacteria, viruses, fungi and parasites (Bilen et al. 2020) that confirmed by Binaii et al. (2014) and Mohammadi et al. (2018). In the present study, it can be justified that there was no significant difference in the amount of these cells due to the absence of the disease and lack of pathogens.

ALP, AST and ALT activities are bio-indicators of liver histological damages. Based on our findings, there were no significant differences in hepatic metabolic enzymes in fish fed different levels of major plantain extract and the control group. Changes in biochemical indices reflect an abnormal physiological state (Osman et al. 2010) and stress conditions (Roohi et al. 2017). Similar results were documented by other researchers who reported herbal extracts did not lead to changes in the amount of liver enzymes in their studied species, because these plant extracts did not have harmful and toxic substances for the species body, so, no adverse outcomes in liver observed (For more information see; Binaii et al. (2014), and Latif et al. (2021). Our results showed supplemented diet with major plantain extract was not effective on serum TP and Al levels in common carp as well. TP concentration is considered as a main indicator of innate immune system status in fish. Diminution of TP can reflect liver disease or liver tissue destruction (Roohi et al. 2017). Also, albumin is transport protein that bind to various ligands and carry them around and play a key role in maintain asthmatic pressure and innate immune system (Yeganeh et al. 2015). In line our results, Osman Sabri Kesbiç et al. (2019) were published herbal-supplemented diet had not significant effect on serum albumin level in common carp. In the present study, due to no change in the level of TP and albumin concentrations, along with no change in the levels of liver metabolic enzymes in the treatments, it can be indicated that major plantain extract had no adverse effect on fish health status.

Serum globulin level in treatment fed with 0.1% major plantain extract was remarkably higher than other treatments. Awad & Awaad (2017), reported that some medical plant extract can improve the level of

globulin in some species. To confirm our results Mohammadi et al. (2020) documented addition of 0.2% and 0.4% ginger extract (*Zingiber officinale*) in common carp diet caused a sharp increase in globulin level. Glucose level in control and treatment fed with 0.5% medical plant extract were as the same. Since, glucose level is considered as stress indicators in fish (Osman et al, 2010) and changes in serum glucose level is sign of malnutrition or kidney damage (Roohi et al. 2017). So, it is concluded that adding major plantain extract more than 0.5% reduces stress and probably kidney damage. Similar to our results, Rashidian et al. (2020) reported that addition of alcoholic extract of oak (*Quercus brantii*) acorn more than 600 mg cause in glucose level decreasing.

Ig is coated by lymphocytes or epithelial cells that detect antigens such as bacteria and increase disease resistance. No significant differences were observed in Ig level fed with different levels of major plantain extract in all treatments and the control group. To the best of our knowledge there is no published study about the effects of dietary supplemented with major plantain in common carp, so, we can benefit from the results of similar cases. In agreement with our study, Safari et al. (2016) reported that herbal-treated dietary had no significant effect in terms of Ig level in common carp. In contrast, previous studies revealed elevation of the total Ig in Indian major carp (*Labeo rohita*) fed root (*Achyranthes aspera*) extract containing diet (Rao et al.,2004), common carp fry fed Persian hogweed (*Heracleum persicum*) (Hoseinifar et al. 2016), loquat (*Eriobotrya japonica*) (Hoseinifar et al. 2018), *Labeo rohita* (Hamilton) fed *Ocimum sanctum* (Tulsi) (Das et al. 2015). No increase observed in total Ig level in the present study may be justified by inability of major plantain extract to influence on immunity system in common carp. However, no negative effects were observed.

CONCLUSION

In conclusion, major plantain extract had no negative effects in all treatments. Based on our results, major plantain extract at the level of 0.1% had more positive effects on some hematological indices and the immune responses; therefore, it can be recommended supplementing diet with major plantain extract to promote immune system in farmed fish.

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مقاله کامل

اثر سطوح مختلف عصاره بارهنگ کبیر (*Plantago major*) بر پارامترهای هماتولوژی، بیوشیمی و ایمونولوژی در بچه‌ماهی کپور معمولی (*Cyprinus carpio*)

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چکیده:

بارهنگ کبیر یکی از گیاهان دارویی بوده که در طب سنتی برای درمان بیماری‌ها مورد استفاده قرار می‌گیرد. پژوهش حاضر، با هدف بررسی تأثیر سطوح مختلف عصاره بارهنگ کبیر بر پارامترهای هماتولوژی، بیوشیمی و ایمنی در بچه‌ماهی کپور معمولی انجام شد. بدین‌منظور، تعداد ۲۴۰ قطعه بچه‌ماهی سالم کپور معمولی با میانگین وزنی ۲/۰۹±۱۸/۶۹گرم در ۳ تیمار شامل: ۰/۱ ، ۰/۵ و ۱ درصد عصاره بارهنگ کبیر در کیلوگرم غذا و یک گروه شاهد (بدون عصاره بارهنگ کبیر) هر کدام با سه تکرار، توزیع گردیدند. تغذیه بچه‌ماهیان هر روز به میزان ۳ درصد وزن بدن به‌مدت ۴۵ روز به طول انجامید. در پایان آزمایش، نمونه‌های خون توسط سرنگ از ساقه دمی ماهی گرفته شد و برخی از فاکتورهای خونی (گلبول قرمز، هماتوکریت، هموگلوبین، متوسط حجم گلبول قرمز، متوسط هموگلوبین گلبول قرمز، متوسط غلظت هموگلوبین گلبول قرمز، گلبول سفید، لنفوسیت، مونوسیت، نوتروفیل)، آنزیم‌های متابولیکی کبدی (آلانین آمینوترنسفراز، آسپارتات آمینوترنسفراز و آلکالین فسفاتاز) و برخی از پارامترهای بیوشیمیایی ایمنی ذاتی سرم (گلوکز، ایمونوگلوبولین کل، گلوبولین، پروتئین کل سرم و آلبومین) مورد ارزیابی قرار گرفتند. نتایج حاصل نشان داد که گلبول‌های قرمز خون، هماتوکریت، حجم متوسط گلبول قرمز، لنفوسیت، مونوسیت و نوتروفیل تفاوت معنی‌داری بین تیمارها و گروه شاهد نداشت ($P>0/05$). غلظت هموگلوبین، هموگلوبین گلبول قرمز، غلظت متوسط هموگلوبین افزایش معنی‌داری در تیمار ۰/۱٪ در مقایسه با سایر تیمارها و گروه شاهد نشان داد ($P<0/05$). همچنین، تعداد گلبول‌های سفید خون در تیمار ۱٪ افزایش معنی‌داری را در مقایسه با سایر تیمارها و گروه شاهد نشان داد ($P<0/05$). نتایج بررسی میزان آلکالین فسفاتاز، آلانین و آسپارتات آمینوترنسفراز، پروتیین کل، آلبومین و ایمنوگلوبولین کل نشان داد که تفاوت آماری بین تیمارها و گروه شاهد وجود نداشت ($P>0/05$). سطح گلوکز در تیمارهای تغذیه شده با ۰/۱ و ۱ درصد عصاره بارهنگ کبیر تفاوت معنی‌داری با یکدیگر نداشت ($P>0/05$). اما به‌طور معنی‌داری بالاتر از دو گروه دیگر بود ($P<0/05$). با توجه به نتایج به‌دست آمده و با توجه به تأثیر مثبت بیشتر عصاره باهنگ کبیر در سطح ۰/۱ درصد بر برخی از پارامترهای خونی و پاسخ‌های ایمنی در ماهی کپور معمولی، می‌توان استفاده از عصاره گیاه بارهنگ کبیر را برای تقویت سیستم ایمنی در ماهیان پرورشی پیشنهاد کرد.

کلمات‌کلیدی: بارهنگ کبیر، هماتولوژی، بیوشیمی، ایمنی، کپور معمولی