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Immunomodulatory Effect of *Ocimum gratissimum* Linn. Leaf Extract on a Common Fish *Clarias batrachus* Linn.

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Abstract

The use of immunostimulants for the prevention of disease in fishes is considered as an attractive and promising area in the field of aquaculture. Immunostimulants are valuable for the prevention and control of fish diseases in aquaculture as they represent an alternative and supplementary treatment to vaccination. They also have additional effects such as growth enhancement and increase in the survival rates of the fishes under stress. Certain medicinal plants are believed to promote positive health and maintain organic resistance against infection by re-establishing body equilibrium and conditioning the body tissues. The present study was designed to evaluate the immunostimulant potential of crude extract of Ocimum gratissimum L. leaf on fish Clarias batracus in both specific and non specific levels. Our results showed that there is not a significant decrease in the amount of Glucose and cholesterol at concentration 2.5% but there is a significant reduction in glucose amount at 5% level in comparison to control. But a significant increase was seen the RBC, WBC, Serum protein and globulin at 2.5% and 5% concentrations of crude extracts in both the 15 and 30 days of treatments in the blood of the fish which may be considered as a sign of improvement in both specific and non specific immune responses. Based on the results it is appropriate to conclude that the plant extract of Ocimum gratissimum may act as a potent Immunostimulant in Clarias batrachus.

Keywords: Immunostimulants, Phytochemicals, *Clarias batrachus* Linn, *Ocimum gratissimum* Linn.

Introduction

Natural plant products present a viable alternative to antibiotics and other banned drugs being safer for the reared organism and humans, as well as, the environment. Herbals can be used not only as remedies but even more so, as growth promoters, stress resistance boosters and preventatives of infections. The herbs can also act as immunostimulants, conferring the non-specific defense mechanisms of fish and elevating the specific immune response. Studies have proved that herbal additives enhance the growth of fishes and protect them from diseases. The herbs can also act as immunostimulants, conferring the non-specific defense mechanisms of fish and elevating the specific immune response [1,2]. Recently, there has been increased interest in the immune stimulating function of some herbs in aquaculture. The non-specific immune functions such as bacteriolytic activity and leukocyte function of fish have been improved by some herbs [2]. Treatment with medicinal plants having antibacterial activity is a potentially beneficial alternative in the aquaculture. These herbs mitigate many of the side effects which are associated with synthetic antimicrobials. Today research has been initiated to evaluate the feasibility of herbal drugs in fish diseases [3]. Thus, the use of herbs is an alternative to antibiotics in fish health management. Many studies have proved that herbal additives enhanced the growth of fishes and protected them from diseases [4,5]. The herbs are not only safe for consumers but also widely available throughout Asia and they also have a significant role in aquaculture [6]. The application of herbal additives in diets often provides cooperative action to various physiological functions.

In recent years, the use of medicinal plant as an effective alternative to antibiotics has gained importance, especially to combat disease problems in fishes [7]. Plant derived phytomedicines have great promise in the treatment of infectious diseases. Herbal products promise a cheaper source for therapeutics, greater accuracy than chemotherapeutic agents and a viable solution for all problems which carp culture. Phytochemical is a natural bioactive compound found in plants, such as vegetables, fruits, medicinal plants, flowers, leaves and roots that work with nutrients and fibers to act as an defense system against disease or more accurately, to protect against disease. Several antimicrobial, antistress, immunostimulant, growth-promoting plant products are significantly influenced the fish/shrimp larviculture [8,9,10,11]. A variety of plant-derived materials such as polysaccharides, lectins, peptides flavonoids, isoflavonoids, phytosterols, polysaccharides, alkaloids, sesquiterpenes, glucans, tannins, vitamins, and a variety of other phytochemical substances have been reported to modulate the immune system [12,13,14,15].

The immunostimulants which have been tested for application to aquaculture include peptides like FK-565 [16,17], glucan extracts from a tunicate [18], lactoferin [19], levamisole [20], chitosan [21], GH [22], Vitamin C [23,24], extra cellular products of *Mycobacterium* spp. and oligonucleotides [25]. These immunostimulants have been reported to increase various aspects of innate immunity such as the number of phagocytes, lysozyme and complement activities as well as serum Ig levels. Some immunostimulants have been shown to protect rainbow trout [26] against furunculosis and to decrease unspecific mortality in rockfish [27].

Ocimum gratissimum Linn. is an important medicinal herb, commonly known as "Ram Tulshi" in India. This plant belongs to Lamiaceae family. The plant is used in folk medicine to treat different common diseases like cold and flues [28,29,30,31]. It has been associated with chemopreventive, anticarcinogenic, free radical scavenging, radio protective, and numerous others pharmacological use [32]. The aqueous leaf extract and seed oil showed antiproliferative and chemopreventive activity on HeLa cells [33]. Ocimum gratissimum has been used for the medicinal purposes from the ancient ages and recently we have found a number of beneficial roles against nicotine-induced toxicity in murine peritoneal macrophages [33,34,35,36]. Hence the present study was designed to evaluate the immunostimulant potential of crude extract of *Ocimum gratissimum* L. leaf on fish *Clarias batrachus* Linn. in both specific and non specific levels.

Materials and Methods

Collection of Test Organisms and their acclimatization

Healthy living specimens of *Clarias batrachus* (Linn.) weighing about 300-310gm and 18-23cm and in length were collected from the grow-out ponds of Central Institute of Freshwater Aquaculture (CIFA) at Kausalyaganga, Bhubaneswar, Odisha, India and acclimatized them into laboratory conditions. They were kept for acclimatization for a period of one week before the experimentation. They were kept for acclimatization for a period of one week before the experimentation. Further the fishes were divided into three groups; two experimental groups along with the control (in duplicate). Three fishes for each group were separated out and kept in rectangular fiber glass cisterns of 10L capacity with 100L dechlorinated fresh water. The water level was maintained at 5L. They were kept at an ambient, uncontrolled temperature of 28 ± 2^0C under natural photoperiod. Water was changed on every alternate day. Fishes were fed with fish food with balanced fish diet prepared in the laboratory. The faecal matter and other waste materials of fishes were siphoned off daily to reduce the ammonia content in water.

Experimental design

The fishes were primarily divided into three experimental groups in three separated chambers. Each chamber contained three fishes. The group-A was kept as control group which were fed with control diet throughout the experimental period of 15 and 30 days. Group-B and Group-C received the prepared fish diet as doses at a rate of 2.5% and 5% respectively. The experiment was conducted for a period of 15 and 30 days (Chart-1). During this period, 50% of the experimental solution was replenished once a week. Fishes were fed @ 5% of body weight with a balance pelleted diet consisting of fish meal (40%), rice bran (23.7%), groundnut oil cake (22.6%), soyabean flour (13.6%), wheat flour (10%), supplemented with required amount of vitamin and mineral mixtures (0.1%), the lab prepared fish diet as doses at a rate of 2.5% and 5% respectively for carrying out the experimental work. The fishes were fed for 30 days with their respective feed and then the hematological and biochemical analyses were carried out after 15 and 30 days of observations respectively.

Preparation of Crude Extracts and Fish feed

The collected leaves were shade dried under normal environmental condition, ground into uniform powder using Thomas-Wiley machine. The powdered leaves of *Ocimum gratissimum* Linn. (50g) were extracted by hydro-distillation method by using Soxhlet apparatus at room temperature. The filtrate was collected and the solvent was removed using rotary evaporator (Buchi SMP, Switzerland). The residue obtained after evaporation was dissolved and the desired amount of doses were prepared in sterile distilled water and stored at -20^0C until used for experimentation.

Collection of blood sample for analysis

The effect of immune system on growth was studied by recording the individual weight of three fishes of each chamber at 0, 15 and 30 days. On days 15^{th} and 30^{th} three fishes from each group were bled with the aid of a $2cm^3$ plastic syringe and were inserted in the caudal vein and blood was drawn by keeping the fish vertically held with the head upwards. Blood samples of about 4milliliters was collected from the caudal peduncle with the syringe, out of which 1ml of the blood was dispensed into ethylene diamine tetra-acetic acid (EDTA) anticoagulant for hematological studies, while 3ml was transferred into a tube containing lithium heparin anticoagulant to obtain plasma for biochemical analysis of the plasma obtained by centrifugation (through medical centrifuge, TGL-20, Shuke, Sichuan, Mainland, CHINA) from the lithium heparinised samples was stored at -20^0C until analyzed.

Experimental Procedure

The mean average weight and length of the fishes of each chamber were determined at the beginning of the experiment and after 15 and 30 days of the experiment. The weight of the fishes was determined by using weighing scale (OHAUS MODEL Cs 5000, CAPACITY 5000 2g), and length was measured by normal scale.

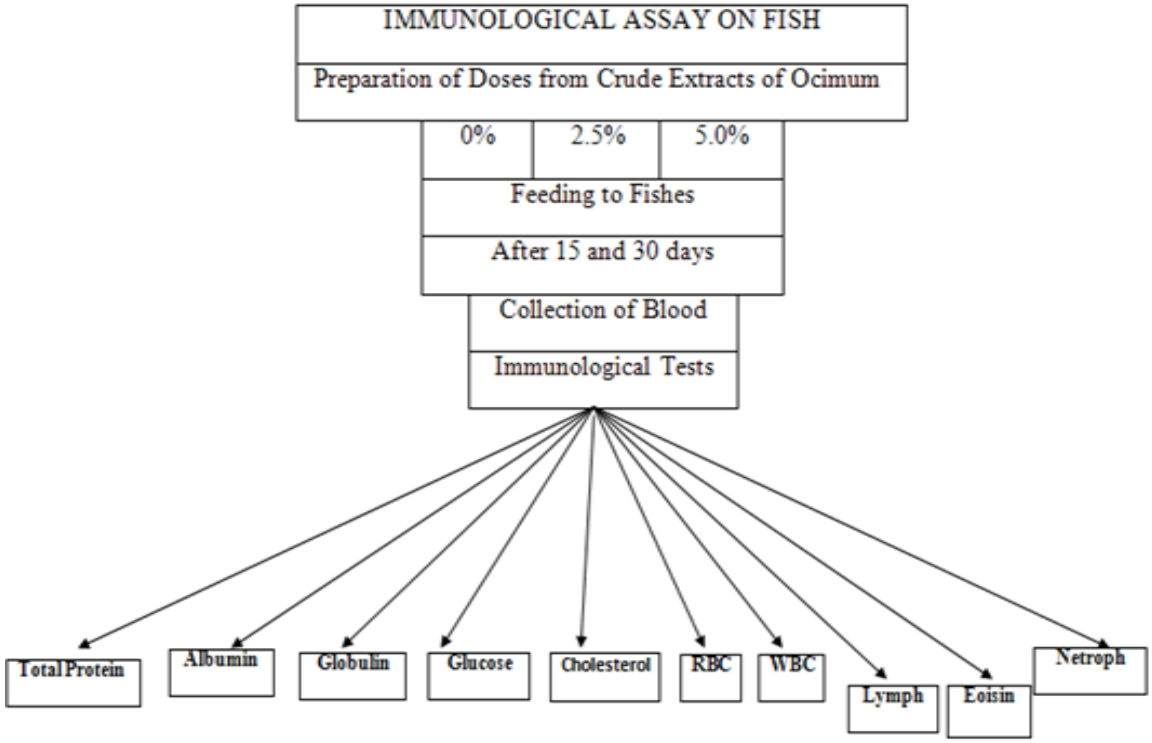

Chart-1: Flow Chart of Experimental Design.

Hematological Studies

Haematological values were measured by following standard methods at 0, 15 and 30 days respectively. Red blood corpuscle (RBC) and White blood corpuscle (WBC) were counted by Neubaur's improved hematocytometer (Superior, Marienfeld, Germany) using Hyem's and Turk's as a diluting field respectively. Differential count was done after selecting about 100 leucocytes from each smear under oil immersion. Percentages of lymphocytes, monocytes, neutrophils and eosinophils were calculated by counting at least 100cells. The thrombocytes were counted from the blood smears prepared [37,38]. The serum total protein concentration was estimated by Biuret colourimetric reaction, according to the method as described by [39,40] and serum albumin and globulin concentration was estimated by bromocresol green colourimetric reaction, according to the method as described by [41,42].

Biochemical Studies

The plasma was analyzed for serum glucose level measured spectrophotometrically by UV-vis spectrophotometer (Microprocessor UV/VIS EI Spectrophotometer model 1371, INDIA) at 505nm by GOD/POD method using glucose kit procured fro Qualigens diagnostics and cholesterol was measured by CHOD/PAP method with the help of a cholesterol kit procured from Crest Biosystems. The total protein following the dye binding method of Bradford using bovine serum albumin (BSA) as a standard, albumin and globulin by the bromocresol green method [43,44,45].

Results

Effect of herbal crude extracts on Body Weight and Body Length

Table 1 show the body weight and length response of the fishes by the repeated administration of the extracts. The initial body weights of fishes from each group (Gr.A, Gr.B and Gr.C) were recorded which are considered as control before carrying out the experimentations and they were as follows: 300.25±0.1gm, 304.12±0.6gm and 305.56±0.5gm respectively. After experimentations of 15 days again the weight of the fishes were weighed from each group (Gr.A, Gr.B and Gr.C) and they were as follows: 302.43±0.5gm, 306.26±0.4gm and 308.56±0.5gm respectively. Likewise after completion of 30 days of experimentations finally the body weights from each group were as follows: 305.20±0.2gm, 309.34±0.5gm and 311.37±0.3gm respectively.

The initial body lengths of fishes from each group (Gr.A, Gr.B and Gr.C) were recorded which are considered as control before carrying out the experimentations and they were as follows: 18.2±0.2cm, 20.5±0.5cm and 21.4±0.6 respectively. After experimentations of 15 days again the lengths of the fishes were

measured from each group (Gr.A, Gr.B and Gr.C) and they were as follows: 19.5±0.4cm, 21.3±0.2cm and 22.3±0.3cm respectively. Likewise after completion of 30 days of experimentations finally the body lengths from each group were as follows: 20.7±0.8cm, 22.8±0.5cm and 23.0±0.6cm respectively (Table 1 and Figure 1).

Table-1: Body Length and Weight of *Clarias batrachus* after 15 Days and 30 Days

Groups	Body Length (cm)			Body Weight (gm)		
	Control	15days	30days	Control	15days	30days
A	18.2±0.2	19.5±0.4	20.7±0.8	300.25±0.1	302.43±0.5	305.20±0.2
B	20.5±0.5	21.3±0.2	22.8±0.5	304.12±0.6	306.26±0.4	309.34±0.5
C	21.4±0.6	22.3±0.3	23.0±0.6	305.56±0.5	308.56±0.5	311.37±0.3

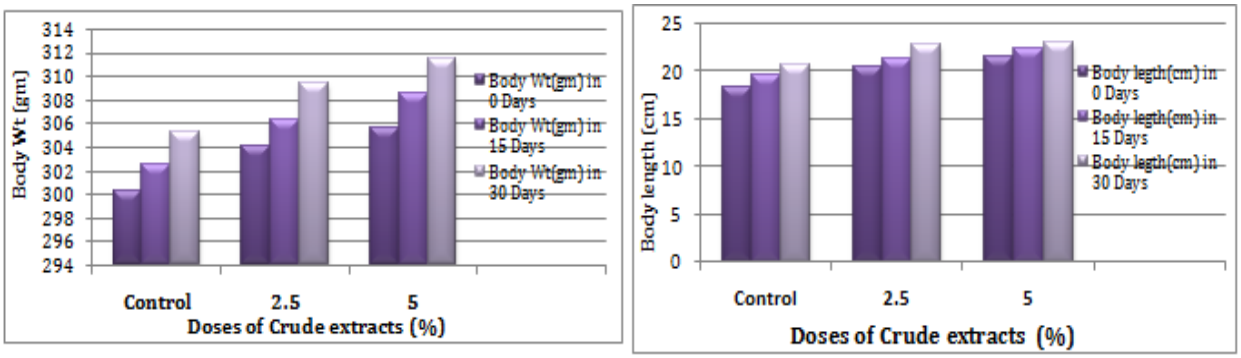

Figure-1: Variation in Body Weight and Length of Fish after 15 and 30 days

Effect of *Ocimum gratissimum* crude extracts on Total protein, Albumin and Globulin

The serum total protein from each group (Gr.A, Gr.B and Gr.C) were found to be 2.25±0.1mg/dl, 2.28±0.3mg/dl and 2.33±0.2mg/dl (at 15 days) and 2.36±0.2mg/dl, 3.11±0.3mg/dl and 3.75±0.5mg/dl (at 30 days of observations) respectively. Whereas the albumin content of Gr.A, Gr.B and Gr.C were 1.32±0.5mg/dl, 1.30±0.2mg/dl and 1.20±0.5mg/dl (at 15days) and 2.32±0.4mg/dl, 1.88±0.5 and 1.05±0.2mg/dl (at 30 days) respectively. The serum globulin value was found to be 1.42±0.4mg/dl, 1.53±0.6mg/dl and 1.62±0.1mg/dl (at 15days) and 1.65±0.5mg/dl, 1.98±0.25mg/dl and 1.60±0.3mg/dl (at 30days) respectively (Table 2 and Figure 2). The total protein and globulin contents of Gr.B and Gr.C increased in comparison to Gr.A in both 15 and 30 days treatments; however the albumin content decreased in Gr.B and Gr.C in comparison to Gr.A in both the treatments.

Table-2: Effect of *Ocimum gratissimum* crude extracts on Total protein, Albumin and Globulin of *Clarias batrachus* after 15 and 30 Days

Groups	15 Days			30 Days		
	Total Protein (mg/dl)	Albumin (mg/dl)	Globulin (mg/dl)	Total Protein(mg/dl)	Albumin (mg/dl)	Globulin (mg/dl)
A	2.25±0.1	1.32±0.5	1.42±0.4	2.36±0.2	2.32±0.4	1.65±0.5
B	2.28±0.3	1.30±0.2	1.53±0.6	3.11±0.3	1.88±0.5	1.98±0.2
C	2.33±0.2	1.20±0.5	1.62±0.1	3.75±0.5	1.05±0.2	1.60±0.3

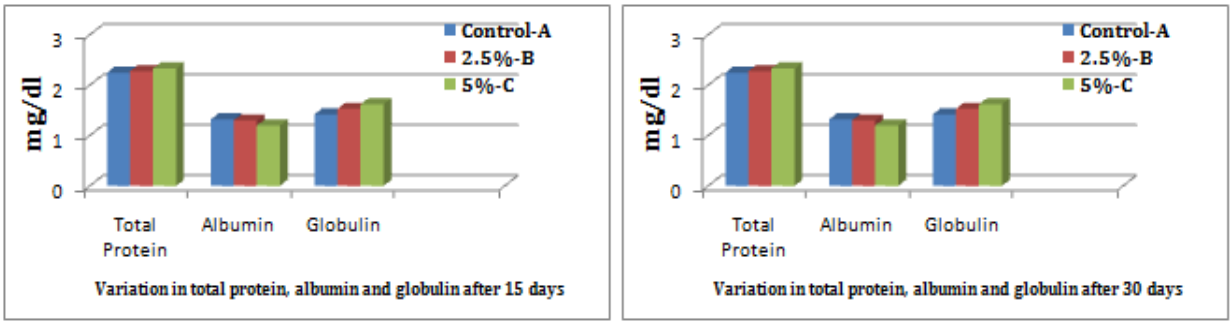

Figure-2: Variation in Total protein, Albumin and Globulin of Fish after 15 and 30 days

Effect of *Ocimum gratissimum* crude extracts Glucose, Cholesterol, RBC and WBC

The serum glucose content of all the experimental fishes (Gr.A, Gr.B and Gr.C) had elevated 50.82±1.20mg/dl, 50.04±1.02mg/dl and 49.65±1.31mg/dl (at 15days) and 51.35±1.2mg/dl, 50.27±1.5mg/dl and 48.43±1.04mg/dl (at 30days) respectively. The serum cholesterol level of the control fish was found to be 156.35±1.08mg/dl, 153.24±0.5mg/dl and 151.21±0.7mg/dl (at 15 days) and 158.14±1.32mg/dl, 153.25±1.20mg/dl and 145.33±1.14mg/dl (at 30days) respectively. The cholesterol content of fishes of both the Gr.B and Gr.C appeared to be lower than control as well as there was a decrease value from Gr.B to group Gr.C. The total number of erythrocytes of control fish had a mean value of 2.175±1.03million/mm^3 where as experiment Gr.B and Gr.C had 2.2025±1.05million/mm^3 and 2.2132±0.8million/mm^3 (at 15days) and 2.2215±1.05million/mm^3, 2.2285±1.04million/mm^3 and 2.2345±1.07million/mm^3 respectively. The WBC counts of fishes of all the three groups (Gr.A, Gr.B and Gr.C) were found to be 4330.32±0.5cells/µl, 4345.0±0.4cells/µl and 4378.5±0.7ells/µl (at 15 days) and 4375.0±1.11cells/µl, 4480.12±1.05cells/µl and 4565.0±1.04ells/µl respectively. There is a significant increase in the amount of RBC and WBC Gr.B and Gr.C respectively in comparison to control (Table 3 and Figure 3,4,5).

Table-3: Effect of *Ocimum gratissimum* crude extracts on Glucose, Cholesterol, RBC and WBC of *Clarias batrachus* after 15 and 30 Days

Groups	15 Days				30 Days			
	Glucose (mg/dl)	Cholesterol (mg/dl)	RBC (Million/m^3)	WBC (Per µl)	Glucose (mg/dl)	Cholesterol (mg/dl)	RBC (Million/m^3)	WBC (Per µl)
A	50.82±1.20	156.35±1.08	2.175±1.03	4330.32±0.5	51.35±1.2	158.14±1.32	2.2215±1.05	4375.0±1.11
B	50.04±1.02	153.24±0.5	2.2025±1.05	4345.0±0.4	50.27±1.5	153.25±1.20	2.2285±1.04	4480.12±1.05
C	49.65±1.31	151.21±0.7	2.2132±0.8	4378.5±0.7	48.43±1.04	145.33±1.14	2.2345±1.07	4565.0±1.04

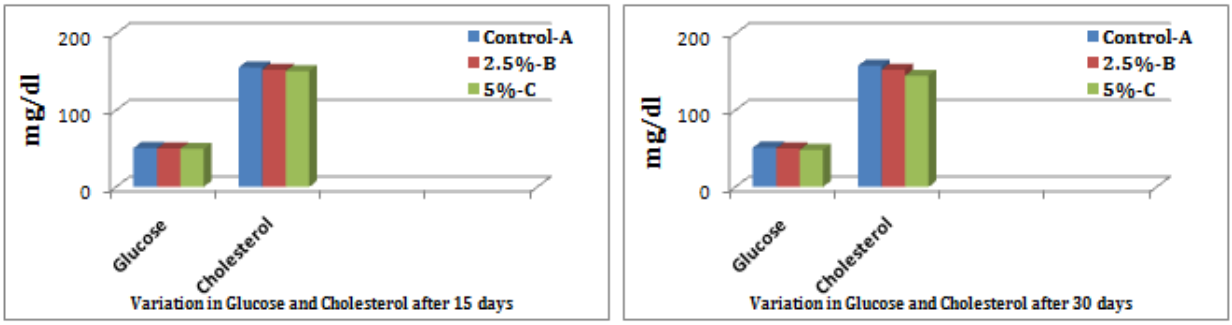

Figure-3: Variation in Glucose and Cholesterol of Fish after 15 and 30 days

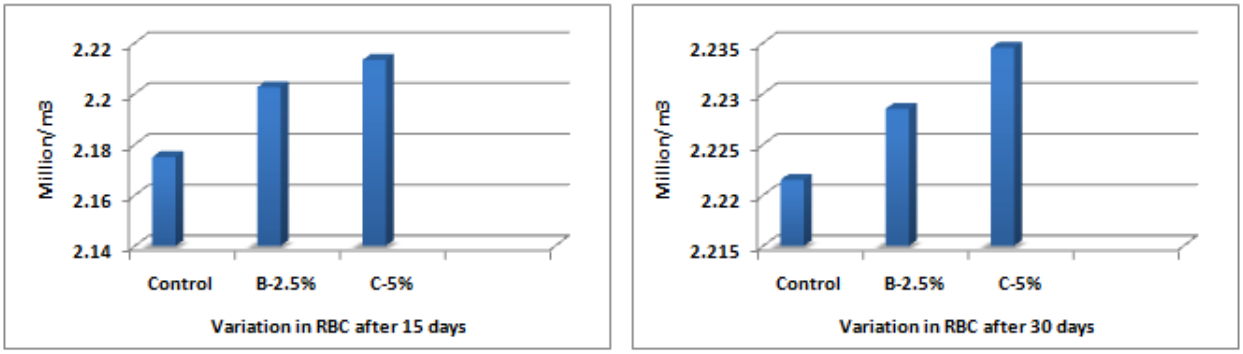

Figure-4: Variation in RBC of Fish after 15 and 30 days

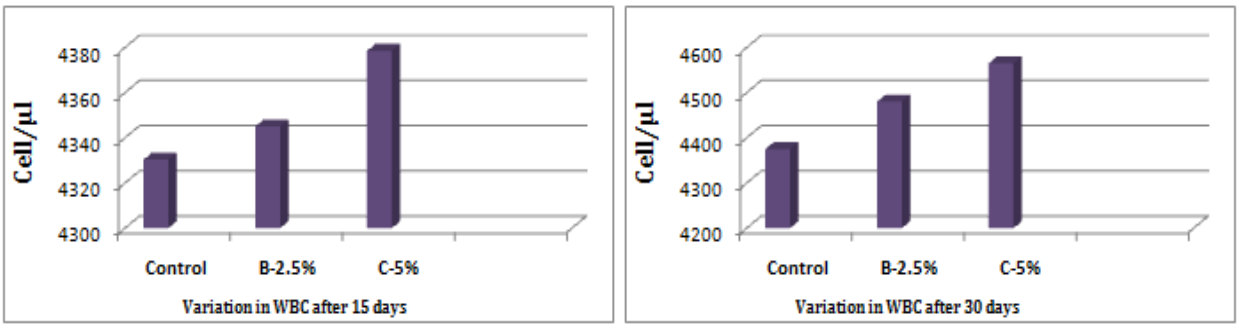

Figure-5: Variation in WBC of Fish after 15 and 30days

Effect of *Ocimum gratissimum* crude extracts on Lymphocytes, Eosinophils and Neutrophils

The phagocytes were found to be abundant in the blood of all treated fishes. The total lymphocytes of all the fishes of each group were found to be 3.4±0.2%(small) 31.1±1.04%(large), 3.0±0.5%(small) 31±0.8%(large) and 3.1±0.2%(small) 32.2±0.5%(large) (at 15days) and 3.6±0.2%(small) 32.1±1.04%(large), 3.7±0.3%(small) 32.8±1.25%(large) and 3.8±0.2%(small) 33.9±1.22%(large) (at 30days) respectively. Whereas the eosinophils were 6.8±0.5%, 6.9±0.2% and 7.0±0.5% (at 15 days) 7.2±0.23%, 7.8±0.12%, 8.1±0.35% (at 30 days) respectively. Similarly in case of Neutrophils they were as follows: 25.7±1.05%, 25.6±1.12% and 26.8±0.6% (at 15 days) and 26.2±1.04%, 27.15±1.34% and 27.85±1.26% respectively. There is a significant increase in the amount of Lymphocytes, Eosinophils and Neutrophils in Gr.B and Gr.C respectively in comparison to control (Table 4 and Figure 6).

Table-4: Effect of *Ocimum gratissimum* crude extracts on Lymphocytes, Eosinophils and Neutrophils of *Clarias batrachus* after 15 and 30 Days

Groups	15 Days				30 Days			
	Lymphcytes (%)		Eosinophils (%)	Neutrophils (%)	Lymphcytes (%)		Eosinophils (%)	Neutrophils (%)
A	3.4±0.2	31.1±1.04	6.8±0.5	25.7±1.05	3.6±0.2	32.1±1.04	7.2±0.23	26.2±1.04
B	3.0±0.5	31±0.8	6.9±0.2	25.6±1.12	3.7±0.3	32.8±1.25	7.8±0.12	27.15±1.34
C	3.1±0.2	32.2±0.5	7.0±0.5	26.8±0.6	3.8±0.2	33.9±1.22	8.1±0.35	27.85±1.26

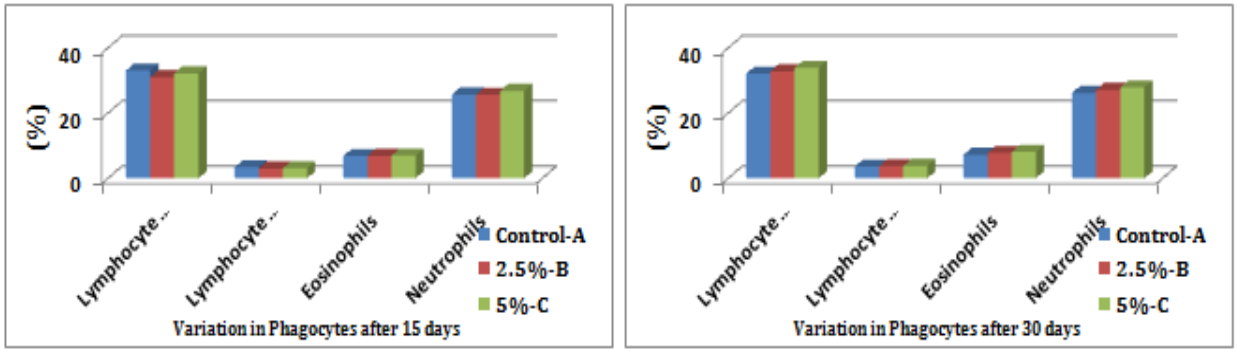

Figure-6: Variation in Phagocytes of Fish after 15 and 30 days

Discussion

It has been shown that herbal based immunostimulants are capable of enhancing nonspecific and specific defense mechanisms and/or reducing losses from viruses, bacteria and/or parasitic infections in carp [46,47,48]. Several plant materials/products such as *Eclipta alba* [49], *Aloe vera* [27], *Ocimum sanctum* [50], *Viscum album*, *Urtica dioica* and *Zingiber officinale* [51], *Solanum trilobatum* [52]. *Astragalus radix* and *Scutellaria radix* [53] and *Achyranthes aspera* [54] have been reported to enhance the immunity of fish. The result showed that there were increasing concentrations of serum total protein levels of test groups i.e. Gr.B and Gr.C in comparison to Gr.A as control. There is a significant increase in the amount of protein and globulin level by increasing concentrations (2.5% and 5%) of crude extracts of *Ocimum gratissimum* (Table 2,3,4) which could be adduced to

possible chronic infection, liver dysfunction, rheumatoid arthritis, systemic lupus, scleroderma, hypersensitivity states, inflammation, dehydration (chronic diarrhea), respiratory distress, hemolysis and alcoholism [55,56,57].

The result from Table 1 showed that a decrease serum albumin contents in 30 days treatment with *Ocimum gratissimum* crude extracts at both 2.5% and 5% concentrations. With reduced levels of serum albumin, fluid may escape into tissues to cause localized oedema and reduce the delivery of nutrients to tissues. Decreased serum albumin usually indicates liver disease of more than 3 weeks duration [58] and it is a reliable prognostic indicator for increased risk of morbidity and mortality [59]. Serum globulins are increased by the stimulation of B lymphocytes differentiation and proliferation by IL-6 andTNF- [60]. Increased serum level of globulins are implicated in chronic infections (parasites, some cases of viral and bacterial infection), liver diseases (biliary cirrhosis, obstructive jaundice), rheumatoid arthritis, multiple myelomas, leukaemias, waldenstrom's macroglobulinemia, autoimmunity (systemic lupus, collagen diseases) and nephrosis [61]. Decrease in serum albumin that is accompanied with increased serum globulin possibly suggests kidney problems, chronic infections, inflammation, cirrhosis etc [62]. The observed differences in the serum albumin and globulin levels supported the explanation of the increase in serum total protein levels: as serum albumin levels are decreased in malnutrition, increased serum IL-6 and TNF- levels [63].

Our results showed that there is not a significant increase in the amount of Glucose and cholesterol at concentration 2.5% but there is a significant reduction in glucose amount at 5% in Gr.B in comparison to control. The reduced level of the liver total cholesterol and LDL-C (Table 2) support the possibilities of the inhibition of de novo cholesterol biosynthesis by the aqueous extract of *A.paniculata* due to the saponin and polyphenol levels as reported by Oyewo *et al.*, [63], the enhanced reverse cholesterol transport and bile acid excretion, and the inhibition the production of apo B, needed for LDL-C production, transport and binding [64]. *Ganoderma lucidium* is another important medicinal herb containing polysaccharides. At relatively higher doses (0.5 and 1%), it has been reported to be effective in modulating immune functions, inhibiting tumour growth [65], preventing oxidative damage [66], protecting the liver and reducing serum glucose levels-while having no toxic effects in animals [67]. An aqueous extracts of *G.lucidum* was found to promote phagocytosis by macrophages in mice immunosuppressed by cyclophosphamide, stimulate the proliferation of lymphocytes induced by concanavalin A or lipopolysaccharidea and influence the gene expression of cytokines [68]. Invariably there is an increase in the RBC and WBC contents of *Clarias batrachus* treated with aqueous extracts of *Ocimum gratissimum*. Similar results were obtained by Dugenci *et al.*, [51] who tested the immunostimulatory effects of various medicinal plant extracts, such as mistletoe (*Viscum album*), nettle (*Urtica dioica*), and ginger (*Zinger officinale*), in rainbow trout. The ginger extract was found to be very effective in enhancing phagocytosis and extracellular burst activity of the blood leukocytes.

The immune system is a complex system, to protect the host from invading and to eliminate diseases. Immunomodulators are being used as an adjuvant in conditions of immunodeficiency in cancer and other immunodeficiency syndrome. In this present study, *Ocimum gratissimum* at 30 days of observation showed increasing immune system in Fish both in specific and non specific level. The herbal immuno modulator containing *Ocimum gratissimum* extracts act as a very helpful in boosting the immune system of the fish *Clarias batrachus*. Our results showed that there is not a significant decrease in the amount of glucose and cholesterol at concentration 2.5% but there is a significant reduction in glucose amount at 5% in comparison to control.

A significant increase was seen the RBC, WBC, Serum protein and globulin at 2.5% and 5% concentrations of crude extracts in both the 15 and 30 days of treatment in the blood of the fish. It may be due to the effect of this bioactive principle of *Ocimum gratissimum* and ascorbic acid to protect murine peritoneal macrophage from deleterious effect of nicotine and, simultaneously, help to restore their normal functions. Literature showed that an ethanolic extracts of *Ocimum gratissimum* leaves (100mg/kg p.0.) appeared to improve the phagocytic function without affecting the humoral or cell-mediated immune system [69]. In addition, 2 weeks administration of *Ocimum gratissimum* results in no significant differences in lymphocyte, eosinophils and monocytes counts between the experimental groups and control group. Earlier studies had shown that the aqueous leaf extract of *Ocimum gratissimum* has different pharmacological actions including antioxidative properties [70,71,72,73]. But the lymphocyte level decreased at the end of the experiment as compared to the control group. This is supported by the findings of Ephraim *et al.*, [74].

Phytochemical screening of the leaf extract of *Ocimum gratissimum* had shown the plant to contain alkaloids, saponins, tannins, alkaloids, anthraquinone, flavonoids, steroids, terpenoids and cardiac glycosides [70, 75,76].

Almost all the phyto-constituents of *Ocimum gratissimum* are known to influence biological system activities. Furthermore, *Ocimum gratissimum* had been shown to possess diverse pharmacological properties which may be attributed to its usefulness in folk medicine. Also, it contains a trace amount of anthraquinone. Eugenol which is the component obtained as essential oil from *Ocimum gratissimum* is about 93.9% [77]. One of the possible reasons behind it may be the antioxidative property of the AE-Og which contains high level of phenolic and flavonoid compound [34].

Conclusion

Herbals can be used not only as remedies but even more so, as growth promoters, stress resistance boosters and preventatives of infections. The use of herbal extract as feed additives can

significantly benefit any organism cultured under intensive condition. Natural plant products present a viable alternative to antibiotics and other banned drugs being safer for the reared organism and humans, as well as, the environment. The present study evaluated on immunomodulatory activity of aqueous leaf extract of *Ocimum gratissimum* on fish *Clarias batrachus* in biochemical and haemological profiles exhibited a significant increase in RBC, WBC, serum protein and globulin at 2.5% and 5% concentrations of crude extracts in both the 15 and 30 days of treatments in the blood of the fish and which may be considered as a sign of improvement in both specific immune response and non specific immune responses in the blood of the *Clarias batrachus* Linn. Based on the results it is appropriate to conclude that there is a great prospectus of using natural products including plant extracts in the treatment of various parasitic diseases of fish.

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