Research Article

Immunomodulatory Activity of Aqueous Leaf Extract of *Ocimum kilimandscharicum* Guerke in *Clarias batrachus*

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ABSTRACT

Plant derived phytomedicines have great promise in the treatment of infectious diseases because of its cheaper source for therapeutics, greater accuracy than chemotherapeutic agents and a viable solution for all problems to combat disease problems in fishes. *Ocimum kilimandscharicum* Guerke has been suggested to possess various pharmacological properties such as analgesic, anti-inflammatory, antimicrobial, antioxidant, antiulcerogenic, cardiac stimulant, chemomodulatory, hepatoprotective, hypoglycemic, hypolipidemic, immunomodulatory and larvicidal activities. The present study was designed to evaluate the immunomodulatory activity of aqueous leaf extract of *Ocimum kilimandscharicum* on fish *Clarias batrachus* in biochemical and haemological profile. It was observed that the herbal diet (prepared by the aqueous leaf extract of *O.kilimandscharicum*) fed fishes exhibited 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 which may be considered as a sign of improvement in both specific and non specific immune responses. The observed immunostimulatory property of *Ocimum kilimandscharicum* has an implication in the maintenance of fish immune health by using the herbal extract as a potential therapeutic measure.

Keywords: Immunostimulants, Phytochemicals, *Clarias batrachus* Linn., *Ocimum kilimandscharicum* Guerke

INTRODUCTION

As aqua cultural production becomes more intensive, the incidence of disease including various infectious diseases has increased as a result of it leading to significant economic losses. Diseases are a crucial factor which inhibits the expansion of aquaculture. In order to address this problem various researches have been carried out on the immune system of fishes.[1-5] Selected innate parameters of the immune system have been examined, such as the complement factor 3(C3)[6,7,8] and lysozyme[9-12]. The specific immune system and ontogeny of various fish species have also been extensively studied,[13-18] IgM[14,16] as well as maternal transfer of immune parameters[19]. It is also well known that the innate immune system in fish can be triggered by various immunostimulants such as levamisole,[20] glucan,[21,22,23] glucan plus vitamin C,[24,25] yeast RNA,[26] lipopolysaccharide,[27,28] growth hormones,[29] zeranol,[30] chitosan,[31] alginate[32] and medicinal plants[33]. Application of chemotherapeutants has created problems with toxicity, resistance, residues and possibly some public health and environment consequences. Their efficacy under aquatic conditions (open-water systems) remains questionable and they can be costly. The use of chemicals in treating health problems has also been complicated by the misleading advice provided to the farmers by feed and chemical companies regarding the use of antibiotics and therapeutic drugs.[34,35]

To overcome this present situation, herbal medicine can be used as an effective phytotherapy against various fish diseases. Several herbal principles have been tested for their growth promoting activity, biochemical and haemalogical studies.[36-42] Natural plant products have been reported to promote various activities like antistress, growth promotion, appetite stimulation, tonic and immunostimulation and to have aphrodisiac and antimicrobial properties in finfish and shrimp larviculture due to the active principles such as volatile oils, tannins, phenols, saponins, alkaloids polysaccharides, polypeptides, terpenoids, steroids etc.[43,44,45]

Ocimum kilimandscharicum Guerke (Camphor Basil in English) is an economically important medicinal perennial herb belongs to the family Lamiaceae and is distributed in East Africa, India and Thailand.[46,47] In traditional medicine, this plant is widely used for the treatment of various ailments including colds, coughs, abdominal pains, measles, anti-ulcer, bronchitis, anorexia, memory disorders and diarrhea.[48] It is also considered a source of aromatic compounds and essential oils containing biologically active constituents that act as insect repellents, particularly against mosquitoes and storage pests[49-53] or show antibacterial[54] and antioxidant activity[55]. Aqueous extract of leaves of *Ocimum kilimandscharicum* contains flavonoids, tannins, saponins, sterols, carbohydrates, proteins and triterpenoids, camphor, 1,8-cineole, limonene, trans caryophyllene, camphene, 4-terpeneol, myrtenol, α-terpineol, endo-borneol, linalool.[55-59] These chemical constituents are mainly responsible for various biological activities. In spite of many reports about the species *Ocimum kilimandscharicum* Guerke there are not sufficient studies

about the immunomodulatory study in animals. So the objective of the present study was to evaluate the development of immunity on both specific and non specific levels by the leaf extract of *Ocimum kilimandscharicum* Guerke on *Clarias batrachus* Linn. a common fish.

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, India and acclimatized them into laboratory conditions. 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^{0}C 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 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. 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 haematological 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 kilimandscharicum* (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^{0}C 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 day 15th and 30th, 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 haematological 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^{0}C 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.

Haematological 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 haematocytometer (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.[60,61] The serum total protein concentration was estimated by Biuret colourimetric reaction, according to the method as described by Koller[62] and Burtis et al.[63] and serum albumin and globulin concentration were estimated by bromocresol green colourimetric reaction, according to the method as described by Doumas et al.[64] and Gendler[65].

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.[66,67,68]

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: 300.25gm, 304.12gm and 305.56gm respectively. After experimentations of 15Days again the weight of the fishes were weighed from each group (Gr.A, Gr.B and Gr.C) and they were as follow: 303.48gm, 307.20gm and 308.90gm respectively. Likewise after completion of 30 days of experimentations finally the body weights from each group were as follows: 306.26gm, 312.31gm and 312.27gm 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: 18.2cm, 20.5cm and 21.4 respectively. After experimentations of 15Days again the lengths of the fishes were measured from each group and they were as follows: 19.8cm, 22.01cm and 22.45cm respectively. Likewise after completion of 30 days of experimentations finally the body lengths from each group were as follow: 21.7cm, 22.8cm and 23.33cm respectively (Table 1).

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

Groups	Body Length (cm)			Body Weight (gm)		
	Control	15 days	30 days	Control	15 days	30 days
A	18.2	19.8	21.7	300.25	303.48	306.26
B	20.5	22.01	22.8	304.12	307.20	312.31
C	21.4	22.45	23.33	305.56	308.90	312.27

Effect of *O. kilimandscharicum* Crude Extracts on Total protein, Albumin and Globulin

The serum total protein from each group were found to be 2.25 mg/dl, 2.00mg/dl and 2.45mg/dl (at 15 days) and 2.36 mg/dl, 2.88 mg/dl and 3.25mg/dl (at 30 days of observations) respectively. Whereas the albumin content of group A, B and C were 1.32 mg/dl, 1.05 mg/dl and 0.75 mg/dl (at 15days) and 1.40 mg/dl, 1.02 mg/dl and 0.86 mg/dl (at 30 days) respectively. The serum globulin value was found to be 1.42 mg/dl, 1.65 mg/dl and 2.30mg/dl (at 15days) and 1.55 mg/dl, 1.98 mg/dl and 2.20 mg/dl (at 30days) respectively (Table 2 and Figure 1). 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 kilimandscharicum* 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	1.32	1.42	2.36	1.40	1.55
B	2.00	1.05	1.65	2.88	1.02	1.98
C	2.45	0.75	2.30	3.25	0.86	2.20

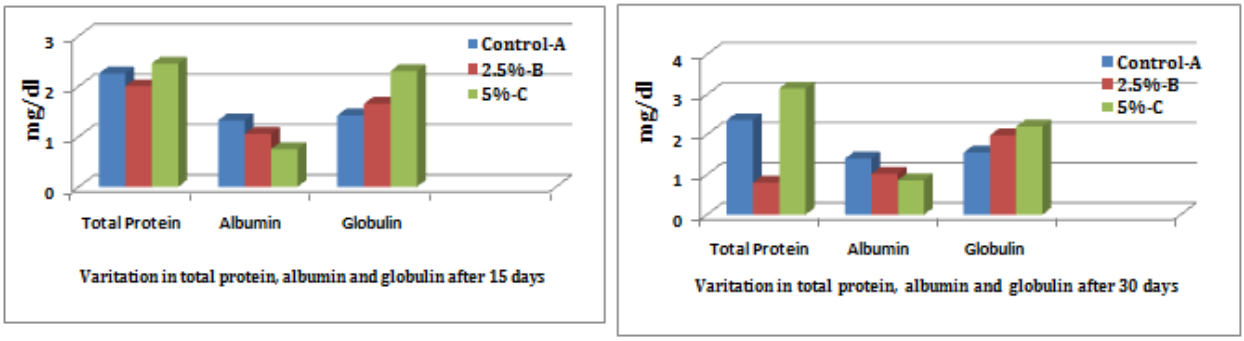

Figure 1: Variation in Total protein, Albumin and Globulin of Fish after 15 and 30 Days

Effect of *O. kilimandscharicum* Crude Extracts on Glucose, Cholesterol, RBC and WBC

The serum glucose contents of all the experimental fishes (Gr.A, Gr.B and Gr.C) had elevated 50.82mg/dl, 51.40mg/dl and 52.05mg/dl (at 15days) and 51.46mg/dl, 49.22mg/dl, 48.04mg/dl (at 30days) respectively. The serum cholesterol level of the control fish was found to be 156.45mg/dl, 152.63mg/dl and 150.55mg/dl (at 15 days) and 157.10mg/dl, 152.34mg/dl and 144.27mg/dl (at 30days) respectively (Figure 2).

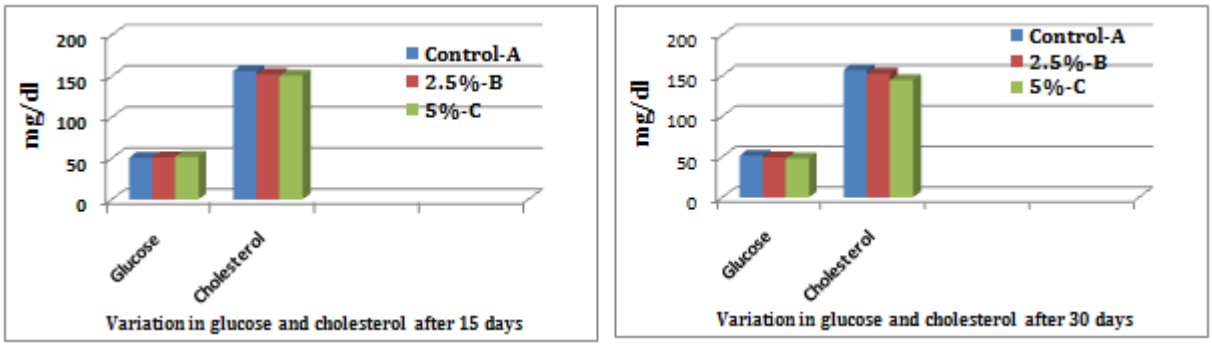

Figure 2: Variation in Glucose and Cholesterol of Fish after 15 and 30 Days

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 Gr.C. The total number of erythrocytes of control fish had a mean value of 2.175 million/mm^3 whereas experiment Gr.B and Gr.C had 2.2100 million/mm^3 and 2.2214 million/mm^3 (at 15days) and 2.2110 million/mm^3, 2.2203 million/mm^3 and 2.2438 million/mm^3 respectively (Figure 3).

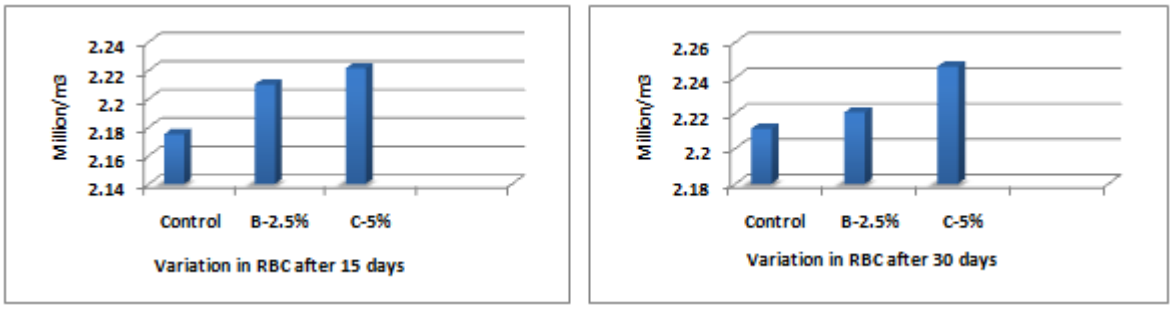

Figure 3: Variation in RBC of Fish after 15Days and 30 days

The WBC counts of fishes of all the three groups (Gr.A, Gr.B and Gr.C) were found to be 4330.32cells/µl, 4388.10cells/µl and 4410.36ells/µl (at 15 days) and 4375.0cells/µl, 4405.12cells/µl and 4483.08ells/µl respectively (Figure 4).

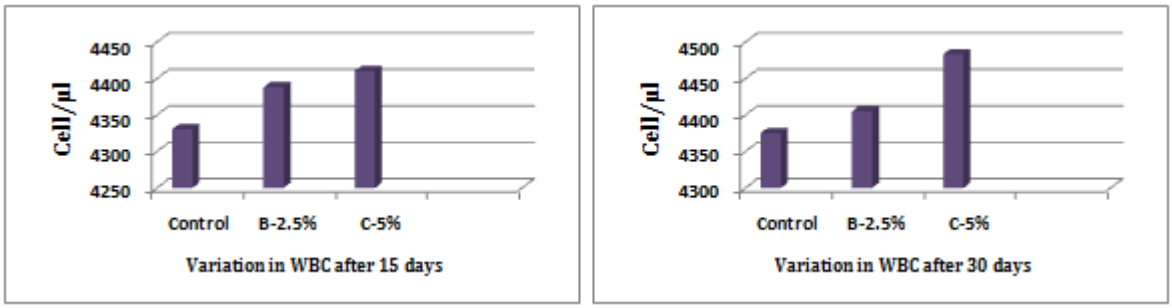

Figure 4: Variation in WBC of Fish after 15 and 30 days.

Table 3: Effect of *Ocimum kilimandscharicum* 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	156.45	2.175	4330.32	51.46	157.10	2.2110	4375.0
B	51.40	152.63	2.2100	4388.10	49.22	152.34	2.2203	4405.12
C	52.05	150.55	2.2214	4410.36	48.04	144.27	2.2438	4483.08

Table 4: Effect of *Ocimum kilimandscharicum* 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	31.1	6.8	25.7	3.6	32.1	7.1	25.2
B	3.6	31.0	6.9	25.93	3.7	32.3	7.2	26.11
C	3.7	32.24	7.2	26.36	3.7	33.7	7.5	26.85

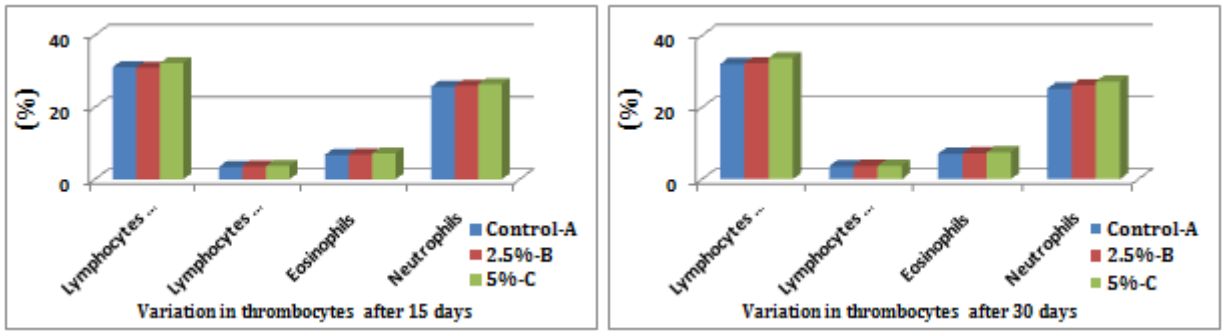

Figure 5: Variation in Phagocytes of Fish after 15 and 30 Days

Effect of *O. kilimandscharicum* Crude Extracts on Phagocytes

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% (small) 31.1% (large), 3.6% (small) 31.0% (large) and 3.7% (small) 32.24% (large) (at 15 days) and 3.6% (small) 32.1% (large), 3.7% (small) 32.3% (large) and 3.7% (small) 33.7% (large) (at 30days) respectively. Whereas the eosinophils were 6.8%, 6.9% and 7.2% (at 15 days), 7.1%, 7.2% and 7.5% (at 30 days) respectively. Similarly in case of Neutrophils they were as follows: 25.7%, 25.93% and 26.36% (at 15 days) and 25.2%, 26.11%, 26.85% respectively. The amount of Lymphocytes, Eosinophils and Neutrophils in Gr.B and Gr.C were decreased at the end of the experiment as compared to the control group (Table 4 and Figure 5).

DISCUSSION

Recently, extensive studies have been initiated to determine the feasibility of using herbal medicines in fish disease management insofar as these products are often without side effects and are biodegradable. Besides, the raw materials are inexpensive, locally available, and can be easily prepared.[69] The present study demonstrated that the dietary supplemented with aqueous leaf extract of *Ocimum kilimandscharicum* enhance growth in 30 days. Several herbs have been tested for their growth promoting activity in aquatic animals.[70,71] The enhanced growth could be due to the growth promoting effect of *Ocimum kilimandscharicum* leaf extracts. A similar observation has been reported by Mathivanan et al.[72] in Broilers and Seung-Cheol et al.[73] also showed that the addition of different single herbal extracts (*Massa medicata*, *Crataegi fructus*, *Artemisia capillaries*, *Cnidium officinale*) or a mixture of all the herbs promoted growth and enhanced some non-specific immunity indicators of red sea bream Pagrus major.

The evaluation of haematological and biochemical characteristics in fish has become an important means of understanding normal, pathological processes and toxicological impacts.[74] Haematological alterations are usually the first detectable and quantifiable responses to environmental changes.[75] Haematological techniques, including erythrocyte count, haemoglobin concentration, haematocrit and leucocyte count have provided valuable knowledge for fishery biologists in the evaluation of fish health[76,77] and in monitoring stress responses[78]. The result of this research programme revealed an enhancement in differential leucocytes (lymphocyte, eosinophils, monocytes, and neutrophils) in the treatment groups, which were fed for 15 and 30 days, especially with the 2.5% and 5% doses.

In this present study, *Ocimum kilimandscharicum* at 15 and 30day of observation showed an increasing immune system in *Clarias batrachus* both in specific and non specific levels. The herbal immuno modulator containing *Ocimum kilimandscharicum* extracts act as a very helpful diet in boosting the immune system in *Clarias batrachus*. The result showed that there were increasing concentrations of serum total protein in all the test groups (Gr.B and Gr.C) in comparison to Gr.A as control. There is a significant increase in the amount of protein and globulin levels by increasing concentrations (2.5% and 5%) of crude extracts of *Ocimum kilimandscharicum* (Table 2,3,4) which could be adduced to possible for the treatment of bronchitis, bronchial asthma, malaria, diarrhea, dysentery, skin diseases, arthritis, painful eye diseases, chronic fever, respiratory distress, insect bite etc.[79] This revealed that the immunostimulant herbals incorporated diets helped to increase the humoral elements in the serum.[80]

Proteins include albumin and globulin; the former of which is synthesized in the liver.[81] Globulin is made up of fractions of α1, α2, β, and γ globulins, which are considered as the source of almost all the immunologically active protein in the blood.[82] Generally, increases in the levels of serum protein and globulin in fish is thought to be associated with a stronger innate response.[83] Although albumin did not increase in most of the treatment groups in the present study, globulin responded similarly to total protein, which certainly increased. It is apparent that many investigators have recorded increases in total protein and globulin in fish serum after administration of herbal compounds/ immunostimulants. For example, feeding diet supplemented with 0.1, 0.5 and 1% garlic and 1, 5 and 10% mango kernel led to higher levels of total protein and globulin in the serum of rohu.[84] Furthermore, Yuan et al.[85] recorded significant increases in total protein and globulin in common carp after administration of diets containing 0.5 and 1% of a herbal mixture of Chinese medicine comprising yellow leader (*A. membranaceus*). Similarly in an another study, the maximum level of total protein and globulin in rohu serum was recorded after feeding for 14 days with 0.5% p flower, with those levels gradually decreasing at 21 and 28 days.[86] The results from Table 1 and Figure 2 showed that a decrease serum albumin contents in 30 days treatment with *Ocimum kilimandscharicum* 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[87], and it is a reliable prognostic indicator for increased risk of morbidity and mortality[88]. But serum globulins values were increased at both 2.5% and 5% concentrations and this may be due to the stimulation of B lymphocytes differentiation and proliferation by IL-6 and TNF-α.[89] 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.[90] Decrease in serum albumin that is accompanied with increased serum globulin possibly suggests kidney problems, chronic infections, inflammation, cirrhosis etc.[91] 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-α levels.[89]

The results of the present study demonstrated that as the value of herbal plant extracts increased in the diet, the value of plasma glucose decreased. This is probably due to the capability of plant extracts to reduce the effects of stressors. It has been shown that glucose level increases in the infected or stressed animals to ward off the infection or stress.[92] Similar to present observation was found in rohu fed with garlic and mango kernel for 60 days showed reduction in glucose levels compared to controls, except in the group fed with 5% mango.[84] This observation may be attributed to the hypoglycaemic activity of some plant extracts to increase the level of serum insulin[93] and to the enhancement of peripheral metabolism of glucose.[94]

The reduced level of the liver total cholesterol and LDL-C (Table 2 and Figure 3) 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.[89], the enhanced reverse cholesterol transport and bile acid excretion and the inhibition of the production of apo B, needed for LDL-C production, transport and binding[95]. *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,[96] preventing oxidative damage,[97] protecting the liver and reducing serum glucose levels-while having no toxic effects in animals[98]. An aqueous extracts of *Ganoderma lucidium* 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.[99]

Invariably there is an increasing RBC and WBC contents of *Clarias batracus* treated with aqueous extracts of *Ocimum kilimandscharicum* at both the groups (Gr.B and Gr.C). It may be due to the effect of this bioactive principle of *Ocimum kilimandscharicum*. In agreement with the present findings Sahu et al.[84,100] reported that WBC and RBC counts were higher in *Labe rohita* fingerlings fed *Magnifera indica* kernel when compared to control. Gopalakannan and Arul[101] also reported that there was an increase in the WBC count after feeding the common carp with immunostimulants like chitin. Similar results were obtained by Dugenci et al.[102] 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 results of this study were in agreement with previous research in which feeding with lupin, mango and stinging nettle led to an increase in erythrocyte and leucocyte count, haemoglobin level and haematocrit, compared to the controls.[103] Similarly, haemoglobin percentage, and erythrocyte and leucocytes count were significantly higher in rohu fed for 20 and 40 days with 5g and 10g of mango kernel and garlic/kg of fish diet, compared with the controls.[84,100] Furthermore, Martins et al.[104] recorded an increase in fish erythrocyte count, Hb content, Hct value, and leucocyte and thrombocyte numbers, after feeding with garlic.

Macrophages and granulocytes are mobile phagocytic cells found in the blood and head kidney, which play a

vital role in promoting synthesis of antibody.[105,106] Neutrophil activity may be an indicator of the non-specific immune response,[107] which exhibits increased production of oxygen radicals released during the oxidative burst process. These reactive species are capable of destroying the invading pathogens.[108,109] An increase in phagocytic activity by immunostimulants has been documented by many authors.[102-112] However, the ideal time and dose of immunostimulants for enhancement of immunity is variable.

In the present study after 15 days of experiment *Ocimum kilimandscharicum* extract treatment the lymphocyte and phagocyte counts were increased in all experimental groups as compared to control group. In addition, 2 weeks administration of *Ocimum kilimandscharicum* it showed that there was no significant differences in lymphocyte, eosinophils and monocytes counts between the experimental groups and control group (Figure 5). Jeney and Anderson[113] recorded an increase in phagocytic activity in rainbow trout after exposure to glucan for 1 day, with the activity peaking at 3-4 days. Earlier studies had shown that the aqueous leaf extract of *Ocimum kilimandscharicum* has different pharmacological actions including antioxidative properties.[55,57,79]

But the amount of Lymphocytes, Eosinophils and Neutrophils in Gr.B and Gr.C were decreased at the end of the experiment as compared to the control group (Table 4). This is supported by the findings of Ephraim et al.[114] The major reason for this enhanced concentrations of lymphocytes and phagocytes in the experimental groups may be their participatory role in immune functions as observed by Kollner et al.[115] However the counts of these cells in Gr.B and Gr.C decreased at 30 days treatment in comparison to the control group which is supported by the findings of Ephraim et al.[114]. Conversely, Yin et al.[111] found that tilapia fed with Scutellaria extract at higher doses (0.5 and 1.0%) had a reduced function in the phagocytic cells whereas when fish were fed with a lower dose, i.e. 0.1%, there was not any effect on phagocytic activities. Certainly, the enhancement in phagocytic activity of macrophages in treatment groups, especially those receiving the 2.5% and 5% doses, could be responsible for the lower mortality recorded after challenge with *Ocimum kilimandscharicum*.

The present results indicated that dietary plant extract supplementation could significantly enhance the immune system of *Clarias batrachus* in some extent and this might be due to the enhancement of the non-specific immune system of fish by herbal plant extracts. In agreement with the present findings, Sahu et al.[84] reported that survival rate after challenging the fish with *A.hydrophila* was enhanced in *Labeo rohita* fed diets containing *Magnifera indica* kernel. Similar findings were recorded by Nya and Austin[116] insofar as there were significant changes of rainbow trout lymphocyte, monocyte and neutrophil counts following feeding with ginger for 14 days. Pachanawan et al.,[117] also reported that survival rate after challenging the fish with *A. hydrophila* was increased in tilapia (*Oreochromis niloticus*) fed diets containing either dry leaf powder of *Psidium guajava* or ethanol extract of *P. guajava* leaf.

CONCLUSION

Recently, extensive research has been initiated to determine the feasibility of using herbal medicines in fish disease management insofar as these products are often without side effects and are biodegradable. Besides, the raw materials are inexpensive, locally available, and can be easily prepared. The results prove that the application of *Ocimum kilimandscharicum* extract at 2.5% and 5% concentrations feed will enhance the growth, immunity on specific and non specific levels and plasma biochemical profile to a significant level in the blood of the *Clarias batrachus*. And based on these data we could consider *Ocimum kilimandscharicum* as a potent Immunostimulant in *Clarias batrachus*. Moreover, these extracts are safe to be used because of its non toxicity and protective activity. The potential for the application of research, our findings to both human and environment health issues makes fish species more attractive and valuable as an alternative model organism for conducting different experimental studies. This work may provide a new perspective for use of medicinal plants as adjuvant therapy added to fish food to prevent diseases. Further studies are needed to evaluate cost-benefit.

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