

IMMUNOMODULATORY ACTIVITY OF AQUEOUS LEAF EXTRACT OF OCIMUM BASILICUM LINN IN CLARIAS BATRACHUS

¹GAYATRI NAHAK AND ²RAJANI KANTA SAHU*

¹KIIT School of Biotechnology, KIIT University, Bhubaneswar, Odisha, India, ²B.J.B. Autonomous College, Bhubaneswar, Odisha, India.
Email: gayatri.science@gmail.com, sahurajani.sahu@gmail.com*

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ABSTRACT

Objective: Basil *Ocimum basilicum* Linn. (Lamiaceae), popularly known as "Sweet Basil" has been used as traditional medicine for household remedy against various human ailments from antiquity. The present study deals with the development of immunity on both specific and non specific levels by the leaf extract of *Ocimum basilicum* Linn. on *Clarias batrachus* a common fish.

Methods: The aerial parts of *Ocimum basilicum* Linn. were extracted with double distilled water and then extracts were screened for their immunomodulatory effects on *Clarias batrachus*. Haematological and biochemical studies were done on specific and nonspecific levels after administering the extracts for 15 and 30 days.

Results: The present study was designed to evaluate the immunomodulatory activity of aqueous leaf extract of *Ocimum basilicum* on fish *Clarias batrachus* in biochemical and haematological profiles. It was observed that the herbal diet (prepared by the aqueous leaf extract of *O. basilicum*) 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 immune response and non specific immune responses. It may be due to the presence phenolic compounds like tannins, saponin, flavonoids, steroid, terpenoids, eugenol, caryophylline, cardiac glycerides etc.

Conclusion: Based on the results it is appropriate to conclude that the plant extract of *Ocimum basilicum* may act as a potent Immunostimulant in preventing and controlling various diseases of human being.

Keywords: Immunostimulants, Phytochemicals, *Clarias batrachus*, *Ocimum basilicum* linn.

INTRODUCTION

A large proportion of world's population depends on traditional medicine because of scarcity, high cost of orthodox medicine and unpleasant side effects [1]. There are currently about 250 000 registered medical practitioners of the Ayurvedic system, as compared to about 700,000 of the modern medicine system [2,3,4]. These are not only used for primary health care not just in rural areas in developing countries, but also in developed countries as well where modern medicines are predominantly used. In addition, herbs have provided us some of the very important lifesaving drugs used in the armamentarium of modern medicine [5,6]. The medicinal plants are rich in secondary metabolites and essential oils of therapeutic importance [7]. The important advantages claimed for therapeutic uses of medicinal plants in various ailments are their safety besides being economical, effective, fewer side effects and their easy availability [8,9,10].

Because of these advantages the medicinal plants have been widely used by the traditional medical practitioners in their day to day practice. Among the plants known for medicinal value, the plants of genus *Ocimum* are rich in phenolic compounds and are very useful for their therapeutic potentials [11,12,13,14]. Numerous laboratory studies have shown various effects of *Ocimum* species including bactericidal, antiinflammatory, antioxidative, antiulcer, antidiarrheal, chemopreventive, hypoglycemic, nervous system stimulation and radiation protection [15-22]. The profound medical effects of this herb may be attributed to its pharmaceutical potentiality due to presence of the active phyto-compounds like flavonoids and polyphenols contents [23-27].

The chemical composition of the essential oil of *O. basilicum* has been under study since 1930s [12] and more than 200 chemical components have been identified. Due to different combinations of the essential oils, various varieties of *O. basilicum* differ in their fragrances. Different chemo varieties are found in different regions of the world. Various investigations through phytochemical

screening of aqueous extract and elemental analysis of *O. basilicum* showed the presence of variety of bioactive compounds glycoside, gums, mucilage, proteins, amino acids, tannins, phenolic compound, triterpenoids steroids, sterols, saponins, flavones and flavonoids which could enhance the curative process of health [28-34].

An extensive review on the use of immunostimulatory uses in fish suggested that herbal extracts can be used in fish culture as an alternative to vaccines, antibiotics or chemotherapeutics agent provide a cheaper source for treatment and greater accuracy than chemotherapeutic agents without causing toxicity [35-38]. Several antimicrobial, antistress, immunostimulant, growth-promoting plant products are significantly influenced the fish/shrimp larviculture [39-42]. A variety of plant-derived materials such as polysaccharides, lectins, peptides flavonoids, isoflavonoids, phytosterols, polysaccharides, alkaloids, sesquiterpenes, glucans, tanins, vitamins, and a variety of other phytochemical substances have been reported to modulate the immune system [43-49].

Basil has a long and interesting history steeped in legend which is a part of religious traditions around the world, from Christianity to Hindu [50,51,52]. It also considered as a good luck charm in some folk medicine system. It is reportedly used in exorcisms, for protection and attracts wealth [53,54,55]. Scientific literatures are continuously reporting herbal drugs having immunomodulatory activity and generally act by stimulating both specific and non-specific immunity [56,57]. Hence the present study deals with the development of immunity on both specific and non specific levels by the leaf extract of *O. basilicum* on *Clarias batrachus* 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 $28 \pm 2^\circ\text{C}$ 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 (Fig. 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 haematological and biochemical analyses were carried out after 15 and 30 days of observations respectively.

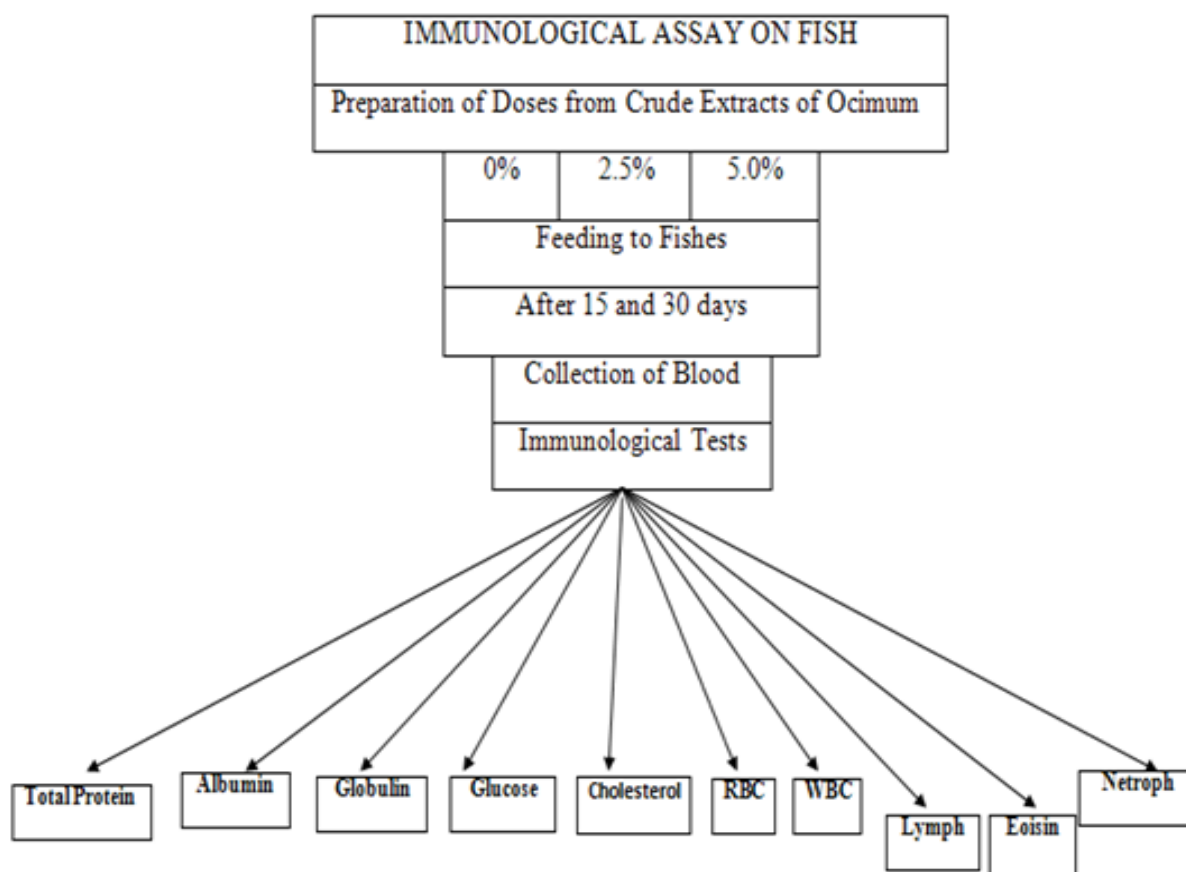


Fig. 1: Experimental Design on Fish

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 basilicum* (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°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 15 and 30, three fishes from each group were bled with the aid of a 2cm^3 plastic syringe was inserted in the caudal vein and

blood was drawn keeping the fish was vertically held with the head upwards. Blood samples of about 4 milliliters 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°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 $5000 \times 2\text{g}$), 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 100 cells. The thrombocytes were counted from the blood smears prepared [58,59]. The serum total protein concentration was estimated by Biuret colourimetric reaction, according to the method as described by [60,61] and serum albumin and globulin concentration was estimated by bromocresol green colourimetric reaction, according to the method as described by [62,63].

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 from 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 [64,65,66].

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: 303.55±0.8gm, 305.62±0.6gm and 306.26±0.5gm 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: 304.66±0.4gm, 306.25±0.8gm and 307.16±0.6gm respectively. Likewise after completion of 30 days of experimentations finally the body weights from each group were as follows: 305.26±0.8gm, 302.31±0.4gm and 307.27±0.2gm 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: 18.5±0.5cm, 21.5±0.4cm and 22.4±0.5 respectively. After experimentations of 15Days again the lengths of the fishes were measured from each group (Gr.A, Gr.B and Gr.C) and they were as follows: 20.80±0.4cm, 21.61±0.2cm and 21.45±0.3cm respectively. Likewise after completion of 30 days of experimentations finally the body lengths from each group were as follow: 21.75±0.7cm, 22.80±0.5cm and 22.33±0.3cm respectively (Table 1).

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

Groups	Doses of Crude extracts (%)	Body Length (cm)	Body Weight (gm)	Body Length (cm)	Body Weight (gm)	Body Length (cm)	Body Weight (gm)
		Control	Control	15-Days	15-Days	30-Days	30-Days
A	Control	18.5±0.5	303.55±0.8	20.80±0.4	304.66±0.4	21.75±0.7	305.26±0.8
B	2.5	21.5±0.4	305.62±0.6	21.61±0.2	306.25±0.8	22.80±0.5	302.31±0.4
C	5.0	22.4±0.5	306.26±0.5	21.45±0.3	307.16±0.6	22.33±0.3	307.27±0.2

Effect of *Ocimum basilicum* 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±0.4mg/dl, 2.55±0.5mg/dl and 3.05±0.8mg/dl (at 15 days) and 2.38±0.7mg/dl, 2.91±0.6mg/dl and 3.15±0.5mg/dl (at 30 days of observations) respectively. Whereas the albumin contents of Gr.A, Gr.B and Gr.C were 1.32±0.2mg/dl, 1.11±0.6mg/dl

and 0.95±0.5mg/dl (at 15days) and 1.44±0.2mg/dl, 1.10±0.3mg/dl and 0.86±0.3mg/dl (at 30 days) respectively. The serum globulin values were found to be 1.42±0.5mg/dl, 1.88±0.1mg/dl and 2.37±0.4mg/dl (at 15days) and 1.52±0.5mg/dl, 2.28±0.5mg/dl and 2.60±0.5mg/dl (at 30days) respectively (Table 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 basilicum* crude extracts on Total protein, Albumin and Globulin of *Clarias batrachus* after 15 and 30 Days

Immune Parameters	Days of observation					
	15 Days			30 Days		
Doses of herbal extracts (%)	0	2.5	5.0	0	2.5	5.0
Total Protein(mg/dl)	2.25±0.4	2.55±0.5	3.05±0.8	2.38±0.7	2.91±0.6	3.15±0.5
Albumin(mg/dl)	1.32±0.2	1.11±0.6	0.95±0.5	1.44±0.2	1.10±0.3	0.86±0.3
Globulin(mg/dl)	1.42±0.5	1.88±0.1	2.37±0.4	1.52±0.5	2.28±0.5	2.60±0.5

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

Immune Parameters	Days of observation					
	15 Days			30 Days		
Doses of herbal extracts (%)	0	2.5	5.0	0	2.5	5.0
Glucose(mg/dl)	50.82±1.12	51.24±1.20	51.55±1.45	51.46±1.44	50.31±1.50	49.24±1.2
Cholesterol(mg/dl)	156.45±1.22	154.00±1.36	153.56±1.30	157.10±1.52	155.68±1.55	153.27±2.0
RBC(Million/m ³)	2.175±1.45	2.1920±1.25	2.2232±1.42	2.2110±1.07	2.2328±1.13	2.2462±4.5
WBC(Per µl)	4330.32±1.88	4352.0±1.08	4400.54±1.33	4375.0±1.65	4411.12±1.24	4421.0±36

Effect of *Ocimum basilicum* crude extracts on 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±1.12mg/dl, 51.24±1.20mg/dl and

51.55±1.45mg/dl (at 15days) and 51.46±1.44mg/dl, 50.31±1.50mg/dl, 49.24±1.2mg/dl (at 30days) respectively. The serum cholesterol level of the control fish was found to be 156.45±1.22mg/dl, 154.00±1.36mg/dl and 153.56±1.30mg/dl (at 15

days) and 157.10±1.52mg/dl, 155.68±1.55mg/dl and 153.27±2.0mg/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 C. The total number of erythrocytes of control fish had a mean value of 2.175±1.45million/mm³ where as Gr.B and Gr.C had 2.1920±1.25million/mm³ and 2.2232±1.42million/mm³ (at 15days) and 2.2110±1.07million/mm³, 2.2328±1.13million/mm³ and 2.2462±45million/mm³ respectively. The WBC counts of fishes of all the three groups (Gr.A, Gr.B and Gr.C) were found to be 4330.32±1.88cells/μl, 4352.0±1.08cells/μl and 4400.54±1.33cells/μl (at 15days) and 4375.0±1.65cells/μl, 4411.12±1.24cells/μl and 4421.0±36ells/μl respectively. There is a significant increase in the amount of RBC and WBC Group-B and C respectively in comparison to control (Table 3).

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

The thrombocytes 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±0.8% (large), 3.3±0.03% (small) 32.4±0.5% (large) and 3.7±0.5% (small) 32.15±0.4%(large) (at 15 days) and 3.6±0.11% (small) 32.1±0.5% (large), 3.7±0.4% (small) 32.5±0.3% (large) and 3.8±0.7% (small) 33.0±0.8%(large) (at 30days) respectively. Whereas the eosinophil values were 6.8±0.5%, 6.9±0.2% and 7.0±0.6% (at 15 days) 7.2±0.8%, 7.3±0.8%, 7.7±0.6% (at 30 days) respectively. Similarly in case of Neutrophils they were as follows: 25.7±0.4%, 25.8±0.5% and 26.45±0.5% (at 15 days) and 26.2±0.5%, 27.00±0.5%, 27.18±0.7% 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).

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

Days of Observation		15 Days					30 Days							
Immune Parameters	Doses of herbal extracts (%)	0	2.5	5.0	0	2.5	5.0	0	2.5	5.0				
Lymphocytes (%)	0	3.4±	31.1±	3.3±	32.4±	3.7±	32.15±	0	3.6±0.11	32.1±0.5	3.7±0.4	32.5±0.3	3.8±0.7	33.0±0.8
Eosinophils (%)	0.2	0.8	0.03	0.5	0.5	0.4		7.2±		7.3±		7.7±		7.7±
Neutrophils (%)	0.5	0.2	0.6	0.5	0.5	0.4		0.8		0.8		0.6		0.6
	25.7±	25.8±	26.45±	26.2±	27.00±	27.18±		26.2±		27.00±		27.18±		27.18±
	0.4	0.5	0.5	0.5	0.5	0.7		0.5		0.5		0.7		0.7

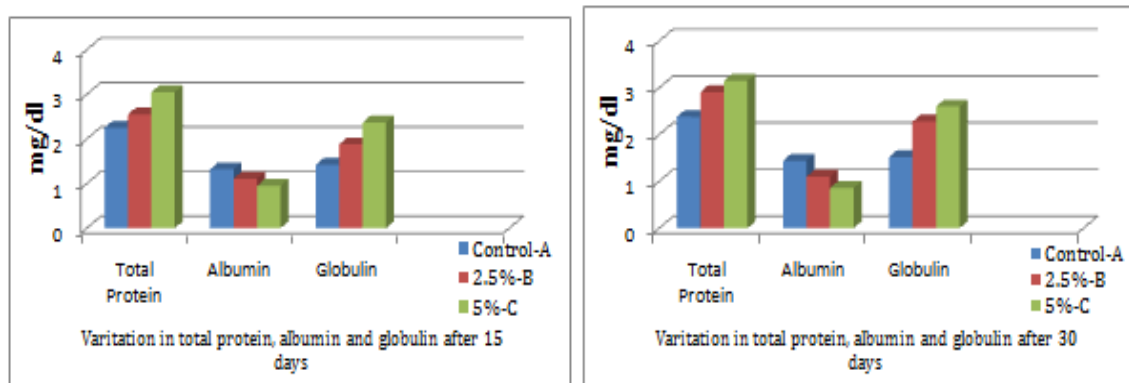


Fig. 2: Variation in Total protein, Albumin and Globulin of Fish after 15 and 30 Days

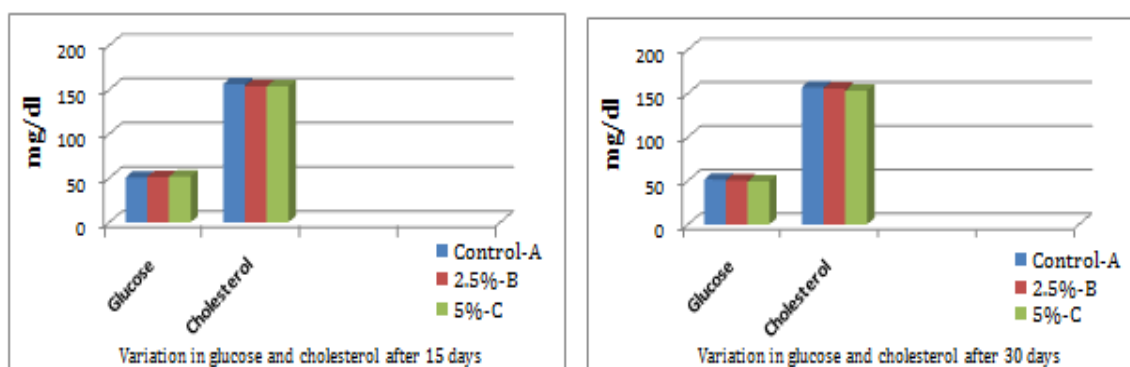


Fig. 3: Variation in Glucose and Cholesterol of Fish after 15 and 30 Days

DISCUSSION

Elaborate scientific investigations, ranging from phytochemistry, pathogenic and insecticidal activities, utilization as a spice and flavouring ingredient etc. have been extensively catalogued for

Ocimum basilicum. The phytochemical and pharmacological studies on the plant indicate that it possesses analgesic, anti-inflammatory, antimicrobial, antioxidant, antiulcerogenic, cardio-stimulant, chemomodulatory, hepatoprotective, hypoglycemic, hypolipidemic, immunomodulatory and larvicidal activities [15-22].

In this present study, *Ocimum basilicum* at 15 and 30day of observations showed an increasing immune system in *Clarias batrachus* both in specific and non specific levels. The herbal immuno modulator containing *Ocimum basilicum* extracts act as a very helpful in boosting the immune system in *Clarias batrachus*. The result showed that there were increasing concentrations of serum total protein in test 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 basilicum* (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 [20, 67]. This revealed that the immunostimulant herbals incorporated diets helped to increase the humoral elements in the serum [68].

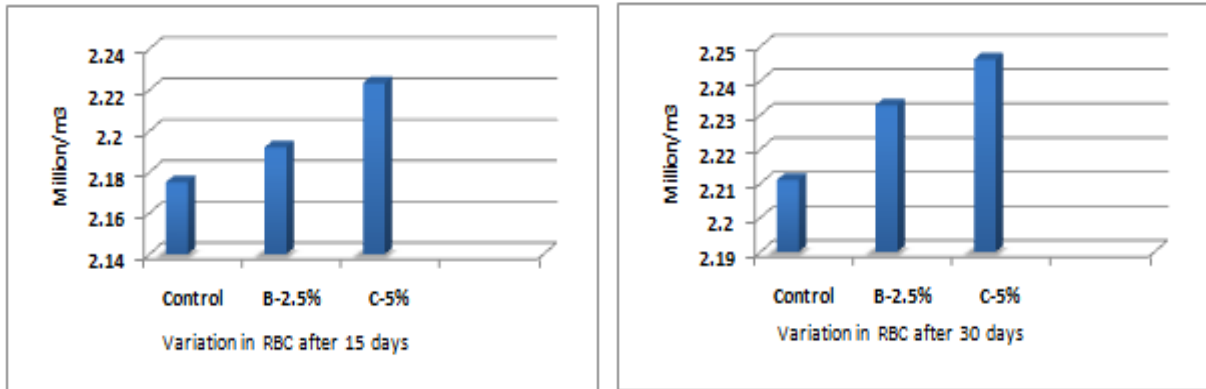


Fig. 4: Variation in RBC of Fish after 15Days and 30 days

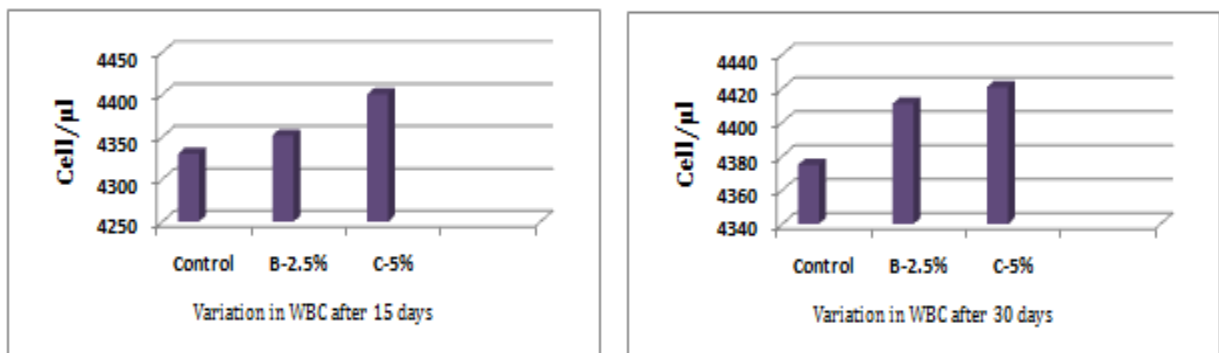


Fig. 5: Variation in WBC of Fish after 15 and 30 days

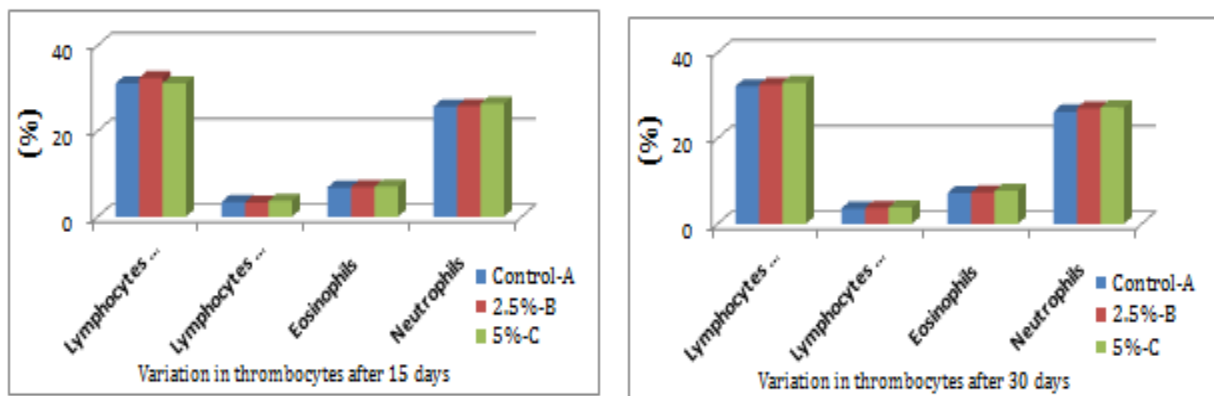


Fig. 6: Variation in Thrombocytes of Fish after 15 and 30 Days

The results from Table 1 and Fig 2 showed that a decrease serum albumin contents in 30 days treatment with *Ocimum basilicum* 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 [69], and it is a reliable prognostic indicator for increased risk of morbidity and mortality [70]. But serum globulins values were increased at both 2.5% and 5% concentrations and this may be due to the presence phenolic compounds like tannins, saponin,

flavonoids, steroid, terpenoids, eugenol, caryophylline, cardiac glycerides etc [23,29-34]. 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 [71]. Decrease in serum albumin that is accompanied with increased serum globulin possibly suggests kidney problems, chronic infections, inflammation, cirrhosis etc [72]. 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 [73].

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 [74]. Similar observation was found in *Labeo rohita* fingerlings [75] and black tiger shrimp, *Penaeus monodon* [74] that glucose levels were reduced after feeding with herbal immunostimulant diets. The reduced level of the liver total cholesterol and LDL-C (Table 2 and Fig 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 [73] 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 [76].

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 [77], preventing oxidative damage [78], protecting the liver and reducing serum glucose levels-while having no toxic effects in animals [79]. 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 lipopolysaccharide and influence the gene expression of cytokines [80].

Invariably there is an increasing RBC and WBC contents of *Clarias batracus* treated with aqueous extracts of *Ocimum basilicum* at both the groups (Gr.B and Gr.C) (Fig 4 and Fig 5). Similar result was found in Wister albino rat which was administrated *O.basilicum* in low and high dose by SRBC titre method where a good increasing values were observed in RBC, WBC, haemoglobin count and antibody [67,81]. In agreement with the present findings, Sahu et al [75] reported that WBC and RBC counts were higher in *Labeo rohita* fingerlings fed *Magnifera indica* kernel when compared to control. Gopalakannan and Arul [82] 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 [83] 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. Thus significant increase in the Total leukocyte counts can be considered as an indicator for improvement in general resistance. Increase in neutrophils in control feed fed fishes may be a non-specific immune response and increase in lymphocyte counts in herbal dose prepared diet fed fishes can be attributed to the specific immune response [84,85,86].

In addition, 2 weeks administration of *O.basilicum* it showed that there was no significant differences in lymphocyte, eosinophils and monocytes counts between the experimental groups (Gr.B and Gr.C) and control group (Fig 6). But the values were gradually increased with duration of time and doses. It is may be due to the presence flavonoids and terpenoids [81,87,88,89]. The result also showed that 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 [90].

Results indicated that dietary plant extract supplementation could significantly enhance the immune system of *Clarias batracus* 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 [75] reported that survival rate after challenging the fish with *A.hydrophila* was enhanced in *Labeo rohita* fed diets containing *Magnifera indica* kernel. Similar results were also reported after feeding tilapia (*Oreochromis niloticus*) with two Chinese medicine herbs and challenging with *A.hydrophila* [91]. Pachanawan et al [92] 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.

The plant *O.basilicum* is considered as one of the most important source of medicine and drugs due to the presence of various phytochemical active compounds like alkaloids, saponins, tannins, alkaloids, anthraquinone, flavonoids, steroids, terpenoids and cardiac glycosides [29-34]. Almost all the phyto-constituents of *O.basilicum* are known to influence biological system activities at some extents. Furthermore, *O.basilicum* had been shown to possess diverse pharmacological properties which may be attributed to its usefulness in folk medicine. Also, it's essential oil contains a variety of phytochemicals such as linalool, α -terpineol, eugenol, β -elemene, α -bergamotene, α -guaiane, germacrene D, cubenol, taucadinol, camphor, bornil acetate, β -cariophyllene etc [93-96] which may be the important possible reason for showing the plant as a strong immune potent for enhancing growth, immunity on specific and non specific levels and in increasing plasma biochemical profile of *Clarias batracus*.

CONCLUSION

The results obtained in the present study indicate that *Ocimum basilicum* Linn. is a potent immunostimulant, stimulating specific and nonspecific immune mechanisms. The present study was evaluated that the immunomodulatory activity of aqueous leaf extract of *Ocimum basilicum* on fish *Clarias batracus* in biochemical and haemological profiles 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 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 batracus* Linn. The immunostimulatory activity of *O.basilicum* could be attributed to the presence of flavonoids (quercetin), alkaloids, tannins, saponin glycosides and phenolic compounds. Therefore, the plant holds promise for being used as a strong immunostimulating agent for human health.

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