ASIAN JOURNAL OF PHARMACEUTICAL AND CLINICAL RESEARCH

INNOVARE ACADEMIC SCIENCES Knowledge to Innovation

Vol 7, Issue 3, 2014

ISSN - 0974-2441

Original Article

IMMUNOSTIMULATORY EFFECT OF *OCIMUM SANCTUM* LINN. LEAF EXTRACT IN *CLARIAS BATRACHUS* LINN.

[1]GAYATRI NAHAK AND [2]RAJANI KANTA SAHU*

[1]KIIT School of Biotechnology, KIIT University, Bhubaneswar, Odisha, India, [2]B.J.B. Autonomous College, Bhubaneswar, Odisha, India

Email: gayatri.science@gmail.com and sahurajani.sahu@gmail.com*

Received: 13 May 2014 Revised and Accepted: 7 June 2014

ABSTRACT

Objective: Recently, there has been increased interest in the immune stimulating function of some herbs in aquaculture. The phytomedicines provide a cheaper source for treatment and greater accuracy than chemotherapeutic agents without causing toxicity. Many studies have proved that herbal additives enhanced the growth of fishes and protected them from various diseases. The herbs are not only safe for consumers but also widely available throughout the world and they also have a significant role in aquaculture. 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.

Methods: The aerial parts of *Ocimum sanctum* 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.

Result: Our results showed that there is no 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. 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. It may be due to the presence of 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 sanctum* may act as a potent Immunostimulant in *Clarias batrachus* Linn.

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

INTRODUCTION

One of the most important sources of immunostimulator which are being explored extensively currently comes from plant derived substances [1]. A large population of India uses plants for its healing, preventive, curative and much therapeutic property together with immunostimulatory property [2]. These natural plant products have been reported to have various properties such as anti-stress, growth promoters, appetisers, tonic and immuno-stimulants [3]. Moreover, these substances also possess other valuable properties; they are nontoxic, biodegradable and biocompatible [4,5]. No herbal-resistance immunity has been found by any pathogen to date. Various medicinal properties of herbs and plants are well documented, however very few commercial remedies exists for use in large-scale aquaculture. Use of expensive chemotherapeutants and antibiotics for controlling disease have widely been criticized for their negative impacts like residual accumulation in the tissue, development of the drug resistance and immunosuppression, thus resulting in reduced consumer preference for food fish treated with antibiotics. Hence, instead of chemotherapeutic agents, increasing attention is being paid to the use of immunostimulants for disease control measures in aquaculture [6]. Immunostimulants comprise a group of biological and synthetic compounds that enhance the non-specific cellular and humoral defense mechanism in animals. These substances such as levamisole and glucan, peptidoglycon, chitin, chitosan, yeast and vitamin combinations as well as various products derived from plants and animals are effective in prevention of diseases [7]. An application of immunostimulants for the prevention of fish diseases are considered as an attractive and promising area as plants are safer, much more effective and cheaper, conventional immunomodulator plants can be explored [8].

In the world there are many herbal plants but the designation of Queen of the herb has been retained generation after generation by *Ocimum sanctum* because of the greater medicinal value possessed by it [9]. From time immemorial to plant a Tulsi sapling in each house has become a part of Indian tradition and heritage. It is undoubtedly considered as a natural vitalizer. Investigations have been carried out from time to time to purify various components of the plant followed by subsequent characterization in terms of bio-pharmacological activities and chemical nature [10]. Several works have been carried out especially by the Indian researchers and scientists in the last two decades to prove the versatile beneficial nature of this particular plant for the benefit of common people [11-15].

Different preparations (dried leaf powder, methanolic, acetonic and petroleum ether extracts) are obtained from leaves of *Ocimum sanctum* on the basis of Gas chromatography and mass spectrophotometery (GC-MS). In dried leaf powder 49 components were found in which major components are 1-Methyl eugenol, 2-Eugenol, 1-Stigmast-5-en-3-ol, 2-Stigmast-5, 22-dien-3-ol, 2-Neophytadiene, 2-Octadecane, 3-β-caryophylene, 3-Methyl eugenol etc (16). The leaf volatile oil contains eugenol (1-hydroxy-2-methoxy-4-allylbenzene), euginal (also called eugenic acid), urosolic acid, carvacrol (5-isopropyl-2-methylphenol), linalool (3,7-dimethylocta-1,6-dien-3-ol), limatrol, caryophyllene, methyl carvicol (also called Estragol: 1-allyl-4-methoxybenzene) while the seed volatile oil have fatty acids and sitosterol. Other than these the seed mucilage of Tulsi contains some levels of sugars and green leaves are the source of anthocyans. Two major sugars of the plants are xylose and polysaccharides [17,18]. The aqueous extract of *Ocimum sanctum* leaves revealed alkaloids, flavonoids, tannins and carbohydrates [19,20].

Ocimum sanctum is popularly called as "Holy Basil" has been claimed to be valuable against a wide variety of diseases which possess antioxidant, antibiotic, antiatherogenic, immunomodulatory, anti-inflammatory, analgesic, antiulcer, chemopreventive and antipyretic properties proven by various researches [21-27]. Although a few studied on the immunomodulatory effects of *O.sanctum* have been reported for various animal species [28]. Since the present study was designed to show the effectiveness of crude extract *Ocimum sanctum* leaves on a common fish *Clarias batrachus* 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, 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 (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 haematological and biochemical analyses were carried out after 15 and 30 days of observations respectively.

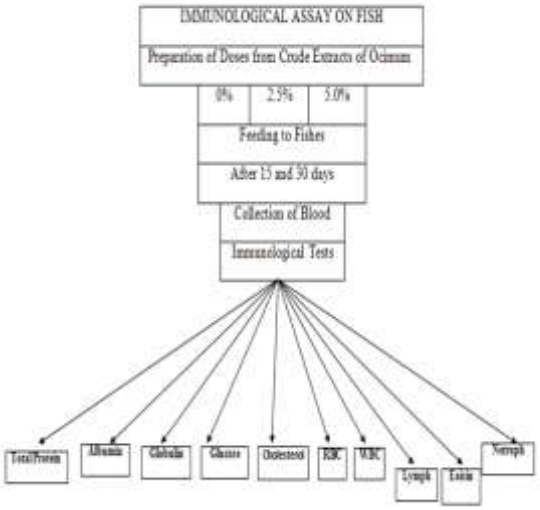

Chart. 1: Flow Chart of Experimental Design

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 sanctum* (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 15 and 30, 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 [29,30]. The serum total protein concentration was estimated by Biuret colourimetric reaction, according to the method as described by Koller, [31] and Burtis et al. [32] and serum albumin and globulin concentration was estimated by bromocresol green colourimetric reaction, according to the method as described by Doumas et al. [33] and Gendler [34].

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 [35,36,37].

RESULTS

Effect of herbal crude extracts on Body Weight and Body Length

Table-1 shows the body weight and length responses 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 15 Days 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 21.4cm respectively. After experimentations of 15 Days 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	15days	30days	Control	15days	30days
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 *Ocimum sanctum* crude extracts on Total protein, Albumin and Globulin

The serum total protein from each group were found to be 2.25 mg/dl, 2.70mg/dl and 3.15mg/dl (at 15 days) and 2.36mg/dl, 3.11mg/dl and 3.75mg/dl (at 30 days of observations) respectively. Whereas the albumin content of Gr.A, Gr.B and Gr.C were 1.32 mg/dl, 1.15 mg/dl and 1.05 mg/dl (at 15 days) and 1.40 mg/dl, 1.00 mg/dl and 1.16 mg/dl (at 30 days) respectively. The serum globulin values were found to be 1.42 mg/dl, 2.12 mg/dl and 2.57mg/dl (at 15 days) and 1.55 mg/dl, 2.28 mg/dl and 2.60 mg/dl (at 30days) respectively (Table 2 and Fig. 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 of 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 sanctum* 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	2.70	1.15	2.12	3.11	1.00	2.28
C	3.15	1.05	2.57	3.75	1.16	2.60

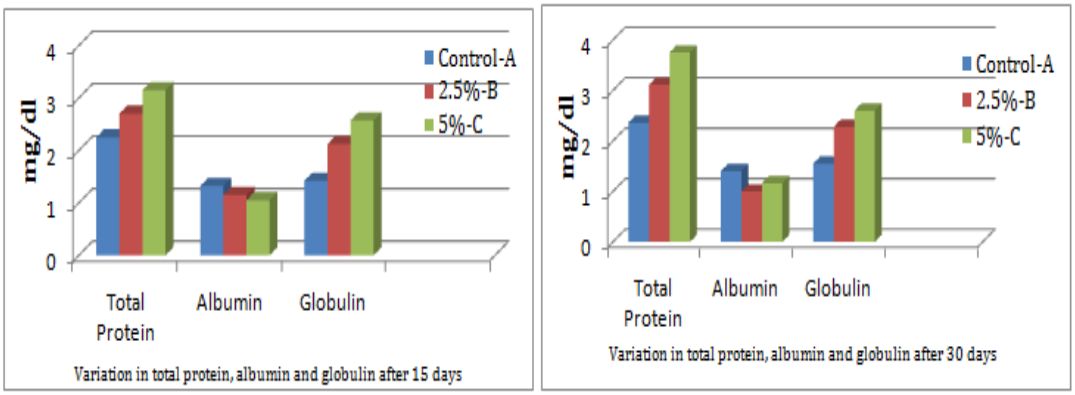

Fig. 1: Variation in Total protein, Albumin and Globulin of Fish after 15 and 30 Days

Table 3: Effect of *Ocimum sanctum* 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	51.44	153.64	2.2520	4385.0	50.31	153.18	2.2608	4397.12
C	51.65	152.71	2.2552	4420.5	48.54	140.47	2.2642	4415.0

Effect of *Ocimum sanctum* 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 50.82mg/dl, 51.44mg/dl and 51.65mg/dl (at 15 days) and 51.46mg/dl, 50.31mg/dl, 48.54mg/dl (at 30days) respectively. The serum cholesterol level of the control fish was found to be 156.45mg/dl, 153.64mg/dl and 152.71mg/dl (at 15 days) and 157.10mg/dl, 153.18mg/dl and 140.47mg/dl (at 30days) respectively (Fig. 2). 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 2.2520 million/mm^3 and 2.2552 million/mm^3 (at 15 days) and 2.2110 million/mm^3, 2.2608 million/mm^3 and 2.2642million/mm^3 respectively (Fig. 3). The WBC counts of fishes of all the three groups (GrA, Gr.B and Gr.C) were found to be 4330.32cells/µl, 4385.0cells/µl and 4420.5ells/µl (at 15 days) and 4375.0cells/µl, 4397.12cells/µl and 4415.0ells/µl respectively (Fig. 4). There is a significant increase in the amount of RBC and WBC in Gr.B and Gr.C respectively in comparison to control (Table 3).

Effect of *Ocimum sanctum* 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% (small) 31.1% (large), 3.4% (small) 31.5% (large) and 3.8% (small) 32.08%(large) (at 15 days) and 3.6% (small) 32.1% (large), 3.7% (small) 32.8% (large) and 3.8% (small) 33.9%(large) (at 30days) respectively. Whereas the eosinophils were 6.8%, 6.9% and 7.2% (at 15 days), 7.1%, 7.4% and 7.8% (at 30 days) respectively. Similarly in case of Neutrophils they were as follows: 25.7%, 26.12% and 26.85% (at 15 days) and 26.2%, 26.34%, 26.88% 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 Fig. 5).

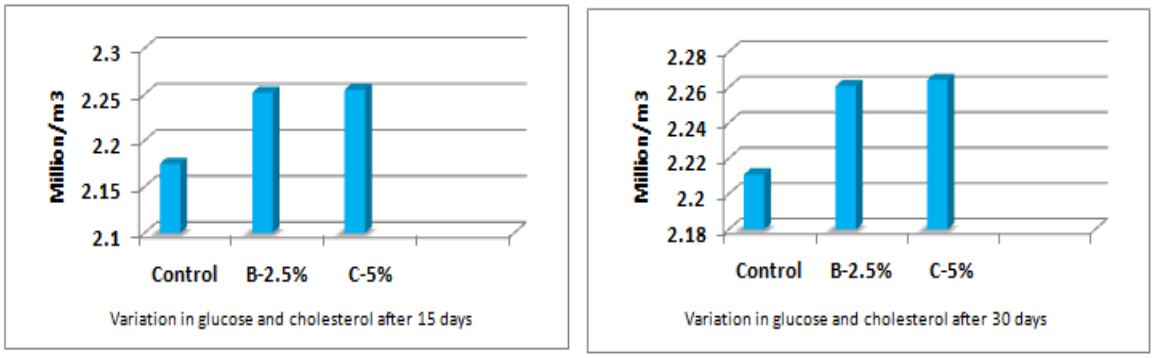

Fig. 2: Variation in Glucose and Cholesterol of Fish after 15 and 30 Days

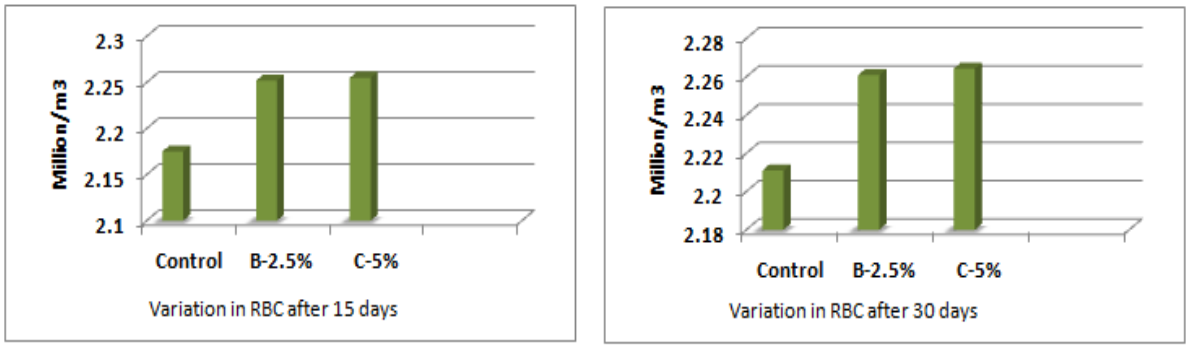

Fig. 3: Variation in RBC of Fish after 15 and 30 Days

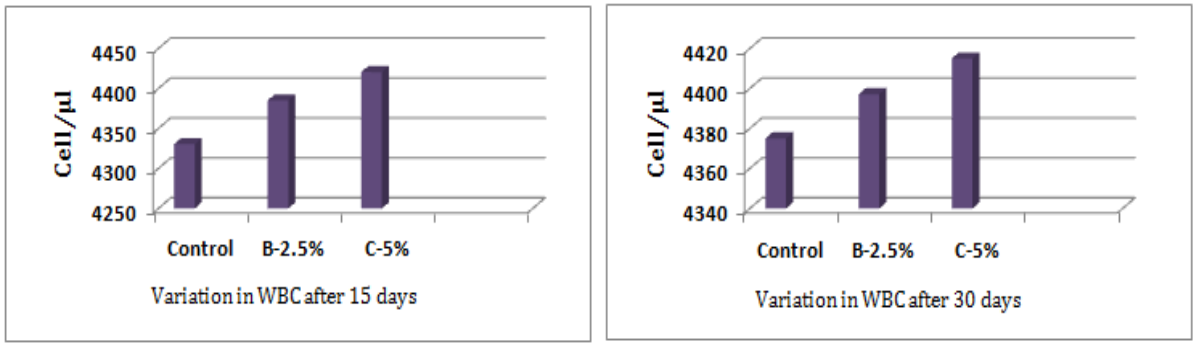

Fig. 4: Variation in WBC of Fish after 15 and 30 Days

Table 4: Effect of *Ocimum sanctum* 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	26.2
B	3.4	31.5	6.9	26.12	3.7	32.8	7.4	26.34
C	3.8	32.08	7.2	26.85	3.8	33.9	7.8	26.88

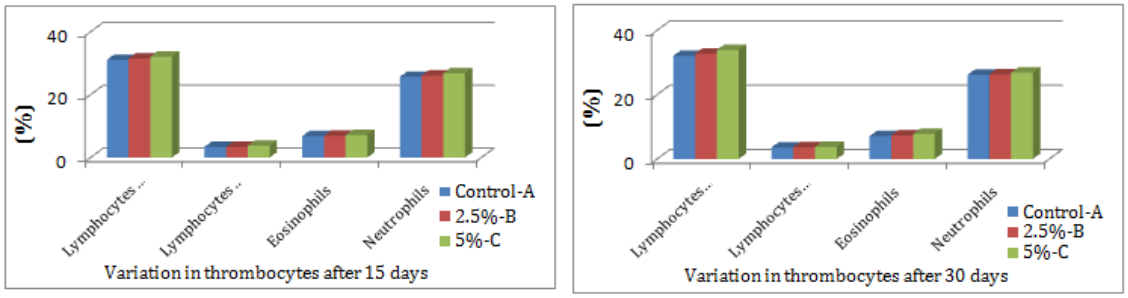

Fig. 5: Variation in Thrombocytes of Fish after 15 and 30 Days

DISCUSSION

Medicinal plant extract as immunostimulant elevate specific and nonspecific differences against pathogens. *Ocimum sanctum* Linn. (Tulsi or Holy Basil) of Family Lamiaceae has been extensively used in Haemeopathy and Aurvedic system of medicines. It is mostly used as chemopreventive, antistress, anticancer and immunostimulatory agent [21-27].

The present study demonstrated that the dietary supplemented with aqueous leaf extract of *Ocimum sanctum* enhance growth in 30 days. Several herbs have been tested for their growth promoting activity in aquatic animals [38,39]. The enhanced growth could be due to the growth promoting effect of *Ocimum sanctum* leaf extracts. A similar observation has been reported by Mathivanan et al. [40] in Broilers.

The evaluation of haematological and biochemical characteristics in fish have become an important means of understanding normal, pathological processes and toxicological impacts [41]. Haematological alterations are usually the first detectable and quantifiable responses to environmental changes [42]. Haematological and biochemical profiles of blood can provide important information about the internal environment of the organisms [43]. The present study on effect of aqueous leaf extract of *Ocimum sanctum* on Haematological parameters such as RBC, WBC, lymphocytes, eosinophils and neutrophils indicated increased counts in treated groups in comparison to the control at the end of the 15 and 30 days of studies. The results are consistent with the results obtained by Alishahi et al. [44] who used common crap treated with dietary *Aloe vera* extracts.

Invariably there is an increasing RBC and WBC contents of *Clarias batracus* treated with aqueous extracts of *Ocimum sanctum* at both the groups (Gr.B and Gr.C). It may be due to the effect of this bioactive principle of *Ocimum sanctum* and ascorbic acid to protect murine peritoneal macrophage from deleterious effect of nicotine and, simultaneously, help to restore their normal functions. In agreement with the present findings, Sahu et al. [45] reported that WBC and RBC counts were higher in *Labe rohita* fingerlings fed *Magnifera indica* kernel when compared to control. Gopalakannan and Arul [46] 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. [47] 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, *Withania somnifera* in Babl/c mice [48] and *Ficus bengalensis* in rats [49] were found to stimulate immunological activities. 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 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.

In 15 days of *Ocimum sanctum* extract treatment the lymphocyte and phagocyte counts were increased in all experimental groups as compared to control group. The major reason for this enhanced concentration of lymphocytes and phagocytes in the experimental groups may be their participatory role in immune functions as observed by Kollner et al. [50]. 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. [51].

The present study demonstrated decreased level of glucose and cholesterol in 30 days of exposures to *Ocimum sanctum* extract in Gr.B and Gr.C in comparison to Gr.A. This is probably due to the capability of plant extracts to reduce the effect of stressors. Similar observation was found in *Labeo rohita* fingerlings [45] and black tiger shrimp, *Penaeus monodon* [50] that glucose levels were reduced after feeding with herbal immunostimulant diets. The dose dependent reduction in serum cholesterol of the treated fishes may be due to the levels of polyphenolic compounds (alkaloids, saponins, tannins, alkaloids, anthraquinone, flavonoids, steroids, terpenoids and cardiac glycosides) in the aqueous extract of *Ocimum sanctum* [19,20] and the inhibition of cholesterol biosynthesis in the liver [53].

In the present study, dietary supplemented *Ocimum sanctum* extract group enhanced total plasma protein and globulin values in comparison to control group. Similar results were reported in rainbow trout fed with garlic, ginger, lipopolysaccharide, *Laurus nobilis*, and *Coggyria coggyria* [54-58]. This study revealed that *Ocimum sanctum* extracts incorporated in fish diet triggered the humoral elements in the serum. Globulin is the main resource of immunoglobulin production, thus its enhancement in serum provide immunostimularory potential [59]. However there was a decrease serum albumin contents in 30 days treatment with *Ocimum sanctum* extracts. With reduced levels of serum albumin, fluid may escape into tissues to cause localized oedema and reduce the delivery of nutrients to tissues [60,61]. 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 [53].

The overall results of the present study proved that the extract of *Ocimum sanctum* induced the innate immunity of fish in all treated groups. Our results suggest the protective ability of *Ocimum sanctum* mediated through cellular and may be non cellular immune mechanisms, as evident from the enhanced haematological parameters such as RBCs, WBCs and lymphocytes and phagocytes. The exact mechanism of action of the leaf extract on immune system of fish is not known. But it has been observed that the immunostimulant might act directly on the immunopoitic cells [62]. The leaf of *Ocimum sanctum* has been shown to contain water soluble phenolic compounds such as alkaloid, glycosides, saponin etc [19,20] that might act as a potential immunostimulant. However the active principle responsible for the immunostimulatory property observed in the present study has to be identified.

CONCLUSION

The results of the present study indicated the beneficial role of *Ocimum sanctum* on a common fish *Clarias batrachus* (Linn.) in augmenting the immunity, growth and survivality as evident from the enhanced haematological and biochemical parameters. Thus there is a great prospectus of using natural products including plant extracts in the treatment of various parasitic diseases of fish. The evaluated data from our study also suggests the use of *Ocimum sanctum* as home remedy for controlling various diseases by increasing the immunity level in the human body. Further studies to evaluate about the safety and efficacy of the extracts in human are needed.

ACKNOWLEDGEMENTS

The authors are thankful to University Grants Commission, New Delhi, India for financial assistance. We are also thankful to Head of the Department of Botany and Principal B.J.B. (A) College for providing necessary facilities for carrying out the experimental work. Finally we are thankful to Sabitri Nahak for helping in computer work without which preparation of the manuscript would not have been possible.

REFERENCES

1. Yeap SK, Omar AR, Ho WY, Ben BK, Ali AM, Alitheen NB. Immunomodulatory effect of *Rhaphidophora korthalsii* on mice spenocyte, thymocyte and bone marrow cell proliferation and cytokine expression. African Journal of Biotechnology 2011; 10(52):10744-1075.
2. Archana, Jatawa S, Paui R, Tiwari A. A review on immunostimulatory plants: A rich source o f Natural Immunomodulator. International Journal of Pharmacology 2011; 7:198-205.
3. Shaikh M. Recent advance on ethanomedicinal plants as immunomodulator agent. Ethnomedicine 2010; 227-244.

4. Anderson DP, Siwicki AK. Basic haemotology and serology for fish health programs. In diseases in Asia Aquaculture II. J Asian Fisheries society 1995; 185-202.
5. Esteban MA, Cuesta A, Ortuno J, Meseguer J. Immunomodulatory effects of dietary intake of chitin on gilthead seabream (*Sparus aurata* L.) innate immune system. Fish Shellfish Immunol 2000; 11:303-315.
6. Sahu MK, Swarnakumar NS, Sivakumar K, Thangaradjou T, Kannan L. Probiotics in aquaculture: importance and future perspectives. Indian J. Microbiol 2008; 48(3):299-308.
7. Anderson DP. Immunostimulant, Adjuvant, and Vaccine Carrier in Fish: Application to Aquaculture. Annual Review of Fish Diseases 1992; (21):281-307.
8. Kumar A, Singh V, Ghose S. An experimental evaluation of in vitro immunomodulatory activity of isolated compound of *Ricinus communis* on human neutrophils. Int. J. Green Pharm 2011a; 5:201-204.
9. Jeba CR, Vaidyanathan R, Kumar RG. Immunomodulatory activity of aqueous extract of *Ocimum sanctum* in rat. Int. J. on Pharmaceutical and Biomed Res 2011; 2:33-38.
10. Kumar A, Rahal A, Chakraborty S, Tiwari R, Latheef SK, Dhama K. *Ocimum sanctum* (Tulsi): a miracle herb and boon to medical science– A Review. International Journal of Agronomy and Plant Production 2013; 4 (7):1580-1589.
11. Sarkar A, Pandey DN, Pant MC. A report on the effect of *Ocimum sanctum* (Tulsi) leaves and seeds on blood and urinary uric acid, urea and urine volume in normal albino rabbits. Ind. J. Physiol. Pharmacol 1990; 34:61-62.
12. Mandal S, Das DN, Dey K. *Ocimum sanctum* Linn – A study on gastric ulceration and gastric secretion in rats. Ind. J. Physiol. Pharmacol 1993; 37:91-92.
13. Sethi J, Sood S, Seth S, Thakur A. Protective effect of Tulsi (*Ocimum sanctum*) on lipid peroxidation in stress induced by anemic hypoxia in rabbits. Ind. J. Physiol. Pharmacol 2003; 47:115-119.
14. Govind P. An overview of anticancer natural products. J. Pharm. Res 2009; 2(12):1799-1803.
15. Kumar A, Rahal A, Verma AK. *In vitro* antibacterial activity of hot aqueous extract (HAE) of *Ocimum sanctum* (Tulsi) leaves. Ind. J. Vety. Medicine 2011b; 31(2):96-97.
16. Wagner H, Norr H, Winterhoff H. Plant adaptogens. Phytomed 1994; 1:63-76.
17. Kelm MA, Nair MG, Strasburg GM, DeWitt DL. Antioxidant and cyclooxygenase inhibitory phenolic compounds from *Ocimum sanctum* Linn. Phytomed 2000; 7:7-13.
18. Shishodia S, Majumdar S, Banerjee S, Aggarwal BB. Urosolic acid inhibits nuclear factor-kappaB activation induced by carcinogenic agents through suppression of I kappaB alpha kinase and p65 phosphorylation: Correlation with down-regulation of cyclooxygenase 2, matrix metalloproteinase 9, and cyclin D1. Cancer Res 2003; 63:4375-83.
19. Gupta SK, Prakash J, Srivastava S. Validation of claim of Tulsi, *Ocimum sanctum* Linn as a medicinal plant. Ind. J. Experimental Biol 2002; 40:765-773.
20. Aswar MK, Joshi RH. Anti-Cataleptic Activity of Various Extracts of *Ocimum sanctum*. Int. J. Pharma. Res. Development–Online IJPRD. 2(1). Ref No. IJPRD/2010/PUB/ARTI/VOV-2/ISSUE-6/AUG/015; 2010.
21. Singh S, Manish T, Dipak M. Biological activities of *Ocimum sanctum* L. fixed oil – An overview. NISCAIR Online Periodicals Repository 2007; 45(5):403-412.
22. Bhanuprakash V, Hosamani M, Balamurugan V, Gandhale P, Naresh R, Swarup D, Singh RK. *In vitro* antiviral activity of Plant extracts on goat pox virus replication. Ind. J. Experimental Biol 2008a; 46:120-127.
23. Bhanuprakash V, Hosamani M, Balamurugan V, Singh RK, Swarup D. *In vitro* antiviral activity of *Eugenia jambolana* Plant Extract on *Buffalopox virus*: Conventional and qPCR Methods. Int. J. Trop. Med 2008b; 2(1):3-9.
24. Joshi B, Sah GP, Basnet BB, Bhatt MR, Sharma D, Subedi K, Pandey J, Malla R. Photochemical extraction and antimicrobial properties of different medicinal plants: *Ocimum sanctum* (Tulsi), *Eugenia caryophyllata* (Clove), *Achyranthes bidentata* (Datiwan) and *Azadirachta indica* (Neem). J. Microbiol. Antimicrobials 2011; 3(1):1-7.
25. Nahak G, Mishra RC, Sahu RK. Phytochemical investigation and *in vitro* antioxidant evaluation of some Ocimum species. Journal of Pharmacy Research 2011; 4(7):2340-2343.
26. Vogel HG. Analgesic, anti-inflammatory and antipyretic activity. In: Vogel, W. H., Scholkens, B. A., Sandow, J., Muller, G. and Vogel, W. F. (eds.), Drug discovery & evaluation Pharmacological Assays, 2nd edition, New York, Springer 2002; 759-767.
27. Mahima Rahal A, Deb R, Shyma K Latheef, Samad HA, Tiwari R, Verma AK, Kumar A, Dhama K. Immunomodulatory and therapeutic potentials of herbal, traditional/indigenous and ethnoveterinary medicines. Pak. J. Biol. Sci 2012; 15(16):754-774.
28. Jadhav HR, Singh A, Bhutani KK. Rationale for immunomodulatory and anti-inflammatory effects of *Ocimum sanctum*: Radical scavenging potential and effect on nitric oxide production. Acta Horticulturae 2005; 678:159-162.
29. Dacie SIV, Lewis SM. Practical haematology (7th edition) J. and A. Churchill Ltd. Livingston, Lodon Melborne and New York, 1991. p.67.
30. Joshi PK, Bose M, Harish D. Changes in certain haematological parameters in a siluroid catfish *Clarias batrachus* (Linn) exposed to cadmium chloride. Pollution Resources 2002a; 21(2):119-131.
31. Koller A. Total serum protein. Kaplan A et al. Clin Chem. The C.V. Mosby Co St. Louis, Toronto Princeton 1984; 418:1316-1324.
32. Burtis CA, Ashwood ER, Bruns DE. eds. In: Tietz textbook of clinical chemistry and molecular diagnostics, 3rd ed AACC, 1999; 1915-1916.
33. Doumas BT, Waston WA, Brigg HG. Albumin standards and the measurement of serum albumin with bromocresol green. Clin. Chem. Acta 1971; 31:87–96.
34. Gendler S. Uric acid. Kaplan A et al. Clin Chem. The C. V. Mosby Co St. Louis, Toronto Princeton 1984; 425:1268-1273.
35. Stoskopf MK. Clinical pathology in fish medicine. W.B. Saunders Company, Hartcourt Brace Jovanourah Inc 1993. p.89.
36. Reinhold JG. Standard Method of Clinical Chemistry. Academic Press New York 1953. p.256.
37. Duncan RM. Multiple range and multiple f-tests. Biometrics 1955; 11:1-42.
38. Citarasu T, Michael Babu M, Raja Jeya Sekar R, Peter Marian M. Developing artemia enriched herbal diet for producing quality larvae in *Penaeus monodon*, Fabricius. Asian Fish. Sci 2002; 15:21–32.
39. Jayaprakas V, Eupharsia J, Growth performance of *Labeo rohita* (Ham.) Livol (IHF-1000), an herbal product. Proc. Indian Natl. Sci. Acad. 1996; 63(2):1–10.
40. Mathivanan R, Edwin CS, Viswananthan K. Effect of *A. paniculata* supplementation on growth and feed conservation efficiency of broilers. Indian J. Poultry Sci. 2008; 43(2):189-192.
41. Sudova E, Piackova V, Kroupova H, Pijacek M, Svobodova Z. The effect of praziquantel applied per as on selected haematological and biochemical indices in Common carp (*Cyprinus carpio* L.). Fish Physiology and Biochemistry 2008; 35(4):599-605.
42. Wendelaar Bonga SE. The stress response in fish. Physiology Reviews 1997; 77(3):591-625.
43. Masopust J. Clinical biochemistry. Karolinum, Praha, 2000. p.832.
44. Alishahi M, Ranjbar MM, Ghorbanpour M, Mesbah M, Razi Jalali M. Effects of dietary *Aloe vera* on some specific and nonspecific immunity in the common carp (*Cyprinus carpio*). Int. J. Vet. Res 2010; 4:189-195.
45. Sahu S, Das BK, Pradhan J, Mohapatra BC, Mishra BK, Sarangi N. Effect of *Magnifera indica* kernel as a feed additive on immunity and resistance to *Aeromonas hydrophila* in *Labeo rohita* fingerlings. Fish Shellfish Immunol 2007; 23:109-118.
46. Gopalakannan A, Arul V. Immunomodulatory effects of dietary intake of chitin, chitosan and levamisole on the immune system of *Cyprinus carpio* and control of *Aeromonas hydrophila* infection in ponds. Aquaculture 2006; 255:179-187.

47. Dugenci SK, Arda N, Candan A. Some medicinal plants as immunostimulant for fish, J. Ethnopharmacol 2003; 88:99–106.
48. Singh MP, Ahirwar J, Muthal N. Evaluation of immunomodulatory activity of aqueous extract of *Ficus bengalensis* aerial roots in wistar rats. Asian Journal of Pharmaceutical and Clinical Research 2011; 4(1):82-86.
49. Davis L, Kuttan G. Immunomodulatory activity of *Withania Somnifera*. Journal of Ethno Pharmacology 2000; 71:193-200.
50. Kollner B, Wasserrab B, Kotterba G, Fischer U. Evaluation of immune functions of rainbow trout (*Oncorhynchus mykiss*)—how can environmental influences be detected? Toxicol. Lett. 2002; 131:83–95.
51. Ephraim KD, Salami HA, Osewa TS. Effect of aqueous leaf extract of *Ocimum gratissimum* on heamatological and biochemical parameters in rabbits. African Journal of Biomedical Research 2000; 3:175-179.
52. Citarasu T, Sivaram V, Immanuel G, Rout N, Murugan V. Influence of selected Indian immunostimulant herbs against white spot syndrome virus (WSSV) infection in black tiger shrimp, *Penaeus monodon* with reference to haematological, biochemical and immunological changes. Fish Shellfish Immunol 2006; 21:372-384.
53. Oyewo EB, Akanji MA, Iniaghe MO, Fakunle PB. Toxicological implications of aqueous leaf extract of *Andrographis paniculata* in Wistar Rat. Nature and Science 2012; 10(2):91-108.
54. Awad ES. Studies on plant based dietary supplements for the control of *Aeromonas hydrophila* infections in rainbow trout (*Oncorhynchus mykiss* Walbaum) [dissertation]. Edinburgh, UK: School of Life Sciences, Heriot Watt University; 2009.
55. Bilen S, Bulut M. Effects of laurel (*Laurus nobilis*) on the nonspecific immune responses of rainbow trout (*Oncorhynchus mykiss*, Walbaum). J. Anim. Vet. Adv 2010; 9(8):1275-1279.
56. Bilen S, Bulut M, Bilen AM. Immunostimulant effects of *Coggyria coggyria* on rainbow trout (*Oncorhynchus mykiss*). Fish Shellfish Immunol 2011; 30:451-455.
57. Nya EJ, Austin B. Use of dietary ginger, *Zingiber officinale* Roscoe, as an immunostimulant to control *Aeromonas hydrophila* infections in rainbow trout, *Oncorhynchus mykiss* (Walbaum). J Fish Dis 2009b; 32:971-977.
58. Nya EJ, Austin B. Use of garlic, *Allium sativum*, to control *Aeromonas hydrophila* infection in rainbow trout, *Oncorhynchus mykiss* (Walbaum). J Fish Dis 2009a; 32: 963-970.
59. Sahu S, Das BK, Mishra BK, Pradhan J, Sarangi N. Effect of *Allium sativum* on the immunity and survival of *Labeo rohita* infected with *Aeromonas hydrophila*. J. Appl. Ichthyol 2006; 23:80-86.
60. Jeremy MB, Tymoczko LJ, Lubert S. The Immune System. Biochemistry.5th Edition, Freeman and Company, NY 2001; 926–945.
61. Lichenstein HS, Lyons DE, Wurfel MM, Johnson DA, McGinley MD, Leidli JC, Trollinger DB, Mayer JP, Wright SD, Zukowski MM. Afamin is a new member of the albumin, alpha-fetoprotein, and vitamin D-binding protein gene family. J. Biol. Chem 1994; 269(27):18149–54.
62. Jeney G, Anderson DP. Enhanced immune response and protection in rainbow trout to *Aeromonas salmonicida* bacteria following prior immersion immunostimulants. Fish Shell. Immunol 1993; 33:51-58.