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Evaluation of the *Eulophia epidendraea* tuber extract responses on Nutritional, Bacterial Disease Challenges and Pathological analysis of Common carp fish, *Cyprinus carpio* (L.)

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Abstract

In the present study, tuber extract of *Eulophia epidendraea* responses on nutritional, challenges on bacterial disease and pathological analysis of common carp fish, *Cyprinus carpio* (L.). Feeding experimental analysis of basal diet with 5% of tuber extract of *Eulophia epidendraea* responses on nutrition, bacterial disease challenges and pathological analysis of common carp fish, *C. carpio* conducted for 42 days. Results of control fed with 5% tuber extract *E. epidendraea* responses on good peripheral haemogram of diseased carps, *C. carpio*. The significant antibacterial response in the experimental fish was observed on 14^{th} and 28^{th} days. The curative effect of the tuber extract of *E. epidendraea* peaked at 14^{th} and 28^{th} days after the feeding regimen, suggesting the development of immunotolerance to the decreased fish which could able to regain better health after feeding the tuber extract.

Keywords: *Eulophia epidendraea;* tuber; Common carp; fish, *Cyprinus carpio* Nutritional; Bacterial; Disease; Challenges ; Pathological analysis, macrophages

Introduction

Aquaculture industries are very leads to great losses and decrease in fish production. Disease management is one of the biggest problems of fish and shellfish culture. In recent years, there has been a growing interest in combating or controlling bacterial fish diseases through alternative source of natural medicine or plant products. Phytochemicals are responsible for several therapeutic agents and pharmacological actions. Thus they play a significance role in proving primary health care for fishes. As part of this, a study was made on the immunomodulatory activity of medicinal plants in animal models (Kapil and Sharma,1997; Thatte *et al*., 1994; Mungantiwar *et al*.,1997; Bafna and Mishra, 2004). Many researchers have been successfully worked on fed with diets containing extracts of several medicinal plants such as *Vitax negundo* and *Allium sativa, Ocimum santum* (Nargis *et al*., 2011; Pavaraj *et al*., 2011). Recently, reviewed on fish diseases control for several medicinal plants such as *Ocimum sanctum, Phyllanthus emblica), Azadirachta indica), Solanum trilobatum Eclipta alba, Zingiber officinale, Allium sativum, Camellia sinensis, Aloe vera, Cynodon dactylon, Achyranthes aspera, Nyctanthes arbortristis, Tinospora cordifolia* and *Picrorhiza kurooa* (Bairwa *et al*., 2012). Immunomodulatory effect of dietary feeding of terpenoid compound was reported (Jitpukdeebodintra *et al*.,2005).

Orchid *Eulophia epidendraea* (Retz.) Fischer belongs to the family Orchidaceae, which has been traditionally used for the treatment of tumour, abscess and healing of wounds (Maridass and Ramesh, 2010). Earlier studies, *β*-sitosterol (I), *β*-sitosterolglucoside (II), *β* – amyrin (III) and lupeol (IV) were isolated and identified from the tuber and also four flavonoids constituents of apigenin, luteolin, kaempferol, and quercetin were identified from the leaves of *E. epidendraea* (Maridass *et al*., 2008). Recently, pharmacological analyses on wound - healing activity and anti diarrhoeal activity of tuber extract of *E. epidendraea* were reported (Maridass and Ramesh, 2010; Maridass, 2011). The present study was undertaken to evaluate the dietary feeding of *Eulophia epidendraea* tuber extract on the disease challenge responses of the fish, *Cyprinus carpio* (L.)

Material and Methods

Solvent Extraction

The tubers of *E. epidendraea* (Retz.) Fischer was air- dried, and powdered. About 250g of the powder was extracted with acetone (56^{o}C) in a

Soxhlet apparatus for 8h. The solvent was evaporated under reduced pressure. After determining the yield, sediment extract was stored at 4°C for further use.

Feed preparation

About 5% of the sediment extract was incorporated with experimental diet along with other ingredients maintaining a protein level of 40% approximately. A control practical diet was prepared by using only the ingredients without tuber extract.

Experimental design

The control, diseased and treated experimental common carps, *Cyprinus carpio* (L.), were acclimated and maintained in the laboratory conditions. The feeding experiment was conducted in 15L troughs. At the start of the experiment, the fish weighing about 15.28 ± 0.8g were divided in to three groups of 60 individual each. Three groups were fed with the experimental diet containing 5%, of *Eulophia epidendraea* tuber extract and a basal diet without plant tuber extract. The feeding trials were conducted for 42 days.

Identification characteristics of pathogen

Staphylococcus aureus was used to observe the defense responses of *Cyprinus carpio* (L.) during the disease challenge study. *Staphylococcus aureus* was identified following the chemical characteristics as shown in Table-1.

Table -1: Biochemical characteristics identified in *S. aureus*

Characteristics	Responses
Catalase activity	+
Coagulase production	+
Thermonuclease production	+
Lysostaphin sensitivity	+
Anaerobic utilization of	
Glucose	+
Mannitol	+

S. aureus was maintained in nutrient agar medium and subcultured every fifteen days and stored at 4°C. Before use, they were subcultured in nutrient broth incubated at 28 ±1°C for 18 - 24 hours. *C. carpio* was exposed to a bacterial suspension of *Staphylococcus aureus* at a concentration of 2.6 ($x10^{-6}$ cells/ml) through water medium and the pathogen level was maintained in the water medium for two days.

Specific Growth rate (%)

The weekly increase in the weight of fish was recorded and the growth (gain in wet weight) and specific growth rate (SGR %) were calculated by using the following formulae (Samuel, 2001).

$$\text{Growth rate (\%)} = \frac{W_2 - W_1}{W_1} \times 100$$

$$\text{Specific Growth Rate} = \frac{\text{Log}W_2 - \text{Log}W_1}{t} \times 100$$

Where, W_1 = Initial body weight
W_2 = Final body weight
T = No. of days of culture

The difference in average growth between the control and experiment fish was tested for significance (0.05%) by performing the t-test the using the Microsoft Excel XP.

Collection of blood sample

The peripheral blood cell counts were made in control and experimental fishes on the 1st, 14th , 28th and 42nd days. The blood sample was collected from caudal vein without using anticoagulants.

Total / differential blood counts

The blood was drawn up to the 0.5 mark in RBC and WBC pipette diluted up to the mark with Dacie's fluid (Modified). TEC and TLCs were counted with a haemocytometer. The results were given as mean ± standard deviations of five fish in triplicates.

Macrophage counts

The kidneys were removed from the same group of 5 fishes and used to determine the number of the macrophages both control and experimental fishes on the 1st, 14th, 28th and 42nd days.

Histological technique

Control and experimental fishes were dissected out and gills, liver and intestine were fixed in Bouin's fluid for 48 hrs. Then the tissues were

washed with water to remove the fixative. The tissues were dehydrated by passing through graded series of ethanol. The tissues were cleared in xylene, embedded in wax (MP 58 – 60^{o}C) and sectioned at 4-6(µm) thickness with the help of a Spencer microtome. The sections were stained with trichrome. The slides were scanned under oil immersion (500X) objective and Photomicrographs were taken in Nickon Microscope (Model Eclipse E - 400).

Statistical analysis

Results were analysed by a Student's *t*- test. Differences were considered statistically significant at $P < 0.05$.

Results

Weight Gain (g)

The average weight gain of normal fish in 42 days was 16.43g for control diet. The weight gain of diseased fish fed with control diet incorporated with 5% of tuber extract of *Eulophia epidendraea* was 16.47g. Diseased fish fed with control diet died in the course of the experiment (Fig.-1). Statistical analysis revealed that weight gain of the fish fed with the tuber extract was significantly higher than the control diet.

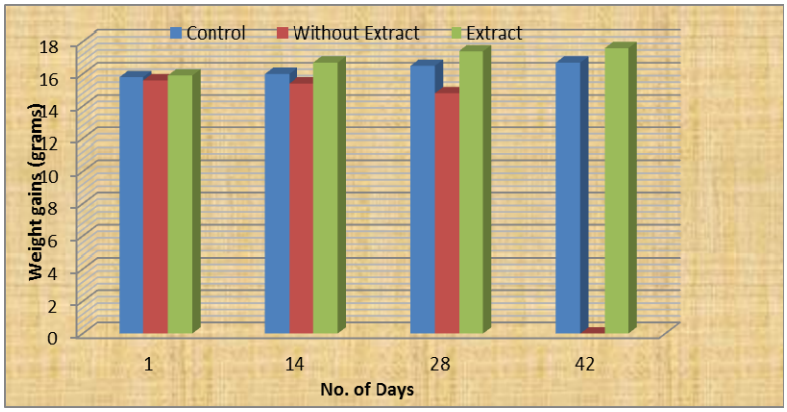


Fig.1. Body mass weight gain of normal, control, diseased (control) and diseased fish fed with tuber extract of *Eulophia epidendraea*

Table -2: Growth responses of control, diseased and treated fishes

Days	Control		Diseased		Treated	
	GR mg/g fish	SGR (%)	GR mg/g fish	SGR (%)	GR mg/g fish	SGR (%)
14	0.46	0.24	-1.52	-33.1	2.4	1.41
28	2.60	0.96	-4.89	-11	3.74	1.40
42	3.80	0.62	-	-	0.73	0.18

Specific Growth rate (SGR %)

In the present study, the result of specific growth rate (g/week) was calculated (Table-2). The specific growth rates of *Cyprinus carpio* (L.) in control diet and the diet incorporated with 5% of tuber extract have been calculated and are presented in Table-2.

Normal *Cyprinus carpio* (L.) fed with control diet showed increasing trend in the growth rate from 0.46 to 3.80mg/g fish/day and the specific growth rate from 0.24 to 0.62%. But the diseased fish on control diet showed negative growth. The infected fish fed with tuber extract showed positive growth till 28 days and later after 42nd day they exhibited decreased growth.

Differential blood cell counts

Infection with *Staphylococcus aureus* initiated several pathological changes on the blood cells of *Cyprinus carpio* (L.). Light microscopic studies revealed pathomorphological changes on the different types of blood cells of infected fish (Plate-1 B-H). Normal erythrocytes of *Cyprinus carpio* (L.) were nucleated, oval cells. Their nuclei were oval and centrally located. The nucleus stained dark purple, the cytoplasm was stained light red (Plate -1A). The peripheral

haemogram responses of carps during 48 day feeding trials with 5% of tuber extract and control diets are presented in Table -3.

Table -3: Responses of TEC and TLC in *Cyprinus carpio* (L.) after administering *E. epidendraea* tuber extracts for 42 days

No.of Days	Erythrocytes (10^6/ mm^3)		Leucocytes (10^4/ mm^3)	
	Control	Treated	Control	Treated
1	2.5 ± 0.08	6.93 ± 0.498	1.93 ± 0.124	5.83 ± 0.37
14	4.63± 0.12	13.0 ± 1.143	2.5 ± 0.081	6.8 ± 0.33
28	4.66 ± 0.09	9.87 ± 0.249	3.0 ± 0.08	4.8 ± 0.45
42	3.5 ± 0.09	8.3 ± 0.294	5.2 ± 0.163	4.1 ± 0.35

Average ± SD Values are expressed in triplicates*

Table - 4: Proportion (%) of monocytes to lymphocytes in *C. carpio* (L.) feeding on experimental diet with 5% tuber acetone extract of *E. epidendraea*

No.of days	Monocytes (%)		Lymphocytes (%)	
	Control	Treated	Control	Treated
1	46.7 ± 6.5	46.0 ± 3.6	43.3 ± 1.25	36.7 ± 1.70
14	47.7 ± 1.2	47.7 ± 1.25	32.3 ± 1.25	37.7 ± 1.90
28	24.7 ± 1.25	26.0 ± 2.16	28.6 ± 0.47	46.0 ± 0.82
42	27.7 ± 1.25	22.7 ± 3.8	35.0 ± 0.82	40.7 ± 1.25

Values are expressed as Mean ± SE from the experiments (no. = 5 fishes).

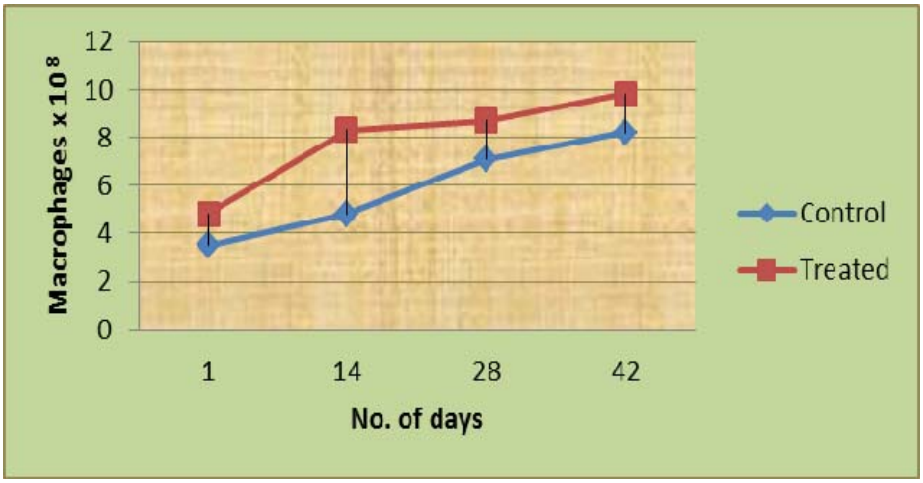


Fig. 2: Influence of tuber extract of *Eulophia epidendraea* on macrophage count of control and *Staphylococcus aureus* infected and treated *Cyprinus carpio* (L.)

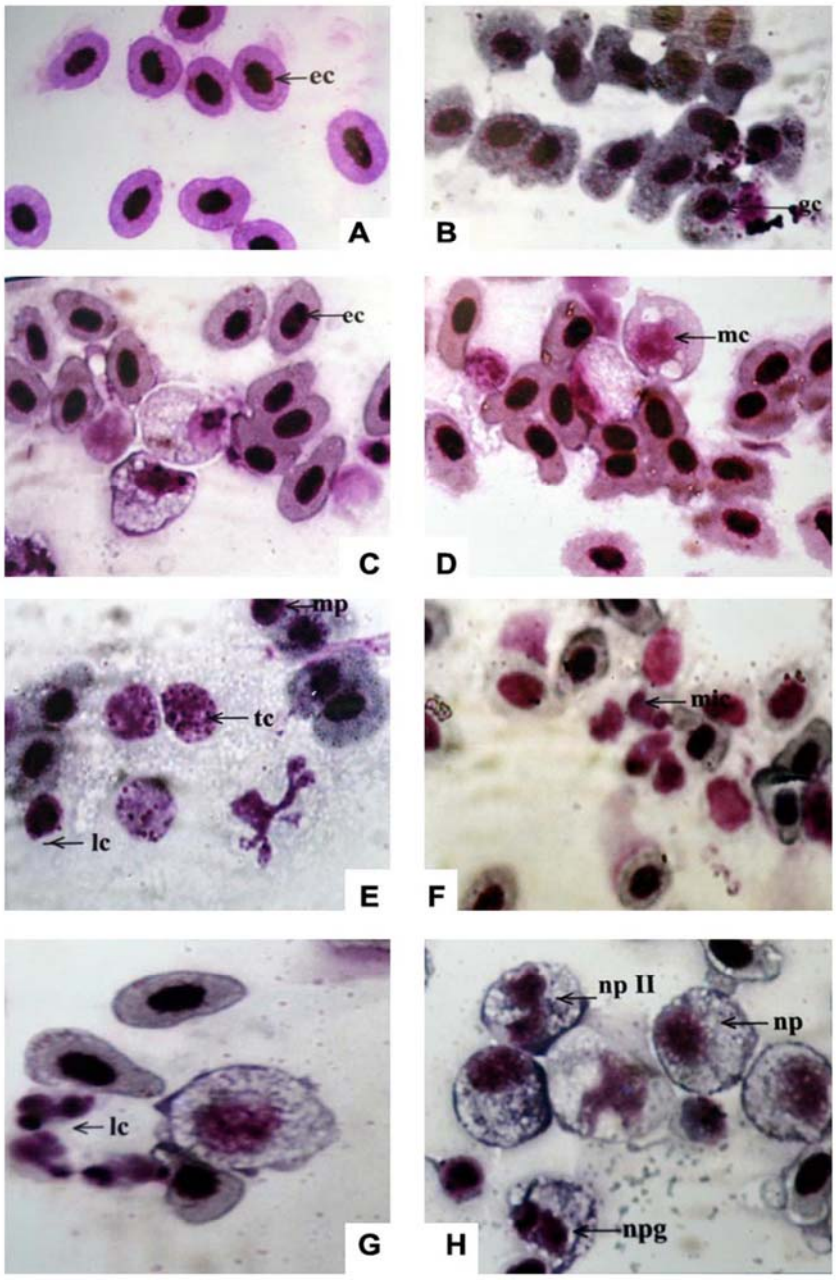
The curative effect of the tuber extract peaked at 14 and 28 days after the feeding regimen, suggesting the development of immunotolerance to the decreased fish which could able to regain better health after feeding the tuber extract.

Monocytes and Lymphocytes

There appeared to be a steady increase in the proportion of lymphocytes to monocytes from 36.7 ± 1.70 (%) immediately after feeding with tuber extract, to 40.7 ± 1.25(%) after 42 days. However, the values were similar to those of the control (Table 4).

Macrophages

The number of macrophages gradually increased in both control and treated group (Fig.2). It was observed that *Cyprinus carpio* (L.) which had received tuber extract contained significant number of macrophages than the control (1E), ($P < 0.05\%$).



ec- erythrocytes; tc-trompocytes; np-neutrophil; mc-monocytes;
mic-microcytes; np-II - neutrophill II; npg- neutrophilicgranulochytes;
Giemsa's strain X200

Plate 1. Peripheral blood of *Cyprinus carpio* L.

Histopathology

Histopathological changes were more evident in the gill, intestine and liver in *Staphylococcus aureus* infected *Cyprinus carpio* (L.) in the laboratory condition (Plate-2-3).

In normal fish the gills were reddish colour while it was become pale red in diseased fish and red in extract- treated fish. In diseased fish, gill filaments revealed the presence of bacteria *S. aureus*.

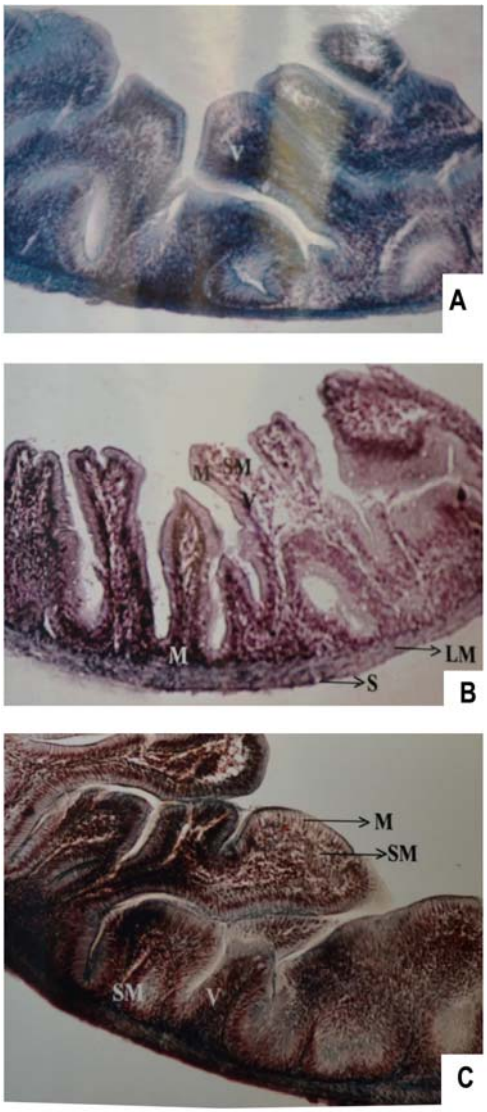


Plate-2: A: Struecture of Normal intestine; B-Diseases intestine showing loss of architecture; C- Extract treated intestine showing normal architecture of villi (X200)

The infected gill epithelium showed marked hyperplasic changes. The feeding of tuber extract resulted in curative gill responses. The infected kidney showed necrotic changes in renal tubules. The renal tubular structure become normal after treatment of tuber extract. The hepatocytes of diseased liver exhibited cytoplasmic vacuoles, karyolysis and pyknosis in the entire parenchyma. While the liver of normal *Cyprinus carpio* (L.) was reddish blue. In diseased fish, liver was greenish black colour. The intestine of control fish exhibited four usual layers serosa, muscularis, submucosa and mucosa. Serosa is the outer thin vascular layer followed by a thick layer of muscularis, consisting of outer longitudinal and inner circular muscle layer. It is followed by highly vascular submucosa having abundant blood cells. In diseased fish, the intestinal mucosa and submucosa were completely destroyed. Disorganization of the columnar epithelial cells and necrotic changes in the epithelial and goblet cells were evident in the infected fish. The treated diseased fish regained normal histological structure of villi and submucosa (Plate-3 B). In the liver, congestion was observed in the sinusoids, with centrilobular necrosis of the hepatocytes. Liver of *S. aureus* infected *C.carpio* (L) exhibited karyolysis and vacuole formation in the cytoplasm of the hepatic cells.

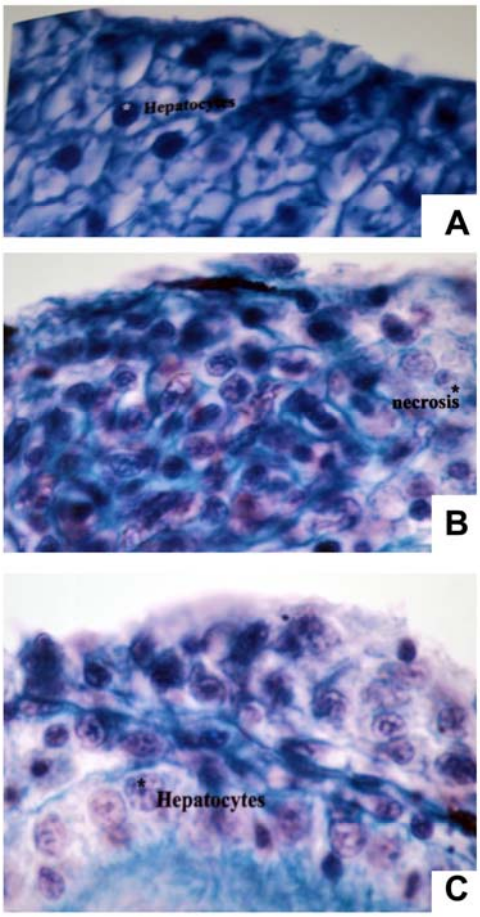


Plate-3: A.Cross section of Liver of normal C.carpio; B.Disese liver of C.carpio, C.Extract treated liver of C.carpio

Discussion

The present investigation revealed the immunomodulatory effects of *E. epidendraea*

extending a support for its usage in traditional medicine. The present study, acetone extract of the tuber had a good tissue regenerative activity against *Staphylococcus aureus*, which were commonly found in wounding sites of skin and infected respiratory organs.

Although the neutrophils, which phagocytose foreign material, presenting a first line of defence against invading bacterial micro-organisms and basophils (and their tissue counterpart, mast cells), which are rich in metachromatic granules, are involved in the mucosal immune response (Bainton, 2001). The macrophage lineage (macrophages being the tissue counterpart of circulating monocytes) is capable of engulfing endogenous cellular debris, foreign inanimate particles and invading micro-organisms, killing them wherever necessary (Douglas, 2001; Lehrer and Ganz, 2001). The macrophages were phagocytically active, and granulocytes participated in acute inflammatory responses in infected *C. carpio* (L.).

Macrophages were obvious in sections of the adult kidney and spleen (Bennett *et al*.,2001; Lieschke *et al*.,2001). They were large cells with large phagosomes, a high cytoplasm to nuclear ratio, diffuse nuclear chromatin, and an agranular but vacuolated cytoplasm. The vacuoles contained phagocytosed material, including pigment and red cell carcasses. These cells were negative for peroxidase activity. A mononuclear peroxidase-negative phagocytic cell was evident in kidney and may resemble a *Zebra fish* macrophage precursor. These cells were large and round, and were characterised by weak cytoplasmic staining, an elongated curved peripherally-located nucleus, a ruffled cell border, and no cytoplasmic granules (Crowhurst *et al*.,2002). Ellis,(1976) reported that development of the macrophages were regarded as an important part of the cellular immune system of fish and function to protect the host by phacocytizing foreign materials including disease causing agent.

In the present study, the feeding of tuber extract of *E. epidendraea* to *Cyprinus carpio* augmented the healthy mass of the lymphoid system, as indicated by the presence of functional white blood cells and by their proportional redistribution in response to foreign antigen. The ability to mount cellular and humoral immune responses has been demonstrated in early stages of all fish classes, including jaw-less fishes (Skjermo *et al*.,1995). Nakahishi, (1986) reported that the cultured two-month old rockfish, *Sebastiscus marmoratus*, were able to mount an antibody response against sheep red blood cells and even showed an allograft response in the same fashion in adults. In the carp, lymphocytes were able to reject allografts as early as day 16 post hatch (Botham and Manning,1981). Protective immunity was also reported in two-week-old rainbow trout . It was reported that the fish defense system was basically similar to mammals(Tatner and Horne, 1983). In mammal, anaphylactic reactions are mediated through B lymphocytes. In teleost fishes B lymphocytes are found (Iwama and Nakanishi, 1996). Phytohemagglutinin (PHA) is a mitogen for T lymphocytes (Ellis,1988). Previously, Te-Feng Tsai *et al*., (2001) reported that the immunomodulatory effect of two Chinese medicinal plants of *Angelica sinensis* and, *Astragalus membranaccu*. It stimulates growth of erythroid precursor cells to differentiate into red blood cells. This result was indicated that the immunostimulants enhanced the mitogen activities caused by concanavalin A or lipopolysaccharides and produced macrophage activating factors(Hardie *et al*.,1991;Te-Feng Tsai *et al*.,2001; Siwicki *et al*.,1996). Complement activity can also be activated by several immunostimulants. Hardie *et al.,*(1991) reported that the fish which received the large amounts of vitamin C showed increased levels of complement activity. Atlantic salmon injected with yeast glucan also showed increased complement activities (Engstad *et al*.,1992). Lysozyme activity is also influenced by the administration of immunostimulants (Engstad *et al*., 1992; Jorgensen *et al*.,1993). No immunopathological studies have been reported do far in *C. carpio* (L.) infected with *S. aureus*. The evidence of macrophage activation in *C.carpio* (L.) by tuber extract of *Eulophia epidendraea* indicated the immunomodulatory influence. The oral administration of tuber extract phytochemicals boosted the general health and immunotolerance of the chosen fish *C.carpio* (L.) against bacterial pathogen.

References

Kapil, A. and Sharma, S.1997. Immunopotentiating compounds from *Tinospora cordifolia*. *Journal of Ethnopharmacology,* 58: 89-95.

Thatte, U.M., Rao, S.G. and Dahanukar, S.A.1994. *Tinospora cordifolia* induces colony stimulating activity in serum. *Journal of Postgraduate Medicine,* 40: 202-3.

Mungantiwar, A.A., Nair, A.M., Shinde, U.A.1997. Effect of stress on plasma and adrenal cortisol levels and immune responsiveness in rats: modulation by alkaloidal fraction of *Boerhaavia diffusa*. *Fitoterapia,* 68: 498-500.

Bafna, A.R. and Mishra, S.H.2004. Immunomodulatory activity of methanol extracts of flower-heads of *Sphaeranthus indicus* Linn. *Ars Pharmaceutica,* 45(3): 281-291.

Nargis, A., Khatun, M. and Talukder, D.2011. Use of medicinal plants in the remedy of fish diseases. *Bangladesh Research Publications Journal,* 5(3): 192-195.

Pavaraj, M., Balasubramanian, V., Baskaran, S., Ramasamy, P.2011. Development of Immunity by Extract of Medicinal Plant *Ocimum sanctum* on Common Carp *Cyprinus carpio* (L.). *Research Journal of Immunology,* 4: 12-18.

Bairwa, M.K., Jakhar, J.K., Satyanarayana, Y. and Devivaraprasad Reddy, A.2012. Animal and plant originated immunostimulants used in aquaculture. *J. Nat. Prod. Plant Resour*., 2(3):397-400.

Jitpukdeebodintra, S., Chantachum, S., Ratanaphan, A. and Chantapromma,K. 2005. Preliminary study on immuno modulatory properties of limonin from lime seeds. *Journal of Food Agriculture and Environment,* 3(2): 109-112.

Maridass, M. and Ramesh, U.2010. Investigation of Phytochemical constituents from *Eulophia epidendraea. International Journal of Biological Technology,*1(1):1-7.

Maridass, M., Raju, G. and Ghanthikumar, S.2008. Tissue - Regenerative responses on tuber extracts of *Eulophia epidendraea* (Retz.) Fischer in Wistar rat. *Pharmacologyonline*,3: 631-636.

Maridass, M.2011. Anti diarrhoeal activity of rare orchid *Eulophia epidendraea* (Retz.)Fisher. *Nature of Pharmaceutical Technology*, 1(1):5-10.

Samuel, M.2001. Studies on the reproductive biology and the dietary nutrient requirement of the chosen freshwater fish. Ph.D., *Dissertation*, Manonmaniam Sundaranar University, Tirunelveli,Tamil Nadu.

Bainton, D.F.2001. Morphology of neutrophils, eosinophils, and basophils. In: Williams Hematology. (Editors Beutler E, Coller B, Lichtman MA, Kipps TJ, Seligsohn U). McGraw-Hill, Sydney. 6th edition. 729-743.

Douglas, S.D. and Ho, W.Z.2001. Morphology of monocytes and macrophages. In Williams Hematology. (Editors Beutler, E., Coller, B., Lichtman, M.A., Kipps, T.J., Seligsohn). 6th edition. McGraw-Hill Company, Sydney,855-863.

Lehrer, R.I. and Ganz, T.2001. Biochemistry and function of monocytes and macrophages. In Williams Hematology. (Editors Beutler, E., Coller, B., Lichtman, M.A., Kipps, T.J., and Seligsohn, U.), McGraw-Hill, Sydney. 6th edition, 865-885.

Bennett, C.M., Kanki, J.P., Rhodes, J., Liu, T.X., Paw, B.H., Kieran, M.W., Langenau, D.M., Delahaye-Brown, A., Zon, L.I., Fleming, M.D. and Look, A.T.2001. Myelopoiesis in the zebrafish, *Danio rerio*. *Blood,*98: 643-651.

Lieschke, G.J., Oates, A.C., Crowhurst, M.O., Ward, A.C. and Layton, J.E.2001. Morphologic and functional characterization of granulocytes and macrophages in embryonic and adult zebrafish. *Blood*, 98:3087-3096.

Crowhurst, M.O., Layton ,J.E. and Lieschke, G.J.2002. Developmental biology of zebrafish myeloid cells. *International Journal of Developmental Biology,* 46: 483-492.

Ellis, A.E., Munroe, A.L. and Roberts, R.J.1976. Defense mechanisms in fish I. A study of the phacocytic system and the fate of intraperitoneally injected particulate materials in the plaice (*Pleuronectes platessa* L.). *Journal of Fish Biology,* 8: 67-78

Skjermo, J., Defoort, T., Dehasque, M., Espevik, T., Olsen, Y., Skjaak-Break, G., Sorgeloos, P. and Vadstein, O.1995. Immunostimulation of juvenile turbot (*Scuphthalmus maximus* L.) using an alginate with mannuramic acid content administrated via the live food organism *Artemia*. *Fish Shellfish Immunology,* 5: 531-534.

Nakahishi, T.1986. Ontogenetic development of the immune response in the marine teleost (*Sebastiscus marmoratus*). *Bull. Jap. Soc. Sci. Fish. Nissuishi,* 52: 473- 477.

Botham, J.W. and Manning, M.J.1981. The histogenesis of the lymphoid organs in the carp (*Cyprinus carpio* L.) and the ontogenetic development of allograft reactivity. *Journal of Fish Biology,* 19: 403- 414.

Tatner, M.F. and Horne, M.T.1983. Susceptibility and immunity to *Vibrio anguillarum* in post-hatching rainbow trout fry (*Salmo gairdneri* R.). *Dev. Comp. Immunol.,* 7: 465- 472.

Iwama, G. and Nakanishi, T.1996. The Fish Immune System. Organ Pathogen, and Environment, Academic Press, San Diego,1-380.

Ellis, A.E.1988. Ontogeny of the immune system in teleost fish. In: General Principles of Fish Vaccination (Editor Ellis, A.E.), Academic Press, New York, 20–32.

Kuby, J.1997. Immunology, WH. Freeman and Company, New York, 285-310.

Te-Feng Tsai, Chiung-Hui Hsieh, Yz-Sheng Lin, Wei-Jern Tsai, Yuh-Chi Kuo,2001. Enhancement of cell proliferation and cytokines production in human peripheral blood mononuclear cells by extracts from blood-enriching Dang-gui decoction contained in BDGD. *Journal of Chinese Medicine,* 12(3): 191-202.

Hardie, L.J., Fletcher, T.C. and Secombes, C.J.1991. The effect of dietary vitamin C on the immune response of Atlantic salmon (*Salmo salar*). *Aquaculture,*95: 201-214.

Siwicki, A.K,, Miyazaki, T., Komatsu, I. and Matsuzato, T.1996. *In - vitro* influence of heat extract from firefly squid *Watasenia scintilans* on the phagocyte and lymphocyte activities in rainbow trout *Oncorhynchus mykiss. Fish Pathology,* 31: 1-7.

Engstad, R.E., Robertsen, B. and Frivole, E.1992. Yeast glucan induces increase in activity of lysozyme and complement-medical haemolytic activity in Atlantic salmon blood. *Fish Shellfish Immunology,* 2: 287-297.

Jorgensen, J.B., Lunde, H. and Robertsen, B.1993. Peritoneal and head kidney cell response to intraperitoneally injected yeast glucan in Atlantic salmon, *Salmo salar* L. *Journal of Fish Diseases*,16: 313-325.

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