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IMMUNOSUPPRESSIVE POTENTIAL OF *PLECTRANTHUS AMBOINICUS* LEAF EXTRACT IN *CARASSIUS AURATUS*

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ABSTRACT

Objective: Leaf extract of *Plectranthus amboinicus* (PA) possess pharmaceutically important phytoconstituents that contribute to significant curative credential of the plant. The objective of the study was to analyse the Immunosuppressive nature of Hydroalcoholic extract of Plectranthus amboinicus (HAPA) by haematological and other related studies in comparison with immunosuppressive and immunostimulatory standards Cyclosporine A (CsA) and Concanavaline A (ConA) respectively. **Methods:** Hematological studies and immunomodulatory assays were performed using EAHAPA fraction of the plant. **Results:** Hematological indices such as RBC, WBC, Differential count, showed significant immunosuppressive potential on fishes, whereas Hb, plasma protein and immunoglobin levels were markedly unaffected by EAHAPA fraction. Immunomodulatory assays – carbon clearance assay, Respiratory burst activity, Neutrophil activity and myeloperoxidase activity showed significant immunosuppressive credential on par with CsA. **Conclusion:** The immunomodulatory studies and hematological indices analysis have revealed that innate immune system of the animal is affected. Further studies on animal model would reveal specific molecular targets involved in the action of plant extract for employing it in the treatment of autoimmune disorders.

KEYWORDS: Immunosuppression, Hematology, *Plectranthus*, Myeloperoxidase, Autoimmune disorder.

INTRODUCTION

Fishes are an important resource for humans worldwide, especially as food. Commercial and subsistence fisherial activities exist as fishing, aquaculture and maintaining as pet animal. Fishes have short generation time, highly fertile and cost little in terms of housing space and daily maintenance owing to their tiny size. In order to accurately extrapolate findings obtained from aquatic models to humans, it is critical to understand the differences and similarities in organ structure, response to injury, and molecular mechanisms between these phylogenetically distant species.[1] Hence, growing interest in using fish models is increasing today. The knowledge of the hematological characteristics is an important tool that can be used as an effective and sensitive index to monitor physiological and pathological changes in fishes.[2]

Modulation of immune response as a potential of therapeutics is a key for scientific researchers all over the world to discover the novel drug for achieving the complete success in transplantation field. Immunosuppression[3] is the required target to be achieved in the path of potential drug discovery for organ transplantation which suffers from acute[4,5] or chronic rejection[6] as a major drawback. The discovery of highly effective immunosuppressive agents has greatly contributed to the progress of organ transplantation.[7] Each immunosuppressive agent has both immune and non immune toxicity. Immune toxicity is the result of the total immunosuppression over a given period of time and is usually nonspecific.[8]

Herbal Immunosuppression: Herbal medicines inhibit cellular and humoral mediated responses which gain interest in treating immune mediated disorders and some autoimmune diseases.[9] Activation of T and B lymphocytes and macrophages and apoptosis of immune effector cells play critical roles in pathogenesis of these disorders.[10,11] All over the world, many scientists has documented the immunosuppressive effects of many herbs and their effective compounds.

***Plectranthus amboinicus* (Lour)** (PA), commonly called as country borage or Indian borage is a native plant of Taiwan. It is a large succulent aromatic perennial herb, shrubby below, hispidly villous or tomentose.[12,13] Juice of leaves mixed with sugar acts as a powerful aromatic carminative, given in colic and dyspepsia.[14] The antidiabetic, antioxidant, Antinociceptive, antihelminthic, hepatoprotective nature of PA were studied and reported by many scientists all over the world. P.amboinicus is considered as a useful medicinal plant because it contains lots of bioactive compounds which actively play a role for immune

response. Therefore medicinal plants are gaining importance in aquaculture diet due to their positive effects with no harmful results. An attempt to study the effect of *P.amboinicus* on hematological indices and Immunomodulatory nature of *C.auratus* is done here.

MATERIALS AND METHODS

Chemicals

CyclosporineA, ConcanavalinA, Benzocaine (anaesthetic) were bought from Sigma Aldrich, Mumbai, India. All other chemicals and solvents were of commercial grade.

Plant Materials

Fresh leaves of *Plectranthus amboinicus* were collected from local garden in Chennai during the month of August. The leaves were authenticated by **Prof. Dr. P. Jayaraman**, Director, Plant Anatomy Research Centre (PARC), Chennai, India. A voucher specimen (No: PARC/2013/2063) was deposited in the institute.

Preparation of the extract

1 Kg of powdered dry leaves was incubated with 70% Hydroalcohol for 48 hrs in a mechanical shaker. The extract was concentrated using rotary evaporator and the distilled solvent was collected. Phytochemical analysis[15] and invitro anti-inflammatory effect of HAPA[16] were studied and the crude extract was now reincubated with Hexane, chloroform, ethylacetate and butanol successively in a separating funnel for 1 hr and the solvent phase formed as a separate layer were respectively removed, lyophilized and stored. The stored samples were analysed qualitatively for flavonoids (Shinoda and Lead acetate tests).

The HPLC analysis of the ethylacetate extract showed the presence of Luteolin, Galangin, quercetin and gallic acid[17] in HPLC analysis**.**

Experimental Fish and their maintenance

Gold fish (mean weight of 160 ± 10g) of both sexes were obtained from ornamental fish breeders of Kolathur fish farm, chennai. A fish specimen submitted was identified and authenticated (File no: **12-28/2007-Tech/334)** by **Dr.K.Ilango**, Scientist – E & Officer-in-charge, Zoological Survey of India, Chennai as *Carassius auratus auratus (Linnaeus, 1758).* The fishes were maintained in glass tanks of 100L capacity and had a minimum acclimatization period of 10 days. No mortality was observed during the acclimatization period. The fish were fed thrice daily with a commercial balanced diet formulated for carp

fishes @3% of their body weight. During the experiment, the temperature ranged from 23 to 26°C, dissolved oxygen (DO) ranged from 5.6 to 7.8 mg/l and pH was 7.82±0.05 and the total ammonium and nitrite were kept below 0.1 and 0.05mg/L, respectively.

Group 1 - Control

Group 2 - Cyclosporine A (in olive oil) (20mg/kg.b.wt) (CsA)

Group 3 - Ethyl acetate fraction of Hydroalcoholic extract of *Plectranthus Amboinicus* (in DMSO) (200mg/kg.b.wt) (EAHAPA)

Group 4 - Concanavalin A (in 0.9% saline) (10mg/kg.b.wt) (ConA)

The induction was done intramuscularly for a period of three days.

Collection of heparinised blood and separation of plasma

The fishes were anaesthesized with Benzocaine (20 mg/litre) before collection of blood samples from fish. Blood was drawn by severing the caudal peduncle of fish by using 1.0 ml hypodermal syringe and 0.45 x 13 mm x 26 mm gauge needles, which was rinsed with heparin sodium (5000 IU/ml) solution before use. Few drops of blood were used for Smear Preparation and remaining blood was immediately transferred to the test tube coated with thin layer of EDTA (as an anticoagulant) in order to prevent haemolysis and clotting of blood. The tubes were left undisturbed and plasma separated was collected using micropipette and stored in -20^0C until use.

Hematological parameters

The **Total erythrocyte count** (RBC) and **White Blood Corpuscles** (WBC) was determined by the method described by **Blaxhall and Daisley**.[18] The differential count (DC) was done by the method of **wintrobe**[19] and expressed as percentage. The levels of plasma protein were estimated by the method described by **Lowry**.[20] Total immunoglobulin (Ig) was determined by the method described by **Anderson and Siwicki**.[21] Hemoglobin (Hb) was determined by the method of **Drabkin and Austin**.[22]

Carbon clearance test

Phagocytic activity of reticulo endothelial systems (RES) was assayed by carbon clearance test. Phagocytic index was calculated as a rate of carbon elimination of reticulo endothelial systems by carbon clearance test. Carbon ink suspension was injected intramuscularly to each animal after 48 hours of the three days treatment. Blood samples (25µl) were then withdrawn from the caudal vein under mild anesthesia at 0, 10 and 20 minutes after injection of colloidal

carbon ink and lysed in 0.1% sodium carbonate solution (3ml). The optical density was measured spectrophotometrically at 660nm.[23] The phagocytic index was calculated using the following formula

K = logOD1 - logOD2 / t2 – t1

where OD1 and OD2 are the optical densities at time t1 and t2, respectively.

Respiratory Burst Activity Of Neutrophils

Respiratory burst activity was assayed by the reduction of Nitroblue Tetrazolium (NBT) **Anderson (1995)**.[21] 0.1 ml of heparinized blood was taken in Eppendorf to which 0.1 ml of 0.2% NBT solution was added. The mixture was incubated for 30 min at 25^{o}C. From the resultant NBT - blood cell suspension, 50 µl was taken, added to 1.0 ml N, N-dimethyl formamide (DMF) in a glass tube and centrifuged at 3000 g for 5 min. The optical density (OD) of the supernatant was measured at 540 nm in UV spectrophotometer.

Neutrophil activity

Estimation of Neutrophil Activity was done by **Secombes**[24] method with modification described by **Stasiak and Baumann.**[25] The heparinized blood was collected in silica coated Eppendorff tubes and puffy coat was separated by centrifuging at 500 rpm for 10 min. 50 µl of the puffy coat was placed into each well of a 24 well flat bottomed microtitre plate (NEST Biotech, China) and incubated at 37°C for 1 h to facilitate adhesion of cells. Then the supernatant was removed and 50 µl of 0.3% NBT was added. After incubation for 1 h, the NBT was removed. The cells were then fixed with 100% methanol and washed thrice with 70% methanol and the plate was air dried. 60 µl of 2 N KOH and 70 µl DMSO were added into each well to dissolve the formazan blue precipitate formed. The turquoise-blue colored solution was then read at 665 nm using UV spectrophotometer.

Myeloperoxidase activity

Head kidney Myeloperoxidase activity was determined by the method described by **Renlund et al.**[26] The head kidney tissue was weighed and placed in 0.5 ml of 0.5% hexa decyl trimethyl ammonium bromide (HTAB) in 50 mM phosphate buffer (pH 6.0). The tissue was minced with scissors for 20 sec on an ice-cold plate and then homogenized for 20 sec. Another 0.5 ml HTAB buffer was added to the homogenate, vortexed and centrifuged at 14000 rpm for 2 min. The supernatant (50 µl) was assayed for MPO activity by adding 1.45 ml O-dianisidine hydrochloride (0.167mg/ml in 5mM Phosphate buffer (pH 6.0) with 0.0005% H_2O_2) and the absorbance was taken at 460 nm 4 times at 30 sec intervals.

Hydrogen peroxide (50-250 n moles) aliquots were treated similarly. The enzyme activity was expressed as **µmoles of hydrogen peroxide degraded/min/mg of protein**.

RESULTS

HEMATOLOGY

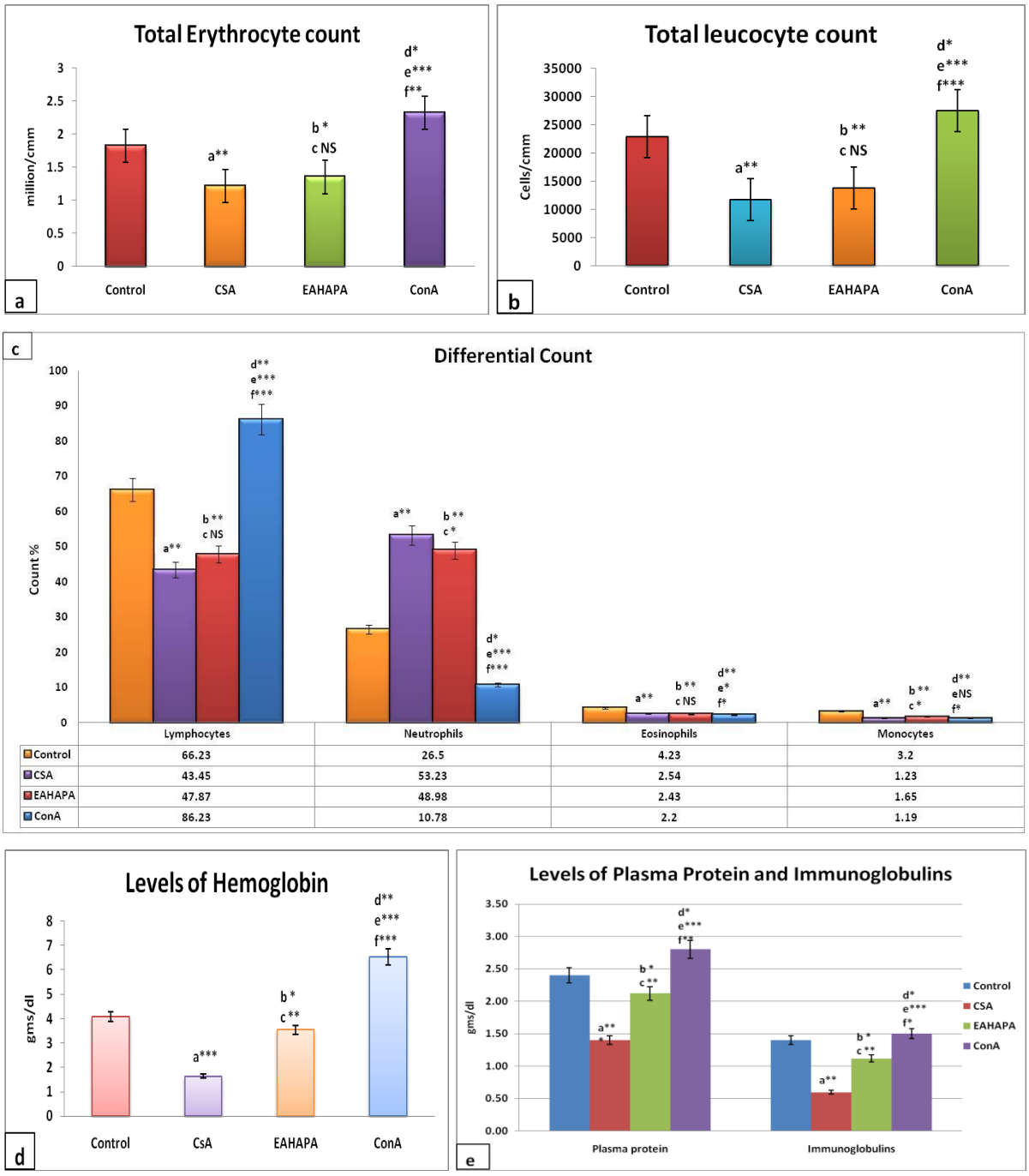

Figure 1a): Total Erythrocyte count b) Total Leucocyte count c) Differential count d) Levels of Hemoglobin e) Levels of Plasma protein and Immunoglobulins in Control and other treated fishes.

IMMUNOMODULATORY ASSAYS

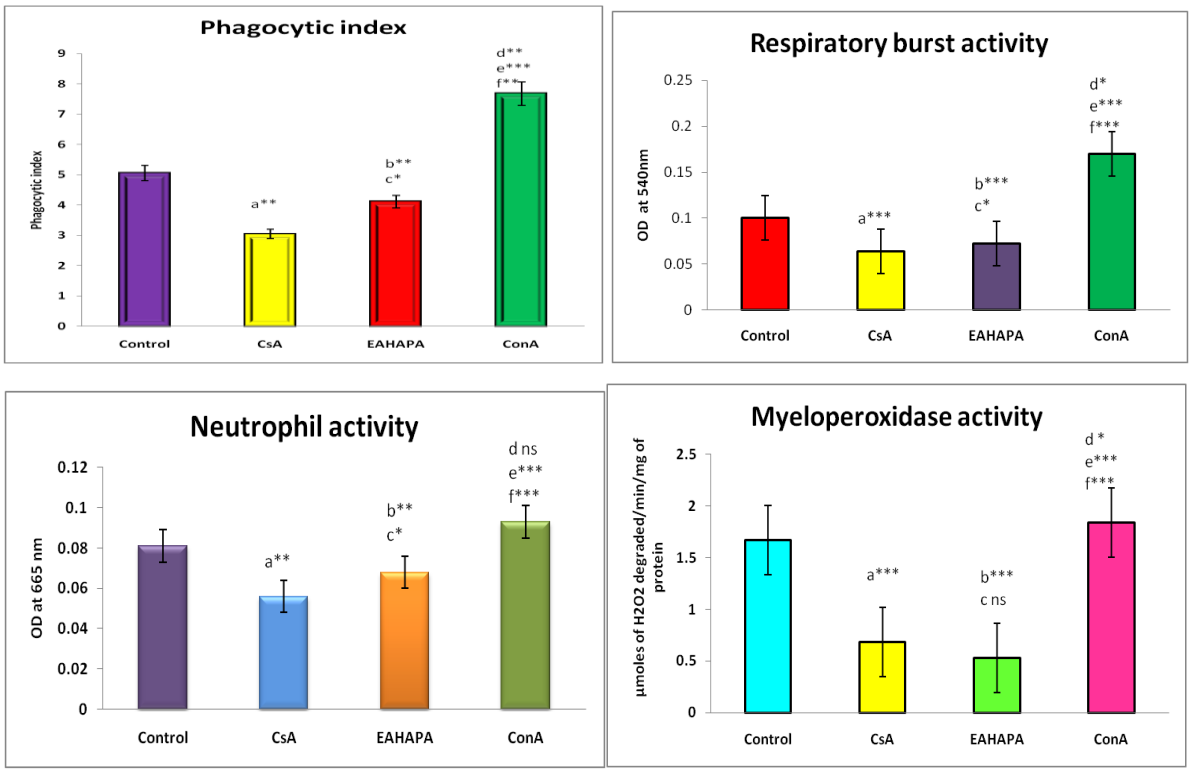

Figure 2a): Phagocytic index b) Respiratory burst activity c) Neutrophil activity d) Myeloperoxidase activity in Control and other treated fishes.

Statistical Analysis

Comparisons were made between a - Control and CsA, b - Control and EAPA, c- CsA and EAPA, d- Control and ConA, e- CsA and ConA, f- EAPA and ConA. Results are expressed as mean ± SE (n=6), *($p<0.05$)** ($p<0.01$))* ** ($p<0.001$) statistical significance difference between control and treated groups. One way ANOVA followed by student's t test using SPSS21 software package.

The hematological study to analyse total erythrocyte count (RBC) in the blood of Control fishes and other treated fishes of *C.auratus* were represented in **Figure 1a**. The study revealed abnormal levels of RBC in CsA and ConA induced fishes when compared to control fishes. A significant decrease ($p<0.01$) and significant increase ($p<0.05$) in the levels of RBC were observed in CsA and ConA induced animals respectively when compared with normal fishes. The levels of CsA and ConA were contrary and showed significant ($p<0.001$) variations. The levels of Ethyl acetate fraction of *Plectranthus amboincius* (EAHAPA) treated animals were also found to be non-significant with that of CsA induced animals and

significant variation ($p<0.01$) with ConA induced animals showing Immunosuppressive potential.

Figure 1b represents total Leucocyte count (WBC) in the blood of Control fishes and other treated fishes of *C.auratus* The study revealed significant decrease ($p<0.01$) and increase ($p<0.05$) in the levels of WBC in CsA and ConA induced animals respectively when compared to control group. The ConA induced animals showed very wide variation with CsA induced animals with notable variation ($p<0.001$) showing immunostimulatory nature. The levels of Ethyl acetate (EA) treated animals were found to be similar with that of CsA (p = ns) induced animals showing Immunosuppressive nature.

Differential count of Leucocytes in control and different treated fishes were represented in **Figure 1c**. CyclosporineA and ethylacetate treated group animals showed significant changes in Lymphocytes ($p<0.01$, $p<0.01$), neutrophils ($p<0.01$, $p<0.01$), eosinophils ($p<0.01$, $p<0.01$) and monocytes ($p<0.01$, $p<0.01$) when compared with control group animals respectively. ConA treated animals showed significant ($p<0.01$) variation with Control fishes in all cell types except Neutrophils ($p<0.05$). Lymphocyte and Neutrophils of ConA treated fishes showed wide variation ($p<0.001$) with CsA treated animals whereas eosinophils and Monocytes showed small variation ($p<0.05$) or no variation. Similarly, Lymphocyte and Neutrophils of ConA treated fishes showed wide variation ($p<0.001$) with EAHAPA treated animals whereas eosinophils and Monocytes showed small variation ($p<0.05$) with them. While, EAHAPA treated animals exhibited similar closely similar results (comparison between CsA and EA – p=ns; $p<0.05$) adding to the immunosuppressive nature.

The Levels of hemoglobin in different fishes including CyclosporineA, EAHAPA and ConA treated groups were presented in the **figure 1d**. Cyclosporine A has caused a significant decrease in the Hb concentration after 3 days of exposure ($p<0.001$), in comparison to the control fishes. Mild alterations ($p<0.05$) were observed in Ethylacetate treated groups when compared to control and significant difference ($p<0.01$) were observed when compared to CsA showing that CsA attributes to Immunosuppressive effect by affecting the Hb levels whereas EAHAPA produces the same effect without affecting the levels of Hb. ConA induced group animals showed wide variation ($p<0.01$) with control group, significant difference ($p<0.001$) with CsA and EAHAPA treated animals adding to the immunostimulatory nature of it.

The levels of Plasma protein and immunoglobulins in different treated groups were presented in the **figure 1e**. CyclosporineA treated fishes showed significantly reduced levels ($p< 0.001$ and $p<0.01$) of plasma protein and Ig when compared to control fishes. Plant extract treated groups showed significant variation ($p<0.01$) with CsA treated groups and very small variation ($p<0.05$) with normal fishes showing that it does not affect the plasma protein and immunoglobulin levels as compared with Cyclosporine despite its immunosuppressive effect. ConA treated fishes showed very small variation ($p<0.05$) with normal fishes, notable variations ($p<0.001$; $p<0.01$, $p<0.05$) with CsA and EAHAPA treated fishes respectively.

Figure 2a represents the phagocytic index levels assessed using carbon clearance rate in Control, CsA, Ethylacetate fraction and ConA treated animals. The CsA treated groups showed significant ($p<0.01$) decreased index when compared to control showing its immunosuppressive potential on phagocytic activity. EAHAPA showing closely similar results ($p<0.05$) with CsA and significant variation ($p<0.01$) with normal fishes adding on to its immunosuppressive credential. CsA and EAHAPA treated group animals showed significant adverse findings with ConA treated group which is an marked immunostimulatory agent ($p<0.001$ and $p<0.01$ respectively).

Figure 2b represents the Respiratory burst levels of Control, CsA , Ethylacetate fraction and ConA treated animals. The CsA treated groups showed significant ($p<0.001$) depleted activity when compared to control. EAHAPA showing similar results ($p<0.05$) with CsA adding to the immunosuppressive credential. CsA and EAHAPA treated group animals showed significant adverse findings with ConA treated group ($p<0.001$ and $p<0.001$ respectively).

Figure 2c represents the neutrophil activity levels of Control, CsA, Ethylacetate fraction and ConA treated animals. The CsA treated groups showed significant ($p<0.01$) depleted activity when compared to control. EAHAPA showing similar results ($p<0.05$) with CsA adding to the immunosuppressive credential. CsA and EAHAPA treated group animals showed significant adverse findings with ConA treated group ($p<0.001$ and $p<0.001$ respectively).

Figure 2d represents the levels of myeloperoxidase activity of Control, CsA, Ethylacetate fraction and ConA treated animals. The CsA treated groups showed significant ($p<0.001$) depleted activity when compared to control. EAHAPA showing similar or non-significant results with CsA adding to the immunosuppressive credential. CsA and EAHAPA treated

group animals showed significant adverse findings with ConA treated group ($p<0.001$ and $p<0.001$ respectively).

DISCUSSION

Blood is an indicator of physiological condition of an animal. Haematological parameters are used as a diagnostic tool by fish biologists and researchers throughout the world. This is so because fish are closely associated with the aquatic environment and the blood will reveal conditions within the body of the fish long before there is any outward manifestation of diseases. Several studies have confirmed that haematological parameters are important to the evaluation of fish physiological status. Blood can be used to study - changes in health status revealing lesions in other organs or tissues, to help breeding, understand infestations and infections or even environmental changes.

The dosage of plant fraction used (200mg/kg b.wt) was also found to be on par with other findings of **Koti and Aparna gore et al.,** and **Rajathi and Suja et al.**[27,28]

Our results were found to be similar to the findings of **Vani and Saharan et al.**,[29] who have also reported significant decrease in total **erythrocyte and leucocyte** count in Indian major carp, Catla catla fingerlings exposed to sub-lethal concentrations of cypermethrin ($1/10^{th}$ of LC50). The erythrocyte membrane contains abundant polyunsaturated fattyacids, which are common targets of oxidative damage by free radical-induced peroxidation. It has been recently reported that the exposure of red blood cells to by-products of lipid peroxidation results in a severe oxidative stress in the cell, which may eventually lead to hemolysis.[30]

Maryam Khaniyan and Negin Salamat et al.,[31] investigated the effects of benzo[a]pyrene (BaP) on immune status of orange spotted grouper (Epinephelus coioides). BaP concentration in the muscle of treated fish reached a maximum level after 4 days ($P < 0.05$) and exposure of fish to BaP resulted in a significant **decrease of total RBC and WBC**, **IgM level,** lysozyme activity, lysosomal membrane stability and antibacterial activity after 4 days and phagocytosis after 7 days of the experiment ($P < 0.05$).

The results of **Gui-Hong Zheng et al.,**[32] also showed that Ni (as Nickel sulfate) exposure to gold fishes, the major constituent of aquatic pollutants, significantly decreased the relative lymphocyte count (by 1–24%) and increased the relative count of monocytes (by 25–111%) and neutrophils (by 10–322%) as compared to controls. The findings of **Akinrotimi and**

Orlu et al.,[33] also revealed decreased levels of lymphocytes and increased levels of neutrophils ($p<0.05$) on exposure of *T.guineensis* to industrial effluents induced stress and have lead to changes in differential count of leucocytes in the blood.

Similar results were observed in previous investigations on Hemoglobin (Hb) by **Zikić and Stajn et al.,**[34] in *C.auratus*, carp fishes[35] and also in mammalian blood[36] during cadmium exposure. Hemolyzed and mild hemolyzed plasma were observed in CsA and EAHAPA treated fishes respectively which indicate destruction of erythrocytes, similar to the findings observed by **Zikić and Stajn et al.,**[34], where exposure to cadmium caused destruction of erythrocyte membranes and hemolysis.

Enis Yonar.,[37] assessed the effect of Lycopene on Oxytetracycline (OTC) induced oxidative stress and immunosuppression in rainbow trout (*Oncorhynchus mykiss*, W.). OTC also appeared to suppress specific and nonspecific immune system parameters, such as the haematocrit, **leucocyte count**, oxidative radical production (nitroblue tetrazolium activity), **total plasma protein, immunoglobulin levels and phagocytic activity** in trout fishes and Lycopene treatment (post and pre) alleviated the stress and immunosuppressive effects induced by OTC.

Phagocytic Index

Fish have several types of phagocytic leukocytes, which are present in blood, the peritoneal cavity, and a variety of tissue locations. Phagocytic functions appear to play a very important role in the protection of fish against pathogens.[38]

The effect of EAHAPA on the Reticulo endothelial system (RES) was evaluated using the carbon clearance test. RES mainly consists of phagocytic cells (macrophages), which specialized in the removal of foreign substances from the blood stream. When colloidal carbon particles in the form of ink are injected directly into the systemic circulation, the rate of clearance of carbon from the blood by macrophage is governed by an exponential equation.[39] Since EAHAPA on par with CsA down regulated the phagocytic index, it can be said that RES was suppressed by *Plectranthus amboinicus.*

Evaluation of immunosuppressive potential of Low fat buffalo milk supplemented with omega-3 fatty acids in mice was done by **Divya and Sathish et al.**[40] The **phagocytic activities** and **lymphocyte proliferation index** of omega-3 milk group showed significantly

reduced levels than control group after 4 and 8 weeks of treatment. The IgG level in serum also reduced after dietary supplementation showing the immunosuppressive nature of low fat buffalo milk similar to our findings.

Respiratory Burst Activity of Neutrophils

Macrophages play an important role in host protection against microorganisms and also present antigen to lymphocytes during the development of specific immunity. When activated, macrophages increase the phagocytic activity and release various materials such as cytokines and reactive intermediates and then carry out non-specific immune responses. Phagocytosis of particles by macrophages is usually accompanied by a burst of oxidative metabolism allowing the generation of reactive oxygen species which can be detected through an assay based on the reduction of NBT.[41]

The modulatory effect of **Chlorpyrifos** and abrogative role of Vit C in tissues of *Clarius batrachus* was evaluated by **Narra and Rajender et al.,**[42] and found that in addition to reduction in levels of RBC, Hb, total protein and Hepatosomatic index noticed, **respiratory burst** activity was also addressed by them similar to our findings in CsA and EAHAPA treated groups.

Neutrophil activity

Polymorpho nuclear leukocytes (neutrophils), derived from bone marrow, circulate in blood and play a vital role in host defense function as part of innate immunity. They are mobile, migrate from blood into tissue where they phagocytose, kill and digest foreign material. Along with macrophages, they also help in removing cell debris after tissue damage.[43-45] Neutrophils are readily activated by cytokines and other inflammatory mediators, accumulate in sinusoids, can extra vasate and attach distressed hepatocytes.[46]

The NBT assay is a quick inexpensive test focusing on the ability of phagocytes to reduce the dye by the production of oxygen radicals. In animals, the oxygen radicals are focused at the destruction of bacterial invaders. The lower optical density in the NBT assay was observed in our study showing the suppressed regulation of neutrophil activity denoted by NBT reduction showing down regulation of nonspecific immune system of the fish by EAHAPA on par with CyclosporineA. Conversely, Injection of *Ocimum sanctum* extract (20µg) into *Tilapia mossambicus* produced higher neutrophil activity[47] and also levamisole when administered

against *Aeromonas hydrophila* infection in Cyprinus carpio fingerlings, increased NBT activity was observed which narrated elevated response of immune system.[48]

Myeloperoxidase Activity

Sahoo and Kumari et al.,[49] recorded physiological normal range of some non-specific immune responses in juveniles of Indian major carps and found a wide variation among the individuals within *L. rohita* in the range of immune parameters studied including NBT activity in the blood.

The head kidney or pronephros has hematopoietic functions, and unlike in higher vertebrates, it is the immune organ involved in phagocytosis[50], antigen processing, production of IgM[51,52] and immune memory through melanomacrophagic centres.[53,54]

Myeloperoxidase, a heme protein secreted by neutrophils and macrophages, which uses the oxidizing potential of H_2O_2 to convert chloride ion into hypochlorous acid (HOCl). HOCl, potent bactericidal agent is a critical component of host defenses against invading bacteria, fungi and viruses.[55] The decrease in stimulation index in CsA and EAHAPA treated groups showed decreased defense capability to disease pathogens.

Aquatic contaminants and pollutants significantly alter the immune responses in fishes. Metal compounds when present are absorbed by fishes and passed up in the food chain and ultimately affect humans through bioaccumulation. Mercury, on exposure to fresh water fish *Channa punctatus Bloch*. was found to inhibit innate immune responses by significantly decreasing NO and Myeloperoxidase (MPO) production indicating intracellular damages[56], similar to the effects caused by CyclosporineA.

CONCLUSION

Unraveling new Immunosuppressants from the herbal kingdom is the key concept of scientists to knock out many serious health ailments. CyclosporineA, even being a standard immunosuppressant, showed toxic and damaging effects on animals adding to adverse or side effects. The Hematological profile analysis including erythrocytes count, leucocyte count, differential analysis and levels of hemoglobin revealed the immunosuppressive effect of plant fraction on par with that of CyclosporineA. The notable findings from Immunomodulatory assays – phagocytic index, Myeloperoxidase activity, respiratory burst and neutrophil activities also prove the suppression of immune response by ethylacetate fraction of plant

extract targeting the non specific and innate immune defense system of the animal. Thus, EAHAPA along with non-toxic nature causes significant suppression of immune system proving its immunosuppressive nature. Further insights into mechanism of immunosuppressive activity of *Plectranthus amboinicus* at molecular level are required to employ the plant for treatment of many hypersensitive reactions and autoimmune disorders.

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