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ORIGINAL ARTICLE

EFFECT OF *CYNODON DACTYLON* ON THE HEAMATOLOGICAL PARAMETERS IN THE BLOOD OF *OREOCHROMIS MOSSAMBICUS*

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ABSTRACT

The present study is aimed to investigate *Cynodon dactylon* activity on the haematological parameters in the blood of *Oreochromis mossambicus*. In the present study, the Red blood cells(RBC), White blood cells(WBC), Haemoglobin (Hb), Hamatocrit (Ht), Mean Cell Haemoglobi (MCH) and Mean Cell Heamoglobin Concentration (MCHC) were observed in the blood of *Oreochromis mossambicus* treated with *Oreochromis mossambicus* extract. The present study showed the RBC counts, WBC counts,Hb, Ht, MCH and MCHC were significantly increased when compared with control fish.

Keywords: *Cynodon dactylon, Oreochromis mossambicus, Haematological Study*

1.INTRODUCTION

India is a biodiversity nation and it has a rich background in medicinal herbs, most of which have been used to treat human and animal diseases. Aquaculture is a fast developing industry in a India. Fish farming and aquaculture industries and also has essential amino acids with minerals like zinc, magnesium, sodium, etc. Development of aquaculture is mainly depended on availability of compatible and suitable diets.

Many of medicinal herbs and their chemical components are used as an immunostimulants which are used in artificial diet preparation, aquaculture research and their practices. Many of the herbal plants have the ability to inhibit the microbial pathogens and activate the immunity (Immanuel *et al* ., 2004; Chansue *et al* ., 2000: Dugenci *et al* .,2003. Immunostimulation is an alternative effective method against vaccination. It may be achieved through only feed supplement. Several ayurvedic medicinal plants are acting as a powerful immunomodulators. Now a -days, supplemental treatment is popular for preventing the diseases in aquative animals. Moreover, they are cheaper, safer and biocompatible. Respiraatory burst activity of phagocytic cells and plasma lysozyme ativity have been significantly increased in Common carp (*Cyprinus carpio*) and large yellow croaker (*Pseudosciena crocea*) after feeding with *Astragalus membranaceus* and *Angelica sinensis* mixed diet (Jian and Wu.2003,2004)Medicinal plants are progressively being estimated as appropriate alternative sources of antibacterial and antiviral agents. The extract of plant *C. dactylon* was found to be highly effective in preventing the growth of *A. hydrophila* infection in *C. catla*. Similarly, *C. dactylon* acts as an antibacterial agent against several pathogenic bacteria (Kaleeswaran *et al* ., 2010).

Cynodon dactylon (L) Pers (Gramineae, Poaceae) is a herbal plant commonly known as 'Arugampul' in Tamil Nadu, India, which is treated as a blessing plant. This grass is widely distributed in India and in almost all parts of the world. Traditionally, the juice of this plant is commonly consumed as a health drink during early morning in south India. It takes an important role in Ayurvedic medicine. *C. dactylon* extract is used to treat hysteria, epilepsy and insanity. Traditional healers used *C. datylon* for purifying blood, anuria, biliousness, conjunctivitis, diarrhea, gonorrhea, itches and stomachache (Muthu *et al* .,2006). The present study evaluates the effect of dietary administration of *C. datylon* ethanolic extract on the potential recovery in *Oreochromis mossambicus*, which is associated with and histological and haematological changes.

THERAPEUTIC USES

The species possesses immense medicinal value and may be applied both externally as well as internally. Being haemostatic refrigerant, healer and beneficial for skin complexion externally it is used in wounds, heamorrhages

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,burning sensation (like urticaria erysipelas) and dispigmentation of skin leaf paste is applied in traumatic wounds and piles, fresh juice of the plant is installed into eyes for catarrhal conditions and when used as nasal drops controls nasal bleeding (Durva, 2011). The plant extract checks uterine bleeding strengthens the uterus averts abortion and augments of foetal growth3 the species is also used in traditional cultures for toothache treat kidney stones. Extract of the whole plants shows antiviral activity against vaccinia virus, whit e spot syndrome virus (Balasubramanian *et al* ., 2008). The grass is reported to be highly nutritional for cattle (Burton,2011). It can also be used as dietary supplements for Carp (Kaleeswaran et al., 2012 ;Immanuel,2001).

Remediation of metal contaminants is an emerging field in the broad area of environmental bio-geo-technology. plant based remedies have always been an integral part of tradition medicine throughout the world. The increasing demand for herbal medicines in recent years due to their fewer side effects in comparison to synthetic drugs and antibiotics, has highlighted the need for conservation and propagation of medicinal plants. The health benefits of a plant based diet are immense. It can prevent, retard or even reverse many life threatening disease processes (Gurumoorthy, 2005).

Medicinal plants have been used in various traditional systems, as they have immune potential against numerous diseases (Kottai Muthu *et al*., 2005).Remediation through plant materials would be cheaper, cost effective and eco-friendly with no deleterious effects. Medicinal plants have been in various traditional systems, as they have immune potential against numerous diseases. Several naturally occurring dietary or non-dietary constituents as well as parts of several species of edible plants having pharmacological activity, influence the antioxidant enzymes and provide protection against free radical induced damage. Plants are a rich source of phytochemicals.

Blood plays a decisive role in the regulation of life processes to make them function properly. An organism must be able to keep its blood composition relatively constant under normal conditions and must also have the ability to change it under extreme conditions such as stress situations. Changes occurring in the haematological characteristic of fishes provide a sensitive measure to assess the health of fish fauna. Further, the fish blood is a valuable diagnostic tool for the investigation of diseases and physiological or metabolic alterations. Haematological techniques including measurements of haematocrit, white Blood Corpuscles, Red Blood Corpuscles and Haemoglobin have proved valuable for fishery biologists in assessing the health status of fish and monitoring stress response.

2.MATERIALS AND METHODS

Procurement and rearing of experimental fishes.

Oreochromis missambicus commonly called African Mouth breeder is widely distributed in the freshwater of India. *Oreochromis mossambicus* was collected from the fish farm located in puthur, 40 km away from the Periyar Arts College, Cuddalore-1. The collected fishes without least disturbance were transported in polythene bags filled half with water. About 50 fishes were put in each bag and water was well aerated, using pressurized air from a cylinder. This mode of transit proved successful, since there was no mortality in all consignments throughout the course of this study.

The fishes brought to the laboratory were acclimatized in fibre aquarium for a fortnight before they were used for the experiment. The fish tanks were kept free the fungal infection by washing with potassium permanganate solution. The fish were disinfected with 0.1% potassium permanganate solution and were maintained for three weeks in well aerated tap water. Test stress, physical damage and mortality. The injured, severely diseased, abnormal and dead individuals were discarded. Feeding was discontinued two days prior to the commencement of the experiments to reduce the additive effects of animal excreta in the test through (Arora *et al* ., 1972). The fishes were exposed to plant extract and control for the period of 15 and 30 days. A control group was maintained with identical environment. The plant extract with water and normal water was renewed every day. The fish were sacrificed from both experimental and control groups on 15and 30 days of exposure periods.

Preparation of Fish Feed

For the present study, two different types of feeds were prepared following Hardy's Square Method (1980)

I. Control feed - Feed 1
II. *Cynodon dactylon* plant extract mixed feed - Feed 2

The control feed was a standard based diet. It was prepared as a mixture of Rice bran (6.30%), Tapioca flour (21.30%), Groundnut oil cake (13.50%), Wheat flour (15.60%), Corn flour (15.20%), Rice pori (4.20%), Soya meal (22.70%), Vitamin and mineral mix (1.10%). The proximate composition of all the dried, powdered ingredients

Feed pellets were prepared by following the methods of Bindu and Sobha (2004). Appropriate quantities of finely powdered ingredients were weighed and mixed thoroughly by adding water. The dough thus prepared was stem cooked for 30 min in a pressure cooker. The cooled dough was fortified with vitamin and mineral mix 1% and was palletized using a hand pelletizer. The extruded pellets were dried overnight in a hot air oven at 60^{o}C. Proximate analysis of diets was carried out using standard methods (AOAC, 1990). (Table 1.).

PLANT EXTRACT PREPARATION

Collection of Plant Material

One medicinal plant that are described in traditional Indian medicine were chosen in this study in consultation with a practitioner of traditional Sidda medicine in the Union territory of Pondicherry. The availability of the plant material in sufficient amounts was also considered in chosen them. The one plant chosen were *Cynodon dactylon*

(Bermuda grass). The plants powder collected from the K.S.R. HERBAL PRODUCTS 2, Sanakkiya Nagar, Manjakuppam, Cuddalore **(MI. NO. 330181100346E)**. The plants materials thus obtained were shade dried and powdered. The powdered material was stored in air tight containers.

Preparation of Extracts

The plant material was extracted with four solvents with different polarities Starting with Hexane (Polarity index 0) followed by **Chloroform** 50ml (Polarity index 4.1), **Ethyl Acetate** 50ml (Polarity index 4.4) and **Methanol** 50ml (Polarity index 5.1),and 250ml of distilled water added. The one plant materials (100gm) were extracted sequentially with 1:1:1 solvent to dry weight ration for 24 hours on a shaker at 200 r.pm. The extracts were then filtered through a Whatman filter paper and the filtrate collected in glass beakers.The plant material was then re-extracted several times for maximum efficiency. The filtrates were dried in a Rotor Vapor Aspirator by applying vacuum and the solvents recovered were reused for extraction. The extracts were dried further by keeping them in vacuum desiccators. They were then stored in airtight containers at 4^{o} C and used for further analyses.

Experimental Procedure

Oreochromis mossambicus weighing 20-25g were divided in to 3 groups and stocked at random into 3 different concrete tanks each tank was assigned a specific type of feed.

The following experimental groups were conducted in the freshwater fish *Oreochromis mossambicus* for the period of 15 and 30 days.

Group I - Fish exposed t tap water and fed with feed -I (Control feed1)

Group II - Fish exposed to tap water and fed with feed 2 (*Cynodon dactylon* extract mixed for 15 days)

Group III - Fish exposed to tap water and fed with feed 2 (*Cynodon dactylon* extract mixed for 30 days)

Hematological Studies

Collection of blood

Blood samples were collected from the control and experimental fish in the ductus cuvier with the help of 24 gauge needle and stores in heparinized glass tube. The haematological Parameters viz., Total Red Blood Corpuscles (RBC), White Blood Corpuscles (WBC), Haemoglobin (Hb), haematocrit (Ht), Mean Cell Haemoglobin(MCH) and Mean Cell Haemoglobin Concentration (MCHC) Were determined by adopting the method of Daecie and Lewis (1984).

Enumeration of Red Blood Corpuscles (RBC)

Blood samples were slowly sucked up by means of the Haemocytometer pipette till the mark 0.5 is reached (marked 0.5: 1.0 and 101). Then the diluting fluid was sucked as far as the mark 101. This produced a dilution of 1 in 200. While this was being done, the pipette was gently rotated so as to start the mixing. The pipette was firmly seized by its ends between the forefinger and thumb and shaken thoroughly for about one minute. The finger was then removed from the pipette and the diluting fluid in the capillary tube blown out. After a few drops of the diluted blood have shaken out, a small drop was transferred to the counting slide.For enumeration of red blood cells at least five sets of sixteen squares were counted. The squares in each set should be gone over systematically in horizontal rows of four at a time. Only those on the upper and on the left- hand lines were counted.

Calculation:

$$\text{Number of RBC/Cu.mm} = \frac{\text{Total no of corpuscles counted}}{\text{Total no of small squares counted}} \times \text{dilution} \times 10$$

Enumeration of White Blood Corpuscles (WBC)

The total WBC count was made with Haemocytometer's Neubuer counting chamber. WBC was counted from the control and treated fish. The blood samples were drawn up to the 0.5 mark in WBC pipette and diluted upto the mark 11 with diluting fluid (Turk's fluid=Gention violet, glacial acetic acid 3ml and distilled water 97ml). This produced a dilution of 1 in 20. The remaining procedures were as the same as above for the RBC counting.For enumeration of leucocytes four sets of sixteen squares were counted out of nine squares. Insteade of going over the squares in rows of four, whole set of a sixteen can easily be counted at one time.

Calculation

$$\text{Number of WBC/Cu mm} = \frac{\text{Total no of leucocytes counted}}{\text{Total no of large square counted}} \times \text{dilution} \times 10$$

Estimation of Haemoglobin (Hb) content in the blood

Heamoglobin content in the blood was estimated using sahli's Haemometer (Superior, Germany) with permanent glass comparison standards and expressed in gm Hb/100ml blood.

Determination of Haematocrit Value (Ht or packed cell Volume)

Haematocrit value of blood was estimated by centrifuging blood in heparinzied haematocrit tubes (Germany) at 7000 rpm/ min for 30 minutes. From the valume of blood taken and packed cell volume after centrifugation haemtocrit per cent was calculated.

Mean corpuscular haemoglobin (MCH)

The Mean Corpuscular Haemoglobin (MCH) content was computed from the calues of haemoglobin content and erythrocyte count using the formula and espressed as pictograms.

$$MCH = \frac{\text{Hb (gm/100ml)} \times 10}{\text{Erythrocyte count (million cells/cu mm blood)}}$$

Estimation of Mean Cell Haemoglobin Concentration (MCHC)

Estimation of mean cell Haemoglobin Concentration (MCHC) was computed from the values of haemoglobin and the haematocrit percentage using the formula and expressed as percentage.

$$MCHC = \frac{\text{Haemoglobin (gm/100ml)}}{\text{Haematocrit}} \times 100$$

Statistical Analysis

The values are expressed as mean ± SE. Data were statistically analysed by Analysis of Variance (ANOVA) along with Duncan's Multiple Range Test (DMRT) (Duncan, 1955) which was applied to find out significant difference between various treatment means and control means for the observed parameters.

3.RESULTS

Heamatological Study

Red blood corpuscles (RBC) in the blood

The RBC count of normal blood of fish feed with feed 1 (Group I) when compared to *Cyanodon dactylon*extract was mixed with feed 2 (Group II& III). The percent decrease over the control was 2.19 and 9.34 for 15 and 30 days respectively. There was significant changes in the fish feed with feed 2 (Group II& III) when compared to feed 1 (Group I) for 15 and 30 days of exposure (Fig.1).

White blood corpuscles (WBC) in the blood

The WBC count of normal blood of fish feed with feed 1 (Group I) when compared to *Cyanodon dactylon*extract was mixed with feed 2 (Group II& III). The percent decrease over the control was 3.65 and 11.02 for 15 and 30 days respectively. There was significant changes in the fish feed with feed 2 (Group II& III) when compared to feed 1 (Group I) for 15 and 30 days of exposure(Fig.2).

Haemoglobin(Hb) in the blood

The Hb of normal blood of fish feed with feed 1 (Group I) when compared to *Cyanodon dactylon*e xtract was mixed with feed 2 (Group II& III). The percent decrease over the control was 7.35 and 14.54 for 15 and 30 days respectively. There was significant changes in the fish feed with feed 2 (Group II& III) when compared to feed 1 (Group I) for 15 and 30 days of exposure(Fig.3).

Fig. 1. Red blood corpuscles counts in the blood of control and experimental fish, *Oreochromis mossambicus*

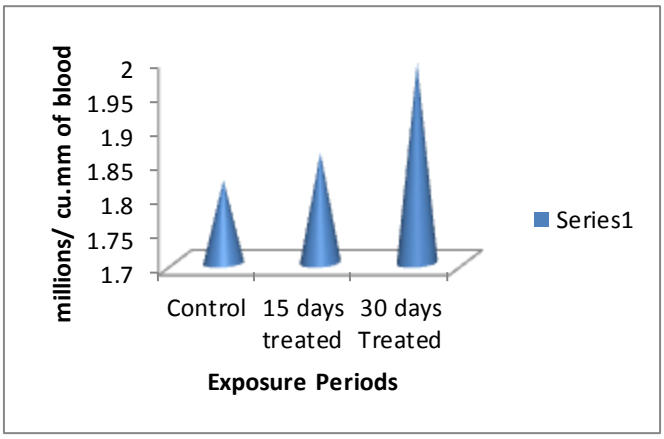


Fig.2. White blood corpuscles counts in the blood of control and experimental fish, *Oreochromis mossambicus*

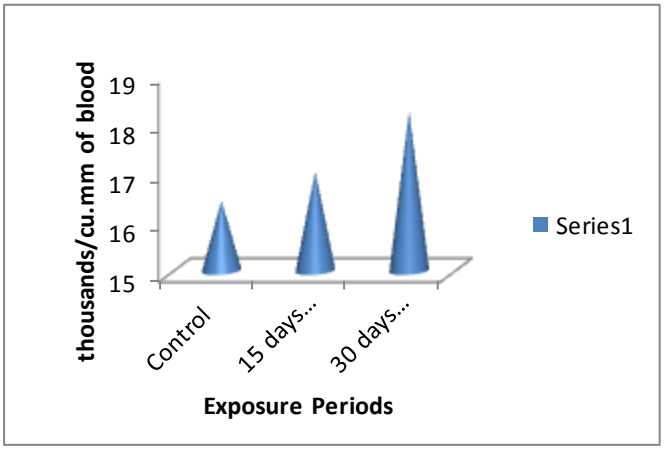
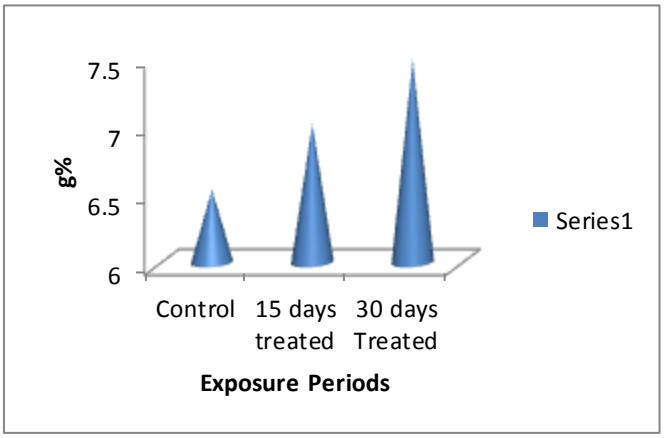


Fig. 3. Haemoglobin level in the blood of control and experimental fish, *Oreochromis mossambicus*



Haematocrit (Ht) in the blood

The Ht of normal blood of fish feed with feed 1 (Group I) when compared to *Cyanodon dactylon* extract was mixed with feed 2 (Group II& III). The percent decrease over the control was 1.59 and 4.63 for 15 and 30 days respectively. There was significant changes in the fish feed with feed 2 (Group II& III) when compared to feed 1 (Group I) for 15 and 30 days of exposure(Fig.4).

Fig.4. Haematocrit level in the blood of control and experimental fish, *Oreochromis mossambicus*

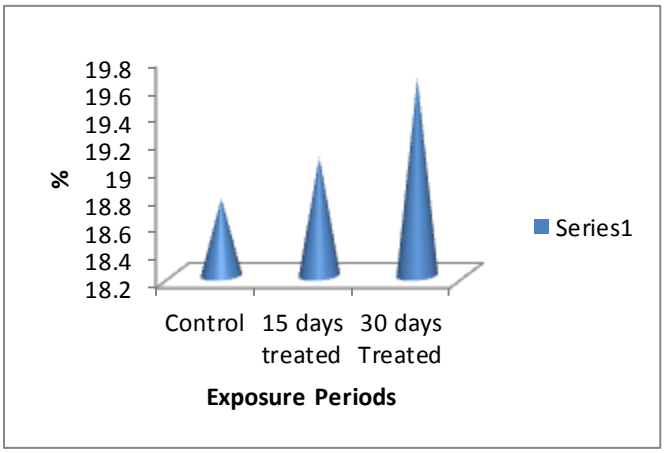


Fig.5. Mean cell haemoglobin level in the blood of control and experimental fish, *Oreochromis mossambicus*

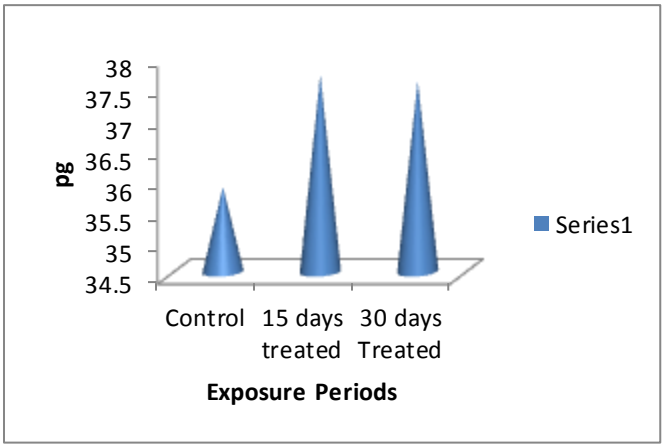
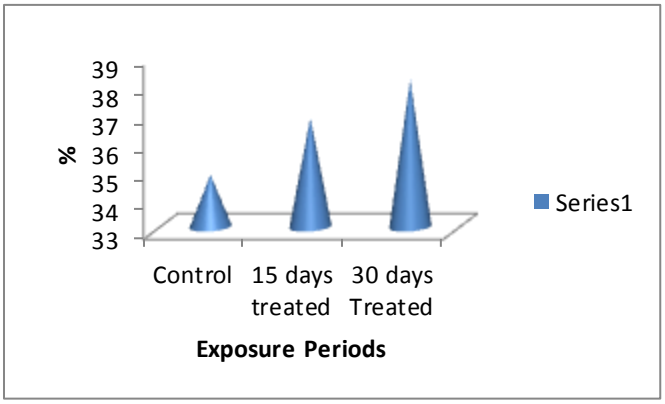


Fig.6. Mean cell haemoglobin concentration level in the blood of control and experimental fish, *Oreochromis mossambicus*



Mean Cell Haemoglobin(MCH) in the blood

The MCH of normal blood of fish feed with feed 1 (Group I) when compared to *Cyanodon dactylon* extract was mixed with feed 2 (Group II& III). The percent decrease over the control was 5.04 and 4.76 for 15 and 30 days respectively. There was significant changes in the fish feed with feed 2 (Group II& III) when compared to feed 1 (Group I) for 15 and 30 days of exposure

Mean Cell Haemoglobin Concentration (MCHC) in the blood

The MCHC of normal blood of fish feed with feed 1 (Group I) when compared to *Cyanodon dactylon* extract was mixed with feed 2 (Group II& III). The percent decrease over the control was 5.66 and 9.48 for 15 and 30 days respectively. There was significant changes in the fish feed with feed 2 (Group II& III) when compared to feed 1 (Group I) for 15 and 30 days of exposure

4.DISCUSSION

Haematological Study

Blood acts as an internal transport and plays a significant role in the regulation of life activities. It may be described as a specialized fluid connective tissue in which the components are suspended. Blood is responsible for transporting oxygen, carbon dioxide, nutrient, food and hormone and also it involves in the production of antibodies. Blood plays a decisive role in the regulation of life processes to make them function properly. An organism must be able to keep its blood composition relatively constant under normal conditions and must also have the ability to change it under extreme conditions such as stress situations. The MCV, MCH and MCHC are corpuscular indices that have particular importance in most animals in describing anemias and can be used in diagnosis and therapy (Coles, 1992). The PCV readings are valuable in determining the effect of stressors on the health of fish and are also used to determine the oxygen carrying capacity of blood (Larsson *et al* ., 1985). The low PCV would indicate anaemia or oligohaemia (Wepener *et al* ., 1992).

In the present study *Oreochromis mossambicus* exposed to *Cyanodon dactylon* extract fed with feed 2 (Group II & III)) shows a significant increase in the Red Blood Corpuscles (RBC), Haemoglobin (Hb), Haematocrit (Ht), Mean Cell Haemoglobin (MCH),Mean Cell Haemoglobin Concentration (MCHC) whereas the white Blood Corpuscles (WBC) increase for 15 and 30 days of exposure periods. Sastry and Sachdeva (1994) have reported decreased haemoglobin, haematocrit level and erythrocyte count in *channa punctatus* after exposure to either copper alone or copper and cadmium for 15 and 30 days. Increasing levels of erythrocyte and haemoglobin content were observed in *A. hydrophila* infected *C. carpio* treated with *Azadiracta indica* (Harikrishnan *et al* ., 2003). During the experimental period, the WBC count also increased in the experimental diet groups compared to the control. This result was supported by the study of Sahoo and Mukherjee (1999) who found that WBC count was increased in rohu (*L. rohita*) fingerlings treated with immunostimulants such as levamisole and ascorbic acid. Sharma *et al* . (2005) have reported that spirulina feed improved toleration of fish towards methylred which may be ascribed to their better health an increase in RBC content.

Harikrishnan *et al*., (2003) reported that the common carp, Cyprinus carpio was treated with an aqueous Azadirachta

indica leaf extract the hematological such as the white blood cell (WBCs: 10^4 mm^{-3}) counts significantly increased from 3.15±0.14 in control fish to 3.6±0.20 on the 10th day of treatment ($P<0.01$) and only in treated fish ($P>0.05$) on the 30th day. The red blood cell (RBC: 10^6 mm^{-3}) counts also significantly decreased to 1.68±0.12 on the 10th day ($P<0.001$) when compared to the control. The hemoglobin (Hb) and hematocrit/packed cell volume (PCV) counts decreased significantly on the 10th day and this value attained a normal level on the 30th day.It is clear that (*Cyanodon dactylon*; Arugamkpul mixed feed 2 (Group II & III) might possess the protective role against chemical induced haematological changes. There is significant effect in the haematological parameters of the control fish feed 2 (Group II & III) when compared to control fish feed 1 (Group I) with feed 1 (Group I). Thus feed 2 mixed feed) (Group II & III) Cyanodon dactylon extract have been found to be effective against lead induced haematotoxicity. The prophylactic effect of dietary garlic application to rainbow trout, infected with *Aeromonas hydrophila*, was confirmed by Nya and Austin (2011). Thanikachalam et.al., (2010) showed that the embedding of garlic peel in feed enhances the hematological parameters even at a low level (0.5%) incorporation and makes *Clarias gariepinus*, fingerlings, higly immunopotent and more resistant to infection by *A. hydrophila*(. Harikrishnan *et al* . 2003).

Kaleeswaran *et al* ., (2012) have reported that the RBC, WBC count increased in 0.5% and 5% concentration of *C. dactylon* mixed diet. Other erythrocyte indices also increased significantly in the experimental diet group of 0.5% and 5% than the 0.05% and control diet group in all experimental period of post immunization. Scott and Rogers (1981) showed a significant increase of hemoglobin leading to elevated oxygen carrying capacity of the individual erythrocyte *I. punctatus*. Yin *et al* .(2006) reported that oral administration of ginger (*Zingiber oficinale*) extract increases the phagocytic capability of cells in rainbow trout (fish), ehile the extract of 4 chinese herbs (*Rheum officinale Andrographis paniculata, Isatis indigotica and Lonicera japonica*) increased the phagocytosis of white blood cells of carp. The prophylactic effect of dietary garlic application to rainbow trout, infected with *Aeromonas hydophila*, was confirmed by Nya and Austin (2011). Thanikachalam *et al* ., (2010) showed that the embedding of garlic peel in feed enhances the hematological parameters even at a low level (0.5%) incorporation and makes Clarias gariepinus, fingerlings, highly immunopotent and more resistant to infection by *A. hydrophila*.

The ethanolic extract of plant *Cynodon dacylon* is very effective immunostimulant in catla catla against A. hydrophila infection. This plan extract could develop or induce the specific antibody in fish against the antigen, especially at the higher (5%) concentration. Chakrabarti and Vasudeva (2006) also stated that prophylactic treatment of *Achyranthes aspera* significantly enhanced the specific antibody response and antigen clearance against BSA. Similarly, Spirulina significantly enhanced the antibody titers to keyhole limpet haemocyanin (KLH) in channel catfish,*I. punctatus*. Supplementation feeds responsible for returning haematological and biochemical parameters to hear normal values and triggering the immune system of the specific and innate immunity of goldfish against *A. hydrophila* when treated with 400 mg/kg or 800 mg/kg of mixed herbal supplementation feeds undoubtedly, and, indicated that the ethanol of triherbal solvent extract seems to be a better immunostimulany, which can have a promising role in aquaculture to prevent diseases and infectious outbreaks (Harikrishnan *et al* ., 2010). Sahu *et al* . (2007) reported that long term dietary administration of mango kernel led to considerably increases immunity and survival of fingerlings of rohu. The group fed with 5 g kernel/kg dry diet showed highest percentage survival (98%). Results indicated that mango kernel stimulates the immunity and makes *Labeo rohita* more resistant to *A. hydrophila*. The total erythrocyte count and haemoglobin level increased from 10 to 30 days of feeding in all the experimental diet groups than in the control. On the other hand, no modulation was observed in the erythrocyte and haemoglobin content of both normal and yeast RNA w-3 fatty acid b carotene treated *C. carpio* treated with *Azadiracta indica* (Harikrishnan *et al* ., 2003). The decreased haemoglobin content may be the result of the swelling of RBC as well as por mobilization of haemoglobin from the spleen and other haemopoietic organs in *Ictalurus punctatus* (Scott and Rogers, 1981). Mukesh *et al* ., (2012) reported that the herbal extracts and animal originated product have a potential application as an immunostimulant in fish culture, primarily because they can be easily obtained, are not expensive and act against a broad spectrum of pathogens. Most of the herbs and herbal extracts can be given orally, which is the most convenient method of immunostimulation. However, the effect is dose-dependent, and there is always a potential for overdosing consequently, dosage optimization is strongly recommended. The use of such plant products as immunostimulants in fish culture systems may also be of environmental value because of their biodegradability.

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