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Hematological assessment of fish *Channa punctatus* (bloch) by feeding the medicinal herb *Arnebia benthamii*

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Abstract
Due to worthy status of blood in clinical pathology, hematological parameters are used as catalog to detect physiological changes following different stress conditions. The present study assessed the effect of herbal medicinal plant Arnebia benthamii of family 'Boraginaceae' on the hematological {(Total leucocyte count (TLC), Total erythrocyte count (TEC), hemoglobin level (HB)} and serum biochemical parameters {(serum glutamate oxaloacetate transaminase (SGOT), serum glutamate pyruvate transaminase (SGPT) and blood glucose)} of fish which was observed at 14 and 28 days of feedings. This investigational trial effect of A. Benthamii on hematological parameters in Channa Punctatus was conducted by feeding the various levels of A. Benthamii as control S0, S1 (0.1%), S2 (0.2%), S3 (0.4%), S4 (0.8%) and S5 (1.6%) for a period of 14 and 28 days. Fish fed with A. Benthamii showed significant enhancement in (TEC), (TLC), (Hb), and also major boost in serum biochemical parameters. Thus, A. Benthamii can be used as a potential hematological enhancer in aquaculture.

Keywords: arnebia benthamii, channa punctatus, hematological assessment, erythrocyte, leucocyte, hemoglobin

Introduction

For improvement of performance and to maintain fish health, herbal plants have been castoff as dietary essences to improve, feed efficiency, weight gain, and disease resistance in cultured fish. The aquaculture sector has revealed a great development in the course of the most recent 30 years with related rise in infection issues as consequence of fast extension and among different factors high stocking densities. Although natural cures have been with us for human treatment from times but there has been moderately little examination on the therapeutic plants to be utilized against fish wellbeing.

Natural plants can be utilized as cures as well as development advertisers, stress obstruction supporters and safeguards of diseases.

Consequently, herbal plants in disease management are gaining success, since they are commercially expertise, eco-accommodating and have negligible symptoms. especially in developing countries relies upon the customary arrangement of medication for an assortment of illnesses. A few hundred genera are utilized restoratively and plants are vital sources for potent and powerful drugs.

Plants are well-off in an extremely wide range of secondary metabolites of phytochemical constituents like tannins, terpenoids, alkaloids and flavonoids, that act against completely different diseases (Pandey, *et al.*, 2010; Ravikumar, *et al.*, 2010). As of late, numerous manufactured and herbal immunostimulants have been accounted for to improve the insusceptible status of fish by upgrading the phagocytic, lysozyme and complement activities and also the immunoglobulin level in contradiction of a number of causative boundaries. (Siwicki AK 1989). A large number of plants have been used in traditional medicine for the treatment and control of many diseases. (Duke JA 1987) [8]. The utilization of regular plant items has been accounted for as anti-stress, for development advancement, appetite stimulation, tonic and immune stimulation, and to have antimicrobial properties in finfish and shrimp larviculture because of the presence of dynamic standard segments, for example, alkaloids, flavonoids, pigments, phenolics, terpenoids, steroids, and basic oils. (Citarasu T, *et al.*, 2001) [5].

Herbal goods are low-priced source for therapeutics and have better accuracy compared with chemotherapeutic agents, and offer a feasible solution for all the problems which aquaculture faces today. Bioactive compounds are present in numerous medicinal plants such as, *Acalypha indica, Phyllanthus niruri, Azadirachta indica, Piper bettle, Mentha piperita*. (Ardo L, *et al.*, 2008) [2]. *Allium sativum* and *Astragulus membraneceus.* (Jegede T. 2012) [11] reportedly enhance growth, innate immune response, and disease resistance against pathogenic bacteria in fish (Zahran E, et al., 2014) [22]. To our knowledge, at present there are limited number of studies on the use of medicinal herbs in aquaculture; these studies briefly implied that, herbal extracts could be certainly possible alternative to artificial antibiotics and other chemotherapeutic drugs in fish culture.

Arnebia benthamii or "Kahzaban", is being used over the years, this medicinal herb grows in the hilly zones of North West Himalaya (Dar et al 2002; Dar and Khuroo 2013) [6, 7]. The marketable drug Gaozaban, has major composition of this species alone which has anti-inflammatory, antibacterial, antifungal, and wound-healing properties (Kaul 1997). With its anti-pyretic properties, it is also used in the treatment of heart problems. (Dar and Khuroo 2013; Ganie *et al.*, 2012) [7, 9].

Therefore, in view of above pharmaceutical properties of *Arnebia benthamii* this study attempted to investigate the effect of this herb on hematological and serum biochemical parameters of fish *Channa punctatus.*

Materials and methods

Disease free mature *Channa Punctatus* having average weight (19.33 g) were procured from Fisher man Amravati Maharashtra. The fishs were acclimatized for 15 days in laboratory condition in 50 L tanks with continuous aeration. Fishes were fed with commercially available diet 3% of body weight twice a day.

Experimental diets

The method of extraction was followed as described by Ajaiyeoba and Fadare 2006. The air-dried herb leaves were grounded with a hammer mill and 200 g of fine powder was soaked in 100 ml of 80% methanol for 72 hours. Herb leaves were properly mixed with methanol and filtered using a sterile muslin cloth after which the extract obtained were air dried and stored at 25 °C until required. Ingredients and formulation of the basal ration was followed as described by Boonyaratpalin 1993. The basal diet contained 50% protein; 9% lipid; 15% ash; 7% moisture and 10% fibre. Test diets were prepared separately and mixed to the feeds in the concentration of 100 (S1), 200 (S2), 400 (S3) 800 (S4) and 1600 (S5) mg/ kg diets. A control diet (S0), lacking of herbal product was also prepared. To prepare the diets, firstly, the ingredients were mixed systematically and 4% gelatin solution containing active principles with suitable concentration was added along with oil ingredients. Sufficient water was added and the pH was adjusted 7 with saturated sodium hydroxide and mixed thoroughly using a mixer for 15 min. The resulting dough was cold extruded through a palletizer with appropriate size. The capsules were dried and kept at room temperature in air close-fitting containers.

Experimental design

The experiment was done in rectangular plastic tubs of 50 L capacity sheltered with pricked lids and the water used for rearing was taken from tap water. The acclimatized fishes were casually circulated into five different experimental groups including control. Fifteen fish were kept in each tub. The experimental trial for effects of *A. Benthamii* on hematological parameters in *Channa Punctatus* was conducted by feeding the various levels of *A. Benthamii* control S0 (*A. Benthamii*), S1 (0.1%), S2 (0.2%), S3 (0.4%), S4 (0.8%) and S5 (1.6%) for a period of total 28 days. The fish were fed with the experimental diet at the rate of 3% of their bodyweight three time a day at 09:00, 13:00 and 18:00 h to estimated satiation for 28 days.

All physico-chemical parameters of water such as oxygen, temperature, and pH. were maintained in optimal range during the experimental period. Total fifteen fishes from each treatment were tested and blood was drawn on 14th and 28th days for various hematological analyses.

Collection of blood sample and separation of serum from blood

Sampled fish were anesthetized with clove oil (Merck, Germany) at 50 ml per liter of water before collecting blood samples from fish. Blood was taken from caudal vein of fish by means of 1.0 ml hypodermal syringe with 24-gauge needle. The amassed blood was directly transferred to the coated EDTA (as an anticoagulant) test tube and shake well to prevent hemolysis and clotting of blood. Serum was collected without using anticoagulant and was separated from blood by keeping the tubes in slanting position for about 2 h and thereafter it was centrifuged at 3500 rpm for 15 min at 4 ºC followed by collection of straw coloured serum with micropipette and stored at -20 ºC for further analysis.

Hematological parameters

The hematological parameters were reviled with the help of Hematology Analyzer, for Clinical and Path Lab (Medonic)

Serum biochemical test

SGOT activity (U/L) and SGPT activity (U/L) in serum were determined by using SGOT and SGPT diagnostic kit (Merck, Germany) respectively. The analytical kits were constructed on Reitman and Frankel method.

Blood glucose was assessed by using glucose diagnostic kit (Merck, Germany) which was based on the method of Nelson and further modified by Oser, 1965 [15].

Statistical analysis

The data were statistically analyzed by statistical package SPSS version 16 in which data was subjected to one-way ANOVA. The significance level was set at $P<0.05$ and the results were expressed as mean ±S.E.M.

Results

Hematological studies showed that the erythrocyte, leucocyte and hemoglobin percentage significantly increased while as in serum biochemical tests, The SGOT and SGPT values declined when treated with low doses but showed significant rise in levels when treated with high doses. Glucose level in treatment groups showed random results which can be a cause of stress.

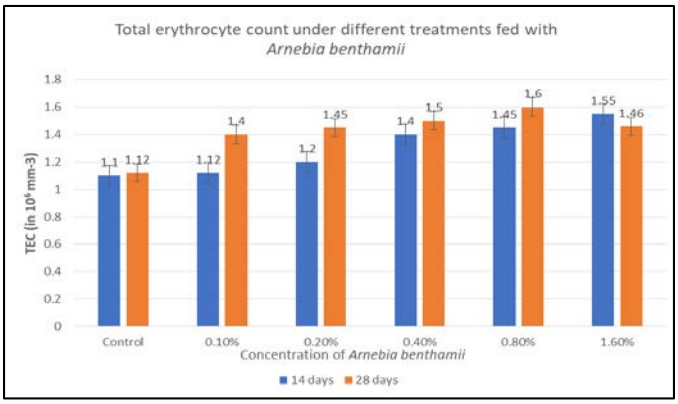
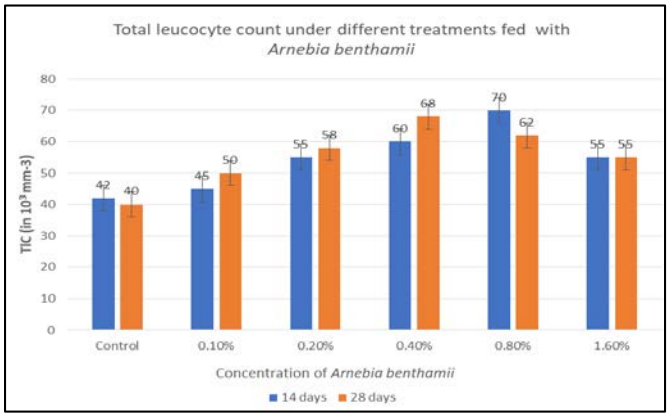


Fig 1



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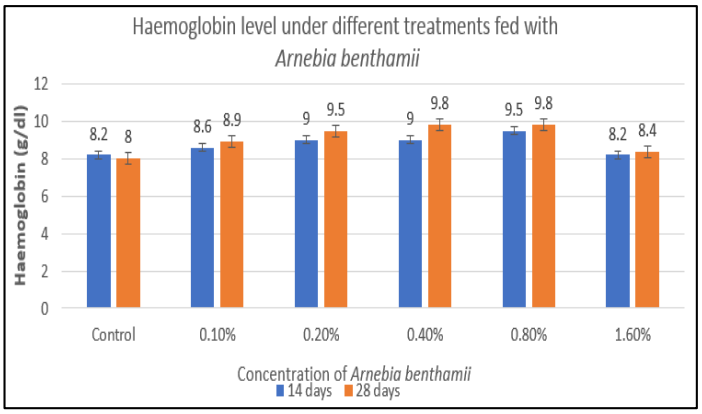

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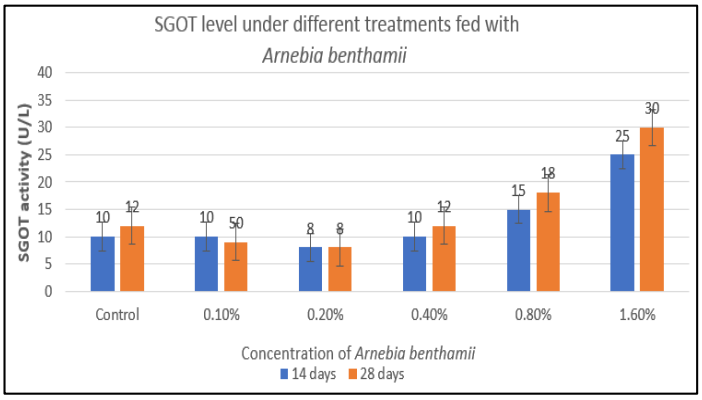
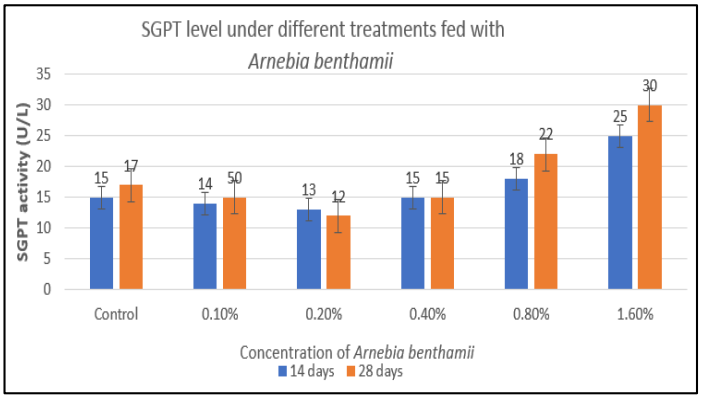


Fig 4



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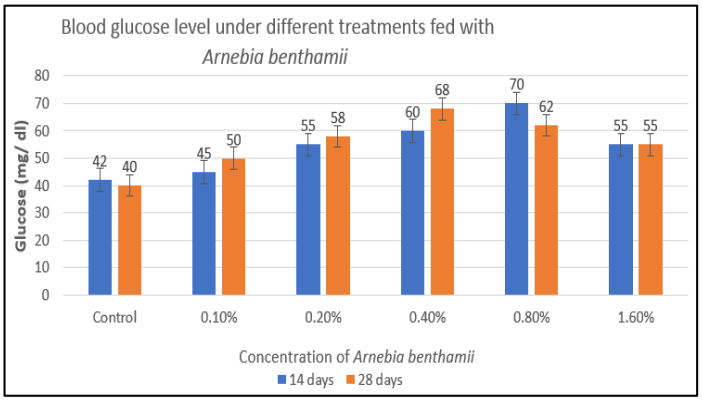

Fig 6

Discussion

In this current study, total erythrocyte counts (TEC) in the treatment groups have showed significantly higher number as compared to control group. When concentration of *A. Benthamii* increased. (Sahu *et al.*, 2007) [12, 19] also found similar results of increasing trend of TEC in Labeo rohita fed with diet containing *A. sativum.* Typically, Red blood cells (erythrocytes) constitute major part of blood cells and number may vary with species to species but they are usually in range between 1.05 to 3.0 $\times 10^6/mm^3$

In this hematological study, WBC counts increased significantly in treated groups. (Choudhury *et al.*, 2005) reported similar result in *L. rohita* fed with the diet having yeast RNA. Total leucocyte counts in *L. rohita*, fed with a diet containing n-3 PUFA was found significantly high (Misra CK *et al.*, 2006) [14]. Similarly, substantial increase in TLC was recorded in *Catla catla* juveniles fed with yeast RNA, u-3 fatty acids and β-carotene (Jha AK *et al.*, 2007) [12].

The function of RBC and the chances of anemia can be determined from the hemoglobin content. In the results it was clear that Hemoglobin content had an increasing trend as contrast with RBC count, which is in support of the findings of (HariKrishnan R *et al.*, 2003) [10].

From the study it was clear that effect *of A. Benthamii* level showed declination in levels of SGOT and SGPT when given in small concentration, but the level significantly increased when treatment groups were given high concentration of *A. Benthamii*; therefore, excessive amount of *A. Benthamii* showed negative impact on liver tissue and this finding supports the finding of higher SGOT and SGPT level by (Rao *et al.*, 2006) [17].

Glucose measurements in this finding exhibited random results; firstly, it showed little increment in glucose level but suddenly it showed a huge difference; this should be an accompaniment of stress tests rather than a main indicator. It has been also reported as an indicator of stress caused by physical factors by (Manush SM, *et al.*, 2005) [13].

Blood glucose level study in this hematological study showed significant variation between the treatment groups and showed high-level compared to control. (Misra *et al.*, 2006) [14] also reported a significant higher blood glucose level in treated group in *L. rohita*. fed with β-glucan.

Conclusion

From this study it has been concluded that medicinal plants *A. Benthamii* demonstrated the eminent influence on histological and serum biochemical parameters as well as the general fish health. Further researches are needed to confirm the efficacy of this medicinal plant in fish farms with special concern about their impact on the physiological and health condition of fish.

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