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The Effect of *Adathoda vasica* Extract on the Haematology and Protein Content of Black Moor- *Carassius auratus*

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Abstract: ***Aquaculture systems are known stressful ecosystems. The stress is buffered by the culture fish via a number of behavioral, physiological or even genetic mechanisms. Adathoda vasica is a well known drug in the Ayurvedic and Unani system of medicine. The leaves contain a small amount of an essential oil, a crystalline acid and a white crystalline alkaloid vasicine. The present study attempts to throw some light on the effect of Adathoda vasica extract on the stress metabolic axis of the ornamental fish Carassius auratus (Black moor), a member of the family Cyprinidae. Elevated levels of TEC, TLC, protein and glycogen at 48 hours and 72 hours in both TD1 and TD2 treatments indicated an improvement in its growth. The fluctuations seen in the parameters studied indicate a positive impetus to the stress metabolism axis. It is possible to conclude on a positive note that Adathoda vasica methanolic extract dose have a growth promoting effect on Carassius auratus.***

Keywords: Aquaculture, *Adathoda vasica*, vasicine, *Carassius auratus*

1. Introduction

Aquaculture can be used as a means to boost the socio-economic status of the people living at the bottom strata of the social order. For increasing the aquaculture outputs, farmers rely heavily on the use of antibiotics. As an alternative to antibiotics, biodegradable products assisting in elevating the immunological status of the culture fish is an active area of research (Murthy, 2000). Kerala, as we know, has a wealth of floristic heritage. Ayurveda offers a hoard of immunostimulant products for the well being of mankind. *Adathoda vasica* is a well known drug in the Ayurvedic and Unani system of medicine. It belongs to the family *Acanthacea*. It is recommended for a variety of ailments mostly those associated with the infections of upper respiratory tracts. The leaves and roots are antispasmodic and anti haemorrhagic. The leaves contain a small amount of an essential oil, a crystalline acid and a white crystalline alkaloid, *vasicine*. Its molecular structure is $C_{11}H_{12}N_2O$ and melting point is 190-191^{0}C.The leaves have the maximum content of *vasicine*, 0.2-0.4%. Broncho-dialation, anti-hemorrhagic, insecticidal and antiseptic properties are attributed to *vasicine*.

Fishes have an immunological set up as delicate as that of man. Aqua culturists have started recognizing these delicate balances lately (Frederickson, *et al*., 1997). *C. auratus* is an important aquarium fish which belongs to the family Cyprinidae. It lives for 10 years and attain a maximum body length of 7cms.. It has an omnivorous feeding habit and has a characteristic eye which projects strongly along the optic axis. The fish come under the Class - *Teleostomi,* Sub class- *Actinopterigii*, Order- *Cypriniformes,* Family- *Cyprinidae*, Genus- *Carassius* and Species - *auratus*.

2. Materials and Methods

2.1 Study design and Sample Collection

To assess the biological impact of the methanolic extract of *A. vasica* on *Carassius auratus*, a minimum effective concentration had to be found. Two doses of *A. vasica* were taken -0.01mg (Test Dose 1) and 0.05 mg (Test dose 2). These doses were given as intramuscular injections using standard micro syringe (Merk). Each experimental set up consisted of 15 fish/ test dose. The same fishes were again subjected to the determination of oxygen consumption, protein content, glycogen content, Total Erythrocyte Count (TEC) Total Leucocyte Count (TLC) and Hb at intervals of 24 hours, 48 hours and 72 hours subsequent to TD-1 and TD-2 injections.

2.2 Collection of Blood Samples

The blood of *C. auratus* was obtained by dorsal gill incision according to the method of Watson et al., (1989). The fish was manually retained in a horizontal position with the ventral side up. The left operculum was excised revealing the gill structures. Excess water and any other residual hemorrhaging resulting from the excision of the operculum was removed by blotting the area with a tissue paper. Using a pair of small surgical scissors, an incision was made to cut the efferent arteries at the junction of the apex of gill arches 1-3 and the buccal cavity (Watson et al., 1989). Blood samples were immediately collected directly in to heparinized capillary tubes placed at the site of the incision.

2.3 Water Analysis

Water samples were collected in 250 ml BOD bottles from the fish tank just after the introduction of fish in to the tank and after 24 hours, 48 hours and 72 hours.

These were analyzed for the amount of oxygen consumption by the fish by employing Winkler's method.

2.4 Alkaloid extraction:

The crude extract was prepared from the *Adathoda vasica* leaves. The leaves were completely dried in the sun and in the current of hot air; and were subjected to powdering in a mixy. The powder taken in a conical flask was soaked in double volume of methanol and stirred. After 24 hours the extract was filtered and then transferred in to a crucible and maintained at 60^0 in the oven till attaining crude powder.

2.5 Protein Content

The total protein content of muscle, liver and head kidney was determined by Folin-ciocalteu method using Bovine serum albumin as standard (Lowry, et al., 1951).

2.6 Glycogen Content

The total glycogen content of muscle, liver and head kidney was determined by the Anthrone method following Sifter et al., (1950)

2.7 Total Blood Cell Count

For determining Total Erythrocyte Count (TEC), the conventional haemocytometer method was used. Using Hendricks fish blood diluting fluid tightly coloured with methylene blue, the sample blood was diluted 200 times in RBC Thoma pipette. Counting was done using a haemocytometer at the high power of a microscope and calculated as Total Erythrocytes Counted 10^6 (TEC 10^6). Total Leucocyte Count (TLC) was determined along with TEC and calculated as Total Leucocytes Counted 10^4 (TEC 10^4). The erythrocytes were in the form of oval or rounded cells with a distinct nucleus .The leucocytes were small or large amorphous whose nucleus stained a slight blue colour. Duplicate counts were made for all samples and the average of the counts was taken in all cases.

2.8 Haemoglobin Concentration

The Hb content was determined by Sahlis acid haematin method. The blood samples were transferred to a Sahli pipette up to the 20 mm mark. Wiped out all the excess blood with a soft absorbant tissue and transferred the sample in to a transmission test tube containing 10 ml of 0.1N HCl. Rinsed the pipette several times in the acid solution and allowed it to stand for 3-5 minutes. The transmission was read in a calorimeter at 540 nm. The value was then converted in to gm%.

3. Results and Discussions

The collected samples are shown in the Figures 1, 2 and 3. The results observed as per the experimental design mentioned in the materials and methods are documented in tables 1 and 2.The oxygen consumption of the experimental fishes recorded a slight increase in both TD1 and TD2 treatments. Haematological parameters especially the WBC counts exhibited greater fluctuations than RBC and haemoglobin contents. An overall increase in the protein and glycogen content of muscle, liver and head kidney is observed. From these three tissues, increase in head kidney protein content and muscle tissue glycogen content is appreciably high in both TD1 and TD2 treatments.

Figure 1: Adathoda vasica plant

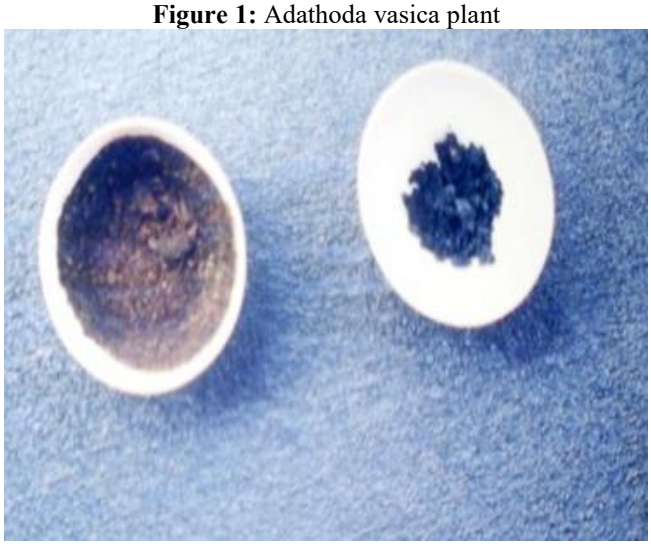

Figure 2: The crude extract of Adathoda vasica

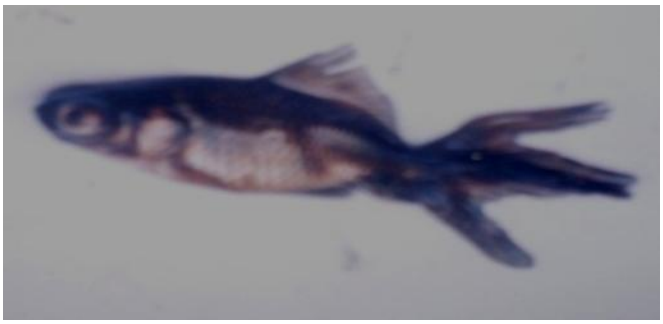

Figure 3: Carassius auratus (Black moor)

The rate of oxygen consumption showed a fluctuation from 0.0017 ± 0.0003 ml /hr /gm wt at 24 hours to 0.0023 ± 0.0008 ml / l /hr /gm wt at 72 hours in TD1 treatments, the control for the same being 0.0019 ± 0.0002 ml /l /hr / gm wt. In TD2 treatments the rate of oxygen consumption ranged from 0.0019 ± 0.0004 ml /l/hr /gm /wt at 24 hours to

0.0024 ± 0.0006 ml /l /hr / gm / wt at 72 hours. The control values being 0.0018 ± 0.0001 ml /l / hr / gm wt.

Table 1: Oxygen consumption of *Carassius auratus* subsequent to TD1&TD2 treatments

Dosage	Normal (mg %)	24hours	48hours	72ours
TD1	0.0019± 0.0002	0.0017± 0.0003	0.0022± 0.0006	0.0023± 0.0008
TD2	0.0018± 0.0001	0.0019± 0.0004	0.0024± 0.0001	0.0024± 0.0006

The total protein of muscle tissue in TD1 treatments ranged from 46-34 ± 0.123 mg% at 24 hours to 55.23 ± 0.31 mg% at 72 hours (control 48.11 ± 0.012 mg%). In case of liver tissue it ranged from 43.40 ± 0.160 mg% at 24 hours to 50.31 ± 1.861 mg% at 72 hours (control 44.34 ± 1.020 mg %). In case of head kidneys the same ranged from 35.85 ± 0.130 mg% at 24 hours to 46.01 ± 1.030 mg% at 72 hours (control 136.79 ± 1.014 mg%).

In TD2 treatments the total protein content of muscle tissue ranged from 49.10 ± 0.184 mg% at 24 hours to 56.04 ± 0.139 mg% at 72 hours (control 48.32 ± 0.014 mg%. The same for liver tissue varied from 45.932 ± 0.016 mg% at 24 hours to 51.42 ± 0.021 mg% at 72 hours (control 45.60 ± 0.129 mg %). In case of head kidney tissue the same ranged from 37.52 ± 0.166 mg% at 24 hours to 47.62 ± 0.120 mg% at 72 hours (control 37.31 ± 0.124 mg %).

The glycogen content in muscle tissues subsequent to TD1 treatment ranged from 0.717 ± 0.013 mg% at 24 hours to 1.893 ± 0.014 mg% at 72 hours (control 0.887 ± 0.011 mg %). The same for liver tissue ranged from 1.030 ± 0.016 mg% at 24 hours to 1.920 ± 0.006 mg% at 72 hours (control 1.120 ± 0.003 mg %). Whereas for head kidney tissue the same ranged from 0.392±0.010 mg% at 24 hours to 0.532 ± 0.016 mg% at 72 hours (control 0.392 ± 0.013). In TD2 treatments the glycogen content of muscle tissue ranged from 0.942 ± 0.002 mg% at 24 hours to 1.432 ± 0.132 mg% at 72 hours (control 0.893 ± 0.014 mg%). The same for liver ranged from 1.468 ± 0.137 mg% at 24 hours to 1.983 ± 0.843 mg% at 72 hours (control 1.401 ± 0.042 mg %). In case of head kidney tissue the same ranged from 0.499 ± 0.032 mg% at 24 hours to 0.593 ± 0.124 mg% at 72 hours (control 0.430 ± 0.011 mg %).

The hematological parameters observed showed the following variations. In TD1 treatment the RBC count ranged from 184000 ± 1600/mm^3 at 24 hours to 200000 ± 1900/mm^3 the control value being 146000 ± 2300/ $mm^{3.}$ .The same for TD2 treatment ranged from 162000 ± 2400/mm^3 at 24 hours to 220, 000 ± 1890/mm^3 at 72 hours the control being 146000 ± 2300/mm^3 .In TD1 treatment the WBC count ranged from 5700 ± 493/mm^3 at 24 hours to 6092 ± 301/mm^3 at 72 hours the control value being 5400 ± 203mm^3 .The same for TD2 treatment ranged from 5810 ± 311/mm^3 at 24 hours to 6152 ± 200/mm^3 at 72 hours and control value for the same being 5400 ± 203/mm^3. In TD1 treatment the, Hb content ranged from 642 ± 0.19 mg Hb/100 ml at 24 hours to 7.54 ± 0.30 mg Hb/100 ml at 72 hours and the control being 6.80 ± 0.03 mg Hb/100 ml. The same for TD2 treatment ranged from 7.42 ± 0.18 mg Hb/100 ml at 24 hours to 8.30 ± 0.02 mg Hb/100ml at 72 hours, the control value being 6.80 ± 0.03 mg Hb /100ml. The candidate tested (*C.auratus*) has responded positively with respect to the hematological parameters studied. Though it is too early to give a comprehensive idea with regard to the plant candidate *Adathoda vasica*, it certainly has a positive physiological effect on the fish *Carassius auratus*.

Table 2: Protein content of various tissues of Carassius auratus subsequent to TD1 and TD2 treatments

Tissues	Normal (mg %)		24hours (mg %)		48hours (mg %)		72hours (mg %)	
	TD1	TD2	TD1	TD2	TD1	TD2	TD1	TD2
Muscle	48.11±0.012*	48.32±0.014*	46.34±0.123	49.10±0.184	52.39±0.603	53.36±0.131	55.23±0.31	56.04±0.139
Liver	44.34±1.020	45.60±0.129	43.40±0.160	45.932±0.016	47.79±0.891	48.63±0.142	50.31±1.861	51.42±0.021
Head kidney	36.79±1.014	37.31±0.124	35.85±0.130	37.52±0.166	40.39±1.423	41.01±0.012	46.01±1.030	47.62±0.20

Table 3: Glycogen content of various tissues of Carassius auratus subsequent to TD1 and TD2 treatments

Tissues	Normal (mg %)		24hours (mg %)		48hours (mg %		72hours (mg %)	
	TD1	TD2	TD1	TD2	TD1	TD2	TD1	TD2
Muscle	0.887±0.011*	0.893±0.014*	0.717±0.013	0.942±0.002	0.912±0.003	1.031±0.103	1.893±0.014	1.432±0.132
Liver	1.120±0.003	1.401±0.042	1.030±0.016	1.468±0.137	1.870±0.041	1.924±0.356	1.920±0.006	1.983±0.843
Head kidney	0.392±0.013	0.430±0.011	0.392±0.010	0.499±0.032	0.484±0.014	0.561±0.005	0.532±0.016	0.593±0.124

The present study was an attempt to throw some light on the effect of *A. vasica* extract on the stress- metabolism axis of the ornamental fish *C. auratus*. The fluctuations seen in the haematological parameters studied shows a positive impetus to the stress- metabolism axis indicating an adaptive stress response. Iversen et al., (1998) has studied the effect of physical stressors on certain hematological parameters. According to Iwama et al.,(1999) elevated levels of TEC and TEC at 48 hours and 72 hours in both TD I and TD 2 treatments indicates an improvement in its growth. The slight rise in Hb content (7.54+0.3 mg 100ml/ blood) probably accounts for the high metabolic activity coupled with the rate of oxygen consumption.

Table 4: Hematological parameters of Carassius auratus subsequent to TD1 and TD2 treatments

Dosage	Normal	24hours	48hours	72hours
TD1	A*:146000±2300	184000±1600*	200000±3600	200000±1900
	B*:5400±203	5700±493	5810±199	6092±301
	C*:6.80±0.03	6.42±0.19	7.86±0.02	7.54±0.3
TD2	A*:146000±2300	162000±2400	200000±2830	220000±1890
	B*:5400±203	5810±311	5911±132	6152±200
	C*:6.80±0.03	7.42±0.18	7.96±0.29	8.30±0.02

4. Conclusion

Adathoda vasica methanolic extract dose have a growth promoting effect on *Carassius auratus.* It is very early to comment on the physiological mode of action of the plant extract, but it certainly opens up an ecofriendly avenue for further scientific investigations.

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Dr. F. George D'Cruz took his B.sc, M.Sc, and Ph.D degrees from the University of Kerala, India. After Post graduation, he joined Fatima Mata National College (at present a leading Autonomous College in Kerala) under the University of Kerala as Lecturer in 1979. In 1998 he took his Ph.D in Zoology, through Part time Research, while serving as a Professor. In Zoology, his area of specialization was Fisheries and Environmental Toxicology, Biodiversity, Wetland Ecology and Fish Diversity. In 2008, he retired as the HOD of Zoology and at present holds the post of honorary director of the Zoology Research Centre, under the Department of Zoology in the same college. There are about 20 Research papers published in Research Journals and more than 50 scientific papers presented in Seminars, Symposia and other Public functions in his credit. At present he is guiding students for their Ph.D in Fish Diversity and Wetland Ecology. He has authored three books and co-authored three other books on various topics like Fisheries, Environmental Biology and Human Evolution, in addition to Scientific Articles in Popular Journals and Print media. There are 16 Radio talks through All India Radio and 15 talks through Radio Benziger, Kollam on various scientific topics under Fisheries, Biodiversity Conservation, Environmental Biology, etc. Dr. F. George D'Cruz's Contributions as Subject Expert Member in various State Government Bodies like District (Kollam) Monitoring Committee Kerala Forestry Project, Department of Forest (1996-2002), District (Kollam) Forest Extension and Information Centre, Department of Forest (1996-2002), Committee for Project Formulation and Implementation, Department of Fisheries (2002-2004), State Council of Educational Research and Training (1999-2005), Wetland Cell (2004-2006), etc., are noteworthy. He also served as the State Level Programme Co-ordinator of the Kerala State Biodiversity Board in Preparing People's Biodiversity Register (2009-2011). He had completed 3 projects on Research and Conservation of the State Government Agencies (Department of Fisheries, Department of Forest and Kollam Corporation).