Blood Creatinine and some Enzyme Levels in Four Species of Indian Carp Fishes Collected from a Local Aquatic Body

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Raghavendra Kulkarni and Pruthviraj C.B.

Department of Studies in Zoology, Gulbarga University, Kalaburgi-585106, India

rskgug@gmail.com

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Abstract. The blood creatinine, some blood enzymes, glutamic oxaloacetic tranaminase (SGOT) and serum glutamic pyruvic transaminase (SGPT) have been determined in four locally available Indian carp fishes such as *Labeo rohita*, *Catla catla*, *Cirrhanus mrigala and Labeo fimbriatus* to assess the kidney functioning in these fishes in relation to the aquatic body and their successful survival. The results indicate that the blood creatinine level is low with more or less uniform values in all the four species of fish. The higher values were observed for the two blood enzymes (SGPT and SGOT) in all the four types of fishes compared to the fish *Notopterus notopterus* from our earlier studies and also reported in other fishes. Thus indicating normal functioning of kidneys and other organs in all the four types of fishes studied which are healthy and are probably not stressed.

1. Introduction

Studies of the haematology and blood biochemistry in different species of fish are of comparative physiological interest. They contribute to a greater understanding of habitat, food selection and mode of life. The blood creatinine is chiefly filtered out by the kidneys (glomerular filtration and proximal tubular secretion). If the filtering of the kidney is deficient, blood levels rise. Therefore, creatinine levels in blood and urine may be used to calculate the creatinine clearance (CrCl), which reflects the glomerular filtration rate (GFR). However, in cases of severe renal dysfunction, the filtration is clinically important because it is a measurement of renal function. The Creatinine is a chemical waste molecule that is generated from muscle metabolism. Creatinine is produced from creatine, a molecule of major importance for energy production in muscles. The kidneys maintain the blood creatinine in a normal range. Creatinine has been found to be a fairly reliable indicator of kidney function. Elevated creatinine level signifies impaired kidney function or kidney disease. As the kidneys become impaired for any reason, the creatinine level in the blood will rise due to poor clearance of creatinine by the kidneys. Abnormally high levels of creatinine thus warn of possible malfunction or failure of the kidneys. The elevated creatinine levels seen in nitrate exposed fish may be an indication of compromised renal function as is observed in mammals. In fishes, creatinine is excreted by the kidneys, but it is not known if blood levels become elevated with impaired renal function [11].

The serum glutamic oxaloacetic transaminase (SGOT), serum glutamic pyruvic transaminase (SGPT) enzyme associated with liver parenchyma cells. SGPT is a transaminase enzyme is found predominately in the liver, with lesser quantities found in the kidneys, heart, and skeletal muscle. As a result, the SGPT is a more specific indicator of liver inflammation than the SGOT, as this may also be elevated in diseases affecting other organs, such as the heart or muscles in myocardial infarction, also in acute pancreatitis, acute hemolytic anemia and acute renal disease. Hence, in the present study kidney functioning is being studied by determining blood creatinine and two important enzymes (SGOT and SGPT) in four species of fresh water fishes collected from a local aquatic body.

The availability of a particular fish species in wild in large numbers reflects that the fish is thriving well in the environment, which is most suitable for its survival, growth and breeding activities. In this context it becomes necessary to identify the suitability of a particular environment

for normal functioning of specific organs or tissues of fish. Hence, the present study is being undertaken to find out the levels of some blood parameters.

2. Materials and methods

Four types of fresh water fishes have been selected for the present study because of their easy availability in a particular aquatic body situated near Gulbarga. The fish feeds on insects, fish, crustaceans and some young roots of aquatic plants. All these fishes are commercially important and are gaining importance as food source in the local area.

Live specimens of the Indian fresh water carp fishes (about 10 fishes each species) were obtained from a local aquatic body called Khaja Kotnoor reservoir situated around 25 km away from Gulbarga, Karnataka State, India and transported in aerated containers to the laboratory. The reservoir is a standing aquatic body with thick vegetation and receives water from surrounding small streams. The local fisherman regularly goes for fishing and collects large number of fish belonging to different species particularly Indian carps which are cultured. The size of the fish varied from 26.5±1 cm in length and 105±10 gm in weight. All sexes were used without discrimination. The fish were then placed belly upwards and blood samples obtained from the caudal circulation with the aid of a heparinized 2 cm disposable plastic syringe and a 21 gauge disposable hypodermic needle. The use of plastic syringe is a necessary precaution with fish blood because contact with glass results in decreased coagulation time [17]. The site chosen for puncture (about 3 to 4cm from the genital opening) was wiped dry with tissue paper to avoid contamination with mucus. The needle was inserted at right angle to the vertebral column of the fish and was gently aspirated during penetration. It was then pushed gently down until blood started to enter as the needle punctured a caudal blood vessel. Blood was taken under gentle aspiration until about 2 ml has been obtained. Thereafter the needle was withdrawn and the blood gently transferred into plastic containers. Blood serum was obtained by centrifugation and then used for the determination of creatinine and the two enzymes, SGOT and SGPT. All determinations were carried out in duplicates for each sample.

The creatinine was determined by modified Jaffe's method Kinetic test without deproteinisation according to the Jaffe's method. (Using commercial kit available in the market), creatinine forms a colored orange complex in an alkaline picrate solution. The difference in absorbance at fixed times during conversation is proportional to the concentration of creatinine in the sample. Serum SGOT and SGPT activity was assayed following modified International Federation for Clinical Chemistry (IFCC) method using commercial kit. Thermo Scientific ICE 3000 series AA Spectrophotometer was used for determination of blood samples.

The experimental data was analyzed statistically by adopting varied statistical methods. Standard deviation and standard error were calculated. The student's' test was carried out to know the levels of significance using the standard formula. All the values of P below 5% level are designated as significant, and the values above 5% level are designated as non-significant [13].

3. Observation

The four fishes are available in the aquatic body (Khaja Kotnoor reservoir) near Gulbarga. They can be collected by using caste net with the help of local fisherman. The normal haematological studies have been carried out to know the blood creatinine and the two enzymes SGOT and SGPT profile of the four fishes and are presented in the Table – 1 together with the standard deviation and standard error of each mean.

Creatinine is end product of creatine metabolism. It is an anhydride of creatine. Creatinine is present in muscle, brain, and blood in free form as well as in the form of creatine phosphate creatinine in the blood of all the four species of fish is ranged from 0.16 -mg/dl with an overall mean of 0.18 mg/dl. The values are presented in the Table -1. The enzymes of cellular metabolism are located within the tissue cells and are present at high concentrations. These enzymes are present at all times in the circulation of normal animal. Their substrates are also present in the circulation; these functional enzymes perform physiologic function in the blood. They are generally synthesized

in the liver but are present in equivalent or higher concentrations in the tissues. In all the four species of fish SGPT range is 27.01 -41.40 U/L with an overall mean of 34.54 U/L and SGOT is 19.00 -28.00 U/L with an overall mean of 22.36 U/L. The values are presented in the Table -1.

Table 1: Showing blood parameters, creatinine and two enzymes (SGPT and SGOT) in four species of fish collected from a local aquatic body.

Sl.No.	Fish species/ Blood Parameters	Creatinine (mg/dl)	SGPT (μ/L)	SGOT (µ/L)
1.	Labeo rohita	0.21 ± 0.0036 (SD=0.0089)	41 .405 ±0.0055 (SD=0.0137)	21.46 ± 0.0036 (SD=0.0089)
2.	Catla catla	0.1616 ± 0.0047 (SD=0.116)	33.76 ± 0.0049 (SD=0.0109)	28.00 ± 0.0033 (SD=0.0081)
3	Cirrhanus mrigala	0.2116 ± 0.0030 (SD=0.0075)	27.01 ± 0.0036 (SD =0.0089)	21.00 ± 0.0040 (SD=0.0098)
4	Labeo fimbriatus	0.1833 ± 0.0033 (SD=0.0081)	36.01 ± 0.0036 (SD=0.0089)	19.01 ±0.0042 (SD=0.0103)

Each value is expressed as mean \pm SD, N = 6. All values are significant P = < 0.01

4. Discussion

The haematological characteristics of a number of cultivable fish species have been studied with the aim of establishing normal value ranges and any deviation from it may indicate a disturbance in the physiological processes [14]. Several of these studies were attempts to determine if significant variations from normal values of these parameters exist that could be attributable to some internal or external factors [7]. The observation in this study agrees with that of [7] who noted wide variations in some hematological parameters (Hb, PCV and RBC) indices of *Clarias isheriensis* from the wild. Similar observations have been made in other fish species and were attributed to intrinsic factors [5]. The creatinine values of fresh water fish *N.notopterus* were found to be higher [9] in comparison to the marine toad fish [12]. The preponderance of creatine over creatinine in the plasma of fish corresponds to the relationship of the two compounds in the urine and emphasizes the relative importance of the former in the creatine-creatinine metabolism of these animals at least in so far as concerns with excretion. Creatinine levels of *N.notopterus* blood serum [9] were found slightly high range of values as observed for other fish, e.g., *Salvelinus fontinalis* (brooktrout), *Salmo trutta* (brown trout), *Oncorhynchus mykiss* (rainbow trout), *Cyprinus carpio* (carp), and *Lepomis punctatus* (spotted sunfish).

In the present study, in all the fish species observed the creatinine level found to be less as compared to the fish *N.notopterus* indicating that the four species fish such as *Labeo rohita*, *Cutla cutla*, *Cirrhanus mrigala and Labeo fimbriatus* are healthy and probably their kidney is functioning normally and also these fishes are not stressed. However, there is variation of values between the species. Besides specificity and ecology of the target population, the method of capture, age of fish, method of drawing blood and diet are all variables that should be considered in accepting study specimens and evaluating results, [2]. In the fish, *N.notopterus* [9] serum enzymes such as SGPT and SGOT and alkaline phosphatase (ALP) were determined and found to be with a range of 15.15 – 17.20 U/L, 12.62 – 16.70 U/L, 35.40 – 69.37 IU/L respectively. Cells contain various enzymes that are related to the function of the cell. The SGOT and SGPT belong to the 'family' of non-plasma-specific enzymes that are localized within the tissue cells of the liver, heart, gills, kidneys, muscles, and other organs; when present in blood serum or plasma, they may provide specific information on organ dysfunction. Elevation of SGPT activity appears to reflect liver disease, and it is more specific for liver disease than SGOT because of the biological location of these two enzymes. However, the activity of enzyme, particularly SGOT, may also be elevated in acute liver

necrosis and in carbon tetrachloride toxicity, but it does not increase in chronic liver necrosis. Most valuable modern diagnostic tools because it has been shown that the physiological values of these parameters are species-specific and age-dependent and variations of some serum parameters, such as SGOT, SGPT, ALP and CK in rainbow trout has been reported to characteristic of spawning time [1].

Measurement on the activity of various enzymes in fishes can be used for confirming maturity and monitoring any changes in the quality of water and related soils [16]. [10] believed that the values of three enzymes may vary in different fish species. In the present study SGOT and ALP activities were found to be higher than those reported in *Acipenser stellatus* [16] whereas the SGPT activity is higher in the fish *N.notopterus* [9]. This variation has been also reported for common carp and gold fish [4], channel catfish, *Ictalurus punctatus* [2]. Such variation in the activities of various enzymes in fish species may be due to sampling technique, analysis method, age of fish, habitat and diet [15]. Stress factors due to the method of capture handling and sampling procedure must also be considered as important factors that can cause intra-species variations in hematological parameters [3].

The fish, Scorpaena porcus a commercial important fish feeds on small fishes and other invertebrates (a carnivorous), a study on the enzymes such as SGOT and SGPT activity were found to be (224.11 \pm 14.09) and very lower for SGPT (28.89 \pm 2.26 IU/L) compared to the values of other reported fishes Esox lucius (252.0 -583 IU/L), Thymallus thymallus (59.98 - 119.97 IU/L), Salmo trutta (71.98 – 719.85IU/L), and Salmo salar (278 \pm 73 IU/L), but were much higher than values reported in Piaractus brachypomus (49.1 IU/L), Pagrus auratus (33 IU/L), Chondrostoma nasus (29.99 – 539.89 IU/L) and Squalius cephalus (29.99 – 329.33 IU/L). The SGOT activities are similar to those reported in *Oreochromis niloticus* (230.5 ± 219.6 IU/L). SGPT activities were higher than the values reported in Esox lucius (4.9 – 11.2 IU/L), Salmo solar (6 IU/L), Salmo trutta (1.19 - 41.99 IU/L), Thymallus thymallus (0.59 - 71.98 IU/L) and Squalius cephalus (5.99 -44.99 IU/L), but were much lower than values reported in *Oreochromas niloticus* (96.5 IU/L), Pagrus auratus (97 IU/L) and Chondrostoma nasus (2.39 -167.96 IU/L). These differences in the enzyme values were suggested to be due to the fish species (Gabriel et.al; 2005). In the four species of fishes in the present study the values of SGPT and SGOT enzymes were lower compared to those mentioned above, the values are comparatively higher than the fish N.notopterus (SGOT- $14.6 \pm$ 1.29 range 12.62 - 16.70) and (SGPT -16.68 ± 0.74 , range -15.15 - 17.20).

Conclusions

The blood creatinine and two enzymes (SGPT and SGOT) level studied in four species of fishes such as *Labeo rohita*, *Cutla cutla*, *Cirrhanus mrigala and Labeo fimbriatus* indicates that the values are more or less normal in comparison to other fishes reported. Thus indicating all the four types of fishes are healthy and probably their kidney and other organs are functioning normally and are not stressed. These carp fishes are cultured in the Khaja Kotnoor reservoir for growth through setting small fingerlings and our study indicates that these fishes are thriving well because of suitability of the environment for growth as there is proper availability of food and other nutrients needed for functioning of organs.

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References

- [1] C.E. Anver, Blood chemistry (electrolytes, lipoprotein and enzymes) values of black scorpio fish (*Scorpaena porcus*, 1758) in the Dardnelles, Turkey, Journal of Biological Sciences. 4(6) (2004) 716-719.
- [2] J. Bentinck-Smith et al., Biochemistry references range for commercially reared channel catfish, The Progressive Fish-Culturist. 49(2) (1987) 108-114.
- [3] R.G. Bouck, R.C. Ball, Influence of capture methods on blood characteristics and mortality in rainbow trout (Salmo gairdneri), Transactions of the American Fisheries Society. 95(2) (1966) 170-176.
- [4] R.A. Brenden, H.W. Huizinga, Pathophysiology of experimental *Aeromonas hydrophila* infection gold fish, *Carassius auratus L.*, Journal of Fish Diseases. 9(2) (1986) 163-167.
- [5] L. Etim, S.B. Ekanem, A. Utim, Haematological profiles of two species of catfish, *Chrysicthys nigrodigitatus* and *Chrysichthys furcatus* from the Great Kwa River, Nig. Global J. Pure Appl. Sci. 5(1) (1999) 1-8.
- [6] U.U. Gabriel, A.D.I. George, Plasma enzymes in *Clarias gariepinus* exposed to chronic levels of Roundup (glyphosate), Environment and Ecology. 23(2) (2005) 271-276.
- [7] U.U. Gabriel, J.K. Alagoa, M.E. Allison, Effects of dispersed crude oil water dispersion on the haemaglobin and haematocrit of *Clarias gariepinus*, J. Aqua. Sci. Environ. Manag. 5(2) (2001) 9-11.
- [8] P. Kori-Siakpere, Haematological characteristics *of Clarias isheriensis*, Journal Fish Biology. 27 (1985) 259-263.
- [9] R.S. Kulkarni, Haematological and serum biochemical parameters in two types of fishes collected from different habitat, International J. of Current Science. 7(12) (2015) 23441-23443.
- [10] V. Luskova, Annual cycles and normal values of haematological parameters in fishes, Acta Sci. Nat. Brno. 31(5) (1997) 70-78.
- [11] D.G. McDonald, C.L. Milligan, Chemical Properties of Blood, In: W.S. Hoar, D.J. Randall, A.P. Farrell (eds.), Fish Physiology, Academic Press, New York, 1992, pp. 56-135.
- [12] A.F. Mensinger, J.W. Patrick, T.H. Roger, Blood chemistry of the Oyste Toad fish, Joirnal of Aquatic Animal Health. 17(2) (2005) 170-176.
- [13] A.M. Mungikar, Biostatical analysis, Saraswati Publ., Aurangabad, 2003.
- [14] C.C. O'Neal, C.R. Weirich, Effects of low level salinity on Production and haematological parameters of channel catfish (*Ictalurus punctatus*) reared in multi-crop ponds, In: Book of abstract. Aquaculture, International Triennial Conference of World Aquaculture Society, Jan. 21-25, 2001, Disney Coronado Springs Resort Lake Buena Vista, Florida, p. 484.
- [15] K. Sakamoto, G.A. Lewbart, T.M. Smith, Blood chemistry values of juvenile Red Pacu (*Piaractus brachypomus*), Veterinary Clinical Pathology. 30(2) (2001) 50-52.
- [16] D. Shahsavani, M. Mohri, H. Gholipour, Determination of normal values of some blood serum enzymes in *Acipenser stellatus* (Pallas), Fish physiology and biochemistry. 36(1) (2010) 39–43.
- [17] C.G. Smith, W.M. Lewis, H.M. Kaplan, A comparative morphologic and physiologic study of fish blood, The Progressive Fish-Culturist. 14(4) (1952) 169-172.