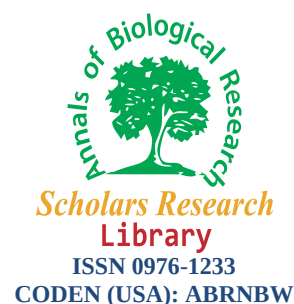




Scholars Research Library

Annals of Biological Research, 2012, 3 (4):1843-1846
(<http://scholarsresearchlibrary.com/archive.html>)



Comparison of Haematological and Biochemical indices in healthy and Monogenean infected Common Carp, *Cyprinus carpio*

Haidar Ali* and K. K. Ansari

P.G. Department of Zoology and Research Studies, M.L.K. (P.G.) College, Balrampur, (U.P.), India

ABSTRACT

The present investigation was carried out to compare the haematological and biochemical parameters in healthy and Monogenean infected *Cyprinus carpio*. The haematological analysis showed significant reduction ($P < 0.01$) in RBC, haemoglobin, haematocrit (PCV) and lymphocyte in monogenean infected fishes, whereas total leucocyte count (TLC), neutrophils, eosinophils and monocytes significantly increased ($P < 0.01$ and $P < 0.05$) in infected test fish. The biochemical analysis showed significant reduction ($P < 0.01$) in bilirubin content and blood glucose, SGOT and SGPT were significantly increased in the same test fish.

Key words: Haematology, Common Carp, *Cyprinus carpio*, Monogenean.

INTRODUCTION

For the last few decades, fishes have been extensively used as protein rich diets for human consumption in India thus contribute to its economy. It is estimated that about 10 million tons of fish required annually to meet the present day demand of fish protein in India against an annual production of only 3.5 million tons [1].

As the lakes, rivers and seas become illegally the end point of the discharge of pollutants [2] the majority of fish diseases might be occurred as a result of parasitic infection or environmental pollution [3]. About 80% of fish diseases are parasitic especially in warm water fishes [4].

Diseases are the most serious limiting factors in aquaculture because of increased density of fish in restricted water where the fish pathogens can easily transmit from one fish to another. Much of economic loss is however, preventable with proper fish health management [5].

Monogenean (flukes) is a group of parasites best described as flatworms, which are commonly found on the gills, skin or fins of fishes and lower aquatic invertebrates. About 100 families of monogenean found on fishes of both fresh and salt waters and at a variety of temperatures. Most monogenean are browsers, moving about the body surface and feeding on dermal (skin) mucus and gill debris. Monogenean has a series of hooks that enable them to attach while feeding. Most species are host-specific, requiring only one host to complete an entire life cycle. In fact, some adult monogenean will remain permanently attached to a single site on the host.

Dactylogyrus and *Gyrodactylus* are the most common monogenean parasites of the fishes. *Dactylogyrus* is usually attached to the gills of freshwater fishes. It reproduces by laying eggs, which are often resistant to chemical treatment. Therefore, weekly treatment over a period of 34 weeks is recommended. *Gyrodactylus* is usually found on the skin and fins of freshwater fishes and produces young ones, so one treatment may be adequate to control the infection.

Haematological and biochemical analysis can provide valuable knowledge for monitoring the health condition of both wild and cultured fishes. Haematological changes depend on the fish species, age, cycle of sexual maturity and health condition [6]. Moreover, haematological tests and analysis of serum constituents have showed useful information in detection and diagnosis of metabolic disturbances and diseases in fishes [7]. Thus this study is aimed to compare the haematological and biochemical parameters of healthy and monogenean infected Common Carp, *Cyprinus carpio*.

MATERIALS AND METHODS

Adult healthy and Monogenean infected *Cyprinus carpio* were collected from Pajawa Tal, located in densely populated area in Balrampur, U.P. (India) and kept separately in plastic aquaria. Blood samples for haematological and biochemical analyses were taken from the caudal vein and collected in a heparinized tube and then stored in a polyethylene cool bags until analyzed.

Total RBC and WBC counts were made by using a Haemocytometer and Neubaur grid method. A 1:100 dilutions of blood samples were made by Rees-Ecker diluting solution [8] in standard Red Cell Dilution Pipettes.

Differential leucocyte count was made by using blood smears and film fixed in Leishman's stain. The concentration of haemoglobin was determined by Cyanometer Haemoglobin method [9]. Haematocrit or PCV estimations were made by macro-haematocrit method [10]. Erythrocyte Sedimentation Rate (ESR) after one hour was determined by the method recommended by Golodets [11]. The level of blood glucose (mg/ml) was estimated by the methods of Folin and Wa [12]. Bilirubin level was analyzed by the urease neutralization method [13]. The level of SGPT and SGOT in serum was estimated by the method described by Frankel [14].

The mean and standard deviation of various haematological and biochemical parameters were calculated with the help of standard formula. Students t-test of the various haematological and biochemical values would be used to test difference between experimental fishes and their respective control $P < 0.01$ and $P < 0.05$ [15].

RESULTS

The haematological and biochemical parameters of healthy and monogenean infected *Cyprinus carpio* were presented in Table. In present study the total RBC count, haemoglobin content, haematocrit (PCV), ESR and total leucocyte count (TLC) were observed $2.68 \pm 0.04 \times 10^6/\text{mm}^3$, 9.50 ± 0.14 gm/100 ml, $27.50 \pm 0.08\%$, 3.92 ± 0.48 mm/hr and $11.12 \pm 0.13 \times 10^3/\text{mm}^3$ in healthy fishes and $1.92 \pm 0.3 \times 10^6/\text{mm}^3$, 7.35 ± 0.42 gm/ml, $20.14 \pm 0.81\%$, 4.11 ± 0.52 mm/hr and $12.25 \pm 0.08 \times 10^3/\text{mm}^3$ in monogenean infected fishes respectively.

The lymphocyte, neutrophils, monocytes, basophils and eosinophils percentage were observed $35.50 \pm 2.32\%$, $50.11 \pm 2.14\%$, $0.79 \pm 0.14\%$, $1.30 \pm 1.21\%$, $11.12 \pm 1.32\%$ in healthy fishes and $32.12 \pm 1.14\%$, $55.21 \pm 0.12\%$, $1.4 \pm 0.52\%$, $1.10 \pm 0.42\%$, $13.20 \pm 1.12\%$ in monogenean infected *Cyprinus carpio* respectively.

Thus the data represent that RBC, haemoglobin, haematocrit and lymphocytes were significantly reduced ($P < 0.01$) in monogenean infected fishes whereas total leucocytes, neutrophils, eosinophils and MCV significantly increased ($P < 0.01$ and $P < 0.05$) in monogenean infected fishes as compared to healthy ones (Table).

The bilirubin content, blood glucose level, Serum Glutamic Oxaloacetate Transaminase (SGOT) and Serum Glutamic Pyruvate Transaminase (SGPT) level were 03.0 ± 0.15 mg/ml, 57.0 ± 1.58 mg/ml, 55.58 ± 0.28 unit/gm and 32.12 ± 0.41 unit/gm, in healthy common carp, whereas 2.50 ± 0.14 mg/ml, 58.0 ± 1.41 mg/ml, 58.32 ± 0.38 unit/gm and 36.18 ± 0.12 unit/gm, respectively in healthy fishes.

Thus bilirubin was significantly reduced ($P < 0.01$) and blood glucose, SGOT and SGPT were significantly increased ($P < 0.05$ and $P < 0.01$) in monogenean infected *Cyprinus carpio* in comparison to healthy individuals (Table).

DISCUSSION

To investigate the fish blood factors and their changes, the normal rate of these factors must be initially measured in healthy fish. In the present study the blood parameters including Hb, PCV and RBC were found higher in healthy common carp. The lower value of these parameters in monogenean infected common carp was in accordance with Shah [16] and Genc [17]. Boon [18] pointed out the erythrocyte count of fish infected with parasite was significantly lower in comparison to those in non-infected fish and they reported no significant difference between the fish

infected with the parasite and non-infected ones in terms of PCV level, but it was reported that after 7 weeks of infection, PCV and plasma protein levels were found decreased.

Table: Haematological and biochemical parameters of both healthy and Monogenean infected *Cyprinus carpio*.

Parameters of Blood	Healthy		Infected		Deviation (%)	t-test
	Range	Mean $\pm$ SD	Range	Mean $\pm$ SD		
RBC ($\times 10^6/\text{mm}^3$)	2.55 – 2.72	2.68 $\pm$ 0.04	1.82 – 2.10	1.92 $\pm$ 0.31	- 28.35%	7.81**
Haemoglobin (gm/100ml)	9.15 – 9.80	9.50 $\pm$ 0.14	7.11 – 7.45	7.35 $\pm$ 0.42	- 22.63%	6.31**
Haematocrit (PCV) %	26.50 – 29.00	27.50 $\pm$ 0.08	18.13 – 22.14	20.14 $\pm$ 0.81	-20.21%	6.98**
ESR (mm/hr)	3.50 – 4.70	3.92 $\pm$ 0.48	3.90 – 5.00	4.11 $\pm$ 0.52	+ 4.84%	1.92
TLC ($1000/\text{mm}^3$)	10.12 – 13.14	11.12 $\pm$ 0.13	11.00 – 12.75	12.25 $\pm$ 0.08	+ 9.22%	4.18*
Lymphocytes %	32.50 – 37.20	35.50 $\pm$ 2.32	30.18 – 34.12	32.12 $\pm$ 1.14	- 9.52%	6.13**
Neutrophils %	49.32 – 52.81	50.11 $\pm$ 2.14	53.14 – 56.18	55.21 $\pm$ 0.12	+ 10.17%	7.38**
Monocytes %	0.68 – 0.81	0.79 $\pm$ 0.14	1.59 – 1.80	1.40 $\pm$ 0.52	+ 43.57%	4.13*
Basophils %	1.00 – 1.50	1.30 $\pm$ 1.21	1.00 – 1.30	1.10 $\pm$ 0.42	- 15.38%	3.21
Eosinophils %	10.10 – 12.14	11.12 $\pm$ 1.32	12.41 – 14.38	13.20 $\pm$ 1.12	+ 15.75%	3.14
Bilirubin (mg/ml)	2.50 – 3.50	3.00 $\pm$ 0.15	2.35 – 2.70	2.50 $\pm$ 0.14	- 16.66%	8.75**
Blood glucose (mg/ml)	54.0 – 59.0	57.00 $\pm$ 1.58	56.12 – 60.18	58.00 $\pm$ 1.41	+ 1.75%	1.11
SGOT (unit/gm)	55.10 – 56.12	55.58 $\pm$ 0.28	55.14 – 61.32	58.32 $\pm$ 0.38	+ 4.92%	3.14*
SGPT (unit/gm)	30.12 – 33.12	32.12 $\pm$ 0.41	32.12 – 37.18	36.18 $\pm$ 0.12	+ 12.64%	7.12**

* = significant ($P < 0.05$); ** = significant ($P < 0.01$)

It is known that WBCs are normally lower in healthy fishes and can be used as a significant indicator for infectious diseases. The leucocytes, neutrophils and monocytes have been increased ($P < 0.05$) in monogenean infected common carp. In the present study, the increase in WBC and neutrophils quantities in infected fishes was accepted as a response of cellular immune system to monogenean infection. Quality and quantity of leucocytes are generally used in the determination of immune reactions and diseases [19]. Shah [16] observed a positive correlation between WBC and prevalence of helminth infection i.e. WBC increased according to intensity of infection. Changes in WBC and differential leucocyte counts have been reported to play important roles in assessing the health status of fish [20]. Sahan [21] reported an increase in s of fish infected with the parasite in European eel, *Anguilla anguilla*. Boon [18] reported that WBC quantities reached the highest level in fishes infected with parasite and there was an adverse relation between the percentage of lymphocyte and basophils.

Further, the Differential Leucocytes Count (DLC) value showed fluctuations. In this study neutrophils, monocytes and eosinophils increased whereas lymphocytes and basophils decreased in monogenean infected fishes. Similar results were found in helminth infected *Schizothorax* spp. and *Cyprinus* spp. [16]. A higher degree of *eosinophilia* was observed in Cat fish, *Clarias batrachus* carrying helminth infections [22]. Similar to this work, a decrease in the percentage of lymphocytes and an increase in the percentage of granulocytes (neutrophils and eosinophils) were seen in European eel *A. anguilla* infected with parasite [21].

The decrease in bilirubin content in monogenean infected fishes points to a possible hepato-dysfunction i.e. malfunction of liver. It causes less secretion of bilirubin into blood and leads to hypobilirubinemia. Hypobilirubinemia was reported in several fishes inhabiting in both natural as well as experimental conditions [23 and 24].

The increase in blood sugar level in monogenean infected *Cyprinus carpio* may be due to increase in the breakdown of liver glycogen or due to decreased synthesis of glycogen from glucose. Hyperglycemic condition in naturally as well as experimentally stressed fishes may be due to impairment in the hormone level in blood involved in the carbohydrate metabolism [23, 24, 25 and 26]. Inhibition of cholinesterase in adrenal medulla which stimulates the breakdown of glycogen to glucose and increase in corticosteroid level which increase the blood glucose level in experimentally stressed fishes and ultimately causes hyperglycemia [27]. Thus hyperglycemia in monogenean infected *Cyprinus carpio* seems to be due to reduced insulin secretion, increased corticosteroid and also stimulation of glyconeogenesis. It is due to metabolization of glycogen deposits of the liver to the site of their active metabolism for the liberation of energy.

The elevated levels of serum transaminases (SGOT and SGPT) are markers of liver functions, that were observed in monogenean infected *Cyprinus carpio*. This increased level of serum transaminases related to disruption of normal metabolism which is due to extensive alterations in the liver histology and indicates liver damage.

Thus, the entire study reveals that the intensity of monogenean infection was responsible for altering the haematology and biochemistry of Common carp, *Cyprinus carpio*. It is further speculated that mechanical damage

caused by monogenean to the host's skin and gills may also lead to side tracking of iron to affected tissues which is otherwise responsible for erythropoiesis. Increased number of TLC and DLC values may be associated with the defense mechanism and immunological responses against infectious diseases caused by monogenean.

Acknowledgement:

The authors are thankful to Principal and management of M.L.K. (P.G.) College, Balrampur for providing laboratory and other required facilities.

REFERENCES

- [1] Shukla, G. S. and Upadhyay (1998). A text book of Economic Zoology. Rastogi Publications.
- [2] Elnwshy, N.; Ahmed, M.; El-Shareif, M. and Abd Elhameed, M., *J. Agric. Soc.* **2007**, 3: 52-54.
- [3] Hussain, S.; Hassan, M. Z.; Mukhtar, Y. and Saddiqui, B. N., *Int. J. Agric. Biol.* **2003**, 5: 660-661.
- [4] Eissa, I. A. M., *Parasitic Fish Diseases in Egypt*, 1st edition, **2002**, p. 52-53.
- [5] Kabata, Z., *Taylor and Francis Ltd.* **1985**, 318pp.
- [6] Hrubec, T. C.; Cardinale, J. L.; Smith, S. A. *Veterin. Pathol.* **2000**, 29(1): 7-12.
- [7] Aldrin, J. F.; Messenger, J. L.; Laurencin, F. B., *CNEXO, Actes Colloq.* **1982**, 14: 291- 326.
- [8] Klontz, G. W. and Smith, L. S., *Methods of Animal Experimentation. Academic Press, New York* **1968**, 3: 325-385.
- [9] Dacie, J. V. and Lewis, S. M., *Practical Haematology*, 5th edition. J and A Churchill. London, **1975**.
- [10] Wintrobe, M. M., *Clinical Hematology*, 4th edition. Lea and Febiger, Philadelphia **1965**.
- [11] Golodets, G. G., *Techniques for Investigation of Fish Physiology*, **1955**.
- [12] Oser, B. L., *Hawk's Physiological Chemistry. XIV edn. Tata McGraw-Hill Publishing Co. Ltd., New Delhi*, **1976**.
- [13] Varley, H., *Practical Clinical Biochemistry Indian edn. Arnold- Heihemann Publishers (India) Pvt. Ltd*, **1975**.
- [14] Frankel, S., In *Gradwohl's clinical laboratory method and Diagnosis S. Frankel, S. Reitman and A. C. Sonnenwirth*, eds. VI edn. CV. Mosby company. St. Louis, **1963**.
- [15] Biradar, R. S., *Course Manual Fisheries Statistics. CIFE, Bombay*, **1988**.
- [16] Shah, A. W.; Parveen, M.; Mir, S. H.; Sorwar, S. G. and Yousuf, A. R., *Pakistan Journal of Nutrition*, **2009**, 8(1): 42-45.
- [17] Genc, E.; Sahan, A.; Altun, T.; Cengizler, I.; Nevsat, E., *J. Vet. Anim. Sci.*, **2005**, 29: 661-663.
- [18] Boon, J. H.; Lokin, C. J. A.; Ceusters, R. and Oilevier, F., *Aquaculture*, **1989**, 76: 203-208.
- [19] Cagiran, H., *J. Fisher. Aquat. Sci.*, **1990**, 7: 57-68.
- [20] Gabriel, U. U.; Ezeri, G. N. O.; Opabunmi, O. O., *Afr. J. Biotechnol.*, **2004**, 3(9): 463-467.
- [21] Sahan, A.; Altun, T.; Cevik, F.; Cengizler, I.; Nevgat, E. and Genc, E., *J. Fisher. Aquat. Sci.* **2007**, 24: 167-171.
- [22] Sinha, K. P., *J. Parasitic Diseases*, **2000**, 24: 167-170.
- [23] Chaturvedi, L. D. and Agarwal, K., *Ad. Biol.* **1993**, 12: 85-92.
- [24] Srivastava, S. K.; Singh, D.; Prakash, S. and Ansari, K. K., *Ecol. Env. & Cons.* **(2007)**, 13(3): 511-514.
- [25] Dalela, R. C.; Rani, S.; Kumar, V. and Verma, S. R., *J. Environ Biol.*, **1981**, 2(2): 79-86.
- [26] Shastry, K. V. and Sunita, K. M., *J. Environ. Biol.*, **1984**, 5(1): 47-57.
- [27] Bhatia, N. P.; Sandhu, G. S. and Johal, M. S., *Poll. Res.*, **2002**, 21(3): 323-327.