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EFFECT OF pH ON PLASMA CHOLESTEROL AND GLUCOSE LEVELS ON FRESH WATER FISH *CYPRINUS CARPIO*

M. Mari Selvan[1], C. Parvathi bai[1], U. Ramesh[2], M. R. Sudhakaran[3] G. Raju[4], P. Parvathiraj[5*], S. Selvakumar[6]

1.Department of Zoology, Manonmaniam Sundaranar University, Tirunelveli, India.

2. Department of Molecular Biology, School of Biological sciences, Madurai Kamaraj University, Madurai, India

3. Department of Zoology, Sri Paramakalyani College, Alwarkurichi, India.

4. Department of Zoology, Pioneer Kumarasamy College, Nagercoil, India.

5. Department of Microbiology, San International College of Arts and Science, Walayar, Coimbatore, India.

6. AJK College of Arts and Sciences, Navakkari, Coimbatore, India.

ABSTRACT: The present investigation was showed that estimate the comparative biochemical profile of *Cyprinus carpio* were treated at pH 4.0 - 5.0 for 28 days. At the level of pH 4.5 and 5.0 fish were survived in 38 days. Moreover in pH 4.0 the fish survived only for 19 days. Biochemical parameters such as plasma cholesterol and plasma glucose level were examined at 7 days interval in total treatment period. Result revealed that the level of plasma cholesterol and plasma glucose was increased significantly during the acid treatment. From the investigation it may not exhume in biochemical parameters of the model fish one of the reason for population decline.

KEYWORDS: *Cyprinus carpio,* pH, Plasma cholesterol, Plasma glucose.

Corresponding Author: Dr. P. Parvathiraj* Ph.D.

Department of Microbiology, San International College of Arts and Science, Walayar, Coimbatore, Tamilnadu, India. Email Address: rajaspkc@gmail.com

1.INTRODUCTION

Water pollution is recognized global threat for animal and plant populations, which is interact with aquatic environments [1, 2]. A pollutant, the creators of pollution like chemicals, heavy metals and agricultural products such as pesticides, chemicals and fertilizers, but also acids [3]. Acidification

is a growing threat to the freshwater ecosystems. Acid mine drainage, industrial effluents and resultant products of acid rains lead to unnatural acidification of freshwater sources [4, 5]. However, accumulation of bio concentration of chemicals in aquatic biota is an important factor in the assessment of the potential hazarded chemicals to the environment [6]. Heavy rainfall flush out peat bogs or strip mining areas and produce a sudden flush of acid water or acid discharges from industrial sources may create temporarily low pH value of the water it leads to increasing the level of fresh water biota mortality [7]. Freshwater fishes are one of the essential food sources in developed as well as developing countries. Fish remain the major source of protein, whereas processed fishmeal plays an important role in the production of meat in human nutritions [8]. Fish have been a popular and useful test organism in aquatic toxicological studies with the logic, that if fish life is protected, the rest of the aquatic food chain is also protected [9]. Toxic substances even in very low concentration which is sub-lethal have been reported earlier, to interfere with basal metabolism and suppressed reproduction, steroidogenesis, lipid metabolism, degenerative changes in gonadotropin cells and reduction in interstitial cells size, gonadotropin levels act as reproductive biomarkers and also as endocrine disruptors [10]. Blood profile indicates metabolism changes and biochemical processes of the organism, resulting from the effect of various pollutants [11]. To monitor the fish health the hematological investigation are very essential, and for better understanding of the mode of action of pollutants more knowledge about the biochemical changes is necessary [12, 13]. The exposure of fish to several types of polluting agents may express the several changes in haematological and biochemical parameter which are frequently used to evaluate fish health. Moreover, limited studies only conducted for understanding effect of pH in fish health profile monitoring. At present investigation was undertaking the effect of acid water on plasma cholesterol and plasma glucose level of the fresh water fish *Cyprinus carpio*.

2. MATERIALS AND METHODS

Healthy specimens of *Cyprinus carpio* were obtained from the fish farm of Tamil Nadu Fisheries Development Corporation, Ltd., Aliyar. Fish were acclimated for 15 days in the laboratory conditions and were fed *ad libitum* with rice bran and ground nut oil cake in the form of dough. The survival / mortality of *Cyprinus carpio* in different pH values were observed and based on it, pH 4.0, pH 4.5 and pH 5.0 were selected for experimental purpose. Treatment was carried out for a period of 28 days. The pH of the experimental water was decreased to the desired level by adding 0.1 N Sulphuric acid drop by drop. Sulphuric acid was used as it is a common mineral acid pollutant in the wild [3, 14]. Fish were fed *ad libitum* during the experimental period. At the end of every 7^{th} day, fish were sacrificed for assay from control and experimental groups up to the 28^{th} day of exposure period. Blood was drawn from the heart region by cardiac puncture using a syringe pre-rinsed with heparin an anticoagulant and was transferred into small clean vials. Then they were centrifuged at 9000 rpm for 20 minutes and clear plasma was collected and used for the analysis of

cholesterol. Plasma cholesterol was estimated by one step method of [15] Plasma glucose was estimated by O-Toluidine method (Single Step method) of [16]. The significance of sample means between control and acid treated fish was tested by using Student's't' test [17].

3. RESULTS AND DISCUSSION

Plasma Cholesterol

In pH 4.0 the fish survived for 19 days whereas in pH 4.5 and pH 5.0 the fish survived more than 30 days. Alterations in the plasma cholesterol level of fish *C. carpio* exposed to different pH levels for 28 days were presented in the Table 1.

Table 1: Changes in the plasma cholesterol level of fish *Cyprinus carpio* exposed to different pH levels each at 7 days interval for 28 days

pH values		Plasma cholesterol level in mg % during different exposure periods							
		7 days		14 days		21 days		28 days	
		C	E	C	E	C	E	C	E
5.0	mean	81.769	160.527	80.128	161.628	80.487	176.087	79.327	115.903
	sd	±1.009	±6.228	±0.831	±1.040	±0.601	±2.884	±0.584	±5.913
	%change	+98.85		+103.71		+121.53		+42.33	
	't' value	11.307*		54.753*		29.324*		5.054*	
4.5	mean	79.928	156.452	79.718	156.406	79.616	124.869	79.648	112.838
	sd	±0.228	±2.957	±0.129	±3.012	±0.091	±3.177	±0.086	±3.093
	%change	+95.74		+98.20		+56.84		+43.67	
	't' value	23.078*		22.749*		12.735*		9.591*	
4.0	mean	79.728	226.667	79.678	113.276				
	sd	±0.048	±4.217	±0.019	±2.605	-			
	%change	+184.30		+44.17					
	't' value	31.169*		11.534*					

Values are mean S.E of five individual observations.

+ denotes percent increase over control.

*Values are significant at 5 % level. Degrees of freedom at 8t 0.05 = 2.306.

At pH 5.0, after 7th day interval, the control fish showed 81.769±1.009 mg % of plasma cholesterol and in the experimental fish the plasma cholesterol was 160.527 ±6.228 which showed 98.85 % increase. And in pH 4.5 and pH 4.0 also there was a significant increase in the plasma cholesterol level after 7th day of exposure period. And after 14 days, a similar trend, in increased plasma cholesterol level over the control was noticed in pH 5.0 (% change+103.71), pH 4.5 (% change+98.20) and in pH 4.0 (% change+44.17), was statistically significant. After 21 days of exposure the plasma cholesterol level was increased in pH 5.0 and also in pH 4.5 .The increase was

significant. And the fish exposed to pH 4.0 survived only up to 16 days and hence the data cannot be collected. After 28 days of exposure period at pH 5.0 the control fish showed 80.327± 0.584 and the experimental fish showed 115.903± 5.913 in which the percent increase was 42.33 and in pH 4.5 similar alterations was noticed and the percent increase over the control was 43.67, which was statistically significant.

Plasma glucose

Fishes were exposed to pH 4.0, pH 4.5 and pH 5.0. In pH 4.0 the fish survived for 19 days whereas in pH 4.5 and pH 5.0 the fish survived more than 30 days. Changes in the plasma glucose level of fish *C. carpio* treated with different pH ranges (pH 5.0, pH 4.5 & pH 4.0) at 7 days interval for 28 days were observed. And the results were indicated in Table 2. The results revealed that there was an increase in plasma glucose level of a stressed fish. At 7^{th} day the control fish gave 120.584 ± 1.364 mg/100ml of plasma glucose whereas in pH 5.0 the experimental fish showed 206.500 ± 3.317 mg/100ml of plasma glucose indicating an increase of 72.52%. At pH 4.5, the acid treated fish showed 216.444 ± 2.846 mg/100ml of plasma glucose over the control 122.403 ±0.246 mg/100ml of plasma glucose which was an elevation of showing 79.11 % increase over that of controls. An elevation of 29.55 % in plasma glucose level of fish from pH 4.0 at the end of 7^{th} day was recorded. However, the increase in the last case was much less when compared to that of fish from pH 5.0 and 4.5. After 14 day of exposure, the plasma glucose level of fish registered 9.64 % increase over that of the control from pH 5.0. Fish that were exposed to pH 4.5 and 4.0 also exhibited a marked increase in the plasma glucose levels over that of controls. At the end of 21 days the plasma glucose level of fish from control showed 120.391±1.196 mg/ 100ml, while fish from pH 5.0 exhibited 127.016±1.058 mg/100ml giving an overall percent increase of just 5.50. On the other hand, the increase in the plasma glucose level from pH 4.5 was higher giving 63.77 %. The fish exposed to pH 4.0 survived only up to 16 days and hence plasma glucose data could not be collected. After 28 days of exposure, the fish from pH 5.0, recorded a significant increase in the level giving 70.56 % increase over that of control. In case of fish from pH 4.5 also, there was a significant increase in the plasma glucose level. Changes in the plasma glucose level of fish treated in different pH ranges have been analyzed statistically using student's' test and they were found to be significant at 5% level.

Table 2: Changes in the plasma glucose level of fish *Cyprinus carpio* exposed to different pH levels

pH values	Plasma glucose level in mg % during different exposure periods								
		At 7th day		At 14th day		At 21st day		At 28th day	
		C	E	C	E		C	E	C
5.0	Mean	120.584	206.500	120.313	130.833	Mean	120.391	207.500	120.313
	sd	±1.364	± 3.317	±1.198	± 1.105	sd	± 1.196	± 3.317	±1.198
	% change	+72.52		+9.64		+ 5.50		+70.56	
	't' value	21.928*		5.771*		3.712*		12.046*	
4.5	Mean	120.403	216.444	122.607	164.407	Mean	127.016	214.444	120. 607
	sd	±0.246	± 2.846	±0.166	±5.230	sd	± 1.058	± 2.846	±0.166
	% change	+79.11		+36.32		+ 63.77		+44.09	
	't' value	29.444*		7.487*		10.357*		19.283*	
4.0	Mean	120.612	156.250	120.664	186.441	Mean		120.612	
	sd	±0.052	±1.768	±0.024	± 4.152	sd		±0.052	
	% change	+29.55		+54.51					
	't' value	18.027*		14.170*					

+ denotes percent increase over control.

*Values are significant at 5 % level.

Degrees of freedom at 8t 0.05 = 2.306.

DISCUSSION

Hematological and biochemical profiles of plasma can provide important information about the internal environment of the organism [19]. Changes in blood glucose have been suggested as useful general indicator of stress in teleost [20]. In our experiments with C. carpi, which were treated with different pH levels showed a significant increase in its plasma cholesterol level. It is generally considered that under acidic conditions more energy is required for maintenance of basic functions than under non-acidic conditions [21]. Plasma glucose appeared to be a sensitive indicator of environmental stress in fish. It has been reported that increase in glucose level is a typical response in carps exposed to various environmental pollutants [22, 23]. Alterations of carbohydrate metabolism towards high circulating glucose levels and gluconeogenesis are consistent responses of fish to acidic conditions [24]. Adequate energy obtained from lipid, were require by organisms to mediate the effects of stress and to serve as energy buffers during periods of unsuitable environmental conditions and malnutrition [25, 26]. Thus, the elevated lipid contents are frequently

associated with increased bioconcentration of lipophilic toxicants, which is usually correlated with enhanced toxicitic compounds [27]. Glucose level increase by basic responding for fish to acute and sub lethal pollutant effects [28]. Since, lipid forms the chief fuel during severe and sustained activities and acidic environment exerts a potent stress condition on the aquatic animals [29]. The elevated phospholipids and cholesterol levels in the tissue are suggestive of their increased synthesis or decreased utilization [30]. Hyper-glycaemia of blood glucose was caused by disorders in carbohydrate metabolism by physical and chemical stresses [31]. A variety of stressors stimulate the adrenal tissue, resulting in increased level of circulating glucocorticoids [32] and catecholamines [33]. Both of these groups of hormones produce hyperglycemia. The increased plasma glucose level reported in this investigation revealed that C. carpio exposed to acid water become hyperglycemic. This may be due to incomplete metabolism of the blood sugar because of impaired osmoregulation [34]. Increase in cholesterol, triglyceride and VLDL in zinc exposed fishes indicates increased lipid content in blood and retardation of fat metabolism which may be due to hepatic dysfunction and hypoxic condition [35].

4. CONCLUSION

From this study, revealed that elevation in the plasma cholesterol and plasma glucose levels of *Cyprinus carpio* exposed to acid stress may be due to hepatic dysfunction or tissue hypoxia, which is the result of pollution reflect disturbance in metabolism and can be used as marker of pollution. The above biochemical alterations of the plasma of the fish may be useful as non-specific biomarkers against acid toxicity. It is interesting to note that the increase in the plasma glucose level is directly related to the hydrogen ion concentration of water and exposure period probably as a biochemical adaptation to acid stress.

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CONFLICT OF INTEREST

Authors have no any conflict of interest.

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