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Impact of acidification on plasma cholesterol and glucose level of fresh water fish cyprinus carpio

P. Parvathiraj, R.Manonmani, A.Kalirajan, M. R. Sudhakaran and M. R. Sudhakaran
Department of Zoology, Sri Paramakalyani College, Alwarkurichi, Tamilnadu, India.

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ABSTRACT
Specimens of fish *Cyprinus carpio* were exposed to pH 5.0, pH4.5 and pH4.0 for a period of 28 days. In pH 5.0 and pH4.5 the fish survived for 30 days. While in pH 4.0 the fish survived only for 16 days. During the above treatment biochemical parameter such as plasma cholesterol and Plasma glucose level was observed an interval of 7 days of the total exposure period. Acid treated fish exhibits a significant increase in the plasma cholesterol levels and Plasma glucose level. The observed biochemical parameter may be used as non-specific biomarker in the field of toxicology.

Introduction

Water pollution is recognized globally as a potential threat to both human and other animal populations which interact with the aquatic environments (Biney et al., 1987; Svensson et al., 1995). A pollutant, the creators of pollution not only includes the chemicals such as heavy metals, pesticides, herbicides, fungicides, etc., but also the acids. Acidification is a growing threat to the freshwater ecosystems. Acidmine drainage, industrial effluents, air pollutants and resultant products of acid rains lead to unnatural acidification of freshwater lakes and streams (Beamish and Harvey, 1972; Dillon et al., 1978). Bioconcentration of chemicals in aquatic biota is an important factor in the assessment of the potential hazard of chemicals to the environment (Karthikeyan et al., 2007).

Alabaster and Lloyd (1982) reported that heavy rainfall may flush out peat bogs or strip mining areas and produce a sudden flush of acid water or acid discharges from industrial sources may temporarily lower the pH value of the water to a lethal level. Changes in the biochemical blood profile indicate changes in metabolism and biochemical processes of the organism, resulting from the effect of various pollutants and they make it possible to study the mechanisms of the effect of various pollutants (Luskova et al., 2002). 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 protected as well (Mahananda et al., 2008). 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 (Fujiya, 1965; Alderdice, 1967).

As the studies on the effect of acidified waters on the metabolism of fresh water animals are very limited, the present study was undertaken to investigate the effect of acid water on plasma cholesterol level of the fish Cyprinus carpio. As the plasma glucose has been widely monitored to study stress in fish (Mazeaud et al., 1977; Donaldson, 1981; Pickering et al., 1982), in the present study an attempt was made to assess the effect of low water pH on the plasma glucose of the fish Cyprinus carpio.

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 (Beamish and Harvey, 1972; Schofield, 1976). 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 upto 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 Wybenga and Pileggi (1970). Plasma glucose was estimated by O-Toluidine method (Single Step method) of Cooper and McDaniel (1970).The significance of sample means between control and acid treated fish was tested by using Student's 't' test (Campbell,1981).

Tele:
E-mail addresses: sudhakaranmr@gmail.com

Results

Plasma Cholesterol

Fishes were exposed to pH 4.0, pH 4.5 and pH 5.0. In pH 4.0 the fish survived for 16 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 Cyprinus carpio exposed to different pH levels for 28 days were presented in the Table 1 and Figure 1.

At pH 5.0, after 7 th day interval, the control fish showed 80.769±1.009 mg % of plasma cholesterol and in the experimental fish the plasma cholesterol was 160.527 ±6.228 which showed 98.75 % 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 +101.71), pH 4.5 (% change+ 96.20) and in pH 4.0 (% change +42.17), was statistically significant.

After 21 days of exposure the plasma cholesterol level was increased in pH 5.0 and also in pH4.5 .The increase was significant. And the fish exposed to pH4.0 survived only upto 16 days and hence the data cannot be collected. After 28 days of exposure period at pH 5.0 the control fish showed 79.327 ± 0.584 and the experimental fish showed 112.903± 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 41.67, 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 16 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 Cyprinus 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 and Figure The results revealed that there was an increase in plasma glucose level of a stressed fish. At 7th day the control fish gave 119.584 ± 1.364 mg/100ml of plasma glucose whereas in pH 5.0 the experimental fish showed 207.500 ± 3.317 mg/100ml of plasma glucose indicating an increase of 73.52%. At pH 4.5, the acid treated fish showed 214.444 ± 2.846 mg/100ml of plasma glucose over the control 120.403 ±0.246 mg/100ml of plasma glucose which was an elevation of showing 78.11 % increase over that of controls. An elevation of 29% 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 8.74 % 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 ± 1.058mg/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 62.77 %. 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 69.56 % 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 analysed statistically using student‘s‘t’ test and they were found to be significant at 5% level.

Discussion

Hematological and biochemical profiles of blood can provide important information about the internal environment of the organism (Masopust, 2000). Changes in blood glucose have been suggested as useful general indicator of stress in teleost (Ramesh and Saravanan, 2008).

In our experiments with Cyprinus carpi, which were exposed to 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 (Rosseland and Stournes, 1994). Nemcsok and Bores (1982) reported that blood 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 pollutants (Hanke et al., 1983). Alterations of carbohydrate metabolism towards high circulating glucose levels and gluconeogenesis are consistent responses of fish to acidic conditions. (Brown et al., 1986).

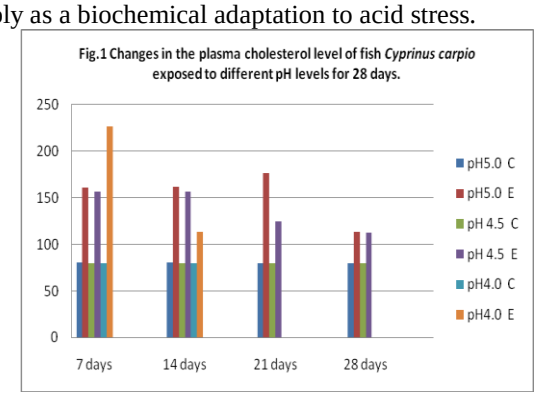
Lee et al., (1983) also stated that adequate energy (lipid) reserves are required by organisms to mediate the effects of stress and to serve as energy buffers during periods of harsh environmental conditions and food shortages (Adams and Mclean, 1985).Thus, the elevated lipid contents are frequently associated with increased bioconcentration of lipophilic toxicants, which is usually correlated with enhanced toxicity of these compounds (Goksoyr et al., 1994). Glucose increase is a general response of fish to acute and sub lethal pollutant effects (Luskova et al., 2002).

Since, lipid forms the chief fuel during severe and sustained activities (George and Jyothi, 1958) and acidic environment exerts a potent stress condition on the aquatic animals. The elevated phospholipids and cholesterol levels in the tissue are suggestive of their increased synthesis or decreased utilization (Harper et al., 1979). Wedemeyer et al., (1981) stated that high levels of blood glucose are caused by disorders in carbohydrate metabolism appearing in the condition of physical and chemical stresses. A variety of stressors stimulate the adrenal tissue, resulting in increased level of circulating glucocorticoids (Hontela et al., 1996) and catecholamines (Nakano and Tomlinson, 1967). Both of these groups of hormones produce hyperglycemia.

Chan and Woo (1978) noted that cortisol has shown to promote catabolism of peripheral tissues via, increased gluconeogenesis; leading to hyperglycemia. The increased plasma glucose level reported in this investigation revealed that Cyprinus carpio exposed to acid water become hyperglycemic. This may be due to incomplete metabolism of the blood sugar because of impaired osmoregulation (Omoregie et al., 1990). 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 (Sen et al .,1992).

It is apparent from this study that the elevation in the plasma cholesterol level 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.



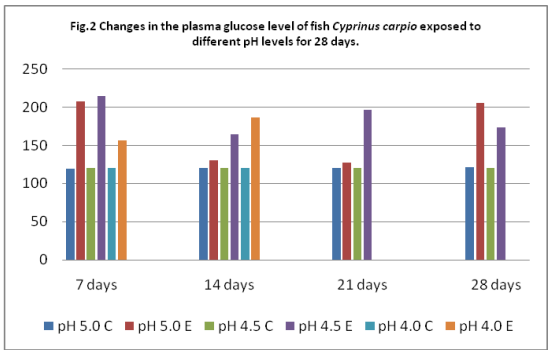


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Table 1: Changes in the plasma cholesterol level of fish Cyprinus carpio exposed to different pH levels each at 7 days interval for 28 days.

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	80.769	160.527	80.128	161.628	79.487	176.087	79.327	112.903
	sd	±1.009	±6.228	±0.831	±1.040	±0.601	±2.884	±0.584	±5.913
	%change	+98.75		+101.71		+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		+96.20		+56.84		+41.67	
	'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		+42.17					
	'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

pH value		Plasma glucose level in mg/100ml at different exposure period (n =25)							
		At 7^{th} day		At 14^{th} day		At 21^{st} day		At 28^{th} day	
		C	E	C	E	C	E	C	E
pH 5.0	Mean	119.584	207.500	120.313	130.833	120.391	127.016	121.322	205.714
	sd	±1.364	± 3.317	±1.198	± 1.105	±1.196	± 1.058	±0.859	±0.207
	% change	+73.52		+8.74		+ 5.50		+69.56	
	't' value	21.928*		5.771*		3.712*		12.046*	
pH 4.5	Mean	120.403	214.444	120. 607	164.407	120.681	196.429	120.754	174.000
	sd	±0.246	± 2.846	±0.166	±5.230	±0.152	±6.540	±0.137	± 2.466
	% change	+78.11		+36.32		+ 62.77		+44.09	
	't' value	29.444*		7.487*		10.357*		19.283*	
pH 4.0	Mean	120.612	156.250	120.664	186.441				
	sd	±0.052	±1.768	±0.024	± 4.152	-			
	% 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

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