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EFFECT OF SUB-LETHAL DOSES OF NONYLPHENOL ON SERUM ORGANIC METABOLITES IN OPHIOCEPHALUS PUNCTATUS (BLOCH, 1793)

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ABSTRACT

Variations in the blood parameters are the direct reflection of unusual metabolism of the cells or tissues in the body of inflicted organisms. In the present study, total protein, cholesterol and glucose in the serum of *Ophiocephalus punctatus* was literally increased on exposure to sub-lethal concentrations of NP LC-50. It is inferred that it may be due dysfunctions of various organs impacted by NP. The NP would have increased the metabolism of proteins, cholesterol and glycogen that led to release of protein, cholesterol and glucose in the circulation for its ultimate use by the other demanding cells and tissues of the body. Cholesterol got shoot up consistently might be due to its synthesis and not getting converted into other steroidal products. Elevation in serum glucose level could be due to glycogenolysis and hepatic and/or pancreatic dysfunction.

Keywords: Nonylphenol, Serum, Metabolites, *Ophiocephalus*.

INTRODUCTION

Fishes belonging to different taxonomic groups adopted variously depending on different prevailing ecological conditions [1], and fluctuations in the blood constituents subjected to change in temperature, ecological conditions, food habits, chemical and environmental stress.

Blood is a medium of transportation of various metabolites in the body of vertebrate species.The blood composition of the fish could be used as one of the indices and biochemical parameters can be measured for abnormalities in the liver and other tissues [2]. Fishes are the sensitive organisms and quickly respond to aquatic changes occurred either due to physical, chemical or biological clue. Some workers suggest that during stress fish need more energy for the detoxification of toxicant and stress increases the physiological process which in turn brings about the mobilisation of proteins to fulfill the demand of energy needs. Hence, in order to overcome the stress the protein synthetic activity increases [3-5].

Cholesterol in the blood has been reported to link with lipid metabolism [6] and due to its versatile use in the body its level in the blood is never constant. It is a basic substrate of steroid and steroid based hormones [7]. It is also one of the important constituent of the plasma membrane which help cell to maintain its shape, size and structure. It is suggested that the cholesterol participate in the rise of total lipid, rise of these energy reserves in response to pollution may be due to excess of energy reserves like that of glucose, triglycerides and cholesterol itself that required by organism to mediate the effects of stress [8]. However their level in the blood has long been used as indicator of stress in fish [9]. Many researchers have reported that the increased in the level of blood glucose in fish is a kind of undesirable conditions and this

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help fish by supplying the energy substrate to the organs that demands energy the need of which quite increased [10] during pathological conditions.

MATERIALS AND METHODS

All the experimental fingerlings of *Ophiocephalus punctatus* selected for present study were purchased from fisherman of Mulchera, District-Gachchiroli (M.S.) India. Fingerlings were brought to the laboratory and treated with 0.01% potassium permanganate for fifteen minutes for two subsequent days to kill the external infectious pathogenic microorganisms to avoid the possible mortality of fish due to microbial infections, and later they were acclimatised in a large aquarium for fortnight. During acclimatization, fingerlings were fed alternate days with boiled egg albumen and dried minced prawn. Fingerlings selected for the experiment had an average length 25±4 cm and weight 80±5 gm. Health of aquarium was timely managed for physiochemical parameters like pH, temperature, dissolved oxygen, conductivity; free carbon dioxide and total alkalinity.

Lethal concentration 50 (LC-50) for nonylphenol (NP) was evaluated [11] and found to be 15.51 ppm [12]. Sub-lethal concentrations (20 and 50% of NP LC-50) were prepared and exposed fingerlings separately for twenty one days. Three aquarium sets were setup. Out of three, first one was used as a control and remaining two containing 20% and 50% of NP LC-50 respectively as experimental tanks. Fingerlings added to these tanks were fed with albumen of boiled eggs and dried minced prawn in alternate days. During experimental tenure dead fingerlings were quickly removed from the aquarium.

At the end of 1st and later at an interval of seven days, fingerlings were taken out from the tanks. The blood samples were collected and after clotting of blood the serum was isolated for biochemical estimation of organic metabolites. The total protein was estimated by the method of Lowry *et al*, [13] cholesterol concentration was determined according to the method of Zlatkis *et al* and total glycogen was estimated according to Seifter *et al* [14, 15]. The estimations were carried out on spectrophotometer 'Labtronics India' and statistical analysis were performed by one way ANOVA by using trial statistical software Prism Graph pad and Microsoft Excel- 2008.

RESULTS

Serum metabolites estimated were total protein, cholesterol and glucose. All these metabolites showed variations with respect to time of exposure in vitro conditions.

Total serum protein

In normal fish *O. punctatus*, the total serum protein was estimated to be in the range of 3.91 ± 0.275 to 4.13 ± 0.03 gm/dl. In an experimental fish, the total serum protein went on increasing significantly from the first day to twenty first days of exposure. The minimum significant ($P<0.05$) increase was noted at very first day on exposure to 20% and 50% of NP LC-50 were 4.22 ± 0.026 and 4.27 ± 0.040 gm/dl. However the maximum significant ($P<0.001$) increase at twenty first days were recorded to be 4.79 ± 0.090 and 4.89 ± 0.042 gm/dl respectively (Table-1). Serum protein percentage increased on first day of exposure to 20% and 50% were noted to be 3.69 and 4.92 gm/dl. However on twenty first days it was calculated to be 17.69 and 20.15 gm/dl.

Serum Cholesterol

In controlled fish *O. punctatus*, serum cholesterol was estimated to be in the range of 202.80 ± 1.643 to 207.6 ± 5.37 mg/dl. When the fish was exposed for long period to both the 20 and 50% of NP LC-50 significant changes in serum cholesterol were prominently observed. At first day of exposure to 20 and 50% of NP LC-50 the concentration of serum cholesterol suddenly shoot up to 209.4 ± 2.3 and 211.2 ± 4.5 mg/dl. As far as percentage increased comparison to control fish was concerned the serum cholesterol increased by 2.02 on exposure to 20% and 2.99 on exposure to 50% of NP LC-50 respectively. The increase was significantly consistent right from first day to twenty first days of exposure (Table-1). The maximum serum cholesterol estimated for both the concentration was 225.2 ± 5.67 and 232.8 ± 8.84 mg/dl, and corresponding increase in serum cholesterol was estimated to be 9.83 and 13.24%.

Serum Glucose

The serum glucose in the normal fish was estimated to be in the range of 66.40 ± 1.140 to 66.80 ±1.304 mg/dl. The minimum serum glucose was observed at first day of long term exposure to both 20 and 50% of NP LC-50 and they were 72.80 ± 1.643 and 74.20 ± 2.864 mg/dl. The consistent increase was noted from first day to twenty first days of exposure while exposing to both the concentrations (Table-1). The increase was in fact significant ($P<0.001$). The maximum increase in the concentration of serum glucose was estimated on twenty first days of exposure to both the concentrations and the corresponding increased were 74.60 ± 1.949 and 78.20 ± 2.588 mg/dl. The percent increase on the first day of exposure to both concentration were calculated to be 9.39 and 11.50%, and on twenty first days 12.10 and 17.51%.

Table 1. Effect of Sub-lethal doses of nonylphenol on serum metabolites.

Sr. No.	Concentrations of NP	Metabolites	Days of Exposure			
			1-Day	7-Days	14-Days	21-Days
1.	Control	Total Protein	4.13 ± 0.030	4.12 ± 0.019	4.11 ± 0.015	3.91 ± 0.275
	20%		4.22 ± 0.026*	4.38 ± 0.064*	4.59 ± 0.120*	4.79 ± 0.090*
	50%		4.27 ± 0.040*	4.45 ± 0.049*	4.74 ± 0.064*	4.89 ± 0.042*
2.	Control	Cholesterol	203.20 ± 1.304	202.80 ± 1.643	207.6 ± 5.37	206.6 ± 1.67
	20%		209.40 ± 2.3	215.40 ± 7.369	219.4 ± 4.72*	225.2 ± 5.67*
	50%		211.20 ± 4.6	220.80 ± 9.73**	224.0 ± 3.46*	232.8 ± 8.84*
3.	Control	Glucose	66.60 ± 1.517	66.80 ± 1.304	66.40 ± 1.140	66.40 ±1.140
	20%		72.80 ± 1.643*	73.40 ± 1.949*	74.00 ± 1.225*	74.60 ± 1.949*
	50%		74.20 ± 2.864*	76.00 ± 2.550*	77.20 ± 1.304*	78.20 ± 2.588*

DISCUSSION

Serum consists of vitamins, hormones, minerals, ions, gas, amino acids, proteins, cholesterol, glucose etc. Besides these things it consists of metabolic wastes, insecticides and some other chemicals that enter the living system from the environment. These chemical may be retained either in its native form or in metabolically altered form. They may be toxic or non-toxic and can be removed from the system by various means.

Total protein content of the serum generally used as an indicator of physiological status of fish in general, because, it is considered to be the most stable components of blood, which impacted by few factors [16]. Proteins are the important organic metabolites that perform multidimensional functions in the living system. Most of the protein in the body is made up of albumen and globulin. The diseases of immune disorders, liver dysfunction and activity of impaired kidney can be monitored by testing the total protein, albumin and globulin of plasma [10]. Banaee et al reported decrease in total protein, albumin and globulin in the plasma of rainbow trout upon exposure to diazinon and this decreased in total protein credited to starvation, malnutrition and chronic diseases. Similar to these reports other authors also suggested decreased in the level of total protein in the fishes exposed to different kind of pollutants and insecticides [17, 18]. Sayed and Hamed reported significant decrease in total protein as compared to control after exposure of fish to 4-nonylphenol [19] and it is supported by the finding in serum of *C. gariepinus* after exposure to pesticides [20-22]. However, Giron-Perez reported that this depletion of total protein and albumin in the plasma of 4-nonylphenol exposed fish may be due to dysfunctions of the liver and kidney [23]. Serum total protein mainly contains both the albumin and globulin. Albumin is believed to perform three basic functions in fish such as osmotic regulation of blood volume, serve as a source of reserve protein and transportation of exo and endogenous chemicals [24, 25].

In the present study, it was observed that the total protein in the serum of *O. punctatus* significantly increased compared to control on long term exposure to sub-lethal concentrations (20 and 50% of NP LC-50). Same was reported on exposure of *O. punctatus* to NP LC-50 for short term durations in African catfish [26], *C. gariepinus.* Significant increase in total protein in the serum of *Orechromis mossambicus* was also reported by Uadhyay *et al*., (2014) but some workers reported that there is no consistency as far as total protein is concerned it may decrease or increase [27-29]. However Hager and Heba also reported increased in serum total protein on exposure to NP in *Oreochromis niloticus* [30].

Cholesterol is important for the synthesis of steroids and also involves in maintaining the structure of biological membrane. Some authors have reported that the increase in the concentration of blood cholesterol can be used as an indicator of hepatic dysfunction [31]. Some authors have suggested the nature and degree of stress in fishes [32-34] caused due to pollutants that result in increase in the level of cholesterol in the blood.

In present work it was observed that the serum cholesterol level increased significantly on long term exposure to sub-lethal concentrations of NP LC-50. Similar results were reported in *O. punctatus* treated for long durations [26]. Sayed and Hamed [21] also suggested the increase in the serum cholesterol in their experiment and reported that the elevation of cholesterol is due to stress conferred by 4-NP for its own intoxication [35].

Glucose is an ultimate chemical use for metabolic need and present in blood in variable quantity to become available for different energy demanding cells and tissues. Banaee suggested that the difference in the concentrations of glucose in the plasma of controlled and insecticides treated fish is the result of stress [36]. However Toal suggested that the increase in glucose level can be used as secondary marker in response to stress [37]. Ceron also reported the increased in glucose concentration in common eel, *Anguilla anguilla* exposed to sub-lethal concentrations of Diazinon for 96 hrs [38]. However Bhatia suggested marked increase in blood sugar on exposure to pollutants.

In the present investigation, it was observed that the serum glucose significantly increased during exposure

to sub-lethal concentrations of NP LC-50 as compared to controlled one. Same results were reported in *O. punctatus* treated with NP LC-50 for short term durations [26] and also suggested the increase could be due to glycogenolysis. The present result is agree with the results of Sayed and Hamid [21] who also reported the increase in serum glucose in African catfish, *Clarias gariepinus* on exposure to 4-nonyphenol for fifteen days. However same results were reported by some authors [20, 18].

It is concluded that the increase in the serum protein, cholesterol and glucose is due to stress induced by nonylphenol that led to dysfunction of various organs such as liver and pancreas.

CONFLICT OF INTEREST

Authors stated that no conflict of interest.

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