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EFFECT OF PETROCHEMICAL EFFLUENT ON BIOCHEMICAL PARAMETERS IN THE FRESH WATER FISH, *LABEO ROHITA*

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Abstract: **Petrochemical wastes discharged to waterways result in a variety of pollution problems. It contains high quantities of dissolved and suspended solids, organic and inorganic chemicals, high BOD and COD, oils and grease, besides toxic metals which causes deleterious effects on the fresh water fish when discharged into water bodies. Biochemical changes are being used as indicators in the measurement of health conditions and toxicological symptoms of organisms. The body components like protein, carbohydrate and lipid play a significant role in body construction and energy production. They are involved in major physiological events and therefore the assessment of biochemical can be considered as diagnostic tool to determine the physiological phases of organisms. The acute toxicity of the petrochemical effluent on the fingerlings of *Labeo rohita* was evaluated to determine its effect on the biochemical values. The fish was exposed to varying levels of the toxicant concentrations using static bioassay to determine the median lethal concentration. The LC_{50} value is 30 ml the fish was exposed to different hours (24, 48, 72 and 96 hours) in lethal concentration of petrochemical effluent. Biochemical characteristics like Protein, Carbohydrate and Lipid were estimated in gill, liver, kidney and muscle. The decrease of biochemical constituents from the control was noted. The results are statistically analysed and most of the values found to be significant.**

Keywords: **Protein, Carbohydrate, Lipid, Petrochemical effluent, *Labeo rohita.***

I. INTRODUCTION

The fresh water habitats have become the depositors of pollutants released from all the anthropogenic activities. Toxicity evaluation is an important and cost effective tool in water quality monitoring as it provides the complete response of test organisms to all the compounds in accumulative way. Toxicity of a substance refers to its capacity to cause adverse effects on living organisms and the term is more commonly used to compare the impact of two or more substances. Toxicity is nothing but a chemicals potency to cause an adverse impact on living organism and is dose and duration dependent. To evaluate the toxic impact of various pollutants, a number of bioassay procedures have been put to use in the study of concentration level of poisonous substances on organisms. Petrochemical effluent from various processing industries have reported to contain high amounts of heavy metals ions such as nickel, iron, lead, zinc, chromium, cadmium, and copper. The presence of these heavy metals in industry waste water is serious concern because they are highly toxic, non-biodegradable and carcinogenic and their continuous deposition into receiving lakes, streams and other water sources within the vicinity causes bioaccumulation in the living organisms [1].

Labio rohita fishes are widely used to evaluate the health of aquatic ecosystems because pollutants build in the food chain and are responsible for adverse effects and death in the aquatic systems. Fish can serve as bioindicators of environmental pollution and can play significant roles in assessing potential risk associated with contamination of aquatic environment

since they are directly exposed to chemicals resulting from agricultural production via surface run off indirectly through food chain of ecosystem. [2] Among the various animal groups, fishes have been identified as being very sensitive to pollutants and have been the most popular test organism because they are presumed to be the best understood organism in the aquatic environment.

Biochemical monitoring techniques like biochemical variable have become alternative and useful for monitoring environmental quality, water pollution and the health condition of aquatic organism. Biochemical parameters are frequently used as an indicator of the general state of health and early warming of stress in fish under stressful condition [3].

II. MATERIALS AND METHODS

Bulk of sample of fishes *Labio rohita* ranging in weight from 14-17 gms and measuring 7-10 cm in length were procured from Aliyar fish farm. Fishes were acclimatized to the laboratory conditions for one month in large plastic tank (200 L).The fishes were fed with adlibitium, rice bran, wheat bran and oil cakes. Appropriate narrow range of concentration 30 ml was used to find the median lethal concentration and the mortality was recorded for every 24 hrs upto 96 hrs. It was found as 30 ml for 96 hrs using probit analysis method [4]. Three groups of fishes were exposed to 30 ml of 96 hrs LC_{50} value concentration of the petrochemical effluent for 24, 48 72 and 96 hrs respectively. Another group was maintained as control.

At the end of the each exposure period, fishes were sacrificed and tissues such as liver, gill, muscle and kidney were dissected and removed. The tissues (10 mg) were homogenized in 80% methanol, Centrifuged at 3500 rpm for 15 minutes and the clear supernatant was used for the analysis of different parameters. Total protein concentration was estimated by the method of [5]. Carbohydrate was estimated by the method of [6]. Cholesterol was estimated based on enzymatic method using cholesterol esterase, cholesterol oxidase and peroxidase [7].

III. RESULT AND DISSCUSSION

In the present investigation the effect petrochemical effluent and biochemical nature of protein, carbohydrate, and lipid in the different tissues such as gill, liver, kidney and muscles of the fish, *Labeo rohita* have been studied and tabulated.

Protein:

The amount of protein estimated different tissues of fish, Labeo rohita subjected to different exposure periods are present in table no.1. Gill tissues showed 1.52, 1.45, 1.20 and 1.00 of protein in 30 ml of petrochemical effluent and 1.80 mg/g of protein in control after 24, 42, 72 and 96 hours of exposures. Liver tissues shows the decreased value of protein content in the liver 1.83, 1.72, 1.60 and 1.32 mg/g in 30 ml of petrochemical effluent and 1.95 mg/g in control after 24,48,72 and 96 hours exposures. In kidney tissues 1.43, 1.30, 1.15 and 1.02 mg/g of protein in 30 ml of petrochemical effluent exposure and 1.67mg/g in control after 24, 48, 72 and 96 hours respectively. The protein content in the muscle is also reduced. In control the protein level is 2.20 mg/g. It decreased to 1.90, 1.72, 1.68 and 1.52 mg/g in 30 ml of petrochemical effluent.

Proteins are an essential part of all organisms. And they do most of the work in cells and are required for the structure, function, and regulation of the body's tissues and organs. In this work the protein content of the *Labeo rohita* at different lethal concentrations decreased in all exposure periods. During the changes in total protein in gill, liver, muscle and kidney. The total protein content decreased significantly in kidney followed by gill and muscle. There was no significant decreases in protein in liver. Total protein content in all the tissues decreased considerably upon acute exposure to petrochemical effluent although the relative decreases varied from tissue to tissue.

In present study, there was decreased in protein content of all organs in lethal concentration of petrochemical effluent. For petrochemical effluent at lethal concentration there was decrease in protein content in gill, liver and muscle except kidney. Significant decrease in total protein content indicates that, stress due to effluent treatment induces proteolysis. Protein decrease may be due to stress in fish as protein is likely to undergo hydrolysis and oxidation through TCA cycle to meet the increased demand for energy caused by the stress [8]. [9] reoported alterations in the protein content on exposure to Cadmium and Lead in certain tissues of fish, *Channa punctatus*.

Studied the toxic effect of arsenic on protein content in the fresh water fish, *Labeo rohita* have revealed that significant reduction of protein level in the tissues like liver and muscle. Fall in the protein content might be attributed to the

diversification of energy demands when the fish is under stress or altered enzyme activities [10] [11]. Decrease in the level of tissue protein may also be due to excessive proteolysis to overcome the metabolic stress, as deposited protein in the cytoplasm can easily be used to replace the loss of proteins that occur during physiological stress [12]. This proteolysis is intended to increase the role of proteins in the energy production during Cadmium stress. These alterations may be due to utilization of amino acids through transamination and deamination which might have supplied necessary ketoacids to act as precursors for the maintenance of carbohydrate metabolism to meet the energy requirements during Cadmium stress [13].

The effect of Thiamethoxam on the growth and liver total protein of this exotic fish, *Oreochromis niloticus*. Their results revealed that various sublethal doses of Thiamethoxam had significant impact on growth and liver total protein of this fish [14]. The weight, length and breadth showed a decreasing trend in all doses of pesticide though there was a variation in protein level at different dosed fish. The impact of Dichlorvos on tissue glycogen, total protein and albumen content in the selected tissues of *Oreochromis mossambicus*. In their study, when *O. mossambicus* is treated with sub lethal doses of Dichlorvos for all the exposure periods, it shows a significant decrease in the liver, kidney and muscle protein content and it is suggested that depletion of tissue total proteins after 7 days exposure period may be due to increased proteolysis thereby contributing to the availability of free amino acids that may be fed to the tricarboxylic acid (TCA) cycle and further possible utilization of its products for metabolic process [15].

Table 1: Effect of Petrochemical Effluent on Protein in the fresh water fish, *Labeo rohita*

Sample	**Exposure periods**				
(mg/g wet tissue)	**Control**	**24HRS**	**48HRS**	**72HRS**	**96HRS**
GILL		1.52± 0.13	1.45± 0.11	1.20 ±0.09	1.00± 0.08
't'value	1.80± 0.10	4.37**	6.77**	8.38**	9.53**
%Change		-0.027	-0.026	-0.021	-0.018
LIVER		1.83 ±0.21	1.72 ±0.14	1.60 ±0.18	1.32 ±0.16
't'value	1.95 ±0.15	5.24**	7.25**	8.62**	10.53**
%Change		-0.035	-0.033	-0.031	-0.025
KIDNEY		1.43 ±0.17	1.30 ±0.18	1.15 ±0.12	1.02 ±0.16
't'value	1.67 ±0.12	6.63**	8.15**	9.56**	10.28**
%Change		-0.023	-0.021	-0.019	-0.017
MUSCLES		1.90 ±0.12	1.72 ±0.14	1.68 ±0.18	1.52 ±0.19
't'value	2.20 ±0.15	6.97**	7.07**	8.15**	8.74**
%Change		-0.041	-0.037	-0.036	-0.033

Values are mean ± SD, n=5,Figures in parenthesis are percentage decrease over control.

*- Significant at 5% (t<0.05) ** -Significant at 1% (t<0.01) , NS- Non significant.

Carbohydrate:

The amount of carbohydrate estimated in the different tissues of fish, *Labeo rohita* subjected to different exposures are presented in table no.2. The carbohydrate level in gill for 24, 48, 72 and 96 hours is reduced to 13.40, 12.00, 10.60 and 10.25 from 13.92 mg/g in control of 30 ml of petrochemical effluent. In liver tissues decreased level of 16.21, 15.43, 14.9 and 13.6 mg/g of carbohydrate in 30 ml of petrochemical effluent and 16.80 mg/g in control after 24, 48, 72and 96 hours respectively. The carbohydrate level in kidney is also reduced. In the carbohydrate level is 15.20 mg/g. It is decreased to 15.00, 14.20, 13.82 and 13.00 mg/g in 30 ml of petrochemical effluent. The carbohydrate level in muscle during control is 14.50 mg/g. It is reduced to 14.30, 14.00, 13.40 and 12.82 mg/g in 30 ml of petrochemical effluent for 24, 48, 72 and 96 hours respectively.

Carbohydrates are one of the main type of nutrient. They are most important source of energy. It also provides energy to all cells in the body. Many cells prefer glucose as source of energy. Carbohydrate typically contributes to structural support, protection and serves as nutrient and stored energy which increase or decrease according to organism need [16]. It is stored as glycogen in fish tissue to supply the energy needs when there are hypoxic condition and lack of food [17]. Carbohydrates are considered to be degraded first under the stress condition of animals. In the present study, significant decrease in the level of carbohydrate has been noticed in the gill and liver of fish which may due to high energy demand required for the hepatic synthesis of detoxifying enzymes [18]. The recorded findings in the present study corroborated with the observations of they have noticed the reused level of tissue carbohydrates in the toxicant exposed animals. They

have also stated that this may be due to the glycogenolysis, possibly by increasing the activity of glycogen phosphorylase to meet the energy need under stressful environment or the toxicant may have an effect of glycogenesis by the inhibiting the carbohydrate metabolism [19]. The decline in carbohydrate level may be due to utilization of stored glycogen possibly through anaerobic glycogenolysis to meet the energy demand under heavy metal stress [20].

Decreasing trend in glycogen and protein content was observed in various organs of fish, *Ctenopharyngodon idellus* on exposure to sublethal concentrations of malathion and fenvalerate by [21]. The effect of profenofos and carbosulphan on biochemical characteristics of the fresh water fish, *Labeo rohita* and reported decreased glycogen content in gill, liver, kidney, brain and muscle [22]. Depletion in biochemical parameters like protein and glycogen in *Labeo rohita* during various periods of exposure to malathion [23].

The changes in carbohydrate, protein and lipid content in fish due to stress induced by various contaminants in the petrochemical effluent. The contaminants can cause its effect at cellular, even at molecular level and ultimately the biochemical alterations. In the present study levels of carbohydrate were found to be decreased significantly in liver and gill of *Labeo rohita* may be due to the discharges of industrial effluent.

Table 2: Effect of Petrochemical Effluent on Carbohydrate in the fresh water fish, *Labeo rohita*

Sample (mg/g wet tissue)	Exposure periods				
	Control	24HRS	48HRS	72HRS	96HRS
GILL	13.92 ± 0.23	13.40± 0.24	12.00± 0.23	10.60 ±0.32	10.25± 0.13
't'value		21.62**	22.86**	24.35**	25.24**
%Change		-1.865	-1.670	-1.475	-1.426
LIVER	16.80 ± 0.20	16.21 ±0.12	15.43 ±0.15	14.9 ±0.33	13.6 ±0.31
't'value		21.17**	21.49**	22.76**	23.23**
%Change		-2.723	-2.592	-2.503	-2.284
KIDNEY	15.20 ± 0.18	15.00 ± 0.37	14.20 ±0.62	13.82 ±0.22	13.00 ±0.23
't'value		24.06**	24.16**	25.42**	27.78**
%Change		-2.28	-2.158	-2.100	-1.976
MUSCLES	14.50 ± 0.29	14.30 ±0.22	14.00 ±0.22	13.40 ±0.35	12.82 ±0.29
't'value		23.40**	25.14**	26.93**	8.10**
%Change		-2.073	-2.03	-1.942	-1.858

Values are mean ± SD, n=5,Figures in parenthesis are percentage decrease over control.

*- Significant at 5% (t<0.05) ** -Significant at 1% (t<0.01) , NS- Non significant.

Lipids:

The amount of lipid estimated in different tissues of fish, *Labeo rohita* subjected to exposures present in table no.3. The lipid content in the gill tissues exposed to 30 ml of petrochemical effluent were 15.52, 15.0, 14.7 and 14.20 mg/g in 16.70 mg/g control experiment after 24, 48, 72 and 96 hours exposures. Liver tissues recorded lipid level of 12.70, 12.10, 11.32 and 11.15 mg/g when exposed to 30 ml petrochemical effluent. It is recorded 13.23 mg/g n control for 24, 48, 72 and 96 hours exposure periods. Kidney tissue was found to contain 14.85 mg/g of lipid in control sample and recorded 14.20, 13.90, 12.85 and 12.00 mg/g in 30 ml petrochemical effluent exposure after 24, 48, 72 and 96 hours respectively.

The amounts of lipids in muscle tissues were 15.00, 14.72, 14.10 and 13.60 mg/g in 30 ml of petrochemical effluent exposure periods of 24, 48, 72, and 96 hours respectively. In control the amount of lipid was found to be 15.20 mg/g. The effect of petrochemical effluent exposure on biochemical changes in the tissues of the fish, *Labeo rohita* showed a significant change in the present study. The mortality of the fish, *Labeo rohita* exposed to different concentration exposure was observe and it showed that the exposure is very toxic to fish even at very low concentration.

Lipid is the most essential part of living organism. Lipids are also important content of living cell. In the total lipid content in various tissues of *Labeo rohita* exposed to petrochemical effluent after acute exposure for 96 hrs. Although the relative decrease varied from tissue to tissue, the present depletion in lipid content was more significant in muscle and kidney, while it was less significant in gill. There was non- significant decrease in liver as compared to control. The depletion in the hepatic total lipid could be due to their active mobilization towards the blood and /or tissue metabolism [24]. The decrease might be due to the utilization of lipid to meet the additional energy requirement under stress [25]. Toxic substances might have accumulated in the brain of fish, causing disintegration of nerve cells, clotting of blood and

reduction in transport of oxygen to brain. Loss of lipids noticed in this study may be due to inhibited lipid synthesis and mobilizing the stored lipid, either through oxidation or gradual unsaturation of lipid molecules [26]. Decrease in biochemical constituents in liver kidney and gill of abamectin treated fish *O. niloticus* [27]. Industrial effluents alter the biochemical composition (glycogen, protein and lipid) of the various organs of fish *Labeo rohita* [28]. Endosulphan mediated biochemical changes in brain, liver and muscle in the fresh water fish *Clarius batracus* was studied by [29]. Thus, the pesticide toxification disturbs the normal functioning of cell that alters the metabolic processes of the organism.

Table 3: Effect of Petrochemical Effluent on Lipid in the fresh water fish, *Labeo rohita*

Sample (mg/g wet tissue)	**Exposure periods**				
	Control	**24HRS**	**48HRS**	**72HRS**	**96HRS**
GILL	16.70 ± 0.34	15.52± 0.25	15.00± 0.17	14.7 ±0.16	14.20± 0.21
't'value		24.79**	25.27**	28.55**	29.12**
%Change		-2.591	-2.505	-2.454	-2.371
LIVER	13.23 ± 0.25	12.70 ±0.12	12.10 ±0.25	11.32 ±0.14	11.15 ±0.23
't'value		21.64**	22.16**	23.26**	25.63**
%Change		-1.680	-1.600	-1.497	-1.475
KIDNEY	14.85 ± 0.15	14.20 ± 0.27	13.90 ±0.34	12.85 ±0.12	12.00 ±0.12
't'value		22.83**	23.58**	25.72**	26.38**
%Change		-2.108	-2.064	-1.908	-1.782
MUSCLES	15.20 ± 0.29	15.00 ±0.22	14.72 ±0.32	14.10 ±0.16	13.60 ±0.11
't'value		26.35**	27.65**	28.42**	29.37**
%Change		-2.28	-2.237	-2.143	-2.067

Values are mean ± SD, n=5, Figures in parenthesis are percentage decrease over control.

*- Significant at 5% (t<0.05) ** -Significant at 1% (t<0.01) , NS- Non significant.

Lipids play a physiological role for storage of energy and vitamins. The depletion in the total lipid in the tissues could be due to their active mobilization towards the blood and /or tissue metabolism. In the present study, a significant decrease in the lipid levels in gill and liver was noticed in the test fish exposed to effluent. It may be suggested that, pollutants inhibited lipid synthesis and started mobilizing the stored lipids either through β-oxidation or through a gradual unsaturation of lipid molecules [30].

IV. CONCLUSION

The present study reveals that biochemical changes induced by petrochemical effluent stress lead to metabolic disturbances inhibit of important enzymes, retardation of growth and reduction in the fecundity and longevity of the organisms. The changes at biochemical levels might result in important of energy requiring vital processes and hence can deteriorate the health status of the fish population.

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