Effects of Multipollutants on the Biochemical Constituents of Muscles in Fishes, Labeo rohita & Catla catla of River Ganga near Patna

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Abstract:
Now a days it has been proved that pollutants exert remarkable impact on the different metabolic activities of animals. Huge increase in the use of pesticides and other pollutants, which are, released intentionally or unintentionally in the gangatic basin, have disturbed the ecology of the Ganga water ecosystem. Among the aquatic faunas besides others, fishes are most sensitive to the pollutants causing serious concern as they change the systematic biochemical status in the tissues of the fishes. As a result it has deviating consequences on the metabolic pathway in the fishes in different tissues. In this investigation we concentrate at the alterations in concentrations of the biochemical constituent units found in the muscles of the fishes under reference due to multipollutant effects. In the present experimental work we found decrease in the glycogen level by 28.5% in the protein level by 25% and in SDH activity level by 3.8%. Contrary to these results we found increased level of ascorbic acid and lactic acid by 28.3% and 56.5% respectively. The level of GOT, GPT & LDH was also found increased by 3.6%, 4.2% and 4.5% respectively. The level of lipid fractions, total lipids and cholesterol was also estimated which was found decrease in their respective levels. The level of phospholipids decreased by 2.6%, glycerophospholipids & sphingosine decreased by 2.1%, triglycerides & sterols decreased by 2.8%. Similarly the level of total lipids decreased by 2.5% and the level of cholesterol decreased by 2.4% in the experimental fishes in comparison to standard fishes. The standardization in the present work was done by the allowing the collected fish for acclimatization conductive to fishes for two months in the lab.

Keywords: *Fishes, Ganga Pollutants*

1. Introduction

The induced-pesticides effect on different biochemical constituents in the muscles of the fishes is a fact. This is on the basis of the experimental works recorded by the researchers who found their respective samples from different sources. The pollutants (Endosulfan, DDT, Formothion, Permetherin etc.) used in the previous experiments by different scientists in one and another or in the same fishes brought about either depletion / decrease/ increase in the level of glycogen, protein, ascorbic acid, lactic acid, lipid fractions, total lipids, cholesterol, enzymatic activities of GOT, GPT, SDH & LDH in the muscles. These findings influences us to investigate whether such effects are there under the influence of multipollutants inherently coming from the surroundings or absorbed by Ganga water time to time from the polluted environment, if any, found there. This consists of the estimation of different biochemical constituents in the muscles of the fishes, *Labeo rohita & Catla catla* collected from the river Ganga near Patna.

2. Material and Methods

The total glycogen was estimated according to the colorimetric method of Kemp *et. al.,* (1954) as modified by Krishnaswamy *et. al.,*(1961). The conc. of protein in the tissues was determined according to the method of Sutherland *et. al.*, (1949) using phenol (Folin – Ciocalteau) reagent. The

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ISSN:(P) 2347-5404 ISSN:(O)2320 771X

method of extraction of ascorbic acid from the tissues was the same as the Kanungo & Patnaik (1964), which was modified from Roe (1954). Cholesterol in the tissues was estimated quantitatively by the method of Zak (1957). SDH activity was measured by the method of Kun and Abood (1949). The method adopted for the assay of LDH was that of Kornberg (1955). GOT & GPT activity was estimated by using GOT substrate and GPT substrate respectively by the following process. 0.1ml of non-hemolysed serum and 0.5ml of GOT substrate/GPT substrate was mixed. This mixture was incubated at 37^0C for 1 hour. After that 0.5ml of 2, 4-DNP solutions was added and let stand for 15 min. at room temp. Now 5 ml of 0.4 N NaOH solution was added. It was mixed well and stands for 20 min. at room temp. After that the optical density was taken at 540nm.

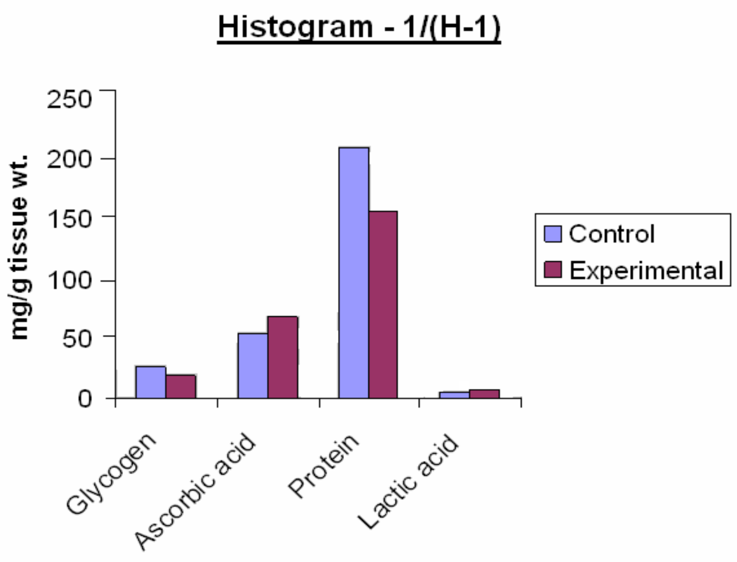
Standards: Standard solutions, containing 0.2, 0.4, 0.6, 0.8, and 1mg/ml Pyruvate, was prepared along with a blank containing no Pyruvate. The colour was allowed to develop in the same manner as the experiments mentioned above.

3. Results

Table 1.
Estimation of glycogen, ascorbic acid, protein and lactic acid in the muscle of control and experimental fresh water fishes Labeo rohita and Catla catla

Tissue Muscle	No. of observations	Control 15	Exp. 15	% Inc./Dec.	t value	P value
Glycogen mg/g tissue wt.	Mean	26.86	19.18	28.5	27.86	P<0.001
	S.D.	±1.005	±0.36			
Ascorbic Acid mg/100g tissue wt.	Mean	53.59	68.38	28.3	50.614	P<.001
	S.D.	±0.78	±0.82			
Protein mg/g tissue wt.	Mean	209.38	156.79	25.0	173.73	P<0.001
	S.D.	±0.56	±1.03			
Lactic acid mg/g tissue wt.	Mean	4.72	7.39	56.5	18.99	P<0.001
	S.D.	±0.14	±0.049			

Graph 1.
Showing the percentage increase or decrease of glycogen, Ascorbic acid, protein and lactic acid in the muscles of control and experimental fresh water fishes Labeo rohita and Catla catla



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ISSN:(P) 2347-5404 ISSN:(O)2320 771X

Table 2

Estimation of lipid fractions (phospholipids, glycerophospholipids & sphingosine and triglycerides & sterols) and total lipids and cholesterol in the muscle of fresh water fishes Labeo rohita and Catla catla

Tissue Muscle	No. of observations	Control 15	Exp. 15	%Inc. /Dec.	t value	P value
Phospholipids mg/g tissue wt.	Mean	11.76	10.82	2.6	2.55	P<0.05
	S.D.	±0.77	±1.2			
Glycerophospholipids and sphingosine mg/g tissue wt.	Mean	15.78	14.63	2.1	2.77	P<0.01
	S.D.	±0.85	±1.36			
Triglycerides and Sterols mg/g tissue wt.	Mean	39.66	38.78	2.8	1.41	P<NS
	S.D.	±1.56	±1.01			
Total Lipids mg/g tissue wt.	Mean	66.87	64.44	2.5	1.09	P<NS
	S.D.	±6.32	±5.83			
Cholesterol mg/g tissue wt.	Mean	47.7	46.75	2.4	2.009	P<NS
	S.D.	±1.3	±1.29			

Graph 2.

Showing the percentage increase or decrease of lipid fractions (phospholipids, glycerophospholipids & sphingosine and triglycerides & sterols) and total lipids and cholesterol in the muscle of fresh water fishes Labeo rohita and Catla catla

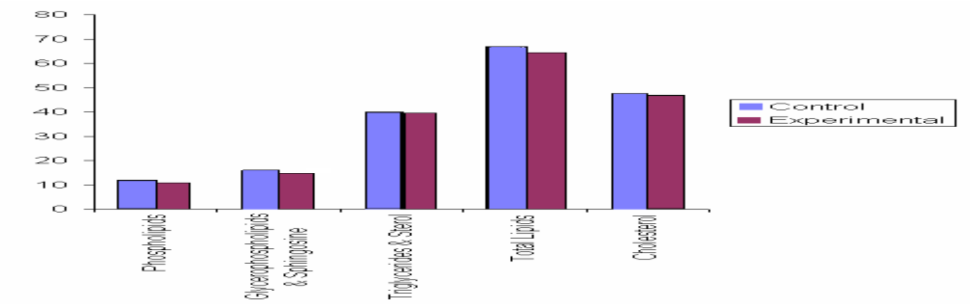


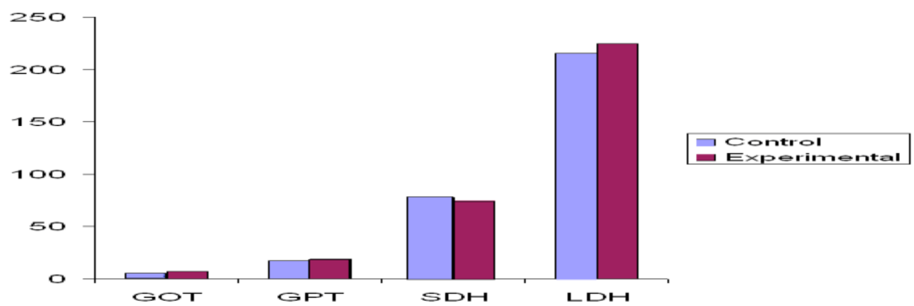
Table 3.

Estimation of enzymatic activity of GOT, GPT, SDH and LDH in the muscle of fresh water fishes Labeo rohita and Catla catla

Tissue Muscle	No. of observations	Control 15	Exp. 15	%Inc. /Dec.	t value	P value
GOT U/g tissue wt.	Mean	5.62	7.21	3.6	4.18	P<0.001
	S.D.	±0.85	±1.2			
GPT U/g tissue wt.	Mean	17.44	19.13	4.2	4.88	P<0.001
	S.D.	±1.02	±0.87			
SDH µg/mg-protein/hour	Mean	77.84	74.76	3.8	6.389	P<0.001
	S.D.	±1.33	±1.31			
LDH U/g tissue wt.	Mean	214.7	224.59	4.5	23.20	P<0.001
	S.D.	±1.29	±1.03			

Graph 3. Histogram-3/H-3

Showing the percentage increase or decrease of enzymatic activity of GOT, GPT, SDH and LDH in the muscle of fresh water fishes Labeo rohita and Catla catla



4. Discussion

Different types of pollutants affect some of the biochemical activities of tissues of fishes in different ways. Here I am limiting myself to biochemical alterations due to multiple pesticides/pollutants in Ganga water to muscles of the fishes under reference in the present investigation. In the present study the glycogen levels were estimated 26.86 mg/g tissue wt. and 19.18 mg/g tissue wt. in control and experimental fishes respectively. This value was decreased by 28.5% which was highly significant ($p<0.001$) in comparison to control fishes. Similar trend was found by Singh and Srivastava (1982) in catfish due to the effect of formation. Sastry and Siddiqui (1982) also found depletion in glycogen content of muscles due to Sevin treatment. Begum and Vijayaraghvan (1999) reported the gradual decrease in muscle glycogen. Similarly, muscle glycogen levels were decreased in *S. fossilis, Mytus vittalus and C. batrachus* due to pesticide induction reported by Verma *et, al.* (1983). The level of ascorbic acid was found increased by 28.3% which was significant, by $p<0.001$. The estimated amount was 53.59 mg/100 gm tissue wt. in control fishes and 68.38 mg/100 gm tissue wt. in experimental fishes. A marked depletion was found in protein level, which was 25% in comparison to control fishes. The protein level recorded in control fishes was 209.38 mg/gm tissue wt. and in experimental fishes 156.79 mg/gm tissue wt. The significant level was $p<0.001$. Ramalingham and Ramalingham (1982) studied the total protein content of muscles in *Tilapia* exposed to sublethal concentration of DDT, Malathion and mercury, they found decline in muscle protein. Das and Mukherjee (2000) reported the decreased level of muscle proteion. Elevated level of lactic acid was found in fishes by 56.5%. It was found 4.72 mg/gm tissue wt. in control fishes and 7.39 mg/gm tissue wt. in experimental fishes. The result was significant by $p<0.001$.

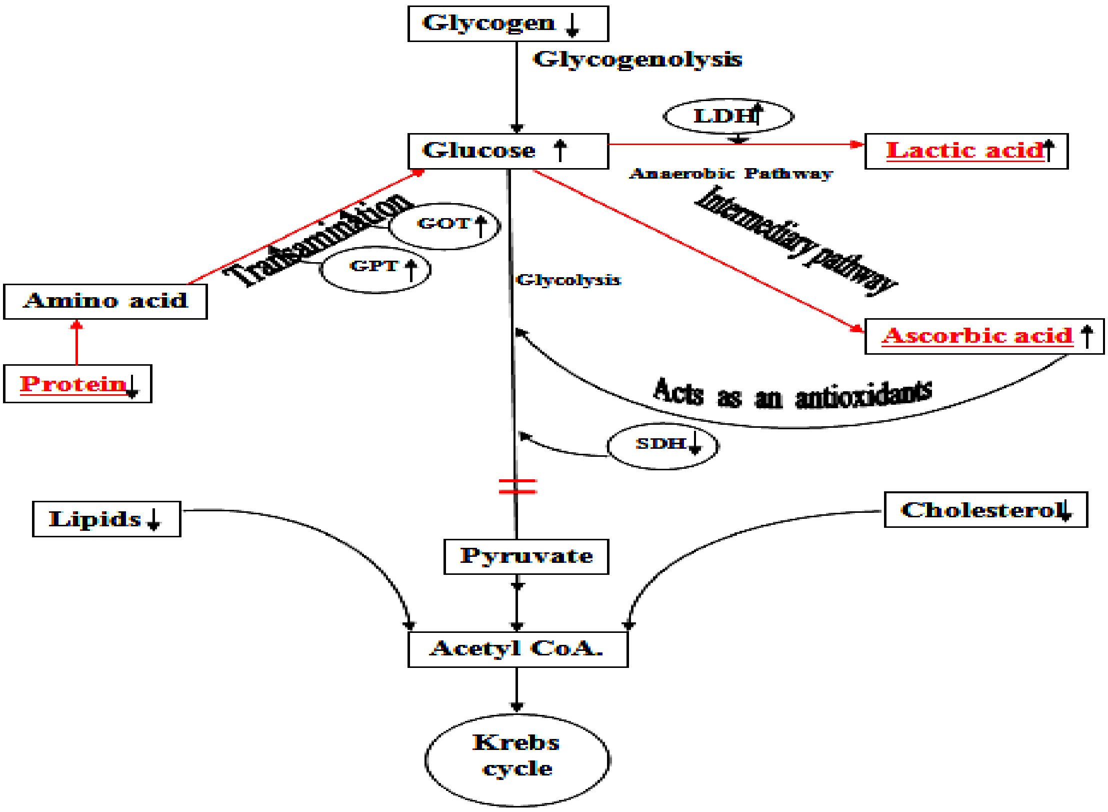
The estimated amount of lipid fractions and total lipids and also cholesterol was not so significant but a decreasing trend was found there. The amount of phospholipids was estimated as 11.76 mg/gm tissue wt. in control and 10.82 mg/gm tissue wt. in experimental fishes. Percentage decrease was 2.6%. The amount of glycerophospholipids and sphingosine was decreased by 2.1% in comparison to control fishes. It was found 15.78 mg/gm tissue wt. in control fish and 14.63 mg/gm tissue wt. in experimental fishes. Estimated amount of triglycerides and sterols in control fishes was 39.66mg / gm tissue wt. and in experimental fishes it was 38.78 mg/gm tissue wt. The percentage decrease was 2.8%. As a result, the amount of total lipids was recorded 66.87 mg/gm tissue wt. in control fishes and 64.44 mg/gm tissue wt. in experimental fishes. So the percentage decrease was found 2.5%. The cholesterol level in control fishes was recorded 47.7 mg/gm tissue wt. and in experimental fishes it was estimated 46.75 mg/gm tissue wt. The percentage decrease of cholesterol level was 2.4%.

The level of GOT activity recorded in control fishes was 5.62 U/gm tissue wt. and it was 7.21 U/gm tissue wt. in experimental fishes. Percentage increase was 3.6% and significance level was $p<0.001$. The estimated level of GPT was 17.44 U/gm tissue wt. in control fishes and 19.13 U/gm tissue wt. in experimental fishes. The level of significance was $p<0.001$ and percentage increase was 4.2%. The recorded levels of SDH activity were 77.84μg/mg – protein / hr. and 74.76 μg/mg- protein/hr. in control and experimental fishes respectively. The percentage decrease was 3.8% and significance level was $p<0.001$. The levels of LDH activity were estimated as 214.7 U/gm tissue wt. in control fishes and 224.59 U/gm tissue wt. in experimental fishes. The level of significance was $p<0.001$ and percentage increase was 4.5%. Sastry and Siddiqui (1982) reported that LDH activity was high in muscle. They found a fall in SDH activity in muscle. Begum and Vijayraghavan (1999) found a sharp rise in the activity level of LDH. Contrary to present report Tripathi and Shukla (1990) reported the endosulfan-induced impact on LDH and in fresh water Catfish. The activity of LDH is skeletal muscle was inhibited. They reported *de novo* synthesis of enzyme during the recovery period.

As being simple workers in this field and after considering the findings of previous researchers and also under the context of findings in the present investigation the author has been influenced to take into account of decrease, increase and inhibition of different enzymatic activities by different kinds of pesticides induced in fresh water fishes, very seriously. This is because of the facts that the author has recorded more logical results, which are supportive of integration and degradation of different metabolic constituent units under reference in the metabolic hierarchy.

Graph 4.

Showing deviated metabolic pathways under the influence of pollutants/pesticides as studied in the present investigation



It has come in several standard text books of biochemistry that the concentration of glucose when goes up, it turns the glycogen synthase on that turns the glycogen phosphorylase off, means these two enzymes have antagonistic activities as and when there is raising of the glucose concentration in the cell. But in the present work glycogen level went down. This decrease in glycogen level may be indicative of possible facts that glucose was converted to lactic acid which was found to be increased here, causing downward trend in the glucose level which in turn may switch the glycogen synthase off and switch the glycogen phosphorylase on. So that glycogen level was found to be lowered. In the normal course of carbohydrate metabolism glycogen depletion after passing through the different stages reach the pyruvate stage, which in turn gives rise to Acetyl-CoA. The Acetyl-CoA enteres the Krebs cycle to follow the aerobic pathway. But here, in the present investigation low level of SDH causing impairment of Krebs cycle as a result enhancement of glucose level through anaerobic pathway has been suggested in the foregoing paragraph. Here also raising of LDH is quite indicative that it converts the pyruvate into lactic acid and its enhancement possibly creates trouble somewhere in the intermediary pathway in conversion of pyruvate to Acetyl-CoA/or its penultimate goal to enter the central meeting ground or pathway/ common central route which is designated as Krebs cycle. Simultaneous rising of ascorbic acid level in the muscles of test fishes led the author to explain it on the basis of alteration in the metabolic pathway. Here it is thought that increase in the concentration of ascorbic acid may be due to intermediary routes in the carbohydrate metabolism as a whole. Now a day it has become an established fact that for ascorbic acid synthesis glucose is necessary, which has also been reported by Rousell (1957) and Briggs (1962). The glucose also acted as a precursor of both hexosamine and hexuronic acid in mammalian tissue as reported by Davidson et al. (1962).

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ISSN:(P) 2347-5404 ISSN:(O)2320 771X

Shukla and Kanungo (1968) reported that the ascorbic acid is a requirement for metabolic activities in rats. This ascorbic acid also reported to be an intermediate of the uronic acid pathway, which is inter linked with hexose monophosphate pathway as has been recorded earlier by Harper (1969). Agrawal et. al., (1978) was of the opinion that ascorbic acid protected the toxic effect of aldrin. Here in the present experiment high level of ascorbic acid and glucose supports the contention of Rousell (1957) and Briggs (1962) that glucose is necessary for the synthesis of ascorbic acid. Here author is in a position to suggest that ascorbic acid might be interfering with the oxidation of glucose through intermediary pathways. This suggestion of the author is based on the very information that ascorbic acid also acts as antioxidants. It is also suggested in the context of previous findings that ascorbic acid acts as antioxidant.

The decrease of amount of protein is supportive of the fact that Succinic dehydrogenase (SDH) brings about disturbances in Kreb's cycle forcing the fish to choose the anaerobic pathway for the production of energy through lactic acid pathway. And under this condition the depletion of protein helps in the maintenance of glucose level through the pathway involving GOT and GPT activity for the transformation of amino acids through transamination to glucose. The author is also happy to inform here that the activity of GOT and GPT has been increased in the present experiment. This finding supports protein depletion and their transformation to glucose through transamination process of amino acid, which caused increase in the level of glucose. The increased glucose level was also recorded here in the present experiment.

The toxic effect of pollutant on lipid fractions/ total lipid content was noted. The concentrations of lipids under references in the present experiments were not significant but effect was there. This effect of lipids may be viewed under the facts that long term effect of pollutants caused depletion in its level which was here in the case of test fish and is found to be true, because the test fish constantly living under the stress of multiple pollutants in the water of river Ganga.

The cholesterol level was also noted to be depleted. This was also the result of action of multiple pollutants in the test fishes for long exposure while living in the polluted water. So this was nothing but the toxic effects of multiple pollutants in test fishes, which brought about decrease of cholesterol concentration is muscles and liver.

Due to continuous living in the stress condition some of the constituent units from the depletion of lipid and cholesterol took the pathway of production of energy through Krebs cycles.

5. Conclusion

In nutshell, it can be concluded that the increased LDH activity and lactate accumulation was observed in muscles of fishes, *Labeo rohita* and *Catla catla* exposed to multipollutants by inhibition of SDH activity in the Kreb's cycle. This shifts anaerobic glycolysis with the concomitant recycling and utilization of its end product viz., lactic acid to maintain the energy potentials. These changes in metabolism facilitate the way for the better adaptation of the fish to reduce the toxic effect when exposed to environmental pollutants.

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