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Fish White Muscle as Biomarker for Riverine Pollution*

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Abstract.- An endangered South Asian freshwater fish, *Tor putitora* netted from polluted part of River Kabul was studied for various muscle biochemical parameters and was compared with control fish caught from non polluted Warsak Dam water reservoir to investigate the remedies caused by the ambient pollution in the fish health. Fish muscle was analyzed for various biochemical parameters like total protein, soluble protein, total cholesterol, total lipid, glucose, free amino acid, DNA, RNA and free fatty acids and enzymes such as amylase, GOT, GPT and LDH. The muscles showed an increase in total muscle proteins (11.8% and 89.6%), total lipid (406.8% and 119.6%), DNA (18.7% and 18.8%) in sample 1 and 2 respectively. Soluble protein, however, remained unaffected in sample 1 but showed a drastic 77.35% increase in sample 2. The muscles showed a decrease in total cholesterol (17.22% and 12.43%), glucose (41.26% and 51.00%) and RNA (23.95% and 40.04%) in sample 1 and sample 2 respectively. However, free amino acids decreased 12.14% in sample 1 and increased 31.3% in sample 2. Similarly free fatty acids decreased 53.5% in sample 1 but showed 97% higher value in sample 2. Among enzymes amylase (36.3% and 42.8%), LDH (21.8% and 45.8%) increased, likewise GOT activity (18.72% and 43.34%) and GPT (45.82% and 48.6%) decreased in sample 1 and 2, respectively, as compared with that of control fish. The increase and decrease in various biochemical parameters and enzymes in the white muscle of test fish samples in comparison with the control, reveals the adverse effect of aquatic pollution on the inhabitant fish health. Pollution stress could be one of the reasons of rapid decline in population of this South Asian endangered freshwater fish.

Key words: Biochemistry, disenzymia, hyperglycemia, hypolipemia, hypercholesterolemia, hypoproteinemia

INTRODUCTION

River Kabul originates from Paghman Mountains in Afghanistan and enters Pakistan at Shalman in the Khyber Agency. Inside Pakistan it flows into Warsak Dam. Below the dam the river is divided into three main channels and several canals irrigating various Districts of NWFP. The city of Peshawar is close to a branch of the River with a population of more than one million. Other large and small towns and a large number of villages are situated at the banks of the River.

About fifty-four fish species have been identified from River Kabul and its tributaries (Rafique, 2001) of which about thirty five are considered as common. The main commercial species are Mullee *Wallago attu* (Schneider, 1801), Sheermai spp. *Ompok bimaculatus* (Bloch, 1794) and *Ompok pabda* (Hamilton, 1822), Gulfam *Cyprinus carpio* (Linnaeus, 1758), Swati *Schizothorax* spp. like *Schizothorax richardsonii plagiostomus* (Heckel, 1838), *Schizothorax progastus labiatus* (MeClleland, 1842), *Schizothorax esocinus* (Heckel 1838), Singhara *Aorichthys seenghala* (Sykes, 1841), Torki *Labeo dyocheilus pakistanicus* (Mirza and Awan, 1976), and Chinese grass carp *Ctenopharyngodon idella*. Mahaseer is hardly 2% of the total catch.

Mahaseer, the king of river fish is both resident and migratory through the river it is found in rivers and streams of hilly regions of Indo-Pakistan sub-continent. The fish has oblong, compressed and streamlined smart body. Anteriorly the head is pointed and behind the anal fin the body becomes narrow. It weights about 10 to 20 kg. It inhabits clear and cold waters of hilly rivers and streams with stony beds, all over Pakistan except northern areas and western Balochistan at an elevation of 200-2000 meter. It has been reported in the River Chenab upto Head Marala, in River Jhelum upto Head Mangla and in River Indus upstream from Kalabagh upto Besham including Tarbela Dam, in River Kabul upto Bagram near Charikar in Afghanistan, in River Swat upto Bagh Dheri. It has also been reported from lower parts of River Panjkora, River Chitral and from Bara River (Mirza, 1986, 2001; Mirza and Alam, 2000).

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It is omnivorous and feeds on aquatic weeds, molluscs, crustaceans, insects and some small trash fishes like *Securicula gora*, *Barilius vagra*, *Crossocheilus latius diplocheilus* etc. (Subhan and Hafeez, 1994). The growth rate of this fish is quite slow. According to Tandon and Johal (1996), it takes about ten years to grow upto 62.6 cm and about 15 years to grow upto 91.97 cm in length.

A survey in NWFP lists about 80 industrial units discharge their untreated effluents directly or indirectly into the river. Among these are: sugar mills, distilleries, ghee (edible oil) factories, textile mills, woolen mills, tanneries, paper and board mills, chemical and pharmaceutical factories, match factories, soap industries, petroleum refinery, photo laboratories, paint and varnish industries and rubber and plastic industries. Unluckily all the above units are without effluent treatment facilities and the effluents from the above units end up in the river, either directly or indirectly through canals or nullahs. These pollutants have not only deteriorated the river water but the sub-surface water of the area as well (IUCN, 1994; Khan *et al*., 1999a; Akif *et al*., 2002). About 12 tanneries discharge their effluents in River Kabul. These effluents contain heavy sediment load, toxic metallic compounds, chemicals, biologically oxidisable materials and large quantities of suspended matter. These effluents with high BOD are responsible for the depletion of dissolved oxygen of the receiving water-body and thus seriously affecting the aquatic life. Such water if used for irrigation causes increased salinity of the soil (Nasreen *et al*., 1995). Effluents from tanneries/leather industries in NWFP drained to River Kabul were studied by Nasreen *et al*. (1995) and found high load of solids, COD, phenols, chromium and sulfides besides being highly colored. Jan *et al*. (2002) studied effluents of selected industries located at small Industrial Estate, Kohat Road, Peshawar and found higher concentrations of TSS, Fe^{3+}, Mn^{2+} and Cr^{6+}. Higher values of the above parameters are of great concern because finally these effluents are drained into River Kabul.

To examine the toxic effects of the fore-said effluents and city sewage on the inhabitant fish population *Tor putitora* being declared endangered by IUCN was selected and analyzed for various muscle biochemical parameters and enzymes.

MATERIALS AND METHODS

Fish samples were collected from two sites (1 and 2) of the polluted portion of the River and were compared with control samples taken from the Warsak Dam reservoir (site 3).

Collection of fish samples

Fishing was done during late night with the help of professional local fishermen. Gill nets (Patti) made locally of nylon were used for fishing as fish gear. Two fish samples were collected from the highly polluted belt of the Main River. One fish sample was collected from the Main River upstream Nowshera-Mardan Road Bridge (Site 1), while the second fish sample was taken downstream Nowshera-Mardan Road Bridge (Site 2) with a distance of 3 km. Both the above samples were considered fish samples from polluted water (test fish sample) and were compared with the third fish sample (control fish sample) collected from the non polluted Warsak Dam (Site 3) about 60 km upstream the polluted part of the River Kabul.

Preservation of fish sample

A portion of fish muscle was taken, washed with distilled water, and shifted to properly marked sterilized polythene bags and then stored in freezer (at –20°C) for further analyses.

Processing of muscle tissue

Muscle tissue after thawing were cut with razor, washed with distilled water and blotted with blotting paper. A weighed portion (about 3 grams) of muscle was homogenized in 3 ml ice-cold saline (0.89% NaCl) solution for saline extract and 3 ml ethanol for ethanol extract in a motor driven teflon glass homogenizer. The homogenate was centrifuged at 4,000 rpm (3,500 ×g) for 45 minutes at 5°C in a refrigerated centrifuge (DAMON/IEC DPR-6000) to get a clear saline supernatant and for 15 minutes at 5°C at the same speed for ethanol supernatant. Aqueous muscle extract in ice-cold saline was used for the estimation of enzymes like glutamate oxaloacetate transaminase (GOT),

glutamate pyruvate transaminase (GPT), amylase, lactate dehydrogenase (LDH) and other biochemical parameters like glucose, free amino acids (FAA) and protein (total and soluble) contents. For the estimation of cholesterol, total lipids and free fatty acids ethanol extract was used, which was extracted from separate, weighed muscle tissue. Total protein content was estimated from the tissue processed for nucleic acid estimation. For this purpose the pellet obtained after extraction of DNA and RNA was crushed with 2.5 ml of 0.5 N NaOH to solublize the protein fraction for estimation.

Biochemical analysis of fish muscle

Muscle ethanol and saline extracts were used for the estimation of the following biochemical parameters to assess the effect of water pollution on the fish muscle. Total and soluble proteins were determined by the method of Lowry *et al*. (1951), cholesterol according to the method of Liebermann and Burchardt described by Henry (1964), glucose contents were determined by O-toluidine method of Hartel *et al*. (1969), free amino acids by the method of Moore and Stein (1954), total lipid by the method of Zöllner and Kirsch as described by Henry and Henry (1974). Nucleic acids were extracted according to the method reported by Shakoori and Ahmad (1973). DNA and RNA contents were estimated according to the method mentioned by Schneider (1957).

Nucleic acid contents of liver tissue were extracted by the method described by Shakoori and Ahmed (1973). Weighed amount of liver tissue was crushed in boiling ethanol. 2-3 washings in methanol: ether (3:1 mixture) followed by three washings in ethanol. The crushed tissue was then desiccated over dry calcium chloride as a desiccant in the vacuum for 24 hours. RNA was extracted in 10% perchloric acid (PCA) after keeping at 4°C for 18 hours, while DNA was extracted after keeping in 10% PCA at 65°C for 30 minutes.

Among enzymes GOT and GPT were determined by the method of Reitman and Frankel (1957), LDH activity by the method based on the method of Cabaud and Wroblewski (1958) and amylase activity according to procedure described by Wootton (1964).

RESULTS

Table I and Figures 1 and 2 show the increase and decrease in various parameters in the white muscles of the test fish samples 1 and 2 in comparison with the control fish samples. The muscles showed an increase in total muscle proteins (11.8% and 89.6%), total lipid (406.8% and 119.6%), and DNA (18.7% and 18.8%) in sample 1 and 2, respectively. Soluble protein, however, remained unaffected in sample 1 but showed a drastic increase (77.35%) in sample 2. The muscles showed a decrease in total cholesterol (17.22% and 12.43%), glucose (41.26% and 51.00%) and RNA (23.95% and 40.04%) in sample 1 and 2, respectively. However, free amino acids decreased 12.14% in sample 1 and increased 31.3% in sample 2. Similarly free fatty acids decreased 53.5% in sample 1 but showed 97% higher value in sample 2.

Among enzymes amylase (36.3% and 42.8%), LDH (21.8% and 45.8%) activities increased, likewise GOT activity (18.72% and 43.34%) and GPT (45.82% and 48.6%) decreased in sample 1 and 2, respectively, as compared with that of fish in control water.

DISCUSSION

Most of the biochemical parameters under investigation showed significant variation in relation to control, showing response to the ambient toxicants. Similarly enzymes also exhibited induction or inhibition in their activities in response to the pollutants.

Tissue biochemical changes can be used as stress indicator of fish health. Fish store energy as either glycogen or lipid and during times of stress, these energy stores are mobilized. Proteins can also be used as an energy source during severe stress (Mayer *et al*., 1992). The analysis of these three types of energy reserves can be used as a general indicator of fish health. Changes in these systems are generally indicative of long-term sub-lethal exposure to a stressor (Mayer *et al*., 1992). Biochemical responses can be affected by environmental factors, such as water pollution, temperature, age, disease, nutritional status, and seasonal changes (Lohner *et al*., 2001).

Table I.- Biochemical components of muscles of *Tor putitora,* caught from Warsak Dam (control) and two sites (site 1 and site 2) of polluted part of River Kabul.

Parameters	Control (n=6)	Site 1 (n=5)	Site 2 (n=5)
Total protein (mg/g)	155.61±1.63*	174.00±27.89	164±2.53*
Soluble protein (mg/g)	44.81±0.99	43.80±2.40	79.4±1.89***
Total cholesterol (mg/g)	3.39±0.13	2.8±0.11**	2.96±0.24
Total lipid (mg/g)	10.14±0.98	51.39±6.34***	22.27±2.48***
Glucose (mg/g)	16.4±4.79	9.64±2.56	8.03±0.46
Free amino acid (mg/g)	0.45±0.05	0.398±0.07	0.59±0.09
DNA (mg/g)	1.16±0.16	1.38±0.19	1.38±0.10
RNA (mg/g)	6.45±0.25	4.91±0.26**	3.9±0.27***
Free fatty acids %	0.43±0.1	0.20±0.07	0.85±0.12*
Amylase (IU/g)	31.61±1.22a	43.08±3.71*	45.14±4.29**
GOT (mIU/mg)	191.5±7.31	155.6±22.68*	108.52±10.67***
GPT (mIU/mg)	654.76±21.7	354.8±18.58***	336.5±16.22***
LDH (IU/g)	5833±317.6	7106±767.5	8551±207.4***

[a]Mean±SEM; Student's 't' test; *P<0.05, **P<0.01; ***P<0.001

For statistical significance enzymes in muscle of test fish samples has been compared with control.

Abbreviations used: DNA, deoxy ribonucleic acids; RNA, ribonucleic acids; LDH, lactate dehydrogenase; GOT, glutamate oxaloacetate transaminase; GPT, glutamate pyruvate transaminase; CPK, creatine phosphokinase. Enzyme units: IU (international unit), transformation of one micromole of substrate in one minute under the conditions of the test.

Control, fish sample from non polluted Warsak Dam; Site 1, polluted portion of River Kabul upstream Nowshera-Mardan Road bridge; Site 2, downstream to Site 1 where Nowshera city sewage also joins the main river.

Total protein concentration increased in both the test samples in the present investigation. The increase in total protein contents shows the acceleration of protein synthesis under the influence of toxicants in response to stress situation. In a similar study Lohner *et al*. (2001) have also reported such tissue protein increase and have attributed with increasing metal levels, possibly due to the synthesis of proteins to sequester the metals.

Soluble protein decreased in sample 1, while increased in sample 2. Similarly free amino acids also decreased in sample 1 and increased in sample 2. The decrease in free amino acids reveals either their incorporation into proteins or their utilization as energy source through the process of gluconeogenesis.

In muscle too like liver, carbohydrates are the main source of energy, as the total proteins, free amino acids and soluble protein contents increased in sample 2. However, decreased free amino acids and soluble proteins in sample 1 showed a shift from carbohydrates to protein as source of energy.

The free amino acids, which normally would be metabolized through kreb's cycle, due to enzyme inhibition, start accumulating in the cells, as is clear from decreased activities of GOT and GPT. This can be correlated with increase in free amino acids concentration in sample 2. Secondly, increased level of amino acids could also be due to its increased absorption from the intestine under the effect of toxicants (Iqbal, 1988). Thus the decrease in amino acids concentration in sample 1 could logically be correlated with decreased absorption from the intestine.

Mujeeb (1985) reported a significant decrease in free amino acids and soluble protein contents after feeding *Cirrhina mrigala* with lead for a period of one week. Same decrease in free amino acids and soluble protein again was reported after feeding lead for a period of two, three and four weeks. In a similar study Mujeeb (1985) also reported a decrease in soluble protein concentration after feeding *Cirrhina mrigala* with lead (0.25 g/ml, 1.0 g/ml) for a period of six weeks and an increase in soluble protein after feeding same dose of lead for a period of seven weeks. In another study of similar nature Shakoori *et al*. (1994) noted an increase in soluble protein concentration after 6 hour feeding of mercuric chloride mixed diet to *Ctenopharyngodon idella*, but later on a significant decrease was

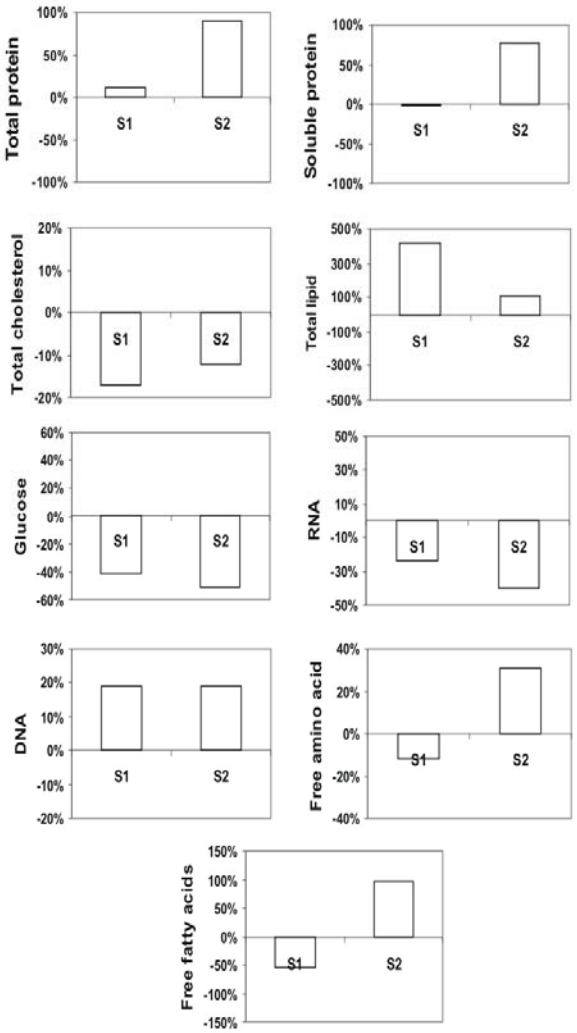

Fig.1. Biochemical responses in the muscle of *Tor putitora* showing % increase (+) or decrease (–) captured from two polluted sites (S1and S2) of River Kabul. Abbreviations used: S1, *Tor putitora* sampled from polluted site 1 of River Kabul upstream to Nowshera- ardan Road Bridge; S2, *Tor putitora* sampled from polluted site 2 of River Kabul downstream to Nowshera-Mardan Road Bridge. DNA, deoxy ribonucleic acids; RNA, ribonucleic acids.

observed after 12, 14 and 18 hour exposure to the toxicant. Soluble protein concentration here can be correlated with time of exposure to the toxicants. This can better explain the decrease of soluble protein in test sample 1 and again an increase in test sample 2, being of more age comparatively.

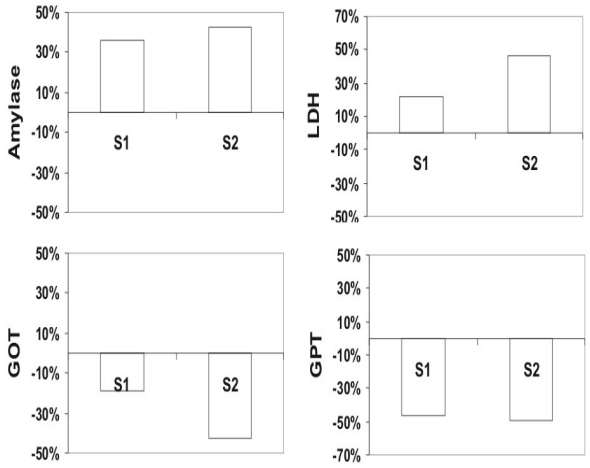

Fig.2. Biochemical responses in the Muscle of *Tor putitora* showing % increase (+) or decrease (–) captured from two polluted sites (S1 and S2) of River Kabul.
Abbreviations used: S1, *Tor putitora* sampled from polluted site 1 of River Kabul upstream to Nowshera-Mardan Road Bridge; S2, *Tor putitora* sampled from polluted site 2 of River Kabul downstream to Nowshera-Mardan Road Bridge.
GPT, glutamate pyruvate transaminase; GOT, glutamate oxaloacetate transaminase; LDH, lactate dehydrogenase

Significant increase in total protein and total lipid in both the samples from polluted water again signals the effect of ambient toxicants as a result of which the metabolism of these two energy reserves is hindered. The depletion of soluble protein and free amino acids in sample 1 confirms the hindrance of protein metabolism by its relative enzymes. The soluble protein seems to be more sensitive to aquatic pollutants. It decreased in sample 1, while significantly increased in sample 2. This suggests that soluble proteins are prominently affected as compared to total protein.

Increase of total protein and glucose in the muscle indicate the presence of metabolic disorders. It seems that the ambient toxicants interfered with the normal metabolic processes occurring in the body. This indicates a very extensive list of conditions including diabetes mellitus, renal failure, hepatic failure like hepatitis etc.

Increase in protein and lipid contents

confirms the presence of hyperproteinaemia and hyperlipaemia. The chemical composition of fish is known to vary with the season in addition to differences in sex, individual, age and body parts. A marked variation is usually shown for lipid contents during spawning period. Also changes in lipid contents of fish are related to increasing and decreasing water temperature. Free fatty acids decreased in sample 1 and increased in sample 2. Increase in free fatty acids in sample 2 correlates with the increase in lipid content in the same sample.

Decrease in glucose level in the muscle reflects the hypoglycemic condition which most probably could be because of renal failure to reabsorb 100% glucose and its redistribution to the tissue cells via the blood capillaries. Possibly the renal impairment could be due to the toxic effects of heavy metals present in the ambient habitat. There are many such reports about renal tubules impairment due to metal intoxication. Renal dysfunction has been reported in children (Friberg *et al.*, 1979). Lead contaminated whisky may also show impairment of the renal tubular transport mechanism (NAS, 1972). Gill and Pant (1983) noted hypoglycemia and diminishes cholesterol levels and Dubale and Shah (1981) found decreased protein in fish chronically exposed to cadmium. Sastry and Sunita (1983) noted similar changes in fish chronically exposed to chromium.

Total cholesterol decreased significantly in both the treated samples which signals the hypocholesterolemic conditions due to metal intoxication. Chromium has been reported for conspicuous changes in blood and tissue metabolite levels with acute and chronic poisoning and it causes hyperglycemia, glycogenolysis in the brain and liver (Pant and Gill, 1984; Campanella *et al.*, 1987; Ginter *et al*., 1989; Gauglhofer, 1984; Muller *et al*., 1989). Decrease in total cholesterol level in both the samples can also be correlated with the inhibition of protein metabolism and switching on the energy production source to some other metabolite. Significant decrease in cholesterol level may also be due to decreased synthesis, which in turn may be due to the lack of cholesterol starting material (acetyl co-enzyme A) partly in the glucose deficient environment and partly due to extra needs of energy for the body to detoxify the toxic compounds, as also stated by Ali (1989).

DNA content in the present study remained unaffected, while RNA content increased significantly. DNA seemed to be resistant to the ambient toxicants. Significant increase in RNA can be logically correlated with the significant increase in protein content. Decrease in muscle RNA and increases in DNA contents are also reported after feeding lead to freshwater fish, *Cirrhina mrigala* (Mujeeb, 1985).

In another study of similar nature the effect of exposure to sub-lethal concentrations of cypermethrin, a synthetic pyrethroid pesticide, muscle of the Indian major carp, *Labeo rohita* was studied. RNA levels decreased, while DNA levels were elevated in muscle homogenates (Das and Mukherjee, 2003). The above study correlates with our study in relation to increase in DNA and decrease in RNA content. In another study by Das and Mukherjee (2003) on *Labeo rohita* a decrease in RNA and increase in DNA level was observed after feeding cypermethrin a synthetic pesticide.

Besides biochemical components the activities of various enzymes have also been affected by the ambient toxicants. Among the muscle enzymes, certain enzymes like GOT and GPT were inhibited in both the treated samples while others like amylase and LDH were inducted showing dysenzymic situation. Amylase activity significantly increased both in sample 1 and in sample 2. GOT activity significantly inhibited both in sample 1 and in sample 2. GPT activity inhibited in both sample 1 and 2. LDH activity increased in sample 1 and significantly increased in polluted sample 2.

The main cellular component to be effected in the result of ambient toxicants was the cell membrane. Ambient toxicants either have increased the membrane permeability causing enhanced leaching out of the enzymes, or reduced the permeability forcing the enzymes to accumulate in the cells. Moreover, cellular damage was another reason for decreased synthesis of enzymes in the living organisms.

Alterations in enzymes are direct measures of histopathological effects in the secretary cells, reflecting dysenzymia. This better explains the

induction of amylase and LDH and inhibition of GOT and GPT activities, which occurred in the present study. Inhibition of GOT and GPT in the present study shows the great inhibition of metabolic activities and blockage of protein metabolism, while increase of LDH shows the compensation of energy from carbohydrates, as the protein metabolism is hindered. Increase in cellular protein and depletion of glucose better support the phenomenon. This is also supported by Shakoori and Ali (1986) in a previous report and later on by Iqbal (1988). The administration of chromium compounds has been reported to produce inhibition of the activities of a number of enzymes and cellular degeneration (Pant and Gill, 1984; Fan, 1987; Mormo, 1987). Rainbow trout, *Oncorhynchus mykiss* exposed to different concentrations of aniline chlorohydrate for 15 days showed decreased GOT activity and increased GPT activity. It was suggested that the increase in GPT activity was due to an increase in cellular permeability. Same decrease in both the transaminases was reported after feeding mercury to promethazine pretreated rabbits (Anjum, 1991). In one of the previous studies sublethal toxicity of sodium arsenite was investigated in teleost fish, *Tilapia mossambica*. The GOT and GPT activities were inhibited (Shobha *et al*., 2001).

The low activities of transaminases could be due to heavy metal impaired cell membrane permeability, which results in leakage of enzymes in general circulation. Other possibility is decreased synthesis of the enzymes in the secretory cells, thus decreasing their concentration inside the cell. Enhanced excretion of enzymes in the urine is another possibility of decreased concentration in the cells as reported by Gumbleton and Nicholls (1988). Low activity could also be due to defective or inactive enzymes, which were unable to catalyze their reactions (Sastry and Sunita, 1983). Probably the transaminases are denatured or inactivated by the ambient toxicants.

However, increased activities of enzymes like amylase and LDH in the tissue could be because of increased secretion by the specialized cells or by changes in the permeability of their cell membranes to stop leach out of the enzymes and keep stored in the cells as already stated by Shakoori and Aziz (1991) and Shakoori and Jaferi (1991). LDH is also raised in acute liver damage. Increase in amylase could be because of pancreatitis.

Decreased levels of these enzymes exhibit heavy metals interference in protein metabolism. It is reported that due to chromium intoxication, the metal is taken up by the plasma and protein fractions and binds with iron binding protein, transferrin (Langard, 1983; Zober *et al*., 1984). Heavy metals are transported and distributed by the blood stream and deposited in different organs and tissues, which interferes with their normal activities.

Amylase activity is increased in muscle in both the treated samples. Exocrine portion of the pancreas is responsible for the secretion of amylase enzyme. Amylase is a neutral enzyme with pH 6.5 and is responsible for the digestion of carbohydrates. The increased activity or level of this enzyme in the body may be due to acute pancreatitis. The second possibility of increased level in the body may be due to increased synthesis in the intestine due to environmental stress, which consequently enhanced starch digestion and transferred itself and the degradation products into the circulatory system and then into muscle cells through assimilation. Ali (1989) has reported a significant increase in amylase activity after feeding rat with aldrin mixed diet (2.5 mg/kg body weight/day) for 6-18 months.

LDH is an active enzyme of glucose metabolism and any effect on its activity directly affects the energy production of the cell. LDH activity indicates the switching over of anaerobic glycolysis to aerobic respiration. The presence of LDH in the muscle is important for both rapid production of lactate as the anaerobic end product of glycolysis and for the conversion of lactate into pyruvate as substrate for gluconeogenesis (Batty and Wardle, 1979).

LDH activity in both the treated samples in the present study have increased significantly, showing either induced synthesis or leakage of the enzyme through the damaged membranes. Level of LDH reflects cytotoxicity. Many investigators reported significant increase in LDH level after heavy metal administration (Itoh and Ozasa, 1985; Siegel, 1988; Wang *et al*., 1989). In another study, freshwater fish, *Labeo rohita* after exposure to a

lethal and sublethal concentration of cypermethrin exhibited elevation in LDH activity (Philip *et al*., 1995). The increase in LDH activity after mercury treatment to freshwater fish is also reported by Stokinger (1980). Many investigators reported an increase in LDH activity after exposing freshwater fish to various pollutants (Mujeeb, 1985; Shakoori *et al*. 1994; Asztalos *et al.*, 1988, 1990).

CONCLUSION

The presence of disenzymia, hypoglycemia, hyperglycemia, hyperlipemia, hypocholesterolemia, hyperproteinemia in the white muscles of the fish under investigation reveals the disturbance of biochemical processes occurring in the fish body by the ambient pollutants, thus disrupting the fish health, growth and more obviously the fish population in the river.

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