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Study on the Impact of Groundwater-Minerals of Urban-Dhubri on Transamination and Mobilization of Thyroid Hormones in Liver of *Heteropneustes fossilis* Bloch

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ABSTRACT

This study coved observations of Lipid Peroxide (LPO) in liver, Aspartate Transferase (AST) and Alanine Transferase (ALT) activities in liver and blood serum; amount of Essential and Non-essential amino acids in liver; Tri-iodothyronine (T_3), Tetra-iodothyronine (T_4) and Thyroid Stimulating Hormone (TSH) in liver and blood serum of Heteropneustes fossilis Bloch exposed to sublethal concentrations of groundwater-minerals of Urban-Dhubri.

LC_{50} values (for 96 hours) for the ground minerals separated from waters of Balur Char area was determined as 61.8 ± 0.39 ppm, for those of D. K. Road was 47.4 ± 0.27 ppm and for those of R. K. Mission Road was 66.9 ± 0.28 ppm. Minerals from the water of R. K. Mission Road was found to be the most toxic and studies carried using the groundwater minerals of R. K. Mission Road.

The study revealed that sub-lethal exposure to groundwater mineral lead to increased Lipid Peroxidation in liver, decreased activities of AST and ALT in liver and increased activities of these enzymes in blood serum. Hypothyroidic condition with decreased T_3 and T_4 contents and increased TSH content were observed both in liver and blood serum on treatment. Increased amount of essential amino acids and decreased amount of non-essential amino acids were marked in liver on exposure.

Key words: *Exposure, Fish, Lipid-peroxide, Transamination, Hypothyroidic.*

INTRODUCTION

The Urban Dhubri is a highly populated area and the only source of drinking as well as washing-water in the urban and semi-urban areas of the town is the ground-water. Though the Mighty Brahmaputra is running through the south-eastern corner of the town, the water of the river remained un-used for the mentioned purposes. The Urban Water-Supply and Sewerage Board though covers the main areas of the town, the water-supply process is yet to begin. If near future it will work, it will go to use the ground-water as per information. The Public Health Engineering Department is also supplying some sub-urban areas of the town, totally depending on ground water.

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The ground-water of the urban and semi-urban Dhubri is highly loaded with various salts of iron, sulphur, calcium, carbonates, bicarbonates and so many unknown compounds. Fluorides and arsenicals may also be present. Detailed information regarding these components is still now very fragmentary. However, the water is usable only after "Sand-Stone-Charcoal Filtration", which been practiced from long back by the domestic, commercial and industrial users. After filtration these compounds have been left in the bare places along with the filtrating materials (i.e. - Sand, Stone and Charcoal) and in the rainy season these are washed out and carried to the water-bodies (the wet-lands). The ground minerals separated during filtration of the Urban Dhubri are thought to be toxic to aquatic animals. This work was designed to study on the impact of groundwater-minerals of Urban-Dhubri on liver, the main metabolic organ of *Heteropneustes fossilis* Bloch on exposure to sub-lethal concentration of the separated groundwater minerals, as this fish is a very resistant representative of the aquatic animals.

Collection of ground-waters from 3 different corners of Urban Dhubri (namely- Balur Char, D. K. Road and R. K. Mission Road) was done and ground minerals were separated. LC_{50} values (for 96 hours) for the species were determined on exposure to these. The experimental fishes were exposed to 2 sub-lethal concentrations of ground-water minerals against Normal-Control Group. Study covered investigation of lipid peroxide in liver, Study on transamination in liver and mobilization of thyroid hormones from blood to liver.

In intoxication, free radicals including $H^{\cdot}$ & $OH^{\cdot}$ are formed which in turn bring lysis of the lipid bi-layer of the cell membrane by oxidative degradation of lipids. It is the process in which free radicals "steal" electrons from the lipids in cell membranes, resulting in cell damage. This process proceeds by a free radical chain reaction mechanism. It most often affects polyunsaturated fatty acids, because they contain multiple double bonds in between which methylene $-CH_2-$ groups lie, that possess especially reactive hydrogen molecules. This phenomenon is known as "lipid peroxidation". As a result of this, the cells of soft tissues like liver are destroyed and the contents of the cell are released to the body fluid (serum)[1]. Study of lipid peroxide in liver homogenate of the experimental fishes were done in this study.

Aspatrate Transaminase (AST) also known as Glutamate Oxaloacetate Transaminase (GOT) and Alanine Transferase (ALT) also known as Glutamate Pyruvate Transaminase (GPT) in liver homogenate and blood serum. The activities of these primary transaminase enzymes- are mainly localized in the hepatocytes. These are the main catalysts of conversion of nonessential amino acids from essential amino acids in this main metabolic organ[2]. Hepatocyte destruction increases their level of activity in blood serum. Reduction of these enzyme-activities in tissues like liver may hamper the normal process of transamination in the organ. Activities of these two primary transaminases were investigated in the liver homogenate as well as blood serum was done.

As per the claim of the medical practitioners here, most of the people of this locality are the victims of hypothyroidism and impurity of the groundwater is thought to be responsible for this. Similar case may happen to aquatic vertebrates including fishes. So, study on mobilization of Thyroid Stimulating Hormone (TSH) and Thyroid Hormones (T3 and T4) in liver in relation to blood were done in this study, as these hormones are of prime importance in synthetic processes, growth and repair of the tissues[3,4,5].

MATERIAL AND METHODS

Separation of the ground minerals from the collected ground-water samples from 3 different corners of Urban Dhubri namely- Balur Char, D. K. Road and R. K. Mission Road were done by gravitation-created centrifugal force in sand-charcoal filter. Separated minerals were washed out with sufficient amount of distilled water and

precipitated again by centrifugation from the water. LC_{50} values for the fish species were (for 96 hours) for the species were determined on exposure to these[6]. LC_{50} values were found to be 61.8 ± 0.39 ppm, for those of D. K. Road was 47.4 ± 0.27 ppm and for those of R. K. Mission Road was 66.9 ± 0.28 ppm. Minerals from the water of R. K. Mission Road was found to be the most toxic and studies carried using the groundwater minerals of R. K. Mission Road.

Healthy adult fishes of either sex were collected from local vendors and treated with 1.5% $KMnO_4$ solution and kept in an aquarium for an week for acclimatization, feeding with commercial fish–food prepared from "Dried Spirulina, Daphnia and Mysis". Later on they were divided into three groups and reared in three aquaria namely Aquarium-I comprising Normal-Control Group, Aquarium –II and III comprise Experimental Groups of fishes exposed to 15 ppm/L and 25 ppm/L separated groundwater minerals. After the experimental period of four weeks fishes were sacrificed by diethyl ether anesthetization. Blood samples were collected by cutting caudal fins and allowed to clot keeping in separate labeled micro-centrifuge tubes. The separated sera of respective blood samples were then 30 times diluted with normal saline and centrifugation at 5000 R.P.M. The supernatants were collected in separate labeled micro-centrifuge tubes and kept in deep freeze. Livers of the study animals were dissected out, washed with normal saline and kept in deep freeze in separate labeled vials. Later on, measured amount of liver samples were homogenized with fixed amount of deionized water. The homogenates were centrifuged at 5000 RPM and supernatants are collected for enzyme and hormone assays and kept in deep freeze. For chromatography of free amino acids, the sample preparation was done in similar way, but with use of 80% ethanol in place of deionized water. The biochemical investigations and assays were done within a few hours of preparation of the samples.

Measurement of Liver Lipid Peroxidate (LLPO) was done by the photometric evaluation of molar extinction co-efficient of thiobarbituric acid[7].

AST activity was measured by using AST (GOT) reagent kit based on UV-Kinetic Enzyme Assay Technique[8]. ALT activity was measured by using ALT (GPT) reagent kit based on UV-Kinetic Enzyme Assay Technique[9].

Separation of the free amino acids were done by Thin Layer Chromatography using Ethanol: 25% Ammonium Hydroxide: Deionized Water (18:1:1) followed by n-Butanol: Glacial Acetic Acid: Deionized Water (13:3:5) and separated amino acids were estimated by Ninhydrin-Photometric Assay[10]. Measurement of the amount of TSH both in liver homogenate and blood serum were done by Enzyme Immuno Assay technique[11].

Measurement of the amount of T3 both in liver homogenate and blood serum were done by Enzyme Immuno Assay technique[12].

Measurement of the amount of T4 both in liver homogenate and blood serum were done by Enzyme Immuno Assay technique[13].

Thiobarbituric acid is procured from CDH (India) LTD. AST (GOT), ALT (GPT), TSH, T3 and T4 assay kits were procured from Benesphera™ (A division of Avantor Preformance Materials India LTD.). Standard Amino Acid Kit was procured from Loba Chemie LTD and Ninhydrine was procured from E. Merck LTD. The other regents and chemicals are procured from Benesphera-Ranchem, (India) LTD.

The photometric observations and biochemical assays were done in a semi automated biochemistry analyzer ("Benesphera C-61" manufactured by Benesphera - Avantor Preformance Materials India LTD.). ELISA well-plate readings were done in an ELISA Reader ("Benesphera E-21" manufactured by Benesphera - Avantor Preformance Materials India LTD.) Necessary kit specifications and dilution factors were preprogrammed in the machines.

RESULTS

Results obtained so far were analyzed statistically[14] with the help of Microsoft Excel and presented in the following tables:

Table 1: Liver lipid peroxide; transaminase activities and amount of thyroid hormones in liver and blood serom of experimental fishes

Study Parameters	Experimental Fish Groups		
	Group-I Normal-Control Group	**Group-II Fishes exposed to 15ppm/L of Ground Minerals**	**Group-III Fishes exposed to 25ppm/L of Ground Minerals**
Lipid Peroxide in Liver (n mol/mg)	12.938 ± 0.011	15.267 ± 0.077 + 18.001% *	22.096 ± 0.149 + 70.783% *
AST (GOT) activity in Liver (IU/L)	578.41 ± 0.408	523.99 ± 0.586 -9.408% *	487.45 ± 0.181 -15.726% *
AST (GOT) activity in Blood Serum (IU/L)	432.59 ± 0.077	497.01 ± 0.360 + 14.863	723.307 ± 0.318 + 67.205
ALT (GPT) activity in Liver (IU/L)	286.58 ± 0.558	267.60 ± 0.649 -6.621% *	223.95 ± 0.658 -21.854% *
ALT (GPT) activity in Blood Serum (IU/L)	39.58 ± 0.050	48.59 ± 0.079 +22.747	72.41 ± 0.067 +82.906
Amount of TSH in Liver (IU/mg)	0.265 ± 0.002	0.286 ± 0.001 + 7.651% *	0.314 ± 0.001 + 18.620% *
Amount of TSH in Blood Serum (IU/mg)	284.58 ± 0.719	312.87 ± 0.835 + 14.156	365.39 ± 0.535 +28.396
Amount of T_3 in Liver (ng/mg)	0.835 ± 0.002	0.773 ± 0.001 - 7.412% *	0.682 ± 0.004 -18.366% *
Amount of T_3 in Blood Serum (ng/mg)	0.97 ± 0.001	0.83 ± 0.002 -13.825	0.55 ± 0.004 -43.728
Amount of T_4 in Liver (ng/mg)	12.547 ± 0.051	11.242 ± 0.260 - 10.593% *	9.425 ± 0.046 - 25.042% *
Amount of T_4 in Blood Serum (ng/mg)	13.59 ± 0.072	12.55 ± 0.082 -7.660	9.547 ± 0.091 -29.729
"*" indicates Significant at p<0.001, "+…%" and "-…%" indicate percent increase and percent decrease respectively.			

Table 2: Amounts of essential and non-essential amino acids in Liver of experimental fishes

Amino acids		Normal-control fishes	Fishes exposed to 5 ppm detergent	Fishes exposed to 10 ppm detergent
Essential Amino Acids	Arginine	1.748±0.008	2.47±0.005 +41.304% *	2.557±0.007 +46.281% *
	Histidine	1.17±0.006	1.243±0.007 +6.239% *	1.32±0.063 +24.017% *
	Isoleucine	0.483±0.008	0.538±0.015 +22.831% *	0.648±0.008 +50.228% *
	Leucine	0.653±0.008	1.238±0.006 +89.59% *	1.354±0.008 +107.351% *
	Lysine	1.551±0.007	1.956±0.007 +26.112% *	2.141±0.006 +38.38% *
	Methionine	1.354±0.006	1.847±0.007 +36.411% *	2.237±0.013 +65.214% *
	Phenylalanine	2.146±0.007	2.697±0.008 +28.006% *	3.066±0.004 +42.870% *
	Threonine	0.747±0.008	0.944±0.006 +26.372% *	1.247±0.006 +66.943% *
	Tryptophan	1.247±0.009	1.853±0.007 +48.596% *	2.164±0.005 +73.536% *
	Valine	3.452±0.008	4.648±0.008 +34.647% *	5.153±0.010 +49.276% *
Non-essential Amino Acids	Alanine	296.660±0.580	278.008±0.410 -6.287 *	256.246±0.676 -13.617% *
	Aspartic acid	66.233±0.183	59.43±0.124 -10.271% *	57.405±0.157 -13.193% *
	Cystine	13.533±0.065	12.546±0.049 -7.293% *	10.490±0.080 -22.486% *
	Glutamic acid	91.585±0.172	87.484±0.067 -4.478% *	84.408±0.138 -6.745% *
	Glycine	112.499±0.352	104.138±0.262 -7.432% *	91.149±0.294 -18.978% *
	Hydroxy proline	43.581±0.097	38.975±0.170 -10.569% *	36.484±0.090 -16.331% *
	Proline	71.534±0.049	66.404±0.086 -7.171% *	58.218±0.172 -18.615% *
	Serine	72.441±0.072	66.380±0.144 -8.367 *	59.332±0.141 -18.097% *
	Tyrosine	19.634±0.047	18.515±0.078 -5.695% *	16.647±0.084 -15.213% *
	Taurine	13.563±0.088	12.586±0.059 -7.203% *	10.355±0.173 -23.653% *
"*" indicates Significant at p<0.001, "+…%" and "-…%" indicate percent increase and percent decrease respectively.				

DISCUSSION

The normal control value of Lipid peroxide in liver was found 12.938 ± 0.011 n mol/mg which increased up to 15.267 ± 0.077 n mol/mg and 22.096 ± 0.149 n mol/mg on treatment with 15 ppm/L and 25 ppm/L of the groundwater-minerals with deviations of 18.001% and 70.783% respectively. These must have worst impact on hepatocyte membranes leading to destruction of those.

The AST (GOT) activity in liver of normal-control fishes was found to be 578.41 ± 0.408 IU/l. Significant decrease ($p<0.001$) of the activity of this enzyme was marked with 9.408% and 15.726% deviations on exposure to 15 ppm/L and 25 ppm/L of the groundwater-minerals respectively. AST (GOT) activity in blood serum of normal-control fishes was found to be 432.578 ± 0.077 IU/l. Significant increase ($p<0.001$) of the activity of this enzyme was marked with 14.863% and 67.205% deviations on exposure to 15 ppm/L and 25 ppm/L of the groundwater-minerals respectively.

The ALT (GPT) activity in liver of normal-control fishes was found to be 286.58 ± 0.558 IU/l. Significant decrease ($p<0.001$) of the activity of this enzyme was marked with 6.621% and 21.854% decrease on exposure to 15 ppm/L and 25 ppm/L of the groundwater-minerals respectively. The ALT (GPT) activity in liver of normal-control fishes was found to be 39.58 ± 0.050 IU/l. Significant increase ($p<0.001$) of the activity of this enzyme was marked with 2.747% and 82.906% decrease on exposure to 15 ppm/L and 25 ppm/L of the groundwater-minerals respectively.

The pictures of these enzyme activitiess in liver and blood serum and the findings of liver LPO indicating huge scale destruction of hepatocytes and release of these transaminases to blood from the liver on exposure of the fishes to the groundwater-minerals even in sublethal exposures.

Significant increase ($p<0.001$) of the amount of essential amino acids in liver are observed in this study. Arginine increases 41.304% and 46.281% , Histidine increases 6.233% and 24.017%, Isoleucine increases 22.831% and 50.228%, Leucine increases 89.59% and 107.351%, Lysine increases 26.112% and 88.039%, Methionine increases 36.411% and 65.214%, Phenylalanine increases 28.006% and 42.870% , Threonine increases 26.372% and 66.934%, Tryptophan increases 48.596% and 73.536% and Valine increases 34.647% and 49.276% from their respective normal control amounts on treatment of experimental fishes with 15 ppm/L and 25 ppm/L of the groundwater-minerals respectively.

Significant decrease ($p<0.001$) of the amount of non-essential amino acids in liver are also observed in this study. Alanine decreases 6.287% and 13. 617%, Aspartic acid decreases 10.271% and 13.617%, Cystine decreases 7.293% and 22.486%, Glutamic acid decreases 4.478% and 6.745%, Glycine decreases 7.432% and 18.978%, Hydroxy proline decreases 10.569% and 16.331%, Proline decreases 7.171% and 18.615%, Serine decreases 8.367% and 18.097%, Tyrosine decreases 5.695% and 18.615%, Taurine decreases 7.203% and 13.653% from their respective normal control amounts on treatment of experimental fishes with 15 ppm/L and 25 ppm/L of the groundwater-minerals respectively.

Significant increase of the amounts of essential amino acids in respect to significant decrease of the amount of non-essential amino acid counterparts in liver are due to hamper in transamination reactions (which mainly occur in liver) catalyzed by transaminase enzymes especially AST (GOT) and ALT (GPT), as these has been released from liver due to huge scale destructions of hepatocytes.

Amount of TSH in liver of normal control fishes were found to be 0.265 ± 0.002 IU/mg which increased up to 0.286 ± 0.001 IU/mg and 0.314 ± 0.001 IU/mg, with deviations of 7.651% and 18.620% respectively on exposure to 15 ppm/L and 25 ppm/L of the groundwater-minerals. TSH content in blood serum of normal control fishes were found to be 0.295 ± 0.001 IU/mg which increased up to 0.326 ± 0.002 IU/mg and 0.357 ± 0.001 IU/mg, with deviations of

10.760% and 21.147% respectively on exposure to 15 ppm/L and 25 ppm/L of the groundwater-minerals.

Amount of T_3 in liver of normal control fishes were found to be 0.835 ± 0.002 ng/mg which decreased up to 0.773 ± 0.001 ng/mg and 0.682 ± 0.004 ng/mg, with deviations of 7.412% and 18.366% respectively on exposure to 15 ppm/L and 25 ppm/L of the groundwater-minerals. T_3 content in liver of normal control fishes were found to be 0.968 ± 0.001 ng/mg which decreased up to 0.834 ± 0.002 ng/mg and 0.545 ± 0.004 ng/mg, with deviations of 13.825% and 43.728% respectively on exposure to 15 ppm/L and 25 ppm/L of the groundwater-minerals.

Amount of T_4 in liver of normal control fishes were found to be 12.574 ± 0.051 ng/mg which decreased up to 11.773 ± 0.001 ng/mg and 9.425 ± 0.004 ng/mg, with deviations of 10.593% and 25.042% respectively on exposure to 15 ppm/L and 25 ppm/L of the groundwater-minerals. T_4 content in liver of normal control fishes were found to be 13.586 ± 0.072 ng/mg which decreased up to 12.545 ± 0.082 ng/mg and 9.547 ± 0.091 ng/mg, with deviations of 7.660% and 29.729% respectively on exposure to 15 ppm/L and 25 ppm/L of the groundwater-minerals.

A hypothyroidic condition was found both in liver and blood serum which might be due to less production of the thyroid hormones in thyroid gland definitely is due to less production of the amino acid tyrosine in liver from its precursor- phenylalanine. Increased amount of TSH might be due to lessening of feedback to pituitary for inhibition.

CONCLUSION

From the analyses of this study it was found that the exposure to the groundwater minerals enhances huge scale destruction of Liver-cells of *H. fossilis* Bloch (due to enhanced lipid peroxidation) even though in very minute amount, if present in the aquatic system. As a result, the contents of the hepatocytes including transaminase enzymes (including AST, ALT etc.) are released to the blood. This plays an adverse impact on the transformation of the essential amino acid to non essential amino acids. As the amino acids are the building blocks of all the structural and the functional proteins (including enzymes, some hormones- including thyroid hormones and protein factors of biochemical pathways), insufficiency of non essential amino acids definitely hamper the normal biochemistry of the exposed fishes; resulting in retardation of growth including damaged tissue, metabolism including reproductive processes even death. As this resistant species is suffered from the exposure to these minerals in sub-lethal amount too, the conditions of the other organisms both in soil and water are definitely more sufferers and it has worst impact to biodiversity in soil and water.

It may be suggested for minimizing use of groundwater both in domestic and official purposes (in Water supply plants by "Urban Water Supply" and "Public Health Engineering Department") and to concentrate on the available, comparatively load-free water of the mighty Brhamaputra, adjacent to the town.

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