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Research Article

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Effect of sub-lethal exposure of detergent on transamination in liver of *Heteropnustes fossilis* Bloch.

Santanu Sarma*
Department of Zoology, Bholanath College, Dhubri-783324, (Assam)
E-mail address: dr.santanusarma111@gmail.com

ABSTRACT

This study deals with the effect of a common laundry detergent (Surf Excel Blue) on transamination in liver of Heteropnustes fossilis Bloch on sub-lethal exposure to it.

Adult fishes of either sexes were reared in three different aquaria namely aquarium-I comprising Normal-Control group, aquarium-II and III comprise Experimental groups of fishes exposed to 5 ppm and 10 ppm of the detergent respectively. Prior to this the LC_{50} of this detergent for this species of fish was experimentally evaluated as 22.51 ± 0.057 ppm. The treatment was done for 30 days in the months of August and September, after which the study on the activities of two primary transaminases namely Glutamate Oxaloacetate Transaminase (GOT) and Glutamate Pyruvate Transaminase (GPT), were done in liver and blood-serum of both the Normal-Control and Experimental fishes. Estimation of the important free amino acids was also done in liver homogenate of the fishes.

The study revealed that sub-lethal exposure to this detergent lead to significant alterations of these transaminase activities and the process of transamination in liver of the experimental fishes.

Key words: *- Detergent, liver, transaminase, amino acid.*

INTRODUCTION

The history of using detergents is as older as human civilization. In Vedic period people used herbal ingredients such seeds of Soap nuts (*Sapindus mukorossi*) for washing garments or hair.[1] From ancient days, chemical additives were recognized for their ability to facilitate the mechanical washing with water. The Italians used a mix of sulfur and water with charcoal to clean cloth. Egyptians added ashes and silicates to soften water. Soaps were the first detergents. The detergent effects of certain synthetic surfactants were noted in Germany in 1917, in response to shortages of soap during World War I. In the 1930s, commercially viable routes to fatty alcohols were developed, and these new materials were converted to their sulfate esters, key ingredients in the commercially important German brand FEWA, produced by BASF, and Dreft, the US brand produced by Procter and Gamble. Such detergents were mainly used in industry until after World War II. By then, new developments and the later conversion of aviation fuel plants to produce tetra-propylene, used in household detergents, caused a fast growth of domestic use in the late 1940s. The use of enzymes for laundry was introduced in the early part of the 1900s by Otto Rohm. Only in the latter part of the century with the availability of thermally robust bacterial enzymes did this technology become mainstream.[2] Laundry detergents of commonly used today mainly comprise oil-based surfactant, sodium carbonate, cellulase, subtilisin (proteolytic enzyme), alkyl – benzene sulfonic acid, sodium salts and oxygen or chlorine bleaching agents.[3] Laundry detergents are one of the main sources of water pollution. The ingredients of detergents or their byproducts are mixed with the aquatic systems either directly on washing the garments, vehicles etc. in the water body or indirectly

with the drainage system. On release to the aquatic systems these ingredients may lead to abrupt alteration of the physico-chemical properties of water and harm the normal physiology of the plant and animals of the system.

Synthetic detergents are reported to be acutely toxic to fish in concentrations between 0.4 and 40 mg/L.[4] Non-ionic and anionic detergents are found in a wide variety of household products, including body soaps, shampoos, dishwashing detergents, various household cleaners, etc. These products are gastrointestinal and ocular irritants with few to no systemic effects. Clinical signs consist of hyper-salivation, vomiting, and diarrhea, and are generally mild and self limiting, although ingestion of large quantities may result in more severe vomiting (+/- blood) in higher beings. Protracted vomiting may also cause dehydration and electrolyte abnormalities. Cationic detergents are contained in fabric softeners, some potpourri oils, hair mousse, algaecides, germicides and sanitizers. Cationic detergents are more toxic than non-ionic/anionic detergents and can cause extensive systemic and local effects at levels as low as 2% or less. Local tissue injury caused by cationic detergents resembles that seen with exposure to alkaline products. In addition, cationic detergents can cause systemic toxicity including CNS depression, coma, seizures, hypotension, muscular weakness and fasciculations, collapse, pulmonary edema, and metabolic acidosis.[5] It has been found that the chronic exposure to four common detergents at sublethal concentration (10 ppm) resulted in reduction of the RBCs (41-58%) in *Gambusia affinis*.[6] Blood characteristics in dielectric, haematological and biochemical terms of the fish *Cyprinus carpio* exposed to a sublethal concentration of sodium alkyl benzene sulfonate were lead to an increase in the electrical conductivity and permittivity of the erythrocytes' interior which was associated with a decline of mean corpuscular haemoglobin.[7] It was also reported that Sodium hypochlorite; a common bleaching agent of detergents enhances Lipid Peroxidation in blood lipoproteins and phospholipid liposomes.[8] A comparative study conducted on the toxicity of some commercial detergents on Nile Tilapia, *Oreochromis niloticus* revealed their adversity to survival of the species.[9] Study on the role of linear alkyl benzene sulfonate using the common detergent “Henko” on *Puntius ticto* revealed the histo-patological lesions on gill archs, rakers and gill filaments.[10] Study on Toxicological impact of house hold detergent “Surf” on digestive tissue on fresh-water fish *Clarius batrachus* (Linn) marked the large scale destruction of the tissues of gastro-intestinal mucosa and liver.[11]

Significant retardation of growth and development in *Lates calcalifer* Bloch larvae along with hypertrophy, hyperplasia, telengeastases, and melanization of the gill was observed on exposure to Alkyl-benzene sulfonate.[12] Congestion and vacuolar degeneration of the liver were also observed on this exposure.

In intoxication, (such as detergent intoxication) free radicals (H^0 & OH^0) 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 -CH2- 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, Kidney and Gill etc. are destroyed and the contents of the cell are released to the body fluid (serum).[13]

The activity of the primary transaminase enzymes GOT (Glutamate Oxaloacetate Transaminase) or AST (Aspartate Transferase) and GPT (Glutamate Pyruvate Transaminase) or ALT (Aspertate Transferase) 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.[14] 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 tranamination in the organ.

Present investigation deals with the effect of a common laundry detergent- “Surf-Excel Blue” on transamination in liver of *H. fossilis* Bloch (on sub-lethal exposure to it), with investigations of the activities of Glutamate Oxaloacetate Transaminase (GOT) and Glutamate Pyruvate Transaminase (GPT)

in liver and blood-serum of both the Normal-Control and Experimental fishes. Estimation of the important free amino acids was also done in liver of the fishes.

MATERIALS AND METHODS

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 7 days 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 5 ppm and 10 ppm of the detergent respectively. Prior to this the LC_{50} of this detergent was experimentally evaluated as 22.51±0.057 ppm. The treatment was done for 30 days in the months of August and September. After the experimental period the 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 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.

GOT activity was measured by using AST (GOT) reagent kit based on UV-Kinetic Enzyme Assay Technique.[15] GPT activity was measured by using ALT (GPT) reagent kit based on UV-Kinetic Enzyme Assay Technique.[16] These reagent kits were procured from Ranbaxy-RFCL, LTD.

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.[17] Standard Amino Acid Kit was procured from Loba Chemie LTD and Ninhydrine was procured from E Merk LTD. The other regents and chemicals were procured from Ranbaxy-Ranchem LTD.

All the photometric observations and biochemical assays were done in a semi automated biochemistry analyzer ("Lab Life Chem-Master" manufactured by Ranbaxy- Diagnova LTD). Necessary kit specifications and dilution factors were preprogrammed in the machine.

RESULTS

Results obtained so far were analyzed statistically[18] with the help of Microsoft Excel and presented in the following tables:

Table-1: GOT (AST) and GPT (ALT) activities in Liver and Blood Serum of experimental fishes

Study Parameters	Experimental Fish Groups		
	Group-I Normal-Control Group	**Group-II Fishes exposed to 5 ppm Detergent**	**Group-III Fishes exposed to 10 ppm Detergent**
GOT activity in Liver IU/L	579.822 ± 3.547	428.456 ± 8.297 -26.106% *	312.547 ± 9.372 -46.096% *
GPT activity in Liver IU/L	284.947 ± 0.856	232.781 ± 0.848 -18.307% *	199.63 ± 1.538 -29.941% *
GOT activity in Blood Serum IU/L	493.719 ± 0.604	567.587 ± 3.064 +14.962% *	871.584 ± 4.466 +76.534% *
GPT activity in Blood Serum IU/L	47.693 ± 0.373	57.023 ± 0.238 +19.563% *	95.416 ± 0.306 +100.036% *

"*" 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	1.67±0.043	2.34±0.071 +40.12% *	2.43±0.056 +45.51% *
	Histidine	1.03±0.028	1.24±0.034 +20.39% *	1.32±0.063 +28.16% *
	Isoleucine	0.32±0.036	0.47±0.048 +46.88% *	0.54±0.072 +68.75% *
	Leucine	0.79±0.043	1.31±0.031 +65.82% *	1.43±0.059 +81.01% *
	Lysine	1.67±0.057	2.07±0.029 +23.95% *	2.34±0.048 +40.12% *
	Methionine	1.48±0.037	2.03±0.042 +37.16% *	2.28±0.098 +54.05% *
	Phenylalanine	2.03±0.042	2.68±0.034 +32.12% *	2.84±0.045 +39.90% *
	Threonine	0.87±0.053	1.08±0.048 +24.14% *	1.23±0.039 +41.37% *
	Tryptophan	1.14±0.091	1.76±0.045 +54.39% *	1.93±0.062 +69.29% *
	Valine	3.79±0.043	4.83±0.032 +27.44% *	5.44±0.048 +43.54% *
Non-essential Amino Acids	Alanine	313.49±0.341	304.39±0.479 -2.905 *	295.43±0.454 -5.76% *
	Aspartic acid	62.46±0.169	61.17±0.097 -2.07% *	57.92±0.084 -7.27% *
	Cystine	12.42±0.073	11.82±0.031 -4.83% *	10.38±0.028 -16.43% *
	Glutamic acid	93.77±0.081	90.34±0.074 -3.66% *	86.49±0.076 -7.76% *
	Glycine	107.69±0.486	93.47±0.643 -13.20% *	89.92±0.662 -16.50% *
	Hydroxy proline	49.32±0.043	41.41±0.062 -16.04% *	38.84±0.071 -21.25% *
	Proline	68.92±0.032	62.38±0.037 -9.49% *	56.97±0.048 -17.34% *
	Serine	78.43±0.094	69.79±0.081 -11.02 *	63.43±0.076 -19.13% *
	Tyrosine	18.46±0.029	18.09±0.058 -2.00% *	17.66±0.039 -4.33% *
	Taurine	14.82±0.086	14.69±0.074 -0.88% *	14.29±0.045 -3.53% *

"*" indicates Significant at $p<0.001$, "+…%" and "-…%" indicate percent increase and percent decrease respectively.

DISCUSSION

The GOT (AST) activity in liver of normal-control fishes was found to be 579.822 ± 3.547 IU/l. Significant decrease ($p<0.001$) of the activity of this enzyme was marked with 26.106% and 46.096% decrease on exposure to 5 ppm and 10 ppm detergent respectively. The activity of this transaminase was found to be 493.719±0.604 IU/l in blood serum of normal-control fishes. The level was increased with 14.962% and 76.534% deviations from normal-control level on exposure of fishes to 5 ppm and 10 ppm detergent respectively. The GPT (ALT) activity in liver of normal-control fishes was found to be 284.947 ± 0.856 IU/l. Significant decrease ($p<0.001$) of the activity of this enzyme was marked with 18.307% and 29.941% decrease on exposure to 5 ppm and 10 ppm detergent respectively. The activity of this transaminase was found to be 47.693 ± 0.373 IU/l in blood serum of normal-control fishes. The level was

increased with 19.563% and 100.036% deviations from normal-control level on exposure of fishes to 5 ppm and 10 ppm detergent respectively. The pictures of these enzyme activities in liver and blood serum indicate huge scale destruction of hepatocytes on exposure of the fishes to the detergent containing water in sub-lethal amounts, (i.e. 5 ppm and 10 ppm).

Significant increase ($p<0.001$) of the amount of essential amino acids in liver were observed in this study. Arginine increased 40.12% and 45.51% , Histidine increased 20.39% and 28.16%, Isoleucine increased 46.88% and 68.75%, Leucine increased 65.82% and 81.01%, Lysine increased 23.95% and 40.12%, Methionine increased 37.16% and 54.05%, Phenylalanine increased 32.12% and 39.90% , Threonine increased 24.14% and 41.37%, Tryptophan increased 54.39% and 69.29% and Valine increased 27.44% and 43.54% from their respective normal control amounts on treatment of experimental fishes with 5 ppm and 10 ppm detergent-water respectively.

Significant decrease ($p<0.001$) of the amount of non-essential amino acids in liver were also observed in this study. Alanine decreased 2.905 and 5.76%, Aspartic acid decreased 2.07% and 7.27%, Cystine decreased 4.83% and 16.43%, Glutamic acid decreased 3.66% and 7.76%, Glycine decreased 13.20% and 16.50%, Hydroxy proline decreased 16.04% and 21.25%, Proline decreased 9.49% and 17.34%, Serine decreased 11.02 and 19.13%, Tyrosine decreased 2.00% and 4.33%, Taurine decreased 0.88% and 3.53% from their respective normal control amounts on treatment of experimental fishes with5 ppm and 10 ppm detergent-water 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 GOT and GPT, as these has been released to serum due to huge scale destructions of hepatocytes.

CONCLUSION

From the analyses of this study it was found that the common detergent “Surf Excel Blue” enhances huge scale destruction of Liver-cells of *H. fossilis* Bloch even though in very minute amount, if present in the aquatic system. As a result, the contents of the hepatocytes including transaminase enzymes (viz. GOT, GPT 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 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, impotency and even death. *H. fossilis* Bloch is one of the “resistant fishes” present in common aquatic habitats. As this resistant fish suffered from the minute amount of the detergent, the other organisms which are comparatively vulnerable inhabiting such type of habitat definitely will be more sufferers. In the introductory part of this article it has been mentioned that almost all the detergents commonly used are of same chemical configurations and all of them if released to the water may play adverse impact of normal physiology of the aquatic organisms and may bring deleterious alterations in the food chains concerned with the ecosystem. It may be advised not to wash garments or vehicles in commercial ponds and also the other water bodies having economic and ecological importance. The drained water also possibly made free from such type of components for the healthy contour of such ecosystems.

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