The Bioscan 8(4): 1343-1345, 2013
AN INTERNATIONAL QUARTERLY JOURNAL OF LIFE SCIENCES
www.thebioscan.in

INFLUENCE OF THE HOUSEHOLD DETERGENTS ON SOME SERUM BIOCHEMICAL PARAMETERS OF FRESHWATER FISH *CHANNA PUNCTATUS* (BLOCH).

GEETANJALI CHOUDHARY* AND B. S. JHA
Department of Zoology, L. N. MithilaUniversity, Darbhanga - 846 008
e-mail: geetanjalichy@gmail.com

KEYWORDS
Nirma
Tide
Glucose
Protein
Cholesterol
GOT, GPT, ALP, ACP,
Channa punctatus.

Received on :
21.06.2013

Accepted on :
29.10.2013

***Corresponding author**

ABSTRACT

The fish *Channa punctatus* exposed for 30 days to sub lethal concentrations of the detergents Nirma (9.50 mg/L) and Tide (37.0 mg/L) registered a significant ($p < 0.001$) increase in serum glucose by 50.72% and 39% respectively whereas serum protein decreased by 43.47% ($p < 0.01$) and 25.87% ($p < 0.05$) under Nirma and Tide exposure respectively. The serum cholesterol elevated by 52.47% ($p < 0.001$) in Nirma and 26.15% ($p < 0.01$) in Tide exposed fish groups. The enzymatic activities of serum glutamate oxaloacetate and glutamate pyruvate transaminases and alkaline phosphatases also increased significantly ($p < 0.001$) under the toxic influence of both the detergents. Contrary to this, the activity of acid phosphatases was found to be significantly ($p < 0.01$) inhibited by 45.2% and 33.12% as a consequence of Nirma and Tide exposure respectively. The magnitude of alterations in the serum biochemical parameters were evidently greater under the influence of Nirma thereby indicating higher sensitivity of the fish to this detergent. This study concludes that both the detergents, even at safe doses, are quite potent to manifest physio-metabolic crisis in the fish and that each of the above biochemical indices may be used as bio-indicator of detergent toxicity in fish.

INTRODUCTION

Contamination of natural water by detergents has become a matter of concern in recent years because of their large scale use in home and industrial applications ,such as , washing powders, dye fasteners , formulation of shampoos, industrial and household cleansing agents, toothpaste, tooth powder, in dispersing oil spills etc (Roy, 1988; Ogundiran *et al.,* 2010). Available reports indicate that entry of detergents into aquatic system build up in the food-chain and are responsible for many hazardous effects and even death of the aquatic organisms, including fishes (Summarwar and Lall, 2013).

Fishes are very good biosensors of aquatic contaminants and as bio-indicator species respond with great sensitivity to changes in the aquatic environment. Scanning of pertinent literature reveals that detergent related works on fish are still very meagre and limited to acute toxicity determination (Lal *et al.,* 1983: Adewoye and Fawole, 2005; Ogundiran *et al.,* 2010); growth and maturity(Chattopadhyaya and Konar, 1984) and histopathological studies (Byrne *et al.,*1989 ; Ogundiran *et al.,* 2010) .Reports pertaining to detergent induced biochemical changes in Indian freshwater fishes are very scanty (Jha, 1999: Kamble and Tapale, 2011) despite the fact that toxic influences in any living tissue are first exerted at biochemical level and therefore, biochemical markers are the earliest indicators of toxic potential of any xenobiotics.

In consideration of these facts, the present study was performed which reflects changes in serum glucose, total protein, cholesterol and enzymatic activities of glutamate oxaloacetate transaminase (GOT), glutamate pyruvate transaminase (GPT) and alkaline phosphatases (ALP) and acid phosphatases (ACP) in the freshwater fish *Channa punctatus* (Bloch) exposed for 30 days to sublethal concentrations of Nirma (9.5mg/L) and Tide (37.0mg/L), the two most commonly used branded detergents.

MATERIALS AND METHODS

The freshwater air-breathing fish *Channa punctatus* (average length 15-18 cm and average weight 35-40g),procured from our University Campus ponds, strictly prohibited for washing, bathing etc, and acclimatized and maintained in laboratory as per procedure described elsewhere (Jha and Jha, 1995) were the experimental animal model in this study. The two household detergents, Nirma (NirmaPvt. Ltd. Company, Ahmedabad) and Tide (Procter and Gamble Home Products Ltd; Mumbai) were the test chemicals for this study. Test concentrations of the two detergents were prepared in tap water (average temperature-25.4^{0}C; pH 7.2; dissolved solids - 12.5 mg/L; suspended solids- 26.0 mg/L; DO -7.6 mg/L; Free CO_2 1.2 mg/L; total hardness as $CaCO_3$-132.2 mg/L and total alkalinity as $CaCO_3$ -119.4 mg/L).

Three rectangular glass aquaria designated as A, B and C, each filled with 10-litres of water were taken. To each of these aquaria 10 numbers of fish were randomly transferred from acclimated fish aquarium. The fish of aquarium B and C were exposed for 30 days to 9.5 mg/L *and* 37.0 mg/L sub lethal concentrations of Nirma and Tide respectively. The sub lethal concentrations were determined by employing the formula $C = 48hr\ LC_{50} \times A/S^2$ (Hart *et al.*, 1945), where C = sub lethal or safe concentration; A = Application factor (0.3) and S = 24hr $LC_{50/48}$hr LC_{50}. No mortality of the fish occurred upto 30 days at the above concentration. Aquarium A served for the control group of fish. The exposure media were renewed every 24 hr to maintain the effective concentrations of the detergents. Fish of each group were fed with equal quantity of chopped goat liver everyday *ad libitum* at 10.30 AM before the renewal of exposure media.

On day 30 of the exposure, fish of each group were anaesthetized with MS 222 (tricane methane sulfonate, Sandoz) for two minutes. Free flowing blood was collected from each fish group in heparinized tubes by severing the caudal peduncle. The blood so collected was stored at 4^0C for 3-5 hr and allowed to clot. Thereafter, the serum was separated by centrifugation at 3000 rpm for 10 minutes and stored at-20^0C until analysis. The estimation of blood glucose was done as per O-toluidine method (Cooper and Mc Danile,1970).Serum protein was estimated colorimetrically using bromocresol green(Lowry *et al.,1951*) and serum cholesterol by method of Zak (1957). The enzymatic activity of glutamate oxaloacetate and glutamate pyruvate transaminases were estimated by employing the method of Reitman and Frankel (1957) and that of alkaline and acid phosphatases(ALP and ACP) by method of Wooten (1964).

Mean and Standard error (± SE) were calculated for values of individual parameters which were subjected to students 't' test (Fisher,1950) to test the level of significance. 'P' values less than 0.05 were considered significant.

RESULTS AND DISCUSSION

Results of various parameters investigated have been presented in Table 1. As is evident from the table, 30 days exposure of the fish to sub lethal concentrations of Nirma (9.5 mg/L) and Tide (37.0 mg/L) induced a significant increase ($p < 0.001$ and $p < 0.01$) in all parameters investigated except protein and ACP which registered significant ($p < 0.01$ and $p < 0.05$) depletion .The results of this study establish higher sensitivity of *Channa punctatus* to Nirma than Tide, the former inducing higher percentage of elevation/depletion than the latter possibly to cope with higher energy demand. Our results are in conformity with those of Ogochukwu and Joseph (2009) and Summarwar and Lall (2013).

Glucose is one of the most sensitive indices of stress .The significant hyperglycaemic response under the toxic influence of the detergents indicates high energy demand and utilization of energy reserves. The elevated blood glucose level is attributed to glycogenolysis or glucogenesis at tissue level especially liver and muscle. In stressful conditions, the chromaffin cells release catecholamines, adrenalin and nor-adrenalin towards blood circulation (Nakano and Tomlinson, 1967; Reid *et al.,*1998) and such stress hormones together with cortisol mobilize and elevate glucose production in fish through glucogenesis and glycogenolysis to cope with the energy demand (Wendelaar- Bonga,1997).

The observed significant decrease ($p < 0.01$ under Nirma and $p < 0.05$ under Tide exposure) of total protein appears to be a consequence of direct action of detergents on hepatocytes since serum proteins have hepatic origin. This may be associated with either partial inhibition of protein synthesis or breakdown of protein into free amino acids (Shakoori *et al.*, 1994) for utilization as supplementary energy source. The detergent induced hypercholesterolemia recorded in the present case is attributed to leakage of cholesterol from liver (Garg *et al.*,1989) or disruption in formation of lipoprotein (Awasthi *et al.*,1984; Sharan *et al.*, 1993) or to decreased rate of steroid biosynthesis as a result of liver damage caused by detergents (Sastry and Sharma,1980).

The increased enzymatic activities of GOT and GPT measured in our study may be correlated with cell-membrane damage or changed permeability caused by detergents leading to selective leakage of enzymes to blood stream as suggested by previous workers (Travlos *et al.*, 1996; Summarwar and Lall, 2013).This contention also gets support from the view of Harper *et al.,*(1981) that the enzymes GOT and GPT are diagnostically most useful to detect cellular damage.

Acid phosphatase hydrolyzes large variety of organic phosphatase esters with the formation of an alcohol and a phosphate ion. The decreased profile of this enzyme estimated in this study is attributed to adverse effect of detergent on cell and its organelles (Jana *et al.*, 1985). ALP is basically a membrane bound enzyme and hence, any perturbation in the membrane property as a result of interaction with detergents could lead to alteration in ALP activity (Hedayati *et al.*, 2010).The increased activity of ALP observed in this study may be attributed to increased synthesis and reduced bilary

Table 1: Changes in serum parameters of the fish C. *punctatus* under the sub lethal toxic influence of the detergents. Values are Mean ± SE of 5 observations

Parameter	Unit	Control	Nirma Exposed	Tide Exposed
Glucose	mg/dL	138.40 ± 0.08	208.60 ± 0.07^c (+50.72)	192.45 ± 0.12^c(+39.05)
Protein	g/dL	4.83 ± 0.07	2.73 ± 0.06^b(-43.47)	3.58 ± 0.08^a(-25.87)
Cholesterol	mg/dL	163.48 ± 0.26	249.27 ± 0.43^c (+52.47)	206.24 ± 0.56^b (+26.15)
GOT	IU/L	49.6 ± 0.08	89.2 ± 0.05^c (+79.83)	78.6 ± 0.09^c(+58.46)
GPT	IU/L	12.03 ± 0.06	20.5 ± 0.06^c(+70.4)	18.3 ± 0.07^c(+52.1)
ALP	IU/L	42.5 ± 0.03	66.5 ± 0.04^c (+56.47)	57.6 ± 0.08^c (+35.52)
ACP	IU/L	15.7 ± 0.05	8.6 ± 0.06^b(-45.2)	10.5 ± 0.08^b(-33.12)

Values are significant at, a = $p < 0.05$; b = $p < 0.01$ and c = $p < 0.001$; Values in parentheses are percent change over control; GOT = glutamate oxaloacetate transaminase; GPT = glutamate pyruvate transaminase; ALP = alkaline phosphatases; ACP = acid phosphatases; IU/L = International unit (transformation of one micromole substrate in one minute under the condition of the test.)

excretion (Kaplan,1986). Ogochukwu and Joseph (2009) also reported increased enzymatic activity of alkaline phosphatase in the fish *Clarias gariepinus* under the stress of Ariel detergent and suggested extensive damage of liver cells and rupture of blood vessels as possible reasons of increased ALP activity for compensatory action of physiological stress.

Thus, our study concludes that the detergents induce severe metabolic crisis in fish and each of the parameters investigated can safely be used as marker or bio-indicator of stressed physiological state of the fish.

REFERENCES

Adewoye, S. O. and Fawole, O. O. 2005. Acute toxicity of soap and detergent effluent to freshwater *Clarias gariepinus* fingerlings. *Ethiop. J. Sci*. **28**: 189 -194.

Awasthi, M., Shah, P.,Dubale, M. S. and Godhia, P. 1984. Metabolic changes induced by organophosphates in piscine organs. *Environmental Research*. **35:** 320-325.

Byrne, P., Speare, D. and Ferguson, H. W. 1989. Effect of cationic detergent on the gills and blood chemistry of rainbow trout *Salmo gairdneri*. Dis. *Aquat. Org*. **6:** 185-196.

Chattopadhyaya, D. N. and Konar, S. K. 1984. Influence of an anionic detergent on fish. *Environ. Ecol*. **2:** 257-261.

Cooper, G. R. and Mc Danile, V. 1970. Manual of routine methods in clinical chemistry for use in Intermediate laboratories. *Std. Methods.Clin. Chem*. **6:** 159-170.

Fisher, R. A. 1950. Statistical Methods for Research workers. 11th Edition. Oliver and Boyd, London.

Garg, V. K., Garg, S. K. and Tyagi, S. K. 1989. Manganese induced haematological and biochemical anomalies in Indian catfish,*Hetropneustes fossilis*. *J. Environ. Biol*. **10:** 349-353.

Harper, H. A., Rodwell, V. W., and Mayes, P. A. 1981. Review of Physiological Chemistry. Lange Medical Publication, California.

Hart, N. B., Doudoroff, P. and Greenbank, J. 1945. The evaluation of toxicity of industrial wastes, chemicals and other substances to freshwater fishes. Atlantic Refining Company. *Phil. Part* **(I):** 317-326.

Hedayati, A., Safahieh, A., Savar, A. and Ghofleh, M. J. 2010. Assessment of aminotransferase enzymes in yellowfin sea bream *(Acanthopagrus latus)* under experimental condition as biomarkers of mercury pollution. *World J. Fish. Mar. Sci*. **2:** 186-192.

Jana, S., Sahana, S. S., Chaudhuri, M. A. and Chaudhury, D. K. 1985. Effect of mercury on inorganic phoshphorous and activities of acid and alkaline phosphatases in freshwater fish *Clarias batrachus*. *Environ. Ecol*. **3:** 2-4.

Jha, B. S. 1999. Impact of chronic exposure of the household detergents, Surf and Key, on tissue biochemistry of the freshwater fish, *Clarias batrachus* (Linn). *Indian J. Environ. and Ecoplan*. **2:** 281-284.

Jha, B. S. and Jha, M. M. 1995. Biochemical effects of nickel chloride on the liver and gonads of the freshwater climbing perch,*Anabas testudineus* (Bloch). *Proc. Nat. Acad. Sci. India*. **65B:** 39-46.

Kamble, S. M. and Tapale, B. K. 2011. Effect of sublethal concentrations of a household detergent on certain biochemical constituents of catfish, *Mystus seenghala*. *Biosci. Biotech. Res. Comm*. **4:** 198-204.

Kaplan, M. M.1986. Serum alkaline phosphatase another piece is added to the puzzle. *Hepatology*. **6:** 526-528.

Lal, H., Mishra, V., Vishwanathan, P. N. and Krishnamurthi, C. R. 1983. Comparative studies on ecotoxicology of synthetic detergents. *Ecotoxicol. Environ. Saf*. **8:** 447-450.

Lowry, O. H., Rosenbrough, N. G., Farr, A. L. and Randall, R. G. 1951. Protein measurements with folin phenol reagent . J .*Biol. Chem*. **193:** 265-275.

Nakano, T. and Tomlinson, N. 1967. Catecholamine and carbohydrate concentrations in rainbow trout *(Salmo gairdneri)* in relation to physical disturbance. *J. Fish. Res. Bd. Can*. **24:** 1701-1715.

Ogochukwu, N. H. and Joseph, C. I. 2009. Toxicity of crude oil product and detergent on serum alkaline phosphatase concentration of *Clarias gariepinus* juveniles. *Animal Research International*. **6:** 1045-1048.

Ogundiran, M. A., Fawole, O. O., Adewoye, S. O. and Ayandiran, T. A. 2010. Toxicological impact of detergent effluent on juvenile of African catfish *(Clarias gariepinus)* (Buchell 1822). *Agric. Biol. J. N. Am*. **1:** 330-342.

Reid, S. G., Bernier, N. J. and Perry, S. F. 1998. The adrenergic stress response in fish: Control of catecholamine storage and release. *Comp. Biochem. Physiol*. **120:** 1-27.

Reitman, S. and Frankel, S. 1957. A colorimetric method for the determination of serum glutamic oxaloacetic and pyruvic transaminase. *Amer. J .Clin. Path*. **28:** 56-63.

Roy, D. 1988. Toxicity of an anionic detergent, dodecyl benzene sodium sulfonate to a freshwater fish, *Rita rita*. Determination of LC_{50} values by different methods. *Ecotoxicol. Environ. Saf*. **15:** 186-194.

Sastry, K. V. and Sharma, K. 1980. Mercury induced haematological changes in *Ophiocephalus (Channa) punctatus*. *Ecotoxicol. Environ. Saf*. **5 :** 171-176.

Shakoori, A. R., Iqbal, M. J., Mughal, A. L. and Ali, S. S. 1994. Biochemical changes induced by inorganic mercury on the blood, liver and muscles of freshwater Chinese grass carp, *Ctenopharyngodon idella*. *J . Ecotoxicol. Environ. Monit*. **4:** 81-92.

Sharan, P., Yadava, V. K. and Yadava, S. C. 1993. Impact of herbicides on serum and muscle cholesterol level of *Channa punctatus*. *Him. J. Env. Zool*. **7:** 124-128.

Summarwar, S. and Lall, D. 2013. Effect of toxins on blood plasma of *Clarias* batrachus. *Indian J. Fundamental and Applied Life Sciences*. **3:** 133-136.

Travlos, G. S., Morris, R. W., Elewell, M. R., Duke, A., Rosenblum, S. and Thompson, M. B. 1996. Frequency and relationship of clinical chemistry and liver and kidney histopathology finding in 13 week toxicity study in rats. *Toxicology*. **107:** 17-29.

Wendelaar-Bonga, S. E. 1997. The stress response in fish. *Physiol. Rev*. **77:** 591-625.

Wooten, L. J. P. 1964. Microanalysis in Medical Biochemistry. 4th Edition. Churchill, London. p.107.

Zak, A. 1957. Estimation of serum cholesterol. *Am. J. Clin. Pathol*. **27:** 583-586.