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

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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 sub lethal concentrations of the detergents Nirma (9.50 mg/L) and Tide (37.0 mg/L).

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.

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

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°C; pH 7.2; dissolved solids -12.5 mg/L; suspended solids - 26.0 mg/L; DO -7.6 mg/L; Free CO₂ 1.2 mg/L; total hardness as CaCO₃-132.2 mg/L and total alkalinity as CaCO₃ -119.4 mg/L).

Three rectangular glass aquaria designated as A, B and C, each filled with 10-litres of water were taken.
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 LC50 x AS2 (Hart et al., 1945), where C = sub lethal or safe concentration; A = Application factor (0.3) and S = 24hr LC50. 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°C for 3-5 hr and allowed to clot. Thereafter, the serum was separated by centrifugation at 3000 rpm for 10 minutes and stored at-20ºC 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<0.05) in all parameters investigated except protein and cholesterol. 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 glycolysis 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 (Awasthi et al., 1984; Sharan et al., 1993) or to decreased rate of gluconeogenesis and glycogenolysis as a result of liver damage caused by detergents (Sastry and Sharma, 1980).

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Unit</th>
<th>Control</th>
<th>Nirma Exposed</th>
<th>Tide Exposed</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucose</td>
<td>mg/dL</td>
<td>138.40±0.08</td>
<td>208.60±0.07 (+50.72)</td>
<td>192.45±0.12 (+39.05)</td>
</tr>
<tr>
<td>Protein</td>
<td>g/dL</td>
<td>4.83±0.07</td>
<td>2.73±0.06 (+43.47)</td>
<td>3.58±0.08 (+25.87)</td>
</tr>
<tr>
<td>Cholesterol</td>
<td>mg/dL</td>
<td>163.48±0.26</td>
<td>249.27±0.43 (+52.47)</td>
<td>206.24±0.56 (+26.15)</td>
</tr>
<tr>
<td>GOT</td>
<td>IU/L</td>
<td>49.6±0.08</td>
<td>89.2±0.05 (+79.83)</td>
<td>78.6±0.09 (+58.46)</td>
</tr>
<tr>
<td>GPT</td>
<td>IU/L</td>
<td>12.03±0.06</td>
<td>20.5±0.06 (+70.4)</td>
<td>18.3±0.07 (+52.1)</td>
</tr>
<tr>
<td>ALP</td>
<td>IU/L</td>
<td>42.5±0.03</td>
<td>66.5±0.04 (+56.47)</td>
<td>57.6±0.08 (+35.32)</td>
</tr>
<tr>
<td>ACP</td>
<td>IU/L</td>
<td>15.7±0.05</td>
<td>8.6±0.01 (+45.2)</td>
<td>10.5±0.08 (+33.12)</td>
</tr>
</tbody>
</table>

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


