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In Vitro Effect of Linear Alkylbenzene Sulphonate (LAS) on Some Enzymes in Liver and Gills of the Teleost *Channa punctatus*

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In recent years there has been a rapid increase in the production of synthetic detergents used in industries and for household purposes. These have become important contributory substances that create pollution in natural water systems. Srivastava et al (1973) observed the presence of synthetic detergents between 2.0 and 14.0 mg/L in the sewage of Delhi, Calcutta, Madras, Kanpur, Nagpur and Jaipur. Agarwal (1979) has also reported that about 200 kg per day of synthetic detergent is discharged into the Nazafgarh drain which opens into the Yammuna river, India. It is evident that synthetic detergents cause toxicity in aquatic environment by adversely affecting the fauna and flora particularly fish (Abel 1974; Henderson et al 1959; Verma and Mohan 1976). Channa punctatus occurs in the natural water systems in India. Our previous study has shown that exposure to LAS causes alterations in enzyme activity. To further understand the mechanisms by which LAS causes toxicity, this fish species was chosen. Effect of LAS was studied on some selected enzymes under in vitro conditions.

MATERIALS AND METHODS

Healthy fish specimens of Channa punctatus were kept in clear rectangular glass aquaria in the laboratory for 2 wk. The fish were fed on Shalimar fish food (Bombay, India) once daily. The size of the Channa punctatus varied from 118-170 mm and weight 70-100g. Normal fishes were sacrificed and the liver and gill arches were excised, rinsed in ice cold physiological saline. The mucosal layer from gill arches was scrapped from underlying cartilage. Homogenates (10%

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W/V) were prepared in 0.25 M sucrose solution using Potter-Elvehjem homogenizer and centrifuged at 900 g for 15 min under cold conditions. The supernatants were used for enzymatic estimations. The activities of acid phosphatase (ACP) and alkaline phosphatase (ALP) (Wootten 1964), glutamic oxaloacetic transaminase (GOT) and glutamic pyruvic transaminase (GPT) (Reitman and Frankel 1967), sorbitol dehydrogenase (SDH) (Asada and Galambos 1963) and 5-nucleotidase (Dixon and Purdom 1954) were estimated employing Sigma diagnostic kits (Sigma, St. Louis, Missouri). The enzyme glucose-6-phosphatase (G-6-pase) was determined according to the method of Swanson (1965).

Different quantities i.e. 4, 10, 20, 40 and 100 mg/L of LAS (pH 7.0) were added to the reaction mixture and pre-incubated for 5 min at 37°C. The reaction was then started by adding substrates. The incubation time was variable for above enzymes. The protein contents of the tissue homogenates was determined by the method of Lowry et al (1951) using bovine serum albumin as standard.

Student's 't' test was used to calculate the significance of difference between the means.

RESULTS AND DISCUSSION

ACP and ALP were inhibited in liver and gills at 20 mg/L LAS and the degree of inhibition increased with the increasing concentrations (Table 1). The activity of GOT in liver was inhibited at 10 ppm and further inhibition was observed at higher concentrations, while in gills it inhibited at 40 and 100 mg/L (Table 2). Likewise, inhibition in the activity of GPT was observed at 40 and 100 mg/L in liver and at 100 mg/L in gills (Table 2). An exposure to LAS at 20, 40 and 100 mg/L caused inhibition in the activities of G-6-pase and SDH, while 5-nucleotidase inhibited at 40 and 100 mg/L in both the organs (Table 3).

The above enzymes play an important role in the metabolic functions. The toxicity of surfactants arises from its action on biological system. Effects of surfactants is generally attributed to their ability to react directly with proteins leading to enzyme inhibition. The inhibition of membrane bound enzymes following *in vitro* treatment of LAS in the present study indicates that probably even the lower concentration of surfactants causes the cellular damage and also inhibits the enzyme activities as well. The data suggest that LAS can affect the lysosomal functions as indicated by inhibition of ACP (Gupta and Dhillon 1983). Inhibition of ALP reflects alterations in protein synthesis and uncoupling of oxidative phosphorylation (Verma et al 1979). The depletion in the activities of GOT and GPT indicates disruption of link between carbohydrate and protein metabolism providing source of keto acids for Krebs cycle and gluconeogenesis.

Table 1. Effect of linear alkylbenzene sulphonate on acid phosphatase (ACP) and alkaline phosphatase (ALP) in liver and gills of Channa punctatus

Enzyme	Conc. (mg/L)	Liver		Gills	
		Sp.act.	% inhibition	Sp.act.	% inhibition
Acid phosphatase*	-	8.00±1.46	-	8.07±2.42	-
	4	8.09±1.35	-	7.97±1.97	-
	10	8.06±1.49	-	5.88±2.14	27.1
	20	5.71±1.27	28.6[c]	5.08±1.53	37.0[c]
	40	4.01±0.93	49.9[a]	2.96±0.64	63.0[b]
	100	1.35±0.40	83.1[a]	1.25±0.39	84.5[a]
Alkaline phosphatase*	-	4.69±2.05	-	10.29±1.47	-
	4	4.60±1.96	-	10.42±1.50	-
	10	4.01±1.22	14.5[c]	10.53±3.12	-
	20	3.14±0.75	33.0[c]	9.78±0.46	4.91
	40	3.14±0.55	33.0[c]	8.54±0.58	17.0[c]
	100	1.94±0.84	58.6[b]	4.88±0.85	62.6[a]

*n moles/min/mg protein. Mean ± SD (5 observations)

[a] $p<0.001$, [b] $p<0.01$, [c] $p<0.05$, when compared to control (Student's 't' test)

Table 2. Effect of linear alkylbenzene sulphonate on glutamic oxaloacetic transaminase (GOT) and glutamic pyruvic transaminase (GPT) in liver and gills of Channa punctatus

Enzyme	Conc. (mg/L)	Liver		Gills	
		Sp. act.	% inhibition	Sp. act.	% inhibition
GOT*	-	13.59±1.59	-	5.29±1.44	-
	4	13.29±1.25	-	5.18±1.45	-
	10	8.74±2.57	35.7[c]	4.51±1.06	14.7
	20	8.64±2.96	36.4[c]	3.99±0.71	24.6
	40	8.71±2.94	35.9[c]	2.29±0.85	56.7[b]
	100	6.08±2.42	55.3[a]	1.92±0.69	63.7[b]
GPT*	-	6.91±1.60	-	3.56±0.82	-
	4	6.70±1.72	-	3.56±1.00	-
	10	5.85±2.75	15.3	3.26±2.23	8.4
	20	6.41±2.27	7.2	3.37±1.09	5.3
	40	4.74±1.62	31.4[a]	3.18±1.54	10.7
	100	4.79±1.79	39.6[a]	1.17±0.10	67.1[a]

*n moles/min/mg protein. Mean ± SD (5 observations)

[a]$p<0.001$, [b]$p<0.01$, [c]$p<0.05$ when compared to control (Student's 't' test)

Table 3. Effect of linear alkylbenzene sulphonate on glucose-6-phosphatase (G-6-pase), sorbitol dehydrogenase (SDH) and 5-nucleotidase in liver and gills of Channa punctatus

Enzyme	Conc. (mg/L)	Liver		Gills	
		Sp.act.	% inhibition	Sp. act.	% inhibition
Glucose-6-phosphatase*	-	0.202±0.003	-	0.175±0.05	-
	4	0.200±0.003	-	0.174±0.04	-
	10	0.201±0.005	-	0.166±0.026	5.1
	20	0.124±0.04	38.6[c]	0.129±0.03	26.3[c]
	40	0.063±0.001	68.8[a]	0.069±0.019	60.6[b]
	100	0.059±0.002	70.8[a]	0.051±0.002	70.8[b]
Sorbitol dehydrogenase**	-	362.97±14.95	-	227.49±67.04	-
	4	350.80±15.27	-	220.98±25.07	-
	10	312.27±24.56	13.9	176.26±22.17	22.5
	20	235.62±16.00	35.1[a]	145.22±8.89	36.2[c]
	40	225.92±79.58	37.7[b]	123.67±13.4	45.6[c]
	100	147.15±24.63	59.4[a]	119.49±10.42	47.5[c]
5-Nucleotidase***	-	0.159 ±0.003	-	0.1596±0.002	-
	4	0.1579±0.003	-	0.1580±0.003	-
	10	0.1576±0.003	-	0.1571±0.003	-
	20	0.1576±0.003	-	0.1502±0.007	-
	40	0.1283±0.001	18.7[a]	0.1297±0.001	18.7[a]
	100	0.1105±0.005	30.0[a]	0.1109±0.006	30.9[a]

*mg Pi/mg protein/hr, **IU/mg protein, ***mg Pi/mg protein/hr

Mean ± SD (5 observations)

[a] $p<0.001$, [b] $p<0.01$, [c] $p<0.05$, when compared to control (Student's 't' test)

An inhibitory effect of LAS on G-6-pase reflects disturbances in glycogen metabolism (Grant and Mehrle 1973) alongwith damage to endoplasmic reticulum (Christensen et al 1973). The decrease in the activity of SDH shows impairment of the oxidation-reduction reactions involving interconversion of fructose and sorbitol after exposure to LAS. The inhibitory effect on plasma membrane marker enzyme, 5-nucleotidase (Bont et al 1969; Chandrasekharan and Narayan 1970; Avruch and Wallach 1971) depicts loss of plasma membrane structure.

The wide spectrum of enzyme inhibitions is suggestive of disruptive effect on lysosome, plasma membrane, carbohydrate metabolism and Krebs cycle. It demonstrates the toxic potentials of surfactants. However, in view of our observations and the fact that detergents interact with proteins (Swisher 1970) and alter membrane permeability (Henderdon et al 1969), it appears that surfactants act as a general cell toxic agent rather than specific inhibitor.

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