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RESEARCH ARTICLE

Levels of protein metabolites in *Cyprinus carpio* (L) on sublethal exposure to synthetic detergent linear alkylbenzene sulfonate (LAS)

■ **S. GOPAL, M. NAGABHUSHAN REDDY AND P. INDIRA**

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SUMMARY : Synthetic detergents deposited in the aquatic environment may accumulate in the food chain and cause ecological damage and even threat to human health. Linear alkylbenzene sulfonate (LAS), an anionic surfactant is now-a-days widely spread in many aquatic environments, where it has a significant potential. During this research sublethal effects of LAS on the levels of total proteins, urea, amino acids and protease activity in various tissues of freshwater fish, *Cyprinus carpio* Linnaeus were studied. The levels of total proteins and urea decreased initially at 24 h in relation to control and up to day 15. After day 15, these levels increased gradually through day 20 and reached nearer to control at day 30. The increase in the levels of total proteins and urea was more in liver followed by muscle and gill. The levels of both free amino acids and protease activity followed a reverse trend to that of both total proteins and urea. It was evident that there was drastic protein utilization through proteolytic activity in all the tissues for releasing extra energy to cope up with the energy crisis developed during the toxic stress of synthetic detergent (LAS). These changes support the ability acquired by the fish exposed to LAS toxicity stress which might have achieved by activating the detoxification process.

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Key Words :
Cyprinus carpio, LAS, Protease activity, Synthetic detergent, Total proteins

Author for correspondence :
M. NAGABHUSHAN REDDY
Department of Zoology, Rayalaseema University, KURNOOL (A.P.) INDIA
Email:mnbhushr@gmail.com

See end of the article for **Coopted authors'**

Proteins are the linear compounds of high molecular weight and are basic units of life. The structure of proteins enables them to act as the catalysts that control the rate of biological reactions. Proteins are heterogeneous group of macromolecules having diverse physiological functions (Lehninger, 1984). Hence, protein profiles can be considered as diagnostic tool in assessing the physiological status of a tissue or an animal as a whole (Harper, 1986; Chopra *et al.*, 2001). The concentration of proteins is a balance between the rate of their synthesis and degradation or catabolism.

Synthetic detergents are one of the resultants of the modem technologies emerged as major contributors to the problem of pollution at this moment, posing a great potential risk than the organic wastes and eutrophicating nutrients. All surfactants are potentially harmful to most of the organisms, aquatic as well as terrestrial, at some level or other and are reported to produce toxic effects (Belanger *et al.*, 2002). Several reports have come to light in recent years explaining that synthetic detergents interfere with various metabolic aspects of organisms and cause death (Toshima *et al.*, 1992). Linear alkylbenzene sulfonate (LAS) is one of the most widely used anionic surfactants in commercial use today (Konnecker *et al.*, 2011). LAS accounts for 28% of the surfactants produced in United States, Western Europe and Japan (Federle and Schwab, 1989). Most detergents are formulated products containing surfactants consist of a hydrophobic and hydrophilic component and have the ability to change the surface properties of water whereby the surface tension of water is reduced. Since the 1970's decade, investigations on the toxic effects of the

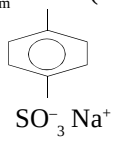
LAS have been conducted with aquatic organisms as fish (Barbieri *et al.*, 2002), crustaceans (Singh *et al.*, 2002) especially in Europe and North America. The chemicals in the LAS category possess properties indicating a hazard for the environment (fish, invertebrates and algae) (SIDS Report, 2005). In India, the investigations on this aspect are scanty. Hence, the authors made an attempt to study the protein metabolism in freshwater fish [*Cyprinus carpio* (L.)] exposed to the toxicity of LAS in order to fill the lacuna to the possible extent.

EXPERIMENTAL METHODOLOGY

Commercial grade of household green arial surf was selected as the representative toxicant for the study of biochemical changes in *C. carpio.*

Chemical name : Linear alkylbenzene sulfonate (LAS)

Structural formula : $CH_3 - (CH_2)_m - CH - (CH_2)_n - CH_3$



$SO^-_3\ Na^+$

Molecular weight : 342.4 g/mol

CAS Registry No. : 68411-30-3

Physical state : Powder

Solubility in water : Partially soluble

Experimental design:

Individuals of *C. carpio* weighing 10 ± 2g of length 5 – 6 cm were collected from the freshwater ponds of the Department of Fisheries, Anantapur, Andhra Pradesh and maintained in the laboratory. Then they were released in aquaria of size 6' x 3' x 3' containing dechlorinated tap water. The fish were fed with groundnut pellets having around 40% protein content once a day. Then LC_{50} was determined using Dragstedt-Behren's method as given by Carpenter (1975). Later, the fish were separated into 6 batches, each consisting of 6 fish and water was renewed every day to provide freshwater rich in oxygen and to prevent hypoxic conditions. As the level of toxicity is reported to vary with the interference of various factors like temperature, salinity, pH, hardness of water, exposure period, density of the animal, size, sex etc., precautions were taken to control all these factors as far as possible by adapting the fish to laboratory conditions for 15 days prior to the experimentation. As a part of it, water from same source was for the maintenance of fish (at temperature of 28 ± 1^0C and pH 7.6 ± 0.4).

Fixation of sublethal concentration:

The knowledge on the concentration of toxicant that kills 50% of the test animals in fixed period of time could become insufficient to assess various responses of the animal to toxicant (Nobbs and Pearu, 1976). Further, studies on acute toxicity have significant limitations such as the adaptation of the test animals to the toxicity, also viewed the need for sublethal studies because distinct changes involving a sequence of events in the responses of test animal could occur during sublethal concentration (Stockner and Aueta, 1976). The 96h LC_{50} obtained to fish was 31.11 mg/L. Taking into consideration the fact that the effect of a synthetic detergent LAS on fish becomes consistent within 24 h of exposure, sublethal concentration was taken to study the protein metabolism in fish *C. carpio.* Hence, about $1/10^{th}$ of the LC_{50} *i.e.* 3.111 mg/L was taken as the sublethal concentration to evaluate further studies.

Fixation of exposure periods:

Since the duration of exposure is having a great influence on the toxicity of synthetic detergent in an organism, 24 h, day 7, day 15, day 20 and day 30 were chosen to observe the short-term (15 days) and long-term (30 days) effects of synthetic detergent LAS on *C. carpio.*

The experiments were carried out both in control fish (freshwater with out LAS) and exposed fish (LAS treated). The animals were starved for 24 h prior to each estimation of protein metabolites to avoid any influence of differential feeding. The fish were sacrificed to death after the period of exposure and gill, liver and muscle were dissected out from each of them and were weighed separately to nearest milligram on a sartorius electronic weighing balance (Model : GK3102) and transferred in to separate microbeakers containing fish ringer solution (Ekberg, 1958). An equilibration time of 15min was allowed to the above tissues to enable them to regain normally from a state of stroke, if any due to handling and dissecting. The entire procedure was carried out in a sterilized cold room (at temperature 15 ± 1°C). The total proteins was estimated by the method of Lowry *et al.* (1957), free amino acids by Moore and Stein (1954), urea by Natelson (1971) and protease activity by Davis and Smith (1955). The experimental data were analyzed by one-way analysis of variance (ANOVA) in which 'p' values '< 0.01' denotes the level of significance. Values expressed are mean ± SEM.

EXPERIMENTAL FINDINGS AND DISCUSSION

The levels of total proteins and urea were initially decreased relative to the control in all the tissues of fish from 24 h up to day 15. This decrease was significant at day 15. Later from day 15 onwards, the levels of total proteins and urea were increased up to day 30 through day 20 nearer to the control (Table 1 and 3).

The levels of free amino acids and protease activity

reciprocal to that of total proteins and urea increased initially relative to control in all tissues of fish at 24 h and this increase continued through day 7 to day 15 with maximum levels at latter period. The levels of free amino acids were gradually decreased through day 20 to day 30. The protease activity followed similar trend to that of free amino acids levels through day 20 to day 30 (Table 2 and 4).

The protein level in the tissue depends upon a dynamic equilibrium between the rates of synthesis and degradation. A significant decrease in tissue proteins and marked elevation of free amino acids was observed in *C. catla* on exposure to lethal and sublethal concentration of fenvalerate (Anita Susan *et al.*, 1999). Decline in total proteins and elevation of total free amino acids in *M. malcolmsoni* on exposure to carbaryl were reported by Bhavan and Geraldine (2002). An increase in proteolytic activity could be due to the damage caused to tissues especially hepatic tissues (Suresh *et al.*, 1992) and an impairment of protein synthetic potentials (Malla Reddy and Philip, 1991). Reddy (1987) suggested an impairment of protein synthesis induced by toxicant stress is also a reason for the quantitave reduction in protein content of various tissues. Toxicant has the capacity to bind plasma

Table 1 : Levels of total proteins (mg/g wet wt. of tissue) in the various tissues of *C. carpio* on exposure to sublethal concentration of synthetic detergent (LAS)

Tissue	Sublethal exposure period					
	Control	24h	Day 7	Day 15	Day 20	Day 30
Gill	99.76±3.99	91.95±3.67	83.94±6.71	60.02±2.40*	75.67±4.35	90.51±5.43
Liver	140.27±5.61	132.45±5.29	122.47±4.89*	92.37±3.69*	112.16±13.45	125.21±10.01
Muscle	136.75±5.47	124.12±4.96	113.06±11.30	93.40±3.73*	107.98±4.31	122.22±9.77

Data presented are mean ±SEM (n=6)
Statistical analysis done by one-way ANOVA
* indicates significance of value at P>0.001 compared with control

Table 2 : Levels of free amino acids (mg amino acid/g wet wt. of tissue) in the various tissues of *C. carpio* on exposure to sublethal concentration of synthetic detergent (LAS)

Tissue	Sublethal exposure period					
	Control	24h	Day 7	Day 15	Day 20	Day 30
Gill	7.21±0.28	7.56±0.30	8.12±0.32*	8.51±0.34*	7.98±0.31	6.89±0.27
Liver	13.62±0.54	13.95±0.55	14.21±0.56	14.98±0.59*	14.09±0.56	13.28±0.53
Muscle	10.24±0.40	10.56±0.42	11.39±0.45	11.89±0.47*	10.75±0.43	10.01±0.40

Data presented are mean ±SEM (n=6)
Statistical analysis done by one-way ANOVA
* indicates significance of value at P>0.001 compared with control

Table 3 : Levels of urea («g/g wet wt. of tissue) in the various tissues of *C. carpio* on exposure to sublethal concentration of synthetic detergent (LAS)

Tissue	Sublethal exposure period					
	Control	24h	Day 7	Day 15	Day 20	Day 30
Gill	0.312±0.01	0.285±0.02	0.234±0.01	0.187±0.01*	0.251±0.01	0.284±0.02
Liver	0.952±0.03	0.861±0.06	0.798±0.03*	0.656±0.02*	0.798±0.03	0.869±0.06
Muscle	0.451±0.08	0.420±0.01	0.363±0.01	0.298±0.01*	0.345±0.01	0.415±0.03

Data presented are mean ±SEM (n=6)
Statistical analysis done by one-way ANOVA
* indicates significance of value at P>0.001 compared with control

Table 4 : Levels of protease activity («M/mg wet wt. of tissue/h) in the various tissues of *C. carpio* on exposure to sublethal concentration of synthetic detergent (LAS)

Tissue	Sublethal exposure period					
	Control	24h	Day 7	Day 15	Day 20	Day 30
Gill	0.285±0.01	0.305±0.01	0.336±0.02	0.395±0.02	0.319±0.01	0.262±0.02
Liver	0.316±0.01	0.385±0.02	0.407±0.02	0.446±0.02*	0.356±0.01	0.301±0.01
Muscle	0.237±0.02	0.267±0.01	0.294±0.02*	0.325±0.02	0.281±0.02	0.220±0.02

Data presented are mean ±SEM (n=6)
Statistical analysis done by one-way ANOVA
* indicates significance of value at P>0.001 compared with control

protein and intracellular proteins (Desai *et al.,* 2002).

Shingadia *et al.* (2006) reported that detergent can dissociate the 80S ribosomes and cause damage to polyribosomes formation; finally it leads to inhibition of the initation of the polypeptide synthesis. As a result altered and declined protein synthesis was observed. It was supported by several toxicological studies on protein metabolism by different metals and toxicants. The depletion in protein content in *S. mossambicus* on exposure to chloropyrifos and endosulfan (Kamble and Muley, 2000) in cat fish, *C. batrachus* on exposure to sublethal toxicity of fluoride (Achyutha Devi and Piska, 2006). Loss of protein in the tissues resulted not only due to impairment of protein synthesis but also due to intracellular leakage of proteins in the blood on account of cellular necrosis, because of mitochondrial accumulation of detergent lead to potential collapse and leakage of ions from the internal matrix, measured as potassium and calcium release from mitochondria (Higgins and Rogers, 1974).

An increase in the content of total proteins of the tissues in the latter half of the sublethal exposure period of synthetic detergent (LAS) may be due to the induction of microsomal enzymes for detoxification of extraneous material and other constituent enzymes of various metabolic segments. Gills are the primary tissue sites of pollutant uptake from ambient water because of their large surface area and their proximity to the internal and external environment (Rajyasree and Sreedhar, 2007). A significant increase in N-O dimethylase activities in malathion exposed gill of *C. batrachus* (Mukhopadhyaya and Dehadri, 1978) are drawn in support of the present results. The decrease in protease activity might be to facilitate the synthesis of necessary proteins and also due to rigidity of lysosomal membranes and the gradual decrease of free amino acid levels could indicate their speedy mobilization for protein synthesis (Stefanoni and Abessa de Souza, 2011). Since ammonia forms the toxic product, it cannot be stored for longer period in the body as it leads to endogenous ammonotoxicity (Wekell and Brown, 1973). Ammonia is the major unwanted nitrogenous end product in the catabolism of proteins (Hoar, 1976). However, the freshwater animals have the ability to convert toxic ammonia to less toxic substances during the periods of stress in order to prevent the intake of water required to eliminate the toxic ammonia (Ramana Rao and Ramamurthy, 1983). Therefore, the toxic ammonia should invariably be converted into a non-toxic product. The major detoxification mechanism operating in animals to combat ammonia toxicity is the formation of urea (Hoar and Randall, 1969). Urea production has additional function in the maintenance of osmotic pressure and in the production of arginine and ornithine. Liver is the main tissue for the operation of urea cycle. Significant decrease in the levels of urea in fish was observed during sublethal concentration of carbaryl and phenthoate (Sambasiva Rao, 1987). This was well evident by the decreased urea levels in this fish subjected to exposure of LAS. Hence, it is concluded that the LAS has a significant toxic effect which can induce changes / alterations regarding the protein metabolism of the fish, *C. carpio.*

Coopted Authors' :

S. GOPAL AND P. INDIRA, Department of Zoology, S.K. University, ANANTPUR (A.P.) INDIA

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