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Study of sodium arsenite induced biochemical changes on certain biomolecules of the freshwater catfish *Clarias batrachus*

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Toxic impact of sublethal concentration (1 mg/L; 5% of 96h LC_{50} value) of sodium arsenite ($NaAsO_2$) on certain biomolecules (proteins, nucleic acids, lipids, and glycogen) of five tissue components (muscles, liver, brain, skin, and gills) of the freshwater catfish *Clarias batrachus* was analysed. The important toxic manifestations include marked decrease in the concentration of proteins (21.72-45.42% in muscles; 3.42-53.94% in liver; 15.39-45.42% in brain; 15.40-4.00% in skin and 11.35-64.13% in gills), DNA (0.55-22.95% in muscles; 8.33-14.06% in liver; 5.30-18.40% in brain; 13.57-52.80% in skin; and 12.38-31.01% in gills), RNA (42.68-76.16% in muscles; 10.68-39.75% in liver; 5.66-29.05% in brain; 7.72-27.93% in skin and 21.47-44.38% in gills) and glycogen (24.00-51.72% in muscles; 49.11-72.45% in liver; 11.49-26.03% in brain; 26.13-38.05% in skin and 17.80-37.97% in gills). Excepting liver where the lipid content increases (15.82-24.13%), the fat content also showed depletion in their concentration (10.40-29.83% in muscles; 8.30-34.45% in brain; 8.94-31.47% in skin and 12.75-28.86% in gills), in the rest of the organ systems.

Foi analisado o impacto tóxico da concentração subletal (1 mg/L; 5% do valor de LC50 de 96h) do arsenito de sódio (NaAsO2) sobre certas biomoléculas (proteinas, ácidos nucleicos, lipídios e glicogênio) de cinco tecidos (músculos, fígado, cérebro, pele e brânquias) do bagre *Clarias batrachus*. As manifestações tóxicas importantes incluiram o decréscimo acentuado na concentração de proteinas (21,72-45,42% nos músculos; 3,42-53,94% no fígado; 15,39-45,42% no cérebro; 15,40-4,00% na pele e 11,35-64,13% nas brânquias), DNA (0,55-22,95% nos músculos; 8,33-14,06% no fígado; 5,30-18,40% no cérebro; 13,57-52,80% na pele e 12,38-31,01% nas brânquias), RNA (42,68-76,16% nos músculos; 10,68-39,75% no fígado; 5,66-29,05% no cérebro; 7,72-27,93% na pele e 21,47-44,38% nas brânquias) e glicogênio (24,00-51,72% nos músculos; 49,11-72,45% no fígado; 11,49-26,03% no cérebro; 26,13-38,05% na pele e 17,80-37,97% nas brânquias). Excetuando o fígado onde o conteúdo de lipídeos aumentou (15,82-24,13%), houve uma depleção na concentração de lipídeos no restante dos sistemas orgânicos (10,40-29,83% nos músculos; 8,30-34,45% no cérebro; 8,94-31,47% na pele e 12,75-28,86% nas brânquias).

Key words: Glycogen, Lipids, Nucleic acids, Proteins, Toxicity.

Introduction

There has been a continuous and alarming influx of arsenic to aquatic environment worldwide from both naturally occurring and anthropogenic sources (Goering *et al.*, 1999). More than one hundred million people are at high risk of elevated arsenic exposure, mainly via drinking water, as well as by the air born metalloid in the areas with coal burning and industrial emissions. Consumption of the arsenic contaminated fishes collected from the polluted waters might also contribute to bioaccumulation of arsenic in human beings. Hence it is of immense importance to know the arsenic induced damages in the different organ systems of fishes used for human consumption. In an effort to achieve this, the toxicity analyses of an arsenic salt (sodium arsenite) on certain important biomolecules (proteins, nucleic acids, lipids, and glycogen) of various organ systems (gills, skin, liver, brain, and muscles) were performed.

In aquatic environment arsenic exists either in arsenite (As^{3+}) or arsenate (As^{5+}) form, which is inter-converted through redox and methylation reactions (Eisler, 1988; Philips, 1994). Of these arsenic in As^{5+} form is the least toxic while As^{3+} has been found to be more harmful both under *in vivo* and *in vitro* conditions (Cervantes *et al*., 1994). The data related to toxicity of arsenic on fishes are scanty (Leah *et al*., 1992; Ghosh *et al*., 2006; Bhattacharya *et al*., 2007; Singh & Banerjee, 2007; Kavitha *et al*., 2010; Kumar & Banerjee, 2012). These data however do not reflect the damage caused to the various biomolecules of physiological importance. Hence in this study efforts have been made to analyse the arsenic induced damage caused to proteins, nucleic acids, lipids, and glycogen of certain organs (Table 1-5) of *Clarias batrachus* a popular catfish of great food value of

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the Indian subcontinent. Because nucleic acids are very important in maintaining the physiological configuration of the fish, arsenic induced alterations in the DNA and RNA levels have also been evaluated.

Material and Methods

Animal care and maintenance

Freshwater catfish *Clarias batrachus* (Linnaeus, 1758) weighing 45±5g were purchased from the local fish market at Chaukaghat Varanasi during month of November 2008. The fish were acclimated in tap water (having dissolved O_2 6.3 mg/L, pH 7.2, water hardness 23.2 mg/L and room temperature 28±3°C) for 21 days under laboratory conditions. Feeding was allowed after every 24h during the entire period of acclimation as well as the experimentation. Water from both control as well as experimental tubs were renewed regularly four hour after every feeding.

Experimental design

Twenty groups of 10 fish each were exposed separately to a sublethal concentration (1 mg/L; 5% of 96h LC_{50} value) (Kavitha *et al*., 2010) of sodium arsenite (Batch N° G270707 Loba Chemie Pvt. Ltd. Mumbai, minimum assay 98.5-102.0%) in large plastic tubs containing 10 liters of the test solution prepared in the tap water following 24h renewal bioassay technique. Tap water was selected because survival duration of the exposed fish did not vary in the test solution prepared in different types of water like distilled, pond and tap waters. Control fish were retained in 10 liters of plain tap water (without having the arsenic salt) under identical laboratory conditions. For toxicity analyses three sets of experiment (control as well as experiment) groups were run. Three fish from all the experimental as well as control aquaria were cold anesthetized and sacrificed by spinal dislocation after the expiry of 0 day, 10, 30, 45, and 60 days of exposure. Entire brain, liver, gills, and small fragments of muscle and skin were dissected out and washed in fish saline before processing.

Biochemical analyses

For preparation of extracts, tissue samples were homogenised in 20% TCA (10% tissue homogenate) and centrifuged at 5000 rpm for 10 minutes at 4°C for estimating of proteins and nucleic acids. The step was repeated once to eliminate the acid soluble substances. Pellet thus obtained was re-dissolved in 95% ethanol and centrifuged again at 5000 rpm for 10 minutes at 4°C . This step was repeated once to eliminate the lipid moieties. The supernatant was discarded and the pellet thus obtained was finally dissolved and retained in 5% TCA for 10 minutes at 90°C and centrifuged at 7000 rpm at 4°C for 10 minutes. The pellet was digested in 0.5N NaOH at 90°C in water bath for 15 minutes and centrifuged at 7000 rpm at 4°C for 10 minutes and the clear solution obtained was used for protein estimation (Lowry *et al*., 1951).

Total DNA was estimated by the diphenylamine method (Schneider, 1957). Calf-thymus DNA was used as a standard. Total RNA was estimated by the orcinol method (Schneider, 1957) using yeast RNA as a standard. For analyses of total lipids tissue samples were subjected to extraction in chloroform methanol mixture (2:1) following the method of Folch & Stanely (1957). Glycogen content was analysed following the method of Carroll *et al*. (1956). The tissue samples were digested in 30% KOH in a water bath at 90°C for 30 minutes. The digested samples were cooled overnight at 4°C and precipitated with 95% ethyl alcohol and centrifuged for 15 minutes at 1200g. This step was repeated twice. Finally the residues containing glycogen were dissolved in distilled water and measured using anthrone reagent. Glucose was used as a standard.

Statistical analysis

Two-way ANOVA followed by Dunnett's *t*-test was performed to detect the level of significance at 5% of error probability ($P<0.05$) between the treated and untreated fish groups by using the SPSS software.

Results

Fluctuations were observed in the amount of protein, nucleic acid, lipid, and glycogen contents in all the five tissues at different time intervals of exposure (Table1-5).

Skin

The amount of proteins in the skin of unexposed control fish ranged between 69.22±0.99 mg/g (in November) to 72.22±0.96 mg/g (in January). Following exposure the protein contents of the skin decreased significantly throughout the period of exposure (Table 1).The DNA content in skin of unexposed control fish fluctuated between 4.74±0.13 to 5.36±0.03 mg/g. In exposed tissue after an initial increase (after 10 days), the DNA content decreased progressively as well as significantly. The cutaneous RNA content of unexposed fish also fluctuated and ranged between 3.90±0.18 to 3.33±0.044 mg/g. The level of RNA in the exposed skin remained below the control level throughout the period of experiment. The decrease was statistically very significant after 60 days. Lipid contents in unexposed controls ranged between 21.32±0.60 mg/g (in November) to 21.64±0.37 mg/g (in January). After an initial increase following 10 days of exposure, the lipid contents of skin progressively decreased throughout the period of exposure. The decrease was statistically very significant after 60 days of exposure period. The amount of glycogen in the skin of unexposed control fish ranged between 4.83±0.197 mg/g (in November) to 4.73±0.069 mg/g (in January). After an initial increase following 10 days of the exposure, the glycogen content progressively decreased throughout the period of exposure. The decrease was statistically very significant after 60 days of exposure (Table 1).

Gills

The amount of proteins in the gills of unexposed control fish ranged between 40.88±1.16 mg/g in November to 43.66±1.01 mg/g in January, when the protein level increased

Table 1. Fluctuations in different biomolecules in the skin of *Clarias batrachus* at different period of exposure to 1mg/L of sodium arsenite. Protein, DNA, RNA, lipid, and glycogen were expressed in mg/gm wet wt of tissue. Data are shown as mean ± SEM (n = 9). (%) Denote changes over respective controls. Two way ANOVA followed by Dunnett's *t*-test. The criterion for significance differences set at (*) $p<0.05$, (**) $p<0.01$ and (***) $p<0.001$ with respective control. (^{a}) Indicates when exposed groups were compared with 0 day controls and (^{b}), when exposed groups were compared with previous exposed group.

Biomolecules	Treatments	Periods (days)				
		0	10	30	45	60
Protein	Control	69.22 ±0.996	71.44 ±0.929	71.55 ± 1.167	71.33±1.054	72.22 ±0.968
	Exposed	69.22 ±0.996	60.44 ±1.692^{***a} (-15.39%)	55.00±0.866^{***a,b} (-23.13%)	44.66±0.897^{***a,b} (-37.39%)	33.22±1.288^{***a,b} (-54%)
DNA	Control	4.74 ± 0.128	4.83 ± 0.101	5.01± 0.076	5.56 ± 0.044	5.36 ± 0.033
	Exposed	4.74 ± 0.128	5.6 ± 0.076^{a} (+15.94%)	4.33 ± 0.072* (-13.57%)	3.4 ± 0.057^{*a} (-38.85%)	2.53 ± 0.060^{*a} (-52.80%)
RNA	Control	3.90 ± 0.180	3.76 ± 0.109	3.37 ± 0.092	3.83 ± 0.192	3.33 ± 0.044
	Exposed	3.90 ± 0.180	3.3 ± 0.028 (-12.23%)	3.11 ± 0.028 (-7.72)	3.21 ± 0.086^{a} (-16.18%)	2.4 ± 0.076^{*a,b} (-27.92)
Lipid	Control	21.32±0.606	21.05±0.580	20.94±0.340	20.81±0.403	21.64±0.374
	Exposed	21.32±0.606	27.30±1.42^{*a} (+29.69%)	26.72±0.469* (+27.60%)	18.95±0.629^{*a,b} (-8.93%)	14.83±0.673*** (-31.47%)
Glycogen	Control	4.83 ±0.197	4.22 ± 0.126	4.63 ± 0.242	4.79 ± 0.118	4.73 ± 0.076
	Exposed	4.83 ±0.197	4.98 ± 0.132** (+18%)	3.42 ± 0.115^{**a,b} (-26.13%)	3.32 ± 0.098^{***a} (-30.68%)	2.93 ± 0.130^{***a,b} (-38.05%)

slightly. In exposed fish the protein contents decreased progressively throughout the period of exposure. All the decreases were statistically significant. The DNA content in gills of unexposed control fish showed periodic fluctuations (between 7.13±0.08 to 6.9±0.13 mg/g). In exposed fish after an initial increase (after 10 days), the DNA content of the gills progressively decreased which were statistically significant. The RNA content in unexposed control fish showed periodic fluctuation (7.03±0.06 to 7.30±0.07mg/g). On exposure the RNA content of gills increased initially and remained above the control level up to 30 days. Subsequently it decreased progressively and got reduced significantly after 45 days (Table 2). The lipid contents in unexposed control fish fluctuated in a narrow range (24.77±0.314 mg/g (in November) to 24.44± 0.68 mg/g (in January). Following exposure the lipid contents decreased progressively throughout the tenure of the experiment. The decreases were statistically significant during the entire period of exposure (Table 2). The amount of glycogen in the gills of unexposed control fish ranged between 4.62±0.166 mg/g to 4.01±0.117 mg/g. After an initial increase (10 days of the exposure), the glycogen content of skin progressively decreased throughout the period of exposure. The decrease was statistically very significant after 60 days (Table 5).

Liver

The amount of proteins in the liver of unexposed control fish fluctuated between 80.78±2.21 mg/g (in November) to 88.78±1.16 mg/g (in January). The protein contents in the liver of exposed fish decreased progressively throughout the period of exposure (Table 3). The decreases after 30 days onwards of exposure were highly significant. The DNA content in liver of unexposed fish fluctuated (between 2.56 ±0.04 to 2.63±0.04 mg/g) throughout the period of experimentation. Following exposure it (DNA content) decreased progressively (Table 3). The RNA content in liver of unexposed control fish showed periodic fluctuations (between 0.90±0.007 to 0.96±0.006 mg/g). Following exposure the RNA content decreased progressively throughout the period of treatment (Table 3). Lipid contents of unexposed control fish fluctuated at different experimental period and ranged between 55.33±0.91 mg/g in the initial stages of the experiment (in November) to 56.06±1.03 mg/g after 60 days (in January). Excepting after 45 days, the lipid contents of exposed fish remained above the control level throughout the period of experimentation. The alterations were also statistically significant at all the stages of exposure. The amount of glycogen in the liver of unexposed control fish fluctuated between 33.58±0.621 mg/g (in November) to 32.311±0.889 mg/g (in January). The glycogen contents in the liver of exposed fish decreased progressively throughout the period of exposure. The decrease in glycogen was highly significant (Table 3).

Brain

The amount of proteins in the brain of unexposed control fish ranged between 35.11±0.89 mg/g (November) to 41.33±1.66 mg/g (January). In the exposed tissue the protein contents after an initial increase (10 days of exposure) decreased steadily throughout the period of exposure (Table 4). The DNA content in unexposed control fish during the 60 days of observation also showed periodic fluctuation (between 1.46±0.04 to 1.63±0.04 mg/g). Following exposure the DNA contents decreased prominently. The RNA content

Table 2. Fluctuations in different biomolecules in the gills of *Clarias batrachus* at different period of exposure to 1 mg/L of sodium arsenite. Protein, DNA, RNA, lipid, and glycogen were expressed in mg/gm wet wt of tissue. Data are shown as mean ± SEM (n = 9). (%) Denote changes over respective controls. Two way ANOVA followed by Dunnett's *t*-test. The criterion for significance differences set at (*) $p<0.05$, (**) $p<0.01$ and (***) $p<0.001$ with respective control. (^{a}) Indicates when exposed groups were compared with 0 day controls and (^{b}), when exposed groups were compared with previous exposed group.

Biomolecules	Treatments	Periods (days)				
		0	10	30	45	60
Protein	Control	40.88 ±1.16	42.11 ±0.934	41.22 ± 0.795	41.44±0.959	43.66 ±1.013
	Exposed	40.88 ±1.16	37.33 ±1.20$^{**\,a}$ (-11.35%)	32.44±0.929$^{**\,a,b}$ (-21.30%)	24.22±0.878^{***a,b} (-41.55%)	15.66±0.799$^{***\,a,b}$ (-64.13%)
DNA	Control	7.13 ± 0.088	7.134 ± 0.101	6.7 ± 0.152	7.13 ± 0.101	6.9 ± 0.132
	Exposed	7.13 ± 0.088	7.76 ± 0.044 (+8.77%)	5.87 ± 0.09 ^{a,b} (-12.38%)	6.7 ± 0.076^{*a,b} (-6.03%)	4.76 ± 0.044^{*a,b} (-31.01%)
RNA	Control	7.03 ± 0.06	6.80 ± 0.076	6.93 ± 0.101	6.66 ± 0.109	7.3 ± 0.076
	Exposed	7.03 ± 0.06	7.83 ± 0.072 ^{a,b} (15.15%)	7.06 ± 0.044 ^{a,b} (1..88%)	5.23 ± 0.116* (-21.47%)	4.06 ± 0.066$^{*\,a,b}$ (-44.38%)
Lipid	Control	24.77 ±0.314	24.47±0.354	25.63±0.457	24.75±0.487	24.44±0.688
	Exposed	24.77 ±0.314	21.35±0.538^{*a,b} (-12.75%)	20.61±0.391^{*a} (-19.53%)	17.57±1.94^{*a,b} (-28.87%)	17.91±0.891^{***a} (-26.72%)
Glycogen	Control	4.62 ± 0.166	4.18 ± 0.176	4.022 ± 0.164	4.544±0.182	4.011±0.116
	Exposed	4.62 ± 0.166	4.98 ± 0.156** (+19.14%)	4.044 ± 0.157 ^{a,b} (+0.55%)	3.744±0.138^{**a} (-17.80%)	2.488±0.143^{***a,b} (-37.97%)

in unexposed control fish during the present tenure of observation also showed periodic fluctuations (between 0.49±0.01 to 0.53±0.002 mg/g). Following exposure the RNA content decreased progressively after 10 days of exposure (Table 4). The amount of lipids in the brain of unexposed control fish ranged between 66.16±0.87 mg/g (in November) to 73.38±0.87 mg/g (in January). In experimental fish the lipid contents of the brain decreased significantly throughout the period of exposure. The decrease was statistically significant throughout the tenure of exposure. The glycogen content in the brain of unexposed control fish ranged between 15.0±0.003 mg/g (in November) to 14.6±0.003 mg/g (in January). In the exposed tissue the glycogen content after a significant increase (after 10 days), decreased steadily

Table 3. Fluctuations in different biomolecules in the liver of *Clarias batrachus* at different period of exposure to 1 mg/L of sodium arsenite. Protein, DNA, RNA, lipid, and glycogen were expressed in mg/gm wet wt of tissue. Data are shown as mean ± SEM (n = 9). (%) Denote changes over respective controls. Two way ANOVA followed by Dunnett's *t*-test. The criterion for significance was set at (*) $p<0.05$, (**) $p<0.01$ and (***) $p<0.001$. (^{a}) Indicates when exposed groups were compared with 0 day controls and (^{b}), when exposed groups were compared with previous exposed group.

Biomolecules	Treatments	Periods (days)				
		0	10	30	45	60
Protein	Control	80.78 ±2.21	81.33 ±1.94	89.33 ± 0.942	83.11±1.388	88.78 ±1.164
	Exposed	80.78 ±2.21	78.55 ± 1.35 (-3.42%)	65.89±0.715$^{***\,a,b}$ (-26.23%)	55.77±1.278$^{***\,a,b}$ (-32.89%)	40.89±1.206^{***a,b} (-53.94%)
DNA	Control	2.56 ± 0.044	2.5 ± 0.028	2.4 ± 0.028	2.43 ± 0.044	2.63 ± 0.04
	Exposed	2.56 ± 0.044	2.53 ± 0.116 (+1.2%)	2.2 ± 0.028 (-8.33%)	2.20 ± 0.028^{a} (-9.46%)	2.26 ± 0.06^{a} (-14.06%)
RNA	Control	0.916 ± 0.007	0.936 ± 0.006	0.91 ± 0.007	0.953 ± 0.007	0.956 ± 0.006
	Exposed	0.916 ± 0.007	0.836 ± 0.015 (-10.68%)	0.736 ± 0.014 (-19.12%)	0.673 ± 0.004^{*a,b} (-29.38%)	0.576 ± 0.013 ^{*a,b} (-39.74%)
Lipid	Control	55.33±0.915	53.69±0.790	53.71±0.760	54.30±0.621	56.06±1.035
	Exposed	55.33±0.915	62.25±2.269* (+15.94%)	66.67±0.748* (+24.13%)	63.92±0.852* (+ 17.72%)	64.93±0.724* (+15.82%)
Glycogen	Control	33.58 ± 0.621	33.23 ± 0.633	33.01 ± 0.569	34.25 ± 0.608	32.311 ± 0.568
	Exposed	33.58 ± 0.621	16.91±0.458^{***a} (-49.11%)	15.17± 0.552^{***a,b} (-54.044%)	13.41 ± 0.577^{***a,b} (-60.84%)	8.9 ± 0.551^{***a,b} (-72.45%)

Table 4. Fluctuations in different biomolecules in the brain of *Clarias batrachus*, at different period of exposure to 1 mg/L of sodium arsenite. Protein, DNA, RNA, lipid, and glycogen were expressed in mg/gm wet wt of tissue. Data are shown as mean ± SEM (n = 9). (%) Denote changes over respective controls. Two way ANOVA followed by Dunnett's *t*-test. The criterion for significance differences set at (*) $p<0.05$, (**) $p<0.01$ and (***) $p<0.001$ with respective control. (^{a}) Indicates when exposed groups were compared with 0 day controls and (^{b}), when exposed groups were compared with previous exposed group.

Biomolecules	Treatments	Periods (days)				
		0	10	30	45	60
Protein	Control	35.11 ± 0.889	34.89 ± 0.824	37.55 ± 0.766	37.66 ± 0.745^{a}	41.33 ± 1.66^{a}
	Exposed	35.11 ± 0.889	35.64 ± 0.776 (+ 2.104%)	31.77 ± 0.571^{**a,b} (-15.39%)	25.33 ± 0.781^{***a,b} (-32.74 %)	22.56 ±1.25^{***a,b} (-45.41%)
DNA	Control	1.466 ± 0.044	1.51 ± 0.028	1.60 ± 0.06	1.53 ± 0.060	1.63 ± 0.044
	Exposed	1.466 ± 0.044	1.43 ± 0.044^{*a,b} (-5.29%)	1.46 ± 0.04^{a,b} (-8.75%)	1.33 ± 0.033^{**a,b} (-12.87)	1.33 ± 0.044** (-18.40)
RNA	Control	0.496 ± 0.0164	0.53 ± 0.005	0.536 ± 0.004	0.553 ± 0.007	0.53 ± 0.002
	Exposed	0.496 ±0.0164	0.50 ± 0.004 (-5.6%)	0.443 ± 0.006^{a,b} (-17.35%)	0.423 ± 0.004^{a,b} (-23.5%)	0.376 ± 0.01^{a,b} (-29.05%)
Lipid	Control	66.16 ± 0.871	66.789±0.708	71.733± 0.712	74.044±0.810	73.389±0.873
	Exposed	66.16 ± 0.871	51.178±0.937** (-23.37%)	65.789±0.703^{*b} (-8.29 %)	55.933±0.758^{***a,b} (-24.46%)	48.11±1.148^{**a,b} (-34.44%)
Glycogen	Control	15.0 ± 0.33	13.9 ± 0.096	14.8 ± 0.144	14.3 ± 0.197	14.6 ± 0.241
	Exposed	15.0 ± 0.33	15.3 ± 0.213^{***a} (+10.07%)	13.1 ± 0.133^{***a,b} (-11.48%)	12.4 ± 0.129^{***a,b} (-13.28%)	10.8 ± 0.115^{***a,b} (-26.02%)

during the rest of the period of exposure (Table 4). The decrease was statistically significant.

Muscles

The amount of proteins in the muscles of unexposed control fish ranged between 82.78±1.42 mg/g (in November) to 83.66±1.10 mg/g (in January). The protein contents of the muscles decreased significantly during the entire period of exposure and the loss ranged between 21.72 to 45.42% (Table 5). The DNA content in the muscular tissue of unexposed fish during the 60 days of observation also fluctuated between 5.63±0.21 to 6.1±0.08 mg/g. In the experimental fish, the decrease was statistically significant only after 45 days of exposure. The RNA content of muscular tissue of unexposed fish during the 60 days of observation also fluctuated but in narrow range (between 5.46±0.06 to 5.16±0.072 mg/g). In exposed tissue after an initial increase (10 days of the exposure) the RNA contents decreased progressively that were statistically very significant. The lipid contents in the unexposed control tissue were more or less constant ranging between 35.45±0.55 mg/g (in November) to 38.11±0.63 mg/g (in January). The pattern of fluctuation (ranging between 35.45±0.55 mg/gm to 26.74±1.04 mg/gm) in exposed fish was more or less similar to that of RNA. The amount of glycogen in the muscles of unexposed control fish ranged between 3.5±0.08 mg/g (in November) to 2.9±0.117 mg/g (in January). The glycogen contents of the muscles decreased significantly during the entire period of exposure. (Table 5)

Discussion

The slime secretion of the skin and gills of fishes protect the fish in many ways. It provides a thick barrier layer which prevents the penetration of the ambient toxicants into deeper layers. In this process the slimy secretion is subsequently sloughed off which costs heavily to the fish as reflected by progressive depletion of protein content of the skin and gills of exposed fish. In the present study the exposure of the arsenic salt was continued for 60 days with maintenance of its concentration by renewing the arsenic solution after every 24 h. Prolongation of exposure damaged the slimy protective layer causing exposure of the different cellular components of the fish to the toxic stress of the arsenic. A review of the tables 1-5 indicates that 60 days of exposure to sodium arsenite causes significant alteration in the protein, nucleic acid, lipid and glycogen contents of skin, gills, liver, brain and muscles of the fish. However the concentrations of these biomolecules fluctuated independently in these tissue components at different periods of exposure. The loss caused by the arsenic salt to the concentration of proteins in the liver was significant after 60 days when it remained only about 54% of the control value (Table 3) indicating great hepatotoxic nature of the arsenic salt. Palaniappan & Vijayasundram (2008) also demonstrated a decrease in protein quantity of liver of *Labeo rohita* fingerlings exposed to higher sublethal doses (41.5mg/L) of arsenic trioxide for 14 days. Their findings are in contrast to the observation of Irwin (1997) who noticed increased amount of proteins in the liver of *C. batrachus* following exposure to the pentavalent arsenic salt, sodium arsenate. Depletion in the protein levels of liver and muscles of fishes exposed to different concentration of several heavy metal salts for various exposure periods have also been noticed by James *et al*. (1991), Jha & Jha (1995), Palanichamy & Baskaran (1995), Redy *et al*. (1998), and Almeida *et al*. (2001).

Table 5. Fluctuations in different biomolecules in the muscles of *Clarias batrachus* at different period of exposure to 1 mg/L of sodium arsenite. Protein, DNA, RNA, lipid, and glycogen were expressed in mg/gm wet wt of tissue. Data are shown as mean ± SEM (n = 9). (%) Denote changes over respective controls. Two way ANOVA followed by Dunnett's *t*-test. The criterion for significance differences set at (*) $p<0.05$, (**) $p<0.01$ and (***) $p<0.001$ with respective control. (^{a}) Indicates when exposed groups were compared with 0 day controls and (^{b}), when exposed groups were compared with previous exposed group.

Biomolecules	Treatments	Periods (days)				
		0	10	30	45	60
Protein	Control	82.78 ±1.42	84.88 ±0.675	76.55 ± 3.84	79.66±1.080	83.66±1.105
	Exposed	82.78 ±1.42	66.44 ±1.740^{***a,b} (-21.72%)	56.88±1.24^{***a,b} (-25.70%)	53.33±1.00^{***a,b} (-33.05%)	45.66±1.536^{***a,b} (45.42%)
DNA	Control	5.63 ± 0.21	5.46 ± 0.08	5.8 ± 0.02	5.36 ± 0.06	6.10 ± 0.08
	Exposed	5.63 ± 0.21	5.43 ± 0.04 (-0.55%)	5.1 ± 0.02 (-12.07%)	5.10 ± 0.07* (-4.85%)	4.7 ± 0.13^{**a,b} (-22.95%)
RNA	Control	5.46 ± 0.142	5.53 ± 0.187	5.6 ± 0.116	5.5 ± 0.175	5.16 ± 0.072
	Exposed	5.46 ± 0.142	6.13 ± 0.072^{*a} (+10.85%)	3.21 ± 0.05^{*a,b} (-42.68%)	2.76 ± 0.044^{**a,b} (-49.82%)	1.23 ± 0.044^{***a,b} (-76.16%)
Lipid	Control	35.45±0.553	35.65 ±0.429	35.46±0.448	37.34±0.540	38.11±0.636
	Exposed	35.45±0.553	39.62±0.580^{*a,b} (+11.14%)	31.77±0.378^{*a,b} (-10.40%)	30.96±0.500^{*a,b} (-17.09%)	26.74±1.036^{***a,b} (-29.83%)
Glycogen	Control	3.46 ± 0.078	3.11 ±0.129^{a}	2.75 ± 0.148^{a}	2.85 ± 0.106^{a}	2.90 ± 0.161^{a}
	Exposed	3.46 ± 0.078	2.32 ± 0.090^{***a} (-25.4%)	2.09 ± 0.063^{**a} (-24%)	1.66± 0.113^{***a,b} (-41.75%)	1.40 ± 0.108^{***a} (-51.72%)

Like liver the protein contents of muscles also decreased (about 45%) significantly after 60 days of exposure (Table 1). Exposure to higher sublethal (21.66 mg/L) value of pesticide dimethoate for 8 days (Begum & Vijayaraghvan, 1996) also causes decrease in protein concentration of muscular tissue of *C. batrachus*. However the decrease caused by organophosphate insecticide exposure was not so extensive as observed under arsenic stress where the depletion was marked progressive throughout the period of exposure.

Exposure to the arsenic salt did not cause significant alteration in the protein level of the brain prior to 30 days of treatment. Although the toxicity of arsenic on the protein content of the brain manifested late, its concentration fell to about 55% of the control value after 60 days. This might perhaps be due to the capacity of arsenic to cross the blood brain barrier and accumulate in the brain causing degenerative changes (Rodriguez *et al*., 2002). Thirumavalavan & Samipillai (2010) also noticed decreased amount of proteins in the brain of *Catla catla* exposed to 0.1 ppm of arsenic trioxide. Arsenic induced protein depletion has also been observed in the brain of rat (Samuel *et al*., 2005).

Following exposure the protein contents of skin and gills decreased substantially. The main reason for protein decrease in these boundary tissues might be due to excessive synthesis followed by sloughing of the slime (made up of glycoprotein) induced by the arsenic stress. For inducing increased secretion of slime in the skin and gills the concentration of RNA also increases (Banerjee, 2007). The other probable reason for the loss of proteins might be due to rejection of damaged cellular components of the skin and gills rendered by the contact stress of different toxicants (Parashar & Banerjee, 2002; Hemalatha & Banerjee, 2003; Chandra & Banerjee, 2005; Devi & Banerjee, 2006; Banerjee, 2007), including an arsenic salt (Singh & Banerjee, 2008). Sloughing of damaged cells from the surface of the skin also causes decreased amount of DNA in the skin exposed to the arsenic solution for prolonged period (Table 1). The contents of both the nucleic acids (DNA and RNA) of the brain and liver decreased throughout the period of exposure indicating malfunction and degenerative changes of these vital organ systems. In rat brain it may also be due to induction of oxidative stress causing DNA damage (Samuel *et al*., 2005) leading to the decreased synthesis of RNA. Other xenobiotics like carbaryl are also known to cause DNA degenerative changes like necrosis and pycnosis in the nerves of the fish brain (Walsh & Ribelin, 1975). While the DNA content in the muscles decreases throughout the period of exposure, it increases in the skin. Sloughing of excessive quantity of slime from the body surface may perhaps be one of the main reason for RNA decrease because the slime secreting goblet cells are known to contain large amount of RNA also (Banerjee, 2007). The DNA of the skin increases but only up to 10 days perhaps due to hyperplasia of the epidermal cells a common phenomenon following exposure to various xenobiotics (Rajan & Banerjee, 1991; Banerjee, 2007), including arsenic (Singh & Banerjee, 2008c). Due to prolonged continuation of exposure the damaged cells get sloughed off causing decreased level of DNA in the skin beyond 10 days of exposure. The RNA contents of the gills increased up to 30 days of arsenic exposure perhaps due to enhanced production of slime as well as hyperplasia (Singh, 2007) of the branchial epithelia. Due to prolongation of exposure, the different components of the gills including the slime secretary cells get severely damaged causing

decrease quantity of RNA beyond 30 days of exposure. The reason for increase in the RNA content of the muscles after 10 days when there is marked decrease in protein concentration can not be properly understood. Decrease in RNA content beyond 10 days along with decrease in protein and DNA might be due to degradation of the muscular tissue.

The pattern of alteration in the lipoidal moieties of different organ systems is not identical at different stages of exposure. While the lipid contents in the gills and brain decreased significantly, in liver, they increased and remained above the control level throughout the exposure tenure. Throughout increase in the lipid contents suggests the fatty degeneration of the liver due to disturbed lipid metabolism caused by the arsenic stress (Santra *et al*., 2007). Begum & Vijaraghvan (2001) observed significant increase of lipids in the liver and other organ systems of *C. batrachus* following exposure to carbofuran. According to these authors the maximum enhancement of lipids in liver is due to the fact that the rate of lipogenesis is more in liver as it is the primary organ being affected by the insecticide toxicity. The other reason for the toxicity induced lipid increase might be due to the increased diversity of acetyl Coenzyme A to acetoacetate units for lipogenesis. According to these authors increased oxidative stress may be due to mitochondrial damage within the hepatocytes that in turn caused decreased mitochondrial oxidation of fatty acids. These fatty acids are shunted towards esterification pathways resulting in accumulation of triglycerides within the hepatocytes which seems be true in the present study with *C. batrachus* also. Arsenic also disturbs the glucose metabolism by uncoupling of oxidation and phosphorylation (Murray *et al*., 2000) causing excessive availability of unutilized glucose molecules in the tissue. These additional molecules of glucose might later get converted into glycogen or fat causing increased glycogen/lipoidal concentration. Excepting 30 days of exposure when the lipid content of the brain increases, its amount decreases at other stages indicating severe impairment caused to the central nervous system (CNS). Oxidative changes in the CNS induced by arsenic exposure in rodents are well documented (Garcia-Chavez *et al*., 2006; Dhar *et al*., 2005; Flora *et al*., 2005; Shila *et al*., 2005a, 2005b). The increase in the lipids in the skin of exposed *C. batrachus* up to 30 days followed by significant decrease indicates disturbed cutaneous lipid metabolism. The significant increase of lipids in the skin after 10 day onwards up to 30 days might be due to their release from the membrane of the damaged cells. According to Jarrett *et al*. (1995) phospholipids of the horny cells of the skin of mammals during cornification are mainly derived from the structural membrane lipids of normal cells. Exposure to several heavy metal salts including zinc chloride (Hemalatha & Banerjee, 1997a, 1997b; Chandra & Banerjee, 2005), lead nitrate (Parasar & Banerjee, 2002; Devi & Banerjee, 2006), arsenic (Singh & Banerjee, 2008a) and manganese (Chatterjee, 2008) are known to cause extensive wear and tear of the gills and skin. On the other hand arsenic causes progressive decrease in the lipid contents of the gills. This is perhaps because arsenic affects the synthesis of lipids necessary as energy reserve (Irwin *et al*., 1997). Another reason for the decreased lipid contents might perhaps be due to loss of structural membrane of the damaged cells caused by their shedding. Excessive sloughing of the slime from the body and gill surface might also decrease lipid contents because lipids have also been demonstrated in the goblet cells (Banerjee & Mittal, 1976; Mittal & Nigam, 1986). Garg *et al*. (2009) also noticed decreased lipid contents in the gills of three Indian major carps exposed to various combinations of three heavy metals (Cd, As, and zinc). In the muscles, lipids also decreased progressively but only after 10 days. The decreased lipid contents of the muscles in the later stages might have occurred due to breakdown of lipids to meet the higher energy requirement induced by toxicity (Neelamegam *et al*., 2006).

In comparison to the proteins, the carbohydrate content of fish tissues are negligible (about 1% or less) (Borrensen, 1995). The disturbance in the glycogen level is one of the important biochemical lesions due to the stress of ambient toxicants (Elumalai & Balsubramanian, 1997). The depletion of glycogen from different tissue system of *C. batrachus* following exposure to trivalent arsenic might be due to enhanced utilization of the glycogen as the immediate source to meet the energy requirement under arsenic stress. According to Kumari & Ahsan (2011) arsenic has definite role in depletion of carbohydrate store which might be a counter active mechanism to fight and survive under toxic environment.

Although the glycogen contents in the muscles and liver in *C. batrachus* decreased significantly throughout the period of exposure, its level in skin, gills, and brain decreased significantly especially after prolonged (60 days) exposure (Table 1-5). Other stress like various heavy metal contamination (Garg *et al*., 2009), cold (Haque *et al*., 2009) exercise (Driedzick & Hochachkka, 1979) or severe hypoxia (Heath & Pritchard, 1965) also cause rapid depletion of stored carbohydrate, primarily liver and muscle glycogen. According to Shastry & Rao (1984) and Naidu *et al*. (1984) the decreases in the glycogen contents in the muscles and liver of the fish following exposure to heavy metals are species specific perhaps due to their metabolic differences and environmental concentration of heavy metals and duration to which the fishes are exposed. Diwan *et al*. (1979) noticed that decrease in glycogen contents in different organ systems following exposure to industrial effluents (loaded with high concentration of Cd, Co, Cr, Pb, and Fe) were different in the same or different fishes. The glycogen content of the brain of arsenic treated *C. batrachus* showed an initial increase (Table 4). The glycogen level however decreased subsequently on further continuation of exposure. Thirumavalavan & Samipillai, (2010) also noticed decrease level of glycogen in the brain of *C. catla* exposed for 21 days to 0.1mg/L of arsenic trioxide.

Due to the toxicity of sodium arsenite the levels of glycogen, lipid, protein, and nucleic acids decreased very significantly in most of the tissues illustrating its severe toxicopathological impact on fish subjects.

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