Kathmandu University Medical Journal (2008), Vol. 6, No. 1, Issue 21, 60-69

Original Article

Arsenic induces oxidative stress, sphingolipidosis, depletes proteins and some antioxidants in various regions of rat brain

Haider SS[1], Najar MSA[2]
[1]Associate Professor, Department of Biochemistry, Nepalgunj Medical College, Chisapani Campus, Nepalgunj, Nepal, [2]Lecturer, Department of Biochemistry, Faculty of Medicine, Great Al-Fateh University, Tripoli, Libya

Abstract
Objectives: To seek an interrelationship, if any, between oxidant stress and neurochemical changes in various rat brain regions after arsenic exposure.
Materials and methods: This study was carried out at the Department of Biochemistry, Al Arab Medical University, Benghazi, Libya. Seventy five male Spraque-Dawley rats were divided into three groups:
Control group: Rats were administered 2 ml of normal saline solution/kg body weight (b.wt.) daily for 20 days by intraperitoneal (i.p.) route.
Arsenic-treated group: Rats received elemental arsenic (as sodium arsenate) 2.0 mg/kg b.wt. daily for 20 days by i.p. route.
Recovery group: Rats received 2.0 mg/kg b.wt. elemental arsenic daily for 20 days by i.p. route and were allowed to recover for 20 days. Rats were sacrificed and brains were dissected into cerebral cortex, corpus striatum, cerebellum and brain stem. Tissue homogenized in respective mediums. And were analyzed for lipid classes, oxidative stress, concentration of proteins, glutathione and ascorbic acid by utilizing standard colorimetric procedures.
Results: Arsenic exposure increased the oxidant stress because lipid peroxidation was enhanced. And decreased the contents of lipid classes, proteins, glutathione and the ascorbic acid in various rat brain regions. However, thins-layer chromatography exhibited regional variations in phospholipids classes.
Conclusion: These results suggested that arsenic-initiated oxidant stress by increasing lipid peroxidation. The losses of lipid classes, ascorbic acid and glutathione may be attributed to peroxidative damage and binding of arsenic with sulfhydryl groups of enzymes. Recovery of animals showed reversibility in most of studied parameters, but gangliosides and cerebrosides over shooted. And speculated "Sphingolipidosis". It is then likely that repeated exposures of humans to arsenic may result in hampering of cell signalling, apoptosis and mutagenesis.

Arsenic, as trivalent arsenite (As^{3+}) or pentavalent arsenate (As^{5+}), is a naturally occurring common environmental contaminant widely distributed in the biosphere[1]. Arsenate contamination of drinking water is a serious environmental problem world wide and a large number of people are at a risk[2]. Arsenate is released from several anthropogenic sources such as mining wastes, mineral debris, glass manufacture, computer chips, wood preservatives, insecticides, and some agrochemicals[3].

The potential carcinogenicity of arsenic has been extensively reviewed[4,5]. Chronic exposure of humans to trivalent arsenic has been implicated with a variety of toxic effects such as peripheral vascular disease, liver injury, neurotoxicity and cancer[6],[7,8,9,10]. Ingestion of toxic doses of arsenic may develop neurological symptoms, such as drowsiness, confusion, fever, convulsions and coma. The most common arsenic-induced neurological lesion is a peripheral neuropathy with a "stocking glove" distribution of dysesthesia. The vascular cerebral lesions occur both in the grey and white matter[11,12,13,14]. Occupational exposure to arsenic fumes displayed the symptoms such as cognitive impairment, dysfunctions of learning, and short term memory[15,16]. The mechanisms of arsenite toxicity are not clearly known. Evidences in support of the role of oxidative damage in arsenic genotoxicity/carcinogenesis are overwhelming[1]. It has been proposed that arsenite induced production of Reactive Oxygen Specie (ROS) results in damage to mitochondrial membrane and subsequently to cell death. This process has been postulated as a central step in neuronal injury[17].

Correspondence
Dr. Syed Saleem Haider
Associate Professor
Department of Biochemistry
Nepalgunj Medical College
Chisapani Campus, Nepalgunj
Nepal
E-mail: syedsaleemhaider@gmail.com

The effects of arsenic on nervous system have received considerably less attention[3]. In this study we planned to investigate the effects of sodium arsenite on the oxidative stress, contents of lipids, proteins, antioxidant defence systems in various regions of the rat brain to seek contribution of arsenic, if any, in peroxidative damage and other neurochemical perturbations. Furthermore, to unravel the effects of recovery on arsenic induced neurotoxicity in various regions of the rat brain.

Materials and methods

Chemicals

Lipid standards, brain lipids, cerebrosides, lysolecithin, phosphatidylserine, and sphingomyelin, N-acetylneuraminic acid, butylated hydroxytoluene, and 2-thiobarbituric acid were purchased from Sigma Chemical Co., St. Louis, Missouri, USA. 1,2,4-Amino naphthol sulfonic acid was obtained from Aldrich Chemical. Cholesterol and galactose were from Merk, Darmstadt, FRG. Sodium arsenite, and all the other chemicals were purchased from BDH Chemicals, England and were of analytical grade.

Animals

Seventy five male Sprague-Dawley rats were obtained from the Central Animal House of Al Arab Medical University, Benghazi, Libya. All animals weighed between 150 to 350 g. The animals were housed in stainless steel cages in groups of 4 in an animal colony. The colony was maintained on a 12 hr light/ dark cycle (dark at 2000 hr). The animals were allowed free access to laboratory pellet diet (National Company of Animal Feeds, Benghazi, Libya) and tap water.

Treatment

Animals were divided into three groups of twenty five each in the following manner.

Group I (Control group): Rats received 2 ml of physiological saline per kg body weight for 20 consecutive days by intraperitoneal route.

Group II (Arsenic-treated group): Rats received 2 mg per kg body weight elemental arsenic (as sodium arsenite) in distilled water (2 ml) for 20 consecutive days by intraperitoneal route. This dose of arsenic was in accordance with the findings of Levvy[18].

Group III (Recovery group): Rats received 2 mg per kg body weight elemental arsenic for 20 consecutive days by intraperitoneal route, were allowed to recover for further 20 days, and were then sacrificed. The weight of rats in all the three groups was recorded daily.

Neurochemical investigations

Overnight fasting rats were sacrificed by decapitation 24 hours after last injection. The brains were removed and placed in deep freezer at -20^{o}C after removing the adherent blood clots from the surface.

Dissection of the brains

The brains were dissected into the cerebral cortex, corpus striatum, cerebellum and the brain stem on a glass plate resting over crushed ice for separation of various regions[19]. The tissues were weighed to the nearest milligram on an electronic balance.

Extraction of lipids from discrete brain areas

Different parts of the brain, weighing between 150 to 300 mg were homogenized in a glass homogenizer with a teflon pestle to a final volume of 6 ml chloroform – methanol (2:1 v/v) according to the method of Folch et al.[20], with a modified procedure. Homogenates were filtered under vacuum through a sintered glass funnel. The final volume of each extract was made up to 10 ml with chloroform – methanol mixture. Thereafter, 2.5 ml of normal saline solution was added to the extracts in each test tube (4:1 v/v). This was shaken vigorously on a mixer and placed overnight at -20^{o}C in a deep freezer for separation of the two layers. Volumes of the upper and lower layers were marked respectively. The aqueous layer was used for the estimation of gangliosides and the lower layer was stored at -20^{o}C for the analysis of total lipids, phospholipids, cholesterol and cerebrosides. The analysis of total lipids was performed according to the method of Woodman and Price[21]. Phospholipids were measured by phosphate determination according to the method of Marinetti[22]. Cholesterol was estimated by Lieberman-Buchard reaction as described by Bloor et al[23]. Cerebrosides levels were determined by the method described for galactolipids and sulfolipids by Roughan and Batt[24]. Gangliosides were determined according to the method of Pollet et al[25]. Protein was measured by the procedure of Lowry et al[26]. The estimation of total and free sulfhydryl group (reduced glutathione) was performed by utilizing the procedure as described by Sedlak and Lindsay[27]. Ascorbic acid was estimated according to the method of Kyaw[28]. This procedure was partly modified by us. The amount of free malondialdehyde formed was estimated according to the method as described by Albro et al[29].

Thin layer chromatography (TLC)

Lipids were separated by one dimensional TLC on silica gel G or H according to the procedure of Ghwarsha[30]. Chloroform-methanol (90: 10: 20, by volume). And chloroform-methanol-acetic acid water (50: 30: 8: 4, by volume) were used as developing solvents for neutral lipids and individual phospholipids, respectively. The streaks were made visible with I_2 vapour or by spraying

ninhydrin and identified by reference to standards chromatographed simultaneously. The chromatographic system did not separate phosphatidylserine from phosphatidylinositol, which were therefore assayed together. After development and identification, the phospholipids spots were scraped from the plate and the phosphorus content was determined[22]. The concentration of individual phospholipids were calculated from total lipid phosphorus and the percentage distribution of phosphorus in the isolated phospholipids fractions was determined.

Statistical analysis

Data were analysed by one-way analysis of variance (ANOVA). When the analysis indicated a significant difference ($P<0.05$) was compared from corresponding controls. Statistical analyses were performed by F-test (variance-ratio test) for homogeneity of variance followed by a t-test[33].

Results

General observations

The signs of arsenic toxicity displayed akinesia, blindness, loss of hair, bleeding from the fragile nails of the limb, skin lesions. And an increased rate of urination with watery stools. Arsenic-induced manifestations, hair loss and skin lesions, remained in the rats even after a recovery period of 20 days.

Arsenic administration to the rats significantly reduced the body weight (-6%, $P<0.001$) compared from the control ones. In recovery group, the animals showed a significant regain in the body weight (+5%, $P<0.02$) (Table 1). The fresh whole brain weight of arsenic-treated rats was significantly, reduced (-9%, $P<0.001$) compared from the controls rat brain weight (Table 2). However, the rats of recovery group showed statistically significant regain in whole brain weight (+6%, $P<0.001$) (Table 2).

Effect of arsenic in various regions of rat brain

On lipid profiles

The results embodied in Table 3 show the effect of arsenic on the concentration (mg g^{-1} or μg g^{-1} fresh tissue) of total lipids, phospholipids, cholesterol, cerebrosides, gangliosides and, proteins in various regions of the brain of control and arsenic-treated rats. ANOVA revealed statistically significant reductions in the levels of total lipids and cholesterol in all the brain regions of arsenic treated rats in comparison from the control groups. The maximum depletion was observed in the cerebral cortex (-41%; $P<0.001$) followed by the corpus striatum (-33%; $P<0.001$), the cerebellum (-32%; $P<0.001$) and brain stem (-27%; $P<0.001$).

The concentration of phospholipids exhibited statistically significant increment only in the cerebellum (+13%; $P<0.01$) and the brain stem (+13%; $P<0.01$). The contents of cerebrosides were substantially elevated in the cerebellum (+51%; $P<0.001$) followed by the brain stem (+48%; $P<0.001$, corpus striatum (+46%; $P<0.001$) and the cerebral cortex (+42%; $P<0.001$). Furthermore, ganglioside levels showed significant increases in their levels in the corpus striatum (+34%; $P<0.001$) followed by the cerebral cortex (+33%; $P<0.001$), the brain stem (+23%; $P<0.001$) and the cerebellum (+20%; $P<0.01$).

Quantitative analysis of lipid classes by TLC

Lipid classes were resolved on silica G or H plates followed by quantitative analysis (Table 4). The cerebroside concentration was remarkably elevated in the following sequence: Cerebellum (-58%; $P<0.001$)) > brain stem (+55%; $P<0.001$),> corpus striatum (+49%; cerebral cortex (+40%; $P<0.001$). Phospholipids exhibited significant increases (+16%<0.01) in the brain stem followed by the cerebellum (+11%), the corpus striatum (+9%) and the cerebral cortex (+2%). However, cholesterol contents were significantly decreased in the cerebral cortex (-28%; $P<0.001$) followed by the corpus striatum (-22%; $P<0.001$), the cerebellum (-17%; $P<0.01$) and the brain stem (-16%; $P<0.001$).

Four lipid classes sphingomyelin (SM), phosphatidyl choline (PC), phosphatidyl serine (PS) plus phosphatidyl inositol and phosphatidyl ethanolamine (PE) were resolved in various brain regions on silica gel-H by TLC. PI was chromatographed with PS. The percent distribution of phospholipids in the various brain regions of control rats was in the following manner PC and PS > SM > PE in the cerebral cortex. PC > SM > PS > PE in the corpus striatum. PS and PC > SM > PE in the cerebellum. SM > PE > PS > PC in the brain stem. The brain regions of arsenic intoxicated rats, however, showed altered sequence in the following way: PC and PE > PS and SM in the cerebral cortex. PS and PE > SM > PC in the corpus striatum. PC > PE > PS and SM in the cerebellum. And in the brain stem the pattern was SM > PS > PE > PC. Furthermore, quantitative analysis of phospholipids streaks from TLC plates confirmed increases in their concentrations following arsenic intoxication. SM-containing phospholipids were remarkably increased ($P<0.001$) in the brain stem followed by cerebellum ($P<0.01$), cerebral cortex and corpus striatum ($P<0.05$). PC-containing phospholipids were increased in cerebellum followed by brain stem ($P<0.001$) and the cerebral cortex ($P<0.05$). PS + PI-containing phospholipids were significantly decreased in the cerebral cortex in the cerebellum and in the brain stem ($P<0.05$). PE-containing phospholipids were increased

significantly in the corpus striatum followed by cerebellum, cerebral cortex and the brain stem (P<0.001) (Table 4).

On proteins levels

Table 6 shows statistically significant reductions (P<0.001) in the protein levels in the following sequence: brain stem (-31%) followed by the corpus striatum (-25%), the cerebellum (-24%) and the cerebral cortex (-17%) following arsenic-intoxication.

On total sulfhydryl groups (TSH) and glutathione (GSH) contents

Table 6 depicts that arsenic toxicity resulted in remarkable decreases (P<0.001) in the TSH groups in the following order: cerebral cortex (-69%) followed by brain stem (-68%), the corpus striatum (-67%), and the cerebellum (-66%). Furthermore, significant depletion (p<0.001) in GSH concentration was discernible in the various brain regions of arsenic intoxicated rats. The corpus striatum exhibited maximum depletion (-57%) followed by the cerebellum (-52%), the brain stem (-51%), and the cerebral cortex (-48%).

On ascorbic acid concentration

There was a remarkable diminution in the concentration of ascorbic acid in various regions of arsenic-treated rats. The order of reduction was corpus striatum (-31%), the cerebral cortex (-27%), the brain stem (-25%), and the cerebellum (-21%). The p value calculated was less than 0.01 in all the brain regions (Table 1).

On oxidative stress (occurrence of lipid peroxidation)

The data in table 5 demonstrates significant increases in occurrence of lipid peroxidations (LPO), measured as malondialdehyde, formed, in all rat brain regions following incubation of homogenates in the absence or in the presence of atmospheric oxygen for 2 hrs in a metabolic shaker. The occurrence of LPO was in the following order: the cerebral cortex (+60%), the corpus striatum (+57%), the cerebellum (+44%) and the brain stem (+37%). The p value was less than 0.01 in all the brain regions.

Effect of recovery (20 days) following arsenic intoxication

On lipid profiles

The concentration of total lipids (Table 3) was significantly increased in various rat brain regions in the recovery group in comparison from arsenic intoxicated group. The corpus striatum showed maximum increases (+21%; p<0.01) followed by the cerebral cortex (+19%; p<0.01), the brain stem (+14%; p<0.01), however only +10% increase was discernible in the cerebellum. These results indicate "restoration" of total lipid concentration following recovery of the animals.

The effect of recovery on phospholipids was insignificant as slight increases in the corpus striatum (+2%), but decreases in the cerebellum (-6%), the cerebral cortex (-4%), and the brain stem (-3%) were evaluated. Furthermore, the concentration of cholesterol exhibited slight increment after recovery in the following sequence: the cerebral cortex (+8%), the cerebellum (+6%), the corpus striatum (+5%) and the brain stem (+3%) (Table 3). Very interestingly, noteworthy findings were, accumulation of the gangliosides and cerebrosides in all the rat brain regions even after recovery period (Table 3), in comparison from arsenic intoxicated rats. The accumulation of gangliosides were in the following order: cerebral cortex (+13%) > brain stem (+8%) > corpus striatum (+2%) and cerebellum (+1%). However, cerebrosides were accumulated in a range of 1 to 2% in all the brain regions following recovery. These findings suggest "irreversible manifestations" or "sphingolipidosis."

Effect of recovery (20 days) on arsenic neurotoxicity

On lipid peroxidation (LPO)

Table 5 shows that arsenic induced increases in occurrence of LPO were significantly inhibited in various brain regions in the following order: the cerebral cortex (-27%; p<0.001), the corpus striatum (-21%; p<0.001), the cerebellum (-4%) and the brain stem (-2%).

On protein, TSH, GSH and ascorbic acid concentrations

The protein levels were significantly increased in the brain stem (+25%; P<0.02), however, insignificant increment in the cerebral cortex (+10%), the corpus striatum (+4%), and in the cerebellum (+3%) was discernible. Table 6 shows that decrement in the TSH in arsenic intoxication was restored in the various rat brain regions in the following sequence The brain stem (+51%; P<0.001) > corpus striatum (+49%; P<0.001), cerebral cortex (+44%; P<0.001) and the cerebellum (+44%; P<0.001). Table 6 also demonstrates statistically significant restoration of GSH levels in the following sequence: Cerebellum (+40%; P<0.001), cerebral cortex (+34, P<0.001), brain stem (+16%; P<0.05) and corpus striatum (+15%; P<0.05). Furthermore, ascorbic acid levels were significantly increased (P<0.001) in the recovery group in the following sequence: brain stem (+26%; P<0.001) > cerebral cortex (+24%; P<0.001) > corpus striatum (+22%; P<0.001) > cerebellum (+20%; P<0.001).

Table 1: Effect of arsenic exposure on body weights and mortality of rats

Group	Treatment	Body weight (gm)		Changes in body weight (g)	P value	Mortality
		Day 1	Day 20			
1	Control (normal saline)	326.5±16.52 (N=32)	330.4±16.64 (N=32)	+4 (g)	N.S.	0/32
2	Arsenic	325.77±16.33 (N=32)	305.02±19.10 (N=30)	-20 (g)	<0.001	2/32
3	Arsenic + Recovery	342.2±2.69 (N=10)	321.7±3.16 (N=10)	-21 (g) -17 (g)	<0.001 <0.02	0/10

N = number of animals in each group. Arsenic (2 mg kg^{-1} body weight) was injected intraperitoneally for 20 consecutive days.
Values represent the mean ± SD statistical analysis by analysis of variance.

Table 2: Effect of arsenic exposure on rat brain weights

Groups	Brain weight (g)
Normal saline	1.79 ± 0.023 (N = 32)
Arsenic	1.632 ± 0.64^{a} (N=30)
Arsenic + Recovery	1.731 ± 0.087^{ab} (N = 9)

N = number of animals in each group. Arsenic (2 mg kg^{-1} body weight) was injected intraperitoneally for 20 consecutive days.
Values represent the mean ± S.D. a = significantly different from control group (P<0.05). b = significantly different from arsenic-treated group (P<0.05).

Table 3: Effect of Arsenic (2 mg/kg^{-1} b.wt.) exposure intraperitoneally consecutively for 20 days on lipid profiles and protein concentration in various regions of rat brain.

Brain regions / parameters	Control (N=8)	Arsenic (N=8)	% of control	Arsenic + Recovery (N=6)	% of Arsenic
Cerebral cortex		**(mg/g fresh weight)**		**(mg/g fresh weight)**	
Total lipids	117.12±7.34	69.53^{a}±9.51	-41	82.92^{ab}±11.28	+19
Phospholipids	48.72±4.71	52.49±3.54	+08	50.45±12.97	-04
Cholesterol	21.19±0.9	16.32^{a}±1.48	-23	17.58^{a}±1.2	+08
Gangliosides*	370.89±28.06	493.00^{a}±22.6	+33	558.31^{a}±33.44	+13
Cerebrosides	23.25±2.35	32.94^{a}±3.89	+42	33.19^{a}±1.89	+01
Proteins	45.45±3.04	37.64±3.71	-17	41.54^{+10}±3.21	+10
Corpus striatum					
Total lipids	118.38±5.25	79.85^{a}±7.68	-33	96.9^{ab}±5.66	+21
Phospholipids	48.44±8.42	54.21±3.88	+12	55.31±1.39	+02
Cholesterol	21.88±1.82	17.04^{a}±1.17	-22	17.84^{a}±1.87	+05
Gangliosides*	355.37±28.22	477.55^{a}±27.41	+34	480.19^{a}±30.03	+01
Cerebrosides	26.58±5.63	38.72^{a}±6.05	+46	37.99^{a}±1.78	+02
Proteins	46.45±2.42	34.88^{a}±2.35	-25	36.21^{a}±3.18	+04
Cerebellum					
Total lipids	122.78±4.93	84.10^{a}±13.45	-32	92.29^{a}±10.66	+10
Phospholipids	57.18±2.24	64.49^{a}±14.37	+13	60.68±13.86	-06
Cholesterol	21.34±1.95	16.84^{a}±0.75	-21	17.93^{a}±2.06	+06
Gangliosides*	396.19±24.98	474.03^{a}±25.35	+20	477.06^{a}±32.11	+01
Cerebrosides	27.00±5.09	40.65^{a}±2.73	+51	41.3±3.91	+02
Proteins	45.21±4.55	34.32^{a}±4.68	-24	35.44^{a}±1.62	+03
Brain stem					
Total lipids	171.84±9.37	125.66^{a}±8.86	-27	143.78^{a}±8.08	+14
Phospholipids	63.29±3.44	71.78^{a}±3.01	+13	69.42±2.98	-03
Cholesterol	27.11±2.89	22.97^{a}±1.65	-15	23.61^{a}±3.10	+03
Gangliosides*	285.35±25.87	350.61^{a}±26.85	+23	379.2^{a}±16.29	+08
Cerebrosides	47.28±6.34	69.77^{a}±8.5	+48	67.98^{a}±1.79	+01
Proteins	45.03±3.03	31.05^{a}±4.38	-31	38.81^{ab}±1.25	+25

*The data are expressed as μg/g of fresh weight. Values represent the mean±S.D. Statistical analysis by one-way analysis of variance.
a = Significantly different from control group (P<0.05). b = Significantly different from arsenic-treated group (P<0.05).

Table 4: Effect of arsenic (2 mg/kg^{-1} b.wt.) exposure intraperitoneally consecutively for 20 days on the major phospholipids classes in the various regions of the rat brain: analysis by thin layer chromatography

Brain regions / Phospholipid class	Control (N=3)	Arsenic (N=3)
	(Percentage of all major total phospholipids)	
Cerebral cortex		
Sphingomyelin	24 ± 2.32	23 ± 5.5
Phosphatidyl choline	26 ± 6.03	24 ± 3.37
Phosphatidyl serine	25 ± 5.09	23 ± 2.19
Phosphatidyl ethanolamine	20 ± 3.5	24 ± 2.28
Corpus striatum		
Sphingomyelin	26 ± 6.32	24 ± 2.62
Phosphatidyl choline	27 ± 7.93	23 ± 3.72
Phosphatidyl serine	24 ± 2.22	25 ± 2.78
Phosphatidyl ethanolamine	20 ± 1.78	25 ± 2.05
Cerebellum		
Sphingomyelin	23 ± 0.62	23 ± 3.93
Phosphatidyl choline	25 ± 3.06	30 ± 1.72
Phosphatidyl serine	22 ± 3.9	23 ± 4.61
Phosphatidyl ethanolamine	23 ± 3.93	26 ± 2.38
Brain stem		
Sphingomyelin	27 ± 1.21	31 ± 4.29
Phosphatidyl choline	21 ± 5.24	22 ± 7.15
Phosphatidyl serine	22 ± 3.13	29 ± 4.4
Phosphatidyl ethanolamine	26 ± 4.13	25 ± 1.12

N = number of samples in each group. The data are expressed as percentage of the sum of all lipid classes.
Values represent the mean ± S.D. Statistical analysis was insignificant by one-way of ANOVA.

Table 5: Effect of arsenic (2 mg/kg^{-1} b.wt.) exposure intraperitoneally consecutively for 20 days on lipid peroxidation in various regions of rat brain

Brain Regions	Control (N=8)	Arsenic (N=7)	% of control	Arsenic + Recovery (N=6)	% of Arsenic
	Malondialdehyde (nanomoles formed g^{-1} fresh tissue)			Malondialdehyde (nanomoles formed g^{-1} fresh weight)	
Cerebral cortex					
Zero hour	22.02±1.97	28.58^{a}±2.69	+30	23.87^{b}±1.01	-16
2 hour	137.45±6.09	219.83^{a}±10.21	+60	159.60^{ab}±10.68	-27
Corpus striatum					
Zero hour	19.26±2.64	23.39^{a}±3.81	+21	20.48$^{b±}$0.78	-12
2 hour	120.37±10.34	188.00^{a}±9.72	+57	148.78^{ab}±8.35	-21
Cerebellum					
Zero hour	17.29±2.64	20.11^{a}±1.43	+16	19.53^{a}±0.78	-03
2 hour	120.25±6.6	172.79^{a}±5.99	+44	165.71^{a}±6.7	-04
Brain stem					
Zero hour	17.2±2.7	19.7^{a}±2.2	+15	19.58^{a}±0.67	-01
2 hour	124.08±4.83	170.34^{a}±8.07	+37	167.38^{a}±8.36	-02

Zero hour = samples not incubated under atmospheric O_2. 2 hour = samples incubated under atmospheric O_2 for 2 hour in a metabolic shaker. N = number of animals in each group. Values represent as the mean ± S.D. Statistical analysis by one-way analysis of variance.
a = significantly different from control group ($P<0.05$) b = significantly different from arsenic-treated group.

Table 6: Effect of arsenic (2 mg/kg^{-1} b.wt.) intraperitoneally consecutively for 20 days on total protein-bound sulfhydryl group, free sulfhydryl group (glutathione) and ascorbic acid in various region of rat brain

Brain Regions	**Control (N=8)**	**Arsenic (N=8)**	**% of control**	**Arsenic + Recovery (N=6)**	**% of Arsenic**
	(millimoles g^{-1} fresh tissue)			**(millimoles g^{-1} fresh tissue)**	
Cerebral cortex					
Total sulfhydryl group	1.74±0.1	0.53^{a}±0.05	-69	0.77^{ab}±0.04	+44
Free sulfhydryl group (glutathione)	0.496±0.022	0.259^{a}±0.022	-48	0.347^{ab}±0.011	+34
Ascorbic Acid*	294.1±7.17	182.57^{a}±11.13	-27	225.5^{ab}±11.03	+24
Corpus striatum					
Total sulfhydryl group	1.63±0.16	0.53^{a}±0.04	-67	0.79^{ab}±0.09	+49
Free sulfhydryl group (glutathione)	0.562±0.038	0.241^{a}±0.026	-57	0.278^{ab}±0.019	+15
Ascorbic Acid*	253.18±5.56	174.63^{a}±7.84	-31	213.00^{ab}±8.36	+22
Cerebellum					
Total sulfhydryl group	1.52±0.18	0.52^{a}±0.05	-66	0.75^{ab}±0.02	+44
Free sulfhydryl group (glutathione)	0.505±0.047	0.240^{a}±0.01	-52	0.337^{ab}±0.040	+40
Ascorbic Acid*	194.57±8.89	153.88^{a}±3.91	-21	184.25^{ab}±9.67	+20
Brain stem					
Total sulfhydryl group	1.61±0.10	0.51^{a}±0.02	-68	0.77^{ab}±0.08	+51
Free sulfhydryl group (glutathione)	0.482±0.029	0.238^{a}±0.02	-51	0.282^{ab}±0.019	+16
Ascorbic Acid*	192.13±8.03	143.94^{a}±6.26	-25	181.63^{ab}±6.00	+26

*The data are expressed as μg g^{-1} of fresh weight. N = number of animals in each group.
Values represent the mean ± S.D. Statistical analysis by one-way analysis of variance.
a = significantly different from control group (P<0.05) b = significantly different from arsenic-treated group.

Discussion

No comparable reports of any previous study dealing with the effect of arsenic on lipid profiles, proteins, ascorbic acid, glutathione and oxidative stress in the specific regions of rat brain are available in literature[3]. The present study is a singular attempt that provides information regarding the effect of arsenic on these neurochemical parameters in specific rat brain regions.

Intraperitoneal administration of sodium arsenate (2 mg arsenic / kg b.wt.) for 20 days was in accordance with the findings of Ledet and Buck[32]. Because maximum arsenic levels accumulate in the nervous tissue after 19 to 21 days. Subacute arsenic intoxication in our study was manifested by akinesia, loss of appetite, significant loss of body weights and subsequent loss in brain weights. Additionally, loss of hairs, dermatitis and hyperkeratosis of the skin, blindness and bleeding from fragile nails of limbs, and increased urine frequency followed by watery stool. This is an indication that arsenic administration in our study has reduced appetite and interfered with the normal metabolism of the nutrients by the tissues.

In this study, the body weight loss and akinesia could be attributed to refusal to take food and deficit of energy (calories). The loss of brain weight in arsenic treated rats was possibly due to arsenic-evoked neuronal cell loss in brain. The dermatitis, hyperkeratosis of the skin and blindness with opacity of cornea in arsenic-treated rats could have been following damage of the mucus membrane. The binding of arsenic to sulfhydryl group of keratin in this study might have lead to loss of hairs and fragility of nails. Previously, such manifestations have been described in man and animals following acute and subacute arsenic exposure[33,34].

Effect of arsenic exposure on total lipids, cholesterol and occurrence of lipid peroxidation in the specific rat brain regions

To the best of our knowledge no information is available on the effects of arsenic exposure on the regional brain lipid profiles. The results of this study demonstrated that arsenic exposure, 2.0 mg/kg b.wt., intraperitoneally for 20 days resulted in significant depletion of total lipids and cholesterol concentration in specific brain regions, such as, cerebral cortex, which exhibited maximum decrement followed by corpus striatum,

cerebellum, and brain stem. The decrease in total lipids was accompanied by an increase in lipid peroxidation, parameter that measures oxidative stress, in specific rat brain regions following arsenic intoxication. The results of this study are in congruence with our previous work where substantial losses of rat brain lipids and occurrence of peroxidative damage were evaluated following exposure to environmental pollutants and heavy metals[35,36]. We had also shown that the exposure of rats to trimethyltin initiates peroxidative brain damage with losses of lipids. And electron microscopic studies showed accumulation of lipofusin in specific rat brain regions.[37] Furthermore, Sasi and Haider[38] had demonstrated similar magnitude of vanadium induced initiation of peroxidative reactions with preferential losses of fatty acids and lipid fractions. And reductions in concentrations of glutathione and proteins as well in specific rat brain regions. In this study, the decreased total lipids concentrations were mainly attributed to enhanced lipid peroxidation in arsenic administered rats. The significant decrements in cholesterol levels in specific regions of rat brain might be due to inhibition of the enzymes of cholesterol synthesis.

Effect of arsenic exposure on phospholipid classes and sphingolipids in various rat brain regions

Our results have demonstrated that arsenic exposure resulted in significant elevations in phospholipids in the cerebrum and brain stem, however, insignificant increases in cerebral cortex and corpus striatum were noticed. We cannot exclude the possibility that arsenic has close resemblance with inorganic phosphate (P_i), therefore, it may have replaced phosphate by attacking the energy rich thioester intermediates in metabolic pathways[39]. Henceforth, synthesis of phospholipids was increased in an attempt to replenish their damage in cell membrane following oxidant stress.

Noteworthy findings in our study were significant increases in the contents of gangliosides in the following order: corpus striatum > cerebral cortex > brain stem and cerebellum. However, sequence of increment in the cerebroside concentration was cerebellum > brain stem > corpus striatum > cerebral in the cortex after arsenic exposure. It is well documented in the literature that accumulations of one of these lipids in the brain might result in human genetic diseases such as Taysach's disease, Gaucher's disease or Nieman Pick's disease[40]. It is thus likely that arsenic might have inhibited the enzymes like β-galactosidases or glucocerebrosidases or galactosidases. These errors consequently lead to "sphingolipidosis" in the studied at brain regions following arsenic intoxication.

Thin layer chromatography, performed in various rat brain regions, further substantiated the notion that arsenic neurotoxicity is a cause of "sphingolipidosis" because sphingomyelin is elevated as well after arsenic exposure. It is speculative that either sphingomyelinase enzyme is inhibited or slow rate of sphingomyelin degradation was responsible for sphingomyelin accumulation. Furthermore, levels of phosphatidylcholine, and phosphatidyl-ethanolamine were decreased. These results suggest that asymmetrical biological membrane lipid-bilayer contributed to differential effects of arsenic on phospholipid-classes in the specific rat brain regions.

Effect of arsenic exposure on the glutathione and ascorbic acid concentration in specific regions of rat brain

In this study, we have evaluated enhancement of lipid peroxidation with concomitant decreases in the levels of TSH groups, GSH levels and ascorbic acid. GSH exhibited maximum depletion in the corpus striatum and minimum in the cerebral cortex, while the cerebellum and the brain stem showed decreases by 51% respectively. On the other hand, ascorbic acid also showed maximum depletion in corpus striatum. And minimum in the cerebellum. Our results are in congruence with the notion that arsenic neurotoxicity might have been overwhelming the studied antioxidants defences following peroxidative damage[41].

Effect of arsenic exposure on protein concentration in the various regions of rat brain

Oxidant stress may cause damage to sulfur-containing enzymes and other proteins. The phenomenon culminates in inactivation, defective cross linking, and protein denaturation[42]. In this study, we have evaluated significantly reduced concentrations of ascorbic acid and proteins in comparison from control ones. Our results are in agreement with the results of Hismaga et al.[43] We have also shown that exposure to elemental vanadium inhibited TSH groups and GSH in various rat brain regions[44]. These findings may be explained in two ways: firstly, arsenic exposure might have oxidized thiol groups in the proteins because both protein-bound and non-protein bound sulfhydryl groups are reduced. Secondly, these events may contribute oxidative modification of glycoprotein carbohydrate moieties.

Implications of recovery on arsenic induced manifestations in the various regions of rat brain

The recovery period of 20 days in our study seemed to be sufficient following arsenic exposure.

Because Thorn et al[45]. and Grecelius[46], have reported that the rats excrete 86% of administered arsenic in urine in 18 days. In our study animals gained body weight and the brain weight as well as. This could be attributed to increase in appetite and food intake by the rats. Loss of hair persisted which provides evidence that arsenic binds with strong affinity to keratin-sulfhydryl groups. Occurrence of lipid peroxidation was retarded in various rat brain regions in comparison from control ones following recovery of arsenic treated rats. Moreover, ascorbic acid and glutathione levels also demonstrated reversibility toward normal values. It is then likely that probably most of the administered arsenic is eliminated from the body of the animals. Partial recovery in the levels of lipids and proteins in our study is a consequence of weight gain. Because rats dietary intake was increased and deficit of calories was made up. Furthermore, occurrence of lipid peroxidation was retarded, therefore, lesser degradation of total lipids was noticed in comparison from control ones.

Interestingly, however, cerebrosides and gangliosides exhibited accumulation in the various regions of the brain in the recovery period in comparison to arsenic ones. These results are referred to as "Irreversible Manifestations". It may be a possibility that the classes of enzyme which catabolize sphingolipids were completely inhibited in various regions of rat brain following arsenic neurotoxicity. The "arsenic-induced sphingolipids", could be responsible in brain encephalopathy. Our results are in good agreement with a WHO report[5].

In summary, the results of the present study suggest that arsenic neurotoxicity in rats initiated peroxidative reactions in membrane lipids of the brain. The depletion of studied antioxidant in this study might be responsible for reduced disposal of oxygen free radicals and peroxides. There is a possibility that they may escape the cellular defence system and eventually cause cellular injury by damaging DNA structure, proteins and lipids. We cannot exclude the possibility that the accumulation of cerebrosides and gangliosides in the various regions of rat brain after arsenic exposure might be responsible in the development of genetic defects, the "sphingolipidosis." Furthermore, it is implicated that arsenic is a de-myelinating agent. And may alter neuronal functions followed by CNS dysfunctions.

Much remains to be learned about this ancient neurotoxicant. It is planned in future to study the effects of arsenic in brain with respect to myelin structure and functions, DNA and RNA levels and to seek a correlation with oxidant stress, and to estimate the levels of antioxidant defence system enzymes.

References

1. Hei TK, Filipic M. Role of oxidative damage in the genotoxicity of arsenic. Free Rad Biol Med 2004;37:574-81.
2. Chowdhury UK, Biswas BK, Chowdhury TR, Samanta G, Mandal BK, Basu GC, et al. Groundwater arsenic contamination in Bangladesh and West Bengal, India. Environ Health Perspect 2000;108:393-7.
3. Rordriguez VM, Jiménez-Capdevillie ME, Giordano M. The effects of arsenic exposure on the nervous system. Toxicol Letters 2003,145:1-18.
4. U.S. Environmental Protection Agency. Special report on ingested inorganic arsenic: skin cancer and nutritional essentiality. Risk assessment form. EPA, Washington, DC, 1987.
5. World Health Organization (WHO). Environmental Health Criteria 19. Arsenic World Health Organization, Geneva, 1981.
6. Abernathy CO, Liu YP, Longfellow HV, Aposhian B, Beck B, et al. Arsenic: health effects, mechanisms of action and research issues. Environ. Health Perspect 1999;107:593-7.
7. Gerr F, Letz R, Ryan PB, Green RC. Neurological effects of environmental exposure to arsenic dust and soil among humans. Neurotoxicology 2000,21:475-87.
8. Kitchin KT. Recent advances in arsenic carcinogenesis: modes of action, animal model systems, and methylated arsenic metabolites. Toxicol Appl Pharmacol 2001,172:249-61.
9. Pi JH, Yamauchi Y, Kumagai G, Sun T, Yoshida H, et al. Evidence for induction of oxidative stress caused by chronic exposure of Chinese residents to arsenic contained in drinking water. Environ. Health Perspect 2002,110:331-6.
10. Tseng CH, Tai TY, Chong CK, Tseng CP, Lai MS. Long-term arsenic exposure and incidence of non-insulin-dependent diabetes mellitus: a cohort study in arseniasis-hyperendemic villages in Taiwan. Environ Health Perspect 2000,108:847-51.
11. Dart CR. Arsenic. In: Sullivan Jr, JB, Krieger GR, editors. Hazardous Material Toxicology. Clinical Principles of Environmental Health, USA: Williams and Wilkins 1992:818-22.
12. Spancer PS, Miller MS, Ross SM, Schwab BW, Sabri MI, et al. Biochemical mechanisms underlying primary degeneration of axons. In: Lajtha A, editor. Handbook of Neurochemistry. Alternations of Metabolites in the Nervous System Vol. 9. 2nd ed. New York: Plenum Press, 1985: 49.
13. Klaassen CD, Eaton DL. Principles of Toxicology. In: Amdur MO, Doull J, Kaassen CD, editors. Casarett and Doull's Toxicology. The Basic Science of Poisoning. New York: Pergamon Press; 1992. P. 12-47.
14. Danan M, Conso F, Dally S, Bertaux L, Chouraki L, et al. Arseneous anhydride poisoning. Peripheral neuropathy and changes in cognitive functions. Ann Med Interne 1985;6:479-81.

15. Hall AH. Chronic arsenic poisoning. Toxicol Lett 2002;128:69-72.
16. Morton WE, Caron GA. Encephalopathy: an uncommon manifestation of workplace arsenic poisoning? Am J Ind Med 1989;15:1-5.
17. Reynolds IJ. Mitochondrial membrane potential and the permeability transition in excitotoxicity. Ann NY Acad Sci 1999;893:33-41.
18. Levvy GA. The toxicity of arsine administered by intraperitoneal injection. Br J Pharmacol 1946;1:287-90.
19. Glowinski J, Iverson IL. Regional studies on catecholamines in the rat brain. I. The disposition of (3H) norepinephrine, (3H) dopamine and (3H) dopa in various regions of the rat brain. J Neurochem 1966;13:665-9.
20. Folch J, Ascoli I, Less M, Meath JA et al. Preparation of lipid extracts from brain tissue. J Biol Chem 1951;191:833-41.
21. Woodman DD, Price CP Estimation of serum total lipids. Clin Chem Acta 1972, 38: 39-43.
22. Marinetti GV. Chromatographic separation, identification and analysis of phosphatides. J Lipid Res 1962;3:1-12.
23. Bloor Wr, Pelkan KF, Allen DM. Determination of fatty acids and cholesterol in small amount of blood plasma. J Biol Chem 1922;52:191-205.
24. Roughan PG, Batt RD. Quantitative analysis of sulfolipids and galactolipids in plant tissues. Anal Biochem 1968;22:74-88.
25. Pollet S, Ermidou S, Lesaux F, Monge M, Baumann N. Microanalysis of brain lipids: Multiple two dimensional thin layer chromatography. J Lipid Res 1979;19:916-21.
26. Lowry OH, Rosenberg NJ, Farn AL, Randall RJ. Protein measurement with Folin phenol reagent. J Biol Chem 1951;193:265-75.
27. Sedlak J, Lindsay F. Estimation of total protein-bound, and non-protein sulfhydryl groups in tissue with Ellman's reagent. Anal Biochem 1968;25:192-205.
28. Kyaw A. A simple colorimetric method for ascorbic acid determination in blood plasma. Clinica Chimica Acta 1978;86:153-7.
29. Albro PW, Cobett JT, Schroeder JL. Application of the thiobarbi-turate assay to the measurement of lipid peroxidation products in microsomes. J Biochem Biophys Methods 1986;13:185-94.
30. Ghwarsha KM. Action of lipolytic enzyme on myelin sheath preparation from the central nervous system. D.Phil Thesis, UK. University of York, Helsington 1981.
31. Kirkwood BR. Essentials of Medical Statistics. Blackwell Scientific Publications, U.K., Oxford; 1989. P. 47-50.
32. Ledet AE, Buck WB. Toxicity of organic arsenials in food stuffs. In: Oehme FW, editor. Toxicity of Heavy Metals in the Environment. USA, Marcel Dekker; 1978. P. 375-90.
33. Gonzales SG. Acute arsenic poisoning in dairy cattle in the Lagoon region. In: Memorias del 1 Semposium International de Laboratories Veterinarios de Diagnosticos. Mexico; 1977. P. 3: 551-60.
34. Pinto SS, McGill CM. Arsenic trioxide exposure in industry. Ind Med 1953;22:281-7.
35. Haider SS, Hasan M. Neurochemical changes by inhalation of environmental pollutants sulfur dioxide and hydrogen sulfide: Degradation of total lipids, elevation of lipid peroxidatioin and enzyme activity in the discreate regions of the guinea pig brain and spinal cord. Industrial Health 1984;22: 23-31.
36. Haider SS, Hasan M, Hasan SN, Khan SR, Ali SF. Regional effects of sulfur dioxide exposure on the guinea pig brain lipids, lipid peroxidation and lipase activity. Neurotoxicology 1981;2:443-50.
37. Hasan M, Haider SS, Bajpai VK. The biochemical and ultrastructural studies on the effects of trimethyltin on regional brain lipid profiles and lipid peroxidation. Indust Health 1984;22:107-16.
38. Sasi MM, Haider SS, El-Fakhri M, Ghwarsha KM. Micro-chromatographic analysis of lipids, protein and occurrence of lipid peroxidation in various brain areas of vanadium exposed rats: A possible mechanism of vanadium neurotoxicity. Neurotoxicology 1994;15:413-20.
39. Burtis CA, Ashwood ER. Tietz textbook of clinical chemistry, London, W.B. Saunders Company 1994;1216-7.
40. Zubay G, editor: Biochemistry, 3rd edition, England, W.C. Brown Publishers, 1993;627-9.
41. Machlin LJ, Bendich A. Free radical tissue damage; protective role of antioxidant nutrients. FASEB J 1987;1:441-7.
42. Serhan C, Anderson P, Goodman E, Dunham P et al. Phosphatide and oxidized fatty acids are calcium ionophores. J Biol Chem 1991;256:2736-41.
43. Hismaga K, Sagar SM, Sharp ER. Ascorbate neurotoxicity in cortical cell culture. Annals Neurol 1992;15:562-5.
44. Haider SS, Kashyap SK. Vanadium intoxication inhibits sulfhydryl groups and glutathione in rat brain. Indust Health 1988, 27: 23-25.
45. Thorne MC, Jackson D, Smith AD. Pharmacodynamic models of selective chemicals in man: Review of metabolic data. London, Butler and Tanner; 1986. P. 9-62.
46. Crecelius EA. Arsenite and arsenate levels in urine. Bull Environ Contam Toxicol 1997;18:227-30.