

Subchronic Early Life Arsenic Exposure at Low Doses Impaired the Biogenic Amine Neurotransmitter and Nitric Oxide Levels in Different Brain Regions of Rats

Lalit P Chandravanshi^{1,2*} and Devendra K Patel³

¹Department of Zoology, Institute of Science, Banaras Hindu University, Varanasi, 221005, India

²Developmental Toxicology Division, CSIR-Indian Institute of Toxicology Research, Post Box No. 80, MG Marg, Lucknow, 226 001, India

³Analytical Chemistry Division, CSIR-Indian Institute of Toxicology Research, Post Box No. 80, MG Marg, Lucknow, 226 001, India

Abstract

Current approaches to risk assessment assume the neurotoxic response for arsenic at the low doses while the mechanism of arsenic-induced developmental neurotoxicity is unknown. Because of this, our previous studies point out that arsenic-induced cholinergic and dopaminergic dysfunctions with associated behavior and also observed the persistent effects were more pronounced in dopaminergic system in early life exposed rats. In continuation of previous studies, rats were exposed to arsenic in drinking water at low doses (2 or 4 mg/kg) during early life. Here, we investigated that alteration in the levels of biogenic amines and their metabolites (norepinephrine (NE), epinephrine (EPN), dopamine (DA), 3, 4-Dihydroxyphenylacetic acid (DOPAC), Homovanillic acid (HVA) and 5-hydroxytryptamine (5-HT) by using reversed phase high performance liquid chromatography in different brain regions of arsenic exposed rats on PD60 as compared to controls and furthermore determine persistent effect of arsenic after withdrawal of exposure. Levels of nitric oxide (NO) were also declines in brain regions of arsenic-treated rats in different brain regions of early life exposed rats. Arsenic has been found to modulate the monoaminergic and nitrergic systems during the critical periods of brain development; however, these modifications get more compromised in corpus striatum than frontal cortex and hippocampus even after withdrawal of exposure on PD90 as compared to controls. Modification at the level of biogenic amines and NO in certain brain regions would provide opportunities for the development of therapeutics tools for minimizing developmental neurotoxicity.

Keywords: Biogenic amines; Nitric oxide; Early life; Corpus striatum; High-performance liquid chromatography

Introduction

Arsenic is a heavy metalloid that can exist in biological systems in many different inorganic and organic compounds with several oxidation states, the most common being the trivalent and pentavalent forms [1]. The majority of the epidemiological studies of arsenic are associated with exposure to inorganic arsenic compounds through drinking water. Pentavalent arsenic has been reported in the surface water and trivalent arsenic in the ground water, which suggests this, is the most common reason for the humans generally being exposed to trivalent arsenic [2]. Arsenic is a major global health alarm due to its wide distribution and adverse health effects on the human body. The people of the large portions of the world are being exposed to arsenic through drinking water or ingestion of food materials and causing serious health effects involving the dermal, gastrointestinal, nervous, hepatic, renal, cardiovascular, respiratory, hematopoietic and ophthalmic systems [3-8]. In contrast to other physiological systems of the body, the nervous system appears to be the most vulnerable and the main target for arsenic-induced neurotoxicity [9]. Central nervous system and the peripheral nervous system both are commonly affected by the arsenic exposure [10], however, the effects of arsenic exposure on the peripheral nervous system are more prominent in adults while the arsenic significantly affects the central nervous system during the crucial phase of brain development.

It has been proved that arsenic can easily cross the placental barrier to the fetus [11] and even moderate exposure to arsenic during pregnancy and early life is linked with various adverse health outcomes, including neurological problems in the fetus [12,13] and children's [14,15]. Further, the various human population-based studies found that arsenic concentrations in urine were inversely associated with

Verbal IQ, Performance IQ and processing speed problems in children's [16-20]. Exposure occurred during gestation is associated with adverse effects on the fetus including premature and low birth weight of infants, spontaneous abortion [21,22], increased risk of birth defects [23] and reduced fetal size even at low to moderate levels of arsenic.

The accumulation property of arsenic in the brain is the prime reason for its prolonged persistence in the brain. The presence of arsenic in the brain causes various dysfunctions of the nervous system through several mechanisms and alters the development of the nervous system. Oxidative stress destroyed the balance between pro-oxidant and antioxidant system and it has been one of the main mechanism in the neurotoxicity and also a loss of brain functions. In our previous studies on early life exposure to arsenic at different concentrations, we reported a behavioural cholinergic and dopaminergic modification with biochemical alterations, such as apoptosis, oxidative stress and antioxidant enzymatic activity of brain tissue [24,25]. Exposure to arsenic in vitro experiments on Murine neuroblastoma cell line caused the enhancement of the reactive oxygen species leading to neuronal cell apoptosis through JNK/ERK-mediated mitochondria-dependent and

*Corresponding author: Lalit P Chandravanshi, Department of Zoology, Institute of Science, Banaras Hindu University, Varanasi, 221005, India, Tel: +91 542 6702523; Fax: +91 542 2368174; E-mail: chandravanshi04@gmail.com

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GRP 78/CHOP-regulated pathways [26]. Moreover, some studies have also confirmed the neuronal apoptosis contributing to the development of many human diseases including neurodegenerative diseases [27,28].

A lot of Information regarding the disturbance on biogenic amines in adults by arsenic at high doses has been reported [29-31], while the information about the effect of arsenic on biogenic amine at low doses during crucial period of brain development is scanty. However, mechanisms underlying the developmental neurotoxicity of arsenic are indistinct and the research on neurobiological consequences of arsenic exposure is important to understand the mechanism of developmental neurotoxicity. Impairment of neurotransmitters functions in the central nervous system (CNS) is closely related to neurodegenerative disease, learning, and memory impairment. Therefore, studying the impairment of biogenic amine and their metabolites in the brain of rats is extremely considerable to understand the developmental neurotoxicity of arsenic. Developmental exposure to arsenic affects the release, synthesis, uptake of neurotransmitter and receptor expression could be deleterious and lead to long-term behavioral deficits [32-34]. Levels of some neurotransmitters and the activity of enzymes related to neurotransmitter metabolism, such as cholinesterase and dopamine b-hydroxylase, in the brain of rodents were found to be altered after exposure to arsenic [34-37].

NO may also regulate the neurotransmitter release and the intercellular neurochemical signaling which is involved in neuronal plasticity, learning and memory [38-40]. Impairment in NO-related function may also contribute to the cognitive deficits in children and adolescents exposed to arsenic [17,41]. Rios et al. [35] have reported the decreased NO markers associated with the morphological changes in the brain of rats exposed to arsenic. Several nitrenergic markers deficit has been observed in blood and brain tissue of rats and rabbits that may be playing a significant role in the arsenic-induced neurotoxicity [42,43]. However, an impact of arsenic exposure on the biogenic amines and NO levels are not understood during crucial periods of brain development. Moreover, it is not understood whether these changes are transient or persistent after the withdrawal of arsenic exposure. Therefore, we aimed to assess the association between impairment of these functions and arsenic exposure at low doses during the early life crucial period of brain development. The present study may be useful in disclosing the mechanism of developmental neurotoxicity of arsenic and suggesting the occurrence of developmental neurotoxicity is associated with changes of biogenic amines and their metabolites and NO in CNS.

Materials and Methods

Animals and treatment

Early life exposure of rats to sodium arsenite: Male Wistar rats (21 days old) were obtained from the central animal breeding colony of CSIR-Indian Institute of Toxicology Research (CSIR-IITR), Lucknow, India. All the animals were maintained on a commercial pellet diet and water ad libitum, housed in plastic cages with bedding at a temperature ($22 \pm 2^\circ\text{C}$) controlled room with a 12/12 h light/dark cycle. All experimental procedures involving animals were carried out strictly accordance with the international care guidelines for animals approved by the Ministry of Environment and Forests (Government of India), New Delhi, India. Further, male rats were randomly divided into three groups on PD22. Group I rats were treated with normal saline (p.o.) identically as in groups II and III and served as controls. Group II and Group III rats were treated daily with one dose per day sodium arsenite 2 or 4 mg/kg body weight, p.o. respectively from PD22 to PD59.

On PD60, control and treated rats were sacrificed and brains were taken out from skull quickly and washed in ice-cold saline. The frontal cortex corpus striatum and hippocampus regions of the brain were carefully separated, following the method as described by Glowinski et al. [44]. The brain regions were kept frozen at -80°C for the estimation of biogenic amines and their metabolites and levels of NO.

To understand whether these changes were transient or persistent after withdrawal of arsenic exposure, early life exposed rats were left for 30 days and monitored daily. Neurotoxic effects of arsenic on biogenic amines and their metabolites and levels of NO were studied on PD60 and PD90.

Estimation of biogenic amines and their metabolites in brain regions of rats

To determine the levels of DA, NE, EPN, 5-HT, DOPAC and HVA in different brain regions of rats, reversed-phase high-performance liquid chromatography coupled with an electrochemical detector (HPLC-ECD) was carried out following the method described by Kim et al. [45] with some modifications. The HPLC system (Waters, Melford, USA) was integrated with a high-pressure isocratic pump (515 HPLC Pump), a sample injector valve, C-18 reverse phase column (250×4 mm, particle size $5 \mu\text{m}$) and an electrochemical detector (464 Pulsed electrochemical detector). The frozen brain tissue sample was weighed and transferred into the tube and added the pre-chilled homogenize buffer solution (0.1 M Perchloric acid and 3,4- dihydroxy benzylamine, an internal standard at a final concentration of 25 ng/ml) at a volume of 1 ml/100 mg sample. Brain tissues were homogenized and kept on ice for 15 min. The homogenate was centrifuged at maximal speed ($36,000 \text{ xg}$) at 4°C for 10 min and the supernatant was collected and transferred to a new sample tube. The supernatant was filtered through nylon filters (0.25 mm, Millipore, USA) and used for the estimation of biogenic amines and their metabolites.

20 μl volume of the sample was injected into a reverse-phase analytical column with a mobile phase (pH 4.2) containing sodium dihydrogen phosphate (0.15 M), ethylenediaminetetraacetic acid (0.25 mM), sodium octyl sulfate (1.75 mM) and 4% methanol at the flow rate of 1.5 ml/min. The concentrations of biogenic amines and their metabolites in different brain regions of rats were determined by using an electrochemical detector coupled with an integrator. Chromatograms were analyzed with the help of Empower2 software (Waters, Melford, USA) and results were expressed in ng/g tissue weight.

Estimation of NO levels in brain regions of rats

NO level in different brain regions of rats was analyzed using NO assay kit procured commercially (Calbiochem, USA). The assay principle is based on the conversion of nitrate to nitrite by the enzymatic action of nitrate reductase. Nitrate is converted into a fluorescent compound 1(H)-naphthotriazole after the addition of 2,3-diaminonaphthalene and sodium hydroxide. The emitted fluorescence was measured spectrofluorimetrically (excitation 430 nm/ emission 450 nm) using a multiwell plate reader (Biotek Synergy HT, USA) [46]. The measured values were expressed in pmoles nitrate/mg tissue weight.

Protein estimation

Protein content in samples was measured following the method described by Lowry et al. [47] using bovine serum albumin as a reference standard.

Statistical analysis

In the present study Graph Pad prism software has been used to analyze data of biogenic amine and their metabolites and levels of NO. One way analysis of variance (ANOVA) followed by Newman-Keuls test has been used to analyzed the data for posthoc comparisons. Values up to $p < 0.05$ have been considered significant.

Results

Effects on biogenic amines and their metabolites in brain regions of rats on PD60 and PD90

The levels of biogenic amines and their metabolites showed dose dependent changes in the frontal cortex, corpus striatum and hippocampus of arsenic exposed rats as compared to controls. In comparison to controls, the administration of both doses of arsenic has increased the levels of DA (16%, $p < 0.05$; 39%, $p < 0.05$), EPN (46%, $p < 0.05$; 59%, $p < 0.01$), NE (18%, $p < 0.05$; 39%, $p < 0.01$;) and decreased the level of DOPAC (19%, $p < 0.05$; 29%, $p < 0.01$), HVA (20%, $p < 0.05$; 36%, $p < 0.01$) on PD60 (Table 1). The arsenic exposed rats showed a trend of recovery in the levels of DA (6%, $p > 0.05$; 14%, $p < 0.05$), NE(5%, $p > 0.05$; 8%, $p > 0.05$), EPN (11%, $p > 0.05$; 15%, $p > 0.05$), DOPAC (6%, $p > 0.05$; 10%, $p > 0.05$), and HVA (9%, $p > 0.05$; 15%; $p > 0.05$) in frontal cortex even at 30 days after withdrawal of arsenic exposure in comparison to respective controls (Table 1). No significant change was observed in the level of 5-HT in frontal cortex of arsenic-treated rats as compared to controls.

A significant increase in the levels of DA (29% $p < 0.05$; 64%, $p < 0.01$), HVA (22%, $p < 0.05$; 49%, $p < 0.01$) and decrease in the levels of NE (22%, $p < 0.05$; 35%, $p < 0.05$), EPN (24%, $p < 0.05$; 35%, $p < 0.05$) and

DOPAC (25%, $p < 0.05$; 36%, $p < 0.01$) were observed in corpus striatum of rats exposed to arsenic (2.0 or 4.0 mg/kg body weight) from PD22-59 on PD60 in comparison to controls (Table 2). A trend of recovery in the levels of DA (19%, $p > 0.05$; 37%, $p < 0.05$), NE(14%, $p > 0.05$; 24%, $p < 0.05$), EPN (16%, $p > 0.05$; 24%, $p < 0.05$), DOPAC(5%, $p > 0.05$; 14%, $p < 0.05$) and HVA (21%, $p < 0.05$; 29%; $p < 0.05$) were seen in the corpus striatum of arsenic-treated rats. The changes were found to persist at the higher dose 30 days after withdrawal of exposure on PD90 in comparison to respective controls (Table 2). The level of 5-HT was not significantly impaired in corpus striatum of arsenic-treated rats.

Exposure with arsenic to rats caused a significant increase in the levels of DA (19%, $p < 0.05$; 28%, $p < 0.05$), NE (17%, $p < 0.05$; 35%, $p < 0.01$), EPN (23%, $p < 0.05$; 59%, $p < 0.05$) and decrease in the levels of DOPAC (26%, $p < 0.05$; 32%, $p < 0.05$) and HVA (26%, $p < 0.05$; 33%, $p < 0.05$) in hippocampus on PD60 as compared to controls (Table 3). The animals were appeared to be recovered from the toxic effects of arsenic 30 days after withdrawal of exposure as no significant variation in the levels of NE (2%, $p > 0.05$; 4%, $p > 0.05$), EPN (10%, $p > 0.05$; 16%, $p > 0.05$), DOPAC (5%, $p > 0.05$; 12%, $p > 0.05$), and HVA (2%, $p > 0.05$; 6%; $p > 0.05$) were observed. The levels of DA (9%, $p > 0.05$; 19%, $p < 0.05$) was found to be significantly remained to persist at the higher dose of arsenic in the hippocampus of arsenic exposed rats 30 days after withdrawal of exposure on PD90 in comparison to respective controls (Table 3). The hippocampal level of 5-HT was not significantly altered in arsenic exposed rats on PD60 and 30 days after withdrawal of arsenic exposure as compared to controls.

Effect on the level of NO in different brain regions of rats on PD60 and PD90

A significant decrease in the levels of NO in frontal cortex (1.57-

Age/ Biogenic amine (s)/ Metabolite (s)	Control	Treatment groups	
		ARS I (2 mg/kg)	ARS II (4 mg/kg)
Postnatal day 60			
Dopamine	1103 ± 69.53	1281 ± 67.60*	1541 ± 81.63*
Norepinephrine	294.90 ± 27.70	348.30 ± 8.05*	411.90 ± 14.70**
Epinephrine	349.40 ± 38.58	513.70 ± 43.08*	555.50 ± 38.97**
Serotonin	956.80 ± 70.18	1004 ± 90.04	1105 ± 106.90
DOPAC	326.50 ± 17.40	264.40 ± 14.19*	229.40 ± 9.50**
Homovanillic acid	124.10 ± 2.88	99.09 ± 6.02*	79.14 ± 2.70**
Postnatal day 90			
Dopamine	1146 ± 132.90	1224 ± 154.50	1335 ± 108.20*
Norepinephrine	227.50 ± 12.44	240.90 ± 9.34	246.60 ± 15.46
Epinephrine	369 ± 23.44	410.30 ± 32.56	427.30 ± 24.69
Serotonin	907.50 ± 98.09	860.40 ± 69.42	844.70 ± 88.32
DOPAC	445.40 ± 19.58	416.80 ± 51.87	401.80 ± 20.42
Homovanillic acid	114.70 ± 20.44	103.90 ± 10.75	97.41 ± 9.64

Table 1: Early life exposure (PD22–PD59) to sodium arsenite and its effect on biogenic amines and their metabolites in frontal cortex of rats on PD60 and PD90.

Rats were exposed to sodium arsenite (ARS I-2.0 or ARS II-4.0 mg/kg body weight/ day, p.o.). Effect on biogenic amine and their metabolites studied on PD60 and 30 days after withdrawal of exposure on PD90. Values are mean ± SEM of five animals in each group. Data have been analyzed by one-way analysis of variance followed by Newman-Keuls test. *Significantly differs from controls ($p < 0.05$); **Significantly differs from controls ($p < 0.01$) and the values are expressed as ng/g tissue weigh

Age/ Biogenic amine (s)/ Metabolite (s)	Control	Treatment groups	
		ARS I	ARS II
	Control	ARS I (2 mg/kg)	ARS II (4 mg/kg)
Postnatal day 60			
Dopamine	5360 ± 456.30	6944 ± 319.80*	8800 ± 818.10**
Norepinephrine	595.90 ± 61.87	460.10 ± 25.47*	386.40 ± 9.69*
Epinephrine	363.80 ± 16.31	274.80 ± 9.79*	234.90 ± 25.73*
Serotonin	779.20 ± 86.87	860.50 ± 125.20	907.80 ± 9.24
DOPAC	3216 ± 183.90	2380 ± 151.60**	2033 ± 152.70**
Homovanillic acid	594.80 ± 44.90	731.40 ± 34.01*	889.40 ± 46.94**
Postnatal day 90			
Dopamine	5068 ± 272	6064 ± 277.90	6986 ± 481.10*
Norepinephrine	403.70 ± 26.10	344.70 ± 10.93	304.40 ± 10.83*
Epinephrine	311.10 ± 40.26	258.70 ± 12.33	234.70 ± 10.56*
Serotonin	663.50 ± 64.17	689.50 ± 97.66	710.10 ± 167.50
DOPAC	4484 ± 187.40	4236 ± 65.44	3818 ± 139.20*
Homovanillic acid	648.70 ± 37.55	790.20 ± 57.85	842.80 ± 58.57*

Table 2: Early life exposure (PD22 – PD59) to sodium arsenite and its effect on biogenic amines and their metabolites in corpus striatum of rats on PD60 and PD90.

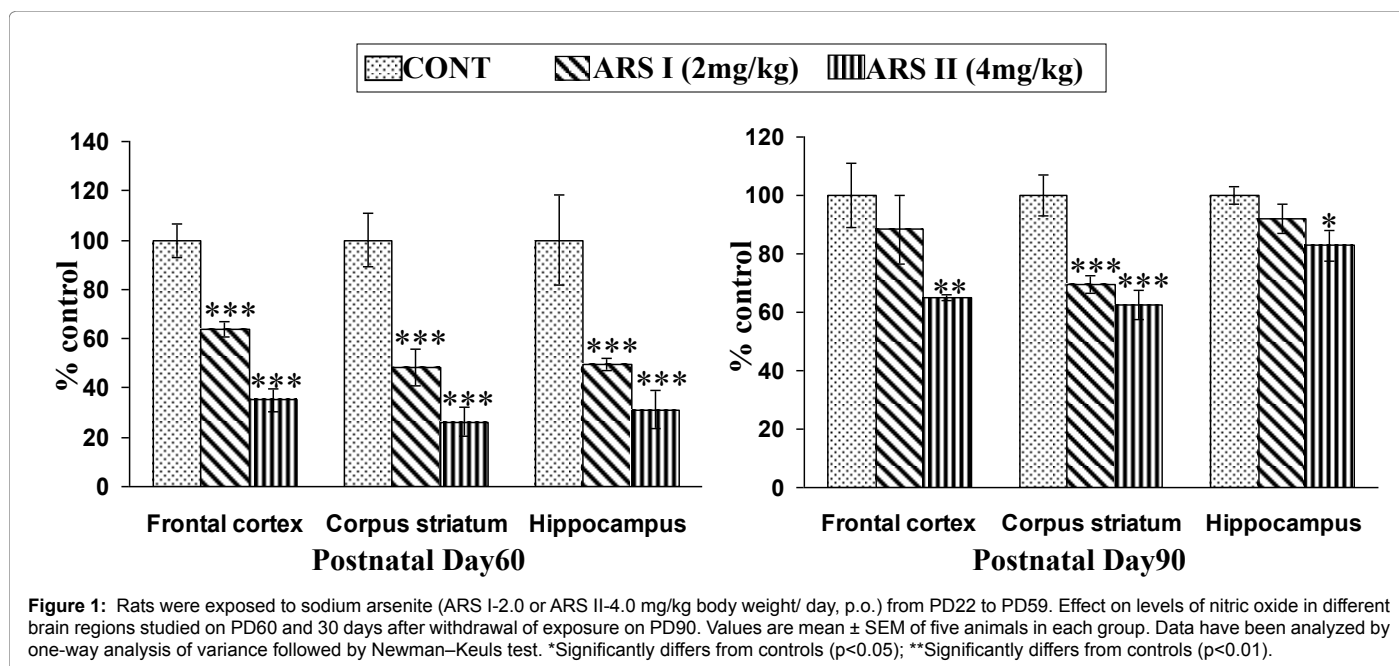
Age/ Biogenic amine (s)/ Metabolite (s)	Control	Treatment groups	
		ARS I	ARS II
	Control	ARS I (2 mg/kg)	ARS II (4 mg/kg)
Postnatal day 60			
Dopamine	450 ± 40.16	511.60 ± 7.64*	579.70 ± 36.54*
Norepinephrine	197.90 ± 7.93	231 ± 12.18*	267.30 ± 3.67**
Epinephrine	278.70 ± 23.17	344.40 ± 27.37*	444.60 ± 40.43*
Serotonin	396.40 ± 47.24	359 ± 42.24	347 ± 12.53
DOPAC	31.55 ± 0.83	23.14 ± 1.72*	21.38 ± 2.47*
Homovanillic acid	33.20 ± 2.35	27.52 ± 1.64*	24.83 ± 1.21*
Postnatal day 90			
Dopamine	466.10 ± 66.77	508.30 ± 27.09	559.10 ± 37.91*
Norepinephrine	224.10 ± 10.82	228.40 ± 11.5	233.90 ± 17.24
Epinephrine	267.30 ± 24.12	296.70 ± 14.15	310.10 ± 19.47
Serotonin	417.30 ± 24.93	403.30 ± 34.81	396.20 ± 63
DOPAC	45.96 ± 2.88	43.41 ± 2.39	40.12 ± 2.60
Homovanillic acid	27.63 ± 3.64	27.11 ± 3.34	25.96 ± 3.31

Table 3: Early life exposure (PD22 – PD59) to sodium arsenite and its effect on biogenic amines and their metabolites in hippocampus of rats on PD60 and PD90.

fold, $p < 0.001$; 1.85-fold, $p < 0.001$), corpus striatum (2.08-fold, $p < 0.001$; 3.81-fold, $p < 0.001$) and hippocampus (2.02-fold, $p < 0.001$; 3.21-fold, $p < 0.001$) were observed in rats treated with arsenic (2.0 or 4.0 mg/kg body weight) in comparison to controls on PD60 (Figure 1). A trend of recovery was found in the levels of NO in frontal cortex and hippocampus but the changes were remained persistent at a higher dose of arsenic, 30 days after withdrawal of exposure. Interestingly, the levels of NO in the corpus striatum (1.43 fold, $p < 0.001$; 1.59 fold, $p < 0.001$) was remained persistent at both doses of arsenic in comparison to controls on PD90 (Figure 1).

Discussion

Brain development during prenatal phase represents only one part of the lengthy nervous system developmental process. While the early life, childhood, and adolescence represent windows of remarkable brain changes and maturational processes. Biogenic amines are one of major groups of the neurotransmitters which play important roles in the CNS as major neurotransmitters, being involved in regulating the neurotransmission in the brain and the regulation of cognitive and motor functions, mood, emotional behaviour, including memory



[31,48,49]. Many investigators have reported the alteration in biogenic amines and their metabolites in various neurophysiological and neuropsychological diseases [4,50]. Many biogenic amines are essential for the shaping, wiring, brain growth events and the normal development of the nervous system during the critical period of the brain development [51]. Impairment in the levels of biogenic amines (DA, 5-HT, NE) in the brain during critical developmental phases of postnatal could be delayed postnatal maturation of the brain along with neurodevelopmental disturbances associated with cognitive deficits [52-54].

The present study was carried out to investigate the impact of arsenic on the levels of biogenic amines and their metabolites and levels of NO during critical periods of brain development of rats. The level of DA significantly higher was found in the arsenic exposed rats as compared to controls during the postnatal period of brain development in the frontal cortex, corpus striatum and hippocampus of rats at both doses of arsenic. Dose-dependent effect of arsenic on rodents has been observed at low and intermediate doses of arsenic produced hyperactivity whereas the high dose produced hypoactivity along with DA release as increase and decrease in extracellular DA levels [29,36,55], it could be a consequence of dysfunction of the dopaminergic neurotransmitter system. Overproduction of DA can lead to hyperactivity and linked with attention deficit hyperactivity disorder [56]. Weaned mice received to arsenic (20, 40, 60 or 100 ppm) showed a significant correlation between arsenic level and regional monoamine levels in the brain and also found the ACTH levels in plasma were significantly associated with NE concentration in pons and medulla (Delgado et al. 2000). Declined levels of DA while an increase in the levels of NE and 5-HT were reported in the whole brain of guinea pigs after the exposure to arsenic (25 mg/l) for 120 days [57].

Further, differential changes in the metabolites of DA (DOPAC and HVA) were observed in selected brain regions due to the arsenic exposure during crucial periods of brain development. Reduced levels of DOPAC were found in selected brain regions of early life arsenic exposed rats. HVA is another metabolite of DA was significantly impaired in frontal cortex and hippocampus of early life arsenic

exposed rats. The hippocampal level of HVA was also impaired at both doses (2 or 4 mg/kg) in early life arsenic exposed rats. Interestingly, these changes were found to recover, but the levels of DA and their metabolites remained more persistent at a higher dose of arsenic in the corpus striatum of rats, after withdrawal of exposure as compared to controls. These findings showed that an intensity of these changes was more striking in the corpus striatum than the frontal cortex and hippocampus, after withdrawal of exposure. Alteration in the levels of DA and their metabolites (HVA, DOPAC), it might be suggested that the catabolic pathway of DA by catechol-o-methyltransferase might have been influenced by arsenic leading to increased DA degradation [58]. Rodriguez et al. [59] were reported the diminished level of DA, HVA and DOPAC in the corpus striatum of rats after the exposure to mining waste containing mainly arsenic. Hyper-locomotor activity at a low dose (3 mg/kg body weight) and while decreased motor activity in those treated with a high dose of arsenic trioxide (10 mg/kg body weight) was observed in mice [60].

In the present study, we observed increased levels of NE in the frontal cortex, hippocampus and decreased level in corpus striatum of early life arsenic exposed rats on PD60. The level of NE was increased may be due to amplifying the activity of the enzyme dopamine hydroxylase, catalyzing the conversion of DA to NE [61]. Alterations in the levels of EPN were also observed in this study, immediately after the arsenic exposure.

Several behavioral and physiological functions such as thermoregulation, mood, sleep, aggression, sexual behaviour, and appetite regulate by the 5-hydroxytryptamine (5-HT) (3, 27). In our study decreased levels of 5-HT were observed in different brain regions of arsenic-treated rats at low doses, however, changes were insignificant. Our findings are in agreement, even at a high dose of arsenic with those of Tripathi et al. [33]. The differential changes in the level of biogenic amines and their metabolites in different brain regions were found might be due to the different duration of exposure and different sensitive period of exposure. These differential changes were more striking in the corpus striatum of the brain after the early life exposure to arsenic and intensity of these modifications were more in the high

dose of arsenic. Based on these finding changes were also more persist in the corpus striatum of rats after the withdrawal of arsenic exposure, suggesting that monoaminergic system in the corpus is found to a more vulnerable target of arsenic-induced neurotoxicity.

The present results indicate that arsenic caused extensive damage to the dopaminergic, adrenergic neurons of the corpus striatum than those of frontal cortex and hippocampus, as the levels of DA, DOPAC, HVA, NE, and EPN remained significantly altered even after withdrawal of exposure. Disturbances in the biogenic amine neurotransmitter levels have also been implicated in the behavioural dysfunction and impaired cognitive performance. Any modifications were observed in DA transmission may negatively effect on several neurological processes and lead to behavioral modifications [36]. Monoamine neurotransmitters (NE, DA, 5-HT) contribute a major role in learning and memory and depressive-like behaviors, therefore any disruption in the neurotransmitter system in the brain after exposed to arsenic might be contributing the abnormal functions of the brain along with impairs various normal physiological functions [34,62]. It has been reported that arsenic interferes with synaptic mechanisms of release of some neurotransmitters, and is responsible for the impairment in various neurotransmitters' systems along with neurobehavioural modifications [30,37,43]. The amino acid neurotransmitter has been investigated in immature brain after the realgar treatment in rats indicate that neurotoxicity by the realgar is linked with the disturbance the levels of amino acid neurotransmitters [63]. The amino acid neurotransmitters are closely linked to the vulnerability of the developing brain.

Neuropathy was developed in that human who is exposed to arsenic from long time [64] and also alters the normal functions of central and peripheral nervous systems due to the inhibit the activities of the neurotransmitters- mediating enzymes and their neurotransmitter-products (DA, 5-HT, NE, and EN). DBH is the most important enzyme in the catecholamine neurotransmitter (catecholamine)-mediating enzyme, which responsible for the catalyzes and biosynthesis of NE from DA. The DBH activity was found to be decreased in the serum of arsenicosis patients [65]. Inhibition of the activity of DBH in serum considered as a possible index of sympathetic nervous functions.

NO is regulating different functions, including neurotransmission, regulation of blood vessel dilation and the immune response. In the central nervous system, NO play important role in the cell signaling associated with cognitive function, maintenance of synaptic plasticity to neuron and neurosecretion [66-68]. NO act as a neuromodulator and diffuses from one neuron to another neuron directly and is involved in brain development [69]. NO activity in the CNS has an important role in both the physiological and pathological condition and also reported a localization of nNOS in different area of the brain, such as the corpus striatum, medulla, cortex, hippocampus, hypothalamus and the cerebellum of rodents [70-72]. Decreased the nitrites and nitrates in the striatum of rats associated with chronic exposure to arsenite and NO production was also decreased in vitro experiment [43]. Our study too showed decreased NO production in frontal cortex; corpus striatum and hippocampus in arsenic exposed rats may be due to the lower protein levels, which could outcome from declined expression and/or increased degradation. Reduced production of NO suggested that alter the pattern of synapse due to the nitregeric deficit during CNS development.

The finding of the present study indicates that production of NO was remained more decreased in the corpus striatum region compare to other brain regions after the withdrawal of arsenic exposure. Tetrahydrobiopterin levels enhance the ubiquitylation of nNOS and

resulting degradation of the protein. Decreased tetrahydrobiopterin levels have been found in arsenic exposed rabbits [42] and this result can also be associated with decreased monoamine levels in arsenic-exposed animals [73]. Rios et al. [35] have been observed that decreased the nitregeric functions affecting the structural organization of the brain targeting the cytoskeletal proteins and membranes at lower concentration (3 ppm equivalent to 0.4 mg/kg body weight/day) of arsenic in rats.

The present study demonstrates that low doses of continuous exposure to arsenic during early life are associated with the disturbance in biogenic amine levels and their metabolites. Because of arsenic exposure, the reduction in the level of NO was found in the developing brain, which could modulate the several neurological risk factors. However, further studies are required to establish the arsenic-induced developmental neurotoxicity and by which mechanism changes were more compromised in the corpus striatum region even after withdrawal of arsenic exposure. The results of the present study drag attention to these CNS effects of arsenic even at low doses, besides the more general damaging effects of arsenic reported at a higher dose of arsenic in other models. Further, the continuous exposure to arsenic during critical periods of brain development may be an important risk factor for increased vulnerability to neurodegenerative diseases.

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