Review Article

Arsenic Toxicity and Neurobehaviors: A Review

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Abstract

Deterioration in public health due to arsenic toxicity is a worldwide concern for clinicians. The subject requires extensive and careful assessment of arsenic toxicity born symptoms, across the geographical boundaries. Arsenic induced deleterious effects have been documented in countries, including India, Bangladesh, Argentina, Australia, China, Hungary, Thailand, Mexico and United States of America, which cover the major part of world population. Arsenic found in soil and drinking water comes from geophysical as well as anthropogenic sources. Humans are exposed to arsenic through food, drinking water and or smelters. Nevertheless, newborns are most sensitive to arsenic insult and if mother is exposed to arsenic at gestational stage, irreversible postnatal cardiac, carcinogenic, behavioral, cognitive and motor disabilities are inevitable. Sufficient data from animal studies on hamsters, mice, rats and rabbits demonstrate arsenic to produce developmental toxicity, which includes malformation, growth retardation and even death. Developmental toxicity characteristically depends on route, dose and the period of gestational exposure. Arsenic exposure induce oxidative stress and decreases ATP production, putting structural and functional maturity of nerve cells at stake, leading to improper brain development and related behaviors.

Chronic exposure during pregnancy produces dose dependent increase in conceptus mortality and postnatal growth retardation. Also, pregnant females exposed to arsenic, express malformations and resorption of pups. Animal studies have not identified an effect of arsenic on fertility in males or females. Only few studies have been performed on human subjects and the data reports spontaneous abortion and stillbirth in more than one of these studies. Interpretation of observed outcome is complicated in human subjects, because they can be exposed to multiple chemicals at a given time.

There is no single drug to stop or minimize arsenic induced deleterious effects. Therefore, our newborns are and will be compelled to live behaviorally retarded and socially handicapped, if the issue is not properly addressed. The present review meticulously discusses the detrimental role of arsenic on postnatal neurobehavioral development. This attempt will add to the existing knowledge on the subject and help to design a better approach to culminate the lethal effects of arsenic.

Keywords: Arsenic, gestational exposure, postnatal exposure, developmental toxicity, CNS vulnerability, neurobehavioral assessment

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1. Introduction

There is increasing global concern over the public health impacts attributed to arsenic. It is found in air, water and soil and could find their way into the human body through: Inhalation, Ingestion and Absorption. Continuous arsenic exposure via drinking water has been reported in many countries of the world; especially in Argentina, Bangladesh, India, Mexico, Thailand, and Taiwan, where a large proportion of ground water is contaminated with a high concentration of arsenic. Arsenic contaminated air, water and food is associated with reproductive and developmental diseases. Also, it causes diseases including cancer, dermal, cardiovascular, hepatic, renal, peripheral vasculature maladies, dysfunction of the endocrine, bladder and kidney [1,2]. The nervous system is highly vulnerable to the toxic effects of arsenic, resulting into multiple neurological effects [3]. The epidemiological studies indicate that behavioural and cognitive functions in children are compromised following gestational and developmental arsenic exposure. Owing to increased exposure associated with food intake patterns and lifestyle of individuals impose higher risk to nervous system [4]. Furthermore, arsenic exposure during neurodevelopment can impair central nervous system (CNS) functioning long after exposure has occurred.

Various epidemiological studies have reported that arsenic exposure in utero increased spontaneous abortion and stillbirth and decreased birth weight [5-8]. However, these studies lack detailed information about exact effects of maternal arsenic exposure. Thus use of novel animal models is essential for investigating arsenic particularly for developmental and reproductive toxicities. Importantly, arsenic can pass through the placenta to the developing fetus. Arsenic toxicity related diseases are however not easily detected and may be acquired during childhood and manifested later in adulthood. Embryonic arsenic exposure poses adverse developmental outcomes. The reproductive and developmental toxicity of inorganic arsenic have primarily been documented through murine studies, suggesting arsenic as a risk to the developing fetus. Recently, arsenic induced toxicity has been examined in situations more similar to human exposures and using broader endpoints, such as behavioral changes and gene expression. Even, at non-maternally toxic levels, inorganic arsenic affects fetal brain development and postnatal behaviours [9]. Number of papers report arsenic exposure to pregnant laboratory animals and occurrence of defective neurobehaviors of offspring. Previous works on the subject emphasise arsenic induced reproductive and developmental toxicity in rats and mice. However, detail consideration on the possible neurobehavioral impairments following arsenic toxicity is missing. Furthermore, plethora of papers on arsenic toxicity and neurobehaviors impel to make this review. In the present attempt we have tried to put the observations on the gestational arsenic exposure and postnatal neurobehaviors at one place. We searched for the following keys: Arsenic source, arsenic toxicity and health issues, mechanism of arsenic toxicity, gestational exposure of arsenic and arsenic and neurobehaviors to systematically place the reported observations together.

Arsenic Source

Arsenic is one of the most toxic metals derived from contamination of drinking water from natural geological sources rather than from mining, smelting, or agricultural sources (pesticides or fertilizers) [10]. Arsenic is a naturally occurring ubiquitous semi-metallic element found in foods and environmental media such as soil, air and water [11]. Arsenic is mainly found in two forms, arsenite (the trivalent form, As III) and arsenate (the pentavalent form, As V), of which trivalent arsenic has been considered to be more toxic than pentavalent arsenic [12]. Both arsenite and arsenate would have similar effects in vivo, as the absorbed arsenate is mostly reduced to arsenite in the body. Pentavalent form of arsenic (arsenate) is reduced to trivalent form of arsenic (arsenite) in the body via methylation in the liver by the enzyme methyltransferase in the presence of Sadenosylmethionine as the methyl donor and glutathione (GSH) as an essential co-factor, resulting in the formation of monomethylarsonous (MMA) and dimethylarsinous (DMA) as end metabolites [13]. The World Health Organization has recommended 0.01 mg/l of arsenic in drinking water as an allowable range for human consumption.

Gestational Exposure of Arsenic and its Consequence

We have observed the effect of dose dependent arsenic exposure in rats, starting gestational day 8 till parturition. Pups were critically assessed for physical development, reflex development, strength and motor coordination but no significant change were observed. In addition to this, our study also focused on malformation and developmental variation points but at the selected dose of arsenic no treatment related variations were observed [14, 15]. As a continuation of these results
Gandhi and Panchal [14], also demonstrates that inorganic arsenic exposure (0, 4.5, 6 and 7.5mg/kg/day/po) in rat gestational day 8 till parturition and offspring’s were examine for physical development, reflex development, and strength and motor coordination. But, prenatal exposures of arsenic at this dose failed to induce neurobehavioral toxicity in rat. Nevertheless, exposure of arsenic gas via inhalation to pregnant mice showed higher concentrations in fetal brain and liver than placenta and maternal liver [16]. Supporting to this fact, another relevant study reported that the inorganic arsenic in the drinking water (36.70 mg arsenic/l in drinking water) from gestation day 15 (GD 15) or postnatal day 1 (PND 1), of pregnant rats could also result in arsenic accumulation in fetuses, particularly the fetal brain [17]. Further, study showed that, pregnant C57BL6/J mice when consumed drinking water containing sodium arsenite from fourth day of gestation until birth the result noted were most striking effects of arsenic particularly on the development of gait and other motor responses [18]. In conclusion, exposure of arsenic in gestational stage causes developmental and behavioural toxicity.

**Arsenic and Nervous System Disorders**

The developing brain is vulnerable to toxic metals that interfere with the critical developmental processes i.e., cell proliferation, migration, differentiation, synaptogenesis, myelination and apoptosis in the central nervous system (CNS) [19]. Arsenic toxicity-induced defects in nervous system includes Peripheral Neuropathy, Alterations in peripheral nerves, Neuiritis retrobulbar neuritis; neuropathy, Encephalopathy, Abnormal EEG’s, numbness in extremities; parathesia, Abdominal Pain, Depression, mood swings, flat affect, impaired facial recognition, Mental retardation, borderline intelligence, Hearing loss and difficulty hearing, Decreases locomotor activity, Convulsions; seizure, Muscle pain; headache and acrodynia [20].

Nevertheless, arsenic exposure has been associated with weakness and pain in the digital extremities, to changes in sensory action potential propagation [21-23] and axonal degeneration in the sural nerve [24]. Disturbances in the functional state of the CNS were reflected as changes in conditioned reflexes and in chronaximetry. Histopathological changes in the brain included pericellular oedema, plasmatic impregnation of the vascular walls, plasmolysis, and karyolysis of the neurons. Several of the effects mentioned, although less marked, were also observed in a group of rats exposed to an aerosol containing 3.7 μg As/m3. Peripheral nervous disturbances, primarily of a sensory type, are frequently encountered in individuals surviving acute poisoning with inorganic arsenic compounds. These disturbances usually become manifest 1-2 weeks after ingestion.

**Arsenic Exposure and Reproductive Toxicity**

Arsenic is well known for its reproductive toxicity. In case of male reproductive system arsenic reported to interfere with spermatogenesis and alters activities of spermatogenetic enzymes [25-27]. Arsenic may act on the brain and/or pituitary or directly on the germ cells. Also, arsenic affects the female reproductive cycle such as it suppresses ovarian steroidogenesis, prolongs diestrus, degenerates ovarian follicular and uterine cells and decreases the plasma levels of estradiol and prosterone [28]. Moreover, arsenic down regulates estrogen receptor and estrogen-responsive genes [29]. Further, low plasma gonadotrophin levels could decrease activities of ovarian 3β-HSD and 17β-HSD, two important regulatory enzymes for steroidogenesis [30]. These observations suggest that low plasma levels of estradiol could be the cause of diestrous. Moreover, arsenic exposure in humans causes reproductive toxicity, including increased incidences of miscarriages, stillbirths, and low birth weights in offspring of women who used drinking water containing 463–1025 μg arsenic per litter. In three-generation study administering 5ppm litter sizes were smaller in the arsenic-treated group than in controls in all three generations [31]. Likewise, in a second generation study by Hazleton et al. 1990, mice administered 13.25mg As/kg/day in diet had reduced litter sizes in both generations, with the difference (8.0 vs. 10.96 pups) being significant in the F2 generation. Further, the weaning index was also significantly affected in the dams. Thus, these results appear to reflect primarily an effect on viability in the conceptus and pups. Importantly, in terms of general toxicity, dam mortality and weight gain were also affected in the high dose group. F2 dams were about 30% smaller than controls from weaning, continuing through gestation and lactation; mortality was also higher than controls [128].
Inorganic arsenic also targets the human nervous system producing peripheral neuropathies and behavioral changes such as lowered intelligence scores on standardized tests that are indicative of central nervous system deficits [32-39]. The chronic exposure of arsenic during pregnancy makes the fetal period more dangerous which creates impact on optimal brain development, thereby leading to impairment of behaviors and skills, including cognitive abilities and social competence that are further developed and fine-tuned during childhood and adolescence, this is collectively defined as gestational and developmental toxicity. It is further conformed by a study by Milton et al. [8] they conducted the cross-sectional study in Bangladesh with 533 women and noted that, women expose to chronic arsenic increases excessive risks for spontaneous abortion and stillbirth. Plethora of evidences suggest a positive relation between consumption of arsenic (contaminated drinking water) and spontaneous pregnancy loss. In another study, Ahmad et al. [5] conducted a cross-sectional study in rural Bangladesh, in which 96 women employing drinking water with arsenic concentrations 0.10 mg/L and reported spontaneous abortion and stillbirth. Indeed, a significant association between maternal exposures to arsenic during pregnancy (individual water arsenic concentrations) on infant survival was observed in the cohort study of 29 and 134 pregnancies in Mat lab, Bangladesh [40]. In this study, authors evaluated the effect of arsenic exposure on fetal and infant survival and observed the dose response of arsenic exposure to risk infant survival. The Von Ehrenstein et al., [36] conducted the clinical study in between years 2001 and 2003 and reported outcomes of pregnancy denominators that increase the risk of stillbirth and neonatal mortality among 202 married women in West Bengal, India. Subjects were exposed to high concentration of arsenic through drinking water. The BRAC, the largest non-governmental organization in Bangladesh, manage and administered via Community Nutrition Center the large cohort studies with individual exposure data in Bangladesh gave pregnancy outcome data for 2000 women. Providing care to all pregnant women in areas with known elevated arsenic concentrations in drinking water, showed a small but statistically significant association between arsenic concentrations and birth defects [41].

A cross-sectional study on arsenic mediated toxicity in 201 children of 10 years age in Araihaazar, Bangladesh, reported that the children’s intellectual function on tests drawn from the Wechsler Intelligence Scale for Children, version III, was reduced in relation to exposure to arsenic in drinking water, after adjustment for socio-demographic covariates and water manganese [38]. This contention is supported by recent study in Ronnskar, province of Sweden, where greater risk for spontaneous pregnancy loss was reported for women with respiratory exposure to presumably high arsenic concentrations, compared to women with minimal exposure while employed at the same metal smelter [42]. It is further supported by the results of several epidemiological studies [43-46].

Gonoshasthaya Kendra, a large nongovernmental organization providing health care to some 600 villages in Bangladesh, collected data on arsenic induced toxicity. The data shows overall stillbirth rate in women was 3.4% and increased with estimated arsenic concentration (2.96% at < 10 µg/l; 3.79% at 10 µg/l to < 50 µg/l; 4.43% at > 50 µg/l), which ultimately concluded that the increased risk of stillbirth is associated with arsenic contamination [47]. In a recent cross-sectional study spontaneous abortion and stillbirth were detected between exposed and unexposed women, study design involved interview of 240 women residing in arsenic endemic villages in West Bengal of India with high level of arsenic in drinking water, as well as 60 women from a village with low level of arsenic in the drinking water were included [48]. Plethora literature focuse on West Bengal of India and Bangladesh, the most common region where arsenic mediated miscarriage was noted timely due to contaminated ground water [49]. However, arsenic mediated spontaneous abortion and stillbirth were also observed in no of areas other than India and Bangladesh. Accordingly, a sympathetic large ecologic study of almost 8000 pregnancies and 500 events was conducted among women residing in an arsenic endemic region of southeast Hungary between 1980 and 1987 [50]. This comparative study reported spontaneous abortion or stillbirth in women expose to high arsenic concentration. The retrospective study was design to investigate the role for arsenic exposure as risk for late fetal and infant mortality. In this study authors investigate the trends in infant mortality between two geographic locations in Chile; Antofagasta, which has a well-documented history of arsenic exposure from naturally contaminated water and second is Valparaiso, comparative low- arsenic exposed city. The result indicates an elevation of the late fetal, neonatal and postneonatal mortality rates for Antofagasta, relative to Valparaiso [51]. Furthermore, a hospital-based case-control study
reported severity of the effects of chronic inhalation of low levels of arsenic on reproduction about study of stillbirths in a central Texas community that included a facility with more than a 60-year history of producing primarily arsenic-based agricultural products and remark as high arsenic exposure is risk for stillbirths [7]. Yang, et al. [52], performed a study to compare the risk of adverse pregnancy outcomes (preterm delivery and birthweight) on females of Lanyang Basin, which is located in the northeastern portion of Taiwan Island. Above study provide sufficient evidence for a potential role for arsenic exposure through drinking water and increasing risk to low birth weight. On other hand, the cross-sectional study examined the possible influence on the development of cognitive function among adolescents due to long-term arsenic exposure. Furthermore, students drinking arsenic-containing well water showed changes in neurobehavior like, the pattern memory and switching attention were noted to be significantly affected [35]. Guo et al., [53], conducted the cross-sectional study and reported as increased odds for spontaneous abortion among 224 Mongolian women residing in villages with arsenic drinking water concentrations >50 ppb, compared to 99 women residing in villages with arsenic concentrations <50 ppb, and statistical difference occurs spontaneous abortion. Arsenic poisoning is associated with neurological toxicity. This contention is supported by the study in which people chronically poisoned by arsenic (through groundwater) suffer from neurological toxicity. The neuropsychological tests showed mildly impaired psychomotor speed and attentive processes, whereas verbal learning and memory were severely impaired. Moreover, delirium and encephalopathy was also noted. This describes that arsenic is associated with neurological manifestations. Peripheral neuropathy is common in persons chronically exposed to As-contaminated drinking water. As-induced peripheral neuropathy may last for several years or even life-long, which leads to rapid severe ascending weakness, requiring mechanical ventilation [54].

Animal Models of Arsenic Toxicity

Various animal studies using different species have been examined to explore the basic mechanism involve arsenic mediated developmental toxicity. Rodriguez et al. (2002) [17] has proved the role of arsenic, when given at dose of 36.70 mg/L in drinking water to sprague-dawley rats. Rats were exposed from GD 15 or postnatal day 1 (PND 1), until approximately 4 months old, result of this study stated developmental deficits like spontaneous locomotor activity and alterations in a spatial learning in rats. This indicates that arsenic causes development and behavioral alteration. A report on the effect of arsenic exposure on pregnant rats and their offspring in which i.p., injections of 30 or 40 mg/kg increased the incidence of and produced neural tube, eye, skeletal, renal, and gonadal defects [55,56]. The malformation incidences were greatest following treatment on GD 9, though dosing on day 8 or 10 was also effective [55]. Burk and Beaudoin [57] have shows that sodium arsenate after single ip administration in wistar rat on GD9, 10 or 11 and at a dose of 30, 40 or 50 mg/kg (foetuses assessed on GD 20) shows multiple significant dose dependent toxic effects. At lower dose 30 mg/kg the significant decrease in fetal weight on treatment day 8 and 9. Further, increased percentage of live fetuses with malformations on treatment day 10 was observed. At dose 40 mg/kg the increased % resorbed or dead fetuses on treatment day 9 and with increased % of live fetuses with malformations on treatment day 9 and 10 with decrease in fetal weight on treatment day 9, 10, and 11. Moreover, at higher dose 50 mg/kg the increased % resorbed or dead fetuses on treatment day 9, 10, and 11 with increased % of live fetuses with malformations on treatment day 9 and 10. Above observations give very clear demonstration about the dose dependent effects of arsenic on developmental phase of rat.

Nervous System Effects

Arsenic caused developmental toxicity in wistar rats, when sodium arsenate was given as single i.p. administration on GD 8 in a dose of 5 mg/kg (foetuses assessed on GD 19) [58]. Arsenic at 5 mg/kg dose significantly increased total abnormalities but had no effect on skeletal retardation and abnormal vertebrae. In a time specific study, when sodium arsenate was given to sprague-dawley rats via single i.p. injection on GD 11.5 at 2-3 p.m at a dose of 50 mg/kg (foetuses assessed 18h after treatment). 50 mg/kg dose of arsenic significantly reduced maternal food intake and body weight gain [59]. Kojima [60], explained the effects of arsenic trioxide when administered via feed during gestation and lactation period in rats at a dose of 0.5, 2.5, 5.0 mg/kg/day and no maternal toxicity were noted. This indicated that arsenic is not toxic at very low doses. Similarly, Stump et al., [61], showed that, when pregnant rats received
Furthermore, Umpierre [62] noted resorption and malformation in rats exposed to sodium arsenite (NaAsO2, 11 mg/kg).

Particularly for Swiss mice, Domingo et al, [63], have demonstrated that after a single i.p. injection of 12 mg/kg of sodium arsenite on GD 10 and at 24, 48, and 72 hr thereafter shows embryotoxicity and teratogenicity. Similarly, in case of CD-1 mice, Baxley et al. [64] demonstrated arsenic toxicity, when given by single dose via oral gavage from 1 day, GD 8-15, at dose of 20, 40, 45 mg/kg (fetuses assessed on GD 18), showed dose dependent toxicity. Arsenic, at 40 mg/kg dose produced significant increase in dead or resorbed fetuses on GD 10 and 12 and no effect on fetal body weight or gross malformations were noted. Further, at higher dose 45 mg/kg, significant increase in dead or resorbed fetuses on GD 10, 12, 13, 14, and 15 were noted. Morrissey and Mottet [65], have shown a toxic effect of sodium arsenate on mice (BALB/c) with time and gestational day dependent effect in which sodium arsenate was given as single i.p. injection on GD 7, 8 a.m., GD 8, 8 a.m. and 2 p.m.; GD 9, 7 a.m. at a dose of 15, 20, 45, 60, 75 mg/kg (doses used varied with time), the result noted as at 45 mg/kg on GD 8, 8 a.m. the significant decrease in fetal weight was noted. Further, at a 45 mg/kg dose on GD 8, 2 p.m. significant increase in incidence of exencephaly was noted. Thus, the toxic effect of arsenic is time and gestational days dependent. In other study, by Earnest and Hood [66] on postnatal endpoints, 40 days after prenatal exposure. Mice treated with sodium arsenite by gavage at a dose of 5 mg/kg or i.p., injection at a dose of 2 or 4 mg/kg on GD 1-17 were evaluated postnatally. Results noted as no effect on postnatal weight or maturational indices, but survival through 30 days of age was reduced. On forty-day-old mice from the group of 5 mg/kg p.o., arsenic treated group made more errors on the initial day of testing in a Lashley III maze. Likewise, various studies also support arsenic mediated resorption and malformation in which, intraperitoneal administration of sodium arsenite (Na2HAsO4 z H2O, 40 or 45 mg/kg1) were noted to increase resorptions and produced eye, jaw, and skeletal malformations. Exencephalic fetuses were seen when dams were dosed on gestational day 8; in addition, some investigators observed neural tube defects after dosing on Day 6, 7, or 8, and perhaps 9 [67-69]. Arsenic is also noted to develop resorption in mice. Intraperitoneal administration of sodium arsenite (10 or 12 mg/kg) in mice increased resorption; produced moderate increase in gross malformations and exencephaly after dosing on gestational day 7, 8, or 9 [70,71]. Furthermore, oral doses of sodium arsenite (40 or 45 mg/kg) in mice increased resorptions and caused a low incidence of malformation and exencephaly was seen after dosing on Day 7, 8, or 9 [64]. In addition to this, the teratogenic actions of sodium arsenate (20 mg/kg i. p.) were noted when administered on GD 9 in pregnant mice [72].

Nevertheless, while focusing on developmental toxicity, it is appreciated to consider studies on hamster, which plays a major role to explore the key factors involved in arsenic mediated developmental toxicity. In hamster (LVC), single i.p. administration of sodium arsenate, at a dose of 20 mg/kg and fetuses assessed on GD 9, 10, 11, 12, 13, 14, or 15. The result showed increased prenatal mortality and increased % malformed fetuses (>90%) [73]. Further, Ferm and Carpenter [74], have noted particularly resorptions and malformations in hamsters. At 5 mg/kg: 16.7% resorptions or death, at 20 mg/kg: 35% resorptions, 48.6% malformations, at 40 mg/kg: all fetuses resorbed in hamsters administered sodium arsenate. Ferm and Hanlon [75], showed that administration of sodium arsenate through subcutaneously implanted mini pumps containing 150, 175, 200, 225, or 250 mg/ml (approximately 74.86, 100, 110, and 130 umol/kg/day), pumps inserted on day 4, 5, 6 or 7 of gestation (fetuses assessed on GD13). In all groups, fetal weight was decreased as length of exposure increased. Resorption rate increased as exposure period increased. Congenital malformations in live fetuses were not correlated with exposure duration but had strong correlation with dose level: 0 mg/ml, <1%; 150 mg/ml, 10%, 175 mg/ml, 24%; 200 mg/ml, 39%; 225 mg/ml, 46%; and 250 mg/ml, 59%. In continuous with pervious study Ferm and Hanlon [76], have shown effect of arsenic in Syrian Hamsters, when sodium arsenate was administered as single ip injection on GD8, 40.1, 48.2, 64.2 umol/kg (fetuses assessed on gd 13). Result reported 20, 38, and 95% neural-tube defects, dose dependently. Folate infuson did not affect the incidence of neural-tube defects. In a studie of Ferm and Saxon [77], have noted arsenic effects in hamsters, when sodium arsenate administered at single iv injection, GD8; 20 mg/kg (fetuses assessed on GD150 20 mg/kg dose showed no malformations in 45/216; other malformations in 110/216 and also 20 mg/kg arsenic increased amniotic fluid volume in fetuses with an/exencephaly. Ferm et al., [78], have demonstrated
that the golden hamsters (golden) when treated with sodium arsenate, dibasic, single iv injection, GD8, 15, 17.5, 20 mg/kg, GD8, 15, 7.5, 20 mg/kg, or GD8, 15, 20, 25 mg/kg (fetuses assessed Gd15). Result showed increased rate of resorption and malformation from 15 to 20 mg/kg. Rates varied from <10% to >90%

Holmberg and Ferm [79], have shown that golden hamsters when administered with sodium arsenate at a dose of single iv injection, GD8, 20 mg/kg and then fetuses assessed on GD13, showed only gross malformations. 48% of the embryos were malformed and 84% were malformed and/or resorbed. This indicates that administration of arsenic at a dose of 8, 20 mg/kg is responsible for the malformation and resorption process. Hood and Harrison [80], perform the experiment on hamsters (LVG), in which the sodium arsenite was given as a single oral dose on GD9 or 10, 2.5 mg/kg; GD8, 11, or 12 5 mg/kg (fetuses assessed on GD15). The dose dependent results are, at a dose of 2.5 mg/kg: No significant effects on number of litters, fetal weight, prenatal mortality, or incidence of malformation and at a dose of 5 mg/kg: No significant effects on number of litters or incidence of malformations. Significant depression of fetal weights on treatment day11 and 12 was noted. Also, significant increase in prenatal mortality on GD 8 and 11. Another study by Hood and Harrison [80], on hamsters g (LVC) shows that administration of sodium arsenite, single oral dose on GD9 or 10, 20 mg/kg; GD8, 11, or 12, 25 mg/kg (fetuses assessed on GD15) and result found was like, maternal mortality: 1/20 (5%) at 20 mg/kg and 6/36 (16.7%) at 25 mg/kg. Control: 1/51 (2%) maternal deaths. 20 mg/kg: No significant effects on number of litters, fetal weight, prenatal mortality, or malformations. 25 mg/kg: No significant effect on number of litters or malformations; significant increase in prenatal mortality on GD 8 and 12; significant decrease in fetal weight on GD12. It is necessary to focus on hamster treated with arsenic 25 mg/kg, which shows prenatal mortality on GD8 and 12. An impressive dose dependent study by Willhite [81], again on golden hamsters treated by sodium arsenite, single iv injection, GD8 and dose of 0, 2, 5, or 10 mg/kg (fetuses assessed on GD14). 2 mg/kg: Slight increase in prenatal mortality and % abnormal live fetuses. 5 mg/kg: "Increased" prenatal mortality and "increased" % abnormal fetuses. 10 mg/kg: "Marked" increase in prenatal mortality and "increased" % abnormal live fetuses. Thus, these studies provide information about dose dependent arsenic toxicity. Accumulating preclinical and clinical evidences suggests that exposure of arsenic cause developmental toxicity. It is needed to include developmental neurotoxicity studies in assessment for developmental toxicity [82].

Arsenic Exposure and Neurobehavioral Effects: Profound Look

Epidemiological studies mainly concentrate on neurobehavioral effects of arsenic. The large data from literature proves that exposure of arsenic is associated with Neurological disorders including encephalopathy, impairments of superior neurological functions such as learning, recent memory, and concentration particularly in patients with occupational exposure to arsenic compounds [83-85]. Bolla-Wilson and Bleecker [83], screened patient routinely for occupational arsenic exposure. A battery of neuropsychological tests was performed 6 weeks, 4 and 8 months after delirium. Elevated levels of arsenic were found in urine (41µg/l) and hair (5.1µg/g). The results showed visuoarperception, vasoconstriction, psychomotor integration, psychomotor speed, verbal learning and memory to be severely impaired while tests of general intellectual abilities and language remained unaffected. Morton and Caron [86] noted the cases of two workers occupationally exposed to arsenic fumes. First worker, complained for increasing forgetfulness, irritability and difficulty maintaining concentration. Biochemically, the concentration of arsenic in urine (24 hr) was found to be 115 µg/l. Further, neuropsychological tests showed moderate impairment of concentration, new learning, short-term memory plus considerable mental confusion and anxiety, however, symptoms improved after 3 months. Second worker reported headaches, irritability, recent memory deterioration, easy fatigue and the arsenic concentration in urine was 300µg/l. Thus, the available biochemical analysis suggests urinary arsenic as positive correlate to altered neurobehavioral changes. Calderon et al. [32], conducted tests in Mexican children living around a copper smelter and exposed to arsenic during their whole life. Urine analysis from children living in the high-arsenic exposure area was 62.9µg As/g and 40.2 g As/g in children living in a lower arsenic exposure area. Thus arsenic in urine is inversely correlated with neuropsychological parameters including verbal intelligence quotient (IQ) scores that encompass higher brain functions such as language, verbal comprehension and long-term memory. Accumulating clinical evidence suggest that arsenic exposure interferes with the neurobehavioral features in humans.
Although developmental exposure to arsenic can produce cognitive deficits in humans, most of the neurobehavioral studies with animal models have focused on locomotor activity [17, 87-91]. Studies observed hypoactivity [88, 89, and 91] and hyperactivity in rats [17] and dose-specific hyper- or hypoactivity in mice [87, 90]. Results on mouse studies are better correlate for human than rats. Tissue disposition in mice appears to better model that of humans [92]. Markowski et al., [93], reported the tissue-specific and dose related accumulation of arsenic in mouse offspring following maternal consumption of arsenic-contaminated water. Hemoglobin binds the metabolite, dimethylarsinic acid, and alters the rate of excretion [94]. Furthermore, developmental studies are perhaps more useful than acute exposures during adulthood since arsenic’s effects on human cognition have been observed primarily in children. For example, Martinez-Finley et al., [95], found that exposure throughout the gestational and lactational periods to low level arsenic in maternal drinking water increased indices of anxiety in mouse offspring during a novel object exploration task. Exposed offspring also performed worse than controls on a radial arm maze task. During an earlier study, a similar arsenic exposure paradigm increased the escape latency during an active avoidance task and increased periods of immobility during a forced-swim task [96]. In both experiments there were changes in hippocampal glucocorticoid receptors suggesting that arsenic disrupted the development of the hypothalamic-pituitary-adrenal axis.

Arsenic exposure could produce behavioral changes through a direct effect on the developing brain since arsenic freely crosses the mammalian placenta and blood–brain barrier [81, 99]. One of the most common fetal malformations in exposed mice is exencephaly [97,98]. Early in gestation, arsenic selectively accumulates in the neuroepithelium [99] and As3+ is retained in brain tissue for longer periods of time [98]. Embryonic exposure has been shown to produce neural tube defects, increase neuronal apoptosis, disrupt neural outgrowth, and reduce overall head size in both mouse and zebrafish models [100,101]. Viable offspring do not show signs of gross toxicity but there is early evidence of neurobehavioral toxicity such as reduced open field locomotion in neonatal rats [102].

It has been reported that As crosses the placental barrier reaching the conceptus after maternal exposure [103,104] and produces malformations, decreased prenatal rate of growth, increased mortality and neural tube defects [61,67,78,81,105-107]. Colomina et al., [108,109], reported that mice litters from dams exposed to sodium arsenite (10 mg/kg/day) on gestational days (GD) 15–18, and to a period of restraint (2 h daily) showed few behavioral deficits with the exception of an increase in pivoting, a type of abnormal gait behavior, decreased pivoting on postnatal day (PND) 9, and delayed pinna detachment and eye opening. A delay in eye opening was found in the group exposed exclusively to sodium arsenite (10 mg/kg/day) on GD 15–18.

Animal studies significantly support arsenic mediated neurobehavioral changes in human. In fact, animal studies rule out the exact picture of arsenic exposure and behavioral changes. Animal studies frame the toxic dose of arsenic, which classified the dose dependent toxic effects. This contention is further supported by the recent dose dependent study of arsenic in which arsenic exposure (0, 1.5, 3.5 and 4.5 mg/kg/day/po) in rat GD 8 till parturition and pups were critically assessed for physical development, reflex development, strength and motor coordination. In addition to this, our study also focused on malformation and developmental variation points but at the selected dose of arsenic no treatment related variations were observed [15]. Importantly, in continuous with these results Gandhi and Panchal [14], also demonstrates that inorganic arsenic exposure (0, 4.5, 6 and 7.5mg/kg/day/po) in rat gestational day 8 till parturition and offspring’s were examine for physical development, reflex development, and strength and motor coordination. This conclude that the inorganic arsenic exposure at low dose in not a toxicant.

The subtle central effects following low-level arsenic exposure have prompted neurobehavioral studies in animals, at exposure levels that do not produce symptoms of overt toxicity. Mice exposed to arsenic trioxide for 14 days showed a biphasic response on locomotor activity (horizontal and vertical parameters). A low dose of arsenic trioxide (3 mg/kg) increased locomotor activity, and a high dose (10 mg/kg) decreased locomotor activity [90].

**Mechanism involved in Arsenic Mediated Reproductive and Developmental Toxicity**

Exposure to inorganic arsenic has been associated with reproductive and developmental toxicity [81], although such effects are dependent upon the route of exposure [110,111]. Intricate molecular and cellular mechanisms are involved in arsenic mediated reproductive and developmental
toxicity. Both arsenate as well as arsenite interfere with the cellular production of energy, but they act via different mechanisms. In case of arsenate, they are structurally similar to phosphate thus interfere with process of glycolysis and ultimately decreases ATP production [112]. Further, arsenate by substituting the phosphate at the ATP-synthase enzyme and thus it uncouple with process of oxidative phosphorylation. At cellular level arsenate decreases ATP production and uncouple with oxidative phosphorylation, which ultimately results in transfer of electrons to oxygen and ADP-arsenate (rather than ATP) is hydrolyzed. Thus, there is net loss of energy produce by electron transport chain [112]. On other hand, arsenite is known to inhibit enzymes of the mitochondrial citric acid cycle, with a resulting decrease in cellular ATP production [112]. Together, arsenate and arsenite ultimately attack on the cell’s energy production system and determine cell survival. Another supportive factor for the toxicity of arsenic is excessive involvement of reactive oxygen species (ROS). Oxidative stress is a relatively new theory of arsenic toxicity and additional data supporting this theory and scientific acceptance of this mode of action have continued to occur. It has been demonstrated that arsenite has the ability to induce the formation of ROS in a wide variety of cells including human epithelial bladder cells and human vascular smooth muscle cells [113]. Barchowsky et al. [50] demonstrated free radicals production in mice after acute exposure to inorganic arsenic. The basic reason behind it is the presence of Fe2+, arsenite undergoes a Fenton reaction and produces ROS resulting in cell damage [114,115]. One of the most prominent toxic effects produced by ROS is DNA damage [114,116,117], which commonly results in apoptosis [114,116,118]. Chronic exposure of arsenite (300 μg/L) in rats resulted in depletion of glutathione and an increased oxidized glutathione and lipid peroxidation in the brain [102,119]. Apoptosis and oxidative stress has been linked to a variety of neuro-degenerative diseases. Furthermore, the effects of arsenic exposure showed changes in brain cell membrane function, which affects neural networking. This indicates additional involvement of oxidative stress during gestational period of rat [102]. However, the mechanism for arsenic mediated production of free radical and related toxicity is still incomplete. Although Yamanaka et al., [120] proposed another link of ROS and arsenic, as arsenic responsible for formation of intermediary arsenic species [120-122]. Other possibilities for the formation of ROS by arsenic lay on the oxidation of AsIII to AsV that under physiological condition will produce hydroperoxide. Worthwhile, recently new molecular mechanism reported, which gives clarity on concept about neurobehavioral changes associated with memory in mice expose to arsenic. In this novel study it has been demonstrate that the increased level of arsenic concentration in cerebellar tissue of the exposed mice in a dose–response manner, down-regulate the expression of Ca2+/calmodulin dependent protein kinase IV (Camk4), a very important regulator in the long-term depression (LTD) pathway, which ultimately impairment the learning and memory in mice [123]. Arsenite mediated neurotoxicity involves induction of apoptosis in cerebral neurons by activating p53 mitogen activated protein kinase (p53MAPK) and JNK3 pathways [124]. Together, a mass evidences suggest that arsenic initially interferes with ATP production and enhance ROS generation leading to DNA damage and cell. Thus, various studies explored the molecular and cellular mechanisms of arsenic mediated neurotoxicity.

Arsenic exposure reduces neuronal cell migration and maturation. It can perturb the embryonic differentiation process by repressing the Wnt/β-catenin signaling pathway [125]. Decrease in the levels of dopamine (DA, 28%), norepinephrine (NE, 54%), epinephrine (EPN, 46%), serotonin (5-HT, 44%), 3,4-dihydroxyphenylacetic acid (DOPAC, 20%) and homovanillic acid (HVA, 31%) in corpus striatum; DA (51%), NE (22%), EPN (47%), 5-HT (25%), DOPAC (34%) and HVA (41%) in frontal cortex and DA (35%), NE (35%), EPN (29%), 5-HT (54%), DOPAC (37%) and HVA (46%) in hippocampus were observed in arsenic (sodium arsenite, 20 mg/kg body weight, p.o., 28 days) treated rats [126]. Also, arsenic treatment increased NO level in corpus striatum (2.4-fold), frontal cortex (6.1-fold) and hippocampus (6.2-fold) (Previous). It is envisaged that, arsenic induce oxidative stress produces increased reactive oxygen species (ROS) and nitric oxide (NO) generation. Increased ROS further caused apoptosis via mitochondrial driven pathway. Oxidative stress cause alterations in antioxidant enzymes, GPx, GST and SOD and neurotransmitter levels in brain, leading to impaired neurobehaviors [127].

**Concluding Remarks**

In conclusion, pregnancy is a unique time when a woman may seek treatment out of concern for the health and well being of her offspring. Substance used during pregnancy can affect the developing fetus
both directly through passage of the toxicant through the placenta and indirectly, through poor maternal health habits and environmental conditions. Arsenic is a potent carcinogen and toxicant, which easily passes the placenta. There is convincing evidence that arsenic induces a number of effects in the foetus, some of which result in foetal loss or growth retardation. Plethora of evidences suggest that the changes induced in foetal or infant life lead to detectable adverse health effects later in childhood as well as in adult life. Arsenic toxicity mediated effects are mainly due to DNA hypomethylation, increased interaction with hormones (e.g. estrogens, immune suppression, neurotoxicity and inhibition of numerous enzymes), fetal and postnatal environment, dose of the agent and the developmental stage of the fetus at the time of exposure. Several population studies looking at effects of prenatal exposure to arsenic show a large variation in outcomes of neurodevelopmental endpoints. The preclinical and clinical evidences suggest that low dose exposure of arsenic could not affect the reproductive and developmental toxicity while high dose exposure is precipitate in neurobehavioral alterations. However, in most cases, the exposure, for example, via drinking water or air continue even after early childhood and cross-sectional studies later in life has not identified critical windows of exposure. A few epidemiological and mechanistic studies indicate that the foetus is highly susceptible to arsenic and adverse effects that give rise to health disorders later in life may occur, even at low exposure levels. Finally, developmental outcomes may be optimized by interventions that occur early in life. Environmental pollutants, heavy metals, addictive substances, drugs, malnutrition, excessive stress and/or hypoxia-ischemia are reported to induce functional maldevelopment of the brain with consequent neurobehavioral disorders. The appropriate animal model and study design might provide valuable tools in the search for biological markers that possibly severe cognitive function in adults or the elderly.

Conflict of Interest: None

References


[12] Yousef MI, El-Demerdash FM, Radwan FME, 2008, Sodium arsenite induced biochemical perturbations in rats: Ameliorating effect of curcumin, Food and Chemical Toxicology, 46(11); 3506–3511.


[20] Reprint from Townsend News Letter for Doctors & Patients, behavioral and functional abnormalities associated with heavy metal toxicity, bioray, Inc. 23151 Alcalde Drive, Suite C-3 Laguna Hills, CA 92653 (949);305-7454.
arsenic exposure and children's intellectual function in Araihazar, Bangladesh, Environ Health Perspect, 112(13); 1329–1333.


administered sodium arsenite in mice, Bull Environ Contam Toxicol, 26;749—756.


[66] Earnest NM, Hood RD, 1981, Effects of chronic prenatal exposure to sodium arsenite on mouse development and behavior, Teratology, 24;53A.


[68] Hood RD, Pike CT, 1972, BAL alleviation of arsenite-induced teratogenesis in mice, Teratology, 6; 235—238.


[70] Hood RD, 1972, Effects of sodium arsenite on fetal development, Bull Environ Contam Toxicol, 7;216—222.


[125] Hong GM, Bain LJ, 2012, Arsenic exposure has been observed to inhibits myogenesis and neurogenesis in P19 stem cells through repression of the β-catenin signaling pathway, Toxicol Sci, 129(1);146-56
[127] Flora SJ, Bhatt K, Mehta A, 2009, Arsenic moiety in gallium arsenide is responsible for neuronal apoptosis and behavioral alterations in rats, Toxicol Appl Pharmacol, 240(2); 236-44.

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