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Inorganic arsenic exposure during pregnancy affects post-natal growth, blood parameters, and organ development of mice offspring

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ABSTRACT

Arsenic is a potentially toxic agent for human health due to its widespread presence in the environment. Arsenic poisoning from drinking contaminated groundwater has become one of Bangladesh's most difficult healthcare problems. However, there is a lack of understanding of the detrimental impact of arsenic toxicity on children of arsenic-exposed parents. This study evaluates the effect of arsenic toxicity on body growth, blood parameters, and organ development of F1 mice. In this study, adult female mice were exposed to sodium arsenite from gestation day 12.5 until parturition, and then the postnatal growth, blood indices, and organ development were assessed. The result showed that from birth to weaning offspring of the sodium arsenite-treated group exhibited significant and weaning to sexual maturity of female offspring significantly slower increase in final body weight, total body weight gain, and rate of body weight gain than that of the offspring of the control group. Hematological tests revealed a significant reduction in RBC and WBC count while significant elevation in platelet count, MCV, MCH, and MCHC in offspring of sodium arsenite exposed female mice than the offspring obtained from control female mice. RBS and triglycerides levels were significantly higher, while alkaline phosphatase level was significantly lower in the offspring of sodium arsenite-exposed female mice than in the offspring obtained from control female mice. In terms of organ-to-body weight ratio, the female F1 mice from the exposed group demonstrated a significantly lighter kidney and heart as compared to that of the female F1 mice from the control group. Visual inspection of the organ morphology showed a slightly affected liver, lungs, and testes. Overall, the study suggests that inorganic arsenic exposure of the parent mice exerts harmful effects on the body growth, hematological, and biochemical parameters, and organ morphology of the offspring.

INTRODUCTION

Arsenic (As) is a naturally occurring omnipresent element that makes up a significant portion of the Earth's crust, 20th in terms of abundance [1]. It is a heavy hazardous toxicant that is naturally present in drinking water and endangers both human and natural ecosystems [2]. Arsenic poisoning affects over 200 million people in at least 105 nations, both advanced and developing, primarily through drinking water [3-5]. Arsenic exposure through groundwater has been a serious threat to health in Bangladesh. According to World Health Organization (WHO), Bangladesh's arsenic disaster is "the largest recorded poisoning of a population in history" [6]. In 61 districts of Bangladesh out of 64, As contaminated drinking water poses a chronic health risk to a large number of people [2]. According to the world health organization guidelines, the recommended level of As in drinking water is 10 micrograms per liter; however, in Bangladesh, this threshold is 50 micrograms per liter [7-9]. Surprisingly in Bangladesh,

a significant amount of the groundwater is contaminated with As at a concentration of 100 to 2000 ppb, which is higher than the WHO-recommended level [10]. Long-term inorganic arsenic (iAs) exposure from drinking water has been shown to increase the prevalence of a wide range of health illnesses, such as persistent illnesses, precancerous skin lesions, and skin, liver, lungs, spleen, kidney, and bladder malignancies, and also non-cancerous health hazards like diabetes mellitus, cardiovascular disease, neurological deficits, respiratory issues, and reproductive complications [11-14]. Numerous pathogenic events, including oxidative damage, inflammation, mitochondrial dysfunctions, ER stress, apoptosis, altered protein homeostasis, and aberrant calcium signaling, are brought on by exposure to As and its metabolites [15]. Fatty changes and cirrhosis in the liver, as well as severe hepatic vessel congestion, congestion in the central veins, and hemorrhage in the hepatic lobules and lobular tissues were also observed [16, 17]. In the liver, there was severe congestion, cytoplasmic vacuolation, and bile duct hyperplasia [18]. Further studies reveal the blood biochemical parameters of As-exposed humans and animals were changed and associated with liver and kidney function. Biochemical and hematological analyses have the potential to assess the nature of the action of toxicants, and knowledge of toxicant physiology can also aid in the prediction of significant sub-lethal consequences. Blood parameter (hematological and biochemical) studies are crucial in determining the operational and organizational condition of a person exposed to a toxic substance [19, 20]. It has been revealed that iAs can cross the placenta and enter the system of the fetus after being administered during pregnancy. This proved transplacental exposure to As is a viable exposure pathway [20]. Consequences of early-life exposure are a growing issue because there is evidence of harmful effects of gestational or even earlier exposure in parents' lives (F0 generation) to various environmental toxicants, which can result in adult-onset disease in the offspring (F1 generation) or a multigenerational (F2 generation) or transgenerational way (F3 and beyond) [21-23]. Both the F1 and the offspring of F1 of C3H mice are more likely to develop hepatic tumors after being exposed to As during pregnancy [2, 24].

In Bangladesh, now a significant health concern is the physical and mental health of As affected children. There are many people from a variety of occupations, notably farmers, who remained in the As-polluted region and drank As- contaminated water for a prolonged period. Children of those families who want to carry on their family business must remain in the same region from one generation to the next. Thus, there is a risk of health hazards for children from As-contaminated parents. However, there is a lack of understanding of the adverse effect of arsenic toxicity on children of As exposed parents. Consequently, the current study has been conducted through the mouse model with the precise goals to observe the post-natal growth, investigate the arsenic-induced hematological and biochemical changes and evaluate the organ development anomalies of offspring due to arsenic toxicity. The study has been focused for the first time on hematological alterations and gender differences in body growth from weaning to sexual maturity and the relative organ weights of male and female F1 offspring separately.

MATERIALS AND METHODS

Animal selection, housing, and care

Swiss albino mice of either sex, 6-8 weeks of age, or weighing 25-30 g were obtained from the Animal Resources Facility (ARF) of the International Centre for Diarrhoeal Disease Research, Bangladesh (ICDDR, B). The housing was kept at a temperature of 26±2ºC with a relative humidity of 30–70% and provided the animals with complete access to food and water. The animals were housed under-regulated lighting (12-hour light/dark cycle). They were kept in an animal house with plenty of ventilation. Five mice per cage for female mice and one mouse per cage for male mice were selected randomly and housed in plastic cages with wood-cube bedding. Provide bedding that was sanitary and cozy within the cage. Drinking water and sleeping materials (wood-cob) were transformed every morning to provide a relaxing environment. They were provided regular food in the form of pellets. Water and food were always available. Every day, the amount of water consumed was recorded. Before the beginning of the research, the mice were maintained for a week to acclimate to the new surroundings, and the cages were marked with the appropriate introduction. With the approval of the HSTU Laboratory Animal Care and Use Committee, all animal experiments were carried out by the Guidelines for the Care and Use of Laboratory Animals implemented by the Department of Genetics and Animal Breeding (HSTU/VAS/GAB/2009/437-A).

Estrous detection and breeding

Evaluation of the estrous cycle in experimental animals, the vaginal cytology method was implemented to identify the stages of estrous cycles. Female mice were randomly selected and mated with male mice from separate cages once proestrus was proven to produce time-pregnant females. The mating strategy was two females and one male. The next morning, the female vulva was examined for the presence of a sperm plug. The detection of a sperm plug was labeled embryonic day 0.5 (E0.5) of gestation. Females who tested positive for plugs were considered “pregnant” and were immediately separated into separate cages for further treatment.

Treatment options and arsenic exposure

Pregnant female mice were randomly assigned to one of two experimental groups (n=6 per group; repeated at least 3 times), namely (1) the control group, which received filtered water, and (2) the sodium arsenite group, which received 10 micromolar arsenic as sodium arsenite. Sodium arsenite (S7400; Sigma-Aldrich, St. Louis, MI, USA) was dissolved in distilled water and given to pregnant female mice daily ad libitum in drinking water from 12.5 days of gestation to lactation. The arsenic concentration used in this study was determined by previous research into the effects of arsenic on the placenta formation [25].

Postnatal body growth of F1 offspring

The effect of Na-arsenite on the post-natal growth of mice was evaluated based on the growth of offspring from birth to weaning (4 weeks of age) and weaning to sexual maturity (8 weeks of age). After parturition, litter size (number of pups), litter weight, and weight/pups (separately male, and female) were observed. Then pups were observed until sexual maturity to see the body growth from weaning up to sexual maturity. Body weight gain and rate of weight gain were calculated using the following

formula: Body weight gain = (final body weight – initial body weight) and Rate of weight gain = (final weight – initial weight)/day.

Blood sampling and organ collection

After 8 weeks sexually mature F1 mice were deprived overnight and given mild diethyl ether (W509043; Sigma-Aldrich, St. Louis, MI, USA) anesthesia the next morning to draw blood. Due to the need for a significant volume of blood, a cardiac puncture was used to collect the blood. Blood was then preserved in a collecting tube (lithium heparinized tube (BD 366667; Thermo Fisher, Waltham, Massachusetts, USA), clot activator tube) for hematological and biochemical examination. Various internal organs including the liver, lung, kidney, spleen, heart, uterus, and testis were carefully collected for further analysis.

Hematological analysis

Complete blood count (CBC) including red blood cells (RBC), white blood cells (WBC), differential blood count (neutrophils, eosinophils, basophils, monocytes, and lymphocytes), platelets (PLT), hemoglobin (Hb), packed cell volume (PCV), erythrocyte sedimentation rate (ESR), trichloroethylene (TCE), mean corpuscular volume (MCV), mean hemoglobin concentration (MCHC), mean corpuscular hemoglobin (MCH), a standard deviation of red cell distribution width (RDW-SD) and coefficient of variation of red cell distribution width (RDW-CV) were determined using automated hematology analyzer (BC-20; Mindray, Shenzhen, P.R. China). The comparison of hematological parameters between the exposed and control groups was done.

Biochemical evaluation

Each sample was given a minimum of 30 minutes to clot (maximum of 60 min). Following clotting, the sample was centrifuged for 10 minutes at 6000 rpm. Then the serum was taken by pipette and mixed with available reagents (RANDOX, Crumlin, Country Antrim, United Kingdom) for analyzing random blood sugar (RBS), alkaline transaminase (ALT), alkaline phosphatase (ALP), triglyceride and total cholesterol, and uric acid by using a biochemical analyzer (18200; HUMAN, Wiesbaden, Germany).

Evaluation of different organs and organ-to-body weight ratio

After removing organs, all these organs were weighed individually using a digital balance. The organ-to-body weight ratio was calculated by dividing each animal's weight by its body weight. This was called relative organ weight. The organ-to-body weight ratio was calculated by the following formula: organ-to-body weight ratio = (organ weight/body weight) ×100. The control and treated group organs were evaluated to compare the effect.

Organ morphology

The organs were indisputably visualized to evaluate gross morphology. The criteria of gross morphological examination were based on the organs' position, shape, size, color, and consistency. The organs were kept separately in different Petri dishes according to

morphological features. Organ size was determined using a centimeter scale. Then the organs were compared between the control and exposed groups of offspring.

Statistical analysis

Every experiment was carried out at least three times. The results of body growth, relative organ weight, and hematological and biochemical parameters were expressed as a ratio against each control and $NaAsO_2$ exposed and are expressed as Mean ± Standard Error of Mean (SEM). Single-factor analysis of variance (ANOVA) was used to analyze the statistical differences between the control and exposed groups where significances were considered at $p<0.05$. Furthermore, the Student-Newman-Keuls test was used to compare the two groups. Differences were considered significant at the level of $p<0.05$.

RESULTS

Evaluation of body growth of F1 mice (from birth to weaning)

The results revealed that the offspring of $NaAsO_2$ treated mice had significantly ($p<0.002$, $p<0.001$, $p<0.001$ consecutively) slower increases in final body weight (Figure 1A), total body weight gain (Figure 1B), and rate of body weight gain (Figure 1C) than the offspring of control mice that weren't given any treatment. The current study also looks at how gender influences the growth of offspring. In the case of males, the rate of weight gain was significantly ($P<0.005$, $p< 0.02$ respectively) higher than females in both the control and treated offspring. (Figure 2).

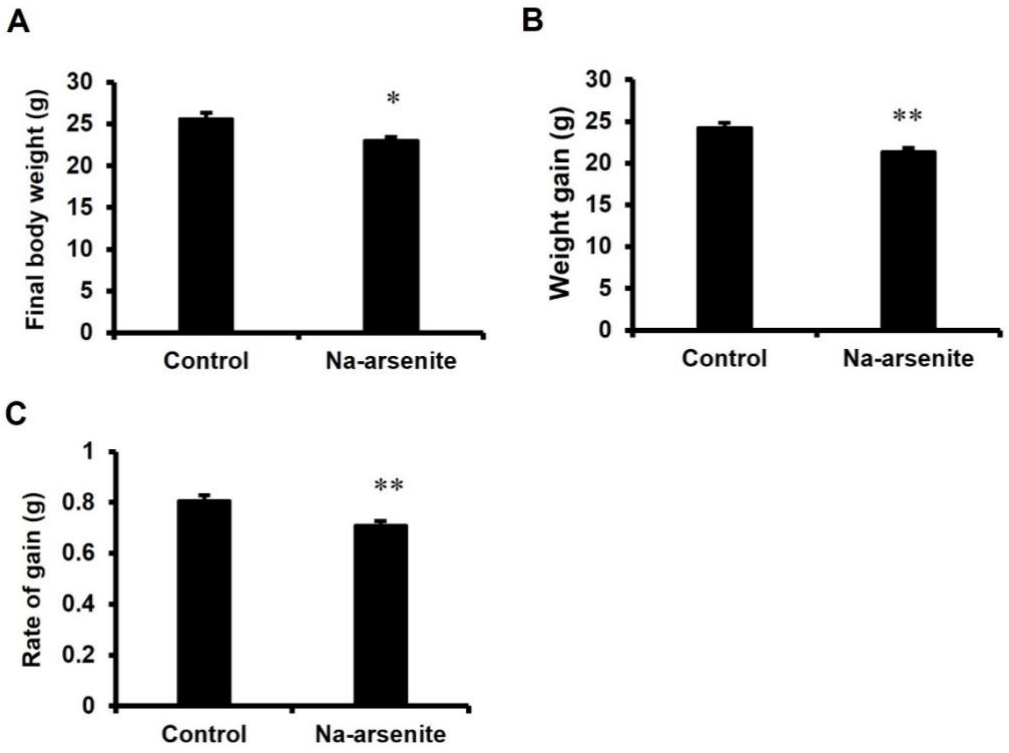

Figure 1. Impact of sodium arsenite ($NaAsO_2$) on body growth (from birth to weaning) of offspring. In control and $NaAsO_2$ exposed mice, body growth after birth to weaning (4 weeks) of offspring from control and treatment group mice was assessed and compared according to final body weight (A), body weight gain (B), and rate of body weight gain (C). Data are presented as mean ± SEM (n=3). *p<0.002, **p<0.0001 indicates statistical significance from the control mice.

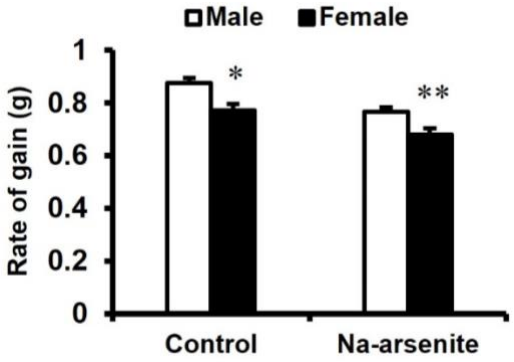

Figure 2. Implications of gender differences in the rate of body weight gain from birth to weaning. In both control and $NaAsO_2$ exposed mice, the rate of body weight gain from birth through weaning was assessed and compared in male and female pups. Values are presented as mean ± SEM (n=3). *p<0.005, **p<0.02 indicates statistical significance from the male offspring.

Gender-specific effects of Na-arsenite on body growth (weaning to sexual maturity) in F1 mice

To investigate the sex-specific effects of Na-arsenite on body growth (weaning to sexual maturity) in arsenic-exposed male and female groups of offspring, following weaning offspring were monitored daily up to maturity. In this study, the body weight of F1 mice (male and female respectively) was measured and compared between the control and Na-arsenite exposed group of F1 mice. The results showed that Na-arsenite ($NaAsO_2$) exposed female offspring had significantly (*p<0.01) slower increases in initial body weight, final body weight, body weight gain, and rate of body weight gain than the control group of female offspring (Table 1). But in the case of arsenic-treated male offspring body weight was significantly (*p<0.01) lighter in initial body weight and final body weight than the control group of male offspring. Although the body weight gain and rate of body weight gain were comparatively lower than the control group, the differences were not at a significant level (Table 2).

Table 1. Effect of arsenic on body growth (weaning to sexual maturity) in female offspring.

Treatment	Initial body weight (g)	Final body weight (g)	Body weight gain (g)	Rate of body weight gain (g/day)
Control	24.64±0.71	38.12±0.95	13.48±0.76	0.38±0.02
Na-arsenite	22.12±0.54*	32.55±0.53*	10.42±0.69*	0.29±0.01**

Body weight was compared and assessed between the control and $NaAsO_2$ exposed group of female offspring. Values are presented as mean ± SEM (n=3). *p=<0.01, *p=<0.0001 indicates statistical significance from the control group.

Table 2. Effect of arsenic on body growth (weaning to sexual maturity) of male offspring.

Treatment	Initial body weight (g)	Final body weight (g)	Body weight gain (g)	Rate of body weight gain (g/day)
Control	27.76±0.52	51.53±0.77	23.77±1.08	0.67±0.03
Na-arsenite	24.65±0.77*	45.97±1.38*	21.32±0.80	0.60±0.02

Body weight was compared and assessed between the control and $NaAsO_2$ exposed group of male offspring. Values are presented as mean ± SEM (n=3). *p=<0.01 indicates statistical significance from the control group.

Toxic effect of arsenic on organ development of mice offspring

The relative weight (% of body weight) of various organs (liver, Lung, kidneys, Spleen, Heart, Uterus, and Testis) were measured and compared between exposed and control groups of F1 mice in this study. The current study observes the various organ development influenced by gender. In the case of female offspring, the Kidney and

Heart weight of the Na-arsenite exposed group was significantly (*p<0.01) reduced compared to nonexposed females (Table 3). However, no statistically significant difference existed between the control and treated groups in male offspring (Table 4).

Table 3. Effects of $NaAsO_2$ on organ development of F1 female mice.

Treatment	Organ to body weight ratio (%)					
	Liver	Lung	Kidney	Spleen	Heart	Uterus
Control	5.48±0.14	0.64±0.01	1.10±0.03	0.39±0.02	0.41±0.01	0.45±0.03
Na-arsenite	5.39±0.13	0.66±0.03	0.99±0.01*	0.70±0.32	0.37±0.00*	0.40±0.02

The organ-to-body weight ratio was assessed and compared between the control and $NaAsO_2$ exposed group of female offspring. Values are presented as mean ± SEM (n=3). *p<0.01 indicates the statistical significance from the control offspring.

Table 4. Effects of $NaAsO_2$ on organ development of F1 male mice.

Treatment	Organ to body weight ratio (%)					
	Liver	Lung	Kidney	Spleen	Heart	Testis
Control	5.84±0.14	0.51±0.03	1.27±0.04	0.34±0.03	0.37±0.01	0.48±0.02
Na-arsenite	6.09±0.15	0.51±0.02	1.26±0.03	0.41±0.02	0.38±0.01	0.49±0.01

The organ-to-body weight ratio was assessed and compared between the control and $NaAsO_2$ exposed group of male offspring. Values are presented as mean ± SEM (n=3). There is no significant difference between the control and treated male offspring.

Arsenic-induced toxicity on organ morphology

The impact of Na-arsenite on different organs was assessed after reaching the sexual maturity of F1 mice (Figure 3). Visual observation of the Morphological characteristics of different organs showed slight discoloration of the liver (Figure 3A, 3H) and lungs (Figure 3B, 3I) and substantially were affected in $NaAsO_2$ treated offspring compared to non-treated control offspring. Interestingly, the size of the Testis (Figure 3G, 3N) was comparatively shorter than the group of control offspring. On the contrary, normal morphological features of the kidney (Figure 3C, 3J), spleen (Figure 3D, 3K), heart (Figure 3E, 3L), and uterus (Figure 3F, 3M) were observed both in control and $NaAsO_2$ treated offspring.

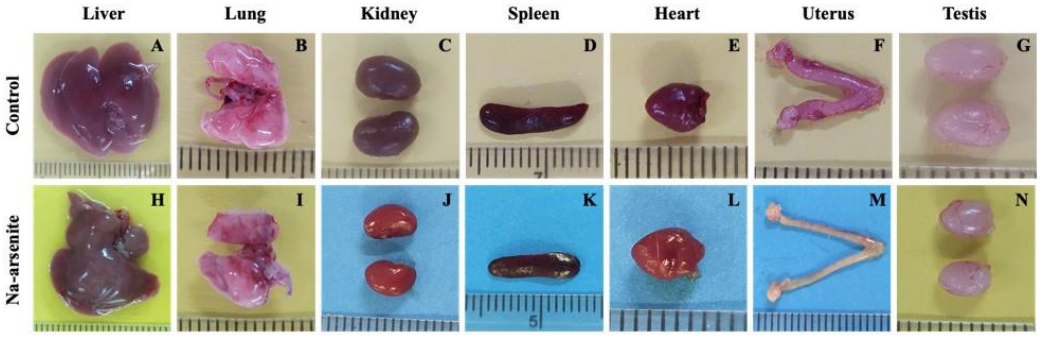

Figure 3. Effects of Na-arsenite ($NaAsO_2$) on different organs of F1 mice. Offspring (control and $NaAsO_2$ exposed) after reaching sexual maturity were euthanized and organs namely the Liver (A, H), Lung (B, I), Kidney (C, J), Spleen (D, K), Heart (E, L), Uterus (F, M), and Testis (G, N) were collected and photographed. Each organ of the control and $NaAsO_2$ exposed F1 mice were assessed based on the morphology of the organ.

Effect of $NaAsO_2$ exposure on hematological alterations in mice offspring

The present study aimed to measure the changes in hematological parameters of the blood tissue and compared the sodium arsenite exposed and the control group of mice offspring. The result showed that the Platelet count, MCV, MCH, and MCHC were significantly (*p<0.02, ***P<0.05 respectively) higher in the Na-arsenite exposed group of offspring compared to the control group of offspring (Table 5). Whereas TC of WBC,

and RBC count, were significantly (*p< 0.02, **p<0.03 respectively) decreased in sodium arsenite-treated offspring than in control offspring (Table 5).

Table 5. Haematological parameters in Mice offspring exposed to $NaAsO_2$.

Parameters	Treatments	
	Control	Na-arsenite
TC of WBC (/cmm)	3366.66±296.27	1933.33±185.59*
Neutrophils (%)	7.33±1.45	8.66±1.45
Lymphocytes (%)	85.66±2.96	82.66±3.48
Eosinophils (%)	2.66±0.66	3.66±0.88
Monocytes (%)	4.33±0.88	5±1.15
HB (g/dl)	13.4±0.20	13.03±0.49
TCE (/cmm)	92.66±29.94	73±22.94
Platelet count (/cmm)	389333.33±67261.51	835666.66±105527.77*
RBC count (million/cmm)	8.55±0.10	6.75±0.53**
PCV (%)	37.83±0.28	31.3±2.57
MCV (fl)	44.3±0.55	46.3±0.20*
MCH (Pg)	15.66±0.12	19.46±1.06*
MCHC (g/dl)	35.33±0.38	42±2.45***
RDW SD (fl)	29.86±0.53	31.56±2.12
RDW CV (%)	15.9±0.45	16.03±1.16

Hematological parameters of mice offspring are assessed and compared between control and $NaAsO_2$ exposed offspring. Values are presented as mean ± SEM (n=3). *p<0.02, **p<0.03, ***p<0.05 indicates statistical significance from the control offspring. TC: total count; WBC: White blood cell; Hb: Hemoglobin; TCE: Trichloroethylene; RBC: Red blood cell; PCV: Packed cell volume; MCV: Mean corpuscular volume; MCH: Mean corpuscular hemoglobin; MCHC: mean corpuscular hemoglobin concentration; RDW-SD: red blood cell dimension width-standard deviation; RDW-CV: Red cell distribution width-coefficient of variation.

Effect of $NaAsO_2$ exposure on biochemical alterations in mice offspring

The study also revealed the serum biochemical alteration in sodium arsenite-exposed mice offspring and compared to exposed and nonexposed groups (Table 6). The results showed that the RBS and Triglyceride were significantly (*P<0.05, ***P<0.0008 respectively) increased in the arsenic-treated group than in the control but in the case of alkaline phosphatase was significantly (***p<0.02) reduced from the nonexposed group of offspring (Table 6).

Table 6. Serum biochemical alteration in mice offspring exposed to Na-arsenite.

Parameters	Treatments	
	Control	Na-arsenite
RBS (mmol/L)	10.5±0.68	14.86±1.41*
ALT(SGPT) U/L	190±55.30	76±22.59
Total Cholesterol(mg/dl)	197.33±8.68	229.33±10.72
Alkaline Phosphatase(U/L)	183.33±46.93	24±6.08**
Triglyceride(mg/dl)	354.33±6.17	507.33±15.64***
Uric acid(mg/dl)	9.13±0.99	8.16±1.02

Serum biochemical analysis and comparison between control and $NaAsO_2$ exposed offspring. Data are presented as mean ± SEM (n=3). *p<0.05, **p<0.02, ***p<0.0008 indicate statistical significance from the control offspring. RBS: Random blood sugar; ALT: Alanine transaminase; SGPT: Serum glutamic pyruvic transaminase.

DISCUSSION

Arsenic toxicity is very threatening in pregnant women and animals, and it is very easy to get arsenic toxicity from drinking water in Bangladesh. There have been several studies on arsenic toxicity in pregnancy, but it is important to investigate arsenic's effect on their progeny from generation to generation. We reported that arsenic exposure during pregnancy affects post-natal growth, blood parameters, and organ development

in F1 generation mice offspring. Arsenic quickly crosses the placental barrier, affecting fetal growth and offspring later in life. Several studies have raised concerns about the effect of arsenic in drinking water on human pregnancy [26]. In this study, we evaluated arsenic-induced toxicity on body growth and assessed hematological and biochemical alteration and organ morphology of offspring after Na-arsenite exposure during embryonic day 12.5 of gestation to lactation in adult female mice. A decrease in body weight is a well-known indicator that one's normal health is deteriorating. Arsenic-caused toxicological consequences and metabolic dysfunctions pose substantial threats to the fitness [27]. Body growth of arsenic-treated offspring did not increase when compared to the control group of offspring in the current study, which showed the final body weight, body weight gain and rate of body weight gain are significant ($P<0.002$, $P<0.001$, $P<0.001$ respectively) decrease throughout birth to weaning of mice, which is comparable to the toxicological effects of arsenic on humans and is associated with low birth weight and height [28] and growth of the F1 offspring of a mouse model [29] and depletion of body weight in later life of offspring [30, 31]. The growth impairments shown in the F1 offspring are thought to be caused by nutrient deficiency in the dam's breast milk, particularly TGs [29]. Body growth in offspring is influenced by gender. The observations throughout the weaning to sexual maturity reveal a noticeable reduction ($p<0.01$, $p<0.0001$ respectively) in body weight, and rate of body weight gain in Na-arsenite-exposed female offspring, compared to the non-exposed female control, which is in line with the observation of postnatal development of female offspring [32]. It also reduced in males, but the differences were not at a significant level which is similar to the previous report [30]. The negative effects of arsenic on normal metabolic and developmental processes, as well as excessive protein breakdown in tissue, may be responsible for the slower rate of weight gain in arsenic-treated mice, which is very similar to our current study [31]. Hematological and biochemical parameters are reliable indicators for assessing the health of animals and humans with arsenic poisoning [33, 34]. Toxic substances have a negative impact on hemopoiesis, as well as the metabolism and activity of mature blood cells. This could result in abnormal, reduced, or inhibited blood cell synthesis. In this study, we found that the RBC and WBC counts were significantly lower in Na-arsenite-exposed offspring than in control mice offspring [20, 35]. Anemia and leucopenia are common poisoning symptoms caused by acute, immediate, and chronic arsenic exposures, which may result from a direct cytotoxic or hemolytic impact on blood cells as well as erythropoiesis impairment [36]. Reduction of WBC count may be related to a decrease in the individual's nonspecific immunity following arsenic exposure. Arsenic's apoptosis effect on plasma cells may be responsible for the WBC reduction [37]. This study's findings revealed a significant rise in platelet counts in $NaAsO_2$-exposed offspring compared to non-exposed offspring, which is consistent with earlier research [38]. Thrombocytosis, or elevated platelet counts, may be a sign of immediate-type hypersensitivity to arsenic, according to this study. We found that MCV, MCH, and MCHC levels were significantly higher in the arsenic-exposed group than in the control groups of offspring, which is consistent with the findings in carp exposed to arsenic [39]. The rise in MCV, MCH, and MCHC levels could be due to macrocytic type anemia. Analysis of serum biochemical parameters is recommended to provide early warning signs of significant changes in stressed animals, and it is especially useful for identifying the target organs of toxicity as well as the overall health state of the animals [40]. Furthermore, biochemical analyses were used to demonstrate the toxicity of various tissue systems. Arsenic has the potential to cause biochemical disruptions as well as toxicological effects, both of which pose significant health risks [41]. The results of this serum biochemical analysis study revealed that random blood sugar (RBS) levels

were significantly higher in Na-arsenite-exposed offspring compared to control offspring [41, 42]. A prior study found that hyperglycemia is caused by increased glucose production and decreased glucose utilization [43]. Arsenic caused diabetes mellitus in chronically exposed individuals who consumed arsenic or its methylated metabolites. According to some research, insulin resistance and b cell dysfunction are both pathologically linked to oxidative stress. Continuous arsenic exposure causes oxidative stress, which causes structural damage to the pancreatic islets and the production of amyloid proteins, which not only limit insulin release into the blood but also slowly destroy the insulin-secreting b cells. In addition, the triglyceride (TG) level of the exposed group was significantly higher than the non-treated or control group of offspring which is supported by those researchers, where TG levels were significantly enhanced in the rat after $NaAsO_2$ exposure [42, 43]. This finding resulted from the disruption of lipid metabolism caused by arsenic stress. Increased oxidative stress may be caused by mitochondrial damage within the hepatocytes, resulting in decreased fatty acid oxidation within the mitochondria. Triglycerides accumulate within the hepatocytes as a result of these fatty acids being diverted into esterification pathways [40]. ALP is a liver damage marker enzyme. Surprisingly, the present study revealed that ALP reduced significantly in sodium arsenite-treated offspring than a control group, which contrasts with the study of parental exposure of As associated biochemical alteration in F1 offspring of mice [2]. A possible reason might be the effect of environmental variation, the selected dose of As, selected time, and duration of exposure differences, which might exert the dissimilarity of ALP level. A reduction in kidney and heart weight was also observed significantly in Na-arsenite-exposed female offspring compared to the control group of female offspring. Studies have reported a significant reduction in the weight of kidneys [44, 45] and heart [46] of mice and rats exposed to arsenic. This could be brought on by the oxidative damage caused by tubular cells. The lowering amounts of contractile proteins in the heart tissue may be to blame for the decrease in the relative organ weight [47]. An earlier study demonstrated that oxidative stress and cardiac injury could cause myocardial sarcomeric proteins to deteriorate [48]. To develop As nephropathy and damage to renal tubular cells, which results in a decrease in relative kidney weight, oxidative stress is a crucial element [49]. They reported that oxidative stress is a critical element in the development of As nephropathy and damage to renal tubular cells, which results in a decrease in relative kidney weight [49]. Morphological characteristics of various organs showed that the liver and lung in the $NaAsO_2$ exposed group were slightly discolored compared to the control group [24, 50]. Since degenerative changes were noted in the testicular tissue of mice treated with arsenic, it is interesting to note that the observations revealed the size of the testis was less than the group of control offspring due to the loss of germ cells [51]. Further study is required to determine the mechanism of Arsenic toxicity-enhanced effects in F1 mice, although the hematological and biochemical data of this study are well matched by the toxic effect of arsenic on offspring in later life after Na-arsenite exposure during embryonic day 12.5 of gestation. For the first time, this study focused on the effect of gestational exposure to inorganic arsenic on gender differences in body growth from weaning to sexual maturity, organ-to-body weight ratio separately in males and females, and hematological alterations of F1 offspring in Swiss albino mice. However, this investigation did not examine the histology of the liver, lung, kidney, heart, and testis. In this regard, we have no reluctance to declare that this may be a specific limitation of our study.

CONCLUSION

Offspring from As-exposed female mice in mid-gestation are particularly sensitive to arsenic poisoning on post-natal growth (birth to weaning and weaning to sexual maturity), haematological and biochemical alterations, as well as organ morphology, in the later life of the offspring. Although in this study it was observed that Na-arsenite possesses a deleterious effect on the F1 generation of offspring, further experimentation on the multigenerational and transgenerational effects of As will be needed to learn more about the specific health implications and the way that arsenic affects the health of children.

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AUTHOR CONTRIBUTIONS

Conceptualization and design of the research, MRI and SS; methodology, MRI; experimental investigation, MKB, SS, MHM, and MRI; sample resources, MRI; writing-original draft preparation, MKB and MRI; writing-review and editing, MKB, SS, MHM, and MRI; supervision, MRI; project administration, MRI. All authors have read and agreed to the published version of the manuscript.

CONFLICTS OF INTEREST

There is no conflict of interest among the authors.

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