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# Adverse effects of arsenic exposure on uterine function and structure in female rat

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ABSTRACT

The present investigation examined the adverse effects of arsenic exposure on uterine function and structure of female rat at 56 days of age, exposed to different doses (50, 100, and 200 ppm) of sodium arsenite in drinking water at immature age (28 days) for 28 days. Dose-dependent decrease ($P<0.001$) was observed in mean uterine weight and length in all treated groups compared to control. Higher arsenic deposition was found in uterine tissue against increased doses of arsenite. Arsenite treatment altered the histomormphology of the uterus. Uterine epithelium in 50 ppm group was lined by cuboidal cells instead of columnar cells observed in control epithelium. In 100 and 200 ppm groups, no demarcation was observed between epithelial cells and endometrial stroma. No basement membrane was seen in these groups; even in 50 ppm, basement membrane was disturbed. The endometrial stroma in 100 and 200 ppm groups was very dense in appearance and contained irregular-shaped cells. In myometrium, loosening of cells was observed in 100 and 200 ppm groups. Dose-dependent decrease ($P<0.001$) was observed in mean uterine diameter, epithelial height, thickness of endometrium, myometrium, and in plasma levels of estradiol, progesterone, FSH and LH in all the treatment groups compared to control. In summary, arsenic is a major threat to female reproductive health acting as a reproductive toxicant and as an endocrine disruptor, restricted the function and structure of uterus, by altering the gonadotrophins and steroid levels, not only at high dose concentration but also at low (50 ppm) levels, when they become mature.



## 1. Introduction

Arsenic is present in measurable quantities in nature (Holson et al., 2000a), e.g., in water, soil, and air from natural and anthropogenic sources (Hughes, 2002). High concentrations of arsenic in water have been reported in Asia (Singh et al., 2007), Bangladesh (Heck et al., 2006; Hall et al., 2007), China (Singh et al., 2007), India (Mazumder, 2003), Pakistan, Taiwan (Mukherjee et al., 2006), Europe (Hungary), and America (Argentina, Mexico) (Bates et al., 2004; Steinmaus et al., 2006).

Arsenite and arsenate are recognized to cause acute and chronic toxicity to a wide variety of organisms including humans (Kitchin, 2001). Organs most affected by arsenic are those involved in absorption, accumulation and/or excretion (Duker et al., 2005). Toxicity of arsenic depends on its chemical form as well as on oxidative stress (Mandal et al., 2004). Chronic exposure to inorganic arsenic (iAs) has been associated with loss of body weight (Nandi et al., 2005), cancer of the skin and internal sites (Kapaj et al., 2006), burning sensation of eyes, solid swelling of legs, liver fibrosis, chronic lung disease, gangrene of toes, (Mazumder, 2003), metabolic disorder such as diabetes (Longnecker and Danials, 2001; Tseng et al., 2002), and dysfunction of endocrine system (Rahman et al., 1998; Tseng et al., 2000), nervous system (Del Razo et al., 2001), and reproductive system (Borzsonyi et al., 1992; Hopenhayn-Rich et al., 2000; Singh et al., 2007).

Limited evidence suggests that arsenic may have adverse human reproductive effects (Hopenhayn et al., 2003b), including higher risk of low birth weight, spontaneous abortions, preeclampsia, congenital malformations, and infant mortality (Ahmad et al., 2001; Hopenhayn et al., 2003a; Milton et al., 2005; Kapaj et al., 2006). Arsenic produces toxic effects on female reproductive system in rodent models also (Chattopadhyay et al., 1999, 2001). Reproductive tract function in the female is controlled primarily by the interaction of the ovarian sex steroids estradiol and progesterone. In the uterus, estradiol-17b (E2) initiates a series of biochemical responses in uterine cells in preparation for the possibility of pregnancy, including cell hypertrophy and hyperplasia (Nephew et al., 2000). Chattopadhyay et al. (2001, 2003) exposed adult rats to 0.4 ppm of sodium arsenite for 28 days, and found loss in uterine weight, reduction in uterine peroxidase activity along with diminished levels of estradiol, LH and FSH. All the uterine dysfunctions were reversed when the rats were supplemented either with ascorbic acid (Chattopadhyay et al., 2001) or with

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sodium selenite (Chattopadhyay et al., 2003). Arsenic toxicity was studied in mice also. Pregnant mice were administered 42.5 and 85 ppm of sodium arsenite from day 8 to 18 of gestation. Dams delivered normally and the offspring were observed for 104 weeks (Waalkes et al., 2004) and 90 weeks (Waalkes et al., 2003). Female offspring showed increased incidence of pre-neoplastic lesions of the reproductive tract, including hyperplasia of the uterus and oviduct after arsenic exposure. Arsenic is known to produce oxidative stress (Kitchin, 2001), and it has recently been established that uterine endometrium degeneration is associated with the increased production of reactive oxygen species (ROS) such as superoxide radicals, hydrogen peroxide, and hydroxyl radicals (Beltran-Gracio et al., 2000).

The present study was designed to investigate the adverse effects of arsenic on the function and structure of uterus in female rat (56 days old) exposed to different doses (50, 100, and 200 ppm) of sodium arsenite at immature age (28 days) for 28 days.

## 2. Materials and methods

### 2.1. Chemicals

All the chemicals used in this experiment were obtained from Sigma.

### 2.2. Animals

Adult male and female Sprague-Dawely rats were obtained from National Institute of Health (Islamabad, Pakistan) and were kept under standard laboratory conditions, fed standard diet and received tap water ad libitum at the Animal House of Quaid-i-Azam University, Islamabad. After mating dams were allowed to give birth and the resulted offspring (when 28 days old) were used in this study.

### 2.2. Treatments

Female rats were randomly divided into two main groups: control ($n = 8$) and treated group. Treated group was further subdivided into three groups ($n = 8$) according to the different doses (50, 100, 200 ppm) of the sodium arsenite they received in drinking water. Control group received plain drinking water. Treatment at three different dose levels was initiated at 4th week (28 days) of age and continued up to 8th week (56 days) of age. The total treatment time period was 28 days. All of the animals were sacrificed at 56 days of age.

### 2.3. Blood collection and tissue sampling

Blood was collected from dorsal aorta of each rat and plasma samples were separated and stored at −20 °C until used for hormone analysis. Both uterine horns were weighed, one was stored at −70 °C for the estimation of arsenic deposition and the other was processed for routine histological procedure. Randomly selected sections (5 μm) from each animal stained with hematoxylene and eosine were used to study the histomorphology and morphometry of the uterine tissue.

### 2.4. Atomic absorption

Deposition of arsenic content was estimated in uterine horn of each animal by atomic absorption spectrophotometric method. Samples were digested with nitric acid in a microwave digester (MARS) at 210 °C. The digested samples were filtered and diluted by adding deionized water. Then arsenic concentration was estimated using a hydride generating system fitted with a flame atomic absorption spectrophotometer (Varian (AA240FS).

### 2.5. Hormone analysis

Plasma levels of estradiol, progesterone (Biochek, Inc.) and FSH (Biocode Hycel) were estimated by ELISA kits. LH was estimated by RIA kit (Biocode Hycel). Rat specific kits were used for FSH and LH estimation. Absorbency of hormones for ELISA was read in Microplate Reader (Platos R496, AMP Diagnostics). Radioactivity for LH was counted in Beckman (LS5801) liquid scintillation counter.

### 2.6. Statistical analysis

Values were expressed as Mean ± SEM. To evaluate the effect of different doses of sodium arsenite *Tukey's test was used*. Linear Regression Analysis of Variance was used to assess the dose-dependent trend against increased doses of sodium arsenite. All the data were analyzed using Graph PadPrism version 5.00.

## 3. Results

### 3.1. Morphology

Both the uterine horns of 100 and 200 ppm treated groups were thinner than in control and 50 ppm treated group. Even uterine horns of 200 ppm group were thread-like in appearance compared to 100 ppm group. Blood supply was observed to be very low in 100 and 200 ppm groups compared to control (Table 1).

### 3.2. Weight and length

Highly significant ($P<0.001$) dose-dependent decrease was observed in mean uterine weight and length in all the treatment groups compared to the control. With 100 and 200 ppm treatment uterine weight reduced significantly ($P<0.001$) compared to 50 ppm treatment. Even 200 ppm showed significant ($P<0.001$) reduction in mean uterine weight and length compared to 100 ppm treatment (Table 1).

Regression analysis of variance showed highly significant decrease in uterine weight ($P = 0.0030$) and length ($P = 0.027$) against increased doses of sodium arsenite.

### 3.3. Atomic absorption

Compared to control, non-significant increase of mean elemental arsenic deposition was observed in 50 ppm treatment group. In contrast, significant increase in mean arsenic deposition was found in 100 ppm ($P<0.01$) and 200 ppm ($P<0.001$) treatment groups compared to control. Between the treatment groups, significant elevation in mean arsenic deposition was observed in 100 ppm ($P<0.05$) and 200 ppm ($P<0.001$) treatment groups compared to 50 ppm treatment group. Even 200 ppm treatment group had significant ($P<0.001$) increase of mean arsenic deposition compared to 100 ppm treatment group (Table 1).

Non-significant dose-dependent increase ($P = 0.1082$) was found by regression analysis of variance for mean arsenic deposition against increased doses of sodium arsenite.