Effect of Dietary Co-Administration of Sodium Selenite on Sodium Arsenite-Induced Ovarian and Uterine Disorders in Mature Albino Rats

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The subchronic treatment of mature female Wistar-strain albino rats in diestrous phase with sodium arsenite at a dose of 0.4 ppm/100 g body weight/rat/day via drinking water for period of 28 days (seven estrous cycles) caused a significant reduction in the plasma levels of leuitinizing hormone (LH), follicle-stimulating hormone (FSH), and estradiol along with a significant decrease in ovarian activities of delta five, 3 beta-hydroxysteroid dehydrogenase (Δ5,3β-HSD), and 17 beta-hydroxysteroid dehydrogenase (17β-HSD) followed by a reduction in ovarian and uterine peroxidase activities. A significant weight loss of the ovary and uterus was also observed after this treatment, along with a prolonged diestrous phase and a high accumulation of arsenic in the plasma and these organs. Moreover, sodium arsenite was also responsible for ovarian follicular and uterine cell degeneration characterized by a high number of regressing follicles and a reduction in the uterine luminal diameter, respectively, in comparison with the controls. A dietary supplementation of sodium selenite at the dose of 0.6 mg/100 g body weight/rat/day for a period of 28 days along with arsenic treatment minimized the gonad weight loss significantly and increased the activities of the ovarian steroidogenic enzymes as well as the ovarian and uterine peroxidase at the control level. Selenium was also able to increase the plasma levels of LH, FSH, and estradiol toward the control level. Vaginal smears showed normal estrous cyclicity in sodium selenite-supplemented arsenic-treated rats along with lower arsenic levels in the plasma and gonadal tissue in comparison with arsenic-only–treated rats. Histological sections of ovary and uterine tissues in the control and experimental groups confirmed that sodium selenite supplementation was able to prevent arsenic-induced histopathological changes in the ovary and uterus. Plasma levels of norepinephrine and dopamine in the midbrain and diencephalon decreased significantly, whereas the serotonin level was increased significantly after 28 days of sodium arsenite treatment. All of these parameters were, in most cases, unchanged from the control level when sodium selenite was co-administered with sodium arsenite. Arsenic intoxication was also associated with increased liver weight and elevation in the activities of hepatic and renal acid phosphatase, alkaline phosphatase, and transaminases, but selenium co-administration was not able to change these toxic effects of arsenic. The results of our experiments indicate the significant protective action of sodium selenite on arsenic-induced toxicity in the female reproductive system, while there was no significant protective effect of selenium on arsenic-induced toxicity in other organs.

Key Words: sodium arsenite; sodium selenite; steroidogenesis; brain monoamines; peroxidase; ovarian follicles.

Arsenic, a nonessential trace element and a potent toxic mutagenic and xenobiotic metalloid, has been growing rapidly during the last 5 years as a major pollutant of drinking water in several districts of West Bengal, India (Mazumder et al., 1988; Saha, 1991); Bangladesh (Chatterjee et al., 1995; Nickson et al., 1998); and Northern Chile, Thailand, Taiwan, China, Inner Mongolia, Mexico, Argentina, Finland, and Hungary (Chappell et al., 1997). From a geochemical aspect, the supply of excess oxygen during the withdrawal of ground water from tube wells appears to be responsible for the hydrolysis of ferric-arsenate and ferric sodium arsenite. The hydrolysis of ferric-arsenate produces poisonous arsenic acid and ferric hydroxide, whereas ferric sodium arsenite breaks down into arsenious acid, which is one of the major causes of ground water pollution (Roy, 1999). Adverse health effects are observed in locations where arsenic leaches into well water or thermal springs from the substratum or percolates into surface water from soil rich in volcanic sediment (Chappell et al., 1997). Its frequent uses as herbicides, insecticides, rodenticides, food preservatives, and a by-product of used fossil fuel (Baxley et al., 1981; Flora et al., 1995) are challenging the aquatic environment. Arsenic exposure results in endemic arsenic dermatosis along with hyperkeratosis, gangrene, and skin cancer (Chowdhury et al., 1997; Mazumder et al., 1988; Saha, 1995). Arsenic has been claimed to be of clinical utility in the treatment of syphilis, amoebiasis, and certain other tropical diseases (Klaassen, 1990) and also has been used in Fowler solution in the treatment of arthritis (Klaassen, 1990), but recently arsenic intoxication in experimental animals has been associated with hepatic tumors (Waalkes et al., 2003), the inhibition of testicular steroidogenic function (Sarkar et al., 1991), and spermat-
Arsenic exposure is also reported to result in structural changes in the thymus of pregnant and newborn mice (Skal’naia et al., 1995), and long-term exposure of arsenic is associated with abortion, low birth weight, and reduced lactation (Donald et al., 1995) as well as with embryonic cells toxicity in vitro (Lee et al., 1985). Survey reports from the Ukraine, Taiwan, and Bangladesh revealed that the intake of arsenic-contaminated drinking water caused reproductive disturbances in women (Zadorozhnaja et al., 2000), adverse pregnancy outcomes (Yang et al., 2003), and spontaneous abortion (Ahmad et al., 2001). Acute arsenic exposure may promote immediate gastrointestinal tract infection (Goebl et al., 1990), whereas chronic effects may exert degenerative, inflammatory, and neoplastic changes of the respiratory, haematopoetic, cardiovascular, and nervous systems (Neiger and Osweiler, 1989). There is a lack of literature data related to the effect of arsenic on ovarian steroidogenic functions, particularly at the dose levels occurring in drinking water in wide areas of India and in other countries where this trace element is present in the range above the admissible limit of 0.01 ppm, according to the World Health Organization (Rahman et al., 2003). Recently we established that arsenic is responsible for the inhibition of ovarian steroidogenesis (Chattopadhay et al., 1999) as well as an elevation of adrenocortical steroidogenesis (Ghosh et al., 1999) when the level of arsenic is within the range in drinking water of West Bengal.

Free radicals and reactive oxygen species are produced in the metabolic processing of arsenical compounds (Tabacova et al., 1992; Yamanaka et al., 1990), which may lead to DNA single-strand breakage and DNA–protein cross-link via the formation of apurinic/apyrimidinic (AP) sites through a Schiff-based reaction between amino groups of nuclear protein, particularly histone (H1) and nonhistone proteins and aldehyde groups of AP sites in DNA (Kato et al., 1994).

On the other hand, the potential of dietary antioxidants (such as vitamin C, vitamin E, and β-carotene) to reduce the activity of radical-induced reactions during any kind of stress has drawn increasing attention in recent years (McCall and Balz, 1999). In addition, the in vivo detoxification of arsenic is one of the challenging issues in this decade. Some researchers have shown that selenium may play an important role in arsenic detoxification from living cells. Dietary supplementation with sodium selenite significantly reduces the clastogenic and genotoxic effects of arsenic (Beckman and Nordenson, 1986; Biswas et al., 1999). An arsenic-induced chromosomal aberration of cultured lymphocytes was reduced in smelter workers after treatment with selenium (Hu, 1989); there are data that suggest that selenium is able to antagonize arsenic-mediated inhibition of DNA synthesis in human peripheral lymphocytes (Hu et al., 1996). Alteration of heme oxygenase in human red blood cell (RBC) by sodium arsenite is prevented by selenium (Takehata et al., 1991). Concomitant administration of selenium and gallium arsenide suggested that selenium has some beneficial role in the prevention of the appearance of signs of gallium arsenide toxicity by obstructing the inhibition of blood 3-aminolevulinic acid dehydratase as well as hepatic malondialdehyde formation and finally blocking the accumulation of gallium and arsenic (Flora et al., 1999). We have already established that antioxidants like vitamins E and C are able to reduce arsenic-mediated ovarian and uterine toxicity (Chattopadhay et al., 2000, 2001). However, there is a paucity of information regarding the role of selenium in arsenic-mediated ovarian-gonadotropic and steroidogenic dysfunction, even though selenium is an essential dietary component for the maintenance of mammalian reproduction (Basini and Tamanini, 2000; Bleau et al., 1984; Kaur et al., 1999). Therefore the present study was aimed at examining whether selenium in the form of sodium selenite has any significant role on arsenic-mediated changes in steroidogenic status and ovarian-uterine dysfunction.

**MATERIALS AND METHODS**

**Animal selection and care.** Fifty-four adult female albino rats (8 weeks of age) of the Wistar strain having a regular 4-day estrous cycle and weighing 150–160 g were selected for this experiment. The animals were maintained under standard laboratory conditions (14 h light:10 h dark and 30 ± 2°C) with free access to food and water. The Principles of Laboratory Animal Care (NIH publication no. 85–23, revised 1985) were followed throughout the experimental schedule.

**Drug treatments, selection of dose, study of estrous cycle, and sample collection.** Sodium arsenite and sodium selenite were obtained from Sigma Chemical Co. (St. Louis, MO). The animals were divided into three equal groups, with 18 animals per group, and their initial body weights were noted along with a record of their daily water consumption (about 10 ml/day). The animals of the first group received an oral daily dose of 0.4 ppm sodium arsenite dissolved in 10 ml of distilled drinking water for 28 days. Another group of animals received the same dose of sodium arsenite followed by dietary supplement of 0.6 mg of sodium selenite (mixed with 10 g of a standard diet)/100 g body weight/day. The control group was supplied with 10 ml of distilled water orally as a vehicle for 28 days. Each of the control and experimental groups was provided with separate water bottles, where pure water for the control rats and arsenic-contaminated water for experimental rats was given. The animals sucked the water from the respective bottles. Vaginal smears were collected twice daily (8:00 A.M. and 5:00 P.M.) from all of the animals. The smears were stained by eosin-haematoxylin and examined microscopically. The feeding habits of all of the animals were observed throughout the experimental schedule. All of the animals were sacrificed at the diestrus phase 20 h after the last arsenic treatment. The body weights of all of the animals were recorded on the day of sacrifice. Blood was collected from all of the animals from the dorsal aorta using a heparinized syringe (21-gauge needle) after a light ether anesthesia. Plasma samples were separated by centrifugation and stored at –20°C until all of the samples had been used for the determination of plasma levels of gonadotrophins and estradiol. The ovaries and uterus were dissected out, and the organs’ weights were measured on a single-pan electronic balance. The level of arsenic was analyzed in all of the organs by atomic absorption spectrometry. The ovaries from six animals of each experimental group were used for biochemical assay of ovarian delta five, 3 beta-hydroxysteroid dehydrogenase (Δ5,3β-HSD) and 17 beta-hydroxysteroid dehydrogenase (17β-HSD) activities, whereas one ovary and one uterine horn from another six animals of each group were kept at –20°C for biochemical measurement of peroxidase activities. The other ovaries and uterine horns of each animal of the above-mentioned groups were used for
histological study. The plasma, ovaries, and uterine horns of the remaining six animals of each group were kept ready to measure the elementary arsenic content. The brain of the animals of each experimental group was dissected out and very quickly transferred to a petri dish covered by ice. The whole midbrain and diencephalon were separated out in intact condition and immediately transferred at −20°C for spectrophotometric estimation of brain monoamines. The livers and kidneys of all groups of animals were dissected out and frizzed for the assay of acid and alkaline phosphatase and assays of glutamic oxaloacetic transaminase (GOT) and glutamic pyruvate transaminase (GPT).

**Assay of ovarian Δ₃,β-HSD and 17β-HSD activities.** To study ovarian Δ₃,β-HSD and 17β-HSD activities, two ovaries from each of the six animals of each group were homogenized separately, maintaining chilling conditions (4°C) in 20% spectroscopic-grade glycerol containing 5 mM of potassium phosphate and 1 mM of EDTA at a tissue concentration of 10 mg/ml homogenizing mixture in a homogenizer (Remi RQ-127A, Mumbai, India). This mixture was centrifuged at 10,000 g for 30 min at 4°C in a cold centrifuge (Avanti™-M 30, Beckman, USA). The supernatant was mixed with 1 ml of 100-μM sodium pyrophosphate buffer (pH 8.9) and 40 μl of 30 μg of dehydroepiandrosterone (DHEA), making the incubation mixture a total of 3 ml. Δ₃,β-HSD activity was measured after the addition of 0.5 μM of NAD to the tissue supernatant mixture in a spectrophotometer (U-2001, Hitachi, Japan) at 340 nm against a blank (without NADP) (Talalay, 1962). For ovarian 17β-HSD activity measurements, the same supernatant fluid (1 ml) of homogenizing mixture was added with 440 μM of sodium pyrophosphate buffer (pH −10.2), bovine serum albumin (25 mg of crystalline BSA), and 0.3 μM of testosterone, making the incubation mixture a total of 3 ml. The enzyme activity (17β-HSD) was measured (Jarabak et al., 1962) after the addition of 1.1-μM nicotinamide adenosine dinucleotide phosphate (NADP) to the tissue supernatant mixture in a spectrophotometer at 340 nm against a blank (without NADP). One unit of enzyme activity is equivalent to a change in absorbency of 0.001/min at 340 nm.

**Estimation of serotonin (5-HT), dopamine (DA), and norepinephrine (NE).** Norepinephrine, dopamine, and serotonin were extracted after homogenizing the midbrain or diencephalon separately in acidified ice-cooled butanol (Das et al., 1990). These homogenizing mixtures were centrifuged separately at 4000 rpm. After centrifugation, a portion of butanol extract was processed for fluorescence development (Kent Shellenger and Gordon, 1971). The fluorescence of 5-HT was measured in the Perkin Elmer MFP 44B Spectrophotometer with the excitation wavelength set at 385 nm and the emission spectra at 490 nm (Kent Shellenger and Gordon, 1971). A reading for DA was made at 4°C at 325 nm activation peak and fluorescence at 380 nm uncorrected (Kent Shellenger and Gordon, 1971).

The remaining portion of butanol extract was shaken with 0.1-M phosphate buffer, (pH 6.5) and then centrifuged. After processing for the development of fluorophores (Kent Shellenger and Gordon, 1971), the fluorescence of NE was read with the excitation wavelength at 380 nm and the emission spectra at 495 nm (Kent Shellenger and Gordon, 1971).

**Study of ovarian and uterine peroxidase.** Ovarian and uterine peroxidase activities were measured spectrophotometrically (Sadasivan and Manickam, 1996). These tissues were homogenized separately in 0.1-M phosphate buffer solution (pH −7.0) at a tissue concentration of 10 mg/ml. Next, 20-nM guaiacol was mixed with 0.1-ml supernatant collected from a homogenate. In the presence of 0.3 ml of 12.5 mM H₂O₂, the time was noted when the absorbency was increased (436 nm) by 0.1.

**Biochemical assay of alkaline phosphatase and acid phosphatase.** For a quantitative estimation of alkaline phosphatase in the liver and kidney, these organs were homogenized separately in a Potter Elvihjem homogenizer using ice-cold homogenizing medium (0.22-M Tris, HCl buffer pH 7.5) at a tissue concentration of 20 mg/ml. 0.25ml of homogenate then was added immediately in a centrifuge tube containing 1-ml buffer (1-MM p-nitrophenol phosphate in 1-M Tris buffer, pH −8.0). The mixture was incubated at 37°C for 30 min in a water bath. The assay was based on the formation of p-nitrophenol phosphate (PNPP). The activity was measured spectrophotometrically at 420 nm using a visible spectrophotometer (Malmy and Horecker, 1966). Serum alkaline phosphatase was measured using the same buffer and 0.1 ml of serum (Malmy and Horecker, 1966).

For determination of the acid phosphatase activity, the same homogenizing medium was used and the tissue concentration was as above. The acid phosphatase activity was measured in an acetate buffer at pH 4.5 using p-nitrophenol phosphate as a substrate (Vanha-Perttula and Nikkanen, 1973). Serum acid phosphatase was measured using the same buffer and a substrate with 0.1-ml serum added (Vanha-Perttula and Nikkanen, 1973).

**Biochemical assay of transaminases.** The liver and kidney GOT and GPT were measured by homogenizing their tissues in a phosphate buffer at pH 7.4 according to the method of Goel (1988).

**Radioimmunoassay (RIA) of LH and FSH.** The plasma levels of LH and FSH were measured by RIA (Moudgal and Madhwa Raj, 1974) using reagents supplied by the Rat Pituitary Distribution Program and NIDDK (Bethesda, MD). Carrier-free ¹²⁵I for hormone iodination was obtained from Bhabha Atomic Research Center (Mumbai, India). Pure rat FSH (NIDDK-rFSH-I-5) and LH (NIDDK-rLH-I-5) were iodinated using the Chloramine-T (Sigma Chemical Co., St.Louis, MO) (Greenwood et al., 1963). NIDDK anti-rat FSH-S-11 and NIDDK anti-rat LH-S-5 were used as anti-sera after final dilutions of 1:2500 and 1:10,000, respectively. Goat anti-rabbit γ-globulin was used as the second antibody. It was obtained from Indo-Medicine (Friendswood, TX). The intraassay variations were 5% and 6% for LH and FSH, respectively. All samples were run at a time to avoid interassay variation.

**Radioimmunoassay of estradiol.** The plasma level of estradiol was assayed by a radioimmunooassay technique (Hannig et al., 1974). Methodological loss during extraction was monitored by adding 10,000 cpm [¹²⁵I-2β-3H(N)] estradiol before extraction with 4 ml of diethyl ether twice. The samples were assayed in duplicate. The anti-sera to estradiol were purchased from Endocrine Science (Tarzanna, CA), and it had 40% cross-reactivity with estrone. Using dextran-coated charcoal, free and bound estradiols were separated. The intraassay variation was 6.5%. All of the samples were run at the same time to avoid interassay variation. Since chromatographic purification of the samples was not performed, the values reported are the sum of estradiol and estrone.

**Histological study of ovarian-uterine tissue and quantification study of folliculogenesis.** For the fixation of the ovary and uterine horn, Bouin’s fluid was used. Graded dehydration of the tissue was done by 70 to 100% alcohol in subsequent steps. Xylene was used as the clearing agent. The tissues were embedded in paraffin (58.6°C). Sections of paraffin blocks were cut by a rotary microtome (5 μ). The sections were stained by eosin and haematoxylin and observed under a microscope. The quantification study of folliculogenesis was performed according to Patil et al. (1998). Diameters and morphologies of the follicles were used to classify the follicles as:

- **Class I:** Small preantral follicle (SPAF) (>94 μm);
- **Class II:** Large preantral follicle (LPAF) (94–260 μm);
- **Class III:** Small antral follicle (SAF) (261–350 μm);
- **Class IV:** Medium antral follicle (MAF) (351–430 μm);
- **Class V:** Large antral follicle (LAF) (431–490 μm);
- **Class VI:** Graafian follicles (GF) (>491 μm).

The follicles under regeneration were classified depending on the degree of regeneration. In stage IA, pyknosis in some granulosa cells is observed; in stage IB, degenerative changes occurred in the entire granulosa cell layer; stage IIA was characterized by meiosis in metaphase I (pseudomaturation) and degenerating cumulus cells; and stage IIB was characterized by oocytes floating in the antrum with few pyknotic bodies.

The diameter of the uterine lumen along with the uterine epithelium height and the diameter of the endometrium and myometrium were measured under a microscope with the help of an ocular and stage micrometer at 10 × 10× magnification.

**Determination of elemental arsenic content.** The arsenic contents in the plasma and ovarian and uterine tissues were measured by atomic absorption
spectrometry (Locke, 1979; Nurenberg, 1982). Twenty-five mg of ovarian tissue, 50 mg of uterine tissue, and 0.5 ml of plasma were allowed to fix in formaldehyde and dried at 150°C for 20 min to evaporate the formaldehyde. Next, samples were transferred inside quartz beakers, which had been washed with 1:1 HNO₃ and H₂SO₄ solution. The samples were digested with a 2-ml pure nitric acid mixture inside a digestion chamber at 150°C for 20 min. Until a small volume was obtained, the beakers were capped and enclosed samples were boiled. Digested samples were filtered and diluted up to 25 ml with deionized distilled water. The same deionized water was used as blanks, and a reading was taken in a Varian AA-575 ABQ model of the atomic absorption spectrometer.

**Statistical analysis.** Results of the experiments were expressed as mean and standard error of different groups. The differences between the mean values were evaluated by ANOVA followed by multiple Student’s t-test (Zar 1996). The values for \( p < 0.05 \) were considered significant.

### RESULTS

**Food Consumption and Body and Organ Weights**

No differences in food consumption were seen in any of the groups of animals throughout the experimental schedule. The body weights of arsenic-treated and sodium selenite–supplemented rats did not differ significantly from the controls (Table 1). After 28 days of arsenic treatment, there was a significant reduction in the wet weight of ovaries and uterus in comparison to the control group (Table 1). After dietary supplementation with sodium selenite, the wet weight of the above female sex organs of the arsenic-treated group was insignificantly different from the control group. The liver weight was increased significantly in the arsenic-treated group when compared to the controls. Sodium selenite supplementation did not show any significant differences from the sodium arsenite-treated group (Table 1). There was no significant alteration found in the kidney weights between the control and experimental groups (Table 1).

**Vaginal Smear Study**

In the control group, regular estrous cycles of 4 days were noted whereas, in the arsenite-treated group, a constant diestrus phase was observed after 18 ± 2 days of arsenic ingestion. No estrous cycle irregularities were observed upon selenium supplementation to the arsenic-treated animals.

**Ovarian Steroidogenic Key Enzymes Activities**

Twenty-eight days of arsenic treatment significantly suppressed ovarian \( \Delta^{5},3\beta\)-HSD and \( 17\beta\)-HSD activities in comparison with the controls. The concomitant administration of sodium arsenite with a sodium selenite–mixed diet resulted in enzymatic activities similar to the control level (Fig. 1).

**Plasma Gonadotrophins and Estradiol**

Arsenic treatment for 28 days resulted in a marked reduction in the plasma levels of LH, FSH, and estradiol (Fig. 2) with respect to the control group, whereas selenium supplementation along with sodium arsenite treatment resulted in hormonal levels near the basal levels, similar to the control group.

### TABLE 1
Effect of Sodium Selenite on Reproductive and Nonreproductive Organs’ Weight in Sodium Arsenite-Treated Mature Rats Compared with Control

<table>
<thead>
<tr>
<th>Group</th>
<th>Initial body weight (g)</th>
<th>Final body weight (g)</th>
<th>Ovarian weight in pair (mg)</th>
<th>Uterine weight (mg)</th>
<th>Liver weight (g %)</th>
<th>Kidney weight (g %)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vehicle-treated control</td>
<td>152 ± 6</td>
<td>171 ± 6</td>
<td>94.55 ± 7.9</td>
<td>162.2 ± 7.4</td>
<td>3.32 ± 0.22</td>
<td>0.85 ± 0.01</td>
</tr>
<tr>
<td>0.4 ppm sodium arsenite-treated</td>
<td>160 ± 7</td>
<td>170 ± 6</td>
<td>55.20 ± 5.1*</td>
<td>105.98 ± 4.8*</td>
<td>4.79 ± 0.25*</td>
<td>0.83 ± 0.02</td>
</tr>
<tr>
<td>0.4 ppm sodium arsenite-treated + 0.6-mg sodium selenite</td>
<td>157 ± 5</td>
<td>175 ± 5</td>
<td>88.79 ± 7.0</td>
<td>149.61 ± 6.8</td>
<td>4.53 ± 0.25*</td>
<td>0.84 ± 0.02</td>
</tr>
</tbody>
</table>

*Indicates significant difference.

Note. Mean ± SE, \( N = 18 \), \( p < 0.001 \) (ANOVA followed by multiple-comparison two-tailed t-test).

**FIG. 1.** Changes in ovarian steroidogenic key enzyme activities after selenium supplementation in arsenic-treated rats. Data represent mean ± SE, \( N = 6 \), \( p < 0.001 \), and ANOVA followed by multiple-comparison two-tailed t-test. The asterisk (*) on the bar indicates significant difference.
Ovarian and Uterine Peroxidase Activities

Ovarian and uterine peroxidase activities were markedly diminished after arsenic exposure in comparison with the controls. No such effect on these enzymes was observed when sodium selenite was supplied along with sodium arsenite treatment (Fig. 3).

Enzymatic Changes in Liver and Kidney

Acid phosphatase, alkaline phosphatase, GOT, and GPT activities were elevated in the livers and kidneys after arsenic treatment, in comparison to the controls (Table 2). Sodium selenite supplementation in sodium arsenite-treated rats did not induce significant alteration in these enzymatic activities as compared to the control levels (Table 2).

Brain Monoamines in Midbrain and Diencephalon

As described in Table 3, significantly reduced NE and DA levels followed by an elevated 5-HT level in the midbrain and diencephalon were observed after sodium arsenite exposure alone. In the arsenic-treated rats that received dietary supplementation of sodium selenite, these parameters were similar to the control levels (Table 3).

Histological Changes of Ovary and Uterine Horn

Histological examination of the ovary showed a significant reduction in primary classes of preantral and antral follicles followed by a reduced number of graafian follicles and corpora lutea along with an increasing number of regressing follicles after arsenic treatment in comparison with the control rats (Table 4). A significant protection was found after sodium selenite supplementation with a significantly higher number of matured follicles as well as a significantly lower number of regressing follicles in comparison to sodium arsenite-treated group (Table 4).

Degeneration of the uterine endometrium, myometrium, and epithelial layers was characterized by a notable reduction in the thicknesses of these uterine layers after sodium arsenite treatment. No such reduction of these cellular layers was seen when a dietary supplementation of selenium was given along with arsenic (Table 4).

Arsenic Content in Plasma and Female Sex Organs of Rat

The group treated with arsenic alone showed a significant elevation in arsenic deposition in the ovary and uterus as well as in the plasma when compared with the control group (Table 5). However, with selenium supplementation, the elementary arsenic content in the above sex organs and plasma was comparable to the controls (Table 5).
plasma inhibit gonadotrophin secretion (Christian, 1964; Luton et al., 1977). There may also be the possibility of the elevation of plasma levels of glucocorticoids, which may suppress the sensitivity of gonadotroph cells to GnRH and therefore may prevent gonadotrophin secretion (Kamel and Kubujak, 1987; Ogle, 1977; Ringstrom and Schwartz, 1985). Since body weight gain was not altered significantly in arsenic-treated rats in comparison to the controls, this deleterious effect of arsenic on the female reproductive system may be due to the toxic effect of arsenic itself on this specific system, and not to the bad health of the animals. Consistent diestrous may be due to low plasma levels of estradiol in arsenic-treated rats after 18 ± 2 days (Parshad et al., 1989). Since ovarian weight is regulated by gonadotrophins (Kulin and Reiter, 1973; Tagatz et al., 1979), whereas uterine weight is regulated by estradiol (Eldan, 1983), the weight loss of these female sex organs in arsenic-treated rats may be due to the possibility of low plasma levels of gonadotrophins and estradiol. Protection of the plasma gonadotrophins levels after selenium co-administration in sodium arsenite-treated rats may be due to the stimulatory

### DISCUSSION

The results of the present investigation demonstrate the adverse effect of sodium arsenite treatment on female gonadal steroidogenic activity. In ovarian steroidogenesis, Δ^5,3β-HSD and 17β-HSD are the key regulatory enzymes (Hinshelwood et al., 1994); the suppressed activities of these steroidogenic enzymes by arsenic is in agreement with the previous findings where arsenic treatment was associated with inhibition in testicular steroidogenesis (Sarkar et al., 1991). A decrease in the plasma levels of estradiol in sodium arsenite-treated rats may occur due to the inhibition of ovarian steroidogenic enzyme activity, because these enzymes are responsible for the regulation of ovarian estradiol synthesis (Hinshelwood et al., 1994). This alteration in steroidogenic enzymes activity in arsenic-treated rats may be the result of low plasma levels of FSH and LH, since these are the regulators of ovarian 17β-HSD activities (Odell et al., 1963). The possibility of low plasma levels of gonadotrophins in this experiment may be due to the elevated level of plasma glucocorticoids in arsenic-treated rats (Ghosh et al., 1999) as high levels of ACTH in plasma inhibit gonadotrophin secretion (Christian, 1964; Luton et al., 1977). There may also be the possibility of the elevation of plasma levels of glucocorticoids, which may suppress the sensitivity of gonadotroph cells to GnRH and therefore may prevent gonadotrophin secretion (Kamel and Kubujak, 1987; Ogle, 1977; Ringstrom and Schwartz, 1985). Since body weight gain was not altered significantly in arsenic-treated rats in comparison to the controls, this deleterious effect of arsenic on the female reproductive system may be due to the toxic effect of arsenic itself on this specific system, and not to the bad health of the animals. Consistent diestrous may be due to low plasma levels of estradiol in arsenic-treated rats after 18 ± 2 days (Parshad et al., 1989). Since ovarian weight is regulated by gonadotrophins (Kulin and Reiter, 1973; Tagatz et al., 1979), whereas uterine weight is regulated by estradiol (Edman, 1983), the weight loss of these female sex organs in arsenic-treated rats may be due to the possibility of low plasma levels of gonadotrophins and estradiol. Protection of the plasma gonadotrophins levels after selenium co-administration in sodium arsenite-treated rats may be due to the stimulatory

### TABLE 2

Effect of Sodium Selenite on Hepatic and Renal Enzymatic Activities in Sodium Arsenite-Treated Mature Rats Compared with Control

<table>
<thead>
<tr>
<th>Group</th>
<th>Alkaline phosphatase (mg of PNP/mg of tissue/h)</th>
<th>Acid phosphatase (mg of PNP/mg of tissue/h)</th>
<th>GOT (unit/g of tissue)</th>
<th>GPT (unit/g of tissue)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Liver</td>
<td>Kidney</td>
<td>Liver</td>
<td>Kidney</td>
</tr>
<tr>
<td>Control</td>
<td>15.81 ± 0.96</td>
<td>17.7 ± 1.13</td>
<td>9.57 ± 0.52</td>
<td>12.32 ± 1.11</td>
</tr>
<tr>
<td>Sodium arsenite-treated</td>
<td>25.76 ± 1.87*</td>
<td>29.36 ± 3.60*</td>
<td>16.66 ± 1.02*</td>
<td>25.16 ± 2.12*</td>
</tr>
<tr>
<td>Sodium selenite supplement</td>
<td>23.91 ± 2.33*</td>
<td>30.67 ± 1.99*</td>
<td>18.22 ± 2.34*</td>
<td>25.59 ± 1.76*</td>
</tr>
</tbody>
</table>

*Indicates significant difference.

### TABLE 3

Changes in the Monoamines Level in Midbrain and Diencephalon after Oral Co-Administration of Sodium Selenite in Arsenic-Treated Rats

<table>
<thead>
<tr>
<th>Mode of treatment</th>
<th>NE (ng/g of tissue)</th>
<th>DA (ng/g of tissue)</th>
<th>5-HT (ng/g of tissue)</th>
<th>NE (ng/g of tissue)</th>
<th>DA (ng/g of tissue)</th>
<th>5-HT (ng/g of tissue)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vehicle-treated control</td>
<td>405 ± 25.22</td>
<td>517 ± 26.21</td>
<td>345 ± 26.05</td>
<td>433 ± 30.34</td>
<td>556 ± 31.15</td>
<td>418 ± 28.56</td>
</tr>
<tr>
<td>0.4 ppm sodium arsenite</td>
<td>234 ± 29.47*</td>
<td>326 ± 28.37*</td>
<td>845 ± 28.58*</td>
<td>195 ± 28.26*</td>
<td>405 ± 32.36*</td>
<td>823 ± 33.50*</td>
</tr>
<tr>
<td>0.4 ppm sodium arsenite + 0.6-mg selenite</td>
<td>387 ± 30.41</td>
<td>476 ± 29.56</td>
<td>349 ± 29.07</td>
<td>463 ± 31.44</td>
<td>543 ± 33.34</td>
<td>451 ± 27.76</td>
</tr>
</tbody>
</table>

*Indicates significant difference.

**Note.** Mean ± SE, N = 18, p < 0.001 (ANOVA followed by multiple-comparison two-tailed *t*-test).
TABLE 4

Effect of Sodium Selenite on Number of Different Generations of Follicle and Uterine Histometry in Sodium Arsenite-Treated Mature Rats Compared with Control

<table>
<thead>
<tr>
<th>Group</th>
<th>Number of different generation of ovarian follicle</th>
<th>Thickness of different layers of uterus (µm)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>SPAF</td>
<td>LPAF</td>
</tr>
<tr>
<td>Vehicle-treated control</td>
<td>42.83 ± 2.01</td>
<td>16.33 ± 1.40</td>
</tr>
<tr>
<td>Sodium arsenite-treated</td>
<td>4.30* ± 0.62</td>
<td>4.33* ± 0.56</td>
</tr>
<tr>
<td>Sodium selenite supplement</td>
<td>6.00** ± 0.40</td>
<td>4.50* ± 0.22</td>
</tr>
</tbody>
</table>

Note. Mean ± SE, N = 6, p < 0.001 (ANOVA followed by multiple-comparison two-tailed t-test).

*Indicates significant difference of arsenic-treated group in respect to control; **indicates significant protection in sodium selenite-supplemented group in respect to sodium arsenite-treated group.
TABLE 5
Elemental Arsenic Content in Plasma, Ovary, and Uterus after Arsenic Treatment in Combination with Sodium Selenite Supplement in Rat Compared with Control

<table>
<thead>
<tr>
<th>Group</th>
<th>Arsenic content</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Ovary (μg/g of tissue)</td>
</tr>
<tr>
<td>Control</td>
<td>2.79 ± 0.19</td>
</tr>
<tr>
<td>Sodium arsenite-treated</td>
<td>6.81 ± 0.14*</td>
</tr>
<tr>
<td>Sodium selenite supplement</td>
<td>1.37 ± 0.08**</td>
</tr>
</tbody>
</table>

Note. Mean ± SE; N = 6, p < 0.001 (ANOVA followed by multiple-comparison two-tailed t-test).
*Indicates significant difference of arsenic-treated group in respect to control; **indicates significant protection in sodium selenite-supplemented group in respect to sodium arsenite-treated group.

The decrease in peroxidase activity in the follicular fluid or the high concentration of superoxide radical may be one of the major factors that are responsible for follicular regression (Cassano et al., 1999; Paszkowski et al., 1995). Peroxidase is one of the major components of the antioxidant system (Hochstein and Atallah, 1988; Sun, 1990; Susa et al., 1996). The low levels of peroxidase activity in ovarian tissue found in our study after sodium arsenite treatment support these data. The normal levels of these parameters after selenium supplementation may be due to the protection against arsenic-induced oxidative stress (Helzlsouer et al., 1996). Exogenous administration of selenium was reported to have some stimulatory effect on ovarian peroxidase activity (Apostolaki et al., 1998). The pronounced reduction in elemental arsenic content in the reproductive organs and plasma after dietary intake of selenium may be due to the possibility of trapping arsenic, thereby resulting in a low uptake of arsenic in these organs. An inverse relationship between selenium and arsenic has been reported in the literature (Beckman and Nordenson, 1986; Flora et al., 1999; Rostkowski-Nadolska et al., 1999).

Selenium may also act on the granulosa cells by modulating their proliferation and estradiol synthesis (Basini and Tamanini, 2000). The protection of folliculogenesis in our selenium-supplemented group also supports the recovery in ovarian steroidogenesis and normal functioning of the hypothalamic–hypophysial–ovarian axis. Selenium supplementation prevents the ovarian and uterine weight loss induced by arsenic, and this may be due to the rapid distribution of selenium to the ovary and uterus, which in turn may activate the selenium-dependent peroxidase activity (Sundstrom et al., 1989), which is more pronounced in the ovary and uterus (Baiza-Gutman et al., 2000; Sundstrom et al., 1989).

Uterine tissue degeneration after arsenic treatment may result from decreased ovarian estradiol as uterine growth depends on the ovarian estradiol secretion (Patil et al., 1998). Moreover, there also may be a possibility of diminished progestogesterone action on estradiol-primed uterus, which may obstruct the transition of the uterine epithelium from the proliferative to the secretory state (Patil et al., 1998). It has recently been established that uterine endometrium degeneration is associated with the increased production of reactive oxygen species such as superoxide radicals, hydrogen peroxide, and hydroxyl radicals (Beltran-Garcia et al., 2000). This is supported by our findings of a significant decrease in uterine peroxidase activity after sodium arsenite treatment in this experiment.

Dietary administration of selenium ameliorates the inhibitory effect of sodium arsenite on the uterus and prevents a reduction in uterine size, endometrial and myometrial thickness, and height of epithelial layer. This protective effect may be the result of selenium preventing the decrease in estradiol level induced by arsenic alone, which in turn may be responsible for the normalization of uterine peroxidase activity (Farley et al., 1992; Wang and Qi, 1993), as peroxidase activity is responsible for the maintenance of uterine sensitivity and receptivity (Baiza-Gutman et al., 2000).

Since selenium is unable to prevent an elevation in the weight of the liver and to protect the arsenic-mediated changes in the activities of renal and hepatic acid phosphatase, alkaline phosphatase, and transaminases (such as GOT and GPT), it therefore may be stated that a preventive effect of selenium on arsenic-induced toxicity is specific for reproductive organs.

For the preventive action of selenium on arsenic-induced disorders in female reproductive system, the following two
hypotheses may be formulated. One is that selenium may modulate the hypothalamic–pituitary–ovarian axis by the stimulation of dopaminergic or adrenergic neurons or by the inhibition of serotonergic neuronal activities and by its direct stimulatory effects on ovarian steroidogenesis that maintain ovarian folliculogenesis. An alternative hypothesis is that arsenic-induced reproductive toxicity may be due to the induction of oxidative stress or free radical generation, and, since selenium is an important dietary antioxidant, it may prevent this toxicity by its free radical scavenging action (Helzlsouer et al., 1996; Sreekala et al., 1999). Further studies are needed to clarify the mechanism of selenium prevention of the arsenic-induced disorders in the female reproductive system.

REFERENCES


