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Adverse Effects of Arsenic Exposure on the Mammary Glands of Adult Female Rats

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Abstract.- The present study was designed to investigate the negative effects of arsenic exposure on mammary glands and hormonal profile of female adult rats. Two different doses of sodium arsenite 100ppm and 200ppm were given in drinking water for 28 days. Dose-dependent decrease was observed in the mean body weight gain in both treated groups as compared to control. A significant decrease ($p<0.0001$) was observed in the mean weight of mammary glands in treated groups as compared to control. Arsenite treatment also altered the histomorphology of mammary glands. Epithelial cells of lactiferous ducts were no more compactly arranged and became irregular in shape instead of compactly arranged cuboidal cells as in control. Myoepithelial cells were scarcely present in 100ppm and 200ppm treated group as compared to proper layeres in control. Stroma was also disturbed containing irregular, loosely arranged cells in 100ppm and 200ppm treated groups. Dose-dependent decrease ($p<0.001$) was observed in morphometric measurements of mean epithelial height of lactiferous ducts, nipple epithelium, nuclear diameter and number of terminal end buds. Plasma levels of estradiol and prolactin were significantly decreased ($p<0.001$) in both treated groups as compared to control. These findings indicate that arsenic is a toxicant which restricts the development and alters the morphology of mammary glands by affecting the estradiol and prolactin levels.

Key words: Arsenic, mammary glands, steroidogenesis , female rats**.**

INTRODUCTION

In nature Arsenic (As) exists in several oxidative states but its pentavalent (arsenate) and trivalent (arsenite) forms are more abundantly present and are significantly toxic. A large proportion of underground water around the world is intoxicated with high concentration of arsenic which is considered an important health hazard (Brinkel *et al*., 2009). Short and long term exposure of rats and human beings to arsenic is known to result in a wide variety of health effects (Nandi *et al*., 2005) including liver fibrosis, chronic lung disease, gangrene of toes (Mazumder, 2003), cancer of skin and internal sites (Hopenhayn-Rich *et a*l., 2000; Kapaj *et al*., 2006), metabolic disorder such as diabetes (Longnecker and Daniels, 2001; Tseng *et al*., 2002), gastrointestinal tract infection (Goebel *et al*., 1990), 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).

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Arsenic at very low concentrations as 0.01Mm, approximately 0.07ppb is a potent endocrine disruptor, altering gene regulation by the closely related gluccorticoids, progesterone, and androgen steroid receptors (SRs) (Davey *et al*., 2007). The activated receptor is unable to stimulate the correct cascade of signals in the presence of arsenic that usually result from hormone binding, particularly the ability to run on certain hormone-responsive genes (Kaltreider *et al*., 2001). Arsenic as endocrine disruptor also disrupts estrogen receptor (ER) both *in vivo* and in cell culture (Davey *et al*., 2007). Endocrine disrupting chemicals interfere with the normal function of an organism's endocrine system whether they are natural or synthetic (Dickerson and Gore, 2007). Hormonal regulation can be suppressed after exposure to arsenic and hormone mediated gene transcription, verbal IQ and long term memory can also be affected (Kapaj *et al*., 2006). Rats are comparatively more tolerant to arsenic than other animal species and are appropriate model for reproductive and developmental toxicity studies of arsenic (Wang *et al*., 2004). Arsenic is distributed and accumulated not only in erythrocytes of rats, but it is also distributed to other tissues of the body (Wang *et al*., 2004).

Some literatures are available on adverse

effects of arsenic exposure on reproductive organs is available on female adult rats but no data was found on analysis of the histological changes in the mammary gland. In the present study it was intended to investigate the adverse effect of arsenic on morphology of rat's mammary gland and hormonal secretion after exposure to two different doses (100ppm, 200ppm) of sodium arsenite for 28 days.

MATERIALS AND METHODS

Fifteen adult female Sprague-Dawley rats were divided into three groups (n=5 each). The rats were kept under standard laboratory conditions, fed standard diet and received drinking water *ad libitum*. One group served as control while the other two groups received 100ppm and 200ppm doses of sodium arsenite (Merck) respectively in drinking water daily for 28 consecutive days.

Blood sampling

Blood was collected from the dorsal aorta using a haperinized syringe (21-guage needle) after a light ether anesthesia to all animals of control and treated groups. Plasma was separated by centrifugation at 3000 rpm for 10 minutes and then stored at -20 ◦C until hormonal analysis

Tissue sampling and histology

At the end of 28 days of treatment, rats were dissected and mammary glands were removed. Weights of the gland were taken with the help of Sarotorious Digital Balance (Germany). Tissues were then immersed in fixative sera. Following dehydration in the descending ascending grades of ethyl alcohol, tissues were clarified in cederwood oil and then embedded in paraffin. The 5µm thick sections were cut out of paraffin block by using Reichert Microtome (USA). Sections were then affixed to pre-cleaned albuminized glass slides and stretched at 60 ^{0}C on Fisher slide warmer (USA). Hematoxylin and Eosin (H&E) staining was carried out and the slides were then examined under a Nikon optishot research microscope equipped with an automatic micro photographic system (Leica, Germany). Five tissues were collected from each animal and five slides were studied from each tissue. For histological and morphometrical consequences of Arsenic exposure, the epithelial height, nuclear diameter of epithelial cells, ductular diameter, thickness of nipple epithelium, number of TEBs (terminal end buds) were noted.

Hormonal analysis

Plasma level of estradiol (E) and prolactin (PRL) were estimated by commercially available Enzyme immunoassay kits (Amgenix international. Inc. USA).

Statistical analysis

All the data were expressed as mean±SEM. The difference between the mean values of all experimental groups was evaluated by ANOVA followed by Tukey test. The values $P \leq 0.05$ were considered significant. The data were analysed by using Graph Pad Prism version 5.0.

RESULTS

Body weight and mammary gland weight

After administration of two different doses of sodium arsenite 100ppm and 200ppm there was a significant ($P<0.001$) decrease in the mean body weight and mammary gland weight in both cases as compared to control groups (Table I).

Table I.- Effect of two doses (100ppm and 200ppm) of sodium arsenite on body weight and mammary glands weight of rats after 28 days of treatment.

Groups	Body weight (g)		Mammary gland weight (g)
	Day 1	Day 2	
Control	201 ± 2.26	237 ± 4.41	0.039 ± 0.01
100ppm	200 ± 1.33	182 ± 2.04***	0.005 ± 0.001
200ppm	200 ± 4.07	159 ± 4.68***	0.001 ± 0.000

Values are expressed as Mean ± SEM
*** p< 0.001 vs control

Morphometrical analysis

Epithelial height of lactiferous ducts, epithelial height of nipple of mammary gland, nuclear diameter of epithelial cells of lactiferous ducts, number of tubular end buds (TEBs) and diameter of lactiferous ducts were studied for morphometrical analysis. Sodium arsenite treatment

Table II.- Effects of two doses (100ppm and 200ppm) of sodium arsenite on epithelial height of ducts, nipple of mammary glands, nuclear diameter of epithelial cells of ducts, number of tubular end buds (TEBs) and diameter of lactiferous ducts of mammary glands of rats after 28 days.

Groups	Epithelial height of ducts (µm)	Epithelial height nipple (µm)	Nuclear diameter of epithelial cells (µm)	Number of TEBs	Diameter of lactiferous duct (µm)
Control	155.29 ± 6.04	28.43 ± 2.76	4.7 ± 0.31	4.96 ± 0.45	21.6 ± 3.6
100ppm	105.00 ± 4.36***	29.42 ± 1.85	3.25 ± 0.25**	2.85 ± 0.44**	23.5 ± 2.7
200ppm	72.77 ± 4.55***	14.03 ± 1.42***	2.05 ± 0.13***	0.92 ± 0.20***	24.0 ± 2.6

Values are expressed as Mean ± SEM
**p<0.01, ***p<0.001 vs respective control group

(both 100ppm and 200 ppm) caused significant decrease in the measurement of these structures as compared to control group except diameter of lactiferous ducts which were non significantly (P>0.05)changed after 28 days treatment (Table II).

Hormone assay

Plasma estradiol was significantly lower (P< 0.01) in both treated groups (100ppm and 200 ppm) as compared to the control group. Plasma estradiol was significantly lower (P<0.05) in 200 ppm treated compared to 100 ppm treated group (Fig. 1A). Significant (P<0.01) dose-dependent decrease in mean plasma concentration of prolactin was also observed in both sodium arsenite treated groups (100 ppm and 200 ppm) as compared to control group. Further dose-dependent (P< 0.001) reduction in prolactin level was observed in 200ppm group compared to 100 ppm treated group (Fig. 1B).

Histomorphology

In control rats histomorphological study revealed that the mammary gland was divided into two compartments, the epithelial (or parenchymal) compartment and the stromal (or mesenchymal) compartment. The parenchymal compartment of the mammary gland was composed of different epithelial structures with distinct morphologies and functional and proliferative activities, comprising the luminal epithelium of ducts, ductules, terminal end buds, blunt end buds, and alveolar buds, as well as the underlying myoepithelial layer. Histological section of control group showed extensive branching of lactiferous duct and solid or semisolid bulbous clusters of immature epithelial cells at the ends of ducts terminal end buds (TEBs). They were

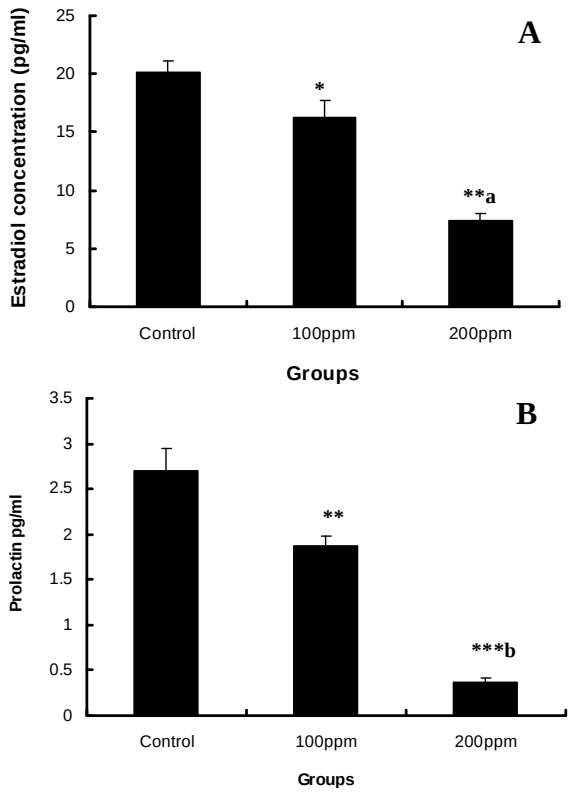

Fig. 1. Effects of sodium arsenite on mean plasma estradiol level (A) and prolactin level (B) in adult female rats after 28 days of treatment. *P<0.05,**P<0.01 vs control, aP<0.05 vs 100 ppm treated group (ANOVA followed by Tukey test).

most abundant at the most distal regions of the epithelium in control group resulting in duct elongation while in 100 ppm and 200 ppm Sodium arsenite treated groups both these structures decrease in dose dependant manner (Fig. 2).

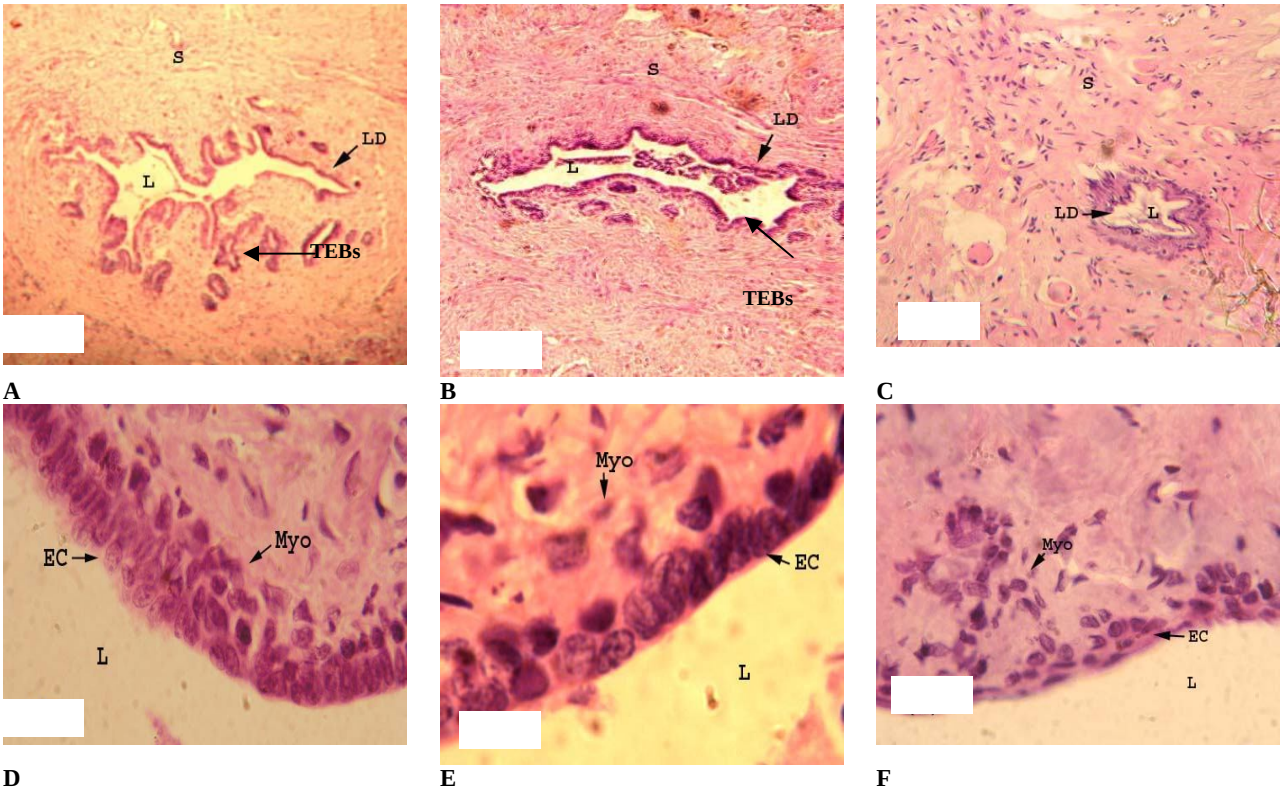

Fig. 2. Histological structure of lactiferous ductular (LD) region and terminal end buds (TEBs) of mammary gland of control and sodium arsenite treated rats after 28 days of treatment (A, C) control showing extensive branching of duct (B) 100ppm treated group; C, E, 200ppm treated group (Magnification in A, B, C, 20X; D, E, F, 40X. EC, epithelial cells and Myo, myoepithelial lining

Luminal epithelium and myoepithelium layers compactness showed marked alteration in both treated groups as compared to control (Fig. 3). The connective tissue stroma surrounding the ducts, ductules and alveolar lobules showed intensely eosinophilic, dense fibrous connective tissue having spindle shaped nuclei in the cross section of control group while in case of 100 ppm the stromal tissue was compact but the stromal cells showed disorganized cellular arrangement unlike the control group and the little spaces and vacuole were also observed and in 200 ppm apparently the stroma was not as dense as in control group and more vacuolation appeared in it (Fig. 3).

DISCUSSION

Arsenic is a potent toxic substance and considered to be an important global health threat due to its adverse health effects. In the present study a significant ($P<0.001$) decrease in body weight gain was observed following treatment with different doses of sodium arsenite (100ppm and 200ppm). It was also suggested previously that arsenite treatment caused loss in body weight in rats (Nandi *et al.*, 2005). One possible reason for the reduction of body weight may be the low food consumption as was reported by Wagstaff (1978) that when female Holtzman rats were treated with different doses of arsenic trioxide (As_2O_3) in feed for 15 days, body weight and food consumption were decreased.

It was also found that sodium arsenite treatment disrupted the structure of mammary gland after 28 days. It caused reduction in the branching of lactiferous ducts. Significant ($p<0.001$) dose-dependent decrease in mean number of TEBs was observed in the sodium arsenite treated groups as compared to control. This is in line to the findings

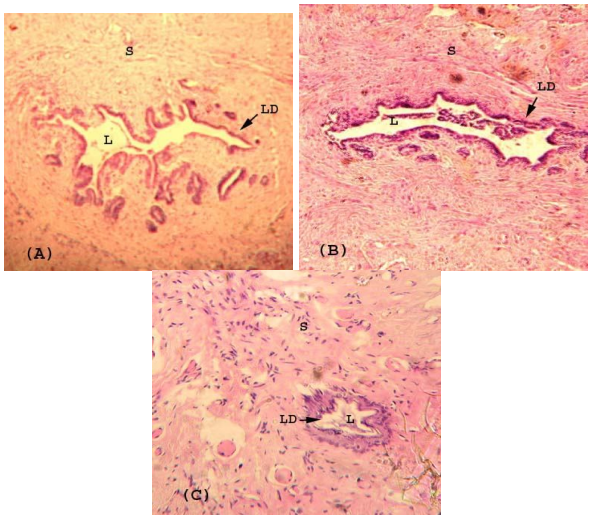

Fig. 3: Photomicrograph of lactiferous ductular (LD) region and terminal end buds (TEBs) from mammary gland of control and sodium arsenite treated rats after 28 days of treatment (A) control showing extensive branching of duct (B) 100ppm treated group (C) 200ppm treated group (H&E stain, 20X).

of Fenton *et al*. (2002), who described an early-detected and persistent reduction in the primary branching and number of TEBs in virgin rats when they were exposed to carcinogen TCDD. A decrease in lobule formation of 50-day old rats following heavy metal exposure was observed in one study (Brown *et al*., 1998). Brown and Lamartiniere, (1995) observed reduced number of TEBs as well as decreased number of proliferating cells in TEBs, terminal ducts and lobules under toxicant condition. One possible reason for the under development might be the low levels of prolactin (PRL) because it is required for the normal differentiation of mammary gland and the gland development is stunted in prolactin receptor knock-out mice (Brisken, 2002; Horseman, 1999; Ormandy *et al*., 1997). In this study sodium arsenite caused a significant ($p<0.01$) reduction in the mean plasma level of PRL compared to control. Likewise, an inverse relationship between the PRL and heavy metals was reported among pregnant women in Canada (Takser *et al*., 2005).

Sodium arsenite treatment also affected the epithelial height of lactiferous ducts and nipples. Significant ($p< 0.001$) dose-dependent decrease was observed in lactiferous ductular epithelial height in 100ppm as well as 200ppm treated groups as compared to control. This is in an agreement with Chattopadhyay *et al*. (2003), who observed the same kind of parameters in the uterus of adult rats treated with sodium arsenite. In the high dose group of present study lactiferous ductular epithelial cells were not well defined as compared to cuboidal cells of control group. The possible reason for this may be the low levels of estrogen because it plays an important role in differentiation of mammary glands. It was observed that sodium arsenite treatment caused reduction in the plasma levels of estradiol. Significant ($P<0.001$) dose dependent decrease in mean plasma concentration of estradiol was observed in both treated groups. It is because arsenic can alter steroidogenesis by its action as endocrine disruptor chemical (EDC). EDCs can altered the hypothalamic-pituitary-gonadal function, estrogen and androgen synthesis and their receptor-mediated effects in mammals and other animals (Gray *et al*., 2004). Arsenic as potent EDC alters the gene regulation by closely related receptors of gluccocorticoids, mineralocorticoids, progesterone, androgen and estrogens at a concentration as low as 0.01 M or 0.7ppb (Davey *et al*., 2007). Very low doses of arsenic enhance the hormone mediated gene transcription, whereas higher doses act as suppressor to these hormones. So the arsenic may alter some common target or process in the steroid receptor mechanism of action (Davey *et al*., 2007). All steroid receptors are affected by arsenic in a similar manner and showed a complex dose-dependent response to arsenic (Stoica *et al*., 2000; Bodwell *et al*., 2004; Kaltreider *et al*., 2001).

It may be concluded that arsenic causes a significant decrease in the body weight and mammary gland weights. It also restricts the development and alters the morphology of mammary glands by affecting the plasma estradiol and prolactin concentrations in adult female rats.

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