REVERSIBLE TOXICITY OF FLUORIDE AND ARSENIC IN OVARY OF MICE

DD Jhala, SB Nair, NJ Chinoy[a]
Ahmedabad, India

SUMMARY: Sodium fluoride (NaF) (5 mg/kg body weight) and arsenic trioxide (As_2O_3) (0.5 mg/kg body weight), administered alone and in combination, were studied for their effects on ovarian histology and steroidogenesis in mice. The beneficial effects of antioxidant vitamins C and E and calcium phosphate supplementation for recovery after treatment were also examined. The NaF + As_2O_3 treatment (30 days) revealed a significant decline in protein levels and in the activities of 3β- and 17β-hydroxysteroid dehydrogenases (HSDs) in the mouse ovary concomitant with a significant accumulation of cholesterol. The affected ovarian steroidogenesis also correlated with altered histology. Withdrawal of the combined treatment (30 days) produced incomplete recovery. On the other hand, supplementation with vitamins C or E or calcium during the withdrawal period led to recovery from the induced effects, which were therefore transient and reversible by antidotes. These findings are significant in relation to humans living in endemic regions of high fluoride and arsenic.

Keywords: Arsenic and ovary; Calcium; Cholesterol; Fluoride and ovary; Protein; Vitamin C; Vitamin E; 3β- and 17β-Hydroxysteroid dehydrogenases (HSDs).

INTRODUCTION

Fluoride and arsenic are distributed ubiquitously in our environment. Fluoride, under certain conditions, can affect virtually every phase of human metabolism. Arsenic, through consumption in food and water, can cause hyperpigmentation and keratosis, or blackfoot disease, which leads to gangrene in the extremities.[1] Combined arsenic and fluoride poisoning is an exceptional disease in the world. Unfortunately, many wells dug in China, Bangladesh, and India have an abundance of fluoride as well as arsenic.

The effects of fluoride and/or arsenic on the reproductive organs as well as fertility impairment are not fully understood, and the data are conflicting. Fluoride intake by female rats and mice is reported to be fetotoxic[2-4] and to reduce fertility in mice.[5] Although Tao and Suttie[6] reported that, with adequate intake of iron, fluoride had little effect on reproduction in mice, chronic exposure to inorganic arsenic[7] and organic fluorine[8] are associated with increased still births, along with a greater risk of miscarriages, premature births, and infant mortality in humans.[9] Treatment of mice and rats with combinations of fluoride and arsenic has also been found to affect reproduction and development.[10,11]

[a]For correspondence: Reproductive Endocrinology and Toxicology Unit, Department of Zoology, School of Sciences, Gujarat University, Ahmedabad 380 009, India.
E-mail: zooldeptgu@satyam.net.in

The present study was undertaken to investigate the effects of fluoride and arsenic administration, alone and in combination, for 30 days on the ovary of mice. Recovery from the induced effects after withdrawal of treatment and after administration of the antioxidant vitamins C and E and calcium phosphate, alone and in combination, during the withdrawal period, was also investigated in the light of earlier work.

MATERIALS AND METHODS

The animals and groups, experimental protocol, and the various treatments and doses are described in detail in a recent earlier report.[12] Histology of the ovary was carried out by the standard haematoxylene-eosin (HE) staining on 5 μ sections.

Biochemical parameters: After the respective treatments, the animals were sacrificed by cervical dislocation. The ovary was excised, blotted free of blood, weighed on a torsion balance (Ohaus, USA), and utilized for determining protein,[13] cholesterol,[14] and 3β- and 17β-hydroxysteroid dehydrogenases (HSDs) (E.C.1.1.1.51)[15] by the techniques cited.

Statistical analysis: For each biochemical parameter, a minimum of 5–6 replicates were done, and the data were statistically analysed by Student's t test and ANOVA.

RESULTS

Histology: The ovary of control mice (Group I) showed all the characteristic features of normal tissue (Figure 1).

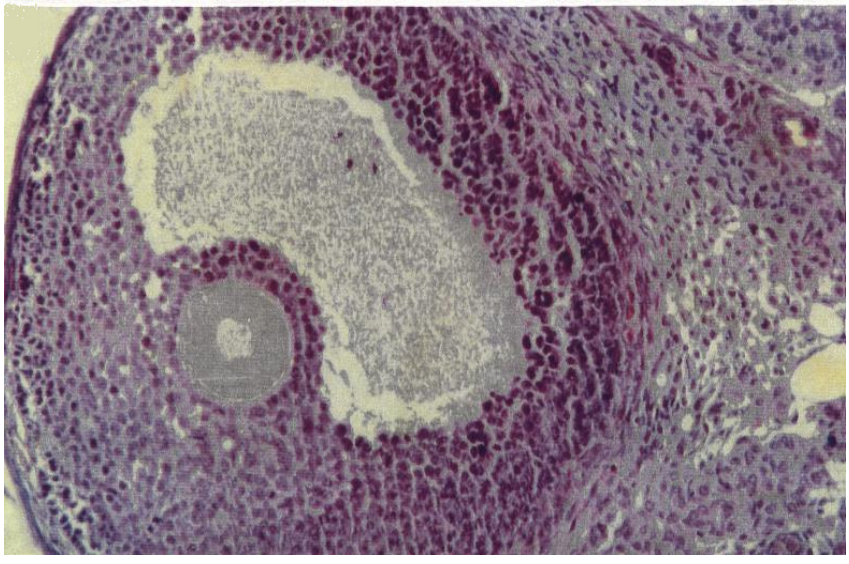

Figure 1. Transverse section of ovary of control mice showing mature Graafian follicle. HE staining (X 250).

After sodium fluoride treatment (Group VI), the ovary showed vacuolization of stroma, atretic follicles, and pyknotic follicular cells (Figure 2).

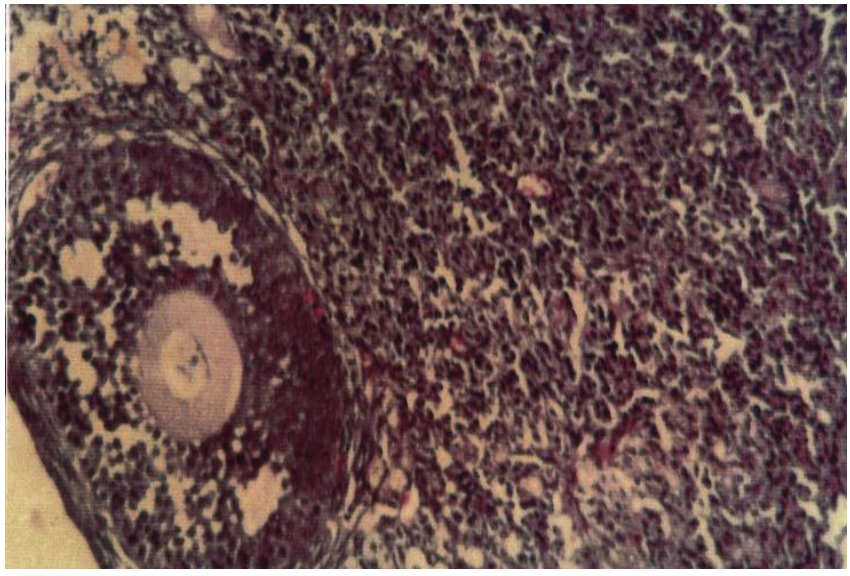

Figure 2. Transverse section of mouse ovary showing pyknotic follicular cells and necrosis of stromal tissue after NaF treatment. HE staining (X 240).

After As_2O_3 treatment (Group VII), the corpus luteum and stroma showed vacuolization and atrophy, and some atretic follicles (Figure 3).

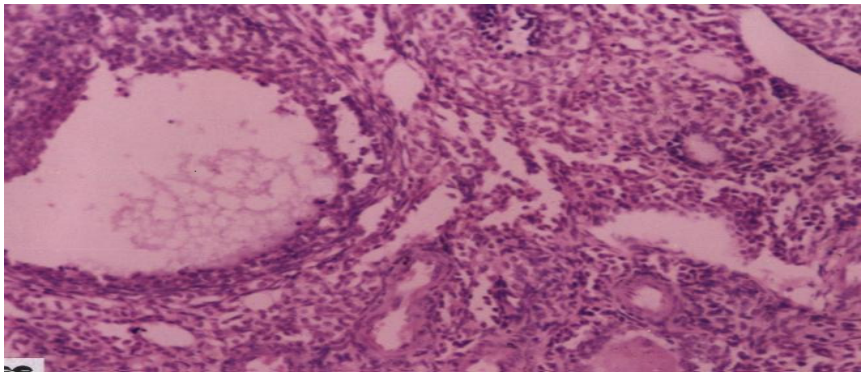

Figure 3. Transverse section of ovary of mice treated with As_2O_3 showing atretic follicles and pyknotic follicular cells. HE staining (X 250).

The degenerative changes were more pronounced in the ovary of mice after combined treatment with NaF + As_2O_3 (Group VIII) (Figure 4).

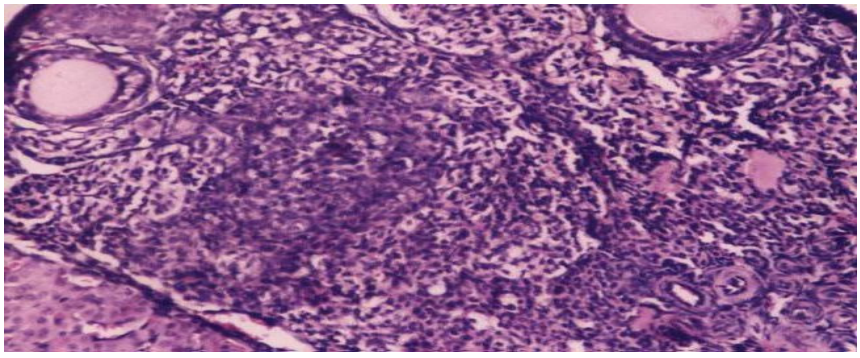

Figure 4. Transverse section of ovary of mice treated with NaF + As_2O_3 showing atretic follicles and necrosis of the stromal tissue. HE staining (X 240).

Merely withdrawing the NaF + As_2O_3 treatment (Group IX) was not conducive for recovery. However, administration of vitamin C, calcium, and vitamin E, alone (Groups X, XI and XII) or in combination (Group XIII), brought about significant recovery (Figure 5).

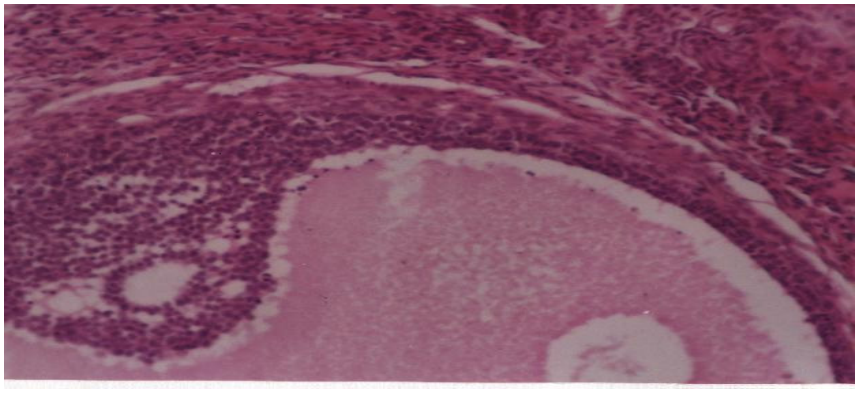

Figure 5. Transverse section of ovary of mice of Group XIII showing recovery and normal Graafian follicle. HE staining (X 230).

Protein and cholesterol: All three treatments in Groups VI, VII, and VIII brought about a significant ($P<0.001$) decrease in protein levels along with a significant ($P<0.001$) increase in ovarian cholesterol as compared to control Groups I–V. However, withdrawal of the treatment (Group IX) brought about partial recovery of protein and cholesterol ($P<0.02$) as compared to the combined treatment Group VIII. All the antidote treatments (Group X–XIII) were

able to restore ovarian protein and cholesterol levels very significantly ($P<0.001$) as compared to Group VIII (Table 1).

Table 1. Protein, cholesterol (mg/100 mg fresh tissue wt) and activities of 3β- and 17β-hydroxysteroid dehydrogenases (HSDs) (nano moles of androstenedione formed/mg protein/minute) in ovary of mice in Groups I-XIII[a]

Group	Treatment	Protein	Cholesterol	3β-HSD	17β-HSD
I	Control + distilled water	11.16 ± 0.27	1.64 ± 0.01	0.90 ± 0.02	0.349 ± 0.027
II	Control + olive oil	11.28 ± 0.32	1.84 ± 0.05	0.91 ± 0.01	0.350 ± 0.023
III	Control + ascorbic acid (AA)	11.92 ± 0.30	1.67 ± 0.02	0.89 ± 0.02	0.318 ± 0.016
IV	Control + calcium phosphate (Ca)	11.64 ± 0.32	1.62 ± 0.01	0.89 ± 0.02	0.317 ± 0.016
V	Control + vitamin E (Vit. E)	10.96 ± 0.34	1.79 ± 0.03	0.92 ± 0.01	0.371 ± 0.012
VI	NaF	09.88 ± 0.20§	1.82 ± 0.03§	0.70 ± 0.03§	0.258 ± 0.020‡
VII	As_2O_3	06.14 ± 0.20§	2.38 ± 0.10§	0.50 ± 0.04§	0.102 ± 0.004§
VIII	NaF + As_2O_3	04.69 ± 0.24§	3.00 ± 0.13§	0.25 ± 0.02§	0.032 ± 0.002§
IX	Withdrawal of Group VIII treatment	05.75 ± 0.37†	2.44 ± 0.20†	0.31 ± 0.01†	0.073 ± 0.002§
X	Withdrawal of Group VIII treatment + AA	10.02 ± 0.12§	1.82 ± 0.02§	0.80 ± 0.01§	0.303 ± 0.003§
XI	Withdrawal of Group VIII treatment + Ca	10.03 ± 0.20§	1.83 ± 0.02§	0.85 ± 0.02§	0.301 ± 0.012§
XII	Withdrawal of Group VIII treatment + Vit. E	10.15 ± 0.11§	1.78 ± 0.02§	0.83 ± 0.02§	0.304 ± 0.008§
XIII	Withdrawal of Group VIII treatment + AA, Ca & Vit. E	11.07 ± 0.16§	1.68 ± 0.01§	0.86 ± 0.03§	0.345± 0.008§

[a]Data are expressed as mean ± S.E. * P<0.05; † P<0.02; ‡ P<0.01; § P<0.001; NS Not significant.

Comparisons between: Group I to Groups VI or VII or VIII individually; Group VIII to Groups IX or X or XI or XII or XIII individually.

Table 1a. ANOVA of various parameters

Parameter	Source of variation	SS	df	MSS	F-cal	F-tab
Protein	Between Groups	701.4360	12	58.4530	85.2090	1.8358
	Within Groups	80.2615	117	0.6860		
Cholesterol	Between Groups	22.3351	12	1.8613	118.2747	1.8358
	Within Groups	1.8412	117	0.0157		
3β-HSD	Between Groups	6.2759	12	0.5230	113.1108	1.8358
	Within Groups	0.5410	117	0.0046		
17β-HSD	Between Groups	1.6379	12	0.1365	85.7239	1.8358
	Within Groups	0.1863	117	0.0016		

SS=Sum of squares; df=degree of freedom; MSS=Mean sum of squares;
F-cal=Fisher calculated; F-tab=Fisher tabulated.

3β- and 17β-Hydroxysteroid dehydrogenases (HSDs): The ovarian 3β-HSD and 17β-HSD activities were significantly reduced ($P<0.001$; $P<0.01$ by NaF in 17β-HSD) by all the three treatments in Groups VI, VII, and VIII as compared to the control Group I. In the withdrawal group (Group IX) significant recovery ($P<0.02$) occurred in 3β-HSD and 17β-HSD activity ($P<0.001$). The recovery was highly significant ($P<0.001$) in the antidote treated groups (Groups X–XIII) as compared to Group VIII (Table 1).

DISCUSSION

As reported earlier by us, histological and structural alterations in mice ovary were seen after fluoride treatment[16] and after combined treatment with fluoride and arsenic.[10]

The results of the present study revealed significant decline in the levels of total protein in ovary of treated mice in agreement with data of others[17-19] for NaF-treated rabbits and our earlier data[10,20] in mice administered arsenic alone and in combination with fluoride. It is likely that fluoride affects the rate of cellular protein synthesis,[21] whereas arsenite (As^{3+}), with its high affinity for thiol groups in protein, is able to inhibit more than 200 enzymes.[22]

Decline in protein levels could also be related to the possible inhibition of DNA synthesis by fluoride and by arsenic. The lack of adequate protein turnover would have an adverse effect on the available enzymes, receptors, struc-

tural proteins, and ovarian growth. Thus the decrease in the number of primary, secondary, and Graafian follicles seen in the current study could be due to a lack of available proteins necessary for cell division, growth, and differentiation of germ cells during oogenesis or else inhibition of the hypothalamus[23] or pituitary by fluoride, and hence a decrease in follicle-stimulating hormone (FSH) secretion as reported for prolactin[24] in rats.

The increase in ovarian cholesterol levels in the present investigation by fluoride and/or arsenic treatment and in serum of fluorotic guinea pigs[25] suggest alterations in its metabolism, probably due to a simultaneous decline in the activities of 3β- and 17β-HSDs which would affect ovarian steroidogenesis.[10,26] A reported decrease in the activity of 3β-HSD in adrenal gland of rabbits treated with fluoride[27] implies that in fluoride intoxication, adrenal steroidogenesis would also be impaired. Vitamins C and E as well as calcium, administered alone and in combination to the animals during the withdrawal period, resulted in reversal of the induced toxic effects and enabled full recovery due to their specific modes of action. Vitamin C, besides being an antioxidant,[28] is known to inhibit phosphodiesterase, thus causing an increase in cAMP levels[29] (a second messenger) which in turn activates several enzymes and influences cell metabolism. In the present study, 3β- and 17β-HSDs were found to recover after treatment of the fluoride and/or arsenic intoxicated mice with by vitamin C, which has also been shown to ameliorate fluoride-induced embryotoxicity in pregnant rats,[30] whereas a diet low in vitamin C increases the adverse effects of fluoride in monkeys.[31]

Calcium, like vitamin C, activates several enzymes via its interaction with cAMP.[10,29,32] Lower levels of calcium in a system cause a depletion of cellular α-tocopherol[33] and affect its metabolism and those of its esters.[34]

Like vitamin C, vitamin E is also a potent biological antioxidant and has the ability to prevent cell injury by maintaining sulphydryl groups of membrane-binding proteins and by quenching free radicals.[35] The cytosolic vitamin C enhances the radical-quenching property of vitamin E[10] and has synergistic antioxidant effects.[36] Thus the interaction of these antidotes (vitamins C and E plus calcium) in sodium fluoride and arsenic trioxide treated mice ovary resulted in reversal of induced toxicity in all the parameters studied.

The data reported here show that arsenic and fluoride induced toxicity in mice ovary is transient and reversible by use of appropriate antidotes. These results have important implications for the mitigation of fluoride and arsenic toxicity in endemic populations and emphasize the value of key dietary factors.[37]

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