FLUORIDE+ALUMINIUM INDUCED TOXICITY IN MICE TESTIS WITH GIANT CELLS AND ITS REVERSAL BY VITAMIN C

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SUMMARY: Administration of sodium fluoride (NaF, 10 mg/kg bw) together with aluminium chloride ($AlCl_3$, 200 mg/kg bw) to adult male mice for 30 days resulted in structural alterations in the testis with formation of giant cells. These changes along with lower protein levels affected spermatogenesis. Steroidogenesis was also altered since the activities of 3β- and 17β-hydroxysteroid dehydrogenases were inhibited with accumulation of cholesterol. Although withdrawal of treatment for 30 days resulted in some recovery, administration of vitamin C along with the toxicants produced significant recovery.

Keywords: Aluminium and testis; Cholesterol; Fluoride and testis; Giant cells; 3β- and 17β-Hydroxysteroid dehydrogenases (HSDs), Protein; Vitamin C.

INTRODUCTION

The effects of aluminium chloride on testis of mice were reported earlier.[1,2] Similarly, giant cell formation with disruption of spermatogenesis and steroidogenesis in testis of mice treated with fluoride and/or arsenic has also been reported for the first time.[3] These induced effects were reversed by vitamins C and E and by calcium.[3]

However, toxicity of combined or simultaneous administration of fluoride and aluminium, withdrawal of treatment, and vitamin C therapy ($NaF+AlCl_3$+vitamin C) have not been studied so far. Hence, this investigation was conducted on mice given fluoride+aluminium for 30 days followed by withdrawal of treatment for an additional 30 days, and then simultaneous treatment with the toxicants+vitamin C to note recovery, if any.

MATERIALS AND METHODS

Animals: Swiss strain adult male mice (*Mus musculus*) weighing between 20 and 30 g were procured from the National Institute of Occupational Health (NIOH), Ahmedabad, India under Registration number as described elsewhere.[3] The mice were housed in an air-conditioned animal room at 26±2 ºC with 10–12 hours of light/day and were maintained on standard chow and distilled water provided *ad libitum*.

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Exposure treatments: The mice were divided into four groups each containing 10–12 animals and treated according to the following experimental protocol:

Group	Treatment and dose (10–12 animals in each group)	Duration (days)	Day of autopsy
I	Control+distilled water (DW)	-	a
II	NaF–treated (10 mg/kg bw)+$AlCl_3$–treated (200 mg/kg bw)	30	31st
III	Same as in Group II then withdrawal for an additional 30 days	30+30	61st
IV	Same as in Group II+Vitamin C (15 mg/animal/day) for 30 days	30+30	61st

[a]Sacrificed along with treated mice.

All treatments were given orally with a hypodermic syringe attached to an angular needle. Animals in Group I served as control (untreated). Sodium fluoride (NaF) (Loba Chemie, Mumbai, 99% purity) and aluminium chloride ($AlCl_3$) (anhydrous) (SD Fine Chem Ltd, Boisor–401501, India, 99.5% purity) were administered to Group II, III and IV animals at a dose of 10 mg/kg bw and 200 mg/kg bw, respectively, for 30 days. These doses were established on the basis of LD_{50} values of 54.4 mg/kg bw for NaF and 4 g/kg bw for $AlCl_3$, respectively.[1,2,4] To study the reversibility of the induced effects, the treatment of Group II animals was withdrawn for 30 days. These were Group III animals which continued to be maintained on standard food and water *ad libitum.* During the 30-day withdrawal period, animals in another group (Group IV) were administered vitamin C (vit C) (15 mg/animal/day) (Loba Chemie, Mumbai, 99% purity) i.e. NaF+$AlCl_3$+vit C. The doses of vitamin C were based on earlier work.[3] At the end of each treatment, the animals were weighed on an animal weighing balance (Ohaus, USA) and sacrificed by cervical dislocation. The testes were dissected out carefully, blotted free of blood, and utilized for study of various parameters.

After the respective treatments of Groups I to IV, the testes were used for studying its histology by haematoxyline and eosin (HE) staining and some biochemical parameters. The levels of protein[5] and cholesterol[6] as well as the activities of 3β- and 17β-hydroxysteroid dehydrogenases (HSDs) (E.C. 1.1.1.51)[7] in testis of mice in Groups I to IV were determined using standard methods.

Statistical analysis: A minimum of 5 or 6 replicates were assayed for all biochemical parameters, and the data were statistically analyzed by Student's t test.

RESULTS

Testis histology: The control mice testis showed normal seminiferous tubules with various stages of spermatogenesis, sperm bundles in the lumen, and interstitial Leydig cells (Figures 1 and 2). The NaF and $AlCl_3$ treated mice testis revealed disorganized germinal epithelium, denudation of cells in the lumen, some of which were giant cells. Sperm bundles were absent (Figures 3 and 4). Withdrawal of treatment (Group III) showed partial recovery as compared to Group II as some tubules had sperm, but giant cells in the lumen persisted (Fig-

ures 5 and 6). Administration of vitamin C along with NaF and $AlCl_3$ to mice (Group IV) for 30 days resulted in complete recovery in testis histology (Figures 7 and 8).

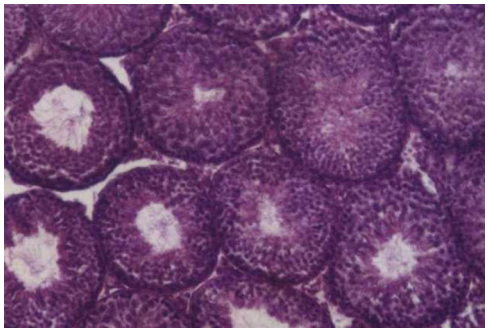

Figure 1. Transverse section of testis of control (Group I) mice showing seminiferous tubules and Leydig cells. HE staining (X 150).

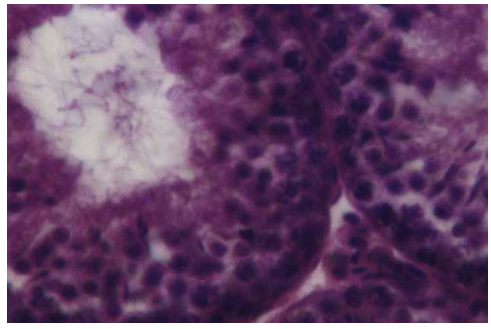

Figure 2. Transverse section of testis of control (Group I) mice. HE staining (X 750).

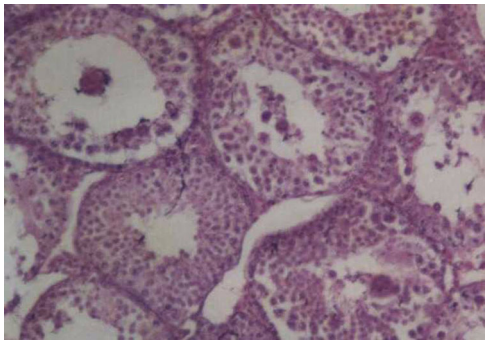

Figure 3. Transverse section of testis of NaF+$AlCl_3$ (Group II) treated mouse showing disorganized germinal epithelium and giant cells in lumen. HE staining (X 160).

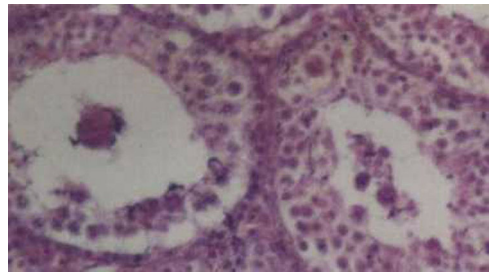

Figure 4. Transverse section of testis of NaF+$AlCl_3$ (Group II) treated mouse. HE staining (X 900).

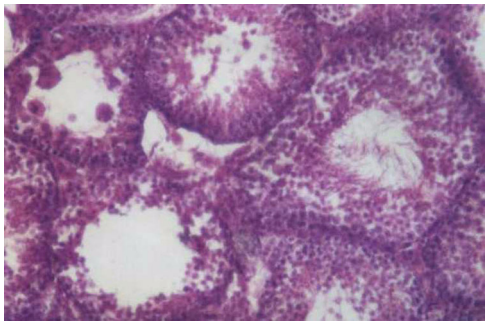

Figure 5. Transverse section of testis of Group III mouse after withdrawal of treatment. HE staining (X 160).

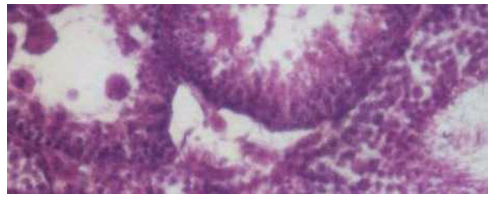

Figure 6. Magnified view of Figure 5. HE staining (X 900).

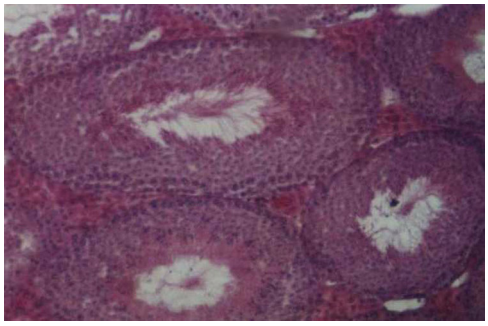

Figure 7. Transverse section of testis of Group IV mouse after NaF+$AlCl_3$+vitamin C treatment showing recovery. HE staining (X 190).

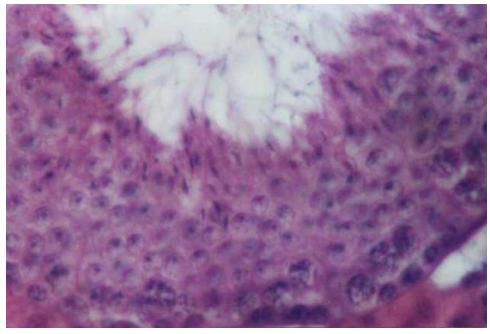

Figure 8. Magnified view of Figure 7. HE staining (X 900).

Biochemical assays: The levels of protein and activities of 3β- and 17β-HSDs decreased significantly ($p<0.001$) in testis of Group II mice after 30 days treatment compared to controls (Group I) (Table). Recovery was significant in these parameters (protein, $p<0.02$; 3β-HSD, $p<0.02$; 17β-HSD, $p<0.05$) in Group III mice after withdrawal of treatment (Table) compared to Group II. All these parameters recovered significantly ($p<0.001$), almost to normal levels, after 30 days of treatment with vitamin C (Group IV) compared to Group II (Table).

Cholesterol levels in testis of NaF+$AlCl_3$ treated mice (Group II) increased significantly ($p<0.001$) as compared to control Group I (Table). The recovery in Group III mice was significant ($p<0.05$) compared to Group II after 30 days withdrawal. However, a much more significant recovery ($p<0.001$) occurred in Group IV mice testis after 30 days treatment with NaF+$AlCl_3$+vitamin C compared to Group II (Table).

Table. 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 testis of Groups I-IV mice[a]

Group	Treatment	Protein	Cholesterol	3β-HSD	17β-HSD
I	Control + distilled water	13.20 ± 0.17	0.62 ± 0.01	0.33 ± 0.01	0.22 ± 0.01
II	NaF + $AlCl_3$	9.82 ± 0.25§	0.75 ± 0.02§	0.22 ± 0.02§	0.14 ± 0.02§
III	Withdrawal of Group II treatment	10.87 ± 0.43†	0.69 ± 0.02*	0.29 ± 0.02†	0.20 ± 0.02*
IV	Withdrawal of Group II treatment + vitamin C	12.51 ± 0.04§	0.64 ± 0.02§	0.32 ± 0.02§	0.21 ± 0.02§

[a]Data are expressed as mean ± SE; * $p<0.05$; † $p<0.02$; ‡ $p<0.01$; § $p<0.001$; where no sign = not significant
Comparisons: Group I with Group II; Group II with Group III; Group II with Group IV individually.

DISCUSSION

According to the dose regimen of sodium fluoride and aluminium chloride for adult male mice used in the present study for 30 days, marked changes in the histology of their testes occurred, including disorganized epithelium and denudation of cells in the lumen of seminiferous tubules that hampered spermatogenesis. Similar effects have been reported by administering either fluoride and/or aluminium alone or in combination in different doses and durations to mice.[3,8-10] Kamboj and Kar[11] reported reduced weight of the mice testis, shrinkage of seminiferous tubules, and spermatogenic arrest at the primary spermatocyte or spermatogonial stage when treated with daily subcutaneous injection of 27.4 mg/kg aluminium sulphate for 30 days. Short-term aluminium chloride exposure to rats and guinea pigs caused gonadal toxicity, whereas, in chronic exposure, the sperm density and motility were affected,[12] in agreement with the work of others.[1,2,13]

The occurrence of giant cells in the lumen of mice testis after 30 days of treatment with fluoride and arsenic has been reported for the first time elsewhere.[3] In the present study also, with fluoride+aluminium combined treatment, similar giant cells were observed denuded off from the spermatogenic epithelium into the tubular lumen. These giant cells could be the result of faulty or failed chromosomal replication or cell division. This again, to the best of our knowledge, is the first report of this effect. The treated animals also manifested decreased protein levels, probably correlated with structural changes contributing to faulty spermatogenesis in agreement with earlier data.[3,9,10]

The significantly reduced activities of 3β- and 17β-HSDs in mice testis after fluoride+aluminium treatment for 30 days correlates with the significant accumulation of cholesterol. This, in turn, would reduce circulating testosterone levels[3] and alter the metabolism of all androgen-dependent reproductive organs in the animals studied.[1,2,13-16]

Withdrawal of treatment for 30 days resulted in significant recovery in all the parameters investigated including histology. However, in Group IV mice treated simultaneously with $NaF+AlCl_3$+vitamin C, complete amelioration of the induced toxic effects was observed, evidently due to the antioxidant and reducing properties of the vitamin which is known to increase C-AMP levels promoting cellular growth and metabolism.[3]

The present study therefore confirms the beneficial effects of dietary factors in mitigating fluoride+aluminium toxicity in testis of mice. The importance of antioxidants in the diet for recovery from fluoride toxicity has also been stressed by Susheela.[17]

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