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ScienceDirect
Reproductive Toxicology 22 (2006) 606–612
Reproductive Toxicology
www.elsevier.com/locate/reprotox

Management of fluoride induced testicular disorders by calcium and Vitamin-E co-administration in the albino rat

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Received 12 November 2005; received in revised form 23 April 2006; accepted 1 May 2006
Available online 12 June 2006

Abstract

Fluoride contamination of drinking water can disrupt male gametogenesis and steroidogenesis and induce testicular oxidative stress. Treatment of rats with sodium fluoride at the dose of 20 mg/kg/day for 28 days resulted in significant diminution of testicular Δ^5,3β-hydroxysteroid dehydrogenase (HSD) and 17β-hydroxysteroid dehydrogenase (HSD) activities and low plasma levels of testosterone, follicular stimulating hormone (FSH) and leutinizing hormone (LH). Spermatogenesis inhibited after sodium fluoride treatment has been assessed here by the quantification of different generation of germ cells like spermatogonia A (ASg), preleptotene spermatocyte (PLSc), midpachytene spermatocyte (MPSc) and step 7 spermatid (7Sd) at stage VII of seminiferous epithelial cycle. Furthermore, fluoride treatment was associated with low activities of testicular, prostatic and epididymal catalase (CAT), superoxide dismutase (SOD) and peroxidase along with elevation of malondialdehyde (MDA) and conjugated dienes (CD) in those tissues. Co-administration of calcium and Vitamin-E with fluoride resulted in a significant recovery from testicular disorders and oxidative stress in the testis and male accessory sex organs. The results of this study demonstrate that fluoride exposure, at the dose available in drinking water in contaminated areas, led to inhibition of testicular gametogenesis and steroidogenesis in association with oxidative stress in the testis and male accessory sex organs, which are protected significantly by dietary agents like Vitamin-E and calcium.
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Keywords: Sodium fluoride; Testicular androgenesis; Spermatogenesis; Oxidative stress; Calcium; Vitamin-E

1. Introduction

Fluoride levels in drinking water may be a problem in some regions [1]. The permissible limit of fluoride in drinking water is 1 ppm [2–4]. In India, for example, at least eight states are affected by fluoride pollution [5]. In most affected areas fluoride is present in drinking water at the levels of 10–20 ppm [5,6] and as high as 38 ppm [7].

Although fluoride has pharmaceutical value [2] there are reports concerning fluoride-induced health disorders. Acute fluoride toxicity is associated with nausea, salivation and abdominal pain [2]. Chronic fluoride toxicity is associated with premature aging, arthritic pain, skeletal and dental fluorosis, osteosclerosis and crippling fluorosis [8]. Autoimmunity and impairment in cognition and memory [9,10] are also associated with fluoride intoxication. Fluoride intoxication also results metabolic disorders [11], renal toxicity [12,13], muscular atrophy [14,15], decalcification and fragility of bone [16]. There are few reports about the undesirable effects of fluoride on the reproductive system. Fluoride treatment led to impaired fertility [17], low birth rate [18], sperm deflagellation [19], and low sperm count [20]. We previously reported that sodium fluoride treatment inhibited testicular androgenesis and gametogenesis [20] through oxidative stress [21]. More recently, we reported that fluoride intoxication diminished both the humoral and cellular immunity [22].

To date there has not been dietary management of reproductive toxicity induced by fluoride. Some reports suggest partial recovery of fluoride toxicity during calcium supplementation [23,24]. We also reported the partial protection of fluoride toxicity by Vitamin-E co-administration [21]. The present study investigated the possible protective effects of calcium and/or Vitamin-E co-administration on fluoride induced testicular disorders in rats. The dose of fluoride selected here was derived in

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doi:10.1016/j.reprotox.2006.05.001

our previous studies [20,22] and also is relevant for effects on human reproduction parameters *in vitro* and *in vivo* [19,23].

2. Materials and methods

2.1. Animals and exposures

Thirty-six adult male albino rats having body weight 150 ± 10 g were used for the present experiment. Animals were house 6 per group and maintained under standard laboratory conditions (14.00 h light: 10.00 h dark, 25 ± 2 °C). They were acclimated in the animal facility for 3–5 days before use. The rats were fed commercial rodent pellets and given water *ad libitum* throughout the experiment. Animal feed was monitored throughout the experimental schedule and final body weights of the animals were recorded on the day of sacrifice. Blood was collected from the dorsal aorta using a heparinized syringe (21 gauge needle) after light ether anesthesia. The principle of laboratory animal care (NIH publication no. 85-23, revised, 1985) was followed throughout the experimental protocol. This protocol was reviewed and approved by our institutional ethical committee.

Dosing for all test compounds was delivered by oral intubation in 0.1 ml distilled water using 20 mg/kg body weight per day [20,22] for 28 days unless noted otherwise. Six rats were assigned to the untreated control group (group I). Another six were dosed with sodium chloride at 20 mg/kg/day as the vehicle control group (group II). The remaining 24 animals were exposed to 20 mg/kg/day sodium fluoride. Six rats not subjected to either co-administration (calcium or Vitamin-E) comprised the fluoride-treated group (group III). Six fluoride-treated rats were subjected to 20 mg/kg/day calcium chloride given 4 h after each sodium fluoride treatment (group IV). Another six fluoride-treated animals were subjected to Vitamin-E co-administration at the dose of 20 mg/100 g/day for 28 days given 4 h prior to sodium fluoride treatment (group V) The remaining six fluoride-treated animals received Vitamin-E at the aforementioned dose (4 h prior to fluoride treatment) and calcium chloride at the aforementioned dose 4 h after sodium fluoride treatment as the Vitamin-E + calcium co-administered group (group VI).

2.2. Biochemical assays

Plasma samples were separated by centrifugation and stored at −20 °C until determination of testosterone. Testes, prostate and epididymides were dissected, weighed and used for biochemical assay of antioxidant enzymes and free radical products. The right testis of each animal was used for biochemical assay of the activities of Δ^5,3β-HSD and 17β-HSD, testicular antioxidant enzymes and products of free radicals (MDA and CD). The left testis of each animal was fixed in Bouin's fluid and paraffin blocks were prepared followed by sectioning and staining for staging of the seminiferous epithelial cycle as described below.

Testicular Δ^5,3β-HSD and 17β-HSD activities were measured biochemically after minor modification of standard methods [24,25] as described [20]. One unit of each of the above mentioned enzyme activity was equivalent to a change in absorbance of 0.001/min at 340 nm. Plasma testosterone was assayed by immunoenzymatic method using an ELISA reader and a standard protocol [26]. The absorbance of the standard and unknown were recorded by using selective filter of 450 nm and differentiating filter of 630 nm. The testosterone kit of IBL-Germany was used here. The supplied testosterone antibody cross-reacted to dihydrotestosterone at 8.5%; the intra-run precision had a co-efficient variation of 5.8%. inter-assay variation was not considered here since all the samples were run simultaneously. Plasma FSH and LH were assayed by radioimmunoassay using reagent supplied by NIAMDD rat pituitary distribution program according to the established method [27]. Plasma samples were analyzed in duplicate and gonadotrophins were expressed as μg/l plasma. The intra-assay variation was 8% and 10% for LH and FSH, respectively. All samples were run concurrently to avoid concern of inter-assay precision.

Activity of catalase (CAT) was measured biochemically [28]. For the evaluation of CAT activities in testis, prostate and epididymis the tissue samples were homogenized in 0.05 M Tris–HCl buffer solution (pH 7.0) at the tissue concentration of 50 mg/ml. The homogenates were centrifuged at 10,000 × *g* at 4 °C for 10 min. For assay, 0.5 ml of H_2O_2 and 2.5 ml of distilled water were mixed and absorbance was monitored spectrophotometrically at 240 nm. The peroxidase activities in the testis, prostate and epididymis were determined as described by others [29]. Tissues were homogenized in 0.1 M sodium phosphate buffer saline (pH 7.4) to give a tissue concentration of 100 mg/ml. The homogenate was centrifuged at 10,000 × *g* for 15 min at 4 °C; 0.1 ml of supernatant was mixed with 3 ml phosphate buffer (pH 7.4) at 25 °C, 0.05 ml of 20 mM H_2O_2 solution (0.042%). The cuvette was placed in spectrophotometer and left until the absorbance increased by 0.05. The time in min (Δt) required for the absorbance to increase by 0.1 was noted at 246 nm.

For assay of superoxide dismutase (SOD) activity the testis, prostate and epididymis were homogenized in cold 100 mM Tris–cacodylate buffer to give a tissue concentration of 50 mg/ml. Samples were centrifuged at 10,000 × *g* for 20 min at 4 °C. SOD activity of the supernatant was estimated by measuring the percentage inhibition of the pyragallol auto-oxidation [30]. One unit of SOD activity was defined as the enzyme activity that inhibits the auto-oxidation of pyragallol by 50%. The reaction mixture was prepared by mixing 0.8 ml of TDB, 40 ml of 7.5 mM NADPH, 25 ml of (100–50 mM) EDTA–$MnCl_2$ and 0.1 ml of supernatant in Tris–HCl buffer (pH 7.4) containing 0.16 M KCl. SOD activity in this mixture was monitored spectrophotometrically based on the rate of oxidation of NADPH.

For estimation of lipid peroxidation from the concentration of MDA [31] and CD [32], the testis, prostate and epididymis were homogenized (50 mg/ml) in 0.1 M ice-cold phosphate buffer (pH 7.5). The homogenates were centrifuged at 10,000 × *g* at 4 °C for 5 min for assay of the supernatant [31,32]. For MDA [31], the homogenate of 0.5 ml was mixed with 0.5 ml of normal saline (0.9%, w/v NaCl) and 2 ml of TBA–TCA mixture (0.392 g thiobarbituric acid in 75 ml of 0.25N HCl with 15 g trichloroacetic acid, volume brought to 100 ml by 95% ethanol) and heated at 100 °C for 10 min. This mixture was cooled at room temperature and centrifuged at 4000 × *g* for 10 min. The supernatant was monitored spectrophotometrically at 535 nm. For CD [32], the lipids were extracted with chloroform–methanol (2:1) followed by centrifugation at 1000 × *g* for 5 min. The chloroform layer was evaporated to dryness under a stream of nitrogen. The lipid residue was dissolved in 1.5 ml of cyclohexane and the absorbance was measured at 233 nm that indicates the amount of hydroperoxide formed.

2.3. Staging the seminiferous cycle

The left testis of each animal was fixed in Bouin's fluid, dehydrated by a graded series of alcohol and embedded in paraffin (56–58 °C). Paraffin blocks were serially cut at 5 μm and stained in hematoxylin and eosin. The germ cells were counted only in one of the stage of epithelial cell cycle of seminiferous tubule, e.g. stage VII according to the method of Leblond and Clermont [33]. This particular stage is composed of type A spermatogonia (ASg), preleptotene spermatocytes (PLSc), midpachytene spermatocytes (MPSc), step 7 spermatids (7Sd) and step 19 spermatids (19Sd). The relative number of each variety of germ cells, i.e. ASg, PLSc, MPSc and 7Sd (except 19Sd, which cannot enumerated precisely) at stage VII of the epithelial cell cycle were counted in 20 round tubular sections in each rat and averaged to represent that animal. The nuclear count (crude count) of the germ cells was corrected for differences in diameter [34]: true count = (crude count [time] section thickness)/(section thickness + nuclear diameter of germ cells). In both treated and control groups, the tubular shrinkage in sections was determined from the average number of Sertoli nuclei-containing prominent nucleoli in section of seminiferous tubules of treated rats divided by the average number of such Sertoli nuclei in the section of seminiferous tubules of the control [35,36].

2.4. Statistical analysis

For statistical evaluation, we performed one-way analysis of variance (ANOVA) followed by post-hoc multiple two-tail '*t*'-test comparison with Bonferroni modification [37]. Differences were considered significant when $P \leq 0.05$.

3. Results

Among these six treatment groups, there was no significant alteration in body weight gain across the experiment. After

Table 1
Effect of calcium and calcium + Vitamin-E co-administration on body weight, testicular somatic, prostato-somatic, and epididymal-somatic indices in sodium fluoride-treated albino rats

Group	Initial body weight (g)	Final body weight (g)	Testicular somatic index (g%)	Prostato-somatic index (g%)	Epididymal somatic index (g%)
I	125 ± 10 a	148 ± 11 a	1.35 ± 0.03 a	0.198 ± 0.004 a	0.45 ± 0.03 a
II	122 ± 10 a	145 ± 11 a	1.36 ± 0.04 a	0.193 ± 0.004 a	0.46 ± 0.02 a
III	120 ± 11 a	141 ± 12 a	1.82 ± 0.04 b	0.132 ± 0.002 b	0.29 ± 0.04 b
IV	124 ± 9 a	144 ± 11 a	1.52 ± 0.02 c	0.168 ± 0.005 c	0.37 ± 0.04 c
V	124 ± 9 a	144 ± 11 a	1.33 ± 0.05 a	0.192 ± 0.003 a	0.44 ± 0.05 a
VI	124 ± 9 a	144 ± 11 a	1.36 ± 0.06 a	0.197 ± 0.006 a	0.46 ± 0.03 a

Values are represents mean ± S.E.M. $n = 6$. In each vertical column, the mean with different letters (a, b, c) differ from each other significantly, $P < 0.05$ (ANOVA followed by multiple two tail t-test).

sodium fluoride treatment there was a significant diminution in the relative weight of prostate by 31%, epididymis by 37% and elevation in testicular weight by 34% with respect to vehicle controls (Table 1). Relative weights of each organ was the control level after Vitamin-E or calcium + Vitamin-E co-administration. Calcium co-administration resulted in a partial but significant recovery in above parameters whereas testicular weight was elevated only by 11% along with diminution in prostatic weight by 13% and of epididymis by 20% in comparison to the vehicle control (Table 1).

Activities of testicular Δ^5,3β-HSD and 17β-HSD were decreased significantly in fluoride-treated rats with respect to untreated or vehicle controls (Fig. 1). The diminution in activities of Δ^5,3β-HSD, 17β-HSD after fluoride treatment were 35% and 31% with respect to vehicle rats. After calcium chloride supplementation there was a significant elevation in the levels of those enzymatic activities; in that case, the diminution values were 24% and 17%, respectively. After Vitamin-E co-administration, partial but significant recovery was noted in above parameters, which was near to the calcium

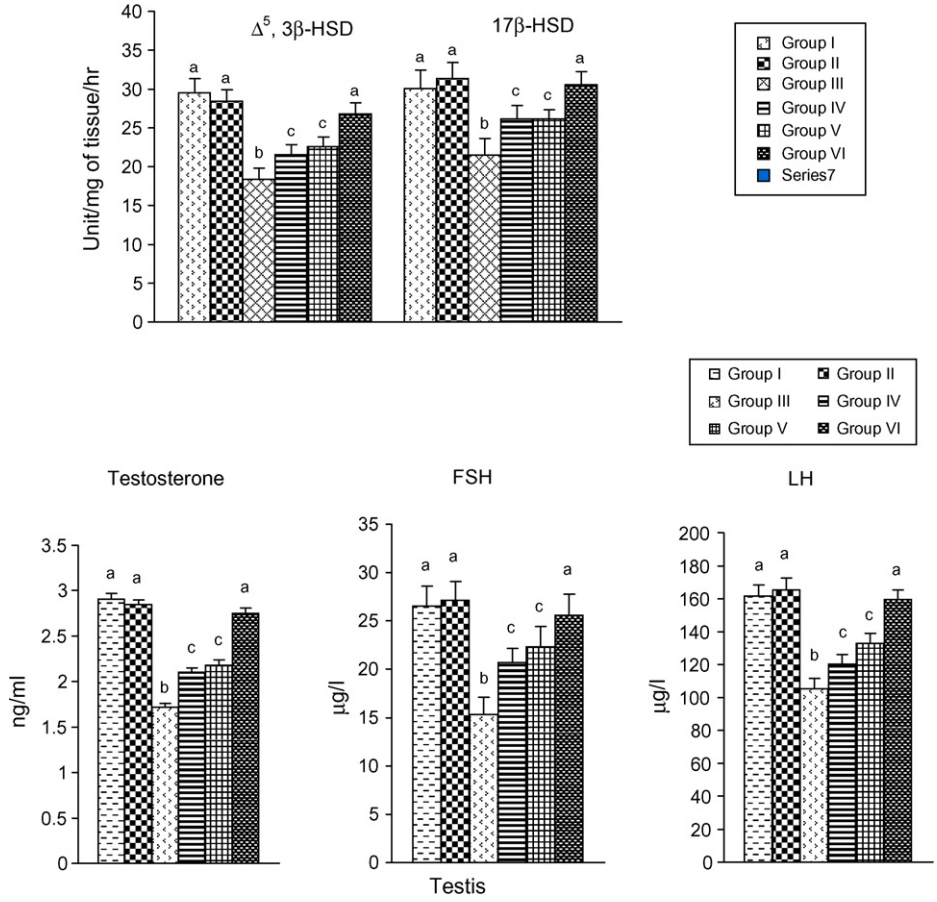

Fig. 1. Effect of calcium or Vitamin-E or calcium + Vitamin-E co-administration on Δ^5,3β-HSD, 17β-HSD, testosterone, FSH and LH in sodium fluoride-treated albino rat (mean ± S.E.M. $n = 6$). Within the same duration of treatment, bars with different superscript (a, b, c) differ from each other significantly $P < 0.05$ (ANOVA followed by multiple two tail t-test).

co-administration group. In contrast, calcium chloride + Vitamin E co-administration to sodium chloride treated rats resulted in a significant protection and the above parameters resolved to the control level (near-complete recovery).

Plasma testosterone, FSH and LH were decreased significantly in fluoride-treated rats when compared to controls. The levels of diminution were 40%, 44% and 36%, respectively (Fig. 1). After calcium chloride co-administration there was a partial but significant recovery in above parameters and the diminution levels were 26%, 24%, and 27%, respectively. Vitamin-E co-administration to sodium fluoride-treated rats also resulted in a significant but partial recovery, like the calcium co-administration group, and it was not restored to the control level. The degree of recovery was not significant different when the data were compared between the calcium chloride co-administered group (group IV) and Vitamin-E co-administered group (group V). On the other hand, co-administration of Vitamin-E + calcium chloride to sodium fluoride-treated rats resulted in a significant recovery restoring all of the above hormones to the control level (near-complete recovery) (Fig. 1).

Activities of testicular, prostatic and epididymal CAT were decreased significantly after sodium fluoride treatment with respect to controls. The inhibition was 28%, 35% and 44%, respectively, in comparison to the vehicle control group. Partial but significant recoveries were noted for these tissues after calcium chloride co-administration to fluoride-treated rats. The percentage of inhibition when compared to vehicle controls was 10.5%, 12% and 44%, respectively. After Vitamin-E or Vitamin-E + calcium chloride co-administration to fluoride-treated rats we observed significant recovery in CAT activity compared to vehicle treated control; the percentage of recovery was near complete (Fig. 2).

Activities of peroxidase in testis, prostate and epididymis were decreased significantly after sodium fluoride treatment when compared to vehicle controls. The degree of inhibition in terms of percentage was 32%, 32% and 49% (Fig. 2). After co-administration of calcium chloride to sodium fluoride-treated rats resulted in a significant but partial recovery and the inhibition levels were 18%, 14% and 23% with respect to vehicle controls. Vitamin-E or Vitamin-E + calcium chloride co-administration to sodium fluoride-treated rats resulted a significant and near-complete recovery to the control level in all three tissue samples (Fig. 2).

Activities of SOD in testis, prostate and epididymis were decreased significantly after sodium fluoride treatment when compared to vehicle controls (Fig. 3) and the degree of inhibition in terms of percentage was 53%, 54% and 53% when compared to vehicle controls. After calcium chloride co-administration in sodium fluoride-treated rats we observed partial but significant recovery and the percentage of inhibition in the activities of above enzyme in all of these three tissue samples were 32%, 31% and 27% when compared to vehicle controls. Vitamin-E co-administration also resulted in a significant but partial recovery in above parameters in all the tissues but not to the control value (Fig. 3). There was no significant difference in SOD activities in testis, prostate and epididymis between calcium

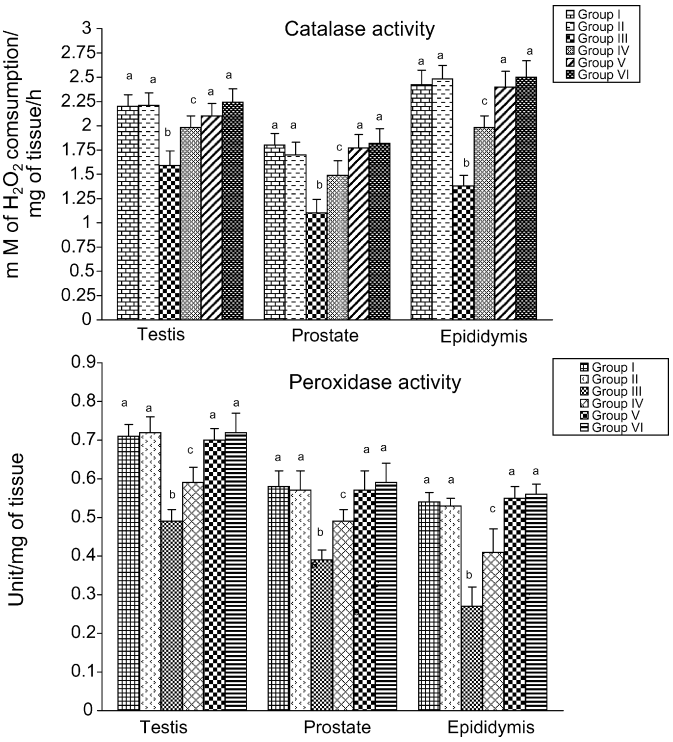

Fig. 2. Effect of calcium or Vitamin-E or calcium + Vitamin-E co-administration on catalase and peroxidase activities in testis, prostate and epididymis in sodium fluoride-treated albino rat (mean ± S.E.M. $n = 6$). Within the same duration of treatment, bars with different superscript (a, b, c) differ from each other significantly $P < 0.05$ (ANOVA followed by multiple two tail t-test).

chloride versus Vitamin E co-administered group. In contrast, after Vitamin E + calcium chloride co-administration to sodium fluoride-treated rats we observed a significant and near-complete recovery of above parameter to the control level (Fig. 3).

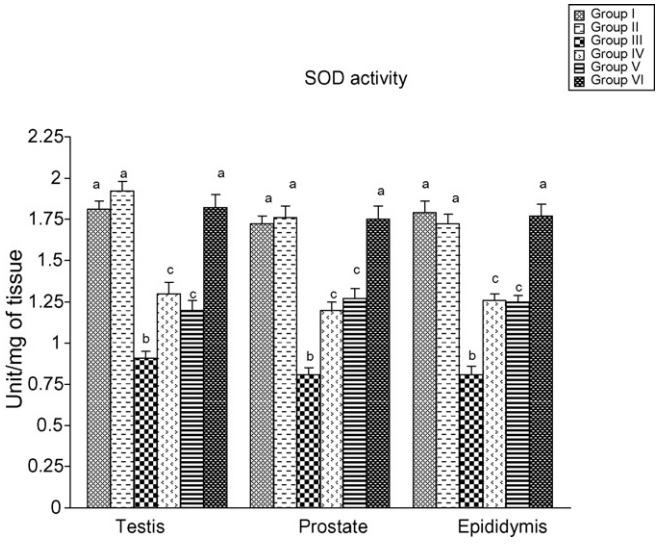

Fig. 3. Effect of calcium or Vitamin-E or calcium + Vitamin-E co-administration on SOD activity in testis, prostate and epididymis in sodium fluoride-treated albino rat (mean ± S.E.M. $n = 6$). Within the same duration of treatment, bars with different superscript (a, b, c) differ from each other significantly $P < 0.05$ (ANOVA followed by multiple two tail t-test).

The quantity of MDA in testis, prostate and epididymis increased significantly after sodium fluoride treatment when compared to both the control groups (Table 2). The elevation in above parameters was 45%, 75% and 72.5%, respectively. After calcium chloride co-administration to sodium fluoride-treated rats we observed a partial but significant protection where the degrees of elevation in testis, prostate and epididymis were 23%, 26.5% and 28%, respectively, compared to vehicle control. Complete recovery in all of these tissue samples was noted after Vitamin-E or calcium + Vitamin-E co-administration (Table 2) to fluoride-treated rats. There was no significant difference when the comparison was made between vehicle treated control with Vitamin-E or Vitamin-E + calcium chloride co-administered groups (Table 2).

The levels of CD in testis, prostate and epididymis were significantly elevated after sodium fluoride treatment (Table 2). The degrees of elevation in the sodium fluoride-treated group with respect to vehicle controls were 55%, 33% and 32%, respectively. Calcium chloride co-administration to fluoride-treated rats resulted in a partial but significant recovery where the degree of elevation with respect to vehicle control was 33%, 18.6% and 6.6%, respectively. In contrast, complete recovery in above parameters in all tissue samples was noted after Vitamin-E or calcium + Vitamin-E co-administration (Table 2). There was no significant difference when the comparison was made between vehicle controls versus Vitamin-E or Vitamin-E + calcium chloride co-administered groups (Table 2).

Numbers of different generation of germ cells at stage VII were decreased significantly after sodium fluoride treatment when compared to the control (Table 3). The percentage of inhibition in the number of ASg was 55.5%, in PLSc-36.87%, in MPSc-38.5% and in 7Sd it was 45% in respect to vehicle control. In contrast, after calcium chloride or Vitamin-E co-administration to sodium fluoride-treated rats, we observed a partial but significant recovery in the count of germ cells when compared to sodium fluoride-treated rats (Table 3). Percentages of diminution in the count of above germ cell in calcium chloride co-administered rats with respect to vehicle treated control were 30.5%, 16%, 18%, and 23%, respectively. Significant and complete recovery was noted in the count of above germ cells after Vitamin-E + calcium co-administration to sodium fluoride-treated rats (Table 3).

4. Discussion

Fluoride treatment led to inhibition in testicular steroidogenesis, monitored here by diminution in the activities of testicular Δ^5,3β-HSD and 17β-HSD, which are key androgenic enzymes [38,39], along with low plasma level of testosterone consistent with our previous report [20]. This may be due to the low levels of plasma FSH and LH observed since both hormones are important regulators of testicular androgenesis [40]. Another possibility for the inhibition in androgenesis is oxidative stress imposed by sodium fluoride in testicular tissue because free radical generation during oxidative stress can interfere with the activities of steroidogenic enzymes [41] consistent with our previous study [21].

Table 2
Effect of calcium, Vitamin-E or calcium + Vitamin-E co-administration on malondialdehyde and conjugated dienes level in testis, prostate and epididymis in sodium fluoride-treated albino rat

Group	Malondialdehyde (nM/mg of tissue)			Conjugated dienes (nM/mg of tissue)		
	Testis	Prostate	Epididymis	Testis	Prostate	Epididymis
I	51.00 ± 3.40 a	29.7 ± 2.12 a	33.2 ± 2.51 a	145.92 ± 6.14 a	129.55 ± 5.33 a	151 ± 2.3 a
II	52.00 ± 3.60 a	31.7 ± 2.12 a	34.2 ± 2.51 a	148.79 ± 5.14 a	133.45 ± 4.33 a	151 ± 2.3 a
III	75.49 ± 4.20 b	55.4 ± 3.03 b	59.0 ± 3.11 b	230.81 ± 7.16 b	173.95 ± 5.08 b	199 ± 2.1 b
IV	64.01 ± 3.8 c	40.1 ± 2.75 c	43.9 ± 2.02 c	198.49 ± 5.05 c	158.37 ± 6.21 c	161 ± 2.9 c
V	53.40 ± 4.23 a	31.4 ± 1.06 a	35.0 ± 2.11 a	150.45 ± 5.11 a	130.11 ± 5.51 a	152 ± 3.2 a
VI	50.06 ± 4.89 a	30.1 ± 2.79 a	32.9 ± 2.02 a	148.45 ± 6.11 a	132.11 ± 5.50 a	153 ± 3.2 a

Values are represents mean ± S.E.M. $n = 6$. In each vertical column, the mean with different letters (a, b, c) differ from each other significantly, $P < 0.05$ (ANOVA followed by multiple two tail t-test).

Table 3
Effect of calcium, Vitamin-E or calcium + Vitamin-E co-administration on different generation of germ cells at stage VII of seminiferous epithelial cycle in sodium fluoride-treated rat

Group	Stage VII seminiferous epithelial cell cycle (different generation of germ cells)			
	ASg	PLSc	MPSc	7Sd
I	0.56 ± 0.04 a	17.12 ± 0.41 a	18.11 ± 0.63 a	62.73 ± 1.82 a
II	0.59 ± 0.03 a	16.92 ± 0.42 a	17.52 ± 0.65 a	64.02 ± 2.10 a
III	0.28 ± 0.02 b	10.68 ± 0.35 b	10.78 ± 0.52 b	35.02 ± 2.10 b
IV	0.41 ± 0.03 c	14.32 ± 0.51 c	14.32 ± 0.48 c	49.52 ± 2.20 c
V	0.42 ± 0.03 c	13.95 ± 0.52 c	13.95 ± 0.42 c	52.39 ± 1.50 c
VI	0.54 ± 0.04 d	16.32 ± 0.46 a	17.82 ± 0.62 a	60.54 ± 2.50 a

Values are represents mean ± S.E.M. $n = 6$. In each vertical column, the mean with different letters (a, b, c, d) differ from each other significantly, $P < 0.05$ (ANOVA followed by multiple two tail t-test).

Staging the generation of germ cells at stage VII was regarded as a measure of spermatogenesis as a whole [42]; generation of these germ cells is hormone dependent [43]. Diminution in the count of germ cells at stage VII in fluoride-treated rat may be due to low level of testosterone [44] and FSH [45] as well as oxidative stress in the testis [46], which have been indicated here by testicular antioxidant enzyme activities and quantification of products of free radicals like MDA and CD.

From the above findings two hypotheses may be framed to explain the mechanism of toxicity induction in testicular tissue by fluoride: indirect effect of fluoride on testis by the modulation of pituitary-testicular axis; direct oxidative stress imposition in testicular tissue. To further ascertain the mechanism and possible dietary management of fluoride toxicity the fluoride-treated animals were subjected to calcium or calcium + Vitamin-E co-administration. Calcium is a chelating agent for fluoride that results insoluble calcium-fluoride generation [47] and thereby reduces fluoride toxicity [48,49]. After calcium co-administration, partial recovery was noted in fluoride-treated rats that may be due to interference of oxidative stress imposed by fluoride. Since full recovery to control levels was not observed we suggest that fluoride may induce alteration in pituitary-testicular axis. Vitamin-E is also a potent antioxidant [50] and androgenic stimulant [51]. Furthermore, Vitamin-E significantly protects fluoride induced oxidative stress in testicular tissue [21]. This agent also resulted in significant but incomplete recovery and as well implies direct (pituitary independent) or indirect (pituitary dependent) mechanisms of fluoride induced reproductive disorders.

Vitamin-E + calcium co-administration led to more complete recovery in the fluoride-treated rats, restoring the measured parameters to the control level. We might explain this recovery as interference with free fluoride availability via the formation of insoluble calcium fluoride that is less effective in imposing oxidative stress on male reproductive organs, as well as the direct stimulatory activity of Vitamin-E on steroidogenesis [51]. Although we tested these agents by oral intubation, both could be delivered in the diet as an acceptable low cost remedy that requires no technical support and likely has few side effects. So it is reasonable to speculate that calcium and Vitamin-E supplements might help safeguard male reproductive health in communities where fluorosis is a concern.

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