AMELIORATION OF FLUORIDE TOXICITY BY VITAMINS E AND D IN REPRODUCTIVE FUNCTIONS OF MALE MICE

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SUMMARY: Studies on the beneficial effects of vitamins E and D supplementation on functions of caput and cauda epididymides, their spermatozoa, vas deferens and seminal vesicle of sodium fluoride (NaF) treated (10 mg/kg body weight) male mice *(Mus musculus)* were carried out. The NaF treatment resulted in significant decrease in the body and epididymis weight but those of vas deferens and seminal vesicle were not affected. NaF treatment brought about alterations in epididymal milieu as elucidated by the significant decrease in levels of sialic acid and protein as well as activity of ATPase in epididymides. As a result, the sperm maturation process was affected leading to a significant decline in cauda epididymal sperm motility and viability. This caused a significant reduction in fertility rate. The cauda epididymal sperm count was also significantly reduced. The data obtained suggest that fluoride treatment induced significant metabolic alterations in the epididymides, vas deferens and seminal vesicles of mice. The withdrawal of NaF treatment (30 days) produced incomplete recovery. On the other hand, sup-plementation of vitamins E or D during the withdrawal period of NaF treated mice was found to be very beneficial in recovery of all NaF induced effects, thus elucidating their ameliorative role in recovery from toxic effects of NaF on the reproductive functions and fertility. On the whole, a combination of vitamins E and D treatment was comparatively more effective than that with vitamin E or D alone. Therefore, vitamin therapy could be beneficial for the amelioration of fluoride induced changes in reproductive functions.

Key words. Epididymis; Fluoride toxicity; Reversibility; Seminal vesicle; Vas deferens; Vitamin D; Vitamin E.

INTRODUCTION

Fluoride is one of the potent toxicants to which humans are exposed. Extensive data on skeletal and dental fluorosis are available.[1] However, the effect of fluoride on the structure and metabolism of several soft tissues has been reported recently. Messer *et al*[2] reported that low levels of fluoride in food rendered mice infertile, while a high fluoride diet improved their fertility. These reports were contradicted by Tao and Suttie,[3] whose experiments showed that fluoride did not play any essential role in reproduction. Later Kour and Singh[4] reported that the testicular spermatogenic process was affected in mice administered fluoride at a dose of 500 and 1000 ppm in drinking water. Li *et al*[5] claimed that fluoride did not have adverse effects on spermatogenesis or sperm morphology. Earlier, our laboratory reported that ingestion of 10 or 20 mg sodium fluoride in mice caused alterations in the histology of reproductive organs, morphology of sperm and induced biochemical changes.[6,7]

Recent investigations[8,9] showed that fluoride interferes with the structural and functional integrity of testis, internal milieu of epididymis, vas deferens and also affected the metabolism and morphology of spermatozoa of mice, rats and rabbits and reduces fertility. The above results clearly indicate that some

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reproductive organs. The present study was undertaken because of the above conflicting reports and paucity of data on the toxic effects of fluoride on male reproductive system and its possible reversal by vitamins E and D.

MATERIALS AND METHODS

Healthy, adult male mice *(Mus musculus)* of Swiss strain, weighing between 25 to 30 g obtained from the National Institute of Occupational Health, Ahmedabad, were used for the experiments. They were housed in an air conditioned animal house at a temperature of 26±2°C and exposed to 10 to 12 hours of daylight.

The animals were divided into seven different groups (see protocol in Table 1) and caged separately. Group I (control) mice were maintained on standard diet and water *ad libitum*. Group II (control) mice were fed olive oil orally with vitamins E and D dissolved in the oil. Sodium fluoride (NaF) (Loba Chemie, Bombay, 99% purity) was administered orally to Group III-VII mice at a dose of 10 mg/kg body weight for 30 days using a feeding tube attached to a hypodermic syringe. The dose of NaF was based on the LD_{50} of male mice which is 54.4 mg F^- /kg body weight.[10] Group III mice were sacrificed on the 31st day. NaF treatment of Groups IV-VII animals was withdrawn after 30 days and the animals were maintained on standard diet and water *ad libitum* for a further 30 days to study any reversibility of the induced effects. Additionally, during this 30 day withdrawal period, Group V mice were administered vitamin E (Roche Products Ltd, Bombay), Group VI mice received vitamin D_3 (Teva Pharmaceuticals Ind. Ltd, Israel), and Group VII animals received a combination of vitamins D and E. The doses of vitamins D and E were 0.002 μg/day/animal and 2 μg/day/animal respectively. These doses were based on earlier studies.

Table 1. Experimental Protocol

Group	Treatment and dose	Duration (days)	Day of autopsy	No. of animals used
I	Untreated control	-	Sacrificed with treated	20
II	Vehicle (olive oil) treated control	-	Sacrificed with treated	20
III	Sodium fluoride (NaF, 10 mg/kg body weight)	30	31st	20
IV	NaF as in Group III, on 31st day NaF withdrawal for further 30 days	30 + 30	61st	20
V	NaF as in Group III, on 31st day NaF withdrawal + vit.E for further 30 days	30 + 30	61st	20
VI	NaF as in Group III, on 31st day NaF withdrawal + vit.D for further 30 days	30 + 30	61st	20
VII	NaF as in Group III, on 31st day NaF withdrawal + vit.E + vit.D for further 30 days	30 + 30	61st	20

The control and treated animals were sacrificed by cervical dislocation. The caput and cauda epididymides, vas deferens and seminal vesicle were carefully dissected out, blotted free of blood, weighed on a Roller Smith (USA) torsion balance and utilized for the various parameters as follows:

Biochemical studies

Sperm motility and count: The cauda epididymal sperm suspension was prepared in normal saline. The percent motility and count of cauda epididymal spermatozoa of normal and all treated groups of mice were determined by the method of Prasad *et al*[11] and expressed as percentage motility and millions/ml respectively.

Sperm viability: The ratio of live:dead spermatozoa of control and all treated groups of mice were determined using 1% trypan blue as described in the method of Talbot and Chacon.[12] The number of stained and unstained spermatozoa were scored in 10-20 separate fields and percentages (%) were calculated.

Fertility test: Conducted by cohabiting estrous or proestrous female animals with treated male animals in a ratio of 2:1 on the 31st day of treatment according to the WHO protocol MB50.[13] The vaginal smear was checked the next morning for the presence of sperm, which indicated that mating had occurred. This was day one of pregnancy. The animals were autopsied on day 16 of pregnancy and the implantation sites were counted and compared with the control.

Protein: The protein levels of caput and cauda epididymides and vas deferens of control and all treated animals were estimated by the method of Lowry *et al*[14] at 540 nm on a Spectronic-88 Bausch and Lomb spectrophotometer. Protein was expressed as mg/100 mg fresh tissue weight.

Adenosine Triphosphatase (ATPase) (E.C. 3.6.1.3): The ATPase activity in epididymides of control and all treated groups of mice were assayed following the method of Quinn and White[15]. The activity was expressed as μ moles of ip released/mg protein/30 minutes.

Sialic acid: Sialic acid in the epididymis of both control and treated animals was determined by the method of Jourdian *et al*[16] and was expressed as μg/mg fresh tissue weight.

Phosphorylase (E.C. 2.4.1.1): Phosphorylase activity in vas deferens of control and treated mice was determined by the method of Cori *et al*[17] and the enzyme activity was expressed as μg phosphorus released/mg protein/15 minutes.

Glycogen: The glycogen concentrations in vas deferens of control and treated mice were estimated by the method of Seifter *et al*[18] and expressed as μg glycogen/100 mg fresh tissue weight.

Fructose: Fructose concentration in the seminal vesicles was estimated by the method of Foreman *et al*[19] and was expressed as μg/mg tissue weight.

Statistics: For all biochemical estimations, a minimum of 8 to 10 replicates were done for each parameter. The data were statistically analysed using student's t test and Analysis of Variance (ANOVA) followed by Scheffe's test for least significance.

RESULTS

Body weight: The body weight of the NaF treated Group III animals decreased ($P<0.001$) after 30 days of treatment in comparison to both the control Groups I-II. After 30 days of NaF withdrawal, the body weight of Group IV mice did not recover as compared to control Group I. On administration of vitamins E or D alone and in combination, the body weights of Groups V-VII animals recovered significantly ($P<0.001$) compared to control Group II (Table 2).

Organ weight: The weights of caput and cauda epididymides in Group III mice declined significantly (P<0.01) compared to control Groups I-II. In Group IV (withdrawal) the recovery was less and the difference was not significant as compared to control, whereas significant recovery was obtained in Group V (vit E), VI (vit D) and VII (vit E + vit D). However, weights of seminal vesicle and vas deferens were not affected by different treatments (Table 2).

TABLE 2. Body (g) and organ weight (mg) of control and treated groups of mice

Groups	Body Weight (g)	Organ Weights (mg)			
		Caput Epididymis	Cauda Epididymis	Vas deferens	Seminal Vesicle
I	35.6 ± 0.19	14.0 ± 0.17	12.0 ± 0.4	11.6 ± 0.35	56.0 ± 0.37
II	36.5 ± 0.21	14.8 ± 0.33	12.6 ± 0.6	12.0 ± 0.20	56.9 ± 0.29
III	30.4 ± 0.24**	11.2 ± 0.30*	9.0 ± 0.5*	13.2 ± 0.33^{NS}	58.4 ± 0.16^{NS}
IV	33.5 ± 0.20^{NS}	13.0 ± 0.28^{NS}	10.6 ± 0.4^{NS}	12.4 ± 0.21^{NS}	57.2 ± 0.21^{NS}
V	37.6 ± 0.23**	15.0 ± 0.28*	13.8 ± 0.17*	12.8 ± 0.23^{NS}	57.0 ± 0.19^{NS}
VI	37.2 ± 0.20**	14.2 ± 0.20^{NS}	13.4 ± 0.21*	12.6 ± 0.19^{NS}	57.6 ± 0.22^{NS}
VII	37.8 ± 0.26**	15.2 ± 0.23*	13.9 ± 0.13*	11.9 ± 0.16*	57.9 ± 0.17^{NS}

Values are Mean ± S.E. • P<0.01 ** P<0.001 NS = Non Significant
Groups: I = Untreated Control; II = Vehicle (Olive oil) treated control; III = NaF treated; IV = NaF withdrawal for 30 days; V = NaF as in III, withdrawal on day 31 and vit E for further 30 days; VI = NaF as in III, withdrawal on day 31 and vit D for further 30 days; VII = NaF as in III, withdrawal on day 31 and vits E + D feeding for further 30 days

Sperm motility: The motility of cauda epididymal sperm of Group III NaF treated mice decreased significantly (P<0.001) as compared to control Groups I-II. At the end of the withdrawal phase, sperm motility recovered in Groups IV-VII, but was still lower than control Groups I-II. Group IV had significant but relatively low recovery (P<0.01). Sperm motility of Group V on vitamin E, and Group VII on vitamins E and D recovered comparatively better than Group VI on vitamin D but all three groups recovered significantly better (P<0.001) than Group IV (Tables 3A and 3B).

Sperm count: The sperm count in the cauda epididymis of Group III NaF treatec mice declined significantly (P<0.001) compared to control Groups I-II. In Grouj IV (withdrawal) significant (P<0.01) recovery was obtained, whereas vitamin I or D treated animals showed very significant (P<0.001) recovery as compared tc the NaF treated (Group III) mice which was again more with vitamin E. In Grouj VII the recovery was most significant (P<0.001) (Tables 3A and 3B).

Sperm viability: Cauda epididymal sperm viability (live:dead ratio) was signifi cantly reduced (P<0.001) by NaF treatment as compared to control groups I-II However, significant (P<0.001) recovery was obtained in Groups IV, V and V (withdrawal, vit E, vit D). In the latter two groups, recovery was more than i Group IV. In Group VII almost complete recovery was noted and was comparabl to normal values (Table 3A).

Table 3A. Cauda epididymal sperm motility, count, viability and fertility rate of control and treated groups of mice

Parameters	Group I Untreated Control	Group II Vehicle treated	Group III NaF	Group IV Withdrawal	Group V Vitamin E	Group VI Vitamin D	Group VII Vit E + Vit D
Sperm motility (%)	75.28 ± 1.58	76.19 ± 1.34	26.31 ± 0.55**	43.73 ± 0.79*	67.42 ± 0.73**	52.46 ± 0.91**	68.66 ± 0.92**
Sperm count (millions/ml)	42.33 ± 0.45	43 ± 0.81	22 ± 0.81**	28.6 ± 0.55*	35.5 ± 1.70**	31.66 ± 1.37**	38.0 ± 0.59**
Sperm viability (%) Live : dead	72.15:27.85 ± 0.50	73.62:26.38 ± 0.54	13.89:86.11 ± 0.44**	39.67:60.33 ± 0.31**	66.52:33.48 ± 0.42**	59.23:40.77 ± 0.33**	69.98:30.0 ± 0.39**
Fertility Rate (%)	95 - 100 +ve	95 - 100 +ve	0**	27.97 ± 3.4** +ve	72.31 ± 0.77** +ve	65.47 ± 2.36** +ve	78.13 ± 0.93** +ve

Values are Mean ± S.E. * P<0.01 ** P<0.001 +ve = positive
Groups I and II compared to Group III (NaF)
Group III (NaF) compared to Groups IV, V, VI, VII

TABLE 3B. ANOVA of cauda epididymal sperm motility and count

Source of variation	df	SS	MSS	f(cal)	f(tab)
<u>Sperm motility</u>					
Groups	6	12629.82	2104.97	13.40	2.36
Residual	35	5495.46	157.01		
<u>Sperm count</u>					
Group	6	2048.41	341.40	279.83	2.26
Residual	35	42.74	1.22		

Significance at 5% level
df = Degree of Freedom SS = Sum of Squares MSS = Mean Sum of Square

Fertility rate: Fluoride treatment led to a significant ($P<0.001$) inhibition of fertility rate as compared to control Groups I-II. The fertility was restored significantly ($P<0.001$) upon withdrawal of treatment for 30 days as compared to NaF treated animals (Group III), but ingestion of vitamin E or D in Groups V and VI resulted in very significant ($P<0.001$) restoration of fertility which was more in Group V (vit E) and in Group VII (vit E + vit D) (Table 3A).

Protein: The protein levels in the caput and cauda epididymides and vas deferens decreased significantly ($P<0.001$) after 30 days of NaF treatment in Group III as compared to control Groups I-II. The protein levels in Group IV (withdrawal) showed insignificant recovery after 30 days except in cauda epididymis where recovery was comparatively more ($P<0.01$). However, administration of vitamins E or D (Groups V-VI) resulted in significant recovery in protein levels in caput and cauda epididymides ($P<0.001$) and vas deferens ($P<0.01$). In Group VII the protein levels recovered to almost the same levels as in control mice (Tables 4A, 4B).

ATPase: Activity of ATPase in caput and cauda epididymides of NaF treated mice showed a significant ($P<0.001$) decrease in comparison to both the control Groups I-II. The recovery was less in Group IV (withdrawal) but significant ($P<0.001$) recovery was obtained in caput and cauda epididymides in Groups V and VI (vitamins E and D treated). Combination of vitamin E + vitamin D treatment brought about more recovery than vitamin E or vitamin D alone (Tables 5A and 5B).

Sialic acid: Levels of sialic acid in caput and cauda epididymides declined significantly ($P<0.01$) after NaF treatment as compared to both the control Groups I and II. However, by withdrawal of treatment (Group IV) and subsequent administration of vitamin E or D (Groups V, VI), significant ($P<0.01$) recovery was noted as compared to control mice. The recovery was most significant ($P<0.001$) in Group VII where combination of vit E and vit D was given (Tables 6A and 6B).

Glycogen: A significant ($P<0.001$) accumulation of glycogen in the vas deferens of NaF treated mice was observed as compared to control Groups I-II. In Group IV the recovery was not significant. On the other hand, significant recovery was obtained after vitamin E ingestion (Group V) ($P<0.001$) and in Group VI ($P<0.01$) as compared to NaF treated mice. However, in Group VII (vit E and vit D) significant ($P<0.001$) recovery was observed (Tables 7A and 7B).

Phosphorylase: NaF treatment resulted in significant ($P<0.001$) suppression of vas deferens phosphorylase activity as compared to control Groups I-II. However, after withdrawal of treatment and administration of vitamins E or D (Groups V-VI), the activity of phosphorylase was restored back to normal (Groups IV, V, VI). The recovery was comparatively less in Group IV but was significant ($P<0.001$) with both vitamin treatments. Combined treatment of vit E and vit D was the most effective in restoring enzyme activity to almost normal levels (Tables 7A and 7B).

Fructose: Fructose levels in seminal vesicle increased significantly ($P<0.001$) after NaF treatment for 30 days as compared to control Groups I-II. The recovery was significant ($P<0.01$) by withdrawal (Group IV) of treatment. On administration of vitamins E or D (Groups V-VI) the levels of fructose recovered significantly ($P<0.001$). Moreover, the levels of fructose in vit E + vit D treated mice (Group VII) were also significantly ($P<0.001$) restored to normal as compared to NaF treated mice (Tables 7A and 7B).

TABLE 4A. Protein levels (mg/100 mg fresh tissue weight) in caput and cauda epididymides and vas deferens of control and treated groups of mice

Organs	Group I Untreated Control	Group II Vehicle treated	Group III NaF	Group IV Withdrawal	Group V Vitamin E	Group VI Vitamin D	Group VII Vit E + Vit D
Caput Epididymis	11.43 ± 0.28	12.55 ± 0.14	8.47 ± 0.20**	9.25 ± 0.16^{NS}	11.00 ± 0.24**	10.61 ± 0.19**	12.0 ± 0.18**
Cauda Epididymis	14.10 ± 0.19	15.18 ± 0.37	8.52 ± 0.44**	9.89 ± 0.12*	12.92 ± 0.33**	11.48 ± 0.16**	13.62 ± 0.29**
Vas Deferens	14.09 ± 0.09	14.43 ± 0.21	8.87 ± 0.20**	9.82 ± 0.17^{NS}	11.79 ± 0.26*	11.77 ± 0.15*	12.98 ± 0.18**

Values are Mean ± S.E. * $P<0.01$ ** $P<0.001$ NS = Non Significant
Groups I and II compared to Group III (NaF)
Group III (NaF) compared to Groups IV, V, VI, VII

TABLE 4B. ANOVA of caput and cauda epididymis and vas deferens protein

Source of variation	df	SS	MSS	f(cal)	f(tab)
Caput epididymal protein					
Groups	6	81.75	13.62	38.91	2.36
Residual	35	10.86	0.35		
Cauda epididymal protein					
Groups	6	2047.43	341.23	494.53	2.36
Residual	35	24.49	0.69		
Vas deferens protein					
Group	6	135.94	22.65	266.47	2.44
Residual	28	2.38	0.085		

Significance at 5% level
df = Degrees of Freedom SS = Sum of Squares MSS = Mean Sum of Square

TABLE 5A. Adenosine triphosphatase activity (μ moles of ip released/mg protein/15 minutes) in caput and cauda epididymides of control and treated groups of mice

Organs	Group I Untreated Control	Group II Vehicle treated	Group III NaF	Group IV Withdrawal	Group V Vitamin E	Group VI Vitamin D	Group VII Vit E + Vit D
Caput Epididymis	1.99 ± 0.04	2.01 ± 0.06	0.93 ± 0.07**	1.10 ± 0.03^{NS}	1.69 ± 0.08*	1.65 ± 0.06*	1.78 ± 0.05**
Cauda Epididymis	2.08 ± 0.07	2.12 ± 0.09	0.978 ± 0.03**	1.23 ± 0.05*	1.87 ± 0.08**	1.79 ± 0.10**	1.96 ± 0.08**

Values are Mean ± S.E. * $P<0.01$ ** $P<0.001$ NS = Non Significant
Groups I and II compared to Group III (NaF)
Group III (NaF) compared to Groups IV, V, VI, VII

TABLE 5B. ANOVA of caput and cauda epididymal ATPase

Source of variation	df	SS	MSS	f(cal)	f(tab)
Caput epididymal ATPase					
Groups	6	5.42	0.90	18.06	2.42
Residual	28	1.53	0.05		
Cauda epididymal ATPase					
Group	6	5.82	0.97	161.66	2.42
Residual	28	0.17	0.006		

Significance at 5% level
df = Degrees of Freedom SS = Sum of Squares MSS = Mean Sum of Square

TABLE 6A. Sialic acid levels (μg/mg tissue wt) in caput and cauda epididymides of control and treated groups of mice

Organs	Group I Untreated Control	Group II Vehicle treated	Group III NaF	Group IV Withdrawal	Group V Vitamin E	Group VI Vitamin D	Group VII Vit E + Vit D
Caput Epididymis	4.72 ± 0.03	4.89 ± 0.02	3.76 ± 0.07*	4.14 ± 0.02 NS	4.45 ± 0.05*	4.34 ± 0.02*	4.60 ± 0.06**
Cauda Epididymis	5.56 ± 0.06	5.59 ± 0.09	4.48 ± 0.07*	5.05 ± 0.04*	5.37 ± 0.04*	5.18 ± 0.02*	5.47 ± 0.03**

Values are Mean ± S.E. * P< 0.01 ** P< 0.001 NS = Non Significant
Groups I and II compared to Group III (NaF)
Group III (NaF) compared to Groups IV, V, VI, VII

TABLE 6B. ANOVA of cauda and caput epididymal sialic acid

Source of variation	df	SS	MSS	f(cal)	f(tab)
Caput epididymis					
Groups	6	5.59	0.93	31.05	2.36
Residual	35	1.11	0.03		
Cauda epididymis					
Groups	6	6.32	1.05	87.77	2.36
Residual	35	0.45	0.012		

Significance at 5% level
df = Degrees of Freedom SS = Sum of Squares MSS = Mean Sum of Square

TABLE 7A. Showing glycogen concentration and phosphorylase activity in vas deferens and fructose level in seminal vesicle of control and treated groups of mice

Organs	Parameters	Group I Untreated Control	Group II Vehicle treated	Group III NaF	Group IV Withdrawal	Group V Vitamin E	Group VI Vitamin D	Group VII Vit E + Vit D
Vas Deferens	Glycogen (μg/100mg fresh tissue wt)	776.54 ± 20.65	815.85 ± 19.76	1281.29 ± 15.36**	1181.29 ± 5.36^{NS}	858.35 ± 11.56**	1058.86 ± 19.92*	829.67 ± 13.63**
	Phosphorylase (μg phosphorus/mg protein/15 min)	53.17 ± 1.45	54.0 ± 1.32	36.76 ± 0.88**	42.30 ± 1.01*	51.07 ± 1.75**	45.22 ± 1.06**	52.01 ± 1.68**
Seminal Vesicle	Fructose (μg/mg tissue wt)	14.84 ± 1.11	14.98 ± 1.16	20.83 ± 1.20**	17.62 ± 0.99*	16.37± 0.90**	16.97 ± 1.01**	15.23 ± 0.93**

Values are Mean ± S.E. • $P < 0.01$ ** $P < 0.001$ NS = Non significant

TABLE 7B. ANOVA of glycogen, phosphorylase and fructose

Source of variation	df	SS	MSS	f(cal)	f(tab)
Glycogen (vas deferens)					
Groups	6	1293659.4	215609.9	8.20	2.44
Residual	28	735336.6	26262.02		
Phosphorylase (vas deferens)					
Groups	6	5697.17	949.52	5.96	2.42
Residual	28	4458.4	159.22		
Fructose (seminal vesicle)					
Group	6	123.12	20.52	18.48	2.42
Residual	28	31.19	1.11		

Significance at 5% level df = Degrees of Freedom SS = Sum of Squares MSS = Mean Sum of Square

DISCUSSION

The present investigation was carried out to explore the effects of fluoride (NaF) and the possible ameliorative role of vitamins E and D ingestion on epididymis, vas deferens and seminal vesicle of mice during withdrawal period.

The sodium fluoride treatment caused a decrease in the body weight. Similar results were reported by others in rats and mice fed with different concentrations of fluoride.[8,20] An insignificant reduction in weight of epididymis occurred but those of seminal vesicle and vas deferens were not affected.

In the present study the levels of protein in caput and cauda epididymides and vas deferens showed a significant decrease after 30 days of NaF treatment. This decrease might be due to impairment of protein metabolism/synthesis. Earlier studies carried out from our laboratory and elsewhere have also reported a dose dependent decrease in protein levels in the serum and reproductive organ of mice, rats and rabbits.[7,8,21]

Sialic acid is an important constituent of mucopolysaccharides and sialomucoproteins which are essential for the maturation of spermatozoa in epididymis and maintenance of the structural integrity of their membranes.[22] The levels of sialic acid in caput and cauda epididymides were decreased in the present study. Hence it is likely that the structural integrity of acrosomal membrane of the sperm might have been altered. A decline in sialic acid concentration of NaF treated mouse and rat epididymis has also been reported earlier.[8]

Adenosine triphosphatase activity in caput and cauda epididymides showed a decline with greater propensity in cauda epididymis. Alterations in the activity of ATPase in NaF treated mouse and rat epididymis and rabbit spermatozoa have been reported.[8,21] It was reported[23] that fluoride acts directly on the motile apparatus without substantially affecting other metabolic pathways as it inhibits the dynein ATPase in sperm. According to Hodge and Smith,[24] NaF toxicity involves inhibition of enzyme activity, particularly those in which divalent metal cations act as cofactors. In the present study too, the alterations in ATPase activity might be related to the fact that it is either a Mg^{2+} or a Ca^{2+} activated enzyme.

Reports from our laboratory have revealed that the glycogen concentrations were enhanced in vas deferens of fluoride treated rats and mice.[7,25] These results are in agreement with the observations of the present investigation, wherein glycogen was found to accumulate in vas deferens of NaF treated mice. The increase in glycogen could be correlated with the decrease in the activity of phosphorylase in the vas deferens by NaF ingestion. Fluoride has been reported to alter carbohydrate metabolism mainly by causing allosteric inhibition of some key enzymes in glycolysis and tricarboxylic acid (TCA) cycle.[26]

The increase in level of fructose in seminal vesicle after 30 days of NaF exposure further supports our observation on alteration in carbohydrate metabolism of fluorotic mice. As fructose has a vital role in providing energy to the sperm, it is evident that the increased fructose level might influence sperm metabolism.

Fluoride is known to inhibit sperm motility, glycolysis and respiration process. It has been demonstrated[27] that bovine sperm treated with 30 mM fluoride became immobile within two minutes. Human spermatozoa lost their motility *in vitro* in the presence of 250 mM NaF within 20 minute incubation.[28] In the present study too, a significantly low sperm motility was obtained by NaF ingestion.

The sperm density of cauda epididymis in NaF treated group of mice also declined significantly. The decrease could be correlated with the testicular spermatogenic arrest following fluoride ingestion in mice, rats and rabbits.[8,21]

The evaluation of NaF treated spermatozoa stained with trypan blue showed a large number of dead sperm, probably due to loss of membrane permeability, which might be another major factor in the decline in sperm motility.[25]

The above mentioned alterations in sperm motility, density and metabolism might be the outcome of the altered and hostile internal milieu of the epididymis of NaF treated mice since it is known that normal epididymal structure and its internal microenvironment are important for sperm maturation and for maintaining them in a viable, motile state.[22,29,30]

The reduction in sperm motility, count, viability and changes in their metabolism led to the significant decline in fertility of treated mice as also reported earlier in mice, rats and rabbits.[8,21,31]

In the present study, NaF treatment affected numerous androgen dependent parameters in the epididymis, vas deferens and seminal vesicle to a variable extent. Hence, it is evident that fluoride affects male reproductive organs and fertility.

In withdrawal group of animals (Group IV) the NaF induced effects were not restored completely to normal state after 30 days. However, in Groups V, VI, VII of animals treated with vitamin E or vitamin D alone and in combination in the withdrawal period, almost complete recovery from fluoride toxicity was obtained. The extent of recovery was more pronounced with vitamin E as compared to D and was most significant with the combined treatment.

In rats, the main symptoms of vitamin E deficiency are degeneration of the testis, abnormalities of gestation, regression in the ovary and changes in ovulation.[32] At the cellular-molecular level, vitamin E is believed to exert its protective effect primarily through destruction of cell-damaging free-radical oxygen species.[33] In the present study too, vitamin E ingestion was beneficial for recovery from fluoride toxicity.

According to earlier reports, elevated levels of vitamin D mitigated the symptoms of fluorosis. Studies carried out by Gupta *et al* 1996[34] have revealed that the treatment of vitamins C, D and calcium showed a significant improvement in the skeletal, clinical fluorosis and biochemical parameters in children consuming water containing 4.5 ppm of fluoride.

The above reports and earlier work carried out by Chinoy and associates have elucidated that therapeutic agents like amino acids, vitamin C and Ca^{2+} could mitigate fluoride induced effects.[8,35,36]

Thus, in conclusion, sodium fluoride has a definite effect on reproduction. However, the fluoride induced effects are reversible and transient and could be effectively reversed by withdrawal of treatment and subsequent supplementation of vitamins E and D. Thus vitamins E or D may be used as therapeutic agents for the mitigation of fluoride induced toxicity in endemic areas all over the world. Hence, these results have important implications for amelioration of fluorosis in endemic regions.

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