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Evaluation of vitamin E and calcium effects on fluoride toxicity-induced fertility impairment

Ashraf M. Emara¹*, Rabab S. El-Kelanya, Thanaa A. Elmasryb and Mahmoud Elkarebc

¹Faculty of Medicine, Departments of Forensic Medicine & Clinical Toxicology, Tanta University, Tanta, Egypt; ²Faculty of Pharmacy, Department of Pharmacology and Physiology, Tanta University, Tanta, Egypt; ³Faculty of Medicine, Tanta University, Tanta, Egypt

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Chronic fluoride (Fl) toxicity is a serious public health problem globally where drinking water contains more than 1 ppm of Fl. Sodium fluoride (NaF) produced male reproductive system toxicity. The aim of the present study was to evaluate the amelioration of Fl toxicity-induced fertility impairment by vitamin E and calcium during the withdrawal period. The study was carried out on 70 adult male albino rats divided into five main groups: group I control; subdivided into group Ia (maintained on standard diet and water ad libitum for 60 days) and group Ib (maintained on standard diet and water ad libitum for 120 days), group II was administered NaF and subdivided into group IIa (administered NaF for 60 day and sacrificed) and group IIb (administered NaF for 60 day then maintained on standard diet and water ad libitum for a further 60 days), and treated groups III, IV, and V were administered NaF. Rats were maintained during withdrawal from NaF, on vitamin E (10 mg kg⁻¹ day⁻¹ for 60 days), calcium (50 mg kg⁻¹ day⁻¹ orally for 60 days), and both vitamin E and calcium, respectively. The duration of NaF administration was 60 days at a dose 20 mg kg⁻¹ day⁻¹ for all treated groups. The following parameters were determined: body and organ weights, sperm motility, sperm morphology, sperm viability, fertility test, and hormone assays: testosterone, in vitro testosterone production, luteinizing hormone, and follicular stimulating hormone. The combined administration of vitamin E and calcium during withdrawal from NaF showed significant improvement from chronic Fl-induced toxicity on male reproductive organs.

Keywords: fluoride toxicity; fertility impairment; vitamin E; calcium

Introduction

Fluorosis is a serious public health problem globally where drinking water contains more than 1 ppm of fluoride (Fl) (Krishnamachari 1986; Das 1996). Fluorosis is a well-defined clinical entity characterized by toxic effects of high Fl intake on teeth, bones, and soft tissues (Susheela 1999). Some studies reported biochemical changes in the composition of bone, urine, and plasma, as well as hormonal alterations in fluorosis (Krishnamachari 1986; Das 1996). Furthermore, Fl is also known to cross the cell membrane and enter soft tissues. Impairment of soft-tissue function was demonstrated in Fl-intoxicated animals.
Sodium fluoride (NaF) produced adverse effects on testicular activity related to oxidative stress as evidenced by increased activity of peroxidases and catalases due to inhibition of 2-androgenesis-regulator enzymes DELTA (5) b-HSD and 17beta-HSD (Ghosh et al. 2002; Giachini and Pierleoni 2004). Ghosh et al. (2002) found that NaF induced significant decrease in the relative weight of testis, prostate, and seminal vesicle without alterations in body weight gain. Chinoy and Narayana (1989) reported that NaF exposure produced morphologic anomalies in sperm and a significant decline in sperm motility. NaF may also result in significant increase in follicle stimulating hormone (FSH) and a reduction in free testosterone levels (Ortiz-Perez et al. 2001) and luteinizing hormone (LH) (Sprando et al. 1996).

Oral administration of NaF resulted in significant reduction in feed consumption and lowered concentrations of glucose and protein in rats (Verma and Guna-Sherlin 2002). Administration of vitamin E produced significant amelioration in feed consumption, as well as glucose and protein levels.

Calcium may play a beneficial role in regulation of the reproductive system. Ekambaram and Paul (2002) concluded that calcium carbonate prevented the toxicity of Fl by maintaining serum Fl at a less toxic level. In addition, the toxic effects of Fl are reversible if exposure is withdrawn for 2 months. However, evidence for the effect of calcium, vitamin E and a combination of both on Fl toxicity-induced fertility impairment in male albino rats has not been fully investigated. Hence, in the present study, calcium, vitamin E, and a combination of both were administered to NaF-treated rats during the withdrawal period to assess their efficacy in preventing Fl-induced toxicity on the reproductive system.

Materials and methods

The study was carried out on 70 adult male albino rats (Rattus norvegicus) weighing approximately 130–155 g. Rats were fed on the standard lab diet and water ad libitum, housed under constant environmental conditions with regard to temperature and light, and divided into seven groups:

1. Group Ia (control) consists of 10 rats and were maintained on standard diet and water ad libitum for 60 days and group Ib consists of 10 rats and were maintained on standard diet and water ad libitum for 120 days.

2. The remaining 50 rats were administered sodium fluoride (NaF, UltraPure Research Grade, >99.9%) (20 mg kg\(^{-1}\) body weight per day) dissolved in water (Chinoy and Sequeira 1992) (LC Laboratories, a division of PKC Pharmaceuticals, Inc., USA) and given by oral gavage and classified as follows:

   a. Group II consists of 20 rats. Ten rats (group IIa) were administered NaF for 60 days then sacrificed on the 61st day, and 10 rats (group IIb) were administered NaF for 60 days, they were then maintained on standard diet and water ad libitum for a further 60 days to study any reversibility of the induced reproductive toxicity.

   b. Group III consists of 10 rats and was administered NaF for 60 days. Rats were then maintained on vitamin E (gel capsules evacuated by syringe) (MEPACO, Egypt) (10 mg kg\(^{-1}\) day\(^{-1}\) orally) (Marubayashi et al. 1986) for a further 60 days.
(c) Group IV consists of 10 rats. They were administered NaF for 60 days. Rats were then maintained on calcium (Calcium gluconate ampoules, MEPACO, Egypt) (50 mg kg\(^{-1}\) day\(^{-1}\) orally) (Ekambaram and Paul 2002) for a further 60 days.

(d) Group V consists of 10 rats. They were administered NaF for 60 days. Rats were then maintained on vitamin E and calcium in the same dose and forms previously described for a further 60 days.

**Body and organ weights**

Body weights for all treated rats and their corresponding control rats were measured at the end of the experiment. The left testis, epididymis, and vas deferens of all animals were trimmed of fat and fascia and weighed.

**Sperm motility**

Sperm samples were collected from the distal cauda of the epididymis and used for computer assisted sperm analysis (CASA) as previously described (Slott et al. 1993) with the exception that the medium used for motility analysis was as follows: Hanks balanced salts solution (Gibco Invitrogen Co., Grand Island, NY), buffered with 4.2 g L\(^{-1}\) HEPES and 0.35 g L\(^{-1}\) NaHCO\(_3\) and containing 2 g L\(^{-1}\) BSA, 0.9 g L\(^{-1}\) D-glucose, 0.1 g L\(^{-1}\) sodium pyruvate, and 0.025 g L\(^{-1}\) soybean trypsin inhibitor, pH 7.4, at 37°C (Klinefelter, Laskey, and Roberts 1991).

**Sperm morphology**

The cauda sperm suspensions used for the motility assays were diluted 1 : 10 with 10% neutral buffered formalin in Dulbecco’s PBS with 5% sucrose, and the spermatozoa were evaluated for individual sperm morphology (Kempinas et al. 1998).

**Sperm viability**

The ratio of live:dead spermatozoa of the control and all treated groups of mice were determined using 1% trypan blue as described in the method of Talbot and Chacon (1981).

**Fertility test**

Conducted by cohabiting female animals with the control and treated male animals in a ratio of 2 : 1 according to the WHO Protocol MB-50 (1983).

**Hormone assays**

**Testosterone levels**

Blood collected at necropsy was held for 45 min at room temperature, and serum was obtained after centrifugation (3000 \(\times\) g, 15 min, 4°C) in centrifugation tubes (Vacutainer; Becton Dickinson, Rutherford, NJ). Serum was stored in a deep freezer (–20°C) until total
testosterone was assayed by radioimmunoassay (RIA) using a coat-A-Count Kit (Diagnostic Products Corp., Los Angeles, CA) (Klinefelter, Laskey, and Roberts 1991).

**In vitro testosterone production**

The testis of each animal was removed and decapsulated, and the parenchyma was sliced into approximate 50 mg pieces. Each piece was weighed and placed into a medium containing 1 mL Medium 199 (M199). The M199 was buffered with 0.71 g L\(^{-1}\) sodium bicarbonate (\(\text{NaHCO}_3\)) and 2.1 g L\(^{-1}\) HEPES, and contained 0.1% BSA (Schwartz-Mann, Orangeburg, NY) and 25 mg L\(^{-1}\) soybean trypsin inhibitor, pH 7.4. Testosterone production was assessed by incubating parenchyma in duplicate, either with or without maximal HCG stimulation (100 mIU mL\(^{-1}\)), for 2 h at 34°C. After centrifugation (5 min, 10,000 \(\times\) g), medium was frozen until testosterone assay.

**LH levels**

LH levels were analyzed by LH enzyme-linked immunosorbent assay (LH ELISA) Kit (ANOGEN, Ontario, Canada) (Goldman et al. 1986).

**Serum FSH levels**

FSH levels were measured by RIA using the standard NIDDK RP-2. The sensitivity for FSH assay is 0.8 ng mL\(^{-1}\). The coefficients of variation are 5.3 and 12.4% for the FSH assay. The kits are available from Diagnostic Systems Laboratories (Webster, TX).

**Statistics**

Data are expressed as mean \(\pm\) SD. Statistical comparison between different groups was done using one-way analysis of variance (ANOVA), followed by Tukey–Kramer multiple comparison test to judge the difference between various groups. Significance was accepted at \(p < 0.05\).

**Results**

While the animals appeared healthy and active throughout the experiment, the final body and testis weights were significantly decreased in the group administered NaF (group IIa) compared with control (Ia) (Table 1). The group not treated during withdrawal (group IIb) and the group treated with calcium during the withdrawal period (group IV) showed significant decreased final body and testis weights compared with control (Ib). In contrast, the group treated by vitamin E during the withdrawal period (group III) and the group treated with a combination of vitamin E and calcium during the withdrawal period (group V) showed no significant change in final body and testis weights (Ib). Groups treated by vitamin E and with a combination of vitamin E and calcium (groups III and V, respectively) displayed significant increase in final body and testis weights compared with the nontreated withdrawal group (IIb). The group treated with calcium alone (group IV) showed no significant change in final body and testis weights compared with the nontreated withdrawal group (IIb) (Table 2).

The weights of epididymis and vas deferens were significantly decreased in the group administered NaF (IIa) compared with control (Ia). The nontreated withdrawal group and
the groups treated with vitamin E, calcium, and combination during withdrawal period (groups III, IV, and V, respectively) showed significant decrease in the weights of epididymis and vas deferens compared with the control (Ib). The group treated with calcium during withdrawal period (group IV) displayed no significant change in the weights of epididymis and vas deferens compared with nontreated withdrawal group (IIb). In contrast, groups treated with vitamin E and combination of vitamin E and calcium during withdrawal period (groups III and IV, respectively) showed significant increase in the weights of epididymis compared with the nontreated withdrawal group (IIb) (Table 2).

In the control group, a pattern of spermatozoal motility from circular, in the caput epididymidis, to progressively forward, in the cauda epididymidis, was observed. This progression was reflected in the sperm motion parameters measured by CASA where sperm from the cauda epididymides had higher straight-line velocity and straightness. In contrast, treatment with NaF produced a significant decrease in the straight-line velocity, straightness, and linearity of sperm from the cauda epididymidis compared with control (Ia). There was also a significant decrease in % motile and progressively motile sperm (approximately 22 and 29%, respectively) from the cauda epididymides compared with

**Table 1. Effect of chronic F1 toxicity on various parameters.**

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Group Ia</th>
<th>Group IIa</th>
</tr>
</thead>
<tbody>
<tr>
<td>Final body weight (g)</td>
<td>194.98 ± 4.72</td>
<td>183.22 ± 7.09*</td>
</tr>
<tr>
<td>Weight of the testis (mg)</td>
<td>1692.60 ± 21.76</td>
<td>1518.90 ± 23.20*</td>
</tr>
<tr>
<td>Weight of the epididymis (mg)</td>
<td>511.03 ± 8.67</td>
<td>486.23 ± 3.16*</td>
</tr>
<tr>
<td>Weight of the vas deferens (mg)</td>
<td>115.00 ± 2.21</td>
<td>105.41 ± 2.78*</td>
</tr>
<tr>
<td>Velocity parameters</td>
<td></td>
<td></td>
</tr>
<tr>
<td>VAP (µm s⁻¹)</td>
<td>156.77 ± 1.40</td>
<td>157.89 ± 1.17</td>
</tr>
<tr>
<td>VSL (µm s⁻¹)</td>
<td>102.00 ± 2.09</td>
<td>71.20 ± 2.81</td>
</tr>
<tr>
<td>VCL (µm s⁻¹)</td>
<td>352.01 ± 1.60</td>
<td>350.52 ± 1.30</td>
</tr>
<tr>
<td>Head motion parameters</td>
<td></td>
<td></td>
</tr>
<tr>
<td>ALH (µm)</td>
<td>22.03 ± 0.81</td>
<td>22.19 ± 0.70</td>
</tr>
<tr>
<td>BCF (Hz)</td>
<td>21.98 ± 1.00</td>
<td>22.29 ± 0.71</td>
</tr>
<tr>
<td>Derived parameters</td>
<td></td>
<td></td>
</tr>
<tr>
<td>STR (%)</td>
<td>66.98 ± 1.11</td>
<td>58.68 ± 1.24*</td>
</tr>
<tr>
<td>LIN (%)</td>
<td>32.89 ± 1.67</td>
<td>25.46 ± 0.75*</td>
</tr>
<tr>
<td>Sperm motility</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Motile sperm (%)</td>
<td>95.00 ± 4.90</td>
<td>72.45 ± 2.89*</td>
</tr>
<tr>
<td>Progressively motile sperm (%)</td>
<td>84.22 ± 4.02</td>
<td>59.45 ± 3.46*</td>
</tr>
<tr>
<td>Sperm count (x10⁶ per testis)</td>
<td>175.22 ± 7.59</td>
<td>92.60 ± 5.44*</td>
</tr>
<tr>
<td>Normal shaped sperm (%)</td>
<td>90.68 ± 6.05</td>
<td>84.28 ± 1.99*</td>
</tr>
<tr>
<td>Sperm with tail defects (%)</td>
<td>4.92 ± 8.92</td>
<td>12.15 ± 1.64*</td>
</tr>
<tr>
<td>Sperm with head defects (%)</td>
<td>3.00 ± 0.12</td>
<td>3.57 ± 0.37*</td>
</tr>
<tr>
<td>Sperm viability (live: died) (%)</td>
<td>77.72 ± 4.08 : 20.20</td>
<td>43.21 ± 3.02 : 9.93</td>
</tr>
<tr>
<td></td>
<td>20.20 ± 1.89</td>
<td>9.93 ± 1.71*</td>
</tr>
<tr>
<td>Fertility rate (%)</td>
<td>90.33 ± 2.45</td>
<td>35.40 ± 4.09*</td>
</tr>
<tr>
<td>Parenchymal testosterone (ng mg⁻¹)</td>
<td>523.67 ± 31.89</td>
<td>335.93 ± 20.96*</td>
</tr>
<tr>
<td>Serum testosterone (ng mL⁻¹)</td>
<td>4.01 ± 0.76</td>
<td>1.11 ± 0.11*</td>
</tr>
<tr>
<td>Serum LH (ng mg⁻¹)</td>
<td>0.25 ± 0.06</td>
<td>0.08 ± 0.24</td>
</tr>
<tr>
<td>Serum FSH (ng mL⁻¹)</td>
<td>6.08 ± 0.12</td>
<td>7.02 ± 0.24*</td>
</tr>
</tbody>
</table>

Notes: Average-path velocity (VAP), straight-line velocity (VSL), curvilinear velocity (VCL), amplitude of lateral head displacement (ALH), beat cross frequency (BCF), straightness (STR), Linearity (LIN). Values represent mean ± SD (n = 10). *Significant from control group Ia, p < 0.05.
control (Ia) (Table 1). At the end of the withdrawal phase, the nontreated withdrawal group (IIb) and the group treated with calcium (IV) displayed significant decrease in the straight-line velocity and straightness of sperms from the cauda epididymidis compared with control (Ib). The straight-line velocity of sperms from the groups treated with vitamin E, calcium, and a combination (groups III, IV, and V, respectively) during the withdrawal period showed significant increase compared with the nontreated withdrawal group (IIb). Groups treated with vitamin E and a combination of vitamin E and calcium displayed significant rise and the group treated with calcium (IV) showed no marked change in straightness of sperms compared with the nontreated withdrawal group (IIb). Linearity of sperms of the groups treated with vitamin E, calcium, a combination of vitamin E and calcium and nontreated withdrawal (III, IV, V, and IIb respectively) showed significant reduction compared with control (Ib). Average-path velocity, curvilinear velocity, amplitude of lateral head displacement, and beat cross frequency showed no marked differences in all experimental groups (nontreated and treated groups) compared with control (Ib) and nontreated withdrawal group (IIb). Sperm motility (% motile and progressively motile sperms) displayed significant fall in the nontreated withdrawal group, groups treated by vitamin E, calcium, or a combination of vitamin E and calcium (III, IV, and V, respectively) compared with control Ib. Groups treated with vitamin E, calcium, or a combination of vitamin E and calcium (III, IV, and V, respectively) showed significant elevation in sperm motility (% motile and progressively motile sperms) compared with nontreated withdrawal group (Ib) (Table 3).

The present study showed that NaF treatment led to significant reduction in sperm count compared with control (Ia) (Table 1). Groups (I Ib, III, IV, and V) not treated during withdrawal, treated with vitamin E, calcium, or a combination of vitamin E and calcium during the withdrawal period showed significant decrease in sperm count compared with control (Ib). Groups treated with vitamin E and a combination of vitamin E and calcium during the withdrawal period displayed a marked increase and in the group treated with calcium there was no change in the sperm count compared with the nontreated withdrawal group (IIb) (Table 4).

The % of normally shaped sperms showed significant decrease in the group administered NaF (IIa) compared with control (Ia). The nontreated withdrawal group (IIb) and the group treated with calcium during withdrawal period (IV) displayed a significant fall and in groups treated with vitamin E or treated with a combination of

### Table 2. Effect of chronic Fl toxicity on final body weight, testis weight, epididymis weight, and vas deferens weight.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Final body weight (g)</th>
<th>Weight of the testis (mg)</th>
<th>Weight of the epididymis (mg)</th>
<th>Weight of the vas deferens (mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group Ib (control)</td>
<td>211.45 ± 8.04</td>
<td>1721.60 ± 25.61</td>
<td>574.93 ± 6.64</td>
<td>119.54 ± 2.41</td>
</tr>
<tr>
<td>Group IIb (withdrawal)</td>
<td>195.98 ± 4.61</td>
<td>1623.50 ± 34.56</td>
<td>489.82 ± 4.39</td>
<td>106.16 ± 3.20</td>
</tr>
<tr>
<td>Group III (vitamin E)</td>
<td>217.80 ± 7.05</td>
<td>1699.00 ± 11.50</td>
<td>503.39 ± 4.42</td>
<td>108.74 ± 2.75</td>
</tr>
<tr>
<td>Group IV (calcium)</td>
<td>196.12 ± 5.60</td>
<td>1639.50 ± 42.52</td>
<td>492.33 ± 5.26</td>
<td>107.10 ± 3.06</td>
</tr>
<tr>
<td>Group V (Vitamin E and calcium)</td>
<td>221.88 ± 6.45</td>
<td>1707.50 ± 14.77</td>
<td>511.67 ± 5.75</td>
<td>109.67 ± 1.92</td>
</tr>
</tbody>
</table>

Notes: Values represent mean ± SD (n = 10).

\( ^a \)p < 0.05 vs. control group Ib.

\( ^b \)p < 0.05 vs. withdrawal group IIb.
Table 3. Effect of chronic Fl toxicity on motion parameters of cauda epididymal sperm.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Velocity parameters</th>
<th>Head motion parameters</th>
<th>Derived parameters</th>
<th>Sperm motility</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>VAP ($\mu$m s(^{-1}))</td>
<td>VSL ($\mu$m s(^{-1}))</td>
<td>STR (%)</td>
<td>STR (%)</td>
</tr>
<tr>
<td>Group I (control)</td>
<td>158.49 ± 0.93</td>
<td>101.65 ± 1.05</td>
<td>76.72 ± 0.91</td>
<td>67.72 ± 0.91</td>
</tr>
<tr>
<td>Group IIb (withdrawal)</td>
<td>157.94 ± 0.90</td>
<td>76.81 ± 5.02(^a)</td>
<td>62.03 ± 0.90(^a)</td>
<td>62.03 ± 0.90(^a)</td>
</tr>
<tr>
<td>Group III (vitamin E)</td>
<td>156.45 ± 2.80</td>
<td>101.10 ± 0.76(^b)</td>
<td>67.14 ± 0.86(^b)</td>
<td>67.14 ± 0.86(^b)</td>
</tr>
<tr>
<td>Group IV (calcium)</td>
<td>157.34 ± 1.72</td>
<td>89.58 ± 1.80(^ab)</td>
<td>62.45 ± 1.18(^a)</td>
<td>62.45 ± 1.18(^a)</td>
</tr>
<tr>
<td>Group V (vitamin E and calcium)</td>
<td>157.43 ± 1.25</td>
<td>100.56 ± 1.01(^b)</td>
<td>66.86 ± 0.77(^b)</td>
<td>66.86 ± 0.77(^b)</td>
</tr>
</tbody>
</table>

Notes: Average-path velocity (VAP), straight-line velocity (VSL), curvilinear velocity (VCL), amplitude of lateral head displacement (ALH), beat cross frequency (BCF), straightness (STR), Linearity (LIN). Values represent mean ± SD ($n = 10$).

\(^a^p < 0.05\) vs. control group Ib.

\(^b^p < 0.05\) vs. withdrawal group IIb.
Table 4. Effect of chronic Fl toxicity on sperm parameters and fertility rate.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Sperm count ($\times 10^6$ per testis)</th>
<th>Normal shaped sperm (%)</th>
<th>Sperm with tail defects (%)</th>
<th>Sperm with head defects (%)</th>
<th>Sperm viability (live : died) (%)</th>
<th>Fertility rate (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group I (control)</td>
<td>175.19 ± 6.54</td>
<td>92.91 ± 2.50</td>
<td>4.96 ± 0.73</td>
<td>2.42 ± 0.38</td>
<td>75.96 ± 3.19 : 23.34 ± 0.94</td>
<td>96.50 ± 1.65</td>
</tr>
<tr>
<td>Group IIb (withdrawal)</td>
<td>120.25 ± 4.94&lt;sup&gt;a&lt;/sup&gt;</td>
<td>89.35 ± 1.26&lt;sup&gt;a&lt;/sup&gt;</td>
<td>9.39 ± 1.47&lt;sup&gt;a&lt;/sup&gt;</td>
<td>3.60 ± 0.32&lt;sup&gt;a&lt;/sup&gt;</td>
<td>52.78 ± 3.55&lt;sup&gt;a&lt;/sup&gt; : 36.64 ± 0.97</td>
<td>53.88 ± 6.03&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Group III (vitamin E)</td>
<td>163.28 ± 9.00&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>90.69 ± 2.35&lt;sup&gt;b&lt;/sup&gt;</td>
<td>4.98 ± 0.82&lt;sup&gt;b&lt;/sup&gt;</td>
<td>2.81 ± 0.57&lt;sup&gt;b&lt;/sup&gt;</td>
<td>59.35 ± 1.55&lt;sup&gt;ab&lt;/sup&gt; : 30.53 ± 2.44&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>86.60 ± 6.16&lt;sup&gt;ab&lt;/sup&gt;</td>
</tr>
<tr>
<td>Group IV (calcium)</td>
<td>117.36 ± 6.78&lt;sup&gt;a&lt;/sup&gt;</td>
<td>88.93 ± 1.57&lt;sup&gt;a&lt;/sup&gt;</td>
<td>9.46 ± 1.08&lt;sup&gt;a&lt;/sup&gt;</td>
<td>3.27 ± 0.44&lt;sup&gt;a&lt;/sup&gt;</td>
<td>52.46 ± 1.86&lt;sup&gt;a&lt;/sup&gt; : 030.49 ± 1.76&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>55.48 ± 6.12&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Group V (vitamin E and calcium)</td>
<td>163.15 ± 6.67&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>92.75 ± 1.66&lt;sup&gt;b&lt;/sup&gt;</td>
<td>4.85 ± 0.71&lt;sup&gt;b&lt;/sup&gt;</td>
<td>2.66 ± 0.76&lt;sup&gt;b&lt;/sup&gt;</td>
<td>69.76 ± 1.2&lt;sup&gt;ab&lt;/sup&gt; : 25.47 ± 1.12&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>84.94 ± 4.24&lt;sup&gt;ab&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Notes: Values represent mean ± SD ($n = 10$).
<sup>a</sup><sup>p</sup> < 0.05 vs. control group Ib.
<sup>b</sup><sup>p</sup> < 0.05 vs. withdrawal group IIb.
vitamin E and calcium during withdrawal period (III and V, respectively) there was no change in % of normally shaped sperms compared with control (Ib). Groups treated with vitamin E or a combination of vitamin E and calcium during withdrawal period showed significant elevation while in the group treated with calcium (IV) there was no marked change in % of normally shaped sperms compared with the nontreated withdrawal group (IIb) (Table 4).

There was a marked increase in sperm with either an abnormal head or an abnormal tail in the group administered NaF (IIa) compared with control (Ia). In rats not treated during withdrawal and those treated with calcium during withdrawal period (IIb and IV, respectively) there was a significant rise in abnormalities of the tail and head of sperms. Groups treated with vitamin E and a combination of vitamin E and calcium during withdrawal period showed no marked change in abnormalities of the tail and head of sperms compared with control (Ib). In contrast, in groups treated with vitamin E and a combination of vitamin E and calcium during withdrawal period (III and IV, respectively) there was a significant fall in abnormalities of the tail and head of sperms but no significant change was observed in the group treated with the vitamin E during withdrawal period compared with the nontreated withdrawal group (IIb) (Table 4).

Cauda epididymal sperm viability (live:dead ratio) displayed significant decrease in rats administered NaF (IIa) compared with control (Ia). However, significant reduction in sperm viability was obtained in the nontreated withdrawal, treated with vitamin E, calcium, and a combination of vitamin E and calcium groups during the withdrawal period (IIb, III, IV, and V). In contrast, in groups treated with vitamin E, calcium, and a combination of vitamin E and calcium during the withdrawal period there was a significant increase in sperm viability compared with the nontreated withdrawal group (IIb) (Table 4).

The sodium fluoride-treated group (IIa) showed significant reduction in fertility rate compared with control (Ia). In the groups treated with vitamin E, calcium, and a combination of vitamin E and calcium during withdrawal period and those not treated during withdrawal (IIb, III, IV, and V) a significant decrease in fertility rate was noted. Groups treated with vitamin E and a combination of vitamin E and calcium during withdrawal period displayed significant rise, but there was no marked change in fertility rate in the group treated with calcium compared with the nontreated withdrawal group (IIb) (Table 4).

The parenchymal (with or without maximal HCG stimulation) and serum testosterone levels in the NaF-treated group (IIa) were significantly decreased (Table 1). Groups treated with vitamin E, calcium, and a combination of vitamin E and calcium during the withdrawal period and the nontreated withdrawal group (III, IV, V, and IIb, respectively) showed significant decrease in the levels of parenchymal (with or without maximal HCG stimulation) and serum testosterone levels. In groups treated with vitamin E and a combination of vitamin E and a calcium during the withdrawal period (III and V, respectively) there was a significant increase but in the group treated with calcium (IV) no significant change in their parenchymal (with or without maximal HCG stimulation) and serum testosterone levels were found compared with the nontreated withdrawal group (IIb) (Table 5).

Sodium fluoride administration did not significantly affect levels of serum LH (Ia) (Table 1). All rats treated with vitamin E, calcium, a combination of vitamin E and calcium during withdrawal period and the nontreated withdrawal groups (III, IV, V, and IIb) showed no significant change in levels of serum LH (Table 5).
This study revealed that serum FSH levels in NaF-treated rats were significantly increased (Ia) (Table 1). Nontreated withdrawal and treatment with calcium groups showed significant increase in the levels of in serum FSH (Ib). In groups treated with vitamin E and a combination of vitamin E and calcium during withdrawal period there was no significant change in the FSH levels. However, a significant decrease in serum FSH was noted compared with the nontreated withdrawal group (IIb). The group treated with calcium (IV) showed no significant change in serum FSH compared with the nontreated withdrawal group (IIb) (Table 5).

**Discussion**

In the present study, body weight was significantly decreased in the NaF-treated group (IIa), while in the group treated with vitamin E during the withdrawal period (III) there was no marked change in body weight. These findings support that reported by Verma and Guna-Sherlin (2002) who reported that, NaF produced significant reduction in body weight and feed consumption as well as serum concentrations of glucose and protein in rats. Verma and Guna-Sherlin (2002) also found that administration of vitamin E produced significant amelioration in body weight and feed consumption, as well as in serum glucose, protein, sodium, and potassium levels.

In the present work, the weights of testis in the NaF-treated group were decreased significantly. Ghosh et al. (2002) also found that NaF treatment at 20 mg kg\(^{-1}\) day\(^{-1}\) for 29 days resulted in significant reduction in the relative wet weight of the testis. These results may be associated with depletion of the testicular structural, nuclear, and total proteins (Shashi and Kaur 1992).

Results of the present study showed significant decrease in weight of the epididymis in NaF-treated rats. The weight of the epididymis significantly increased after administration of vitamin E during the withdrawal period. Chinoy and Sharma (1998) also noted that NaF treatment resulted in significant decrease in the epididymal weight due to significant metabolic alterations and vitamin E was beneficial in restoration of epididymal weight.

In reproductive toxicology studies, altered sperm motility is a valuable indicator of toxicity arising from a prominent effect on the epididymis or sperm within the epididymis. In the current study, treatment with NaF produced a significant fall in % of motile sperms.
from the cauda epididymidis. At the end of the withdrawal phase, there was a significant increase in % of motile sperms from the cauda epididymidis compared with NaF alone (IIa) and significant decrease compared with control (Ib) suggesting a partial recovery. These results were in agreement with Narayana and Chinoy (1994) and Pushpalatha, Srinivas, and Sreenivasula Reddy (2005) who found altered lysosomal enzyme activity and glutathione levels together with morphologic anomalies resulted in a significant decline in sperm motility following NaF treatment. Chinoy and Sharma (1998) demonstrated that NaF treatment produced alterations in epididymal milieu as evidenced by 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. Narayana and Chinoy (1994) also found that withdrawal from NaF treatment produced partial recovery. Groups treated with calcium during the withdrawal period (IV) showed significant increase in % of motile sperms from the cauda epididymidis compared with the nontreated withdrawal group (IIB). Wennemuth, Babcock, and Hille (2003) postulated that calcium is considered a regulator of sperm motility, a participant in capacitation, and an essential second messenger for the acrosome reaction. The present study demonstrated that in the group treated with vitamin E during the withdrawal period a significant increase in motile sperms from the cauda epididymidis was noted compared with the nontreated withdrawal group (IIB). Chinoy and Sharma (1998) found that vitamin E played a beneficial role in reversibility of reduction in sperm motility induced by NaF treatment.

This study showed that NaF treatment led to significant decrease in sperm count. These results are in agreement with the study of Pushpalatha, Srinivas, and Sreenivasula Reddy (2005). The findings that the activity levels of testicular steroidogenic marker enzymes; 3beta-hydroxysteroid dehydrogenase (3beta-HSD) and 17beta-hydroxysteroid dehydrogenase (17beta-HSD) were significantly decreased in NaF-treated rats indicate decreased steroidogenesis and in turn spermatogenesis may explain our observations. Cauda epididymal sperm viability (live : dead ratio) was significantly reduced in the NaF-treated group. Chinoy and Sharma (1998) and Pushpalatha, Srinivas, and Sreenivasula Reddy (2005) also reported that NaF induced reduction in sperm viability. There was an increase in sperm with either an abnormal head or an abnormal tail in the NaF-treated rats. Pushpalatha, Srinivas, and Sreenivasula Reddy (2005) also found that NaF induced an increase in sperm abnormalities.

Sodium fluoride treatment produced significant reduction in fertility rate. Groups treated with vitamin E (III) and a combination of vitamin E and calcium (V) during the withdrawal period showed significant increase in fertility rate compared with the nontreated withdrawal group (IIB) but in rats there was a significant decrease compared with control group (IIa). These results indicate partial recovery. Chinoy and Sharma (1998) found that NaF treatment produced alterations in epididymal milieu as evidenced by significant decrease in levels of sialic acid and protein as well as activity of ATPase. As a result, the sperm maturation process was affected leading to a significant decline in cauda epididymal sperm motility and viability. This resulted in a significant reduction in fertility rate. Chinoy and Sharma (1998) also reported that withdrawal of NaF treatment (30 days) produced partial recovery. On the other hand, supplementation with vitamin E during the withdrawal period in NaF-treated mice was found to be beneficial in recovery from all NaF-induced effects, thus suggesting an ameliorative role in recovery from toxic effects of NaF on reproductive functions and fertility.

Our study revealed that serum testosterone levels in the NaF-treated group (IIa) were significantly reduced compared with control (Ia). Ortiz-Perez et al. (2003) reported
reduction of serum testosterone levels after exposure to F1 and subsequent F1-induced adverse effects on both Sertoli cells and gonadotrophins. In the group treated with vitamin E during the withdrawal period there was a significant increase in the levels of parenchymal (with or without maximal HCG stimulation) and serum testosterone compared with the nontreated withdrawal group (IIb). Our result is in agreement with that of Chris (1982) who found that vitamin E supplements led to elevation in the testosterone levels (testicular tissue and plasma).

In the present study, all experimental treated groups showed no significant change in the levels of serum LH. Tokar and Savchenko (1977) found that blood LH levels were increased only in those patients with fluorosis who had long contact with F1 compounds (over 15 years). Sprando et al. (1997) also reported that in rats administered drinking-water containing 0, 25, 100, 175, or 200 mg NaF L$^{-1}$ for 14 weeks no changes in LH were noted. This study showed that serum FSH levels in the NaF-treated group (IIa) were significantly increased compared with control group (Ia). The present findings were in agreement with the findings of Ortiz-Perez et al. (2003). Data indicate that calcium and vitamin E together exert an ameliorative effect on NaF-induced fertility impairment in male albino rats.

References


