

Evaluation of the effect of vitamin D on sodium fluoride-induced toxicity in reproductive functions of male rabbits

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ABSTRACT

Background: Fluorosis is an endemic problem in many countries of the world. **Aims and Objectives:** To see the effect of fluoride on the reproductive system and to see the role, if any, of vitamin D analog paricalcitol on it. **Materials and Methods:** Sixty rabbits were divided into four equal groups. Group I was fed on standard diet, Group II vehicle-treated control, Group III on sodium fluoride (NaF) 20 mg/kg body weight, and Group IV on NaF + paricalcitol. **Result:** In Group III (fed on NaF) significant decrease in sperm count ($P < 0.001$) and motility ($P < 0.001$) was seen compared with control. With vitamin D analog paricalcitol, there was a significant improvement in the sperm count ($P < 0.001$) and motility ($P < 0.01$). All of these significant changes were evident by histopathological evaluation of testicular biopsies. **Conclusion:** Vitamin D analog paricalcitol treatment showed a significant improvement in reproductive functions.

KEY WORDS: Fluoride; Paricalcitol; Sperm; Histopathological Evaluation

INTRODUCTION

Fluorosis is an endemic public health problem in many nations around the world. The guideline of World Health Organization is that 1.5 ppm of fluoride is the desirable upper limit in drinking water and the recommended levels are 0.5 to 0.8 mg/L.^[1] Extensive data on skeletal fluorosis are available.^[2] However, the effect of fluoride on the structure and metabolism of several soft tissues has been reported recently and convincing evidence from fluorosis patient is now available to demonstrate the damage or involvement of human spermatozoa.^[3] Chinoy and Sequeira^[4] reported that sodium fluoride (NaF) treatment in mice caused

alteration in histology of reproductive organs, morphology of sperm, and induced biochemical changes. In fluoride-treated male rats there was a decrease in sperm motility.^[5] In an Indian study, infertility was reported among married men in a highly endemic area with fluoride concentration up to 38.5 mg/L.^[6] A significant diminution of relative weight of the testis, prostate, and seminal vesicle, without alteration in the body weight occurs with NaF treatment in a dose of 20 mg/kg/day for 29 days.^[7] In a study performed by Susheela and Kumar,^[8] it was seen that chronic fluoride toxicity caused regression of seminiferous tubule and cessation of the spermatogenesis in the rabbit. Fluoride treatment leads to oxidative stress as indicated by an increased level of conjugated dienes in the testis, epididymis, and epididymal sperm with respect to control.^[9] Vitamin D hormone plays an important role in reproduction.^[10,11] Sood et al.^[12] suggested that deficiency of vitamin D produces retardation of spermatogenesis because of disturbances in sertoli cell functions, and these changes are reversible and can be corrected by supplementing an optimal dose of vitamin D.^[11] Vitamin D is believed to exert its protective

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effect primarily through destruction of free radical oxygen species.^[12,13] Chinoy et al.^[14] reported that the supplementation of vitamin D during the withdrawal period of NaF-treated mice was found to be very beneficial in recovery of all NaF-induced effects on the reproductive functions and fertility. However, not many studies are available to show the effect of vitamin D in fluorosis.^[15] Considering that fluorosis is a public health issue and the very fact that the fluoride exposure has a definite effect on reproduction, the following study was planned to observe the effect of fluoride on the sperm count, motility, and histopathological evaluation of testicular changes and to evaluate the ameliorative role of vitamin D analog paricalcitol on testicular functions.

MATERIALS AND METHODS

This study was conducted in the department of pathology. Sixty healthy, adult New Zealand white male rabbits were used for the study. All the rabbits were of same age group, with weight range of 1.5–2.5 kg. The rabbits were housed in a well-ventilated animal house and caged separately, at a temperature of 29°C and 32°C and exposed to 10 to 12 h of daylight. They received food, water ad libitum. The rabbits were divided into four equal groups of 15 each. NaF was given using a feeding tube attached to a hypodermic needle in the dose of 20 mg/kg body weight/day.^[16] The rabbits were given vitamin D in the a dose of 1000 IU/kg body weight/day in addition to normal diet for 28 days and were sacrificed on the 29th day.^[17,18] These doses were based on previous studies.^[13]

–Group I: (Untreated control) rabbits were maintained on standard diet and water ad libitum.

–Group II: (Vehicle-treated control) rabbits were fed on olive oil and vitamin D dissolved.

–Group III: Rabbits were given NaF for 28 days.

–Group IV: Rabbits were given NaF with vitamin D for 28 days.

The control and the test groups were anesthetized with intravenous injection of urethane (0.5–1.5 g/kg of body weight). Incision was made on the scrotum of the rabbit and the testis and epididymis was carefully exposed, removed, and were subjected to the following biochemical and histopathological studies.

Biochemical Examination (Sperm Count and Sperm Motility)

From each separated epididymis, the cauda part was removed and placed in a beaker containing 10 mL diluting solution (sodium bicarbonate 5 g and formalin neutral 1 mL in 100 mL of distilled water). Each section was quickly macerated with a pair of sharp scissors and left for a few minutes to liberate its spermatozoa into the solution. Sperm count was done under the microscope using a new improved Neubauer Hemocytometer and the sperm count was calculated. Semen drop was placed on the slide and two drops of warm 2.9% sodium citrate was added. The slide was covered with a coverslip and examined under the microscope using a 40 × objective for sperm motility.^[19]

Histopathological Examination

Testis was removed after being freed from surrounding tissue. The tissue was kept in Bouin's solution for fixation. After 1 week,

Table 1: Sperm count and motility in different groups of study

Parameters	Sperm count × 10 ⁶	Sperm motility (%)
Group I (control)	165.77 ± 8.76***	75.21 ± 3.64***
Group II (vehicle treated)	161.44 ± 9.35	71.82 ± 4.64
Group III (NaF fed)	125.64 ± 12.86**&ast	58.62 ± 9.72***
Group IV (NaF + Vit D)	148.63 ± 6.22 ^{a,b,*****}	68.42 ± 3.43 ^{a**}

NaF, sodium fluoride.

** means statistically significant value.

*** means more significant.

(a): means in relation to group 2.

(b): means in relation to group 3.

the tissue was washed for 24 h under running tap water, then dehydrated through ascending grades of alcohol, cleared in xylene, and embedded and blocked in paraffin. Sections of 4–5 µm thickness were taken and stained with hematoxylin and eosin (H&E), and were examined under the microscope.

RESULT

Table 1 shows that the sperm count of Group III NaF-treated mice was significantly reduced compared with control Group I ($P < 0.001$). Group III showed a significant decrease in sperm motility ($P < 0.001$) as compared with the control Group I. In Group IV (NaF with Vitamin D), there was a significant improvement and recovery ($P < 0.01$) that was near normal.

Group I showed normal spermatogenesis with some spermatozoa attached to the inner aspect of the lumen of the seminiferous tubules (H&E at 400 ×) (Figure 1).

Group II showed normal spermatogenesis with different stages of differentiation and maturation (H&E x 400) (Figure 2).

Group III showed evidence of loss of differentiation and maturation of spermatocytes with few mature spermatozoa and necrotic sloughs (H&E at 400 ×) (Figure 3).

Group IV showed improvement in the spermatogenic activity and maturation with excess mature spermatozoa in the lumen of tubules (H&E at 400 ×) (Figure 4).

DISCUSSION

The observation of receptor site for 1,25-dihydroxyvitamin D₃ (1,25 [OH]₂ D₃) in both the seminiferous tubules as well as interstitial tissue in the testis of adult rats and also in human testis together with an increase in receptor level was correlated with testicular maturity that demonstrated the possible effect of vitamin D in testicular function.^[20] Also vitamin D helps in the absorption of calcium, which is an ideal antidote for fluoride because of its binding with fluoride to form insoluble complex calcium fluoride. Moreover, Ca⁺⁺ helps in the activation of some enzymes in the sperm.^[21] Fluoride toxicity affects spermatogenesis through different pathways, by directly inhibiting androgen receptor (AR) mRNA expression in sertoli cells and causing decrease in AR through which testosterone acts, also by reducing testicular zinc levels

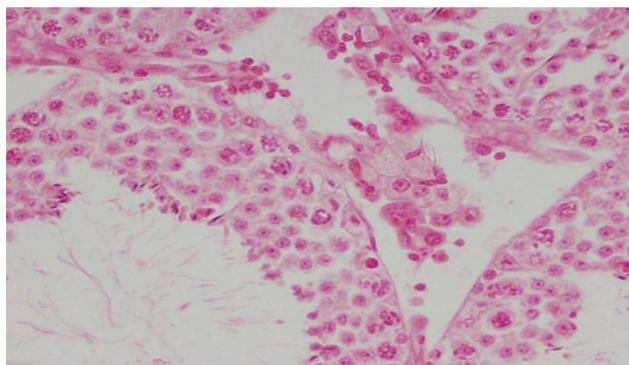


Figure 1: Group I showed normal spermatogenesis with some spermatozoa attached to the inner aspect of the lumen of the seminiferous tubules (H&E x 400).

that impair the angiotensin-converting enzyme activity, and by reducing testosterone levels by diminishing positive signals for its production.^[22] Other mechanism factors responsible for the arrest of spermatogenesis might be the lack of available proteins necessary for cell division, growth, and differentiation of germ cells and increased levels of oxidants that damage DNA.^[23] As per this study, there is a significant decrease in the sperm count as compared with the control group on exposure to 20 mg/kg body weight NaF for 28 days. Our observations were similar to the studies conducted by Salo et al.^[19] and China et al.^[21] who reported that there was a decline in sperm count as compared with control in rats exposed to NaF 10 mg/kg body weight for 50 days or with 10 mg/kg fluoride for 30 days in male mice, respectively. This study also showed a significant ($P < 0.01$) improvement in the sperm count when fluoride was combined with vitamin D compared with NaF-treated Group III. In another study, Sood et al.^[20] reported a significant decrease in sperm count in vitamin D-deficient rats as compared with the control, and injection of vitamin D improved sperm count after 1 month.

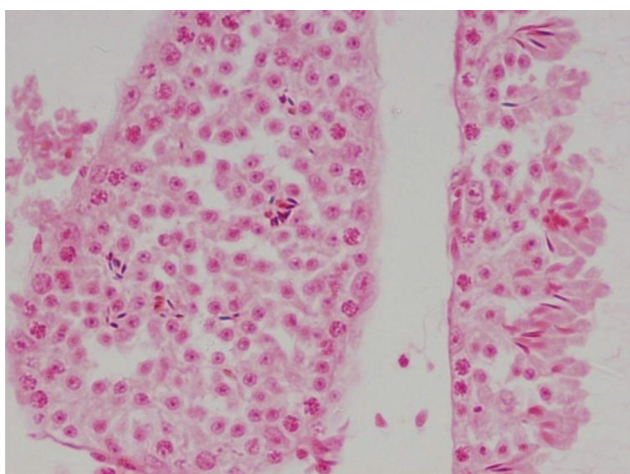


Figure 2: Group II - shows normal spermatogenesis with different stages of differentiation and maturation (H&E x 400).

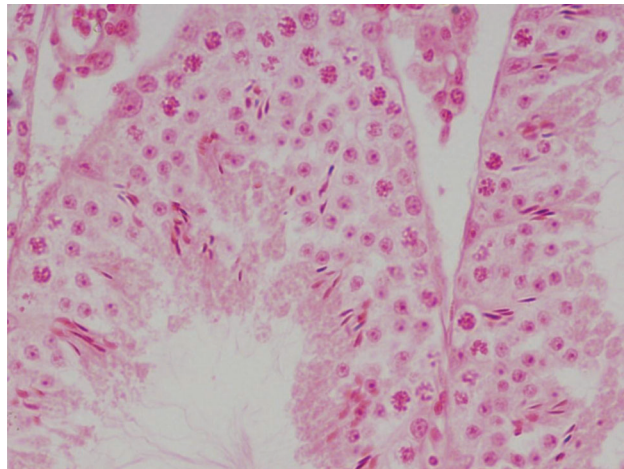


Figure 3: Group III shows evidence of loss of differentiation and maturation of spermatocytes with few mature spermatozoa & necrotic sloughs (H&E x 400).

In this study, there was a significant decrease in sperm motility ($P < 0.001$) in group fed with fluoride 20 mg/kg for 28 days as compared with control. Huang et al.^[24] reported a significant reduction in sperm motility of mice fed on 100, 200, and 300 mg NaF/L for 8 weeks as compared with the control group. Chinoy and Sharma^[25] reported that in group fed on vitamin D during the withdrawal period of 30 days after exposure to 10 mg/kg body weight NaF for the same period showed a statistically significant improvement in sperm motility as compared with control. Effect of fluoride on the metabolism and motility in vitro had also been demonstrated, the sperms became immotile within 2 min at 30°C. Similarly, human spermatozoa lost their motility in vitro in the presence of 250 mM fluoride within 20 min of exposure. However, it has been postulated that fluoride could act directly on motile apparatus without affecting other metabolic systems, also fluoride binds

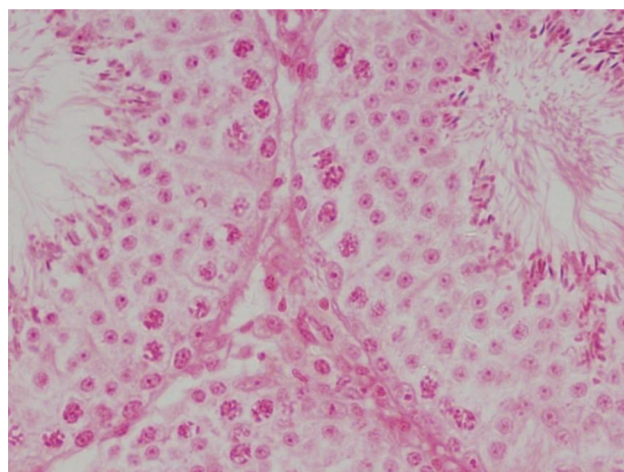


Figure 4: Group IV shows improvement in the spermatogenic activity & maturation with excess mature spermatozoa in the lumen of tubules. (H&E x 400).

with cofactors such as Mg, Ca, Zn, and Se, thus inhibiting glycolysis, respiration, and motility of the sperms. Thus, there could be decline in fructose level because of the alteration in carbohydrate metabolism after fluoride treatment.^[26] Another reason for decreased sperm motility was decreased level of androgen carrier proteins involved in sperm motility.^[23] There was a lack of differentiation and maturation of spermatocytes with marked inflammatory infiltration in the interstitial tissue with no mature spermatozoa detected in the lumens of the seminiferous tubules in Group III (fluoride 20 mg/kg for 30 days) as compared with various stages of differentiation and maturation seen in detected spermatozoa attached in the control group and group IV (fed on NaF and Vitamin D analog paricalcitol). Our results were similar to earlier studies, which showed that 28 days of treatment with NaF (10 mg/kg body weight) in mice resulted in sloughing off of the spermatogenic cells in the lumen of seminiferous tubules of the testes leading to their epithelial disorganization and complete absence of spermatogenesis.^[27] Opposite to this Sprando *et al.*^[28] showed that rats fed on 250 ppm fluoride for 10 weeks showed no distinguishable change in testicular histology from their control group. Histopathological evaluation of testis supported the improvement in the spermatogenic activity in Group IV compared with the Group III (fed on fluoride for 30 days). Therefore, we concluded that vitamin D analog paricalcitol treatment had a better prospective effect in ameliorating fluoride-induced reproductive injury.

CONCLUSION

Vitamin D analog paricalcitol treatment showed a significant improvement in reproductive functions.

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