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Prevention of fluoride-induced testicular disorders in male Sprague-Dawley rats by co-administration of Vitamin E and/or ginger oil.

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ABSTRACT

Elbohi KM, Abd El-Rahman SS**. Prevention of fluoride-induced testicular disorders in male Sprague-Dawley rats by co-administration of Vitamin E and/or ginger oil. Online J Vet Res 13(1):1-11, 2009.* This study was conducted to investigate the ameliorative influence of vitamin E and ginger oil on the adverse effects of sodium fluoride (NaF), a water pollutant that really existed in many areas of the world on male fertility parameters. Five groups of male Sprague-Dawley rats were used. Rats of Group 1 (G1) were kept as control and the other four groups were treated with 20mgNaF/kg/day for 28 days by oral gavage. Rats of group 2 (G2) was kept on NaF treatment only. While, the other three groups were treated as follow; Vitamin E co-administration at a dose of 20mg/100g/day, ginger oil co-administration at a dose of 20 ml/kg/d and both Vitamin E and ginger oil co-administration at the same doses for groups G3, G4, G5 respectively. Results demonstrate that fluoride-induced histological alterations in male genital organs including inhibition of both spermatogenesis and spermiation, necrosis of spermatogonial cells, abnormal spermatid forms and spermatid giant cell appearance. Sperm count as well as sperm motility declined significantly ($p<0.001$).Significant reduction ($P< 0.001$) in sperm viability and a high percent of abnormal spermatozoa were also observed. Moreover, significant decrease ($P<0.001$) in serum and testicular testosterone levels was noticed. Co-administration of Vitamin-E and ginger oil with fluoride resulted in a significant protection from induced testicular disorders, especially when co-administrated together. It was concluded that, fluoride exposure led to inhibition of testicular gametogenesis and steroidogenesis which were protected significantly by dietary agents like Vitamin-E and ginger oil.

Key Words: fluoride toxicity, male fertility, ginger, vitamin E.

INTRODUCTION

Fluorine (F), a member of the halogen family, which includes chlorine, bromine and iodine, is a pale yellow gas which is extremely reactive combining with other elements to form fluorides. Fluorine is known as constituent of bones, teeth, soft tissues and body fluids. A high incidence of caries in humans has been correlated by many investigators to a low fluoride intake. So, in many countries fluoride is added into the main water supplies and this process is called fluorination. Hence, fluoridation of water supplies is practiced in many places in the hope of reducing the incidence of dental caries. The permissible levels of fluoride as recommended by WHO [1] are 1.5 mg/L. Also, Apha et al [2] mentioned that Fluoride is beneficial especially to young when present within permissible limits of 1.0 – 1.5 mg F litre-1 for calcification of dental enamel.
Although fluoride has pharmaceutical value, there are reports concerning fluoride-induced health disorders [3]. Many years have passed since domestic water fluoridation was adopted to reduce the incidence of caries in developed countries; since then people exposed to an additional dose of fluorides ingested with drinks and foods prepared with such waters. But problem has emerged of possible adverse effects on health associated to them, so that in some countries fluorine integrator selling is allowed only with preventive medical prescription[4].

The effect of fluoride on male and female fertility has become an area of growing concern. Various studies showed that fluoride can cause adverse effects on both male and female fertility. [5, 6] . Sodium fluoride has been tested in fertility studies in several species of laboratory animals. Although the evidence is equivocal. [7,8.] The reported reproductive toxic effects include increase in numbers of abnormal spermatozoa, loss of spermatogenesis [9], and interference with steroidogenesis [5]. Moreover, the commonly observed effects of Fluorides in animals include decreased testosterone levels [10, 11], reduced fertility [10], low birth rate [12] , damaged spermatozoa [13] , reduced sperm counts [11], and sperm deflagellation.[13]. Ghosh,[11] reported that sodium fluoride treatment inhibited testicular androgenesis and gametogenesis through oxidative stress. More recently, Das et al,[14] reported that fluoride intoxication diminished both the humoral and cellular immunity.

However, in order to gain further insight into the effect of sodium fluoride on male fertility, the present study was undertaken to investigate the effects of sodium fluoride on fertility in male rats as well as examine the protective role of vitamin E and/or ginger oil in ameliorating the effects on male fertility. The concentrations of NaF used in this study were chosen according to previous studies [15]. The oral route of exposure was chosen to mimic human exposure and to reflect the impact on fertility of the sustained blood levels of fluoride that would occur from water consumption throughout the day.

MATERIALS AND METHODS

***Animals*:** Forty-five adult male Sprague-Dawley rats were used in the experiment. They were raised in the animal house unit in the Faculty of Veterinary Medicine, Zagazige University. The rats were fed commercial rodent pellets and given water ad libitum throughout the experiment. The diet had very low levels of fluoride (traces), and the tap water contained 0.3 ppm F, presumably therefore both food and water sources were negligible. Therefore, our calculations of F intake were based solely on the sodium fluoride (NaF) given to the animals by gavage. Animals were acclimated in the lab conditions for 2 weeks before use.

***Chemicals*:** Sodium fluoride (Sigma Chemical Company, St Louis, MO, USA), vitamin E (E.P.I.C.O, Egypt) and ginger oil (Bader Company, Egypt) were used in this experiment.

***Experimental design*:** Animals were divided into 5 groups, of 9 animals each. Rats of group 1 (G1) didn't receive any treatment as a control. The other four groups were subjected to Sodium fluoride treatment (NaF) at a dose level of 20 mg/kg/day by gavage [15]. One of the later four groups was kept on NaF exposure only (G2), while the other 3 groups were treated as follows; Vitamin E co-administration at a dose of 20mg/100g/day, ginger oil co-administration at a dose of 20 ml/kg/day and both Vitamin E and ginger oil co-administration at the same last mentioned dose rates for groups G3, G4, G5 respectively. The co-administration treatments were given 4 hours prior to NaF treatment. All the treatments continued for 28 days. At the end of the experimental period, control and treated animals of all groups were euthanised by cervical dislocation prior to which blood samples were collected from each animal.

Body and organ weights: At the end of the experimental period (4 weeks + 10-day mating period), animals were euthanised. The testis, the caput and cauda epididymis, vas deferens, seminal vesicle and prostate gland were dissected out and blotted free of blood. Body weight and weights of paired testes, epididymis, seminal vesicles and prostate gland of NaF treated rats and their control counterparts were recorded using (Roller Smith (USA) torsion balance).

***Preparation of testicular homogenates*:** One of the two testes of each rat was weighed and washed twice with phosphate buffered saline (PBS: NaCl 8.0 g, KCl 0.2 g, $Na_2HPO_4 \cdot 12H_2O$ 2.8 g, KH_2PO_4 0.2 g/L). The tissue was homogenized in 1.0 ml of PBS. The homogenate was centrifuged at 3500 rpm for 15 min at 4ºC, and the supernatant was suitably diluted with PBS (usually 1:10) before use for radioimmunoassay of testicular testosterone levels.

***Determination of serum and testicular testosterone levels*:** Blood samples were left for serum separation, and then centrifuged at 3000 r.p.m. for 15 minutes. Serum samples were collected and kept frozen at -20 ^{o}c for performing hormonal analysis. Serum and testicular testosterone were determined using testosterone kits (Gamma Trade International Co.) by RIA (Radio Immune Assay Technique) coated tubes. The inter-assay variation was 6.5% for testosterone. All the samples were run at the same time to minimize such variation.

***Sperm motility and count*:** The cauda epididymal sperm suspension was prepared in normal saline. The ratio of normal and abnormal sperm (%), motility percent (%) and count of cauda epididymal spermatozoa (millions/ml) of control and all treated groups of rats were determined by the method of Prasad et al,[16], Also live/dead ratio was evaluated and expressed as percent according to method mentioned by Zhang et al [17].

Histopathological examination:Specimens from testis, epididymis, seminal vesicles, and prostate gland were fixed in Bouin's fluid then routinely dehydrated by a graded series of alcohol then processed and embedded in paraffin. Paraffin blocks were serially sectioned at 4-5 um thickness. Paraffin sections were stained with Hematoxylin and Eosin [18].

Statistical analysis: Data were expressed as mean ± SE. Differences between control and NaF-exposed groups were analyzed using Student's t-test [19]. A p-value less than 0.01 was considered significant.

RESULTS

Exposure of adult male rats to NaF at a concentration of 20mg/kg/d for 4 weeks by gavage had adverse effects on both the examined male fertility parameters and tissue histology. Vitamin E co-administration resulted in partial but statistically significant protection from loss of the above mentioned parameters. While, ginger oil co-administration resulted in statistically significant protection. The co-administration of both Vitamin E and ginger oil together resulted in highly significant recovery in the measured parameters back nearly to normal compared to the other treated groups.

Body and organ weights: No significant alteration was observed in the body weight gain after NaF administration as compared to control sets. While, an elevation in both testicular as well as seminal vesicle weight in NaF treated rats was observed as compared with the other groups, with a significant diminution in prostate and epididymis weights. Vitamin E and/or ginger oil co-administrations resulted in significant recovery in the previously mentioned weight abnormalities. The marked recovery was in G5 Vitamin E and ginger oil co-administration as presented in Table 1.

Table 1: Body weight (g) and organ weight (g) of control and the different treated groups of Sprague-Dawley rats

Groups	Body weight (g).	Testicular weight (g).	Epididymal weight.	Seminal vesicle weight.	Prostate weight,
Control (G1).	157.7±12.32	1.9±0.3	1.1±0.3	1.8±0.36	0.7±0.1
NaF. (G2).	138.4±0.38	2.5±0.4*	0.2±0.4**	2.1±0.19*	0.3±0.04**
Vit E co-administration (G3).	144.2±0.19	1.6±0.6*	0.6±0.23**	1.4±0.18*	0.5±0.1*
Ginger oil co-administration (G4).	149.6±0.28	1.8±0.1	0.7±0.20*	1.3±0.19*	0.6±0.2
Vit E and ginger oil co-administration (G5).	150.2±0.16	1.9±0.2	0.9±0.21*	1.5±0.22	0.8±0.2

Values are Mean ± S.E. * P<0.01 ** P<0.001 , NaF: sodium fluoride., Vit E: vitamin E.

Serum and testicular testosterone levels:As shown in Table 2 and (Fig. 1) NaF administration to male rats significantly altered both serum and testicular testosterone by lowering their levels as compared with control rats. It was obviously noticed that, Vitamin E or ginger oil and both co-administration treatments resulted in significant restoration of serum as well as testicular levels of testosterone. But the most significant restoration was in co-administration treatment.

Table 2 Serum and testicular levels of testosterone (ng/dl) of control and treated Sprague-Dawley Rats.

Groups.	Serum testosterone level.	Testicular testosterone level.
Control.	245.03 ±13.04	218.40 ±22.45
NaF.	136.54 ± 5.74**	111.21 ±18.32**
Vit E co-administration.	193.05 ±14.64*	178.45 ±15.32**
Ginger oil co-administration.	219.69 ±19.27*	189.28 ±6.38*
Vit E and ginger oil co-administration.	224.19 ±18.64	203.19 ±7.19

Values are Mean ± S.E. * P<0.01 ** P<0.001, NaF: sodium fluoride.,Vit E: vitamin E.

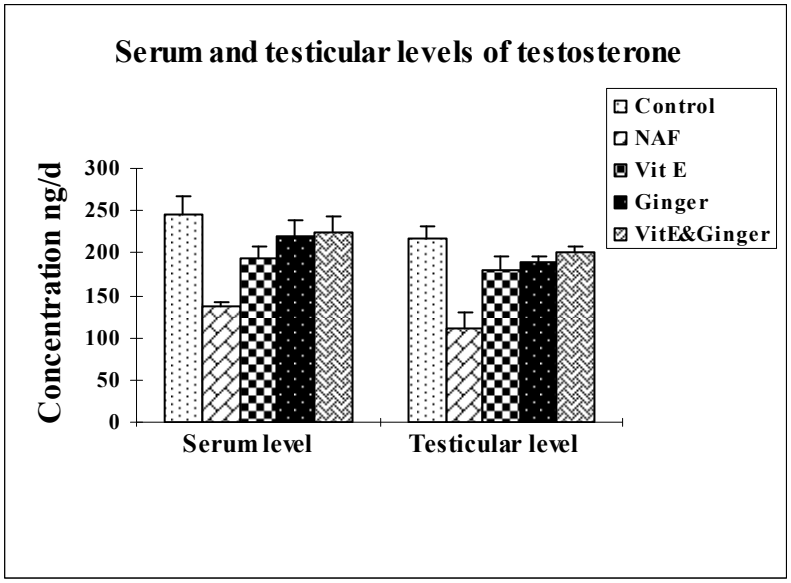

Figure 1: Serum and testicular levels of testosterone in control and the different treated groups

***Sperm count, motility, viability and abnormality*:** The sperm count in the cauda epididymis of different experimental groups is presented in (Table 3). From that table we can notice that both of the sperm count and sperm motility declined significantly ($p<0.001$) in G2 (NaF) treated rats compared to the control group. While, both of them were recovered in G3. Significant recovery was obtained in G4, (ginger oil co-administrated rats). But, the most potent recovery was noticed in both materials co-administrated rats (G5).

As presented in (Table 3) NaF administration to male rats resulted in a significant reduction ($P< 0.001$) in sperm viability (live: dead ratio) and high percent ($P< 0.001$) of abnormal spermatozoa compared to control group. Both of major and minor sperm abnormalities were noticed compared to normal rat spermatozoa (Fig. 2A). Bent tail, double head and broken head were observed as examples of major sperm abnormalities (Fig.2B, C, D respectively). While, minor abnormalities were expressed as distal protoplasmic droplet and coiled tail (Fig. 2E, F). However, sperm viability as well as the percent of abnormal spermatozoa was significantly restored in G4 and G5 ginger oil, and Vitamin E and ginger oil co-administration respectively. The later group (G5) showed the most significant restoration followed by G4.

Table 3 Cauda epididymis sperm count, motility, abnormality, viability and fertility rate of control and different treated groups.

Groups.	Sperm count (million/ml).	Sperm motility (%).	Sperm abnormality. (%).	Sperm viability. Live: dead %.	Fertility rate (%).
Control.	16.52± 0.40	89.28± 0.55	8.21±0.57	80.15 ±0.55	95-100
NaF.	9.48± 0.72**	44.81± 0.73**	21.34±0.12**	27.49 ±0.54**	8.4±0.6**
Vit E co-administration.	11.6± 0.65**	69.42± 0.37**	13.22±2.12*	59.34 ±0.65**	68.48±0.3*
Ginger oil co-administration.	13.5± 1.34*	72.54 ±1.24*	11.61±1.25*	67.63 ±0.43*	75.42±0.77*
Vit E and ginger oil co-administration.	15.66± 0.81	85.1.70 ±0.48	9.15±2.15	74.47 ±0.39	89.7±0.23

Values are Mean ± S.E. * P<0.01 ** P<0.001 , NaF: sodium fluoride., Vit E: vitamin E.

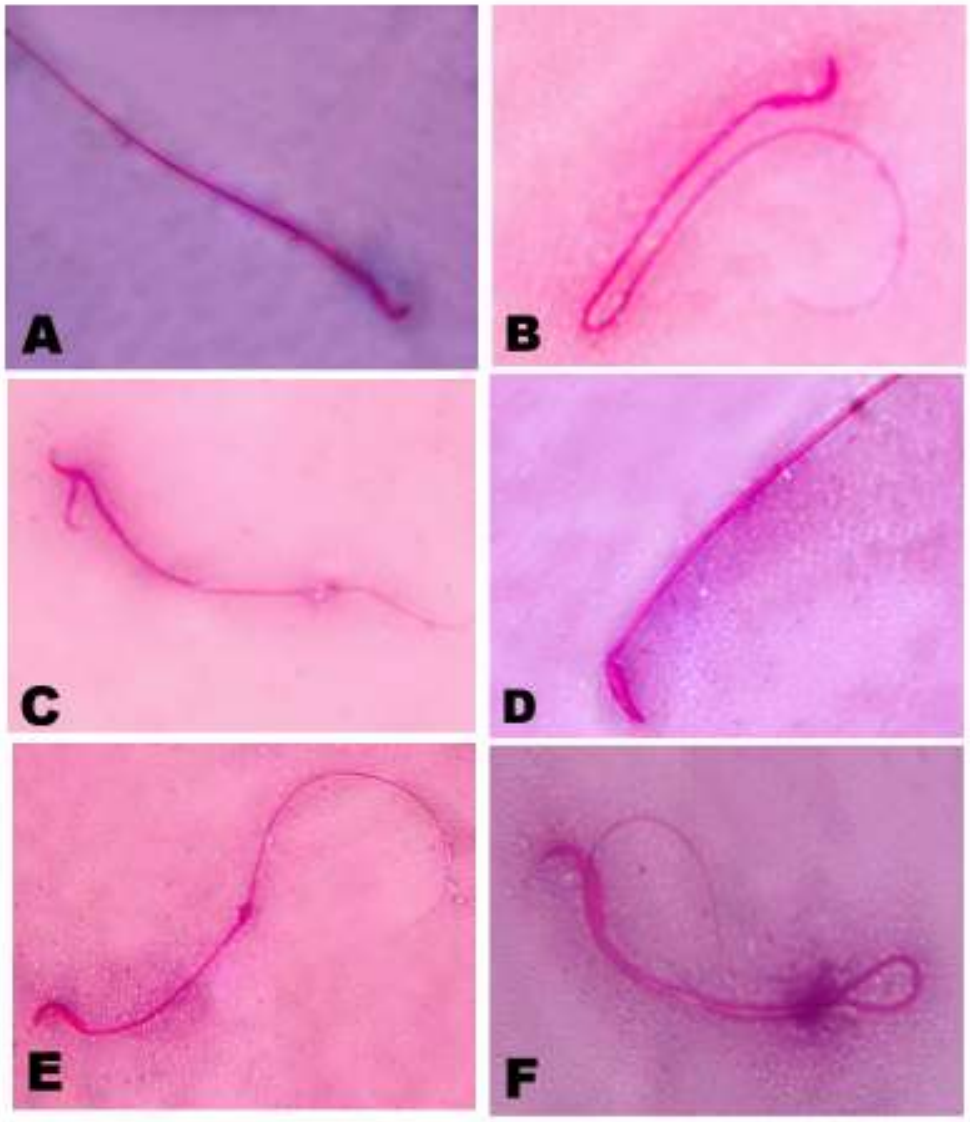

Figure2: A, Normal rat sperm, notice hook –shape head appearance. **B,** Dead sperm of G2 NaF treated rat showing bent tail. **C,** Dead sperm of G2 NaF treated rat showing double head. **D**, Dead sperm of G2 NaF treated rat showing broken head. **E,** Dead sperm of G2 NaF treated rat showing distal protoplasmic droplet. **F,** Dead sperm of G2 NaF treated rat showing coiled tail.

***Histopathological results*:** Microscopical examination of different tissue sections from testis, seminal vesicles and prostate gland of NaF treated Sprague-Dawley rats revealed marked tissue alterations in the previously mentioned organs. Testicular tissue showed variable degrees of defective spermatogenesis and altered spermiation (Fig. 3A). The former was represented by marked degeneration and necrosis of the spermatogonial cells in most of the seminiferous tubules (Fig. 3B), which characterized by pyknosis, karyolysis and variation in the staining intensity. Loss of germinal epithelium was obvious in seminiferous tubules which appeared as if it is lined by sertoli cells with few or no spermatogenic cells particularly mature spermatid (Fig. 3C). While, inhibited spermiation was characterized by appearance of abnormal spermatid forms including presence of elongated and hooked spermatid in the same seminiferous tubule together with

younger spermatid (Fig. 3D). Many spermatid coagulum (Fig. 3E) and marked spermatid giant cells (Fig. 3F) were obvious. Scattered tubules revealed retention of spermatids at the periphery of the tubules with absence of radial orientation. Interstitial oedema as well as Leydig cell degeneration and necrosis were also evident (Fig. 4A). The aforementioned histological alterations in the testicular tissue were less evident in rats co-treated with Vitamin E and ginger oil.

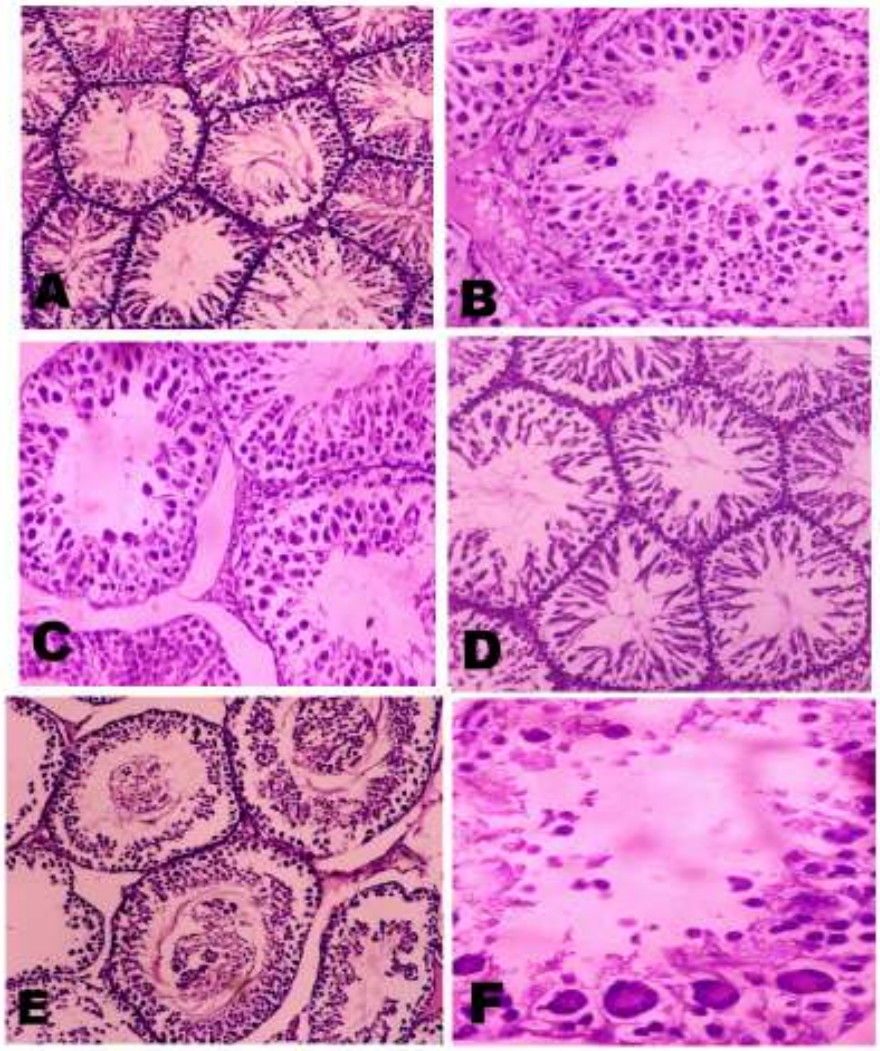

Figure 3: Testis of NaF treated rat showing: **A,** Variable degrees of defective spermatogenesis and altered spermiation in most of the seminiferous tubules. (H&E X200). **B,** marked degeneration and necrosis of the spermatogonial cells in most of the seminiferous tubules.(H&E X 400). **C**, Few spermatogenic cells with absence of mature spermatid and appearance of large number of sertoli cells within the seminiferous tubules. (H&E X 400). **D,** Inhibited spermiation characterized by appearance of abnormal spermatid forms as elongated and young spermatids in the same seminiferous tubule.(H&E X 200). **E**, Many spermatid coagula. (H&E X 200). **F,** Multiple spermatid giant cells within the single seminiferous. (H&E X 400).

Ginger oil was more effective and showed a good degree of regenerative process in the germinal epithelium, of most of the spermatogonial cell layers with marked division and mitotic activity (Fig. 4B). Both co-

administration treatments resulted in more potent recovery in testicular tissue represented by normal spermatogenesis process with appearance of active sperms in the lumen of some seminiferous tubules although few histopathological changes still existed (Fig. 4C).

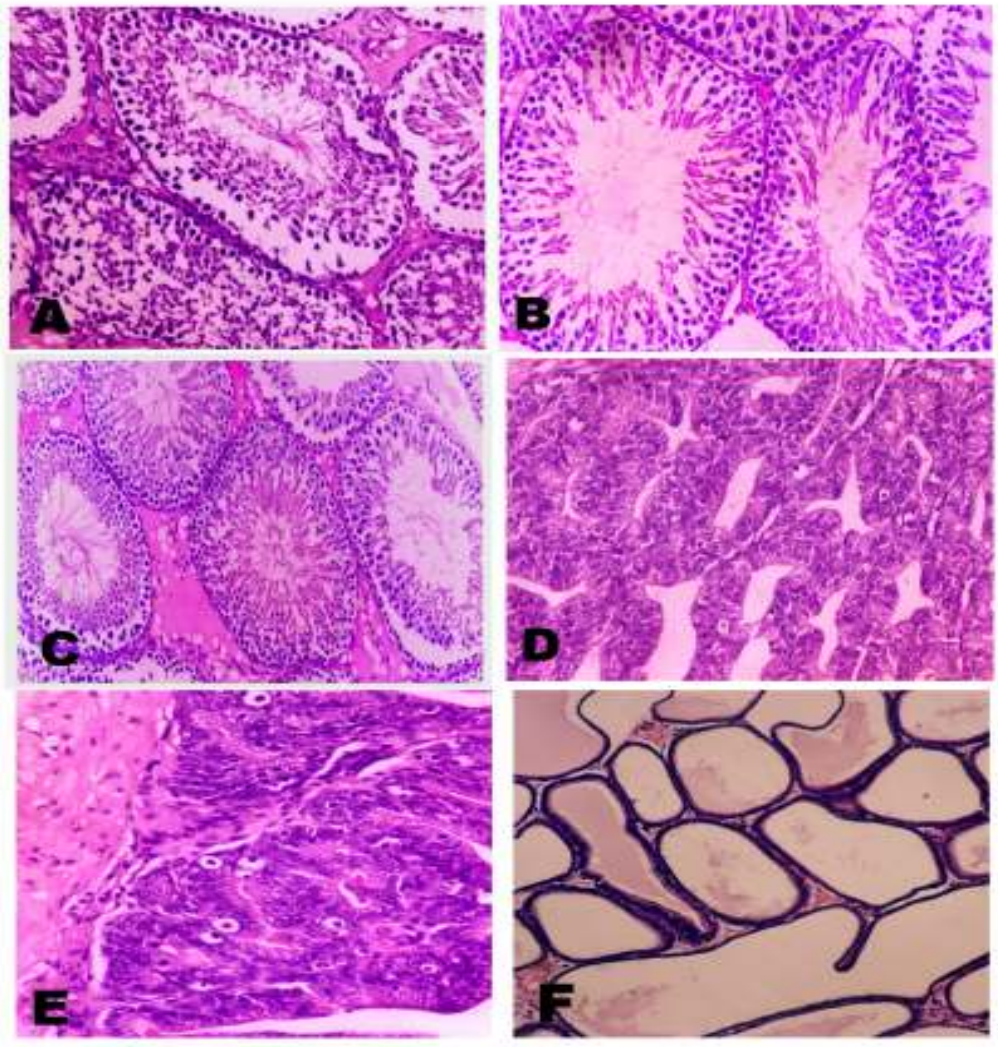

Figure 4 A, Testis of NaF treated rat showing scattered interstitial oedema as well as leydig cells degeneration and necrosis. (H&E X200). **B,** Testis of rat co-treated with ginger oil showing regeneration of the spermatogonial cell layers with marked mitotic activity. (H&E X400). **C,** Testis of rat co-treated with Vit E and ginger oil presenting active spermatogenesis with appearance of active sperms in the lumen of some seminiferous tubules. (H&E X 400).**D:** Seminal vesicle of NaF treated rat revealing; shortening of the branching acini with variation in the staining intensity of the epithelial lining. (H&E X 200). **E,** Seminal vesicle of NaF treated rat revealing; widely extended degenerative changes particularly vacuolar degeneration. (H&E X200). **F,** Prosatic acini of NaF treated rat showing; flattening of the epithelial lining as an early atrophic change.(H&E X 100).

Concerning the epididymis, various degenerative changes of the epididymal lining epithelium as well as presence of cell debris, giant cells and immature germ cells were seen in the epididymal duct. Seminal vesicles of such animals showed shortening of the branching acini which extended into the lumen of the gland. The epithelial lining of these acini were smaller in size with variation in staining intensity (Fig. 4D). Extended degenerative changes particularly vacuolar degeneration (Fig. 4E) was observed among the lining epithelium of the seminal vesicle acini, in addition to detached epithelial cells were also seen in the lumen. Prostate acini showed a vacuolar degeneration as well as necrosis and detachment of the acinar epithelial lining together with cystic dilatation of the prostatic acini. Early signs of atrophic changes were evident, including flattening of the epithelial lining the prostatic acini (Fig. 4F).

DISCUSSION

This study demonstrates that exposure of adult male Sprague-Dawley rats to NaF at a concentration of 20mg/kg/d for 28 days resulted in a significant ($P < 0.001$) reduction in fertility. This was characterized by adverse effects on the male fertility parameters recorded and changes in histological morphology. Although each of Vitamin E and ginger oil treatments provided significant levels of protection against these adverse effects, use of both together gave the most significant results. Although body weight showed no significant changes, there was a significant increase ($P<0.001$) in weights of both testis and seminal vesicles accompanied with significant diminution ($P<0.001$) in weights of both prostate and epididymis in G2 (Na F) treated group.

The previous alterations in organ weights may be related to fluoride-induced degenerative changes. Similar weight alterations were mentioned by Ahmed et al,[20] who attributed weight changes to an alteration in the pattern of testosterone secretion. They reported that this relative weight increase is transient and is reversed with longer exposure to NaF. The impaired fertility in G2 (NaF) exposed animals was presented by distinct decrease in sperm count, sperm viability and serum as well as testicular testosterone levels, together with significant inhibition of fertility rate and increased percent of abnormal spermatozoa, a result which was supported by findings of Wan et al,[21] and Zhang et al,[22]. The increased percentage of abnormal spermatozoa, evidenced testicular and epididymal alteration.

The aforementioned impaired fertility parameters were evidenced by the altered testicular histology. Male reproductive function is well-known to be evaluated by the indexes used in this study [23] which are directly related to the structure and function of the testis[21] and are regulated by hypothalamic-pituitary-testicular axis. Testosterone which is produced by Leydig cells in the testis plays an important role in this regulation process [24]. Hence, the decreased levels of testosterone detected in this study could be attributed to the effect of fluoride by altering the previously mentioned hormone axis as well as its toxic effect on Leydig cells which presented histopathologically as degeneration and necrosis. This hypothesis is partially supported by the work of Das et al,[14] who explained the mechanism of fluoride toxicity in testicular tissue by either its indirect effect on the testis through modulation of pituitary-testicular axis or due to direct oxidative stress imposition on testicular tissue.

Reddy et al,[25] reported that when female rats were exposed to NaF during gestation and lactation, the serum testosterone levels of their male off-spring were significantly decreased. Subsequently, the sperm count, sperm motility and sperm viability in those male off-springs were also decreased. Microscopical examination of testicular sections from NaF exposed rats revealed deleterious alterations to spermatogenesis and defective spermiation with variable degrees of spermatogonial degeneration and necrosis that resulted in loss of the spermatogonial layers and the seminiferous tubules appearing as if lined by sertoli cells. This disturbed morphology is attributed to be the underlying cause of the adverse effects on the observed impaired fertility parameters. Das et al ,[14], recorded inhibited spermatogenesis and

steroidogenesis in association with oxidative stress in the testis and male accessory sex organs after sodium fluoride treatment of male rats. Moreover, the observed toxic changes in the epithelial cells of the seminal vesicle and prostate gland may be related to the oxidative stress effect of fluoride exerted on these cells so altering their structure and function.

The results of this study showed that the adverse toxic effects of fluoride could be reduced by pretreatment with Vitamin E and/ or ginger oil. Either administration alone or in-combination resulted in significant protection of fertility related parameters. Although the response was not to the extent of full restoration to the level of the control animals, both Vitamin E and ginger oil resulted in significant protection.

Zhang et al,[22] stated that fluoride can affect oxygen metabolism and increase the reactive oxygen species. Vitamin E is well-known as a potent antioxidant and androgenic stimulant. Co-administration with fluoride may correct the altered fluoride induced oxygen metabolism and decrease the release of the dangerous reactive oxygen species with its adverse effects on both testicular histology and function. Ginger also has a potent antioxidant activity by scavenging free radicals; in addition to reducing the level of serum malondialdehyde acting as a lipid peroxidation marker as well as increasing the serum level of antioxidant enzyme, superoxide dismutase [15]. Moreover, Amin et al,[26] demonstrated that ginger increased the activity of testicular antioxidant enzymes, superoxide dismutase, glutathione and catalase and reduced the level of malondialdehyde.

Testosterone which is secreted by Leydig cells in the testis plays an important role in the testicular cell proliferation and differentiation. Fluoride can adversely affect the production of testosterone [27]. Decreased serum levels of testosterone in humans and experimental animals due to fluoride exposure have been reported by Aardema and Tusutsui,[28] and Beck,[29] with skeletal fluorosis. Chong et al,[30] postulated that fluoride diminished positive signals for testosterone formation needed for continued growth of germ cells. This concurs with work by Das et al,[31] who reported that Vitamin E significantly protects fluoride-induced oxidative stress in testicular tissue. The effects of Vitamin E imply direct (pituitary independent) or indirect (pituitary dependant) mechanisms of fluoride-induced reproductive disorders. Combined co-administration of Vitamin E and ginger oil led to more protection against altered parameters by fluoride as compared to the control levels. This could be claimed to their combined effects on decreasing the oxidative stress imposed by fluoride. Moreover, Chrubasik, [32] reported that ginger oil has an antioxidant and immunomodulatory effects. Thus the combined antioxidant power of both Vitamin E and ginger oil may have a significant effect in decreasing the drastic effects of fluoride on testicular morphology and function.

In summary, our results showed that Vitamin E and ginger oil co-administration led to more protection in the fluoride-treated rats. We might explain this protective effect as interference with free fluoride availability so decreasing its oxidative stress on male reproductive organs. Although we tested these agents by oral intubations, 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 Vitamin-E and ginger oil supplements might help safeguard male reproductive health in communities where fluorosis is a concern.

REFERENCES

1. International Standards for drinking water, WHO, Geneva (1988). Links.
2. APHA, AWWA, WEF, 1995. Standard Methods for the Examination of Water and Wastewater, 19th Edition APHA, AWWA, WEF, Washington, DC.
3. Robert CH. Agent affecting calcification: calcium, parathyroid hormone, calcitonin, Vitamin D and other compounds. In: Goodman LS, Rall TW, Murad F, editors. Goodman Gillman's the pharmacological basis of therapeutics.7th ed. New York: Macmillan Publishing Co.; 1980. p. 1496–522.
4. Giachini M, Pierleoni F. Fluoride toxicity. Minerva Stomatol. 2004 Apr; 53(4):171-7. PubMed
5. Freni SC. Exposure to high fluoride concentration in drinking water is associated with decreased birth rates. J Toxicol Environ Health 1994; 42:109–12. Links.

6. Susheela AK and Jethanandani P. Circulating testosterone levels in skeletal fluorosis patients. J Toxicol Clin Toxicol 1996; 34:183-9. PubMed
7. Sprando RL, Collins TFX, Black TN, Rorie J, Ames MJ, O'Donnell, M. Testing the potential of sodium fluoride to affect spermatogenesis in the rat. Food Chem Toxicol 1997;35:881-90 PubMed
8. Narayana MV and Chinoy NJ. Effect of fluoride on rat testicular steroidogenesis. Fluoride 1994;27:7-12 PubMed .
9. Pati PC and Bhunya SP. Genotoxic effect of an environmental pollutant, sodium fluoride, in mammalian in vivo test system. Caryologia 1987; 40:79-87 PubMed .
10. Bataineh NH and Nusier MK. Impact of 12-week ingestion of sodium fluoride on aggression, sexual behavior, and fertility in adult male rats. Fluoride 2006; 39(4):293 PubMed -301.
11. Ghosh D, Das (Sarkar) S, Maiti R, Jana D, Das UB. Testicular toxicity in sodium fluoride treated rats: association with oxidative stress. Reprod Toxicol 2002; 16:385– PubMed ;90.
12. Hoffman DJ, Pattee OH, Wiemeyer SN. Effects of fluoride on screech owl reproduction: teratological evaluation, growth, and blood chemistry in hatchlings. Toxicol Lett 1985;26(1):19 PubMed -24.
13. Chinoy NJ and Sequeira E. Effects of fluoride on the histoarchitecture of reproductive organs of the male mouse. Reprod Toxicol 1989; 3:261–;8.
14. Das SS, R. Maiti R , Ghosha D. Management of fluoride induced testicular disorders by calcium and Vitamin-E co-administration in the albino rat. Reprod Toxicol 2006; 22. 606-6–
15. Saber A. Sakr. Ameliorative Effect of Ginger (Zingiber officinale) on Mancozeb Fungicide Induced Liver Injury in Albino Rats. Australian Journal of Basic and Applied Sciences 2007; 1(4): 650-656. .[Abstract/Free Full Text].
16. Prasad MRN, Chinoy NJ, Kadam KM. Changes in succinate dehydrogenase level in rat epididymis under normal and altered physiologic conditions. Fertility and Sterility 1972; 23 180-190.
17. Zhang XS, Zhu JL, Zhou YF, Jiang XZ, Wang YL. Study on the effects of lead acetate on spermatogenesis in male mice. Acta Acad Med Shanghai 1993; 20(1):5-10. Links.
18. Bancroft JD, Stevens A, Turner DR . Theory and practice of histological techniques. 4th Ed. Churchill Livingstone, New York, London, San Francisco, Tokyo.1996.
19. Snedecor, G.W. and Cochran, W.G. Statistical methods. 1980, 7th Ed. Allid pacific, Bombay.
20. Ahmad S A, Ahmed M E, Homa D. Reproductive toxic effects of ingestion of sodiuem fluorid in female rats. Fluoride 2000; Vol.33 No 279-84. Research Report 79. PubMed
21. Wan SX, Zhang JH, Wang JD. Fluoride-induced changes in the expression of epidermal growth factor and its receptor in testicular tissues of young male rats. Fluoride 2006; 39(2):121 PubMed - 5.
22. Zhang JH, Liang C, Ma JJ, Niu RY, Wang JD. Effects of sodium fluoride and sulfur dioxide on sperm motility and serum testosterone in male rats. Fluoride 2006; 39(2):126 PubMed -31.
23. Zhao ZL, Wu NP, Gao WH. Influence of fluoride on contents of testosterone and cholesterol in rat. Fluoride 1995; 28(3):128 PubMed -30.
24. Holstein AF, Schulze W, Davidoff MS. Understanding spermatogenesis is a prerequisite for treatment. Reprod Biol Endocrinol 2003; 1:10
25. Reddy PS, Pushpalatha T , Reddy PS. Suppression of male reproduction in rats after exposure to sodium fluoride during early stages of development. Naturwissenschaften 2007; 94(7):607 PubMed -11.
26. Amin A, Hamza AA, Kambal A, Daoud S. Herbal extracts counteract cisplatin-mediated cell death in rat testis. Asian J Androl. 2007 Dec 20.
27. Chinoy NJ. Studies on effects of fluoride in 36 villages of Mehsana district, North Gujarat. Fluoride 1992; 25(3):101 PubMed - 10.
28. Aardema MJ and Tsutsui T. Sodium fluoride-induced chromosome aberrations in different cell cycle stages. Mutat Res 1995; 331(1):171 PubMed -2.
29. Beck MA. Selenium and host defence towards viruses. Proc Nutr Soc1999; 58(3):707 PubMed -11.
30. Chong H, Ruiyan N , Jundong W. Toxic effect of sodium fluoride on reproduction in male mice. Research report Fluoride 2007; 40(3)162–168 .[Abstract/Free Full Text].
31. Das SS, Maiti R, Ghosha D. Induction of oxidative stress on reproductive and metabolic organs in sodium fluoride treated male albino rats: protective effect of testosterone and Vitamin E co-administration. J Toxicol Mech Method 2005; 15(4):271-7. PubMed
32. Chrubasik S, Pittler MH, Roufogalis BD. A comprehensive review on the ginger effect and efficacy profiles. Phymed.2004, 07.009. PubMed