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Histological Study of The Effects of Sodium Fluoride and The Protective Role of Vitamin E on The Testis of Albino Rats

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ABSTRACT

Sodium fluoride is a prophylactic agent against dental caries commonly used for water fluoridation, treatment of osteoporosis and as a disinfectant. Adverse effects on various organs were reported following exposure to sodium fluoride.

The present study aims to evaluate the histological alterations triggered by administration of sodium fluoride on the testis of Wistar rats and the defensive ability of vitamin E to ameliorate fluoride toxicity.

In this study, forty male Wistar albino rats (Rattus norvegicus), were used, their weight ranging from 250 to 350 gm, all animals were acclimatized for one week before the experiment in suitable cages with standard food pellets and fresh water adlibitum. The period lasted for doing the experiment was four weeks. The animals were grouped equally as following: Group I served as control group given normal saline by oral intubation once daily for one month, Group II given 20 mg/kg/day sodium fluoride through oral gavage once daily for one month. Group III was given received vitamin E by oral intubation in a dose of 3 mg/kg/day followed by giving NaF after 24 hours using the same dose of group II daily for one month. Group IV rats received vitamin E alone for one moth with the same dose. Rats were sacrificed after ether inhalation, the specimens of testis were stained with H and E stain and prepared for light microscopic examination. Biochemical assessment of serum Malondialdehyde and Superoxide dismutase as biomarkers of lipid peroxidation and immunohistochemical determination of caspase-3 activity were done and the results obtained were statistically analyzed.

Analysis of the recorded data of body weight revealed very high significant reduction in the body weights of rats of group II equated to the control group. In group II, we observed a significantly higher Malondialdehyde with a significantly reduced Superoxide dismutase in comparison to the control group. Conspicuous alterations in the testicular histological architecture were observed in NaF recipient group as thickening of tunica albuginea, vacuolar degeneration of spermatogenic cells. A significant rise in the caspase-3 was observed in rats treated with fluoride alone. Concomitant treatment with vitamin E with fluoride showed a considerable improvement in the histological architecture, biochemical tests, and significantly reduced the caspase-3 activity to near normal. Such finding emphasizes the defensive antioxidant influence of vitamin E against oxidative toxicity induced by fluoride which encourages the use of vitamin E supplementation to minimize the harmful effects of fluorosis.

Keywords: Histological, Sodium fluoride, Testis, Protective, Vitamin E

Introduction

Fluorides are natural widely distributed compounds in the soil and water, fluoride anions are broadly used in the agriculture, industry and medicine (1). Sodium fluoride (NaF) is a convinced and effective dental caries prophylactic agent, it is also used for water fluoridation, and as disinfectant. Moreover, it is used for treatment of osteoporosis with calcium and vitamin D (2). Fluoride might induce both beneficial and hazardous effects; numerous studies have indicated that low concentration fluoride is safe and essential for humans and domestic animals particularly for development and growth of bones and teeth (3). The amount fluoride in drinking water is acceptable as 0.5-1.5 mg /L. However, excessive consumption of fluoride results in fluorosis and reduced antioxidant defense mechanism (4).

In the recent years, chronic toxicity of fluoride has been reported following prolonged exposure to drinking water, toothpaste, mouth washes, and industrial fumes (5). Fluoride can be distributed to various organs like liver, kidneys, skin and reproductive system because it has the ability to cross the cell membrane, thus chronic exposure to fluoride might induce obvious gastrointestinal disturbances,

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neurological disorders, and reproductive system dysfunctions (6).

Several investigations denote to a correlation between long periods of exposure to sodium fluoride and impaired reproductive function with modification in the histological structure of gonads in mice, rabbit and even in chicken embryonic gonads (7). Previous studies have genotoxicity, suggested that excessive intake of sodium fluoride is associated with immunotoxicity, cytotoxicity, and oxidative damage with subsequent apoptosis and the harmful effects of NaF can be ameliorated by antioxidant agents (8).

Recently, interesting increased consideration has been concentrated on the consumption of nutritional substances to modify the toxicity of environmental chemicals. Vitamin E (α-tocopherol) is a natural antioxidant that inactivates detrimental effects of free radicals generated via stressful oxidative damage by stabilizing the molecular composition of cellular membranes, terminating lipid peroxidation and preventing the harmful effects of reactive oxygen species (9).

Programmed cell death namely, apoptosis aids in eradicating the cells that are not more necessary, it involves altered mitochondrial function with sequential release of cytochrome C (10). The events of apoptosis are harmonized by a category of the family of cysteine proteases. Caspase 3 is a specific lysosomal enzyme involved in the detection of apoptotic cells, it affects the mitochondria directly or indirectly and it have been shown to mediate and amplify the release of cytochrome C and Bax translocation (11).

Apoptosis might proceeds in either intrinsic or extrinsic pathways both diverge at the effective caspase-3 (12). Caspase-3 is a major mediator of apoptosis thus it is considered as an attractive target in treatment of certain conditions that involve an excess of apoptosis like autoimmune, heart diseases, neurodegenerative diseases like Alzheimer's, even that selective activation of caspase-3 in malignant cells is possible (13). Expression of caspase-3 have been proved to be a favorable dependent prognostic factor for several diseases and used as an indicator for the worth of different modalities of therapy (14).

Many literature reviews capacious information about skeletal and endemic dental fluorosis but few awareness is available regarding the testicular toxicity of fluoride. The current work aimed to shed light on the histological changes that might occur on the tissue of testis of rats after experimental exposure to toxic dose of sodium fluoride (NaF) and to investigate the potential efficacy of vitamin E in eliminating the fluoride toxicity.

Materials and Methods

The current study is a randomized controlled experimental research. The ethical approval of the experiments was conducted by the MREC (Medical Research Ethics Committee) in the College of Medicine, University of Mosul at 22/2/2022.

Materials

Drugs: Sodium fluoride: Zymafluor®1mg tablets Manufactured by Mylan Company in France, Paris purchased from Al-Ahed Pharmacy in Mosul

Vitamin E® 400 IU, in the form of soft gels capsules manufactured by Adrien Gagnon SKU: 063935015013 30 made in Canada purchased from Al-Ahed Pharmacy in Mosul.

Animals: Forty active and healthylooking male Wistar albino rats of the same age ranging from 2.5 to 3.5 months, their weights ranging from 200 to 250 grams, were purchased from the Animal House in the Veterinary College, University of Mosul. The rats were acclimatized for a period of one week before the experiment using proper cages under stern attention, hygienic circumstance and aeriation with a light dark 12:12 cycle with average temperature 24±26ºC with standard rodent food pellets and fresh water adlibitum. The weights of animals were measured and recorded at the beginning and at the end of the experiment using a digital weighing scale digital balance. The duration of the experiment was four weeks.

Lethal dose (LD50), Pilot studies were justified and the comprehensive doses of sodium fluoride and vitamin E were calculated on the basis of previous studies which is equivalent to the human therapeutic doses (15). The work was done from January 2022 to August 2022.

Animal Grouping: The rats were equally subdivided into 4 groups as following:

- Group I serves as a control group include 10 rats, given normal saline 1ml /kg /day by oral intubation as a single dose daily for one month.
- Group II are the NaF recipient group received 20 mg/ kg/day sodium fluoride once daily for one month period by oral gavage using a metal oropharyngeal cannula and syringe.
- Group III: Each rat was given vitamin E in a dose of 3 mg/kg/day which equals to 0.6ml by oral intubation daily followed by giving NaF after 24 hours using the same dose of group II for one month.

- Group IV: received vitamin E alone for one month with the same dose orally by gavage.

The weights of all the animals were estimated again and recorded at the completion of work. Following ether inhalation, the rats were killed and specimens of testis were excised washed in normal saline solution (0.9%) and dried on filter paper, then fixed in Bouin's solution for 24 hours, then processing into paraffin blocks was done in consistence with the method mentioned by Bancroft and Gamble, 2008 (16). Paraffin blocks were trimmed into slices of 5-µm thickness by Reichert's Rotatory Microtome then staining with Harris Hematoxylin and Eosin (H&E) stain to be ready for light microscopic examination.

Biochemical testing: Laboratory investigations for the assessment of serum Malondialdehyde (MDA) and Superoxide dismutase (SOD) as biomarkers of lipid peroxidation were assessed by spectrophotometer to investigate the reactivity of thiobarbituric acid (TBA) following the technique described by Gawel (2004) (17). Blood was obtained from all rats on finishing the experiment through cardiac puncture using wide bore needle and 10 ml syringe, emptied into sterile serum separator tubes then left to be coagulated at 4°C for 30 minutes, then centrifugation for 10 minutes at 2500 rpm in order to isolated the serum which was kept at temperature of −80°C. Thereafter, in a boiling water bath 0.5 ml of supernatant was added to 0.5 ml of 1% TBA for 30 minutes waiting for estimation of MDA using a special colorimetric assay kit (E-BC-K025-S) and SOD using (E-BC-K019-S) colorimetric kit. The results were expressed as µmol/L.

Morphometric assessment: Morphometric study to estimate some variables among the four groups like the diameter of seminiferous tubules, thickness of tunica albuginea and height of germinal epithelium. Besides, enumerating Sertoli cells per each tubule and Leydig cells per field. These measurements were conducted by using a digital image camera (Scope Image 9.0- China) accompanied by an image processing software which was standardized to all lenses of MicroscopeOlympus-CX31 by assistance of 0.01mm stage micrometer (ESM11/Japan). Measurements were expressed as mean±SD of all the parameters.

Immunohistochemistry (IHC) for evaluation of Caspase-3 activity: Caspase-3 activity was determined consuming streptavidinabiotin peroxidase technique. The specimens were processed into paraffin blocks and slices 4 µm thick were cut and laid on positively charged slides. Paraffin wax was removed then sections were rehydrated in downgraded concentrations of alcohol (100%, 80%, 70%, and 50%) each for 2 minutes, then eroded in distilled water. To block the endogenous activity of peroxidase, tissue sections were gestated in hydrogen peroxide at concentration of 3% for 10 min then double washing in phosphate buffer saline (PBS). The retrieval of antigen was done by boiling tissue sections for 10 min in citrate buffer at pH 6.0 using a microwave oven then cooling down to room temperature then incubation of tissue sections with mouse monoclonal antibody against caspase-3 (Clone 31A1067, Catalog # MC0123, Medaysis, USA) overnight at 4ºC at a dilution of 1/200. .Therereafter, washing in phosphate-buffered saline then incubation of the tissues with biotin-conjugated secondary antibody (Universal Staining Kit) and then with biotinstreptavidin complex for 15 minutes at room temperature washed and exposed to diaminobenzidine tetrahydrochloride solution (DAB) until the positive control showed brown staining after 5-10 min. then quickly washed in distilled water and lastly, counterstained with hematoxylin. The Caspase-3 expression appeared as brownish cytoplasmic and membranous deposits besides nuclear staining in the lymphocytic cells.

Statistical Analysis: For statistical analysis of the recorded results, version 22.0 SPSS was used and descriptive statistics of the groups were given as mean ± SD using Shapiro-Wilk test was used to determine whether the data were distributed normally or not. The presence of significant differences between the groups was evaluated using Bonferroni multiple comparisons for post-hoc analysis with significant level set as $P < 0.05$ considering that P-values <0.05 were statistically significant, while P-values <0.01 were viewed as high statistical significance (18).

Results

With frequent observation, the animals in all the groups remained active having a good appetite throughout the experimental period except those of group II who appeared to be slow and less animated with less appetite later in their course of treatment.

Body Weight: The initial and final body weight of rats of the control group shows no significant change while the body weight of group II rats showed very high significant reduction compared to the control group (P-value=0.001). Meanwhile,

the body weight of group III rats who treated with fluoride combined with vitamin E improved significantly when compared with group II but no significant differences in their body weight as equated to the control group (P=0.110). However, the mean body weights of group IV rats revealed no significant changes as compared to control group (Table 1).

Biochemical Results: Measurement of serum levels of the enzymes of lipid peroxidation Malondialdehyde (MDA) and antioxidant parameters in the homogenates of testis showed that the level MDA in group II received NaF alone were significantly higher than control group but a statistically significant decrease in SOD activity (P≤0.05) was noticed in the same group. Co-administration of vitamin E prior to NaF reserved the levels of MDA to the normal values. Giving vitamin E alone reestablished the SOD activity and MDA concentration to be close to normal (Table 2).

Morphometric Results: The measurements were demonstrated as mean±SD, the diameter of ST/µm showed high significant decrease (P=0.002) in group II (181.32±9) when compared with the control group (225.04±18.8), while there was no significant differences in group III (203.15±6.1) as compared to the control group (Table 1). The height of germinal epithelium/µm revealed high significant rise (P=0.001) in group II (14.4±2.2) as compared with the control group (5.2±1.05) and non-significant difference in group III (6.1±1.19) as compared with the control group. The thickness of tunica albuginea is significantly increased in group II (P=0.001) as compared to control and returns to near normal values in group III. Meanwhile, a significant reduction in the number of Sertoli cells and Leydig cells in group II as compared to control (P≤0.05), and their number returns to near normal in group III (Table 3).

Histological Results: The microscopic examination of testis from control group (group I) revealed typical histological appearance of seminiferous tubules lined with germinal epithelium which is composed of spermatogonia sited on the basement membrane, primary and secondary spermatocytes, spermatozoa and Sertoli cells appeared as oval shaped cells resting on basement membrane, Leydig cells appeared in the interstitial space (Fig. 1).

Considerable disorganization of the histological arrangement was observed in the sections of testis taken from NaF recipient group as thickening of tunica albuginea, infiltration of amorphous eosinophilic substance in the interstitial tissue with edematous stroma and degeneration of Leydig cells (Fig. 2) and vacuolar degeneration of spermatogenic cells (Fig.3). The sections of testis taken from animals following administration of vitamin E combined with fluoride exhibited substantial improvement in the histological architecture as compared to group II with orderly arrangement of germinal cells and accumulated spermatozoa inside the seminiferous tubules (Fig.4). The microscopic examination of testis from the animals given vitamin E alone showed normally organized spermatogenic cells lining the seminiferous tubules similar to the control group (Figure 5).

Immunohistochemical Findings: The appearance of caspase-3 in the four experimental groups was regarded as positive when brownish nuclear staining was verified and evaluated according to the intensity of immunostaining. There was a weak expression of caspase-3 in the control group (Fig.6). A significant rise in the caspase-3 activity in the rats received fluoride alone (Fig.7) when compared with the control. However, simultaneous administration of vitamin E significantly reduced the caspase-3 activity to near normal. Group VI revealed weak reaction to caspase-3 in the nuclei of germinal cells in comparison with the control group.

Discussion

Fluoride denotes a hazardous environmental and toxic industrial pollutant mainly ingested from water. Prolonged exposure to fluoride results in widespread health problems including noxious reproductive outcome (19).

The significant reduction in the animal weight in the NaF treated groups as compared to the control group might be attributed to alteration in the sequence of metabolic processes induced by sodium fluoride causing inactivation of some enzymes that interrupted the glycolysis, antioxidative pathways and synthesis of proteins and lipids, these changes together with a reduced food consumption and malabsorption due to widespread pathological changes in the digestive tract result in reduction in the body weight such finding is supported by previous studies on various animals like Choudhury et.al, (2018) (20) who reported that exposure to fluoride significantly reduced the body weight of rabbits as compared with control group but differ from the finding of Arpita et.al, (2012) (21) who stated that NaF doesn't affect the body weight of animals.

Table 1. Comparison of Body Weight (In Gram) Among The Control and Experimental Rats

	Group I	Group II	Group III	Group IV	P-value	
Initial Body weight (Mean ± SD)	231 ± 5.2	221.6± 8	208 ± 2	225± 4	I vs. II=(VHS)	0.001
					I vs. III = (NS)	0.110
					I vs. IV = (NS)	0.310
Final Body weight (Mean ± SD	235±7.2	167± 4	182.2 ± 3	215 ± 3	II vs. III = (S)	0.020
					II vs. IV = (NS)	0.415

SD= Standard deviation; S=Significant (P≤0.05); NS=Non-significant (P>0.05); VHS= Very High Significant (P<0.01); vs. =versus

Table 2. Comparison of Serum Level of The Enzymes of Lipid Peroxidation

	Group I	Group II	Group III	Group IV	P-value	
MDA (µmol/L)	0.9±0.7	5.4±0.1	1.3±2	±0.2 1.1		
					I vs. II=(VHS)	0.001
					I vs. III = (NS)	0.123
					I vs. IV = (NS)	0.334
SOD (µmol/L)	3±0.7	0.4±0.8	2.4±0.1	2.4±0.9	II vs. I= (S)	0.010
					II vs. III = (S)	0.020
					II vs. IV = (NS)	0.420

SD= Standard deviation; S=Significant (P≤0.05); NS=Non-significant (P>0.05); VHS= Very High Significant (P<0.01); vs. =versus

The present investigation demonstrates the deleterious effects of exposure to fluoride but the precise mechanism of toxicity induced by fluoride is still unidentified, However, the disorganization of histological architecture induced by NaF in the current study, as vacuolar degeneration of spermatogenic cells, massive coagulative necrosis and sloughing of germinal cells may be attributed to the oxidative damage induced by NaF via excessive generation of free radicals and peroxidative interruption of the continuity of phospholipids components of cellular membrane with subsequent spread of tissue damage (22). The oxidative testicular tissue damage in the fluoride treated animals as evidenced in this study as coagulative necrosis, edematous stroma, and degeneration of Leydig cells are intensely validated by the results of Orta et al., (2022) (23) who studied the detrimental influence of low concentration of environmental fluoride on Leydig cell and male infertility.

Previous workers reported that reactive oxygen species (ROS) are generated in the spermatogenic pathway following exposure to NaF and they established that oxidative injuries could be exacerbated in the testis of rats following exposure to fluoride due to persistent inflammation which activates Myeloperoxidase (MPO) enzyme with consequent excessive generation of ROS (24). In agreement with our results, some literatures evidenced that oxidative stress status triggered by fluoride in rats is a key pathological mechanism which is blamed for male infertility and reproductive dysfunction due to lipid peroxidation of cellular membrane, DNA damage and reduced motility of the spermatozoa (25). Moreover, other studies revealed that fluoride impaired the autophagy process causing apoptosis of Sertoli cells which might explain male reproductive toxicity (26). Similarly, in their study Dibyendu et al. (2021) (27) had observed a significant decline in the enzymatic activities following exposure to NaF in comparison with the controls which may further exacerbate oxidative damage and overproduction of ROS.

The elevated MDA and the reduced SOD observed in the testis of fluoride recipient rats in the current study verifies previous reviews concerning the role of oxidative stress and the

Table 3. Morphometric Measurments of Some Parameters Among The Experimental Groups

	Group I	Group II	Group III	Group IV	P-value
Diameter of seminiferous tubule/μm	225.04±18.8	181.32±9	203.15±6.1	243.62±7.7	0.002*
Height of germinal epithelium/μm	59.72±6.7	38.64±2.5	47.7±2.9	78.86±7.5	0.001**
Thickness of Tunica albuginea./μm	43.48±3.6	90.88±5.4	68.15±4.1	53.56±5	0.001**

SD= Standard deviation; S=Significant (P≤0.05); NS=Non-significant (P>0.05); VHS= Very High Significant (P<0.01); vs. =versus

Table 4. Morphometric Counting of Sertoli And Leydig Cells/ Field Among The Experimental Groups

	Group I	Group II	Group III	Group IV	P-value
No. of Sertoli cells/ tubule	20.16±1.2	12.66±1.6	18.57±1.3	20.16±0.7	0.01
No. of Leydig cells/ field	12.42±0.8	6.66±1.6	10±1.3	11.33±0.9	0.01

SD= Standard deviation; S=Significant (P≤0.05); NS=Non-significant (P>0.05); VHS= Very High Significant (P<0.01); vs. =versus

Fig. 1. Magnified section of germinal epithelium showed spermatogonia (red arrow), spermatocytes (yellow arrow), spermatozoa (blue arrow), Sertoli cells (white arrow), and Leydig cells in the interstitial space (H&E X 400)

Fig. 2. Section of testis of group II showed thickening of tunica albuginea (white arrow), infiltration of amorphous eosinophilic substance in the interstitial tissue (yellow arrow) with edematous stroma (black arrow) and degeneration of Leydig cells (red arrow) (H&E X200)

degree of lipid peroxidation as biomarkers of endemic fluorosis (28).

The observed defensive effect of vitamin E and the improvement of histological damage appeared as orderly rearrangement of germinal with mild peritubular deposition of amorphous eosinophilic material in rats received simultaneous administration of vitamin E with NaF in the current study was strongly indicated by previous workers who supported the use of vitamin E to reduce mortality and morbidity and enhance the quality of life (29). Such finding declares the protective antioxidant role of vitamin E against oxidative damage when it was used in combination with potentially hazardous pesticides in rat erythrocytes (30). Similarly, the oxidative stress prompted by fluoride in the testicular tissue was significantly attenuated by supplementation with dietary CoQ10 which ameliorates testicular damage probably via potential improvement of testicular dismutase and catalase enzyme activities and consequent reserve of reproductive function in rabbits exposed to fluoride (31).

Furthermore, The notable reverse in the level of MDA and SOD as biomarkers of oxidative stress in the rats treated with vitamin E prior to NaF confirms the findings that vitamin E relieves the oxidative damage by improving the antioxidant enzymes activities and consequently stabilizing the level of oxidants mainly H_2O_2, superoxide radicals

Fig. 3. Section of testis of group II showed vacuolar degeneration of spermatogenic cells (H&E X400)

Fig. 4. Section of testis of group III showed orderly arrangement of spermatogonia (black arrow), spermatocytes (yellow arrow), accumulated spermatozoa in the lumen of seminiferous tubules (red arrow) (H&E X400)

and hydroxyl radical (32). A review of the update findings that concentrated on the improvement of oxidative stress and apoptosis induced by fluoride was published using natural and synthetic chemicals with various mechanisms of action to counteract the oxidative damage (33). The role of antioxidants in ameliorating the oxidative damage on some reproductive organs in rats exposed to chronic sodium fluoride toxicity was previously documented by administration of folic acid (34).

Apoptosis is an irreversible programmed cell death, it plays a vital role in the chronic degenerative diseases. In the present study, the significant increase in the caspase-3 activity in the group treated with fluoride alone when compared with the control group validates the detected alteration in the histological architecture and biochemical results and it indicates the induction of apoptotic cell death.

Fig. 5. Section of testis of group IV showed normally organized spermatogenic cells lining the seminiferous tubules similar to the control group (arrow**)** (H&E X400)

Fig. 6. Section of testis of control group showed weak expression of caspase-3 in the nuclei of spermatogenic cells (black arrows) (X400)

In agreement with our findings, some authors have declared that fluoride cause enhancement of caspase-3 which is a key regulator of both intrinsic and extrinsic apoptosis cascade mammals by using in vivo and in vitro studies (35). Similarly, in their experimental trial Ouyang et al., 2021 (36) noticed that NaF induces cell apoptosis and autophagy by regulating caspase 3 signaling pathway leading to poor growth and development of the exposed organisms. Other workers summarized that induction of apoptosis by NaF is promoted by up-regulating of caspase-3 protein expression and down-regulating of mitochondria membrane potential, Bcl-2 expression and such effect of NaF was time and dose dependent (37). Recent review provides an update evidence confirming the results of our study about the interesting dichotomous role of caspase-3 in maintaing the growth, homeostasis and evolution of both normal and malignant cell biology (38).

Fig. 7. Section of testis of group II showed significant increase in the caspase-3 activity in the nuclei of spermatogenic cells (black arrows) (X400)

The obvious reduction in the caspase-3 activity following simultaneous administration of vitamin E in group III rats clearly suggestive of its efficacy in suppressing the apoptotic cell death provoked by fluoride. Such result supports the finding of former researches who stated that vitamin E has potent effects to conserve the integrity of testis and reduce the adversative effects of oxidative stress on sperm mainly owing to its antioxidant and anti-apoptotic properties (39).

Furthermore, mounting evidences emphasized that VE combined with lycopene can effectively reduce oxidative stress and apoptosis of spermatogenic cell induced by coal burning fluorosis via the inhibition of phosphorylation signaling pathway (40).

The worth of the results of this experimental research is to encourage the use of vitamin E supplementation to minimize the harmful effects of fluorosis.

Based on the results, the current work concluded that exposure to NaF induces adverse effects on the histological architecture of testis of albino rats mainly due to oxidative stress and excessive formation of reactive oxygen species causing degeneration of seminiferous tubules, Vacuolar degeneration of spermatogenic cells, thickening of tunica albuginea, infiltration of amorphous eosinophilic substance in the interstitial tissue with edematous stroma and absence of Leydig cells. Co-administration of vitamin E effectively ameliorates fluoride mediated damage by antagonizing the free radicals, augmenting the antioxidant status and suppressing pro-inflammatory mediators thus improving the oxidative damage and restoring the testicular tissue to normal.

Such findings recommended that fortunately vitamin E being commonly used and easily available nutritional factor may be a favorable supplement to diminish the expected reproductive health problems for the safety of populations at risk of chronic exposure to fluoride particularly in developing countries.

We need further researches of various comparative doses of vitamin E to discover the precise effective dose. Furthermore, whole oxidant-antioxidant balance and cell apoptosis should be further studied to clarify what cell types are more sensitive to fluoride and the exact role of vitamin E.

Conflict of Interest: Regarding the current manuscript, the authors declare that there is no conflict of interest.

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