MSD

Medical Science and Discovery
ISSN: 2148-6832

Effects of Fluoride Exposure on Thiol/ Disulphide Homeostasis, Testicular Oxidative Stress and Histopathological Changes in Rats

Yilmaz Kocak[1]*, Gokhan Oto[2], Volkan Kosal[3], Fikret Altindag[4], Ahmet Ufuk Komuroglu[5], Ezgi Onay[2], Rezzan Temelli Goceroglu[2]

1 Dept of Physical Therapy and Rehabilitation, Faculty of Health Sciences, Yüzüncü Yıl University, Van, TR
2 Dept of Pharmacology, Faculty of Medicine, Yüzüncü Yıl University, Van, TR
3 Dept of Reproduction and Artificial Insemination, Faculty of Veterinary, Yüzüncü Yıl University, Van, TR
4 Dept of Histology and Embryology, Faculty of Medicine, Yüzüncü Yıl University, Van, TR
5 Vocational Higher School of Healthcare Studies, Van Yüzüncü Yıl University, Van, TR

*** Corresponding Author:** Yılmaz koçak **E-mail:** yilmaz_kocak77@hotmail.com

ABSTRACT

Objective: This study investigated the effects of fluoride-induced toxicity on the reproductive system. For this purpose, testicular oxidative stress, thiol/disulpide homeostasis, sperm motility, density, and histopathology of testicular tissue were evaluated.

Material and Methods: In the experiment, 4 groups were formed, with eight animals (n=8) in each group. Group 1, control/saline, groups 2, 3, and 4 were administered orally sodium fluoride (NaF) at doses of 25, 50, and 100 ppm, respectively, for 30 days. Then the rats were sacrificed and testicular tissue samples were evaluated by sperm examination and histopathologically. Biochemical parameters malondialdehyde (MDA), catalase (CAT), advanced oxidation, and protein products (AOPP) were measured spectrometrically. Thiol/disulphide homeostasis was studied with a fully automated analysis method.

Results: NaF exposure increased MDA and AOPP levels and decreased CAT levels. Disrupted thiol/disulphide homeostasis. Histopathological examination revealed integrity between germinal cells and tubular atrophy. NaF showed reduced sperm motility and density and increased abnormal sperm count. The findings of the NaF 100 ppm group were statistically significant when compared with the Control and other NaF groups ($p<0.05$).

Conclusion: The findings concluded that fluoride might cause pathological conditions and infertility in testicular tissue.

Keywords: Fluoride, Thiol/ disulphide homeostasis, Oxidative stress, Testis, Rat

INTRODUCTION

Fluorine is one of the essential elements necessary for human health. It is found in different concentrations in the environment, consumed foods, and in drinking water. However, if the element fluorine is taken in excessive amounts, it can cause acute and chronic fluorosis poisoning in humans. It causes malformations in the digestive, skeletal, and endocrine systems in living beings exposed to high amounts of fluorosis (1–3). Developing countries have revealed their harmful effects on the environment with excessive fossil fuel consumption, an increase in power plants for electricity generation, and excessive accumulation of fluorine in soils and waters in economies that use fluorine-containing pesticides used in agriculture. The increase in fluorine pollution has been shown to cause toxicity in all living things and plants (4).

Fluoride has been shown in studies to prevent dental caries and protect bone integrity if it is taken in sufficient quantities. On the contrary, high exposure to fluoride causes dental fluorosis, causing discoloration of tooth enamel and permanent joint pain, reducing the quality of life of individuals (1). It has also been reported that fluoride can reduce insulin secretion and cause insulin resistance in increasing doses (5). Flouride exposure causes a decrease in thyroid hormones and contributes to the formation of endocrine system diseases (6). Recent studies have shown that fluorine can trigger oxidative stress and increase lipid peroxidation by increasing the intracellular balance (7). Flor, a small element as a molecule, can easily pass through the cell membranes and disrupt oxygen metabolism and lead to the formation of free radicals. This causes oxidative stress (OS).

Research Article

Received 01-03-2023

Accepted 09-03-2023

Available Online: 11-03-2023

Published 30-03-2023

http://dx.doi.org/10.36472/msd.v10i3.904

OS is the deterioration of oxidant and antioxidant balance. This balance disorder causes damage to the cell, protein and lipid oxidation, leading to the deterioration of functions in various organs (8).

Fluoride toxicity has become a worldwide public health problem, causing various diseases associated with oxidative stress. In this context, the effects of fluoride on testicular oxidative stress, thiol/disulphide homeostasis, spermatozoa, and histopathological changes were investigated.

MATERIALS AND METHODS

Animals

Animal material (n=32) of this study was obtained from Van Yüzüncü Yıl University Experimental Medicine Research and Application Center. Male Wistar Albino Rats had a body weight of 250-300 g. Animals were housed in standard plastic cages, in a room at 22+/- 2 degrees, lit at a rhythm of 12 hours of light to 12 hours of dark, with free food and water intake.

Experimental procedure

In the study, 4 groups of eight animals (n=8) in each group were formed and the experimental procedure was designed as follows (9,10).

Group 1 (Control): (n=8) Normal saline was given.

Group 2 (Fluoride 25 ppm): (n=8) Rats were administered 25 ppm oral sodium fluoride (NaF) for 30 days.

Group 3 (Fluoride 50 ppm): (n=8) Rats were administered 50 ppm oral NaF for 30 days.

Group 4 (Fluoride 100 ppm): (n=8) Rats were administered 100 ppm oral NaF for 30 days.

Homogenization of tissues

Experimental animals were intraperitoneally administered ketamine/xylazine (44mg/kg/33mg/kg) anesthesia combination, intracardiac blood was drawn and sacrification due to hypovolemia was performed. Blood samples were centrifuged at + 4 ºC for 10 minutes and serum was obtained. Testicular tissues were taken from post-mortem rats, paying attention to the cold chain, and weighed. The samples were turned into 20% tissue homogenates with the help of a glass homogenizer in ice molds using phosphate buffer (pH 7.4). Tissue homogenates were taken into previously labeled tubes and centrifuged at 5000 g at +40C for 5 min. The resulting supernatants were divided into Eppendorf and stored in a deep freezer at -80 °C until the study.

Sperm examination

Motility examination: Sperm epididymis was punctured and placed on a slide on a heating plate set to 38°C. The coverslip was left at a 45° angle, and motility rates were determined at x40 magnification (%) of the microscope (11).

Density examination: Sperm samples were homogenized by adding 0.1 ml to Eppendorf containing 0.5 ml of Hayem solution. The number of sperm in 1 ml was calculated by counting on the Thoma slide (11).

Abnormal sperm rate: The semen obtained with epididymis puncture was taken into Eppendorf with 0.5 ml Hancock solution, and at least 400 spermatozoa were examined at x40 magnification to determine the abnormal sperm ratio (11).

Biochemical analysis

Malondialdehyde (MDA) (12), Catalase (CAT) (13), and advanced oxidation protein products (AOPP) (14) were measured spectrometrically. Thiol/disulphide homeostasis (TDH) was carried out with the method developed by Erel and Neselioğlu (15). Total thiol (TT) and native thiol (NT) levels were measured in a spectrophotometer. Disulphide level (DS) was calculated using the (TT-NT)/2 formula.

Histopathological examination

Testes taken from sacrificed rats were fixed in 10% buffered formaldehyde. Routine histological tissue follow-up procedures were performed on the fixed tissues using an automatic tissue tracking device (LEICA ASP300S). The testis was embedded in paraffin and made into a block. Sections of 5 µm were taken from paraffin blocks in the microtome. Sections taken were stained with Hematoxylin-Eosin dye and examined under a light microscope (Olympus BX53, Japan). The histopathological evaluation was evaluated as normal (negative), low injury (+), moderate injury (++), and severe injury (+++) according to the observed pathological finding.

Statistical analysis

Shapiro-Wilk (n<50) and Skewness-Kurtosis tests were used to determine whether the data of the study were normally distributed, and parametric tests were applied as a result of the test. Descriptive statistics are expressed as mean, standard deviation number (n), and percentage (%). "One Way ANOVA" followed by "Tukey" post hoc test was used to compare the groups. The statistical significance level was accepted as $p<0.05$. SPSS (IBM SPSS for Windows, ver.26) statistical package program was used for the analysis.

RESULTS

Semen findings

In this study, sperm motility examination, density analysis, and abnormal sperm ratio of rats exposed to fluoride are shown in **Table 1**. In the semen examination of the rats, it was observed that the sperm motility and number decreased, and the number of abnormal cells increased in the fluoride-treated groups. Especially the NaF 100 group was statistically significant compared to the control, NaF 25 and 50 ppm groups ($p<0.05$).

Histopathological findings

Histopathological views of the testicular tissue of rats stained with Hematoxylin Eosin are presented in Figure 1. Control and fluoride groups were compared. The control group was of normal histological structure and had 6-8 layers of Sertoli and germ cells within the germinal epithelium. Dense sperm cells with intact tails were found in the tubule lumen. Leydig cells are present in the interstitial space. The germinal epithelium had 5-6 layers in the NaF 25 and 50 groups, and the germinal epithelium in the NaF 100 group had 3-4 layers.

http://dx.doi.org/10.36472/msd.v10i3.904

Compared to the control group, sperm cells in the seminiferous tubule lumen decreased slightly in the NaF 25 and 50 groups, while it was moderately reduced in the NaF 100 group. The integrity between the cells forming the germinal layer of the NaF 100 group was impaired and tubular atrophy was observed. In addition, an increase in the interstitial area was observed in the NaF 25, 50, and 100 groups compared to the control group. The histopathological score of the testis is shown in **Table 2**.

Biochemical findings: Testicular tissue MDA and AOPP levels in rats caused a dose-dependent increase in fluoride groups. In particular, the Fluoride 100 ppm group showed a significant increase compared to the control group ($p<0.05$, Table 3, Figure 2). CAT levels were decreased in the fluoride 50 and 100 ppm groups compared to the control group. TT and NT levels of fluoride groups were found to be lower and DS levels higher when compared to the control group. The highest dose of the fluoride group was statistically significant ($p<0.05$, **Table 3**, **Figure 2**).

Table 1. Mean (±S.D.) Motility, Density, and Abnormal Sperm Rate results in the fluorosis model

Sperm examination	Control	NaF 25 ppm	NaF 50 ppm	NaF 100 ppm
Motility (%)	85,37±4,37[a]	83,73±6,19[a]	80,33±6,52[a]	67.76±7.16[b]
Density (x109 /ml)	2,34±0,12[a]	2,22±0,17[a]	2,1±0,09[a]	1,79±0,08[b]
Abnormal sperm rate (%)	16,28±3,46[a]	17,82±2,24[a]	19,25±4,75[a]	34,89±4,66[b]

Table 2. NaF-induced testicular tissue histopathological score (NaF: Sodium Fluoride)

Groups	Number of germinal epithelial layers	Sperm count in the tubule lumen	Tubular atrophy
Control	6-8	Regular number	-
NaF 25 ppm	5-6	Slightly decreased	-
NaF 50 ppm	5-6	Slightly decreased	+
NaF 100 ppm	3-4	Moderately decreased	++

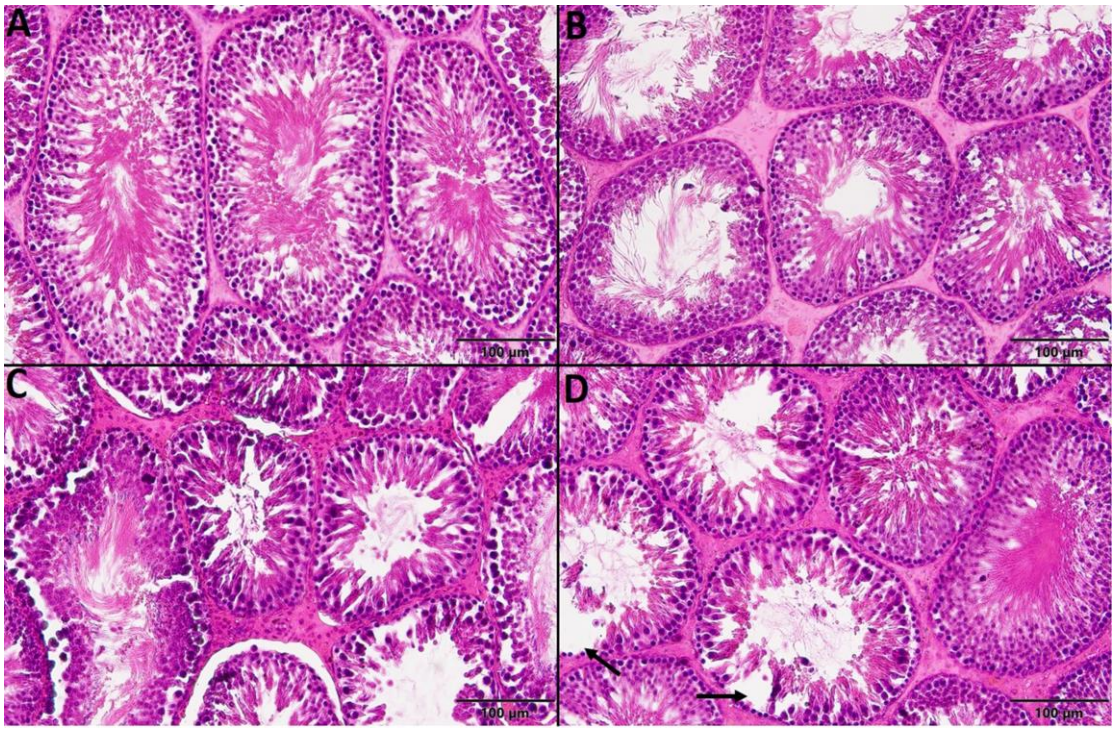

Figure 1. Microscopic images of testis of rats treated with fluoride. NaF: Sodium Fluoride, A: Control, B: NaF 25 ppm, C: NaF 50 ppm, D: NaF 100 ppm. Arrow (↑): Tubular atrophy. H-E. 400x.

Table 3. Comparison of the values of biochemical parameters measured in testicular tissue

Groups	Control (Mean ± SD)	NaF 25 ppm (Mean ± SD)	NaF 50 ppm (Mean ± SD)	NaF 100 ppm (Mean ± SD)
MDA (nmol/grtissue)	3.29±0.43[b]	3.52±0.45[ab]	3.81±0.24[ab]	3.92±0.38[a]
AOPP (mM/gr tissue)	10.11±0.99[ab]	10.82±1.49[b]	11.01±0.65[ab]	11.76±1.01[a]
CAT (K/gr tissue)	528.41±57.76[a]	511.30±39.18[ab]	418.65±77.94[bc]	360.70±98.11[c]
TT (µmol/gr tissue)	56.95±4.62[a]	52.68±4.57[ab]	50.90±4.59[ab]	49.41±6.81[b]
NT (µmol/gr tissue)	49.23±4.25[a]	40.31±4.85[b]	37.99±2.86[b]	36.23±3.60[b]
Disulphide (µmol/gr tissue)	3.85±1.59[a]	6.18±0.76[ab]	6.45±1.59[b]	6.59±2.39[b]

NaF: Sodium Fluoride, MDA: Malondialdehyde, AOPP: Advanced oxidation protein products, CAT: Catalase, a,b,c p: values with different letters are significant when compared with each other ($p<0.05$).

http://dx.doi.org/10.36472/msd.v10i3.904

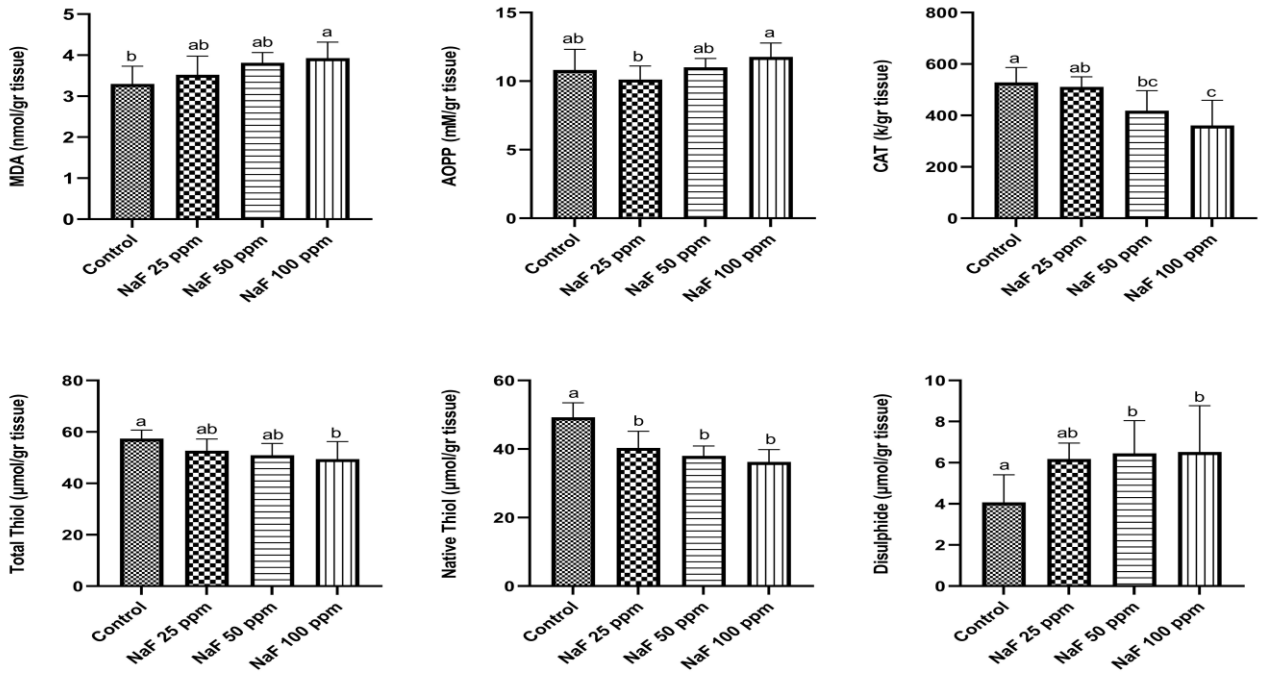

Figure 2. Comparison of the values of the measured parameters in testicular homogenates. MDA: Malondialdehyde, AOPP: Advanced oxidation protein products, CAT: Catalase, NaF: Sodium Fluoride. Values are shown as mean ± SD. n = 8 animals per group. a,b,c,p: values with different letters are significant when compared with each other ($p<0.05$).

DISCUSSION

Consumption of high amounts of fluoride has been cited as the main cause of fluorosis in humans. Fluoride is an environmental pollutant found in drinking water and consumed agricultural nutrients. Overexposure to fluoride has been reported to cause cellular damage to teeth, and skeletal, urogenital and digestive system organs (16). Today, it has been reported that in regions where fluoride concentration is high, it adversely affects the male reproductive system and may be closely related to decreased birth rates (17). In addition, in vivo studies in experimental animals have shown that fluoride crosses cell membranes and causes toxicity in reproductive organs (16,18,19). Although adverse effects of overexposure to fluoride on testis have been reported, the underlying mechanism of this toxicity has not been fully elucidated. Therefore, in the present study, the effects of oxidative stress on testicular tissue, thiol/disulphide homeostasis and some sperm parameters were investigated in rats exposed to fluoride for 30 days. Fluoride doses were determined by considering the fluoride amounts in endemic regions (25,50 and 100 ppm). In the current study, a decrease in sperm motility, a decrease in sperm density, and an increase in abnormal sperm counts were observed in the semen examination of rats exposed to fluoride. While these changes were observed in all fluoride-treated groups, they were especially more pronounced in the highest-dose fluoride group. Indeed, in a previous study, it was shown that fluoride concentrations (10, 20, and 25 mg/kg) gradually increased over 30 days, impaired spermatozoal plasma membrane integrity, and decreased sperm motility and total sperm count in rats (18).

Yıldırım et al. (2018) found in their study that there was a decrease in sperm motility and density in rats exposed to 30 mg of fluoride for 90 days. Although the current study is compatible with the literature, it has been concluded that the duration and amount of fluoride exposure reduce sperm motility and density. The histopathological results of this study show that increasing fluoride concentrations in parallel with sperm semen examination cause damage to sperm tissue. The increase in the testicular tissue interstitial area of fluoride groups and the integrity between the cells forming the germinal layer in the fluoride group in which high doses were applied and tubular atrophy occurred. These findings are consistent with previous observations (16,19,20). Decreased sperm motility and density, atrophy in the tubular area in histopathological findings, and disruption of the integrity of germinal cells consistently showed the effect of fluoride toxicity on testicular tissue. In the pathogenesis of the damage caused by fluoride, excessive free radical production may cause oxidative stress (OS). OS occurs as a result of the deterioration of the balance between the ability of the organism's defense mechanism to get rid of these excessively accumulated free radicals (21). It contributes to the formation of various diseases by causing cellular damage with the formation of lipid peroxidation depending on OS (19). Research shows that the body's antioxidant defense mechanism can inhibit the harmful effects of OS. (21–23). In this context, in the study, the MDA level, which is a marker of lipid peroxidation of OS resulting from fluoride, increased in the fluoride groups, while the 100 ppm dose was significantly higher in terms of MDA level. Similarly, AOPP increased in the group with the highest fluoride dose.

CAT level decreased at both medium and high doses of fluoride. The findings showed that fluoride exposure induces oxidative stress and depletes the antioxidant enzyme. This is an indication of the toxic effect of fluoride accumulating on the testicles (24). It has been reported in previous studies that fluoride increases lipid peroxidation and causes mitochondrial damage in the cell (25,26). These results indicate that fluoride accumulation in testicular tissue may affect fertility (24).

The novelty of this study was to evaluate thiol/disulphide hemostasis in the testicular tissue of rats exposed to fluoride using a colorimetric method. Dynamic thiol-disulphide homeostasis is an antioxidant defense mechanism that protects cells against OS occurring in the organism. In the presence of OS, thiols react with oxidizing agents and form reversible disulphide bonds in proteins. (27). When OS is inhibited, the disulphide bonds are converted back to thiol groups. This antioxidant defense system prevents mitochondrial dysfunction, apoptosis, and protein oxidation in the cell, thereby protecting thiol/disulphide homeostasis (15). Environmental toxic substances or pathological diseases that trigger OS, this balance causes an increase in disulphide level and a decrease in thiols (28). The present study determined that TT and NT levels decreased and DS levels increased with increasing concentrations in fluorine groups. These findings are in line with studies showing that fluoride induces OS (19, 29). No studies showed thiol/disulphide hemostasis on the testicular tissue of fluoride. That's why the results of the experiment are unique. Disruption of thiol/disulphide homeostasis indicates that it can be used to evaluate OS caused by pathological diseases (15).

CONCLUSION

In summary, this study shows that exposure to fluoride at high concentrations can trigger oxidative stress and decrease sperm motility and density, increasing abnormal sperm cell count. Evidence has been presented showing that fluoride toxicity may impair thiol/disulphide homeostasis and cause damage to testicular tissue. The findings concluded that fluoride might cause pathological conditions and infertility in testicular tissue. As a result, the possible effect of fluoride-induced toxicity on dysfunctions in the male reproductive system should not be ignored and should be supported by more detailed studies to understand its mechanism.

Acknowledgments: This study was supported by the Pharmacology of Department, Medical Faculty, Van Yuzuncu Yil University.

Conflict of interest: The authors declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article. This research did not receive and a specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Author Contributions: YK, GO, EO: Conceptualization, Project administration, Writing – original draft, Methodology, Resources, Data curation. **VK, AUK, FA:** Methodology, Investigation, Resources, Data curation. **RTG, EO, VK:** Methodology, Resources, Data curation. **FA, AUK, VK, YK:** Formal analysis, Conceptualization, Investigation.

Ethical approval: All procedures followed were in accordance with the ethical standards of the responsible committee on human experimentation (institutional and national) and/or with the Helsinki Declaration of 1964 and later versions. Informed consent or substitute for it was obtained from all patients for being included in the study. Written consent was obtained from each patient to use their hospital data. This study was conducted with the approval of the local ethics committee of Van Yüzüncü Yıl University (decision date 30.06.2022 and numbered 11).

REFERENCES

1. Srivastava S, Flora SJS. Fluoride in Drinking Water and Skeletal Fluorosis: a Review of the Global Impact. Curr Environ Heal reports. 2020;7(2):140–6.
2. Ozsvath DL. Fluoride and environmental health: a review. Rev Environ Sci Bio/Technology. 2008;8(1):59–79.
3. Hacıoğlu C, KAR F, Kanbak G. Rat brain synaptosomes: In vitro neuroprotective effects of betaine against fluoride toxicity. Med Sci Discov. 2018;90(7):192–7.
4. Zuo H, Chen L, Kong M, Qiu L, Lü P, Wu P, et al. Toxic effects of fluoride on organisms. Life Sci. 2018;198:18–24.
5. Johnston NR, Strobel SA. Principles of fluoride toxicity and the cellular response: a review. Arch Toxicol. 2020;94(4):1051–69.
6. Singh N, Verma KG, Verma P, Sidhu GK, Sachdeva S. A comparative study of fluoride ingestion levels, serum thyroid hormone & TSH level derangements, dental fluorosis status among school children from endemic and non-endemic fluorosis areas. Springerplus. 2014;3(1):1–5.
7. Feng D, Huang H, Yang Y, Yan T, Jin Y, Cheng X, et al. Ameliorative effects of N-acetylcysteine on fluoride-induced oxidative stress and DNA damage in male rats' testis. Mutat Res Toxicol Environ Mutagen. 2015;792:35–45.
8. Wang YX, Xiao X, Zhan XA. Antagonistic effects of different selenium sources on growth inhibition, oxidative damage, and apoptosis induced by fluorine in broilers. Poult Sci. 2018;97(9):3207–17.
9. Pandiyan T, Prabu M. Ameliorative effect of epigallocatechin gallate on sodium fluoride-induced oxidative stress-mediated metabolism in rat. Int J Pharmacol Toxicol. 2014;2(2):76–85.
10. Zhang S, Niu Q, Gao H, Ma R, Lei R, Zhang C, et al. Excessive apoptosis and defective autophagy contribute to developmental testicular toxicity induced by fluoride. Environ Pollut. 2016;212:97–104.
11. Hafez ESE, Hafez B. Reproductive Cycles. Reprod Farm Anim. 2016;55–67.
12. Dubovskiy IM, Martemyanov V V., Vorontsova YL, Rantala MJ, Gryzanova E V., Glupov V V. Effect of bacterial infection on antioxidant activity and lipid peroxidation in the midgut of Galleria mellonella L. larvae (Lepidoptera, Pyralidae). Comp Biochem Physiol Part C Toxicol Pharmacol. 2008;148(1):1–5.
13. Lartillot S, Kedziora P, Athias A. Purification And Characterization Of A New Fungal Catalase. Prep Biochem. 1988;18(3):241–6.
14. Hanasand M, Omdal R, Norheim KB, Gøransson LG, Brede C, Jonsson G. Improved detection of advanced oxidation protein products in plasma. Clin Chim Acta. 2012;413(9–10):901–6.
15. Erel O, Neselioglu S. A novel and automated assay for thiol/disulphide homeostasis. Clin Biochem. 2014;47(18):326–32.
16. Zhang S, Jiang C, Liu H, Guan Z, Zeng Q, Zhang C, et al. Fluoride-elicited developmental testicular toxicity in rats: Roles of endoplasmic reticulum stress and inflammatory response. Toxicol Appl Pharmacol. 2013;271(2):206–15.
17. Long H, Jin Y, Lin M, Sun Y, Zhang L, Fluoride CC-, et al. Fluoride toxicity in the male reproductive system. Fluoride. 2009;42(4):260–76.
18. Pal P, Mukhopadhyay PK. Fluoride induced testicular toxicities in adult Wistar rats. Toxicol Mech Methods. 2021;31(5):383–92.
19. Ghosh D, Das S, Maiti R, Jana D, Das UB. Testicular toxicity in sodium fluoride treated rats: association with oxidative stress. Reprod Toxicol. 2002;16(4):385–90.

20. Long H, Jin Y, Lin M, Sun Y, Clinch C. Fluoride toxicity in the male reproductive system. Fluoride. 2009;42(14):260–76.

21. Birben E, Sahiner UM, Sackesen C, Erzurum S, Kalayci O. Oxidative Stress and Antioxidant Defense. World Allergy Organ J. 2012;5(1):9–19.

22. Bulduk B. Effect of chitosan application on lung tissue in rats with experimental fluorine toxicity. J Heal Sci Med. 2022;5(4):969–72.

23. Pamplona R, Costantini D. Molecular and structural antioxidant defenses against oxidative stress in animals. Am J Physiol Regul Integr Comp Physiol. 2011;301(4):843–63.

24. Altindağ F, Özdek U. Protective effects of chitosan and chitosan oligosaccharide on sodium fluoride-induced testicular damage in male rats: A stereological and histopathological study. Kafkas Univ Vet Fak Derg. 2021;27(2):183–9.

25. Sun Z, Li S, Guo Z, Li R, Wang J, Niu R, et al. Effects of Fluoride on SOD and CAT in Testis and Epididymis of Mice. Biol Trace Elem Res [Internet]. 2018;184(1):148–53.

26. Chaithra B, Sarjan HN, Shivabasavaiah. A Comparative Analysis of Fluoride-Contaminated Groundwater and Sodium Fluoride-Induced Reproductive Toxicity and Its Reversibility in Male Rats. Biol Trace Elem Res. 2020;197(2):507–21.

27. Ferrer-Sueta G, Manta B, Botti H, Radi R, Trujillo M, Denicola A. Factors affecting protein thiol reactivity and specificity in peroxide reduction. Chem Res Toxicol. 011;24(4):434–50.

28. Erdoğan H, Çamkerten İ, Çamkerten G, Ural K, Erdoğan S, Günal İ, et al. The Effect of Hot-Iron Disbudding on Thiol-Disulphide Homeostasis in Calves. J Fac Vet Med Kafkas Univ. 2019;25(3):335–9.

29. Nabavi SM, Nabavi SF, Eslami S, Moghaddam AH. In vivo protective effects of quercetin against sodium fluoride-induced oxidative stress in the hepatic tissue. Food Chem. 2012;132(2):931–5.