

Testicular histopathological changes following sodium fluoride administration in mice

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Abstract

It has been revealed that excessive fluoride intake on long-term is associated with toxic effects and can damage a variety of organs and tissues in the human body, including the male reproductive system. However, the molecular mechanisms of fluoride-induced male reproductive toxicity are not well understood. The study wants to get news concerning the effects of sodium fluoride on testicular tissues when this substance is administrated to a population of mice. The study was conducted on NMRI mice descending from the pregnant females treated with 0.25 mg and 0.5 mg sodium fluoride by daily gavage, from the beginning of pregnancy until the lactation is ceased. Then, the mice, males and females, were divided in six groups, three groups descending from the pregnant females treated with 0.5 mg sodium fluoride (Groups A, B and C) and three groups from the pregnant females with 0.25 mg sodium fluoride (Groups D, E and F). From the moment when the lactation is finished until the adulthood, the animals received the following treatments: homeopathic (a CH7 solution of sodium fluoride – Groups A and D), allopathic–homeopathic (0.25 mg% sodium fluoride administered like drinking water *ad libitum* and CH7 solution of sodium fluoride – Group E; 0.5 mg% sodium fluoride administered like drinking water *ad libitum* and CH7 solution of sodium fluoride – Group B), and allopathic administration of sodium fluoride (0.25 mg% sodium fluoride like drinking water *ad libitum* – Group F; 0.5 mg% sodium fluoride like drinking water *ad libitum* – Group C). When the males reached the adulthood, the administration of sodium fluoride was stopped and, by randomization, they were selected for euthanasia. The euthanasia was realized by cervical dislocation. The testes for the histopathological examination were preserved in a 10% formalin solution. The preparation of samples for optical microscopy was realized with Hematoxylin–Eosin staining. The results indicate that sodium fluoride administered in different doses, even at homeopathic dose or at allopathic–homeopathic dose, determined vacuolar dystrophy of epididymal epithelial cells, vacuolar dystrophy of linear seminal cells and necrosis.

Keywords: sodium fluoride, histopathology, mice, testis.

Introduction

The administration of fluoride during human growth and development has raised numerous questions about the effects on various organs. Because the sodium fluoride is administrated from birth to the adulthood in humans, for 18 years, some of the studies want to get conclusions regarding the effect of sodium fluoride following long-term oral administration. The exposure to sodium fluoride revealed various modifications on the reproductive organs of animals or humans. Recent studies found that the exposure at fluoride doses of 3–27 mg/day induces a subclinical reproductive effect in humans, explained

by the toxicity of fluoride in both Sertoli cells and gonadotrophs [1]. When the toxicity in reproductive system in male rats was studied [2] the researchers discovered that fluoride causes disorders in the oxidative system and antioxidative system in rat testicle.

Also, other studies [3] revealed that the quantity of sperm in fluorotic groups is lower than those of control rats, and decreased with the increase of dosage.

It was studied the presence of apoptotic spermatogenic cells when fluoride is administrated [4]. The fluoride causes lower estradiol level responsible for the presence of apoptotic spermatogenic cells.

Our study aimed to investigate the possible histo-

pathological changes in the reproductive system of male mice, when fluoride is administered in three doses: allopathic dose (0.25 mg% natrium fluoride and 0.5 mg% natrium fluoride, administered *ad libitum* like drinking water), homeopathic dose (CH7) and allopathic–homeopathic dose.

Materials and Methods

The study was performed on NMRI-type mice. All researches were conducted in accordance with the European Directive 86/609/EEC/24.11.1986, the European Convention on the Protection of Vertebrate Animals (2005) and the Romanian Government Ordinance No. 37/2002 regarding the protection of animals used for experimental and other scientific purposes [5, 6].

The administration is realized in three different doses: homeopathic, allopathic and allopathic–homeopathic. The substances administrated are different doses of natrium fluoride: 0.25 mg% natrium fluoride, 0.5 mg% natrium fluoride, and CH7 homeopathic solutions.

The animals for the experiments are descendants from two kinds of maternal parents: pregnant females (mothers) treated with 0.25 mg natrium fluoride and pregnant females (mothers) treated with 0.5 mg natrium fluoride, daily administered by gavage, during pregnancy and lactation.

The experimental animals were classified in six groups, males and females, compared with males and females control.

The descendants are divided into Groups 1A, 1B, 1C, 2D, 2E, 2F, males and females. Groups 1A, 1B, 1C are descendants from the mothers with 0.5 mg natrium fluoride allopathic administration and Groups 2D, 2E, 2F are descendants from the mothers with 0.25 mg natrium fluoride allopathic administration.

The classification of experimental groups and the doses administrated for every group are highlighted in Table 1:

- Groups 1A and 2D – Homeopathic administration by gavage with one channel micropipette of a CH7 dilution of a natrium fluoride solution;

- Groups 1B and 2E – Homeopathic administration by gavage with one channel micropipette of a CH7 dilution of a natrium fluoride solution with allopathic administration of 0.5 mg% natrium fluoride *ad libitum* (Group 1B), respectively of a CH7 dilution of a natrium fluoride solution with allopathic administration of 0.25 mg% natrium fluoride *ad libitum* (Group 2E), males and females;

- Groups 1C and 2F – Allopathic administration of 0.5 mg% natrium fluoride *ad libitum* (Group 1C), respectively allopathic administration of 0.25 mg% natrium fluoride *ad libitum* (Group 2F), males and females.

When mice reached the adulthood, they were selected by randomization for euthanasia. For the histopathological examination, the animals were killed by cervical dislocation, the testes from every specimen selected for the examination was taken and introduced in a 10% formalin solution. The preparation of samples for optical microscopy was realized with Hematoxylin–Eosin staining [7].

Table 1 – The classification of experimental groups by dosage

No.	Groups (males and females)	Dose of administered natrium fluoride
1.	Control group	None
2.	Group 1A	Homeopathic administration by gavage with one channel micropipette of a CH7 dilution of a natrium fluoride solution.
3.	Group 1B	Homeopathic administration by gavage with one channel micropipette of a CH7 dilution of a natrium fluoride solution with allopathic administration of 0.5 mg% natrium fluoride <i>ad libitum</i> .
4.	Group 1C	Allopathic administration of 0.5 mg% natrium fluoride <i>ad libitum</i> .
5.	Group 2D	Homeopathic administration by gavage with one channel micropipette of a CH7 dilution of a natrium fluoride solution.
6.	Group 2E	Homeopathic administration by gavage with one channel micropipette of a CH7 dilution of a natrium fluoride solution with allopathic administration of 0.25 mg% natrium fluoride <i>ad libitum</i> .
7.	Group 2F	Allopathic administration of 0.25 mg% natrium fluoride <i>ad libitum</i> .

Results

The results of histopathological examination of the testicular tissues are classified by groups and dosage (Table 2).

Table 2 – The aspect of testes revealed at the histopathological examination for every group

No.	Groups	Pathological aspects
1.	Control group	Mature testes; seven germinative layers.
2.	Group 1A	Vacuolar dystrophy in seminal cells.
3.	Group 1B	Active testes, six germinative layers, smaller cell nuclei; vacuolar dystrophy in seminal cells.
4.	Group 1C	Vacuolar dystrophy in seminal cells; one layer of cells in the seminal tubules.
5.	Group 2D	Vacuolar dystrophy in seminal cells; variable germinative layers (five, four, three).
6.	Group 2E	Vacuolar dystrophy in seminal cells and necrosis; vacuolar dystrophy in epithelial cells of epididymis.
7.	Group 2F	Vacuolar dystrophy in stromal cells; vacuolar dystrophy in epithelial cells of epididymis; vacuolar dystrophy in seminal tubules.

We observed vacuolar dystrophy in seminal cells for the homeopathic dose and for the allopathic–homeopathic dose descending from pregnant females with 0.5 mg natrium fluoride daily administration by gavage. For the allopathic dose for the same descendants, we found vacuolar dystrophy in seminal cells and one layer of cells in the seminal tubules. For the other groups, descendants from the pregnant females with 0.25 mg natrium fluoride daily administration by gavage, the vacuolar dystrophy is also present and it is revealed in the epithelial cells of epididymis and in seminal tubules.

The testis exists in the scrotum and presents convoluted seminiferous tubules enclosed by the tunica

albuginea. Tunica albuginea is a fibrous capsule of connective tissue. The seminiferous tubules are lined by seminiferous epithelium. The Sertoli cells are the sustentacular cells located at the base of epithelium, at regular intervals. Those cells have a large oval nuclei. The spermatogonia are the least mature cells and they are located at the base of the epithelium. The spermatozoa, the most mature cells are released at the lumen. The Leydig cells are the interstitial cells; those cells have abundant eosinophilic cytoplasm and blood vessels and the Leydig cells located in the interstitial space and also the tunica albuginea at increasing detail (Figure 1).

The atrophic seminiferous tubules exhibit decreasing number of seminal layers. The Leydig cells are dystrophic. The basement membrane is not uniform. Decreasing number of spermatozoa can be observed (Figure 2).

Diffuse atrophy of testicular tissue – only three seminal layers in a few seminal tubules, the base membrane is not uniform and the zone with the atrophy is free of spermatozoa (Figure 3).

Atrophy with decreasing of seminal layers in the seminal tubules is noticed, the spermatozoa are absent

in the tubules, we can observe only spermatocytes and spermatogonia (Figure 4).

We observed the absence of spermatozoa in the center of a few seminal tubules, the Leydig cells are decreasing and are dystrophic, and the base membrane is not uniform (Figure 5). The layers of seminal cells are present in diminishing number compared to the control group.

Severe tissue destruction, with absence of seminal layers and complete cessation of spermatogenesis was highlighted in Figure 6.

Severe tissue destruction, with absence of seminal layers also, no spermatozoa is present in the dystrophic areas (Figure 7).

Severe tissue destruction, with decrease of number of seminal layers has been revealed; no spermatozoa is present in the dystrophic areas (Figure 8).

No mature spermatozoa in the lumen of the seminiferous tubules were seen (Figure 9). In addition, a disorganization of epithelium of seminiferous tubules was observed.

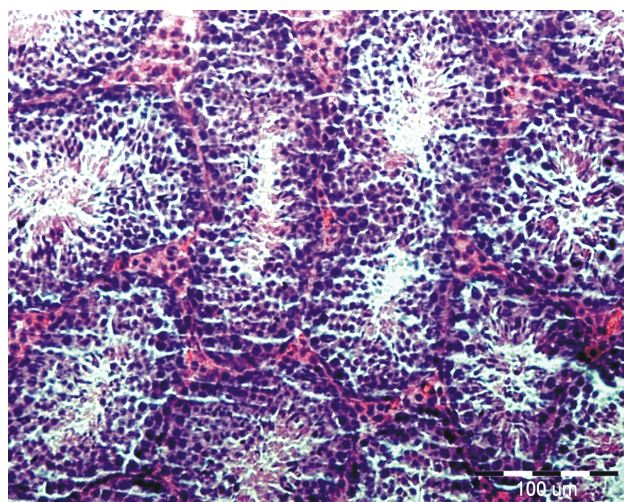


Figure 1 – Normal testicular tissue: control group. HE staining, ob. $\times 20$.

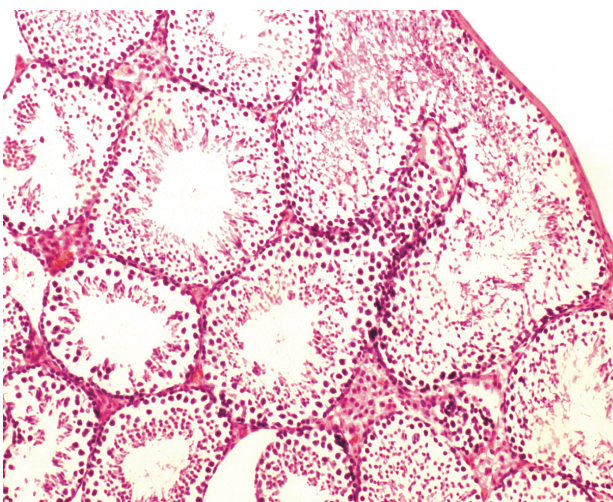


Figure 2 – Diffuse testicular atrophy. The decrease of number of linea seminalis layers. HE staining, ob. $\times 10$.

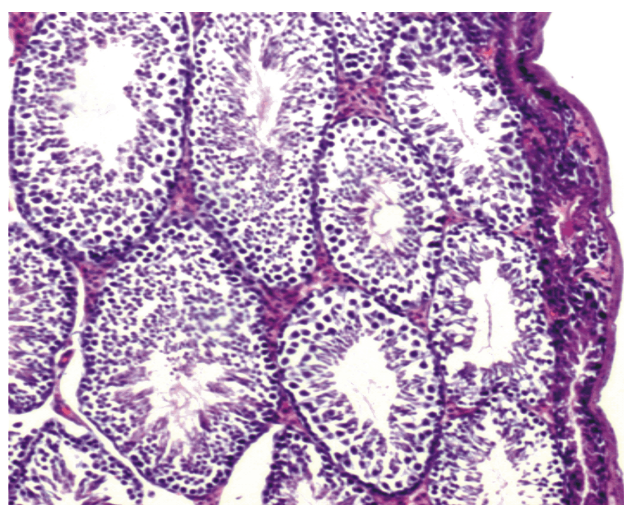


Figure 3 – Diffuse atrophy of testicular tissue. Seminal tubules with reduced number of layers of seminal cells. HE staining, ob. $\times 10$.

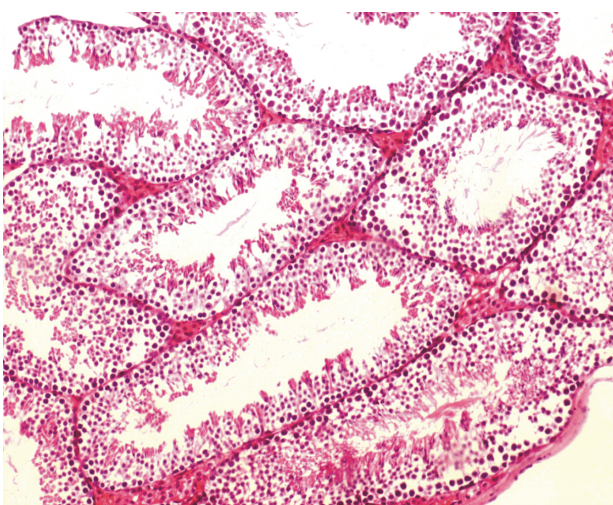


Figure 4 – Diffuse testicular atrophy. HE staining, ob. $\times 10$.

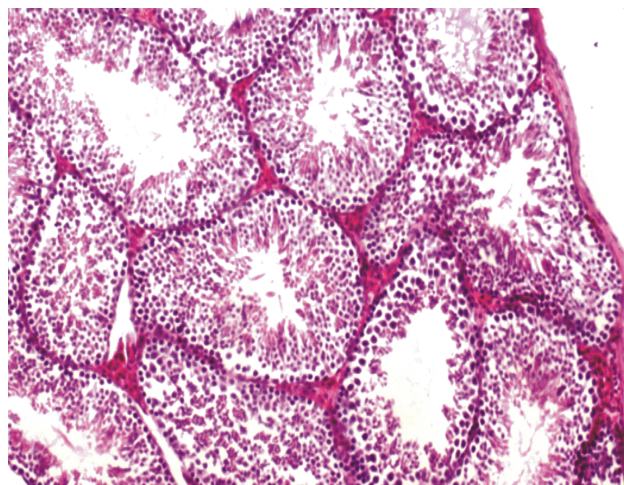


Figure 5 – Multifocal testicular atrophy. HE staining, ob. ×10.

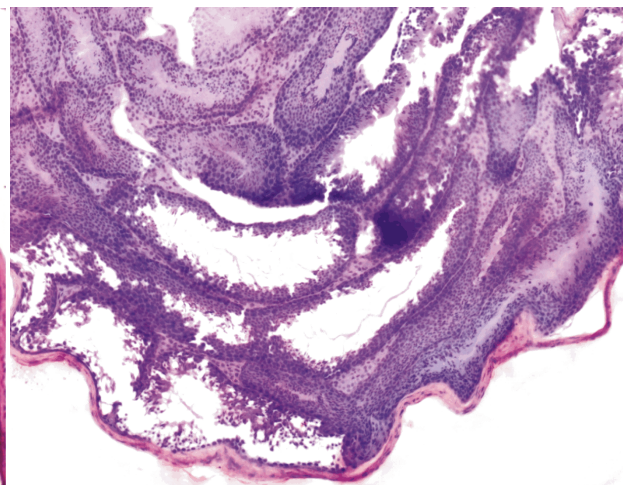


Figure 6 – Focal testicular atrophy. HE staining, ob. ×10.

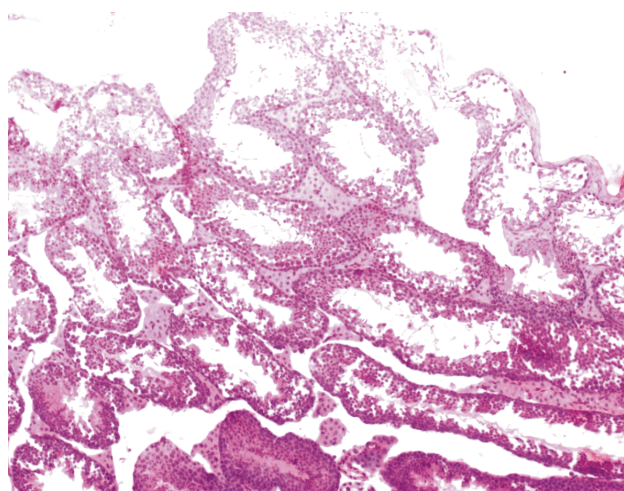


Figure 7 – Diffuse testicular atrophy. HE staining, ob. ×10.

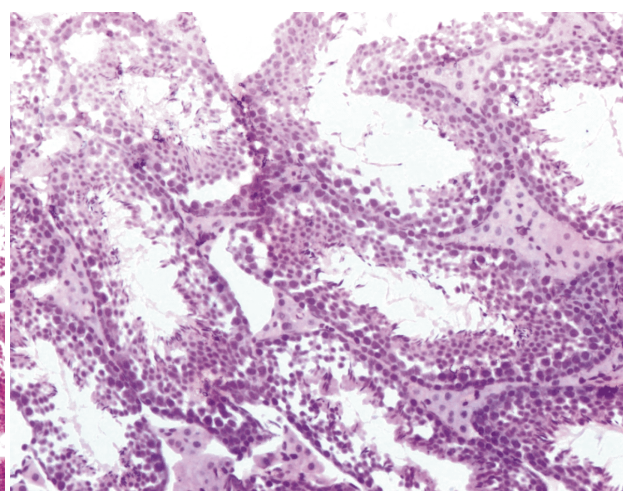


Figure 8 – Multifocal testicular atrophy. HE staining, ob. ×10.

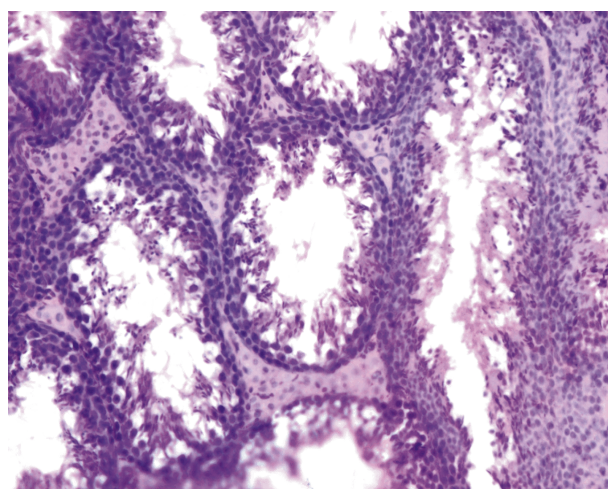


Figure 9 – Diffuse testicular atrophy. HE staining, ob. ×10.

Discussion

The study aimed to the evaluation of histopathological changes following sodium fluoride administration, at different dosages, both before birth until adulthood to a population of mice NMRI type. The function of the

studied organs is affected at different levels depending on the dose administered.

The dystrophy observed for all doses could be responsible for the altering the function of procreation. The effect on the number of germinative layers of the testicle revealed that this function could be altered at different doses, but is more altered at the allopathic dose. Even though we found alteration at the homeopathic dose, we recommend supplementary studies in the field of genetics and longevity.

Also, the placental influence of sodium fluoride could be responsible for the main modifications observed at the histopathological examination. It has been reported that the administration of sodium fluoride in drinking water at concentration of 2, 4, 6 ppm for six months to male rats affected their fertility and reproductive system [8]. Other researchers found that fluoride is unable to induce genotoxicity in tested cell types (testis) [9].

The pathological aspect of testicular tissue following the administration of fluoride at three types of doses revealed that the influence on the development of the gonads is very important for all doses administered. Histological examination of the testicular tissue revealed at the control group normal histological features such

as mature testes and seven germinative layers. The seminiferous tubules have a seminiferous epithelium.

The Sertoli cells, that are sustentacular cells, exists at the base of the seminiferous epithelium at the regular intervals – for the control group, feature not specific for the experimental groups with different doses of sodium fluoride.

The Leydig cells, interstitial cells located between the seminiferous tubules, have for the control group abundant eosinophilic cytoplasm. For the experimental group, the Leydig cells have not so abundant eosinophilic cytoplasm and only a few blood vessels.

In the mice treated with different doses of sodium fluoride, we found different aspects of the testes from the point of view of the histological modifications and we can discuss about a real evidence of different kind of injuries at that level. Some studies found that the administration of fluoride has adverse effects on the reproductive functions of male rabbits, and these effects are more pronounced with the increasing duration of exposure [10].

For the doses of 100, 200 and 300 ppm of sodium fluoride in drinking water, for four to 10 weeks, some researchers found that the fertility was significantly reduced at all three concentrations by exposure to 10 weeks, but not for four weeks [11]. Also, it is found that when drinking water is contaminated with sodium fluoride exists some adverse effects on the male reproductive system and these are associated with indicators of oxidative stress [12]. Sodium fluoride could decreased the activity levels of testicular steroidogenic marker enzymes 3β -hydroxysteroid dehydrogenase (3β -HSD) and 17β -hydroxysteroid dehydrogenase (17β -HSD) indicating decreased steroidogenesis and in turn spermatogenesis in rats exposed to sodium fluoride [13].

The examination of the testes in the groups descending from the pregnant females treated with 0.5 mg sodium fluoride revealed at the homeopathic dose vacuolar dystrophy in seminal cells.

For the allopathic–homeopathic dose, it is revealed active testicles and only six germinative layers, smaller cell nuclei and vacuolar dystrophy in seminal cells.

For the allopathic dose, the testes prelevated from the mice descending from the pregnant females treated with 0.5 mg sodium fluoride revealed vacuolar dystrophy in seminal cells and only one layer of cells in the seminal tubules. The effects on the testicular tissue are very aggressive. We conclude that the function of procreation is definitely affected. Some studies revealed at the dose of 10 mg/kg body weight of sodium fluoride administered to albino rats for 50 days, modifications on the structure and metabolism of sperm. The sperm acrosomal hyaluronidase and acrosin were reduced. Also, it was observed that the spermatozoa suffers a process of deflagellation and an activity of the enzymes more reduced than normal [14].

The histopathological examination of testicular tissue for the descendants from pregnant females with 0.25 mg sodium fluoride daily administration by gavage revealed for the group with homeopathic dose that the modifications at this level consist in vacuolar dystrophy in seminal cells

and variable germinative layers [3–5]. Other researchers found that the alterations in protein profile are more significant in the testis than the cauda epididymis [15].

For the allopathic–homeopathic dose for the same descendants, the histopathological aspects revealed vacuolar dystrophy in seminal cells and necrosis and vacuolar dystrophy in epithelial cells of epididymis.

For the allopathic dose for the mice with 0.25 mg% sodium fluoride, we found vacuolar dystrophy in stromal cells and also vacuolar dystrophy in epithelial cells of epididymis and vacuolar dystrophy in seminal tubules.

The dystrophic aspect of the testis is more important in the group treated with the allopathic dose compared to other doses, no depending on the allopathic dosage is given: 0.25 mg or 0.5 mg sodium fluoride. It is important to achieve more research in the field of genetics regarding this type of substance administered to humans.

Histopathological observation revealed some moderate to severe changes (vacuolar dystrophy in seminal cells; variable germinative layers [3–5]; vacuolar dystrophy in seminal tubules) in the testicular tissue on mice at different doses of sodium fluoride.

We observed severe injuries for the allopathic doses, such as vacuolar dystrophy at seminal cells and seminal tubules and with diminishing of germinative layer.

Conclusions

The study indicates that at different doses of sodium fluoride, the histopathological aspect of testicle on male mice is modified and is characterized by vacuolar dystrophy in seminal cells, vacuolar dystrophy in seminal tubules and diminishing of germinative layer. We found a disorganization of epithelium of seminal tubules, which caused a complete absence of spermatogenesis in the testis. The most important modification is observed at the allopathic dose. The pathological analysis revealed disruption of spermatogenic cells in the seminiferous tubules. The consequence is the degeneration of spermatozoa. Also, at the homeopathic dose, we found alterations of the testicular tissue such as vacuolar dystrophy in seminal cells. The same alterations were found for the allopathic–homeopathic dose. Multiple pathological disorders discovered on males reproductive organs when sodium fluoride is administered indicate problems in the reproductive process and in to get healthy descendants.

References

- [1] Ortiz-Pérez D, Rodríguez-Martínez M, Martínez F, Borja-Aburto VH, Castelo J, Grimaldo JI, de la Cruz E, Carrizales L, Díaz-Barriga F, *Fluoride-induced disruption of reproductive hormones in men*, Environ Res, 2003, 93(1):20–30.
- [2] Xiao YH, Sun F, Li CB, Shi JQ, Gu J, Xie C, Guan ZZ, Yu YN, *Effect of endemic fluoride poisoning caused by coal burning on the oxidative stress in rat testis*, Zhongguo Yi Xue Ke Xue Yuan Xue Bao, 2011, 33(4):357–361.
- [3] Xu R, Shang W, Liu J, Cheng X, Ba Y, Huang H, Cui L, *Experimental study on fas expression of spermatogenic cell in male rats induced by fluorine*, Wei Sheng Yan Jiu, 2010, 39(3):358–360.
- [4] Jiang CX, Fan QT, Cheng XM, Cui LX, *Relationship between spermatogenic cell apoptosis and serum estradiol level in rats exposed to fluoride*, Wei Sheng Yan Jiu, 2005, 34(1): 32–34.

- [5] Mogoşanu GD, Popescu FC, Busuioc CJ, Părvănescu H, Lascăr I, *Natural products locally modulators of the cellular response: therapeutic perspectives in skin burns*, Rom J Morphol Embryol, 2012, 53(2):249–262.
- [6] Mogoşanu GD, Popescu FC, Busuioc CJ, Lascăr I, Mogoantă L, *Comparative study of microvascular density in experimental third-degree skin burns treated with topical preparations containing herbal extracts*, Rom J Morphol Embryol, 2013, 54(1):107–113.
- [7] Baconi DL, Bărcă M, Manda G, Ciobanu AM, Bălălaşu C, *Investigation of the toxicity of some organophosphorus pesticides in a repeated dose study in rats*, Rom J Morphol Embryol, 2013, 54(2):349–356.
- [8] Gupta RS, Khan TI, Agrawal D, Kachhawa JB, *The toxic effects of sodium fluoride on the reproductive system of male rats*, Toxicol Ind Health, 2007, 23(9):507–513.
- [9] Lowry R, Steen N, Rankin J, *Water fluoridation, stillbirths, and congenital abnormalities*, J Epidemiol Community Health, 2003, 57(7):499–500.
- [10] Kumar N, Sood S, Arora B, Singh M, Beena, *Effect of duration of fluoride exposure on the reproductive system in male rabbits*, J Hum Reprod Sci, 2010, 3(3):148–152.
- [11] Elbetieha A, Darmani H, Al-Hiyasat AS, *Fertility effects of sodium fluoride in male mice*, Fluoride, 2000, 33(3):128–134.
- [12] 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(4):385–390.
- [13] Pushpalatha T, Srinivas M, Sreenivasula Reddy P, *Exposure to high fluoride concentration in drinking water will affect spermatogenesis and steroidogenesis in male albino rats*, Biometals, 2005, 18(3):207–212.
- [14] Narayana MV, Chinoy NJ, *Reversible effects of sodium fluoride ingestion on spermatozoa of the rat*, Int J Fertil Menopausal Stud, 1994, 39(6):337–346.
- [15] Chinoy NJ, Shukla S, Walimbe AS, Bhattacharya S, *Fluoride toxicity on rat testis and cauda epididymal tissue components and its reversal*, Fluoride, 1997, 30(1):41–50.

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Received: May 18, 2013

Accepted: December 10, 2013