Effects of Sub Lethal Concentrations of Sodium Fluoride on Sperm Activity and on the level of Sex Hormones of Adult Male Albino Rats

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ABSTRACT

In recent years, fluorosis caused many problems in humans and animals bodies. Dental fluorosis, skeletal fluorosis, bone fractures, decreased birth rates, adverse effects on the male genital system, like damaging the structure of testes and epididymis and loosing fertilization ability can be considered as good examples of fluorosis. Thus, the current study aimed to determine the toxicity of sodium fluoride (NaF) on the activity of sperm and the level of sex hormones including testosterone, follicle stimulating hormone, and luteinizing hormone. For achieving this purpose, thirty adult albino male rats, aged between 90-100 days, were divided randomly into two treated groups with 10 rats for each group which were treated with 150, 300 ppm of sodium fluoride,respectively. In addition, 10 rats were kept as a control group. Sodium fluoride was offered to the treated groups in the drinking water to evaluate the toxic effect of NaF on male reproductive system, sperm concentration, sperm motion, and sperm velocity compared with control group. The findings revealed a significant decrease in the sperm concentration, sperm count, sperm motion, sperm velocity and the level of sexual hormones in comparison with the control group. It can be concluded that NaF may reduce the efficiency of male reproductive system, and reduce the levels of sexual hormones in rats.

Keywords: male genital system, sperm viability, male hormone, rats, NaF.

Introduction

Fluoride is a natural element that is widely distributed throughout the environment. Fluoride anions exist in drinking water and in the ground water, and these materials can be transmitted through winds and volcanic activity (**1**, **2**).

Fluoride usually prevails in two forms (organic and inorganic), the organic compounds are like methyl fluoride, per fluorooctanes sulfonic acid, and the inorganic compounds are like calcium fluoride, hydrogen fluoride and sodium fluoride (**3**). Knowingly, fluorine is considered as the most effective caries preventing agent (**4**), the permissible amount of fluoride in drinking water is 0.5 - 1.5 mg/L (**5**, **6**). Contexually, other applications of fluoride include mouth rinses, toothpaste, dental products, industrial products, pesticides and dietary supplements (**6**). However, excessive consumption of fluoride results in fluorosis, reduce antioxidant defense mechanism and increase oxidative stress of organs (**7**).

Several epidemiological investigations refer to the toxic effects of fluoride on male genital organs (**8**). Renewing the database of each previously studied issue like fluorosis had a major benefit in accurate judgment and evaluation of its risk. Thus, the toxic effects of sodium fluoride can be ameliorated by using antimatter material such as pomegranate seed oil (**9**).

The purpose of the current work was to determine the toxic effects of NaF on male genital organs of adult albino rats that experimentally subjected to different concentrations of it.

Materials and Methods

Animals

The present study utilized thirty male albino rats. The age was between 90 - 100 days with weight between 200 - 250 gm. The animals were placed in cages and held at room temperature in animal

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Pathology and Poultry Diseases, College of Veterinary Medicine, University of Mosul, Mosul, Iraq. Received: 2020, Accepted: 2020, Published: 28 December 2020.

house, College of Veterinary Medicine, University of Baghdad. The ration and water were given *ad libitum*.

Experimental design

The experimental design was based on the session scheduled by the Department of Pathology and Poultry Diseases at the College of Veterinary Medicine, University of Baghdad, held on 4-12-2019, the ethical approval was followed for conducting the experiment. The study lasted three months. Rats were randomly divided into three groups. Ten animals were allocated for each group. Group one was kept as a control group. This group received taps water for 90 days, while groups two and three received 150 ppm and 300 ppm of NaF (Central drug house (p) LTD.7/28 India) for 90 days within drinking water. All animals were sacrificed at the 91st day of the experiment. Determination of sub lethal dose of NaF was applied according to authors (**10**).

After scarifying of the experimental animals, the left caudal epididymis was taken and put in a Petri dish containing 3 µL of normal saline. The epididymis was dissected by a surgical scissors to several species.

The samples were transferred to the laboratory and 0.1 µL of the liquid was taken and put on a special slide for examination (semen analysis series of casa 10) and by using X40 (Mshot microscopic, Germany, software). The parameters followed in this study were sperm motility, high motility, moderate motility, sluggish, immortality, progressive, non-progressive, active sperm, velocity of curved line, velocity of straight line, average of moving sperm, density in rectilinear, linearity, wobbling, straight forward and so on.

Collection of blood for hormonal assay

At the end of the experiment, all animals were injected with ketamine 5 mg/kg-xylazine 50 mg/kg) (**11**). The blood was collected from the orbital sac and instantly was discreted by centrifuge for 3 minutes at 3000 rpm, Biomerieux-FranceMini VIDIS kit was used and the methods were followed on the combination between an enzyme immunoassay sandwich and final fluorescent detection (**12**).

Sperm motion analysis

Two microliters of phosphate–buffered saline was used for sperm motion analysis. The left caudal epididymis was instantly put and cut into about 150 pieces using surgical scissors for liberating the spermatozoa from the epididymal tubes. For further study, epididymal semen suspension (ESS) was instantly brooded at 37^{0}C. Computer assisted semen analysis (CASA) (Biomerieux, France) was used by placing 5 µl of the suspension on a glass slide (**13**).

Sperm count analysis

In order to make a dilution ratio of 1:1000, one ml of epididymis's semen suspension was added to 199 µL of normal saline. The total concentration of sperm was estimated by Neubauer haemocytometers (**13**).

Hormonal analysis

Sex hormone *i.e.* testosterone (TS), follicle-stimulating hormone (FSH), luteinizing hormone (LH) were determined after separation of sperm of the male rats using mini vidas commercial kit (code number 09345B, Bio merieuxmarcy I Etoile-France) for all experimental samples.

Statistical analysis

All variable data were analysed by one–way ANOVA with post-hoc Duncan's test. The statistical values were presented as mean ± standard error (SD) (**14**).

Results

The effects of different NaF concentrations in drinking water on the kinetic motility of the spermatozoa and sex hormone were widely studied.

Sperms motility

The results showed a significant reduction in all motility parameters of the treated groups. However, high, moderate, and sluggish sperm motility values were much lower ($p \leq 0.05$) in the groups given 150 ppm and 300 ppm of sodium fluoride than those values of the control groups.

Also, a significant decrease ($p \leq 0.05$) in the immotility values was noticed in the treated groups

given different doses of sodium fluoride as compared to the non–treated groups (Table 1). It was observed that numbers of abnormal, irregular and disfigured sperms were higher in groups treated with different concentrations of sodium fluoride as compared with those of control untreated groups. There was a proportional increase in the rats of abnormal sperm with concurrent increase in the levels of sodium fluoride administered with drinking water (Table 2).

Table 1. Effects of different sub lethal concentrations of sodium fluoride given with drinking water on general sperm motility of male rats (%), mean ± SE

Groups / Parameter	Control	150 ppm	300 ppm
Motility%	78.56 ± 2.36 a	63.56 ± 2.01 b	55.38± 2.07 c
high M	23.83 ± 1.40 a	6.83± 1.24 b	5.33 ± 0.71 b
moderate M	21.75 ± 1.89 a	15.70 ± 1.56 b	9.65 ± 1.21 c
Sluggish M	6.88± 1.00 c	14.26± 1.14 b	19.50 ± 1.68 a
Immotility	14.33 ± 0.90 c	26.86± 2.25 b	32.60 ± 2.12 a

(a-c) Different letters mean a significant decrease between the groups at the probability p≤0.05.

Table 2. Effects of different sub lethal concentrations of sodium fluoride given with drinking water on rates of motility of sperm, mean ± SE.

Groups	Progressive	Non-progressive	Rate of motility
Control	17.28 ± 3.27 a	4.33 ± 0.91 a	12.11 ± 1.47 c
150 ppm	10.85± 0.83 b	1.83± 0.40 c	21.05 ± 2.31 b
300 ppm	3.68 ± 0.32 c	2.50 ± 0.22 b	27.85 ± 2.52 a

(a-c) Different letters mean a significant decrease between the groups at the probability p≤0.05.

Sperms count

Examination of semen specimen of the group treated with 150 and 300 ppm revealed a significant decrease in the parameters of mean total semen, sperm count and active sperm in comparison with these of control group (Table 3).

Table 3. Effects of different sub lethal concentrations of sodium fluoride given with drinking water on various sperm characteristic (%), mean ± SE.

groups / parameters	control	150 ppm	300 ppm
Total sperm count/millions /ml	70.60 ± 2.88 A	54.53 ± 0.71 b	46.50 ± 1.52 c
Conc. of sperm/10^6	3.53± 0.14 a	2.72±0.03 b	2.35 ± 0.01 c
Active sperm/mls	79.50 ± 0.48 a	55.83 ± 1.16 b	53.16 ± 1.01 b

(a-c) Different letters mean a significant decrease between the groups at the probability p≤0.05.

Velocity of sperms

Sperm velocity showed a decrease in both lines (curved and straight) of the treated groups with 150 and 300 ppm of NaF as compared with the untreated group. Also, Table 4 pointed out that both treated groups have a significant (p≤0.05) reduction of sperm number in rectilinear movement in comparison to the control groups. However, values related to average movement parameters of the treated groups differ significantly from those of the control group (Table 4).

Table 4. Effects of different sub lethal concentrations of sodium fluoride given with drinking water on the various velocity of sperm and rectilinear movement, mean ± SE.

groups / parameters	Control	150 ppm	300 ppm
Velocity of curved line ML/S	39.35±1.549 A	25.0±2.42 b	22.56±3.63 b
Velocity of straight line %	60.73±2.98 A	25.50±17.09 b	18.36±1.67 c
Average of movement degree	172.47±17.29 A	351.65±13.76 b	411.15±21.75 c
Sperm No. in rectilinear movement	13.16 ± 0.79 A	4.83± 0.65 b	2.83± 0.79 c
Sperm Density in Rectilinear movement 10^6/ml	0.12±0.00 A	0.067±0.01 b	0.03±0.01 c

(a-c) Different letters mean that there is a significant decrease between the groups at the probability p≤0.05.

Movement of sperms

Results in Table 5 shows a significant ($p \leq 0.05$) reduction in the linearity, straight in rectilinear movement, wobbling, beating cilia frequency, and number of sperms in rectilinear movement in the NaF groups in contrast to the control groups.

Table 5. Effects of different sub lethal concentrations of sodium fluoride on the movement of sperm, mean ± SE.

groups / parameters	Control	150 ppm	300 ppm
Linearity%	45.52 ± 2.51 A	34.51 ± 1.74 b	21.32 ± 0.84 c
Straight forward	21.75 ± 1.72 A	11.34 ± 1.00 b	9.77 ± 1.12 b
Wobbling	258.25 ± 6.37 A	188.56 ± 15.19 b	146.46 ± 7.03 c
Beating cilia frequency time/s	15.83±0.90 A	9.60±0.66 b	5.66±0.84 c
Number in fast rectilinear movement/10^6	1.30±0.12 A	0.66±0.06 b	0.30±0.11 c

(a-c) Different letters mean a significant decrease between the groups at the probability $p \leq 0.05$.

Effects of NaF in drinking water on the sex hormone

From Table 6, it is clear that there is a significant reduction ($p \leq 0.05$) in all levels of male sex hormones including testosterone (TS), follicle stimulating hormone (FSH), and luteinizing hormone (LH), in the treated groups as compared with the untreated control group. However, another similar significant decrease was seen in the level of TS hormone of the treated group given 300 ppm of sodium fluoride as compared to the treated group given 150 ppm of the same compound (Table 6).

Table 6. Effects of sub lethal concentrations of sodium fluoride on sex hormone, mean ± SE.

Groups	TS(ng/ml)	FSH(ng/ml)	LH(mlU/ml)
Control	2.72n± 0.15n a	1.22 ± 0.13 a	0.94 ± 0.18 a
150 ppm	1.82 ± 0.18 b	0.49± 0.08 b	0.64 ± 0.08 ab
300 ppm	0.85 ± 0.12 c	0.32 ± 0.05 b	0.43 ± 0.13 b

(a-c) Different letters mean a significant decrease between the groups at the probability $p \leq 0.05$.

Discussion

Oxidative stress, reproductive abnormalities, and infertility may be triggered by different environmental toxicants. Fluoride is well-known to have adverse effects on various living organisms as a toxic pollutant for the environment (**15**, **16**). Fluoride is naturally present in all surfaces and well waters. Based on a variety of factors, such as volcanic rocks and minerals, it may be very high in ground water. Drinking water is the major source of fluoride, contributing to the dental exposure (17). High level of fluoride causes toxic effects on the male genital system, such as injury of reproductive organs, tissues and cells (**17**), disturbance and dysfunction of endocrine reproductive system (**16**), and the expression of steroid genesis (**17**).

Sperm quality is one of the most significant male reproductive genital indexes. Fluoride-induced sperm quality changes have been seen in *vivo* and in *vitro* in many species, such as guinea pig, rats, chicken and rabbit (**18**). Experimental outcomes vary, however, some studies suggest that spermatogenesis in rats is not affected by exposure to sodium fluoride (**19**), on the other hand, experimental studies propose that low sperm quality and quantity may be caused by giving fluoride leading to reduced fertility (**20**). A fall in sperm viability and a high occurrence in sperm

abnormality were obtained in the current study of the treated groups with 150, 300 ppm of NaF for 90 days and subsequent findings were recorded by several researchers (**21**).

The process by which fluoride affects the motility of sperm has not been explicitly described. However, it has been postulated that sodium fluoride could directly act on sperm motility without influencing other metabolic processes (**22**). One possible technical issue could be due to reduction in the amount of fructose level, which provides capacity for motility in the vas deferens and seminal vesicle with consequent changes in carbohydrate metabolism (**23**).

It was postulated that fluoride may suppress function of many enzymes, the first work of fluoride is that binding with cofactors such as Se, Zn, Ca, and Mg, thereby inhibiting glycolysis, breathing, and sperm motility, or it may cause insoluble complex with phosphates or magnesium in enzyme like alkaline phosphatase, and acid (**23**) leading to adverse action. At the present study, there was a significant reduction in sperm motility and progressive motility in the treated groups with sodium fluoride for 90 days as compared to the control groups. However, such finding correspond with those of several researchers (**24**) who confirmed that exposure of male mice to 25, 50 and 100 mg/L of sodium fluoride for 90 days decreased motility of sperm due to inhibition of mitochondrial aspiration.

Not only does the smooth progress of spermatogenesis involve a suitable environment in the testis, but various sex hormones are also involved (**25**), Leydig cell–generated Ts, which regulated spermatogenesis in the initial stage. Any reduction in the content of Tscan leads to fail of spermatocyte and infertility (**26**).

Previous studies have shown that exposure to fluoride in Leydig cell can inhibit the development of TS, influencing spermatogenesis (**25**). In the current work, the level of TS is decreased in the treated groups with 150, 300 ppm of NaF, this reduction may be due to structural injury to Leydig cells of the treated groups. Ma *et al* recorded that fluoride can mainly affect Ts synthesis through Leydig cell structure damage. Moreover, several hormones such as FSH, LH, and E2 are co-operated in maturation and spermatogenesis, under the influence of FSH. Sertoli cells of testes synthesize estrogen and androgen. However, estrogen can affect the secretion of androgen and gonadotropin through the regulation and maturation of sperm and lately spermatogenesis. Similar studies, however, have shown that fluoride can inhibit the circulation of FSH, secretion of E2, and distribution of LH and TS in the axis of hypothalamus-pituitary-axis (**25**). The current result indicated that the level of sex hormones were decreased in the treated groups, this reflects that sodium fluoride can disturb the secretion of TS, LH, and FSH, with subsequent blockage of maturation and inhibition production of sperm, leading to dysfunction of the physiology of the reproductive system.

Conclusions

From the present results we conclude that treatment with NaF for 90 days causes toxic effects on the male genital system, reflected by impairment of sperm motility and quality, distributing the secretion of sex hormones and decrease their levels, and finally causes reproductive dysfunction

Acknowledgment

The authors desire to thank and express their appreciation to the College of Veterinary Medicine, University of Baghdad and University of Mosul for their support.

Conflict of interest

The Authors have no conflict of interest about the study

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تاثير التركيز دون المميت الوسطي لمادة فلوريد الصوديوم عل كفاءة الحيوانات المنوية والهرمونات الجنسية لذكور الجرذان البيضاء البالغة

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الخلاصة

في السنوات الاخيرة سبب التسمم بالفلور العديد من المشاكل في جسم الانسان والحيوان، ويمكن اعتبارتسمم الاسنان بالفلور، والفلور الهيكلي ،وكسور العظام، وانخفاض معدلات المواليد، والاثار السلبية على الجهاز التناسلي الذكري مثل تحطم تركيب الخصية والبربخ وفقدان القدرة على الاخصاب امثلة جيدة على التسمم بالفلور ،وهدفت الدراسة الحالية الى تحديد سمية فلوريد الصوديوم على نشاط الحيوانات المنوية ومستوى الهرمونات الجنسية بما في ذلك هرمون التستوستيرون، والهرمون المحفز للجريب، والهرمون اللولتيني. تم تقسيم ذكور الجرذان التي تراوحت اعمارها 90-100 يوماً بصورة عشوائية الى مجموعتين معالجتين وبواقع 10 جرذان لكل مجموعة وعلى التوالي ،عوملت المجموعتان باستخدام 150، 300 جزء بالمليون من فلوريد الصوديوم على التوالي، بالإضافة الى 10 جرذان كمجموعة ضابطة. اعطيت المجاميع المعالجة مادة فلوريد الصوديوم في الماء لدراسة تأثيرها السمي على الجهاز التناسلي، وعلى تركيز الحيوانات المنوية، عدد الحيوانات المنوية، حركة الحيوانات المنوية ،وسرعتها مقارنة بمجموعة السيطرة، وكشفت النتائج عن وجود انخفاض معنوي في تركيز الحيوانات المنوية، عدد الحيوانات المنوية، حركة الحيوانات المنوية ،كثافة الحيوانات المنوية ومستوى الهرمونات الجنسية مقارنة مع مجموعة السيطرة، يمكن الاستنتاج من الدراسة ان مادة فلوريد الصوديوم تقلل من كفاءة الجهاز التناسلي الذكري ومستوى الهرمونات الجنسية في الجرذان .

الكلمات المفتاحية: الجهاز التناسلي الذكري، حيوية الحيامن، الهرمون الذكري، الجرذان، فلوريد الصوديوم.