

## Environmental Toxicity of Sodium Fluoride on Fertility in Male Rats and the Protective Role of Inulin Extract

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Received: 10 October 2017 /Accepted: 2 December 2017

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### Abstract

The aim of this work to investigate the effects of sodium fluoride (NaF) on male reproductive function and evaluate the protective effects of inulin against sodium fluoride (NaF) induced male reproductive toxicity in adult male albino rats.

In this study, forty adult male albino rats were used, they were divided randomly into four groups: a control group (1), which was given distilled water, and three treatment groups, which was received daily oral doses of; sodium fluoride (10 mg/kg.b.w.) of group (2), inulin (10 mg/kg) + sodium fluoride (10 mg/kg) of group (3), and inulin (10 mg/kg.b.w.) of group (4) for 3 months.

The sperm quality, complete sperm motility, morphology and sperm count were assessed. The levels of follicle-stimulating hormone (FSH), luteinizing hormone (LH), estradiol (E2), testosterone (T), and progesterone (P) were used as indicators of male reproductive health. The whole testis tissues isolated was used for histopathological studies.

The results showed that sodium fluoride induces severe toxic changes in the male reproductive system and inulin could provide these toxic effects.

**Keywords:** sperm quality, Sodium Fluoride, Inulin testosterone, picture of the blood.

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### Introduction

Fluoride is an essential element to human body; naturally exists in water, soil, and food. Human are exposed to fluoride from a number of sources, including drinking water, dental products and other commodities (Long *et al.*, 2009; Edzwald, 2011). Human are exposed to fluoride from a number of sources, including drinking water, dental products. Fluoride is a ubiquitous element in the environment, as a widespread natural pollutant, fluoride normally

enters human body through drinking water, food, industrial pollution, drugs, cosmetics (Zhanga *et al.*, 2013).

The increasing exposure to environmental pollutants and chemicals contributes as a major cause for reproductive health problems. Approximately 15% of human couples are affected by infertility, and about half of them can be attributed to men that the quality of human sperm is decreasing because of environmental pollutants and chemicals (Ortiz-Perez *et al.*, 2003; Spittle, 2009). Literature

search revealed that fluoride was shown to decrease fertility and negatively affects sperm function, including morphology, motility, capacitation, and the acrosome reaction (Kim *et al.*, 2015).

Epidemiological investigations have established a statistically significant relationship between excessive fluoride exposure to male infertility and low birth rates (Freni, 1994; Long *et al.*, 2009). Furthermore, animal experimental evidence the deleterious impact of fluoride on sperm morphology and motility, capacitation, hyper activation, and spermatogenesis (Sun *et al.*, 2010; Zhu *et al.*, 2014). Additionally, numerous clinical investigations and animal studies revealed that fluoride is a testicular toxicant that disrupts reproductive hormones levels, induces structural and functional defects in sperm and consequently reduced fertility (Lu *et al.*, 2014; Sun *et al.*, 2014; Dong *et al.*, 2016).

Epidemiological studies indicated that environmental exposure to fluoride was associated with male and low birth rates in endemic areas of fluorosis. The potential relationship between long-term fluoride exposure and fertility impairment has attracted concern (Long *et al.*, 2009; Spittle, 2009). The importance of reproductive health on offspring development has also prompted an epidemiological investigation into the apparent connections between excessive fluoride exposure and male infertility and low birth rates (Freni, 1994; Ortiz-Perez *et al.*, 2003).

Inulin is a water-soluble storage polysaccharide and belongs to a group of non-digestible carbohydrates called fructose. Inulin is extensively available in about 36,000 species of plants, amongst, chicory roots are considered as the richest source of inulin. Commonly, inulin has been a part of our daily food intake for centuries contributing to nutritional properties and exhibits significant technological benefits (Shoaib *et al.*, 2016).

Although, the role of inulin has also been explored in the past few years but nutritional and health benefits along with food applications, specifically its use as functional food was focused in this review. Therefore, the goal of this study was to investigate the effects of sodium fluoride (NaF) on male reproductive function. The protective effects of inulin was evaluated against sodium fluoride (NaF) induced male reproductive toxicity in adult male albino rats.

## Experimental Methodology

The present study investigated the environmental toxicity of sodium fluoride (NaF) on fertility in male rats. The sperm quality, complete sperm motility, morphology and sperm count, sperm abnormality were assessed. The levels of follicle-stimulating hormone (FSH), luteinizing hormone (LH), estradiol (E<sub>2</sub>), testosterone (T), and progesterone (P) were used as indicators of male reproductive health.

### Experimental Protocol

Forty adult male albino rats were used in this study, 250-350 g in weight along with the supplies of their standard diet and maintained in controlled conditions at 24±22C, and 12hr. light/12 hr. dark cycle. All animals were housed in stainless-steel cages suspended in stainless-steel racks for 90 days with relative humidity ranging from 30% to 55%. Briefly, the albino rats were divided randomly into four groups of 40 each: a control group (1), which was given distilled water, and other three treatment groups, which was received daily oral doses of; sodium fluoride (10 mg/kg.b.w.) of group (2), inulin (10mg/kg) + sodium fluoride (10 mg/kg) of group (3), and inulin (10 mg/kg.b.w.) of group (4) for 3 months (Table 1).

Table (1): Demographic Characteristics of Controls and Treated Animals.

Groups	No	sex	Body weight gain (g)	Dose	Treatment
Group 1	10	♂	255.32± 29.22	----	Control
Group 2	10	♂	263.08± 16.46	10 mg/kg.b.w	Sodium fluoride (Naf)
Group 3	10	♂	252.50± 41.31	10 mg/kg.b.w Naf. +10 mg/kg.b.w Inuli	Sodium fluoride + Inulin
Group 4	10	♂	269.75± 29.37	10 mg/kg.b.w	Inulin

The whole testis tissues isolated was homogenized in cold saline solution and the homogenate was kept frozen at -20°C until being analyzed, weighed and the testis were stored in 10% buffer formalin and later used for histopathological studies .

### Semen Analysis

Semen analysis was done for each animal in the studied groups. These include sperm count/million, abnormalities, Sperm vitality and sperm

quality Each semen specimen was taken by cutting tail portion of epididymis of male rats liberating sperm in 1ml of phosphate buffered saline solution for at least half an hour.

### ***Serum Sampling and Detection of Hormones Levels***

After the administration of NaF for 90 days, the rats were sacrificed. Blood were then collected from the hearts and placed in a refrigerator for about 2hr. Serum was stored at -20°C until analysis. The serum hormone levels of FSH, LH, E2, TH and PH were measured using radioimmunoassay kits and automated analyzer.

### ***Statistical Analysis***

Results were expressed as means  $\pm$  SE. Statistical significance was calculated using one-way analysis of variance (ANOVA). All the statistical analysis was carried out with the use of SPSS 22 software. Differences were considered Significant at  $P \leq 0.05$ .

## **Results and Discussion**

### ***Semen Quality***

Table 2 indicates the results at the survival periods of 90 days after the administration of sodium fluoride (NaF) and inulin for treated male albino rats. The sperm count of ( $6.05 \times 10^6/\text{ml}$ ) when compared to that of control group in male rats ( $23.21 \pm 5.56 \times 10^6/\text{ml}$ ), and decreased in group 3 ( $16.82 \pm 6.13 \times 10^6/\text{ml}$ ) compared to the controls. Whereas, it was decreased weak in the sperm count of group

4 (inulin) ( $20.82 \pm 9.87 \times 10^6/\text{ml}$ ) compared to the controls.

The sperm motility of group 2 (NaF) had decrement significantly to (26%) when compared to that of (non-treated) control group of male rats (72.86%), and decreased in group3 (43%) compared to the controls. Whereas, weak decreased in the sperm count of group 4 (64.80%) compared to the control. The sperm motility of group 2 had decrement significantly (38.4) when compared to that of control group (90), and decreased in group 3 (85) compared to the controls. Whereas, there was weak decreased in the sperm count of group 4 (88) compared to the controls. That indicated that fluoride exposure caused a decrease in spermatozoa, these results are in agreement with **Izquierdo-Vega et al. (2008)**.

Fluoride exposure affects the spermatozoa motility, motility was significantly reduced in spermatozoa from fluoride treated rats, compared with the control group ( $81 \pm 6.8$  vs.  $92 \pm 5.6$ ,  $p=0.027$ ), respectively. The spermatozoa concentrations were ( $106$  cells/ml) ( $97 \pm 0.95$  vs.  $41.1 \pm 14.2$ ,  $p=0.027$ ), respectively and the spermatozoa. Viability was ( $95 \pm 1.5$ ,  $30.6 \pm 13.5$ ), respectively. These results are in agreement with **Sun et al. (2016)** that fluoride lowered sperm motility mainly by damaging mitochondrial respiration rather than glycolysis. Similar observation was investigators by **Lu et al. (2014)** that NaF affects the mice sperm chemo-toxic function and decrease the number of sperms. In contrast, **Eraslan et al. (2007)** demonstrated there were no effects of NaF exposure on the sperm number and morphology as well as body weight and organ absolute and relative weights, in spite of the presence of a mild degree of dental fluorosis in the higher dose.

Table (2): Effect of NaF, on Sperm Count, Motility and Viable of Male Albino Rats Comparing to the Protective Effects of Inulin against Sodium Fluoride (NaF) as well as Controls.

Parameters		Groups			
		Control Group (1)	Sodium fluoride group (2)	Sodium fluoride and Inulin group (3)	Inulin group(4)
Count $10^6/\text{ml}$	Mean $\pm$ SD	23.21 $\pm$ 5.56	6.05 $\pm$ 1.48	16.82 $\pm$ 6.13	20.82 $\pm$ 9.87
	Pvalue	---	0.01	0.47	0.95
	%ofchange	---	-73.93	-27.53	-0.44
Motility%	Mean $\pm$ SD	72.86 $\pm$ 25.7	26 $\pm$ 20.73	43 $\pm$ 32.71	64.80 $\pm$ 29.2
	Pvalue	----	0.04	0.426	0.974
	of%change	-----	-64.31	-40.98	11.06-
Viable/Dead	Mean $\pm$ SD	90 $\pm$ 5.19	38.4 $\pm$ 31.67	85 $\pm$ 5.00	88 $\pm$ 6.64
	P value	----	0.002	0.644	0.912
	of % change	----	-57.33	-5.55	-2.22

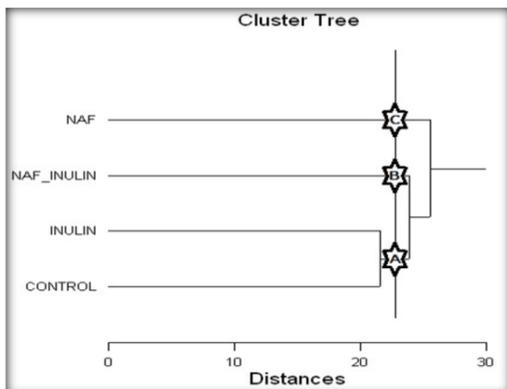
$P < 0.05$ . (n=5). \* Significant at  $P < 0.05$  Abbreviations; C, Control; NaF; sodium fluoride, NaFI; sodium fluoride and Inulin, I; Inulin

Results from previous studies indicated a statistically significant relationship between excessive F exposure to male infertility and low birth rates (Freni, 1994; Long *et al.*, 2009), which is supported by our previous study that high F administration induced poor mice reproductive outcomes (Sun *et al.*, 2011).

#### Analysis According to Count and Motility of Sperms

The application of cluster analysis based on the similarity different count and motility values of sperms of different control and treated groups (4 variables) led to the recognition of three groups. The high similarity index in parameters values between each type appears within the same group, while differential index parameter values separate them in different groups (A, B and C).

Group A comprises two groups; control and inulin treated group. Group B comprises one site; NaF-Inulin treated group. Group C comprises one site; NaF treated group. From the results, control and Inulin treated group drains showed high similarity in the different count and motility values of sperms **Figure (1)**.



**Fig. (1):** Analysis of different rat groups according to count and motility of sperms of treated and control rats.

**Sperm Counts:** The changes in the Sperm Count in the different treated groups. High

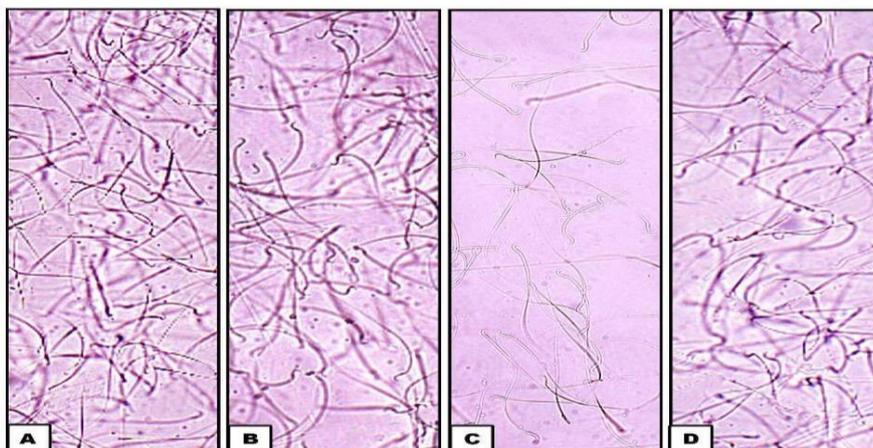
sperm count about as over  $23.2-20 \times 10^6/\text{ml}$  semen in (A-B), moderate sperm count about  $16.82 \times 10^6/\text{ml}$  semen IN (D) and low sperm count about  $6.05 \times 10^6/\text{ml}$  semen IN (C)(100X).

#### Evaluation Hormones Level in Treated Rats and Controls

**Table 3** represents the comparison between the treated group with Sodium fluoride comparing to the protective effects of inulin against sodium fluoride (NaF) as well as controls.

It was established that there was a significant decrease in the concentration of the testosterone, luteinizing hormone LH and follicle stimulating hormone (FSH) in comparing with controls. These results are in accordance with previous research with rats where showed fluorosisation marked reduction in testosterone hormone (Susheela and Jethanandani, 1996; Huang *et al.*, 2007). Confirmatory results derived by Reddy *et al.* (2007), where serum testosterone follicle stimulating hormone and luteinizing hormone, were significantly altered in rats after exposure to Na F. In addition, testicular disorders have been reported to be associated with Na-F-induced oxidative stress in reproductive organs along with possible adverse effects of fluoride on pituitary testicular axis. Similar findings were reported by (Ghosh *et al.*, 2002; Wan *et al.*, 2006; Han *et al.*, 2015; Li *et al.*, 2016).

However, progesterone and estradiol ( $E_2$ ) levels showed a significant increase in comparing with controls. Whereas, the decline in serum hormone established in the protective effects of inulin against sodium fluoride (NaF) induced male reproductive toxicity of adult male albino rats. However, the reduction in the testosterone level is correlated with the testicular spermatogenic day's dysfunction were also found in a study of Chandra *et al.* (2007).



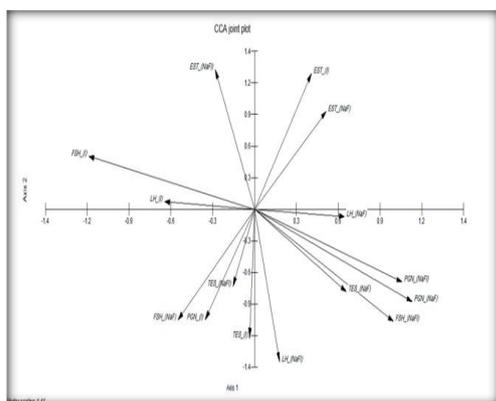
**Fig. (2):** Photomicrograph of semen represents three different sperm count throughout the different experimental groups showing the numerical sperm values. Abbreviations: (A). Control group ,(B) Inulin group,(C) sodium fluoride group ,(D) sodium fluoride and inulin group.

Table (3): Effect of NaF, on serum (LH, TESTO, FSH, E2, PGH) of Male Albino Rats received daily oral dose of sodium fluoride (NaF) and inulin comparing to Controls

Parameters		Groups			
		Control group	Sodium fluoride group	Sodium fluoride and Inulin group	Inulin group
LH (MU/ml)	Mean± SD	0.013±0.009	0.010±0.011	0.0125±0.009	0.0125± 0.009
	P value	----	0.732	1.000	1.00
	Of %change	----	-23.07	-3.85	-3.85
TESTO (pg/ml)	Mean± SD	630.22±118.11	177.03±36.84	117.52±8.59	585.05± 172.78
	P value	-----	0.00	0.00	0.56
	Of % change	-----	-71.91	-81.35	-7.17
FSH (MU/ml)	Mean± SD	0.12±0.08	0.11±0.02	0.08±0.05	0.09±0.02
	P value	----	0.738	0.242	0.550
	Of % change	-----	-8.33	-33.33	-25
E <sub>2</sub> (ng/L)	Mean± SD	14.86±2.48	24.07±4.57	21.22±6.00	19.82± 6.20
	P value	----	0.024	0.099	0.189
	Of % change	-----	+61.98	+42.8	+33.4
PGN (ng/ml)	Mean± SD	1.03±0.04	0.80±0.19	0.76±0.17	1.04± 0.12
	P value	-----	0.044	0.021	0.885
	Of % change	-----	-22.33	-26.21	+0.97

Each value represented the mean± SD. P<0.05. (n=5). \* Significant at P<0.05

**Note:** The levels of LH (reference range =1.7–8.6IU/l), FSH (reference range=1.5–12.5IU/l), and testosterone (reference range=2.5–8.0ng/l) immunometric assay using commercial kits and automated analyzer



**Fig. (3):** Canonical Corresponding Analysis (CCA) ordination diagram of serum hormones of male tested rats (arrows) according to the gradient of the same variables of control sample.

### Histopathological Examination

In this prospective study, we observed the testis of control and inulin groups, show normal morphological appearance of seminiferous tubules in various shapes and sizes with stratified epithelium. Each tubule showed regular arrangement of the spermatogenic cells (spermatogonia, spermatocytes and spermatids).

Spermatogonia and Sertoli cells rested on the basement membrane, surrounded by a concentric my fibroblast layer. Many metamorphosed spermatids and spermatozoa were observed near the lumen of the tubules. Leydig cells were situated in between the tubules.

Sertoli cells were present in all somniferous tubules (Figs. A, B).

After 90 days of sodium fluoride treatment, the testes showed degenerative changes and arrest of spermatogenesis. Also showing, decreases in the diameter of the seminiferous tubules and in the spermatogonial population and number of spermatozoa. Spermatozoa and elongated spermatids were rare. In the group treated with sodium fluoride, focal disorganization of the seminiferous tubules associated with a marked loss of many of the germ cells and the lack of sperms were noted. Degenerated Leydig cells were observed. Sodium fluoride -treatment showed mild sloughing of germ cells from the basement membrane. Enlarged intercellular spaces between germ cells and Sertoli were observed. Morphologically, there was disruption of the seminiferous epithelium, as indicated by large intercellular spaces between Sertoli cells, and between Sertoli and germ cells (Figs. C, D)

The testis shows no histopathological changes in groups treated with sodium fluoride and inulin. The testes show normal spermatogenic cells and many sperms were noticed. Increase in the diameter of seminiferous

tubules, spermatids and Leydig cells were seen. Supplemented group with inulin had well-defined seminiferous tubules lined by spermatogonia, primary and secondary spermatocytes, spermatids (in various phases of spermatogenesis) and the spermatozoa were easily identified. The interstitial cells were highly developed; being relatively large and irregular in shape (Fig. E). Control rats received distilled water showing normal seminiferous. Tubules with all stages of spermatogenesis, Normal inter-tubular Spaces with connective tissue, the lumen filled with spermatozoa.

In the current study, the testicular tissues of rats received sodium fluoride revealed degenerative changes and arrest of spermatogenesis. Also showing, decreases in the spermatogonial population and number of spermatozoa. These findings were go parallel with the results of **Sun *et al.* (2011)** who observed that fluoride exposure induced a marked change in testis structure including the disarrangement of spermatogenic cells and the reduction in luminal sperm. Further confirmation of the present results comes from the study of **Pushpalatha *et al.* (2005)** who mentioned that fluoride induces the changes in spermatogenic process.

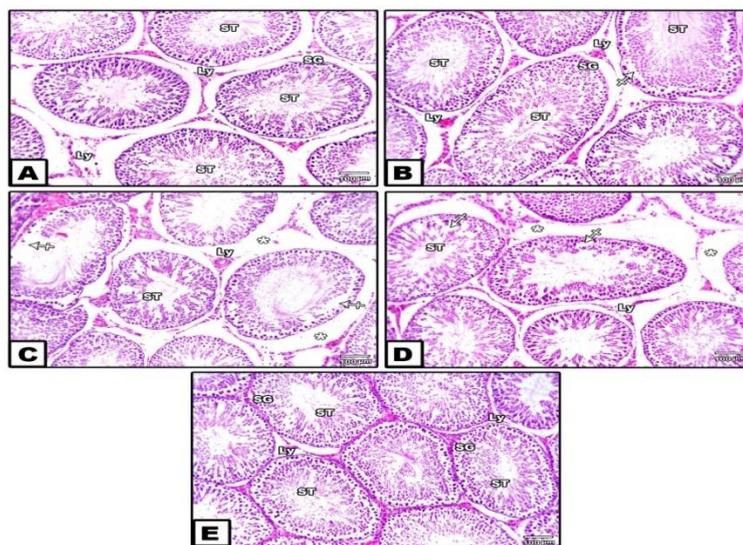


Fig.(4) :( A-E). Photomicrographs of histological sections of rat testis.

(A). Control showing normal arrangement of germ cells.

(B). Inulin supplemented group showing normal morphological appearance of seminiferous tubules with normal spermatogenic cells.

(C,D). Sodium fluoride-treatment showing massive reduction of spermatogenic, detached spermatogenic cells, lumen devoid of spermatozoa and shrinked seminiferous tubule with incomplete spermatogenesis.

(E). sodium fluoride& inulin

Abbreviations; **ST**-Seminiferous Tubules, **SG**-Spermatogonia, **Ly**- **Leydig** cells, Cross arrows-Sloughing of germ cells, Star-Enlarged intercellular spaces or Damage of **Leydig** cells.hematoxylin and eosin staining.

Similar observations were investigated by (Guzick *et al.*, 2001) showing sperm with any abnormal morphology could result in the low function, adversely impacting on the successful fertilization. In addition, that NaF adversely affected the sperm dysfunction and sperm chromatin structure in dose dependent mice (Sun *et al.*, 2014). In the present data, decreased level of spermatogenesis and qualitatively affected the morphological structure of the testicular tissue were observed (Dvoráková-Hortová *et al.*, 2008).

The results reported in histopathological analysis of testis in Na-F treated males, where severe necrotic degenerative changes in seminiferous epithelium of testicular tissues, deficiency of sperms in lumina, enlarged intercellular spaces between germ cells and Sertoli, numbers of spermatid giant cells were also detected. The results are in agreement with earlier reports (Wan *et al.*, 2006; Gupta *et al.*, 2007; Tiwari and Pande, 2009 and El-Iethy *et al.*, 2011). Germ cells, in comparing with somatic cells, are more susceptible to oxidative germ cells are intimately associated with the free radical generating phagocytic Sertoli cells; germ cell plasma membrane contains a higher amount of polyunsaturated fatty acids that are vulnerable to oxidation by free radicals (Lenzi *et al.*, 2000).

In the present study from histological observations of testis revealed that the number of sperms decreased, spermatogenic cells disarranged, supported previous reports. That NaF markedly induced histopathological lesions of testis in different degree (Gupta *et al.*, 2007 and Han *et al.*, 2015). In contrast, Sprando *et al.* (1997) reported that NaF treatment did not adversely affect spermatogenic activity in animals. This toxic effect of fluoride on reproduction is due to inhibition of enzyme activity; particularly of enzymes whose cofactor is the action of a bivalent metal like Mg, Ca, Zn, and Se.

Analysis of testicular histology induce shriveled seminiferous tubule and the diminution of mature sperm in the lumen of the seminiferous tubule. This was in conformity with (Ghosh *et al.*, 2002) which elucidated that fluoride treatment was associated with a reduction in mature sperm in the lumen of the somniferous tubule. Histopathological alterations in testis of the present study manifesting principally as somniferous tubule atrophy, impaired spermatogenesis and germ cell loss. This was in conformity with (Zhang *et al.*, 2013) who

reported that mitochondrial injury and degeneration after fluoride exposure were observed.

The previous investigation indicates that sufficiently high fluoride intake through drinking water; may the fertility of male rats may be inhibited after NaF exposure. The secretion of E<sub>2</sub>, P, T, LH and FSH was suppressed in rats exposed to NaF. These results indicate that the reproductive function of male rats exposed to NaF is inhibited. The study investigated the role of Inulin in reversing sodium fluoride-induced inhibited the fertility of male rats. From the results, control and Inulin treated group drains showed high similarity in the different count and motility values of sperms.

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## المخلص العربي

### عنوان البحث: التأثيرات البيئية لفلوريد الصوديوم على الخصوبة في ذكور الجرذان والدور الواقى لمستخلص الإنبولىن

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التعرض للتأثير السمي لفلوريد الصوديوم من عدد من المصادر، بما في ذلك مياه الشرب ومستلزمات العناية بالأسنان والمواد التي تستخدم لطب الأسنان والمشروبات والأغذية المحضرة بالفلورايد والأدوية والمبيدات لها لعدد من المشاكل الصحية، بما في ذلك الجهاز التناسلي .

لذلك فإن الهدف من هذا البحث هو دراسة التأثيرات المختلفة المحتمل حدوثها نتيجة تأثير فلوريد الصوديوم NaF على الجهاز التناسلي لذكور الجرذان والقدرة الانجابية. وتقييم الدور الواقى لمستخلص الإنبولىن ضد سمية فلوريد الصوديوم . تم تقسيم عدد أربعين من ذكور الجرذان إلى أربع مجموعات، عشرة جرذان لكل مجموعة كالتالي : المجموعة الأولى تلقوا الماء المقطر فقط واستخدمت كمجموعة ضابطة. وتلقت المجموعة الثانية لكل فأر من المجموعة ١٠ مجم/كجم/ وزن الجسم/ يوم من فلوريد الصوديوم. وتلقت المجموعة الثالثة ١٠ مجم/كجم/ وزن الجسم/ يوم من فلوريد الصوديوم و ١٠ مجم/كجم/ وزن الجسم/ يوم من مستخلص الإنبولىن. اما المجموعة الرابعة تلقوا جرعات من الانبولىن ١٠ مجم/كجم/ وزن الجسم/ يوم من مستخلص الإنبولىن عن طريق الفم لمدة ٣ أشهر.

تم تقييم جودة الحيوانات المنوية وقد أظهرت نتائج هذه الدراسة زيادة في مستوى هرمون Testosterone في المجموعة الرابعة (اعطيت الإنبولىن) ، وعدد الحيوانات المنوية والحركية بالمقارنة مع المجموعة الضابطة . تم الكشف علي مستويات هرمونات (FSH) ، (LH) ، (E2)، Testosterone، و Progesterone (P) كمؤشرات لصحة الخصوبة للذكور. أظهرت النتائج إنخفاض في معدل هرمون ،و عدد الحيوانات المنوية وحركتها في المجموعة الثانية (تناولت فلوريد الصوديوم ) عند مقارنتها بالمجموعة الضابطة . وكذلك أظهر الفحص المجهرى لأنسجة الخصية ، موت الخلايا المبرمج الظاهري، و خلل في ترتيبها بالانبيبات المنوية. ونستنتج من ذلك أن التعرض لفلوريد الصوديوم في ذكور الجرذان قد يؤدي إلى مشاكل بالجهاز التناسلي الذي قد يؤدي الى صعوبة في الانجاب.