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Toxicopathologic Effects of Sodium Fluoride on Male's Reproductive System of Rabbits

KEYWORDS: Sodium fluoride, toxic effects on male's reproductive system of rabbits, histopathology.

Dr. Enaam Bader Falih
Asst. Prof. Pathology department/Veterinary Medicine College of Baghdad University

Dr. Zainab Ismail Ibrahim
Lecture Pathology department/Veterinary Medicine College of Baghdad University

ABSTRACT *Objectives: recently appeared that NaF had serious problems on the embryonic development due to its cytotoxic effect, so the purpose of the present study to estimate the chronic toxic effects of NaF on the male genital system of wild rabbits. Study: eighteen males of wild rabbits, divided into three groups; 1st group from six males of them were administered only single dose 50 ppm / NaF, 2nd group six males orally administered 50 ppm of NaF /45 days, 3rd group given PBS as negative control group. For histopathological study all animals sacrificed post time of toxicity and for cytogenetic study. Results: in testes there was atrophic necrosis and thickening of the basement membranes of seminiferous tubules, edematous fluid and hyalinization, vacuolar degenerative changes in sertoli cells, prostatic hyperplasia in chronic form of toxicity was recorded. Conclusions: long time administration of NaF had severe necrotic changes on testicular tissues and diminished the spermatogenesis.*

Introduction:
Sodium fluoride is one of important origin of fluoride (F^-) which rarely found free in nature (Martel and Cassidy, 2004). Fluorosis is the term of chronic fluoride toxicity; NaF had serious problems on the embryonic development due to its cytotoxic effect (ATSDR, 2010), including immunotoxic, sperm motility, and serum testosterone rats (Less, 2005). The significant role of fluoride is prevention of human dental caries was reported late in the third decade off this century and the adjustment of fluoride content of drinking water since the late 1940's are important for human health and well-being (Banana, 2007).

Fluoridated water may be having its most devastating effects on the most vulnerable, those in utero and infants less than one year old, whose brains are most sensitive to developmental neurotoxins such as fluoride (Murray, 2006). The results of researches explained that all the effects of NaF may causes (decreases in testosterone levels, reduced sperm motility, altered sperm morphology, reduced sperm quantity also increased oxidative stress and reduced capacity to breed) these changes observed in one study used high dose of fluoride (WHO, 2011). The changes in sperm quality induced by fluoride have been demonstrated in vivo and in vitro in many species, including the rat, mouse, rabbit, gerbil, guinea pig, bank vole, chicken and even people (Wan et al., 2006). However, experimental results differ. Some reports indicate that sodium fluoride does not affect sperm quality in rats (Collins et al., 2008) where as other experimental studies suggest that fluoride can cause low sperm quality and diminished fertility (Ghosh, 2002 & Wan et al., 2006). So the current study aimed to study the effects of NaF on male reproductive system of rabbits in acute and chronic stages by cytogenetic and histopathological examinations.

Materials and Methods:
1. Chemical: Sodium fluoride (99.99 % NaF) "Riedel-Dehaën. Germen", the toxic dose was prepared according to (Chouhan, and Flora, 2008).
2- Lab animals: (n=18) male rabbits aged about (6-8) weeks and weighed about 1-1.25 Kg were housed in the animal house of Veterinary Medicine College-Baghdad University, under optimal conditions from temperature and diet during the experiment.
3. Experimental Design: Eighteen of male rabbits aged (6-8) weeks and weighed (1- 1.25 Kg), were randomly divided into three groups and treated as following:

Group1: (n=6) was administered 50 ppm/ml NaF via stomach tube/single dose/rabbit.

Group 2: (n=6) was administered 50 ppm/ml NaF via stomach tube daily/45 days.

Group 3: (n=6) was administered sterile PBS orally/daily as control group.

All the animals group (1, 2 and 3) post NaF administration were sacrificed for cytogenetic study (micronucleus test) from bone marrow specimens of treated rabbits, and preserved their genital organs (testis, epididymis, prostate gland also) in 10% formalin for histopathological examination (Allen et al., 1977) (Luna, 1968).

4-Stastical analysis: Use as analysis of unidirectional (One-way Anova) and the analysis of bi-directional in the analysis of the humoral immune response. To analyze the data statistically Use the statistical ready SPSS (2008), and to study the moral differences between the averages of use Dunkin polynomial test (Duncan, 1955).

Results:
1- Micronuclei (MN):
Table (1); showed the number of micronuclei were increased significantly ($P < 0.01$) in first group (93.0±0.01) while in the second group was (77.32 ± 0.01) in compared with control group (82.4 ± 0.5).

Table- 1: Effect of NaF on micronuclei formation in male rabbit.

Group	Mean.± SE
1- acute dose	93.00 ± 0.01 a
2- chronic dose	77.32±0.01 b
3- control group	82.40 ± 0.5 a

2- Histopathology examination:
The testicular tissue showed variable degree of destructive

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lesions; characterized by vacuolization of sertoli cells, hyalinization of basement membrane and interlobular edema (Figure-1&2) which observed at single dose while the main findings at 45 days were severe thickening of tunica albugenia resulting in testicular atrophy with luminal cellular debris accompanied with hypo spermatogenesis (Figure- 3&4) and there was clumping of leydig cells appeared with deeply eosinophilic cytoplasm and rounded nuclei (Figure-5&6).

Disorganization of epithelial lining in both epididymis (Figure-7&8), and prostate with clumping sterocilia where seen at single dose while there was severe hyalinization of epididymis tissue with focal MNCs aggregations at 45 days post administration of NaF (Figure-9&10).

The prostate gland at acute toxic (single dose), showed evidence of variable degree of acinus epithelial hyperplasia accompanied with severe dilation resulted in giant acini and papillary projection formation (Figure-11&12). Infiltration of mononuclear cells "MNCs" mainly plasma cell, some acini filled with eosinophilic proteincions material especially in chronic groups (Figure-13&14).

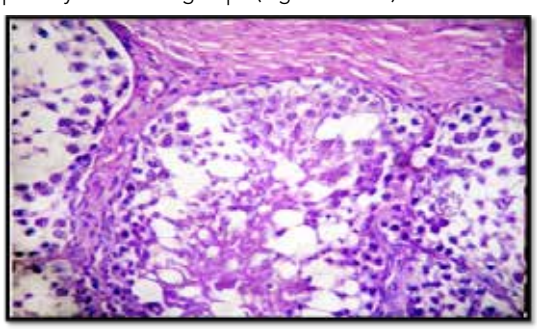

Figure-1: Histological section in the testis of rabbit treated with 50 ppm NaF/single dose; showed thickening of basement membranes of seminiferous tubules (H&E stain, 400X).

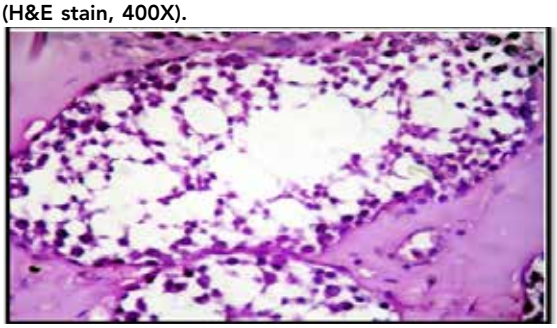

Figure-2: Histological section in the testis of rabbit treated with 50 ppm NaF/single dose; hypo spermatogenesis and intertubular edema (H & E stain, 400 X).

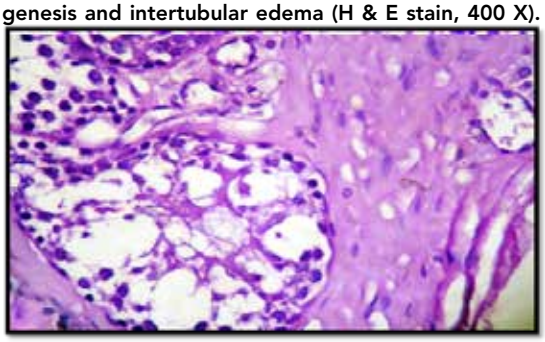

Figure-3: Testis treated with 50 ppm (NaF)/45 days; severe thickening of tunica albugenia of testis and hyalinization with atrophi somniferous tubules which contain cellular debris H & E stain, 40 X"

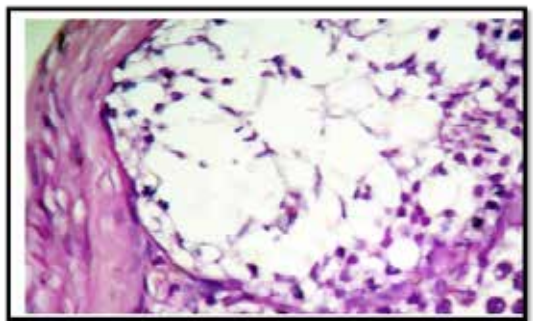

Figure-4: Testis treated with 50 ppm (NaF)/45 days; severe thickening of tunica albugenia of testis and hyalinization and no evidence of spermatogenesis (H & E stain, 400 X).

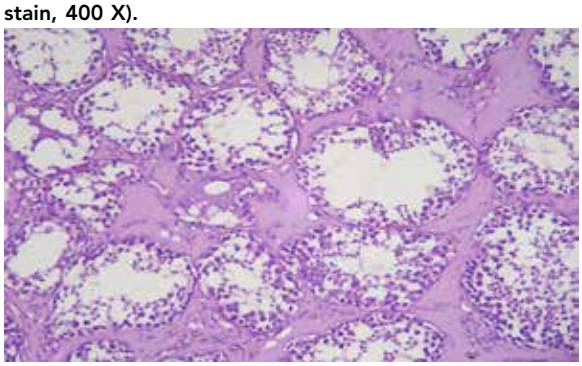

Figure-5: Histopathologic section of testis in rabbit treated (45 days), showed hypospermatogenesis with hyalinized stroma (H&E stain, 40X).

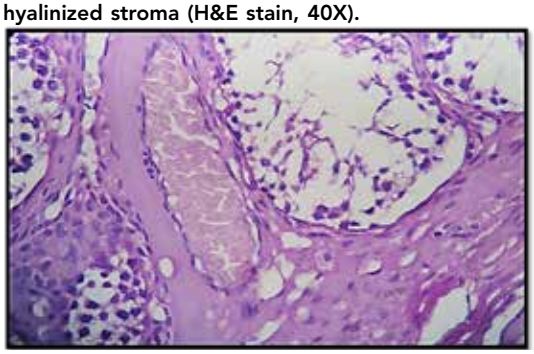

Figure-6: Histopathologic section of testis in rabbit treated (45 days), with focal prolioferated of leydig cells (left) around seminiferous tubules and edema (H&E stain, 40X).

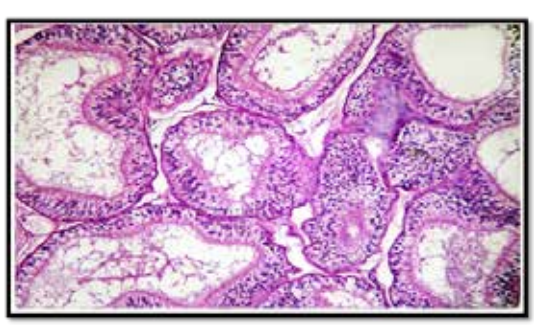

Figure-7: Histological section in the caput epididymis of rabbit administered 50 ppm (NaF)/45 days, show disorganization of pseudostratifed epithelial lining associated

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with vacuolization and nuclear

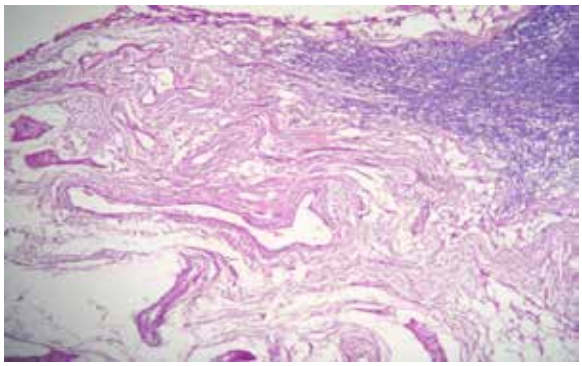

Figure-8: Histopathologic section of epididymis in rabbit treated with 50 ppm (NaF)/45 days showed fibromuscular hyperplasia in stroma and heavy infiltration of mononuclear cells (arrow),(H&E stain, 400X).

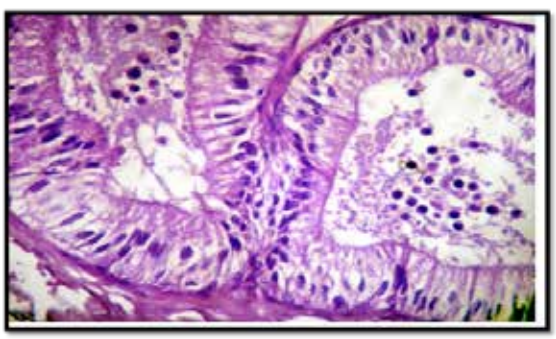

Figure-9: Histological section in the caput epididymis of rabbit, show the lumen of tubules contained inflammatory cells (MNCs) (H & E stain, 400X).

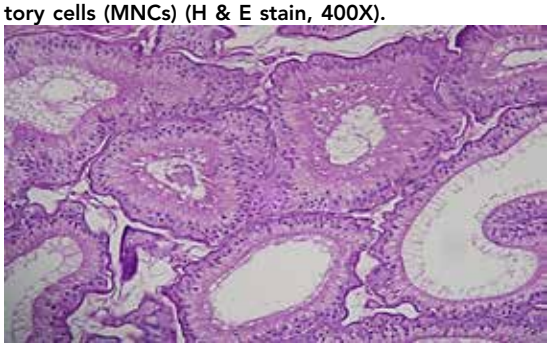

Figure-10: Histopathologic section of epididymus in rabbit treated NaF/45 days, with hypertrophy of lining epithelia and haylinized eosinophilic sperms (H&E stain, 400X).

Figure-11: Histopathologic section of prostate in rabbit treated with (sigle dose), showed fibromuscular hyperplasia of stroma caused wrinkling of mucosal epithelia of acini (H&E stain, 400X).

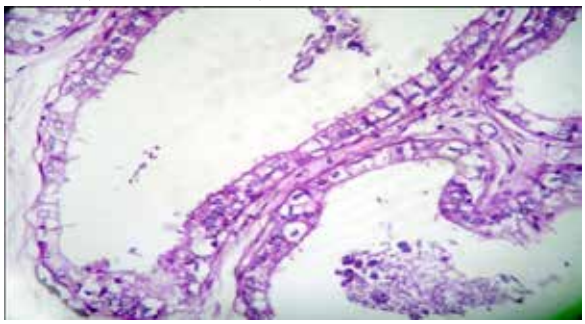

Figure-12: Histological section in the Prostate of rabbit (single dose); hyperplastic epithelial of acini accompany with severe dilation of acini with papillary projection lining (H & E stain, 400 X).

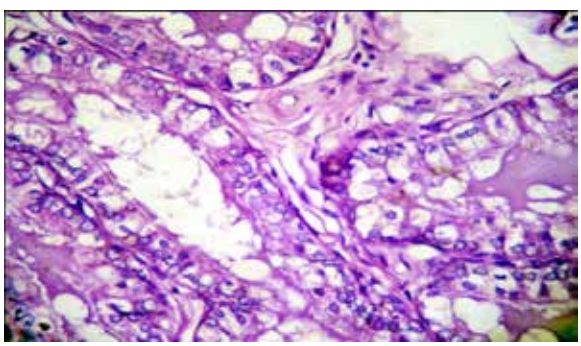

Figure-13: Histopathologic section of prostate in rabbit treated NaF/45 days, the acini filled with eosinophilic proteincions material (arrow) in addition to infiltration of "MNCs" (H & E stain, 400 X).

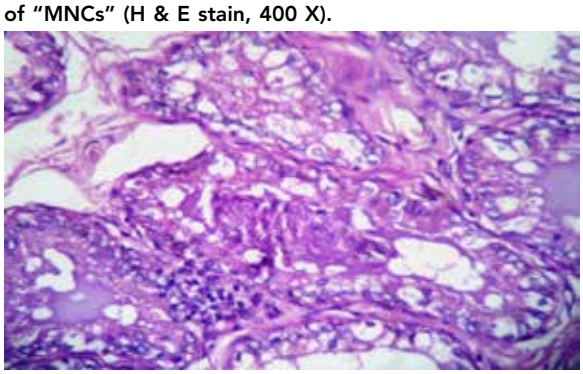

Figure-14: Histopathologic section of prostate in rabbit treated (45 days), with severe vacuolation and focal MNCs aggregation in stroma (arrow)(H&E stain, 40X).

Discussion:

The administration of NaF single dose in rabbits caused variable histopathological changes from thickening the basement membranes of seminiferous tubules due to the oxidative stress of fluoride ions on a decrease levels of testosterone hormone and effects on activities of testicular tissue (Murrray, 2006), which was compatible with high values of micronucleus formation, by both 3β & 17β hydroxy steroid dehydrogenase (3β&17β HSD) which play important role in testis, prostate, and seminal vesicle. The current resulst agreed with (Ghosh et al., 2002) who indicated that fluoride at a dose encountered in drinking water in contaminated areas exerts an adverse effect on the male reproductive system of rat and this effect was associated

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with indicators of oxidative stress.

The atrophic necrosis of seminiferous tubules was in acceptance with (Yang et al., 2002) explained that fluoride stimulated free radicals which increased lipid peroxide (LPO) levels, and decreased the activities of glutathione peroxidase (GSH-Px) and ATPase in testis and epididymis that picked up by mitochondria producing swelling and distortion of mitochondrial cristae, uncoupled energy metabolism, inhibited cellular respiration, and altered calcium kinetics follow; the organelles mediating cellular energy metabolism.

The present results of chronic dosage of NaF which effected on the reproductive organs (testis, epididymis and prostate gland) suggested the possible effect of anti-androgenic agent, which may have altered the physiology and metabolism of reproductive organs that concluded by (Tiwari and Pande, 2011) that the fluoride exposure to rats could induced alterations in normal architecture of the reproductive organs, and both described severe histopathological lesions of chronic dosage on parts of reproductive system of young rats; from hypo-spermatogenesis of seminiferous tubules in testes, in epididymis the lumen of ductules contained few sperms and thickening of surrounding stroma.

The infiltration of inflammatory cells (mononuclear cells) in testis and epididymis may due to severe irritation of fluoride ions on the tissue parenchyma that causes apoptosis and necrosis of spermatic cell (Yang et al., 2002) who investigated that the fluoride toxicity had effectives role on spermatic cell.

Conclusions:

The present results indicated that NaF toxicity was dose related and duration effect of the toxic dose on male's reproductive organs; testis, epididymis and prostate gland.

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