The Antioxidative Action of Dehydroepandrosterone (DHEA) on the Testicular Histology in adult mice treated with two doses of Nitrofurantoin

Tahani S.S.Al-Azawi* and Mohammed H. Asker**
Dept.of Physiology&Pharmacology/College of Veterinary Medicine University of Baghdad *PdF,PhD,MScMSc student**

Abstract:

The antioxidant role of Dehydroepiandrosterone(DHEA) was investigated against two dose of Nitrofurantoin (NFT). Seventy-two adult albino mice were divided in to six equal groups. (G1) control, (G2) received 2 mg/kg B.w of DHEA, (G3) received oral dose of 1.5 mg/kg B.W NFT, (G4) received oral dose of 3 mg/kg B.W of NFT,(G5) received DHEA and 1.5 mg kg/B.w NFT and (G6) received DHEA and 3 mg/kg B.w NFT. Histopathological examination of testes was studied after 30 and 60 day of the experiment and revealed a great number of testicular damage including interstitial hemorrhage and vacuoles, abnormal immature spermatozoa, atrophy in germ cells with severe damage and necrosis of tubules in Nitrofuantoin treated mice. In conclusion, this study showed that NFT has deleterious effect on the reproductive function of male mice and DHEA can protect this system against this harmful effect induced by NFT through its antioxidative activity.

Keywords: Nitrofurantoin, DHEA, male fertility, Histology.

وظيفة للديهايدروابي اندروستيرون كمضاد للاكسدة على نسيج الخصى في الفئران البالغة المعالجة بجرعتين من النايتروفيورانتوين

تهاني سلمان شعوبي العزاوي ومحمد حيدر عسكر
فرع الفسلجة والادوية/كلية الطب البيبطري /جامعة بغداد

المستخلص

تمت دراسة تاثير الفعل المضاد للاكسدة للدايهايدروابي اندروستيرون(DHEA) في ذكور الفئران البالغة المعالجة بجرعتين من النايتروفيورانتوين.

تم تقسيم اثنان وسبعون من الفئران الى ستة مجاميع متساوية:G1- سيطرة,وG2 جرعت ب 2 ملغم/كفم من وزن الجسم يوميا ب DHEA.وG2،G3-جرعت ب 1,5و3 ملغم/كغم من وزن الجسم يوميا ب Nitrofurantoin على التوالي.G5،G6- جرعت ب 1.5و3 ملغم/كغم من وزن الجسم ب Nitrofurantoin بالاظافة الى 2 ملغم/كغم من وزن الجسم DHEA يوميا على التوالي.تم اجراء الفحص النسيجي للخصى بعد قتل الحيوانا ت في نهاية الفترة 30و60 يوم من التجربة. اظهرت النتائج تلف كبير في خصى الفئران المعالجة ب Nitrofurantoin مع وجود نزيف وفجوات وكذالك عدد كبير من النطف المشوهة وغير الناضجة.تم الاستنتاج بان DHEA قادر على حماية الجهاز التناسلي الذكري من الاجهاد التاكسدي المتسبب عن المعالجة ب Nitrofurantoin.

المفتاح : نايتروفيورانتوين ، ديهايدروابي اندروستيرون،خصوبة الذكور ، الفحص النسيجي

Introduction

Infertility can be classified into primary and secondary, a male without biological off spring can be said to have primary infertility, whereas a male who is unable to impregnate his partner but who already has biological children is referred to as having secondary infertility [14]. Infertility affects approximately 15% of couples trying to conceive, and a male factor contributes to roughly half of these cases. Oxidative stress (OS) has been identified as one of the many mediators of male infertility by causing sperm dysfunction. OS is a state related to increase cellular damage triggered by oxygen and oxygen-derived free radicals known as reactive oxygen species ROS [1]. Excessive amounts of ROS affect redox balance and results in oxidative stress. Oxidative stress adversely affects cellular functions in various ways and has been evidently linked to the development of testicular dysfunction and a number of other diseases. Antioxidants preserve adequate function of cells against disturbances of homeostasis, including processes involving oxidative stress. Furthermore, antioxidant supplementation would theoretically protect or prevent peroxidative damage to testicular structures and may be helpful in male infertility [3] . Nitrofurantoin (NFT) 1-(5-nitrofurfurylideneamino) hydantoin, is a synthetic antibacterial Nitrofuran derivative It occurs as lemon yellow crystals, or fine powder, and is very slightly soluble in water or alcohol. However, solubility of the drug in water and urine increases, mainly used for the treatment and prophylaxis of uncomplicated urinary tract infections. However it has been shown that this drug may have mutagenic and carcinogenic effects and its long-term use is associated with adverse effects on liver, lung, cardiovascular, and reproductive systems [11]. The mechanism of action of Nitrofurantoin is by the reduction via bacterial flavoproteins to reactive intermediates, which inactivate or alter bacterial ribosomal proteins and other macromolecules. As a result, the vital biochemical processes of protein synthesis, aerobic energy metabolism, DNA synthesis, RNA synthesis, and cell wall synthesis are inhibited. Gram-negative organisms cause approximately 90% of uncomplicated UTI, and 80% to 90% of those gram negative organisms are *Escherichia coli.* Nitrofurantoin has a broad spectrum of activity against most gram-negative bacilli and many gram-positive organisms [11] [15]. Several case–control studies indicated that exposure to NIT during early pregnancy is associated with congenital malformations [12]. NFT at therapeutic doses can cause mitochondrial dysfunction, especially through inhibition of complex, thus, it seems that the toxicity of NFT can be partly mediated by mitochondrial complexes inhibition [9] [3]. Dehydroepiandrosterone (DHEA) is a very important prohormone secreted in large amounts by the adrenals in humans and other primates, but not in lower species. It is secreted in larger quantities than cortisol and is present in the blood at concentrations only second to cholesterol. All the enzymes required to transform DHEA into androgens and/or estrogens are expressed in a cell specific manner in a large series of peripheral target tissues [9] . DHEA secreted by the adrenal cortex (Zone Reticulars) in response to ACTH [13] . Dehydroepiandrosterone (DHEA)administration to female rate prior and during gestation improve fertility and pregnancy rates with increase number of alive newborn [11] .

Sansone [17] . studied the effects of DHEA on erectile function in a prospective, randomized, double blind, placebo-controlled trial and demonstrated that DHEA significantly improves erectile function. DHEA has been found to inhibit rat prostate carcinogenesis and the proliferation of human prostate cancer cell lines [13]. Our recent study revealed a significant protective role of DHEA on testicular function and semen evaluation of mice treated with NFT [2]. Thus the present study was conducted to study the antioxidant effect of DHEA on testicular histology of mice treated with NFT.

Materials and Methods:

This experiment was carried out at the University of Baghdad. Healthy adult male Swiss Albino mice were obtained from the Drug Control Center/Ministry of Health and housed in animals house of Biotechnology Research Center/Al-Nahraine University. Seventy two adult male mice were used in this study, their ages were ranged between 7-9 weeks, and their weight was around 23-27 gram. These animals were kept under suitable environmental conditions of 20-25 C^0 in an air conditioned room and photoperiod of 12 hours daily. The animals were divided randomly into six groups of twelve mice each. Each six mice were housed in a plastic cage of dimensions 12×15×30 cm, seventy two adult male mice were used in this experiment and were divided equally into six groups they were treated daily and orally as follows for 60 day G1-control, G2-received 2mg/kg B.w DHEA,G3 and G4 treated with 1.5 and 3 mg/kg B.w NFT respectively .G5 and G6 received DHEA and NFT at two doses respectively. At the end of 30 and 60 day, six mice from each group were sacrificed. Testes were dissected free of connective tissues, dried and weighed then submitted to preservation in formalin 10%. Histological preparation and staining with Hematoxylene and Eosine stain were performed according to **luna** [10].

Results:

Control group:

The Longitudinal section to the testes of control group shows seminiferous tubules with various stages of spermatogenic series (Fig.4-1). The majority of these tubules are lined by undifferentiated germ cells consist mainly from spermatogonia with slight smaller secondary spermatocytes . The examiner tubules also showed the presence of an elongated spermtide with recognized spermatozoa .In the luminal tubules also manifestation showed several number of sertoli cells that appear near together with single cell of interstitial cell (Leydig cell).

DHEA group after 30 and 60 day:

The principle finding of DHEA treated group showed well organized tubules and most spermatogenic cells lined are seen .This is accompanied with leydig cells prominence (Fig.4-2). The general characteristic of the seminiferous tubules at this period they are filled with highly mature sperms. The figure shows multiple aggregation of sertoli cells seen toward the lumen of tubule, together with increase in sperm density and quantity in the lumen (Fig.4-3).

Nitrofurantoin group (1.5 mg\Kg) B.W. after 30 and 60 day:

The germinal epithelium of affected tubules appeared disrupted with necrotic cells in the lumen with giant cells formation (Fig.4-4) While other sections showed sever necrosis of some tubules with eosinophilic necrotic mass appear near the basal compartment together with sever exfoliated spermatocyte. As well as evidence of interstitial hemorrhage with few leydig cells and the tubules show occlusion of the lumen by necrotic spermatids together with large vacuoles appear at the basal part .The majority of tubules appeared lined with partial disorganized spermatogonia cells composed mainly of few spermatogonia type B and moderated sertoli cells together with few number and slight spermatozoa appeared in the lumen with large mononuclear cells (figure4-5).

Nitrofurantoin group (3mg\Kg) B.w after 30 and 60 day:

There was extensive blood vessel congestion and dilation in the interstitial tissue and tubules have only few numbers of spermatogonia together with short immature spermatozoa (fig.4-6) . Although there is no evidence of spermatogenesis, the tissue around some tubules is paked with interstitial leydig cells that exhibited slight cytoplasm vacuolation and the tubules contain number of short immature spermatozoa and many of germ cells appeared atrophied.The section show sever extensive damage, necrotic in many of affected tubules with sever atrophy of their germinal epithelial with center clumping of rounded spermatides. There is complete absence and arrest of spermatogensis also the histopathological finding revealed sever necrosis of leydig cells that appeared with more eosinophilic cytoplasm with central nuclei (fig.4-7).

The protective role of DHEA against 1.5mg/ kgB.w Nitrofurantoin on testes after 30and 60 day:

Variable degree of cellular proliferation were recorded in the affected tubules resulted in considerable thickening of their germinal epithelia layer. Most of them composed of both spermatogonia and spermatocyte (this indicated both of proliferative and meiotic division) with slight evidence of immature spermatozoa as well as most tubules are dilated (Fig.4-8) . There is a slight thickening of basement membrane together with an increase in number of rounded and elongated spermatieds, with evidence of spermatogenesis observed in this group (fig. 4-9).

The protective role of DHEA against 3mg/kg B.w Nitrofurantoin on testes after 30 and 60 day:

Some tubules showed no clear pathological changes in the germinal epithelia also with evidence of mature spermatogenic cells, in both germinal epithelia of some tubules and leydig. While in other sections hyperatrophy of some seminiferous tubules resulting from sertoli cell hyperplasia accompanied with slight increase in number of leydig cells. The evidence of mature spermatozoa in some tubules with early phase of spermatogenesis recorded in surrounding other tubules due to presence of a number of rounded spermatide(Fig. 4-10). Extensive fibroblasia was recorded in tunica albugina that covered many of seminiferous tubules with slight cellular infiltration together with slight degeneration changes of surrounding tubules that appear more elongated. However, there is evidence of spermatogenesis in some tubules which

showed high density of germinal epithelia and their lumen are filled with mature spermatozoa Fig . (4-11) .

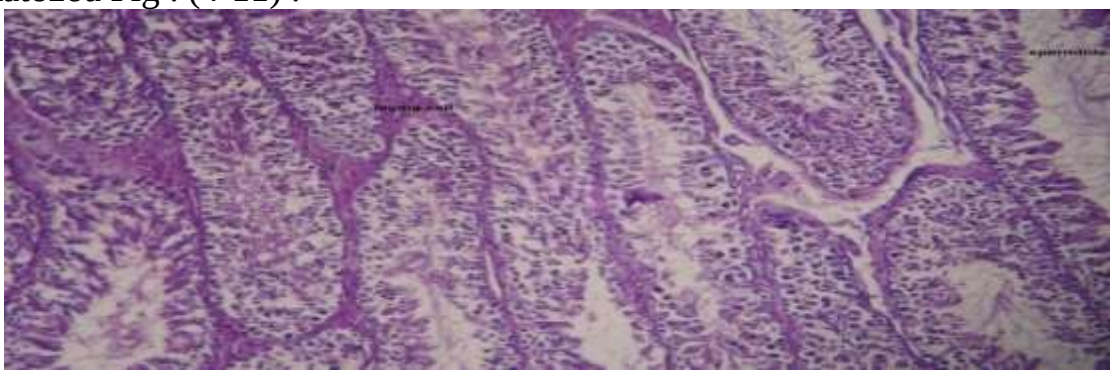

Fig. 4-1 Light microscope photograph of testes of mice (control group); shows seminiferous tubules and spermatogenic series. All tubules show spermstogenic cells in late phase, (H&E stain, 200X).

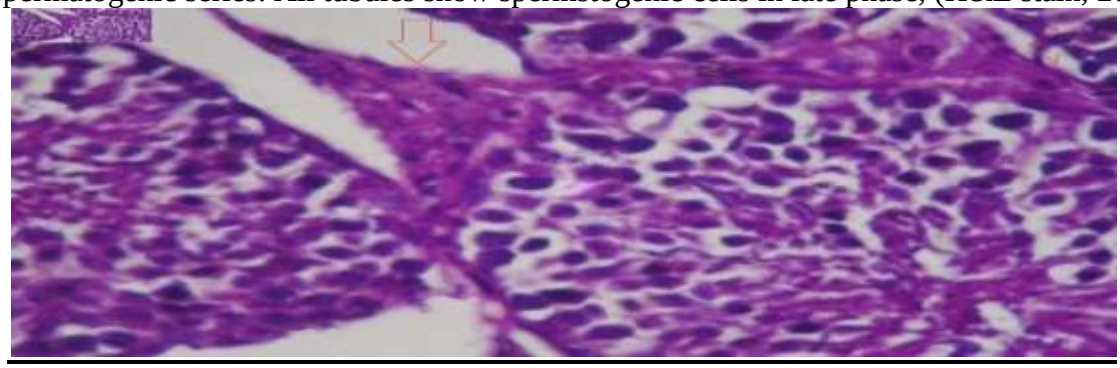

Fig. 4-2 Light microscope photograph of testes of mice received 2 mg /kg B.w. DHEA after 30 day; shows the tubules are well organized &most spermatogenic cell

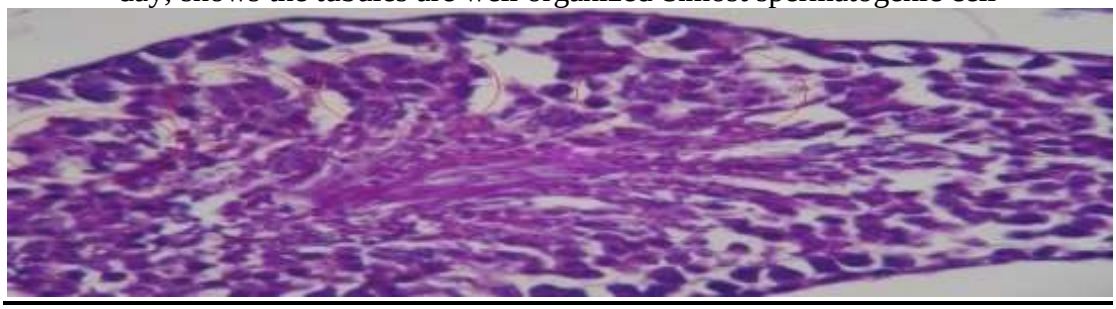

Fig.4-3 Light microscope photograph of testes of mice received 2 mg kg.B.W. DHEA after 60 day(G2) shows multiple aggregation of sertoli cells (H&S stain ,400X).

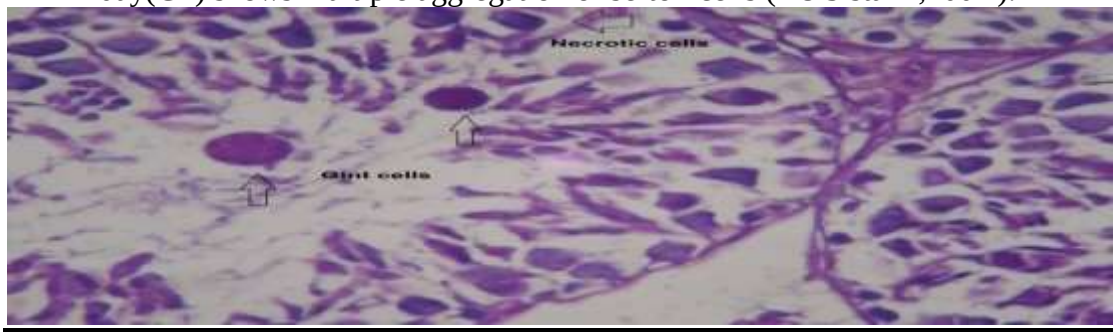

Fig.4-4 Light microscope photograph of testes of mice treated with 1.5 mg/kg B.w Nitrofurantoin after 30 day (G3) shows necrotic cells in the lumen with giant cells formation(H&E stain, 400X).

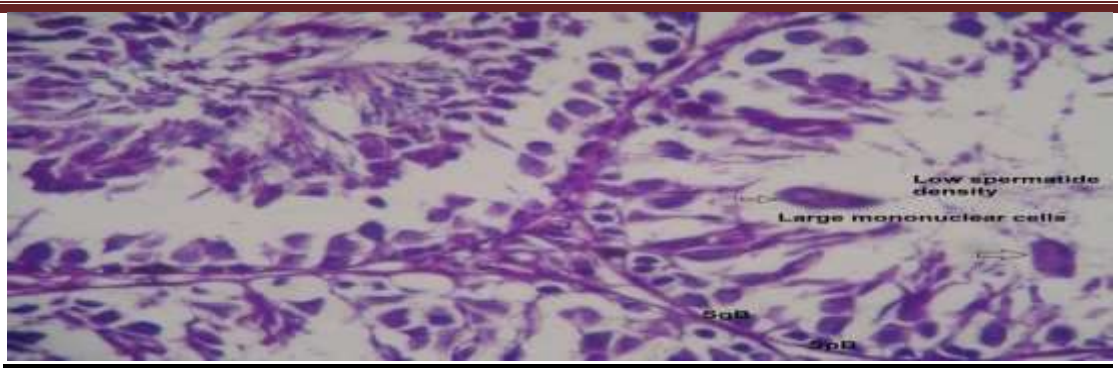

Fig.4-5 Light microscope photograph of testes of mice treated with 1.5 mg/kg B.w Nitrofurantoin after 60 day (G3) shows giant cell with lower sperm density ,large mononuclear cells ,spermatogonia B(sgB), (H&E stain,400X).

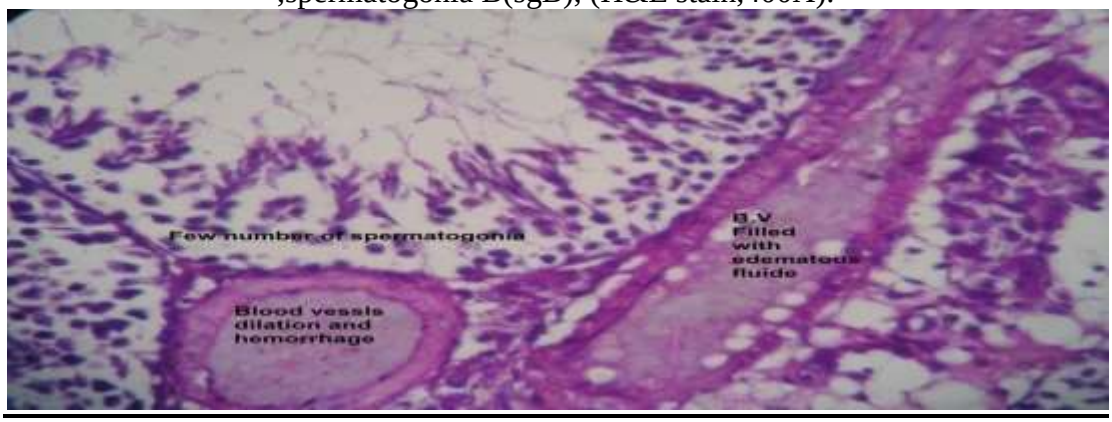

Fig.4-6 Light microscope photograph of testes of mice treated with 3 mg/kg B.w Nitrofurantoin after 30 day (G4) shows short spermatozoa and blood vessels dilation and its lumen filled with edematous fluid(H&E stain ,400X).

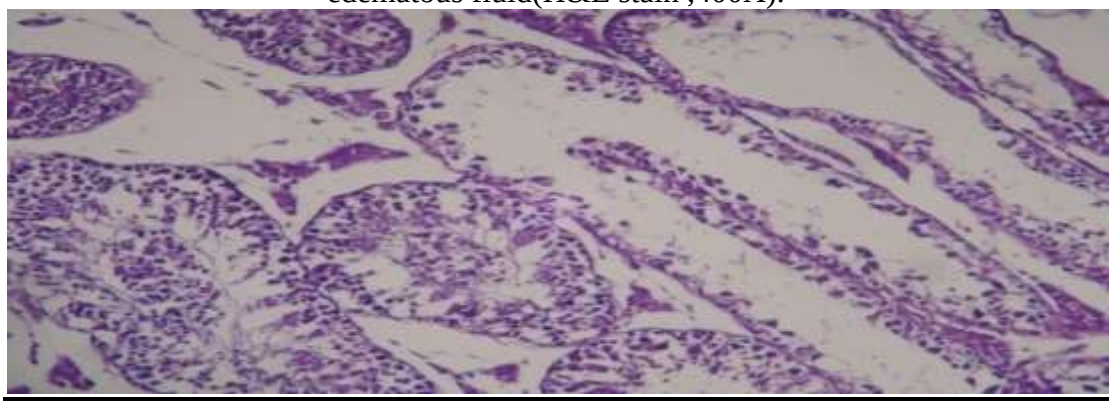

Fig.4-7 Light microscope photograph of testes of mice treated with 3 mg/kg B.w Nitrofurantoin after 60 day (G4) shows completel empty of seminiferous tubules which are only lining by sertoli cells with damaged affected tubules (H&E stain, 200X).

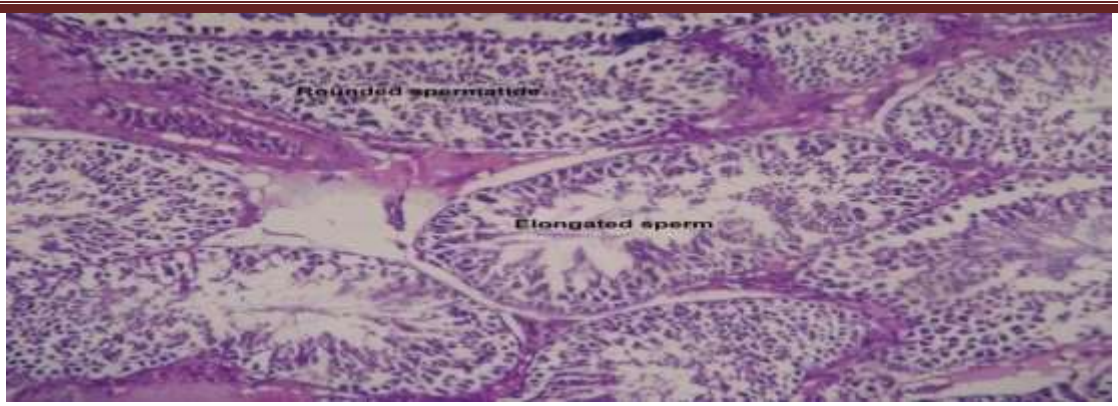

Fig.4-8 Light microscope photograph of testes of mice treated with 1.5 mg/kg B.w Nitrofurantoin and DHEA 2mg/kg B.w after 30 day (G5) shows evidence of mature spermatozoa and some elongated and rounded spermatozoa in the lumen (H&E stain, 200X).

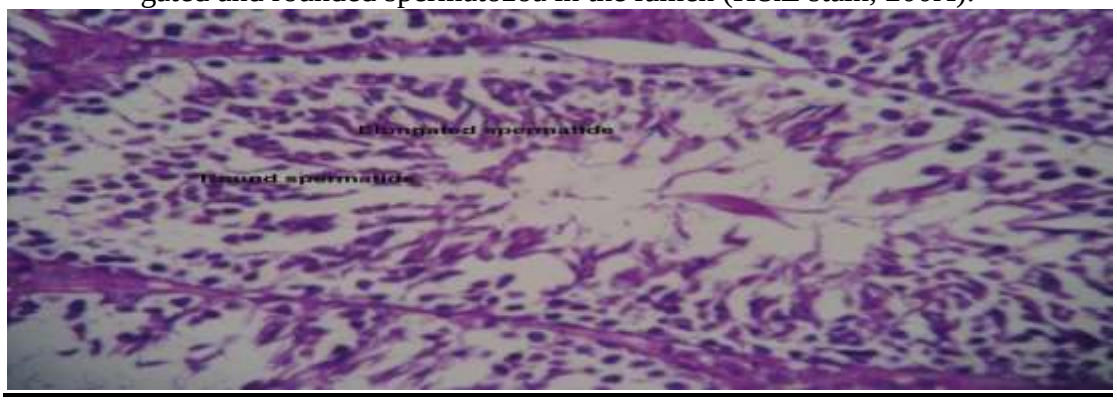

Fig.4-9 Light microscope photograph of testes of mice treated with 1.5 mg/kg B.w Nitrofurantoin and DHEA 2 mg/kg B.w after 60 day (G5) shows rounded and elongated spermatide (H&E stain 400X).

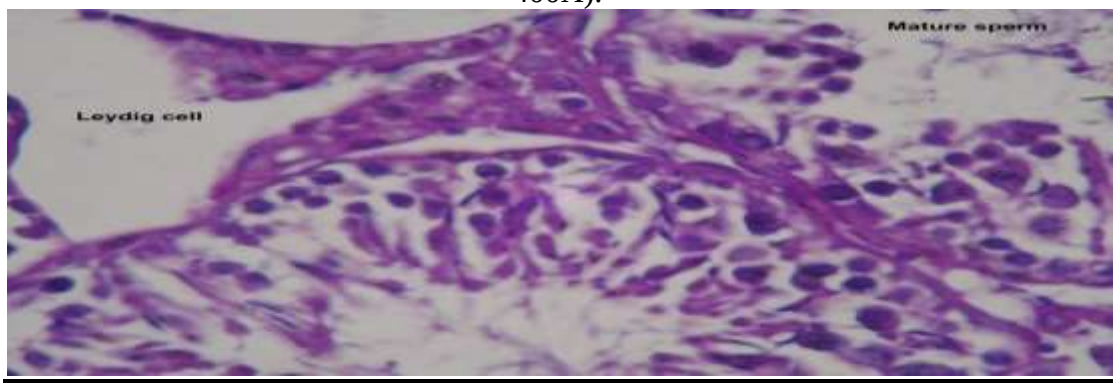

Fig.4-10 Light microscope photograph of testes of mice treated with 3 mg/kg B.w Nitrofurantoin and DHEA 2 mg/kg B.w after 30 day (G6) shows no clear change in leydig cells and mature sperm (H&E stain 400X).

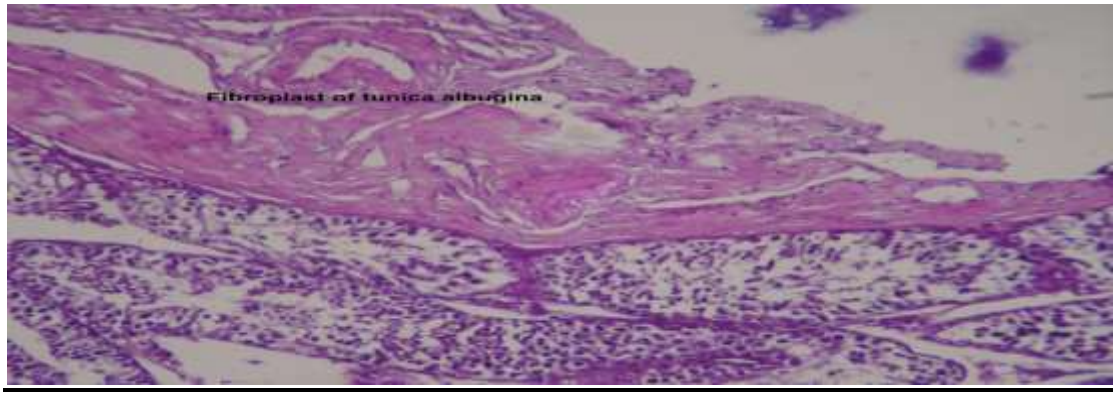

Fig.4-11 Light microscopic photograph of of mice treated with 3mg /kg B.w Nitrofurantoin and DHEA 2 mg/kg B.w after 60 day (G6)shows slight cellular infiltration and fibroblast of tunica albugina (H&E stain 200X).

Discussion:

There is a clear document that DHEA in the used dose could provide a protective role against Nitrofurantoin toxicity. However, the physiological reason behind increased sperm concentration, forward motility and viable sperm in groups received DHEA could be attributed to its antioxidative activity. Our pervious finding demonstrate a normal level of serum catalase which is major antioxidant enzyme in mammals with increase this level in group recived Nitrofurantoin. Moreover, mice received DHEA showed a significant increase in semineferous tubules diameter with normal structure of testes [(2).] DHEA exhibits two opposed effects on lipid peroxidation; depending on its concentration it acts either to limit or to induce oxidative stress. Furthermore, both for its pro-oxidant and for its antioxidant effects, the synthesis of accessory proteins, or other nuclear factors that modulate the oxidative processes, cannot be excluded. Whatever are the mechanism and the active compound, it should be emphasized that the threshold concentration at which the pro-oxidant activity of DHEA prevails is not far in excess of that showing antioxidant effects. This is an important point since DHEA, depicted as the 'youth hormone', is taken daily by millions of people throughout the world. The protective effect of low doses of DHEA on lipid peroxidation and cell viability might, however, be of clinical relevance [(7).]

On the other hand, Nitrofurantoin treated mice in our study revealed marked testicular damage, with hemorrhage and decreases of semineferous tubules diameters with much depletion of germ cells in them. Nitrofurantoin have been reported to exert adverse effects on male fertility, however, there are few human data on the majority of these medications. High doses of Nitrofurantoin have been reported to cause early maturation arrest at the primary spermatocyte stage [(8).] Moreover, the occurrence of giant cell in testes of mice treated with Nitrofurantoin may be considered to be an expression of germ cell degeneration [(18).] Nitrofueantoin or any other oxidant agent increase lipid peroxidation and thus the disruption of blood –testes-barrier will induce testicular inflammation [(19).]This inturn, with cases infiltration of white blood cells and giaint cells formation. From the other hand, the dilation of blood vessels in the present study could be attributed to the activation of nitric oxide (as vasodtilator) to get

ride of increase ROS. ROS is known to play a critical role in inhibition of steroidogenesis [(18).] Nitrofurantoin has been shown to result in spermatogenic arrest, decreased sperm counts and sperm immobilization at high concentrations. This effect is likely to be caused by an inability of testicular cells to use carbohydrates and oxygen [(16).]

In conclusion, we have demonstrated that DHEA supplementation to mice resulted in regulation of steroid hormone levels and antioxidant parameters. Regardless of the mechanism, these data highlight an important inter-relationship between DHEA treatment and the host response against Nitrofurantoin. This has significant biological relevance for delaying animal aging and improving old animal production performance. Further studies are warranted to confirm the efficacy of DHEA treatment in prevention of aging.

References:

1- **Agarwal, A.; Virk,G.;Ong,C. and Plessis,S.S. (2014).** Effect of Oxidative Stress on Male Reproduction.World J Mens Health. 32(1):1–17.

2- **Al-Azawi,T.S.S and Asker,M.H. (2016).** The Protective Role of Dehydroepandrosterone(DHEA) on the Reproductive Function in Adult Male Mice Treated with Nitrofurantoin. Journal of Natural Sciences Research.6 (22): 82-89.

3- **Al-Azawi,T.S.S and Obiad,M.A. (2016).** Fertility Indices of Rats in Response to Dehydroepiandrosterone (DHEA) Administration. Journal of Natural Sciences Research. 6(6): 2224-3186.

4- **Aprioku,J.S. (2013).** Pharmacology of Free Radicals and the Impact of Reactive Oxygen Species on the Testis.J Reprod Infertil.14(4):158–172.

5- **Arnold, J.T.; Le H.; McFann, K.K. and Blackman, M.R. (2005).** Comparative effects of DHEA vs. testosterone, dihydrotestosterone, and estradiol on proliferation and gene expression in human LNCaP prostate cancer cells. Am J PhysiolEndocrinolMetab. 288(3):573–84.

6- **Comhaire,F.A.; Mahmoud,A.M.; Depuydt,C.E.; Zalata,A.A. and Christophe, A.B. (1999).** Mechanisms and effects of male genital tract infection on sperm quality and fertilizing potential: the andrologist's viewpoint, Hum. Reprod. Update 5: 393–398.

7- **Gallo,M.; Aragno,M.; Gatto,V.; Tamagno,T.; Brignardello,E.; Manti.R.; Danni,O. and Boccuzzi,G. (1999).** Protective effect of dehydroepiandrosterone against lipid peroxidation in a human liver cell line. European Journal of Endocrinology 141:35–39. Infertility, Hum. Reprod. 14:2801–2807.

8- **Hargreaves, C.A.; Rogers, S.; Hills, F.; Rahman, F.; Howell, R.J. and Homa, S.T. (1998).** Effects of co-trimoxazole, erythromycin, amoxycillin, tetra-

cycline and chloroquine on sperm function in vitro. Human Reproduction.13:1878-1886

9- **Labrie, F.; Luu-The, V.; Bélanger, A.; Lin, S.X.; Simard ,J.;Pelletier, G.andLabrie, C. (2005).** Is dehydroepiandrosterone a hormone.J Endocrinol. 187(2):169-96.

10- **Luna, L. (1993).** Histopathologic Methods and color Atlas of special stain and tissue Artifacts .Amer. Histolabs Pub Dept (1):767.

11- **Majumder,S and Rahmatullah,M. (2015).** Efficacy of old antibiotics against commonly isolated bacterial isolates in a tertiary care hospital. Journal of Chemical and Pharmaceutical Research.7(4):1392-1396.

12- Muller, A.E,.;Verhaegh, E.M,; Harbarth, S.; Mouton, J.W.; Huttner, A. (2016). Nitrofurantoin's efficacy and safety as prophylaxis for urinary tract infections: a systematic review of the literature and meta-analysis of controlled trials. Clin Microbiol Infect. (16):30304-4.

13- **Neunzig,J. and Bernhard,R.(2014).** Dehydroepiandrosterone Sulfate (DHEAS) Stimulates the First Step in the Biosynthesis of Steroid Hormones.PLoS One. 9(2):89727.

14- **Omide,M.;Niknahad,H and Mohammadi-Bardori,A. (2016).** Dithiothreitol (DTT) rescues mitochondria from Nitrofurantoin in rat. Journal of Biochemical and moleceular Toxicology. :1-11.

15- **Onyije, F.M. (2012).** Drug: A Major Cause of Infertility in Male Asian J. Med. Pharm. Res. 2(2):30-37.

16- Peter,H. and Rebecca, L. (2016). Updated Nitrofurantoin Recommendations in the Elderly: A Closer Look at the Evidence. The Consultant Pharmacist. 31 7: 381-384(4).

17- **Samplaski,M.K and ,A.K. (2015).** Adverse effects of common medications on male fertility. Nature Reviews Urology. 12(7):401-13.

18- **Sansone,A.;Romanelli,F.;Gianfrilli,D. and Lenzi,A. (2014).**Endocrine evaluation of erectile dysfunction.Endocrine.46(3):423-30.

19- **Sharma,R.F.; Pasqualotto,F.F.; Nelson,D.R.; Thomas Jr.A.G. and Agarwal,A. (1999).** The reactive oxygen species-total antioxidant capacity score is a new measure of oxidative stress to predict male infertility, Hum. Reprod. 14:2801–2807.

20- **Singh, S.K and Chakravarty, S. (2001).** Effect of nitrofurazone on the reproductive organs in adult male mice. Asian Journal of Andrology. 3: 39–44.