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The Protective Role of Dehydroepandrosterone(DHEA) on the Reproductive Function in Adult Male Mice Treated with Nitrofurantoin

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Abstract:
The aim to investigate the protective role of daily oral administration of the male reproduction system of mice treated with Nitrofurantoin. Forty eight adult albino mice were divided in to four equal groups as(G1)control , (G2) received 2mg/kg B.w of DHEA,(G3)received Nitrofurantoin at 200mg/kg B.w,(G4)received DHEA and Nitrofurantoin. All parameters were estimated after 30 and 60 day of the experiment. The result revealed the significant decrease testicular index, seminiferous tubules diameters, serum Testosterone and DHEAs level, sperm motility, viability and concentration in Nitrofurantoin treated mice with significant increase in sperm abnormality, serum catalase and peroxynitrite concentration. The opposite result were show in DHEA treated mice. Conclusion, DHEA at 2 mg/kg.B.w has a protective role in male reproduction system of Nitrofurantoin treated mice. This is suggested to be due to its potent antioxidative activity which is able to protect against Nitrofurantoin toxicity.
Keywords: Nitrofurantoin, DHEA, male fertility, catalase, peroxynitrite.

Introduction:

Dehydroepiandrosterone (3β-hydroxy-5-androsten-17-one, DHEA), secreted by the adrenal cortex, gonads, brain, and gastrointestinal tract and its sulfated metabolite DHEA-s are the most abundant endogeneous circulating steroid hormones **(Prough et al.,2016).** It derived from cholesterol and it is androgen precursor **(Ross and Pawlina, 2011).** Dehydroepiandrosterone sulphate DHEA(s) is the most abundant sex steroid, with concentrations more than 100-fold higher than any other sex steroid **(Labrie, 2004).** Zonareticularis (ZR) mainly secreted dehydroepiandrosterone (DHEA), DHEA sulfate and androstenedione (the precursor to testosterone) in male ***(Dunn et al.,2011 ,Neunzig and Bernhard.,2014).***The androgen precursor dehydroepiandrosterone (DHEA) is increasingly used to supplement treatment protocols in patients undergoing in vitro fertilization (IVF). Due to differences in androgen metabolism, however, responses to DHEA supplementation vary between patients. In addition to overall declines in steroidogenic capacity with advancing age, genetic factors, which result in altered expression or enzymatic function of key steroidogenic proteins or their upstream regulators, might further exacerbate variations in the conversion of DHEA to testosterone **(Gleicher et al., 2013).** Moreover, its role in improving fertility has been reported that DHEA supplementation Increase number of myopically active oocytes, increase ovarian steroidgenesis and decrease follicular atresia **(Grimely et al., 2006 Gleicher and Barad, 2011).** *DHEA at 2mg/kg B.w was reported to increase fertility gestation, lactation induces in female rats* ***(Al-Azawi and Obaid., 2016).*** In male, DHEA supplementation has a role in improving testosterone level in blood **(Arlt, 2004).** In the USA, DHEA is sold as a food supplement, requiring no prescription. DHEA was also used by male for erectile dysfunction (ED), and healthy female how has low level of certain hormone to improve well –being and sexuality **(Abraham et al., 2013).** Nitrofurantoin, 1- (5-nitrofurfurylideneamino) hydration, is a synthetic antibacterial Nitrofuran derivative. Nitrofurantoin is used extensively in the treatment and prophylaxis of urinary tract infections (UTI) such as cystitis due to susceptible pathogens in human and animals. Nitrofurantoin is bactericidal in urine at therapeutic doses. The mechanism of the antimicrobial action of Nitrofurantoin is unusual among antibacterials **(Davial., 2014).**The mechanism of action of Nitrofurantoin is unique and complex. The drug works by damaging bacterial DNA, since its reduced form is highly reactive This is made possible by the rapid reduction of Nitrofurantoin inside the bacterial cell by flavoproteins (Nitrofuranreductae) to multiple reactive intermediates that attack ribosomal proteins, DNA respiration, pyruvate metabolism and other macromolecules within the cell. Nitrofurantoin exerts greater effects on bacterial cells than mammalian cells because bacterial cells activate the drug more rapidly. It is not known which of the actions of Nitrofurantoin is primarily responsible for its bactericidal activity. **(Ona et al ., 2009).** The nitro group coupled onto the heterocyclic furan ring represents the specific active site of the drug and has to be activated by microbial Nitroreductases **(Koulaouzidis et al., 2007).** For the first time in Iraq, our recent study in the Department of Physiology and Pharmacology, had explained the role of DHEA in the Pituitary-Adrenal-ovarian Axis and revealed a strong significant action of 2 mg/kg B.W DHEA on female rats reproductive system and fertility

(Obaid, 2016). Therefore, this experiment aimed to study the protective role of DHEA on the reproductive function of male mice treated with Nitrofurantoin.

Materials and Methods:

Forty eight healthy adult male swiss albino mice at 7-9 weeks age and 23-27 gram were used. They were divided in to four groups as twelve mice each and as follows: G1-control received distilled water. G2-received 2 mg/Kg B.w according to **Al-Azawi: and Obaid (2016).**G3-recived 200 mg/Kg B.w of Nitrofurantoin .G4-recives DHEA and Nitrofurantoin. All groups were subjected to daily oral administration for 60 days. At the end of the first month, six mice from each groups were sacrificed after the clinical observation ,weighing and collection of blood samples by cardiac puncture. Testes were isolated for weighing, semen collection **(Esteves et al., 2011).**measurement of seminiferous tubules diameters. The same protocol was reported after the end of the second month.

$$\text{Testicular index (\%)} = \frac{\text{weight of both testes (gm)}}{\text{Body weight of animal (gm)}} \times 100$$

-Seminiferous tubules diameters (Mm) were performed by tacking the mean of vertical and horizontal diameters for each seminiferous tubules **(Franca et al., 2000).**

-Serum catalase and peroxynitrite concentration were determined according to **Cowell et al (1994) and Beckman et al (1992)** respectively.

-Serum Luteinizing hormone (LH) and Follicular stimulation hormone (FSH) level were based on immunoassay system using antigen-antibody interaction and fluorescence technology as mentioned by **Goldstein and Kosasa (1975) and Kim et al(2011)** respectively.

-Serum free testosterone (T) and Dehydroepindrosteron sulfate (DHEAs) assay were based on the competition principal by using ELISA according to the methods described by **Frite et al (2008) and Sato et al (2016)** respectively.

-sperm motility, viability, abnormality and concentration were estimated according to **Bearden and Faquag (1992).**

Statistical analysis of the experimental results was performed according to **SAS (2010).**

Results:

Clinical observation-Group of mice that received Nitrofurantoin for 60 day showed over weight, hair loss, tumor like masses in the pelvic area, wooden-like-appearance tail. On dissection, the liver appeared pale in color with red spots as well as small size testes with accumulation of fat around them.

Effect on Testicular index (%):

Table (1) shows a significant ($p \leq 0.05$) increase in the testicular index of mice received DHEA as compared with control and those received Nitrofurantoin for the two periods of time i.e after 30 and 60 days. At the same time, there is a significant ($p \leq 0.05$) decrease in testicular index of the group of mice received Nitrofurantoin On the other hand, administration of DHEA at 2mg/kg B.w with Nitrofurantoin to mice in G4 caused a significant ($p \leq 0.05$) increase in testicular index as compared to mice received Nitrofurantoin alone.

Effect on seminiferous tubules diameters (мm):

Table (1) represents the mean values of the seminiferous tubules diameters in groups of the experiment. The table reveals that there is no significant ($p \geq 0.05$) difference between groups of mice received DHEA at 2mg/kg B.w and control. From the other hand, mice treated showed a significant ($p \leq 0.05$) decrease in seminiferous tubules diameters .This decrease is greatly dependent on the dose and duration of Nitrofurantoin treatment. At the mean time, the administration of DHEA with Nitrofurantoin caused a significant ($p \leq 0.05$) increase in seminiferous tubules diameters . However, DHEA administration caused a significant ($p \leq 0.05$) increase in diameter after 60 days compared to that after 30 days (G2).

Effect on serum catalase and peroxynitrite concentration.

From the results obtained in table (1) it is clear that DHEA administration to mice significantly ($p \leq 0.05$) decreases the level of serum catalase and peroxynitrite as compared to all treated groups. This table also shows that Nitrofurantoin treatment to mice cause a significant ($p \leq 0.05$) increase in serum catalase and peroxynitrite level after 30 and 60 days of experiment. However, administration of DHEA at 2mg/kg B.w with Nitrofurantoin significantly ($p \leq 0.05$) decreases the concentration of catalase and peroxynitrite after 30 and 60 days of the experiment.

The protective role against Nitrofurantoin on LH, FSH, Testosterone ,DHEAs concentration in serum:

There is a significant ($p \leq 0.05$) increase in LH,FSH, Testosterone and DHEAs level in the groups of mice received DHEA at 2mg /Kg B.w (G2)as compared to control and groups received Nitrofurantoin at 200mg/kg B.w .On the other hand, administration of DHEA plus Nitrofurantoin to mice in group produce a significant

increase in LH level with decreases in FSH in comparison to control.

The protective role of DHEA against Nitrofurantoin on sperms motility, viability, abnormality and concentration:

The administration of DHEA at 2 mg/kg B.wto mice induced a high motility ,viability and concentration with a decrease in abnormality percent of sperms which are significantly ($p \leq 0.05$) higher than control and other groups in the experiment Table (3). From the otherhand, mice treated with Nitrofuratoin showed a significant ($p \leq 0.05$) decrease in these parameters in comparison to control and other groups. The some parameter show a significant increase in group of mice received DHEA with Nitrofuratoin after 30 and 60 days of the experiment.

Table (1)-The protective role of DHEA against 2 doses Nitrofurantion on testicular index, seminitferous tubules diameters serum catalase, peroxynitrite concentration.

Parameter	Group / Day	G1 Control	G2 DHEA 2mg/kg B.w	G3 Nitrofurantoin 200mg/kg B.w	G4 DHEA& Nitrofurantoin	LSD
testicular index	30	B C0.42 ± 0.01 a	A 0.52 ± 0.05 a	BC 0.40 ± 0.07 a	BC 0.37 ± 0.03 a	0.027
	60	B 0.43 ± 0.01 a	A 0.52 ± 0.06 a	D 0.18 ± 0.03 b	BC 0.40 ± 0.01 a	
seminitferous tubules diameters	30	B 182.67 ± 6.11 a	BC 166.84 ± 4.91 b	C 151.05 ± 5.20 A	A 215.05 ± 11.74 a	22.883
	60	B 174.55 ± 9.07 b	B 192.62 ± 1.00 a	C 133.01 ± 7.86 A	B 183.72 ± 4.87 b	
catalase	30	D 1.55 ± 0.07 a	D 1.47 ± 0.05 a	A 7.42 ± 0.03 B	C 2.84 ± 0.04 a	0.3843
	60	E 1.50 ± 0.02 a	E 1.48 ± 0.02 a	A 8.33 ± 0.17 A	D 2.50 ± 0.02 a	
Peroxynitrite	30	D 2.13 ± 0.07 a	D 2.07 ± 0.05 a	A 8.24 ± 0.03 B	C 3.49 ± 0.06 a	0.3388
	60	D 2.06 ± 0.02 a	D 1.98 ± 0.04 a	A 8.64 ± 0.22 A	C 3.35 ± 0.24 a	

Values represent mean ± SE (N=6). Different capital letters denote a significant difference between groups ($p \leq 0.05$). Different Small letters denote significant difference between periods ($p \leq 0.05$)

Table(2)The protective role against Nitrofurantoin on LH, FSH, Testosterone ,DHEAs concentration in serum:

Parameter	Group / Day	G1 Control	G2 DHEA 2mg/kg B.w	G3 Nitrofurantoin 200mg/kg B.w	G4 DHEA& Nitrofurantoin	LSD
LH (mlU/ml)	30	C 1.28 ± 0.04 a	B 1.55 ± 0.02 a	C 1.39 ± 0.05 A	B 1.36 ± 0.09 a	0.1585
	60	C 1.16 ± 0.08 a	B 1.40 ± 0.08 a	B 1.33 ± 0.03 A	B 1.48 ± 0.03 a	
FSH(mlU/ml)	30	E 3.58 ± 0.27 b	B 19.20 ± 0.16 b	B 18.66 ± 0.33 A	D 12.80 ± 0.34 a	0.9984
	60	F 5.55 ± 0.48 a	A 27.46 ± 0.54 a	C 18.10 ± 0.27 A	E 10.60 ± 0.56 b	
Testosterone (pg/ml)	30	D 1.64 ± 0.04 a	A 6.52 ± 0.26 a	C 2.51 ± 0.11 A	CD 1.87 ± 0.02 a	0.3403
	60	D 1.88 ± 0.02 a	A 6.74 ± 0.27 a	C 2.42 ± 0.03 A	D 1.67 ± 0.02 a	
DHEA(s) Ng\m	30	C 0.75 ± 0.04 a	A 1.95 ± 0.05 a	D 0.56 ± 0.04 A	BC 0.83 ± 0.02 a	0.1194
	60	B 0.74 ± 0.04 a	A 2.10 ± 0.05 a	C 0.50 ± 0.07 A	B 0.78 ± 0.04 a	

Values represent mean ± SE (N=6). Different capital letters denote a significant difference between groups ($p \leq 0.05$). Different Small letters denote significant difference between periods ($p \leq 0.05$).

Table(3)The protective role of DHEA against Nitrofurantoin on sperms motility, viability, abnormality and concentration:

Parameter	Group / Day	G1 Control	G2 DHEA 2mg/kg B.w	G3 Nitrofurantoin 200mg/kg B.w	G4 DHEA& Nitrofurantoin	LSD
Motility (%)	30	B 74.33 ± 1.76 a	A 84.34 ± 1.76 a	E 55.33 ± 2.33 A	D 61.00 ± 1.15 a	5.6032
	60	B 74.33 ± 2.72 a	A 89.00 ± 1.15 a	D 59.66 ± 2.40 A	D 60.00 ± 2.88 a	
Sperms viability (%)	30	C 87.00 ± 0.57 a	D 91.00 ± 0.57 a	A 56.34 ± 2.33 A	B 25.33 ± 1.45 b	3.7994
	60	D 87.34 ± 1.45 a	E 92.00 ± 1.00 a	A 49.67 ± 1.45 A	B 67.00 ± 1.52 a	
Sperms abnormality (%)	30	D 14.67 ± 0.88 a	E 8.65 ± 0.89 a	A 28.34 ± 1.20 B	B 23.00 ± 1.53 a	3.1900.
	60	C 14.33 ± 0.89 a	C 11.32 ± 0.87 a	A 33.33 ± 0.87 A	B 21.67 ± 0.67 a	
Sperms concentration (sperm $\times 10^7$/ml)	30	B 2.76×10^7 ± 8.81×10^5 a	A 3.60×10^7 ± 11.54×10^5 a	D 0.92×10^7 ± 26.00×10^5 A	C 2.30×10^7 ± 17.32×10^5 a	
	60	B 2.76×10^7 ± 17.631×10^5 a	A 3.86×10^7 ± 12.011×10^5 a	D 0.58×10^7 ± 11.28×10^5 A	D 0.82×10^7 ± 3.71×10^5 b	

Values represent mean ± SE (N=6). Different capital letters denote a significant difference between groups ($p\leq0.05$). Different Small letters denote significant difference between periods ($p\leq0.05$).

Discussion:

Clinical signs:

The administration of Nitrofurantoin to the mice in the present study produce some signs which may be attributed to oxidative effect, Under anaerobic conditions, Nitrofurantoin is permanently reduced to nitroso and/or hydroxylamine forms **(Leskovac and Popovic, 1980).**which may result in binding to cellular macromolecules (DNA and protein) **,**the covalent binding to macromolecules is apparently greatest in the kidney, liver, ileum, lung, and heart of rats **(Boyd et al., 1979).** Toxicity and DNA damage may increase as oxygen

tension decreases **(Russo et al., 1982).**The result of our study confirm that Nitrofurantoin causes infertility through its direct effect on testes which result in damaging it and reduce the concentration of sperm and viability

Testicular index:

The current study clearly demonstrated the effect of DHEA on body, testes weight, testosterone and semen parameters in male mice. These results showed that supplementation of DHEA decreased body weight gain in the absence of any reduction in feed intake. Furthermore, in our early research, we found that female rats administrated with DHEA for 6 weeks exhibited decreased body weight and body mass index (BMI) with a dose dependent manner **(Obaid, 2016).** A possible explanation is as follows: DHEA is an activator of peroxisome proliferators activated receptor α (PPARα), activation of PPARα induces transcriptional up-regulation of fatty acid transport proteins that facilitate fatty acid entry into cells and the enzymes involved in the β-oxidation of fatty acids, resulting in the decreased expression of fatty acid synthesis **(Ma et al., 2008).**

Dehydroepiandrosterone (DHEA), a precursor for steroid sex hormones produced in adrenals and gonads, has been reported to exert both pro-oxidant properties at pharmacological doses **(Mastrocola et al., 2003)** and antioxidant properties **(Aragno et al., 1993).** Nitrofurantoin commonly prescribed antibiotics may adversely affect fertility. Classically, high doses of Nitrofurantoin have been shown to cause maturation arrest in the testis, most likely by preventing testicular cells from using carbohydrates and oxygen, However, low-dose, short-term therapy with Nitrofurantoin has not been shown to have the same advers effects **(Schlege et al.,1991).**

Semineferous tubules diameter

Mice received DHEA showed a significant increased in semineferous tubules diameter with normal structure of testes. DHEA exhibits two opposed effects on lipid peroxidation; depending on its concentration it acts either to limit or to induce oxidative stress. The threshold concentration at which the pro-oxidant activity of DHEA prevails is not far in excess of that having an antioxidant effect. Either effect of DHEA on lipid peroxidation **(Gallo et al 1999).**On the other hand, Nitrofurantoin treated mice in our study revealed marked testicular damage, with hemorrhage and decreases semineferous tubules diameters with much depletion of germ cells in them. Nitrofurantoin have been reported to exert adverse effects on male fertility **(Hargreaves et al., 1998).**

Catalase and peroxynitrite:

The present study showed a normal serum level of catalase and peroxynitrite in groups control and subjected to DHEA administration, DHEA has been shown to exert an antioxidant effect. Animal and in vitro tissue studies have shown that treatment with a DHEAS replacement lowers the oxidative stress levels. **(Camporez et al., 2011).** Recent claims suggest that oxidative stress is associated with aging and that the degree of oxidative damage controls the rate of aging **(Peppa, 2008).).** It is known that DHEA exhibits an antioxidative activity, including the reducing of lipid peroxidation in the rat brain, inhibition of phorbol myristate acetate (PMA)-induced superoxide production in human neutrophils and rat peritoneal macrophages ,In addition, DHEA has been shown to inhibit glucose-6-phosphate dehydrogenase, which is required for NADPH generation, a necessary component in the production of reactive oxygen intermediate (ROI) ,Reactive oxygen intermediates have been shown to activate the NF-kB transcription factor ,This transcription factor upregulates the transcription of a variety of adhesion molecules (e.g. ICAM-1, VCAM-1), cytokines (TNF, IL-1, IL-6) and enzymes (iNOS) ,Certain stimuli such as TNF-a and LPS induce NF-kB activation through the production of ROIs **(Sprague and Khalil,2009).**

Luitinizing hormone (LH) and Follicular stimulating hormone (FSH):

Our previous study **(Obaid, 2016)** reported an increase in serum levels of ACTH, FSH and estradiol in adult female rats after DHEA administration. However, these findings was coincided with a significant increase of different stages of follicles and corpus luteum in the ovary. However, LH and FSH both are glycoprotein hormones secreted from anterior pituitary gland under hypothalamic control and have a considerable influence on endocrine gland especially adrenals and gonads **(Chahal, 2007).** These hormones stimulate the testes to produce testosterone and spermatozoa by affecting on leydig and sertoli cells respectively. The results of the current study revealed a significant increase in testosterone level and sperms concentration after DHEA administration. Moreover, DHEA is an endogenous steroid that originates from the zone reticularis of the adrenal gland as well as from glands and converted into estrogen and androgens **(Hinson et al, 1999).** This aspect could clearly be observed in our previous and present studies and suggest that DHEA is an active drug with androgen activities. Concerning the effect of Nitrofurantoin on serum LH and FSH level, the literature review lack such studies. Our explanation is that Nitrofurantoin induce direct damage to the testes, which inversely decreases testosterone level accompanied by inhibition of spermatogenesis. This is clearly observed in the groups of mice which received 100 and 200 mg/kg B.w in our experiment. Low testosterone level may cause an increase in LH and FSH secretion from the anterior pituitary gland **(Chahal, 2007).**

Testosteron and DHEAs:

The current study reported a significant increase in serum level of testosterone and DHEAs Although, several modes of action of DHEA are possible, the most likely is that DHEA and DHEAs serve as a source of active androgens and estrogens in some subset of androgen-responsive tissues, This aspect could clearly be observed in

the present study, which demonstrat that in mice treated with 2mg/kg B.w DHEA the serum level of testosterone increased around 3.5 times more. This observation shows that the DHEA is an active drug with androgen possibilities as reported in the literature too **(Hinson et al, 1999).**

A significant amount of DHEA conversions occur inside ovaries, testes, brain and various peripheral cells such as skin and bone and the resulting hormones are metabolized locally, inside the cells/tissues. **(Labrie,2004).**The increase in DHEAs concentration in our experiment by DHEA supplementation may increase DHT (dihydrotestosterone) levels. This is due to both its conversion to testosterone and the fact that DHEA upregulates the activity of 5-alpha-reductase (5AR) enzyme, which further converts testosterone to DHT **(Reiter,2001).** However, the decrease in testosterone and DHEAs serum level in groups received Nitrofurantoin could be attributed to its oxidant action Oxidative stress in any tissue results from an imbalance between the production of reactive oxygen species (ROS) and their efficient removal by available antioxidant systems**(Papa and Skulachev, 1997).** Therefore, the oxidative action of Nitrofurantoin was documented in our experiment by the testicular damage and spermatogenesis arrest.

Sperm motility, viability, abnormality, and concentration:

The aim of this study was to find out the protective role of DHEA against Nitrofurantoin treated mice. Although the last fifty years has given great attention to the benefits of DHEA as it a "Youth hormone" and a million of people tack it even with out prescription, the literature review lack the effect of DHEA on semen evaluation. The present study reported a highly significant increase in the individual progressive forward movement of sperms in group that received DHEA at 2mg/kg B.w The same group showed an increase in alive sperms and concentration with a significant decrease in primary and secondary abnormalities.

Obaid (2016) revealed a significant increase of estradiol concentration in DHEA administration rats. It is well known that DHEA is the most abundant steroid secreted from adrenal gland and converted in to androgen and estrogens. Thus, taking into account the widespread localization of aromatase and estrogen receptors in testicular cells, it is obvious that, besides gonadotrophins and androgens, estrogens produced locally should be considered to be physiologically relevant hormones involved in the regulation of spermatogenesis and spermiogenesis **(Carreau et al 2011).** Oxidative stress due to excessive production of reactive oxygen species (ROS) has been associated with defective sperm function and infertility **(Agarwa et al., 2014).** Reactive oxygen species are known to affect cellular lipids, proteins and DNA. Oxidative stress to the sperm DNA can have profound implications for normal embryonic development and long-term health of progeny **(Evenson et al., 2002).** In conclusion, we have demonstrated that chronic DHEA treatment of 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.

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