

Histological and Ultrastructural Study of the Effect of Nandrolone on the Testis of Adult Male Albino Rat

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Abstract: The present study was designed to investigate the changes produced by the anabolic steroid nandrolone decanoate on the testicular tissue, and to clarify the reversibility of these changes. Sixty adult male Wistar albino rats were divided in 6 groups, treated with oil vehicle (C), therapeutic (T) and high (H) doses of nandrolone decanoate respectively for eight weeks. Each regimen was divided into effect (A) and recovery (B) groups, in which rats were sacrificed two days and eight weeks after the last injection, respectively. The histological findings of the present work showed severe degeneration of seminiferous tubules structure, there were irregular basement membrane, arrest of spermatogenesis in different levels, apoptosis of germ cells, disarranged intratubular cells, reduction of Leydig cells and interstitial tissue edema. The statistical analysis showed a highly significant difference between the control group and the other groups as regards seminiferous tubules diameter, the high dose effect and recovery groups showed a statistical significant lower values than the therapeutic dose effect and recovery groups respectively. As regards the epithelial thickness measurement, a highly significant lower value in the high dose effect group and a significant lower values in the other three treated groups in relation to the control group were found. The ultrastructural findings included Apoptotic and completely degenerated cells, shranked or pale degenerated nuclei, irregular nuclear membrane, degenerated cytoplasm, loss of cytoplasmic organelles, cytoplasmic vacuolization, many fat globules and lysosomes and thick irregular basement membrane. From the present work, it is concluded that, nandrolone administration causes a dose dependent, long lasting testicular tissue damage even in a therapeutic dose, leaving a testicular stigma which may lead to permanent hypofertility or infertility.

[Ahmed F. AlDomairy. **Histological and Ultrastructural Study of the Effect of Nandrolone on the Testis of Adult Male Albino Rat.** *J Am Sci* 2013;9(5):96-105]. (ISSN: 1545-1003). <http://www.jofamericanscience.org>. 14

Key words: nandrolone - anabolic steroid - testis - ultrastructure – rat

1. Introduction

Improving physical appearance and athletic ability have always been a man dream. Anabolic steroids are used at high doses by athletes for improving athletic records and muscle mass. Unfortunately, the abuse of these agents has significantly increased (Feinberg et al., 1997).

Anabolic steroids are synthetic derivatives of testosterone and are important pharmacologically for their use in the treatment of various medical conditions such as growth deficiency, osteoporosis and some blood disorders (Clark et al., 1997). Some trials used nandrolone as a hepatocytes growth promoter after partial hepatectomy (Sileri et al., 2005), and in reversing denervation atrophy after delayed nerve repair (Isaacs et al., 2011). However, it was found that anabolic steroids administration causes much greater tissue damage than expected (Takahashi et al., 2004) and even spatial learning and memory deficits (Tanehkar et al., 2013).

Low and high doses of nandrolone decrease sperm quality and quantity in rats. A controversy of the improvement of these parameters after drug discontinuation is found. According to Karbalay-Doust et al. (2007), these parameters were improved

after discontinuing nandrolone, but not recovered completely.

Many mechanisms were suggested for nandrolone actions including increased androgen and estrogen levels (Huie, 1994), reduction of total and free testosterone levels (Lucia et al., 1996), alteration of insulin like growth factor and their subsequent influence on hypothalamic-pituitary-gonadal axis (Lewis et al., 2002), impairment of antioxidant enzymes activity and the resultant oxidative stress (Chaves et al., 2006), enhancement of germ cells apoptosis (Shokri et al., 2010), and modification of Sertoli cell membrane depolarization (Cavalari et al., 2012). Some or all these factors may consequently lead to infertility.

Aim of the Work:

The aim of the present study was to investigate the effect of nandrolone on the testis of adult male albino rat using histological and ultrastructural techniques, trying to explore the role of the drug in the development of testicular abnormalities which in turn may lead to fertility obstacles, and to clarify the reversibility of these changes after drug discontinuation.

2. Material and Methods

Animals:

The present study was carried out on 60 adult male Wistar albino rats weighing between 228 and 356 g obtained from animal house, Faculty of Medicine, Cairo University. The rats were maintained in a controlled environment with a free access to food and water.

All the rats were left untreated for 2 weeks for acclimation, each rat was housed in individual cage, the rats were then divided into six groups without body weight statistical differences, each contained 10 rats. A sham control rats were subjected to weekly deep intramuscular injection of an oily vehicle for eight weeks and then classified into (CA) group sacrificed two days and (CB) group sacrificed eight weeks after the last injection. Each rat in the following two groups was subjected to a weekly injection of 0.25 mg/kg nandrolone decanoate for eight successive weeks, This dose is equivalent to the therapeutic dose in man, then, a therapeutic dose effect (TA) group rats were sacrificed two days after the last injection, while a therapeutic dose recovery (TB) group were sacrificed after another eight weeks. The last two groups were injected weekly by 10 mg/kg of the same drug for the same duration, again, a high dose effect (HA) group was sacrificed after two days, and a high dose recovery (HB) group was sacrificed after eight weeks of the last injection.

As previously suggested by **Kolasa et al. (2004)**, the eight weeks injection duration was selected according to spermatogenic period in rats, which is approximately 48-56 days. Therefore, 8 weeks of nandrolone injection or deprivation seems to be a reasonable period for assaying its effect especially on germ cells.

All the rats were sacrificed by cervical dislocation, dissected and testicular specimens were collected. Minute parts of the specimens were excluded to be fixed in gluteraldehyde for three hours in room temperature & processed to be examined by transmission electron microscope (TEM). The rest of the specimens were fixed in Bouin's solution for 36 hours at 4°C and processed to be stained by hematoxylin and eosin.

Drug:

The drug used was nandrolone decanoate (Deca-Durabolin®) in the form of 25 mg/ml oily solution, produced by Nile Co. For Pharmaceutical and Chemical Industries. If needed, the drug was diluted in oil vehicle. The drug was injected deeply intramuscular.

Paraffin section preparation:

The paraffin sections were subjected to haematoxylin and eosin staining for general architecture of the studied specimens. Each slide contained specimens from both testis of a single rat. The sections preparation was performed at the National Cancer Institute, Cairo University.

Image Analysis:

Image analysis was performed in Histology department, Faculty of Medicine, Cairo University. Sections were examined microscopically using an eyepiece of X10 and an objective piece of X10, i.e. at magnification of X100. Serial fields were examined to get 10 readings per slide as regards the cross sectional diameter of seminiferous tubules, and another 10 readings for epithelial height, a mean value is calculated per slide then measurement of the mean value per each group (n=10) was done.

Data was obtained by using (Leica Qwin 500 image analyzer computer system; England). The image analyzer consisted of an Olympus BX40 microscope, colored Panasonic video camera, colored monitor, hard disc of Leica IBM personal computer connected to the microscope, and controlled by the Leica Qwin 500 software.

Using the menu of Interactive measurements, measuring of epithelial height was done by drawing a straight line across the epithelium, to be measured in micrometers. Also using the same menu, diameter of tubules was measured by drawing two straight lines perpendicular to each other across the transversely cut tubules, to get the average reading for each tubule.

Statistical methodology:

Statistical analysis of tubal diameter and epithelial height was performed using Statistical Package for Social Sciences, Version 14.0 (SPSS, Inc., Chicago, USA) for Windows. The results were expressed as mean \pm SD.

Kolmogorov-Smirnov test of normality was done to assess normality of continues variables before starting the analysis. Differences among the groups were analyzed with Univariate ANOVA and Bonferroni post hoc test. P-values less than 0.05 were considered significant and less than 0.001 were considered highly significant (**Dawson and Trapp, 2004**).

Transmission electron microscope (TEM):

Samples of about 0.5 mm \times 0.5 mm were taken from the testis. The tissue was fixed in 3% gluteraldehyde, post fixed in 1% osmium tetroxide, rinsed three times in distilled water, dehydrated through gradual alcohols and embedded in epon with

an identification label. Semithin sections stained with toluidine blue were prepared for tissue orientation and examined with light microscope. Ultrathin sections were mounted on grids, stained by uranyl acetate and lead citrate, then examined with Seo transmission electron microscope and the electron micrographs were saved by Videotest computer program. All the preparation and examination steps were performed in Electron Microscopy unit, Egyptian Military Central Laboratories.

3. Results

Histological results:

The control group (C) showed the usual features of seminiferous tubules with 4-6 cell layers thickness inside, competent basement membrane with multiple Sertoli cells based on it, the lumen of the tubules was masked by the abundant amount of formed sperms, and there were numerous extratubular Leydig cells (**fig1**).

Therapeutic dose effect group (TA) showed a patchy effect, with many irregular seminiferous tubules and basement membranes, some seminiferous tubules showed 2-3 layers and wide empty lumen, others showed a thick cellular mass of 6-8 layers but only reaching the stage of round spermatid (**fig. 2**), other tubules were disarranged and included many apoptotic cells, the spaces between the tubules showed few Leydig cells (**fig. 3**).

Therapeutic dose recovery group (TB) showed partial regeneration, most of the seminiferous tubules were regular with regular basement membrane, the tubules showed variable cell layer thickness (1-5), some sperms and variable sized lumen, with variable spaces in between, the spaces between the tubules showed oedema and some Leydig cells (**fig. 4**).

High dose effect group (HA) showed severe testicular tissue damage, seminiferous tubules showed few cells, most of the cells were Sertoli cells, spermatogonia and primary spermatocytes with variable sizes and shapes, scanty Leydig cells appeared outside the tubule, some seminiferous tubules showed few cells inside it, some parts of the basement membrane didn't show any attached cells (**fig. 5**), other sites showed cells detached from it and rare sperms (**fig. 6**).

High dose recovery group (HB) showed variable sized seminiferous tubules with patchy effect, few cells appeared inside it, sometimes indefinite, generally the layers didn't exceed 2-3 cell layers, with scanty or no sperms, the spaces between the tubules showed oedema and some Leydig cells (**fig. 7**).

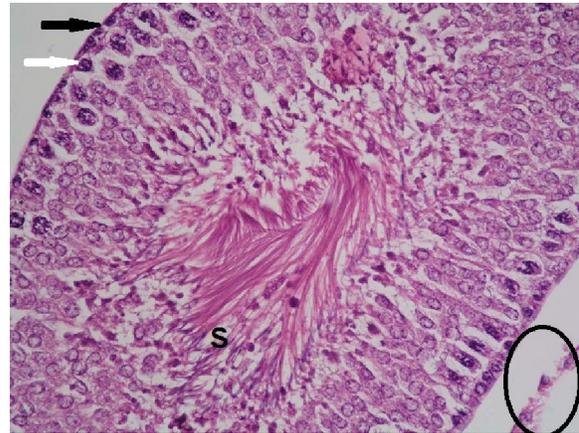


Fig. (1): photomicrograph of a section of testis of control group (C) showing normal seminiferous tubules with 4-6 cell layers thickness inside, competent basement membrane (black arrow) with multiple Sertoli cells based on it (white arrow), the lumen of the tubules is masked by the abundant amount of formed sperms (S) and also showing extratubular Leydig cells (circle) (Hx. & E.; ×400).



Fig. (2): photomicrograph of a section of testis of therapeutic dose effect group (TA) showing patchy effect, the basement membranes is irregular (black arrows), one seminiferous tubule shows a thick cellular mass in the form of 6-8 layers but reaching only the stage of round spermatid (white arrow), other tubules shows 2-3 layers and wide empty lumen (L), the spaces between the tubules shows few Leydig cells (circle) (Hx. & E.; ×400).

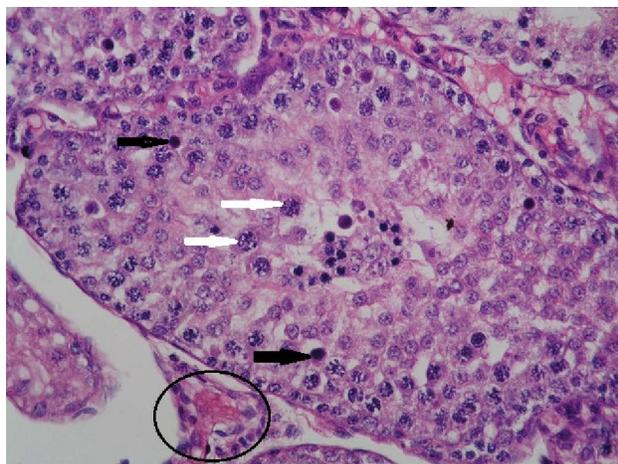


Fig. (3): photomicrograph of a section of testis of therapeutic dose effect group (TA) showing a seminiferous tubule filled with disarranged cells, some primary spermatocytes are in the middle of the cellular mass away from the basement membrane (white arrows), many apoptotic cells appear (black arrows), outside the tubules there are some Leydig cells (circle) (Hx. & E.; $\times 400$).



Fig. (4): photomicrograph of a section of testis of therapeutic dose recovery group (TB) showing a seminiferous tubules with regular basement membrane (arrow), and variable cell layer thickness (1-5) layers, some sperms appear inside the tubules (S) and some Leydig cells appear outside it (circle) (Hx. & E.; $\times 400$).

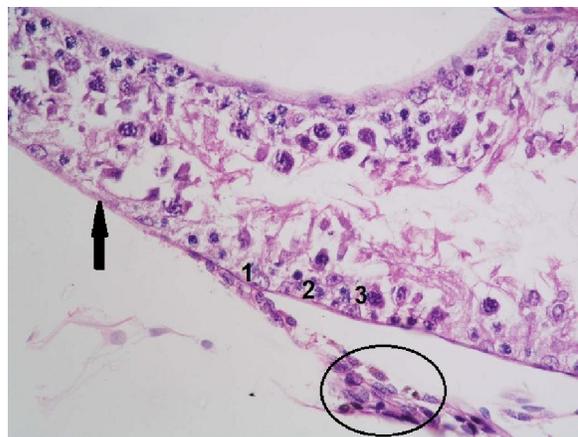


Fig. (5): photomicrograph of a section of testis of high dose effect group (HA) showing a seminiferous tubules with few cells inside it, some parts of the basement membrane doesn't show any attached cells (arrow), most of the cells are Sertoli cells (1), spermatogonia (2) and primary spermatocytes (3) with variable sizes and shapes, few Leydig cells appear outside the tubule (circle) (Hx. & E.; $\times 400$).

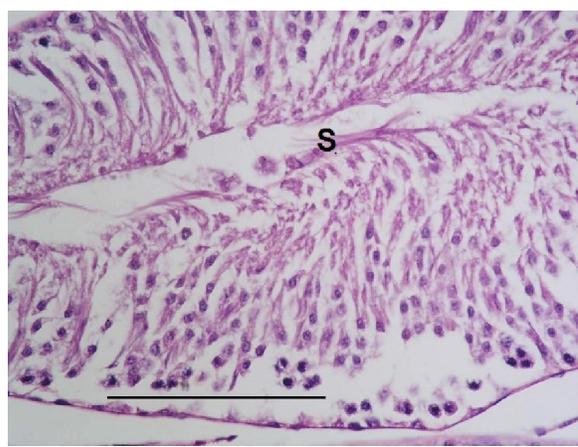


Fig. (6): photomicrograph of a section of testis of high dose effect group (HA) showing a seminiferous tubules with the cells detached from the basement membrane (line) and rare sperms (S) (Hx. & E.; $\times 400$).

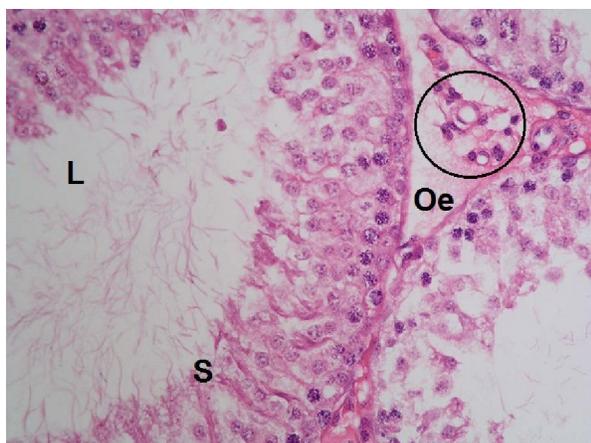


Fig. (7): photomicrograph of a section of testis of high dose recovery group (HB) showing patchy effect, a seminiferous tubule with 2-3 cell layers, a wide lumen (L) and scanty sperms (S), and another tubule showing few cells inside, between the tubules oedema (Oe) and some Leydig cells appear (circle) (Hx. & E.; $\times 400$).

Image analysis and statistical results:

Because of the much comparable results of the 2 control groups, a mean of them was calculated and compared to other groups. The tubal diameter measurements by image analysis technique showed that the mean of control group (C) was 331.1 micrometers. The therapeutic dose effect group (TA) tubal diameter mean was 247.1 micrometers, while that of therapeutic dose recovery group (TB) was 230.8 micrometers. High dose effect group (HA)

showed a mean of 201.7 micrometers, while high dose recovery group (HB) showed a mean of 193.4 micrometers (**table 1 and fig. 8**).

Statistical analysis of the tubal diameter showed a highly significant differences ($P < 0.001$) between the control group (C) and each of the other four groups (TA, TB, HA and HB). A highly significant difference was found also between TA group and HB group, a statistically significant differences ($P < 0.05$) were found between TA and HA groups and between TB and HB groups. The other differences were statistically non significant (**table 2**).

The epithelial height measurements by image analysis technique showed that the mean of control group (C) was 147.7 micrometers. The therapeutic dose effect group (TA) epithelial height mean was 127.4 micrometers, while that of therapeutic dose recovery group (TB) was 130.7 micrometers. High dose effect group (HA) showed a mean of 116.5 micrometers, while high dose recovery group (HB) showed a mean of 123.6 micrometers (**table 1 and figure 8**).

Statistical analysis of the epithelial height showed a highly significant difference ($P < 0.001$) between the control group (C) and the high dose effect group (HA). A statistically significant differences ($P < 0.05$) were found between the control group and TA, TB and HB groups, and between TB and HA groups. The other differences were statistically non significant (**table 3**).

Table (1): comparison between control group (C), therapeutic dose effect group (TA), therapeutic dose recovery group (TB), high dose effect group (HA) and high dose recovery group (HB) according to the mean values of tubal diameter and epithelial height in micrometers.

	C	TA	TB	HA	HB	P value
	n=20	n=10	n=10	n=10	n=10	
Tubal diameter Mean \pm SD	331.1 \pm 30.8	247.1 \pm 21.0	230.8 \pm 20.7	201.7 \pm 25.5	193.4 \pm 17.5	<0.001
Epithelial height Mean \pm SD	147.7 \pm 17.0	127.4 \pm 9.6	130.7 \pm 13.4	116.5 \pm 18.1	123.6 \pm 10.6	<0.001

Univariate ANOVA

SD: standard deviation

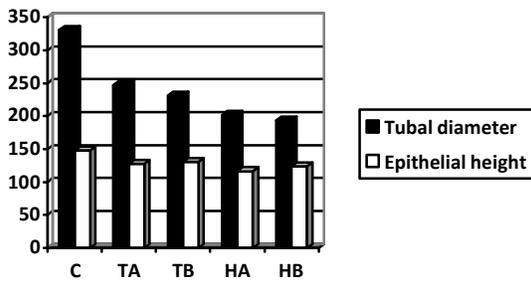


Fig. (8): Bar chart showing comparison between control group (C), therapeutic dose effect group (TA), therapeutic dose recovery group (TB), high dose effect group (HA) and high dose recovery group (HB) according to mean tubal diameter and mean epithelial height in micrometers.

Table (2): statistical differences between control group (C), therapeutic dose effect group (TA), therapeutic dose recovery group (TB), high dose effect group (HA) and high dose recovery group (HB) as regards tubal diameter (P values).

Tubal diameter	C	TA	TB	HA	HB
C		<0.001	<0.001	<0.001	<0.001
TA			1.000	0.002	<0.001
TB				0.083	0.009
HA					1.000
HB					

post hoc test

<0.05: significant, <0.001: highly significant

Table (3): statistical differences between control group (C), therapeutic dose effect group (TA), therapeutic dose recovery group (TB), high dose effect group (HA) and high dose recovery group (HB) as regards epithelial height (P values).

Epithelial height	C	TA	TB	HA	HB
C		0.024	0.043	<0.001	0.005
TA			0.409	0.926	1.000
TB				0.004	0.112
HA					1.000
HB					

post hoc test

<0.05: significant, <0.001: highly significant

Ultrastructural results:

The EM findings of all the treated groups showed degenerative effects to all types of cells of the testis, the different groups shared common features but varied in severity between them.

Apoptotic and completely degenerated cells (**fig. 9**) were more common in the effect groups (TA and HA), most of the survived cells showed shrunk irregular (pyknotic) nuclei (**fig. 10**), or pale degenerated ones (**fig. 15**), clumped chromosomes (**fig. 12**), irregular nuclear membrane (**fig. 10, 13, 14 and 15**), degenerated cytoplasm and loss of cytoplasmic organelles (**fig. 10**), cytoplasmic vacuolization (**fig. 12, 14 and 15**), lipid globules (**fig. 10**), many lysosomes (**fig. 12 and 14**), multiple nucleoli (**fig. 13**) and thick irregular basement membrane (**fig. 15**).

Some cells especially from TB group showed less severe changes and even showed increase in the number of healthy mitochondria (**fig. 11**).

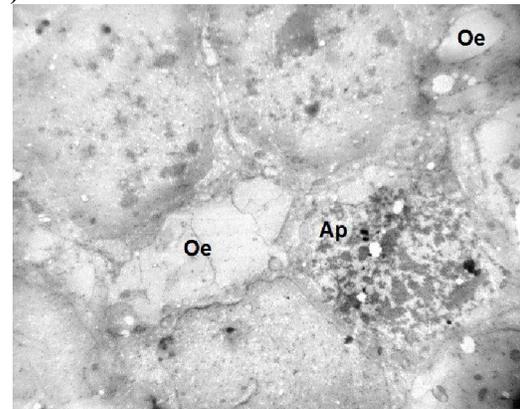


Fig. (9): Electronmicrograph of a section in the testis of a therapeutic dose effect group (TA) showing apoptotic cell (Ap) between other degenerated cells and intercellular and extracellular edema (Oe), all the cells shows no nuclear structure (× 2000).

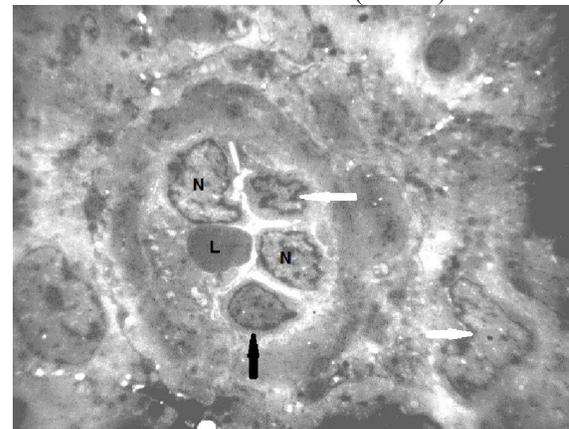


Fig. (10): Electronmicrograph of a section in the testis of a therapeutic dose effect group (TA) showing some degenerated cells with shrunk irregular nuclei (N) surrounded by irregular nuclear membrane (white arrows) and rim of degenerated cytoplasm (black arrow), loss of cytoplasmic organelles and lipid globule (L) (× 1500).

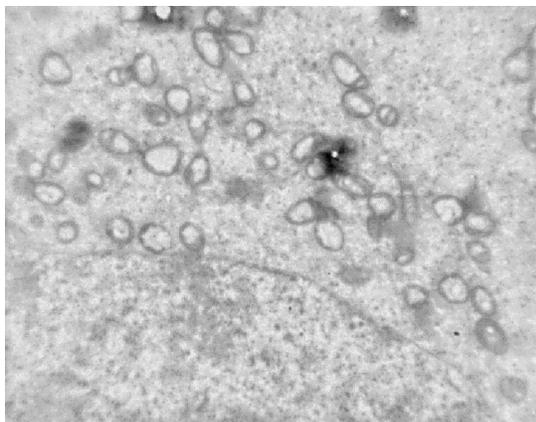


Fig. (11): Electronmicrograph of a section in the testis of a therapeutic dose recovery group (TB) showing many healthy mitochondria ($\times 6000$).

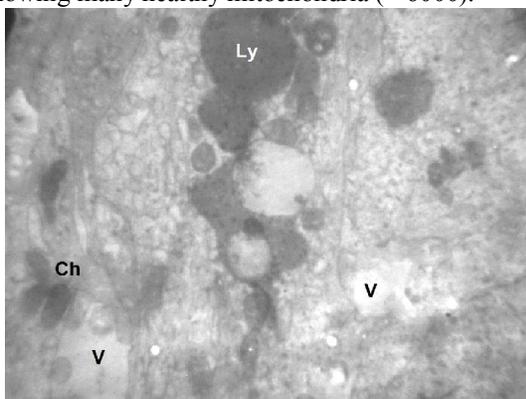


Fig. (12): Electronmicrograph of a section in the testis of a therapeutic dose recovery group (TB) showing part of cytoplasm containing many vacuoles (V) and lysosomes (Ly) and part of nucleus showing clumped chromosomes (Ch) with intranuclear vacuolization (V) ($\times 6000$).

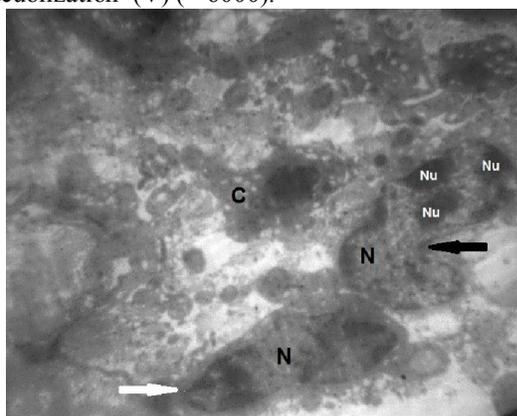


Fig. (13): Electronmicrograph of a section in the testis of a high dose effect group (HA) showing shrinkage of intratubular cell (C) and two other cells with large nuclei (N), cytoplasmic rim (white arrow), irregular nuclear membrane (black arrow) and many nucleoli (Nu) ($\times 3000$).

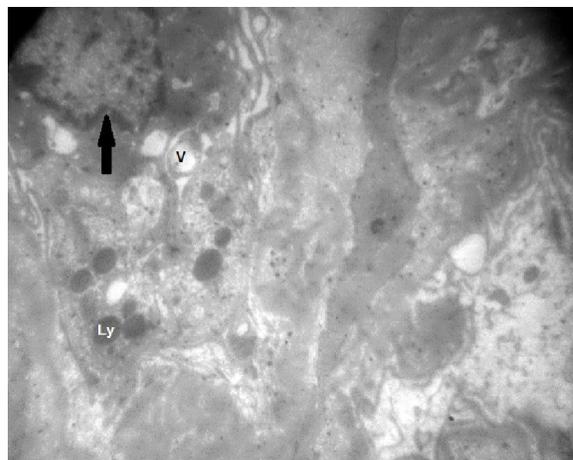


Fig. (14): Electronmicrograph of a section in the testis of a high dose effect group (HA) showing Leydig cell with many lysosomes (Ly), cytoplasmic vacuoles (V) and irregular nuclear membrane (black arrow) ($\times 4000$).

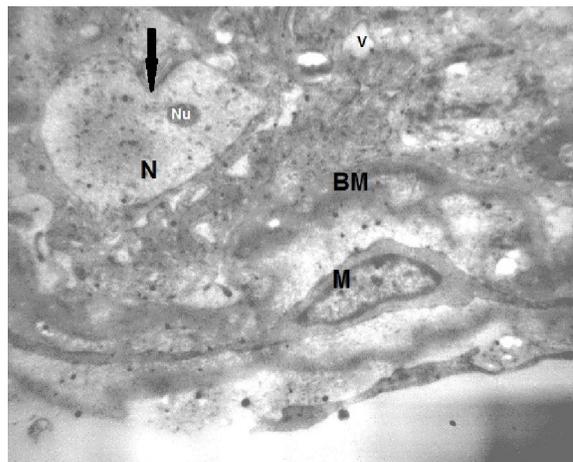


Fig. (15): Electronmicrograph of a section in the testis of a high dose recovery group (HB) showing thickened irregular basement membrane (BM) with a myoid cell outside it (M), it also shows a spermatogonia with pale degenerated nucleus (N), nucleolus (Nu), irregular nuclear membrane (black arrow) and many cytoplasmic vacuoles (V) ($\times 6000$).

4. Discussion

The result of the present study revealed that the use of nandrolone severely affect the tubal diameter, even in the therapeutic dose and after its discontinuation, known from the highly significant differences between the control and the other groups. Furthermore, it was also found that the dose of the drug used affects the tubal diameter significantly, the high dose effect and recovery groups showed a statistical significant lower values than the therapeutic dose effect and recovery groups respectively, indicating a dose dependent effect of the

drug. Although statistically non significant, both recovery groups showed a lower tubal diameter values than their effect groups, indicating the long lasting effect of the drug even after its discontinuation for a long period. These results contradict the findings of **Noorafshan et al. (2005)** who stated that high (but not therapeutic) dose of Nandrolone decreases the length (but not the diameter) of seminiferous tubules. The differences in the findings may be referred to the way of measurement, **Noorafshan et al. (2005)** measured the least diameter in each tubule, while in the present work the measurement of the tubular diameter was done in two perpendicular directions regardless the lowest diameter respecting the different oblique cuts of the tubules in histological sections.

The differences between the two effect groups and between their recovery groups were non significant as regards the epithelial height, the highly significant lower value in the high dose effect group and the significant lower values in the other three groups in relation to the control group, point to the dose dependent effect of the drug. On the contrast to the tubal diameter, the epithelial thickness showed a partial recovery after discontinuing the treatment, however, this recovery was statistically non significant, it only suggests a non progressive effect of nandrolone after its discontinuation as regards the affection of seminiferous tubule epithelium. Although in agreement with their findings, the use of the image analysis technique measuring the epithelial thickness appears to be more accurate in determining its affection than the spermatogenic layer measurement used by **Shokri et al. (2010)**. It is also much more accurate than the rough Johnsen's method (**Johnsen, 1970**), which depends on an overall evaluation of the types of cells present in seminiferous tubules. The inability of the recovery groups to regain its normal values and the worse values in the case of seminiferous tubules diameter indicates a long lasting effect of the drug.

The histological findings of the present work showed an affection and sometimes even complete destruction of the seminiferous tubules, there were irregular basement membrane, arrest of spermatogenesis in different levels (round spermatids in therapeutic dose effect group and primary spermatocytes in high dose effect group), apoptosis of germ cells, disarranged intratubular cells, reduction of Leydig cells and interstitial tissue edema. These findings explain the previous findings of **Karbalay-Doust et al. (2007)** who found that low and high doses of nandrolone decrease sperm quality and quantity in rats in the form of low sperm count, decreased motile sperm fraction and decreased sperms with normal morphology. It also support the

findings of **Shokri et al. (2010)** who found germ cell apoptosis and decreased number of germ cell layers and **Takahashi et al. (2004)** who found a reduction in Leydig cell numbers after nandrolone administration.

Collectively, androgen dependent organs are significantly damaged following anabolic steroid administration, many macroscopic and microscopic findings are usually noticed (**Bahrke et al., 2000**). The variability of the histological effects of the anabolic steroid must be re-evaluated according to the different types and regimens of anabolic androgenic steroids used. It is known that all anabolic steroids retain some androgenic properties. Testosterone has an anabolic/androgenic ratio of 1/1, while nandrolone has a ratio of 8:1 (**Yu-Yahiro et al., 1989**). This may explain the contradiction between the continuous spermatogenesis after high amounts of testosterone in rat (**Ludwig, 1950**) and in man (**Knuth et al., 1989**), the affected sperm parameter in men using methandienone (**Holma, 1977**), spermatogenic arrest and depletion in Leydig cells in rats injected by oxandrolone (**Grokkett et al., 1992**) and the reduction in Sertoli and Leydig cell numbers after using testosterone (**Feinberg et al., 1997**) and nandrolone (**Takahashi et al., 2004**).

The more aggressive effect found in the high dose groups in comparison to the therapeutic dose groups may be explained by the wide range of dose difference. **Noorafshan et al. (2005)** stated that high doses (but not therapeutic doses) of Nandrolone decreases the mean volume of testis. It is not uncommon to use such a high dose, a use of 10-100 fold of the therapeutic dose was previously recorded and high dose (100 times the normal dosing) is necessary for investigating the pathological effects of drug abuse (**Walder and Hailine, 1989**).

As regards the reversibility of the effect of the drug on testicular tissue, in the present work, the recovery groups showed -in some fields- minimal spermatogenesis activity and scanty sperms production. However, this regeneration was not satisfactory and need to be evaluated by fertility indices. This finding supports the previous findings of **Karbalay-Doust et al. (2007)**, who found that sperm parameters in rats were improved, but not recovered completely after discontinuing nandrolone for 14 weeks, and **Noorafshan et al. (2005)** who found structural changes in rat testis after stopping the drug for the same period. On the other hand, these results contradict the previous results of **Holma (1977)** which showed complete recovery after discontinuing methandienone treatment for 3 month, and the conclusion of **Hickson et al. (1989)** who stated that testicular atrophy, decreased spermatogenesis and altered sperm morphology have

all been reversible. Again this controversy may be explained by different anabolic steroid studied.

The ultrastructural findings of all the treated groups showed degenerative effects of all types of cells of the testis, the different groups shared common features but varied in severity. With the severest effect at the high dose effect group (HA) and the lowest at the therapeutic dose recovery group (TB). For example, some cells from therapeutic dose recovery group (TB) showed less severe changes and even showed increase in the number of healthy mitochondria. However, the mitochondrial findings varied among experiments using nandrolone, **Soares and Duarte (1991)** noticed a mitochondrial content decrease with many swollen and disrupted mitochondria in rat soleus muscle after six weeks of treatment, **Satoh et al. (2000)** found that nandrolone results in increased mean cross sectional area of mitochondria in the muscle fibers of diaphragm after 4 weeks of treatment. This finding was reversible after 4 more weeks, While **Naraghi et al. (2010)** found vesicular-like cristae in the mitochondria of the testis in rats treated with nandrolone for 8 weeks. In the present work, mitochondrial disruption among other cytoplasmic organelles was noticed in different treated groups. These findings may be affected by the tissue type used and the duration of treatment.

The other ultrastructural changes included apoptotic and completely degenerated cells specially in the effect groups (TA and HA), shrunk pyknotic or pale degenerated nuclei, clumped chromosomes, irregular nuclear membrane, degenerated cytoplasm and loss of cytoplasmic organelles, cytoplasmic vacuolization, many fat globules, increased lysosomes and thick irregular basement membrane. The results showed common characteristics shown by **Naraghi et al. (2010)** who reported cytoplasmic vacuolization, numerous fat droplets, many lysosomes, apoptotic germ cells, thick wavy multilaminar basement membrane and decreased Leydig cells number and size. The difference between the two studies is that the present work examined the effect of therapeutic and high doses of nandrolone and follows these changes after recovery while **Naraghi et al. (2010)** examined the high dose effect with or without accompanying exercise, and found more obvious changes in combined high dose of nandrolone and exercise. This leads to a suggestion that the nandrolone administration is the milestone factor in the ultrastructural changes in the testicular tissue rather than duration, dose or accompanied exercise.

Conclusion:

Based on this study, nandrolone administration causes a dose dependent, permanent or at least long lasting testicular tissue damage when given to the adult male albino rat, even in a therapeutic dose, leaving a testicular stigma, which may lead to permanent hypofertility or infertility. The present work may provide a suitable base for the future studies dealing with the effect of nandrolone on fertility and identifying the mechanisms of nandrolone in producing testicular changes. Apoptotic indices and oxidative stress measurement seems to be a convenient assist.

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