Effect of Anabolic Steroid “Nandrolone” on Testes of Adult Albino Rats: Immunohistochemical and Ultrastructural Study

MOHIE M. IBRAHIM, M.Sc.; HANY M.A. SONPOL, Ph.D.; MONA A. EL SHAHAT, Ph.D.; ADEL A. ELHAWARY, Ph.D. and ADEL A. BONDOK, Ph.D.
The Department of Anatomy and Embryology, Faculty of Medicine, Mansoura University

Abstract

Background: The Anabolic-androgenic steroids (AAS) are synthetic compounds based on the testosterone hormone structure. They have been abused by athletes to increase the muscular mass.

Aim of Study: This study was performed to investigate the biochemical and histological changes in testis of adult albino rat model using different doses of one of the injectable forms of the anabolic steroid nandrolone decanoate (ND).

Material and Methods: Forty adult albino rats were divided into four groups (ten rats per group), injected IM once a week for ten weeks as follow; group I (control) were injected with 0.1ml of peanut oil, group II, group III and group IV rats were treated respectively with ND in a dose 2.5, 5 and 10mg/kg body weight solved in 0.1ml peanut oil.

Results: Results of this study showed that the body weight significantly decreased only in group IV compared to the control group, a significant decrease in the testis weight in group III and IV while insignificantly changes in group II compared to group I. The blood level of testosterone, LH and FSH hormones insignificantly decreased in group II and III but significantly reduced in group IV compared to group I. Histological findings revealed decrease in the number of Leydig cells in the interstitial tissue, change in the shape of the seminiferous tubules, irregularity of the basement membrane with some areas detached from the germ cells, The germ cells showed vacuolated cytoplasm and decrease in number with subsequent dilatation of intercellular spaces and finally decreased sperms appearance in the lumen of the seminiferous tubules. These changes were noticed in all groups treated with ND to a variable degree being more evidenced in group III and IV and to less degree in group II compared to the group I.

Conclusion: The results of this study showed that fine structures of the testis were affected by ND.


Introduction

IMPROVING physical appearance and athletic ability have always been a man dream. Historically, athletes have used plants, natural and synthetic agents to increase their performance [1]. The commonest abused drugs are (AASs), which 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 [2]. Nandrolone deconate is a synthetic (AASs) promoting muscle growth. It has a stronger anabolic capacity than the natural testosterone. ND is frequently used to treat many clinical problems, such as HIV-associated muscle wasting, growth deficiency and anemia associated with chronic kidney failure. However, despite such therapeutic benefits, chronic administration of ND results in unfavorable outcomes, including hepatic and cardiovascular toxicities [3]. Prominent side effects of ND misuse are also found in the reproduction of male and female. For examples, prolonged treatment of ND in the male leads to altered testicular morphology, a decrease of testosterone secretion, and reduction of sperm count and quality [4]. It is suggested that such detrimental effects of ND on the reproductive tract would be due to a disruption of feedback regulation on hypothalamic-pituitary-gonadal axis by the exogenous agent [5].

Aim of the work: The aim of the present study was to investigate the effect of ND on the testis of adult male albino rat using biological and histological techniques.

Material and Methods

Animals used: The present study was conducted on 40 adult albino rats weighing 200-250gm. The animals were housed in plastic cages (56 x 39 x
19 cm) bedded with wooden chips in groups of five rats per cage. Rats were given ad libitum access to food and water, kept in a good ventilated and relatively humid room with temperature controlled at 24°C ± 3 and 12/12 hours dark/light cycle was preserved. This study was conducted in Mansoura Experimental Research Center, 2018. Drug Used (ND): ND ampoules (Nile Pharmaceutical Company) have been obtained from the local pharmacy in Mansoura, Egypt. According to the manufacturer, each one ml of the ampoule solution contains 25 mg of ND solved in peanut oil.

Experimental design: After one week of acclimatization, the rats were divided randomly into four groups, each group consisted of ten rats: Injected IM once a week for ten weeks as follow; group I (control) were injected with 0.1 ml of peanut oil, group II, group III, and group IV were treated respectively with ND in a dose 2.5, 5 and 10 mg/kg body weight solved in 0.1 ml peanut oil. The doses and the duration of ND administration simulate one cycle of (AAS) abuse by athletes. The duration of administration was corresponding to the spermatogenic period in rats which is approximately 48-56 days [6].

Sample collection and processing:
1- Serum: One ml of blood was collected by hematocrit tube from retro-orbital plexus at the end of experiment. Blood samples were centrifuged at 3000 rpm for 15 min to separate the serum. Serum levels of testosterone, FSH and LH hormones were measured by radioimmunoassay (RIA) using special kits as described in the instructions provided with the kits [7].

2- Body and testis weight determination: The next day after the final drug administration, the animals were weighed, anaesthetized with Ether, then sacrificed by cervical decapitation. The testes and epididymis of both sides were carefully removed through a lower abdominal incision. The testes were then separated from the epididymis and weighed using digital electronic balance [8].

3- Sperms suspension for count and morphology: Sperms were collected from the tail of the epididymis by cutting it into small pieces into 10 ml of normal saline in small petri dish, homogenized by using manual glass homogenizer. The number of sperms was measured by using a hemocytometer using a light microscope. Sperm count was expressed as number of sperms per milliliter [9]. Sperm suspension was examined for the presence of abnormal sperm morphology in the form of angulated bent sperms, fork headed sperms, broken sperms, short or coiled tail sperms [10].

4- Processing of the testes for histological study: The testes were fixed in Bouin’s fixative for 24 h. They were cut longitudinally into two halves and fixed again in fresh Bouin’s fluid for another 24 h. Samples then processed to obtain 5 µm thick paraffin sections for both routine H&E staining and caspase-3 immunohistochemical staining. The other parts of both testes were cut into small fragments to be fixed in 2.5% buffered gluteraldehyde and processed to obtain semi- and ultra-thin sections for the electron microscopic study [11].

5- Statistical analysis: Data were analyzed with SPSS version 21. The normality of data was first tested with one-sample Kolmogorov-Smirnov test. ANOVA test was used to compare more than two groups. Considering the mean ± SEM (standard error of mean) variability between the different groups with fixed significant p-value is less than 0.05 and highly significant is less than 0.001.

6- Ethical Approval: All the experiment steps were carried according to the rules and regulation of the Medical Research Ethics Committee (MREC) of Mansoura, Faculty of Medicine.

Results

1- Changes in the Body Weight and Testicular Weight (Table 1):

Injection of ND at a dose of 2.5 mg/kg led to insignificant decrease of body weight from 347 ± 5.5 gm (mean ± SEM) in control group (I) to 325.0 ± 8.09 gm in group II, group III and group IV revealed a significant decrease in the body weight to 285.0 ± 16.7 gm and 251.67 ± 6.17 gm respectively (Fig. 1).

Insignificant decrease of testicular weight from 2.2 ± 0.15 gm in group I to 2.18 ± 0.11 gm in group II. In group III and IV the testicular weights were 1.73 ± 0.14 gm and 1.43 ± 0.09 gm respectively which showed a significant decrease compared to group I (Fig. 2).

2- Biochemical Changes (Table 2):

Changes in testosterone level Testosterone level was 0.33 ± 0.008 ng/ml and 0.34 ± 0.015 ng/ml in group II and III respectively which showed insignificant decrease compared to 0.341 ± 0.007 ng/ml in group I. Testosterone level in group IV showed a significant decrease to 0.306 ± 0.005 ng/ml (Fig. 3).
Changes in FSH level:

FSH level was 0.280±0.015 mIU/ml and 0.256±0.01 1 mIU/ml in group II and III respectively which showed insignificant decrease compared to 0.289±0.015 mIU/ml in group I. But its level in group IV was 0.216±0.014 mIU/ml which showed a significant decrease compared to group I (Fig. 4).

Changes in LH level:

LH level was 0.299±0.008 mIU/ml and 0.308±0.011 mIU/ml in group II and III respectively which showed insignificant decrease compared to 0.297±0.006 mIU/ml in group I. LH level in group IV was 0.264±0.007 mIU/ml which showed a significant decrease compared to group I (Fig. 5).

Table (1): Changes in the body weight and testicular weight.

<table>
<thead>
<tr>
<th></th>
<th>Group I</th>
<th>Group II</th>
<th>Group III</th>
<th>Group IV</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body weight (gm) Mean ± SEM</td>
<td>347±5.5</td>
<td>325.0±8.09</td>
<td>285.0±16.7*</td>
<td>251.67±6.17*</td>
</tr>
<tr>
<td>Testis weight (gm) Mean ± SEM</td>
<td>2.2±0.15</td>
<td>2.18±0.11</td>
<td>1.73±0.14*</td>
<td>1.42±0.09*</td>
</tr>
</tbody>
</table>

*Significant difference, \( p<0.05 \).

Table (2): Biochemical changes regarding Testosterone, FSH, and LH hormones.

<table>
<thead>
<tr>
<th></th>
<th>Group I</th>
<th>Group II</th>
<th>Group III</th>
<th>Group IV</th>
</tr>
</thead>
<tbody>
<tr>
<td>Testosterone (ng/ml) Mean ± SE</td>
<td>0.341±0.007</td>
<td>0.33±0.008</td>
<td>0.34±0.015</td>
<td>0.306±0.005*</td>
</tr>
<tr>
<td>FSH (mIU/ml) Mean ± SE</td>
<td>0.289±0.015</td>
<td>0.280±0.015</td>
<td>0.256±0.011</td>
<td>0.216±0.014*</td>
</tr>
<tr>
<td>LH (mIU/ml) Mean ± SE</td>
<td>0.297±0.006</td>
<td>0.299±0.008</td>
<td>0.308±0.011</td>
<td>0.264±0.007*</td>
</tr>
</tbody>
</table>

*Significant difference, \( p<0.05 \).
Histopathological Study:

1- Hematoxylin and eosin-stained sections:

The seminiferous tubules showed a normal arrangement of germ cells and Sertoli cells in the control group as the spermatogonia lined the periphery of the seminiferous tubules, it was a relatively small cell, situated next to the basal lamina. The primary spermatocytes were large spherical cells located in the middle third of the wall of seminiferous tubules above the spermatogonia. Spermatids are smaller than spermatocytes and are located higher in the epithelium. They are rounded cells, often deformed to polygons by their close packing. Also, seminiferous tubules in control group showed multiple Sertoli cells close to a regular basement membrane (Fig. 6-B). Seminiferous tubules in the sections of control group usually showed abundant number of sperms in the lumen. The interstitial tissue revealed large and numerous Leydig cells which occurred mostly in clusters of cells with spherical, oval, or irregular shape (Fig. 6-A). In group II, most of the seminiferous tubules maintained the normal arrangement of their seminiferous epithelium and adequate amount of interstitial tissue in between the tubules. The interstitial tissue exhibited groups of Leydig cells and blood capillaries (Fig. 6-C). Some seminiferous tubules showed different shapes, irregular basement membrane and fewer spermatozoa in their lumen (Fig. 6-D). In group III, the stained sections revealed changes in the shape of the seminiferous tubules which appeared condensed and showed irregular germ cells arrangement, also these affected tubules had few sperms in their lumen (Fig. 7-A). The basement membrane showed wide areas without attached cells indicating the low number of germ cells. Dilated intercellular spaces between germ cells are also noticed. The interstitial tissue revealed few and small sized Leydig cells in between the seminiferous tubules (Fig. 7-B). In group IV, multiple seminiferous tubules appeared irregular in shape with empty lumen (Fig. 7-C). Marked reduction in the germ cells causing dilatation of intercellular spaces. Germ cells showed vacuolated cytoplasm, primary spermatocytes lied close to the basement membrane. The interstitial tissue in this group was nearly absent between seminiferous tubules with few Leydig cells (Fig. 7-D).

Fig. (6): A photomicrograph of sections of a rat testis. (A): Control group showing normal semineferous tubules with basal arranged spermatogonia (SG) amount of sperms (ST) in the lumen. Interstitial Leydig cells (LC) distributed between the tubules, (B): Control group showing normal stages of spermatogenesis including spermatogonia (SG), primary spermatocytes (SC), Spermatids (SP), multiple Sertoli cells (SE), abundant sperms in the lumen (ST), intact basement membrane (BL) and extra-tubular Leydig cells (LC), (C): Group II showing normal seminiferous tubules with basal spermatogonia (SG), intraluminal spermatozoa (ST) and groups of Leydig cells (LC) in the interstitial tissue. Minimal changes in the form of empty lumen of some tubules (crossed arrow), irregular basement membrane (arrowhead) and wide spaces between the tubules (astrik) and (D): Group II showing normal seminiferous tubule with basal spermatogonia (SG), spermatocyte (SC), spermatid (SP), intraluminal spermatozoa (ST) and groups of Leydig cells (LC) in the interstitial tissue. The changes appeared in form of empty lumen (crossed arrow), irregular basement membrane (BL). (A and C are H&E X100; B and D are H&E X400).
Fig. (7): A photomicrograph of sections of a rat testis. (A): Group III showing condensed seminiferous tubules with irregular shapes (arrowhead) and decrease of the interstitial tissue (astrik) in between with few Leydig cells (LC). The basement membrane showed spaces between the basal spermatogonia (arrow). Some tubules appeared with empty lumen (crossed arrow) or few sperms (ST) in the lumen, (B): Group III showing basement membrane with detached areas (BL) and areas without attached spermatogonia (SG). There is apparent decrease in the number of spermatocytes (SC) and the lumen empty from sperms (ST). Wide increased intervals between germ cells (astrik) and small sized Leydig cells (LC) in between the seminiferous tubules are noticed, (C): group IV showing seminiferous tubules with irregular shape (arrow), wide areas of their basement membrane are devoid of spermatogonia (arrowhead), and their lumen are empty (crossed arrow). Notice wide spaces (astrik) appear between the tubules with few Leydig cells (LC) and (D): Group IV showing vacuolated germ cells with condensed chromatin (arrow), basal small spermatogonia (SG), lost basement membrane (BL), degenerated spermatocytes (SC), small round spermatids fill the lumen indicating arrest of spermatogenesis, few sertoli cells (SE) and wide space between the tubules (astrik) (A and C are H&E X100; B and D are H&E X400).

2- Caspase III-stained sections: Fig. (8)

Immunohistochemical detection of caspase 3 reaction appeared as brown granules which represent antibody binding sites, contrasted against a blue hematoxylin background. In the control group weak reaction noticed in the interstitial tissue (Fig. 8-A). Examination of caspase 3 stained sections of the testis in group II showed mild positive immunostaining reaction (Fig. 8-B), group III showed moderate reaction (Fig. 8-C) while group IV showed marked reaction (Fig. 8-D).

3- Ultrastructural result by electron microscopic examination:

By EM examination, the ultrastructure of the rat testis in the control group displayed the Sertoli cells and the spermatogenic cells with their intracellular features typical to those described in the normal process of active spermatogenesis. Spermatogonia were seen rested on thin regular basement membrane with oval nuclei containing abundant heterochromatin and their cytoplasm contained normal mitochondria (Fig. 9-A).

Sertoli cells located close to the basal lamina with triangular nuclei containing prominent nucleolus and dense chromatin body. The primary spermatocytes with large spherical nucleus containing clumps of heterochromatin scattered all over the nucleoplasm and cytoplasm contained peripherally arranged mitochondria (Fig. 9-A).

The results of the EM examination exposed degenerative changes to all kinds of the cellular elements of the testis in the different treated groups, they shared common degenerative changes but differed in severity. In group II the spermatogonia appeared close to irregular basement membrane with oval nuclei and abundant heterochromatin.
Sertoli cells showed a well-defined triangular nucleus and small sized vacuoles in its cytoplasm which filled the intervals between the spermatogenic cells. In addition to the previous findings, the primary spermatocytes showed a rounded appearance their nuclei were large, rounded, invested completely with a nuclear membrane, and filled with homogenously apparent chromatin. The cytoplasm was granular in nature with multiple small-size vacuolated mitochondria (Fig. 9-B). In group III, the histological changes that were apparent including the spermatagonia as they lost their normal architecture, position and appeared away from the basal lamina, with irregular-shaped nuclei showed patches of condensed chromatin. Together with the intervals between the spermatogonia were widened. The primary spermatocytes decreased in size, with irregular shrunken nucleus containing condensed chromatin. Sertoli cells appeared containing ill-defined nucleus, swollen mitochondria, and vacuolated cytoplasm. Also, the basal lamina appeared irregular in shape associated with enlarged myoid cells (Fig. 9-C). In group IV, the spermatogonia lost their normal histological pattern, showing the characters of the degenerating cells as they were irregularly shrunken with folded pyknotic nuclei containing visible condensed chromatin. The intercellular spaces between them were widened, Sertoli cells were also shrunken, containing small sized vacuolated mitochondria showing ruptured cristae and degenerated nuclei. The primary spermatocytes distorted with irregular nucleus; its cytoplasm showed small vacuolated mitochondria which lost its normal peripheral arrangement (Fig. 9-D).
Discussion

In the present study, ND decanoate injection at a dose of 2.5mg/kg led to insignificant decrease of body weight and testicular weight. This result agreed with Saddick [12], who showed that chronic administration of ND significantly decreased the body weight in male rats, this significance may be due to the more prolonged duration. The present study revealed that (ND) injection at a dose of 2.5mg/kg led to an insignificant decrease in the serum level of testosterone, FSH and LH. These results corresponded to the work of [13] on rats received 2.5mg/kg ND decanoate intramuscular injection weekly during the experimental period of 13 weeks and the reported serum levels of testosterone, FSH and LH which were significantly reduced when compared with control normal rats. In the present study, ND decanoate injection at a dose of 5.0mg/kg led to significant decrease of body weight, testicular weight. These results agreed with Mohamed and Mohamed [14], who noticed that ND administration to rats led to significant decrease of body weight, testicular weight. Also, injection of ND in a dose 5.0mg/kg led to insignificant decrease of testosterone, FSH and LH levels. These results agree with Barone et al. [15], who showed that middle dose ND administration to mice led to decreased testosterone level. These occurred may be due to Low levels of LH may contribute to low levels of testosterone production as LH stimulates testosterone production in Leydig cells. In the present study, ND injection at a dose of 10.0mg/kg led to significant decrease of body weight, testicular weight. These results agree with Mesbah et al. [16], who noticed that ND administration to rats led to significant decrease of body weight, testicular weight. The testicular concentrations of testosterone are necessary to maintain normal length of the seminiferous tubule and the reduction in tubule length may be one reason for the reduction in testis weight [17]. In the present study, ND injection at a dose of 10.0mg/kg resulted in significant decrease in the serum level of testosterone, FSH and LH.
These results agree with Maravelias et al. [18], Wesson and McGinnis [19] and Shahraki et al. [20] who showed that chronic administration of ND at high dose decreased plasma level of FSH, LH, testosterone, and decreased spermatogenesis in male rats in male rats.

Like other tissues, Androgens act on the testis by activating androgen receptor (AR) transcription. Inside Sertoli cells, testosterone binds to ARs at the beginning of its activation, cause activation of the receptor to maintains spermatogenesis and inhibits apoptosis of germ cells [20]. 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 (especially in high dose group III & IV). These results agree with Tahtamouni et al. [21] who stated that, AR expression is maintained by endogenous testicular androgens, absence of testosterone is known to lead to disruption of spermatogenesis.

ND injected with low or high doses, can lead reduce of testosterone, which cause the maturation arrest at the primary spermatocyte level and spermatid level). Also, in this study there was, apoptosis of germ cells, disarranged spermatogenic cells. These findings support the findings of [22,23] regarding the apoptosis of the germ cell and decreased number of germ cell layers in rats treated with ND.

This study revealed reduction in the number and size of Leydig cells and minimal interstitial tissue in between the seminiferous tubules which matched with Takahashi et al. [24] who found a reduction in Leydig cell numbers after ND administration. The close relationship between Leydig cells and blood vessels suggested that these cells are at high risk of exogenous toxicants. In the present study, there were a remarkable decrease in the number and size of the spermatogenic cells and depletion of intact cells in treated groups, especially in groups III and IV animals. These results were consistent with Naraghi et al. [25] who demonstrated pyknotic changes and severe depletion of intratubular cells following treatment by AASs. The low number of the spermatogenic cells within the tubules of the treated animal was associated with enlarged and dilated intracellular spaces within the tubules. These results agree with Noorafshan et al. [26] who observed that there was disruption of the seminiferous epithelium with broad spaces between the cellular components the seminiferous epithelium of the treated animals with AAS associated with presence of copious vacuoles frequently associated with degenerating germ cells.

In the present study, the seminiferous tubules showed the features of the disrupted spermatogenesis which includes the progressive decrease in the sperm appearance in the lumen which may indicate the arrest of the maturation in different cell stages. These results were in the same side with [25,27] in reporting the histological affection of testis was in the form of seminiferous tubules containing widely separated germinal cells with darkly stained nuclei and vacuolated cytoplasm and that the lumen of the tubules had scanty number of sperms and sloughed germ cells.

Conclusion:

Administration of ND decanoate exerts a clear effect on testicular structure including degenerated changes of germ cells, Sertoli cells, and Leydig cells. These are accompanied by deleterious effects of ND on the feedback regulation of hypothalamo- pituitary-gonadal axis which decreases the serum levels of testosterone, FSH and LH.

References


تأثير الستيرويد الابناثي (الناندرولون) على خصائص الفئران البيضاء البالغة.
دراسة كيميائية مناعية وبيئية دقيقة

مقدمة: المنشأتان الابناثية الأنتروجينية هي مركبات اصطناعية مركبة على أساس هرمون التستوسترون. قد تم إساءة استخدامها لفترات طويلة من قبل الرياضيين لزيادة الكتلة العضلية.

الهدف من الدراسة: أجريت هذه الدراسة لبحث التغيرات البيو كيميائية والنسيجية في خصائص الفئران البيضاء البالغة باستخدام جرعات مختلفة من أحد الأشكال القابلة للحقن من المنشأتان الابناثية الأنتروجينية وهو مركب الناندرولون ديكوتات (ND).

الطريق والمواد: تم تقسيم أربعة فئران عشوائياً بالغة إلى أربع مجموعات (عشرة فئران لكل مجموعة)، وكانت بالفعل مرة واحدة في الأسبوع لمدة عشة أسابيع. تم حقن المجموعة الأولى بـ 0.1 مل من زيت الفئران السواني وحقن المجموعة الثانية وعمليا مجموعة المجموعة الثالثة والمجموعة الرابعة على التوالي باستخدام مركب ND بجرعات 0.5 و 10 ملغ/كامل من زيت الفئران السواني.

النتائج: وزن الجسم انخفض بشكل ملحوظ فقط في المجموعة الرابعة مقارنة بالمجموعة الأولى، وانخفاض كبير في وزن الخصبة في المجموعة الثالثة والرابعة بينما تغيرات طفيفة في المجموعة الثانية مقارنة بالمجموعة الأولى. التغير في شكل الأذنيب المنوية، وعدم انتظام الفوانيس مع بعض المناطق المتميزة من الخلايا الجنسية. أظهرت الخلايا الجنسية تحلل ستيتيلزامين وانخفاض عددها مع توسع المساحات بين الخلايا، وانخفاض الزيوت النارية في تجفيف الأذنيب المنوية. لوحظت هذه التغيرات في جميع الفئران المختبر معالجتها بدرجات متفاوتة أكثر وضوحًا في المجموعتين الثالثة والرابعة ودرجة أقل في المجموعة الثانية مقارنة بالمجموعة الأولى.

الاستنتاج: أظهرت نتائج هذه الدراسة أن الهياكل المنوية للخليج تتأثر بمركب الناندرولون ديكوتات.