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ORIGINAL ARTICLE

Effect of Anabolic Steroid Nandrolone on Prostate of Prepubertal Albino Rats: Histological and Immunohistochemical Study

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ABSTRACT

Background: Nandrolone decanoate (ND) is a synthetic substance derived from testosterone and considered as an anabolic androgenic steroid (AAS). It participates in improving muscular gains associated with exercises. It could cause destructive impacts on the male reproductive system which needs to be evaluated. This study aims to investigate the effects of nandrolone decanoate on the posterolateral lobe of the prostate in albino rats at prepubertal stage and to assess the potential reversibility of these effects after cessation.

Methods: Twenty-six albino rats were used, categorized into three main equal groups. Group I (control group) that was subdivided into two equal subgroups, Group II (treated group) in which rats were received nandrolone decanoate weekly by intramuscular injection (IM) in a dose 10 mg/kg for four weeks and Group III (recovery group) were received nandrolone decanoate weekly via IM injection by a dose of 10 mg/kg body weight for four weeks as in Group II followed by recovery period (without any treatment) for another four weeks. For biochemical, histological and immunohistochemical study, blood samples and tissues from posterolateral lobe of prostate were obtained.

Results: Treated group showed biochemically a highly significant decrease in both serum testosterone as well as PSA and increase in tissue MDA compared to control group. Histologically, the acini were lined by flattened cells and showed weak PAS reactions. Ultrastructural examination of the same group showed nuclei of acinar cells were heterochromatic and rested on irregular basal lamina that had focal interruption and immunohistochemically, they had a negative nuclear immunoexpression for androgen receptors (ARs). However, the recovery group showed amelioration of biochemical, histological and immunohistochemical results.

Conclusions: Nandolone decanonate causes significant biochemical, histological and immunohistchemical changes in the prepubertal dorsolateral lobes of the prostates and its stoppage caused remarkable improvement.

Keywords: Nandolone deconate; Prepubertal; Prostate; Recovery

INTRODUCTION

Steroids that mimic testosterone's effects while also increasing virility and mass of muscles are known as anabolic-androgenic steroids (AASs). Renal problems, blood abnormalities, growth insufficiency, osteoporosis, and delayed puberty in men are among the significant conditions that AASs are used to treat [1].

There is an endogenous androgen in male bodies in a ratio of 1:50 called nandrolone, which is also known as 19-nortestosterone. The medical forms of this compound include esters for example nandrolone decanoate (Deca-Durabolin) and nandrolone phenylpropionate (Durabolin) [2]. Nandrolone decanoate is the most commonly administered AASs that used among athletes and it is also used in and in competitive, criminal circles as well as aggressiveness circumstances [3].

The greatest accessory organ in the male reproductive system is the prostate. This organ produces one-third of the alkaline seminal fluid. Androgens, which are produced in the testes, are

essential for the development and maintenance of the prostate gland [4]. Androgens trigger the development of prostate in both humans and rodent. The cytodifferentiation and maturation phase of rodent prostates continues until pre-puberty so named late organogenesis of the prostate. Any outside influences change the hormonal balance at this point of development could have significant implications in adulthood [5]. Unlike rats and mice, which have a more lobular prostate, humans have a more compact structure. Albino rat prostate is characterized by its four distinct lobes, which are anterior, lateral, ventral, as ell as dorsal lobes. The lobes are categorized regarding to relative positional relation to the urinary bladder [6]. The ventral lobe is different from others that does not have a human homologue [7]. While the rat's dorsolateral prostate lobe and the human prostate's peripheral zone are homologous [8]. This study directed to assess the effects of nandrolone decanoate on posterolateral lobe of albino rat's prostate during the prepubertal age and to evaluate the potential reversibility of these effects after cessation.

METHODS

Materials

Animals:

Twenty-six healthy albino rats were used, taken from the Animal House, Faculty of Medicine, Zagazig University. This study was performed in accordance with the National Institutes of Health's Guidelines for Animal Research and was authorized by the Institutional Animal Care and Use Committee (ZU-IACUC) at Zagazig University (ZU-IACUC/3/F/70/2023). All animals were kept in animal house for one week for acclimatization to new environment before the experiment. All animals were housed in a clean stainless-steel cage and maintained in same environment with respect to temperature, lighting, and food. They were allowed to eat and drink as much as they wanted.

Chemicals:

Nandrolone Decanoate:

The trade name of the drug was Deca-Durabolin and manufactured in the year 2023 by El-Nile Company for Pharmaceuticals and Chemical Industries**.** In packaging of 1ml ampoules, each unit contains 25mg of it in an oily formulation (17b-hydroxy-19-nor4-androstene-3-one). The selection of dosage and method of administration was determined by the usage patterns of athletes in fitness centers. 1.5 ml of peanut oil was used to dissolve it **[9]** and given by intramuscular injection in a dose of 0.06 ml for each rat according to body weight.

Peanut oil*:* obtained from Imtenan Egypt company.

Experimental design:

Rats weighing from 60 to 70 gm and aged 35 days were classified into three groups. This age in experimental albino rat equals human prepubertal age (11-14 years) according to Ghasemi et al. [10].

Group I (control group): Involved ten rats that were equally subdivided into two subgroups (5rat each): Negative control group (Ia): Did not receive any treatment throughout the experiment. Positive control group (Ib): Received peanut oil once weekly by intramuscular injection for 4 weeks.

Group II (treated group): Involved eight rats that were received nandrolone decanoate IM injection once weekly for four weeks in a dose of 10 mg/kg body weight [11].

Group III (recovery group): Involved eight rats that were received nandrolone decanoate IM injection once weekly for four weeks with a dose of 10 mg/kg body weight (as in Group II) followed by a recovery period (without any treatment) for another four weeks [12].

An intraperitoneal dose of thiopental sodium (50 mg/kg) was administered to the animals in each group to put them to sleep at the time of scarification [13]. Intracardiac perfusion was performed by 300 ml of 2.5% glutaraldehyde in a 0.1 mol/L cacodylate buffer at a pH of 7.3 through the heart apex for a duration of five minutes to facilitate the fixation of the prostates. The urogenital complex was exposed by a midline lower abdominal incision. We removed the prostate glands and immediately after collection, the right dorsolateral prostatic lobes were dissected and prepared for examination under both light and electron microscopes. Parts of dorsolateral prostatic lobes were frozen at -80°C to evaluate the levels of malondialdehyde (MDA) enzymes in a prostatic tissue homogenate.

Methods:

General observation: The amount of food and water had been consumed by rats and their overall appearance were monitored throughout the duration of the experiment.

Biochemical study: Using capillary tubes, three milliliters of blood were collected from orbital veins and the serum were separated through the process of centrifugation [14]. For assessment the serum testosterone hormone, using an ELISA and the ELISA reader was used to quantify the levels of mouse and rat testosterone using commercial Randox kits (catalog number: EK7014). Assessment of prostatic specific antigen (PSA) by utilizing ELISA kits and a compact VIDAS automated analyzer [15].

Assessment the concentration of tissue malondialdehyde (MDA) (as a measure of oxidative stress) colorimetrically in prostate homogenates according to Hortu et al. [16].

Histological study:

For light microscopic study: The specimens from dorsolateral prostatic lobe (1cm) were submerged in 10% formalin saline and prepared by paraffin technique to make 5-µm-thick paraffin sections. They subsequently were stained by hematoxylin and eosin (H&E) as a routine method for studying the general architecture [17] as well as periodic acid schiff (PAS) reagent for the detection of mucopolysaccharide [18].

For transmission electron microscope study: The specimens from dorsolateral prostatic lobe (1 mm^2) were fixed in 2.5% glutaraldehyde for 24 hours then washed in 0.1 M phosphate buffer. They were post-fixed at room temperature in 1% osmium tetroxide then dehydrated in progressively stronger concentrations of ethyl alcohol before being immersed in epoxy resin. Semi-thin sections were stained using toluidine blue, then the ultrathin sections underwent staining with uranyl acetate and lead citrate [19]. In the Electron Microscope Unit of the Faculty of Science at Zagazig University in Egypt, specimens were examinated and captured using a transmission electron microscope (JEOL JEM100CX, Jeol Ltd, Tokyo, Japan).

Immunohistochemical study: Anti-androgen receptors is rabbit polyclonal antibodies (catalog number ZR-334 from Thermo Fisher Scientific Corporation in Fremont, CA 91006, USA). The slides were stored at 4°C overnight in the presence of polyclonal primary antibodies. Subsequently, the slides were rinsed and treated with 0.3% H2O2. Antigen retrieval was performed by heating the tissue sections in a citrate buffer (0.01 M, pH 6.0) using a water bath set to 95°C for a duration of 30 minutes. Following this, the sections were incubated overnight at 4°C with primary antibodies. Subsequently, the sections were treated with biotinylated horse anti-mouse IgG for 30 minutes, followed by incubation with the avidin-biotin peroxidase complex. The resulting reaction was visualized using DAB solution. The negative control sections were processed in above mentioned steps but the primary antibody was not added in this step (Instead PBS) was used. Positive results were indicated by nuclear brown coloration to androgen receptors (ARs) of acinar cells. The slides were analyzed and captured using the Lecia ICC50 camera.

Image analysis and morphometric study: Five slides from five distinct specimens in each group were analyzed, and from each slide, five randomly chosen, non-overlapping fields from sections within each group were taken. Measurements included acinar epithelium heights, optical density of periodic acid Schiff (PAS) reactions, and the number of positive immunoexpression in nuclei of acinar cells for androgen receptor (ARs). Using image analysis software and light microscopy, images were processed at a total magnification of X 400 at the Image Analysis Unit of the Human Anatomy and Embryology Department, Faculty of Medicine, Zagazig University.

Statistical analysis: The mean ± SD was used to express all the data. The 13.00 edition of the Statistical Package for the Social Sciences (SPSS) software, which is based in Chicago, Illinois, USA, was used to conduct the statistical analysis. One way Anova Test was used for comparing biochemical variations between groups. A p-value of less than 0.05 was deemed to indicate statistical significance.

RESULTS

General observation:

During the experiment, rats were in good general condition, good appetite and normal physical activity with no mortality recorded. All rats in subgroups Ia and Ib showed identical results of biochemical analysis, light, electron microscope examinations, as well as morphometric analysis. So these rats of subgroups Ia and Ib were referred as the control group.

Biochemical results:

Serum testosterone hormones levels: Statistical analysis revealed highly significant decrease in treated group when compared to both control and recovery groups. However, no significant difference was found between control and recovery groups (Table 1, Figure 1A).

Tissue malondialdehyde (MDA) enzymes levels: Statistical analysis revealed a highly significant increase in treated group compared to both control and recovery groups. Moreover, also a highly significant increase was recorded in recovery group compared with control group (Table 1, Figure 1B).

-Serum prostatic specific antigen (PSA): Statistical analysis revealed highly significant decrease in treated group compared to control group. However, no statistically significant difference was found between control group and recovery groups (Table 1, Figure 1C).

Histological results:

Hematoxylin and eosin (H&E):

Examination of hematoxylin and eosin (H&E) stained sections of dorsolateral prostatic lobe of control group revealed the presence of small acini variable in shape and contour having multiple epithelial folds. The acini were lined by simple cuboidal epithelium with basal rounded nuclei and had apical acidophilic brush borders. The lumina of acini were filled with acidophilic prostatic secretions. They were surrounded by fibromuscular stroma contained blood vessels and smooth muscle cells (Figs. 2a&2b). The treated group showed that many acini were lined by flattened cells with flat nuclei and poorly infolded mucosa. Moreover, they had widened lumina that devoided from secretions and some atrophied acini were also seen. Fibromuscular stroma was narrow and contained congested blood vessels (Figs. 2c&2d). On the other hand, Secretory cuboidal epithelium lining the acini of the recovery group had rounded nuclei and apical acidophilic brush borders. Prostatic secretions filling the lumina of the acini were also observed. Some acini had flattened epithelial lining with no secretions in their lumina and others were atrophied. Fibromuscular stroma in-betweens were quite wide (Figs. 2e&2f).

Periodic acid Schiff results:

Examination of Periodic Acid Schiff (PAS) stained sections of the control group revealed strong positive reactions in acinar basement membranes, apical brush borders and their luminal secretions (Fig. 3a). On contrary, treated group showed weak positive reactions in acinar basement membranes, apical brush borders and their luminal secretions (Fig. 3b). The sections from recovery group showed strong reactions in acinar basement membranes, apical brush borders and their luminal secretions (Fig. 3c).

Transmission electron microscopic results:

Ultrathin sections from acinar cells of dorsolateral lobes of the prostate of control group appeared with euchromatic nuclei rested on basal lamina and surrounded by smooth muscle cells. Their cytoplasm contained rough endoplasmic reticulum and secretory vesicles with variable electron densities. The cells had apical microvilli and apical cellular junctions (Fig. 4a). Treated group revealed acinar cells with heterochromatic nuclei and rested on irregular basal lamina that had focal interruption and surrounded by several layers of smooth muscles. The cytoplasm contained dilated cisterna of rough endoplasmic reticulum and electron dense bodies. There was separation between acinar cells and underlying basal lamina. The acinar cells had disrupted microvilli and there were residual bodies and some disintegrated cells with necrotic nuclei in their cytoplasm were also noticed (Fig. 4b). Moreover, recovery group revealed acinar cells with euchromatic nuclei and rested on regular basal lamina that surrounded by smooth muscle cells. Their cytoplasm contained rough endoplasmic reticulum with dilated cisterna and secretory granules of variable electron densities. apical microvilli were also noticed (Fig. 4c).

Immunohistochemical results:

Regarding the immunohistochemical analysis of prostatic acinar cells, control group sections revealed strong positive immunoexpression in nuclei of acinar cells for androgen receptors (ARs) (Fig. 5a). Negative immunoexpression in nuclei of acinar cells for ARs was shown in treated group. (Fig. 5b). Regarding recovery group, the majority of nuclei of acinar cells exhibited positive immunoexpression but few nuclei showed negative immunoexpression (Fig. 5c).

Image analysis and morphometric results:

Statistically, there were a highly significant decrease in acinar epithelial heights, optical density of PAS reactions as well as the count of positive nuclear immunoexpression for ARs in treated group compared to control and recovery groups. However, no significant difference between control group and recovery group (Table 2 & Figs 1D, 1E &1F).

Table 1: Comparison between the prepubertal studied groups regarding statistical analysis of biochemical results

Mean± S.D	Control	Treated	Recovery		P Value
Serum T	3.297 ± 0.129	2.165 ± 0.23	3.13 ± 0.084	117.131	P_1 <0.001** P_2 <0.001** P_3 =0.117
MDA	3.83 ± 0.23	13.07 ± 0.3	11.04 ± 0.38	1988.33	P_1 <0.001** P_2 <0.001** P_3 =0.002*
PSA	0.583 ± 0.077	0.168 ± 0.055	0.45 ± 0.174	27.35	P_1 <0.001** P_2 <0.001** P_3 =0.075

T:Testosterone **MDA**:Malondialdehyde **PSA**:Prostate-Specific Antigen

One way ANOVA test p>0.05 is no statistically significant *p<0.05 is statistically significant **p≤0.001 is statistically highly significant

p1 difference between control group and treated group
p2 difference between recovery group and treated group
p3 difference between control group and recovery group

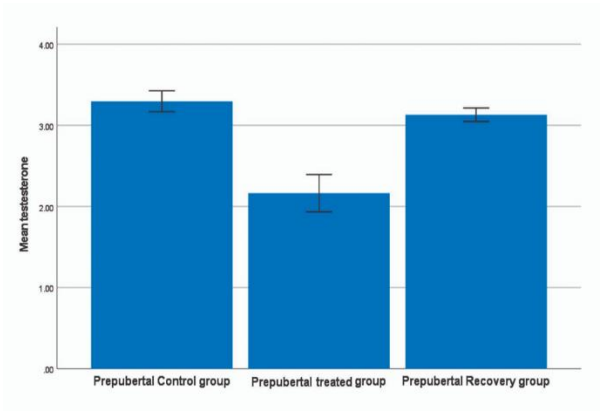
Table 2: Comparison between the prepubertal studied groups regarding statistical analysis of morphometical results

Mean± S.D	Control	Treated	Recovery	F	P Value
Acinar pithelial heights	23.377 ± 2.777	8.433 ± 2.741#	20.747 ± 1.873	81.52	P_1 <0.001** P_2 <0.001** P_3 = 0.113
Optical density PAS reactions	200.749 ± 1.218	143.42 ± 7.156#	195.296 ± 4.013	349.268	P_1 <0.001** P_2 <0.001** $P_{3=}$ 0.081
AR numbers	92.88 ± 4.883	40.63 ± 4.173#	90.25 ± 1.669	26.079	P_1 <0.001** P_2 <0.001** P_3 =0.374

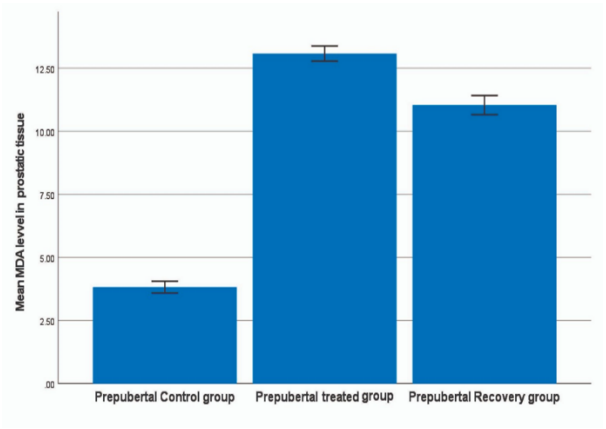
PAS: Periodic acid–Schiff **AR:** Androgen receptors

One way ANOVA test p>0.05 is no statistically significant **p≤0.001 is statistically highly significant

p1 difference between control group and treated group
p2 difference between recovery group and treated group
p3 difference between control group and recovery group



(A)



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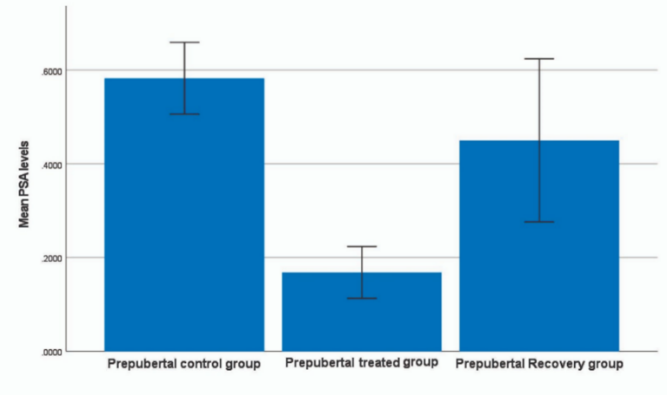
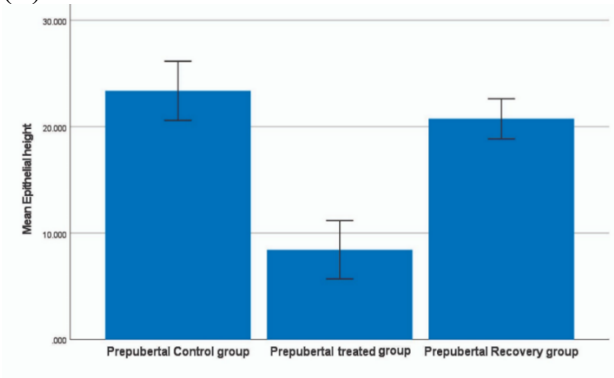
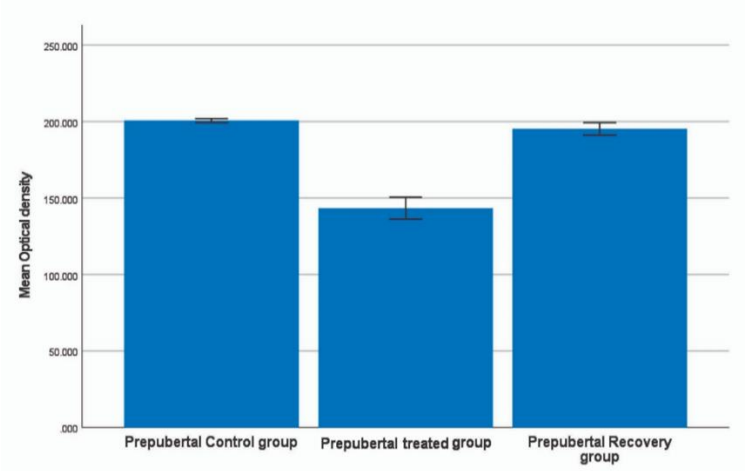


(C)



(D)



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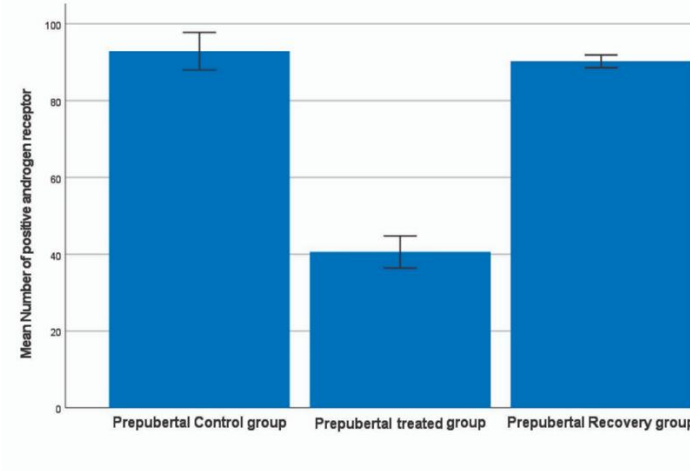

(F)

Figure 1: Bar Charts in different studied groups are showing (A): The mean serum testosterone level in, (B): The mean tissue MDA level, (C): The mean PSA level, (D): The mean acinar epithelial height of prostate, (E): The mean optical density of PAS, (F): The mean number of positive nuclear ARs immunoexpression

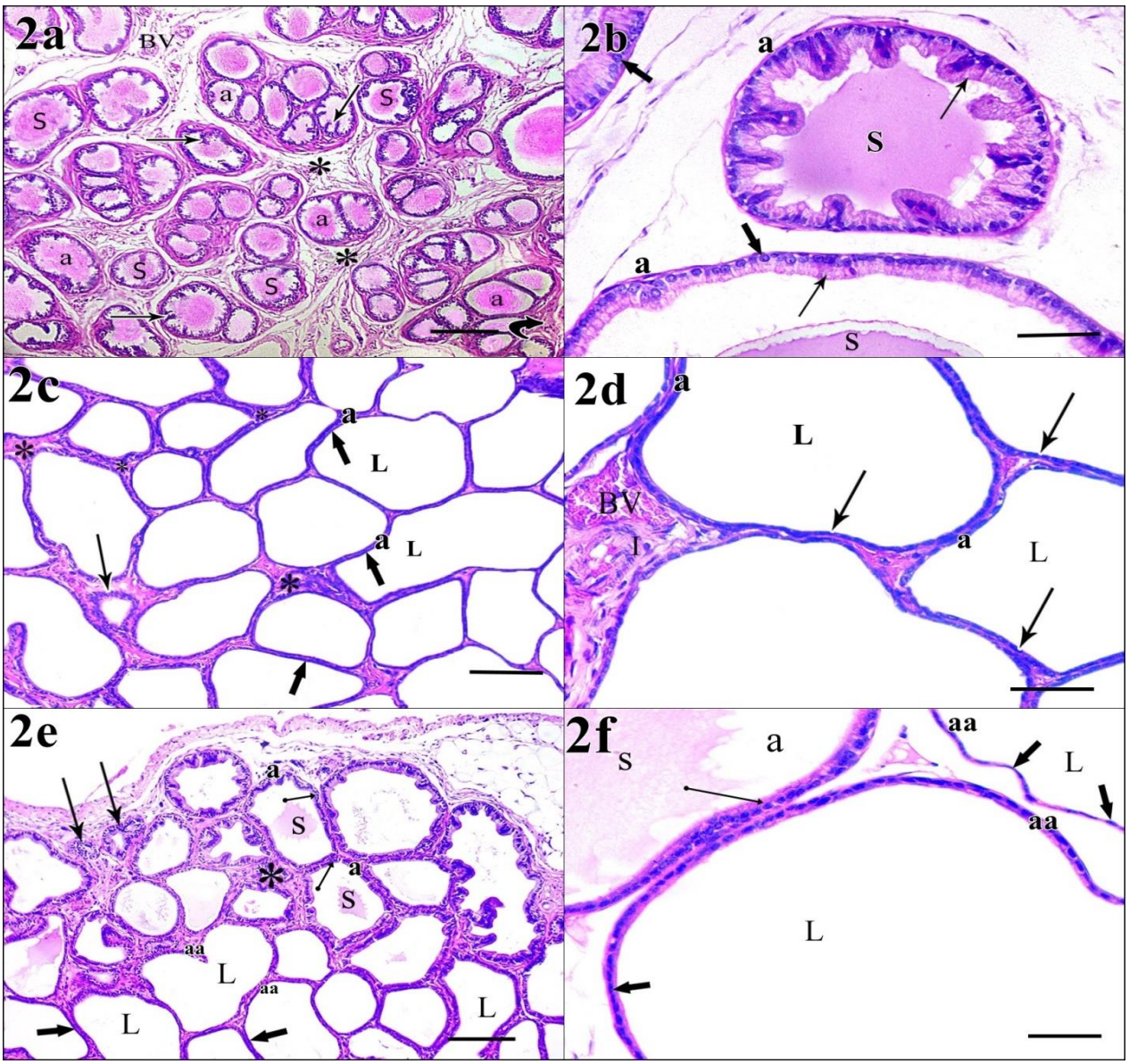

Figure 2: A photomicrograph of H&E stained sections in dorsolateral lobe of prostate of prepupertal albino rat: (2a): The control group showing small acini variable in shape and contour (a) have multiple epithelial folds (arrows). The lumen of each acinus is filled by prostatic secretion (S). Fibromuscular stroma is seen in-between acini (Asterisk) contains blood vessels (BV) and smooth muscle cells (Curved arrow). (H&E, x100 - Scale bar 20 µm). (2b): The control group showing acini (a) are lined by simple cuboidal epithelium with apical acidophilic brush borders (thin arrow) and basal rounded nuclei (thick arrow). The lumina of acini are filled by prostatic secretions (S). (H&E, x400 -Scale bar 30 µm). (2c): The treated group showing many acini (a) with flattened epithelium (thick arrows) and have wide lumina devoid of secretion (L). Some atrophied acini (thin arrow) and narrow fibromuscular stroma in-betweens (Asterisk) are also noticed.(H&E, x100 -Scale bar 20 µm). (2d): The treated group showing many acini (a) are lined with flattened epithelium with flat nuclei (arrows) and lumens have no secretion (L). Fibromuscular stroma is seen in-between acini and contains congested blood vessels (BV) (H&E, x400- Scale bar 30 µm). (2e): The recovery group showing acini (a) are lined by cuboidal epithelium (pointed arrow) and contain luminal secretion (S). Other acini (a) have flattened epithelium (Thick arrows) with no secretions (L) and others are atrophied (Thin arrows). Stroma in-between acini is relatively wide (asterisk). (H&E, x100- Scale bar 20 µm). (2f): The recovery group showing acinus (a) is lined by secretory cuboidal epithelium with rounded nuclei (pointed arrow) and the lumen filled with prostatic secretion (S). other acini (aa) are lined by flattened epithelium with flat nuclei (Thick arrows) and the lumens devoid of secretions (L). (H&E, x400 -Scale bar 30 µm)

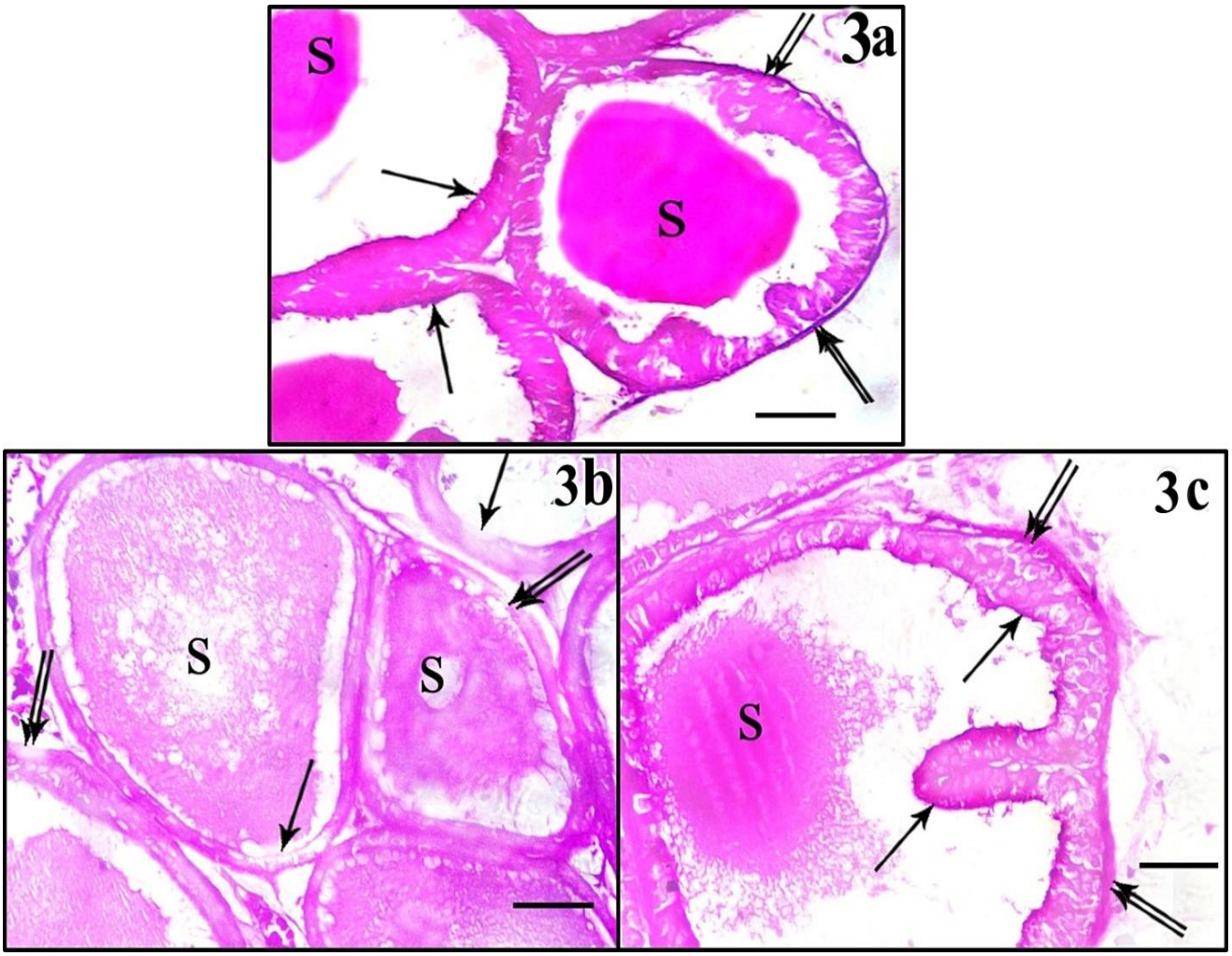

Figure 3: A photomicrograph of PAS reactions of sections in dorsolateral lobe of prostate of prepubertal albino rat: (3a): The control group showing strong PAS positive reactions in acinar basement membranes (double arrows), apical brush borders (arrows) and luminal secretions (S). (PAS x400 -Scale bar 30 µm) (3b): The treated group showing weak PAS-positive reactions in acinar basement membranes (double arrows), apical brush borders (arrows) and luminal secretions (S). (PAS x400 -Scale bar 30 µm) (3c): The recovery group showing strong PAS-positive reaction in acinar basement membranes (double arrows), apical brush border (arrows) and luminal secretion (S). (PAS x400-Scale bar 30 µm).

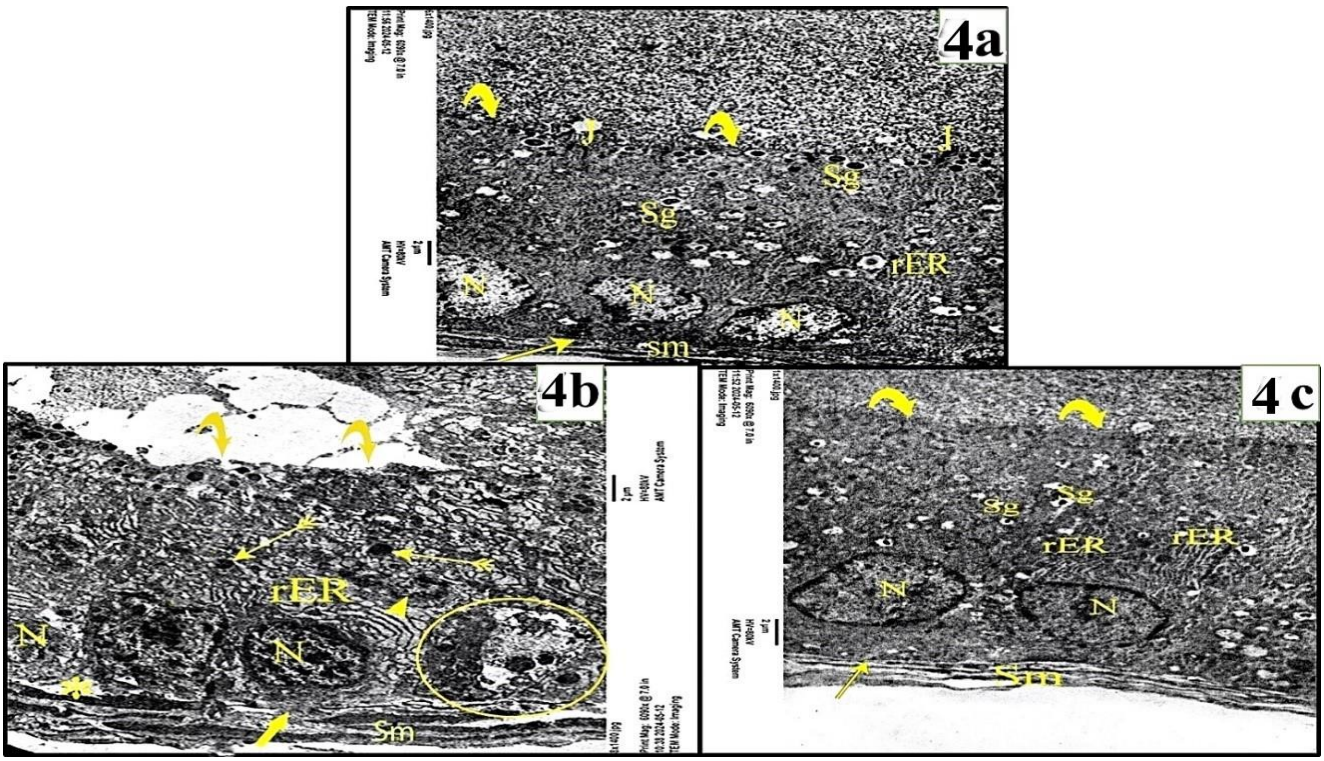

Figure 4: Transmission electron micrograph of ultrathin sections from dorsolateral prostatic lobe of prepubertal albino rat: (4a): The control group showing acinar cells with euchromatic nuclei (N) rest on regular basal lamina (arrow) that surrounded by smooth muscle cell (sm). The cytoplasm contains rough endoplasmic reticulum (rER) and secretory granules of variable electron densities (Sg). The acinar cells have apical microvilli (curved arrows) and apical cellular junctions are also noticed (J). (Orig. Mag. X6090) (4b): The treated group showing acinar cells with heterochromatic nuclei (N) and rest on irregular basal lamina with focal interruption (arrow) surrounded by several layers of smooth muscle cells (Sm). The cytoplasm contains dilated cisterna of rough endoplasmic reticulum (rER) and electron dense bodies (tailed arrows). Acinar cells have disrupted microvilli (curved arrows) and separation between them and basal lamina (asterisk) is noticed. Residual body (circle) and some disintegrated cells with necrotic nuclei (arrowhead) can be seen also. (Orig. Mag. X6090).(4c): The recovery group showing acinar cells with euchromatic nuclei (N) rest on regular basal lamina (arrow) that surrounded by smooth muscle cells (Sm). The cytoplasm contains rough endoplasmic reticulum with dilated cisterna (rER) and secretory granules of variable electron densities (Sg). The acinar cells exhibit apical microvilli are also seen (curved arrows). (Orig. Mag. X6090).

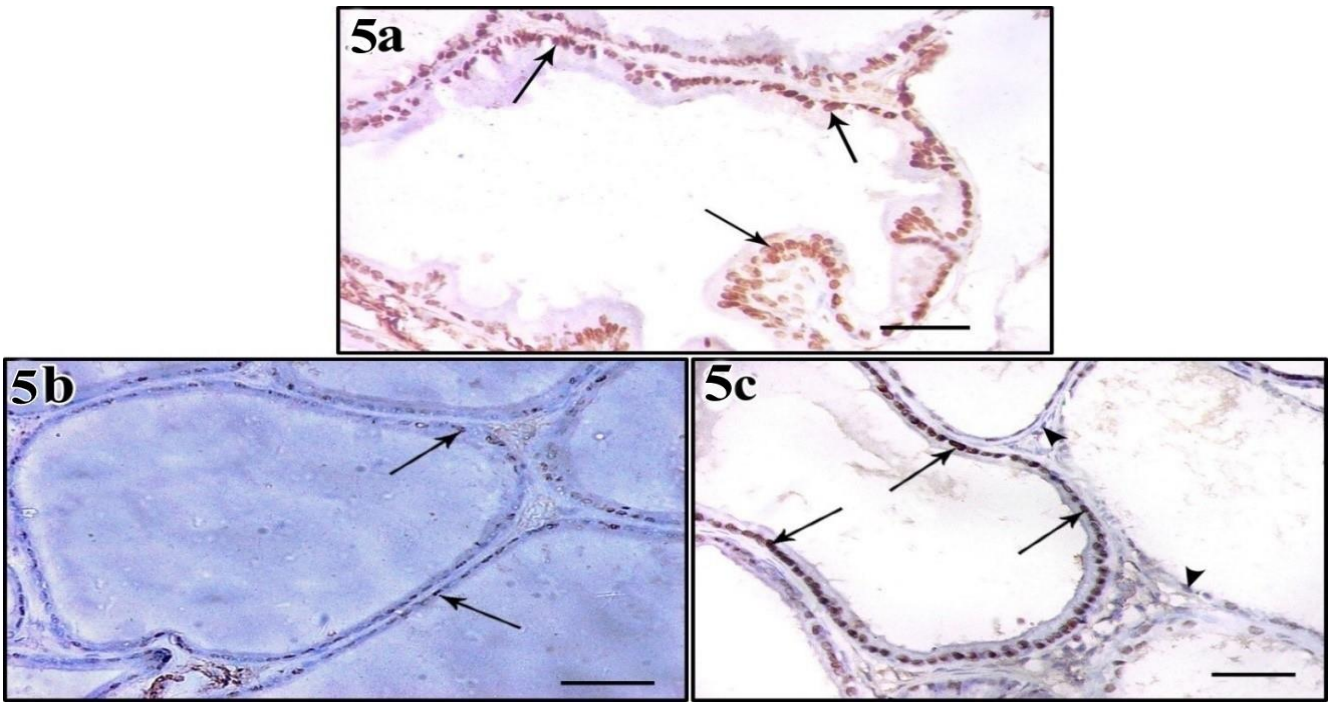

Figure 5: A photomicrograph of immunoperoxidase technique for ARs in dorsolateral lobe of prostate of prepubertal albino rat:(5a): The control group showing a strong positive immunoexpression in nuclei of acinar cells (arrows). (Immunoperoxidase technique for ARs, x400 Scale bar 30 µm). (5b): The treated group showing a negative immunoexpression in nuclei of acinar cells (arrows). (Immunoperoxidase technique for ARs, x400 Scale bar 30 µm) (5c): The recovery group showing positive immunoexpression in nuclei of acinar cells (arrows) but some cells have a negative nuclear immunoexpression (arrowheads). (Immunoperoxidase technique for ARs, x400 Scale bar 30 µm).

DISCUSSION

Anabolic androgenic steroids (AAS) are synthetic derivatives of the male sex hormone testosterone [20]. Medical professionals prescribe AAS to treat conditions like breast cancer, libido dysfunction, male primary and secondary hypogonadism, and chronic kidney disease [21]. Many athletes, bodybuilders, and others who want to look better use these substances because they mimic the anabolic (muscle-building) and androgenic (masculinizing) effects of testosterone [22]. According to Patanè et al. [3], stated that one of the most widely AAS in the world is nandrolone decanoate. This research aimed to examine the impact of nandrolone decanoate on the posterolateral lobe of the prostate in prepubertal albino rats, as well as to evaluate the possible reversibility of these effects following the discontinuation of the treatment.

In the present work, hematoxylin and eosin-stained sections from the treated group revealed that prostatic acini were lined by flattened cells with flat nuclei and poorly infolded epithelium .Acinar lumina devoided from secretions and some atrophied acini were seen. This finding was confirmed by morphometric statistical analysis, which showed a highly significant decrease in acinar epithelial heights in comparison with control group. These findings were in keeping with Ramzan et al. [23] and Pinto et al. [24] who found that folds of prostatic epithelium were decreased concomitantly with epithelial heights due to a reduction in testosterone levels. They mentioned that since prostatic epithelial height is known to be androgen-dependent, atrophic alterations such as flattening of the epithelium, atrophy, and decreased secretion may occur when plasma testosterone levels fall.

Statistical analysis of the biochemical study showed a highly significant reduction in testosterone levels in the same group. These results were in accordance with Pan and Kovac [25] and Jannatifar et al. [26] who found that animal studies have demonstrated that nandrolone reduces endogenous testosterone levels, which is thought to be a consequence of a negative feedback loop that inhibits the hypothalamic-pituitary-gonadal (HPG) axis. According to their findings, nandrolone lowers testosterone levels by acting as an androgen receptor agonist; since it is not converted to

dihydrotestosterone endogenously. It functions as negative feedback to the HPG axis. According to Ahmed [27], Jannatifar et al. [26] and Salerno et al. [28], the primary source of testosterone in male testes is the Leydig cell, which is markedly affected by the administration of nandrolone. This has been linked to a decrease in testosterone levels as testosterone is mainly secreted by Leydig cells.

Flattening of epithelium and atrophic acini were also caused by oxidative stress induced by nandrolone decanoate, as mentioned by El-Mesalmy et al. [29] and Memudu and Dongo [30]. In the current study, the occurrence of oxidative stress is confirmed by tissue MDA enzymes levels, which revealed a highly significant increase among the treated group. This finding was also supported by Ibrahim and Khafaga [31], who reported that oxidative stress and damaged proteins and lipids are linked to elevated MDA levels. The rise in MDA is a reflection of the rise in reactive oxygen species generation, inflammatory and cytotoxic processes that follow.

Saddick [11] found that elevated levels of MDA following nandrolone administration induced by oxidative stress because nandrolone is a lipid derivative, and its administration raises the lipid peroxidation products. Agaga et al. [32] stated that oxidative stress triggers producing inflammatory mediators, which explains why the fibromuscular stroma contained congested blood vessels and inflammatory cells that found in current study.

Biochemical results revealed a highly significant decrease was found in PSA level in the treated group than control group. This result contributes to the theory that the prostatic secretory function was decreased due to epithelium flattening and atrophy. This hypothesis was supported by Asare et al. [33] and Dhurvey et al. [34], who stated that degenerative changes, epithelium flattening, and atrophy result in reduced PSA secretion and complete absence of prostatic glandular secretion.

When comparing the treated and control groups' morphometric optical densities, a statistically significant decrease was found. Both Youssef and Mohamed [35] and Salman et al. [36] suggested that a decrease in the activity of the enzymes involved in glycogen storage and a loss in glycogen content as a result of the breakdown of glycogen molecules into glucose could be the cause of the lowering of the PAS-positive reaction. Memudu and Dongo [30] stated that nandrolone deconate causes a weak PAS reaction in the basement membrane and secretions due to the disruption of epithelial cells, which affects the function of the gland.

When comparing the treated group to the control group, immunohistochemical results revealed statistically a highly significant decrease in ARs immunoexpression in nuclei of acinar cells. Shathly et al. [37] explained that the lack of AR immunostaining was caused by lowering plasma testosterone concentrations because androgens are necessary for the proliferation of epithelial cells, the regulation and secretion of protein synthesis, and they also influence the expression of the AR in the acinar epithelial cells.

Ultrathin sections examination in treated group displayed acinar cells separated from the underlying basal lamina and rested on an irregular basal lamina. The cells also featured disrupted microvilli and a dilated rough endoplasmic reticulum cisterna. According to Sonpol et al. [38], nandrolone decanoate administration resulted in degenerative alterations that caused cells to separate from their basement membrane. The sloughing of these cells was attributed by Mahmoud et al. [39] to alterations in the expression of intercellular adhesion molecules. Treated group revealed acinar cells with heterochromatic nuclei. The cytoplasm contained dilated cisterna of rough endoplasmic reticulum, residual bodies and some disintegrated cells with necrotic nuclei. The cells also had disrupted microvilli. Mohamed and Rateb [40] revealed that the appearance of heterochromatic nuclei and cellular degeneration may result from loss of key proteins involved in homeostasis or apoptosis inhibitor synthesis (such as Bcl-2) that is disrupted within the enlarged cisternae. Hasanluyi et al. [41] stated that dilated rER, few and empty apical secretory vesicles, and disrupted microvilli were a manifestation of the gland's hypofunction.

Concerning the recovery group in the present study, the improvement of prostatic acinar epithelial heights was confirmed statistically non-significant difference was found between the control and recovery groups. Also acini revealed nearly normal architectures. They were lined by simple cuboidal epithelium with rounded nuclei and apical acidophilic brush borders were also noticed. These results were in line with Shalaby and Bahey [42]. In contrast, Simao et al. [43] disagreed with these results and stated that the treatment with nandrolone doses promoted permanent tissue alterations leaving the animals unable to restore their reproductive cycle, even after a long period of treatment discontinuation.

The same group showed non statistically significant difference between the control and recovery groups

in serum testosterone levels. These results were in line with Shalaby and Bahey [42] who found an improvement of biochemical alteration after four weeks of nandrolone withdrawal.

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There was a statistically significant increase in tissue MDA levels in the recovery group than the control group. According to Omobowale et al. [44], the cells in this group were unable to recover from the damage caused by free radical formation and oxidative stress, which is why oxidative stress indicators stayed elevated in comparison to control group.

Concerning periodic acid-Schiff (PAS) stained sections, a strong reaction was in acinar basement membranes, apical brush borders, and luminal secretions. These results were confirmed by morphmetrical statistical analysis. Memudu et al. [46] stated that recovering PAS reactions was due to restoring carbohydrate granules.

Immunohistochemical results showed that most nuclei of acinar cells exhibited positive ARs immunoexpression, and only a few nuclei revealed negative immunoexpression. This result agreed with Simão et al. [47], who stated that following the 30-day nandrolone recovery period, the androgen receptor (AR) was prominently visible in cell nuclei. However, Andrade et al. [48] disagreed with this result, stating that the nuclei of the epithelial cells showed decreased AR expression following a recovery period.

The examination of ultrathin sections of the same group revealed rER with dilated cisterna in the cytoplasm of acinar cells. Zaghloul et al. [49] classified it as a stress situation known as "ER stress," which triggers a tightly controlled program called an unfolded protein response (UPR). The main goal of this program is to restore the physiological functioning of this organelle and restore normal ER function. Hamed et al. [50] stated there was an improvement, but full recovery is still out of reach.

Conclusions:

Nandrolone decanoate harms the prostates of prepubertal rats due to its negative effects on the histological structure as well as functions which probably affects the fertility. These histological changes partially alleviated by arresting nandrolone decanoate intake. So**,** we recommend the use of nandrolone decanoate should be limited except for medical purposes. Also, Further studies needed to study the effect of nandrolone decanoate on different ages and to detect safe doses at these different ages.

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