Original Article

Histological and Immunohistochemical Studies of the Effects of Administration of Anabolic Androgenic Steroids Alone and in Concomitant with Training Exercise on the Adult Male Rats Skeletal Muscles

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ABSTRACT

Background: Anabolic androgenic steroids (AAS) abuse is considered as a public issue as a result of their widespread use to enhance muscular building.

Aim of the Work: The aim of the study is to clarify the hazards effects that occurred in the skeletal muscles of adult male albino rats as a result of administration of Nandrolone Decanoate histologically as well as immunohistochemically and the possible role of the interaction between (AAS) and training exercise.

Materials and Methods: Forty adult male albino rats were divided randomly into four groups; group I (control group), group II (steroids treated group), group III (trained group) and group IV (steroid treated-trained group) . Both groups II and IV were administered 5 mg/kg body weight, of Nandrolone Decanoate twice a week by intramuscular injection for 5weeks. Group III and Group IV were subjected to training protocol in the form of 4 sets of 10 jumps into water for 5 weeks. At the end of the experiments the rats were sacrificed and their quadriceps muscles were processed for histological and immunohistochemically procedures.

Results: Group II (steroid treated group) had hypertrophy of muscle fibers and disrupted striations with wide spacing between them. In addition to areas of degeneration and congested blood vessels. Group III had hypertrophy of muscle fibers. Group IV exhibited normal histological appearance of the muscle fibers. Minute areas of focal degeneration and congested blood vessels were observed.

Conclusion: Administration of (AAS) was presented with noticeable degenerative changes in the quadriceps muscles of the adult male albino rats . These hazards effects could be attenuated in the group of rats that were subjected to (AAS) in concomitant with training exercise.

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INTRODUCTION

Anabolic androgenic steroids (AAS) that are considered as one of the synthetic testosterone derivatives, are widely used as therapeutic agents for the treatment of numerous chronic catabolic disorders[1]. AAS as a structural derivatives of testosterone, they had been programmed to enhance anabolic effects; building of the muscles[2]. Nowadays the abuse of (AAS) is considered as a public issue as a result of their widespread use at fitness centers[3].

The action of (AAS) are displayed through the induction of protein synthesis and the suppression of its breakdown[4]. Usage of (AAS) over long period and in a high dose has been associated with masculinization[5].

When (AAS) has been applied in a therapeutic dose, it displayed beneficial results. However their benefits has been overshadowed as a result of the consequence abuse of (AAS)[6]. Nowadays the abuse of (AAS) has attracted considerable attention and has been blamed for the majority of the numerous hazardous effects, among them the most popular were atherosclerosis, hypertension, endocrine disturbance, hepatic and renal toxicity[7]. Furthermore, the misuse of (AAS) has been associated with neurotoxic effects in the form of behavioral disorders[6]. Over the past ten years the number of adolescents used (AAS) that had been recorded grew greatly[8].

The aim of the current study is to clarify the hazards effects that occurred in the skeletal muscles of adult male albino rats as a results of administration of Nandrolone Decanoate (one of AAS) histologically as well as immunohistochemically and the possible role of the interaction between (AAS) and training exercise.

MATERIALS AND METHODS

Chemicals and drugs

In this study, the anabolic androgenic steroid (AAS); Nandrolone Decanoate (Decadurabolin; Organon, Roseland, USA) was used. Nandrolone decanoate is

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known commercially as Deca-Durabolin. It is available in the form of ampoules and each ampoule contains 25 mg of Nandrolone Decanoate. The ampoule was dissolved in 10ml of sesame oil and then injected intramuscularly to rats in a dose of 5 mg/kg body weight[9]. In this study the intramuscular injection was chosen as a route of administration as this is the most common method of administration used by athletes[10].

The primary antibodies used in this study were anti-PCNA (Proliferating cell nuclear antigen) (Zymed, San Francisco, California, USA) and Bcl2 (Thermo scientific company, USA).

Experimental design

Forty adult male Wistar albino rats (4 months of age), weighing 160-200 g were obtained from Animal House, Faculty of Medicine, Assuit University and used in this work. The rats were housed in clean cages with bedding of fine woods with 12:12 hr light: dark cycle. Food and water were available ad libitum. The rats were divided randomly into four groups (ten rats per group); group I (control group), group II (steroids treated group), group III (trained group) and group IV (steroid treated-trained group). Both groups II and IV were administered 5 mg/kg body weight, of Nandrolone Decanoate as an anabolic androgenic steroid (AAS) twice a week by means of intramuscular injection into the hip region for five weeks[11]. As regard groups I and III, they received similar volume of the calculated dose of nandrolone decanoate that were formed of mixture of benzyl alcohol and peanut oil[11].

This experiment was done in accordance with the guidelines of animal ethics committee, that were accomplished with the internationally accepted principles for laboratory animal's use and care.

Training Protocol

The training protocol of this experiment was done according to Cunha *et al*., 2005[11]. The rats of group III (trained group) and group IV (steroid treated-trained group) were allowed to swim in a tank contained water for 30 min/day during the first week of the experiment. The dimensions of this tank were 60 cm width, 75 cm length and 80 cm depth. This maneuver was done for the adaptation of the experiment environment. During the second to the sixth week, the exercise training was performed as follow; the animals' exercise training included 4 sets of 10 jumps from the tank bottom to its surface with about thirty seconds recovery times in between the sets. This exercise training was done one time per day for five days. For the augmentation of the intensity of the exercise, an additional external load was used during the second week. The rate were allowed to carry extra load, about 50% body weight tied to the chest of the animals. During the third and fourth weeks the same regimes was used but the extra load was about 60% body weight. During the fifth and sixth weeks the allowed extra load was about 70% of body weight.

Histological and immunohistochemically studies

At the end of the experiments the rats of the different groups were sacrificed under inhalation of ether. The quadriceps muscles of both sides were dissected to be processed for histological and immunohistochemically procedures.

Light microscopic study

For the light microscopic study, specimens of quadriceps muscles were processed to paraffin wax and sections of about 6um thickness were obtained for the histological study by using hematoxylin and eosin[12].

Transmission electron microscopic study

The quadriceps muscles' specimens of different groups were processed for ultrastructural study. Immediately the specimens were fixed in a solution of phosphate buffered glutaraldehyde (2.5%). The specimens were then dehydrated by means of ascending grades of alcohol (30, 50, 70, 90 and 100% for 120 minutes). Then they were embedded in epon araldite mixture. Semithin sections (0.5μm thickness) were prepared and stained by toluidine blue. Ultrathin sections (80-90nm) from the chosen areas were cut using ultra-microtome and then stained by lead citrate and uranyl acetate[13]. Finally the sections were examined and photographed by transmission electron microscope (Joel- JEM- 100 CXII; Joel, Tokyo, Japan) in the Assiut Electron Microscopic Unit.

Immunohistochemically study

The tissues sections were dewaxed and then treated with 10% hydrogen peroxide for the reduction of the endogenous peroxidase. The sections were then boiled in10 mM citrate buffer for about 25 minutes. The muscle sections were processed to be incubated overnight with the 1ry antibodies: Bcl2 (a mouse monoclonal antibody; clone c-2). Following this, the sections were taken to be incubated with the 2nd antibody (biotinylated goat anti-rabbit immunoglobulin) and followed by streptavidin–biotin complex. The site of the reaction could be visualized by adding diaminobenzidine HCl, that was converted into brown precipitate by peroxidase. Mayer's hematoxylin was used to stain the slides[14].

As regard the Proliferating cell nuclear antigen (PCNA) immunoreactivity, the muscle sections were deparaffinized and washed in in phosphate-buffered saline (PBS). To suppress the peroxidase activity, 0.3% H_2O_2 in methanol was used for 30 minutes. Following this, the sections were washed by PBS and incubated with blocking solution for about ten minutes. Subsequently, the sections were incubated with the anti-PCNA primary antibody 60 minutes and then were further incubated with streptavidin peroxidase for about ten minutes. Finally, the sections were counterstained with hematoxylin for one minute to be examined[15].

Morphometric procedure and statistical analysis

The thickness of the quadriceps muscle fibers was measured in the different studied groups. Sections stained by toluidine blue (X1000) were used. Five non overlapping fields were chosen from each slide to be measured. CX41 optical microscope (Olympus, USA) was used. The used microscope was equipped by Olympus digital camera that was attached to a computer. The measured data were presented as mean ± standard deviation (SD). Statistical analysis was achieved via SPSS software, version 13.00 (Chicago, Illinois, USA).The results of the different groups were compared by the aid of One-way analysis of variance and a post-hoc least significant difference test. $P \leq 0.05$ was considered significant.

RESULTS

Histological Results

Group I (control group)

Light microscopic examination of a longitudinal section of quadriceps muscles stained with H&E displayed the appearance of regularly arranged well organized parallel muscle fibers with multiple peripheral oval vesicular nuclei just beneath the sarcolemma. Evident transversely striated pattern of muscle fibers was noticed. The flat nuclei of fibroblast were found in the endomysium between the muscle fibers (Figure 1A). Sections stained with toluidine blue showed regularly arranged muscle fibers with well-defined transverse striations. The nuclei were oval, vesicular and peripherally situated. In addition flat nuclei of the spindle shaped cells in the interstitium could be observed (Figure 2A)

Electron microscopic examination of a longitudinal section in the quadriceps muscle revealed regular arrangement of myofibrils forming sarcomere with alternative dark (A) and light (I) bands bisected by Z lines. A peripheral oval euchromatic nucleus beneath the sarcolemma and sarcoplasmic reticulum existed in the region of I band. Around Z lines, paired mitochondria were arranged between myofibrils. In addition, some mitochondria also presented at the periphery of the fibers. Multiple glycogen granules arranged in rows between the myofibrils (Figure 3A).

Group I showed strong positive Bcl2 immunoreactivity that was demonstrated by diffuse cytoplasmic staining in the myocytes (Figure 5A). Negative immunohistochemically stained sections for PCNA could be noticed in this group (Figure 6A) .

Group II (steroid treated group) H&E stained sections declared apparent hypertrophy of muscle fibers and disrupted striations with wide spacing between them. Many large vacuoles replaced and interrupted the continuity of the skeletal muscle fibers. Furthermore areas of complete degeneration, densely stained nuclei and congested blood vessels (C) were noticed (Figures 1B and 1C). Sections stained with toluidine blue presented with apparent hypertrophy of skeletal muscle fibers which appeared disrupted with loss of transverse striations. Areas of degeneration and wide spacing between muscle fibers could be noticed . Centrally located deeply stained nuclei were noticed among the disorganized muscle fibers with disrupted transverse striations. Dilated congested blood vessels were presented (Figures 2B,2C).

Electron microscopic examination of this group showed wide spaces between myofibrils with localized areas of myofibrillar loss, disruption of Z lines and mitochondria with destroyed cristae. The nuclei had irregular outline and peripheral chromatin condensation. Some myofibrils appeared with fragmentation and increased intermyofibrillar space. Others showed disorganization, lysis and disruption of Z lines. Congested blood vessels, Collagen fibrils and swollen mitochondria were also observed (Figures 3B,3C,3D).

Faint cytoplasmic immunoreactivity for Bcl2 antibody in the myocytes of group II was obvious (Figures 5B) . Numerous diffuse brown- positive immunoreaction for PCNA were obvious in this group (Figure 6B).

Group III (trained group) H&E stained section of group III had apparent hypertrophy of muscle fibers which appeared parallel to each other. The muscle fibers were well organized with distinct striations. Peripheral vesicular nuclei with normal appearance formed nuclear chain. Frequent dilated congested blood vessels and extravasated RBCs between muscle fibers were noticed (Figure 1D). Sections stained with toluidine blue had well organized parallel muscle fibers with evident transverse striations. Apparent hypertrophy of muscle fibers might be observed. Flat peripheral oval vesicular nuclei and congested blood vessels were noticed (Figure 2D).

Ultrastructural examination of this group showed parallel arrangement of myofibrils with dark (A) and light (I) bands and sarcoplasmic reticulum. The mitochondria were arranged in pairs around the Z line and at the periphery of the fibers . Multiple glycogen granules and dilated congested blood vessels were noticed (Figure 4A).

Group III displayed positive Bcl2 immunoreactivity (Figure 5C). This group appeared with no expression of PCNA (Figure 6C).

Group IV (steroid treated-trained group) Muscle sections stained by H&E exhibited normal histological appearance of the muscle fibers. The muscle fibers were more or less similar to that of the control group. Partial splitting of some fibers and apparently normal peripheral vesicular nuclei could be noticed. Some muscle fibers appeared with apparent hypertrophy with nearly normal transverse striations (Figure 1E). Sections stained with toluidine blue of group IV showed nearly normal muscle fibers which appeared regularly arranged with distinct transverse striations and peripheral elongated oval vesicular nuclei. Minute areas of focal degeneration and congested blood vessels were observed (Figure 2E).

Electron microscopic examination of the muscle fibers of this group had relatively normal pattern and arrangement of myofibrils with light and dark bands and clear successive Z lines and decreased intermyofibrillar space. Focal areas of degenerated myofibrils were noticed. Flattened elongated oval euchromatic nucleus under the sarcolemma were present. Nearly normal mitochondria were observed but few swollen mitochondria at the periphery of the fibers and also few electron dense mitochondria in between the myofibrils could be noticed. In addition Satellite cell with euchromatic nucleus and collagen fibrils were obvious (Figures 4B,4C).

Immunoreactivity for bcl2 antibody appeared as mild to moderate cytoplasmic reaction (Figure 5D). Few positive nuclear immunoreactions for PCNA as regard this group was present (Figure 6D).

Morphometric and statistical results

The means thickness of the quadriceps muscles were presented with significant differences (P<0.05) between group I (control group) and the other groups; group II (treated group), group III (trained group) and group IV (steroid treated-trained group). Non-significant difference (P>0.05) was observed when compared group II (steroid treated group) and group IV (steroid treated-trained group) (Table 1, Histogram 1).

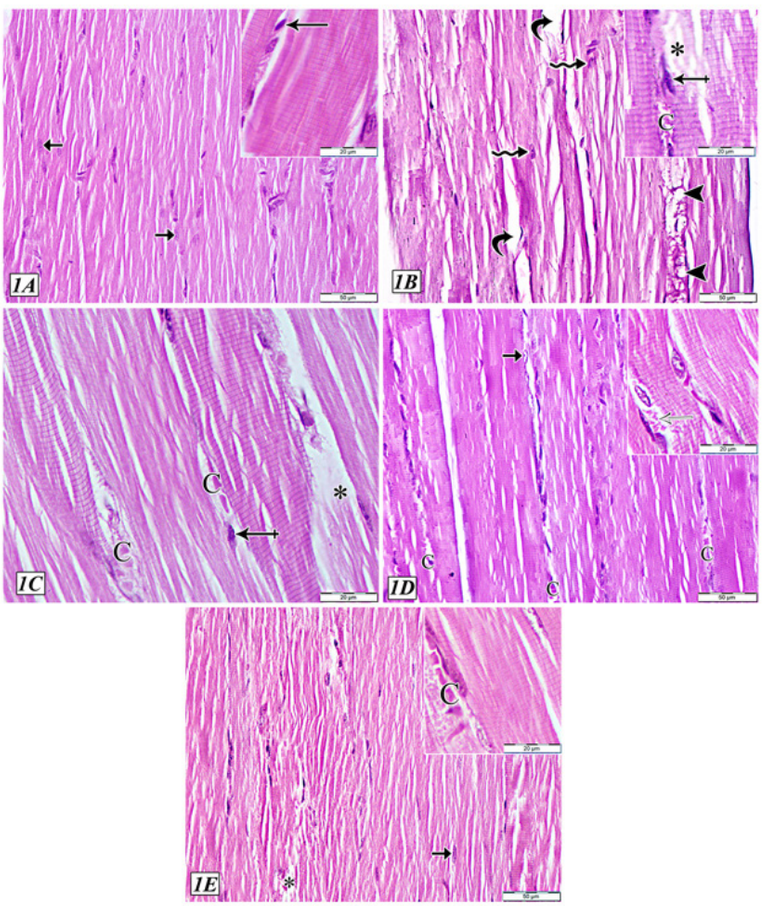


Fig. 1: A photomicrograph of a longitudinal section of quadriceps muscle .1A: group I showing regularly arranged well organized parallel muscle fibers with multiple peripheral oval vesicular nuclei just beneath the sarcolemma (short arrows). Inset: Higher magnification showing evident transversely striated pattern of muscle fibers . The flat nuclei of fibroblast (arrow) are noticed in the endomysium between the muscle fibers. H&E; X400, 1000. 1B: group II showing apparent hypertrophy of muscle fibers and disrupted striations with wide spacing between them (curved arrows). Note many large vacuoles (arrow heads) replace and interrupt the continuity of the skeletal muscle fibers . Centrally located nuclei are observed (wavy arrows). Inset: Higher magnification showing areas of complete degeneration (asterisk). Densely stained nuclei (crossed arrow) and congested blood vessels (C) are noticed. H&E; X400, 1000. 1C : group II showing disrupted striations of muscle fibers with areas of complete degeneration (asterisk). Note densely stained nuclei (crossed arrow) and congested blood vessels (C). H&E; X400. 1D: group III showing apparent hypertrophy of muscle fibers which appear parallel to each other. Note normal appearance of peripheral vesicular nuclei forming nuclear chain (short arrow). Frequent dilated congested blood vessels (C) are noticed. Inset: Higher magnification showing well organized muscle fibers with distinct striations. Note extravasated RBCs between muscle fibers (arrow). H&E; X400, 1000. 1E: group IV showing muscle fibers more or less similar to that of the control group, associated with partial splitting of some fibers (asterisk). Note apparently normal peripheral vesicular nuclei (short arrow). Inset: Showing nearly normal transverse striations with apparent hypertrophy of muscle fibers. Note vascular congestion (C). H&E; X400, 1000.

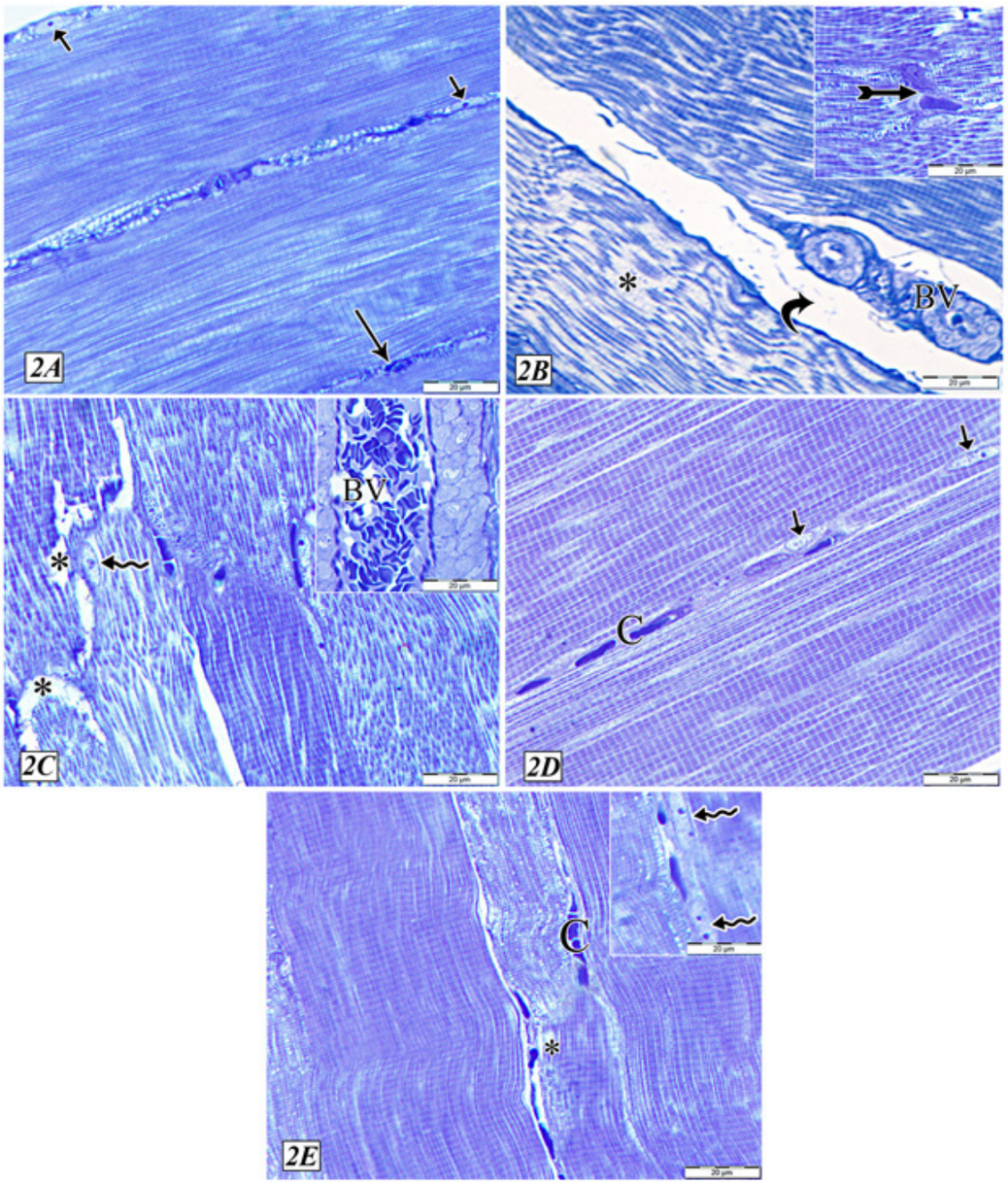


Fig. 2: A photomicrograph of a longitudinal section of quadriceps muscle. 2A: group I showing regularly arranged muscle fibers with well-defined transverse striations. The nuclei are oval, vesicular and peripherally situated (short arrows). Note flat nuclei of the spindle shaped cells in the interstitium (arrow). Toluidine Blue; X1000. 2B: group II showing apparent hypertrophy of skeletal muscle fibers which appear disrupted with loss of transverse striations. Note areas of degeneration (asterisk) and wide spacing between muscle fibers (curved arrow). Dilated congested blood vessels (BV) are observed. Inset: showing centrally located deeply stained nuclei (tailed arrow) among disorganized muscle fibers with disrupted transverse striations. Toluidine Blue; X1000. 2C: group II showing disruption of striation of muscle fibers with focal areas of degeneration (asterisks). Centrally located nuclei can be seen (wavy arrow). Inset: showing dilated congested blood vessels (BV).Toluidine Blue; X1000. 2D: group III showing well organized parallel muscle fibers with evident transverse striations. Note apparent hypertrophy of muscle fibers. Flat peripheral oval vesicular nuclei (short arrows) and congested blood vessels are observed (C). Toluidine Blue; X1000. 2E: group IV showing nearly normal muscle fibers which appear regularly arranged with distinct transverse striations. Note minute areas of focal degeneration (asterisk) and congested blood vessels (C). Inset: showing peripheral elongated oval vesicular nuclei (wavy arrows). Toluidine Blue; X1000.

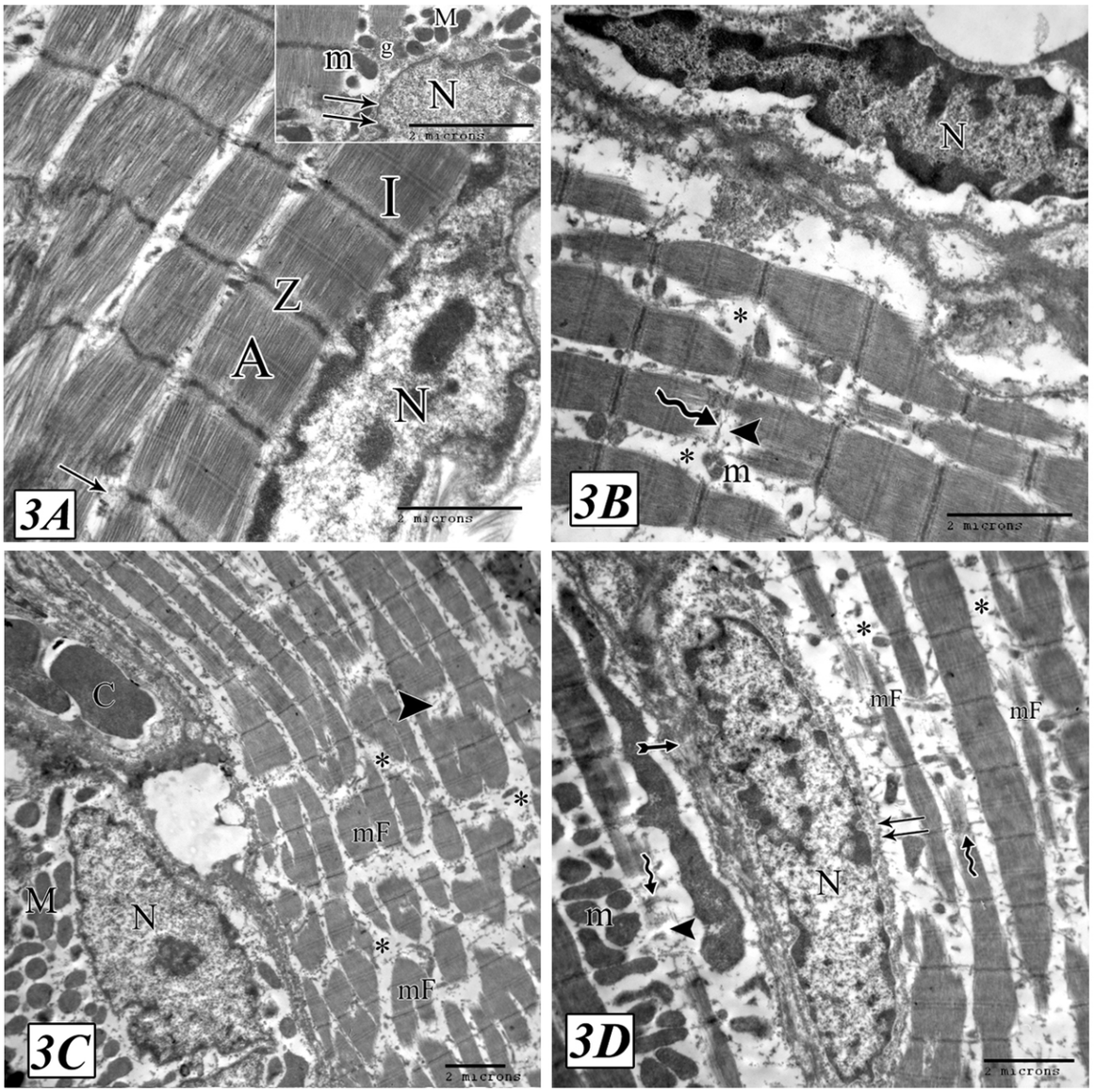


Fig. 3: An electronmicrograph of a longitudinal section in the quadriceps muscle . 3A: group I showing regular arrangement of myofibrils forming sarcomere with alternative dark (A) and light (I) bands bisected by Z lines (Z). Note a peripheral oval euchromatic nucleus (N) beneath the sarcolemma and sarcoplasmic reticulum (arrow) exist in the region of I band. Inset: showing paired mitochondria (m) arranged around Z lines between myofibrils. Mitochondria (M) also present at the periphery of the fibers. There is multiple glycogen granules (g) arranged in rows between the myofibrils. Note the nucleus (N) with indentation of the nuclear envelope (double arrow).TEM; X 10,000. 3B: group II showing wide spaces between myofibrils (asterisks) with localized areas of myofibrillar loss (arrow head), disruption of Z lines (wavy arrow) and mitochondria with destroyed cristae (m). Note the nucleus (N) with irregular outline and peripheral chromatin condensation. TEM; X 10,000. 3C: group II showing fragmentation of myofibrils (mF) with increased intermyofibrillar space (asterisks) and localized areas of myofibrillar loss (arrow head). Congested blood vessels (C) and swollen mitochondria (M) are observed. Note the nucleus (N). TEM; X4800. 3D: group II showing disorganization and lysis of myofibrils (mF) and disruption of Z line (wavy arrows) with wide intermyofibrillar space (asterisks) and localized areas of myofibrillar loss (arrow head). Note ballooning of the mitochondria (m). Collagen fibrils (tailed arrow) and a hetrochromatic nucleus (N) with indentation of the nuclear envelope (double arrow) are observed. TEM; X7200

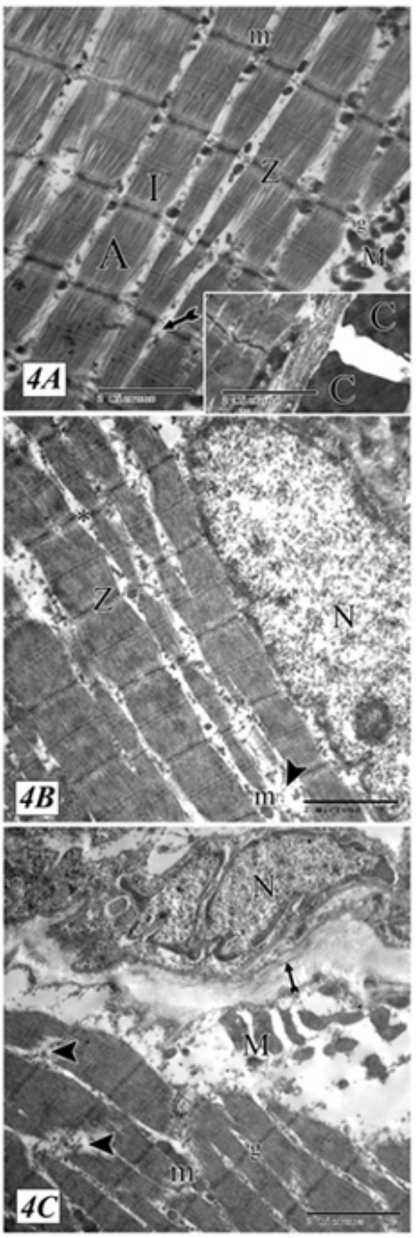


Fig. 4: An electronmicrograph of a longitudinal section in the quadriceps muscle. 4A: group III showing parallel arrangement of myofibrils with dark (A) and light (I) bands and sarcoplasmic reticulum (tailed arrow). The Z line (Z) is noticed. Note the arrangement of the mitochondria (m) in pairs around the Z line. There are mitochondria (M) at the periphery of the fibers and multiple glycogen granules (g). Inset: showing dilated congeśted blood vessels (C). TEM; X10,000. 4B: group IV showing relatively normal pattern and arrangement of myofibrils with light and dark bands and clear successive Z lines (Z) and decreased intermyofibrillar space (aśterisk). Focal areas of degenerated myofibrils are noticed (arrow head). Note few electron dense mitochondria (m) in between the myofibrils. Note the presence of flattened elongated oval euchromatic nucleus (N) under the sarcolemma. TEM; X10,000. 4C: group IV showing apparently normal arrangement of muscle fibers with glycogen granules (g) and minimal focal areas of degeneration (arrow heads). Nearly normal mitochondria (m) with few swollen mitochondria (M) at the periphery of the fibers are seen. Satellite cell contains euchromatic nucleus (N) is observed. Note collagen fibrils (tailed arrow) . TEM; X10,000

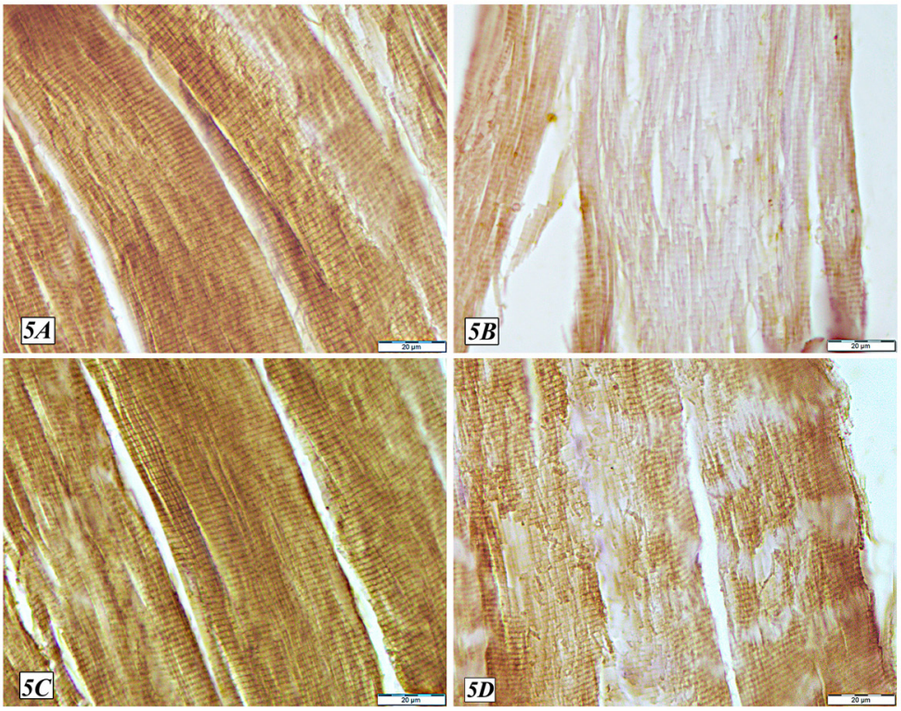


Fig. 5: A photomicrograph of a longitudinal section of quadriceps muscle. 5A: group I showing strong positive Bcl2 immunoreactivity demonstrated by diffuse cytoplasmic staining in the myocytes. Bcl2; X1000 . 5B: group II showing faint cytoplasmic immunoreactivity for Bcl2 antibody in the myocytes . Bcl2; X1000 .5C: group III showing positive Bcl2 immunoreactivity. Bcl2; X1000 .5D: group IV showing mild to moderate cytoplasmic immunoreactivity for bcl2 antibody. Bcl2; X1000

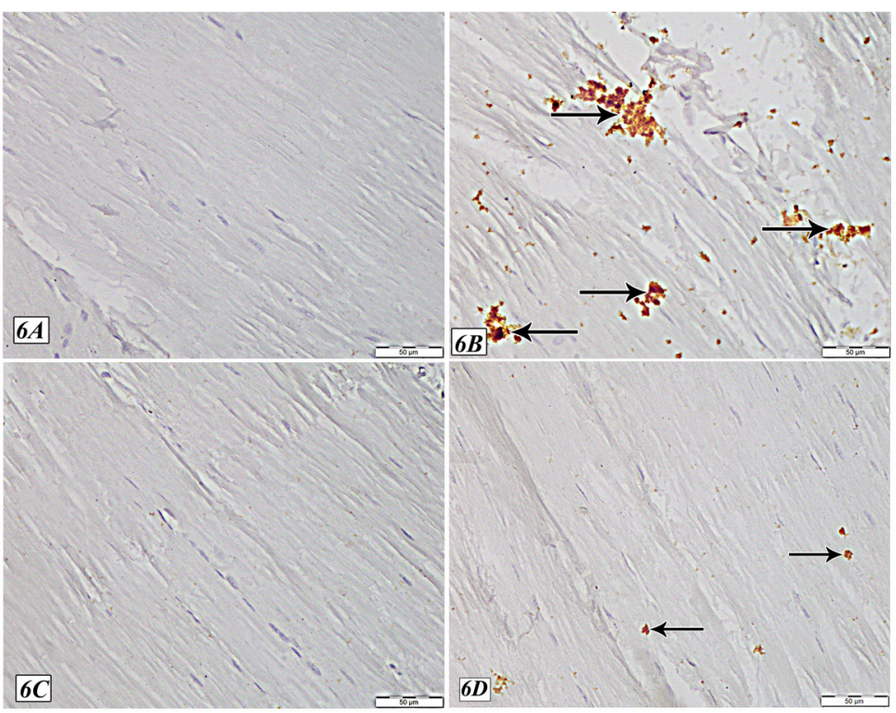
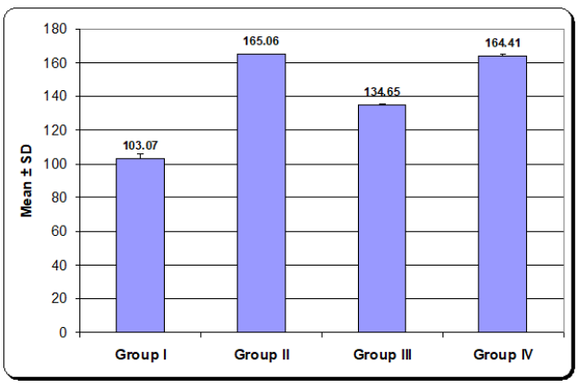


Fig. 6: A photomicrograph of a longitudinal section of quadriceps muscle. 6A: group I showing negative immunohistochemically stained sections for PCNA. PCNA; X400. 6B: group II showing numerous diffuse brown- positive immunoreaction for PCNA (arrows). PCNA; X400 . 6C: group III showing no expression of PCNA. PCNA; X400 . 6D: group IV showing a few positive nuclear immunoreactions for PCNA (arrows). PCNA; X400.

Table 1: Shows the mean thickness (µm) of the muscle fibers in the different groups

	Group I	Group II	Group III	Group IV	*P-value*[1]	*P-value*[2]	*P-value*[3]	*P-value*[4]
Mean ± SD	103.07±2.67	165.06±0.46	134.65±0.62	164.41±1.00	0.000*	0.000*	0.000*	0.223
Range	99.3-105.0	164.6-165.8	133.8-135.1	163.1-165.7				

*P-value*1: Comparison between Group I & Group II
*P-value*2: Comparison between Group I & Group III
*P-value*3: Comparison between Group I & Group IV
*P-value*4: Comparison between Group II & Group IV



Histogram 1: Shows the mean thickness (µm) of the muscle fibers in the different groups

DISCUSSION

The misuse of (AAS) has great attention as one of the worldwide health problem and their unprescribed usage has continued to massively increased especially among athletes and also among men in order to enhance the figure of their body. The interaction between the (AAS) and the exercise training and their resulting effects on the tissues adaptation and the body structure remains not fully understood and under investigation[16]. The aim of the current study is to clarify the hazards effects that occurred in the skeletal muscles of adult male albino rats as a results of administration of Nandrolone Decanoate (one of AAS) histologically as well as immunohistochemically and the possible role of the interaction between (AAS) and training exercise.

In this study Nandrolone Decanoate was chosen, as a previous study done by Wimalawansa *et al*.,1999[17] showed its anabolic effects as regard the rat skeletal muscles. In addition, Nandrolone Decanoate had been known by its long biological half-life[2]. Furthermore, Nandrolone Decanoate had been known to have great anabolic properties in comparison to its androgenic effects, so it was considered as the most frequently abused AAS[18]. It has been reported that the skeletal muscle fibers were established to be target tissues as regard the (AAS) anabolic effects and presented with increased number following exposure to (AAS)[19].

The current work focused on the adult male rat as an animal model to study as about 98% of AAS users were male[20]. Females rarely used such type of drugs as a result of their recorded androgenic effects; breast enlargement ,masculinization and deepening of voice[21].

Light microscopic examination of steroid treated rats (group II) declared apparent hypertrophy of muscle fibers and disrupted striations with wide spacing between them. Many large vacuoles replaced and interrupted the continuity of the skeletal muscle fibers. Furthermore areas of complete degeneration, densely stained nuclei and congested blood vessels were noticed. The results of the current works were in line with Elgendy *et al*., 2018[22]. They stated that following intramuscular injection of Nadrolone decanoate in a dose of 5 mg/kg body weight weekly for 8 weeks, muscular hypertrophy with widening of the spaces in between were observed. The results of the current work were also supported by Dela Cruz *et al*., 2012[23]. They added that (AAS) induced muscular hypertrophy was owing to the ability of the testosterone to enhance the myocytes to keep contractile protein with subsequent muscle growth. The results of the present work were in harmony with Sinha-Hikim *et al*., 2002[24]. They notice that the increase in the size and length of the muscle following administration of (AAS) were performed in a dose dependent manner. They explained that the muscular hypertrophy occurred as a result of increased differentiation of mesenchymal stem cells as well as increased myonuclear number.

It has been reported that 5-α-reductase played a major role in the metabolism of (AAS). This enzyme converted (AAS) to an active compound; dihydrotestosterone (androstnolone). The (AAS) or after conversion to dihydrotestosterone had the capacity to bind androgen receptors. The resulted steroid-receptor complex enhanced the synthesis of protein through interaction via DNA and RNA[25]. The molecular mechanism involved in the growth of the muscle fibers in response to (AAS) included the biological activity of the growth factor myostatin (MSTN); one of the members of the transforming growth factor-b family of proteins (TGF-ß) that was affected by (AAS)[26]. Mosler *et al*., 2012[27] added that exaggerated hypertrophy of the muscle was greatly related to (MSTN) gene mutation.

Ultrastructure study of group II (Steroid treated rats) showed wide spaces between myofibrils with localized areas of myofibrillar loss, disruption of Z lines and mitochondria with destroyed cristae. The nuclei had irregular outline and peripheral chromatin condensation. These observed degenerative changes were supported by the previous study done by Zelleroth *et al*., 2019[18] who reported increased cellular cytotoxicity and decreased cellular viability following (AAS) administration to mixed

cortical cultures taken from embryonic rats. They observed evidences of reduced mitochondrial activity and increased level of lactase dehydrogenase. It has been reported that (AAS) were capable of the induction of apoptosis in different tissue and organs[28]. The (AAS) induced cell death was mediated by two signaling pathway ; an intrinsic (mitochondrial pathway) and an extrinsic (death receptor pathway)[29].

Supraphysiological dose of Nandrolone Decanoate enhanced the production of tumor necrosis factor-α (TNF-α) and interleukin-1beta (IL-1β) in the human peripheral blood lymphocyte cultures. (TNF-α) was known as cytotoxic cytokine that are responsible for the initiation of cellular apoptosis by the disturbance of the mitochondrial membrane and the subsequent release of cytochrome C[30].

H &E stained section of group III (trained group) had apparent hypertrophy of muscle fibers which appeared parallel to each other. Peripheral vesicular nuclei with normal appearance formed nuclear chain were noticed. As regard the sections stained with toluidine blue, well organized parallel muscle fibers with evident transverse striations and apparent hypertrophy of muscle fibers might be observed. The results of the current study were supported by Dela Cruz *et al*., 2012[23] who studied the possible effects of resistance exercise on the skeletal muscles in adult male albino rats. They concluded that physical exercise could be associated with the behavior of the skeletal muscles with subsequent enlargement. Song *et al*., 2006[31] studied the effects of exercise training to suppress the apoptotic signaling in the rat skeletal muscle declared that regular training exercise presented with large muscle mass and also suppress the increased connective tissue associated with age.

Light and electron microscopic examination of the muscle specimens of the trained rat (Group III) were appeared with the presence of dilated and congested blood vessels. Similar to that Prior etal., 2004[32]; Kojda and Hambrecht, 2012[33] observed the intimate association between the vascular improvement of the muscles and the physical exercise. They stated that the molecular mechanism of the increased muscular vasculature was owing to VEGF (vascular endothelial growth factor). In the same line with that Wagatsuma *et al*., 2005[34] reported the elevated level of VEGF in the cardiac muscle of the rat after physical exercise. These results were also supported by Lloyd *et al*., 2003[35] who studied the behavior of the rat skeletal muscles in response to exercise training. They declared the occurrence of angiogenesis with the activation of angiopoietin and VEGF pathway in the rat skeletal muscle. They added that the VEGF/VEGF receptor mRNA abundance and changes in the ratio of angiopoietin 2 to angiopoietin 1 were recorded to precede the noticed angiogenesis.

Light microscopic examination of steroid treated-trained group (group IV) showed normal histological appearance of the muscle fibers. The muscle fibers were more or less similar to that of the control group. Partial splitting of some fibers and apparently normal peripheral vesicular nuclei could be noticed. As regard the muscle behavior in response to (AAS) administration, a questioner was present as regard the muscular hypertrophy in response to testosterone when given to non-exercising person and whether this effect could be augmented when testosterone was given in concomitant with exercise[36]. In the same line with this opinion, Mosler *et al*., 2012[27] reported that the molecular mechanism for the muscular growth in response to the (AAS) and training together was not fully understood. However, Filho *et al*., 2006[37] reported that the administration of (AAS) in concomitant with physical exercise might enhance the skeletal muscles power and their hypertrophy.

Previous researches demonstrated that administration of (AAS) could induced type I and type II muscular hypertrophy and increase muscle mass and strength[5,38]. The muscular behavior was intimately related to the redox state. Skeletal muscle produced reactive oxygen species (ROS) and in the exercising muscle the (ROS) production was greatly noticed. The produced (ROS) might alter the oxidant–antioxidant balance within the cells with the occurrence of muscle fatigue[39]. It has been reported that there was experimental evidence as regard the testosterone ability to regulate the antioxidant enzymes in the cells[40]. Delgado *et al*., 2010[41] stated that administration of (AAS) had the ability to enhance the antioxidant system within the skeletal muscle, however it was not able to enhance the training effects as regard this system.

Congested blood vessels were observed in the steroid treated-trained rats. In harmony with the result of the current work Kahal and Allem, 2018[42] declared the presence of congested renal blood vessels following (AAS) abuse to adult male mice.

In the present study, the histological results were correlated with the immunohistochemically results. Positive Bcl2 immunoreactivity was presented in group I (control group) and group III (trained group), while the steroid treated group (group II) exhibited Faint cytoplasmic immunoreactivity for Bcl2 antibody in the myocytes. Finally mild to moderate cytoplasmic reaction for Bcl2 antibody was noticed in group IV (steroid treated-trained group). Cellular apoptosis could be regulated through numerous pathways, one of the considerable critical pathway is the mitochondrial -mediated pathway including the Bcl2 family[43]. Bcl2 was a 26-KD a protein encoded by a gene that was associated with chromosomal translocation[44]. Iyer *et al*., 2019[45] explained the ability of Bcl2 to inhibit apoptosis through the binding between Bcl2 and proapoptotic proteins . In the same line with these results, Yi *et al*., 2003[46] reported that Bcl2 interacted to the nuclear membrane and mitochondria to maintain their integrity and therefore could control the opening of the mitochondrial permeability transition pore (MPTP) with subsequent inhibition of calcium transmembrane flow and so preventing apoptosis.

Previous researches had been focused on the important of the satellite cells as regard the skeletal muscle regeneration following injury[47]. They were represented as myogenic stem cells found in the adult skeletal muscles[48]. Under steady condition, the satellite cells were inactive, however in response to stressful condition, they activated, proliferated and fused with each other or the damaged muscle fibers to form new fibers, at this point they were known as muscle precursor cells[49]. The control mechanism of the muscle repair process was not fully understood, however disruption of the sarcolemma and basal lamina integrity could be a trigger factor for the activation of the satellite cells[48]. Proliferating cell nuclear antigen (PCNA) was known as an acidic nonhistone auxiliary protein of DNA polymerase and it could be detected during DNA synthesis[49]. It has been known as a marker of cellular proliferation including the satellite cells[48]. This could explain the results of the current study as regard immunoreactions for PCNA . The immunohistochemically stained sections for PCNA demonstrated Negative reaction in group I, Numerous diffuse brown-positive immunoreaction for PCNA in group II, no expression of PCNA in group III and group IV had few positive nuclear immunoreactions for PCNA.

CONCLUSION

In conclusion , administration of Nandrolone Decnoate as one of the (AAS) was presented with noticeable degenerative changes in the quadriceps muscles of the adult male albino rats. These hazards effects could be attenuated in the group of rats that were subjected to (AAS) in concomitant with physical exercise (steroid treated-trained group). As regard the trained group , apparent hypertrophy of muscle fibers was noticed without histological degenerative changes. So the desired benefits muscular effects of (AAS) could be achieved in the same way with exercise and without the associated (AAS) risk for health. Further researches are recommended, using variable doses of (AAS) and different protocol of physical exercise in order to fully understand the interaction between them to gain the wanted benefits with minimal health risks.

CONFLICT OF INTERESTS

There are no conflicts of interest.

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الملخص العربى

دراسات هستولوجية وهستوكيميائية للتأثيرات الناتجة عى اعطاء الاستيرويدات البنائية الاندرجينية منفردة ومتزامنة مع التمرين التدريبى على ا لعضلات الهيكليه لذكور الجرذان البيضاء البالغة

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المقدمة: الاستخدام السيء للاستيرويدات البنائية الاندرجينية يعتبرقضية عامة وذلك بسبب انتشاره الواسع لبناء العضلات.

الهدف من البحث: هذه الدراسه تهدف الى توضيح التأثيرات الضارة التى حدثت للعضلات الهيكليه لذكور الجرذان البيضاء البالغة نتيجه لاعطاء مادة (Nandrolone Decnoate) هستولوجيا وهستوكيميائبا والتأثير المحتمل للتفاعل بين التمرين التدريبى و الاستيرويدات البنائية الاندرجينية.

مواد وطرق البحث: تم تقسيم أربعين من ذكور الجرذان البيضاء البالغة عشوائيا إلى أربع مجموعات: المجموعة١ (المجموعة الضابطة)، المجموعة٢ (المجموعة المعالجة بالاستيروىد)، المجموعة٣ (المجموعة المدربة) والمجموعة٤ (المجموعة المدربة مع المعالجة بالاستيروىد). وقد تم اعطاء المجموعات ٢ و ٤ مادة (Nandrolone Decnoate) مرتين اسبوعيا بجرعة ٥ مجم / كجم من وزن الجسم عن طريق الحقن العضلي لمدة ٥ أسابيع. المجموعات ٣ و ٤ تم وضعها تحت بروتوكول تدريبى فى صورة ٤ جلسات ، كل جلسة تشمل ١٠ قفزات فى الماء لمدة ٥ أسابيع. فى نهاية التجربة تمت التضحية بالفئران وأخذ للعضلات ذات الاربع رؤوس وتمريرها للاجراءات الهستولوجية والهستوكيميائبة.

النتائج: قد اظهرت المجموعة المعالجة بالاستيروىد وجود تضخم فى الياف العضلات ووجود خلل فى نظام ترتيب الالياف مع اتساع المسافات بينهم . بالاضاغة الي وجود مناطق من الانحلال واحتقاتن الأوعية الدموية. في المجموعة٣ لوحظ وجود تضخم فى الياف العضلات . المجموعة٤ اظهرت ان الالياف العضلية لها تركيب هستولوجي طبيعى مع وجود مناطق دقيقة من الانحلال واحتقاتن الأوعية الدموية.

الخلاصة: اظهرت هذه الدراسة ات ان تعاطي الاستيرويدات البنائية الاندرجينية قد تسبب فى حدوث تغيرات انحلالية ملحوظة فى العضلات ذات الاربع رؤوس لذكور الجرذان البيضاء البالغة . كما لوحظ أن هذه التأثيرات الضارة قد قلت في المجموعة المعالجة بالاستيروىد مع التدريبات الرياضية.