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*Original Paper*

# Histological Evaluation of the Effect of Some Steroid Hormones on the Structure of Muscles in Adult Male Albino Rats

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## ABSTRACT

This study aimed to histologically evaluate the effects of selected steroid hormones on skeletal muscle tissue in adult male albino rats. The experiment was conducted at the College of Veterinary Medicine, Tikrit University, using 32 adult male1 albino rats aged 10–12 weeks and weighing 150–180 g. The animals were randomly allocated into eight groups, with four rats in each group. Three dosage regimens were investigated, including therapeutic, prophylactic, and toxic doses. Each regimen involved the combined administration of testosterone with boldenone or testosterone with trenbolone.Histological examination revealed that the negative control group (tap water) exhibited normal skeletal muscle architecture characterized by regularly arranged muscle fibers, eosinophilic cytoplasm, and peripherally located nuclei. In contrast, the positive control group (DMSO) showed mild focal degeneration and partial necrosis of muscle fibers accompanied by slight leukocytic infiltration, which was attributed to mechanical stress associated with the injection procedure.Administration of the therapeutic doses of testosterone combined with either boldenone or trenbolone produced anabolic changes, as demonstrated by well-organized and compact muscle fibers arranged in parallel bundles, eosinophilic cytoplasm, spindle-shaped nuclei, and mild leukocytic infiltration, indicating a limited inflammatory response. The prophylactic doses resulted in hypertrophy of muscle fibers while preserving the normal histological architecture and fiber organization, together with fibrotic hypertrophy.Conversely, toxic doses of both steroid hormone combinations produced severe histopathological alterations in skeletal muscle tissue, including disruption of muscle fiber architecture, prominent cytoplasmic clefts, fragmentation of muscle bundles, focal necrosis, and marked degenerative changes.These findings indicate that steroid hormones act as a double-edged sword. At therapeutic and prophylactic doses, they promote skeletal muscle growth and fiber hypertrophy, whereas excessive or toxic doses induce severe histological damage and structural deterioration of skeletal muscle tissue.

**Keywords:** Steroid hormones, Testosterone, Boldenone, Trenbolone, Skeletal muscle, Histopathology

## 1-INTRODUCTION

Steroid hormones are an essential group of biologically active compounds that may be natural or synthetic. They function as chemical messengers in the body and regulate a wide range of physiological processes, including development, growth, reproduction, and metabolism. All steroid hormones are derived from cholesterol and are produced by endocrine glands. The major classes of steroid hormones include sex hormones, glucocorticoids, and mineralocorticoids [1,2].Steroid hormones are characterized by their ability to readily diffuse across cellular membranes and bind to specific nuclear receptors, thereby regulating gene expression [3]. Among this broad group are the

 

androgenic hormones, which represent a major class involved in the development of male characteristics and the promotion of anabolic effects such as stimulation of protein synthesis and enhancement of muscle mass [4].

Anabolic-androgenic steroids (AAS) are among the most widely misused substances and include a broad range of natural and synthetic androgens. The prevalence of AAS misuse has become a global concern, with an estimated prevalence of approximately 6% among males. Furthermore, between 15% and 25% of males attending fitness centers are estimated to use these compounds at some point in time [5]. In many countries, access to these substances is largely unrestricted through numerous online platforms or illegal sales by private individuals, often within sports and fitness centers [6]. Anabolic steroids are synthetic chemical derivatives of the male hormone testosterone and are used medically because of their ability to support muscle growth and recovery, while athletes frequently use them for cosmetic purposes and performance enhancement[7]. Testosterone is the principal natural androgen and the most abundant androgen in males. It serves as the primary model for the development of more potent synthetic derivatives such as boldenone and trenbolone, both of which belong to the anabolic-androgenic steroid class [8].The use of synthetic androgens, particularly boldenone and trenbolone, has expanded beyond their medical and scientific applications in treating conditions such as muscle wasting and hypogonadism. Their use has become an increasingly widespread phenomenon among athletes and gym-goers worldwide, driven by the desire to achieve rapid muscular development and improved physical performance [9]. These synthetic steroids, similar to natural testosterone but differing in potency and efficacy, exert their effects through binding to androgen receptors within the nuclei of target cells, including muscle cells. This interaction activates the transcription of genes responsible for protein synthesis while suppressing catabolic processes [10]. However, androgen receptors are not restricted to muscle tissues; they are widely distributed throughout various organs and systems, including the liver, heart, brain, and reproductive system, which explains the numerous systemic adverse effects associated with anabolic steroid use [11].

Recent scientific evidence indicates that anabolic-androgenic steroids, especially potent derivatives such as boldenone and trenbolone, exert diverse effects on body tissues and organs. They promote muscle mass accretion through modulation of gene expression, enhancement of protein synthesis, suppression of protein degradation, and acceleration of the repair of damaged muscle fibers [12,13].

 

Due to the widespread use of anabolic agents and hormonal compounds in fitness centers without medical consultation, as well as the limited regulation of these substances and their methods of administration, the present study was conducted to evaluate the histological effects of certain steroid hormones on the muscular structure of adult male rats.

## 2-MATERIALS AND METHODS

### Study Location

The study was conducted at the Animal House of the College of Veterinary Medicine, Tikrit University. The experiment included 32 adult male albino rats (Albino Male Rats), aged 10–12 weeks and weighing 150–180 g. The animals were housed in pre-prepared clean plastic cages under suitable hygienic and controlled environmental conditions.

### Preparation of Stains

The stains used for histological sections included hematoxylin and eosin (H&E). These stains were prepared according to the method described by [14].

### Experimental Design

The experimental animals were randomly divided into eight groups, with four rats in each group of approximately similar body weight, as follows:

**Group I:** Consisted of 4 rats and served as the negative control group. [15]

**Group II:** Consisted of 4 rats and received DMSO; it served as the positive control group.[15]

**Group III:** Consisted of 4 rats and received testosterone (25 mg/kg) and boldenone (2.5 mg/kg) as a preventive dose.[15,16,17].

**Group IV:** Consisted of 4 rats and received testosterone (50 mg/kg) and boldenone (5 mg/kg) as a therapeutic dose.[15,16,17].

**Group V:** Consisted of 4 rats and received testosterone (100 mg/kg) and boldenone (7 mg/kg) as a toxic dose. .[15,16,17].

**Group VI:** Consisted of 4 rats and received testosterone (25 mg/kg) and trenbolone (1 mg/kg) as a preventive dose. .[15,18].

**Group VII:** Consisted of 4 rats and received testosterone (50 mg/kg) and trenbolone (5 mg/kg) as a therapeutic dose. .[15,18].

**Group VIII:** Consisted of 4 rats and received testosterone (100 mg/kg) and trenbolone (10 mg/kg) as a toxic dose. .[15,18].

 

**Preparation of Preventive, Therapeutic, and Toxic Doses**

The doses were prepared according to the body weight of each animal and the concentration of each compound using the dilution equation, as follows:

**First: Preparation of Testosterone Doses**

The preventive, therapeutic, and toxic doses of testosterone were prepared according to the method described by [15], with some modifications. The concentrations were diluted using DMSO after converting the required doses into microliters by applying the dilution formula.

**Second: Preparation of Boldenone Doses**

The preventive, therapeutic, and toxic doses of boldenone were prepared according to the methods described by [16,17], with some modifications, followed by dilution using DMSO.

**Third: Preparation of Trenbolone Doses**

The preventive, therapeutic, and toxic doses of trenbolone were prepared according to the method described by [18], with some modifications and dilution using DMSO.

**Experimental Procedure**

The experiment was conducted after dividing the animals into eight groups, each containing four rats. All husbandry requirements were provided throughout the study period. The tested treatments were administered via intramuscular injection for eight weeks. The injections were given twice weekly, every Saturday and Tuesday, using insulin syringes.

**Dissection of Animals**

At the end of the experimental period, the animals were anesthetized using chloroform and dissected through a longitudinal abdominal incision using a sharp scalpel and scissors. The required organs were carefully removed after eliminating the surrounding connective tissues and then fixed in 10% formalin for preservation.

**Histological Study**

Histological sections of the liver and skeletal muscles were prepared according to the method described by [19]. The procedure included fixation, washing, dehydration, clearing, infiltration, embedding, and tissue sectioning. The organs were sectioned using a rotary microtome at a thickness of 3 μm. The sections were floated on a water bath at 45°C, mounted on glass slides, dried at 37°C, stained with hematoxylin and eosin, and finally photographed using a microscope-mounted digital camera.

 

**3-RESULTS AND DISCUSSION**

The results shown in Figure (1) illustrate the skeletal muscle tissue in the negative control treatment (tap water). The muscle tissue contained skeletal muscle fibers, and each muscle fiber contained cytoplasm filled with myofibrils, with the nuclei positioned laterally. Between the muscle fibers, loose connective tissue (endomysium) appeared, containing some fibroblasts and macrophages, which were also observed in the perimysium of muscle bundles.

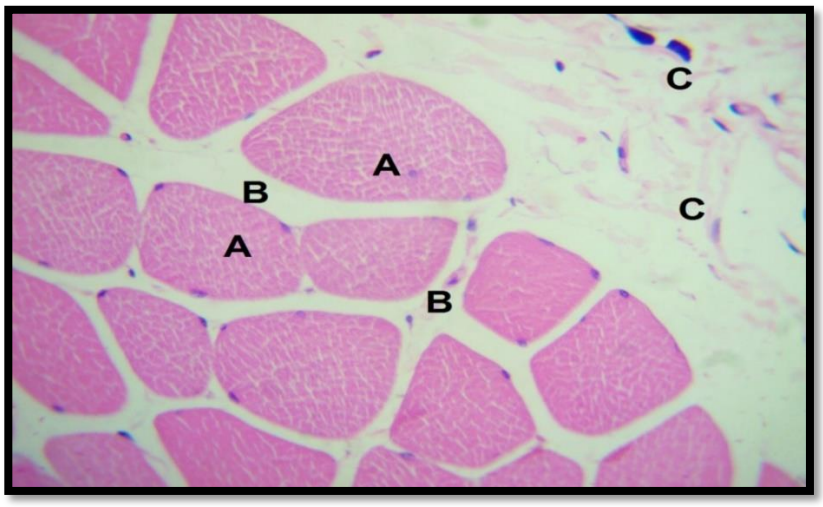


**Figure (1): Skeletal muscle tissue in the negative control treatment (tap water). A – Skeletal fibers with cytoplasm filled with myofibrils; B – Muscle fiber fascia (endomysium); C – Muscle bundle fascia (perimysium) containing macrophages (H&E 400×)**

The results shown in Figure (2) demonstratesm the skeletal muscle tissue in the positive control treatment (DMSO). The muscle tissue contained skeletal muscle fibers with partial necrosis in some of those fibers. The perimysium of the muscle bundles contained infiltration of inflammatory white blood cells and was surrounded by numerous blood vessels.

 

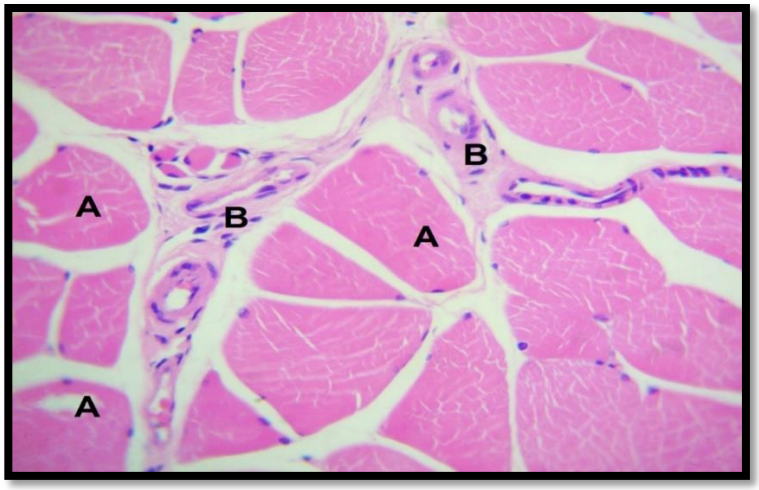


**Figure (2): Skeletal muscle tissue in the positive control treatment (DMSO). A – Skeletal tissue with partial necrosis of some muscle fibers; B – Muscle fiber fascia (endomysium) containing white blood cell infiltration and small blood vessels (H&E 400×).**

Figure (3) shows muscle tissue treated with Testosterone (25 mg/kg) and Boldenone (2.5 mg/kg) at the a preventive dose. The skeletal muscle fibers appeared as densely packed bundles arranged in parallel. The fascia of each muscle fiber contained white blood cells, and vacuolar necrosis was observed in the cytoplasm of some muscle fibers. The perimysium of muscle bundles was covered by loose fibrous connective tissue.

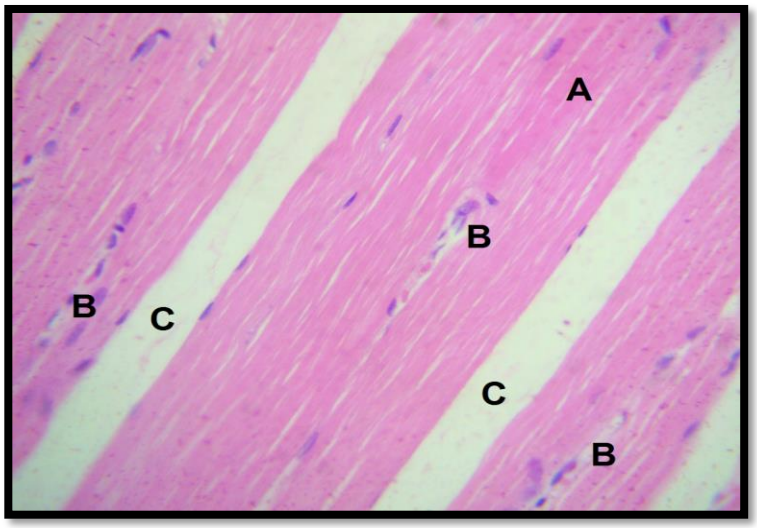


**Figure (3): Skeletal muscle fibers in the Testosterone (25 mg/kg) and Boldenone (2.5 mg/kg) treatment. A – Densely packed muscle fiber bundles; B – Vacuolar necrosis in muscle fiber cytoplasm with white blood cells; C – Muscle bundle fascia composed of collagenous fibrous tissue (H&E 400×).**

 

Figure (4) shows muscle tissue treated with Testosterone (50 mg/kg) and Boldenone (5 mg/kg) at the a therapeutic dose. The skeletal muscle fibers appeared longitudinally and in parallel arrangement, of varying sizes, with normal shape, acidophilic cytoplasm, and laterally positioned spindle-shaped nuclei. The muscle fiber fascia was composed of loose connective tissue continuous with the bundle fascia.

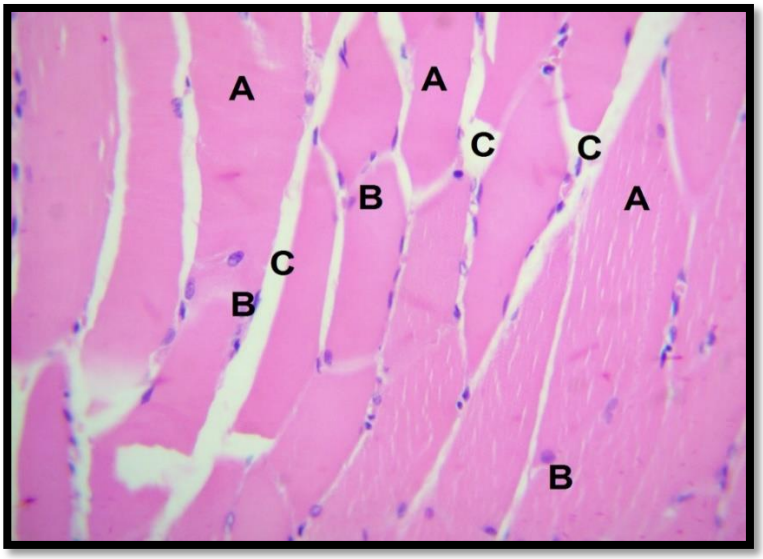


**Figure (4): Skeletal muscle fibers in the Testosterone (50 mg/kg) and Boldenone (5 mg/kg) treatment. A – Longitudinally extending skeletal muscle fibers; B – Nuclei of muscle fibers; C – Muscle bundle fascia (H&E 400×).**

Figure (5) shows muscle tissue treated with Testosterone (100 mg/kg) and Boldenone (7 mg/kg) at the toxic dose. The skeletal muscle tissue appeared disorganized with narrow clefts in the cytoplasm of muscle fibers, in addition to disruption of their continuity. The perimysium contained necrotic collagen fibers surrounded by white blood cells with spindle-shaped fibroblasts. White blood cells and macrophages were also found in the muscle fiber fascia.

 

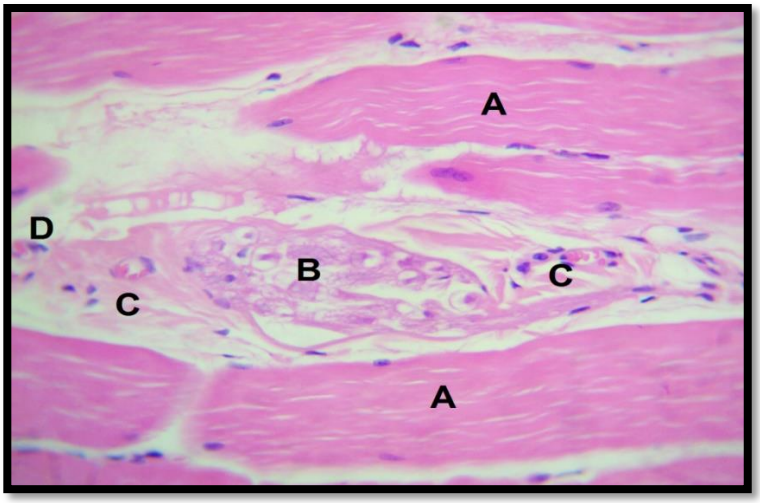


**Figure (5): Skeletal muscle tissue in the Testosterone (100 mg/kg) and Boldenone (7 mg/kg) treatment. A – Disintegration of muscle fibers with narrow clefts in the cytoplasm; B – Muscle bundle fascia with necrosis of collagen fibers; C – White blood cells; D – Macrophages (H&E 400×).**

The results of the current study demonstrated the effect of testosterone and boldenone on skeletal muscle tissue. The muscle tissue in the negative control group (tap water) showed normal structure and composition, represented by skeletal muscle fibers with cytoplasm, lateral nuclei, myofibrils, along with rare fibroblasts and macrophages. These results are consistent with the histological structure of muscles under normal conditions in rat models, where lateral nuclei are a characteristic feature of mature muscle fibers, and the presence of macrophages and fibroblasts is a normal occurrence. These findings are consistent with previous studies [20, 21]. Regarding the results of the positive control (DMSO), they showed slight and partial necrosis in skeletal muscle fibers with inflammatory infiltration. These results are attributed to a type of mechanical stress resulting from repeated injections of DMSO, which causes partial necrosis. Additionally, dimethyl sulfoxide (DMSO) affects the membrane of muscle fibers, and the presence of inflammatory infiltration indicates a local immune response at the injection site [22].

The results of the therapeutic dose of testosterone and boldenone showed densely packed fiber bundles arranged in parallel, with leukocyte infiltration in fiber fasciae and localized necrosis in the cytoplasm. This necrosis and leukocyte infiltration is attributed to mechanical stress caused by repeated injections of hormones into the muscles at the same site. The regularity and compactness of fibers and the presence of parallel bundles is due to the anabolic androgenic effects of these hormones

 

(AAS), which play a role in increasing muscle size and hypertrophy through activation of androgen receptors and interaction with accompanying co-stimulators. These hormones affect the expression of these receptors through cellular metabolic pathways with anti-catabolic effects and interference in the genetic expression of corticosteroid receptors, which explains the compaction of muscle fibers at therapeutic doses [23].

The mechanism by which these hormones build muscle involves their effect on increasing muscle fiber numbers and diameters, increasing satellite cells, and upregulating expression of Insulin-like Growth Factor-1 (IGF-1). The hormones enter the muscles and act on androgen receptors (AR), stimulating genes related to muscle growth and increasing IGF-1 within muscles, which in turn stimulates muscle growth and proliferation of satellite cells, resulting in protein synthesis and muscle hypertrophy [24].

The results of the current study are consistent with previous reports [20, 25], which indicated that low therapeutic doses of androgens stimulate compaction and regularity of skeletal muscle fibers with increased protein content leading to hypertrophy. The leukocyte infiltration is attributed to a local response due to the injection rather than to the effects of therapeutic hormone doses. A recent study also indicated that low doses of anabolic steroids stimulate protein synthesis through the ad pathway and reduce catabolism and corticosteroid inhibition, resulting in muscle fiber hypertrophy and regularity without fibrosis [23]. However, the results of the current study did not agree with a previous study [26], which found no inflammatory infiltration in the fasciae of skeletal muscle fibers in rats injected with low (therapeutic) doses of AAS; instead, the muscles were hypertrophied and regular. This difference may be due to the rats' more efficient immune system, or to differences in injection site and repetition frequency, which caused local mechanical stress and minimal localized necrosis [27].The prophylactic dose results of testosterone and boldenone showed muscle fibers with wider diameter, acidophilic lateral spindle-shaped nuclei, and macrophage infiltration. These findings are attributed to the anabolic effects of androgenic hormones through binding to androgen receptors and activating several pathways including the IGF-1 synthesis pathway, mTOR pathway, and MAPK pathway. Testosterone also exerts anabolic effects directly in muscles via androgen receptors, activating several genes expressed in satellite cells, leading to fibrillar protein synthesis and muscle fiber hypertrophy [28].

These results are consistent with previous studies [20, 23], which showed that moderate doses of anabolic steroids activate IGF-1/Akt/mTOR pathways, stimulating muscle protein synthesis and

 

causing muscle fiber hypertrophy with intact structure. However, these results were not consistent with another study [26], which demonstrated that moderate doses of AAS do not cause hypertrophy and increase in muscle fiber diameters histologically observable in a short period without intensive physical training. This discrepancy may be explained by some studies suggesting that increased muscle fiber diameter with AAS doses may be due to fluid retention within muscle fibers rather than hypertrophy from increased protein content [27].

On the other hand, the toxic doses of testosterone and boldenone showed severe histological effects at the level of muscle fibers, causing muscle degeneration, tissue disintegration, cytoplasmic clefts, and disruption of muscle fiber continuity. All of these result from the effects of high toxic doses of anabolic steroids combined with ischemia and reperfusion. White blood cells migrate to damaged muscle fibers, causing elevated cytokines, free radicals, and prostaglandins, resulting in further myolysis and focal necrosis in muscle fibers. Vascular congestion in capillaries is attributed to blood flow disturbances resulting from increased blood viscosity combined with decreased perfusion associated with this toxic dose [29]. These results are consistent with previous reports [20, 30], which showed that high (toxic) doses of AAS cause collapse of sarcolemma membrane integrity, leading to leakage of muscle fiber contents and attraction of white blood cells and macrophages. It was also shown that toxic (high) doses of boldenone cause oxidative stress within skeletal muscle fibers through free radical production, damaging myosin and actin filaments with collapse of fibrillar structure [31]. On the other hand, results for the toxic dose did not agree with another study [26], which found that rats injected with high doses of anabolic steroid hormones showed a rapid constructive and regenerative response without necrosis, with formation of thin newly formed regenerating fibers alongside damaged muscle fibers, indicating a regenerative capacity dependent on satellite cells. This difference may be due to the use of younger rats with higher regenerative capacity, in addition to differences in injection duration, doses, and injection site.

Figure (6) shows muscle tissue treated with Testosterone (25 mg/kg) and Trenbolone (1 mg/kg) at the Preventive dose. The skeletal muscle tissue was composed of densely packed muscle fiber bundles with acidophilic cytoplasm; fiber ends contained spindle-shaped nuclei; white blood cells were distributed in the muscle fiber fascia.

 

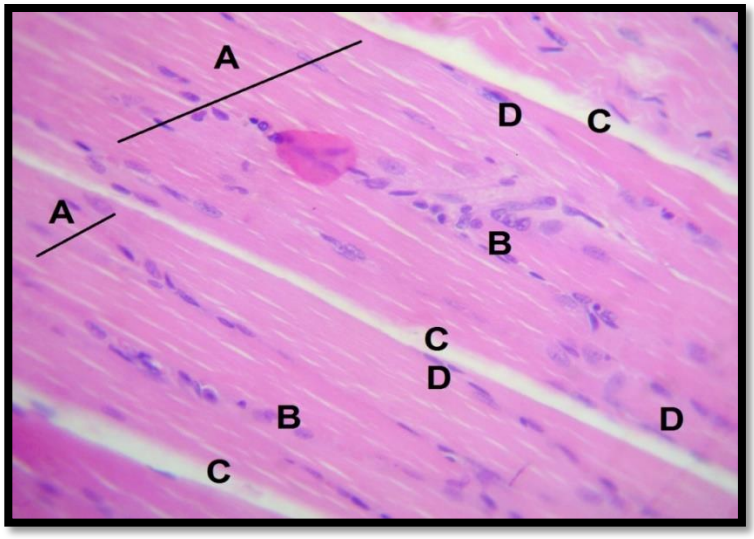


**Figure (6): Skeletal muscle tissue in the Testosterone (25 mg/kg) and Trenbolone (1 mg/kg) treatment. A – Muscle fiber bundles; B – White blood cells in muscle fiber fascia; C – Muscle bundle fascia; D – Muscle fiber nuclei (H&E 400×).**

Figure (7) shows the skeletal muscle tissue with Testosterone (50 mg/kg) and Trenbolone (5 mg/kg) at the Therapeutic dose, appearing as muscle bundles, each containing loose fibrous connective tissue with fine capillaries and fibroblasts.

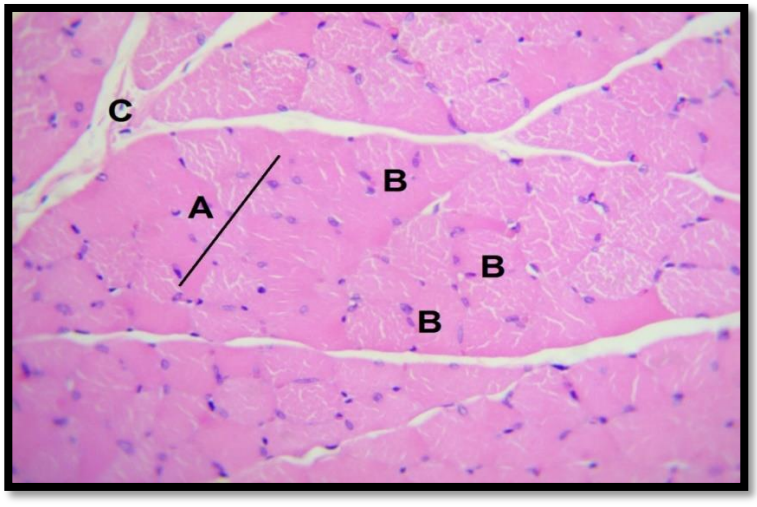


**Figure (7): Skeletal muscle fibers in the Testosterone (50 mg/kg) and Trenbolone (5 mg/kg) treatment. A – Compacted muscle bundle fibers; B – Muscle fiber cell nuclei; C – Muscle bundle fascia containing loose connective tissue (H&E 400×).**

 

Figure (8) shows muscle tissue treated with Testosterone (100 mg/kg) and Trenbolone (10 mg/kg) at the toxic dose. Large blood arteries with thickened walls were found in the muscle fascia, with a blood clot inside their lumen, and the fibrous tissue around the muscles showed massive infiltration of white blood cells and macrophages. The skeletal muscle fibers were densely packed together with white blood cell and macrophage infiltration.

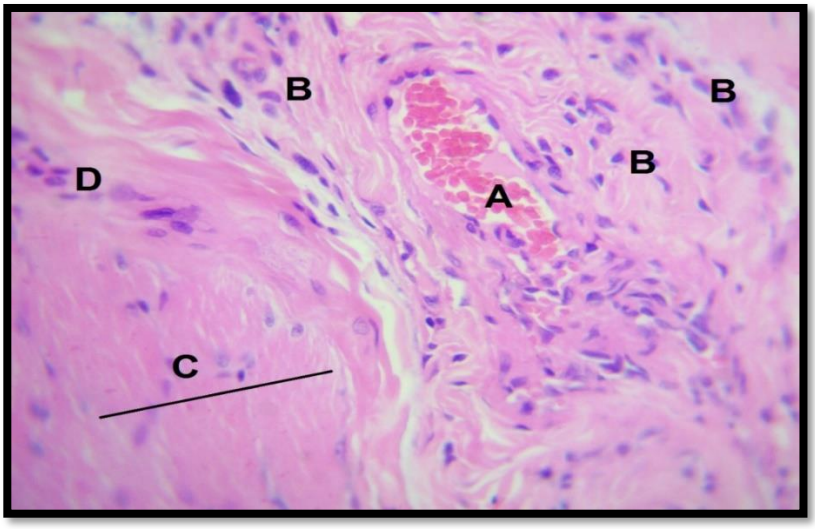


**Figure (8): Skeletal muscle tissue in the Testosterone (100 mg/kg) and Trenbolone (10 mg/kg) treatment. A – Artery surrounding the skeletal muscle fascia, filled with blood; B – Infiltration of white blood cells and macrophages; C – Compaction of skeletal muscle fibers; D – Macrophages (H&E 400×)**

The results of the effect of steroidal hormones (testosterone and trenbolone) on skeletal muscle tissue reflect a dual pattern of anabolic and harmful effects according to each dose. At the therapeutic dose, muscle fibers appeared as densely packed consistent bundles with acidophilic cytoplasm and spindle-shaped nuclei, consistent with the anabolic response of these hormones which increases muscle fiber proteins and tightens muscle bundle structure. The dense acidophilic cytoplasm reflects increased protein production and increased myofibrillar density as a response to anabolic steroid hormones. Testosterone and trenbolone bind to androgen receptors (AR) in muscle fiber cells and satellite cells, stimulating genes responsible for the production of fibrillar proteins such as myosin and actin, which explains the increased density and acidophilic staining [32]. The spindle-shaped lateral nuclei are a normal characteristic of skeletal fibers, reflecting adaptation due to fiber growth and longitudinal extension as a result of anabolic androgenic effects. The presence of white blood cells in muscle fascia

 

is due to a slight inflammatory response related to tissue remodeling, a normal phenomenon accompanying muscle fiber growth, where macrophages mediate the breakdown of remnants of old proteins. This is consistent with a previous study [33], which found that anabolic steroidal hormones at low doses activate muscle tissue remodeling, accompanied by slight inflammatory infiltration of white blood cells. These structural variations in skeletal muscle tissue may also be attributed to findings demonstrating that androgenic steroids at low doses stimulate multiple pathways including the Wnt pathway and Notch pathway within muscle fiber cells and satellite cells, leading to stimulation of protein production and increased myofibrillar density [32].

On the other hand, the results of the current study did not agree with a previous report [34], which demonstrated that low (therapeutic) doses of androgens do not cause any visible histological changes under light microscopy in rat muscles and did not cause any white blood cell infiltration or inflammation. This may be due to the current study's reliance on combining anabolic steroids, as this combination is common in sports environments and among bodybuilders, whereas the disagreeing study administered each hormone separately, in addition to differences in experimental duration and dosing method.

The presence of dense collagen material and fibroblasts in the muscle fascia reflects the role of trenbolone and testosterone in stimulating the collagen pathway and fiber fibrosis in parallel with muscle hypertrophy. Testosterone at supraphysiological doses stimulates fibroblasts to synthesize collagen (types I and III) through the TGF-β pathway, explaining the increased collagen content in skeletal muscle fibers. These results are consistent with previous findings [35].Regarding the toxic dose of testosterone and trenbolone, histological sections showed severe changes in muscles including arteries with thickened walls, thrombosis, macrophage and white blood cell infiltration. The arterial wall thickening is attributed to the toxic dose causing dual vascular disturbance: on one hand raising blood pressure and viscosity through increased red blood cell production, and on the other hand causing direct fibrosis in blood vessel walls, thickening of the tunica media, and abundant collagen deposition [36, 37]. Regarding the necrosis and clefts in the cytoplasm of muscle fibers at the toxic dose, these stimulants produce very high amounts of reactive oxygen species (ROS) that the defensive system (including glutathione, catalase enzymes, and superoxide dismutase SOD) within muscle tissue cannot counteract. This oxidative excess destroys mitochondrial membranes and other membrane pumps such as calcium pumps, causing a collapse in calcium regulation within muscles and auto-proteolytic breakdown ending in cellular degeneration and necrosis [35].

 

## CONCLUSION

Our study evaluating the effect of some steroidal hormones on muscle tissue revealed dose-dependent effects of these hormones, reaching their most severe manifestation at high doses. The results of our experiment also demonstrated that low therapeutic doses of androgens — testosterone combined with boldenone, and testosterone combined with trenbolone — exerted positive anabolic effects on muscle tissue, as evidenced by increased protein content and compaction of muscle fibers. The prophylactic (intermediate) doses showed an increase in muscle fiber diameter with hypertrophy and structural integrity of the tissue, indicating that this dose level is optimal. The toxic doses or high concentrations of androgens, however, caused severe effects represented by tissue deterioration, fiber disintegration with cytoplasmic clefts, and inflammatory infiltration, as well as arterial wall thickening and thrombus formation. The experiment also demonstrated that repeated injection at the same site produces local mechanical stress with localized degeneration, regardless of the concentration or dose used

### Authors' Contributions

The author solely contributed to the conception and design of the study, experimental work, collection, histological analysis, manuscript preparation, and final approval of the manuscript.

### Ethical Considerations

Not applicable

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