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The effect of anabolic steroid on Animals

A thesis submitted to the Council of the College of the Science In a partial Fulfillment of the Requirements for the Degree of bachelor of Biology

By

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بسم الله الرحمن الرحيم

(قُلْ آمِنُوا بِهِ أَوْ لَا تُؤْمِنُوا ۚ إِنَّ الَّذِينَ أُوتُوا الْعِلْمَ مِن قَبْلِهِ إِذَا يُتْلَىٰ عَلَيْهِمْ يَخِرُّونَ لِلْأَذْقَانِ سُجَّدًا وَيَقُولُونَ سُبْحَانَ رَبِّنَا إِن كَانَ وَعْدُ رَبِّنَا لَمَفْعُولًا وَيَخِرُّونَ لِلْأَذْقَانِ يَبْكُونَ وَيَزِيدُهُمْ خُشُوعًا).

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توصية الأستاذ المشرف

أشهد أن أعداد البحث الموسوم (**The effect of anabolic steroid on Animals**) قد جرى تحت إشرافي وهو جزء من متطلبات نيل درجة بكالوريوس علوم في علوم الحياة.

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الاهداء

إلى الرحمة المرسلة هدى للعالمين...إلى سيد الخليقة وإمام المرسلين...إلى نورو ضياء الحق وخاتم النبيين

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ايام مضت من عمرنا بدأناها بخطوة وها نحن اليوم نقطف ثمار مسيرة أعوام كان هدفنا فيها واضحا وكنا نسعى في كل يوم لتحقيقه والوصول له مهما كان صعبا

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Contents

Abstract

The current study investigated the histological effects of nandrolone decanoate (ND) on skeletal muscle and liver tissues in male mice. The experiment was conducted at the University of Misan, where ten male mice (60–80 g, 10–12 weeks old) were divided into two groups: a control group (n=4) treated with distilled water and a treatment group (n=6) receiving ND (10 mg/kg/day via intramuscular injection) for 15 days. Tissues were processed for histological analysis using hematoxylin and eosin (H&E) staining .

Results revealed severe structural alterations in the ND-treated group, including skeletal muscle atrophy, infarction, disorganized myonuclei, inflammatory infiltration, and myofibril degeneration. Liver tissues exhibited hepatocyte necrosis, vacuolated cytoplasm, portal triad fibrosis, vascular congestion, and sinusoidal dilation. These findings align with previous studies linking anabolic-androgenic steroids (AAS) to tissue damage, hepatotoxicity, and metabolic dysfunction. The current study underscores the irreversible risks of AAS misuse, particularly in non-therapeutic contexts.

Chapter One

Introduction

Steroid hormones are generally complex lipophilic molecules synthesized from the precursor cholesterol molecule in endocrine cells in the adrenal cortex, testes, ovaries, and placenta. They are mostly formed as steroid hormone precursors and only after stimulation of the secreting cells of the above-mentioned organs are they converted into active hormones and penetrate from the parent cell by simple diffusion as their intracellular concentration increases. The hypothalamo–pituitary axis is then the superior system for their production and control of endocrine glands by means of tropical hormones. Steroid hormones act on the paracrine and endocrine pathways, where they are released by endocrine cells into the interstitial space and specifically affect cells in the immediate vicinity. In the body, they play a crucial role in regulating and communicating across cells, tissues, and organs throughout an individual's life (Cole *et al.*, 2019). The mechanisms of action of individual steroid hormones differ significantly from each other, especially depending on their physical and chemical properties. Hydrophilic hormones act primarily on the cell surface, where they bind to receptors in the plasma membrane. On the contrary, hydrophobic steroid hormones bind to free plasma proteins and are able to diffuse freely across cell membranes and thus activate specific intracellular hormone receptors. The effects of hormones can then be generally divided into long-term and short-term effects. Long-term effects are mediated by high-affinity intracellular receptor proteins that are localized in specific target tissues for each steroid hormone. The interaction of the steroid–receptor complex with hormone-responsive genes leads to tissue-specific expression of proteins that either directly or indirectly generate the body's biological responses attributed to steroid hormones. This mode of cellular action is generally referred to as the genomic effect of hormones, is slow, and occurs with a time delay of hours or even days . On the other hand, non-genomic action is any mode of action in which gene transcription is not performed directly.

These are mostly short-term and rapid reactions that involve specific G protein-coupled receptors located on the cell membrane. They contain many second messengers, including cAMP and diacylglycerol, kinases, and ion flux. Short term side effects include sexual and reproductive disturbances, fluid retention, increased hunger, general loss of energy, and development or worsening of infections, and severe acne is often reported in humans. Short-term side effects are reversible in most cases (Lösel, & Wehling , 2003 ; Echeverria, & Picard , 2010).

Anabolic steroids have often been used in livestock fattening around the world for their ability to accelerate muscle growth and thereby speed up and ensure fattening and profit from meat sold. Currently, some countries outside the EU approve the use of a number of preparations based on steroid hormones for fattening cattle and sheep. No type of anabolic is approved to promote growth in dairy cows, pigs, or poultry. It is estimated that 80%–90% of cattle in fattening outside the EU are treated with at least one type of growth-promoting anabolic agent. After their possible negative impact on human health began to be investigated, some states began to limit their use and defined it in their legislation, there are still very few studies investigating the anabolic effect directly in farm animals; most studies are conducted on laboratory animals such as mice and rats, or the effects of steroids are investigated in human medicine. It turns out that the use of AAS also brings economic and environmental benefits too. Webb *et al.*2017 determined the environ mental and economic impacts of cattle raised with different levels of growth-promoting technologies, non-hormonally treated and implanted. The implanted technology reduced the carbon footprint by 8%, energy consumption by 6%, water consumption by 4%, and reactive nitrogen losses by 8% (Kreutzer *et al.*, 2008 ; Webb *et al.*, 2017).

Regular intake of anabolic steroids causes changes in the organization at genetic, metabolomic, and structural levels directly in animal tissues. Increased protein synthesis,

fat loss, and a better sensory profile of final meat products are the targeted changes for which anabolic steroids are used in animal fattening. Like natural hormones, synthetic steroids imitate hormonal functions and interfere with the endocrine system of animals. In addition to endocrine changes, steroids have been shown to have carcinogenic, immunotoxic, mutagenic, and teratogenic effects, and the changes are often irreversible. The risks always depend on the age, sex, and individual tolerance of the animals to these substances . As the use of steroid substances in fattening animals poses a potential risk to consumers, their presence needs to be monitored and new detection methods de veloped to prevent the prevalence of public health risks. Many anabolic substances that can theoretically be misused in meat production have not been studied for toxic effects on humans yet (Hirpessa *et al.,* 2020 ; Benedetto *et al.,* 2021)

The Most Commonly Used or Misused Anabolic Steroids are (1) Boldenone, a 1(2)-dehydrogenated analogue of testosterone, differs chemically from testosterone by a double bond between C1 and C2. It is characterized by high anabolic and low androgenic activity and due to its effects muscles grow slower but is of better quality than most testosterone derivatives, it is approved as a veterinary drug in many countries, especially for the treatment of horses, calves, and lambs, the use of boldenone is banned by humans and food-producing animals and for doping in race horses (Aly *et al.,* 2021) . (2) Chlortestosterone, clostebol, is a 4-chlorinated derivative of testosterone. Chlorination prevents the steroid from being converted to dihydrotestosterone and at the same time makes it unable to convert to estrogen. Its use is prohibited in humans and animals, with the exception of some approved veterinary drugs that can be used in specific indications, mainly for dermatological use (Rahnema *et al.*,2015).(3) Nandrolone, 19-nortestosterone, is an anabolic steroid with a chemical structure similar to testosterone. Due to the lack of a methyl group at C19, it has a better binding affinity for androgen receptors and an increased rate of onset of anabolic activity, and thus also faster effects on muscle growth.

Nandrolone and trenbolone have the highest anabolic:androgenic ratio of all AAS. It is most often used in the form of esters, especially as nandrolone decanoate and nandrolone phenylpropionate (Pan & Kovac, 2016). (4) Stanozolol is a synthetic 17-alkylated derivative of 5-dihydrotestosterone. It was previously widely used in human and veterinary medicine, especially for its very high ratio of anabolic: androgenic activity, which means that it has anabolic activity with minimal androgenic undesirable effects (National Center for Biotechnology Information, 2022). (5) Trenbolone is an androgenic anabolic derivative of nandrolone, specifically nandrolone with two added double bonds in the steroid nucleus. It is most often used in the form of esters, especially as trenbolone acetate (TBA) and trenbolone enanthate (TBE). TBA is a very strong anabolic steroid with strong anabolic effects and therefore has great potential for use in fattening. A number of side effects of its use have been described, including aggression, increased blood pressure and cholesterol levels, skin rashes, negative effects on the thyroid gland, decreased sexual function, and testicular atrophy. Most of these effects are irreversible (Post et al., 2004). Regular intake of anabolic steroids causes changes in the organization at genetic, metabolomic, and structural levels directly in animal tissues. Increased protein synthesis, fat loss, and a better sensory profile of final meat products are the targeted changes for which anabolic steroids are used in animal fattening. Like natural hormones, synthetic steroids imitate hormonal functions and interfere with the endocrine system of animals. In addition to endocrine changes, steroids have been shown to have carcinogenic, im-munotoxic, mutagenic, and teratogenic effects, and the changes are often irreversible. The risks always depend on the age, sex, and individual tolerance of the animals to these substances, as the use of steroid substances in fattening animals poses a potential risk to consumers, their presence needs to be monitored and new detection methods developed to prevent the prevalence of public health risks. Many anabolic substances that can theoretically be misused in meat production have not been studied for toxic effects on humans yet (Benedetto *et al.*, 2021).

Skeletal muscle is the most economically important tissue in animal production and the application of anabolic steroids is mostly targeted at it. The increase in body parameters is best processed in beef cattle after the use of steroidal implants. Implanting increased average daily gain by 21%andimprovedfeedefficiencyby 11% in feedlot cattle. In addition, carcass weight was increased by 7% due to implanting. Moreover, a 5% increase was also reported in ribeye size, a 7% decrease in fat cover, a 5% decrease in marbling score, and a 17%decrease in percent of carcasses grading (Johnson *et al.,* 1998). The AAS affects the genitals, especially in males, in a completely different way than the muscles. Thus, they do not cause their growth, but numerous studies have shown that AASs have a strong toxic and degenerative impact, the application of anabolics has an impact mainly on the testicles, where it causes disturbances of have a strong toxic and degenerative impact, the application of anabolics has an impact mainly on the testicles, where it causes disturbances of spermatogenesis, epithelial degeneration, and overall disorganization of the anatomical and histological structure. The membranes of the seminiferous tubules are deformed, the layers of the germinal epithelium inside the tubules are reduced, and the number and mobility of sperm are reduced (Tousson *et al.*, 2012). In addition to the above-mentioned tissues, in which the manifestation of AAS is the most intensively studied, anabolics also affect other tissues in the body. These are mostly significant degenerative changes and disorganization of the histological structure. A negative effect on the heart muscle, kidneys, liver, and bones is described (Kahal & Allem 2018). Since the liver is the primary site of steroid clearance, concerns regarding the toxic effects of chronic administration of anabolic steroids have been present since the early use of anabolic steroids in the 1950s. Anabolic steroids have been implicated in four distinct forms of liver injury, transient serum enzyme elevations , acute cholestatic syndrome , chronic vascular injury to the liver (peliosis hepatis) , and hepatic tumors including adenomas and hepatocellular carcinoma. The esterified injectable steroids, including testosterone cypionate and testosterone enanthate, seem to have few adverse

effects on the liver and have only rarely been implicated in causing cholestasis, but their long-term use may increase the risk of hepatic tumors and nodular transformation . Orally administered steroids, which have the 17-α-alkyl group modification, are generally well tolerated, have limited virilizing activity, and have been extensively evaluated as a means of increasing weight gain and muscle development in catabolic states, as well as improve athletic performance . However, they have been shown to have more adverse effects on the liver compared with the parenteral administration of esterified testosterone . Overall, considering the presence of millions of illicit anabolic steroid users, the number of reports of hepatotoxicity is quite low (Kuipers, 1998. ; Chitturi & Farrell , 2013).

Chapter Two

Material and Method

The present research was carried out in the animal house and the scientific research laboratory / Department of Biology / Science College / University of Misan. Ten male mice weighing (60-80 g) and (10-12 week) . Veterinarian will examine them both before and after the operation to assess their condition and overall health. They were given the standard rodent and kept in separate cages with tap water. Animals distributed into two categories As the following Group 1: (four animals) Control group, animals treated by D.W intramuscular injection and served as control. Group 2: (Six animals) (ND) treated group. The mice give nandrolone decanoate (10 mg / kg), IM injection once per day for (15 days). Animals were sacrificed at the end of the experiment, their organs the Skeletal muscle and the liver removed, fixed in 10% neutral formation for 24 hours, washes dehydrates increasing alcohol concentrations, cleared in xylene, and then embedded in paraffin. After that, specimen sectioned. The tissues section stained by H and E stain, using the light microscope for histological analysis (Taghreed & Faehaa , 2024).

Chapter Three

Results

Light microscopic observations on sections from skeletal muscles related to the control group showed normal parameters, such as cylindrical long multinucleated tubes; preserved myofibers extended and arranged as longitudinal, regular, and parallel fibers; vesicular and peripherally situated myonuclei; and perimysium connective tissues fill with intercellular spaces and separated muscle fibers. On the other hand, the sections from normal skeletal muscle showed normally preserved myofibrils, with clear striation and peripheral nuclei's. Moreover, showed the strands of collagenous fibers extended between muscles bundles. The regular muscle bundles were surrounded by dense connective tissue known as the epimysium. The septa were extended from epimysium to surround the fibers bundle (Figure 1).

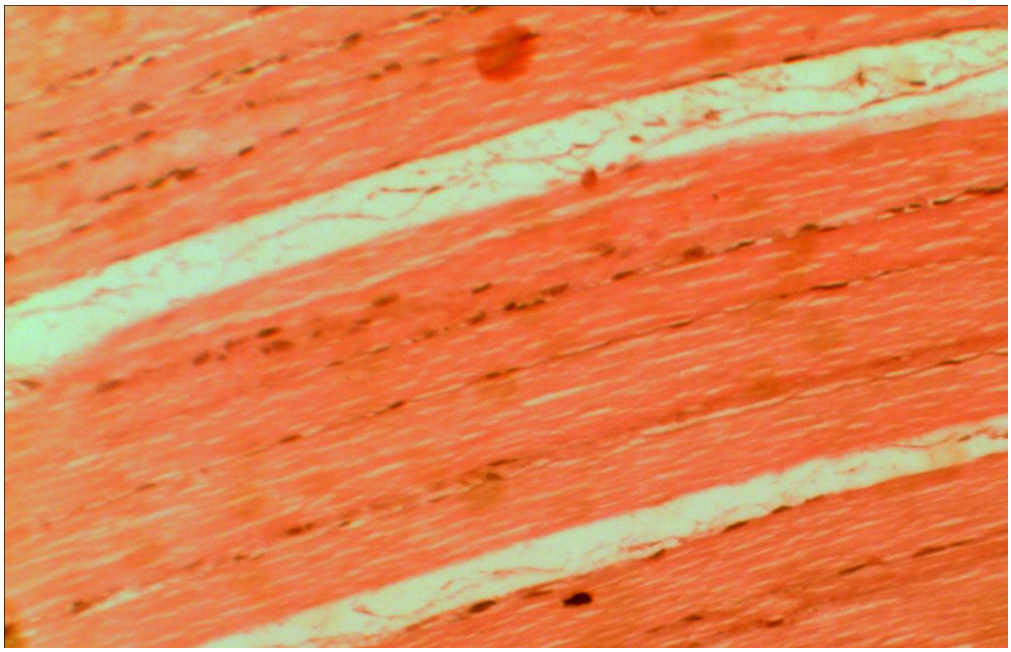

Figure (1)

The sections from the mice skeletal muscles, obtained from the treatment group showed divers changes, including atrophied myofibers, infarction, irregularly arranged myonuclei, disappearance of nuclei from their normal peripheral position with acute skeletal muscular infarction, and infiltration of accumulated inflammatory cells. Most muscle fibers in the treatment group had wavy shapes, and the others had taper endings and barely surface; moreover, their striation was less obvious. On the other hand, the sections showed the extension of dense perimysium among muscle fibers. Moreover, the section depicts the dense collagenous fibers with heavy inflammatory cells . Furthermore, the sections from the treatment group showed fibers with completely myonecrosis, as well as a significant increase in the spaces in the connective tissues that separate muscle fibers. In the connective tissues that separate the muscle fibers, pale areas were clearly distinguishable which revealed the damage of myofibrils and discontinuity. Other sections showed degenerated myofibrils, severe atrophied muscle fibers, damaged pale areas, and deeply stained nuclei, it is revealed that some of these myonuclei are detached from their normal peripheral location (Figure 2, 3).

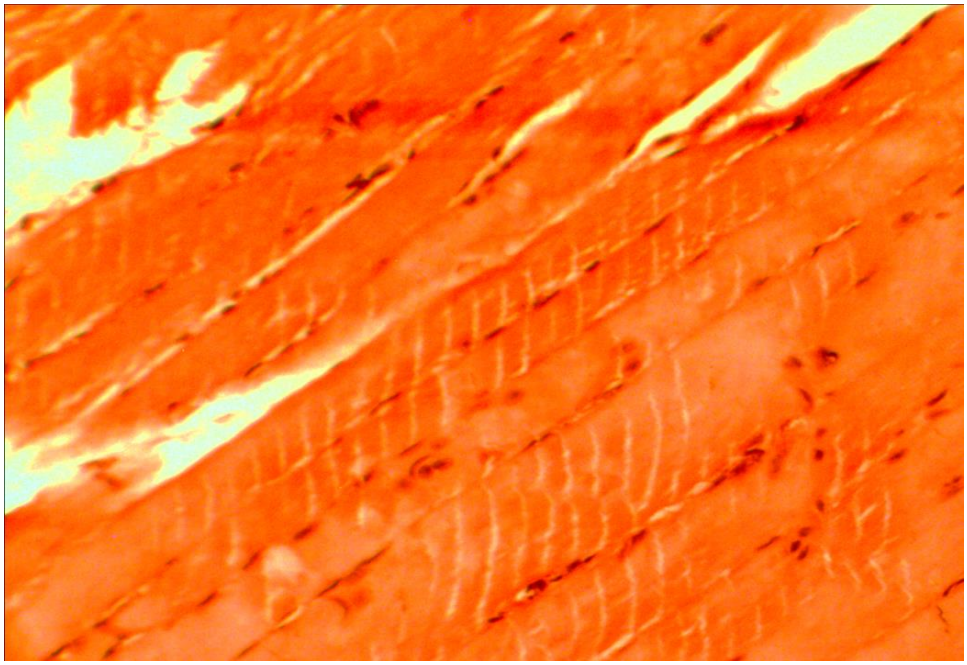

Figure (2)

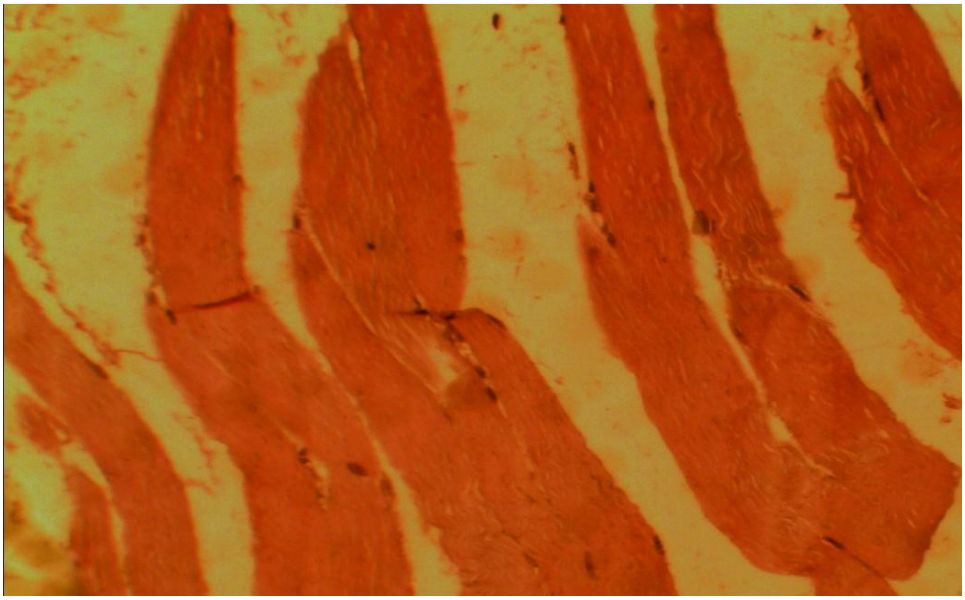

Figure (3)

Light microscopic observations on sections from Liver related to the control group showed the hepatic lobules composed of polygonal and regular hepatocytes with normal cytoplasm and rounded vesicular nuclei. Such cells made up hepatic sinusoids and cords, present between each normal cord. Some Kupffer cells were distributed in the sinuses, the hepatocytes had only one nucleus, and others had binucleated status located around the central vein (Figure 4).

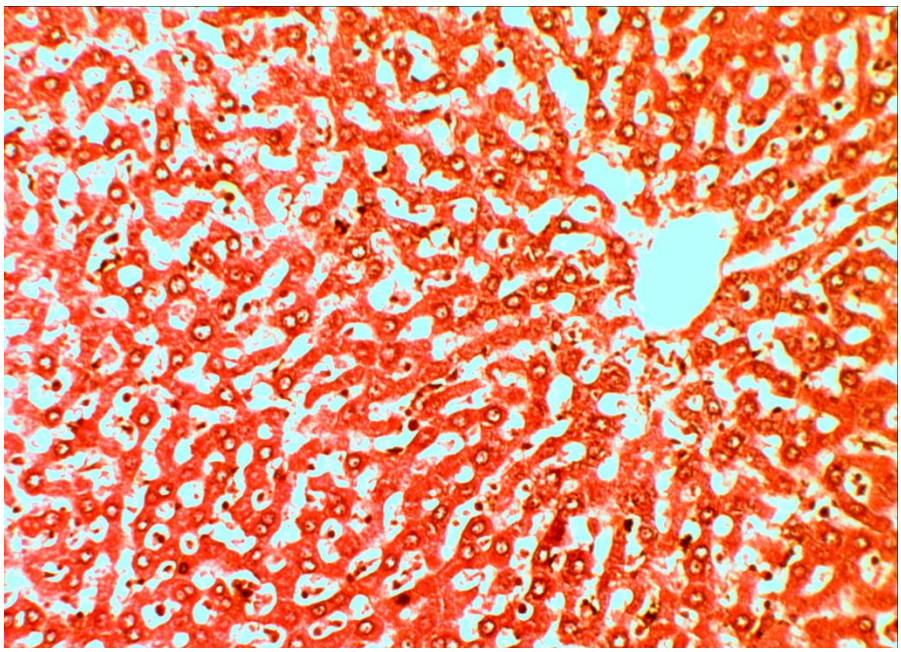

Figure (4)

The sections from the mice Liver, obtained from the treatment group, the observations consisted of the distorted structure of hepatocytes surrounding the central vein, focal hepatocyte necrosis, vacuolated cytoplasm, degeneration, inflammatory cell infiltration, aggregation of inflammatory and blood cells in the portal canal, epithelial layer hyperplasia of bile duct lining, edematosis with mild bleeding in the venous lumen, degeneration of the endothelial lining of the portal vein, portal vein and duct congestion, portal triad and duct fibrosis, portal triad referring to the thickness of the hepatic artery with bleeding, absence of normal tissue stroma, thrombosis around the central vein, irregular dilated sinus hyperplasia of the bile duct, rupture extended to all hepatic structures.(Figure 5 , 6) .

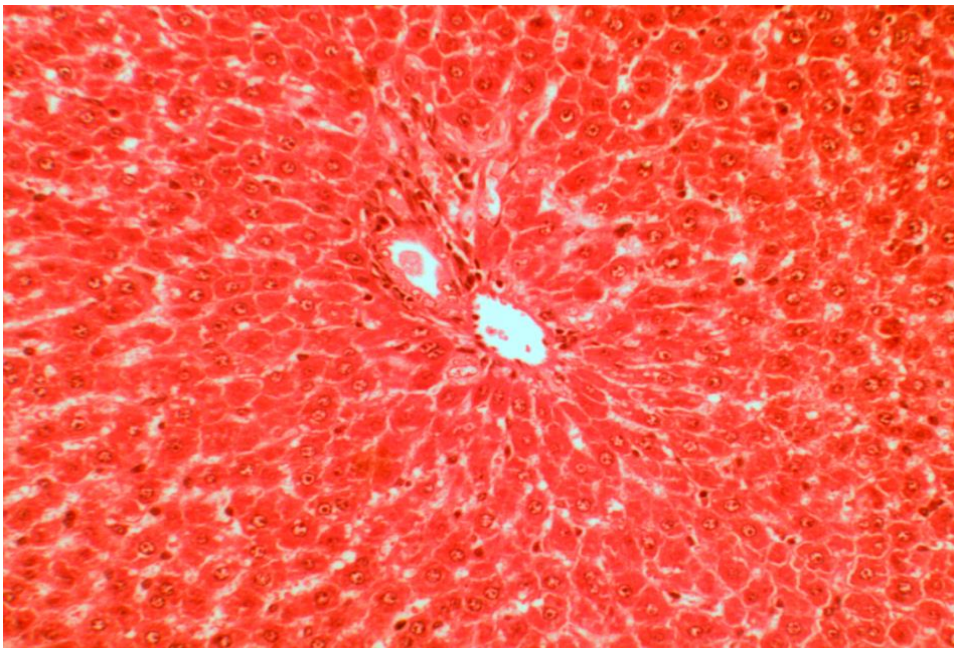

Figure (5)

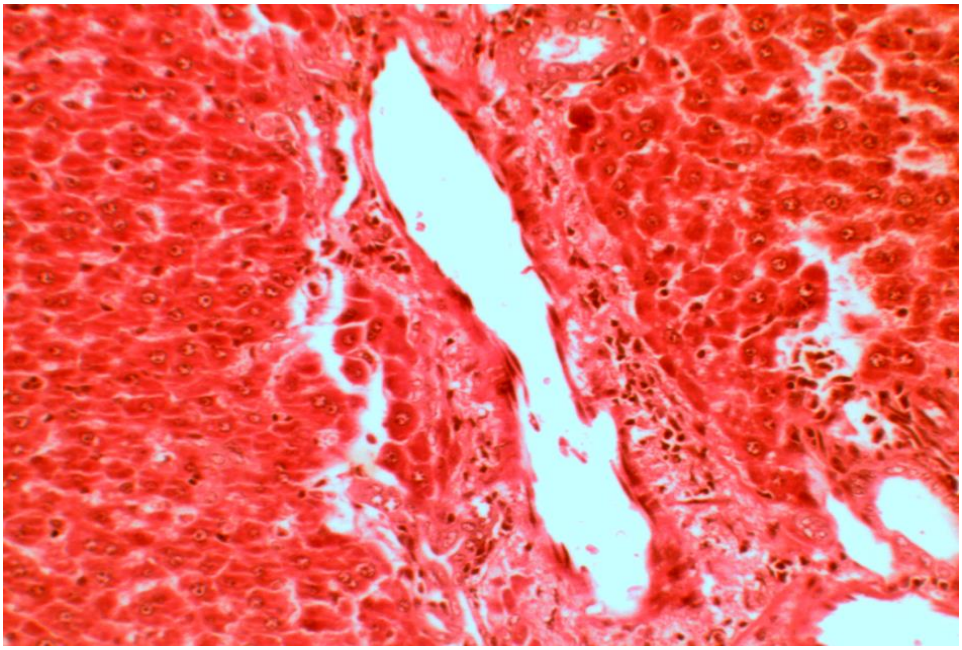

Figure (6)

Chapter Four

Discussion

Histologically observations on skeletal muscle tissue sections in the control group of mice showed muscle fibers with normal structure. These fibbers were parallel and aligned with peripheral myonuclei. The perimysium connective tissues furnish the intercellular area that separated muscle fibers, and these results clarified that all rats' administration with neutral solution did not show any changes. These findings are similar to the recorded data from a study conducted by Anto Michel *et al.,* 2018 who mentioned the normal structure of the skeletal muscles.

Skeletal muscle tissues from the mice treatment group showed obvious alterations, such as distortion, mild atrophy, infarction, and irregularly arranged myonuclei. Most muscle fibers were wavy and had barely surfaces. The striation was less clear, and all these changes were due to the effect of nandrolone decanoate , which was confirmed by the grading of degeneration of muscle fibers. These findings are in agreement with the results of a previously published study by Felipe Cantore Tibúrcio *et al.,* 2023 who reported that Nanodrolone induced adverse morphological changes in the soleus muscle, such as splitting fibers, irregular myofibrils, altered sarcomeres, increase in the number of central nuclei and type I muscle fibers (slow- twitch), and increase in collagen deposition. Although major alterations have not yet been found regarding the NMJs,

According to the observations of liver sections related to control mice treated with normal saline, the liver lobule had typical architecture with no alterations, confirming no injury caused by normal saline, consistent with Aboonabi *et al.,* 2014 who mentioned the normal hepatic structure mammals. Each lobule is made up of radiating plates, strands of cells forming a network around a central vein .

Based on evidence obtained from the animals treated with Nanodrolone , several induced damages were recorded in the liver sections. Such changes might be attributed to

Nanodrolone, the recorded histological changes were as follows: necrosis, congestion and degeneration These findings are in agreement with the results of a previously published study by Ana Petrovic *et al.*, 2022 who reported that in AAS use is closely linked to hepatotoxicity and serious hepatic conditions such as cholestasis, peliosis hepatis, and benign and malignant hepatic tumors, as well as steatohepatitis and dyslipidemia with multiple studies supporting a causal association. Another study by Mark & Niedfeldt, 2018 that reported anabolic steroids can potentially cause a multitude of negative effects on the liver. These may include transient elevations of transaminases, acute cholestatic syndrome, chronic vascular injury to the liver (peliosis hepatis), benign adenoma and hepatocellular carcinoma, or TAFLD.

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