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Deca Durabolin (*Nandrolone Decanoate*) Impair Structure and Function of Liver, Prostate and Pituitary of Male Wistar Rats

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Abstract

Anabolic-androgenic steroids have been widely used by athletes for performance and body building. The present study was carried out to illustrate the cytotoxic effects of nandrolone decanoate on liver, gonadotroph, somatotroph and corticotrophin cells and their correlations to prostate structure and function. Deca-durabolin (nandrolone decanoate) was intramuscular administered (every three days for three months) to male Wistar rats (100 g body weight) at two dose levels; low (4 mg/kg) and high dose-treatment (8 mg/kg body weight). At the end of treatment, the liver, prostate and pituitary were dissected and subjected for histological and transmission electron microscopy and isoenzyme electrophoresis of alkaline and acid phosphatase, glucose-6-phosphate dehydrogenase, sorbitol dehydrogenase, maleic dehydrogenase and lactic dehydrogenase.

The present finding revealed pathological alterations in both liver and prostate. The histopathological structure characterized by leukocytic infiltration at low doses and hepatic necrosis in liver. Meanwhile the prostate gland exhibited marked atrophy of the follicles. Their epithelial lining cells showed marked hyperplasia and flattening as well as nuclear pyknosis especially of the high dose-treatment. The assayed isoenzyme electrophoresis was altered post drug-treatment especially alkaline and acid phosphatase and lactic dehydrogenase. At transmission electron microscopy, there is a marked reduction of the secretory granules within cytoplasm of gonadotroph cells somatotroph and corticotrophin cells associated with striking accumulation of fat globules in between the damaged cells. These manifested depletion of the related hormones secreted by these cells. The authors concluded that athletes and body building men have be aware of using these drugs without regular physical examination to avoid their drastic effects on their body organs.

Keywords: Liver; Pituitary gland; Transmission electron microscopy; Hormones; Anabolic-androgenic steroids

Introduction

Anabolic-androgenic steroids (AAS) are synthetic chemical derivatives of testosterone, used in high doses by athletes to improve athletic ability, physical appearance, and muscle mass. Among of which are anadrol, oxandrin, dianabol, winstrol, deca-durabolin, durabolin, depo-testosterone, equipoise and tetrahydrogestrinone It is rapidly absorbed from the small intestines and metabolized into mostly inactive compounds in the liver [1]. Myocardial infarction and sudden cardiac death among athletes has been closely associated to the use of anabolic-androgenic steroid [2]. Increased level of low-density lipoprotein and decreases in high-density lipoproteins were reported after anabolic-androgenic steroid treatment [3,4] as well as total cholesterol [5]. Long-term anabolic-androgenic steroids-treatment has been linked to many different liver injuries such as cholestasis, peliosis, adenoma and hepatocellular carcinoma [6-8]. Several studies have reported a great association of nandrolondecanoate-treatment and hormonal disturbances in human and experimental animals. Nandrolondecanoate (ND) was found to decrease the levels of serum testosterone, androstenedione and FSH and the ratio of testosterone/oestradiol as well as increase oestrone in patients with rheumatoid arthritis [9].

Also, ND-treatment increased circulating levels of both corticosterone and ACTH levels in brain regions of rats [10], estral acyclicity and degeneration of follicles and an absence of corpus luteum in the ovaries of rat [11], decreased total serum T3, free T4, and TSH in male rats [12] and consequently decreased the expression of GABAB receptor subunits, GHR, IGF-1, and IGF-2 mRNA expression in the pituitary and overexpression of the GABAB2 [13]. There is a less of work concerned this subject of study. The present study aimed to focus on the histopathological alterations on liver and anterior lobe of pituitary and their reflection on prostate structure and function.

Materials and Methods

Deca-durabolin (nandrolone decanoate) treatment

Deca durabolin is a long acting nandrolone decanoate (17β-Hydroxyestra-4-en-3-one). Applied low dose was 4 mg/kg body weight, meanwhile high dose was 8 mg/kg body weight. The applied dose was dissolved in 0.2 ml corn oil and intramuscularly every two consecutive days for three months treatment. Control received the applied dose of corn oil free from the used drug.

Experimental work

Twenty four fertile male Wistar albino rats weighing approximately 100 g body weight, obtained from Hellwan Breeding Farm, Ministry of Health and used for experimentation. Free excess of standard diet and water was allowed ad-libitum. They were keeping in good aerated room with 12 hour light and dark cycle. The rats were arranged into four groups (n=6) such as control, corn oil-treated group, Low dose-DECA-treated group (4 mg/kg body weight) and high dose-DECA-treated group (8 mg/kg body weight). At the end of treatment, the groups were anesthetized, sacrificed and the liver, prostate and pituitary glands were separated and subjected for the following examination.

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Light microscopy

Liver and prostate of the studied groups were dissected, fixed in 10% phosphate-buffered formalin (pH 7.4) and processed for histological investigations. Serial 5 µm thick sections were cut and stained with haematoxylin and eosin and examined under bright field light microscopy.

Transmission electron microscopy

The pituitary gland of the animal groups were fixed in 2% glutaraldehyde in 0.1 M cacodylate buffer (pH 7.4) and post fixed in 1% osmium tetraoxide at 4°C. After critical washing in buffer, the specimens dehydrated in higher concentration of ethyl alcohol, cleared in propylene oxide and embedded in epoxy resin. Ultrathin sections were cut with a LKB Ultratome IV (LKB Instruments, Bromma, Sweden), mounted on grids and stained with uranyl acetate and lead citrate, and examined on a Joel 100CXl transmission electron microscope (Musashino 3-chome; Akishima, Tokyo, Japan).

Isoenzyme electrophoresis

Fresh samples of liver and prostate of both control and experimental groups were homogenized using 0.1 M Tris-HCl (pH 7.5) containing 20% sucrose and their protein content was determined according to Lowry et al. [14] and electrophoresis was carried out according to Laemmli [15]. The protein bands were stained with Coomassie blue R-250 (60 mg/L) in an acidic medium. For visualization of the tested enzymes, electrophoresis of tissues were carried out in the selected incubated medium for each kind of the assessed enzyme as follows.

Alkaline phosphatase electrophoresis (ALPase): AlPase isoenzymes (p-nitrophenyl phosphate substrate) were determined according to a previous study [16]. Known weights of fresh liver specimens were homogenized in 1 mL cold bi-distilled water and centrifuged at 300 g for 5 min at 4°C. An amount of 50 mL of clear supernatant samples was mixed with 20 mL of protein dye (1% bromophenol blue) and 20 mL of 2% sucrose. Thirty mL of the mixture per gel slot was used for enzyme electrophoresis. The isoenzymes were investigated by polyacrylamide gel electrophoresis for 20 min at 180 volts. Then the stained gels were fixed in 7% acetic acid (v/v). The AlPase plates may be inspected visually for the presence of the isoenzyme bands, and documented via scanning on HP Deskjet F370 All-in-One computer assembly.

Acid phosphatase isoenzymes: Staining was carried out using 30 mg naphthol-AS-MX-phosphate as substrate and 50 ml incubation buffer, 0.25 ml 0.1 M $MgCl_2$, 0.25 ml $MnCl_2$ 10%, 5 ml NaCl 20%, and 30 mg fast blue [17].

Glucose-6-phosphate dehydrogenase: The stacking gel (2.8% acrylamide) was prepared in 50 mm Tris-Pi (pH 6.3) and 20% sucrose and the separating gel (5% acrylamide) in 0.75 M Tris-Pi (pH 8). The electrophoretic buffer contained 5 mM Tris, 80 mm aspartate, and 20 µM $NADP^+$ at pH 7.4. Gels were stained for G6PD activity at 30°C in a solution which contained, in a volume of 20 mL, 1.2 mmol Tris-Pi (pH 8.5), 25% (v/v) glycerol, 30 µmol glucose-6-P, 4 g mol $NADP^+$, 6 mg p-nitro blue tetrazolium, and 0.5 mg phenazine methosulfate [18].

Sorbitol dehydrogenase: The method was carried out according to Harris and Hopkinson [19]. The staining solution composed of D-sorbitol (200 mg), pyruvic acid sodium salt (50 mg), pyrazole (50 mg), nicotinamide adenine dinucleotide (20 mg), PMS 92.5 mg), MTT (5 mg) and 0.2 M tris Hcl pH 8.0.

Malic dehydrogenase isoenzymes: Staining solution was carried out by mixing 50 mN TriseHCl (pH 8.5) 50 ml, nicotinamide adenine dinucleotide (NAD) 10 mg, maleic acid 1 ml (after neutralized with NaOH), nitro blue tetrazolium chloride (NBT) 10 mg and phenazine methosulphate (PMS) 2 mg [20].

Lactic dehydrogenase isoenzymes: LDH isoenzyme was determined according to Lehnert and Berlet [21]. After electrophoresis, the gels were incubated with H_2O 18.4 ml, 1 M Tris 4 ml, tetrazolium-blue 12 ml, phenazine methosulphate 4 ml, Na-lactate 4 ml and NAD 1.3 ml to develop color reaction for 20 min. In the color reaction, NAD and lactate serve as substrates, phenazine-methosulphate is the primary electron acceptor and tetrazolium-blue is the final electron acceptor.

Results

Histopathological observations

Liver: The liver of control rats possess normal histological structure feature. It is composed of anastomosing plates, usually one layer thick running radially from the central vein. Hepatic sinusoids are localized in between the hepatic cords and contained fine arrangement of Kupffer cells (Figure 1A).

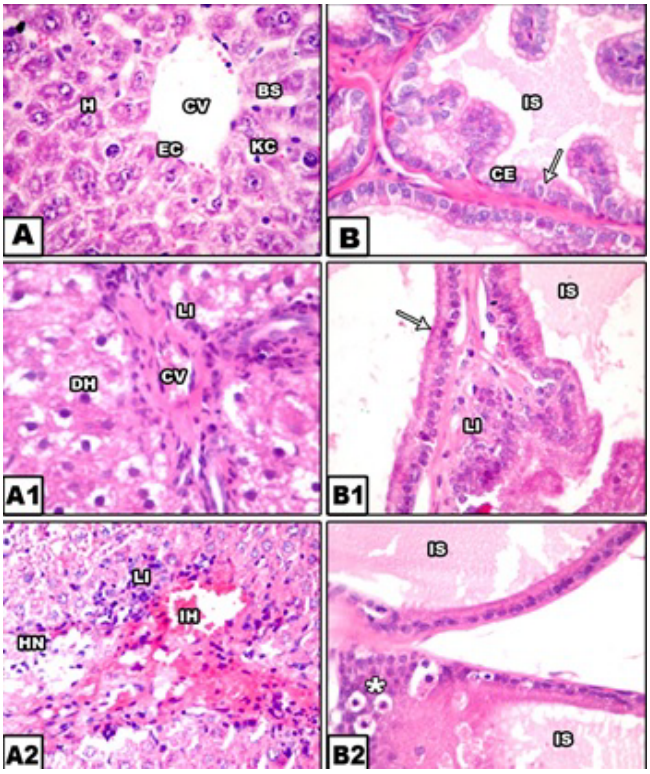

Figure 1: Photomicrographs of histological sections of liver (A-A2) and prostate gland of male rats. (A) Control liver. (A1) Low-dose-treatment showing vacuolated hepatocytes and leukocytic infiltration. (A2) High dose-treatment showing internal hemorrhage, hepatic necrosis and leukoctic infiltration. (B) Control prostate gland showing normal follicle. (B1) Low dose-treatment showing pyknotic lining epithelium and inter follicular leukocytic infiltration. (B2) High dose-treatment showing flattening of lining epithelium and vacuolar degenerated cells .X 400,HX-E. abbreviations; BS, blood sinusoids; CE, cuboidal epithelium; CV, central vein; DH, degenerated hepatocyte;EC, endothelial cell; H, hepatocyte; HN, hepatic necrosis; IH, internal haemorrhage;IS, internal secretion; LI, leukocytic infiltration; Star refer to vacuolar degenerated cells.

Low dose-treatment showed hepatocytes with abnormal cytological features of ballooning and vacuolar degeneration. Many of the nuclei appeared karyolysed. The central blood vessel become congested and densely infiltrated by leukocytes (Figure 1 A1).

On the other hand, high dose-treatment revealed massive damage of hepatic tissue. Dense leukocytic infiltration was detected. Periportal and mid-lobular hepatic necrosis were detected. Different pattern of necrotic and pyknotic hepatocytes were remarked (Figure 1A2).

Prostate gland: The control is composed of tubulo-alveolar glands. Each acini is lined by cubical epithelium of varying height and enclosed lumen containing hyalinized eosinophilic secretion. Some of the epithelium lining layer project inward forming papillary projections (Figure 1B). Low dose-treatment exhibited a marked atrophy and congestion of the alveoli and enclosed reddish inclusions suspected glandular secretion. The epithelial lining become atrophied and hyperplastic. Leukocytic infiltration was detected in the intertubular space (Figure 1B1). Low dose-treatment showed massive flattening of the epithelial cell lining the acini which become enclosed with reddish hyalinized inclusion. Some of the alveolar lining epithelium become vacuolated (Figure 1B2).

Transmission electron microscopy: In control, the somatotroph cells exhibit irregular-shaped nuclei with abundant euchromatin and peripheral alignment of heterochromatin on the nuclear envelope. Mitochondria are spherical and distributed throughout the cytoplasm. Rough endoplasmic reticulum is distributed in the peripheral cytoplasmic regions. Free ribosomes are distributed throughout the cytoplasm. Secretory granules are densely distributed around the nucleus and of uniform size. The gonadotroph cells include both follicle and luteinizing hormone cells. It is detected aligned near to the blood vessel. It is appeared as large ovoid to polyhedral. Heterochromatin aligned in the peripheral margin of nuclear envelope as well as dispersed throughout the nucleoplasm. The cytoplasm contains electron-dense secretory granules of varying sizes. The corticotroph cells are elongated with eccentric nuclei. Heterochromatin is in the form of granules arranged throughout the cytoplasm, rough endoplasmic reticulum and polysomes are abundant within the cytoplasm. Mitochondria and rough endoplasmic reticulum are more abundant in the cytoplasm of the gonado-and somatotroph cells (Figures 2 A-A5).

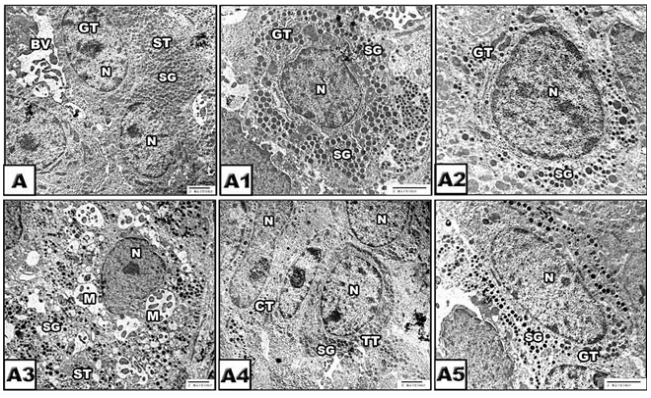

Figure 2: Transmission electron micrographs of anterior lobe of control pituitary gland showing gonadotroph cells with large cytoplasmic secretory granules (A-A2, A5), somatotroph with finely granulated secretory granules (A, A3) and corticotroph with spherical nuclei and less secretory granules (A4). Abbreviations: BV: Blood Vessel; CT: Corticotroph Cell; GT: Gondotroph Cells; M, mitochondria; N, nuclei; SG, secretory granules;; ST: Somatotroph Cell; TT: Thyrotrophic Cell.

Low dose-treatment damaged the studied pituitary cells such as compacted nuclear chromatin manifesting apoptosis and reduction of the cytoplasmic granules especially gonadotrophs and somatotrophs (Figures 3 A-A3).

High dose-treatment exhibited massive degeneration of the anterior pituitary cells. The cells possessed pyknotic nuclei, missing of secretory granules and abundant distribution of lysosomes. The blood vessels showed damage of their endothelial lining cells. Increased deposition of fat globules within the pituitary cells reflecting the most stricking pathological observation (Figures 4A-A5).

Isoenzyme electrophoresis: From (Figure 5), the expression of alkaline phosphatase isoenzyme inn liver and prostate of male subjected of both low and high dose-treatment. In liver, the control expressed two isoenzyme fractions of alkaline phosphatase. Both low and high dose-treatment exhibited decreased expression of the isoenzyme fractions. However high dose-treatment altered mobility of the isoenzyme fraction 1. Concerning acid phosphatase isoenzyme fractions I and II, there is no alterations of their rate of mobility, except decreased expression in high dose-treatment. Glucose 6 phosphate dehydrogenase expressed three isoenzyme fractions I, II and II. There is no change of the isoenzyme fractions except altered mobility of the isoenzyme fraction I in high dose-treatment. Sorbitol dehydrogenase expressed three isoenzyme fractions. In both low and high dose-treatment, there is no change of the isoenzyme mobility rate, however there is apparent decreased expression of the isoenzyme fractions. Malic dehydrogenase expressed three isoenzyme fractions. There is no change of the expression isoenzyme fractions, however, the rate of mobility was slightly altered in fraction I. Lactic dehydrogenase expressed three isoenzyme fractions. Compared to control, treatment decreased the expression of the isoenzyme fractions and altered their rate of mobility (Figure 5).

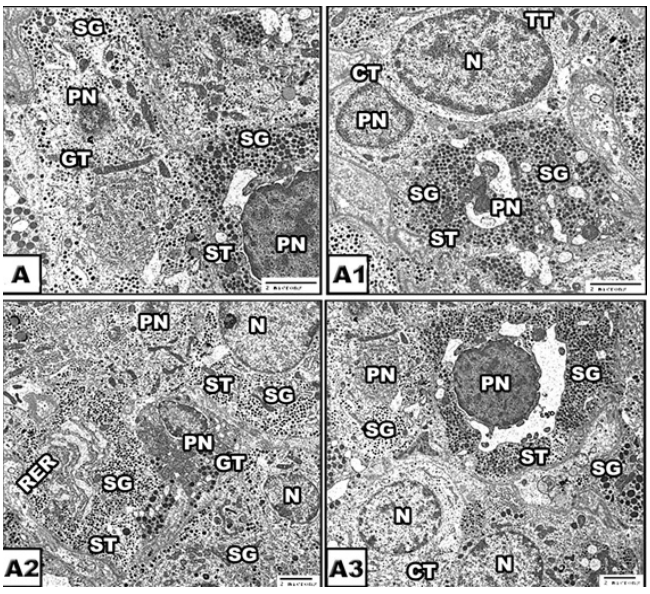

Figure 3: Transmission electron micrographs of anterior lobe of low dose-treated pituitary gland showing gonadotroph cells having pyknotic nuclei and comparatively decreased secretory granules (A-A2), somatotroph with pyknotic nuclei and degranulated cytoplasmic granules (A, A3) and corticotroph with pyknotic nuclei (A1). Abbreviations: CT: Corticotroph Cell; GT: Gondotroph Cells; N: Nuclei; PN: Pyknotic Nuclei; RER: Rough Endoplasmic Reticulum; ST: Somatotroph Cell; TT: Thyrotroph Cell.

In prostate, alkaline expressed two isoenzyme fractions in control. Low dose-treatment impaired the expression of the isoenzyme fractions, meanwhile no alteration was remarked at high dose-treatment. Also, acid phosphatase expressed two isoenzyme fractions which were altered only at low dose-treatment. Glucose 6 phosphate dehydrogenase, sorbitol dehydrogenase and maleic dehydrogenase expressed three isoenzyme fractions and showed no alteration of the applied dose-treatment. Lactic dehydrogenase expressed three isoenzyme fractions. Compared to the control, there was a marked decrease of the isoenzyme fractions (Figure 5).

Discussion

Our findings revealed that the nandrolone decanoate-treated exerted drastic alterations in hepatic tissues manifested ballooning and vacuolar degeneration of hepatocytes associated with leukocyte infiltration at low doses and periportal and mid-lobular hepatic necrosis at higher doses. Hypertrophy of Kupffer cells are detected along the hepatic sinusoids.

The present findings agree with the work of Karbalay-Doust and Noorafshan [22] on mice subjected for nandrolone decanoate-treatment.

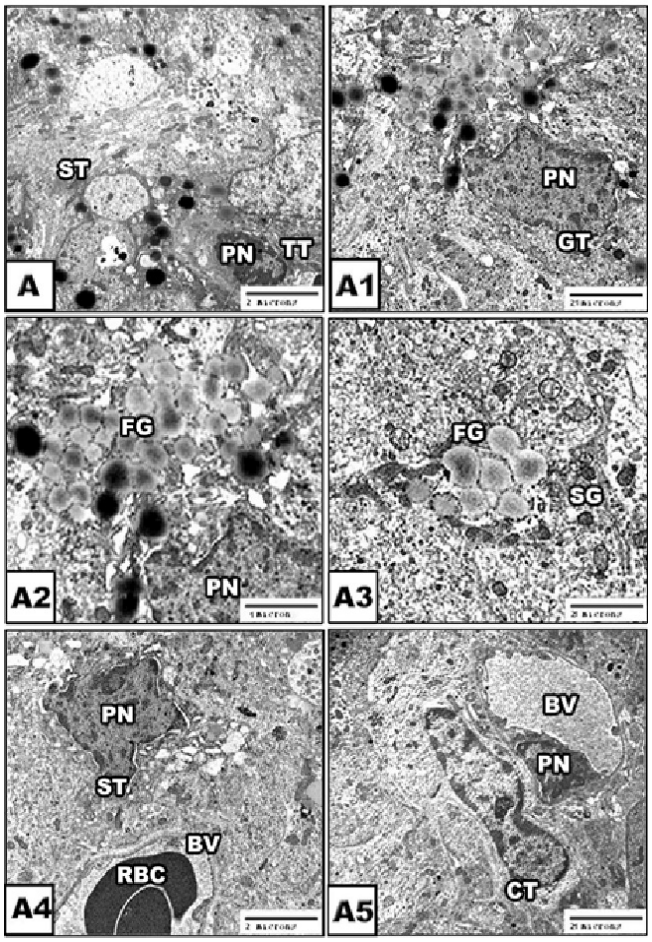

Figure 4: Transmission electron micrographs of anterior lobe of high dose-treated pituitary gland showing massive degeneration and apoptic cell death of different cells. Note the presence of increase deposition of fat globules in-between pyknotic gonado-and somatotroph cells manifesting suspected tumor formation (A2, A3). Abbreviations: BV: Blood Vessel; CT: Corticotroph Cell; FG: Fat Globule; GT: Gondotroph Cells; PN: Pyknotic Nuclei; RBC: Red Blood Cell; ST: Somatotroph Cell; TT: Thyrotroph Cell.

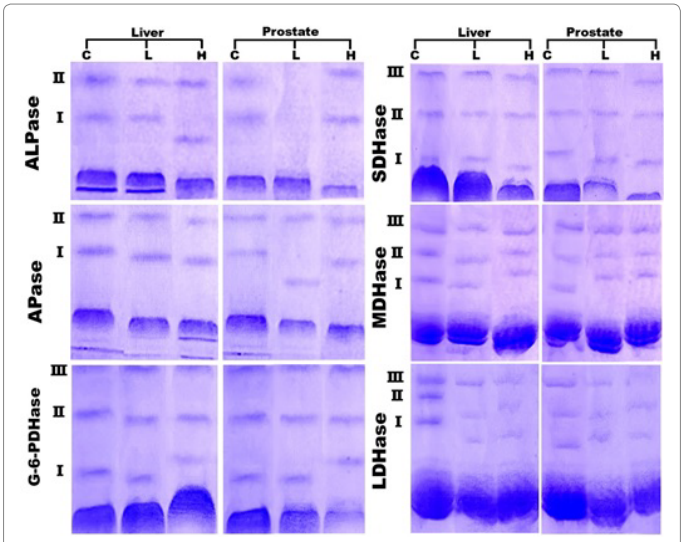

Figure 5: Isoenzyme electrophoresis of alkaline phosphatase (ALP ase), acid phosphatase (APase). Glucose 6 phosphate dehydrogenase (G6PDHase), sorbitol dehydrogenase (SDHase), maleic dehydrogenase (MDHase) and lactic dehydrogenase (LDHase) of liver and testis of control (C) and low dose (L) and high dose-treatment (H).

Vierira et al. [23] reported numerical increase of Kupffer cells in the liver parenchyma, and periportal fibrosis.

The observed histopathological alteration of liver coincides with disruption of liver isoenzyme fractions of alkaline phosphatase and sorbitol and lactic dehydrogenase. Similar findings were mentioned by Vieira et al. [23] who reported increased transaminases, alkaline phosphatase and decreased total proteins in serum of rat treated with nandrolone decanoate for 5 weeks. Many authors reported that alkaline phosphatase [24], sorbitol dehydrogenase [25] and lactic dehydrogenase [26] are predicted markers of hepatic damage.

The development of hepatic lesions may be attribute to the adverse effects of nandrolone decanoate-treatment (1 mg/100 g body weight, 8 weeks) on reduction the activities of mRNA levels of NADPH oxidase (NOX), catalase, glutathione peroxidase (GPx) and total superoxide dismutase (SOD), and thiol and carbonyl residue proteins [27]. Also, the drug-treatment induced histopathological changes in prostate glands manifested by congestion of the alveoli and hyperplasia of their epithelial lining cells. Leukocytic infiltration was detected in the intertubular space at low doses. Higher dose-treatment induced either flattening or vacuolar degeneration of the epithelial lining cells. Similar histopathological alterations were reported by Vargas et al. [28] in rats treated with 10 mg. Kg^{-1} body weight.

Gomes et al. [29] reported increased prostate lesions with TLR4 activation coincides with overexpression of TLR2, TLR4, NOX1, Nrf2, TNF-α, and P38MAPK in rat received ND-treatment. The observed prostate damage was confirmed by altered isoenzyme fractions alkaline and acid phosphatase and lactic dehydrogenase Alkaline [30] and acid phosphatase [31] and lactic dehydrogenase [32] alterations were found to predict prostate damage. Also, there is a detected decrease of secretory granules within the cytoplasm of somatotroph cells responsible for growth hormone secretion. Several studies have suggested an association between growth hormone and anabolic

androgenic steroids Male Wistar rats treated wiyh ND (15 mg/kg) every third day during three weeks increased the level of oestrone and decreased testosterone and androstenedione in the different among the groups [33]. The observed depletion of secretory granules in gonadotropin cells which in turn altered its hormonal secretion and consequently decreased the testosterone level showed a great linkage to prostate damage [34,35] as mentioned by ND-treatment.

It is also known that the gonadotropin-releasing hormone facilitates the endocrine hormone release and consequently regulates cell proliferation. Reduction or Inhibition of pituitary gonadotrophs led to a reduction a phosphotyrosine phosphatase (PTP) and initiate the mutagenic signal transduction of growth factor receptors and promote cancer cell proliferation [36].

Also, it is known that the gonadotroph cells are responsible for secreting two kinds of secretion follicle stimulating hormone and luteinizing hormone. The follicle stimulating hormone is responsible for promoting spermatogenesis meanwhile luteinizing hormone is important in stimulating the Leydig cells for secreting testosterone. Hypofunction of gonadotrophy by the observed reduction of its content of secretory granules led to impairment of testosterone section. Testosterone is metabolized via CYP19/aromatase into estradiol-17β which is important for the prostate gland through its interfering with growth and differentiation. Reduction testosterone secretion disrupts the normal function of prostate and develops the prostate lesions [1].

Conclusion

Finally the authors concluded that athletes and body building men be aware of using these drugs without regular physical examination to avoid their drastic effects on their body organs.

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