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Reversal of the hepatic damage induced by the supraphysiological dose of nandrolone decanoate after its withdrawal in the adult male rat

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ABSTRACT

Nandrolone decanoate is an anabolic-androgenic steroid that is frequently used at a very high dose to improve the physical performance. Recently, this drug has been abused by athletes to augment their muscle mass and improve their physical performance. However, this could have an impact on other body systems with the potential increase in its harmful effect. Therefore, the aim of this study was to evaluate the effect of administering a supraphysiological dose of nandrolone decanoate on the hepatic functions and structure of the adult rat and to test the potential reversibility after nandrolone withdrawal. Thirty adult male rats were equally divided into; control group, nandrolone-treated group (10 mg/kg/IM/weekly) for four weeks and recovery group (received nandrolone for four weeks followed by four weeks recovery). The results showed that nandrolone treatment led to a significant increase in the body weight gain and in the levels of serum alanine and aspartate transaminases. Moreover, the liver sections from nandrolone-treated rat showed; dilatation and congestion in the blood vessels, inflammatory cellular infiltration with hepatic fibrosis, severe vacuolar cytoplasmic degeneration, apoptotic hyperchromatic nuclei and partial loss of mitochondrial cristae in the hepatocytes. In addition, nandrolone treatment resulted in significant increase in the apoptotic index and the area percentage of GFAP positive stellate cells in the liver tissues. Importantly, withdrawal of nandrolone for 4 weeks rescued these biochemical and histological changes. In conclusion, our results showed that supraphysiological dose of nandrolone has hepatotoxic effects in the adult rat and showed that these toxic effects are reversible after treatment withdrawal.

1. Introduction

Anabolic androgenic steroids (AAS) are synthetic hormone derivatives of testosterone, which have either anabolic or androgenic effects according to the target tissue (Simao et al., 2015). AAS are usually used for the treatment of chronic debilitating diseases such as; AIDS, chronic lung disease, renal or hepatic failure, cancer and in cases of burns and recovery after surgical operations (Karbalay-Doust and Noorafshan, 2006; Kicman, 2008). Moreover, AAS are used as androgen replacement therapy in post-menopausal women (Arlt, 2006).

Beside the use of AAS in disease conditions, they are most frequently used in higher doses and for long durations among athletes such as; weightlifters, bodybuilders, and swimmers to improve their physical performance, strength and muscle mass (Nieschlag and Vorona, 2015; Piacentino et al., 2015). The abuse of these drugs is not only restricted to athletes, but also among members of general populations especially adolescents (Matrisciano et al., 2010). These individuals may take doses up to hundred times more than the therapeutic range, which lead to severe adverse effects on different body systems (Maravelias et al., 2005, Shahidi 2001). As a result of the multiple severe effects of high doses of AAS, they were banned by the International Olympic Committee (Mazzoni et al., 2011).

Nandrolone decanoate (ND) is one of the most commonly used AAS all over the world (Eklof et al., 2003). It has been used as a treatment of; human immunodeficiency virus-associated muscle wasting (Saha et al., 2009), prostate cancer and benign prostate hyperplasia (Kuhn, 2002). However, the average doses of ND used by the bodybuilder and the athletes (200–400 mg/week) are usually several times higher than the recommended (50 mg/week) therapeutic doses (Mazzeo and Ascione, 2013). The prolonged administration and/or using high doses of ND were shown to compromise the function and structure of different organs such as; testis, kidney, heart, and liver (Frankenfeld et al., 2014; Jannatifar et al., 2015).

The liver injury is the most common and serious side effect resulting from the abused use of ND. These side effects include; changes in the lipid profile, total protein level, and elevated levels of liver transaminases and alkaline phosphatase in a dose-dependent manner (Samieinasab et al., 2015). Moreover, prolonged administration of ND

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resulted in the development of cholestatic jaundice, liver tumors and peliosis hepatis with the potential increased risk of internal hemorrhage (Neri et al., 2011; Quaglio et al., 2009).

Despite the harmful effect of ND on different body systems, relatively few studies have looked at the potential reversibility following its withdrawal (Belardin et al., 2014; Karbalay-Doust et al., 2007). Therefore, the aim of this study was to identify any biochemical and structural changes that take place in the rat liver after the treatment with ND and to test the possible recovery after its withdrawal.

2. Materials and methods

2.1. Animals and experimental design

The present study was carried out on 30 adults male Wistar rats (6–7 weeks old) with bodyweight of 150–180 grams each. animals were housed in clean properly ventilated cages under similar conditions and had free access to the rat standard laboratory diet and water throughout the experimental duration.

The animal work has been conducted in accordance with the guidelines for the use of animals in research that were established by Tanta University, Egypt. These guidelines comply with the international guidelines set by national institutes of health guide for the care and use of laboratory animals (NIH Publications No. 8023, revised 1978). Following one week of acclimatization in the animal house, the rats were randomly divided into three main groups: **Group I (Control group):** included 10 rats that were kept without any treatment throughout the experiment. **Group II (Nandrolone decanoate-injected group):** included 10 rats that were treated with ND by deep intramuscular injection at a dose of 10 mg/kg once weakly for 4 weeks. The dose of ND was meant to be equivalent to the most common supraphysiological dose used by the bodybuilders (Andreato et al., 2013; Mirkhani et al., 2005; Mohamed and Mohamed, 2015). ND was purchased from El-Nile Company for Pharmaceuticals and Chemical Industries, Cairo, Egypt in the form of oily solution for injection. **Group III (Recovery group):** included 10 rats that received a weekly intramuscular injection of 10 mg/kg ND for 4 weeks (as in group II), followed by recovery period (without any treatment) for another 4 weeks.

2.2. Animal body weight

The rat body weight was measured prior to injection and weakly thereafter until the end of the experiment.

2.3. Liver enzymes

At the end of the experiment, all rats were fasted overnight then anesthetized with ethyl ether (Ha and Kim, 2013). Blood samples were immediately collected from the retro-orbital plexus using capillary tubes into clean dried centrifuge tubes. To separate the serum, the tubes were spun at 3000 rpm for 15 min and the serum samples were carefully separated using Pasteur pipettes and frozen at −20 °C until the time of biochemical analysis (Margoni et al., 2011). Serum alanine transaminase (ALT) and aspartate transaminase (AST) were measured with commercially available kits (Spin react, Spain) that were obtained from Bio-diagnostic, Egypt.

2.4. Histological analysis

Livers of the experimental animals were carefully dissected, washed with a cold saline solution and the upper parts of the right lobes were dissected for histological analysis. Some liver specimens were overnight fixed in 10% formol-saline at 4°C, followed by dehydration in ascending grades of alcohol and cleared with xylol. Impregnation was done in pure soft paraffin for two hours at 50 °C followed by embedding in hard paraffin (Chandler and Roberson, 2009). Sections of 5 µm thickness from each block were deparaffinized in xylol and rehydrated in descending grade of alcohol and water for either staining with hematoxylin and eosin (H&E) to study the general histological structure of the liver or with Mallory's trichrome to stain the collagen fibers (kiernan, 2008).

2.5. Immunohistochemistry of active caspase-3 and glial fibrillary acidic protein (GFAP)

The immunohistochemistry was done according to (El-Esawy et al., 2018). In summary, the deparaffinized liver sections were exposed to heat-induced antigen retrieval in 10 mmol/l sodium citrate buffers at pH 6 for 10 min in a microwave. After cooling for 20 min., the sections were rinsed in phosphate buffer saline (PBS) twice and incubated with 3% hydrogen peroxide in methanol for 10 min to block the endogenous peroxidase activity. The nonspecific protein binding was blocked by incubating the sections in 10% normal goat serum in PBS for 1 h at room temperature. The sections were then incubated at 4°C overnight in the diluted primary antibodies (rabbit-anti- cleaved caspase-3, Cell Signaling Technology) and (rabbit-anti-glial fibrillar acidic protein, GFAP, Dako). The sections were washed 3 times (15 min each) in PBS and were then incubated with the diluted secondary antibody (1:200) in for 2 h at room temperature followed by washing in PBS. The avidin-biotin complex was added to the sections for 20 min. The sections were then incubated with diaminobenzidine (DAB) as chromogen followed by staining with Mayer's hematoxylin as a counterstain. Lastly, the slides were dehydrated, cleared and mounted with DPX. All slides were examined and photographed using Leica light microscope with a built-in camera.

2.6. Transmission electron microscope analysis

Processing samples for electron microscope were done according to (Woods and Stirling, 2008). To summarize, very small pieces of liver tissues ($1mm^3$) were fixed in 2.5% phosphate-buffered glutaraldehyde for two hours at 4 °C. After rinsing in PBS, the specimens were then post-fixed in prepared 1% phosphate buffer osmium tetroxide for one hour at 4 °C. After that, the specimens were dehydrated in ascending grades of alcohol at 4 °C. Sections were then immersed in propylene oxide and were embedded in epoxy resin mixture. Semithin sections (0.5–1 µm thick) were cut by the automatic LKB ultramicrotome, mounted on glass slides, stained with toluidine blue and examined under the light microscope to select the suitable areas. Ultrathin sections (80–100 nm thick) were cut and contrasted with uranyl acetate and lead citrate for examination under JEOL-JEM-100 transmission electron microscope (Japan).

2.7. Morphometric results

The analysis of images was performed using Image J software (4.8) from three non-overlapping sections from each animal (Abramoff et al., 2004). The following parameters were measured;

- The area percentage of collagen fibers as a proportion of the total area of the field from Mallory's trichrome stained liver sections at X400 magnification (Ross et al., 2016; Safer, 2017).
- The apoptotic index through counting (caspase-3 positive cells divided by the total cells number in the field) X100 from caspase-3 immunolabelling liver sections at X 400 magnification (Bressenot et al., 2009).
- The area percentage of GFAP positive hepatic stellate cells as a proportion of the total area of the field from GFAP immunolabelling liver sections at X400 magnification (Bahey et al., 2015).