



Original article

The impact of nandrolone decanoate administration on ovarian and uterine tissues in rat: Luteinizing hormone profile, histopathological and morphometric assessment

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ABSTRACT

The study had been conducted to evaluate the effects of nandrolone decanoate (abused repeated doses) on female rat's ovary and uterus during administration and withdrawal. The study included 18 rats that were divided into control group ($n = 6$) and treated group ($n = 12$). The treated group was injected intramuscular (IM) with nandrolone decanoate (7 mg/kg body weight) for three consecutive days, for two weeks. The study stated that nandrolone decanoate increases the weights of body, ovary, and uterus. Moreover, it has a tendency of bringing upon modifications in the biochemical, histopathological, and morphological makeup of the female reproductive aspects. In conclusion, nandrolone decanoate has been identified as deleterious element for the female rats, and it is suggested that keen observations must be made on the human abusers to control and manage the possible pathologies.

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1. Introduction

The use of androgenic anabolic steroids (AASs) is observed commonly among athletes, youth, and adults in contrast to the common perception. In clinical settings, these compounds are identified as the synthetic analogues of testosterone. Clinically, AASs are usually recommended for the treatment of various pathological conditions; including renal insufficiency, endometriosis, hereditary angioedema and breast cancer (Belardin et al., 2014).

Drug administration has been practiced since many years to enhance the ergogenic effects. AASs are amongst common 'drug of illicit nature' that are mainly taken by athletes along with other stimulants. Barceloux and Palmer (2013) have identified AAS abusers as the poly-drug users. AASs are typically administered via intramuscular route. The drug use cycle lasts for about 6–12 weeks, which is observed with abstinence between the periods to avoid any adversarial outcome (Barceloux and Palmer, 2013).

Women usually have irregularities in menstrual cycles, enlargement of clitoris, voice deepening, and growth of facial hairs with AASs usage. At cellular levels, testosterone has been identified as an inhibiting factor for the endothelial nitric oxide synthase (eNOS) that can ultimately reduce the production of nitric oxide (NO) in the cells and induce oxidative stress (Skogastierna et al., 2014).

Nandrolone decanoate is a popular AASs, which is used by athletes to boost their physical performance and body image. This compound is also known to induce aggression, depression, and many other adverse effects (Rainer et al., 2014). A study indicated that subcutaneously injection of nandrolone decanoate (7.5 mg/kg BW/week) for 30 consecutive days increases the count of atretic follicles and reduces the ovarian oocytes in female rats and mice (Camargo et al., 2014). The objective of this experimental study was to evaluate the effects of nandrolone decanoate (abused dose) among female rat's on the ovaries and uterus during administration and withdrawal for 3 consecutive days per week for two weeks by means of histopathological and morphometric assessment.

2. Material and methods

2.1. Chemicals

Nandrolone decanoate (17 β -hydroxy-19-nor-4-androstene-3-one) was purchased from local pharmacy. It is an injectable

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solution, containing 25 mg of the androgen in 1 ml, available as oily solution.

2.2. Animal models

Eighteen female adult rats with regular estrous cycle were obtained from the animal house of King Fahad Medical Research Center (KFMRC) with the approval of local university ethical committee on the study protocol. The average weight of the rats was 132 g. The settings, within the animal house, had been established for two weeks at the requisite laboratory conditions. The control group (n = 6) received IM injections of 0.1 ml of mineral oil as vehicle for 3 consecutive days each week for two weeks. The treated animals (n = 12) received a daily IM injection of ND diluted in vehicle for 3 consecutive days each week for two weeks. The dose employed in this study simulated the abusive dose of steroid utilized by youth at fitness centers. The first injection was administered at the estrus phase of the cycle in all animals. Blood samples were obtained from rats of the treated group after the first week (n = 3) and second week (n = 3) post-injection. While blood samples were obtained from the withdrawal group (n = 6) after the first (n = 3) and second week (n = 3) after stopping the ND injection.

2.3. Method and experimental protocol

The body weight; of both the control and treated groups, were recorded before the injection (initial), treated, and withdrawal. Moreover, the weight and size of ovary and uterus were also recorded at the end of the experiment. Blood samples from all the rats were obtained from the retro-orbital vein of the eye using capillary tube. The sera was separated for the estimation of luteinizing hormone (LH) using Rat Eliza LH kit (MyBioSource, San Diego, CA, USA).

Rats were exterminated by ethyl ether inhalation before dissection of the pelvis to obtain the ovaries and uterus. The organs were removed from all groups weighed and immediately fixed in 10% formalin, dehydrated, embedded in paraffin and sectioned at 4–5 μm . The stains used in the observations included Hematoxyline, Eosin and Mallory trichromatic stain. The slides were examined under light microscope for histopathological and morphometric assessment. The thickness of myometrium was measured using Figi software (Fiji co., Fiji Island).

2.4. Statistical analysis

The Statistical Package for Social Science (SPSS 20 for Windows) program was used for data analysis. The numerical data are presented as the means \pm standard error (SE). One-way analysis of variance (ANOVA) was used for the comparison of variables between control and other groups. Statistically significant results were considered at a P-value < 0.05.

3. Results

The initial body weight of adult control rats was (107 \pm 16.325 g), while in both treated and withdrawal groups it was around 132 \pm 16.28 g. The evaluation observed a marked increase by first week of the experiment, which has been about 147.6 \pm 17.18 g, followed by a decrease in the second week, which was 126 \pm 11.1 g. The withdrawal group presented that the body weight has returned closer to the initial body weight by the second week of experiment (Table 1).

The weight of ovary and uterus of adult control rats was 0.82 \pm 0.98 g in the 1st week. It has been observed that the weight

of organs was increased by 1.9 \pm 1.38 g in the first week of experiment, followed by a decline by 1.15 \pm 1.46 g in the second week in the ND treated groups. Moreover, withdrawal group displayed an increase in the weight of ovary and uterus by 1.44 \pm 1.22 g during second week of post administration (Table 2).

The LH profile in the test group of rats showed marked drop to 0 IU/ml as compared to the control group (1 IU/ml). However, withdrawal of the drug administration in the second week resulted in marked increase of LH hormone to 3.2 IU/ml (Fig. 1).

The thickness of myometrium of control group was found to be 120.33 \pm 12.25 μm . At the first week and second week; post injection, it became 144.97 \pm 0.56 μm and 179.80 \pm 5.33 μm , respectively. During the period of withdrawal, the thickness had been found to be decreasing to 166 \pm 6.0 μm and 157.07 \pm 8.67 μm , in first and second week of the experiment, respectively (Table 3).

Histopathological observations revealed that ovaries from the control group has a normal cortical follicles appearances at different stages of development (arrows), corpus luteum structures could also be observed (Fig. 2A).

Table 1

Body weights (grams) of rats injected IM, with 7 mg/kg body weight of nandrolone decanoate in all studied groups.

Groups	Initial (g)	1st week (g)	2nd week (g)
Control (n = 6)	107.00 \pm 16.325	107.00 \pm 10.30	102.30 \pm 10.11
Treated (n = 6)	132.00 \pm 16.28*	147.60 \pm 17.18*	126.00 \pm 11.10*
Withdrawal (n = 6)	132.00 \pm 16.28	125.34 \pm 8.00	131.34 \pm 11.45

* Significant versus initial weight of the same group.

Table 2

Weight of ovary and uterus (grams) of rats injected IM with 7 mg/kg body weight of nandrolone decanoate in all studied groups.

Groups	1st week (g)	2nd week (g)
Control (n = 6)	0.82 \pm 0.98	0.80 \pm 0.98
Treated (n = 6)	1.90 \pm 1.38*	1.15 \pm 1.46*
Withdrawal (n = 6)	1.39 \pm 1.00	1.44 \pm 1.22

* Significant at P < 0.01; 0.05.

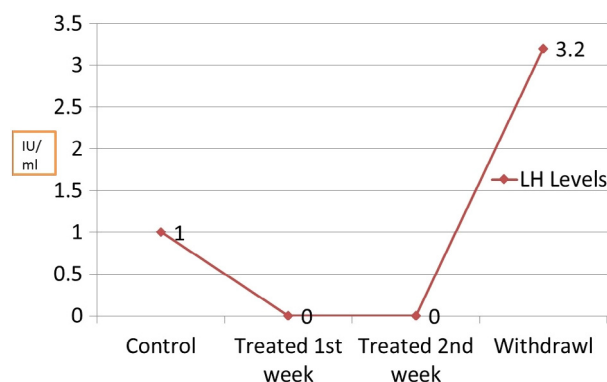


Fig. 1. LH fluctuation in the sera of rats injected IM with 7 mg/kg body weight of nandrolone decanoate in all studied groups.

Table 3

Thickness of myometrium (μm) of rats injected IM with 7 mg/kg body weight of nandrolone decanoate in all studied groups.

Groups	Initial (μm)	1st week (μm)	2nd week (μm)
Control (n = 6)	120.33 \pm 12.25	–	–
Treated (n = 6)	–	144.97 \pm 0.56	179.80 \pm 5.33*
Withdrawal (n = 6)	–	166 \pm 6.00	157.07 \pm 8.67*

* Significant at P < 0.01; 0.05.

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