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Physiological And Histological Toxic Effects Of Different Doses Of Nandrolone Decanoate On The Kidney Of Male Mice

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ABSTRACT

Anabolic androgen steroids are widely used among athletes to increase muscles mass. Some patients with chronic kidney disease are also used these drugs. But the effect of these drugs on the kidney structure receives less attention.The current study was performed to investigate the histopathological effects of nandrolone decanoate injection in kidney tissues of adult male mice. Thirty adult male mice (balb / c) were randomly divided into three groups (10 mice in each group): two groups treated were injected subcutenously in the distled region of femur with 50 and 100 mg / kg b.w of Nandrolone Decanoate every 2 days for 4 weeks , while the control group was injected with 0.9% normal sline solution. Two physiological parameters were performed including urea and creatinine levels. The results showed that there was a significant increase (P <0.05) in kidney weights and also significant increase (P <0.05) in levels of urea and creatinine in all experimental groups compared with control group. All injected groups showed clear histological changes in the structure of the kidneys compared with the control group. These changes included a significant increase (P <0.05) in diameters of the Bowman's capsule, proximal, distal and collecting tubules in the experimental groups compared with control group. Moreover, certain pathological changes have been observed such as enlargement and increased cellularity of the renal glomeruli, degeneration of the lining epithelium of renal tubules, blood hemorrhage inside the tubules and congestion of the renal blood vessels with infiltration of the inflammatory cells. In this paper harmful toxic effects observed in the kidney's tissues.

Keywords: Nandrolone deconoate, Histological changes, kidney tissue,Renal tubules, Urea , Creatinine

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INTRODUCTION

Anabolic androgenic steroids (AASs) is the common name for male sex hormone-related synthetic substances (such as testosterone) That promotes the development of male sexual characteristics in males and females (androgenic effects) and the growth of skeletal muscles (anabolic effects) [1]. Athletes can abuse these compounds to increase the mass of their muscles by increasing protein synthesis [2]. The Anabolic steroids are an important therapeutic target for the treatment of diseases such senile osteoporosis [3] and hypogonadism [4,5]. Together with other hormones, these steroids are used to treat certain types of anemia such as Fanconi's anemia [6], and to stimulate skeletal growth in pre-pubertal boys with pituitary dwarfism (delayed puberty) [7]. Additionally, the use of AAS may lead to severe adverse effects like kidney failure, hepatic failure, cholestasis, low levels of high-density lipoprotein (HDL), acne, vulgaris and gynecomastia [8,9]. AASs also induced side effects in both humans and experimental animals which disrupts the production of testosterone and gonadotropins [10]. In the past several decades it has been known that anabolic steroid use by athletes is increasing [11], these steroides induced chemical abnormalities seen in athletes are accompanied by histological changes. Although nandrolone decanoate can cause acute renal failure as a side effect but it is widely used in patients with end stage renal failure as adjuvant therapy to the parenteral nutrition [12].We used animals in the present study to assess the pathology resulting from the use of anabolic steroids. The main aim of this research is to determine the histopathological toxic effect of deconoated nandrolone on the kidneys in different doses.

MATERIALS AND METHODS

Animals and experimental design

Thirty Swiss male *Mus musculus* mice, 3 months old, weighing (25-30) gm under controlled temperature (25 ± 2) ° C and 12h light: 12h dark in metal cages in this study. Standard foods and water were given to animals *ad libitum*. The animals were brought from the animal house of the College of Science / University of Babylon The animals were randomly distributed in three groups (n = 10 per group) between the control and treatment groups. The mice were injected subcutaneously (in distled region of femur) with 50 mg / kg and 100 mg / kg of nandrolone decanoate every two days for 4 weeks, and during the same treatment period, 0.9% of normal saline solution was administered to the control animals.

Blood collection and biochemical tests Blood was obtained by heart puncture at the end of the treatment time and serum was isolated by centrifugation (3000 rpm for 15 minutes) to estimate levels of urea and creatinine in the blood.

Kidney function was assessed by estimation of urea and creatinine in the blood. Kits of urea and creatinine were obtained from Spinreact, S.A.Ctra.Spain. Creatinine was measured using the described method by [13]. Urea was measured using the enzymatic methods [14].

Histological examination

Kidney was isolated and fixed in formalin solution(10%) for 24 hours and dehydrated through increasing concentrations of ethanol and washed then embedded in paraffin and cut into section 4-5 µm thickness, stained with hematoxylin and eosin [15]. Then the sections were examined by using compouned microscope and the

measurments were acording by using ocular micrometer after calibrated with stage micrometer . Ten readings for the diameter of Bowman's capsule, diameter of proximal, distal tubules and collecting tubules was measured for each animal.

Statistical Analysis: The statistical program SPSS (Version 16) by One-way ANOVA was used to analyze the experimental data . The data are presented as mean± standard error. The significance level was determined at (P <0.05).

Results & DISCUSSION

Drug-related kidney disease is common and responsible for a variety of pathological effects on the kidneys. The kidney is structurally a complex organ, and any damage to the kidney are as complex as its structure. Toxic substances such as drugs or other chemicals may cause interstitial and tubular disorders [16]. The administration of nanodrolone caused a significant increase (P <0.05) in the kidney weights of the experimental groups compared with the control group figure (1). These results were agree with the results of [17] , who found that there was an increase in the size and weight of the kidney in mouse treated with nandrolone decanoate and this is due to increased glomerulus size and tubular hypertrophy .

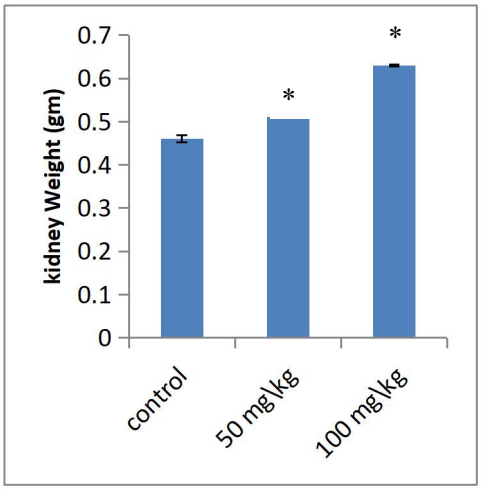


Figure (1):kidney weight (gm) in control and experimental groups , results are shown as mean ± SEM, * P <0.05 compared with the control group.

in addition, another study found that the kidney weight had increased by about 30 % in animals treated with nandrolone [18,19]. The current investigation showed significant (P<0.05) increase in urea and creatinine levels in experimental groups compared with the control figure(2) and (3). This increase in serum urea and creatinine levels may be due to the negative effects of anabolic steroids on the kidneys [6]. These results appear to support the results obtained by [20], who observed a kidney dysfunction in young athletes who had been taking anabolic drugs in high doses for months.The result of histological examination in this study supports this result because they showed negative effects of nanodrolone deconoate on kidney tissue, and this affect kidney function represented by elevated in serum urea and creatinine.

This increase can be attributed to a decrease in the glomerular filtration rate due to histopathological changes that occurred in the kidney tissue [21]. These findings are consistent with the results of [22] who showed a significant increase in the levels of creatinine and urea between the high-dose groups of ND, which refers to the negative impact of ND on renal function.

these parameters are essential for the normal function of kidney and any change in these parameters may indicate renal dysfunction [23] .from a functional perspective, It has been documented that exposure to AASs has contributed to elevated serum creatinine , uric acid and blood urine nitrogen [24,25].

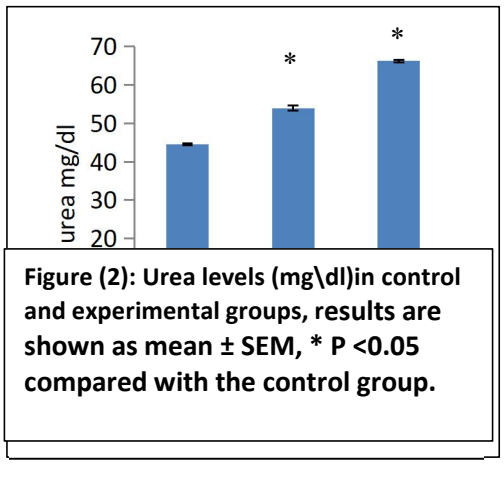


Figure (2): Urea levels (mg\dl)in control and experimental groups, results are shown as mean ± SEM, * P <0.05 compared with the control group.

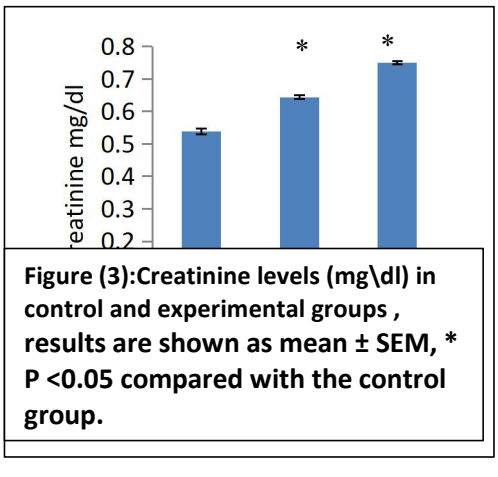


Figure (3):Creatinine levels (mg\dl) in control and experimental groups , results are shown as mean ± SEM, * P <0.05 compared with the control group.

The present study revealed that the diameter of Bowman's capsule, diameter of proximal tubules , distal tubules and collecting tubules were significantly higher (p <0.05) in experimental groups compared with the control group (4),(5),(6)and(7), this increase, may be attributed to nandrolone effects . These results agreed with [26] who found that androgens when administered to overiectomized rats leads to tubular hypertrophy and increase in the size of the glomerulus.

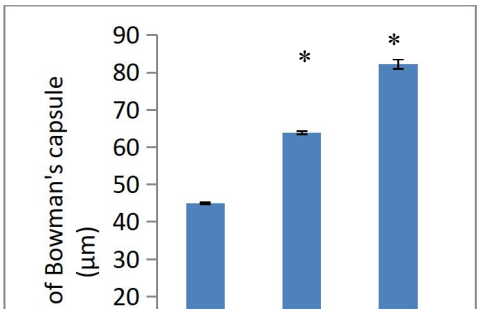


Figure (4):Diameter of Bowman's capsule (µm) in control and experimental groups , results are shown as mean ± SEM, * P <0.05 compared with the control group.

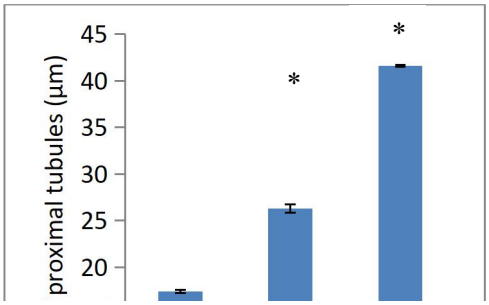
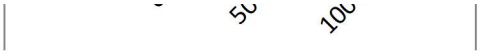


Figure (5):Diameter of diameter of Proximal tubules (µm) in control and experimental groups , results are shown as mean ± SEM, * P <0.05 compared with the control group.



It is believed that anabolic steroids have an effect by binding to androgen receptors at the cellular level, translocate to chromatin- binding sites, promot gene transcription, and stimulate mRNA production and thus increase protein synthesis [27]. Androgens work on a number of organs including the liver, kidneys, heart mus].

The main function of androgens is to stimulate cell growth through hypertrophy and hyperplasia [30]. In addition to its effect on a variety of androgen regulating genes that are expressed in the proximal tubule, some regulatory effects have been recorded on the genes that are expressed in the distal parts of the renal tubules [31].

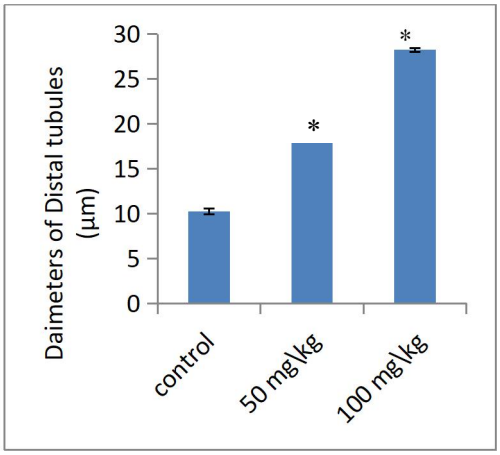


Figure (6):Diameter of diameter of Distal tubules (µm) in control and experimental groups , results are shown as mean ± SEM, * P <0.05 compared with the control group.

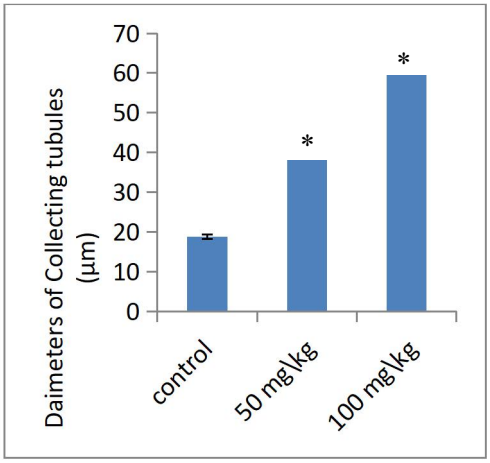


Figure (7):Diameter of diameter Collecting tubules (µm) in control and experimental groups, results are shown as mean ± SEM, * P <0.05 compared with the control group.

The results of current study indicate significant renal damage following androgenic-anabolic steroid administration including :- enlargement and increased cellularity of the renal glomeruli , degeneration of the epithelium lining of renal tubules and blood hemorrhage inside the tubules and congestion of renal blood vesseles with infiltration of inflammatory cells.

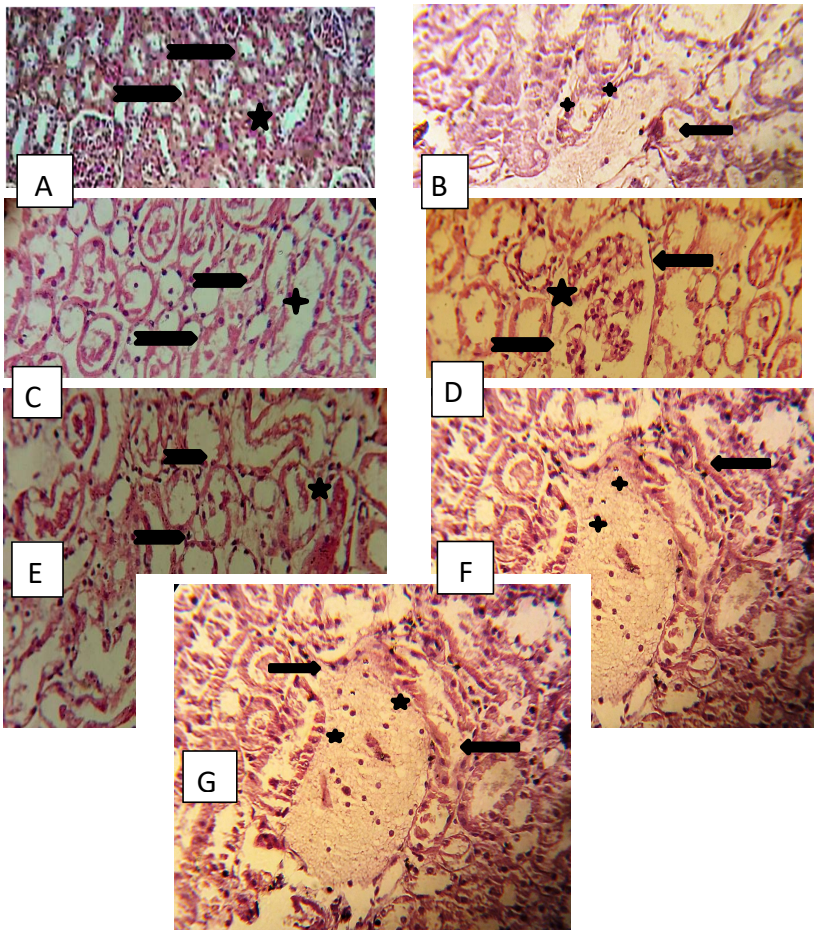


Figure (8) : Light micrograph of kidney section from tested mice stained with H.E. (400X) illustrated: (A) Normal kidney histoarchitecture (control group) , (B) ND -treated mice at 50mg/kg show enlargement of the renal glomeruli ( ) congestion of renal blood vessels with infiltration of inflammatory cell ( ).(C) ND -treated mice at 50mg/kg show degeneration of the epithelium lining of renal tubules ( ) (D,E,F,G) ND -treated mice at 100 mg/kg show enlargement ( ) and increased cellularity of the renal glomeruli , degeneration of of the lining epithelium of renal tubules ( ) , blood hemorrhage inside the renal tubules and renal glomeruli ( ) and congestion of the renal blood vessels with infiltration of the inflammatory cell ( )

These findings were consistent with [32] they stated that pathological changes in the rat kidney after administration of high doses of nandrolone decanoate. And this may be due to the toxic effect of the main metabolite of nandrolone decanoate on tubules. Androgens may induce renal disorders through altering the amounts of cytokinases and growth factors. It is believed that transforming growth factor plays a pivotal role in nephropathy by inducing the proliferation of mesangial cells [33]. The proliferation of glomerular cells observed in our study is probably the result of nandrolone effects. The current histopathological results confirm the findings of [34] who documented severe nephritis changes in body builders who used Deca Durabolin in high doses for 10 weeks. For instance, the study conducted by [35] showed that the toxic effects of long-term use of these drugs on target organs such as liver and kidneys induced by the accumulation of certain toxic testosterone metabolites such as (17α-19-nortestosterone and 17α-testosterone).

Moreover, other researchers such as [36,37,38] found that certain types of anabolic steroids can cause large necrosis and damage to the target organs in the form of chromatin condensation, breaking the DNA strand and cytoplasmic contraction.

CONCLUSION

from this histopathological damages that athletes' prolonged use of steroids (nanoderolone) can cause significant destruction to the natural structure of the kidneys which can lead to severe kidney disorders such as renal failure in the late stages.

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