MORPHOMETRICAL ANALYSIS OF LIVER HEPATOCYTE IN RABBITS TREATED WITH NANDROLONE DECANOATE: PART TWO

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ABSTRACT

Background: The use of anabolic steroids among competitive athletes to enhance their physical performance is widespread. Numerous reports have noted hepatotoxicity secondary to anabolic steroid use. Androgenic-anabolic steroid will disturb the regular endogenous production of testosterone and gonadotrophins.

Objective: To study the effect of nandrolone decanoate on the number, the long diameter of hepatocytes, and the long diameter of nucleus using morphometrical method.

Materials and Methods: Twenty eight rabbits were divided into 4 groups and each group includes 7 animals. Group (1) was the normal control group that was injected with normal saline. Group (2) was injected with 2mg/kg body weight of nandrolone decanoate. Group (3) was injected with 4mg/kg body weight of nandrolone decanoate. Group (4) was injected with 6mg/kg body weight of the drug. The drug was administered weekly for three months by deep intramuscular injection in the thigh. The number hepatocytes, their long diameter and the long diameter of the nucleus were estimated by using Visopan Projection Microscope.

Results: The results showed that the number of hepatocytes in group (3) was significantly higher (28.43±7.83) at P≤ 0.05 compared to other groups. The long diameter of hepatocytes of group (2) which received the lowest dose was normal, while group (3) and (4) showed significant increase in the long diameter of hepatocytes which was (10.71±1.98) in group (3), and (12±1.91) in group (4) compared to normal control group which was (8.86± 1.22). Group (4) had the longest diameter of the nucleus of hepatocytes which was (4.57±1.13).

Conclusion: On the basis of these results, nandrolone decanoate may cause several morphometrical changes in the hepatocytes of treated rabbits.

INTRODUCTION

Liver is a target of anabolic steroid action. It is morphologically and functionally modulated by sex hormones.[1] Anabolic steroids, like other drugs, have been the subject of controversy. They have been frequently linked in the media to dangerous side effects and high mortality rates.[2] But, they are used widely in medicine with an accepted side effect profile, providing patients are monitored for possible complications.[3-6] The main untoward effects of androgenic-anabolic steroid abuse that male athletes most often self-report are an increase in sexual drive, increased body hair and increment of aggressive behavior. Androgenic-anabolic steroid administration will disturb the regular endogenous production of testosterone and gonadotrophins.[7] The use of drugs to enhance physical performance has been observed for several years. Today, individuals continue to use a variety of substances, including androgenic-anabolic steroids, in the hope of enhancing their performance.[8] Experimentally, the effects of androgenic-anabolic steroids on the liver are well documented because these compounds induce several proteins, like carbonic anhydrase-III, and repress the expression of others, e.g. the marker protein-2 and the low affinity glucocorticoid binding sites.[9] Nandrolone decanoate has a specific binding site in male rat liver microsomes. Although, the role of this binding site is, at present, unknown, the fact that adult male rat liver presents a potential membrane receptor for androgenic-anabolic steroids offers new insights into its hepatic effects.[10]

The aim of this study is to identify the morphometrical changes of the hepatocytes in rabbits treated with the anabolic steroid nandrolone decanoate.

MATERIALS AND METHODS

Twenty eight rabbits were divided into 4 groups, 7 animals each. Group (1), the normal control group, was injected with normal saline. Group (2) was injected with nandrolone decanoate (Deca-Vinone, Hikma pharmaceuticals, Amman-Jordan) 2mg/kg body

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weight. Group (3) was injected with nandrolone decanoate 4mg/kg body weight. Group (4) was injected with 6mg/kg body weight of the drug. The medicine was administered weekly for three months by deep intramuscular injection in the thigh. Both the exposed and the control animals were killed by ether inhalation 2 weeks after the last injection. Pieces of 1-2 cm in length were taken from each liver. Each piece was cut into slices about 3-4 mm thickness and fixed in 10% formalin solution, and then the tissue dehydrated in graded alcohol, cleared by xylol, and embedded in paraffin. Two paraffin blocks from each portion was done and thin sections of 5 microns were made using Reicheart Rotary Microtome. Sections were mounted on slide and stained with hematoxylin and eosin stain.[11] The number of the hepatocytes, the long diameter of the hepatocytes, and the long diameter of the nucleus were estimated using Visopan Projection Microscope. A 500X magnification achieved with 40/0.65 objective lens was used, each division of the measuring rule corresponds to a length of 2 microns=0.002 mm. The unit area used for counting the hepatocytes was 0.0144mm².

Statistical analysis

The statistical analysis was done between different groups of rabbits using Duncan's test at P≤0.05 as statistically significant.[12]

RESULTS

The number of Hepatocytes

The number of hepatocytes in the control group was 22.14±2.61, and it was 22.57±3.74, and 20±3.16 in groups (2), and(4) respectively. In group (3) it was 28.43±7.83 which was significantly higher at P≤0.05 compared with other groups (**Table-1)**.

Table 1. Number of hepatocytes in the studied groups.

Groups	No. of hepatocytes (mean ±SD)
1	22.14±2.61(b)
2	22.57±3.74(b)
3	28.43±7.83(a)
4	20±3.16 (b)

Different letters vertically means significant difference at P≤0.05.

The Long Diameter of Hepatocytes

In the control group (1), the long diameter of the hepatocytes was 8.86±1.22(**Table-2**, **Figure-1**) and it was 8.62±1.51, 10.71±1.98 and 12±1.91 in groups (2), (3) and (4) respectively(**Table-2)**. In group (2) the hepatocytes were more or less the same as the control group and not affected by the drug (**Figure-2**). While in groups (3) and (4) there was severe ballooning degeneration of the hepatocytes and this was more evident especially in group (4) (**Figure-3)**.

Table 2. Long diameter of hepatocytes in different groups of rabbits.

Groups	Long diameter of hepatocytes (mean ±SD)
1	8.86±1.22(b)
2	8.62±1.51 (b)
3	10.71±1.98(a)
4	12±1.91 (a)

Different letters vertically means significant difference at P ≤0.05.

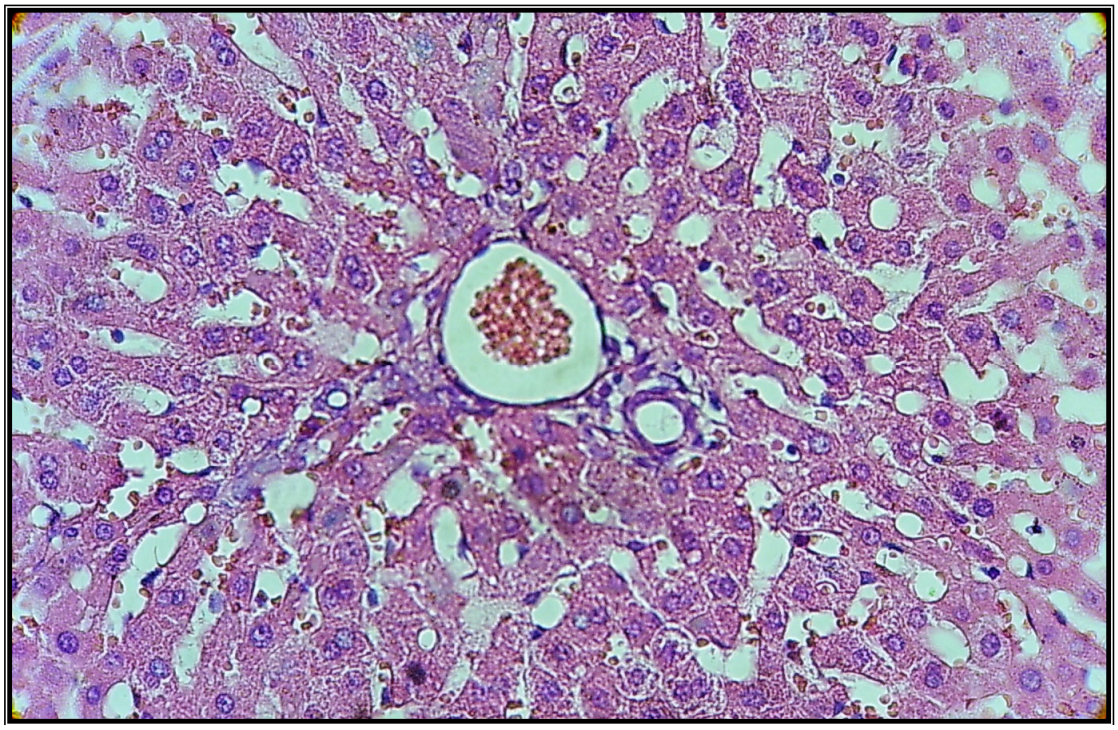

Fig 1. Photomicrograph of liver of group 1 showing the normal hepatocytes. (H&E X 400).

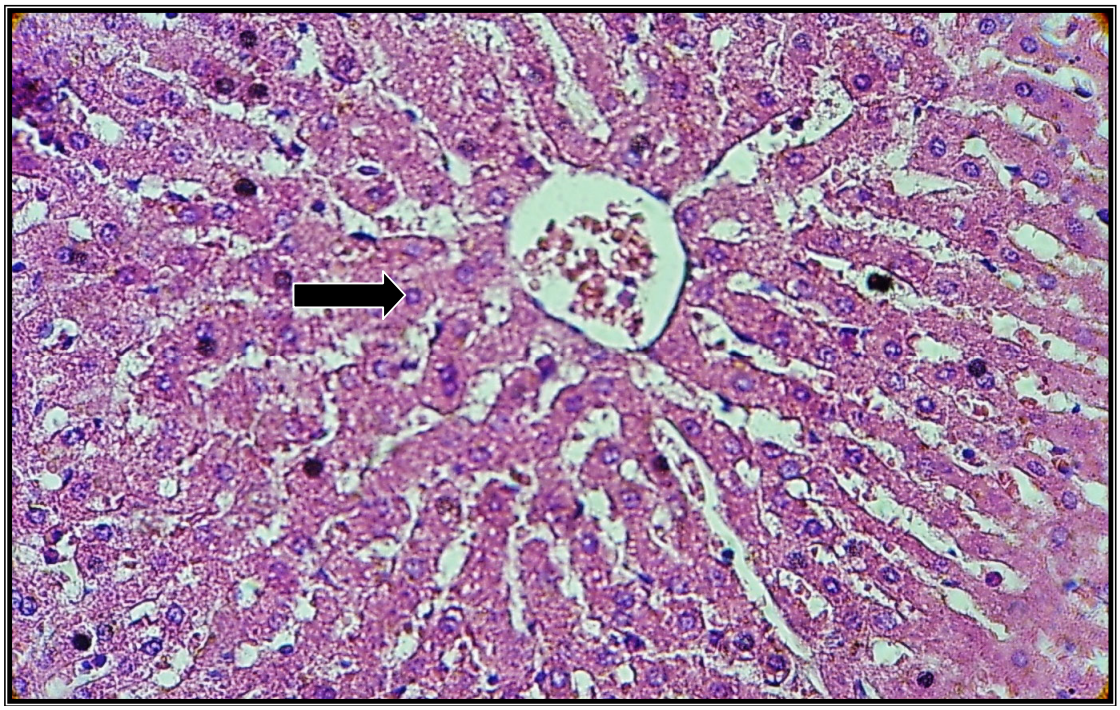

Fig 2. Photomicrograph of liver of group 2 showing the central venule congested with blood and mild swelling of hepatocytes(black arrow). (H&E X 400).

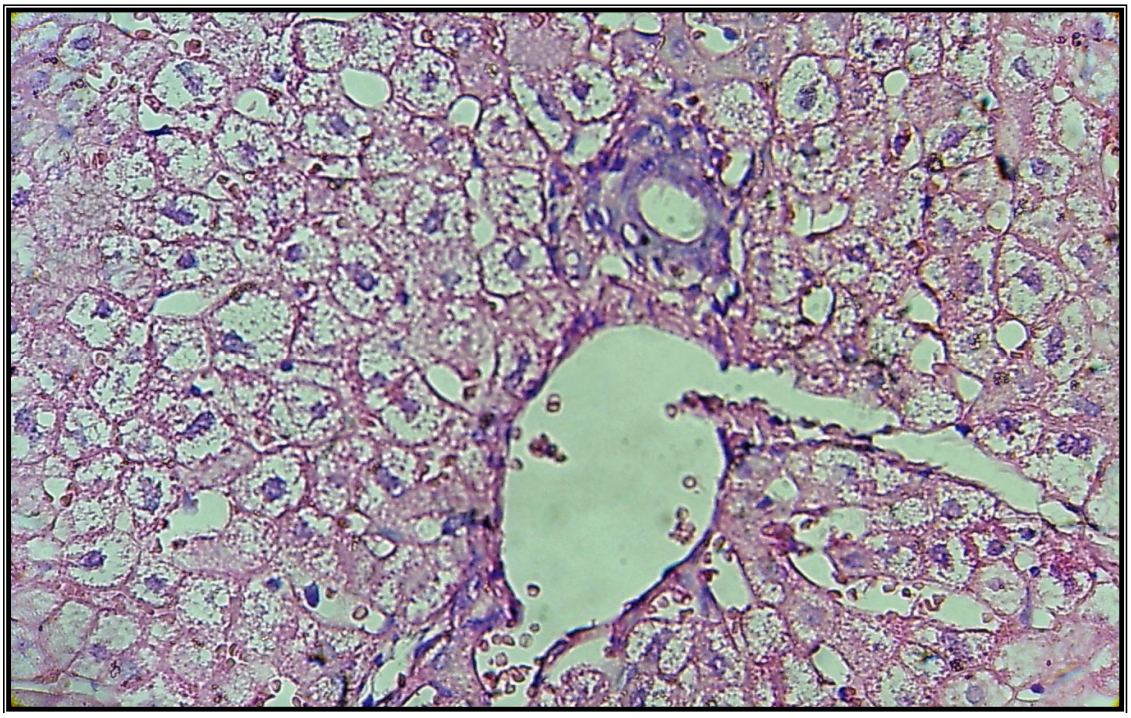

Fig 3. Photomicrograph of liver of group 3 showing the portal area with ballooning degeneration of most hepatocytes. (H&E X 400).

The Long Diameter of Nucleus

The long diameter of the nucleus in the control group was 3.29±0.49. It was 3.43±0.53, 3.14±0.38, and 4.57±1.13 in groups (2), (3) and (4) respectively(**Table-3)**. So there was no significant difference in the long diameter of nucleus of hepatocytes among these groups, except in group (4) which had the longest diameter.

Table 3. Long diameter of the hepatocyte nucleus in different groups of rabbits.

Groups	Long diameter of nucleus(mean ±SD)
1	3.29±0.49(b)
2	3.43±0.53 (b)
3	3.14±0.38(b)
4	4.57±1.13 (a)

Different letters vertically means significant difference at P≤0.05.

DISCUSSION

The number of hepatocytes

The number of hepatocytes in group (2) was 22.57± 3.74 which was nearly normal and not affected by the drug. But, in groups (3) and (4) the number of hepatocytes was 28.43±7.83 and 20±3.16 respectively. This means that groups received higher doses showed more increase in the hepatocytes number. But, in group (3) the number of hepatocytes was significantly higher at P≤0.05 compared to other groups. These results were in agreement with the results of Mayol *et al.* [13] who studied the effect of ethinylestradiol on the hepatic tissue. They pointed to the possibility that anabolic steroids can exert an adaptive response on liver tissue. Some studies have postulated the association of liver tumor promotion with growth and monooxygenase induction in this organ, suggesting that the liver response to administration of steroids might be adaptive.[14] This suggests that the dose (4mg/kg) may be the maximum effective dose which stimulates the maximum proliferative and regenerative response of the hepatocytes.

The Long Diameter of Hepatocytes

The long diameter of hepatocytes in group (2) was 8.62± 1.51 which was nearly normal. But, in groups (3) and (4) the long diameter of hepatocytes was 10.71±1.98 and 12±1.91 respectively. These results showed that groups (3) and (4) were affected by the drug. There was

a significant increase in the long diameter of hepatocytes in these two groups and this was evident especially in group (4). Hepatic dysfunction is a known side effect of treatment with anabolic steroids.[15] They have a direct toxic effect on the hepatocytes and they lead to severe ballooning degeneration of the hepatocytes which is dose dependent effect. The results showed that (group 2) which received only 2mg/kg body weight, had the same diameter of hepatocytes as the control group and this means that this dose did not affect the diameter of the hepatocytes. In group (3) which received 4mg/kg body weight of nandrolone decanoate, the long diameter of the hepatocytes was significantly higher than both the control group and group (2). This might have occurred due to the ballooning degeneration that affected the majority of the hepatocytes of this group. While in group (4) the long diameter of the hepatocytes was significantly highest when compared to other groups. This is because this group received the highest dose (6mg/kg body weight) and the ballooning degeneration was more severe and almost all hepatocytes were affected.

The Long Diameter of Nucleus

The long diameter of the nucleus in groups (2) and (3) was 3.43±0.53 and 3.14±0.38 respectively. While in group (4) it was 4.57±1.13. This means that the long diameter of the nucleus in groups (2) and (3) was not increased and it was nearly the same as the control group. But group (4) showed a significant increase in the long diameter of the nucleus and this might have occurred due to the direct effect of the drug on the nucleus. These results were in agreement with the findings of Boada *et al.* [16] who found that nandrolone stimulates DNA synthesis and cell replication. It, therefore, stimulates the regenerative mechanism of hepatocytes, the active nuclear division and mitosis. Anabolic steroids could act as a liver promoter in two ways: either exerting a cytotoxic effect that induces a regenerative response, or inducing an adaptive response in liver cells with cellular hypertrophy and proliferation of hepatocytes. [13]

On the basis of these results, nandrolone decanoate can cause several changes in the number, the long diameter of hepatocytes, and the long diameter of nucleus hepatocytes of treated rabbits.

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