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ORIGINAL ARTICLE

Study the Effect of the Anabolic Androgen Methandrostenolone on Some Physiological Parameters in Local Male Rabbits

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ABSTRACT

This study amid to detect the effect of anabolic androgenic Methandrostenolone (Dianabol) on the level of liver enzymes, urea and creatinine in addition to its effect on the level of estrogen in local rabbit. In this experiment, we used (30) adult male rabbits, distributed randomly into three groups, each group 10 animals. Control group(C): Animals were dosed with distilled water only. First treatment group (T1):Animals were dosed orally with Methandrostenolone (Dianabol) within a dosing cycle that continued for (8) weeks in an ascending manner: (0.076, 0.152, 0.304, 0.38, 0.456, 0.532, 0.608, 0.684) mg/kg B.W/d. Second treatment group (T2): Animals were administered the same anabolic androgen orally within a course of doses that lasted for (8) weeks in a hierarchical manner:(0.076, 0.152, 0.304, 0.38, 0.304, 0.152, 0.076, 0.076) mg/kg B.W/d. After the end of the experiment period, the results showed that the level of (AST, ALT, Creatinine, and Estrogen) increased significantly (P≤0.05) compared to the control group, and the level of these parameters increased significantly(P≤0.05) in the first treatment group compared with the second group. As for the level of (ALP and Urea) in the two treatment groups, it increased significantly (P≤0.05) in comparison with the control group, and there were no significant differences (P>0.05) when comparing the level between the two treatment groups. It is inferred from the results of our study that taking (Dianabol) has a clear negative effect on the liver, kidneys and estrogen in male rabbits, and that this effect increases with the increase in the concentration of the dose used.

Keywords: Methandrostenolone, Liver enzymes, Kidney parameters, Estrogen and rabbit

INTRODUCTION

The use of performance-enhancing drugs is not new, as its use dates back to ancient times when Greek Olympic athletes and Roman gladiators at the time used herbs and plants to enhance their strength **(1)** Nowadays, most young people use steroid hormones for body building **(2)**, **S**teroid hormones that had anabolic ability divided into several types depending on its chemical structure **(3).** The most common type is Dianabol(Methandrostenolone) its chemical composition contains a methyl group at (17 alpha carbon atom) and carbon double bond at (c1-c2) **(4).** Abusing Dianabol can cause negative side effects such as liver disease, kidney disease, estrogen imbalance, water retention in the muscles, and other damages **(5)**. It was used as a drug in 1958, but because of the damage it might cause to its users, the US Food and Drug Administration withdrew all forms of Methandrostenolone from the US market in 1985, but its production continues to this day in some countries with loose regulations for medicinal drugs **(6)**.

MATERIAL AND METHODS:

animals of experiment: In our experiment, we used 30 adult male rabbits, their weights (1600-2100) grams, age (16-19) months, a room was equipped with a large air window, cages made of iron clips with dimensions (95 cm x 70 cm x 65 cm) were used, In each cage, five randomly distributed animals were placed in it , the room temperature was (24-28)c.

Androgen anabolic (Dianabol): Dianabol tabs 5mg manufactured by the British company (BIOSAFE) was purchased from a bodybuilding gym, tabs are ground into a fine powder to make an easy orally dose suspension.

Experience design: The study included 30 male domestic rabbits, distributed randomly into three equal groups (each group 10 animals) treated as follows:

The first group (C): represents the control group, is dosed with distilled water for the duration of the experiment, which is (8) weeks.

Treatment group (T1): In this group, the animals were dose with the anabolic androgen Dianabol by following the weekly ascending method in Dianabol administration as follows:

- Animals dosed Dianabol at a concentration of 0.076 mg/kg/d for 7 consecutive days.
- Animals dosed Dianabol at a concentration of 0.152 mg/kg/d for 7 consecutive days.
- Animals dosed Dianabol at a concentration of 0.304 mg/kg/d for 7 consecutive days.
- Animals dosed Dianabol at a concentration of 0.38 mg/kg/d for 7 consecutive days.
- Animals dosed Dianabol at a concentration of 0.456 mg/kg/d for 7 consecutive days.
- Animals dosed Dianabol at a concentration of 0.532 mg/kg/d for 7 consecutive days.
- Animals dosed Dianabol at a concentration of 0.608 mg/kg/d for 7 consecutive days.
- Animals dosed Dianabol at a concentration of 0.684 mg/kg/d for 7 consecutive days.

Treatment group (T2): The animals were dosed with the anabolic androgen Dianabol in a pyramidal manner as follows:

- Animals dosed Dianabol at a concentration of 0.076 mg/kg/d for 7 consecutive days.
- Animals dosed Dianabol at a concentration of 0.152 mg/kg/d for 7 consecutive days.
- Animals dosed Dianabol at a concentration of 0.304 mg/kg/d for 7 consecutive days.
- Animals dosed Dianabol at a concentration of 0.38 mg/kg/d for 7 consecutive days.
- Animals dosed Dianabol at a concentration of 0.304 mg/kg/d for 7 consecutive days.
- Animals dosed Dianabol at a concentration of 0.152 mg/kg/d for 7 consecutive days.
- Animals dosed Dianabol at a concentration of 0.076 mg/kg/d for 7 consecutive days.
- Animals dosed Dianabol at a concentration of 0.076 mg/kg/d for 7 consecutive days.

Sacrifice of Animals: After 24 hours of the last dosing process, the animals were anesthetized by exposing them to inhalation of an amount of chloroform, then blood was drawn from the heart directly by Cardiac Puncture using medical syringes with a capacity of (5 ml), Then (4 ml) of blood is placed in a gel tube, then the blood is left for (30) minutes until it clots, and then it is transferred to a Centrifuge device at a speed of (3000 revolutions / minute) for 15 minutes to separate the serum, then the serum is divided into several Eppendorf tubes Capacity (1.5 ml) and kept in the refrigerator at a temperature of (-20) °C for the purpose of conducting biochemical tests.

Biochemical Tests: Liver enzymes, urea and creatinine were measured using a device (REFLOTRON PLUS) manufactured by the French company ROCHE, which allows the measurement of 16 types of blood tests. As for measuring the level of (Estrogen) in the blood serum, it was done using the (Minividas) device

manufactured by the French company Biomerieux, with several ready-made analyzes.

Statistical Analysis: After data collection and tabulation, statistical analysis program SPSS V.25 was used. Where the data were statistically analyzed according to the One-Way ANOVA test, and the averages of the experiment groups were compared using the Least Significant Difference (LSD) test at a significant level of 0.05**(7).**

RESULTS

Liver Enzymes: The results showed in Table (1) a significant(P≤0.05) increase in the levels of ALT and AST for the first (T1) and second (T2) treatment groups that dosed with anabolic androgen (Dianabol) compared with the control group, While when comparing the level of these two enzymes between the two treatment groups, we found that their levels increased significantly (P≤0.05) in the first treatment group compared with the second treatment group.

The results shown in Table (1) indicated an increase in the level of ALP enzyme in the two treatment groups up to a significant(P≤0.05) degree compared with its level in the control group, while when comparing the ALP enzyme level of the treatment groups among themselves, the results of the analysis statistic did not show any significant(P>0.05) difference.

Urea, Creatinine and Estrogen: The results shown in Table (2) indicated a significant (P≤0.05) increase in the urea level of groups of animals that were dosed anabolic androgen compared with the control group, While when comparing the level of urea between the two treatment groups, the results did not show a significant(P > 0.05) difference between them.

The results of the statistical analysis showed a significant(P≤0.05) increase in the creatinine level in the first treatment group compared to the creatinine level in the second treatment group, and the treatment of animals of these two groups with anabolic androgen seems to have caused a significant(P≤0.05) increase in the creatinine level Compared with its normal level in the control group as appeared in Table (2).

The results shown in Table (2) showed a significant(P≤0.05) increase in the estrogen level for the first and second treated groups compared with the control group, while when comparing the estrogen level for the two treated groups among themselves the results of the statistical analysis showed a rise in the estrogen level in the first treatment group significantly (P≤0.05) increased compared with the second treatment group.

Table 1: Effect of anabolic androgen (Dianabol) on (ALT,AST.ALP)

Standards The group	ALT iu\L	AST iu\L	ALP iu\L
C	10.00±0.31 c	14.00±0.31 c	81.20±1.06 b
T1	15.00±0.31 a	18.00±0.31 a	88.40±1.43 a
T2	13.20±0.37 b	16.40±0.24 b	85.60±1.12 a
LSD	0.847	0.74	3.06

The values are mean ± standard error.
Different letters within the same column indicate significant differences (p≤0.05) between the two groups

Table 2: Effect of anabolic androgen (Dianabol) on (Urea,Creatinine, Estrogen)

Standards The group	Urea mg\dl	Creatinine mg\dl	ESTROGEN pg\ml
C	26.00±2.30 b	0.66±0.05 c	14.50±0.76 c
T1	40.80±0.58 a	1.78±0.03 b	18.26±0.51 a
T2	38.80±0.73 a	0.98±0.03 a	15.97±0.35 b
LSD	3.61	0.142	1.44

The values are mean ± standard error.
Different letters within the same column indicate significant differences (p≤0.05) between the two groups

DISCUSSION

The liver is the organ responsible for the detoxification of many chemical preparations, including anabolic androgens such as Dianabol, so this androgen can cause harmful effects to the liver by causing chemical changes **(8)** or histological changes such as the occurrence of histological degenerations with acute hepatitis and cellular swelling of hepatocytes in addition to that the damaged liver tissue may be transformed from highly specialized tissue to deformed connective tissue and thus may end up with cirrhosis **(9),** The liver has the ability to convert the anabolic androgens entering the body into (dehydroepiandrosterone) or (androsterone) and then excrete them with the bile to the intestines and then to the kidneys to be excreted dianabol metabolites through the urine to the outside of the body removing dianabol toxins can harm liver in varying degrees of severity depending on several factors, including the duration of use and the dose concentration, and thus liver enzymes may be released from the damaged liver cells into the blood serum leading to raise their level more than normal limit **(10)**, This corresponds to studies of **(13)** and **(14)** that concluded that the severity of liver damage by dainabol depend on duration and dose concentration, that could explain the increase in liver enzymes in the first treatment group compared to the second group.

Successive studies have been conducted on the explanation of the increase in the level of liver enzymes, as a result of damage to the membrane of hepatocytes or at least the increase in the permeability caused by anabolic androgens as Dianabol **(11)**.

Creatinine and Urea: Our results showed that the concentration of urea and creatinine increased significantly in two treatment groups compared to the control group, and the reason for their high levels could be due to the anabolic androgen Dianabol that was taken. Because the high level of urea may be attributed to the functional imbalances of the kidneys caused by this androgen through its deposition in the collecting tubules which are responsible for filtering urea from the kidneys to the outside so the decrease in its functional effectiveness by this androgen lead to accumulation of urea in the blood **(15).** Low glomerular filtration rate (GFR) due to anabolic androgens administration may be another reason for the high level of urea and creatinine in the blood, as it affects the function of the glomeruli and makes them unable to filter urea and get rid it outside the body **(16).** Both kidneys damage by Dianabol and high level of urea can affect Body tissues in general and kidney tissues in particular and this leads to an increase in creatinine level because it is the responsible organ for excreting creatinine out of the body through urine, and therefore these tissue damage may lead to functional decline of the kidneys, which leads to the accumulation of creatinine and not being excreted to the outside, and thus increasing its level in the blood **(6)**,Increase muscle mass by Dianabol stimulation could be another reason for increase creatinine level as a by-product of energy consumption by muscles **(17)**.In addition, liver tissues damage may occur by Dianabol could be another reason that explains the increase in creatinine in the blood, as the liver is among the organs that synthesized of creatinine normally **(6)**.

Estrogen: The results of current study showed a significant increase in the estrogen level in the treated animals compared to the control group animals. The reason for this rise may be attributed to Dianabol, which was dosed to the two treatment groups, as it contains in its chemical composition (c-alpha-methyl), so Dianabol is one of the androgens that can produce estrogen in high proportions by aromatase enzyme or The so-called cytochrome p450, As this enzyme can catalyze the oxidation reaction of the structural androgen Dianabol, the carbon atoms (c1-2) and (c-alpha-methyl) are oxidized to be turning part of it into (17-β-estradiol), which is the most effective form of estrogen**(18).**

Monooxygenase Enzyme could also be another enzyme that may be involved in converting structural androgens into estrogen

by attacking (C2) in the chemical structure of androgen and initiating the generation of the phenolic ring which is the synthetic unit of estrogen **(19)** and this is in agreement with a recent study which was demonstrated that many anabolic androgens derived from testosterone can be converted into estrogen through a series of enzymatic reactions by the (cytochrome p450 enzyme). The significant increase in estrogen level between the two treatment groups may be attributed to the increase in the concentration of the total dose used in the ascending method **(20).**

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