A Study on The effect of Testosterone on Liver and Kidney functions of Male Rabbits

Mohamed A. Elesawi (1), Soaad A. M. Moftah (2), Ibrahim S. Eldurssi (1), Somya A. Aregeb (1), Mohamed A. El-Mabrouk (1), Abdulsalam M. A. Bolhaj (1)* and Ebtesam M. M. Gheth (1)

1- Zoology Department, Science Faculty, Omar Al-Mukhtar University, El-Beida-Libya
2- Zoology Department, Faculty of Art and Science, University of Benghazi, Elmarj Campus, Libya
P.O.BOX 919 - El-Beida-Libya

ABSTRACT

The purpose of this study was to examine the effect of two supraphysiological doses of testosterone on the liver and kidney functions of male rabbits. Fifteen (15) adult male rabbits were divided into 3 groups: a control group (received 100 µl sesame oil), a low dose group (received 6 mg testosterone/kg body weight), and a high dose group (received 12 mg testosterone/kg body weight). The rabbits were injected intramuscularly once a week for 6 weeks. After the end of the treatment period, the rabbits were sacrificed and blood samples were collected for analysis. Injection of testosterone resulted in a significant increase in the level of this hormone in the sera of the treated rabbits. The hormone caused increases in the levels of the liver function enzymes with the increase in dose; however, only the high dose caused statistically significant increases. Similar trend was observed with the effect of the hormone on the concentrations of creatinine and urea, where only the high dose had a statistically significant effect. These results clearly indicate that the use of this hormone with high doses for long periods could cause damage to the liver and kidney.

Key words: Testosterone, liver enzymes, creatinine, urea, rabbits.
INTRODUCTION

The use of testosterone and related steroids is a widespread phenomenon among top athletes, amateurs, and a large part of the population who simply desire to improve their appearance (Kadi, 2008; Wilson, 1988). The popularity of testosterone and related steroids among drug users is due to the powerful effects of these substances on muscle strength and mass (Bhasin et al., 2001). Testosterone regulates many physiological processes, including muscle protein metabolism, some aspects of sexual and cognitive functions, secondary sex characteristics, erythropoiesis plasma lipids, and bone metabolism (Bhasin and Bremner, 1997; Wilson, 1988). Most studies dealing with the effect of testosterone have used physiological doses for replacement therapy. However, most athletes use doses probably ten times the physiological dose. Therefore, this study was carried out to investigate the effect of two high doses of testosterone on liver and kidney functions.

MATERIALS AND METHODS

Animals:

Twelve-week old, healthy Egyptian male rabbits (total 15) (weighing between 1.2-1.6 kg) were obtained from a local breeder and were maintained in individual cages in the animal care center of the faculty of Veterinary medicine. The animals were maintained under normal temperature and light cycles. The rabbits were given water and food ad libitum. The animals were kept and maintained under these conditions for 4 weeks prior to the experiment.

Chemicals:

Testosterone (cidotestone, 250 mg/ml ampoule for intramuscular injection, made by Chemical Industries Development, Egypt) was obtained from a local pharmacy. Sesame oil (Almadina Company, Ajdabia-Libya) was also obtained from a local pharmacy.

Experimental Procedure:

The rabbits were weighed and divided randomly into 3 groups (5 rabbits in each group): 1- control group (received 100 μl sesame oil), 2- low dose group (received 6 mg testosterone / kg body weight), 3- high dose group (received 12 mg testosterone / kg body weight) (Gui et al., 2008; Zhao et al., 2013). The rabbits were injected with testosterone intramuscularly using 1 ml syringe once a week for 6 weeks (Aydilek and Aksakal, 2005; Zhao et al., 2013). After the end of the 6 weeks period, the rabbits were weighed, slaughtered and blood samples were taken from the 3 groups.
Biochemical and Hormonal Parameters:

Blood samples were centrifuged at 4000rpm, for 5 minutes, and the serum was used to measure the levels of the following:

Creatinine:

This was determined using ready to use kit from Archem Diagnostics Industry. Creatinine reacts with picric acid in alkaline environment to form a color complex. The red color developed is measured photometrically at 500-520 nm. The method in brief is as follows: 100 ul of the sample were mixed with 800 ul of reagent 1 (120 mmol/L carbonate buffer and 360 mmol/L sodium hydroxide) and 200 ul of reagent 2 (7.8 mmol picric acid) and aspirated immediately to photometer.

Urea

Urea + H₂O in the presence of the enzyme urease gives 2 NH₃ + CO₂. Ammonium ions in the presence of sodium salicylate and sodium hypochlorite form a green color (dicarboxylindophenol). The intensity of the color is proportional to the concentration of urea. The absorbance is measured photometrically at 590 nm. The amount of urea in the samples was measured using a ready to use kit (Biomaghreb). 10 ul of the sample were mixed well with 800 ul of reagent 1 (2 mmol/l EDTA, 60 mmol/l sodium salicylate, 32 mmol/l sodium nitroprussiate, 30000 U/l urease, and 60 mmol/l phosphate pH 6.7) and 200 ul of reagent 2 (40 mmol/l sodium hypochlorite and 150 mmol sodium hydroxide). The samples were aspirated immediately and read photometrically.

Alkaline Phosphatase (ALP):

This enzyme was measured using a ready to use kit from Archem Diagnostics. The ALP hydrolyzes 4-NPP to release 4-nitrophenol, under alkaline conditions. The 4-nitrophenol formed is detected spectrophotometrically at 405 nm to give a measurement of alkaline phosphatase activity in the sample. 20 ul of the sample were added to 800 ul of reagent 1 (1mol/L diethanolamine pH 10.4 and 0.50 mmol/L magnesium chloride) and 200 ul of reagent 2 (10 mmol/L p-nitrophenylphosphate). The mixture was mixed well and aspirated immediately and the change in color was read spectrophotometrically (Young, 1990).

Alanine Aminotransferase (ALT):

ALT was measured using a ready to use kit from Archem Diagnostics. The enzyme catalyzes the transaminase reaction between L-alanine and 2-oxoglutarate. The pyruvate formed is
reduced to lactate in the presence of lactate dehydrogenase (LDH). As the reactions proceed, NADH is oxidized to NAD. The disappearance of NADH per unit time is followed by measuring the decrease in absorbance at 340 nm. 100 ul of the sample were added to 800 ul of reagent 1 (120 mM Tris-buffer, pH 7.15, 500 mM L-alanine, and 1700 U/L LDH) and 200 ul of reagent 2 (18 mM 2-Oxoglutarate and 0.18 mM NADH), mixed well and aspirated immediately and the color change read spectrophotometrically (Young, 1990).

Aspartate Aminotransferase (AST):  
The AST catalyzes the transaminase reaction between L-Aspartate and 2-Oxoglutarate. The 2-Oxalactate formed is reduced to malate in the presence of malatedehydrogenase (MDH). As the reaction proceed, NADH is oxidized to NAD. The disappearance of NADH per unit time is followed by measuring the decrease in absorbance at 340 nm. 100 ul of the sample were added to 800 µl of reagent 1 (80 mmol/L Tris-buffer, pH 7.8, 240 mmol/L L-Aspartate, and 1200 U/L MDH) and 200 ul of reagent 2 (15 mmol/L 2-Oxoglutarate and 0.18 mmol/L NADH), mixed well and aspirated immediately and the change in color was measured spectrophotometrically (Young, 1990).

Hormone:  
Testosterone was measured automatically using Elecsys2010 (RD/Hitachi Immunoassay System 2010 from Roche Diagnostics/Hitachi, Japan).

Statistical Analysis:  
Statistical analysis was performed using a computer run package (Graph Pad Prism7). One way ANOVA followed by Tukey's HSD test was performed to show the statistical significance among the means of the groups. Results were expressed as mean ± Standard error of the mean (SEM), N = 5. P-value below 0.05 was considered to be statistically significant.

RESULTS  
None of the rabbits in this study exhibited overt clinical signs of toxicity in response to treatment with testosterone.

The injection of the rabbits with testosterone (T) resulted in a significant increase in the level of this hormone in the serum of the treated animals in comparison with the sesame oil injected controls. The results are shown in figure1. This figure clearly shows a huge
significant difference (p < 0.0001) between the control group (1.362 ± 0.78 U/ml) and the
group treated with 6 mg T (12.37 ± 1.90 U/ml) and the group treated with 12 mg T (14.82 ±
0.12 U/ml). Even though the group treated with 12 mg T had slightly higher level of T than
the 6 mg T treated group, this increase was not significantly different.

The results of the kidney function tests (creatinine and urea) are represented in figures
2 and 3, respectively. With regard to creatinine, the concentration in the serum of the control
group was 0.64 ± 0.07 mg/dl. This value increased to 0.7 ± 0.04 mg/dl in the 6 mg T treated
group, but this rise was not significant (p = 0.83). In the 12 mg T treated group the
concentration was 0.92 ± 0.09 mg/dl. This value was statistically higher (p = 0.04) than that of
the control group, but not statistically different from that in the 6 mg T treated group. The
mean concentrations of urea were 10.4 ± 0.81 mg/dl and 10.2 ± 0.58 mg/dl in the sera of the
control group and the 6 mg T treated group, respectively. There was no significant difference
between these two means. The 12 mg T caused a big increase in the mean concentration of
urea (15.6 ± 1.03), which was significantly different from the mean of the control group (p =
0.0022) and that of the 6 mg T treated group (p = 0.0016).

The results of the liver function testes (AST, ALT and ALP) are represented in
figures 4, 5 and 6, respectively. The mean level of AST in the serum of the control group was
49.8 ± 7.8 U/l. Even though there was an increase in the level of AST in the 6 mg T treated
group (88.2 ± 10.95 U/l), this was not statistically different (p = 0.2006) from the mean level
of the control group. The 12 mg T increased significantly (p = 0.0005) the mean level of AST
(162 ± 21.85 U/l). This was also significant (p = 0.0108) when compared with that of the 6
mg T treated group. The mean ALT level of the control group was 72 ± 9.3 U/l. Although the
mean of ALT increased in the 6 mg T treated group to 142.2 ± 32.9 U/l, this, however, was
not statistically different (p = 0.180) from that of the control group. The mean level of the 12
mg T treated group (181 ± 29.5 U/l), on the other hand, was statistically different (p = 0.030)
from that in the control group, but not different (p = 0.56) from that in the 6 mg T treated
group. The same trend was observed with ALP. In the control group the mean level of ALP
was 125.6 ± 24.3 U/l, and in the 6 mg and 12 mg T treated groups the levels were 173.8 ±
20.4 and 269.2 ± 41.0, respectively. There was no significant difference between the levels in
the control and the 6 mg T treated group. The increase in ALP level observed in the 12 mg T
treated group was significantly different from that of the control group (p = 0.014) but not
from that of the 6 mg T treated group (p = 0.014). From the results, it can be observed that
there was an increase in the levels of the enzymes with the increase in dose, though in some
cases were not statistically significant. However, this may indicate a dose-dependent effect.
DISCUSSION

In this experiment, the testosterone concentrations used were 6 mg/kg and 12 mg/kg, based on the study by Gui et al. (2008) who used these concentrations to study the effect of testosterone on blood lipids in castrated rabbits and based on the study by Zhao et al. (2013) who used 6 mg/kg to study the effect of this hormone on hematological profiles and blood lipids in castrated rabbits. These doses caused a huge increase in the level of testosterone in the serum of treated rabbits.

Testosterone was found to increase the levels of creatinine and urea in the serum of treated rabbits. It is possible that the increase is due to damage caused by testosterone to the filtration mechanism of the kidney. Muraoka (2000) found that testosterone replacement in middle-aged rats increased the rate of apoptosis of renal tubule cells and decreased glomerular filtration rate (GFR), but did not affect serum creatinine and urea. Yassin et al. (2017), on the other hand, found that injection of testosterone undecanoate into hypogonadal men decreased creatinine and urea, and increased GFR. Hu et al. (2011) studied the effects of testosterone on renal function in salt-loaded rats. They found that testosterone caused creatinine retention, high blood pressure and renal injury. Injection of testosterone into a 14-year old with hypogonadal resulted in an increase in serum creatinine and urea levels (Filler et al., 2016). It is believed that testosterone causes renal injury by promoting apoptotic damage in human renal tubular cells (Verzola et al., 2004). Furthermore, Seachrist et al. (2000) reported that testosterone replacement in castrated rats increased blood pressure and renal pathology.
Figure 1: The concentration of testosterone in the serum of the control group injected with sesame oil and in the rabbits treated with testosterone (6 mg/kg and 12 mg/kg) once a week for 6 weeks. Results are mean ± SEM (n = 5). Similar letters indicate no significant difference, while different letters indicate significant difference between the means.

Figure 2: The concentration of creatinine in the serum of control and testosterone (6 mg/kg and 12 mg/kg) treated rabbits after a 6 weeks treatment period. Results are mean ± SEM (n = 5). Similar letters indicate no significant difference between the means. Different letters indicate significant difference.

Figure 3: Urea concentration in the serum of the control group and the testosterone (6 mg/kg and 12 mg/kg) treated groups after a 6 weeks treatment period. Results are mean ± SEM (n = 5). Similar results indicate no significant difference between the means and different letters indicate significant difference.

Figure 4: The level of aspartate aminotransferase (AST) in the serum of the control rabbits and rabbits treated with testosterone (6 mg/kg and 12 mg/kg) for 6 weeks. Results are mean ± SEM (n = 5). Similar letters indicate no significant difference between the means. Different letters indicate significant difference.

Figure 5: The level of the enzyme alanine aminotransferase (ALT) in the serum of control and testosterone (6 mg/kg and 12 mg/kg) rabbits after 6 weeks of treatment. Results are mean ± SEM (n = 5). Similar letters indicate no significant differences between the means. Different letters indicate significant difference.

Figure 6: The level of alkaline phosphatase (ALP) in the serum of testosterone (6 mg/kg and 12 mg/kg) treated and control rabbits after 6 weeks of treatment. Results are mean ± SEM (n = 5). Similar letters indicate no significant differences between the means. Different letters indicate significant differences.
The levels of liver function enzymes were also affected by the doses of testosterone used. The levels of the enzymes increased in a dose-dependent manner. This may indicate damage to liver tissue. Alen (1985) who investigated the hepatic effects of testosterone self-administration in power athletes during 26 weeks of training, reported that liver enzymes even though were higher than those of the control group, they remained within the normal range. In rhesus monkeys, testosterone enanthate injection did not change the level of ALP, but increased the levels of AST and ALT (Tyagi et al., 1999). Hild et al. (2010) reported that a 10 mg/kg testosterone oral dose had no significant effect on the liver enzyme ALT, but there was a slight but significant increase in serum AST. The reason for the no effect or the slight effect of testosterone could be due to the short experimental period which was 14 days only. However, injection of a single dose (500 mg) of testosterone into obese middle-aged men had no effects on liver function tests (Rebuffe et al. 1991). The results of these studies, even though contradictory, clearly indicate that testosterone has an effect on the liver and kidney function enzymes, which could result from injuries to the hepatocytes and nephrons, respectively; therefore, it is recommended that those who use testosterone for purposes other than medical should routinely test for changes in the liver and kidney function enzymes.
REFERENCES


