Effect of Sustanon® 250 on Body Weight Gain, Total Serum, Muscle Protein and Reproductive Efficiency in Male and Female Rats

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Abstract

The present study was conducted to determine the effect of different doses of Sustanon® 250 abusing on body weight gain, total serum, muscle protein and reproductive efficiencies in male and female rats. Thirty two male and thirty two female of Albino rats were used. They were divided into four groups (each group consisted of 8 males and 8 females). The first group (G1) served as control group in which the rats dosed by intramuscular injection of 10 μl olive oil weekly for 12 weeks, the other three treated groups (G2, G3 and G4) in which the rats dosed weekly by intramuscular injection of 50m, 100 and 150 mg Sustanon® 250/ kg BW weekly for 12 weeks respectively. All animals were weighted before scarified to determine total serum protein and muscle protein. With regard to the reproductive efficiency another group of 16 male and 32 female rats were used to determine the reproductive efficiency, from which 4 male and 8 female were dosed by intramuscular injection with 10 μl olive oil weekly for 12 weeks, they are considered as control animals. The rest rats were dosed by intramuscular injection of 150 mg Sustanon® 250 /Kg BW weekly for 12 weeks. Then they were divided into four groups: group 1 (4 non treated males with 8 non treated females), group 2 (4 treated males with 8 treated females), group 3 (4 treated males with 8 non treated females) and group 4 (4 non treated males with 8 treated females). The results of this study showed that the Sustanon® 250 seems to reduce significantly (p≤ 0.05) the body weight in both sexes, through its effect on liver caused increasing total serum protein in treated rats, whereas the muscle protein synthesis was significantly increased in treated male rats but was not affected in treated females. The reproductive parameters were investigated which revealed the fertility percentage as 100% in G1, whereas the female rats in G2, G3 and G4 showed no pregnancy at all which indicated complete infertility. In conclusion, the present study revealed that the abusing of Sustanon® 250 cause serious physiological changes in the most body structures, particularly the reproductive organs, thus affected the fertility.

Keywords: Sustanon, reproductive efficiency, body weight, total serum protein and muscle protein

Introduction

Testosterone promotes protein synthesis and growth of those tissues with androgen receptors. The effect can be classified as androgenic and anabolic, although the distinction is somewhat artificial, as many of the effects can be considered as both. Anabolic effects include growth of muscle mass and strength (Rabkin et al., 2000; Kenny et al., 2001) increased bone density and strength, and stimulation of linear growth and bone maturation (Behre et al., 1997). Testosterone also increases protein synthesis in muscles (Chen et al., 2008). Animals based researches has also suggested that testosterone plays a role in the increasing of glycogen synthesis in tissues and storage (Mooradian et al., 1987). Like males female also need testosterone to maintain bone density and muscle mass through their lives.

At the cellular level, in particular of muscles, the hormonal balance of the animal is of prime importance and this can be exploited to increase growth and feed conversion efficiency of farm animals (Heitzman, 1980). Testosterone is anabolic in many species and physiologically elevations in testosterone concentrations stimulate protein synthesis resulting in improvements in muscle size, body mass and strength (Bhasin et al., 2001). Testosterone promotes nitrogen retention in muscle therefore allowing the muscles to hold more protein and enabling repair and growth of those muscles. Testosterone binds to the androgen receptor to promote receptor dependant mechanisms for muscular growth and fat loss in males and females (Toth and Zakar, 1982).

Androgens have been shown to stimulate local production of insulin-like growth factor I (IGF-I) to increase muscle mass (Chen et al., 2005; Chambon et al., 2010), by increasing protein synthesis and increasing muscle fiber size (Goldspink and Harridge, 2004). AAS-induced increment of muscle tissue can be attributed to hypertrophy and the formation of new muscle fibers, in which key roles are played by satellite cell number and ultrastructure, androgen receptors and myonuclei (Hartgens and Kuipers, 2004). Males typically have less adipose tissue than females. Other findings indicated that androgens inhibit the ability of some fat cells to store lipids by blocking a signal transduction pathway that normally supports adipocyte function (Singh et al., 2006). Conversely Bhasin et al. (1996) found that men given testosterone without exercise had a significant increase in body weight. The aim of the study was to determine the effect of different doses of Sustanon® 250 abusing on
body weight gain, total serum, muscle protein and reproductive efficiencies in male and female rats.

**Material and methods**

A total of one hundred and twelve rats were used in this study, from which 64 rats involved 32 male and 32 female rats were randomly divided into four equal groups, after the acclimatization period each group consisted of 8 adult male rats and 8 adult female rats as following: Control group (G1) injected intramuscularly with 10μl of olive oil weekly for 12 weeks. Group 2 (G2), group 3 (G3) and group 4 (G4) were injected intramuscularly with different doses of sustanon® 250 of 50, 100 and 150 mg/kg body weight (BW) weekly for 12 weeks respectively. All studied rats were weight and then sacrificed at the end of the 12 weeks of treatment period.

Blood samples were collected via cardiac puncture according to the method of Hoff and Rlatg (2000). Then the blood samples were dropped directly from the heart by using 5 ml disposable syringe then put in plane tube to be centrifuged at 3000 rpm for 15 minutes to obtain the plasma. The gastrocnemius muscle was obtained by scalpel from both legs of each rat, piece of this muscle of each animal put in petri dish to use for muscle protein determination.

**Reproductive Efficiency Method**

This part of experiment aimed to study the reproductive effects caused by weekly intramuscular injection of high dose of sustanon®250 (150 mg / kg BW to 48 rats which consisted of 16 male rats and 32 female rats were divided into two groups, which named reproductive control group (RCG) that consisted of eight males and 16 females rats injected weekly with single dose of 10 μl olive oil.

The second group was treated group also consisted of 48 animals that included 16 males and 32 females rats were injected with single dose of 150 mg /kg BW sustanon®250. At the end of treatment period of 12 weeks, the animals further divided into four subgroups as following: Subgroup 1 (SG1) consisted of four non treated males and eight non treated females. Subgroup 2 (SG2) consisted of four treated males and eight treated females. Then subgroup 3 (SG3) consisted of four treated males with eight non treated females, and lastly subgroup4 (SG4) consisted of four non treated males and eight treated females.
The animals in the treated group were left for 12 weeks for mating before measuring the parameters to determine the reproductive efficiency by number of pregnant females, fertility percent, number of life birth, weight of life birth, abnormalities of life birth, number of corpora lutea and number of implanting sites.

Percentage of fertility determined by the following formula:

\[
\text{Fertility \%} = \frac{\text{No. of pregnant females}}{\text{No. of females enter the study}}
\]

**Statistical Analysis**

The results were expressed as mean ± SD. The comparisons between groups were performed with analysis of variance (ANOVA) by using computerized SPSS program (Statistical Program for Social Sciences). P<0.05 was considered to be at least limit of significance. Least significant different test (LSD) was calculated to test difference between means (groups) for (ANOVA) SPSS (1998).

**Results**

According to the body weight gain, the results indicated that a remarkable reduction in body weight gain of male and female rats occur due to sustanon®250 injection 50, 100 and 150 mg /kg BW compared with control once. While the high dose of sustanon®250 of 150 mg /kg BW showed decrease the body weight gain compared with other treated male groups, however such significant differences was not seen in treated female (Table 1).

**Table (1): The Effect of Sustanon®250 injections on weight and body weight gain in male and female rats (mean ± SD)**

<table>
<thead>
<tr>
<th>Groups</th>
<th>BW gain/g male</th>
<th>BW gain/g female</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control 0.1ml olive oil</td>
<td>42.75±2.217</td>
<td>48.75±6.55</td>
</tr>
<tr>
<td>Group 1 50mg/kg BW</td>
<td>23.0±4.082</td>
<td>36.75±6.238</td>
</tr>
<tr>
<td>Group 2 100mg/kg BW</td>
<td>23.25±2.217</td>
<td>32.0±2.944</td>
</tr>
<tr>
<td>Group 3 150mg/kg BW</td>
<td>15.25±2.872</td>
<td>31.25±2.5</td>
</tr>
<tr>
<td>LSD</td>
<td>0.675</td>
<td>10.750</td>
</tr>
</tbody>
</table>

Different small letters (a, b and c) represent significant difference between groups at (p≤0.05)
Using sustanon®250 in doses of 100 and 150 mg /kg BW showed reducing in the serum total protein significantly at p≤ 0.05 in males and females compared with dose 50 mg /kg BW and the control group. Higher dose of sustanon®250 at 150 mg /kg BW in male rates showed increasing muscle protein percentage compared with other sustanon®250 doses and control groups, but there were not any effect of any doses on muscle protein percentage in females compared with other treatment and control groups (Table 2).

**Table (2): Effect of Sustanon®250 injection on serum total protein and muscle protein in male and female rats (means ± SD)**

<table>
<thead>
<tr>
<th>Groups</th>
<th>Male (Serum Total protein g/dl</th>
<th>Muscle protein %</th>
<th>Female (Serum Total protein g/dl)</th>
<th>Muscle protein %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control group (0.1ml olive oil)</td>
<td>73.243± 3.231 a</td>
<td>80.992± 0.403 b</td>
<td>76.907± 5.905 a</td>
<td>74.853± 4.594 a</td>
</tr>
<tr>
<td>Group1 (50mg/kg BW)</td>
<td>72.467±2.804 a</td>
<td>81.460± 2.193 b</td>
<td>75.793± 3.332 a</td>
<td>67.510± 9.117 a</td>
</tr>
<tr>
<td>Group2 (100mg/kg BW)</td>
<td>68.032±1.746 b</td>
<td>81.314± 0.109 b</td>
<td>66.745± 4.738 b</td>
<td>66.472± 4.857 a</td>
</tr>
<tr>
<td>Group3 (150mg/kg BW)</td>
<td>67.760± 0.953 b</td>
<td>85.798± 0.954 a</td>
<td>65.156± 2.239 b</td>
<td>75.940± 5.299 a</td>
</tr>
<tr>
<td>LSD</td>
<td>4.210</td>
<td>5.305</td>
<td>8.937</td>
<td>9.430</td>
</tr>
</tbody>
</table>

Different small letters (a and b) represent significant difference between groups at (p≤0.05)

The treated groups, which included either treated male mated with non-treated female or treated female mated with non treated males or mated treated male with treated females, showed no pregnancies in all treated groups indicating complete infertility compare with control one, in same time SG1 that consisted of non-treated male and non-treated female showed 100% fertility and pregnancy (Table 3).

**Table (3): The reproductive efficiency results of mating rats after 12 weeks of intramuscular injection of 150 mg / kg BW of Sustanon®250 means ± SD**

<table>
<thead>
<tr>
<th>Groups</th>
<th>No. of pregnant females</th>
<th>Fertility %</th>
<th>No. of life birth</th>
<th>Wt. of life birth (grams)</th>
<th>No. of Implanting sites</th>
<th>No. of Corpora lutea</th>
</tr>
</thead>
<tbody>
<tr>
<td>SG1 (4ntm+8ntf)</td>
<td>8</td>
<td>100%</td>
<td>5.25±0.75</td>
<td>5.1±0.35</td>
<td>5.1±0.20</td>
<td>6±0.20</td>
</tr>
<tr>
<td>SG2 (4tm+8tf)</td>
<td>0</td>
<td>0%</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>SG3 (4tm+8ntf)</td>
<td>0</td>
<td>0%</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>SG4 (4ntm+8tf)</td>
<td>0</td>
<td>0%</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

tm :treated male, ntm: non treated male, tf: treated female, ntf: non treated female
Discussion
The present study revealed that the injection of sustanon®250 led to significantly decrease in body weight gain for both sexes of experimental animals. The decreased body weight gain in treated rats could be explained by the fact that an excessive concentration of testosterone can inhibit growth and weight gain due to decreased appetite, electrolyte imbalance and increased lipid oxidation as a result of increased activity of the enzyme carnitine palmitoyl transferase (Lee et al., 2003). A study reported by Singh et al. (2003) indicated that androgens inhibit the ability of some fat cells to store lipids by blocking a signal transduction pathway that normally supports adipocyte function. This might reduce the body weight gain. Hickson et al. (1990) and Zhou et al. (1994) reported that the androgens regulate mesenchymal multipotent cell differentiation by promoting myogenic differentiation, inhibiting adipogenic differentiation and inhibiting preadipocyte differentiation into adipocytes. Anabolic steroids can also decrease fat by increasing basal metabolic rate (BMR), since an increase in muscle mass increases BMR (Singh et al., 2003).

The serum total protein concentration in the treated male and female groups received of sustanon®250 of 100 and 150 mg/kg BW were significantly lower compared with the least dose group 50 mg/kg BW and the control group. The decreased serum total protein in treated rats could be explained through the testosterone effect on liver resulting in liver damage, thereby reduced the protein synthesis and increase the protein catabolism (Barrett et al., 2010), which is supported by (Lok et al., 2010).

On the other hand, the percentage of muscle protein was significant higher in high dose treated male group 150 mg/kg BW compared with the other treated groups 50 and 100 mg/kg BW and control male group. The elevation of muscle protein synthesis in treated animals of the present study could be explained by the role of testosterone in stimulate fractional muscle protein synthesis and improving the reutilization of amino acids by the muscle (Shabsigh et al., 2004).

The androgen promote myogenic differentiation and inhibit adipogenic differentiation after a series events started by binding androgen to the androgen receptors on the cell membrane of the myocytes (Singh et al., 2003). Another reason to increase the percentage of muscle protein is that the testosterone promotes nitrogen retention in muscle therefore allowing the muscles to hold more protein and
enabling repair and growth of those muscles (Hoseini et al., 2009). According to Lobley et al. (1990) the testosterone administration is induced a significant improvement in nitrogen retention due exclusively to a decrease in urinary nitrogen elimination.

According to Wu et al. (2010) the increased level of muscles proteins could be resulted from the reduction in muscle breakdown which occurs through anabolic steroids inhibiting the action of glucocorticoids that promote the breakdown of muscles. This higher percentage of muscle protein was in response to testosterone administration to hypogonadal men (Brodsky et al., 1996) and castrated lambs (Lobley et al., 1990). The absence of the effect of testosterone on female muscle protein synthesis might refer to the anti-anabolic effect of female sex steroids on muscle protein synthesis. There is evidence from study of Toth et al. (2001) on rats that progesterone and estrogen inhibit the muscle protein synthesis. Specifically, to ovariectomized rats the rate of the muscle protein synthesis was higher than in sham-operated, intact controls and ovariectomy with either progesterone or estrogen replacement prevented the increase (Toth et al., 2001).

The reason for an affected of female muscle might be due to the amount of testosterone that is injected being less than the amount which should affect the muscle protein synthesis. The testosterone doses injection used in the present study could be the main cause. It seems that the sustanon®250 defiantly had negative effects on fertility percentage of treated males and / or females. The oxidative stress that induced by sustanon®250 affects natural fertility (Agarwal et al., 2004). Other studies have suggested that reactive oxygen species (ROS) are involved in various causative factors of female infertility as peritoneal factor, tubal factor, endometriosis and unexplained infertility (Szczepanska et al., 2003; Agarwal et al., 2008). According to Polak et al. (2001), the antioxidants concentrations in women with unexplained infertility is less than those in fertile women, the above authors found that increased ROS –induced lipid peroxidation damage resulting in infertility. In conclusion, the present study revealed that the abusing of Sustanon® 250 cause serious physiological changes in the most body structures, particularly the reproductive organs, thus affected the fertility.
References


