Histological and physiological effects of Sustanon on the liver and Kidneys of male rats

Ekhlas Abd Hamza*  Kareem Hameed Rashid**
Veterinary Medicine College, University of Al Qasim green.
*E-mail: ekhlasalalwany@yahoo.com
College of Sciences, University of Babylon, Babylon, Iraq . **

Abstract
This research on male rats was carried out to investigate the possible effects of three different doses of artificial testosterone (Sustanon) on the histology and function of liver and kidneys of adult male rats. In this experiment 48 adult male rats divided into three groups 1, 2 and 3. These groups were injected with 0.05, 0.1, 0.2 mg/kg of sustanon respectively for a period of 42 days. Blood samples were collected at the end of the experiment for the determination of the levels of liver enzymes. Male rats were then dissected out, liver and kidneys were removed, weighed and fixed in 10% formalin for histological study, and 12 adult males were used in parallel to these groups as control group. The results showed that there were significant changes in the levels of liver enzymes in all treated groups in comparison with those of the control. All injected groups showed clear histological changes in the basic structure of liver and kidneys in comparison with those for control group. These changes included the increased number of hepatocytes in liver and also some distortion in renal tubules of the kidneys. Changes like cellular hyperplasia and cell degeneration were also observed in all groups of this study especially those of high doses of sustanon. It was concluded from the results of this study that the injection of sustanon at the doses of 0.05, 0.1 and 0.2 mg/ kg in adult male rats had influenced the histological structure of liver and kidneys and also the levels of liver enzymes.

المستخلص
أجريت الدراسة للتحري عن التأثيرات المحتملة لثلاث جرع من الثعابين الصناعي (الستانون) في التركيب النسجي ووظيفة الكبد والكلى في ذكور الجرذان البالغ. استخدم 48 جرذ قسمت إلى ثلاث مجاميع حقن بالجرع 0.05 و 0.1 و 0.2 ملغم/ كغم من وزن الجسم على التوالي ولمدة 42 يوم. تم سحب الدم عند نهاية المعاملة لتحديد مستوى انزيمات الكبد. كما تم تشريح الحيوانات لاستخراج الكبد والكلى وحفظها في 10% فورمالين. أظهرت النتائج تغيرات معنوية في مستوى الإنزيمات الكبدية اضافها إلى تغيرات نسجية في الكبد والكلى شملت زيادة في عدد الخلايا الكبدية وتشوهات في النسيج الكلوي وخاصة عند الجرع العالي. نستنتج من هذه الدراسة ان حقن السستانون يؤثر في التركيب النسجي للكبد والكلى ومستويات الإنزيمات الكبدية في ذكور الجرذان البالغ.

Introduction
Nowadays many youth and athletes are using large doses of anabolic steroids (8). These synthetic steroids have similar structure to testosterone (18)(16). Androgens are used for the enhancement of muscle growth (18) because high doses are
used to obtain a rapid and massive increase in the skeletal muscle size and efficiency during competition (17). Sustanon used in this study is one of these steroids which has many other useful uses like treatment of osteoporosis, male infertility (12). In regard to sustanon, it has special distinguished properties and structure compared to other steroids. It is consisted of four different testosterone ester compounds in oily estate ensuring testosterone presence into the blood for a period of 3 – 4 weeks (3). These drugs are given to horses and dogs to improve their physical performance (19), and they may also cause Toxicant Associated Steatohepatitis (TASH) disease (21). They cause a marked and fast growth in muscle mass and strength, therefore, many athletes prefer to use sustanon to get quick results. Other studies reported serious effects of anabolic drugs abuse such as left ventricle enlargement, liver disease and jaundice, kidney and testicular disorder which may lead to infertility, hypertension (13)(5). Due to the rare studies on the effects of sustanon on liver and kidneys, this study was done to find out the possible histological changes in these organs.

Materials and methods

Animals:

Three months old albino rats were obtained from the animal house of Sciences College. They were of body weight ranged between 250-350 gm. Animals had free access to laboratory chows and tap water, maintained on a 12:12 hour light – dark cycle and housed in an animal room where the temperature of 22-26°C was controlled. 48 rats were randomly divided into 3 experimental groups of 12 rats in each group and a same number for a control group. Group I received the regular rate diet and considered as negative control group, group II, III, IV were injected intramuscularly (IM) with sustanon supplied as 250mg/ml ampoules obtained from Organon OssHoland company. The doses of 0.05, 0.1 and 0.2 mg/kg of bodyweight were given to group 11, 111 and 1V respectively for a period of 42 days on weekly basis. Six rats from each group were left for 7 days after ceasing treatment and were given normal diet and water only. Blood samples were taken from the heart after the scarification of animals. Serum was obtained by centrifugation at 3000 RPM for 15 minutes and stored at -4°C for enzyme assays. ALT, AST were analyzed by using commercial kits from Elab-science Company.

Sustanon ampoules manufactured by Organon OssInc. Holland were obtained from a local pharmacy in Hilla-Iraq. Each ampoule contained 1mL of oily solution of Sustanon. According to the manufacturer, 1mL of Sustanon consisted of four testosterone ester compounds which included testosterone propionate, testosterone phenylpropionate, testosterone isocaproate and testosterone decanoate.

Experimental Design

The rats were divided randomly into four groups, each group consist of 12 rats, first group served as control group and the other three served as the experimental groups. The control group was injected with (Nacl 0.09%) once a week for six weeks. The first, second and third experimental groups were injected weekly at the doses of 0.05, 0.1 and 0.2mg/kg respectively with sustanon-seasme oil suspension for a period of six weeks.
Enzymes levels measurement & Histological study

The levels of ALT, AST enzymes were measured by using Elisa kit supplied by Spin React Company, according to the procedure given with the kit (22).

At the end of the experiment the rats were dissected out and samples of kidneys and livers were taken, fixed in 10% formalin for 24 hrs for the histological study according to (2).

Statistical Analysis

The results of this study were analyzed statistically using SPSS software (9) this analysis calculates the arithmetic mean and standard error (Mean ± S.E.) comparison between the averages in different dosage intervals using less difference between middle L.S.D. (Least Significant Difference), and under level probability 0.05.

Results

1- Biochemical analysis

The results of biochemical analysis for blood serum in figure 1 reveals significant differences in the levels of ALT enzyme in rats injected with the doses of 0.05, 0.1, 0.2 mg/kg of body weight sustanon as compared with the control group for the period of 42 days, and the levels of ALT enzyme in all treated groups with sustanon were increased. While figure 2 show significantly increased levels of AST enzyme in rats treated with the same doses of sustanon for the same period as compared with the control group.

![Figure (1): ALT levels in male rats injected by different doses of sustanon](image-url)
Figure (2): AST levels in male rats injected with different doses of sustanon.

2-Histological study

A- Liver
The histopathological study of males rats liver revealed mild to severe changes in liver histology at the dose of 0.05 mg/kg of sustanon. These changes included increased number of hepatocytes, slight changes in hepatocytes cytoplasm (figure 4). In 0.1 mg/kg sustanon injected group, hyperplasia in liver lobules was observed, increased nuclear density with an increase in hepatocytes eosinophilic granules (figure 5), while the group of the dose of 0.2 mg/kg of sustanon showed hyperplasia of hepatocyte in addition to increased nuclear density and also an increase in hepatocyte eosinophilic granules (figure 6), in comparison with the control group (figure 3).

B- Kidney
The histological study of male rats kidney showed histopathological changes in the kidney at the different doses of sustanon ranged from mild to severe. In the group of the dose of 0.05 mg/kg of sustanon, there were no obvious pathological changes as compared with the control group (figure 8). In 0.1 mg/kg sustanon injected group there were slight change in the tubular lining epithelial cells (figure 9). Meanwhile the (0.2) mg/kg of sustanon group revealed distortion in renal tubules, orange-red amorphous material in some of the renal tubules (figure 10), in comparison with the control group (figure 7).
Fig 3: liver of control group showing normal hepatocytes. H&E stain 400X.

Fig 4: liver of 0.05 mg male rats showing increased number of hepatocytes. H&E stain 400X.

Fig 5: liver of 0.1 mg injected male showing hepatocytes eosinophilic granules. H&E stain 400X.
Fig. 6: liver of 0.2 mg susstanon injected male showing hyperplasia and hepatocytes eosinophilic granules. H&E stain 400X.

Fig. 7: kidney of control group showing normal glomeruli and normal renal tubules. H&E stain 100X.
Fig. 8: kidney of 0.05 mg sustanon injected group showing no obvious changed in the glomeruli and renal tubules. H&E stain 100X.

Fig. 9: kidney of 0.1 mg sustanon injected group showing slight changes in the lining epithelia of the renal tubules. H&E stain 100X.
Discussion

The increases of AST and ALT enzymes in rats injected with the doses of sustanon used in this study means that the damage of hepatocytes may have been due to the destruction of mitochondria and therefore the release of these enzymes outside the hepatocytes. ALT increase is more indicative of liver damage than AST (26) because AST increase is indicative of muscle and hepatocytes damage due to the fact that this enzyme is found in muscles too (10). This result was different from that obtained from other results when serum parameters used for monitoring hepatic function did not change considerably after the injection with these steroids (4). Another result in this study was that hepatocytes apoptosis similar to what was seen in the use of blodenon, which is another anabolic androgenic steroid, was noticed (15). This steroid caused apoptosis by the increase of P53 protein responsible for such death. This result may suggest that sustanon could have caused apoptosis through the destruction of mitochondria. The misuse of these steroids for long periods may lead to morphological changes in the liver cells (24). On the other hand, alkylation of the molecule could cause a decreased liver function (12) and then greater hepatic toxicity (21).

It is clear from the results of this study that the high doses of sustanon caused serious disorders in the kidneys during the 6 weeks of exposure to sustanon which may have resulted in less ability to excrete nitrogenous compounds. The increased potassium and sodium ions observed by (1) in rats injected with sustanon meant a lower kidney functional ability which was considered as another sign of lowered kidney function. The kidney results of this study seem to support results obtained by Habscheid (11) who recorded a kidney dysfunction in a youth athlete, who had taken high doses of anabolic drugs for months, and may lead to renal failure. The results of this study were similar to those obtained by Hoseini (16) who noticed marked abnormalities in the kidney structure caused by high doses of steroids and were different
from those seen by Conway (6) who suggested that sustanon had very rare if any effects on kidneys. In regard to the mechanism of action of these effects on the kidney as a result of high doses of sustanon is still unknown. Nevertheless, a few hypotheses are suggested. Welder (25) and Draisci (7) for example supposed that testosterone toxic metabolites may cause these effects.

References


