

Pathological effects of anabolic steroid (Sustanon®) on liver of male rats

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Abstract

The present pathological study on the male rats aims to investigate the effects on liver tissue induced by repeated administration of three doses of sustanon for four periods. The experiment was done on the 100 adult male rats randomly divided into five groups 20 rats in each group. The first group is considered as a negative control treated with diet and water only. The second group is considered a positive control treated weekly for 60 days with sesame oil intramuscularly while groups III, IV and V treated with diluted sustanon in 5, 10 and 20 mg/kg body weight intramuscularly weekly for 60 days respectively. Blood was collected in a period 15, 30 and 60 days after treatment for measurements liver function tests ALT (alanine aminotransferase) and AST (aspartate aminotransferase) enzymes. Then the animals were dissected to take samples in a period 15, 30 and 60 days after treatment for histopathological examination, then 5 rats were left in each group in the diet and water for 30 days after last treatment for examination the above mentioned parameters. The results revealed the presence of significantly increasing of liver enzyme activation represented by ALT and AST at level $P < 0.05$ compared with control groups. The value of these levels were higher in group V in a day 60 after treatment and its continue to increase even after stopping treatment and remained on diet and water only for 30 days. Pathologically, all treated groups with sustanon revealed gross and histopathological changes in liver tissue, there were enlargement and congestion gross. Histopathologically, the liver sections elucidate cellular swelling, vacuolar degeneration in the cytoplasm of hepatocytes in addition to fatty change and programmed cell death in all groups during a period 15, 30 and 60 days these changes continue even after stopping the treatment for 30 days but portal fibrosis has been observed. It has been concluded from this study that *sustanon* in concentration 5, 10, 20 mg /kg of body weight in a periods 15, 30 and 60 have ability to induce hepatotoxic effect on liver of male rat and these effects irreversible and progressive for 30 days after stopping the drug administration.

Keywords: Anabolic steroid; Sustanon; ALT; AST; Liver

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التأثيرات المرضية للستيرويد البنائي (السستانون) على كبد ذكور الجرذان

انتصار رحيم الكناني و انتصار خزعل الحمداي

فرع الامراض وامراض الدواجن، كلية الطب البيطري، جامعة الموصل، الموصل، العراق

الخلاصة

ان الدراسة المقدمة تهدف الى الكشف عن التأثيرات المرضية في نسيج الكبد والمحدث باعطاء ثلاثة جرعات من السستانون وعلى اربعة فترات متتالية. اجريت التجربة على ذكور الجرذان قسمت عشوائيا الى خمسة مجاميع عشرون جرذا لكل مجموعة. المجموعة الأولى عدت مجموعة سيطرة سالبة تم معاملتها بالماء والعلف، المجموعة الثانية فقد عُدَّت مجموعة سيطرة موجبة تم حقنها بزيت السمسم أسبوعياً لمدة ٦٠ يوماً عن طريق الحقن بالعضل، بينما المجاميع الثالثة والرابعة والخامسة فقد تم معاملتها بمركب السستانون المخفف وبالتركيز ٥ و ١٠ و ٢٠ ملغم/ كغم من وزن الجسم على التوالي أسبوعياً ولمدة ٦٠ يوماً عن طريق الحقن العضلي، تم سحب الدم خلال الفترات ١٥ و ٣٠ و ٦٠ يوماً بعد المعاملة لغرض قياس انزيمات وظائف الكبد المتمثلة بناقل أمين الاسبارتيت Aspartate aminotransferase enzyme (AST) وناقل أمين الالانين Alanine aminotransferase enzyme (ALT) بعدها تم تشريح الحيوانات واخذ نماذج من الكبد بواقع

خمسة عينات من كل مجموعة وعند الفترات المذكورة اعلاه. تركت خمس جردان من كل مجموعة على الماء والعلف لمدة ٣٠ يوماً بعد آخر معاملة تم سحب الدم و اجراء القتل الرحيم Euthanesia للحيوانات واخذ نماذج الكبد لمعرفة هل ان قياس انزيمات وظائف الكبد والتغيرات المرضية تقدمية ام رجعية. بينت النتائج وجود ارتفاع معنوي في مستوى كل من فعالية إنزيمات وظائف الكبد المتمثلة بناقل أمين الاسبارتيت (AST) Aspartate aminotransferase enzyme وناقل أمين الالانين (ALT) Alanine aminotransferase enzyme. وكانت قيم هذه المستويات أكثر شدة عند المجموعة الخامسة في اليوم ٦٠ بعد المعاملة وعند مستوى معنوية $P < 0.05$ مقارنة بمجموعتي السيطرة (الأولى والثانية) واستمرت بالارتفاع حتى بعد ترك الحيوانات ٣٠ يوماً بعد آخر معاملة على الماء والعلف فقط. واما من الناحية المرضية فقد أظهرت كافة المجاميع المعاملة بالسستانون تغيرات مرضية عيانية ونسجية في نسيج الكبد حيث أوضحت النتائج عيانياً تضخماً واحتقاناً شديدين في الكبد، نسجياً أظهرت المقاطع النسجية وجود تغيرات تمثلت بالتورم الخلوي والتكس الفجوي فضلاً عن التغيرات الدهنية في هولي الخلايا الكبدية فضلاً عن الموت الخلوي المبرمج وفي كافة الجرعات وعند الفترات ١٥ و ٣٠ و ٦٠ يوماً بعد المعاملة واستمرت هذه التغيرات وصاحبها التليف في الباحة البابية بعد ترك الحيوانات لمدة ٣٠ يوماً على الماء والعلف بعد آخر معاملة. نستنتج من هذه الدراسة بان للسستانون وعند الجرعة ٥ و ١٠ و ٢٠ ملغم/كغم من وزن الجسم وخلال الفترات ١٥ و ٣٠ و ٦٠ يوماً كانت له تأثير مباشر على كبد ذكور الجرذان واستمرت حتى بعد انقطاع المعاملات لمدة ٣٠ يوماً وتغذيتها على الماء والعلف فقط ترافقها تغيرات على مستوى وظائف الكبد وكانت هذه التغيرات تقدمية.

Introduction

The phenomenon of abusing Anabolic androgenic steroids (AAS) by many youth and athletes is a serious health phenomenon which increase rapidly in recent years (1,2). Anabolic androgenic steroids are group of synthetic compounds related to testosterone structurally (3,4) androgens play a central role in the biology on medical practice in status as muscle wasting or debilitation (3). The high doses of these anabolic steroids such as metandienone, stanozolole, oxandrolone and sustanon, in order to obtain and produce a rapid and huge increasing in the skeletal muscle mass and to improve their performance during the sport competition (5).

For instance, sustanon (*Androgenicum prolongatum*) is one of these AAS which has many useful therapeutic usages, it is clinically used to treat cases of osteoporosis, male hypogonadisms and infertility (6). In regard to sustanon it is characterized by a very unique and distinguish pharmacological structure and properties comparing to other AAS drugs, it consists of an oily mixture of four different testosterone ester compounds which provide a continuous release of testosterone into the blood serum for a duration of 3 – 4 weeks (7,8).

These drugs were given to race horses and dogs who wish to improve physical performance (1,8,9). Anabolic androgenic steroids suggest could be a possible new risk factor for causing a disease termed toxicant associated steatohepatitis (TASH) (10). It has the ability to give a significant increase in muscle mass and strength, these are the main reasons why many bodybuilders prefer to include sustanon dosage in the cycle because it can produce immediate results as a fast acting steroid. Besides this significant, there were many side effects produced from using sustanon. For example since that compound easily converted

to estrogen so increase growth of breast tissues, this condition called gynecomastia (11).

Previous studies also reported many serious adverse effects resulted from abusing these anabolic drugs which induce cardiovascular disorder (particularly enlargement of the left ventricle) which can lead to sudden death, acute hepatitis and jaundice, testicular dysfunction, which lead to infertility, hypertension, behavioral disorders (12-20).

By reviewing the literature it was observed that hepatic histological changes induced by sustanon have been limited studies so, this study conducted to document and report the histological and biochemical alteration in liver enzymes of graded doses of sustanon in different periods on hepatic tissue.

Materials and methods

Ethical approval

The study was approved by Section of Animal Ethics Committee in the College of Veterinary Medicine, University of Mosul.

Male albino rats were obtained as three months old from laboratory animal house of veterinary medical college, body weight 250-350 gm. Animals had free access to laboratory chow and tap water. They were maintained on a 12:12 hour light – dark cycle and housed in an animal room where the temperature (22-26°C) was controlled.

100 male rats were randomly divided into 5 groups of 20 rats each. Group I received the regular rate diet and maintained as negative control group, group II were intramuscular (IM) injected with sesame oil (which used to dilute sustanon for injection), group III, IV, and V treated with IM injection of sustanon (*Androgenicum prolongatum*) in concentration 250mg/ml (obtained from Organon Oss Holland company) about (5, 10 and 20 mg/kg of body weight), respectively for 60 days one dose weekly. Five rats

from each group remained for 30 days (30 days after stopping of treatment additives on normal diet and water only).

Blood samples were taken from eye vein, after overnight fast for 12 hours and biochemical analysis performed in fresh heparin treated serum.

Serum were collect at 15, 30 and 60 days after treatment then at 30 days after stopping the administration of sustanon and only dieting on normal diet and water. 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 Biolabo French Company.

Sustanon obtained from organon Oss Holand company (250 mg/ml) and the volume used is 5 ml. and we use sesame oil from Bailasan company/mosul/Iraq.

Procedures

For light microscopy livers of the animal from different groups were examined for gross fixed then perfused in 10% neutral buffered formaline for 48-72 hours. The specimens were dehydrated in ascending grades of alcohol, clearing in xylene and embedded in paraffin wax, 4-6 Microne thickness sections were obtained and stained with Harris haematoxylin and eosin (H&E) for microscopic examination (21).

Statistical Analysis

We use SPSS program Sixteen edition we use analysis of Varians ANOVA (one way analysis) and Duncan's test to

test the significant differences between the groups at level $P < 0.05$ (22).

Results

Results of the blood serum biochemical analysis are listed in table 1 and 2, a significant difference in the level of ALT enzyme in the blood serum of male rats treated with sustanon in a dose 5, 10, 20 mg/kg body weight compare with control groups in a periods 15, 30 and 60 days after treatment at level $P < 0.05$ was observed, and the highly value are recorded in GV (20 mg/kg) reach 56.82 ± 1.29 IU/L (table 1).

The percentage level of ALT enzyme in all treated groups with sustanon in a dose 5, 10, 20 mg/kg B.W. revealed increased in all group treated but the highest percentage showed in group V which reach 32%, 34% and 34% in a periods 15, 30 and 60 days respectively (Figures 1).

While table 2 revealed a significantly increasing and difference in a level of AST enzyme in blood serum of male rats treated with sustanon at a dose 5, 10, 20 mg/kg/BW at level $P < 0.05$ in a period 15, 30 and 60 days after treatment, also the highly values were recorded in GV (20 mg/kg) B.W. at a day 60 which reach 69.50 ± 1.40 IU/L, and the percentage of level of AST enzyme in all treated groups of A. *p.* illustrated a significantly increased in their percentage and the highly percentage was recorded in group V which reach 32%, 35% and 36% via the periods 15, 30 and 60 days respectively (Figures 2).

Table 1: Level of ALT enzyme IU/L in the blood serum of male rats treated with sustanon in a dose (5, 10, 20) mg/kg B.W. compare with control groups (G₁ and G₂)

Groups	Treatments	Value / Days		
		Day 15	Day 30	Day 60
Group 1	Negative control	10.75±0.70	10.66±0.56	11.53±0.39
I	(Food & Water)	C a	D a	D a
Group 2	Positive Control Treated	10.00±0.96	10.54±0.80	10.46±0.65
II	with sesame oil	C a	D a	D a
Group 3	Treated with sustanon in	34.11±2.27	35.68±2.37	40.30±0.72
III	dose 5 mg/kg	B b	C ab	C a
Group 4	Treated with sustanon in	37.77±1.54	46.78±1.72	46.46±1.47
IV	dose 10 mg/kg	B b	B a	B a
Group 5	Treated with sustanon in	42.74±0.78	54.68±1.28	56.82±1.29
V	dose 20 mg/kg	A b	A a	A a

The value expressed by Mean ± Standard Error, Capital litter which are different vertically means there are significant difference between groups at level $P < 0.05$. Small litter which are different horizontally means there are significant difference within the groups at level $P < 0.05$. N= 5.

The table 3 revealed a significantly difference in a level of ALT enzyme in blood serum of male rats treated with sustanon in dose (5, 10 and 20) mg/kg body weight after 30

days from discontinuation of treatment at level $P < 0.05$ compare with control groups (20 mg/kg B.W.) compare with 60 days after treatment. On other hand table 3 reveals

a significant increases and differences in a level of AST in blood serum of male rats treated with sustanon in dose 5, 10, 20 mg/kg B.W. after 30 days discontinuation of treatment with A. p.at level $P < 0.05$ as compared with

control groups (GI and GII) while highly value recorded in group V (20 mg/kg B.W.) as compared with its level at a day 60.

Table 2: Level of AST enzyme IU/L in the blood serum of male rats treated with sustanon in dose (5, 10, 20) mg/kg B.W. compare with control groups (G₁ and G₂)

Groups	Treatments	Value / Days		
		Day 15	Day 30	Day 60
Group 1 I	Negative control (Food & Water)	10.40±0.80 B a	10.80±0.79 D a	10.66±0.56 E a
Group 2 II	Positive Control Treated with sesame oil	37.44±3.80 AB b	13.23±0.78 D a	13.96±0.21 D a
Group 3 III	Treated with sustanon in dose 5 mg/kg	37.45±1.49 AB b	40.58±0.89 C ab	43.54±0.83 C a
Group 4 IV	Treated with sustanon in dose 10 mg/kg	44.10±1.76 AB b	52.21±1.69 B a	54.15±0.87 B a
Group 5 V	Treated with sustanon in dose 20 mg/kg	51.08±2.36 A c	63.54±1.26 A b	69.50±1.40 A a

The value expressed by Mean ± Standard Error. Capital litter which are different vertically means there are significant difference between groups at level $P < 0.05$. Small litter which are different horizontally means there are significant difference within the groups at level $P < 0.05$. N = 5.

Table 3: Level of ALT and AST enzyme IU/L in the blood serum of male rats treated with sustanon in concentration (5, 10, 20) mg/kg B.W. compare with control groups (G₁ and G₂)

Groups	Treatments	Value / Days			
		ALT		AST	
		Day 60	Day 30 after last treatment	Day 60	Day 30 after last treatment
Group 1 I	Negative control (Food & Water)	11.53±0.39 D a	11.93±0.57 A a	10.66±0.56 E a	10.88 ± 0.82 D a
Group 2 II	Positive Control Treated with sesame oil	10.46±0.65 D a	10.48±0.95 A a	13.96±0.21 D a	12.19 ± 0.58 D a
Group 3 III	Treated with sustanon in dose 5 mg/kg	40.30±0.72 C b	47.79±2.32 B a	43.54±0.83 C a	52.00 ± 3.11 C a
Group 4 IV	Treated with sustanon in dose 10 mg/kg	46.46±1.47 B b	56.75±1.33 C a	54.15±0.87 B b	64.88 ± 1.52 B a
Group 5 V	Treated with sustanon in dose 20 mg/kg	56.82±1.29 A a	61.58±1.97 D a	69.50±1.40 A a	73.52 ± 1.15 A a

The value expressed by Mean ± Standard Error. Capital litter which are different vertically means there are significant difference between groups at level $P < 0.05$. Small litter which are different horizontally means there are significant difference within the groups at level $P < 0.05$. N = 5.

Pathological changes

Macroscopic Changes

Rats liver treated with 5 mg/kg/B.W. of sustanon showed swelling association with pin point hemorrhage in a period 15, 30 and 60 days of IM injection. This lesion continue and more evidence at 30 days after stopping treatment as compared with control group moreover in a dose 20 mg/kg B.wt. Severe enlargement with fatty in

consistency associated with petechial hemorrhage on ventral surface of hepatic tissue was observed (Figure 3), at 15 and 30 days and more evidence at 60 days post treated, but after leaving animal without treatment with sustanon for 30 days it showed granules in consistency associated with sever congestion (Figure 4), as compared with control groups.

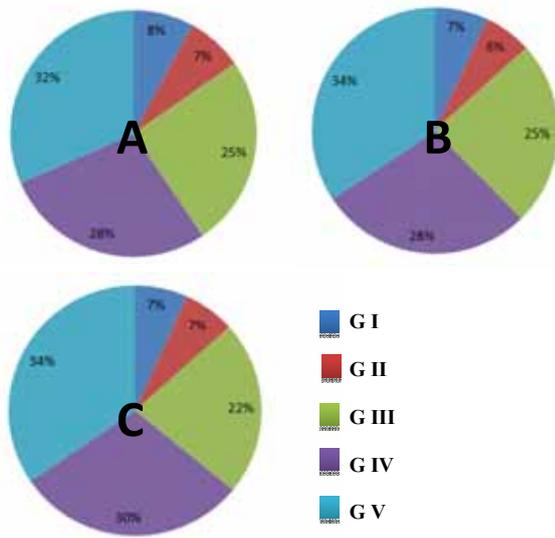


Figure 1: Percentage of ALT in serum of male rats of all groups treated with sustanon at day 15 (A), 30 (B) and 60 (C) as compared with control groups.

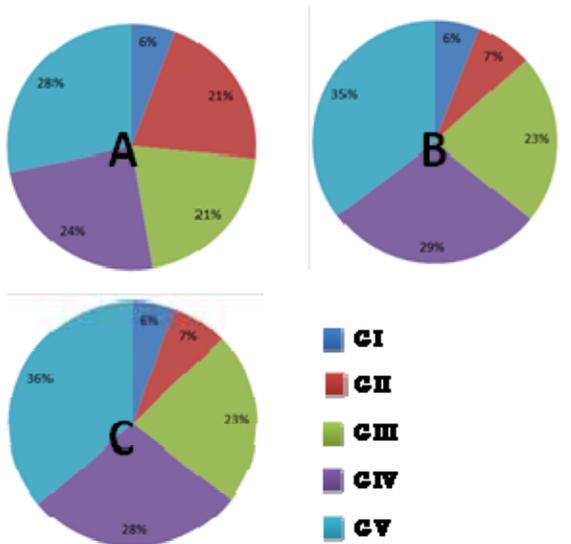


Figure 2: Percentage of AST in serum of male rats of all groups treated with sustanon at day 15 (A), 30 (B) and 60 (C) as compared with control groups.

Microscopic changes

Histopathological section of rats liver showed no histological lesions in control groups, while in group treated with sustanon in a dose 5 mg/kg Bwt after 15 days illustrated congestion of blood vessels (artery and vein) in

portal area, sinusoidal congestion and dilation in addition to privascular of mononuclear inflammatory cells. Vacuolar degeneration and cell swelling, also observed. Some sections revealed fatty change hypertrophied kupffer cells and pyknotic nuclei of hepatocytes (Figuer 5). At 30 days all lesions observed at 15 days continous, in addition to programmed cell death of some hepatocytes (Figuer 6).

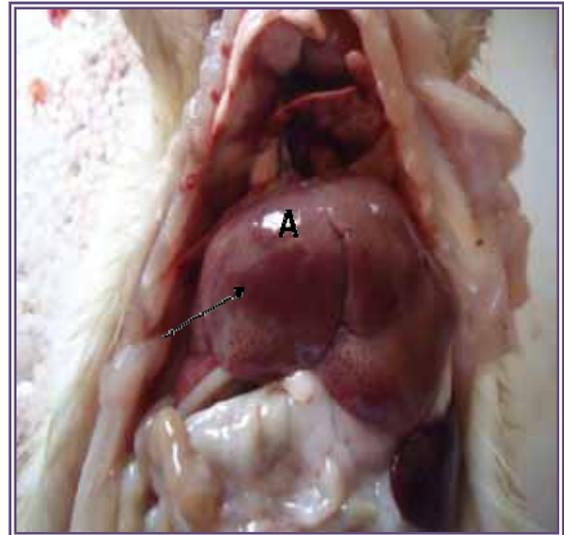


Figure 3: Rats liver treated with sustanon in a dose 10 mg/kg Bwt in a day 30 showed enlargement and sever congestion (A) and pinpoint hemorrhage (arrows).

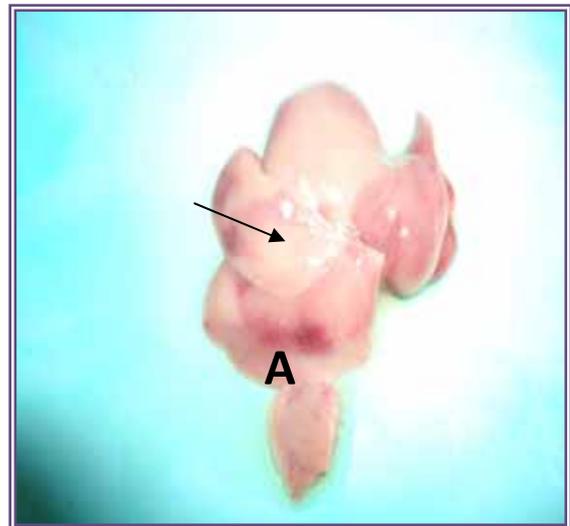


Figure 4: Rats liver treated with 20 mg/kg Bwt in a day 15 showed sever palness (arrow) with fatty consistency and pin point hemorrhage (A).

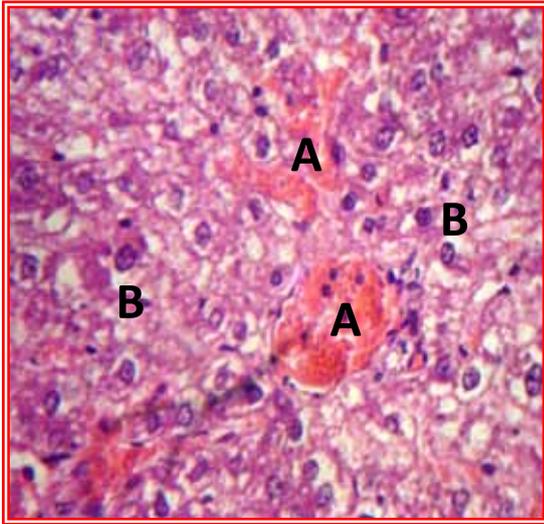


Figure 5: Histological section of rat liver treated with sustanon in a dose 5 mg/kg of body weight after 15 days showed sever congestion of central vein and sinusoids (A) vacuolar degeneration in the cytoplasm of hepatocytes (B). Stain: H&E, 560X.

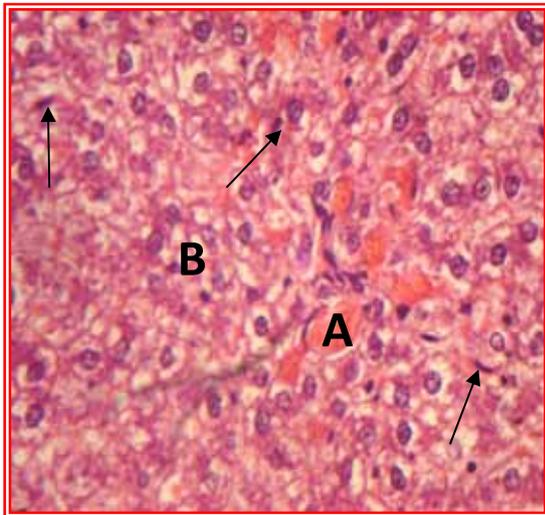


Figure 6: Histological section of rat liver treated with sustanon in a dose 5 mg/kg of body weight after 30 days showed sever congestion in sinusoids (A) vacuolar degeneration (B), swelling of kuffer cell (arrow). Stain: H&E, 560X.

While at 60 days sections of liver revealed loss the normal architectures of lobules in addition to congestion of blood vessels and programmed cell death of hepatocytes. Rat treated with 10 mg/kg Bwt at 15 days also showed same changes but more sever. At 30 – 60 days figures

showed sever congestion of blood vessels and diffuse vacuolar degeneration in addition to congestion necrosis, and infiltration of mononuclear inflammatory cells with programmed cell death (Figuer 7).

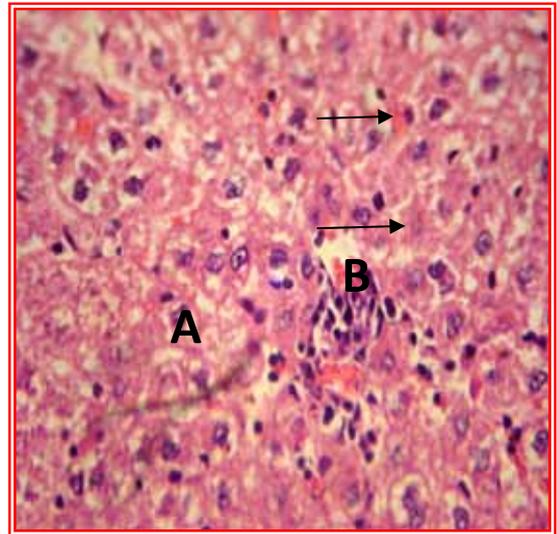


Figure 7: Histological section of rat liver treated with sustanon in a dose 10 mg/kg of body weight after 60 days showed sever vacuolar degen eration (A) and foci of coagulation necrosis (B) programmed cell death (arrow). Stain: H&E, 560X.

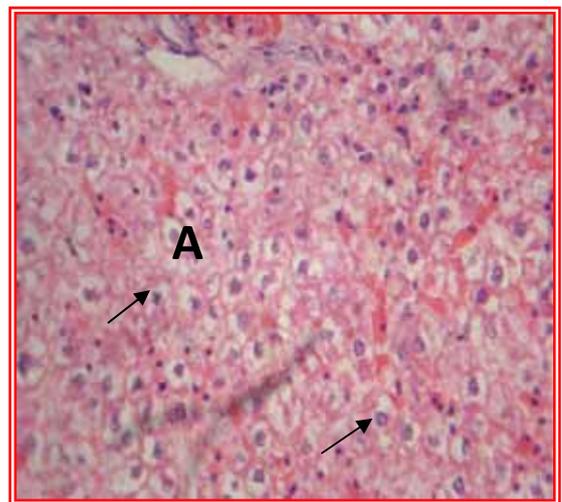


Figure 8: Histological section of rat liver treated with sustanon in a dose 20 mg/kg of body weight after 15 days showed loss of normal arrangement of hepatic cords and sever vacuolar degeneration in the cytoplasm of hepatocytes (A) congestion of sinusiod (arrow). Stain: H&E, 450X.

Rats administrated with 20 mg/kg b.wt. in a day 15 showed lobular and centrolobular necrosis, hypertrophy and hyperplasia of kupffer cells, while at 30 – 60 days, all lesions continue in addition to hyperplasia of epithelial lining bile duct (Figure 8).

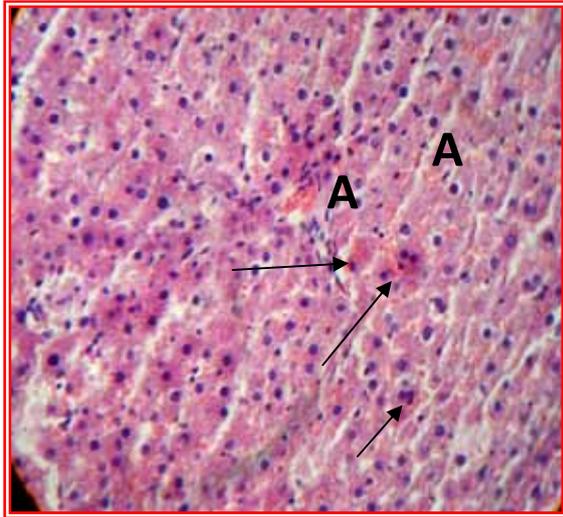


Figure 9: Histological section of rat liver treated with sustanon in a dose 10 mg/kg of body weight after 30 days of stopping of treatment, Showed dilatation of sinusoids (A) with programmed cell death of hepatocytes (arrows). Stain: H&E, 450X.

In animal left without treatment with *A. p.* for 30 days and feeding on normal diet and water only liver showed portal fibrosis (collagen proliferation, newly capillaries and newly canaliculi), bile pigment deposition in addition to loss of the normal arrangement of hepatic cord and apoptotic hepatocytes (Figures 9-11).

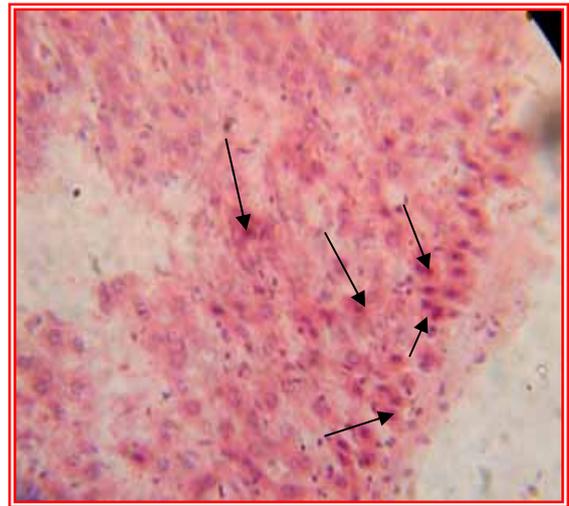


Figure 11: Histological section of rat liver treated with sustanon in a dose 20 mg/kg of body weight after 30 days of stopping of treatment, show programmed cell death of hepatocytes (arrows). Stain: H&E, 450X.

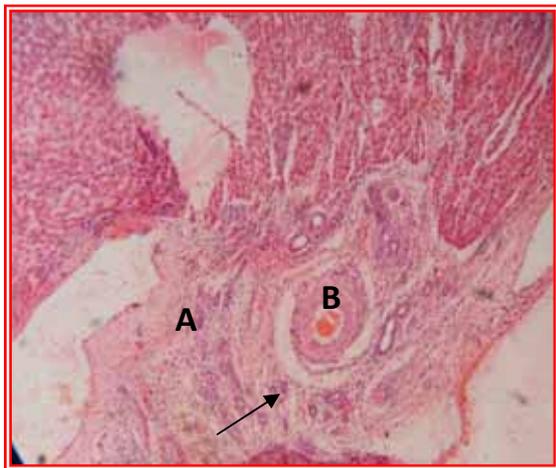


Figure 10: Histological section of rat liver treated with sustanon in a dose 20 mg/kg of body weight after 30 days of stopping of treatment, showed sever fibrosis in portal of area (A) with congestion of blood vessels (B) and proliferation of bile lacnini (arrows). Stain: H&E,200X.

Discussion

The result of this study showed that IM injection of sustanon at 5, 10, 20 mg/Kg BW for 15, 30 and 60 days induce hepatotoxicity in male rats of groups III, IV and V as verified by biochemical and histological finding, and marked elevation in level of ALT and AST in blood serum of male in group III, IV, V as compared with control groups and these elevation continued after stopping treatment for 30 days, suggestion that these changes reflected the occurrence of liver injury. The present results support the previous study of (23) who reported that administration of sustanon in a dose 5 mg/kg b.w. subcutaneously for 10 weeks cause increase level of ALT as compared with control group. The elevation of ALT and AST in blood serum with increasing the dose means damages of hepatocytes was take place through the damaging of mitochondria, which lead to leakage this enzyme outside of hepatocytes and reach to blood ALT is more specific for liver injury than AST (24). Using anabolic steroid randomly for long time can induce hepatotoxicity and morphological alterations of the liver cells.

On the other hand, AST activity was increased in animal treated with sustanon, it may indicate the presence of muscle damage as well as hepatocytes because these enzyme is specific for muscle so it means that damage of muscle also take place (25).

This study was not in agreement with other studies shows that serum parameters commonly evaluated in athletes to test hepatic function are not substantially modified by administration of the anabolic steroids particularly sustanon cause alteration in liver function due to alteration in morphological and histological structure of hepatocytes. The pathological changes revealed hypertrophy, congestion in central vein and sinusoid (particularly in 20 mg/kg Bwt for 30 to 60 days) indication for chronic venous congestion takeplace, also fatty changes in consistency was observed, and these changes reflect to injury of liver cell occurrence due to toxic hypoxia producing from different doses of sustanon like other anabolic steroid (16). Congestion and hemorrhage take place due to increase hydrostatic pressure in blood cause vessels accumulation of red blood cell in the lumen of blood vessels, which allow blood cells to diapedesis and leakage out the blood vessels to cause bleeding. Moreover, sustanon may have ability to cause lipid peroxidation via reactive oxygen species which lead to fatty changes in addition to hypoxia.

Histopathological lesions also observed in this study started in 5 mg/kg Bwt, and reach to 20 mg/kg B.wt. in 15, 30, 60 days which continues after stopping treatment for 30 days, this means that sustanon have ability to induce progress liver cell injury because its considered as a first organ responsible for metabolism of sustanon compound. So, this injury induced by accumulation of toxic metabolite (Xenobiotic) from sustanon metabolism that includes 17, α -19-Nortesterone, 17- α -testosterone (27).

On the other hand, figures showed cell swelling, sinusoid dilation and congestion in addition to centrolobular necrosis, these lesions occurs due to that sustanon like other anabolic androgenic steroid have ability to cause injury of hepatocytes through the effect on mitochondria particularly mitochondrial membrane and inhibit mitochondrial respiration which lead to swelling of mitochondria and cause leakage of ALT and AST (28).

An interesting finding in this study was a presence of programmed cell death in hepatocytes, like other anabolic androgenic steroid for example blodenone undecyleante (29) they illustrated that this drug have ability to cause programmed cell death by using immunohistochemistry through increasing in a protein P₅₃ which responsible for programmed cell death (so this observation need more studying in future). Also, our study suggested that sustanon have ability to cause programmed cell death through the ability to damage cell DNA and mitochondria.

Moreover, this study showed presence of fibrosis in portal area particularly in animal lefted for 30 days after stopping the treatment, this means that liver go to healing the damaged cells by fibrosis in portal area not regeneration of other hepatocytes because the damage is powerful and strong and may need more than 30 days for regenerations because sustanon remain in tissue for 4 weeks after stopping administration (29).

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