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CONCOMITANT OCCURRENCE OF OXIDATIVE STRESS WITH SUSTANON IN MALE RAT

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ABSTRACT

The present study was conducted to investigate the effect of androgenic–anabolic steroids Sustanon 20 mg/ kg on oxidative stress. Twenty male rats were divided into four groups treated for 4 weeks as once weekly treatment. The 1st group considered as control group, the 2nd group injected with sustanon 20 mg/kg intramuscularly, the 3rd group injected with sustanon intramuscularly 20 mg/kg and then orally dosage of vitamin E 100 mg\kg, and 4th group got orally dosage of vitamin E 100 mg/kg. The results revealed significant decrease in glutathione level in the serum and tissue of brain and liver in sustanon group as compared with control, while there is significant increase in malondialdehyde level in the serum of sustanon group as compared with control The result showed no different between the groups treated with sustanon alone and with sustanon and vitamin E.

All the pathological changes have been recorded for the sustanon group only while there were no pathological changes for other groups. The gross pathological changes showed congestion of heart and liver in sustanon group.. The microscopic changes of heart showed vacuolar degeneration of cardiac cells and edema between it. Liver showed necrosis of hepatic cells, infiltration of inflammatory cells. Lungs revealed pneumonia with thickening of wall of alveoli and bronchioles by inflammatory cells, and emphysema of alveoli.

We conclude from this study that sustanon in high dose have an effect on increase oxidative stress, macroscopic and microscopic pathological changes in heart, liver and lung, and no effect of vitamin E in this study.

INTRODUCTION

Several clinical studies showed that administration of anabolic-androgenic steroids (AAS) in the pharmacological doses has a beneficial effect in many cases and is considers safe drug (1). On the other hand, the long-lasting administration of high doses of steroids can seriously harm health (2,3). The effects of high doses of AAS for a long time may cause various side effects (4). One of those harmful effects is induced oxidative stress due to disturbance in the balance between oxidants and antioxidants (5). Persistent oxidative stress may lead to pathological cases and development sever pathologies (over 150 disorders) (6).

Overdoses of AAS cause cardiovascular disorders such as hypertrophy of the left ventricle of the heart, arterial hypertension, cardiac arrhythmia, blood clotting, coronary blood flow disability, myocardium inflammation, acute coronary inefficiency, cardiac infractions, arterial sclerosis, circulatory failure and heart attacks which may end of sudden death (2). It can also cause some mental instability in the form of a maniac-depressive states expressed by uncontrolled nervousness or deep depression associated with some suicidal inclinations (3). Oral synthetic steroids, such as stanozolol, reduce high-density lipoprotein (good fat) and increase low-density lipoprotein cholesterol (bad fat) more than injected testosterone at similar doses(7). Sustanon is described as a long acting anabolic androgenic drug(8). Regardless of these unique pharmacological structure and properties of Sustanon, only few studied attempted to investigate the adverse effects induced by overdose of Sustanon abuse.

This is absolutely not correct as there are many studies which confirmed the occurrence of irreversible adverse effects among the abusers such as cardiac disorders and chronic hepatitis (9,10,and 11). Testosterone replacement therapy has also been shown to reduce circulating levels of inflammatory mediators, such as TNF-α and IL-1β as well as total cholesterol in patients with established coronary artery disease (CAD) and testosterone deficiency (12,13).

Primary aims of this study to assess the effects of toxic dose of sustanon and concomitant with oxidative stress, the role of vitamin E, and histopathological changes of heart, lung and liver.

MATERIALS AND METHODS

Experimental design and treatment:

Twenty male albino rats, weighing between 250-300 g were maintained in clean plastic cages in the laboratory animal room (23 ± 2 °C). Daily dark/light cycle (12/12 hrs) rats were randomly divided in to four groups treated for 4 week administered as once weekly. The first group considered as control, the second group deeply injected with 20 mg/kg sustanon (19) intramuscularly and third group injected with 20 mg/kg sustanon intramuscularly then orally dosage with vitamin E 100 mg animal , fourth group orally dosage with vitamin E 100 mg animal (21).

Chemicals and dose preparations:

Sustanon 250% from Organon Oss Holand company, it was dissolved in sesame oil for dose preparations; volume of dose is 2 ml/kg.

Oxidative stress parameter Malondialdehyde (MDA) content as indicator of lipid peroxidation was determined in the serum, by a colorimetric method according to (14).

The reduced form of glutathione (GSH) was determined in the tissue and serum by colorimetric method according to (15).

Collection of blood samples and organs:

Measurements: At the end of the treatment period (4weeks), each animal of the four groups was anesthetized by inhalation of drops of Diethyl Ether in a piece of cotton. Blood samples were collected from eye choroid venous plexus according to (16). Five ml of blood have been collected and immediately placed in clean tubes and then centrifuged for serum collection, three serum samples were separated from each animal sample and also animals was anatomical examine for study morphological changes and then organs (heart, liver, brain and lungs) were collected for biochemical and histopathological examination.

Histopathological Examination

For light microscopic investigations, specimens from heart, liver, and lungs were fixed in 10% phosphate buffer formalin, dehydrated in alcohols and embedded in paraffin. Five micron tissue sections were stained with hematoxylin and eosin stain for general histopathological examination. One slide was prepared for each rat.

Statistical Analysis

The results were expressed as means ± S.E.M. All data were done with the Statistical Package for Social Sciences (SPSS 11.0 for windows). The results were analyzed using one way analysis of variance (ANOVA) between different treatment groups. Statistical significance was set at $p < 0.05$.

RESULT

There were significant decreased in the serum GSH observed among treated Groups when compared with control group, were as serum MDA level was increased when compared with control group (Table 1).

Table 1: Effect of Sustanon on glutathione and malondialdihyde in serum of rats

Groups	GSH (Micromole/ml)	MDA (Nanomal/ml)
control	2.55 ± 0.07	0.9 ± 0.13
Sustanon 20 mg kg^{-1}	1.2 ± 0.08*	2.79 ± 0.09*
Sustanon 20 mg kg^{-1} + vit E 100 mg $animal^{-1}$	1.03 ± 0.12*	2.29 ± 0.13*
vit E 100 mg/ animal	2.8±0.04	1.0±0.1

Number of animals in each group was five (n = 5), data are expressed as (Mean±SE.); *: Significant difference ($p < 0.05$) comparing to control group.

Brain GSH were significant decreased in treated groups when compared with control group, were as liver GSH level was decreased in sustanon group when compared with control group (Table 2).

Table 2 Effect of Sustanon on glutathione in tissue of Rats

Groups	Brain	Liver
control	2.8 ± 0.07	2.9 ± 0.15
Sustanon 20 mg kg^{-1}	1.5 ± 0.08*	1.4 ± 0.09*
Sustanon 20 mg kg^{-1} + vit E 100 mg $animal^{-}$	1.4 ± 0.12*	2.6 ± 0.12
vit E 100 mg/ animal	2.4 ± 0.12	2.6 ± 0.12

Number of animals in each group was five (n = 5), data are expressed as Mean SE. *: Significant difference ($p < 0.05$) comparing to control group.

Pathological Changes

The gross examination showed that the heart was congested and enlarged and liver congestion (Fig. 1), whereas the lung showed inflammation, congestion and purulence formation (Fig. 2).

The microscopic changes of heart showed vacuolar degeneration of cardiac cells, hyalinization of cardiac muscles fibers and edema between it, infiltration of inflammatory cells, and congestion (Fig. 3). Liver showed necrosis of hepatic cells, infiltration of inflammatory cells, and congestion of central vein (Fig. 4). Lungs showed pneumonia with thickening of wall of alveoli and bronchioles by inflammatory cells, emphysema of alveoli, and congestion of pulmonary blood vessels (Fig. 5).

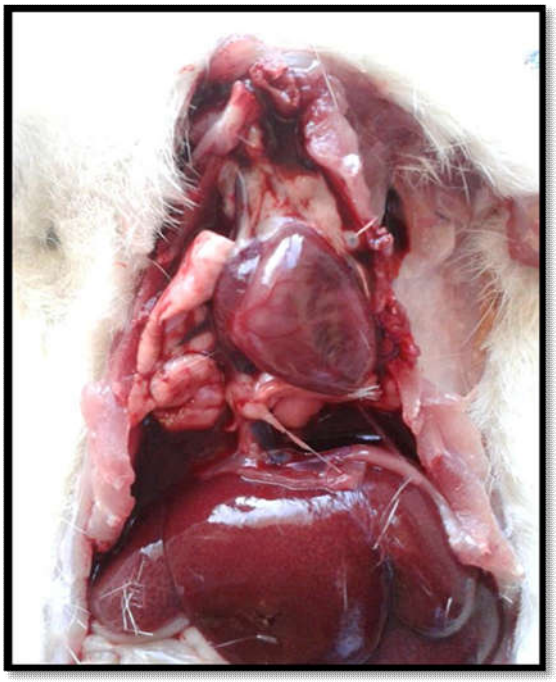

Fig. 1: Gross appearance of rat (sustanon group) showed the heart congested and enlarged (A) and liver congestion (B).

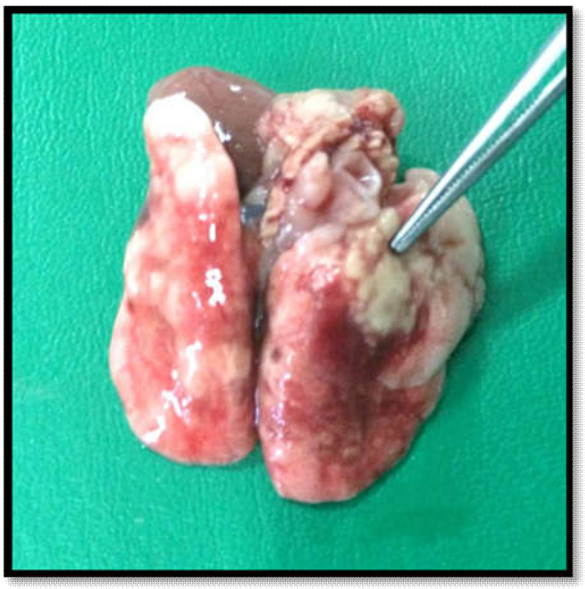

Fig. 2: Gross appearance of rat (sustanon group) showed the lung with inflammation, congestion and purulence formation.

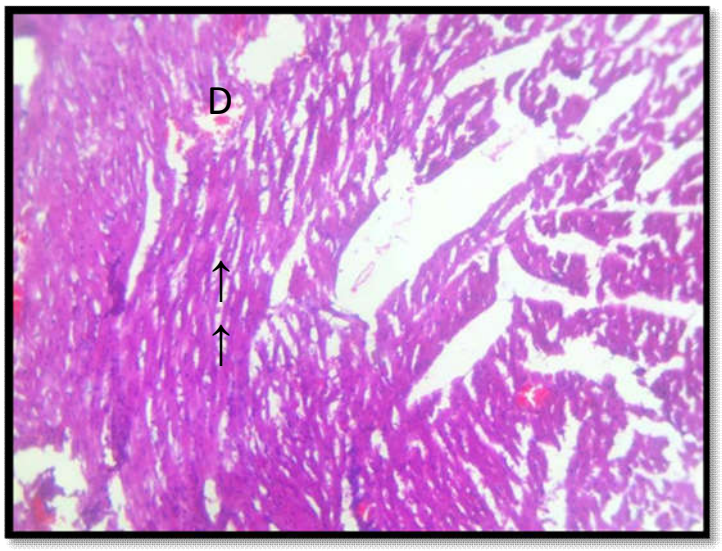

Fig. 3: Microscopic appearance of heart of rat (sustanon group) showed vacuolar degeneration of cardiac cells ↑, hyalinization of cardiac muscles fibers↑↑ and edema between it, infiltration of inflammatory cells (C), and congestion (D). H&E stain. 105X.

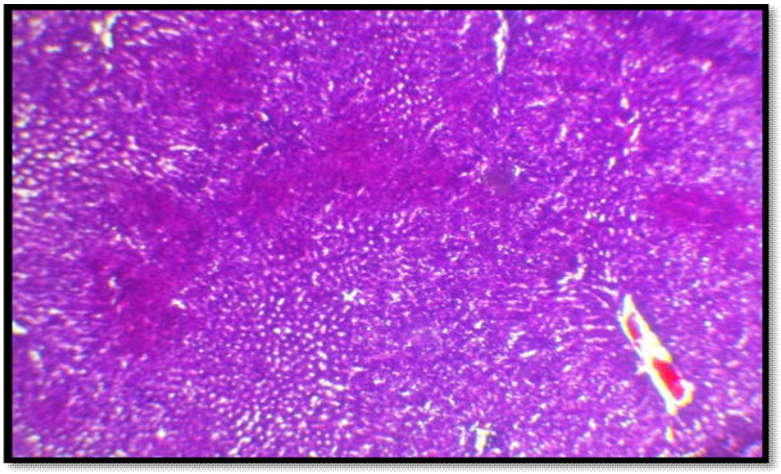

Fig. 4: Microscopic appearance of liver of rat (sustanon group) showed necrosis of hepatic cells (A), infiltration of inflammatory cells (B), and congestion of central vein (C). H&E stain. 195X.

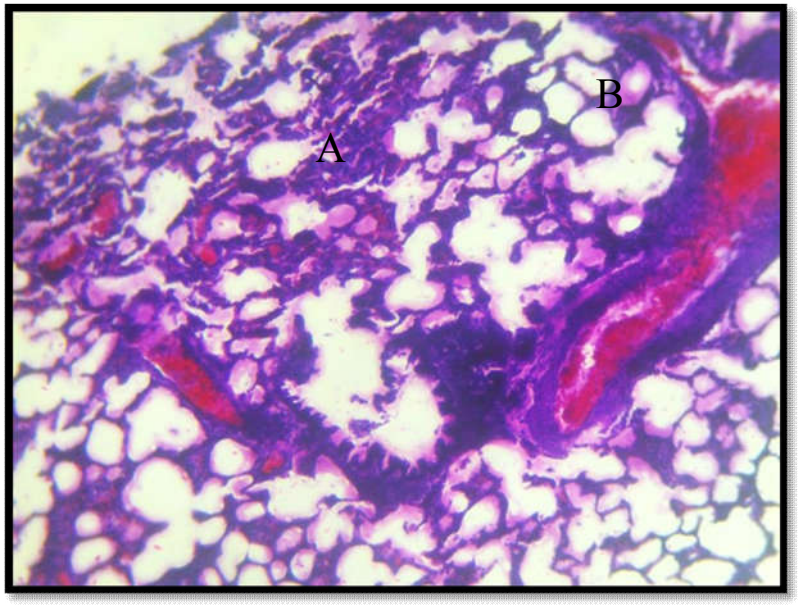

Fig. 5: Microscopic appearance of lung of rat (sustanon group) showed pneumonia with thickening of wall of alveoli and bronchioles by inflammatory cells (A), emphysema of alveoli, and congestion of pulmonary blood vessels (B). H&E stain. 105X.

DISCUSSION

Based on the available literature, the current study is believed to be as one of the prior studies which investigated the oxidative stress and its relationship with heart, lung and liver histopathological changes induced by high dose of Sustanon. However, there are many studies which confirmed the irreversible adverse effects such as cardiac disorders and chronic hepatitis (1,2). The histopathological changes is concomitant with increase malodialdihid and decrease glutathione in sustanon groups that is agreement with (17) Animals body prevent oxidative damage, ranging from antioxidant enzymes to low molecular weight antioxidants, and also specific cellular components that repair molecules which is oxidative damaged. A defect in the balance between pro-oxidants and antioxidants lead to oxidative damage (17).

Pathogenesis of overgrowth of skeletal muscles and heart muscle hypertrophy in subjects taking large doses of AAS is a complex process. It consist of many factors including the insulin-like growth factor 1 (ILGF1) and tissue growth factor 1 (TGFβ1)

(1) that may be the cause for heart hypertrophy in our study in group treated with sustanon.

In high doses, androgens tend to raise LDL cholesterol levels and lower HDL cholesterol levels. That's one of the things that gave testosterone its bad reputation. but in other circumstances, the situation is very different men with the lowest testosterone levels tend to have the highest cholesterol levels. Anabolic androgenic steroids suggest could be possible new risk factor for causing a disease termed toxicant associated steatohepatitis (TASH) (18). The possible reason for ineffective role for vitamin E may be law dose which use in experiment The severe lung injury that report in our study may be because of heart injury or decrease the immunity of lung because of sustanon treatment (20) and hepatopathologic changes agreement with another study (19) that showed Sustanon for 15-30-60 day induce hepatotoxic effect in male rat. These changes are irreversible and progressive for one month after end of drug treatment (19).

CONCLUSION

The high dose of sustanon can cause severe defects representing by increase oxidative stress and pathological damages of heart, lung and liver. Also there is no effect of vitamin E in decrease of oxidative stress effect done by over dose of sustanon.

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ترابط حدوثية الاجهاد التأكسدي مع السستانون في ذكور الجرذان

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الخلاصة

أجريت الدراسة الحالية لمعرفة تأثير السستانون ٢٠ ملغم/كغم على الإجهاد التأكسدي. تم تقسيم ٢٠ من ذكور الجرذان إلى أربع مجاميع عولجت لمدة ٤ أسابيع مرة واحدة اسبوعيا. المجموعة الأولى اعتبرت مجموعة سيطرة ، المجموعة الثانية حقنت بـالسستانون ٢٠ملغم/كغم حقناً عضلياً المجموعة الثالثة حقنت عضلياً بالسستانون ٢٠ مجم / كغم ثم جرعت مباشرة عن طريق الفم بفيتامين E 100ملغم/كغم، اما المجموعة الرابعة فقد جرعت عن طريق الفم بفيتامين E 100 ملغم/كغم . أظهرت النتائج انخفاضا كبيرا في مستوى الجلوتاثيون في مصل الدم والأنسجة في الدماغ والكبد ، في حين أن هناك زيادة كبيرة في مستوى بيروكسيد الدهن في مصل المجموعة المعاملة بعقار السستانون.

أظهرت النتائج عدم وجود اختلاف بين المجموعات التي عولجت بالسستانون وحده والمجموعة المعاملة بالسستانون مع فيتامين E كما أظهرت التغيرات المرضية العيانية احتقان القلب والرئة اما التغييرات المجهرية للقلب فقد اظهرت التنكس الفجوي في الخلايا العضلية القلبية والوذمة البينيه. كما حدث نخر في الخلايا الكبدية ، وارتشاح الخلايا الالتهابية، بينما أظهرت الرئتين وجود التهاب رئوي مع تثخن جدار الاسناخ والقصيبات الهوائية بالخلايا الالتهابية والنفاخ الرئوي للاسناخ. .

نستنتج من هذه الدراسة أن السستانون في الجرع العالية يكون له تأثير في زيادة الإجهاد التأكسدي مع حدوث تغيرات عيانية وميكروسكوبية في القلب والرئة، في حين لم يحدث أي تأثير لفيتامين E في هذه الدراسة.

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