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Estimation of some immunological parameters in rats primed by androgenic anabolic steroid(sustanon)

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Abstract

Sustanon is one of androgenic anabolic steroids ,which uses especially among young people and adolescent , and such drug has many long-term negative side effects , therefore , it has become a major health problem .This study was conducted to examine the effect of intramuscular injection of androgenic anabolic steroid (sustanon) on some immunological parameters in albino rats. Three doses of sustanon (0.05 , 0.1 , 0.2) mg/kg/day were injected respectively for six weeks. Immunological changes included a significant decrease ($P < 0.05$) in the levels of IL-1 β for males treated group in comparison with the control group , while there was a significant increase ($P > 0.05$) in its level for females treated group , compared with the healthy subject group . Meantime , there was a significant increase ($P > 0.05$) in the concentrations of IL-6 and C-reactive protein (C-RP) of both gender groups , compared with the control groups .

Keywords: Sustanon ,C-RP , IL-1 β , IL-6 , rats .

Introduction

Sustanon is one of AAS which includes many practical therapeutics treatments , medically it is used to treat several cases such as osteoporosis , hypogonadisms in male and infertility [10]. Sustanon characterized by an especially unique and

differentiate pharmacological structure and properties compare to the other AAS drugs, it is composed of an oily mixture of four different testosterone ester compound which provides a permanent release of testosterone into the blood serum for length level from 3 – 4 weeks[1] , furthermore , these drugs were given in order to race horse and dogs who wish to enhanced physical presentation [8], it can be increase muscle mass and strength, body builders , sustanon acts as fast steroid, in addition to this benefits , there was many side effect produce by using sustanon ,because it converted to estrogen thus increase growth of breast tissues, this state called gynecomastia [14]. Also many earlier studies recorded adverse effects from using of this drug including , cardiovascular disorder (mainly amplification of the left ventricle) , that leads to sudden death, also acute hepatitis with jaundice, testicular dysfunction result to infertility, hypertension, behavioral disorders [11,3]. Exogenous testosterone attenuated the production of pro-inflammatory cytokines such as TNF , IL-1 β , and IL-6 in human macrophages and dendritic cells [6] , while others anti-inflammatory cytokines such as IL-10 are stimulated in the presence of testosterone [12]. C –reactive protein (C-RP) is secreted by hepatocytes in response to elevated level of IL-6 , a cytokine associated with raised C-RP levels in unstable angina , furthermore its levels in apparently healthy men and women are associated with increased risk of future cardiovascular events , independent of established lipid and non-lipid coronary risk factors and elevated C-RP levels are associated with increased of exogenous testosterone and risk of death or infection [19] . Therefore , the aim of this study to find out the effects of intramuscular injection of sustanon on some immunological parameters in rats .

Materials and methods

Preparation of Sustanon (250 mg) Concentrations :

Sustanon ampoules (manufactured by N.V. Organon Oss Inc. Holland) have been obtained from the local pharmacy in - Iraq. Each ampoule contains 1 mL of oily solution of Sustanon. According to the manufacturer, 1 mL of Sustanon consists of four testosterone ester compounds which include testosterone propionate, testosterone ephenylpropionate, testosterone isocaproate and testosterone decanoate (Khder *et al.* , 2012).

Laboratory Animals :

Forty eight albino rats (*Rattus rattus*) , which divided into two main groups , the first group of males (24 rats) were divide into four subgroups (6 replication for each) , the first , second and third treatment sub groups injected sustanon (0.05 , 0.1 , 0.2) mg/kg/day respectively for six weeks , while the fourth subgroup was considered as control group which injected by physiological normal saline (0.9%NaCl) , the second major group of females (24 animals) has primed with the same drug and doses , and same periods , their weight ranged between 200-250 gm.

Blood sampling

Through the course of the study, the animals were anesthesia by using chloroform and sacrificed. About 5 ml of blood was collected directly by heart puncture with sterile syringes , and keep to stand into sterile plastic tubes for 30 minutes at room temperature to allowing clotting. The clotted blood was centrifuged at 3000 rpm for 10 minutes, and serum was collected for estimate the levels of Interlukin-1B(IL-1B) ,Interlukin-6(IL-6) , and C-Reactive Protein(C-RP) .

Immunological assay

The levels of IL-1 β , IL-6 and C-reactive proteins , were estimated by ELISA kit of rat , according to the procedures that provided by (Boaster Company , China) .

Statistical analysis:

The results of the study were analyzed statistically using SPSS software (Genstat) version (1995) this analysis calculates the arithmetic mean and standard error (Mean ± S.E.) comparison between the averages in different dosage intervals using less Ich difference between middle L.S.D. (Least Significant Differences) , and under level probability 0.05.

Results

1- levels of IL-1β : The results of this study showed that changes in the levels of IL-1β in sera of laboratory animals . There was a significant decreased (P< 0.05) in the concentration of this cytokine in males groups treated with sustanon (0.05 , 0.1 , 0.2) mg / kg of body weight , which reached 41.50 ±3.75 , 46.13 ±2.18 , and 64.06±0.75 ng/ml , compared with the control groups (75.13±6.53 ng/ml) , as well as there was significant increases (P >0.05) in females treated groups which reached 84.73 ±1.44,82.73 ±6.02, and 49.33 ±5.46 ng/ml compared with healthy groups (49.33±5.46 ng/ml) , L.S.D.(0.05)=10.433 , also the results showed that a significant differences among males and females treatment groups (0.05, 0.2) mg / kg of body weight . Furthermore ,there were no significant differences between t males and females in treated groups (0.05 , 0.1) mg / kg of body weight , compared within other groups , as shown in figure (1)

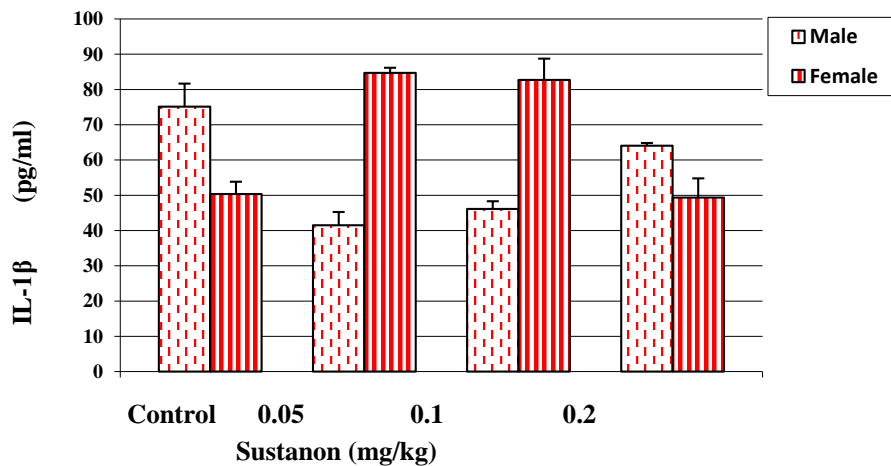
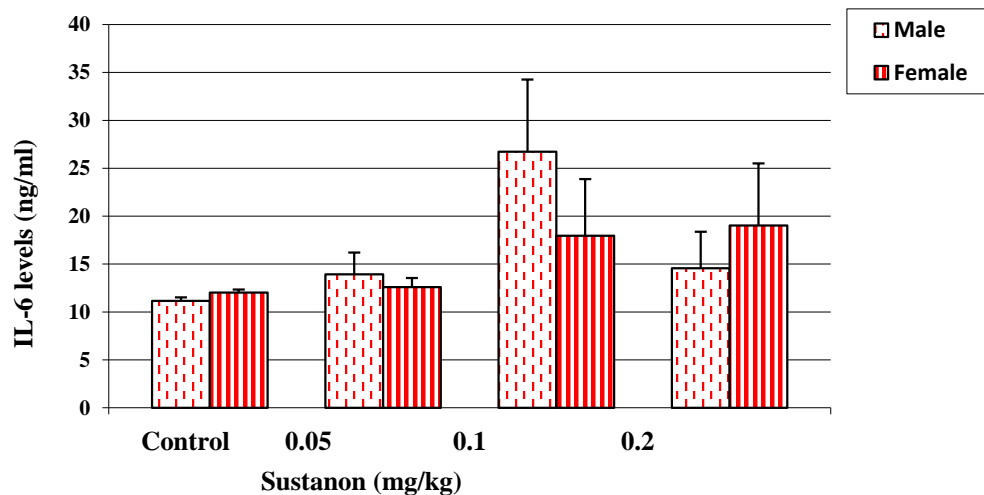


Figure (1): levels of IL-1β in rats primed by different concentrations of sustanon. .

L.S.D.(0.05)=10.433

2- levels of IL-6 : The current study illustrated that cha

nges in the levels of IL-6 between treatment and control groups . The results showed that a significant increases (P> 0.05) in the levels of IL-6 in males groups treated by sustanon (0.1)mg/kg of body weight, which reached 26.73± 7.53 ng/ml compared with control group (11.16±0.37ng/ml) , as well as there was significant increased (P >0.05) in females groups treated by sustanon (0.2) mg/kg of body weight which reached 19.93± 6.48ng/ml , in contrast with the control group (12.03±0.31 ng/ml) , L.S.D.(0.05)= 7.854. Also the results revealed that a significant differences among males treated groups (0.1 , 0.2) mg / kg of body weight , compared with the other groups , while there was no significant differences between females treated groups, compared with the other groups , as shown in figure (2) .



L.S.D.(0.05)= 7.854

Figure (2): levels of IL-6 in rats injected by different concentrations of sustanon .

3- levels of C-RP : The results revealed that changes in the levels of C-RP in experimental animals. There was a significant increase ($P > 0.05$) in males and females treated groups by sustanon (0.05, 0.1, 0.2) mg / kg of body weight, which reached 9.60 ± 0.30 , 14.74 ± 0.47 , 22.39 ± 0.32 ng/ml in males respectively, in comparison with control group (2.38 ± 0.61 ng/ml), while the concentrations of this cytokine in female groups were reached 9.63 ± 0.37 , 15.04 ± 0.92 , 24.10 ± 2.31 ng/ml, compared with control group (4.21 ± 0.71 ng/ml), L.S.D.(0.05)= 2.416. Also the data showed that a significant differences among treatment males and females groups (0.05, 0.1, 0.2) mg / kg, when compared with other groups, as illustrated in figure (3).

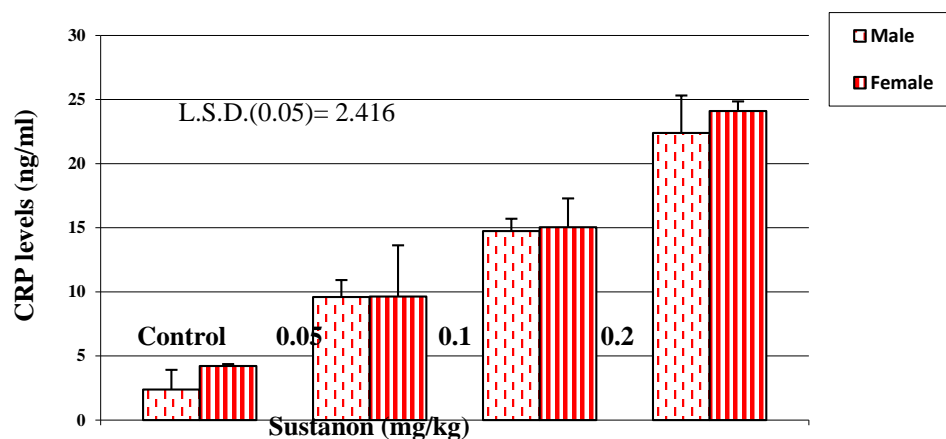


Figure (3): levels of C-RP in rats injected by different concentrations of sustanon .

Discussion:

Three different doses of sustanon, which have been chosen regarding the doses used by athletes, were administered to the rats in the current study. The results in figure (1), revealed that a significant decrease in the levels of IL-1 β in males

treated groups , compared with control groups, in contrast , there was a significant increase in females treated groups , compared with control groups. The males rats primed by sustanon may cause immunosuppressive , reduction of cytokine production , high level of exogenous testosterone weaken immune competence in male because testosterone has anti-inflammatory properties, which propose there was a relationship between the male sex hormone and immune response , while in women there was a higher blood levels of signaling proteins that immune cells pass back and forwards to start inflammation , required for activation of immune-system [9] .These evidence confirm why IL-1 β decreased in males and increased in females, affect immune responsiveness as evidenced by a decrease in antibody secretion [18]. Similarly, [17] have shown that testosterone uptake inhibits the proliferation and differentiation of B lymphocytes. While others suggested that AAS enhance immune function [4] . In contrast, the nature of their effects on the immune system depends on the type and dose of AAS used, as well as timing of administration. Nevertheless, it has been shown that different AAS can act in either immunosuppressive or immune-stimulatory manner [13] .The results of the present study demonstrated that a significantly increases in the levels of IL-6 in both males and females treated groups , than control groups figure (2) . There was a positive correlation between sustanon (AAS) and IL-6 concentration , because high level of artificial testosterone were given to laboratory animals stimulate production of IL-6 as a pro-inflammatory cytokine , that leads to stimulate immune response by T cells and macrophages . [7] investigated that the levels of IL-6 increased when exposure to infection and after injury or other inflammation as a pro-inflammatory cytokine. In contrast IL-6 play an important role in muscles tissues , cytokine produced from muscles , it considers a first cytokine that arise in blood circulation through exercises , therefore , using of AAS by athletes caused elevated of its concentrations which lead to muscle contractions . While, [6] has suggested that testosterone suppresses pro-inflammatory cytokines and may up-regulate anti-inflammatory cytokines .Figure (3) revealed that a significant elevation in the concentration of C-RP in treated groups , in contrast with control groups, these results may be due to many causes , one of them is that C-RP secreted by hepatocytes in response to any inflammatory events in vivo , and raised of C-RP regard a first signed of cardiovascular disorder , this events may cause through using of testosterone as AASs by athletes [16] . The data explained that elevated levels of IL -6 in the present study may leads to elevated of C-RP concentration in the blood stream, this results was agreement with Biasucci *et al.*, (1996) who revealed that raised levels of C- reactive protein (C-RP) was associated with raised of interleukin-6 (IL-6) , because C-RP up regulates of some pro- inflammatory cytokines as IL-6 . Furthermore , C-RP stimulate peripheral blood monocytes to produce tissue factor (TF), necessary for the body developed during exposure to exo- endogenous agents , acts as chemotaxis and pro-coagulant [20] during injury or inflammation by using exogenous testosterone . Additionally, using of AAS by athletes caused increases of body mass index with decreased of high density lipoprotein , waist circumference and height of C-RP, which is a significant correlation between C-RP levels and rate of metabolic activity [5].

Conclusions:

The present study concludes that increasing the doses of sustanon drug in rats may cause clear changes in the levels of the study parameters .

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