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The Physiological Effects on Hormones levels and Kidneys Functions Induced by The Anabolic Androgenic Drug (Sustanon) in Male Guinea Pigs

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Abstract: Problem statement: In recent years, the intentional abuse of anabolic androgenic drugs by athletes has increased rapidly in many countries to become a serious negative phenomenon. Athletes, specially in power sports, abuse these drugs such as Sustanon in high doses to obtain a rapid and huge increasing in the muscles mass and to improve their performance during the sport competitions. This abuse causes severe adverse effects among the abusers. **Approach:** The present physiological study was performed to answer the following question: could Sustanon abuse induce marked abnormal alterations in some hormones levels and in the kidneys functions?. The current study was needed due to the lack of sufficient information about the hormonal and nephritic adverse effects caused by Sustanon abuse in particular. This study on male Guinea pigs aimed to investigate the potential abnormal alterations in the levels of some hormones and in the kidney functions induced by repeated administration of two Sustanon doses. The treated animals were injected intramuscularly (i.m.) once a week with Sustanon doses as follow (15 mg Kg^{-1} in group G II and 30 mg Kg^{-1} in group G III) for a continuous treatment period of 6 weeks. **Results:** The results of the blood biochemical measurements showed statistical significant decreases in means of the serum levels of: Triiodothyronine (TT3), Thyroxine (TT4) and Insulin specially in the treated group (G III) (0.42±0.38 ng mL^{-1}, 3.79±0.15 μg dL^{-1}, 0.62±0.43 μI.U mL^{-1}, respectively) compared to the control group according to (t-test). This was accompanied by significant increase in mean of cortisol serum levels in the treated group (G III) (63.44±4.90 μg L^{-1}). At the same time, marked significant increases were recorded in the means of the serum levels of the blood compounds related to the kidney functions (Blood urea, Uric acid, Creatinine, Albumin and Na^+ and K^+) in the treated group (G III) (53.9±2.70, 4.76±0.62, 1.63±0.24 μg dL^{-1}, 3.38±0.14 gm dL^{-1}, 10.73±1.65 and 143.8±9.55 μmol L^{-1}, respectively) compared to control group according to (t-test). The results have been described and discussed in full details within the text. **Conclusion/Recommendations:** The current hormonal results proved the occurrence of severe abnormal alterations in the serum levels of the thyroid gland hormones, Cortisol and Insulin under the influence of Sustanon treatment. This indicated that abusing Sustanon for long periods by athletes can cause serious pathological hormonal disorders in the abusers. In addition, the present nephritic results can be considered as strong proof that abusing Sustanon for long periods by athletes can induce severe defects and disorders in the kidney functions. This may lead to cause serious pathological damages in the kidneys structure in the late stages. Due to these serious adverse effects, it is recommended that much more official and medical restrictions should be applied to prevent abusers from obtaining these drugs in order to decrease the continuation of this negative health problem. In addition, the study recommends also that athletes and youths should be provided with much more medical information and enlightenment about the health risks and the complications of abusing these drugs through media.

Key words: Anabolic androgenic drugs abuse, Sustanon, kidney functions, hormonal alterations

INTRODUCTION

Abusing anabolic androgenic drugs by athletes is a serious negative phenomenon which is documented by a number of investigators in many countries around the world including: USA. Canada, UK. Australia and in some Arab countries as well[1-5]. Unfortunately, many athletes, specially in the power sports like bodybuilding and weight lifting, administrate illegally high doses of these drugs to obtain huge increasing in the muscles mass and also to improve their performance during the international sport competitions[2,6,7]. Actually, these anabolic androgenic drugs such as Sustanon, Metandienone, Stanozolo and Deca-Durabolin are

useful medical drugs which possess multiple clinical therapeutic benefits[3,8]. For example, Sustanon is one of these anabolic androgenic drugs and it is clinically used to treat many cases of osteoporosis, male hypogonadism and infertility, absence of male libido and it also used as supportive therapy in treating dwarfism cases in children[9,10]. In fact, Sustanon is characterized by a very unique pharmacological structure and properties comparing to the other anabolic drugs. Sustanon consists of four different testosterone ester compounds which provide a continuous release of testosterone into the blood producing a stable testosterone level in the blood serum for long duration extending to 3-4 weeks[9]. This distinguish properties increases the anabolic effectiveness duration of Sustanon[10-12]. Hence, Sustanon is described as a long acting anabolic androgenic drug[13]. This long acting effects of Sustanon justifies why it is more attractive to be abused by athletes[2,12]. Regardless of these unique pharmacological structure and properties of Sustanon, only few studied attempted to investigate the adverse effects induced by Sustanon abuse, whereas other anabolic androgenic drugs such as Deca-Durabolin have been excessively studied by a number of investigators[14,15]. However, regarding the existence of this negative phenomenon in our Arab countries, the recent field study of Tahtamouni[5] in Jordan is believed to be one of the best studies which provided a strong evidences about the existence and the spread of this phenomenon in our Arab countries. Tahtamouni study[5] was carried out on random samples of collages students and athletes in Amman city in Jordan. According to this study it was found that the percentage of the anabolic drugs abusers among the students was 4.2%, whereas the percentage of the abusers among the athletes was found 26%. In addition, it was found that most of the abusers were used to administrate these drugs for long periods. The study also found the ages of the abusers between 19-28 years old. Moreover, it is believed that one of the main factors responsible for the continuation of this abuse problem among the athletes and the youth is the absence of the official restrictions on pharmacies selling these anabolic drugs in many countries around the world[7,4]. For example, during our present study we did not face any restrictions or difficulties in obtaining Sustanon from the pharmacies in Jeddah, Saudi Arabia without any prescriptions. Overall, in regard the adverse effects caused by abusing these anabolic androgenic drugs, number of previous studies reported some of these effects including: cardiovascular disorders (particularly enlargement of the left ventricle) which can lead to sudden cardiac death, acute hepatitis and Jaundice, testicular dysfunction, infertility, hypertension, behavioral disorders in the form of sexual overstimulation and abnormal increase in the aggressive behavior[3.9,16-20]. However, by reviewing the literature of Sustanon abuse in particular, it was noticed that only very few studies have investigated the hormonal and nephritic adverse effects induced by such abuse among athletes[15,21]. The study of Modlinski and Fields[22] provided an explanation of the absence of sufficient information about some of the adverse effects such as the nephritic effects, as it indicated that because anabolic drugs abuse is elicited and occurred secretly among the athletes, most of our knowledge about the adverse effects of these drugs is obtained mainly from cases reports which reached the hospitals in some countries, therefore, our knowledge about some of these adverse effects such as the hormonal and nephritic effects are still very limited. The importance of the current physiological study comes from the lack of sufficient information about the hormonal and nephritic effects induced by Sustanon abuse in particular, as this prior study explored these adverse effects of Sustanon abuse in particular. In summary, the objective of this study was to investigate the adverse effects of two doses of Sustanon on the thyroid gland hormones, parathyroid hormone, Cortisol and Insulin, as well as on the kidney physiological functions.

MATERIALS AND METHODS

Sustanon doses: Sustanon ampoules (manufactured by N.V. Organon Oss Inc. Holland) have been obtained from the local pharmacies in Jeddah city, Saudi Arabia. Each ampoule contain 1 mL of oily solution of Sustanon. According to the manufacturer this 1 mL of Sustanon consists of the following four testosterone ester compounds: Testosterone propionate, testosterone phenylprpropionate, testosterone isocaproate and testosterone decanoate. During the present study two doses of Sustanon have been selected (15 and 30 mg Kg^{-1} of the animal body weight) based on the previous studies of Segura[23] and Johansson[24] which were also performed on Guinea pigs. The doses preparation was performed according to the method of Segura[23].

Experimental animals and treatment: Twenty four adult male Guinea pigs (*Cavia porcellus*) (Dunkin Hartely strain) weighing 700-750 g were used in this study. The animals have been obtained from the animal house unit in King Fahed Medical Research Center in King Abdul Aziz University, Jeddah. Each animal was housed in a wide proper plastic cage and kept under constant normal temperature (22°C), 12 h dark/light

cycle[25]. Animals were daily provided with distilled water and diet. Prior to the experiment start, animals were left for two weeks to acclimatize. After this acclimatization period, animals were divided into 3 groups (8 animals in each). The first group (G I) was the control group, whereas the second group (G II) and the third group (G III) were the treated groups. In these two treated groups (G II) and (G III), each animal was injected intramuscularly (i.m.) once a week with a single dose of (15 mg Kg^{-1} in (G II) and 30 mg Kg^{-1} in (G III), respectively) for a continuous treatment period of 6 weeks.

Collection of blood samples and biochemical measurements: At the end of the treatment period, each animal of the three groups was anesthetized by inhalation of drops of Diethyl Ether in a piece of cotton. Blood samples were then collected via cardiac puncture according to the method of Hoff and Rlatg[26]. Two blood samples were collected from each animal and were immediately placed in two separate Lithium heparin tubes. One blood sample tube was for measuring the levels of the following hormones in the serum: Thyroid gland hormones (triiodothyronine TT3 and thyroxine TT4), parathyroid hormone, cortisol and insulin. While the second blood sample tube was for the measurements of the blood parameters related to kidney functions (blood urea, uric acid, creatinine, albumin and Na^+ and K^+ levels). The blood samples tubes were surrounded by small ice pieces and were sent within less than half an hour to a specialized medical laboratory (AL-Mamlaka Medical Laboratories, Jeddah) to perform the requested blood biochemical measurements. In the laboratory the blood samples were centrifuged at (3000 rpm) for 5 min. to separate the serum. The levels of the hormones and the blood compounds related to the kidney functions were measured using two different computerized blood analyzers. The hormones levels in the serum samples were measured using Abbott Axsym analyzer (manufactured by Abbott Laboratories, IL, USA.), whereas the blood compounds of the kidney functions were measured using Vitros Chemistery automatic blood analyzer (model no.350, manufactured by Jonson and Jonson Co. UK). All the mentioned blood biochemical measurements were performed according to the methods of Tietz[27] and Kaplan[28].

Statistical analysis: Data of the blood biochemical measurements were expressed as (Mean±SE). Statistical analysis was performed using Sigma stat program (v.10) in which (student t-test) has been used to determine the significant differences between each treated group and the control group for each blood parameter[29].

RESULTS

Hormonal effects of (15 mg Kg^{-1}) dose: As shown in Table 1, the repeated treatment with this dose did not induce a significant changes in the means of the following hormones: Triiodothyronine (TT3), Thyroxine (TT4), Parathyroid hormone, Insulin (0.60±0.46 ng mL^{-1}, 4.65±0.27 μg dL^{-1}, 2.87±0.16 pg mL^{-1} and 1.73±0.17 μI.U mL^{-1}, respectively) compared to the control group according to (t-test). On the other hand, significant increase was recorded in the mean of Cortisol under the influence this dose (41.57±2.92 μg L^{-1}) compared to the control group according to (t-test, $p \leq 0.05$).

Hormonal effects of (30 mg Kg^{-1}) dose: The repeated treatment with this dose for 6 weeks induced marked significant changes in the serum levels of the tested hormones as shown in Table 1. Under the influence of this dose, significant decreases were recorded in the means levels of the following hormones: Triiodothyronine (TT3), Thyroxine (TT4) and Insulin in the treated animals of group (G III) (0.42±0.38 ng mL^{-1}, 3.79±0.15 μg dL^{-1}, 0.62±0.43 μI. U mL^{-1}, respectively) compared to the control group according to (t-test, $p \leq 0.05$). On the other hand, significant increase was observed in the mean of cortisol levels (63.44±4.90 μg L^{-1}) compared to the control group according to (t-test, $p \leq 0.01$). Whereas, in regard the mean levels of Parathyroid hormone, the dose did not induced any significant changes in its levels (2.92±0.32 pg mL^{-1}). Overall, it can be concluded from the present hormonal results that the repeated administration of high doses of Sustanon can induce marked abnormal alterations and disorders in the serum levels of the mentioned hormones in the form of significant decreases in the levels of Triiodothyronine (TT3), Thyroxine (TT4) and Insulin, as well as significant increase in the mean of the serum level of Cortisol.

Effect of Sustanon doses on the kidneys physiological functions:

Nephritic effects of (15 mg Kg^{-1}) dose: As showed in Table 2, the repeated treatment with this dose induced significant increases in the means levels of the following compounds in the serum:- Blood urea, Uric acid and Creatinine (27.45±1.98, 2.29±0.39 and 1.24±0.17 μg dL^{-1}, respectively) compared to the control group according to (t-test, $p \leq 0.05$). Whereas,

Table 1: Effects of two Sustanon doses on the serum levels of some hormones in the treated groups (G II and G III) compared to the control group (G I) in male Guinea Pigs

Hormones levels	Control group (G I)	Treated group (G II)	Treated group (G III)
Triiodothyronine (TT3) (ng mL^{-1})	0.54±0.016	0.60±0.46	0.42±0.38*
Thyroxine (TT4) (µg dL^{-1})	5.78±0.34	4.65±0.27	3.79±0.15*
Parathyroid hormone(pg mL^{-1})	2.95±0.26	2.87±0.16	2.92±0.32
Cortisol (µg L^{-1})	26.89±1.12	41.57±2.92*	63.44±4.90**
Insulin (µ I.U mL^{-1})	1.79±0.23	1.73±0.17	0.62±0.43*

Number of animals in each group was eight (n = 8), data are expressed as (Mean±SE.); *: Significant difference ($p \leq 0.05$) comparing to control group according to (t-test); **: Highly significant difference ($p \leq 0.01$) comparing to control group according to (t-test)

Table 2: Effects of two Sustanon doses on the blood parameters related to the kidney functions in the treated groups (GII and GIII) compared to the control group (GI) in male Guinea Pigs

Blood parameters	Control group (G I)	Treated group (G II)	Treated group (G III)
Blood urea (µg d L^{-1})	19.25±1.14	27.45±1.98*	53.9±2.70**
Uric acid (µg d L^{-1})	1.18±1.25	2.29±0.39*	4.76±0.62**
Creatinine (µg d L^{-1})	0.72±0.54	1.24±0.17*	1.63±0.24**
Albumin (gm d L^{-1})	2.15±0.16	2.06±0.32	3.38±0.14*
K^+ (µmol L^{-1})	9.60±0.71	9.03±0.76	10.73±1.65*
Na^+ (µmol L^{-1})	141.53±11.58	138.0±10.52	143.8±9.55*

Number of animals in each group was eight (n = 8), data are expressed as (Mean±SE); *: Significant difference ($p \leq 0.05$) comparing to control group according to (t-test); **: Highly significant difference ($p \leq 0.01$) comparing to control group according to (t-test)

no significant differences were observed in the means levels of the following compound and electrolytes: Albumin, K^+ and Na^+ (2.06±0.32 gm dL^{-1}, 9.03±0.76 and 138±10.52 µmol L^{-1}, respectively) compared to the control group according to (t-test).

Nephritic effects of (30 mg Kg^{-1}) dose: The repeated treatment with this dose for 6 weeks produced highly significant increases in means of Blood urea, Uric acid and Creatinine (53.9±2.70, 4.76±0.62 and 1.63±0.24 µg dL^{-1}, respectively) compared to the control group according to (t-test, $p \leq 0.01$) as shown in Table 2. These high significant elevations in means of these three compounds reflected severe defects in the kidneys functions as a result of treatment with this high dose of Sustanon. Further more, similar significant increases were observed in the means of the following compound and electrolytes: Albumin, K^+ and Na^+ (3.38±0.14 m dL^{-1}, 10.73±1.65 and 143.8±9.55 µmol L^{-1}, respectively) compared to the control group according to (t-test, $p \leq 0.05$).

These significant increases in the serum levels of K^+ and Na^+ indicated the presence of a case of salt retention as a result of the treatment with this dose, this salt retention may reflect disability of the kidneys to excrete these electrolytes out side the body. Meanwhile, it was noticed from the results that the effects on the kidney functions were dose dependent.

DISCUSSION

Based on the available literature, the current study is believed to be as one of the prior studies which investigated the hormonal and the nephritic adverse effects induced by Sustanon abuse. However, it is important at the beginning to mention that part of the complexity of the anabolic drugs abuse problem is the conflict between their necessary clinical therapeutic benefits on one side and the risky health effects which result from abusing them by the athletes on the other side[7,11]. In addition, it is believed that one of the factors responsible for the continuation of this problem is the wrong belief among athletes abusers that the adverse effects of these drugs are reversible effects and it can be easily treated later on after the sport competitions. 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[10,17,20].

Moreover, to realize how serious this health problem is, we have to know that governments in some countries spent millions to stand against this phenomenon, for example, in USA a great institute has been established to face this problem called (the association against steroids abuse) which provide free online medical information to the public regarding the health hazards of abusing these drugs[7,13]. However, regarding the present hormonal results induced by the Sustanon doses, the results seem to support the previous findings of Karila study[10] which confirmed that anabolic androgenic drugs abuse in general, decreases the serum levels of the Triiodothyronine (TT3) and Thyroxine (TT4) and insulin. The study reported also that the high doses of these drugs usually cause a marked decrease in the hormonal-binding proteins which carry these hormones in the blood circulation and this could explain the observed decreases of these hormones in the serum.. Furthermore, the current hormonal results correspond with previous results of Fortunato study[30] which reported similarly significant decreases in the serum levels of the thyroid gland hormones: Triiodothyronine (TT3) and Thyroxine (TT4), as well as in the level of TSH secreted from the pituitary gland due to abusing some types of these drugs. However, Nasrollah and Shahidi study[31] explained that the wide hormonal alterations induced by

abusing these drugs are believed to result from the wide interactions between testosterone and the other hormones in the body. On the other hand, regarding the current nephritic results related to the kidney functions caused by Sustanon doses, it is clear from these results that abusing high doses of Sustanon produced severe defects and disorders in the kidney functions due to the long exposure of kidney cells to the toxic metabolites of Sustanon for 6 weeks. Therefore, there is no doubt that these nephritic results reflect the occurrence of kidney dysfunctions and disability to excrete these nitrogenic compounds out side the body. Similarly, the significant increases observed in the electrolytes levels: K^+ and Na^+ in the treated animals indicated the presence of salts retention due to kidney disability to excrete them out side the body, which is considered as an additional proof that high doses of Sustanon induced acute defects and disorders in the kidney functions[28,32]. Meanwhile, compared to the previous studies, the present nephritic results seem to support the previous findings of Habscheid study[21] which reported a kidney failure in 28 year old athlete abuser who administrated high dosage of anabolic androgenic drugs for several months. Habscheid study[21] concluded that abusing anabolic androgenic drugs in general, for long periods can induce acute renal failure associated with hepatic disorders in the form of hepatitis and cholestasis. Similarly, the present result correspond also with the observations of Hoseini study[33] which reported marked histopathological abnormalities in the kidney structure caused by high doses of anabolic drugs in mice. On contrary, the results appear not match with Conway study[34] as they believed that Sustanon is a very safe anabolic androgenic drug and its adverse effects are very rare. However, regarding the exact mechanism of action which can explain these abnormal hormonal and nephritic adverse effects induced by Sustanon abuse in particular, it is still unknown. In spite of that, a few hypotheses are available related to the anabolic androgenic drugs in general. For example, Welder[35] and Draisci[36] believed that these adverse effects result from the accumulation of certain toxic metabolites of testosterone such as (17α-19-nortestosterone and 17α-testosterone). In addition, some investigators such as Zaugg[37] suggested that abusing anabolic androgenic drugs causes cellular apoptosis in the target cells by inducing severe marked damages in the DNA structure. Furthermore, other investigators as Behrendt and Boffin[38] documented that abusing anabolic androgenic drugs produce serious cellular damages in the mitochondria which are accompanied by cristae destruction. These mitochondrial damages will definitely lead to cellular death due to the role of the mitochondria in supplying the cell with energy.

CONCLUSION

At the end of this study, it can be concluded that the present hormonal results proved that abusing Sustanon for long periods by the athletes can cause marked abnormal hormonal disorders in the levels of the thyroid gland hormones, Cortisol and Insuline. At the same time, the present nephritic results also proved that abusing Sustanon for long periods can cause severe defects and disorders in the kidney functions which may lead to produce serious pathological damages in the kidneys structure in late stages. Finally, it is recommends that much more official and medical restrictions should be applied to prevent the athletes and the youth abusers from obtaining these drugs in order to decrease the continuation of this negative health problem.

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