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Genotoxic and Cytotoxic Effects of Testosterone Undecanoate on Bone Marrow and Germ Cells in Mice

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ABSTRACT

Anabolic androgenic steroids are synthetic compounds used for treatment of numerous medical condition as well as being used widely by athletes performance enhancers and bodybuilding. The aim of the present study was evaluate the genotoxic and cytotoxic effects of testosterone undecanoate in a mouse model, chromosomal aberrations, mitotic index of bone marrow cells, mitotic index of spermatocytes sperm head abnormality. This effect were determined after the administration of a 0.01mg of testosterone undecanoate via oral gavage for 30 days. Result revealed that testosterone undecanoate caused a slight increase in the frequency of chromosomal aberrations and a significant reduction of mitotic index of bone marrow cells. Despite that, there was no significant increase in percentage of sperm head abnormality in male mice. Those findings were parallel to a significant reduction in mitotic index of spermatocytes when compared to negative control group and positive control group. This study documents that testosterone undecanoate has cytotoxic effects on bone marrow and germ cells of male mice.

Keywords: Testosterone undecanoate, Chromosome Aberration, Mitotic index, Cytotoxic.

1. INTRODUCTION

Anabolic androgenic steroids (AAS) synthetic compound were isolated for the first time in the thirties of the last century and used for treated numerous medical condition such as hypogonadism, deled puberty, aplastic anemia and testosterone deficiency [(1)].

AAS are used in widely by athletes to improve both their appearance and athletic ability. With the dramatic increase of use AAS by athletes for non-medical reasons, the impact of these substance on body composition have attracted the attention of researchers and numerous studies have been published concerned side effect of these substance. Many reports suggest that the use of AAS causes collateral effects on other organs such as hepatic toxicity, jaundice, renal disorders, hypertension, reproductive system toxicity and behavioral changes[(2,3)].

To date, studies concerning genotoxic activity of those substances are scarce. Previous studies demonstrate that testosterone combined with estrogen induced genotoxic effects in noble rats[(4)]. Bugarin *et al*.[(5)] reported that the consumption of AAS increased the frequency of micronuclei mucosa of body builders. In view of such findings, the present study aimed to evaluate the genotoxic and cytotoxic effects of testosterone undecanoate in mouse model.

Materials and Methods

Thirty six adult male albino mice (*mus musculs*), approximately 8-12 weeks old and weighing (25± 2) g, were used. The mice were housed in plastic cages and maintained under standard laboratory conditions in the

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animal house annex of the Department of Biology, University of Almustansiriyah. Animals were provided with standard laboratory diet and water *ad libitum*. All animals recruit to the present study left for 7 days before starting the experiment to the adapt to the new place and received humane care in accordance with guidelines prepared by the Institutional Animal Care and Use Committee, Western University of Health Sciences, USA.

Animals were randomly divided into three groups each with 12 mice The mice in Group l were orally given (0.01mg/Ml) of testosterone undecanoate (Catalent France Beinheim) for 30 days. The mice in group II was orally given single dose of cyclophosphamide (Finland) (50mg/kg body weight) for 7 days and considered as positive control[6]. Others were administered orally with distilled water daily for 30 day and considers as negative control.

Chromosomal preparation (in bone marrow cells)

After 30 days of treatment all animals were injected intra peritoneally with colchicine (2.5 mg/ kg / body weight) after two hours animals scarified and chromosomes from bone marrow cells were prepared following the method recommended by Agarwal et al.[7].

To scoring the different types of chromosomal aberrations, 100 proper metaphase spreads were examined microscopically for each animal.

Mitotic index was determined by scoring 1000 cells from each animal, and expressed as percentage.

Mitotic index in Spermatocytes

Testis were obtained from the same animals to calculate mitotic index in Spermatocytes (germ cells) spermatocytes were prepared by using the methodology of Brewen and Preston[8].

Sperm head abnormality

To examine any morphological abnormality of sperm head the sperm suspension was prepared from treated animals by cutting the caudal epipedeymis of testes in petridish containing 5 ml of pre warmed (37C°) serial saline solution two drops of suspension was spread on clean microscope slide air dried, stained with 1% eaosin. one thousand sperm for each animals were examined for abnormality in sperm head shape according to criteria of the Wyrobeck and Bruce [9].

Statistical Analysis

All statistical analysis was conducted using statistical analysis system- SAS.[10] Least significant difference – LSD test was used to test the difference in the mean count of among study groups and subgroups. The value of $P<0.05$ was considered statistically significant.

Results

After 30 days of administrated the male mice with testosterone undecanoate there were a significant decrease in mitotic index of bone marrow cell as shown in Figure (1) The mitotic index value calculated in animals treated with testosterone undecanoate (1.75± 0.21) was significantly lower than the mitotic index value calculated in the negative control group (2.71± 0.24) and positive control group (2.17±0.36) ($P < 0.05$).

Male Mice receiving cyclophosphamide (positive controls group) displayed a significant increase in the percentage of chromosome aberrations in mouse bone marrow cells ($P<0.05$) the mean of chromosome aberration in positive control group was (3.72± 0.90) which was significant higher than mean of chromosome aberration in negative control group . In contrary, treated group showed a slightly higher mean count of chromosomes aberration (2.44±0.32) when compared to negative controls (1.31±0.40) the difference was statistically not significant ($P>0.05$) Table (1).

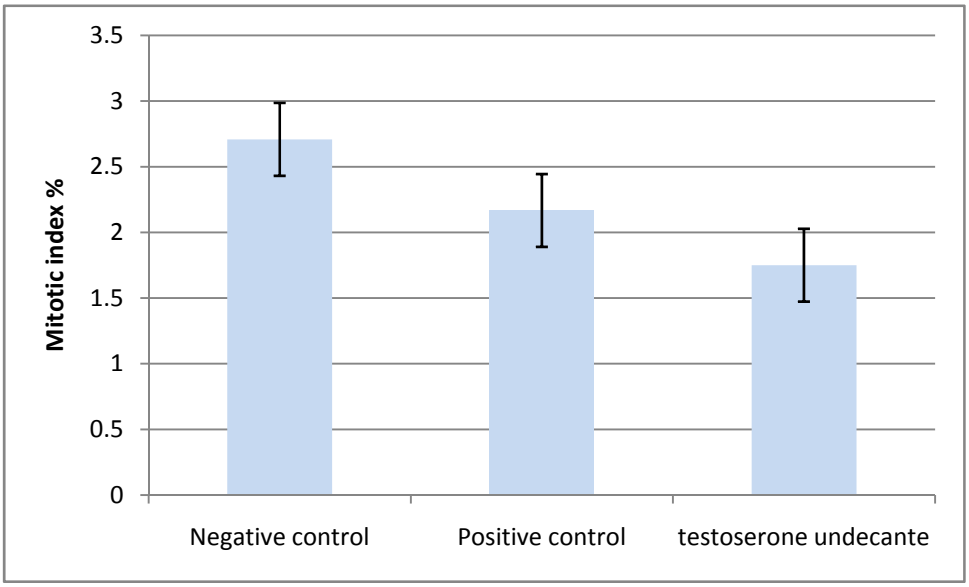

Figure 1: Means of mitotic index of bone marow cells in male mice after 30 days of administration of Testosterone undecanoate and in mice of the control groups

Table 1. Means± S.E. of total chromosome aberration in male mice after 30 days of administration of Testosterone undecanoate and in mice of the control groups

Treatments groups	Total chromosome aberration Mean ±S.E.
Negative control	**1.31±0.40[a]**
Positive control	**3.72± 0.90[b]**
Testosterone undecanoate	**2.44±0.32[a,b]**

Values with different letters (a, b) are significantly different form one another (P < 0.05).

Figure (2) shows that the mitotic index value of spermatocytes in male mice treated with testosterone undecanoate was(2.54 ± 0.05). This was significantly lower than mitotic index value calculated in the negative control group (9.91± 078) and even positive control group (8.17± 0.69) (P < 0.05).

Numerous forms of sperm heads, i.e., hooked…, dwarf, triangular, amorphous, banana shaped etc. were recognized in all groups. highest mean value of sperm head abnormality was detected in positive control group (10.49 ± 1.64) which was significantly higher than mean value detected in treated group (6.28 ± 1.11) and negative control group (5.97 ± 1.12) (P< 0.05) table (2).

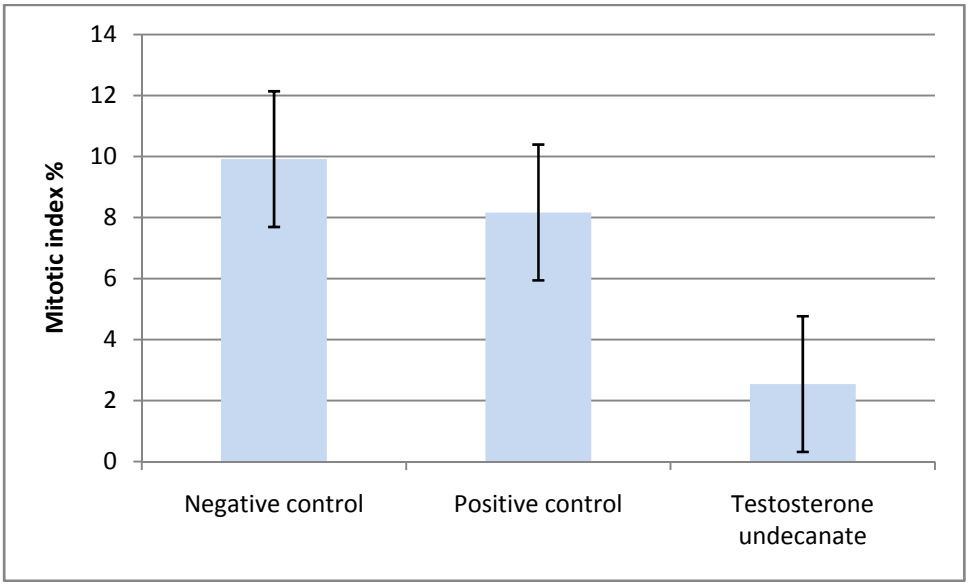

Figure 2: Means of mitotic index of spermatocytes cells in male mice after 30 days of administration of Testosterone undecanoate and in mice of the control groups

Table 2. Means± S.E. of total sperm head abnormality in male mice after 30 days of administration of Testosterone undecanoate and in mice of the control groups

Treatments groups	**Total abnormality Mean ±S.E.**
Negative control	5.97 ±1.12[b]
Positive control	10.49± 1.12[a]
Testosterone undecanoate	6.28±1.11[b]

Values with different letters(a, b) are significantly different form one another (p < 0.05).

Discussion

The present study showed that administration of testosterone undecanoate to male mice for 30 days caused a slight increase in the frequencies of chromosome aberration and significant reduction in mitotic index of bone marrow cells. These result suggest that testosterone has potential genotoxic effect in somatic cells. Similar positive results obtained by do Carmo *et al.*[(11)] who found that a chemically modified testosterone hormone (nandrolone) have genotoxic effects on liver, bone marrow, brain and peripherial blood cells of mice.

Mitotic index is used to indicate cytotoxicity of many compounds. A depression mitotic index reflects the inhibition of cell cycle and affects the cell division negatively[(12)]. In the present study, testosterone undecanoate significantly decreased MI compared to control. But the increase in the frequency of CAs was not significant. This finding suggests that when cell suffer

genetic damage inducing some degree of blockade of the cell cycle, or hindering the onset of prophase by induce death of interphase nucleus[13]. Moreover, one possible mechanisms by which testosteron derivatives induce genotoxic effects it is formation of free radicals after metabolic activation that have ability to induce damage to DNA and other cellular constituents[14].

previous studies demonstrate that numerous chemicals which induce cytotoxic and genotoxic effects in bone morrow cells have genotoxic and /or cytotoxic effects in germ cells as well. sperm head abnormality assay serve as reliable and highly sensitive parameter to detect mutagens and carcinogens moreover it is used to assessment heritable genetics damage fertility and spermatogenic changes[15].

According to result of present study there are no significant increase in percentage of sperm head abnormality in male mice treated with testosterone undecanoate these finding parallel to a significant decrease in mitotic index of spermatocytes when compared with negative control group or positive control group.

Significant decrease in mitotic index of spermatocyte cell indicate that the testosterone undecanoate have cytotoxici effects in germ cells. Moreover, according to previous studies treatment with testosterone or AAS lead to suppression of testicular testosterone production as result of suppression of both gonadotropin-releasing hormone and luteinizing hormone production[16,17]. Testosteron have important role in attachment of germ cells in seminiferous tubules. Depletion of intratesticular testosterone level result in deattachment of germ cells from semiferious epithelium and may induce programmed cell death and consequently, male infertility[18].

It is known that the length of spermatogenesis in mice is approximately 35 days, induction of lower frequency of abnormal sperm after 4 weeks of treatment reflected that post-meiotic cell was less sensitive to testosterone treatment and low frequency of sperm head changes can be related to preceding cycles of spermatogenesis or as consequence a cytotoxic activity of testosterone undecanoate as mention in previous section.

The present study showed that treated male mice with Cyclophosphamide drug for 7 days (Positive control group) led to a significant increase in percentage of chromosomal aberration, decrease in mitotic index of bone marrow and spermatocytes cells and increased in frequency of sperm head defects . This result in line with previous studies.

Cyclophosphamide is an alkalyting agents which interact with transcription and translation of nucleic acids and indication structural chromosome aberration in somatic and germ cells[19]. It also disrupting the cell division as result of cumulative DNA damage[20].

Ray et al.[21] reported that treatment with CP induce the physiological modification such as decrease testosterone hormone and alteration in activity of (Testicular – β – hydroxyl steroid dehydrogenase) and 17 – β - hydroxyl steroid dehydrogenase.

In summary the results of present study demonstrate that testosterone undecanoate has cytotoxic effects in bone marrow and germ cells of male mice. We recommended that further research should be conducted to evaluation of the genotoxic and cytotoxic effects of anabolic steroid such as testosterone undecanoate in athletes and bodybuilders who frequently misused these chemicals.

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التأثيرات السمية الخلوية والوراثية لعقار testosterone undecanoate في خلايا نقي العظم والخلايا الجنسية للفئران

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ملخص

الستيرويدات الاندروجينة البنائية هي مركبات مصنعة تستخدم لعلاج العديد من الحالات المرضية، بالإضافة إلى ذلك تستخدم بصورة واسعة من قبل الرياضيين لتحسين الأداء وبناء الأجسام.

الهدف من الدراسة الحالية تقييم الآثار السمية الوراثية والخلوية لعقار testosterone undecanoat باستخدام الفئران المختبرية، التشوهات الكروموسومية، معامل انقسام خلايا نقي العظم، معامل انقسام الخلايا الجنسية وتشوهات رووس النطف. حددت هذه التأثيرات بعد إعطاء الفئران المختبرية 0.01 ملغم/مل من عقار testosterone undecanoate عن طريق الفم ولمدة 30 يوم. أظهرت النتائج أن المعاملة عقار testosterone undecanoate أدى إلى زيادة ضئيلة بنسبة التشوهات الكروموسومية وانخفاض معنوي لمعامل انقسام خلايا نقي العظم بالرغم من ذلك لم تكن هنالك زيادة معنوية في نسب تشوهات رؤوس النطف لذكور الفئران. هذه النتائج كانت مترافقة مع انخفاض معنوي لمعامل انقسام الخلايا الجنسية (spermatocytes) عند مقارنته مع مجموعه السيطرة الموجبة والسالبة. توصلت الدراسة الحالية إلى أن عقار testosterone undecanoate يمتلك تأثيرات سمية خلوية (cytotoxic) لخلايا نقي العظم والخلايا الجنسية في ذكور الفئران.

الكلمات الدالة: testosterone undecanoate، التشوهات الكروموسومية، معامل الانقسام، السمية الخلوية.

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