

## Research Article



## Cytogenetic and Biochemical Studies of Testonon Effects as a Body Building Agent in Rats.

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### ABSTRACT

Testonon is used as testosterone replacement therapy in male hypogonadal disorders and an effective steroid which give the user high-quality muscles and body strength gains. This study aimed to evaluate the Testonon effects as bodybuilding agent on rat's genetic materials, sperm morphology and body functions performance. A Cytogenetic analysis, DNA fragmentation, sperm morphology assay and biomarker analysis were used to achieve these tasks. Experimental animals were divided into three groups. The Testonon injection was at doses taken by bodybuilders for six weeks. The results revealed that the total number of chromosomal aberrations in somatic and germ cells in the groups injected with Testonon were significant difference compared to the control group. The groups injected with Testonon showed a significant difference in DNA fragmentation compared to the control group. The results of sperm morphology assay illustrated that Testonon injection at different doses caused significant increases in total abnormal sperms head and tail compared to the control group. The results of biomarker analysis showed significantly different in the levels of testosterone, Lipid peroxidation, acid phosphatase, ALT, AST, urea and cholesterol for the groups injected with Testonon compared to the control group. In conclusion, the results revealed that Testonon injection at doses used by bodybuilders had mutagenic effects on rat's somatic and germinal genetic materials, cytotoxic effect on liver tissue and increased the sperm morphology abnormalities, the risk of heart attack, liver damage, kidney failure, hyperparathyroidism and benign prostate hyperplasia. These are alerts for the forbidden use of Testonon as bodybuilding agent.

**Keywords:** Testonon, bodybuilding, cytogenetic analysis, sperm morphology, DNA fragmentation, biomarker

### INTRODUCTION

Anabolic steroids, technically known as anabolic-androgenic steroids (AAS), are drugs that have similar effects to testosterone in the body. They are used as testosterone replacement therapy in male hypogonadal disorders<sup>1,2</sup> and in conjunction with other hormones to promote skeletal growth in prepubertal boys with delayed puberty<sup>3</sup>.

AAS are also used as effective steroids which give the user high-quality muscles and body strength gains<sup>4,6</sup>.

The adverse effects that belong to anabolic steroids function have been reported as the increase in the risk of cardiovascular diseases or coronary artery diseases<sup>7,8</sup>, kidney disease<sup>9</sup>, testicular problems<sup>10</sup> and liver dysfunction<sup>11</sup>.

Hassan et al.,<sup>5</sup> study the doping and effects of AAS on histological, ultrastructural, and echocardiographic of heart in athletes and experimental animals.

Their results showed that athletes who use steroids have smaller left ventricular dimensions with thicker walls and impaired diastolic function.

Ultrastructural examination showed disturbance in the banding pattern of the cardiac muscle fiber with disintegration, loss of striations, dehiscent intercalated disc, and interrupted Z-bands.

Rasul and Aziz<sup>6</sup> reported a histological effect on testis of animals injected by AAS, which included the degeneration

of the germinal epithelium and the appearance of multinucleated giant cells and several variable sized vacuoles in a stage-like changes.

Testonon is a blend of four different testosterone esters. Testonon injection is called a depot injection, where it forms a reservoir of the medicine. The Testosterone is gradually released from the reservoir into the bloodstream. This potent fusion of anabolic and androgenic properties is the reason why AAS is a powerful steroid and is so popular among athletes as well as bodybuilders<sup>4</sup>.

Regulatory authorities all over the world require data on the genotoxic potential of the drugs chemical compounds, as part of the safety evaluation process<sup>12</sup>.

Cytogenetic analysis of somatic and germ cells and DNA fragmentation have been used to detect the genotoxic effects of any agent which can damage the genetic structure of living organisms and thus cause problems such as mutations, cancer or birth defects<sup>13,14</sup>.

In addition, biomarker analysis is used to evaluate the safety and predict effectiveness of biological body functions and provide informative links between mechanism of action and clinical effectiveness, connect animal and human studies, and serve as surrogate endpoints<sup>15</sup>.

To evaluate the Testonon effects as a bodybuilding agent on rat's genetic materials and body functions performance. A Cytogenetic analysis, DNA fragmentation,



sperm morphology assay and biomarker analysis were investigated to achieve these tasks.

## MATERIALS AND METHODS

### Drug

Testonon was supplied by El Nile pharma company (Cairo, Egypt) as solution for injection. Each ampule contains 250 mg/mL of compound mixture of 4 testosterone esters (Testosterone-Propionate 30mg, Testosterone-Phenylpropionate 60mg, Testosterone-Isocaproate - 60mg, and Testosterone-Decanoate - 100mg, eq. to a total of 176 mg of testosterone). Testonon is used by bodybuilders and power lifters at doses 500 and 1000 mg/week for six weeks<sup>16</sup>.

### Animals

A total of 30 adult male albino rats, weighing 150-160 g, were provided by the animal house of the National Research Centre (Cairo, Egypt).

Food and water were provided ad libitum. Animals were divided into three groups. The first group (G<sup>st</sup>, 10 animals) was treated with Testonon at dose 7.14 mg/kg b.w. The second group (G<sup>nd</sup>, 10 animals) was treated at dose 14.28 mg/kg b.w. These doses are equivalent to the Testonon injection doses used by the bodybuilders (500mg or 1000mg/week, respectively). The third control group was 10 animals. The drug was administered once per week intramuscular injection for six weeks (<http://www.anasci.org>). The tested drug doses for lab animals were calculated according to Paget and Barnes<sup>17</sup>.

### Chromosome Preparations

Animals were injected intraperitoneally with 0.3 mL of colchicine (0.05%). They were sacrificed two hours later to prepare the chromosomes of bone marrow and testis cells. Femoral bone marrow cells were flushed with isotonic saline solution (0.9%) and centrifuged for 10 minutes. The cell pellet was re-suspended with hypotonic solution (0.075 M KCl) and incubated at 37°C for 20 minutes. The cells were fixed with methanol-glacial acetic acid (3:1) and spread onto cold slides dipped in 70% ethyl alcohol. The slides were air dried<sup>18</sup> and stained with 10% Giemsa stain solution (diluted with phosphate buffer; pH 6.8) for 30 minutes, then the slides washed twice in phosphate buffer, air-dried, and examined under a light microscope.

Chromosomes of testis were prepared according to Russo<sup>19</sup>. The tubules of testis were squashed in isotonic solution (2.2% sodium citrate), then centrifuged and treated with hypotonic solution (1% sodium citrate) for 15 minutes at room temperature. The cells were fixed with methanol-glacial acetic acid (3:1) and spread onto cold slides dipped in 70% ethyl alcohol. The slides were air dried and stained with 10% Giemsa stain solution (diluted with phosphate buffer, pH 6.8) for 15 minutes, then the slides washed twice in phosphate buffer, air dried and examined under light microscope.

### Sperm Morphology Assay

The sperms were prepared according to Wyrobek and Bruce<sup>20</sup>. The epididymes were excised and minced with fine scissors in physiological saline. The smears were prepared on clean slides. The slides were stained with a mixture of normal saline and 1% eosin-Y for 45 minutes. The slides were air dried and examined under light microscope.

### Quantitation of DNA Fragmentation

DNA fragmentation was quantified by diphenylamine (DPA) as described by Paradones<sup>21</sup> in the liver cells of all investigated groups. The percentage of DNA fragmentation was taken as the ratio of DNA absorbance reading in the supernatant to the total amount of DNA in pellet and supernatant. Absorbance was measured at 600 nm using a UV double beam spectrophotometer (Shimadzu, 160A).

### Biomarker Assay

The sera were separated from blood samples of the experimental animal groups using cooling centrifugation and stored at -20°C until used. The biomarker analysis was as the following:

1. Liver function: AST (GOT) and ALT (GPT) activity was determined according to Reitman and Frankel<sup>22</sup>.
2. Kidneys function: Urea and creatinine contents were determined according to the methods described by Patton and Grauch<sup>23</sup> and Bartels<sup>24</sup>, respectively.
3. Lipid profile: Total lipids, triglycerides, cholesterol, HDL-c, and LDL-c were determined according to the methods of Eckel<sup>25</sup> and Wiel and Seided<sup>26</sup>, respectively using the Bio-diagnostic kit, while Stan bio- lab kit was used for lipoproteins. LDL-c value was calculated as the following equation: value t = Triglycerides content/5<sup>27</sup>.
4. Lipid peroxidation as malondialdehyde (MDA) was determined according to Satoh<sup>28</sup> using Bio-diagnostic kit (Egypt).
5. Acid phosphatase activity was determined by the method described by Kind and King<sup>29</sup> using Bio-diagnostic kit.
6. Testosterone was determined according to Carbayo<sup>30</sup> using Immunospec. Kit.

### Scoring and Statistical Analysis

For each animal, 100 well-spread metaphases and spermatocyte cells were examined from each animal at each dose for chromosomal aberrations (structural and numerical). Chromosomal aberrations were scored by following the guideline of Brusick<sup>31</sup> and Prestone<sup>18</sup>. One thousand sperm was counted for each animal at each dose for scoring different types of sperm abnormalities. The sperms were assessed for morphological



abnormalities according to the criteria of Wyrobek and Bruce<sup>20</sup>.

The results of chromosomal aberrations, sperm abnormalities, DNA fragmentation and biochemical analysis were analyzed using the arithmetic mean, standard deviation and variance [ANOVA], Posthoc multiple comparisons tests<sup>32</sup>. The differences were considered statistically significant when p-value  $\leq 0.05$ .

## RESULTS

### Chromosomal Analysis of Bone Marrow Cells

The screened chromosomal aberrations in bone marrow cells included breaks, deletions, fragmentation, gaps, centric fusions and end to end associations. The frequency of different types of chromosomal aberrations in rat's bone marrow cells treated with different doses of testonon is presented in Table 1 and Figure 1.

Bone marrow cells of the first group treated ( $G^{st}$ ) at dose 500mg/week showed significant increases in total number of structure chromosomal aberrations with or without gaps at  $p \leq 0.05$  compared to the control group. Chromosomal aberrations of breaks, gaps, and centric fusion were increased in  $G^{st}$  compared to the control group but without significant difference, while end to end association showed significantly different in  $G^{st}$  compared to the control group.

The second group ( $G^{nd}$ ) of animals treated at dose 1000 mg/ week showed high significance difference in structural chromosomal aberrations without gaps ( $10.16 \pm 0.47$ ) compared to the control group ( $4.25 \pm 0.47$ ). Centric fusion was observed only in  $G^{nd}$  with significant different at  $p \leq 0.05$  compared to the  $G^{st}$  and control group while end to end associations were recorded in  $G^{st}$  and  $G^{nd}$  only compared to the control group.

Comparative study of treated animals in  $G^{st}$  and  $G^{nd}$  revealed that the total number of chromosomal aberrations was a significant difference in  $G^{nd}$  ( $10.16 \pm 0.70$ ) compared to  $G^{st}$  ( $7.3 \pm 0.76$ ). Chromosomal aberrations of gaps, breaks, deletions and end to end associations showed non-significant different between the two treated groups while centric fusion was significantly increased in  $G^{nd}$  at  $p \leq 0.05$  compared to  $G^{st}$ .

### Chromosomal Analysis of Spermatocyte Cells

The screened chromosomal aberrations in rat's spermatocyte cells included autosomal univalent, sex univalent, translocation and ring. Table 2 and Figure 2 showed the frequency of chromosomal aberrations induced by different doses of Testonon in rat's spermatocytes. The spermatocytes of animals treated at dose 500 mg/ week of Testonon showed increases in the number of autosomal univalent, sex univalent, translocation and ring compared to the control group.

All these increases were statically non-significant compared to the control group while the total number of

chromosomal aberrations was a significant difference at  $p \leq 0.05$  ( $6.33 \pm 0.33$ ) compared to the control group ( $2.7 \pm 0.33$ ).

The structural abnormalities of spermatocytes in animals treated at dose 1000 mg/week of Testonon showed that the autosomal univalent, translocation and total number of chromosomal aberrations were high significant difference compared to the control group (Table 2).

Comparative study of treated animals in  $G^{st}$  and  $G^{nd}$  revealed that the total number of chromosomal aberrations was a significant different in  $G^{nd}$  ( $9.0 \pm 0.57$ ) compared to  $G^{st}$  ( $6.33 \pm 0.33$ ), while the other abnormalities between the treated groups were statically non-significant.

### Sperm Morphology

The screened sperm abnormalities included amorphous, without hook, banana shape, sperm with two heads and tail abnormalities. The frequency of different types of sperm abnormalities with different doses of Testonon is illustrated in Table 3 and Figure 3.

Sperm abnormalities of animals treated at dose 500 mg/ week of Testonon observed increases in the number of sperm without hook and banana shape but without significant difference compared to the control group while sperm abnormalities of amorphous, total head abnormalities, total tail abnormalities and total abnormal sperms (head and tail) were significantly different at  $p \leq 0.05$  compared to the control group.

In  $G^{nd}$ , sperm abnormalities of amorphous, banana shape, sperm with two heads, total head abnormalities, total tail abnormalities and total abnormal sperms (head and tail) were high significant difference compared to the control group.

Comparative study of treated animals in  $G^{st}$  and  $G^{nd}$  revealed that sperm abnormalities of amorphous, sperm with two heads, total head abnormalities and total abnormal sperms (head and tail) were significant different in  $G^{nd}$  compared to the  $G^{st}$ .

The other sperm abnormalities between the treated groups were statically non-significant.

### DNA Fragmentation

DNA fragmentation in control and treated animals is shown in Table 4 and Figure 4. DNA fragmentation recorded significantly different in treated animals ( $G^{st}$  and  $G^{nd}$ ) compared to the control group.

The mean of DNA fragmentation result in  $G^{st}$  was  $8.65 \pm 0.26$  with 2.05% change compared to the control group ( $6.6 \pm 0.48$ ), while the mean of DNA fragmentation in  $G^{nd}$  was  $10.64 \pm 0.39$  with 4.04% change compared to the control group ( $6.6 \pm 0.48$ ).

Comparative study of treated animals in  $G^{st}$  and  $G^{nd}$  illustrated that the mean of DNA fragmentation in  $G^{nd}$  was a significant difference at  $p \leq 0.05$  compared to  $G^{st}$ .



## Biomarker Analysis

Table 5 illustrated the levels of testosterone, acid phosphatase activity, lipid peroxidation, liver and kidneys function in blood of the different experimental animals. Serum testosterone level was used as an indicator of androgenic effect. The serum hormone levels in G<sup>st</sup> and G<sup>nd</sup> were elevated significantly compared to the control group. The increased values were paralleled with dose amount, that 1000 mg/week dose was more effective than 500 mg/week.

Serum activity of acid phosphatase was stimulated significantly in Testonon treated animals relative to normal control. The increased values were 137% and 163% for G<sup>st</sup> and G<sup>nd</sup>, respectively compared to the control group.

Lipid peroxidation had the same previous trend of the both above parameters. Malondialdehyde (MDA) serum level as the indicator of lipid peroxidation was increased significantly in Testonon treated rats compared to normal control.

Kidneys and liver functions of the experimental animals are shown in Table 5. For liver function, the values of serum ALT activity was significantly different in G<sup>st</sup> and G<sup>nd</sup> compared to the control group. The increased activity under the effect of 1000 mg/week of Testonon was more than that of 500 mg/week treatment. AST activity showed the same trends but less than that of ALT activity under the same conditions of Testonon treatments.

Serum level of urea and creatinine as the indicator of kidneys function showed significantly different elevation of urea in G<sup>nd</sup> compared to the control group while non-significant different was observed in G<sup>st</sup> compared to the control group. Creatinine levels showed non-significant changes between the three experimental groups.

Serum lipids profile of the control and Testonon treated experimental rats are shown in Table 6. Cholesterol levels in G<sup>nd</sup> showed significant elevation compared to the control group while non-significant difference was observed in G<sup>st</sup> compared to the control group. The same trend of cholesterol was observed for LDL-c and HDL-c values in serum of the experimental animals. The serum values of total lipids, total triglycerides and vLDL-c observed non-significant difference between control and treated groups.

## DISCUSSION

Testonon acts as a time released steroid which is high in both anabolic and androgenic value. It has a unique blend of one long ester, one short ester and two medium length esters. The purpose for this blend is to provide the user with a quick release of the steroid, but also a long lasting one<sup>2</sup>. The treatment dose of Testonon is 250 mg, the break between the injections four weeks.

Although exogenous testosterone is used to accelerate the growth of an immature prostate and caused

suppression of apoptosis and prevents epithelial cell atrophy (Kim), but administration of suprphysiological doses have been recorded with severe adverse effects<sup>5,6</sup>. Testonon is used by bodybuilders and power lifters at doses between 500-1000 mg/week and commonly used for six weeks<sup>16</sup>.

Our study was concerned with the evaluation of the biological body functions and mutagenic effect of Testonon doses which have been chosen regarding the doses used by bodybuilders on rats. The mutagenic effects of these doses were measured by the cytogenetic analysis of somatic and germ cells and DNA fragmentation assay. The results revealed that chromosomal aberrations were included breaks, deletions, fragmentation, gaps, centric fusions and end to end associations. Comparative cytogenetic analysis between the treated groups of animals and control group revealed that a significant difference was observed in the total number of structure chromosomal aberrations in treated groups compared to the control group (Table 1 and Figure 1). Centric fusion and end to end associations were the obvious aberrations in treated groups compared to the control group. Centric fusion was observed in G<sup>nd</sup> with significantly different compared to the G<sup>st</sup> and control group. Walsh<sup>34</sup> and Riesenbergs<sup>35</sup> mentioned that chromosomal rearrangements are heterozygous and the cause of the chromosome imbalance that led to subfertility and sterility. It was also reported that chromosomal aberration of centric fusion observed with decreases in man and cattle fertility<sup>36-38</sup>. Postiglioni<sup>39</sup> suggested that the changes in chromatin structure may occur during the establishment of centric fusion, altering nucleotide sequences that could be enhancing epigenetic changes. Weinhold<sup>40</sup> reported that the epigenetics can play a role in the development of some cancers that "turns off" genes that help repair damaged DNA, leading to an increase in DNA damage, which in turn, increases cancer risk.

Chromosome is a strand of DNA wrapped around a histone protein core that carries all of the genetic information in an individual, increases in the number of structure chromosomal aberrations caused changes could effect on many genes along chromosomes and disrupt the protein made from those genes<sup>41</sup>. Cytogenetic analysis of mammalian germ cells contributes to the evaluation of genetic risk associated with drug therapy treatment<sup>42</sup>. Exposure of germ cells to mutagenic agents have been reported with genetic alterations in germ cells<sup>43</sup> and reproductive disorders<sup>44</sup>. Comparative study of chromosomal aberrations in rat's spermatocytes between control and treated groups revealed that the total number of chromosomal aberrations showed significant and highly significant different in treated G<sup>st</sup> and G<sup>nd</sup>, respectively compared to the control group (Table 2 and Figure 2).

The cytogenetic results suggested that the administration of suprphysiological doses of Testonon had mutagenic



effects on rat's somatic and germinal genetic materials. This led to impaired or loss of function for a particular gene, and accumulation of mutations which may lead to cancer and/or fertility disorder.

The measurement or evaluation of sperm morphology is an important tool in the diagnosis of a male's fertility potential and in the clinical decision-making for the treatment of patients with infertility problems<sup>45</sup>. It has been reported that the testosterone derivatives caused significant decrease of sperm count<sup>6</sup>. It was also reported that testosterone injection could be led to hormonal disturbance and increased reactive oxygen species (ROS) formation as supported by increased MDA, the imbalance hormones observed with increases in the percentage of dead sperm and low motility<sup>46</sup>. The MDA levels were negatively correlated to sperm count and motility which consistent peroxidation reaction affects<sup>47</sup>. The results of sperm morphology assay revealed that Testonon injection at different doses caused significant increases in the total head abnormalities, total tail abnormalities and total abnormal sperms (head and tail) compared to the control group (Table 3 and Figure 3). These results suggested that Testonon injection for six weeks had a significant effect on sperm morphology and may cause infertile problem.

Apoptosis is a form of programmed cell death characterized by cytoplasmic condensation, plasma membrane blebbing and nuclear pycnosis, leading to nuclear DNA breakdown into multiples of 200-bp oligonucleosomal size fragments. The percentage of DNA fragmentation in cultured cells detected exhibits a direct correlation with the percentage of apoptotic nuclei present in these cultures<sup>48,49</sup>. The results of DNA fragmentation revealed that the DNA fragmentation in treated groups showed a significant difference compared to the control groups. The percentage of DNA fragmentation was significant between the treated groups (Table 4 and Figure 4). These results clarified the cytotoxic effect of Testonon on liver cells at the doses used by bodybuilders.

Testosterone is the most abundant circulating androgen. After six weeks of Testonon treatments, the testosterone levels were consistently higher in serum of treated animals relative to the control animals. These increases were nearly three-fold in  $G^{st}$  and six-fold in  $G^{nd}$  compared to the control group (Table 5 and Figure 5). Several studies documented several possible risks for men taking testosterone-enhancing drugs. These drugs are linked to several heart problems, including heart attacks, possible increase in prostate cancer risk, blood clots, strokes and mini-strokes<sup>50,51</sup>. Therefore, increase the level of testosterone resulted from high doses taken by bodybuilders double the risk of suffering from a heart attack and increase the risk of prostate cancer risk, and blood clots.

Malondialdehyde (MDA) indicator is used to measure the level of oxidative stress. The results of the elevation in MDA content revealed that Testonon injection at doses

500mg/week and 1000mg/week associated with a significant elevation of lipid peroxidation ( $19.32 \pm 1.59$  and  $24.00 \pm 1.99$ , respectively) compared to the control group ( $11.00 \pm 1.02$ ). These results were in agreement with Aydin<sup>52</sup>, Aryal<sup>53</sup> and Rasul and Aziz<sup>6</sup> who reported the high levels of serum testosterone increased lipid peroxidation and decreased levels of antioxidants. Srivastava and Mittal<sup>54</sup> reported that testosterone can be converted to DHT by 5  $\alpha$ -reductase enzyme under the elevation of MDA that could be one of the causes leading to the development of cancer.

Acid phosphatase is produced primarily by the prostate gland, it is found in erythrocytes, platelets, leukocytes, bone marrow, liver, spleen, kidneys and intestine. The levels of this enzyme were elevated with Testonon injection in treated groups compared to the control group (Table 5 and Figure 5). These results were in agreement with Uboh<sup>55</sup> who reported that the increases in testosterone levels were paralleled with stimulation of acid phosphatase activity. Burtis and Ashwood<sup>56</sup> recorded an elevation in the level of acid phosphatase activity in benign prostate hyperplasia, osteoporosis and hyperparathyroidism.

The results presented in Table 5 revealed the effect of testonon injection on ALT and AST activities. The treated animals showed significant elevation in ALT and AST levels compared to the control groups. The results indicated to elevation of liver function related to ALT activity could be due to some liver damage, while elevation of AST level leads to dangerous changes in the structure of the left ventricle of the heart<sup>57,58,59</sup>. Urea and creatinine were used as indicators of kidney function. The results showed non-significant different in creatinine levels between different groups of experimental animals. Urea level at dose 1000 mg/week was observed with a significant difference while the urea level at dose 500mg/week was non-significant compared to the control group (Table 5). Elevation in urea level indicated to significantly impaired in kidney function and a prelude to kidney failure<sup>59</sup>. Cholesterol is the building block of steroid/androgens. Steroid genic cells import cholesterol bound to lipoprotein, which stored in the outer mitochondrial membrane. In the male gonads cholesterol is converted to potent androgens, which converts testosterone to dihydrotestosterone or the back door pathway, which bypasses the testosterone intermediate<sup>60</sup>. The results of lipids profile of different groups of the experimental animals illustrated that cholesterol levels, LDL-c and HDL-c values in  $G^{nd}$  showed significant elevation while  $G^{st}$  was non-significant different in these parameters compared to the control group (Table 6). The total lipids, total triglycerides and vLDL-c were non-significant different between the three experimental groups (Table 6).

These results suggested that the increases in total cholesterol and it's both parts (LDL-c and HDL-c) may be due to that the testosterone pathway was inhibited



by the high levels of serum testosterone as the result of Testonon injection. Nandeesh<sup>61</sup> noted that the circulation of total cholesterol, LDL-c and HDL-c were associated with the high levels of serum testosterone in a series of 50 symptomatic Benign prostatic hyperplasia (BPH). Moyad and Lowe<sup>62</sup> mentioned that high level of total cholesterol (including its lipoproteins)

increased the risk of BPH.

The results of biomarker analysis suggested that Testonon injection at doses used by bodybuilders act to increase the risk of heart attack, liver damage, kidney failure, hyperparathyroidism and benign prostate hyperplasia.

**Table 1:** Chromosomal aberrations induced by Testonon in bone marrow of male rats.

Treatment	Structural abnormalities					Total structural chrom. Aberrations	
	Gaps	Deletions	Breaks	Centric fusions	End to end associations	including gaps	excluding gaps
Control	0.5 ± 0.28 <sup>c</sup>	3.75 ± 0.25 <sup>c</sup>	0.5 ± 0.28 <sup>c</sup>	0.0 ± 0.0 <sup>c</sup>	0.0 ± 0.0 <sup>c</sup>	4.75 ± 0.25 <sup>c</sup>	4.25 ± 0.47 <sup>c</sup>
G <sup>st</sup>	0.5 ± 0.22 <sup>c</sup>	5.83 ± 0.79 <sup>b</sup>	0.66 ± 0.3 <sup>c</sup>	0.0 ± 0.0 <sup>c</sup>	0.83 ± 0.3 <sup>b</sup>	7.83 ± 0.9 <sup>b</sup>	7.3 ± 0.76 <sup>b</sup>
G <sup>nd</sup>	0.83 ± 0.16 <sup>c</sup>	7.5 ± 0.34 <sup>b</sup>	1.0 ± 0.68 <sup>c</sup>	0.66 ± 0.21 <sup>b</sup>	1.0 ± 0.44 <sup>b</sup>	10.8 ± 0.63 <sup>a</sup>	10.16 ± 0.70 <sup>a</sup>

(Mean percentages ± SEM), Within each column, means superscript with different letter are significantly different ( $P < 0.05$ ).

**Table 2:** Chromosomal aberrations induced by Testonon in rat's spermatocytes.

Treatment	Structural abnormalities				Total chromosomal aberrations
	Autosomal univalent	Sex univalent	Translocation	Ring	
Control	1.3 ± 0.33 <sup>c</sup>	1.0 ± 0.0 <sup>c</sup>	0.33 ± 0.6 <sup>c</sup>	0.0 ± 0.0 <sup>c</sup>	2.7 ± 0.33 <sup>c</sup>
G <sup>st</sup>	4.0 ± 0.57 <sup>cb</sup>	1.0 ± 0.0 <sup>c</sup>	1.0 ± 0.0 <sup>cb</sup>	0.3 ± 0.3 <sup>c</sup>	6.33 ± 0.33 <sup>b</sup>
G <sup>nd</sup>	5.66 ± 0.7 <sup>b</sup>	1.3 ± 0.8 <sup>c</sup>	1.6 ± 0.33 <sup>b</sup>	0.3 ± 0.3 <sup>c</sup>	9.0 ± 0.57 <sup>a</sup>

(Mean percentages ± SEM), Within each column, means superscript with different letter are significantly different ( $P < 0.05$ ).

**Table 3:** Sperm abnormalities in male rats of Treated and Control Groups

Treatment	Types of sperm head abnormalities				Total head abnormalities	Total tail abnormalities	Total abnormal sperms (head + tail)
	Amorphous	Without hook	Banana shape	Sperm with Two heads			
Control	1.6 ± 0.3 <sup>c</sup>	1.6 ± 0.66 <sup>c</sup>	0.0 ± 0.0 <sup>c</sup>	0.0 ± 0.0 <sup>c</sup>	3.3 ± 0.88 <sup>c</sup>	14.33 ± 1.4 <sup>c</sup>	17.3 ± 1.4 <sup>c</sup>
G <sup>st</sup>	4.6 ± 0.21 <sup>b</sup>	1.7 ± 0.42 <sup>c</sup>	0.5 ± 0.22 <sup>cb</sup>	0.0 ± 0.0 <sup>c</sup>	6.8 ± 0.54 <sup>b</sup>	20.16 ± 1.1 <sup>b</sup>	27.5 ± 1.0 <sup>b</sup>
G <sup>nd</sup>	9.33 ± 0.71 <sup>a</sup>	2.5 ± 0.56 <sup>c</sup>	1.0 ± 0.25 <sup>b</sup>	0.83 ± 0.30 <sup>b</sup>	13.66 ± 0.66 <sup>a</sup>	24.0 ± 1.7 <sup>b</sup>	37.66 ± 1.3 <sup>a</sup>

(Mean percentages ± SEM), within each column, means superscript with different letter are significantly different ( $P < 0.05$ ).

**Table 4:** DNA Fragmentation in rat's Liver of Treated and Control Groups

Treatment	Percent DNA fragmentation		Change %
	Rang	Mean ± S.E	
Control	5.57 - 7.86	6.6 ± 0.48 <sup>c</sup>	-
G <sup>st</sup>	8.0 - 9.3	8.65 ± 0.26 <sup>b</sup>	2.05
G <sup>nd</sup>	9.5 - 11.29	10.64 ± 0.39 <sup>a</sup>	4.04

(Mean percentages ± SEM), within each column, means superscript with different letter are significantly different ( $P < 0.05$ ).

**Table 5:** The levels of testosterone, acid phosphatase activity, lipid peroxidation as well as liver and kidneys function in blood of the different experimental animals (mean ± SD)

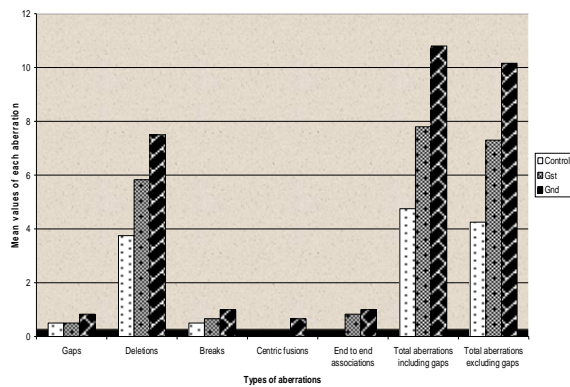
Parameters	Group (1) normal health control		Group (2) rats of 500 mg drug		Group (3) rats of 1000 mg drug	
	Levels of blood	%	Levels of blood	%	Levels of blood	%
Testosterone Mg/L	2.73 ± 0.21 <sup>c</sup>	100	7.98 ± 0.72 <sup>b</sup>	292	15.82 ± 1.51 <sup>a</sup>	579
Acid phosphatase activity U/L	30.00 ± 2.71 <sup>c</sup>	100	41.21 ± 4.01 <sup>b</sup>	137	49.00 ± 4.21 <sup>a</sup>	163
Lipid peroxidation nmol/ml	11.00 ± 1.02 <sup>c</sup>	100	19.32 ± 1.59 <sup>b</sup>	176	24.00 ± 1.99 <sup>a</sup>	218
ALT IU/L	26.23 ± 1.78 <sup>c</sup>	100	29.12 ± 1.99 <sup>b</sup>	111	32.21 ± 2.87 <sup>a</sup>	123
AST IU/L	55.27 ± 4.44 <sup>b</sup>	100	60.16 ± 5.74 <sup>ab</sup>	109	63.07 ± 5.88 <sup>a</sup>	114
Urea mg/dL	30.12 ± 3.01 <sup>b</sup>	100	33.27 ± 3.02 <sup>ab</sup>	110	34.11 ± 2.91 <sup>a</sup>	113
Creatinine mg/dl	0.61 ± 0.05 <sup>a</sup>	100	0.64 ± 0.06 <sup>a</sup>	105	0.66 ± 0.06 <sup>a</sup>	108

% at normal health control \*In column different letters means significant difference at < 0.05

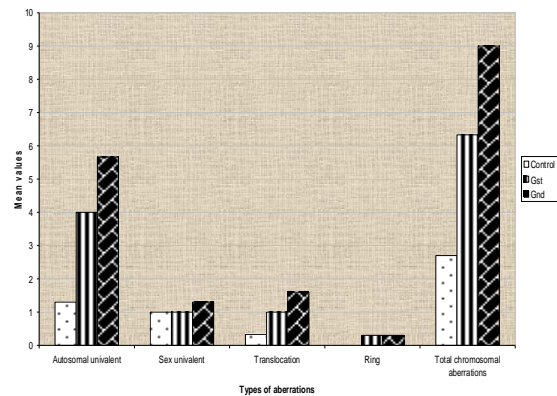
**Table 6:** Blood lipid profile of the different experimental animals (mean ± SD)

Parameters	Group (1) normal health control		Group (2) rats of 500 mg drug		Group (3) rats of 1000 mg drug	
	Mg/dL	%	Mg/dL	%	Mg/dL	%
Total lipid	2.99 ± 21.23 <sup>a</sup>	100	315 ± 24.11 <sup>a</sup>	105	317 ± 23.99 <sup>a</sup>	106
Total cholesterol	113.61 ± 10.23 <sup>b</sup>	100	120.16 ± 11.27 <sup>ab</sup>	106	127.32 ± 12.01 <sup>a</sup>	112
Total triglycerides	111 ± 8.89 <sup>a</sup>	100	114 ± 9.12 <sup>a</sup>	103	116 ± 10.01 <sup>a</sup>	105
LDL-c	37 ± 2.32 <sup>b</sup>	100	40 ± 3.43 <sup>ab</sup>	108	41 ± 2.74 <sup>a</sup>	111
HDL-c	46 ± 3.71 <sup>b</sup>	100	50 ± 3.99 <sup>ab</sup>	109	52 ± 3.92 <sup>a</sup>	113
vLDL-c	22.20 <sup>a</sup>	100	22.80 <sup>a</sup>	103	23.20 <sup>a</sup>	105

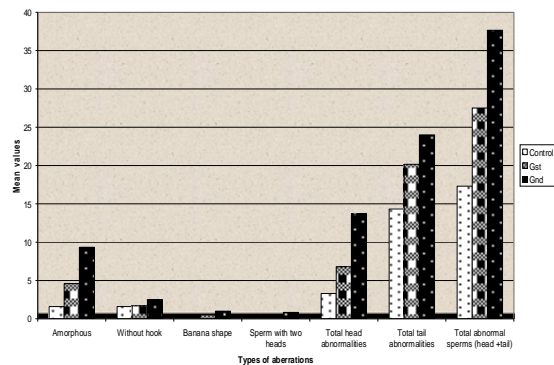
% at normal health control \*In column different letters means significant difference at < 0.05



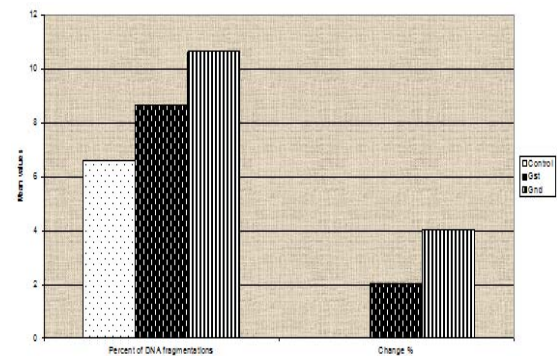
**Figure 1:** Chromosomal aberrations induced by Testonin in bone marrow of male rats



**Figure 2:** Chromosomal aberrations induced by Testonin in rat's spermatocytes.



**Figure 3:** Sperm abnormalities in male rats of Treated and Control Groups



**Figure 4:** DNA Fragmentation in rat's liver of Treated and Control Groups

**CONCLUSION**

In conclusion, the results of cytogenetic analysis, sperm morphology, DNA fragmentation and biomarker analysis revealed that Testonon injection at doses used by bodybuilders had mutagenic effects on rat's somatic and germinal genetic materials, cytotoxic effect on liver tissue and increased the sperm morphology abnormalities, the risk of heart attack, liver damage, kidney failure, hyperparathyroidism and benign prostate hyperplasia. These are alerts for the forbidden use of Testonon as bodybuilding agent.

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