Histopathological and morphometric evaluation in the testis and epididymis of adult rats submitted to a recovery period after treatment with anabolic steroid, alcohol, and/or nicotine

Bianca Ribeiro de Souza, Lucas Solla Mathias, Talita Iara Máximo de Souza, Isabel Cristina Cherici Camargo

ABSTRACT

Objective: Frequently, reproductive toxic substances such as androgenic anabolic steroids, alcohol and nicotine are used in association by adolescents and adults, in an indiscriminate manner. This study investigated the testicular and epididymal tissue of adult rats submitted to a recovery period after treatment with anabolic steroid, alcohol and/or nicotine. Materials and Methods: The animals (n=42) were divided into three control groups simulating the drugs administration routes (CI: distilled water, oral; CII: saline solution, subcutaneous; CIII: water and saline solution) and groups treated with a testosterone esters mixture (T: 7.5 mg/kg body weight - b.w., subcutaneous), alcohol (AL: 3.5 g/kg b.w. of ethanol 25%, oral), nicotine (N: 2.0 mg/kg b.w., subcutaneous), and co-administration of these three substances (T/AL/N). After 15 consecutive days of treatment (once a day), the animals were kept for 30 days in recovery. At the end of this period, the testes and epididymides were collected, weighed and processed for histological and morphometric analysis by light microscope. Results: All groups treated with toxic substances presented histopathological changes in testes and epididymis after the recovery period. There was a significant decrease (p<0.05) in testicular weight and in the morphometric parameters of the testis and epididymis in T and T/AL/N groups. Conclusion: The testis and epididymis of rats treated with anabolic steroid, alcohol and/or nicotine exhibited histopathological changes after a recovery period and the damages were more evident in the groups receiving the anabolic steroid alone or co-administered with other drugs.

KEY WORDS: Testosterone, ethanol, nicotine, male reproduction, histopathology

INTRODUCTION

Frequently, reproductive toxic substances such as androgenic anabolic steroids (AAS), alcohol and nicotine are used in association by adolescents and adults, in an indiscriminate manner [1-3].

AAS are recommended for treatment of sarcopenia and cachexia associated with AIDS, hepatic or renal failure, cancer and severe burns [4,5], but their nonmedical use is increasing during the latest decades. These substances are synthetic derivatives of testosterone that act on androgenic receptors, which are present in several different types of tissues, conferring inseparable anabolic and androgenic effects [6]. Because of its anabolic effect in promoting increased muscle mass, many people use AAS for esthetic reasons [7,8] and this nonclinical use may cause reversible and irreversible side effects [9]. In men and rodents, testicular changes and reduction in the quality of semen are widely reported in the literature as result of AAS use [10-13].

The alcohol is by far the most widely used and abused addictive drug [14], reaching a number of approximately 2 billion people who have the habit of consuming it [15]. It is considered the cause of more than 20 varieties of chronic and acute diseases, which increase the morbidity and mortality rates worldwide [16]. Alcohol causes several damages to the male reproductive system such as a decrease of fertility, reduction in the quality of semen, prostate and epididymis inflammation and prostate cancer [17-21]. According to previous reports [22,23], the damages on the quality of the semen are partially reversible after an abstinence period.
Another deleterious substance for reproductive system is nicotine, considered the most toxic component in cigarettes. Around 20 billion of cigarettes are consumed daily, resulting in an amount of approximately 200 tons of nicotine [24]. According to the World Health Organization, smoking is the main cause of avoidable deaths and every year almost 6 million people die as consequence of tabagism. The nicotine acts as an endocrine disorganizer of male hormones [25,26], which promotes negative effects in the reproduction. The substance reduces the number of spermatozoa [27], increases the number of abnormal gametes and promotes changes in the testicular tissue [28-30].

The side effects promoted by anabolic steroids, alcohol or nicotine in reproductive tissues are well documented in the literature. However, little attention is given on the effects of coadministration of these substances, especially considering a posttreatment recovery period. Then, this study aimed to evaluate for the first time the histological structure and morphometric parameters of the testis and epididymis of adult rats submitted to a recovery period after treatment with anabolic steroid, alcohol, and/or nicotine.

**MATERIAL AND METHODS**

**Animals**

Adult male Wistar rats (Rattus norvegicus), 70 days old and body weight (b.w.) of approximately 320 g, were kept at the Faculty of Sciences and Letters (UNESP; Assis, SP, Brazil) in room under conditions of controlled temperature and luminosity (22-24°C, 12 h light/dark photoperiod, respectively). Commercial feed (multinix™, agroceres; Rio Claro, SP, Brazil) and tap water were provided *ad libitum*. The experimental protocol followed the ethical principles in animal research adopted by the Brazilian Society of Science in Laboratory Animals. The protocols were approved by the Ethical Committee for Animals Use - CEUA (Register 019/2011).

**Drugs**

A testosterone esters mixture (durateston™, Organon Lab., São Paulo, Brazil) containing propionate (30 mg), fenpropionate (60 mg), isocaproate (60 mg), and decanoate (100 mg), was used as an injectable preparation. Nicotine [hydrogen tartrate salt; 1-methyl-2-(3-pyridyl) pyrrolidine] was purchased from Sigma-Aldrich Chemical Company (St. Louis, MO, USA) in the form of a white to off-white powder.

**Experimental Protocol**

The animals were weighed and randomly distributed into seven groups (n = 6/group): Control I (CI): Treatment with 0.3 mL of distilled water, oral route (gavage); Control II (CII): Treatment with 0.3 mL of saline solution, subcutaneous route; Control III (CIII): Simultaneous treatment with saline solution (subcutaneously) and distilled water (orally); testosterone esters mixture (T): Treatment with a dose of 7.5 mg/kg b.w., subcutaneously; alcohol (AL): Treatment with ethanol 25% at a dose of 3.5 g/kg b.w., orally (gavage); nicotine (N): Treatment with a dose of 2.0 mg/kg b.w., diluted in 0.3 mL of saline solution, subcutaneously; coadministration with three substances (T/AL/N), according to the procedures adopted for each substance.

Three control groups were established order to mimic the drugs administration routes and to compare the results of each experimental group with respective control group.

Supraphysiological dose of synthetic steroid was employed simulating the conditions of AAS users that included doses 5-29 times greater than physiologic replacement doses for testosterone [31,32]. The adopted experimental protocol used to choose and administrate alcohol was based on a previous study [33]. In the group treated with nicotine, it was employed the methodology described by Faraday et al. [34] and Hussein et al. [35].

In each group, the animals received the treatment for 15 consecutive days (a single daily dose). Then, they were subsequently submitted to a recovery period of 30 consecutive days, in which they did not receive any treatment.

At the end of the recovery period, the rats were weighed and euthanized at an overdose of anesthetic (thiopental™, Cristalia, São Paulo, Brazil), intraperitoneally. The testes and epididymides were collected, weighed and fixed in Bouin solution. In sequence, the material was dehydrated in crescent solution of ethylic alcohol, clarified in xylene and embedded in Paraplast (Labware-Oxford, St. Louis, MO, USA). The 5 µm thick sections were stained with hematoxylin and eosin for histopathological and morphometric analysis in light microscope.

For the morphometric study, 20 cross-sections of seminiferous tubules of each testicle were randomly chosen for the measurement of the tubular area, germ epithelium height, luminal area, and tubular diameter. In each region (proximal caput, mid-corpus, and proximal cauda) of the epididymis (left and right), it was chosen randomly 10 cross-section of the duct for measurement of the epithelial height, luminal area, and ductal area. All measurements were performed in optical microscopy scope Al-Axio coupled with video-camera AxioCam ICc3 (Carl Zeiss, Germany) and the images were digitalized by the software Axio Vision, version 4.7.2.

**Statistical Analysis**

When the data revealed normality were analyzed by analysis of variance complemented by Tukey test. The results were expressed as mean ± standard deviation (SD). In the absence of normality, the data were analyzed by the Kruskal-Wallis nonparametric analysis complemented by Student-Newman-Keuls test, and the results were expressed as median ± interquartile deviation. Statistical analysis was conducted on GraphPad Prism software, version 5.00. Significance was set at $P < 0.05$. 

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de Souza, et al.: Effects of testosterone, alcohol and/or nicotine
RESULTS

Effects of Treatments on b.w. and Reproductive Organs Weight

There was a significant decrease ($P < 0.05$) on the b.w. in the rats receiving T/AL/N, compared to the CIII, AL and N groups [Table 1]. The absolute and relative weights of testes were smaller in the T group ($P < 0.05$), compared to the CII group. In the T/AL/N group, the absolute weight of the testes was significantly decreased in comparison to the CIII, AL and N groups. The absolute and relative weights of epididymides were similar ($P > 0.05$) in all experimental groups [Table 1].

The Testicular and Epididymal Tissue Presents Histopathological and Morphometric Changes after a Posttreatment Recovery Period

In the groups receiving anabolic steroid [Figure 1c, f and i], alcohol [Figure 1d and g] or nicotine [Figure 1n and h] there were notable changes in the testicular structure, in comparison to the control groups [Figure 1a, b, and j]. In the animals treated with each drug [Figure 1c–i], the seminiferous tubules showed disorganization in the epithelial cytoarchitecture and desquamation of germ cells in the epithelium, which resulted in the presence of immature cells in lumen. In the T-treated group, it was also observed vascular congestion and vasodilatation, interstitial hemorrhage and atrophy of Leydig cells [Figure 1f and i]. The T/AL/N group [Figures 1k and l] exhibited histopathological characteristics similar to the T-treated group, having atrophied seminiferous tubules with epithelial areas devoid of cells, besides vascular congestion and atrophied Leydig cells in the interstitial tissue. Scarcely quantity of gametes was observed into lumen.

Characteristically, the epididymal duct is lined by pseudostratified epithelium with stereocilia, which becomes thin toward the cauda region [Figure 2a and b]. The interstitium is constituted by vascularized connective tissue, containing collagen fibrils and a variety of cells. In the cauda, there was abundant amount of sperm into lumen.

After a recovery period, the main morphological characteristic observed in the epididymis of groups treated with T [Figure 2c and f], AL [Figure 2d and e], N [Figure 2g and h] and T/AL/N [Figure 2i] was the presence of leukocyte infiltrate in an edematous interstitium, sometimes with a hemorrhagic aspect [Figure 2g]. Furthermore, in the group treated with alcohol, it was observed loss of morphological integrity of the duct in the regions of the corpus and cauda [Figure 2e]. Scarcely amount of sperm was observed in the ductal lumen of the T-treated group, whereas in the group receiving T/AL/N there were numerous immature germ cells present in such region [Figure 2i].

The morphometric analysis of epididymides showed that in caput [Table 3], the ductal and luminal area were significantly similar ($P > 0.05$) between the experimental groups. However, the epithelial height increased ($P < 0.05$) in the T and T/AL/N groups, compared respectively, to the CII and CIII groups. In the N and AL groups, the epithelial height was lower than that observed in T/AL/N group.

In the corpus [Table 3], there was a decrease ($P < 0.05$) in the ductal and luminal area of the T and T/AL/N groups, when compared to the CII and CIII groups, respectively. In the AL and N groups, the ductal and luminal area were significantly larger than the T/AL/N group. The epithelial height increased ($P < 0.05$) in the groups receiving anabolic steroid alone or coadministered with alcohol and nicotine, compared to the CII and CIII groups, respectively. There was similar epithelial height between the AL and N groups and their respective controls, but a smaller epithelial height in comparison to the T/AL/N group.

In the cauda [Table 3], the epithelial height was similar in all experimental groups, while the ductal and luminal areas were significantly reduced ($P < 0.05$) in the AL, N, T and AL/N/T groups, compared to their respective control groups.

Table 1: Body weight (g) and absolute (g) and relative (g%) weights of reproductive organs in the different experimental groups, after a posttreatment recovery period

<table>
<thead>
<tr>
<th>Experimental groups</th>
<th>Body weight (g)</th>
<th>Testes weight (g)</th>
<th>Epididymides weight (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CI</td>
<td>396.5 ± 66.2</td>
<td>3.36 ± 0.49</td>
<td>1.43 ± 0.25</td>
</tr>
<tr>
<td>CII</td>
<td>416.5 ± 31.7</td>
<td>3.46 ± 0.36</td>
<td>1.42 ± 0.19</td>
</tr>
<tr>
<td>CIII</td>
<td>459.5 ± 39.2</td>
<td>3.36 ± 0.33</td>
<td>1.48 ± 0.10</td>
</tr>
<tr>
<td>T</td>
<td>407.5 ± 16.5</td>
<td>2.73 ± 0.31</td>
<td>1.44 ± 0.18</td>
</tr>
<tr>
<td>AL</td>
<td>459.5 ± 31.2</td>
<td>3.24 ± 0.14</td>
<td>1.56 ± 0.08</td>
</tr>
<tr>
<td>N</td>
<td>444.0 ± 16.5</td>
<td>3.47 ± 2.73</td>
<td>1.49 ± 0.18</td>
</tr>
<tr>
<td>T/AL/N</td>
<td>369.0 ± 47.0</td>
<td>2.42 ± 0.54</td>
<td>1.29 ± 0.20</td>
</tr>
</tbody>
</table>

*Values expressed as the median±interquartile deviation (Kruskal-Wallis, student Newman-Keuls test). *$P < 0.05$, in comparison between the following experimental groups: Anabolic steroid (T) vs. Control II (CII); coadministration of drugs (T/AL/N) vs. Control III (CIII); *$P < 0.05$, in comparison between the groups: T, AL or N vs. T/AL/N, ANOVA: Analysis of variance, SD: Standard deviation.
DISCUSSION

The deleterious effects promoted by anabolic steroid, alcohol or nicotine in male reproduction are well known. Here, we evaluate the b.w., reproductive organs weight and the histopathological and morphometric parameters of the testes and epididymis of rats treated with each drug or coadministered drugs, considering a period of posttreatment recovery.

In the literature, there are controversies regarding the results on the b.w. of animals submitted to the treatment with anabolic steroids, alcohol or nicotine due to the different experimental protocols adopted, mainly related to the dose and time of treatment. There are few studies that evaluate the effects of such drugs after a period of treatment interruption.

In this study, the alcohol-treated group did not present alteration on the b.w. after a recovery period, similarly to results reported by Emanuele et al. [36]. According to Martinez et al. [37], during the period of treatment with alcohol there is a decrease of food intake, but after treatment interruption a replenishment of food intake occurs, which promotes the b.w. recovery. In this study, the treatment of rats with anabolic steroid or nicotine also did not influence the b.w. after a period of abstinence [38,39]. Interestingly, the coadministration of anabolic steroid, alcohol and nicotine promoted decrease on the b.w. of rats, indicating that the interaction of drugs was harmful to the organism even after a posttreatment recovery period.

Various chemicals substances can affect the weight, structure and/or function of the reproductive organs. In the testes, the

Table 2: Testicular morphometric analysis in the different experimental groups, after a posttreatment recovery period

<table>
<thead>
<tr>
<th>Experimental groups</th>
<th>Tubular area (mm²)</th>
<th>Luminal area (mm²)</th>
<th>Tubular diameter (µm)</th>
<th>Epithelial height (µm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CI</td>
<td>81.2±7.8</td>
<td>17.2±1.8</td>
<td>323.1±13.8</td>
<td>93.4±9.0</td>
</tr>
<tr>
<td>CII</td>
<td>75.2±6.6</td>
<td>15.1±1.8</td>
<td>310.9±15.1</td>
<td>86.3±5.4</td>
</tr>
<tr>
<td>CIII</td>
<td>67.8±2.5</td>
<td>15.1±1.4</td>
<td>295.5±6.3</td>
<td>75.7±4.0</td>
</tr>
<tr>
<td>T</td>
<td>58.6±4.1*</td>
<td>10.8±0.9*</td>
<td>273.5±9.4*</td>
<td>77.3±2.2</td>
</tr>
<tr>
<td>AL</td>
<td>75.4±2.0*</td>
<td>13.7±1.0*</td>
<td>310.0±4.7*</td>
<td>88.0±3.0*</td>
</tr>
<tr>
<td>N</td>
<td>72.2±4.8*</td>
<td>15.0±1.5*</td>
<td>304.4±9.6*</td>
<td>84.6±5.7*</td>
</tr>
<tr>
<td>T/AL/N</td>
<td>53.0±5.0*</td>
<td>9.0±1.5*</td>
<td>258.8±11.4*</td>
<td>74.6±3.5</td>
</tr>
</tbody>
</table>

Values expressed as the mean±SD (ANOVA, Tukey test). *P<0.05, in comparison between the following experimental groups: Anabolic steroid (T) vs. Control II (CII); coadministration of drugs (T/AL/N) vs. Control III (CIII); #P<0.05, in comparison between the groups: T, AL or N vs. T/AL/N, ANOVA: Analysis of variance

Figure 1: Photomicrographs of testes in the rats of groups: CI (a), CII (b), CIII (j), T (c, f, i), AL (d and g), N (e and h) and T/AL/N (k and l). Control group exhibits seminiferous tubules (st) with various layers of germ cells and spermatozoa into the lumen. T, AL and N groups show loss of tubular morphological integrity (c-e, h), necrosis of seminiferous tubules (g, asterisk) and mixture of immature germ cells in the lumen (c, d, g, h). In the T (f and i) and T/AL/N (k and l) groups, there were vascular congestion (v), interstitial hemorrhage (he) and atrophy of Leydig cells (L). Hematoxylin and eosin
germ cells, Sertoli cells, Leydig cells, sperm and/or sex hormones may be negatively influenced by toxicants agents [40,41]. In the epididymis, the direct-acting toxicants can alter the structure and function of the interstitium, the structure and function of the epithelium, and hence the composition of the luminal fluid [42].

Table 3: Epididymal morphometric analysis in the different experimental groups, after a posttreatment recovery period

<table>
<thead>
<tr>
<th>Experimental groups</th>
<th>Ductal area (mm²)</th>
<th>Luminal area (mm²)</th>
<th>Epithelial height (µm)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>CAPUT</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CI</td>
<td>23.5±7.1</td>
<td>12.6±3.1</td>
<td>24.7±1.4</td>
</tr>
<tr>
<td>CI</td>
<td>28.2±1.2</td>
<td>16.2±1.5</td>
<td>21.8±3.0</td>
</tr>
<tr>
<td>CII</td>
<td>24.2±3.3</td>
<td>13.4±2.5</td>
<td>22.9±1.5</td>
</tr>
<tr>
<td>CI</td>
<td>23.4±3.4</td>
<td>9.8±2.2</td>
<td>27.4±3.7*</td>
</tr>
<tr>
<td>AL</td>
<td>28.7±2.9</td>
<td>15.4±4.5</td>
<td>23.6±6.4#</td>
</tr>
<tr>
<td>N</td>
<td>24.6±3.4</td>
<td>13.4±2.8</td>
<td>23.8±2.4#</td>
</tr>
<tr>
<td>T/AL/N</td>
<td>31.4±2.7</td>
<td>16.3±2.1</td>
<td>28.4±2.2*</td>
</tr>
<tr>
<td><strong>CORPUS</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CI</td>
<td>94.4±10.1</td>
<td>71.3±10.0</td>
<td>20.7±2.2</td>
</tr>
<tr>
<td>CI</td>
<td>88.7±3.7</td>
<td>67.5±2.2</td>
<td>19.8±1.2</td>
</tr>
<tr>
<td>CII</td>
<td>85.4±13.1</td>
<td>65.1±17.5</td>
<td>20.3±3.3</td>
</tr>
<tr>
<td>CII</td>
<td>66.0±8.0*</td>
<td>45.2±11.5*</td>
<td>25.0±2.4*</td>
</tr>
<tr>
<td>AL</td>
<td>87.6±3.5#</td>
<td>66.2±4.1#</td>
<td>21.8±1.3#</td>
</tr>
<tr>
<td>N</td>
<td>85.1±9.0#</td>
<td>70.2±11.8#</td>
<td>20.2±1.6#</td>
</tr>
<tr>
<td>T/AL/N</td>
<td>52.0±6.6*</td>
<td>28.8±11.2*</td>
<td>28.5±2.1*</td>
</tr>
<tr>
<td><strong>CAUDA</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CI</td>
<td>245.2±37.3</td>
<td>224.5±36.6</td>
<td>12.8±1.8</td>
</tr>
<tr>
<td>CI</td>
<td>227.6±14.8</td>
<td>210.3±14.1</td>
<td>11.8±1.1</td>
</tr>
<tr>
<td>CII</td>
<td>224.3±24.5</td>
<td>205.1±24.6</td>
<td>13.2±4.4</td>
</tr>
<tr>
<td>CII</td>
<td>163.1±17.0*</td>
<td>144.8±16.7*</td>
<td>12.6±1.4</td>
</tr>
<tr>
<td>AL</td>
<td>193.2±35.5*</td>
<td>172.6±35.8*</td>
<td>12.8±1.0</td>
</tr>
<tr>
<td>N</td>
<td>173.3±10.3*</td>
<td>154.4±10.4*</td>
<td>14.8±2.7</td>
</tr>
<tr>
<td>T/AL/N</td>
<td>156.3±28.3*</td>
<td>136.9±30.9*</td>
<td>13.7±4.0</td>
</tr>
</tbody>
</table>

Values expressed as the mean±SD (ANOVA, Tukey test). *P<0.05, in comparison between the following experimental groups: Anabolic steroid (T) vs. Control II (CII); alcohol vs. Control I (CI); nicotine vs. Control II (CII); coadministration of drugs (T/AL/N) vs. Control III (CIII); #P<0.05, in comparison between the groups: T, AL: Alcohol, or N: Nicotine, vs. T/AL/N, ANOVA: Analysis of variance
In this study, the testicular weight was decreased in the T and T/AL/N groups due to various histopathological changes and seminiferous tubular atrophy observed after a recovery period. It is documented that synthetic androgens cause reduction in the area and diameter of the seminiferous tubules [45,44]. However, none study showed that these effects may remain after the interruption of steroid treatment alone or combined to others drugs. Notably, we observed that the damage was not greater in the gonad tissue of rats treated with coadministered drugs, when compared to the group that only received the steroid. An aspect to highlight in testicular tissue of T and T/AL/N groups was the vasodilatation and vascular congestion, followed by hemorrhage. This result confirms previous report that pharmacological or supraphysiological doses of testosterone or AAS cause vasodilatation [45,46]. Study performed by Simão et al. [47] showed that 30 days of posttreatment recovery was insufficient to reduce vasodilatation in the ovaries of androgenized female rats, confirming the results obtained in this study. There was a remarkable action of exogenous testosterone on the testes, given that the hormone has remained circulating in the organism until 30 days after the end of treatment.

In the rats receiving the alcohol or nicotine alone, the testes weight and morphometric parameters of seminiferous tubules were unchanged after the recovery period, comparatively to the control groups. However, histopathological characteristics were observed in the testes of rats treated with each drug. It is documented that alcohol and nicotine present damages to the testicular tissue, and the gonad weight may be reestablished after the suspension of the drugs, depending on the treatment duration and quantity of drug administered [48-50].

According to Kempinas and Klinefelter [42], toxicant-mediated lesions in the epididymis involve germ cells in the lumen, reuction in epithelial cell height, and altered epithelial morphology. In this study, after a recovery period, the more pronounced changes in the epididymis occurred in the groups receiving the synthetic steroid alone or coadministered with alcohol and nicotine. These changes involved increase in epithelial cells height of caput and corpus regions and decrease in ductal and luminal areas of corpus and cauda. Besides, there were inflammatory infiltrates in the interstitium and the presence of immature germ cells in the lumen. Infiltration of leukocytes also was reported in uterus of rats treated simultaneously with nandrolone decanoate, testosterone, and nicotine [8]. However, in T and T/AL/N groups, the epididymis weight was not changed. The results confirm that coadministration of anabolic steroid, alcohol and nicotine does not intensify the adverse effects of the synthetic steroid on the epididymis.

A previous study [51], showed that simultaneous chronic administration of ethanol and nicotine did not alter the epididymides parameters including weight and volume, but significantly decreased sperm concentration and motility. In this study, the administration alone of alcohol or nicotine did not change the epididymides weight, but promoted atrophy of duct in the cauda portion and infiltration of leukocytes in the interstitium, indicating that treatment interruption has kept the deleterious effects of each drug until the given period of recovery time.

In conclusion, the testes and epididymis of rats treated with anabolic steroid, alcohol and/or nicotine exhibited histopathological changes after the recovery period adopted in the study and the damages were more evident in the groups receiving the anabolic steroid alone or coadministered with other drugs.

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