Ameliorative Effect of Taurine on Nandrolone Decanoate Induced Toxicity on Brain, Heart and Testis in Adult Male Albino Rats

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ABSTRACT

Background: The most prescribed anabolic steroid in gym practice is nandrolone decanoate (ND). It is implicated in sport doping, and criminal circles where personal aggressiveness was evident. Despite its wide use among athletes, its uncontrolled utilization causes serious toxicities of body organs. Taurine is an amino acid that is used as a supplement for bodybuilders and has many cytoprotective properties. Aim: This study aimed to evaluate the role of taurine in attenuation of the toxic effects of ND on biochemical parameters, histopathological and immune-histochemistry changes of hippocampus, heart and testis in adult male albino rats. Materials and Methods: It is an in vivo experimental study where 40 rats were classified into four groups; Control group, Taurine-treated, ND-treated group, and ND+Taurine group. Testicular levels of MDA, SOD, and TNFα, in addition to serum level of CK-MB were measured. Gene expression of testicular BAX, BCL2, MMP-9, Caspase-3, Cyp11a1, and hippocampal Prodynorphin were assessed. Hematoxylin and eosin (H&E), Masson trichrome stainings, immunohistochemical analysis and histo-morphometric studies were also done. Results: Taurine coadministration with ND significantly decreased levels of testicular MDA, TNF-α, serum CK-MB, and mRNA expression of testicular BAX, MMP-9, Caspase-3 and hippocampal Prodynorphin, with significant increase of testicular SOD level, and mRNA expression of testicular Cyp11a1 and a non-significant increase of testicular BCL2. The histopathological findings revealed marked amelioration of testicular and hippocampal toxicity, and significant decrease in collagen fibers in heart. Also, immunohistochemical expression of Ki-67 was increased in testis with decreased expression of GFAP in hippocampus comparable to ND- treated group. Conclusion: Taurine reduced the toxic effects of ND primarily on both the testis and hippocampus, in addition to a partial improvement in the heart.

Keywords: Taurine; Nandrolone Decanoate; Testicular Toxicity; Cardiotoxicity; Neurotoxicity; Prodynorphin Gene Expression.
I- INTRODUCTION

Anabolic-androgenic steroids (AASs) are synthetic forms of testosterone (analogues) that have similar chemical and hormonal properties. They are used in medical practice to treat a variety of diseases, including growth disorders, hereditary edema, blood diseases, osteoporosis, kidney problems, and delayed puberty in males (Ergun-Longmire and Wajnrajch, 2020).

Over the past years, many athletes have utilized illegal substances to increase their physical performance. AASs are among the most frequently misused substances. Moreover, their utilization among non-athletes is considered as a serious public health problem, as abusers minimize and ignore the harmful effects of these drugs (Pope Jr et al., 2014).

Adolescents and bodybuilders are more likely to misuse AAS because of its anabolic properties and ability to boost tolerance to exercise. (Patanè et al., 2020).

As this use of AASs is illicit, a great portion of our data of their negative health impacts is gained from retrospective studies, case reports, or comparisons with studies in other similar patient groups (Hasso, 2009).

Athletes consume these drugs in excessive combinations and doses (10–100 folds) (Naraghi et al., 2010).

When consumed in high concentrations, these substances may modify the hormonal physiology, behavior, and sperm quality in both humans and animals (Torres-Calleja et al., 2001).

The average age at which AASs use begins is about 20 years and only 22 % of the users start before age 20 (Pope Jr et al., 2014). In gym communities, the prevalence of usage among male young adults has been documented to be as high as 9.8 % (Althobiti et al., 2018).

Based on the substitution of the base molecule, AASs fall into three main categories. C-17 esterification is associated with Class I. Class II has a demethylated group at C-19 and maybe C-17 esters. Class III is associated with C-17 alkylation. Nandrolone is a member of the class II AASs, that are derivates of 19-nortestosterone (Pomara et al., 2016).

The most frequently prescribed AAS is nandrolone decanoate (ND), as it has the lowest incidence of the negative toxic effects in comparison to its beneficial effects. Therefore, it had been linked to doping until, the International Olympic Committee (IOC) banned its usage in athletic competitions because of health concerns (Tofighi et al., 2017).

The therapeutical dose of ND as one of AAS ranges between 50 to 100 mg/ week for women and from 100 to 200 mg/ week for men. A person who takes a dosage that is 10-100 times greater than the typical dose may have a variety of negative consequences (Vasavan et al., 2020).

ND is mainly administered by injection, and occasionally, in combination with other AASs. Athletes typically self-administer ND over many weeks prior to sporting events in order to achieve synergistic anabolic benefits with minimum adverse effects and to avoid getting detected on doping tests (Pany et al., 2019; Agriesti et al., 2020).
Noorafshan et al. (2005) and Takahashi et al. (2004) reported that the uncontrolled and extended use of ND results in various structural irregularities in the testes. Male infertility is a major disability caused by ND usage. Furthermore, decreases in testicular size, sperm quantity and motility, and epididymal weights have been recorded as a result of treatment with relatively high nandrolone doses.

Taurine (2-aminoethanesulfonic acid) is a prevalent free amino acid found in tissues of most animal species. Taurine is obtained from exogenous dietary sources or it can be synthesised inside the body from cysteine or methionine mainly in the liver (Ahmed, 2015).

In the male reproductive system and sperm cells, taurine plays various crucial roles. Taurine is a capacitating agent and a sperm motility factor, in addition to controlling cellular osmosis and stabilizing cell membranes. Moreover, it exhibits antioxidant capabilities that safeguard against sperm lipid peroxidation. (Manna et al., 2008).

In animal studies, taurine was proved to be protective against testicular toxicity caused by endosulfan and cadmium (Aly and Khafagy, 2014). It is a semi-essential amino acid (i.e., not essential for humans to consume, but it is beneficial for their health) found in mammalian brains, hearts, retinas, skeletal muscles, and leukocytes. It may be gained from the diet, mostly through eggs, meat, and seafood (Beyranvand et al., 2011).

Furthermore, taurine has been shown to provide a protective role against a variety of pathological conditions, including hypoxia, glutamate-induced neurotoxicity, and inflammation. This protective role is evident through its various actions as a neurotransmitter, neuromodulator, osmoregulator, modulator of intracellular calcium homeostasis, antioxidant, membrane stabilizer, and anti-inflammation factor (El Idrissi, 2008).

Taurine and its analogues have been shown in intact animals to exhibit anti-neurotoxic characteristics, suppress tumor cell growth, and reduce cardiac apoptosis after ischemia, reperfusion, or doxorubicin therapy (Li et al., 2009; Marcinkiewicz et al., 2009). Taurine insufficiency is linked to hyperactivity, anxiety, epilepsy, and depression, thus taurine supplementation helps alleviate these symptoms (Kong et al., 2006).

Previous studies mainly focused on the toxic effects of ND on the testis, and heart. However, there is a lack of studies that also included the brain and the neuronal toxicity of ND. Therefore, the current study was conducted to evaluate the role of taurine in attenuation of the toxic effects of ND on the hippocampus, heart and testis via assessing biochemical parameters, gene expression, histopathologic and immune-histochemistry changes in adult male albino rats.

II- MATERIALS AND METHODS

Site of study:
The study was performed in Zagazig University, Faculty of Medicine, Departments of Forensic medicine and clinical toxicology, Histology and cell biology and Medical Biochemistry.
Type of study:
It is an in vivo experimental study.

Materials:
- Nandrolone decanoate (Deca-Durabolin) as ampoule (25 mg/ml) of oily solution was purchased from Organon Company.
- Taurine as powder (now foods Company, 395 S. Bloomingdale, IL 60108, USA) was purchased from health shop company in Egypt.
- Reagents and commercial kits were supplied from Sigma and Biodiagnostic Chemical Companies.

Experimental Design:
The study used 40 adult male albino rats (200-250 gm), received from the Animal House of Faculty of Medicine, Zagazig University. The rats were provided with full access to water and food during the study, and they were kept in a room-temperature environment with a 12-hour light/dark cycle. This study was designed to mimic sub-chronic intoxication with ND in human. The whole period of the experiment extended to 8 weeks. Rate were divided into 4 main groups:

Group I (Control): consisted of 16 rats subdivided into two equal subgroups (each with eight rats).
- Subgroup Ia (Negative control group), to measure the fundamental values of the tested parameters, each rat got merely a usual food and tap water.
- Subgroup Ib (Positive control group), olive oil (0.1 mL) was injected intraperitoneal (IP) to the rats once weekly (as a vehicle for the ND) (Bordbar et al., 2014).

Group II (Taurine-treated), each 100 mg of taurine powder was dissolved in 4 ml of drinking water and each rat received 1 ml daily, in a dose equivalent to 100 mg/kg/day, by oral gavage for eight weeks (Kalender et al., 2019).

Group III (ND-treated), consisted of 8 rats given once weekly IP injection of ND at a dosage of 39 mg/kg dissolved in olive oil for eight weeks. The used ND dose represents 1/10 LD50 for rats to mimic the abuse dose in body builders. The dose in albino rats is determined by using the conversion table of Paget and Barnes based on the dose in mice (Paget and Barnes, 1964). The ND LD50 of IP injection in mice is > 566 mg/kg (Jurox, 2014).

Group IV (ND + Taurine treated group), included 8 rats received ND and taurine with the same mentioned doses for eight weeks.

Sampling:
At the end of the experiment (24 hours from the last dose) and under complete anesthesia, all animals were euthanized with 25 mg/kg of sodium thiopental IP injection (Abdelrahman et al., 2023). Venous blood samples were sampled from the retro-orbital plexus of animals using micro-capillary glass tubes (Abdelaal et al., 2018), the samples were centrifuged and the supernatant fluid was kept in -20 °C till used for biochemical analysis. Tissue samples from the hippocampus, heart, and testis were collected, homogenized, and kept in -80 °C till used for biochemical analysis, and gene expression. Other tissue samples of the different groups in the experiment were fixed with 10% formal saline for 24 hours, then tissues were subjected to further processing to obtain the paraffin blocks. 6 µm thick sections were cut from the paraffin blocks then mounted, and subjected to staining for histopathologic changes.
Biochemical analysis:

**Estimation of lipid peroxidation and antioxidant activity in the testicular tissue**

MDA and SOD were measured by spectrophotometer using commercial kits in testicular tissue homogenate according to the instructions of the manufacturer (Nishikimi et al., 1972; Satoh, 1978)

**Estimation of Tumor Necrosis Factor – alpha (TNFα) level**

TNFα level was estimated in testicular homogenate via commercial ELISA kit.

**Estimation of cardiac biomarkers**

CK-MB was measured by spectrophotometer in serum according to the manufacturer (Bablok et al., 1988).

**Gene expression analyses**

Hippocampus and testicles were frozen at -80 °C for gene expression. At work, homogenization of the tissues was done and extraction of the total RNA from each homogenate via RNeasy Mini Kit, Qiagen. The absorption ratio (260/280 nm) was used to measure total RNA purity. The QuantiTect Reverse Transcription Kit was used to reverse transcribe the cDNA, as directed by the manufacturer. qRT-PCR was used to assess gene expression using cDNA (5 uL), 10 pmol/µL of each primer, and 10 uL of SYBR Green 2x Master Mix Green (QuantiTect SYBR Green PCR Kits, Qiagen). Mx3005P (Stratagene, CA, USA) was used to perform RT-qPCR. Thermal cycling was performed as follows: first denatured for 10 minutes at 95 °C, 40 cycles of 94 °C for 15 seconds, annealing at 60 °C for 1 minute, and elongation at 72 °C for 30 seconds. The data were standardized against the GAPDH transcript level, and the relative expression was calculated using the 2-ΔΔCt technique. (Livak and Schmittgen, 2001). Table 1 shows the sequences of the primers used in RT-qPCR. All genes were testicular except prodynorphin which was done from hippocampal tissue.

**Table 1:** The sequences of the primers used in reverse transcription-quantitative polymerase chain reaction (RT-qPCR)

<table>
<thead>
<tr>
<th>Target Gene</th>
<th>Primer Sequence</th>
</tr>
</thead>
</table>
| BAX (Ujah et al., 2021) | F: 5'-CGCGTTGGTGCCCTTTACTTTT-3'  
|                  | R: 5'-CAACGCAGCCGCTCACGAGGA-3'                      |
| BCL2 (Ujah et al., 2021) | F: 5'-ATCGCTCTGATGATACGATAC-3'  
|                  | R: 5'-AGAGACAGCCAGGAGAAATCAAC-3'                    |
| MMP9 (Ahmed, 2015)   | F: 5'-AGGGGCAGCAAAGCTGTAGCCT-3'                     
|                  | R: 5'-TTTCAGGTCTCGGGGGAAGACACATA-3'                  |
| Caspase 3 (He et al., 2018) | F: 5'-GATGGAACTGAGATCGATATG-3'  
|                  | R: 5'-CGCAAAGTGACTGGATGAACC-3'                      |
| Cyp11a1 (Ujah et al., 2021) | F: 5'-CTTGGTCAGTGGAGCTAG-3'  
|                  | R: 5'-CGGAAGTGCTGGGTGTTT-3'                         |
| Prodynorphin (Vasavan et al., 2021) | F: 5'-ATGGGCCTGTTCCAGGCTATGC-3'  
|                  | R: 5'-AGTTGTAGATTAGAAGGCTATCC-3'                    |
| GAPDH             | F: 5'-TCACCCACATGGAGAAGGC-3'                         
|                  | R: 5'-GCTAAGCAGTGGTGTCGA-3'                         |
Histological study:

Light microscopic studies (Suvarna et al., 2018):

- **Hematoxylin and Eosin stains (H&E)** for demonstration of the histological structure
- **Masson trichrome staining** for cardiac muscle, for detection of fibrosis
- **Immunohistochemical staining** for:
  - **Ki-67**, an indicator for cellular proliferation in the testis.
  - **GFAP**, a marker for astrocytes in the hippocampus.

Immunostaining always required pretreatment. Firstly, antigen retrieval was done by specimen boiling for 10 minutes in 10 Mm, pH 6 citrate buffer. After that, the sections were cooled at room temperature for 20 minutes. The sections were put in an incubator with the primary antibodies for one hour. Completing the Immunostaining was done by Ultra vision detection system. Finally, Mayer’s hematoxylin was used for counterstaining of sections (Suvarna et al., 2018).

Histo-morphometry:

The following measurements were included:

- Mean area percentage of **Ki-67 positive cells** in immune-stained testicular sections (×400 magnification).
- Mean area percentage of **collagen fibers in Masson trichrome** stained sections of the heart (×400 magnification).
- Mean area percentage of **GFAP positive cells** in immuno-stained hippocampus sections (×400 magnification).

All those measurements were done in 10 non-overlapping fields from different sections of each one of the experimental groups. The analysis of images was performed by Leica Microsystems LTD (DFC 295) software image analysis computer system (Germany) at the Dentistry Research and Equipment Unit, Faculty of Dentistry, Cairo University.

Statistical analysis:

The studied biochemical and morphometrical measurements were statistically analyzed using 28th version of SPSS program (IBM Co., Armonk, NY, USA). Quantitative parametric data were presented as mean and standard deviation (SD). Student T test was used to compare negative and positive control groups. The one-way analysis of variance (ANOVA) test was used to analyze the data, followed by the post hoc test (LSD). We consider the p value <0.05 to be statistically significant.

Ethical Approval:

In this research, all animals were cared for in accordance with the guidelines for animal research issued by the National Institute of Health. The animal ethics committee approved this research, Zagazig University- Institutional Animal Care and Use Committee (Approval number: ZU-IACUC/3/F/444/2022).

III- RESULTS

In the present study, the comparison between the positive and negative control groups revealed statistically non-significant difference regarding all the studied biochemical parameters (testicular levels of MDA, SOD, and TNFα, serum level of CK-MB, and gene expression of testicular BAX, BCL2, MMP-9,
Caspase-3, Cyp11a1, and hippocampal Prodynorphin) and the histological parameters (H&E staining, Masson trichrome staining, immunohistochemical analysis and histo-morphometric studies). Therefore, we used the negative control group as a comparison marker for the other treated groups.

Biochemical Results

1- Estimation of testicular SOD and MDA levels (Table 2, Figures. 1 and 2):

There were statistically significant differences among the four studied groups in SOD level (p<0.01) and MDA level (p<0.001).

Regarding the antioxidant level, SOD level, there was no statistically significant difference in group II (Taurine) in comparison to group Ia (control) (p1 >0.05) with mean values (54.13±6.4, 57.22 ± 5.2 respectively). However, there was a statistically significant difference in the other treated groups; as SOD level was significantly lower in groups III (ND) and IV (ND+ Taurine) in comparison to group Ia (control) (p2 <0.001, p3 <0.05) with mean values of (31 ± 3.1, 47.3 ± 4.5 respectively), but was significantly higher in group IV (ND+ Taurine) than in group III (ND) (p6 <0.01).

Regarding MDA level (oxidant), there was no statistically significant difference in group II (Taurine) in comparison to group Ia (control) (p1 >0.05) with mean values (74.4±8.3, 83.5 ± 10.2 respectively).

However, there was a statistically significant difference in the other treated groups; being higher in groups III (ND) and IV (ND+ Taurine) in comparison to group Ia (control) (p2 <0.001, p3 <0.05) with mean values of (150 ± 6.7, 105 ± 7.1 respectively), but was significantly lower in group IV (ND+ Taurine) than group III (ND) (p6 <0.001).

Table 2 Comparison of testicular antioxidant activity and lipid peroxidation via Superoxide Dismutase (SOD), and Malondialdehyde (MDA) levels among the studied groups using one-way analysis of variance (ANOVA) test, followed by the post hoc test (LSD)

<table>
<thead>
<tr>
<th></th>
<th>Group Ia (Control) (n=8)</th>
<th>Group II (Taurine) (n=8)</th>
<th>Group III (ND) (n=8)</th>
<th>Group IV (ND + Taurine) (n=8)</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>SOD (U/g Tissue)</td>
<td>57.22 ± 5.2</td>
<td>54.13±6.4</td>
<td>31 ± 3.1</td>
<td>47.3 ± 4.5</td>
<td>=0.001; &lt;0.01**</td>
</tr>
<tr>
<td></td>
<td>p1= 0.466; &gt;0.05</td>
<td>p4&lt;0.001***</td>
<td>p6= 0.004; &lt;0.01**</td>
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<tr>
<td></td>
<td>p2&lt;0.001***</td>
<td>p5= 0.129; &gt;0.05</td>
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<tr>
<td></td>
<td>p3=0.04; &lt;0.05*</td>
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<tr>
<td>MDA (nmol/g Tissue)</td>
<td>83.5 ± 10.2</td>
<td>74.4±8.3</td>
<td>150 ± 6.7</td>
<td>105 ± 7.1</td>
<td>&lt;0.001***</td>
</tr>
<tr>
<td></td>
<td>p1= 0.211; &gt;0.05</td>
<td>p4&lt;0.001***</td>
<td>p6&lt;0.001***</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>p2&lt;0.001***</td>
<td>p5= 0.002; &lt;0.01**</td>
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<tr>
<td></td>
<td>p3=0.012; &lt;0.05*</td>
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</tbody>
</table>

Data are presented as mean ± SD, *: Statistically significant as p<0.05, p1: Comparison between GI & GII, p2: Comparison between GI & GIII, p3: Comparison between GI & GIV, p4: Comparison between GII & GIII, p5: Comparison between GII & GIV, p6: Comparison between GIII & GIV SOD: Superoxide Dismutase, MDA: Malondialdehyde
There was no statistically significant difference in group II (Taurine) in comparison to group Ia (control) (p1 >0.05) with mean values (470.4±32.9, 485.5 ± 25.4 respectively).

However, there was a statistically significant difference in the other treated groups; as TNF α level was significantly higher in groups III (ND) and IV (ND+ Taurine) in comparison to group Ia (control) (p2 <0.001, p3 <0.05) with mean values of (750.3±50.6, 580.3± 25.8 respectively), but was significantly lower in group IV (ND+ Taurine) than in group III (ND) (p6 <0.001).

Table 3 Comparison of testicular tumor necrosis factor–alpha (TNF α) levels among the studied groups using one-way analysis of variance (ANOVA) test, followed by the post hoc test (LSD)

<table>
<thead>
<tr>
<th></th>
<th>GIa (Control) (n=8)</th>
<th>GII (Taurine) (n=8)</th>
<th>GIII (ND) (n=8)</th>
<th>GIV (ND+ Taurine) (n=8)</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>TNF α (pg/g tissue)</td>
<td>485.5±25.4</td>
<td>470.4±32.9</td>
<td>750.3±50.6</td>
<td>580.3± 25.8</td>
<td>&lt;0.001***</td>
</tr>
<tr>
<td>p1</td>
<td>0.613; &gt;0.05</td>
<td>p4&lt;0.001***</td>
<td>p6&lt;0.001***</td>
<td></td>
<td></td>
</tr>
<tr>
<td>p2</td>
<td>&lt;0.001***</td>
<td>p5= 0.005; &lt;0.01**</td>
<td></td>
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<tr>
<td>p3</td>
<td>0.011; &lt;0.05*</td>
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</tbody>
</table>

Data are presented as mean ± SD, *: Statistically significant as p<0.05, p1: Comparison between GI & GII, p2: Comparison between GI & GIII, p3: Comparison between GI & GIV, p4: Comparison between GII & GIII, p5: Comparison between GII & GIV, p6: Comparison between GIII & GIV, TNF: Tumor necrosis factor.
3- Estimation of cardiac CK-MB levels (Table 4, Figure 4):

The comparison between the four studied groups in terms of CK-MB level revealed a statistically significant difference (p<0.001) as it was significantly higher in group III (ND) as compared to groups Ia (control), II (Taurine) and IV (ND+ Taurine) with mean values of (580.6 ± 25.4, 365.3 ± 20.2, 340.4±22.6, 380.34 ± 10.2 respectively), however it was comparable between groups Ia, II and III.

![Figure 4 CK-MB levels in the studied groups]

Table 4 Comparison of cardiac biomarker creatine kinase-MB (CK-MB) levels among the studied groups using one-way analysis of variance (ANOVA) test, followed by the post hoc test (LSD)

<table>
<thead>
<tr>
<th>Group</th>
<th>CK-MB IU/L</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ia (n=8)</td>
<td>365.3±20.2</td>
<td></td>
</tr>
<tr>
<td>Iia (n=8)</td>
<td>340.4±22.6</td>
<td></td>
</tr>
<tr>
<td>III (n=8)</td>
<td>580.6±25.4</td>
<td>&lt;0.001***</td>
</tr>
<tr>
<td>IV (n=8)</td>
<td>380.34±10.2</td>
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</table>

$p1=0.174; p2<0.001***$  
$p3=0.393; p4<0.001***$  
$p5=0.043; p6<0.001***$

Data are presented as mean ± SD. *: Statistically significant as p<0.05, p1: Comparison between GI & GII, p2: Comparison between GI & GIII, p3: Comparison between GI & GIV, p4: Comparison between GII & GIII, p5: Comparison between GII & GIV, p6: Comparison between GIII & GIV, CK-MB: Creatine Kinase MB

4- Gene expression:

Testicular gene expression (Table 5, Figure 5):

BAX expression (apoptotic marker) was different significantly among the groups (p<0.01) being significantly higher in groups III (ND) and IV (ND+ Taurine) as compared to group Ia (control) with mean values of (2.1 ± 0.25, 1.5 ± 0.27, 1 ± 0.13 respectively, p2<0.001, p3<0.05 respectively) and was significantly lower in group IV than group III (p6<0.01). However, it was comparable between groups Ia, and II (Taurine).

BCL2 expression (antiapoptotic marker) was different significantly among the groups (p<0.05) being lower in group III (ND) as compared to groups Ia (control), II (Taurine) and IV (ND+ Taurine) with mean values of (0.5 ± 0.16, 0.9 ± 0.19, 1.02±0.15, 0.8 ± 0.2 respectively. This decrease was significant compared to groups Ia and II, but non-significant compared to group IV (p2<0.05, p4 <0.01, p6 >0.05 respectively). BCL2 expression was comparable between groups Ia, II and IV.

MMP-9 expression (inflammatory marker) was different significantly among the groups (p<0.001) being significantly higher in groups III (ND) and IV (ND+ Taurine) as compared to group Ia (control) with mean values of (2.2
± 0.19, 1.5 ± 0.14, 1.1 ± 0.2 respectively, p2<0.001, p3<0.05 respectively) and was significantly lower in group IV than group III (p6 <0.01). However, it was comparable between groups Ia, and II (Taurine).

Caspase-3 expression (apoptotic marker) was different significantly among the groups (p<0.001) being significantly higher groups III (ND) and IV (ND+ Taurine) as compared to group Ia (control) with mean values of (2.8 ± 0.2, 1.6 ± 0.19, 0.89 ± 0.21 respectively, p2<0.001, p3<0.01 respectively) and was significantly lower in group IV than group III (p6<0.001). However, it was comparable between groups Ia, and II (Taurine).

Regarding Cyp11a1 expression (marker for steroidogenesis), it was different significantly among the groups (p<0.01) being lower in groups III (ND) and IV (ND+ Taurine) as compared to group Ia (control) with mean values of (0.4 ± 0.12, 0.7 ± 0.14, 0.9 ± 0.11 respectively, p2<0.01, p3>0.05 respectively) and was significantly higher in group IV than group III (p6<0.05). However, it was comparable between groups Ia, and II (Taurine).

**Table 5** Comparison of testicular gene expression in fold change among the studied groups using one-way analysis of variance (ANOVA) test, followed by the post hoc test (LSD)

<table>
<thead>
<tr>
<th>Gene</th>
<th>Gia (Control) (n=8)</th>
<th>GII (Taurine) (n=8)</th>
<th>GIII (ND) (n=8)</th>
<th>GIV (ND + Taurine) (n=8)</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>BAX</td>
<td>1±0.13</td>
<td>1.1±0.11</td>
<td>2.1±0.25</td>
<td>1.5±0.27</td>
<td>=0.001; &lt;0.01**</td>
</tr>
<tr>
<td>p1= 0.562; &gt;0.05</td>
<td></td>
<td>p4&lt;0.001***</td>
<td>p6=0.007; &lt;0.01**</td>
<td></td>
<td></td>
</tr>
<tr>
<td>p2&lt;0.001***</td>
<td></td>
<td>p5= 0.042; &lt;0.05*</td>
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<tr>
<td>p3=0.017; &lt;0.05*</td>
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<tr>
<td>BCL2</td>
<td>0.9±0.19</td>
<td>1.02±0.15</td>
<td>0.5±0.16</td>
<td>0.8 ±0.2</td>
<td>=0.034; &lt;0.05*</td>
</tr>
<tr>
<td>p1= 0.428; &gt;0.05</td>
<td></td>
<td>p4=0.007; &lt;0.01**</td>
<td>p6=0.071; &gt;0.05</td>
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</tr>
<tr>
<td>p2=0.024; &lt;0.05*</td>
<td></td>
<td>p5= 0.165; &gt;0.05</td>
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<td>MMP-9</td>
<td>1.1±0.2</td>
<td>0.9±0.2</td>
<td>2.2 ± 0.19</td>
<td>1.5 ± 0.14</td>
<td>&lt;0.001***</td>
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<tr>
<td>p1= 0.22; &gt;0.05</td>
<td></td>
<td>p4 &lt;0.001***</td>
<td>p6=0.002; &lt;0.01**</td>
<td></td>
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<tr>
<td>p2&lt;0.001***</td>
<td></td>
<td>p5= 0.004; &lt;0.01**</td>
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<tr>
<td>p3=0.029; &lt;0.05*</td>
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<tr>
<td>Caspase-3</td>
<td>0.89±0.21</td>
<td>1±0.13</td>
<td>2.8 ± 0.2</td>
<td>1.6 ± 0.19</td>
<td>&lt;0.001***</td>
</tr>
<tr>
<td>p1= 0.488; &gt;0.05</td>
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<td>p4 &lt;0.001***</td>
<td>p6&lt;0.001**</td>
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<tr>
<td>p2&lt;0.001***</td>
<td></td>
<td>p5= 0.004; &lt;0.01**</td>
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<tr>
<td>p3=0.002; &lt;0.01**</td>
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<tr>
<td>Cyp11a1</td>
<td>0.9±0.11</td>
<td>1.01±0.11</td>
<td>0.4±0.12</td>
<td>0.7±0.14</td>
<td>=0.001; &lt;0.01**</td>
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<tr>
<td>p1= 0.296; &gt;0.05</td>
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<td>p4 &lt;0.001***</td>
<td>p6=0.016; &lt;0.05*</td>
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<td>p2=0.001; &lt;0.01**</td>
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<td>p5= 0.014; &lt;0.05*</td>
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<td>p3=0.077; &gt;0.05</td>
<td></td>
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</tbody>
</table>

Data are presented as mean ± SD, *: Statistically significant as p<0.05, p1: Comparison between GI & GII, p2: Comparison between GI & GIII, p3: Comparison between GI & GIV, p4: Comparison between GII & GIII, p5: Comparison between GII & GIV, p6: Comparison between GIII & GIV
Taurine Ameliorates Nandrolone Decanoate Toxic Effect

Hippocampal gene expression (Table 6, Figure 6):

Prodynorphin expression was different significantly among the groups (p<0.001) being significantly higher in groups III (ND) and IV (ND+ Taurine) as compared to group Ia (control) with mean values of (2.3 ± 0.3, 1.5 ± 0.12, 1.11 ± 0.12 respectively, p2<0.001, p3<0.05 respectively) and was significantly lower in group IV than group III (p6<0.01). However, it was comparable between groups Ia, and II (Taurine).

Table 6 Comparison of hippocampal gene expression in fold change among the studied groups using one-way analysis of variance (ANOVA) test, followed by the post hoc test (LSD)

<table>
<thead>
<tr>
<th></th>
<th>GIa (Control) (n=8)</th>
<th>GII (Taurine) (n=8)</th>
<th>GIII (ND) (n=8)</th>
<th>GIV (ND + Taurine) (n=8)</th>
<th>p value</th>
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</thead>
<tbody>
<tr>
<td>Prodynorphin</td>
<td>1.11±0.12</td>
<td>1.03±0.11</td>
<td>2.3±0.3</td>
<td>1.5±0.12</td>
<td>&lt;0.001***</td>
</tr>
<tr>
<td>p1= 0.603; &gt;0.05</td>
<td>p2&lt;0.001***</td>
<td>p4 &lt;0.001***</td>
<td>p5= 0.013; &lt;0.05*</td>
<td>p6=0.001;&lt;0.01**</td>
<td></td>
</tr>
<tr>
<td>p2&lt;0.001***</td>
<td>p3=0.03; &lt;0.05*</td>
<td>&lt;0.05*</td>
<td>&lt;0.05*</td>
<td>&lt;0.05*</td>
<td></td>
</tr>
</tbody>
</table>

Data are presented as mean ± SD, *: Statistically significant as p<0.05, p1: Comparison between GI & GII, p2: Comparison between GI & GIII, p3: Comparison between GI & GIV, p4: Comparison between GII & GIII, p5: Comparison between GII & GIV, p6: Comparison between GIII & GIV
Histological Results

Light microscopic studies

1- Testis (Figure 7 and 8):

Hematoxylin and Eosin staining

Hematoxylin and Eosin-stained sections of the testis of the control group and taurine group showed the seminiferous tubules lined by stratified germinal epithelium, and Sertoli cells with large, pale nuclei & a thin regular basement membrane. Little interstitium containing clusters of interstitial cells is also detected. Sperms could be seen in the lumen (Figure 7 A and 7B). The Seminiferous tubules of ND treated group are markedly distorted with irregular basal lamina and lined by disorganized detached germinal epithelium. Some germ cells have dark pyknotic nuclei. Wide interstitium with congested blood vessel is also observed (Figure 7C). Other sections in the same group revealed thickened connective tissue capsule and congested sub-capsular blood vessel. Seminiferous tubules have separated exfoliated germ cells (Figure 7D). The interstitium showed acidophilic vacuolated hyaline material and congested blood vessel between markedly distorted seminiferous tubules (Figure 7E). focal area of the same group revealed markedly disorganized shrunken seminiferous tubule (Figure 7F). Section of the ND + Taurine treated group showed seminiferous tubule were lined by nearly normal stratified germinal epithelium. Sperms could be seen in the lumen. Residual few areas of minimal germinal epithelium separation, slight separation between adjacent cells. Few areas in the interstitium revealed residual acidophilic vacuolated hyaline material and minimally congested blood vessel (Figure 7G).

Immunohistochemical staining for Ki-67

Sections of the testis of control and taurine groups stained with anti-Ki-67 antibody showed strong positive immunoreaction in the nuclei of spermatogonia and primary spermatocytes (Figure 8A and B). Sections of the ND treated group revealed faint positive immunoreaction in few cells (Figure 8C), complete negative reaction is detected in seminiferous tubules of some sections as seen in (Figure 8D). On the other hand, ND + Taurine treated group showed positive immunoreaction in the nuclei of many spermatogonia and primary spermatocytes (Figure 8E).

2- Heart (Figure 9 and 10):

Hematoxylin and Eosin staining

Hematoxylin and Eosin-stained sections of the control and Taurine groups illustrated the normal general architecture of cardiac tissue which is formed of normal elongated, branching and anastomosing muscle fibers with acidophilic cytoplasm show transverse striations and central, oval vesicular nuclei. The intercellular spaces are narrow containing blood capillaries and flat dark nuclei of the fibroblasts (Figure 9A and B). The ND-treated group revealed disturbed general cardiac tissue architecture with degenerated, irregularly arranged myofibers with intensely eosinophilic cytoplasm and small deeply stained nuclei. Wide intercellular spaces are seen between cardiomyocytes. Extravasated red blood cells are observed in some areas (Figure 9C). Some sections in the same group
reveal cardiomyocyte swelling with decreased acidophilia, many cytoplasmic vacuolations, and small deeply stained nuclei. Sever interstitial haemorrhage with red blood cells extravasations are noticed (Figure 9D). In the ND + Taurine treated group, the cardiomyocytes revealed some improvement and preserve nearly normal architecture in some areas with central, oval vesicular nuclei and acidophilic cytoplasm. The intercellular spaces are narrow containing minimally congested capillary. Other areas still have degenerated muscle fibers with small dark nuclei (Figure 9E). Some sections in the same group still show disorganized degenerated cardiac muscle fibers with residual vacuolations and small deeply stained nuclei. Focal area of congested blood capillary was seen (Figure 9F).

**Masson trichrome staining**

Stained cardiac muscle sections of the control and Taurine groups revealed few fine collagen fibers in the connective tissue spaces between cardiac muscle fibers (Figure 10A and B). Sections of the ND treated group showed massive extensive collagen fibers around blood vessels in the interstitium between cardiac muscle fibers (Figure 10C). However, in the ND+ Taurine treated group, mild amount of collagen deposition between some cardiac muscle fibers and around blood vessels could be seen (Figure 10D).

**3- Hippocampus (Figure 11 and 12):**

**Hematoxylin and Eosin staining**

In the control group, H&E-stained sections showed C-shaped hippocampus that is composed of three Cornu Ammonis (CA1, CA2 and CA3) and dentate gyrus (DG) (Figure 11A). A Section in the CA1 area in the hippocampus of both control and taurine groups showed the normal arrangement of cells in three distinct layers; pleomorphic, pyramidal, and molecular layer. The pyramidal cells have large vesicular nuclei. Glial cells are present in the pleomorphic and molecular layers. Blood capillary could be noticed (Figure 11B and C). In the ND-treated group, CA1 area of the hippocampus showed disturbed cellular organization. The pyramidal cells show shrunken pyknotic nuclei. Cellular vacuolations are also present. Glial cells with dark nuclei and wide pericellular area. Wide and dilated blood capillaries could be seen (Figure 11D). CA1 area in the ND+ Taurine-treated group showing apparently normal histological structure. Most of the pyramidal cells appear normal with vesicular nuclei however only few cells with pyknotic nuclei can be still noticed. Glial cells and blood capillaries are noticed (Figure 11E).

**Immunohistochemical staining for glial fibrillary acidic protein (GFAP)**

Sections of the CA1 area of the hippocampus of the control and taurine groups showed few positive GFAP branched astrocytes in different layers (Figure 12A and B). Regarding ND treated group, CA1 area of the hippocampus showed many branched astrocytes with strong positive GFAP in different layers (Figure 12C). In the ND+ Taurine treated group, CA1 area of the hippocampus showed few branched astrocytes with positive GFAP in different layers (Figure 12D).
Figure 7 Hematoxylin and Eosin-stained sections of testes from rats of Group Ia (Control group) (A) and Group II: (Taurine treated group) (B): showing the seminiferous tubules (St) are lined by stratified germinal epithelium (G), and Sertoli cells with large, pale nuclei (green arrow), having a thin regular basement membrane (arrowhead). Little interstitium (I) containing clusters of interstitial cells (red arrow) is also detected. Sperms could be seen in the lumen (blue arrow). (H&E x 400; scale bar 20 μm)

Group III (ND treated group): (C, D, E, F) (C): Seminiferous tubules (St) are markedly distorted with irregular basal lamina (blue arrow) and lined by disorganized detached germinal epithelium (arrows). Some germ cells have dark pyknotic nuclei (arrowhead). Wide interstitium (I) with congested blood vessel (B) is also observed. (H&E x 200; scale bar 50 μm) (D): Sections in the same group reveal thickened connective tissue capsule (C) and congested sub-capsular blood vessel (B). Seminiferous tubules (St) have separated exfoliated germ cells (arrow). (H&E x 400; scale bar 20 μm) (E): Acidophilic vacuolated hyaline material (star) appears in the interstitium containing congested blood vessel (B) between markedly distorted seminiferous tubules (St) is also observed. (H&E x 400; scale bar 20 μm) (F): focal area of the same group reveals markedly disorganized and shrunken seminiferous tubule (red circle). (H&E x 400; scale bar 20 μm)

In Group IV (ND + Taurine treated group) (G): seminiferous tubules (St) are lined by nearly normal stratified germinal epithelium (G). Sperms could be seen in the lumen (blue arrow). Residual few areas of minimal germinal epithelium separation (curved arrow), slight separation between adjacent cells (arrow). Few areas in the interstitium (I) reveal residual acidophilic vacuolated hyaline material (star) and minimally congested blood vessel (B). (H&E x 400; scale bar 20 μm)
Figure 8 Group Ia: (Control group) (A) and Group II: (Taurine treated group) (B): Immunohistochemical stained sections with anti-Ki-67 antibody of the control and taurine treated group testis showing strong positive immunoreaction in the nuclei of spermatogonia and primary spermatocytes (arrows).

Group III (ND treated group) (C and D); (C): Sections in this group reveal faint positive immunoreaction in few cells (arrows), complete negative reaction is detected in seminiferous tubules (St) of some sections as seen in (D).

Group IV (ND+ Taurine treated group) (E): show positive immunoreaction in the nuclei of many spermatogonia and primary spermatocytes (arrows) (Immunoperoxidase technique x 400; scale bar 20 μm)
Figure 9 Hematoxylin and Eosin-stained sections of hearts from rats of Group Ia (Control group) (A) and Group II: (Taurine treated group) (B): illustrate the normal general architecture of cardiac tissue which is formed of normal elongated, branching and anastomosing muscle fibers (mf) with acidophilic cytoplasm show transverse striations and central, oval vesicular nuclei (arrow heads). The intercellular spaces are narrow containing blood capillaries (c) and flat dark nuclei of the fibroblasts (arrow).

Group III (ND treated group): (C, D) (C): reveal disturbed general cardiac tissue architecture with degenerated, irregularly arranged myofibers(mf) with intensely eosinophilic cytoplasm and small deeply stained nuclei (arrow heads). Wide intercellular spaces (stars) are seen between cardiomyocytes. Extravasated red blood cells (arrows) are observed in some areas. (D): Some sections in the same group reveal cardiomyocyte swelling with decreased acidophilia (oval), many cytoplasmic vacuolations (v), and small deeply stained nuclei (arrow heads). Sever interstitial haemorrhage with red blood cells extravasations (arrow) are noticed.

Group IV (ND+ Taurine treated group): (E, F) (E): cardiomyocytes in this group reveal some improvement and preserve nearly normal architecture in some areas with central, oval vesicular nuclei (arrow heads) and acidophilic cytoplasm (mf). The intercellular spaces are narrow containing minimally congested capillary (c). Other areas still have degenerated muscle fibers with small dark nuclei (red arrows). (F): Some sections in the same group still show disorganized degenerated cardiac muscle fibers with residual vacuolations (mf) and small deeply stained nuclei (arrow heads). Focal area of congested blood capillary (arrow) is seen. (H&E x 400; scale bar 20 μm)
Figure 10 Masson trichrome stained sections of the hearts of **Group Ia: (control group)** (A) and **Group II: (Taurine treated group)** (B): reveals few fine collagen fibers in the connective tissue spaces between cardiac muscle fibers (arrows).

**Group III (ND treated group)** (C): shows massive extensive collagen fibers (arrows) around blood vessels in the interstitium between cardiac muscle fibers (arrow).

**Group IV: (ND+ Taurine treated group)** (D): Mild amount of collagen deposition (arrows) between some cardiac muscle fibers and around blood vessels can be seen in this group. (Masson trichrome stain x 400; scale bar 20 μm)
Figure 11 Hematoxylin and Eosin-stained sections of the hippocampi from rats of Group Ia (Control group) (A): showing the hippocampus is C-shaped and consists of three Cornu Ammonis (CA1, CA2 and CA3) and dentate gyrus (DG) (H&E X200, scale bar 50 μm). At higher magnification, the CA1 area in the hippocampus of the Group Ia (Control group) (B) and Group II (Taurine treated group) (C) shows the normal three layers; pleomorphic (PL), pyramidal (P), and the molecular layer (M). The pyramidal cells (arrows) have large vesicular nuclei. Glial cells (curved arrows) are present in the (PL) and (M) layers. Blood capillary (arrow head) can be noticed (H&E X 400, Scale bar 20 μm).

Group III (ND treated group): (D) CA1 area in the hippocampus of the ND-treated group showing disturbed cellular organization. The pyramidal cells show shrunken pyknotic nuclei (arrows). Cellular vacuolations (double arrows) is also present. Glial cells with dark nuclei and wide pericellular area (curved arrow). Wide and dilated blood capillaries (arrowhead) can be seen (H&E X 400, Scale bar 20 μm).

Group IV (ND+ Taurine treated group): (E) CA1 area in the ND+ Taurine treated group showing apparently normal histological structure. Most of the pyramidal cells appear normal with vesicular nuclei (arrows) however only few cells with pyknotic nuclei can be still noticed (notched arrow). Glial cells (curved arrow) and blood capillaries (arrowhead) are noticed (H&E X 400, Scale bar 20 μm)
Figure 12 Immunohistochemical stained sections with anti-GFAP antibody showing: **Group Ia (Control group)** (A) and **Group II (Taurine group)** (B): CA1 area of the hippocampus shows few positive glial fibrillary acidic protein (GFAP) branched astrocytes (arrows) in different layers. **Group III (ND treated group)** (C): CA1 area of the hippocampus shows many branched astrocytes with strong positive glial fibrillary acidic protein (GFAP) (arrows) in different layers. **Group IV (ND+ Taurine treated group)** (D): CA1 area of the hippocampus shows some branched astrocytes with positive glial fibrillary acidic protein (GFAP) (arrows) in different layers. (Immunoperoxidase technique x 400; scale bar 20 μm)
Histo-morphometric and statistical results (Figures 13-15)

1- Ki-67 level as a proliferative marker in the testis:
There was a statistically significant difference between the three studied groups in terms of Ki-67 level (p<0.001) as it was significantly lower in groups III and IV in comparison to group Ia with mean values of (7.99 ± 1.23, 36.45 ± 1.09, 47.87 ± 1.78 respectively, p2 and p3<0.001 respectively) and was significantly higher in group IV than group III (p6 <0.001). It was comparable between groups Ia, and II (Taurine) (p1>0.05) as shown in Figure 13.

2- Evaluation of the area percentage of collagen fibers after using Masson trichrome stain for heart specimen to detect fibrosis:
After using Masson trichrome stain to detect fibrosis in cardiac muscles, area percentage of collagen fibers deposition in cardiac muscles was significantly different between the three groups (p<0.001) being significantly higher in groups III and IV as compared to group Ia with mean values of (19.54 ± 2.21, 9.24 ± 1.57, 2.45 ± 0.58 respectively, p2 and p3<0.001 respectively) and was significantly lower in group IV than group III (p6 <0.001). It was comparable between groups Ia, and II (Taurine) (p1>0.05) as shown in Figure 14.

3- Immunohistochemical staining using GFAP for the hippocampus:
The comparison between the four studied groups in terms of GFAP in hippocampus revealed a statistically significant difference (p<0.001) as it was significantly higher in groups III and IV in comparison to group Ia with mean values of (9.69 ± 1.62, 3.06 ± 0.21, 1.29 ± 0.25 respectively, p2<0.001, p3<0.05 respectively) and was significantly lower in group IV than group III (p6 <0.001). It was comparable between groups Ia, and II (Taurine) (p1>0.05) as shown in Figure 15.
IV- DISCUSSION

A group of steroid hormones known as anabolic steroids are produced through the synthesis of testosterone. They can improve physical strength and muscle mass (Hijazi, 2012). In practice, anabolic steroids are used to treat many cases as AIDS, metastatic breast tumours, burns, trauma, anaemia, and male hypogonadism (Ergun-Longmire and Wajnrajch, 2020).

Due to the fact that it has less side effects than other anabolic steroids, ND was frequently recommended. Then, International Olympic Committee (IOC) has officially abolished it due to its clear negative consequences on health. ND is also employed in various criminal activities and other aggressive competitive scenarios (Bagchus et al., 2005; Novaes Gomes et al., 2014).

According to Patanè et al. (2020), the systems which are most frequently affected systems by ND use are the endocrine, cardiovascular, cutaneous, and psychiatric systems.

In this study, adult male albino rats with ND-induced toxicity to their brains, hearts, and testicles were examined to determine whether taurine had any ameliorative effects.

Testicular toxicity was supported by biochemical analyses (oxidant markers, inflammatory indices, and apoptotic markers), gene expression, and histolopathological examination.

Going hand in hand with our study, Ahmed (2015) discovered that ND decreased glutathione and superoxide dismutase enzymatic levels in the rat testis while increasing testicular malondialdehyde and nitric oxide contents. Additionally, he disclosed that the injection of ND caused testicular inflammation (elevated testicular TNF-α and MMP-9 gene expression), as well as induction of the intrinsic apoptotic pathway (cytochrome c gene and caspase-3 gene expression). Two crucial proteins in the apoptotic enzyme cascade are caspase-3 and BAX. The main protein that inhibits cell apoptosis is called BCL-2 (Liu et al., 2013).

Other studies also examined the impact of ND on the equilibrium of oxidants and antioxidants. Saddick (2021) reported malondialdehyde concentrations increased. Also, Mohamed and Mohamed (2015) evidenced the occurrence of that oxidative damage through sperm parameters, mainly increasing lipid peroxidation and decreasing antioxidant enzyme activities.

After ND intake, the activity of catalase enzyme as another antioxidant enzyme was decreased according to Frankenfeld et al. (2014). Other studies also have explained the possible mechanism of ND-induced toxicity by the disruption of the antioxidant/oxidant balance (Tsitsimpikou et al., 2016; Vasilaki et al., 2016). In Roșca et al. (2020) study, chronic high dosages of nandrolone lowered plasma total antioxidant activity, which suggests the presence of oxidative stress.

Regarding the inflammatory indices, in concordance with our study, ND either directly increases TNF-α production or increases its gene expression (Hughes et al., 1995). TNF-α may then cause leukocyte infiltration, boost the production of reactive oxygen species, and intensify inflammatory reactions (Wang et al., 2008). ROS, TNF-α, and proteolytic enzymes are released by neutrophils once they have entered the tissues, which damages the tissue and hinders spermatogenesis (Lysiak, 2004).
Elevated levels of TNF-α can damage the blood-testis barrier and causes increase in the MMP-9 expression, an enzyme that can start the enzymatic cleavage of collagen in the extracellular matrix and basement membranes, which causes Sertoli cells to separate from the seminiferous tubules and leads to testicular function abnormalities (Li et al., 2006).

In addition, regarding the apoptotic factors, Ali et al. (2018) found ND therapy resulted in a large decrease in antiapoptotic proteins such pPI3K, p-Akt, and BCL-2 and a considerable increase in the pro-apoptosis protein BAX.

Similar results were found by Shokri et al. (2010) who reported that large doses of ND caused an increase in the death rate of spermatogenic cells, which resulted in a decrease in testicular size. Nandrolone had an impact on the redox and apoptotic systems in Joukar et al. (2017). The brain tissue's BAX/BCL-2 ratio also increased.

Regarding the spermatogenesis, Min and Lee (2018) gave the same results like our study and found that even at low doses of ND /week for two weeks caused a reduction in steroidogenic enzymes, including CYP11A1, at the gene expression level. On the other hand, despite the interstitial Leydig cell population's atrophy, Pomara et al. (2016) and Barone et al. (2017) found no expressional change after treatment with ND.

The biochemical data in our study was emphasized by a testis histopathological examination, which also supported the negative effects of ND on rat testis.

Regarding the histopathological study, Saddick (2021) also found that the treated group's testicular weight was lower than the control group's. According to research by García-Manso and Valverde Esteve (2016), after receiving ND treatment, seminiferous tubules' quantity, diameter, and thickness changed.

Hijazi (2012) came to the conclusion that the use of anabolic steroids clearly has an effect on testicular structures, including the deterioration of germ cells and Leydig cells. Seminiferous tubule deformation and testicular shrinkage accompany these changes. In rats treated with ND, in Tahtamouni et al. (2010) study, atrophy in the seminiferous tubules, an aberrant germinal epithelium, and maturation arrest were reported as some of the anomalies in the architecture of the seminiferous tubules.

Noorafshan et al. (2005) and Naraghi et al. (2010) also revealed that ND administration to rats decrease the Leydig cells size and number, increase vacuolization of the cytoplasm, stimulate deposition, increase number of lysosomes in Sertoli cells, and decrease the seminiferous tubules length.

Biochemical tests (level of CK-MB) in the current study indicated that ND caused cardiac damage. According to Ulla et al. (2017), CK-MB is considered as an indicator of myocardial necrosis. In addition, our histological analysis supported these biochemical findings.

Going hand in hand with our study, following ND utilization by athletes, Almaiman et al. (2019) found an increase in creatine kinase enzyme (CK) and the cardiac marker creatine kinase-MB (CK-MB).

According to Ali et al. (2018), ND significantly increased the level of CK-MB, a diagnostic marker of myocardial injury. He also discovered increased heart connective tissue and left ventricular hypertrophy in the ND-exposed
mice. Additionally, an abrupt infarction of the myocardium occurred in a user of AAS in a case described by Huie (1994) suggested a connection between the two.

According to Sretenovic et al. (2016), abusing nandrolone has detrimental effects on the morphology of the heart. Unaware athletes that use high doses of ND during training cause considerable cardiac fibrosis and collagen deposition. Franquini et al. (2013) and Frati et al. (2015) also reported that nandrolone misuse increased left ventricular thickness and connective tissue content.

In the current study, ND induced toxicity on the hippocampus which was confirmed by gene expression and histopathological examination.

Piacentino et al. (2015), Joksimovic et al. (2019) and Hauger et al. (2020) reported that in those who are susceptible, high doses of ND have been shown to impair various central nervous system functions, including memory, aggression, anxiety, and depression. The hippocampus, striatum, and frontal cortex are the regions most commonly damaged (Turillazzi et al., 2011).

Dynorphin is a substance which is found in the paraventricular nucleus of the hypothalamus and in the nucleus tractus solitarii (NTS), an area responsible for the regulation autonomic functions (Przewlocki and Almeida, 2017).

Going hand in hand with this study, Vasavan et al. (2021) discovered neuronal loss, a shallow dentate gyrus with sparse and scattered hilar cells in the granular layer, cell shrinkage, and nuclear condensation. Additionally, due to severe neurodegeneration, the thickness of the CA1, CA2, and CA3 neuronal cell layers was noticeably reduced in the hippocampus.

The ND toxicity group had considerably increased levels of prodynorphin expression in the hippocampus.

According to Novaes Gomes et al. (2014), large ND doses reduce the positive effects of exercise on the hippocampus cell proliferation and apoptotic pathway. This effect was reported with other AASs as well. For instance, the activation of apoptotic and pre-apoptotic cells was shown to be the cause of Stanozolol (another AAS) histopathologic alterations in the hippocampus (Karimoooy et al., 2019). Also another study found that 17β-trenbolone (another AASs) caused hippocampus cellular death and inhibited hippocampal nerve regeneration, indicating that AASs may lower hippocampal volume, the underlying cause of the spatial memory deficits associated with AASs that have been seen in human and animal studies (Ma and Liu, 2015).

According to some studies, administration of ND increased the prodynorphin in male rats' hippocampus (Magnusson et al., 2009). Other studies provide evidence for a possible connection between dynorphin levels and memory impairment (Svensson et al., 2006).

In this study, taurine was used to ameliorate ND induced toxicity on testis, heart, and hippocampus in adult male albino Rats. Regarding the biochemical study, taurine administration improved all parameters of ND toxicity. Also, the histopathological study revealed that taurine administration improved signs of testicular, hippocampal toxicity, in addition to partial improvement in the heart.

Going hand in hand with this study, Ahmed (2015) revealed that taurine's antioxidant, anti-
inflammatory, and antiapoptotic properties completely reversed the harmful effects of ND on rat sperm and testicles. He demonstrated that supplementing with taurine prevented the effects of ND on testicular TNF-α, MMP-9, and ICAM-1. According to Aly and Khafagy (2014), Rezaee-Tazangi et al. (2020) and Oyovwi et al. (2021), taurine can counteract the oxidative stress and apoptosis that many medications produce in the testes.

According to Jakaria et al. (2019), taurine has been shown to have ameliorating benefits against a variety of neurological conditions such as neurodegenerative illnesses, stroke, epilepsy, and diabetic neuropathy. It also has a protective impact against injuries and toxicities to the nervous system.

According to studies by Qaradakhi et al. (2020) and Samadi et al. (2021), taurine also has anti-inflammatory and protective effect on the heart. Previous research on the anti-inflammatory properties of taurine on several organs These findings are also supported by Wei et al. (2008) and Das et al. (2009) studies on the anti-inflammatory effects of taurine on different organs.

The anti-apoptotic effect of taurine was attributed to its antioxidant properties. Taurine has inhibitory effect on cytochrome c release and prevents caspase cascade activation by increasing antiapoptotic protein BCL2 expression and decreasing the pro-apoptotic proteins expression. Das et al. (2009) and Aly and Khafagy (2014) stated that apoptosis caused by TNFα is also inhibited by taurine.

Previous studies demonstrated that taurine deprivation may increase DNA damage and its supplementation may considerably lessen DNA damage through its antioxidant and anti-apoptotic actions (Golubnitschaja et al., 2003; Tsounapi et al., 2012). Taurine may also prevent DNA deterioration by inhibiting TNF and preventing apoptosis subsequently (Das et al., 2009).

V- CONCLUSION

Taurine reduced the toxic effects of ND primarily on both the testis and hippocampus, in addition to a partial improvement in the heart. This Amelioration of the structural, biochemical, and molecular toxic effects of ND was via several mechanisms including; antioxidant, anti-inflammatory and antiapoptotic pathways.

VI- RECOMMENDATIONS

In the light of the results of this study, we recommend:
- Improving health education programs to raise public awareness about the danger of the misuse of ND in gym practice and its harmful effects, particularly on athletes who use it without medical consultation.
- Other studies to determine the adverse effects of ND on various organs of the body and the protective effects of taurine against these toxic effects.

VII-STATEMENTS AND DECLARATIONS

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Competing Interests
The authors have no relevant financial or non-financial interests to disclose.
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Author Contributions
Megahed E.E. and Abdelaal G.M.M.: Creating the research idea, performing the practical part of the experiment, writing the manuscript, and presenting the data. Abdel Moawed D.M.N.: Writing the discussion part and sharing in results data presentation of the biochemical analysis. Abdel Aal S.M. and Mohamed S.R.: Writing the histologic methods, creating the plates of histology photomicrographs with comments, performing the morphometry and statistics of histology results. Talaat A.: Writing the biochemical methods and performing the biochemical analysis. The authors declare that all data were generated in-house and that no paper mill was used.

Consent to publish
All authors revised and approved the final manuscript for publication.

Availability of data and materials
The data that support the findings of this study are available from the corresponding author upon reasonable request.

VIII- REFERENCES


Abdelrahman SA, Raafat N, Abdelaal GM, Aal SMA (2023) Electric field-directed migration of mesenchymal stem cells enhances their therapeutic potential on cisplatin-induced acute nephrotoxicity in rats. Naunyn-Schmiedeberg’s Archives of Pharmacology:1-17 doi:https://doi.org/10.1007/s00210-022-02380-7


Aly HA, Khafagy RM (2014) Taurine reverses endosulfan-induced oxidative stress and apoptosis in adult rat testis. Food and Chemical Toxicology 64:1-9 doi:https://doi.org/10.1016/j.fct.2013.11.007


Ma F, Liu D (2015) 17β-trenbolone, an anabolic–androgenic steroid as well as an environmental hormone, contributes to neurodegeneration.
cell proliferation and apoptotic signaling is impaired by anabolic androgenic steroids. Psychoneuroendocrinology 50:106-117 doi:https://doi.org/10.1016/j.psyneuen.2014.08.009


doi:https://doi.org/10.1507/endocrj.51.425


تأثير تحسيني للتورين على السمية المستحدثة بديكانوات الناندرولون على المخ والقلب والخصية في ذكور الجرذان البيضاء البالغة

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مقدمة:

يدانوات الناندرولون هو السترويد الأكثر انتشاراً في مجال التدريب الرياضي، ويعتبر متورطً في التنشيط الوراثي والدوائر الإجرامية حيث تكون العدوى الشخصية واضحة. على الرغم من استخدامه الواسع بين الرياضيين، فإن استعداده بدون رقابة يستوجب تطبيقه الكيميائي كالة، والدوراء الإجرامية ثياسة واضحة. عقبة التورين، وهو أحد الأحماض الأمينية المستخدمة ككمال غذائي للاعب كمال الأجسام إلا عدد من الخصائص لحماية الخلايا.

الهدف:

تهدف هذه الدراسة إلى تقييم دور التورين في تخفيف التأثيرات السامة لـديكانوات الناندرولون على معايير الكيمياء الحيوية، والتغييرات المرضية التناسجية والمناعية لحصتين المخ، والقلب، والخصية في ذكور الجرذان البالغين.

المتوصيات:

- تحسين برنامج التدريبي الصحي لزيادة الوعي العام حول خطورة تناول سترويد ديكاموات الناندرولون في صلاحيات الرياضة وأثارها الضارة وخاصة في الرياضيين الذين يستخدمونه دون استمرارية طبية.
- عمل دراسات أخرى لمعرفة التأثيرات السامة لـديكانوات الناندرولون على مختلف أجهزة الجسم والآثار الوقائية لـ التورين ضد الآثار السامة الناجمة عن ديكاموات الناندرولون.

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