Original article

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

Elham E. Megahed[1], Dena M. N. Abdel Moawed[1,4], Sara M. Abdel Aal[2], Samar R. Mohamed[2], Aliaa Talaat[3], Ghadeer M. M. Abdelaal*[1]

1. *Forensic Medicine and Clinical Toxicology Department, Faculty of Medicine, Zagazig University, Zagazig, Egypt*
2. *Medical Histology and Cell Biology Department, Faculty of Medicine, Zagazig University, Zagazig, Egypt*
3. *Medical Biochemistry and Molecular Biology Department, Faculty of Medicine, Zagazig University, Zagazig, Egypt*
4. *Forensic Medicine and Clinical Toxicology Department, Faculty of Medicine, Badr University in Cairo, Egypt*

Corresponding author:

Ghadeer Mohamed Mahmoud Abdelaal

***E-mail*:**

ghadeer.mma@gmail.com

ORCID:

0000-0001-8598-1028

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 therapeutic 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.

Traurine (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% formol 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/uL 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)

Target Gene	Primer Sequence
BAX (Ujah et al., 2021)	F: 5'-CGCGTGGTTGCCCTCTTCTACTTT-3' R: 5'-CAAGCAGCCGCTCACGGAGGA-3'
BCL$_2$ (Ujah et al., 2021**)**	F: 5'-ATCGCTCTGTGGATGACTGAGTAC-3' R: 5'-AGAGACAGCCAGGAGAAATCAAAC-3'
MMP9 (Ahmed, 2015)	F: 5'-AGGGGCAGCAAAGCTGTAGCCTAG-3' R: 5'-TTTCAGGTCTCGGGGGAAGACCACATA-3'
Caspase 3 (He et al., 2018)	F: 5'-GTGGAACTGACGATGATATGGC-3' R: 5'-CGCAAAGTGACTGGATGAACC-3'
Cyp11a1 (Ujah et al., 2021)	F: 5'-CTTTGGTGCAGGTGGCTAG-3' R: 5'-CGGAAGTGCGTGGTGTTT-3'
Prodynorphin (Vasavan et al., 2021)	F: 5'-ATGGCGTGGTCCAGGCTGATGC-3' R: 5'-AGTTTGTAGATTTAGAAGCCTTATCC-3'
GAPDH	F: 5'-TCACCACCATGGAGAAGGC-3' R: 5'-GCTAAGCAGTTGGTGGTGCA-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. Afterthat, 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 ngative 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)

	GIa (Control) (n=8)	**GII (Taurine) (n=8)**	**GIII (ND) (n=8)**	**GIV (ND + Taurine) (n=8)**	**p value**
SOD (U/g Tissue)	57.22 ± 5.2	54.13±6.4	31 ± 3.1	47.3 ± 4.5	**=0.001; <0.01****
	p1= 0.466; >0.05 p2<0.001* p3=0.04; <0.05***	**p4<0.001*** p5= 0.129; >0.05**	**p6= 0.004; <0.01****		
MDA (nmol/g Tissue)	83.5 ± 10.2	74.4±8.3	150 ± 6.7	105 ± 7.1	**<0.001*****
	p1= 0.211; >0.05 p2<0.001* p3=0.012; <0.05***	**p4<0.001*** p5= 0.002; <0.01****	**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 SOD: Superoxide Dismutase, MDA: Malondialdehyde

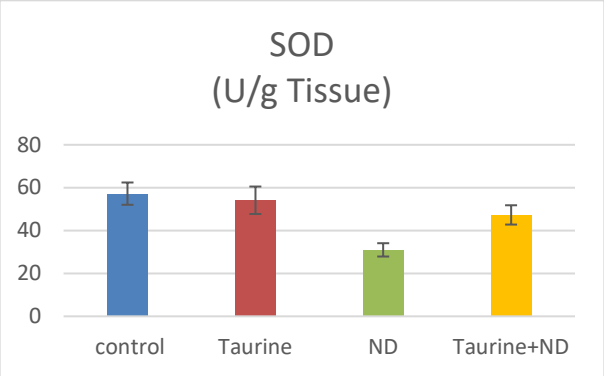

Figure 1: SOD expression in the studied groups

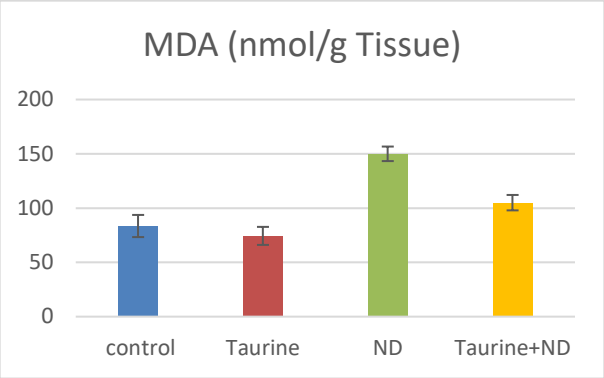

Figure 2 MDA expression in the studied groups

2-Estimation of testicular TNF α levels (Table 3, Figure 3):

The comparison between the four studied groups in terms of testicular TNF α level revealed a statistically significant difference (p <0.001)

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).

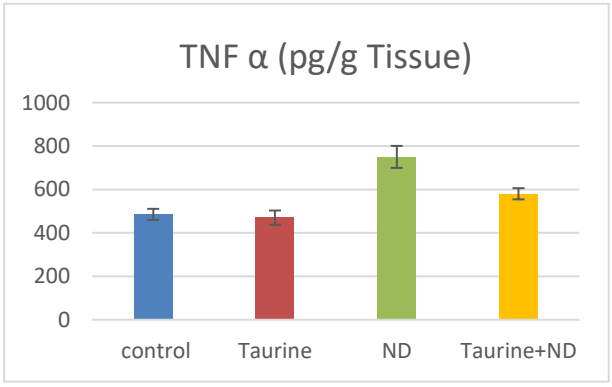

Figure 3 TNF α expression in the studied groups

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)

	GIa (Control) (n=8)	**GII (Taurine) (n=8)**	**GIII (ND) (n=8)**	**GIV (ND + Taurine) (n=8)**	**p value**
TNF α (pg/ g tissue)	485.5±25.4	470.4±32.9	750.3±50.6	580.3± 25.8	**<0.001*** **
	p1= 0.613; >0.05 p2<0.001* p3=0.011; <0.05***	**p4<0.001*** p5= 0.005; <0.01****	**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, TNF: Tumor necrosis facto

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.

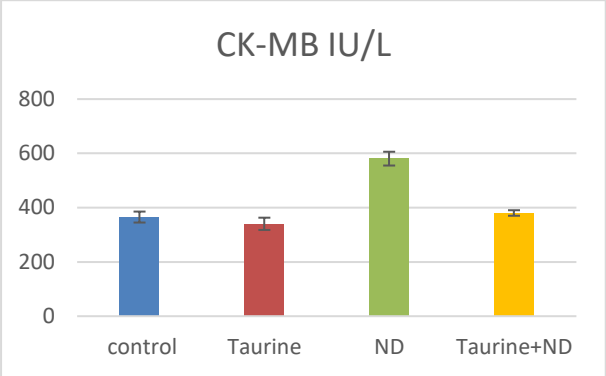

Figure 4 CK-MB levels in the studied groups

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)

	GIa (Control) (n=8)	GII (Taurine) (n=8)	GIII (ND) (n=8)	GIV (ND + Taurine) (n=8)	p value
CK-MB IU/L	365.3±20.2	340.4±22.6	580.6± 25.4	380.34±10.2	**<0.001*** **
	p1= 0.174; >0.05 p2<0.001* p3=0.393; >0.05**	**p4<0.001*** p5= 0.043; <0.05***	**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)

	GIa (Control) (n=8)	**GII (Taurine) (n=8)**	**GIII (ND) (n=8)**	**GIV (ND + Taurine) (n=8)**	**p value**
BAX	1±0.13	1.1±0.11	2.1±0.25	1.5±0.27	**=0.001; <0.01****
	p1= 0.562; >0.05 p2<0.001* p3=0.017; <0.05***	**p4<0.001*** p5= 0.042; <0.05***	**p6=0.007; <0.01****		
BCL2	0.9±0.19	1.02±0.15	0.5±0.16	0.8 ±0.2	**=0.034; <0.05***
	p1= 0.428; >0.05 p2=0.024; <0.05* p3=0.507; >0.05	**p4=0.007; <0.01** p5= 0.165; >0.05**	**p6=0.071; >0.05**		
MMP-9	1.1± 0.2	0.9±0.2	2.2 ± 0.19	1. 5± 0.14	**<0.001*****
	p1= 0.22; >0.05 p2<0.001* p3=0.029; <0.05***	**p4 <0.001*** p5= 0.004; <0.01****	**p6=0.002; <0.01****		
Caspase-3	0.89±0.21	1±0.13	2.8 ± 0.2	1.6 ± 0.19	**<0.001*****
	p1= 0.488; >0.05 p2<0.001* p3=0.002; <0.01****	**p4 <0.001*** p5= 0.004; <0.01****	**p6<0.001****		
Cyp11a1	0.9±0.11	1.01±0.11	0.4±0.12	0.7±0.14	**=0.001; <0.01****
	p1= 0.296; >0.05 p2=0.001; <0.01 p3=0.077; >0.05**	**p4 <0.001*** p5= 0.014; <0.05***	**p6=0.016; <0.05***		

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

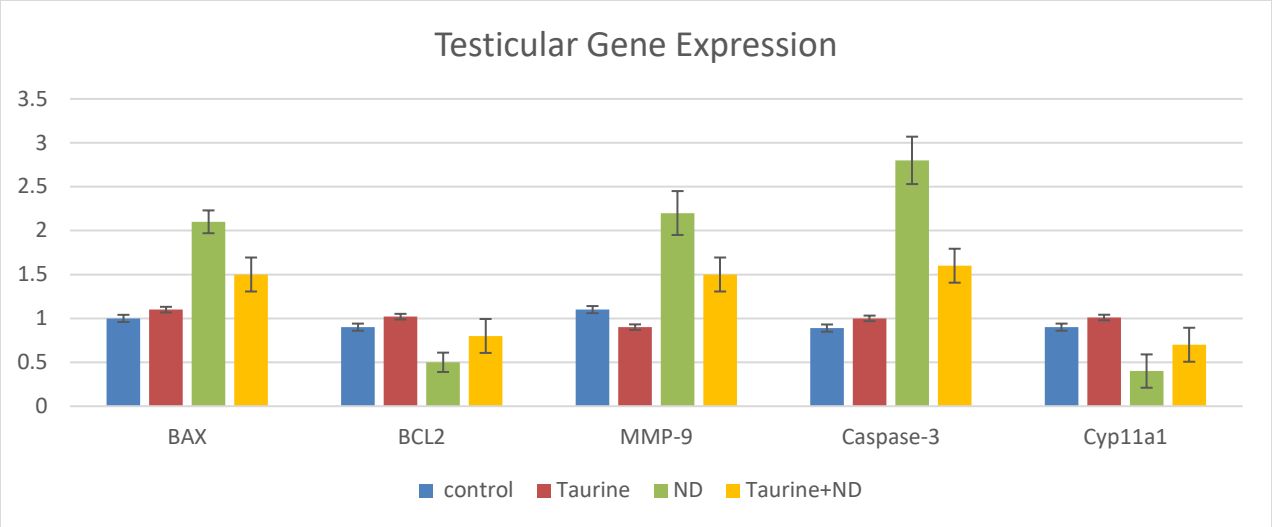

Figure 5 Testicular gene expression of BAX, BCL2, MMP-9, Caspase-3 and Cyp11a1 in fold change of the studied groups

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).

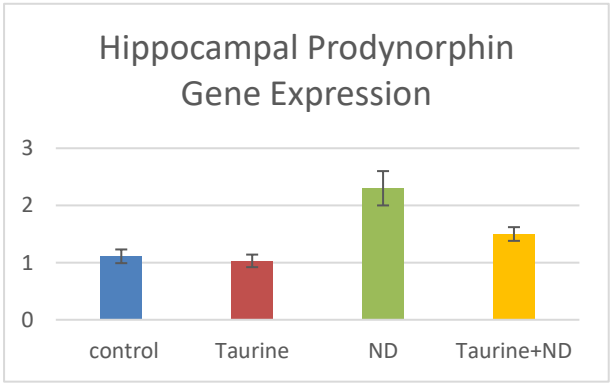

Figure 6 Hippocampal Prodynorphin gene expression in fold change of the studied groups

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)

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

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 cadiomyocyte 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).

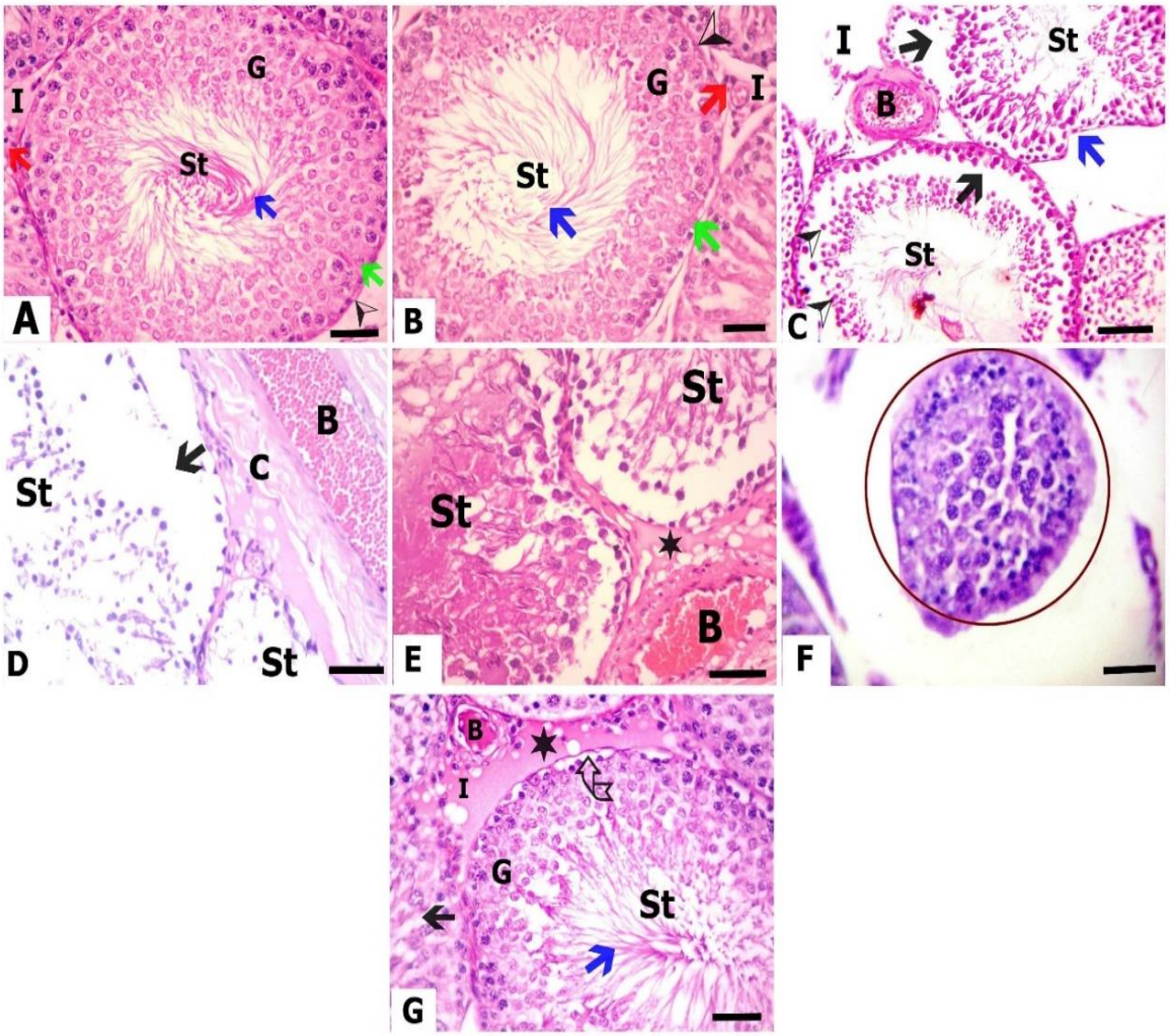

Figure 7 Hematoxylin and Eosin-stained sections of testes from rats of **Group Ia (Control group) *(*A) and Group II: (Taurine treated group) (B)**: showing the seminiferous tubules (St) are lined by stratified germinal epithelium (G), and Sertoli cells with large, pale nuclei (green arrow), having a 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)**

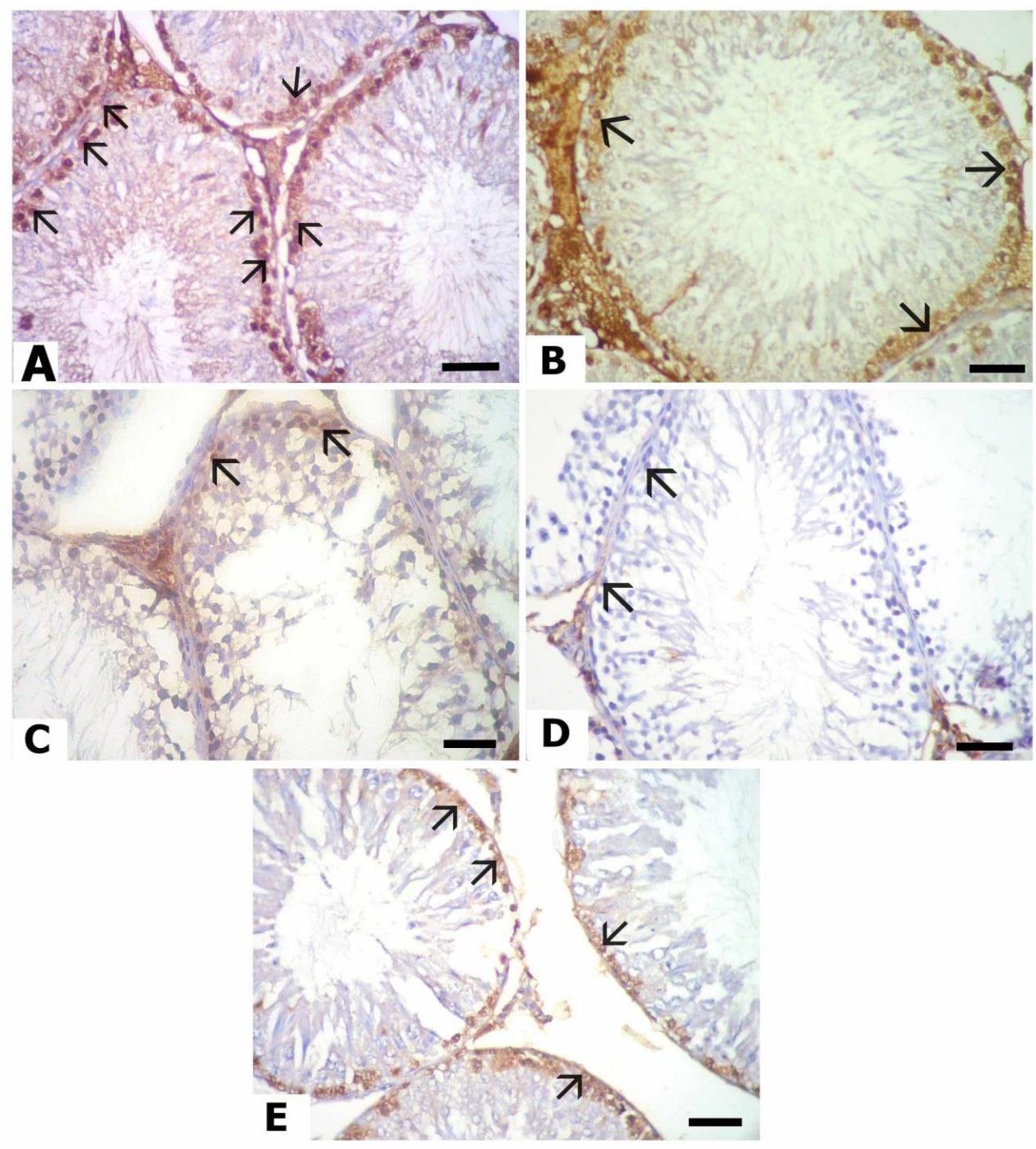

Figure 8 Group Ia: (Control group) (A) and Group II: (Taurine treated group) (B): Immunohistochemical stained sections with anti-Ki-67 antibody of the control and taurine treated group testis showing strong positive immunoreaction in the nuclei of spermatogonia and primary spermatocytes (arrows).
Group 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)**

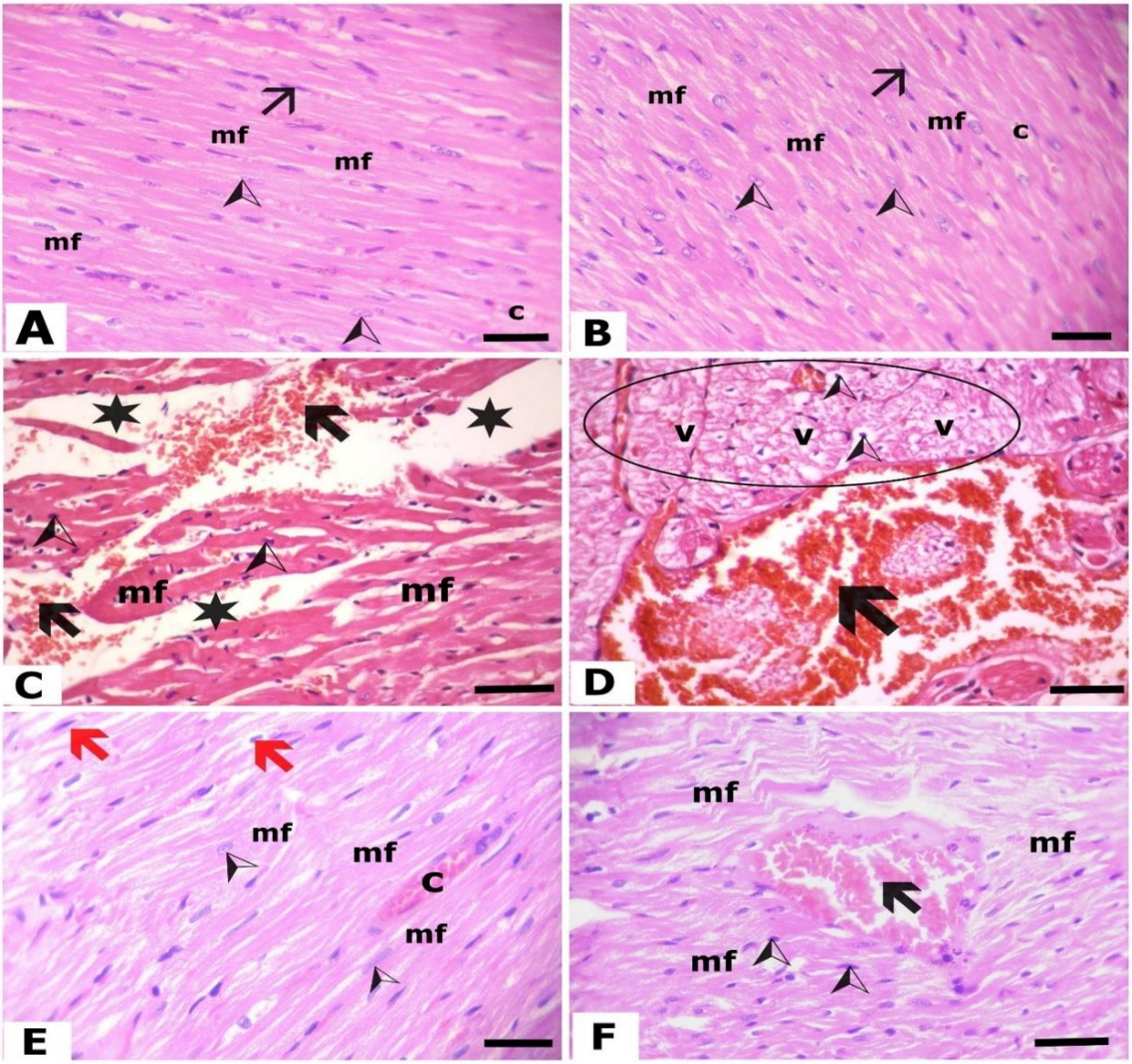

Figure 9 Hematoxylin and Eosin-stained sections of hearts from rats of **Group Ia (Control group) *(*A) and Group II: (Taurine treated group) (B)**: illustrate the normal general architecture of cardiac tissue which is formed of normal elongated, branching and anastomosing muscle fibers (mf) with acidophilic 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 cadiomyocyte 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)**

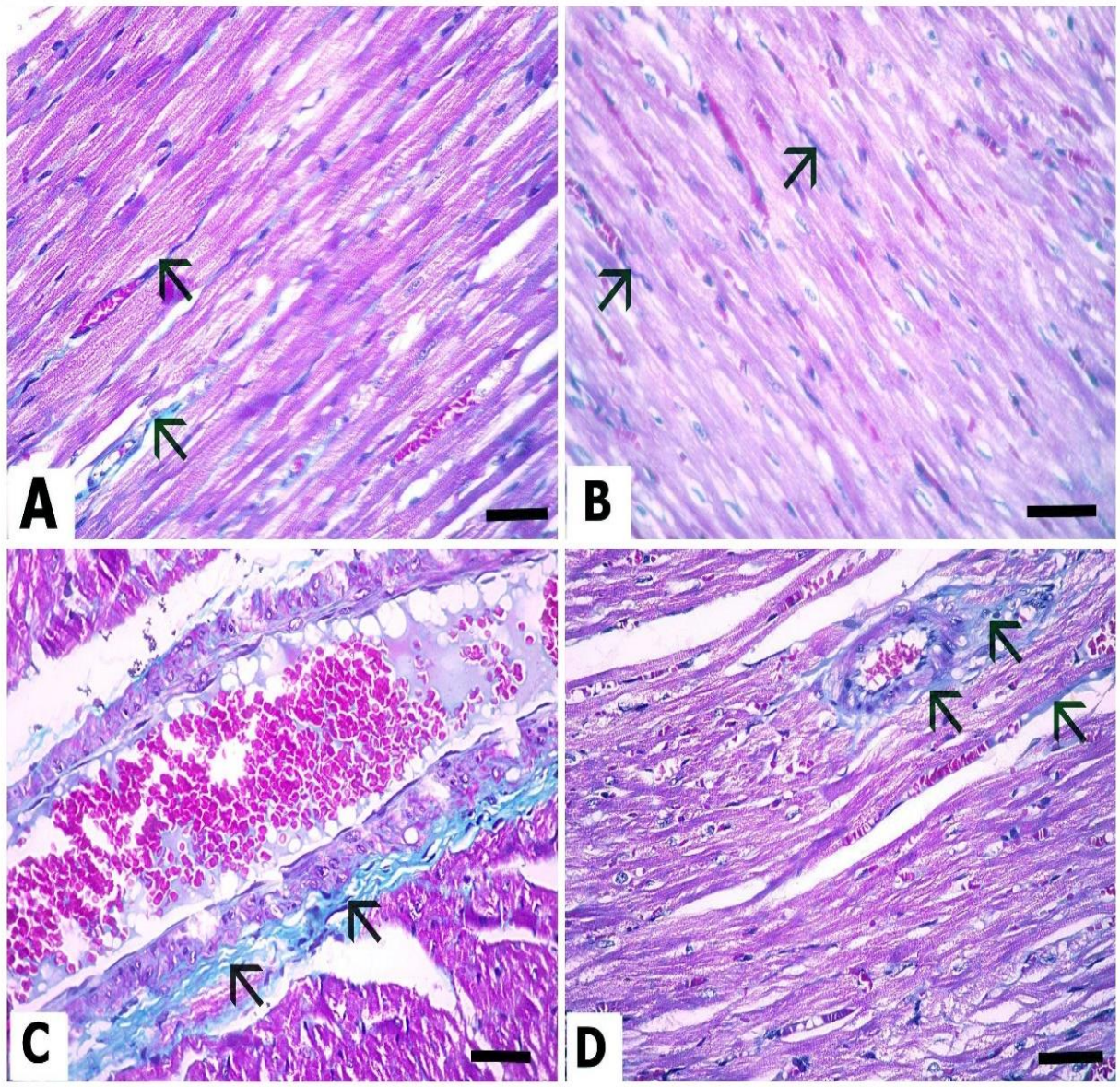

Figure 10 Masson trichrome stained sections of the hearts of **Group Ia: (control group) (A) and Group II: (Taurine treated group) (B):** reveals few fine collagen fibers in the connective tissue spaces between cardiac muscle fibers (arrows).
Group III (ND treated group) (C): shows massive extensive 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)**

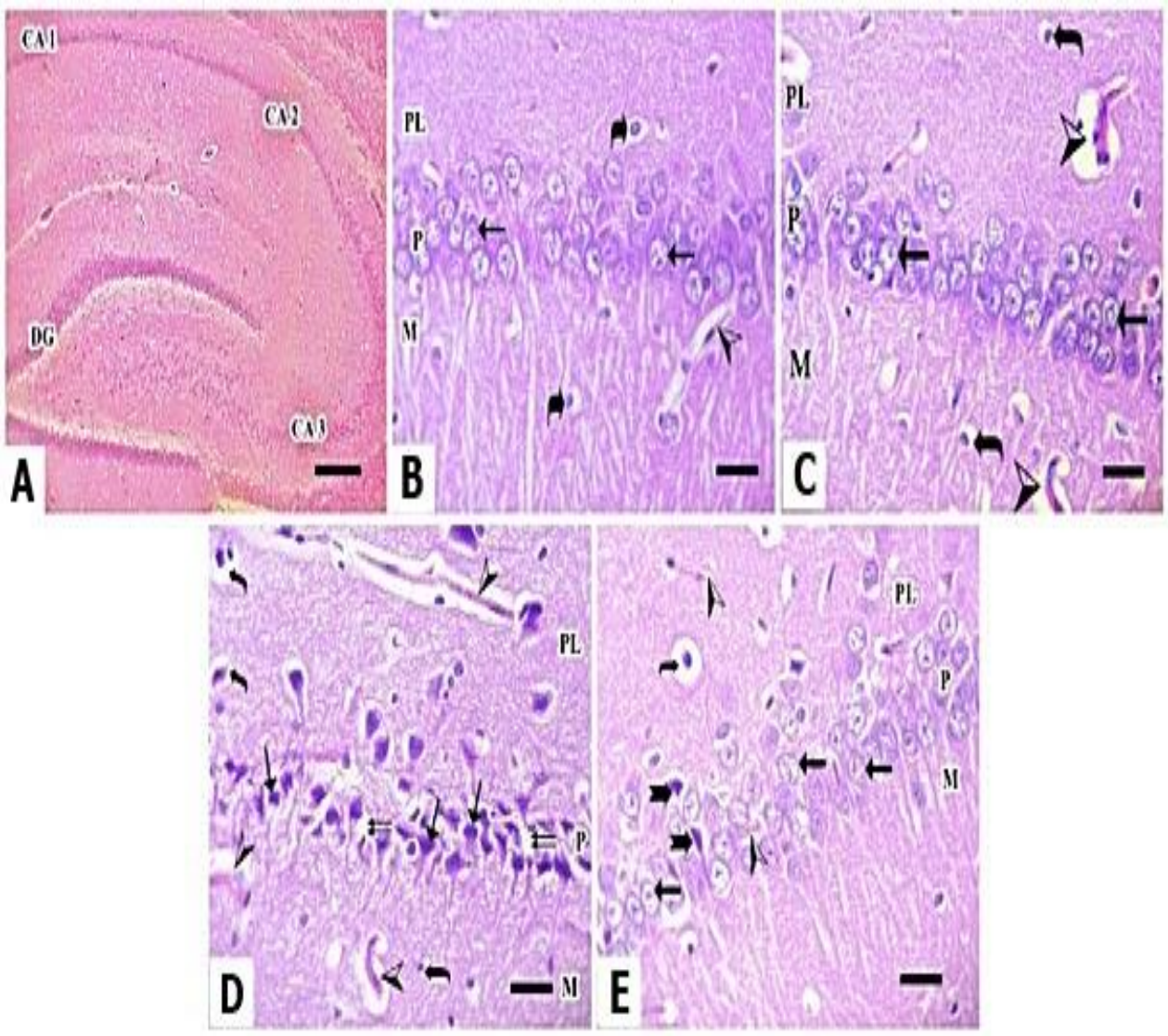

Figure 11 Hematoxylin and Eosin-stained sections of the hippocampi from rats of **Group Ia (Control group)** (**A**): showing the hippocampus is C-shaped and consists of three Cornu Ammonis (CA1, CA2 and CA3) and dentate gyrus (DG) (**H&E X200, scale bar 50 µm). At higher magnification, the CA1 area 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)**

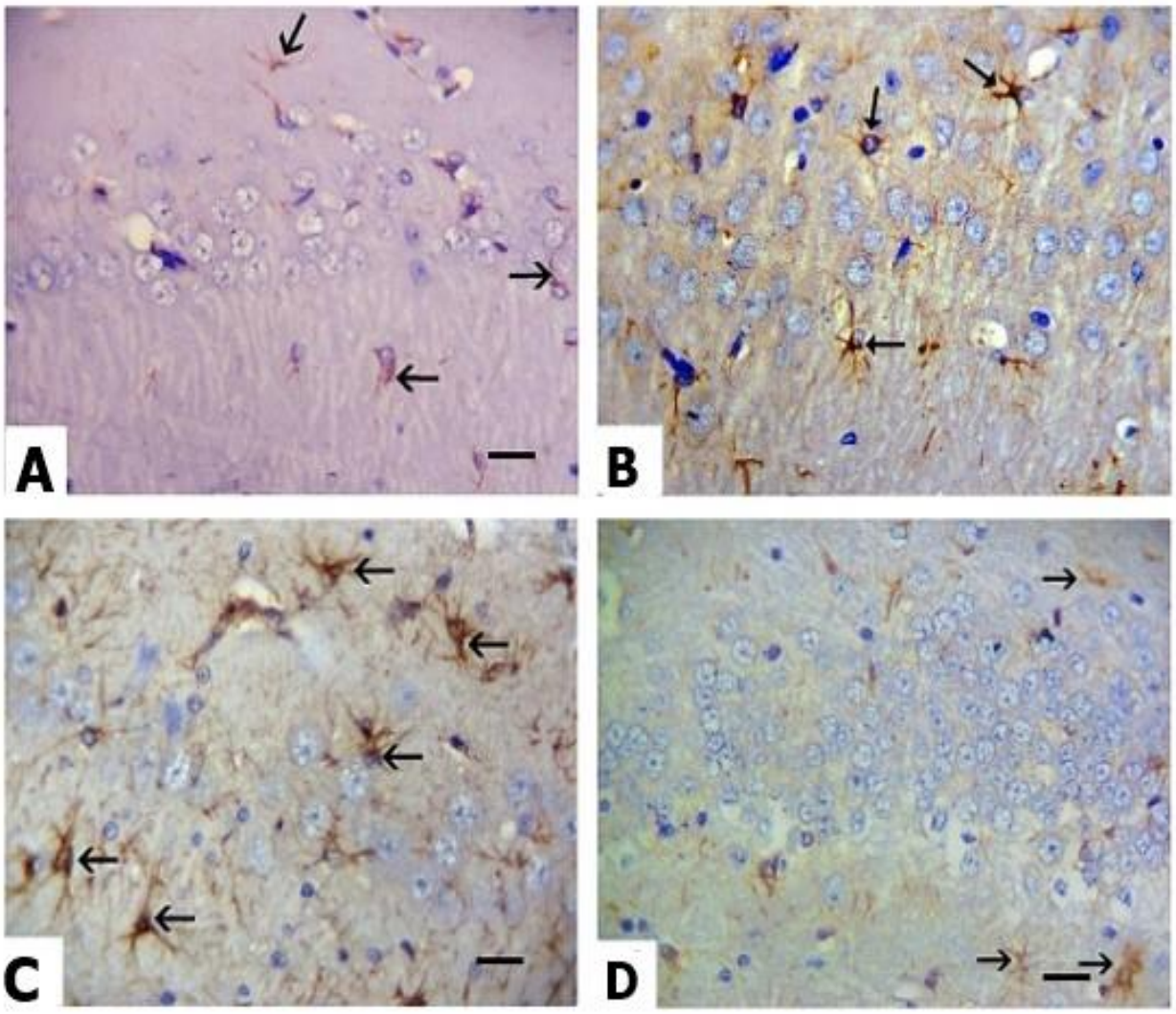

Figure 12 Immunohistochemical stained sections with anti-GFAP antibody showing: **Group Ia :(Control group) (A)** and **Group II (Taurine group) (B):** CA1 area of the hippocampus shows few positive glial fibrillary acidic protein (GFAP) branched astrocytes (arrows) in different layers.
Group III (ND 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 Figure13.

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 thee 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.

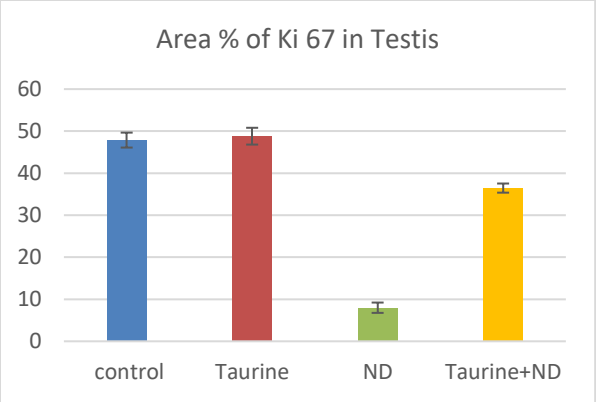

Figure 13 Area percentage of Ki-67 in testicular tissues among the studied groups

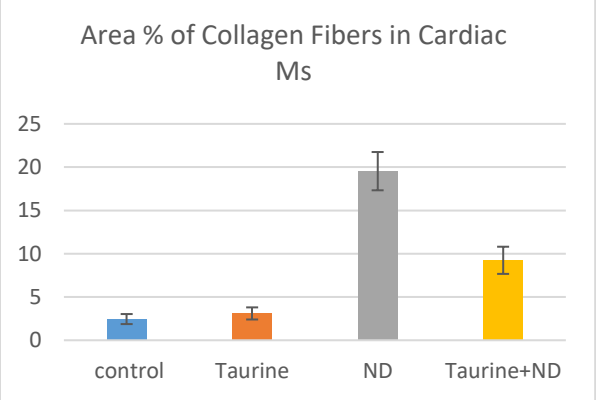

Figure 14 Area percentage of collagen fibers using Masson trichrome stain for heart specimen

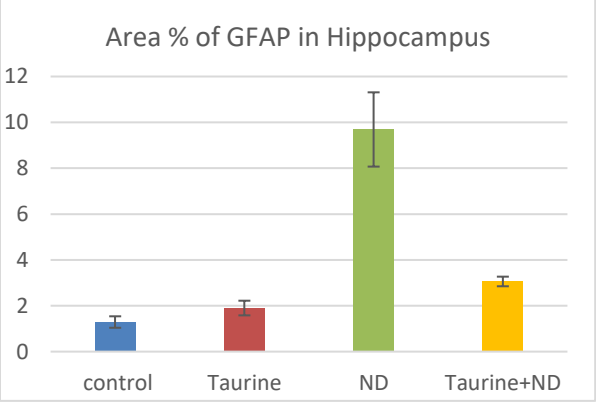

Figure 15 Area percentage of GFAP in hippocampus among the studied groups

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 occurance of that oxidative damage through sperm parameters, maily 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 Roşca 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 occured 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. Franquni 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 (Karimooy 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 properities 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 stuctural, biochemical, and molecular toxic effects of ND was via several mechanisms including; antioxidant, antiinflammatory 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

Funding

The authors declare that no funds, grants, or other support were received during the preparation of this manuscript.

Competing Interests

The authors have no relevant financial or non-financial interests to disclose.

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

Abdelaal G, Sabik LM, Etewa RL, Soliman WI (2018) Protective Effects of Tomato Lycopene and Zingiber officinale Extracts on Gentamicin-induced Nephrotoxicity in Adult Albino Rats (Comparative study). Zagazig Journal of Forensic Medicine 16(1):29-49 doi:https://doi.org/10.21608/zjfm.2018.2441.1006

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

Agriesti F, Tataranni T, Pacelli C, Scrima R, Laurenzana I, Ruggieri V, Cela O, Mazzoccoli C, Salerno M, Sessa F (2020) Nandrolone induces a stem cell-like phenotype in human hepatocarcinoma-derived cell line inhibiting mitochondrial respiratory activity. Scientific Reports 10(1):2287 doi:https://doi.org/10.1038/s41598-020-58871-1

Ahmed MA (2015) Amelioration of nandrolone decanoate-induced testicular and sperm toxicity in rats by taurine: effects on steroidogenesis, redox and inflammatory cascades, and intrinsic apoptotic pathway. Toxicology and applied pharmacology 282(3):285-296 doi:https://doi.org/10.1016/j.taap.2014.12.007

Ali DM, Labib DAA, Mohamed MMN (2018) The protective role of coenzyme q10 against nandrolone decanoate-induced cardiotoxicity in rats: Biochemical, genetic, histological and immunohistochemical study. The Egyptian Journal of Forensic Sciences and Applied Toxicology 18(1):19-34 doi:https://dx.doi.org/10.21608/ejfsat.2018.28292

Almaiman AA, Almaiman SH, Elagamy EI, Al Wutayd O, Almarzuqi M, Alzunaidi R, Alhatlani S, Eid EE (2019) Side effects of anabolic steroids used by athletes at Unaizah Gyms, Saudi Arabia: a pilot study. The Journal of Sports Medicine and Physical Fitness 59(3):489-495 doi:https://doi.org/10.23736/S0022-4707.18.08366-4

Althobiti SD, Alqurashi NM, Alotaibi AS, Alharthi TF, Alswat KA (2018) Prevalence, attitude, knowledge, and practice of anabolic androgenic steroid (AAS) use among gym participants. Materia socio-medica 30(1):49 doi:https://doi.org/10.5455/msm.2018.30.49-52

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

Bablok W, Passing H, Bender R, Schneider B (1988) A General Regression Procedure for Method Transformation. Application of Linear Regression Procedures for Method Comparison Studies in Clinical Chemistry, Part III. Clinical Chemistry and Laboratory Medicine (CCLM) 26(11):783-790 doi:doi:10.1515/cclm.1988.26.11.783

Bagchus WM, Smeets JM, Verheul HA, De Jager-Van Der Veen SM, Port A, Geurts TP (2005) Pharmacokinetic evaluation of three different intramuscular doses of nandrolone decanoate: analysis of serum and urine samples in healthy men. The Journal of Clinical Endocrinology & Metabolism 90(5):2624-2630 doi:https://doi.org/10.1210/jc.2004-1526

Barone R, Pitruzzella A, Marino Gammazza A, Rappa F, Salerno M, Barone F, Sangiorgi C, D'Amico D, Locorotondo N, Di Gaudio F, Cipolloni L, Di Felice V,

Schiavone S, Rapisarda V, Sani G, Tambo A, Cappello F, Turillazzi E, Pomara C (2017) Nandrolone decanoate interferes with testosterone biosynthesis altering blood–testis barrier components. Journal of Cellular and Molecular Medicine 21(8):1636-1647 doi:https://doi.org/10.1111/jcmm.13092

Beyranvand MR, Khalafi MK, Roshan VD, Choobineh S, Parsa SA, Piranfar MA (2011) Effect of taurine supplementation on exercise capacity of patients with heart failure. Journal of cardiology 57(3):333-337 doi:https://doi.org/10.1016/j.jjcc.2011.01.007

Bordbar H, Mesbah F, Talaei T, Dehghani F, Mirkhani H (2014) Modulatory effect of gonadotropins on rats' ovaries after nandrolone decanoate administration: a stereological study. Iranian journal of medical sciences 39(1):44.

Das J, Ghosh J, Manna P, Sinha M, Sil PC (2009) Taurine protects rat testes against NaAsO2-induced oxidative stress and apoptosis via mitochondrial dependent and independent pathways. Toxicology Letters 187(3):201-210 doi:https://doi.org/10.1016/j.toxlet.2009.03.001

El Idrissi A (2008) Taurine increases mitochondrial buffering of calcium: role in neuroprotection. Amino acids 34:321-328 doi:https://doi.org/10.1007/s00726-006-0396-9

Ergun-Longmire B, Wajnrajch MP (2020) Growth and growth disorders. Endotext [Internet]

Frankenfeld SP, Oliveira LP, Ortenzi VH, Rego-Monteiro IC, Chaves EA, Ferreira AC, Leitão AC, Carvalho DP, Fortunato RS (2014) The anabolic androgenic steroid nandrolone decanoate disrupts redox homeostasis in liver, heart and kidney of male Wistar rats. PloS one 9(9):e102699 doi:https://doi.org/10.1371/journal.pone.0102699

Franquni JVM, do Nascimento AM, de Lima EM, Brasil GA, Heringer OA, Cassaro KOdS, da Cunha TVP, Musso C, Silva Santos MCLF, Kalil IC, Endringer DC, Boëchat GAP, Bissoli NS, de Andrade TU (2013) Nandrolone decanoate determines cardiac remodelling and injury by an imbalance in cardiac inflammatory cytokines and ACE activity, blunting of the Bezold–Jarisch reflex, resulting in the development of hypertension. Steroids 78(3):379-385 doi:https://doi.org/10.1016/j.steroids.2012.12.009

Frati P, P Busardo F, Cipolloni L, De Dominicis E, Fineschi V (2015) Anabolic androgenic steroid (AAS) related deaths: autoptic, histopathological and toxicological findings. Current neuropharmacology 13(1):146-159

García-Manso JM, Valverde Esteve T (2016) Consequences of the Use of Anabolic-Androgenic Steroids on Female Athletes' Fertility. In: Vaamonde D, du Plessis SS, Agarwal A (eds) Exercise and Human Reproduction: Induced Fertility Disorders and Possible Therapies. Springer New York, New York, NY, p 229-238

Hasso RA (2009) Histological toxic effect of nandrolone decanoate on the kidney of male rabbits: Part one. The Medical Journal of Basrah University 27(1)

Hauger LE, Westlye LT, Bjørnebekk A (2020) Anabolic androgenic steroid dependence is associated with executive dysfunction. Drug and Alcohol Dependence 208:107874 doi:https://doi.org/10.1016/j.drugalcdep.2020.107874

He X, Sun J, Huang X (2018) Expression of caspase-3, Bax and Bcl-2 in hippocampus of rats with diabetes and subarachnoid hemorrhage. Exp Ther Med 15(1):873-877 doi:10.3892/etm.2017.5438

Hijazi MM (2012) Androgenic anabolic steroidal-based effects on the morphology of testicular structures of albino rats. Pakistan journal of zoology 44(6)

Hughes TK, Fulep E, Juelich T, Smith EM, Stanton GJ (1995) Modulation of immune responses by anabolic androgenic steroids. International Journal of Immunopharmacology 17(11):857-863 doi:https://doi.org/10.1016/0192-0561(95)00078-X

Huie MJ (1994) An acute myocardial infarction occurring in an anabolic steroid user. Medicine and science in sports and exercise 26(4):408-413 doi:https://doi.org/10.1249/00005768-199404000-00002

Jakaria M, Azam S, Haque ME, Jo S-H, Uddin MS, Kim I-S, Choi D-K (2019) Taurine and its analogs in neurological disorders: Focus on therapeutic potential and molecular mechanisms. Redox Biology 24:101223 doi:https://doi.org/10.1016/j.redox.2019.101223

Joksimovic J, Selakovic D, Jovicic N, Mitrovic S, Mihailovic V, Katanic J, Milovanovic D, Rosic G (2019) Exercise Attenuates Anabolic Steroids-Induced Anxiety via Hippocampal NPY and MC4 Receptor in Rats. Frontiers in Neuroscience 13 doi:https://doi.org/10.3389/fnins.2019.00172

Joukar S, Vahidi R, Farsinejad A, Asadi-shekaari M, Shahouzehi B (2017) Ameliorative Effects of Endurance Exercise with Two Different Intensities on Nandrolone Decanoate-Induced Neurodegeneration in Rats: Involving Redox and Apoptotic Systems. Neurotoxicity Research 32(1):41-49 doi:https://doi.org/10.1007/s12640-017-9705-1

Jurox (2014) Reepair injection Material Safety Data Sheet. Section 11: Toxicological information. Jurox Pty Limited, Rutherford, New South Wales, Australia (July 2014):4

Kalender S, Apaydin FG, Kalender Y (2019) Testicular toxicity of orally administrated bisphenol A in rats and protective role of taurine and curcumin. Pakistan journal of pharmaceutical sciences 32(3):1043-1047

Karimooy FN, Bideskan AE, Pour AM, Hoseini SM (2019) Neurotoxic Effects of Stanozolol on Male Rats' Hippocampi: Does Stanozolol cause apoptosis? Biomolecular Concepts 10(1):73-81 doi:https://doi.org/10.1515/bmc-2019-0009

Kong WX, Chen SW, Li YL, Zhang YJ, Wang R, Min L, Mi X (2006) Effects of taurine on rat behaviors in three anxiety models. Pharmacology Biochemistry and Behavior 83(2):271-276 doi:https://doi.org/10.1016/j.pbb.2006.02.007

Li MWM, Xia W, Mruk DD, Wang CQF, Yan HHN, Siu MKY, Lui W-y, Lee WM, Cheng CY (2006) Tumor necrosis factor α reversibly disrupts the blood–testis barrier and impairs Sertoli–germ cell adhesion in the seminiferous epithelium of adult rat testes. Journal of Endocrinology 190(2):313-329 doi:https://doi.org/10.1677/joe.1.06781

Li Y, Arnold JMO, Pampillo M, Babwah AV, Peng T (2009) Taurine prevents cardiomyocyte death by inhibiting NADPH oxidase-mediated calpain activation. Free radical biology and medicine 46(1):51-61 doi:https://doi.org/10.1016/j.freeradbiomed.2008.09.025

Liu G, Wang T, Wang T, Song J, Zhou Z (2013) Effects of apoptosis-related proteins caspase-3, Bax and Bcl-2 on cerebral ischemia rats. Biomed Rep 1(6):861-867 doi:https://doi.org/10.3892/br.2013.153

Livak KJ, Schmittgen TD (2001) Analysis of relative gene expression data using real-time quantitative PCR and the 2− ΔΔCT method. methods 25(4):402-408 doi:https://doi.org/10.1006/meth.2001.1262

Lysiak JJ (2004) The role of tumor necrosis factor-alpha and interleukin-1 in the mammalian testis and their involvement in testicular torsion and autoimmune orchitis. Reproductive Biology and Endocrinology 2(1):9 doi:https://doi.org/10.1186/1477-7827-2-9

Ma F, Liu D (2015) 17β-trenbolone, an anabolic–androgenic steroid as well as an environmental hormone, contributes to neurodegeneration. Toxicology and Applied Pharmacology 282(1):68-76 doi:https://doi.org/10.1016/j.taap.2014.11.007

Magnusson K, Hånell A, Bazov I, Clausen F, Zhou Q, Nyberg F (2009) Nandrolone decanoate administration elevates hippocampal prodynorphin mRNA expression and impairs Morris water maze performance in male rats. Neuroscience Letters 467(3):189-193 doi:https://doi.org/10.1016/j.neulet.2009.09.041

Manna P, Sinha M, Sil PC (2008) Cadmium induced testicular pathophysiology: prophylactic role of taurine. Reproductive toxicology 26(3-4):282-291 doi:https://doi.org/10.1016/j.reprotox.2008.09.009

Marcinkiewicz J, Walczewska M, Olszanecki R, Bobek M, Biedroń R, Dulak J, Józkowicz A, Kontny E, Maślinski W Taurine haloamines and heme oxygenase-1 cooperate in the regulation of inflammation and attenuation of oxidative stress. In: Taurine 7, 2009. Springer, p 439-450

Min T, Lee KH (2018) Effects of nandrolone decanoate on expression of steroidogenic enzymes in the rat testis. Asian-Australasian journal of animal sciences 31(5):658-671 doi:https://doi.org/10.5713/ajas.17.0899

Mohamed HM, Mohamed MA-H (2015) Effect of different doses of nandrolone decanoate on lipid peroxidation, DNA fragmentation, sperm abnormality and histopathology of testes of male Wister rats. Experimental and Toxicologic Pathology 67(1):1-11 doi:https://doi.org/10.1016/j.etp.2014.09.003

Naraghi M, Abolhasani F, Kashani I, Anarkooli I, Hemadi M, Azami A, Barbarestani M, Aitken R, Shokri S (2010) The effects of swimming exercise and supraphysiological doses of nandrolone decanoate on the testis in adult male rats: a transmission electron microscope study. Folia morphologica 69(3):138-146

Nishikimi M, Appaji Rao N, Yagi K (1972) The occurrence of superoxide anion in the reaction of reduced phenazine methosulfate and molecular oxygen. Biochemical and Biophysical Research Communications 46(2):849-854 doi:https://doi.org/10.1016/S0006-291X(72)80218-3

Noorafshan A, Karbalay-Doust S, Ardekani FM (2005) High doses of nandrolone decanoate reduce volume of testis and length of seminiferous tubules in rats. Apmis 113(2):122-125 doi:https://doi.org/10.1111/j.1600-0463.2005.apm1130205.x

Novaes Gomes FG, Fernandes J, Vannucci Campos D, Cassilhas RC, Viana GM, D'Almeida V, de Moraes Rêgo MK, Buainain PI, Cavalheiro EA, Arida RM (2014) The beneficial effects of strength exercise on hippocampal

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

Oyovwi MO, Ben-Azu B, Tesi EP, Oyeleke AA, Uruaka CI, Rotu RA, Aya-Ebi EO (2021) Repeated endosulfan exposure induces changes in neurochemicals, decreases ATPase transmembrane ionic-pumps, and increased oxidative/nitrosative stress in the brains of rats: Reversal by quercetin. Pesticide Biochemistry and Physiology 175:104833 doi:https://doi.org/10.1016/j.pestbp.2021.104833

Paget G, Barnes J (1964) Toxicity tests. Evaluation of drug activities: pharmacometrics 1:135-65

Pany S, Panigrahi SK, Rao EV, Patnaik L, Sahu T (2019) Anabolic androgenic steroid abuse and their health impacts: a cross-sectional study among body builders in a city of Eastern India. International Journal of Preventive Medicine 10 doi:https://doi.org/10.4103%2Fijpvm.IJPVM_524_17

Patanè FG, Liberto A, Maria Maglitto AN, Malandrino P, Esposito M, Amico F, Cocimano G, Rosi GL, Condorelli D, Nunno ND (2020) Nandrolone decanoate: use, abuse and side effects. Medicina 56(11):606 doi:https://doi.org/10.3390/medicina56110606

Piacentino D, D. Kotzalidis G, del Casale A, Rosaria Aromatario M, Pomara C, Girardi P, Sani G (2015) Anabolic-androgenic Steroid use and Psychopathology in Athletes. A Systematic Review. Current Neuropharmacology 13(1):101-121 doi:https://doi.org/10.2174/1570159X13666141210222725

Pomara C, Barone R, Marino Gammazza A, Sangiorgi C, Barone F, Pitruzzella A, Locorotondo N, Di Gaudio F, Salerno M, Maglietta F (2016) Effects of nandrolone stimulation on testosterone biosynthesis in leydig cells. Journal of Cellular Physiology 231(6):1385-1391 doi:https://doi.org/10.1002/jcp.25272

Pope Jr HG, Kanayama G, Athey A, Ryan E, Hudson JI, Baggish A (2014) The lifetime prevalence of anabolic-androgenic steroid use and dependence in Americans: Current best estimates. The American journal on addictions 23(4):371-377 doi:https://doi.org/10.1111/j.1521-0391.2013.12118.x

Przewlocki R, Almeida OF (2017) Stress and opioid systems Hormones, Brain and Behavior; Vol 1 Mammalian hormone-behavior systems. Elsevier Academic Press, p 225-260

Qaradakhi T, Gadanec LK, McSweeney KR, Abraham JR, Apostolopoulos V, Zulli A (2020) The Anti-Inflammatory Effect of Taurine on Cardiovascular Disease. Nutrients 12(9):2847 doi:https://doi.org/10.3390/nu12092847

Rezaee-Tazangi F, Zeidooni L, Rafiee Z, Fakhredini F, Kalantari H, Alidadi H, Khorsandi L (2020) Taurine effects on Bisphenol A-induced oxidative stress in the mouse testicular mitochondria and sperm motility. JBRA assisted reproduction 24(4):428-435 doi:https://doi.org/10.5935/1518-0557.20200017

Roşca A, Iosif L, Tivig I, Mirica R, Căruntu C, Timaru C, Voiculescu S, Zăgrean L, Stoian I (2020) Chronic nandrolone decanoate administration effects on antioxidant parameters in Wistar rats. doi:https://doi.org/10.25083/rbl/26.2/2483.2488

Saddick SY (2021) Effect of Nandrolone decanoate induced-oxidative stress on rat testes, prostate, and seminal vesicle: Biochemical, morphometric and histopathological studies. Saudi Journal of Biological Sciences 28(1):196-203 doi:https://doi.org/10.1016/j.sjbs.2020.09.039

Samadi M, Baeeri M, Haghi-Aminjan H, Rahimifard M, Gholami M, Hassani S, Sattari M, Azarmi Y, Bameri B, Armandeh M, Hooshangi Shayesteh MR, Eghbal MA, Abdollahi M (2021) On the mechanisms of taurine in alleviating electrocardiographic, hemodynamic, and biochemical parameters following aluminum phosphide cardiotoxicity. Food and Chemical Toxicology 154:112347 doi:https://doi.org/10.1016/j.fct.2021.112347

Satoh K (1978) Serum lipid peroxide in cerebrovascular disorders determined by a new colorimetric method. Clinica chimica acta; international journal of clinical chemistry 90(1):37-43 doi:10.1016/0009-8981(78)90081-5

Shokri S, Aitken RJ, Abdolvahhabi M, Abolhasani F, Ghasemi FM, Kashani I, Ejtemaeimehr S, Ahmadian S, Minaei B, Naraghi MA, Barbarestani M (2010) Exercise and Supraphysiological Dose of Nandrolone Deconoate Increase Apoptosis in Spermatogenic Cells. Basic & Clinical Pharmacology & Toxicology 106(4):324-330 doi:https://doi.org/10.1111/j.1742-7843.2009.00495.x

Sretenovic J, Zivkovic V, Srejovic I, Milosavljevic Z (2016) The Effects of High Doses of Nandrolone Decanoate on Cardiac Muscle Tissue. Experimental and Applied Biomedical Research (EABR) 17(4):303-308 doi:https://doi.org/10.1515/sjecr-2016-0021

Suvarna KS, Layton C, Bancroft JD (2018) Bancroft's theory and practice of histological techniques E-Book. Elsevier health sciences

Svensson J, Diez M, Engel J, Wass C, Tivesten A, Jansson JO, Isaksson O, Archer T, Hökfelt T, Ohlsson C (2006) Endocrine, liver-derived IGF-I is of importance for spatial learning and memory in old mice. The Journal of endocrinology 189(3):617-27 doi:https://doi.org/10.1677/joe.1.06631

Tahtamouni LH, Mustafa NH, Hassan IM, Ahmad IM, Yasina SR, Abdallaa MY (2010) Nandrolone decanoate administration to male rats induces oxidative stress, seminiferous tubules abnormalities, and sperm DNA fragmentation. Jordan Journal of Biological Sciences 3(4):165-174

Takahashi M, Tatsugi Y, Kohno T (2004) Endocrinological and pathological effects of anabolic-androgenic steroid in male rats. Endocrine Journal 51(4):425-434 doi:https://doi.org/10.1507/endocrj.51.425

Tofighi A, Shirpoor M, Ansari MHK, Shirpoor A, Zerehpoosh M (2017) The effect of nandrolone treatment with and without enforced swimming on histological and biochemical changes in the heart and coronary artery of male rats. Anatolian Journal of Cardiology 17(3):176 doi:https://doi.org/10.14744%2FAnatolJCardiol.2016.7333

Torres-Calleja J, Gonzalez-Unzaga M, DeCelis-Carrillo R, Calzada-Sanchez L, Pedron N (2001) Effect of androgenic anabolic steroids on sperm quality and serum hormone levels in adult male bodybuilders. Life sciences 68(15):1769-1774 doi:https://doi.org/10.1016/S0024-3205(01)00972-9

Tsitsimpikou C, Vasilaki F, Tsarouhas K, Fragkiadaki P, Tzardi M, Goutzourelas N, Nepka C, Kalogeraki A, Heretis I, Epitropaki Z, Kouretas D, Tsatsakis AM (2016) Nephrotoxicity in rabbits after long-term nandrolone decanoate administration. Toxicology Letters 259:21-27 doi:https://doi.org/10.1016/j.toxlet.2016.06.1122

Turillazzi E, Perilli G, Di Paolo M, Neri M, Riezzo I, Fineschi V (2011) Side Effects of AAS Abuse: An Overview. Mini Reviews in Medicinal Chemistry 11(5):374-389 doi:https://doi.org/10.2174/138955711795445925

Ujah GA, Nna VU, Suleiman JB, Eleazu C, Nwokocha C, Rebene JA, Imowo MU, Obi EO, Amachree C, Udechukwu EC, Mohamed M (2021) Tert-butylhydroquinone attenuates doxorubicin-induced dysregulation of testicular cytoprotective and steroidogenic genes, and improves spermatogenesis in rats. Scientific Reports 11(1):5522 doi:10.1038/s41598-021-85026-7

Ulla A, Mohamed MK, Sikder B, Rahman AFMT, Sumi FA, Hossain M, Reza HM, Rahman GMS, Alam MA (2017) Coenzyme Q10 prevents oxidative stress and fibrosis in isoprenaline induced cardiac remodeling in aged rats. BMC Pharmacology and Toxicology 18(1):29 doi:https://doi.org/10.1186/s40360-017-0136-7

Vasavan SS, Jagadesan V, Sivanesan S, Rajagopalan V (2020) Protective effect of Withania somnifera on nandrolone decanoate-induced biochemical alterations and hepatorenal toxicity in wistar rats. Pharmacognosy Magazine 16(68):218

Vasavan SS, Sivanesan S, Jagadesan V (2021) Withania somnifera ameliorates nandrolone-decanoate-induced brain damage in rats by inhibiting cell death, prodynorphin mRNA expression and acetylcholinesterase activity. 20(3):685-693

Vasilaki F, Tsitsimpikou C, Tsarouhas K, Germanakis I, Tzardi M, Kavvalakis M, Ozcagli E, Kouretas D, Tsatsakis AM (2016) Cardiotoxicity in rabbits after long-term nandrolone decanoate administration. Toxicology letters 241:143-151

Wang L, Du F, Wang X (2008) TNF-α Induces Two Distinct Caspase-8 Activation Pathways. Cell 133(4):693-703 doi:https://doi.org/10.1016/j.cell.2008.03.036

Wei S-M, Yan Z-Z, Zhou J (2008) Taurine reduces testicular ischemia/reperfusion-induced neutrophil recruitment to testis probably by downregulation of pro-inflammatory cytokines and E-selectin. Urology 72(2):464-465 doi:https://doi.org/10.1016/j.urology.2008.01.064

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

الهام الشوادفي مجاهد[1]، دينا محمد نجيب عبدالمعوض[1،4]، سارة محمد عبدالعال[2]، سمر رمزي محمد[2]، علياء طلعت[3]، غدير محمد محمود عبدالعال[1]*

1- قسم الطب الشرعي و السموم الإكلينيكية، كلية الطب البشري، جامعة الزقازيق، مصر
2- قسم الهستولوجيا الطبية و بيولوجيا الخلية، كلية الطب البشري، جامعة الزقازيق، مصر
3- قسم الكيمياء الحيوية الطبية، كلية الطب البشري، جامعة الزقازيق، مصر
4- قسم الطب الشرعي و السموم الإكلينيكية، كلية الطب البشري، جامعة بدر بالقاهرة، مصر

مقدمة: يعد ديكانوات الناندرولون هو الستيرويد الأكثر وصفًا في صالات ممارسة التمارين الرياضية، ويعتبر متورط في التنشيط الرياضي والدوائر الإجرامية حيث تكون العدوانية الشخصية واضحة. على الرغم من استخدامه الواسع بين الرياضيين ، إلا ان استخدامه بدون رقابة يسبب سمية خطيرة لأعضاء الجسم. و التورين هو أحد الأحماض الأمينية المستخدمة كمكمل غذائي للاعبي كمال الأجسام وله العديد من الخصائص لحماية الخلايا.

الهدف: تهدف هذه الدراسة إلى تقييم دور التورين في تخفيف التأثيرات السامة لديكانوات الناندرولون على معايير الكيمياء الحيوية ، والتغيرات المرضية النسيجية و المناعية لحصين المخ ، والقلب ، والخصية في ذكور الجرذان البيضاء البالغة.

المواد والطرق: تم تقسيم أربعين جرذا إلى أربعة مجموعات: مجموعة ضابطة، ومجموعة معالجة بالتورين، ومجموعة معالجة بديكانوات الناندرولون، ومجموعة معالجة بديكانوات الناندرولون + التورين. تم قياس مستويات MDA و SOD و TNFα في الخصية ، بالإضافة إلى قياس مستوى سيروم CK-MB . و تم تقدير تعبير الجينات الخاصة بـ BAX و Bcl2 و MMP-9 و Caspase-3 و Cyp11a1 في الخصية، و ال Prodynorphin في الحصين. و تم أيضًا فحص النسيج الخلوي بإستخدام صبغة الهيماتوكسيلين والأيوسين، و صبغة ماسون ثلاثية الألوان، والتحليل الكيميائي النسيجي المناعي، وقياسات الأنسجة االهيستومورفومترية.

النتائج: أظهرت النتائج أن إعطاء التورين بالإضافة إلى ديكانوات الناندرولون قلل بشكل كبير ذو دلالة إحصائية من مستويات MDA و TNF-α في الخصية و CK-MB في السيروم، و من تعبير mRNA للجينات BAX و MMP-9 و Caspase-3 في الخصية و Prodynorphin في الحصين ، و بزيادة ذات دلالة إحصائية في مستوى SOD وتعبير mRNA لجين Cyp11a1 و زيادة ليست ذات دلالة إحصائية لجين BCL2 في الخصية. كما أظهرت نتائج الدراسة النسيجية تحسنًا ملحوظًا في تأثيرات السمية على الخصية و الحصين، بالإضافة إلى انخفاض له دلالة إحصائية في ألياف الكولاجين في القلب. و أظهر التحليل المناعي للأنسجة انخفاضًا في التعبير لبروتين GFAP في الحصين مع زيادة في التعبير للبروتين Ki-67 في الخصية مقارنةً بالمجموعة المعالجة ديكانوات الناندرولون فقط.

الاستنتاج: خفف التورين من التأثيرات السامة لديكانوات الناندرولون أساسا على كل من الحصين والخصية بالإضافة إلى تحسن جزئي على القلب.

التوصيات: فى ضوء نتائج هذه الدراسة نوصى بالأتى:

- تحسين برامج التثقيف الصحي لزيادة الوعي العام حول خطورة تناول ستيرويد ديكانوات الناندرولون في صالات الرياضة وآثاره الضارة وخاصة على الرياضيين الذين يستخدمونه دون استشارة طبية.
- عمل دراسات أخرى لمعرفة التأثيرات الضارة لديكانوات الناندرولون على مختلف أجهزة الجسم و الآثار الوقائية لمادة التورين ضد الآثار السامة الناجمة عن ديكانوات الناندرولون.