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Original Article

Does Omega-3 Ameliorate Olanzapine-Induced Testicular Toxicityin Adult Albino Rats? A Histo-morphometric Study

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ABSTRACT

Introduction: Olanzapine is one of the most frequently used antipsychotics that had undesirable effects on male sexual function.

Aim of the work: To explore possible protective effect of Omega 3 on olanzapine induced testicular toxicity.

Material and Methods: Forty adult male Albino rats were used and allienated into four equal groups: I (control), II (omega3 treated), III (olanzapine treated) and IV (olanapine +omega 3 treated).Group I received vehicles of olanzapine and omega 3 orally once daily. Group II received 400 mg/kg/day of omega 3 while Group III received olanzapine 0.5 mg/kg/day orally once daily. Group IV received a combination of the same mentioned doses of both drugs. After 14 weeks, testicular specimens were taken and processed for light and electron microscopic study.

Results: Group III showed shrunken degenerated seminiferous tubules (ST) with corrugated basement membrane whose lumina were devoid of spermatozoa. Few degenerated spermatogonia, primary spermatocytes, elongated spermatid, many Sertoli cells (SCs) were seen. The tubules were separated with wide interstitial spaces and congested blood vessels. Ultrastructural results revealed spermatogonia with degenerated nucleus, loss of nuclear membranes and cell boundaries of primary spermatocytes. Elongated spermatids appeared abnormal in shape with defects in acrosomal cap. A high significant decrease in mean ST diameter, height of germinal epithelium while a high significant increase in SCs count, number of caspase 3 immunopostive cells were observed. Group VI showed improvement of the above mentioned results.

Conclusion: Omega 3 could protect against olanzapine induced testicular toxicity therefore; it can be used as an adjuvant therapy with antipsychotic drugs.

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Key Words: Histomorphometry, olanzapine, omega -3, rat, testis.

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INTRODUCTION

Psychiatric diseases have lots of drawbacks on the psychosocial performance and quality of life such as intense disruption in cognition and emotions[1], apathy, reduced social implementation, hallucinations and delusions[2]. Nowadays, prevalence of psychiatric diseases is not limited to adults but recently is observed among children and adolescents. For that reason, secure and efficient therapies are required to treat this group of vulnerable people[3].

The principal etiology of these diseases is largely unidentified, but it is feasible to be a complex interaction between genetic tendency and environmental properties. Adding, it has been evidenced that there is a strong association linking hyperactivity of the dopaminergic pathway along with psychoses[4]. Consequently, medications that hinder dopamine receptors have been used in the handling of this illness[5]. Antipsychotic drugs have been used for the management of schizophrenia[3], bipolar disorder, mania, dementia, and severe anxiety[6]. They can be classified into typical, such

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as chlorpromazine and haloperidol, or atypical, such as clozapine, olanzapine, quetiapine, aripiprazole and risperidone[7,8].

Typical antipsychotics are capable of reducing symptoms of psychosis excluding negative symptoms or cognitive deficits. Atypical second-generation antipsychotics (SGAs) have gained attractiveness due to their value in controlling both negative and positive symptoms of psychoses. Furthermore, SGAs are ideal for the treatment of children and adolescents owing to their lesser rates of extrapyramidal symptoms[9,10,11]. Unfortunately, medications functioning on the central nervous system (CNS) have been proved to have undesirable effects on male sexual functions[12-14]. Mckim, 2007[15] declared that the intake of antipsychotics diminishes the sexual interest, weakens the ejaculation and increases the prolactin level as a result of their action on tuberoin fundibular tract. Also, blockage of dopaminergic receptors consequences in augmented conversion of androgens to estrogens ensuing in impotence, and decreased spermatogenesis[16].

Olanzapine is one of the two most frequently used atypical SGAs that have been more and more applied in management of many psychiatric diseases such as schizophrenia, bipolar disorder especially in children and adolescents[3]. Various researches reported its undesirable histological outcomes on the structure of the seminiferous tubules[5, 17]. Also, this drug has been related to too much weight gain, dyslipidemia, hypertension and metabolic syndrome[18]. It acts through binding to a huge number of neurotransmitter receptors, including serotonin receptors, dopamine receptors, histamine H1 receptors, muscarinic receptors, α- and β-adrenergic receptors[5, 19].

Omega-3 is a polyunsaturated fatty acid that cannot be produced in vivo, so it is considered as one of the crucial polyunsaturated fatty acids which are extracted from fish oil and some nuts[20]. Omega-3 acts as competitive inhibitor of omega-6 fatty acids which are converted to prostaglandins and leukotrienes resulting in a diminution in the production of inflammatory markers as IL-1, IL-6 and TNF-α[21-23]. Also, omega-3 fatty acids appeared to be helpful in treatment of metabolic and cardiovascular disorders found in psychiatric patients[24,25].

Few studies were anxious about unfavorable histo-pathological impacts of olanzapine on the testicular structure and also hardly any researches were planned for the possible protective effect of omega-3 against its lethal action on the testis. So the present work was designed to explore olanzapine induced testicular histomorphometric changes in rats and to evaluate the possible protective effect of Omega-3 using light and electron microscopy.

MATERIAL AND METHODS

• *Animals:*

Forty adult Wistar male albino rats weighing 200-250 gm were used in this study. Rats were purchased and locally bred at the animal house of the Medical Research Center (MRC), Faculty of Medicine, Ain Shams University. Then, they were housed in plastic cages, two rats per cage, and were left for one week before the start of the experiment to adapt to surrounding conditions. Animals were exposed to good ventilation, 12 hours light/dark cycle, allowed free access to rat chow and water ad libitum. All the experiments were done after obtaining the approval of Committee of Animal Research Ethics (CARE) – Faculty of Medicine – Ain Shams University.

Drugs

1- Omega-3 (Super omega®): The drug was obtained as gelatin capsules each containing 1000 mg from Safe for pharmaceuticals Company, Cairo, Egypt ,under license of Majestic Power, USA.

2- Olanzapine (Zyprexa®): The drug was purchased as 5 mg tablets (Lilly S.A., Madrid, Spain) which were dissolved in 10 ml of 0.9% NaCl to get a final concentration of 0.5 mg/ml.

Experimental design

Rats were alienated into four groups (10 rats in each).

Group I (control): Rats were kept on the same prior conditions for 14 weeks. They were further subdivided into two subgroups. Subgroup Ia (5 rats) received corn oil ‘vehicle of omega-3’ orally by gavage once daily. Subgroup Ib (5 rats)

received 0.9% NaCl “vehicle of olanzapine” orally by gavage once daily.

Group II (omega-3 treated group): Animals were given 400 mg/kg B.W. of omega-3 dissolved in corn oil orally by gavage once daily for 14 weeks 26.

Group III (olanzapine-treated group): Includes rats that received olanzapine at a dose of 0.5 mg/kg B.W. /day orally by gavage for 14 weeks 27.

Group IV (olanzapine & Omega-3 treated group): Rats received a combination of olanzapine at a dose of 0.5 mg/kg and Omega -3 at a dose of 400 mg/kg orally by gavage once daily for 14 weeks.

Collection of samples and tissue preparation:

At the end of the experiment, all rats were anesthetized by intraperitoneal injection of sodium pentobarbital (Sigma-Aldrich, St. Louis, MO, USA) at a dose of 60 mg/kg B.W. in 0.9% NaCl[28]. They were sacrificed and scrotal skin was shaved and cleaned with 5% iodine solution. Scrotal five cm midline incision was made by the use of no. 15 blade (Aesculap AG and Co. KG, Tutlingen, Germany). Testicular specimens were obtained from all rats then were processed for both light and transmission electron microscopic study.

Light microscopy study

Specimens were fixed in 10% formalin for one week then washed in water and dehydrated in ascending grades of ethanol, cleared in xylol and embedded in paraffin blocks. Sections of 5 µm in thickness were cut, mounted on glass slides, deparaffinized in xylene and stained with Hematoxylin and Eosin[29]. All sections were examined and photographed by means of light microscope (Olympus, U-MDOB, Olympus, Tokyo, Japan) at Faculty of Medicine, Ain Shams University, Cairo, Egypt.

Staining procedures for immunohistochemistry

Paraffin sections were deparaffinized, in xylene, rehydrated, rinsed in tap water, and immersed in 3% Hydrogen peroxide (H2O2) in phosphate buffer solution (PBS) for 10 min. Sections were incubated overnight at 4°C with a polyclonal rabbit anti-active caspase-3 (Clone No C92-05, Catalog No 55955, Phar-Mingen, San Diego, CA at 1:500 dilutions). After incubation with primary antibodies, the sections were incubated with the appropriate secondary antibody; a biotinylated goat anti-rabbit IgG (Vector Laboratories, Burlingame, CA). Sections were incubated for 30 min at room temperature in horseradish peroxidase-avidin-biotin complex (Vectastain Elite, Vector, CA) then 3,3’-diaminobenzidine in H2O2 (DAB kit, Vector, CA) was add to visualize the brown reaction. Sections were then counterstained with hematoxylin and mounted. Negative controls were obtained by the same procedures but without incubation with primary antibodies[30].

Transmission Electron Microscopic (TEM) study

One mm^3 thick specimens were immersed in 2.5% phosphate-buffered glutaraldehyde solution at 4°C for 24 hours, washed over night for several times with fresh solution of 0.1 M sodium phosphate buffer and post-fixed in osmium tetraoxide for 1–2 hours. Subsequently, they were dehydrated through a graded ethanol series and in propylene oxide and then embedded in resin. The blocks were cut into semi-thin sections (0.5 µm), stained with 1% toluidine blue, examined and photographed. Ultrathin sections (70–90 nm) were cut, mounted on copper grids and stained with uranyl acetate and lead citrate[31] and studied by means of JEOL 1010 transmission electron microscope (JEOL, Tokyo, Japan) at the Mycology and Regional Biotechnology Center, Al Azhar University, Cairo, Egypt.

Morphometric study

Leica Qwin 500 image analyser system (Cambridge, UK) was applied to measure the diameter of ST (µm), height of germinal epithelium (µm) and SCs count in H & E stained sections (x400). Also, number of immune-positive spermatozoa and Leydig cells were calculated from anti- caspase-3 immuno-stained sections (x400). Six randomly chosen fields from six different sections of six different rats were examined in each group. Automatic Calibration of the image analyzer was done to change

the measurement units (pixels) into definite micrometer units. Morphometric analysis was performed in the Image Analyzer Unit, Anatomy Department, Faculty of Medicine, Qassim University, Al Qassim, KSA.

Statistical analysis

Statistical analysis was done using SPSS (Statistical Package for Social Studies – Version 13.0). One-way analysis of variance (ANOVA) was employed to compare means between groups. Bonferroni Post Hoc t test was performed for intergroup comparison. $P > 0.05$, $P \leq 0.05$ and $P \leq 0.001$ were considered non-significant, significant and highly significant, respectively. All data were expressed as mean ± SD. Data were represented in tables and bar charts.

RESULTS

A. Light microscopic results:

1. H&E stain

Group II rat testis showed findings similar to those of the control group. Round to oval seminiferous tubules (ST) were surrounded by well-defined intact basement membrane (BM) and lined by multiple layers of spermatogenic cells in different stages of development. They were formed of spermatogonia which rest directly on the BM, two to three layers of primary spermatocytes and rounded spermatids. The lumina of ST contained eosinophilic threads representing spermatozoa tails (flagellum) giving them the characteristic whorly appearance. Spermatogenic cells and spermatozoa were supported by elongated Sertoli cells. Also, Leydig cells were seen in between the tubules (Fig. 1A, B&C). Group III testis showed shrunken irregular shaped ST with massive degeneration of some tubules. All ST were separated with wide interstitial spaces (Fig. 1D). ST showed complete degeneration of spermatogenic cells leaving empty spaces within the wall and their lumina were devoid of spermatozoa. Corrugated thin BM, few number of spermatogonia, primary spermatocytes, elongated spermatids and many Sertoli cells were detected (Fig. 1E&F). Additionally, congested blood vessels were observed (Fig. 1G). On the other hand, group IV testis showed apparently normal shaped ST which appeared round to oval surrounded with intact BM and separated by narrow interstitial spaces containing Leydig cells. ST with complete layers of spermatogenic cells were detected in different stages of development including spermatogonia, primary spermatocytes, rounded spermatids, abundant amount of spermatozoa and all were supported by Sertoli cells (Fig. 1H&I).

2. Toluidine blue stain

Semithin stained sections of group I and group II rat's testis showed that ST were lined by several layers of spermatogenic cells in different stage of development and surrounded by a well-defined BM. Also myoid cells were observed. Spermatogonia with rounded dark nuclei resting directly on the BM, two to three layers of large primary spermatocytes contained characteristic large rounded nuclei with clumps of chromatin were seen. Moreover, rounded spermatids with rounded nuclei and elongated spermatids with peripheral small condensed nuclei and late stage spermatozoa that were interspersed among the spermatogenic cells and attached to Sertoli cells were noticed. Leydig cells were also seen in between ST (Fig. 2A&B). Group III testis demonstrated nuclear degeneration in many primary spermatocytes with loss of chromatin. Some rounded spermatids showed also loss of chromatin and others had small and condensed pyknotic nuclei. Some Leydig cells contained degenerated nuclei were observed (Fig. 2C). In group IV, ST appeared with nearly normal cell lining included primary spermatocytes, rounded spermatids, elongated spermatids, spermatozoa and Sertoli cells. In addition, most of Leydig cells appeared nearly normal (Fig. 2D).

3. Anti - caspase-3 immunostain

Anti caspase-3 immuno-stained sections of the control (group I) and group II testis showed negative caspase-3 reaction in all cell types of ST and Leydig cells (Fig. 3 A&B). Group III testis showed strong positive caspase-3 immuno-reaction exhibited by nuclei of nearly all spermatozoa and Leydig cells (Fig. 3C). Examination of group IV rat's testis revealed nearly negative caspase-3 immuno-reaction in most of ST cells except few showed positive immune-expression by the nuclei of few spermatozoa and Leydig cells (Fig. 3D).

B. Transmission electron microscopic results:

Control rat's ST showed dark type A spermatogonia with rounded dark nucleus and pale type A with pale oval nucleus and both types were resting on intact thick BM. Many primary spermatocytes with oval nucleus and many late stage elongated spermatids with acrosomal cap over the condensed nucleus and numerous mitochondria were seen (Fig. 4A &B). The lumina of ST showed numerous middle pieces of spermatozoa and some Sertoli cells appear with many extruded extra cytoplasm resulting from the process of spermiogenesis (Fig. 4C). In group II, ST were more or less similar to those of the control group with intact BM and myoid cells. The ST contained many primary spermatocytes with numerous mitochondria in addition to Sertoli cells with triangular nucleus and prominent nucleolus. Also, Leydig cells with rounded nucleus, many mitochondria and cytoplasmic electron dense lipid droplets were seen (Fig. 4D). Early stage elongated spermatids with acrosomal cap over the nucleus and Golgi apparatus were detected. Numerous mitochondria and chromatoid body were also observed (Fig. 4E).

In group III, ST showed shrunken degenerated primary spermatocytes with loss of their nuclear membranes and cell boundaries, rarefaction of the cytoplasm and large vacuoles appeared in between the cells. Spermatogonia with degenerated nucleus and Sertoli cells were detected resting on a thin BM (Fig. 5A). Additionally, elongated spermatids showed rarefaction, areas of loss in their cytoplasm, vacuoles and serration in acrosomal cap (Fig. 5B). Some spermatids showed defect in the formation of acrosomal cap with nuclear elongation, condensation and others appeared small and shrunken and abnormal in shape (Fig. 5C).

In group IV, ST with apparently nearly normal shaped spermatogonia which were resting on a thick intact BM and contained rounded nucleus with normal chromatin density and numerous mitochondria were detected (Fig. 5D). In addition, ST showed adherence and normal arrangement of spermatogenic cells including primary spermatocytes with large rounded nucleus and clumps of chromatin, rounded spermatids with rounded nuclei of normal chromatin density and well developed Golgi and elongated spermatids (Fig. 5E). Some elongated spermatids were observed in early stage of spermiogenesis, their nuclei were small, condensed and covered by acrosomal cap. Also, spermatids in late stage of spermiogenesis with small elongated dark nuclei and beginning of tail appearance were also seen (Fig. 5.F).

Morphometric and statistical results:

No significant difference was observed in the mean of ST diameter, germinal epithelial height and SCs count in group II when compared to group I. Group III showed highly significant decrease in the mean values of ST diameter and height of germinal epithelium as well as highly significant increase in mean number of SCs when compared to group I. In group IV, there was a highly significant increase in mean values of ST diameter, germinal epithelial height and a highly significant decrease in SCs count when compared to group III. Also, no significant difference was observed in group IV when compared to group I in all of the above mean values of the measured parameters (Table 1 & Fig.6).

Regarding, the mean number of caspase -3 immuno-positive spermatozoa and Leydig cells, there was no significant difference in group II when compared to group I. On the contrary, group III showed a highly significant increase when compared to group I while group IV showed a highly significant decrease in the mean number of these immune-positive cells when compared to group III. No significant difference was observed in group IV when compared to group I (Table 1 & Fig. 7).

H&E stain

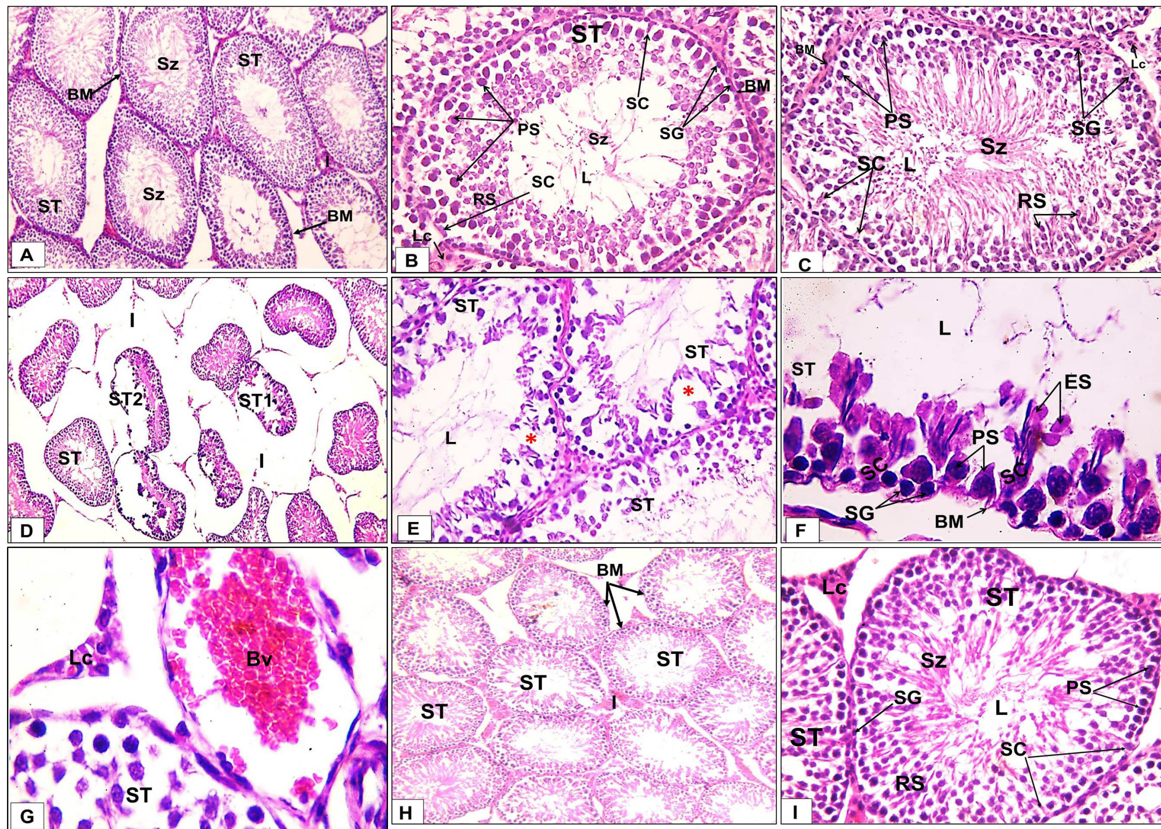

Fig. 1: photomicrographs of H&E stained sections of the control (group I) (A, B) and group II (C) rat testis showing rounded to oval seminiferous tubules (ST) surrounded by well-defined basement membrane (BM↑) and lined by multiple layers of spermatogenic cells which are formed of spermatogonia (SG) resting directly on the BM, two to three layers of primary spermatocytes (PS) and rounded spermatids (RS). The lumen (L) of the tubules contain eosinophilic threads representing flagellum of spermatozoa (Sz) giving them characteristic whorly appearance. Spermatogenic cells and spermatozoa are supported by elongated Sertoli cells (SC). Leydig cells (Lc) are seen in between the ST. Group III testis (D, E, F&G); D) Showing shrunken irregular shaped ST separated by wide interstitial spaces (I) and some tubules (ST1&ST2) are massively degenerated. (E) Extensive degeneration of spermatogenic cells leaving empty spaces (*) within the wall of ST and the lumina (L) are devoid of any spermatozoa. F) Part of ST shows corrugated thin BM, few numbers of spermatogonia (SG) primary spermatocytes (PS), and elongated spermatids (ES). Many Sertoli cells are seen and the lumen of ST is devoid from spermatozoa. (G) showing congested blood vessel (Bv) and Leydig cells (Lc) in between ST. Group IV testis (H&I); H) nearly normal shape ST which appear rounded to oval, surrounded with intact BM and separated by narrow interstitial spaces (I). (I) Showing part of ST lined with complete layers of spermatogenic cells in different stages of development including spermatogonia (SG), primary spermatocytes(PS),rounded spermatids (RS) and abundant amount of spermatozoa (Sz) in the lumen (L) and all are supported by Sertoli cells (SC). (H&E stain; A, D&H x 100; F & GX 1000;B, C, E& Ix400).

Toluidine blue stain

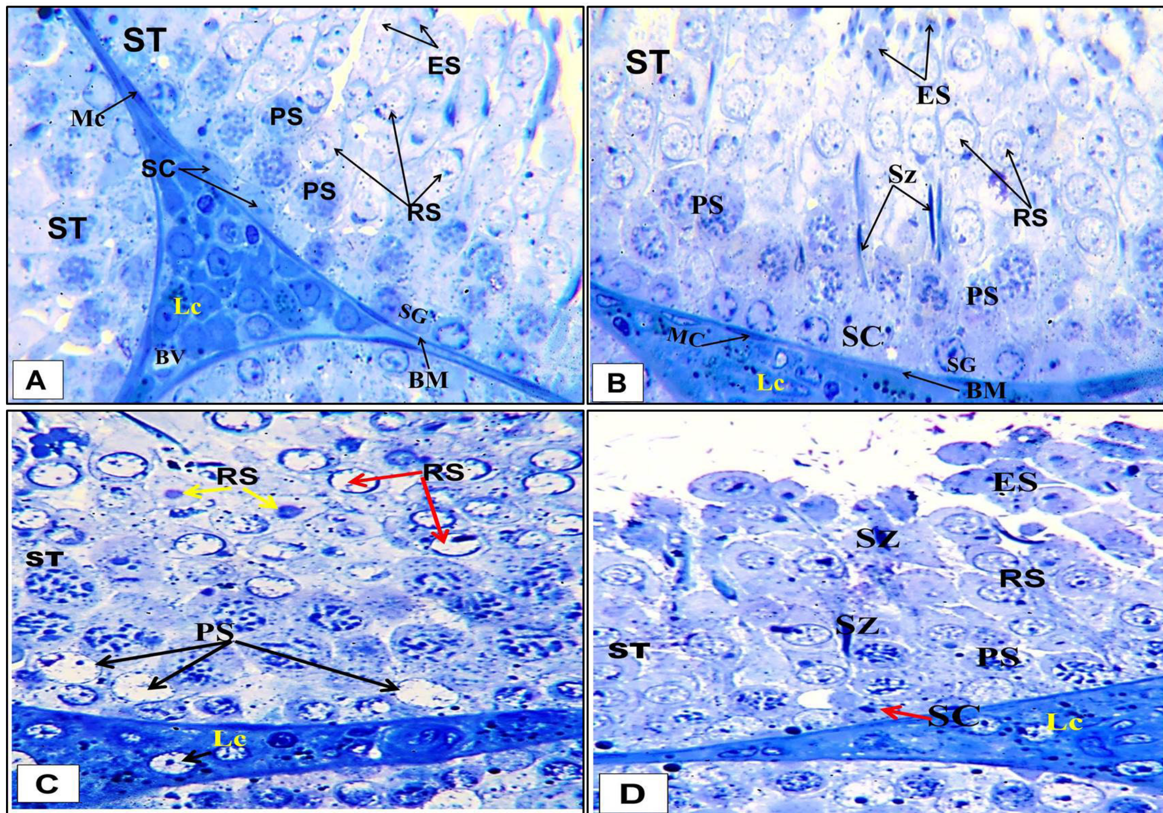

Fig. 2: Photomicrographs of toluidine blue stained semithin sections of the control (group I) (A) and group II (B) rat's testis. Both groups show part of seminiferous tubules (ST) surrounded by well-defined basement membrane (BM↑),myoid cells (Mc) and lined by multiple layers of spermatogenic cells in different stage of development which are formed of spermatogonia (SG) with rounded dark nuclei resting directly on the BM, two to three layers of large primary spermatocytes (PS) that have characteristic rounded nuclei with clumps of chromatin ,rounded spermatids (RS) with rounded nuclei and elongated spermatids (ES) with peripheral small condensed nuclei and late stage spermatozoa (Sz) interspersed among the spermatogenic cells and attached to Sertoli cells(SC). Leydig cells (Lc) are also seen in between ST. C) Group III showing part of ST with nuclear degeneration in many primary spermatocytes (PS) with loss of chromatin (black arrows), some rounded spermatids (RS) show also loss of chromatin (red arrows) and other RS show small and condensed pyknotic nuclei (yellow arrows). Some Leydig cells (Lc) also contain degenerated nuclei. D) Group IV showing apparently nearly normal cells of seminiferous tubules (ST), primary spermatocytes (PS), Sertoli cells (SC) rounded spermatids (RS), elongated spermatid (ES), spermatozoa (Sz). Most of Leydig cells (Lc) also appear nearly normal. (Toluidine blue stain; A, B, C&D x1000).

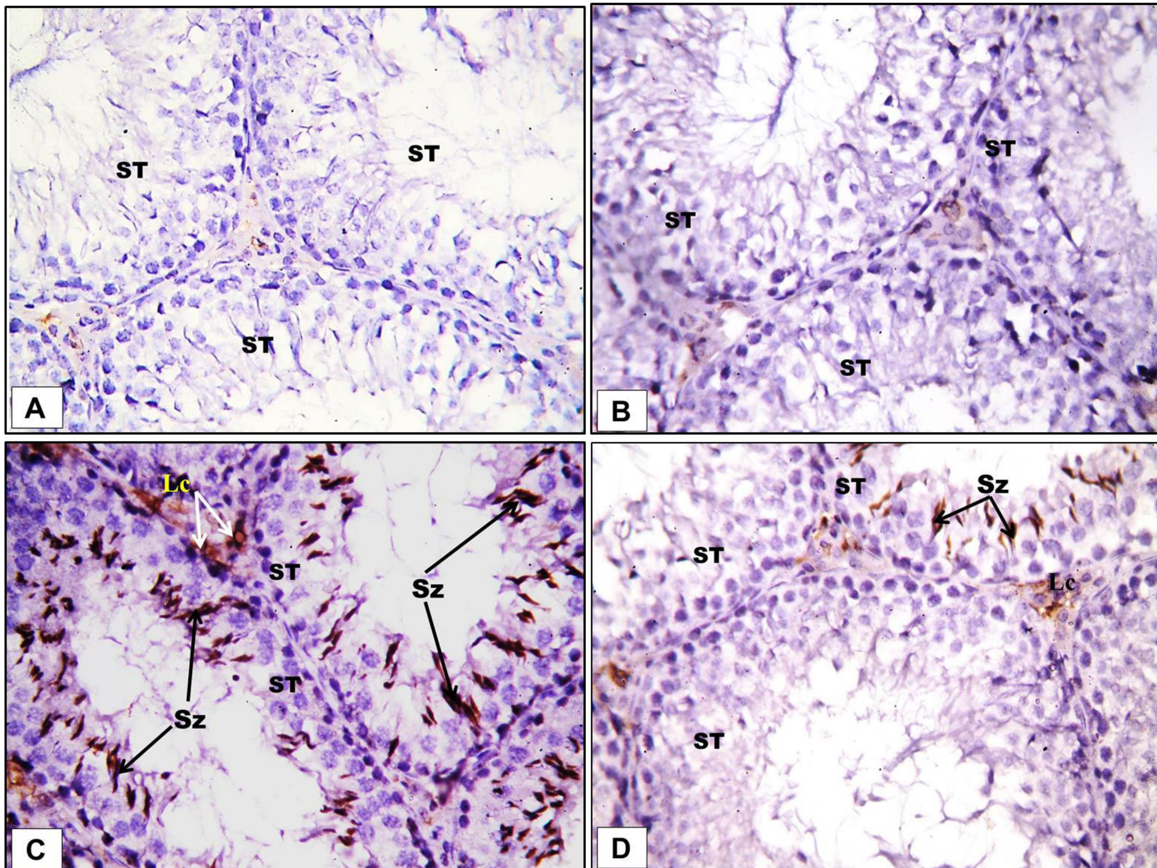

Fig. 3: photomicrographs of anti-caspase immunostained sections of the control (group I) (A) and group II (B) rat's testis. Both groups show negative caspase -3 immunoreaction in all cells of seminiferous tubules (ST) and Leydig cells. C) Group III testis show strong positive caspase-3 immunoreaction exhibited by nuclei of nearly all spermatozoa (Sz) and Leydig cells (Lc). D) Group IV testis showing nearly negative caspase-3 immunoreaction in seminiferous tubules (ST) except one tubule show positive immunoexpression by nuclei of few spermatozoa (Sz) and Leydig cells. (Anti-caspase-3 immunostain; A, B, C&Dx400)

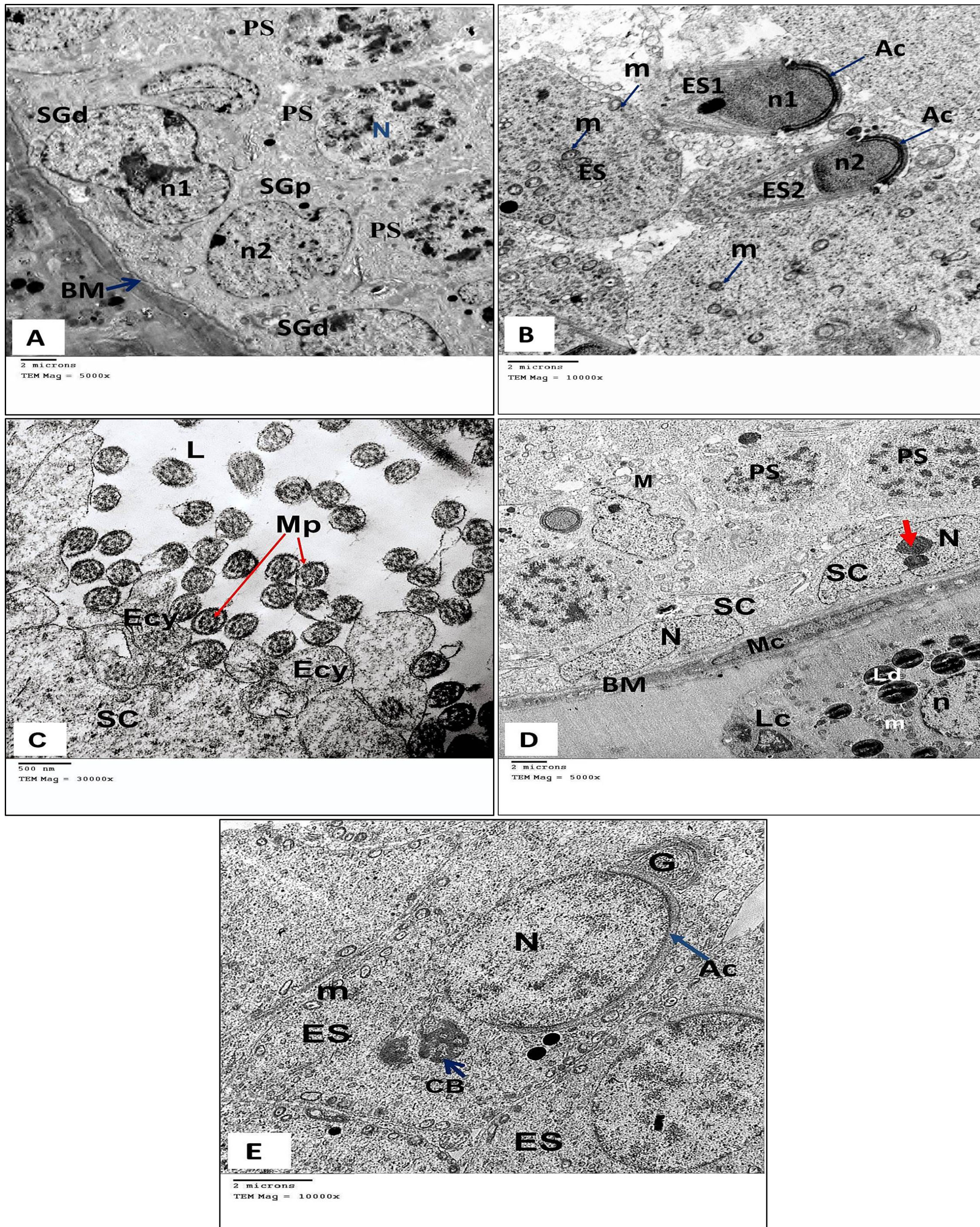

Fig. 4: Electron micrographs of the rat's ST of group I (control) (A,B& C) and group II (D & E); (A): showing dark type A spermatogonia (SGd) with rounded dark nucleus (n1) and pale type A (SGp) with pale oval nucleus (n2) that rest on intact thick basement membrane (BM) and many primary spermatocytes with oval nucleus (N) that are adherent to each other. (B): Many late stage elongated spermatids (ES1&ES2) with acrosomal cap (Ac) over the condensed nuclei (n1&n2) and numerous mitochondria (m). (C) Part of the lumen (L) of seminiferous tubule with numerous middle pieces(Mp) of spermatozoa (red arrows) and part of Sertoli cells (SC) appear with many extruded extra cytoplasm (Ecy) from the process of spermiogenesis. D): showing part of seminiferous tubule that contain Sertoli cells (SC) with triangular nucleus (N) and prominent nucleolus (red arrow) and many primary spermatocytes (PS) containing numerous mitochondria(M). Note the intact basement membrane (BM), myoid cell (Mc), part of Leydig cell (Lc) with rounded nucleus (n), mitochondria (m) and many electron dense lipid droplets (Ld). (E): early stage elongated spermatids (ES) with acrosomal cap over the nucleus (N) and Golgi apparatus (G) is seen close to it. Numerous mitochondria (m) and chromatoid body (CB) are also seen. (TEM; A x5000, B x 10000, C x30000, D x5000&E x10000).

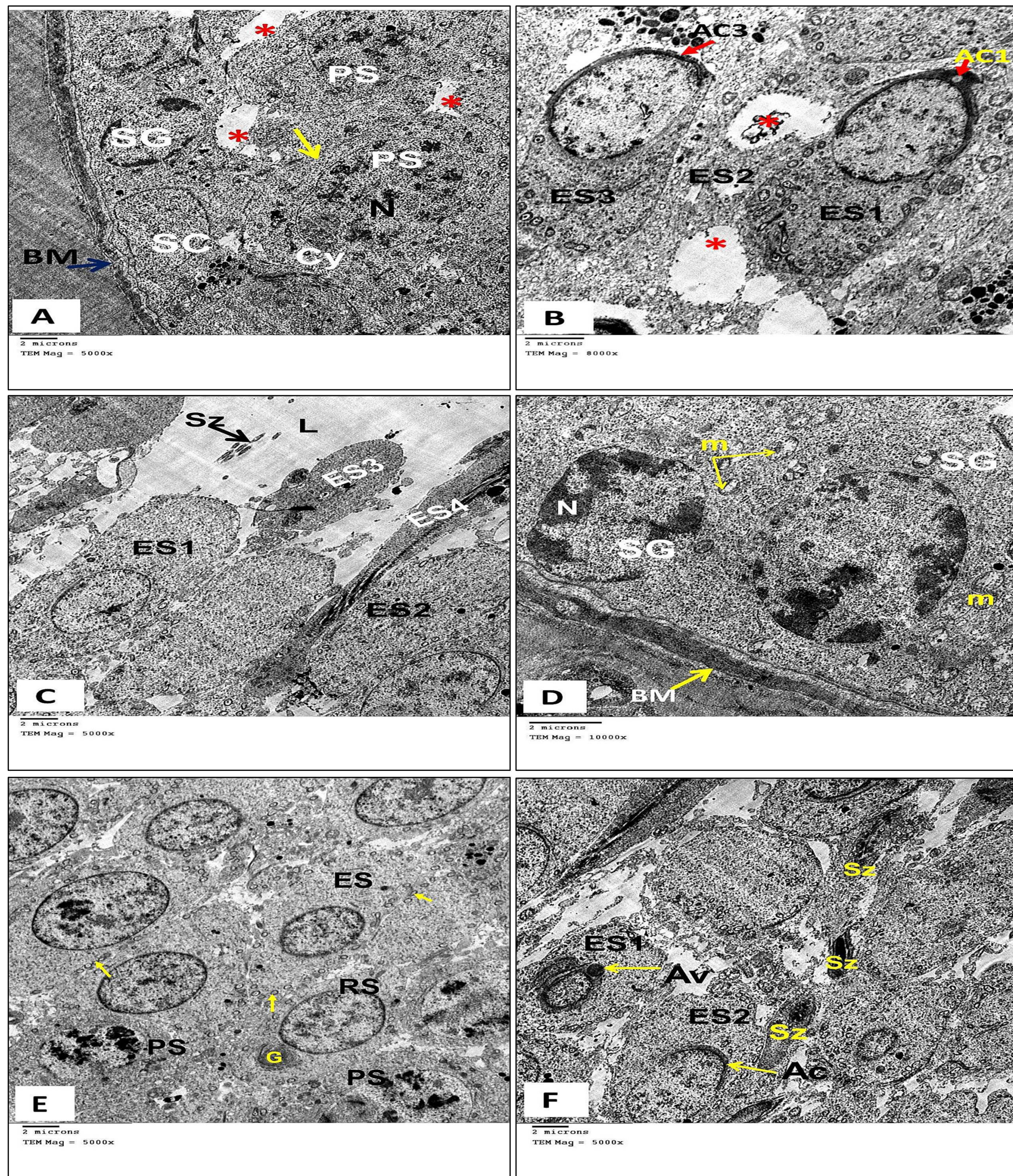

Fig. 5: Electron micrographs of group III (A, B&C) and group IV seminiferous tubules (D, E&F); A): showing shrunken degenerated primary spermatocytes (PS) with disruption of their nuclear (N) membranes and cell boundaries (yellow arrows), rarifaction of the cytoplasm (Cy) and many vacuoles (*) appear in between them. Spermatogonia (SG) with degenerated nucleus and Sertoli cells (SC) are seen resting on thin basement membrane (BM).B): Three elongated spermatids (ES1, ES2&ES3), ES1 shows serrated acrosomal cap(AC1), ES2 has areas of cytoplasmic loss (*) and ES3 shows a vacuole in the acrosomal cap (AC3). C): Four elongated spermatids; ES1&ES2 shows defect in the formation of acrosomal cap and nuclear elongation and condensation. ES3 is small and shrunken and ES4 is abnormal in shape .Group IV D) Part of seminiferous tubule with nearly normal shape spermatogonia (SG) which contains rounded nucleus (N) with normal chromatin density, numerous mitochondria (m) and rests on a thick intact basement membrane (BM). E): Normal shape, adherence and arrangement of spermatogenic cells; primary spermatocytes (PS) have large rounded nucleus with clumps of chromatin, rounded spermatid (RS) with rounded nuclei of normal chromatin density and well developed Golgi (G) is seen close to the nucleus in addition to elongated spermatid (ES).F): Shows two elongated spermatids ((ES1&ES2) in early stage of spermiogenesis where their nuclei are small and condensed. ES1 nucleus is surrounded by acrosomal cap (AC) while an acrosomal vesicle (AV) is seen close to the nucleus of ES2. Also, spermatozoa (Sz) in late stage of spermiogenesis with small elongated dark nuclei and beginning of tail appearance are seen. (TEM; A, B, C, E&Fx5000, Dx10000)

Table 1: Comparison of Mean ST diameter (µm), height of germinal epithelium (µm), number of Sertoli cells/HPF, number of caspase -3 immuno-positive SZ/HPF and number of caspase-3 immuno-positive Leydig cells/HPF in group I, II, III and IV. Values are represented as mean ± SD.nd Leydig cells in group I, group II, group III and group IV. Values are represented as mean ±standard deviation (SD).

	Group I	Group II	Group III	Group IV
ST diameter	923.8±9.39	927±8.75	325.9±10.06 (*P<0.001*)[a]	916.8 ±13.38 (*P<0.001*)[b] (*P= 0.192*)[c]
Height of GE	498.4± 6.25	501 ± 6.79	213.4 ± 6.36 (*P<0.001*)[a]	492.9 ± 6.41 (*P<0.001*)[b] (*P=0.068*)[c]
Number of Sertoli Cells	19.6 ± 1.89	19. 9 ± 1.91	25.5 ± 1.90 (*P<0.001*)[d]	20.2 ± 3.48 (*P<0.001*)[e] (*P=0.638*)[f]
Number of Caspase -3 immuno-positive SZ	7.4±1.34	6.5±1.17	114.2±6.66 (*P<0.001*)[d]	8.5±1.08 (*P<0.001*)[e] (*P=0.690*)[f]
Number of Caspase-3 immuno-positive LC	1.9±0.56	2.4±0.51	22.2±1.61 (*P<0.001*)[d]	2.3±0.67 (*P<0.001*)[e] (*P=0.168*)[f]

a) Highly significant decrease in comparison with group I.
b) Highly significant increase in comparison with group III.
c) Non significant decrease in comparison with group I.
d) Highly significant increase in comparison with group I.
e) Highly significant decrease in comparison with group III.
f) Non significant increase in comparison with group I.

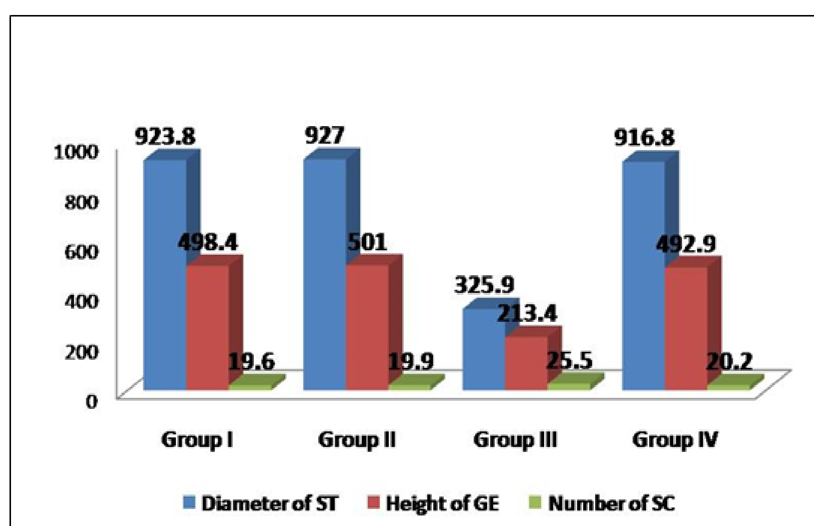

Fig. 6: Bars representing the mean diameter of seminiferous tubules (ST) (µm), height of germinal epithelium (GE) (µm), number of Sertoli cells (SC)/HPF in group I, II, III and IV. Values are expressed as mean ± SD.

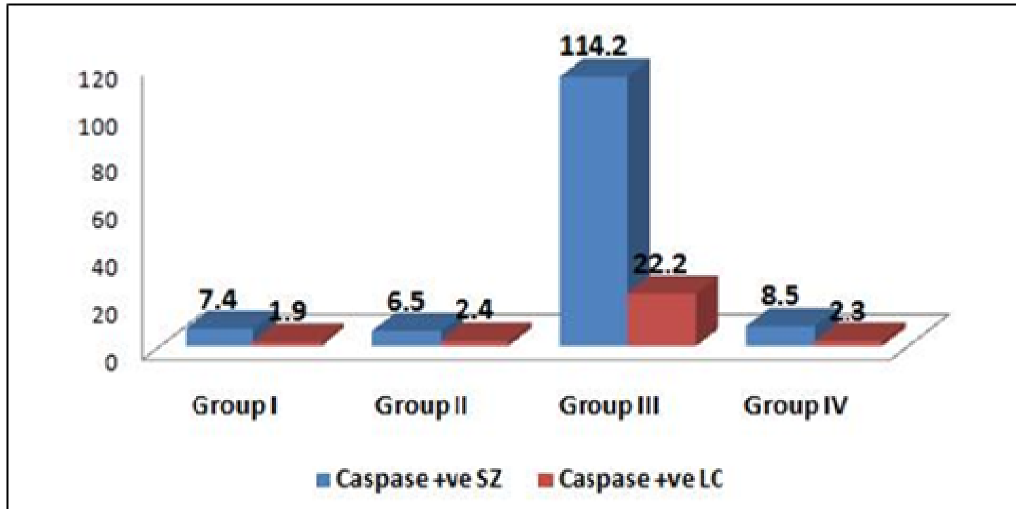

Fig .7: Bars representing the mean number of caspase-3 immuno-positive spermatozoa (SZ)/HPF and the mean number of caspase -3 immuno-positive Leydig cells (LC) /HPF in group I, II, III and IV. Values are expressed as mean ± SD.

DISCUSSION

Nowadays, frequency of psychotic diseases is rising among general population. Patients receiving olanzapine for long period suffered from sexual dysfunction. Few studies examined the histopathological impacts of antipsychotic drugs on the testicular tissue. So, this study was done to evaluate the undesirable structural changes of olanzapine on the rat testes and to assess the possible protective effect of omega- 3.

The current work revealed that olanzapine administration produced high significant reduction in the mean ST diameter, height of germinal epithelium, and increased SCs count compared to control group. All of these data pointed to a diminution of spermatogenic activity. This was in accordance to some authors who found interruption in spermatogenesis plus structural changes in the testis[32]. Olanzapine use can result in an amplified level of prolactin secretion[33- 35] which is capable of inducing galactorrhea, sexual dysfunction and hypogonadism[36]. As a consequence of increased prolactin, inhibition of gonadotropin releasing hormone, follicle stimulating hormone (FSH), luteinizing hormone (LH) and a decline in plasma levels of testosterone occurs[17,32]. Testosterone and FSH are vital for continuation of spermatogenesis[37,38]. Thus, this decline of plasma testosterone levels may result in that unfavorable action on spermatogenesis. Moreover, olanzapine caused a decreased in serum inhibin B level which is strongly linked to Sertoli cell dysfunction and sperm counts[39]. The increase in SCs count may be as a consequence to increased degeneration of cells that are attacked and removed by the Sertoli cells as previously found in progressive testicular atrophy occurring in old age men[40].

Also, group III showed shrunken irregular shaped and degenerated ST with corrugated thin BM, widened interstitial spaces and congested blood vessels. Additionally, there was a degeneration of spermatogenic cells, widened intercellular spaces, and few numbers of spermatogonia, primary spermatocytes, and spermatids with absence of spermatozoa. Similar findings were found due to administration of other atypical antipsychotic drugs as aripiprazole[41] and the antidepressant drug fluoxetine[5]. Dopamine receptors are clearly known to be present in germ cells of ST[42,43] and olanzapine can attach to these receptors thus affecting spermatogenesis causing cellular disorganization and intercellular spacing.

Moreover, germ cells are kept in their places by a secure relationship among their plasma -membranes and Sertoli cell membranes specialized junctions[44]. So, degeneration of germ cells might result from interruption of this link leading to disarrangements, spacing of spermatogenic cells and tubular atrophy. Furthermore, down regulation of cell adhesion proteins in the Sertoli cells causes separation of ST epithelial cells that might lead to affection of the tubular BM, shape and size[30]. Additionally,

interruption of the BM of the ST might be explained by either myoid cells contraction or decline of ST diameter. Epithelial injury stimulates the secretion of substances as oxytocin and prostaglandins with a consequent myoid cells contraction[44]. The wide spaces between the ST were explained by the existence of interstitial oedema[46]. Also, congestion of blood vessels caused by olanzapine may be described by the occurrence of endothelial vasodilatation which is linked to ATP responsive potassium channel[47]. It was reported that antipsychotic drugs reduced human retinal endothelial cell count, viability and migration[48].

Interestingly, these unfavorable outcomes were significantly alleviated in rats treated with omega-3 compared with olanzapine - treated animals. Statistically, there was no significant difference between group I and IV. This was in accordance to the results of one study which investigated the protective role of omega-3 against B -Cyfluthrin induced testicular toxicity[49]. Moreover, intake of omega-3 improved the toxic effects of doxorubicin on the testis and maintained the integrity of spermatogenic structures[50]. These observations could be described by the potent antioxidant and anti-inflammatory effects of omega-3[51]. Previous studies declared that omega-3 fatty acids have anti-inflammatory action, so in this manner altering the formation of inflammatory and chemotactic substances and calming cell mediated immune responses[52]. Intake of omega-3 stimulates spermatogenesis, increases the activity of Sertoli cells removes free radicals and inhibits lipid peroxidation[53,54]. Supplementary findings reported that testicular toxicity induced by anticancer drugs were efficiently attenuated by giving omega -3[55].

The ultrastructural findings in this work proved the histological changes seen by light microscopy. The current study showed extensive vacuolation between the shrunken degenerated primary spermatocytes, degenerated spermatogonia, and vacuolated Sertoli cells and thin BM. Similar findings were found in the ST after administration of anti-schizophrenic drugs[5]. These observations were also seen in rats receiving lithium carbonate which is a well recognized antipsychotic drug[56]. Various studies stated that Sertoli cells vacuolation is related to the degeneration of abnormal germ cells and dilations of the extracellular spaces[41]. Also, degeneration of both spermatogonia and primary spermatocytes may be an indication of pre-apoptosis and inactive cellular DNA[57] resulting in decreased cellular division and germinal epithelium thickness observed in this study.

In the current study, spermatids were small and abnormal in shape with a defect in acrosomal cap formation. Furthermore, spermatids showed variable abnormalities in their nuclei and acrosomes after occlusion of the vas deferens with styrene maleic anhydride[58]. Additionally, Mapanare snake venom and fungicide benomyl -induced obvious ultrastructural changes in the heads and acrosomes of the spermatids. In benomyl treated rats, spermatids showed parallel degenerative changes such as abnormal heads and acrosomes or lack of acrosomes formation[59]. All of the above mentioned results were improved in omega-3 treated rats which can be attributed to its antioxidant effects that reduce reactive oxygen species and protect against oxidative damage. Its antioxidant property offers a guard mechanism through three levels; protection- prevention, interception and repair[60,56].

In the current work, olanzapine augmented the expression of anti-caspase-3 in the spermatozoa and Leydig cells coinciding with some authors who noticed apoptosis of the germinal cells as a result of decreased serum testosterone levels. Apoptosis has a vital function through the elimination of degenerated spermatogonia to avoid the development of atypical sperms[61,62]. Furthermore, apoptosis is used to get rid of spermatocytes that could not complete their mitotic division[63]. On the contrary, omega-3 administration along with olanzapine improved immunohistochemical results, which were only, limited to few spermatozoa and Leydig cells. Moreover, there was no significant statistical difference in mean number of immunopositive cells between group I and IV. These findings were in harmony with a previous work discovered that antioxidant action of omega-3 normalized and protected against the decline in sperm count, motility and viability as well as, the sperm abnormalities[64]. Omega-3 attenuated cancer cells growth and induces death of a diversity of human cancer cell lines[65]. Moreover, omega-3 therapy provided an extremely considerable decline in the percentage of DNA fragmentation[66].

CONCLUSIONS

Omega-3 fatty acids pre-treatment effectively protected against the adverse histomorphometric and apoptotic effects of olanzapine on the rat testes. These findings raise the possibility that omega-3 can be used as an adjuvant therapy to help in preventing harmful effect of olanzapine in clinical application. Additional clinical studies are required to establish the proper way of combination of omega-3 with olanzapine.

CONFLICT OF INTERESTS

There are no conflicts of interest.

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هل أوميجا ـ٣ يخفف من سمية الخصيه المستحثة بالأولانزابين في الجرذان البالغة؟ دراسة هستومورفومتريه

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ملخص البحث

المقدمة: أولانزابين هو أحد مضادات الذهان الأكثر استخدامًا والتي كان لها آثار غير مرغوب فيها على الوظيفة الجنسية الذكرية.

الهدف من البحث: لاستكشاف التأثير الوقائي المحتمل لأوميجا ٣ على سمية الخصية المستحدثه بالأولانزابين فى الجرذان.

المواد والطرق المستخدمة: تم تقسيم أربعين من ذكور الجرذان البيضاء إلى أربع مجموعات متساوية. المجموعةالأولى (الضابطة)، والثانية (أوميجا ٣), والثالثة (الأولانزابين)،الرابعة (أوميجا ٣ + الأولانزابين).تلقت المجموعة الأولى السوائل التى أذيب فيها كلا من أوميجا ٣ والأولانزابين عن طريق الفم مرة واحدة يوميًا. تلقت المجموعة الثانيه ٤٠٠ مل / كجم / يوم من أوميجا ٣ عن طريق الفم بينما تلقت المجموعة الثالثة أولانزبيين ٠,٥ مل / كجم عن طريق الفم مرة واحدة يوميًا.تلقت المجموعة الرابعة مزيج من نفس الجرعات المذكورة من كلا الدوائين عن طريق الفم. وبعد ١٤ أسبوعًا تم أخذ عينات من الخصية وتمت معالجتها للدراسة بالمجهر الضوئى والمجهرالإلكترونى النافذ.

النتائج: أظهرت المجموعه الثالثه انكماش وتحلل الأنيبيبات المنويه والتى كانت تجويفاتها خاليه من الحيوانات المنويه وقد فصلت عن بعضها بمساحات خلالية واسعة. شوهد عدد قليل وتحلل الخلايا المنوية الأم، الخلايا المنوية الأولية ، الحيوانات المنوية الطويلة ، العديد من خلايا سيرتولي، أحتقان الأوعية الدموية و تموج الغشاء القاعدي. لوحظ انخفاض كبير في متوسط قطر الأنابيب المنويه، و انخفاض ارتفاع الغشاء الظهارى الجنينى ، بينما لوحظت زيادة كبيرة معتد بها احصائيا في عدد خلايا سيرتولي، وعدد الخلايا الموجبه المناعيه لcaspase-3. وكشف الفحص المجهرى الدقيق عن صغرو تحلل نواة الخلايا المنوية الأم، وفقدان الأغشية النووية وحدود الخلايا بين الخلايا المنوية الأولية. وقد ظهرت الحيوانات المنوية الطويله صغيرة ، غير طبيعية في الشكل مع وجود عيوب في الغطاء الأكروسومي. وأظهرت المجموعة الرابعة تحسن النتائج المذكورة أعلاه.

الخاتمه: أوميجا ٣ قد يحمى ضد سمية الخصية المستحثه بالأولانزابين, ولهذا يمكن استخدام أوميجا ٣ كعلاج مساعد مع الأدوية المضادة للذهان.