Original Article

Protective Role of Folic Acid Versus Atorvastatin on Experimentally Induced Doxorubicin Toxicity on Testes of Adult Albino Rats: Anatomical, Histological and Immunoassay Study

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ABSTRACT

Introduction: Infertility is a worldwide common health problem that might be induced by many factors and anti-cancerous are among the most common causes. Doxorubicin, and similar drugs are widely used anti cancerous agents mainly in the treatment of solid tumors, lymphoma and leukemia. they are attributable to male infertility due to testicular intoxication, and issue necessitates the concomitant usage of protective agents especially with increase life expectancy of cancer patients associated with better health care.

Aim of the Work: The current study is designed to compare the possible protective effect of both folic acid and Atorvastatin in doxorubicin induced testicular intoxication in adult male albino rats.

Materials and Methods: The experiment was conducted on sixty adult animals divided randomly and equally as control group (including three subgroups, 10 rats EACH, received no treatment, 10 rats received Atorvastatin and 10 rats received Folic acid), Doxorubicin treated group and two more groups that received folic acid and Atorvastatin in the second concomitantly as protective agents.

Results: The results assessed anatomically (body weight, testicular weight and body: testicular weight ratio), biochemical assay (LH, antioxidant and testosterone serum level), and histologically both by light and Transmission Electron Microscopy (TEM). All results suggest that folic acid has a significant protective role on doxorubicin induced testicular intoxication. Administration of Atorvastatin at the used dose has a limited role in protection.

Conclusion: The results recommend the use of folic acid as a routine protective agent for those under doxorubicin treatment especially in males during childhood and young age.

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Key Words: Albino rats, atorvastatin, doxorubicin, folic acid, testicular toxicity.

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INTRODUCTION

Infertility is considered as one of the most common health and life issues. It is known as the inability to achieve natural pregnancy in newly married couples after a year of regular unprotected uninterrupted intercourse. The incidence of infertility varies between populations due to many variants as, dietary habits, life-style, environmental and occupational factors, and infectious diseases. Infertility can either be primary or secondary. One of the causes of primary infertility is the unwise or non-inventible use of various drugs. A wide variety of medications as the antineoplastic agents may be attributable to the condition[1].

Combination of Chemotherapeutic drugs and different doses may lead to high infertility risk, and generally chemotherapy has a major potential to cause testicular injury[2]. The severity of injury is related to the type and dose of drug, age and gender of the patient[3]. Chemotherapy can severely damage the testicular germinal epithelium in same manner as radiation therapy, that leads to oligospermia and/ or azoospermia, without affection of interstitial cell of Leydig or production of testosterone[4]. The most dangerous gonadotoxic drugs are high doses alkylating class, more than 90% of men are treated by such doses experience prolonged azoospermia that also occurs in about 50% of men treated by platinum compounds[5].

As a result of major advances in diagnostic methods, treatment and therapeutic tools, Survival of cancer patient has markedly been improved over the last few decades. the number of young long-term survivors is increasing due to early detection and successful treatment, consequently the strategy of treatment has changed from just cure from the cancer to achieve better quality of life[6].

Ideal anticancer drugs have the ability to eradicate cancer cells without harming normal tissues, however, unfortunately, there is no current chemotherapeutic drug meet this criterion and clinical use of these drugs involves a weighing of benefits against toxicity in a search for a favorable therapeutic index[7]. From chemotherapeutic

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agents, Anthracyclines (Doxorubicin, Daunorubicin, Epirubicin, and Idarubicin) are considered as one of the most effective anti-tumor families in the for eradication of cancer. Specifically, Doxorubicin (DOX), commercially known as Adriamycin, is one of the most effective chemotherapeutic drugs; although it is not commonly used because of major toxic side effects it produces[8]. DOX can causes many adverse effects including reproductive damage in humans and experimental animals[9,10]. Even a low dose of Doxorubicin (1 mg/kg bw) given to adult mice is able to target the germ cells, mainly spermatogonia, leading to seminiferous epithelium depletion[11]. Also, it has been approved that DOX causes significant decrease in reproductive organs weight and reduction of sperm concentration and motility[9,11].

Reduction of the harmful effects of these agents is extremely important and with the era of the use of available and newly emerging protective and antioxidant agents, it would be achievable. Atorvastatin (ATV) is a synthetic potent statin which was first synthesized in 1985 by Bruce Roth[12,13]. It was built on the discovery of fungal metabolite inhibitors Mevastatin, Lovastatin, Pravastatin and Simvastatin. The drug has many useful therapeutic effects. These include antioxidant anti-inflammatory roles as well as endothelial function improvement, increased bioavailability of nitric oxide, cardiac protection, and anticancer activity[14,15].

Folic acid (FA) and folate (the naturally occurring form) are water-soluble vitamins, known as (B9). Folic acid itself is inactive form, but it is needed to be converted to dihydrofolic acid in the liver, then converted to other derivatives. The most important biological form is tetrahydrofolate. Vitamin B9 (folic acid and folate) plays a role in many of biological functions of the body particularly in hemopoiesis of red blood cells. Folic acid is present in a wide variety of foods, such as green-leafy vegetables, liver, bread, yeast and fruits[16].

AIM OF THE WORK

The present study was designed to evaluate the protective value of Atorvastatin versus that of Folic acid in experimentally induced Doxorubicin testicular toxicity in adult albino rats.

MATERIALS AND METHODS

Drugs and Chemicals

Doxorubicin: Doxorubicin was purchased from a local pharmacy in the form of 1 vial of 50mg/ 25 ml. It is manufactured by Ebewe Pharama GmbH Nfg KG-Austria.

Atorvastatin: Atorvastatin was purchased from a local pharmacy in the form of 10 mg capsules. It is manufactured by EIPICO company, Tenth of Ramadan City, Egypt. It was dispensed as a crystalline white powder and was freshly prepared in normal saline.

Folic Acid: Folic Acid was purchased in the form of powder. It is manufactured by MOLBASE Biotechnology company, China. It was freshly prepared in normal saline.

Study Design

The current study was performed out on 60 young adult male albino rats, with average weight 120-200 g, obtained from the animal house of the Human Anatomy Department, Faculty of Medicine, Alexandria University. The animals were handled according to the code of ethics of experimental research adopted by Alexandria Faculty of Medicine. The rats were all kept under standard housing conditions for 2 weeks before the beginning for acclimatization. They were randomly divided into four groups as followed;

Group I (control group): Thirty male rats were divided into 3 equal subgroups:

- Subgroup Ia received intraperitoneal (IP) injections of 5 ml normal saline on 1st, 8th, 15th and 22nd days of the study,
- Subgroup Ib received intraperitoneal (IP) injections of folic acid (10 mg/kg) dissolved in 1 ml normal saline on 1st, 8th, 15th and 22nd days of the study
- Subgroup Ic received intraperitoneal (IP) injections of Atorvastatin (10 mg/kg) dissolved in 1 ml normal saline on 1st, 8th, 15th and 22nd days of the study

Group II (DOX group): Ten male rats received IP injections of DOX (5 mg/kg) dissolved in 1 ml normal saline[17].

Group III (DOX + folic acid): Ten male rats received pretreatment with folic acid (10 mg/kg) dissolved in 1 ml normal saline[18] a day before, IP injections of DOX (5mg/kg).

Group IV (DOX + Atorvastatin): Ten male rats received pretreatment with atorvastatin (10 mg/kg) dissolved in 1 ml normal saline[19] a day before, IP injections of DOX (5mg/kg).

The drug intake was following the same timing schedule of the first group. Saline and Drugs were administered via intraperitoneal injection on the 1st, 8th, 15th and 22nd days of the study for DOX and a day before, following the same schedule for Ator and Folic acid. Animals were euthanized a day after the last dose of the treatment, the abdomen was incised through a midline abdominopelvic incision to expose the reproductive organs.

Animal deaths were reported during the experiment in all groups; one in group II, two in group III and one rat death in group IV. Post-mortem dissection revealed signs of intraperitoneal hemorrhage that might be attributable to these deaths.

A)- Anatomical Study

1. Body weight and growth rate: Individual body weight of all the rats was recorded by using electronic balance on day zero and just before euthanization to study the body weight changes. The recorded data was analyzed as indicated in statistical analysis.

2. Testes weight: Immediately following euthanization, and prior to histopathological examinations, testes were removed, washed, and dried. All testes were then weighed on a digital scale. The recorded data was analyzed as indicated in statistical analysis.
3. Testes weight: body weight ratio: The pre-euthanization body weight for each animal and the post- euthanization testes weight of the same animal was used to calculate the ratio. The ratio is calculated to evaluate if the protective effect of the two drugs is tselectived to the gonads only or a part of the general effect. The resulting data was analyzed as indicated in statistical analysis.

B) - Biochemical Assay

3:5 ml. of blood obtained through cardiac puncture from all animals just after euthanizing. The samples were collected into tubes containing lithium heparin as anticoagulant Blood tubes were centrifuged at 3000 rpm for 10 minutes at 4oC. The plasma was separated, transferred into plain tubes and kept at -80lab freezers until analysis of luteinizing hormone (LH), testosterone, and toxic stress markers including total antioxidant power (TAP). Radioimmunoassay kits were used to determine concentrations of LH, testosterone and TAP. All samples were measured at the same time to minimize errors[20].

C) - Histological Methods

Testes for Histological, Immunostaining, Ultrastructure analysis were pierced with a needle, and placed in Blouin's fixative.

Light microscopic study

specimens were fixed in 10% buffered formalin, then processed in alcohol and xylin, paraffin blocks were prepared and 5-µm thick paraffin sections were cut from each block and stained with Hematoxylin & Eosin. Trichrome staining was separately used on blank slides for evaluation of fibrosis[21]. Morphometric data regarding tubular diameter and thickness of the epithelium were assessed. A graded scale lens was used to measure the mean diameter of seminiferous tubules and the mean thickness of tubular epithelium.

Immunostaining technique

The 5 micron-thick sections, mounted on positively charged, coated slides (Polysine, Bio Optica, Milano, Spain), were deparaffinized in xylene, rehydrated in descending grades of alcohol followed by water. Slides were incubated in 3% H_2O_2 for ten minutes for blocking endogenous peroxidase activity. Epitope retrieval was done using 10 mM citrate buffer solution (pH 6.0) for 15 min in a microwave oven at 60°C. Ultra V block was applied for 3 minutes to block non-specific background staining. The primary antibody,anti-Bcl2 (Dako, Cambridge, UK) monoclonal antibody was applied at a concentration of 1:100 then the sections were incubated overnight at 4 C° in the humidity chamber. Biotinylated Goat anti-Polyvalent antibody was then applied followed by Streptavidin Peroxidase, DAB Plus substrate, and DAB Plus Chromagen. Counter staining was done using Harris hematoxylin. Slides were dehydrated in ascending grades of alcohol, and then cleared in xylene. The antibodies and the detection system was provided by Lab Vision Corporation (Neo Markers, Fremont, USA)[22].

Evaluation of immunostaining results

Bcl2- stained tissue sections of each groups were examinedusing light microscopy, low power examination (x100) to assess the positive/negative staining for Bcl2 then high-power examination was then applied to assess the percentage of the stained spermatogenic cells to the total number of spermatogenic cells, then percent of staining was evaluated according to Yildiz et a1. in a score ranging from 1 to 4 as follows; 0: no staining, 1: <25%, 2: 26-50%, 3: 51-75%, 4: >75% staining

Electron microscopic study

Small pieces of 1 mm3 of the testis were excised, the process of fixation was done in solution of 2% gluteraldehyde then the buffering was done using 0.1 mol/l phosphate at pH 7.4 for 2 hours at 4°C, Post fixation was done in 1% osmium tetroxide. After that they were dehydrated by using rising concentration of ethanol and placed in propylene oxide for 30 min at room temperature, followed by inoculation in a mixture of propylene oxide and resin (1 : 1) for 1 hour and then in a mixture of the previous reagents at 48°C for 1 h. The specimens were embedded in an EM bed-812 resin in BEEM capsules (Pennsylvania) at 60°C for 24 h (Trevor, 1996) Ultrathin sections were cut and stained by couples of stains known as uranyl acetate and lead citrate[23] and were examined with a JEOL transmission electron microscope (JEM 100 cx-11, Germany), , Faculty of Science, Alexandria University, Egypt.

Statistical analysis

All numerical data including; anatomical measurments, biochemical assay data and morphometric results were fed to the computer and analyzed using IBM SPSS software package version 20.0. (Armonk, NY: IBM Corp). The Kolmogorov- Smirnov was used to verify the normality of distribution of variables, ANOVA was used for comparing the four studied groups for normally distributed quantitative variables and followed by Post Hoc test (Tukey) for pairwise comparison. while Paired t-test was assessed for comparison between two periods for normally distributed quantitative variables. Significance of the obtained results was judged at the 5% level[24].

RESULTS

A- Anatomical Study

1- Body Weight

There was no statically significant difference between different groups of control (I a, b &c) and they all showed nearly similar results. There was no statically significant difference between different groups of control. Rats injected with doxorubicin (Group II) showed a significant ($p \leq 0.05$) decline in their body weight as compared with the control rats (Group I). While, rats received folic acid before injection with doxorubicin (Group III) exhibited a significant ($p \leq 0.05$) elevation in the body weight compared with rats administrated with doxorubicin alone (Group II), but very similar to the control group (Group I), as there was no significant difference between group I control and group III (Dox + folic acid). However, rats protected with atorvastatin before the administration with doxorubicin (Group IV) showed no significant ($p \leq 0.05$) difference in weight with group II (DOX) (Table 1, Figure 1a).

2- Testes weight

Testes weight of rats in the control subgroups (I a, b &c) showed nearly similar results. Rats administrated doxorubicin (Group II) showed a significant ($p < 0.05$) decrease in their testes mean weight as compared with those of the control (Group I), While, rats received folic acid before injection with doxorubicin (Group III) exhibited a significant ($p \leq 0.05$) elevation in the testes mean weight compared with rats administrated with doxorubicin alone (Group II), but very similar to the control group (Group I), as there was no significant difference between group I control and group III (Dox + folic acid). rats protected with atorvastatin before the administration with doxorubicin (Group IV) showed significant ($p \leq 0.05$) difference elevation in the testes mean weight with group II (DOX). (Table 2, Figure 1b).

3- Testes Weight: Body Weight Ratio

The lowest ratio between testes weight and body weight was detected in group II (DOX) with mean = (0.60 ± 0.14). (Table 2, Figure 1c).

B) Biochemical Assay

Regarding the level of LH hormone, there was no statically significant difference between different groups of control. Doxorubicin administrated rats (Group II) showed a significant ($p < 0.05$) elevation in the level of LH as compared with the control (Group I), While, rats received folic acid before injection with doxorubicin (Group III) exhibited a significant ($p \leq 0.05$) reduction in the level of LH compared with rats administrated with doxorubicin alone (Group II), but very similar to the control group (Group I), as there was no significant difference between group I control and group III (Dox + folic acid). Rats protected with atorvastatin before the administration with doxorubicin (Group IV) showed significant ($p \leq 0.05$) reduction in the level of LH compared with group II (DOX) (Table 3, Figure 2a).

The level of testosterone hormone, showed no statically significant difference between different groups of control. Doxorubicin administrated rats (Group II) showed a significant ($p < 0.05$) reduction in the level of testosterone as compared with the control (Group I), While, rats received folic acid before injection with doxorubicin (Group III) exhibited a significant ($p \leq 0.05$) elevation in the level of testosterone compared with rats administrated with doxorubicin alone (Group II), but very similar to the control group (Group I), as there was no significant difference between group I control and group III (Dox + folic acid). Rats protected with atorvastatin before the administration with doxorubicin (Group IV) showed significant ($p \leq 0.05$) elevation in the level of testosterone hormone compare with group II (DOX) (Table 3, Figure 2 b).

The total antioxidants power (TAP) level showed also no statically significant difference between different groups of control. Doxorubicin administrated rats (Group II) showed a significant ($p < 0.05$) reduction in the level of total antioxidants as compared with the control (Group I), While, rats received folic acid before injection with doxorubicin (Group III) exhibited a significant ($p \leq 0.05$) elevation in the level of total antioxidants compared with rats administrated with doxorubicin alone (Group II), but very similar to the control group (Group I), as there was no significant difference between group I control and group III (Dox + folic acid). Rats protected with atorvastatin before the administration with doxorubicin (Group IV) showed significant ($p \leq 0.05$) elevation in the level of total antioxidants compare with group II (DOX) (Table 3, Figure 2 c).

C. Histological results

Light microscopic study

Examination of testicular sections of control group showed normal architecture of the seminiferous tubules with complete spermatogenesis. Each tubule was bounded by a regular basal lamina and lined by several layers of spermatogenic cells including spermatogonia interposed by Sertoli cells, spermatocytes, elongated spermatids and the lumen was filled with large number of mature sperms. The interstitial space was narrow contains fibroblasts, average amount of collagen fibers, blood vessels and Leydig cells. With Trichrome staining, testicular seminiferous tubules of the control group showed average thickness of the basement membranes and few collagen fibers seen in the interstitium and in the wall of the blood vessels. Strong and diffuse positive staining for Bcl2 was noted in the nuclei of the lining spermatogenic cells of the seminiferous tubules in the control group, most of which were score 3 (>50%) with average staining density of 90%. (Figure 3).

The examination of testicular sections of group II showed distorted seminiferous tubules with arrested

spermatogenesis. The tubules showed disorganized lining, most of the tubules were only lined by several layers of spermatogonia or showing few layers of spermatocytes. The apical cells are detached within the lumina. The tubules were reduced in diameter. Each tubule was bounded by irregular basal lamina, decreased height of the layers. Vacuolar degeneration was noted as well as numerous degenerated eosinophilic spermatocytes with condensed chromatin admixed with apoptotic bodies and cell debris. Some tubules showed focal hyalinization. The interstitial space was widened. It showed edema and excess collagen fibers, numerous hyperplastic Leydig cells as well as thick walled blood vessels. Testicular seminiferous tubules of group III showed thickened basement membrane and wide interstitial spaces showing dense collagen fibers and thick-walled blood vessels.

Immune staining, Focal positive staining for Bcl2 was noted in the nuclei of the lining spermatogenic cells of the seminiferous tubules, in group II with average staining of 10%, score 1. (Figure 4)

Light microscopic examination of testicular sections of group III showed seminiferous tubules with reduced diameter. Mild vacuolar degeneration was noted in the basal spermatogonia of some tubules. The interstitial space is widened. Testicular seminiferous tubules of this group showed wider interstitial spaces with edema, fibrosis and thick-walled blood vessels. With Bcl2 immunostaining, Diffuse strong positive staining for Bcl2 was noted in the nuclei of the spermatogenic lining in group IV score 3 (>50%), with average staining density of 80%. (Figure 5)

Examination of testicular sections of rats of group IV showed seminiferous tubules with distorted shapes. Some tubules were with complete spermatogenesis, while, other tubules showed detachment of the surface spermatogenic cells within the lumen. Mild vacuolar degeneration is noted in some tubules as well as numerous apoptotic bodies. The interstitial spaces were widened. Testicular seminiferous tubules of group IV showed wider interstitial spaces with edema, fibrosis and thick-walled blood vessels. Focal positive staining for Bcl2 was noted in the nuclei of the basal spermatogonia in group II score 2 (<50%), with average staining density of 40%. (Figure 6)

Morphometric results

Regarding the tubular diameter, there was no statically significant difference between different groups of control. Doxorubicin administrated rats (Group II) showed a significant ($p < 0.05$) reduction in the tubular diameter as compared with the control (Group I), While, rats received folic acid before injection with doxorubicin (Group III) exhibited a significant ($p \leq 0.05$) difference in the tubular diameter compared with rats administrated with doxorubicin alone (Group II), but very similar to the control group (Group I), as there was no significant difference between group I control and group III (Dox + folic acid). Rats protected with atorvastatin before the administration with doxorubicin (Group IV) showed significant ($p \leq 0.05$) difference in the tubular diameter compare with group II (DOX) (Table 4, Figure 7).

Evaluation of the tubular thickness of epithelium, showed no statically significant difference between different groups of control. Doxorubicin administrated rats (Group II) showed slight reduction in the tubular thickness of epithelium, but in general, there was no statically significant difference between all groups.

Electron microscopic examination

TEM examination of the testes of the control group showed that seminiferous tubules lined with Non spermatogenic cells as Sertoli cells and spermatogenic cells as spermatogonia, spermatocytes and spermatids, resting on regular basal lamina. Sertoli cells resting on the basal lamina with their basally located nuclei, having deep indentations and well-defined nucleoli. The cytoplasm contained a moderate number of lysosomes dispersed throughout the cytoplasm. The spermatogonia were broadly applied to the basal lamina with vesicular nucleus. Primary spermatocytes were the largest spermatogenic cells. They were recognized by their large rounded nuclei. Early spermatid had peripherally oriented mitochondria and contains also Golgi apparatus with secretory granules. The structure of the mature spermatozoa was formed of head (H) that contains pyriform condensed nucleus; and tail that contains mitochondria (M). Cut section of middle piece of mature sperm showed nine doublets of microtubules arranged at periphery with one central pair of microtubules in the middle. Leydig cells appeared with irregular nucleus, vesicular mitochondria and lipid droplets. (Figure 8).

In group II, TEM study of testes of rats in these group showed manifestation of severe testicular damage at cellular level. Thickened irregular basal lamina with distortion at some points. Sertoli cells and spermatogenic cells appeared damaged with vacuolated cytoplasm and irregular dark shrunk damaged nuclei. Loss of chromatin and disruption of nuclear membrane were noted. Early spermatids showed abnormal diffraction; abnormal shape of acrosomal cap. Spermatozoa appeared malformed with abnormal heads. Mal-maturation of spermatozoa with failure or abnormal tail formation. Sperms were severely malformed with loss of mitochondria sheath and some of dense fibers. Ring-shaped mitochondria detected in different cells. There were apparent Golgi complex and excessive lysosomal activity. Edema and widened intercellular spaces were marked especially in the interstitium. (Figure 9)

Group III; high dose of doxorubicin and folic acid; TEM examination of testes of rats of this group reveals more or less normal ultrastructure with reservation of most of cells in seminiferous tubules and interstitium. Some sections showed irregularity of basal lamina, defective sperm tails and vacuolation of middle pieces of spermatozoa. (Figure 10).

The ultrastructure of the testes of group IV; High dose of doxorubicin and Atorvastatin, showed also signs of severe affection at the cellular the level with abnormal thickening

of basement membrane. Abnormal differentiation of spermatids and loss of normal contour of their acrosomal cap were also noted. The middle pieces of the sperms showed severe malformation of the microtubules. (Figure 11)

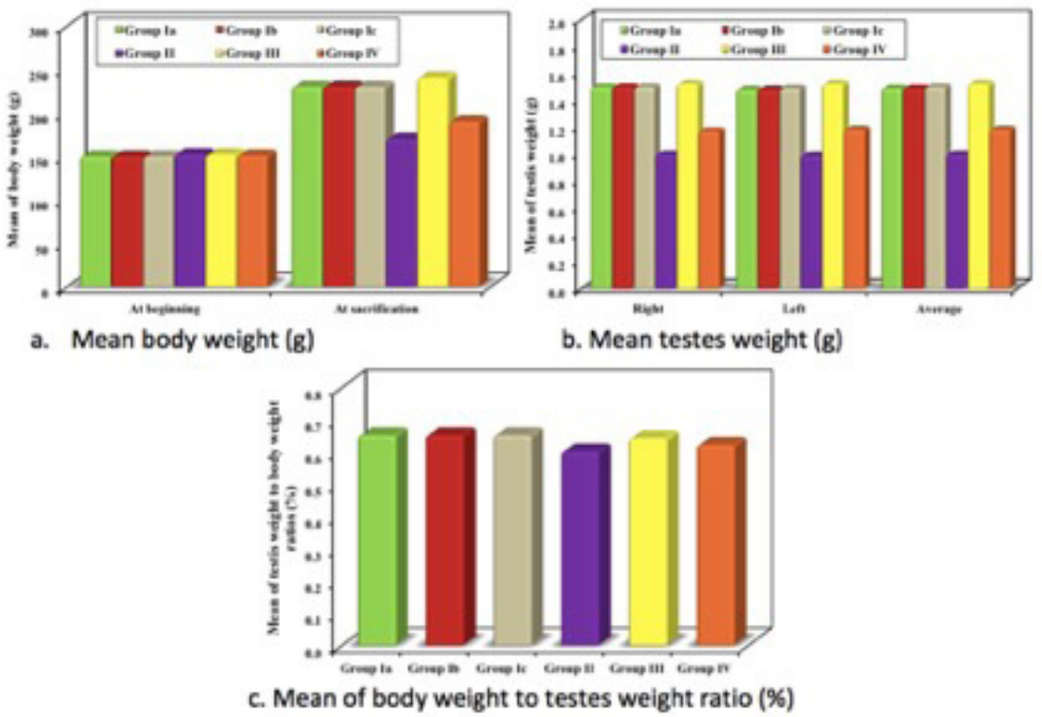

Fig. 1: Comparison between studied groups according to: **a:** Body weight, **b:** Testis weight, **c:** Testes Weight: Body Weight Ratio

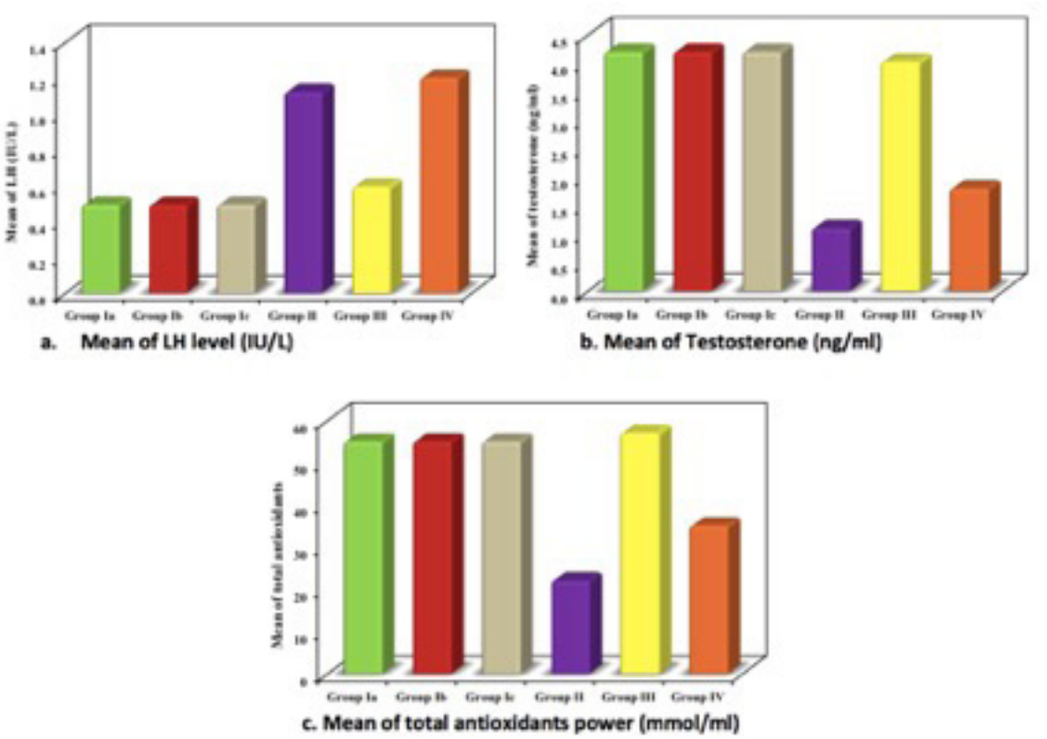

Fig. 2: Comparison between studied groups according to: **a:** Level of LH, **b:** Level of Testosterone, **c:** Level of total antioxidants power (TAP)

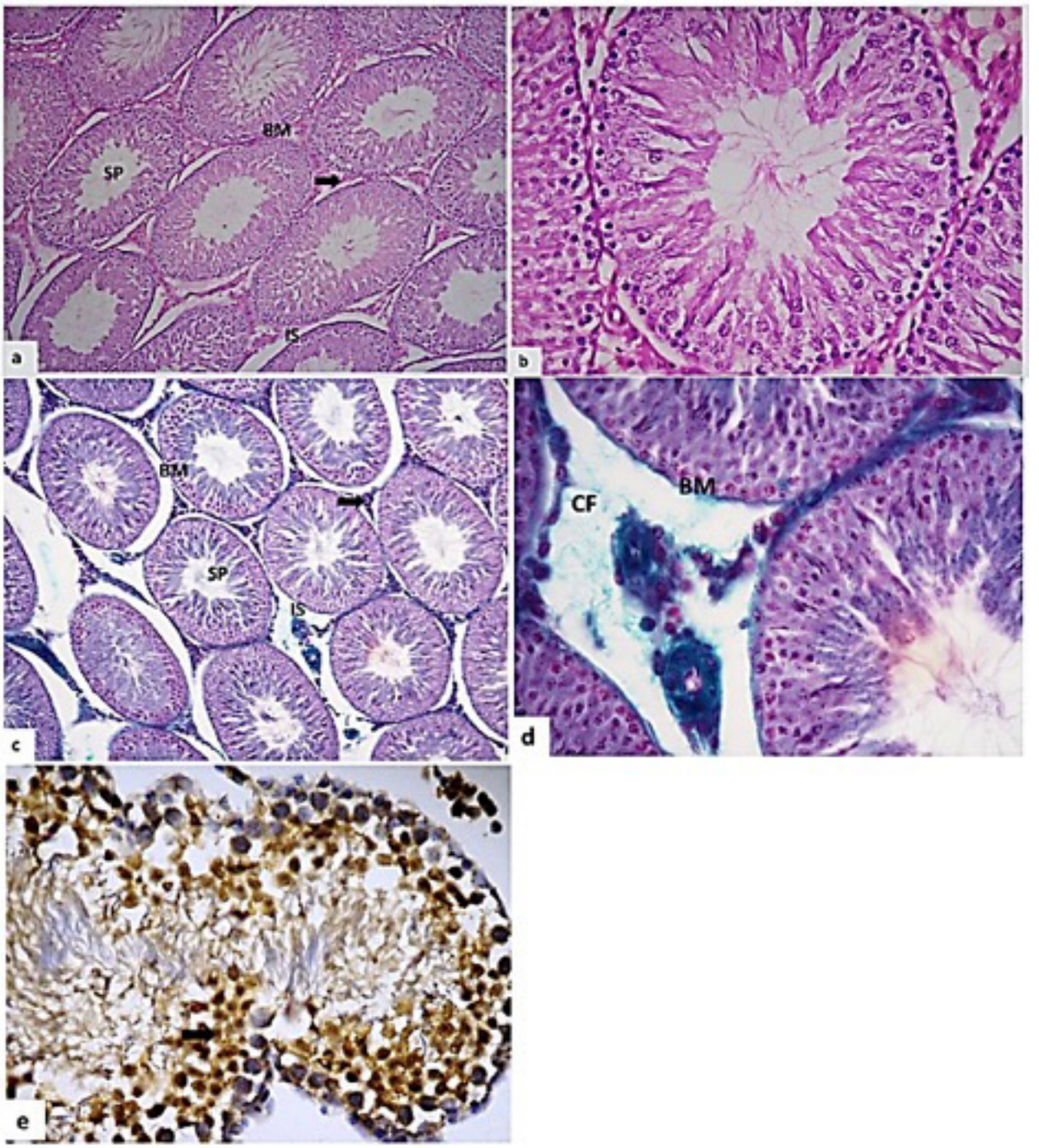

Fig. 3: a: A photomicrograph of the testicular tissue of the control group showing average number, diameter and basement membranes (BM) of seminiferous tubules with different stages of spermatogenesis with mature sperms (SP) and narrow interstitial spaces (IS) containing Leydig cells (arrow). (H&Ex100)
b: A photomicrograph of the testicular tissue of the control group, higher magnification showing complete spermatogenesis and many mature sperms in the lumen. (H&E x400)
c: A photomicrograph of the testicular tissue of the control group, showing average thickness of the tubular basement membranes (BM) with narrow interstitial space (arrow, IS) (trichromex100)
d: A photomicrograph of the testicular tissue of the control group, higher magnification showing average thickness of the tubular basement membranes (BM) ,collagen fibers (CF) are seen in the interstitial space and within the wall of the interstitial blood vessels
e: A section of the testicular tissue of the control group, showing diffuse strong positivity for Bcl2 in most of the lining spermatogenic cells (arrow). (Bcl2x400)

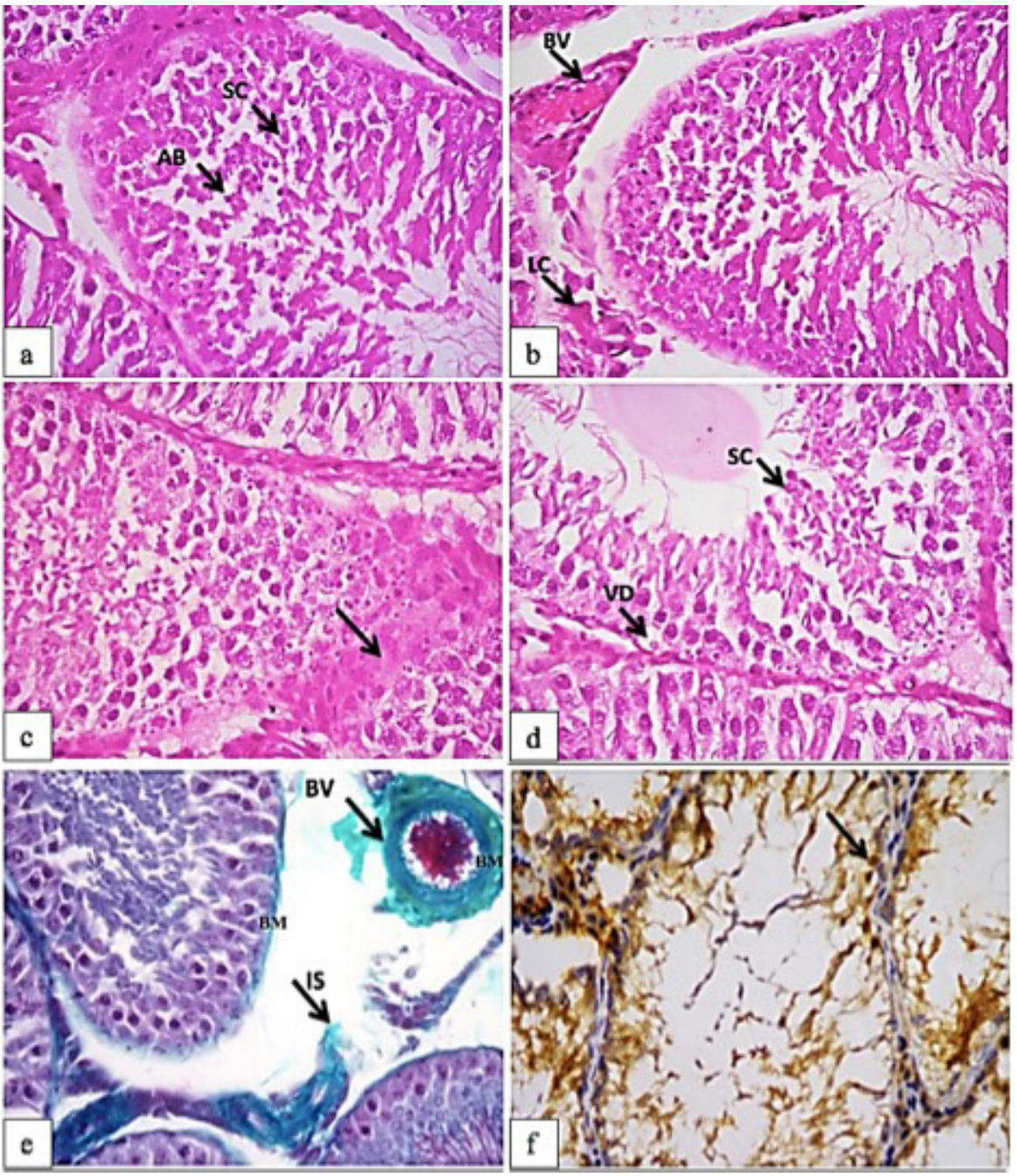

Fig. 4: a: A photomicrograph of the testicular tissue of group II showing seminiferous tubules exhibiting disorganized spermatogenic cells (arrow, SC) many are detached within the lumina, with numerous apoptotic bodies (arrow, AB) (H&Ex400)
b: A section of the testicular tissue of group II with interstitial tissue showing edema, fibrosis, proliferating Leydig cells (arrow, LC) and congested blood vessels, (arrow, BV). (H&Ex400).
c: A photomicrograph of the testicular tissue of group II, the seminiferous tubule shows focal hyaline degeneration. (Arrow). (H&Ex400)
d: A photomicrograph of the testicular tissue of group II showing seminiferous tubules exhibiting detached spermatogenic cells (arrow, SC) within the lumina, with vacuolar degeneration of basal spermatogonia (arrow, VD). (H&Ex400)
e: A photomicrograph of the testicular tissue of group II, showing thick tubular basement membranes (BM), thick walled blood vessels (arrow, BV and wide interstitial space (arrow, IS). (Trichromex400)
f: A photomicrograph of the testicular tissue of group II, showing average nuclear staining for Bcl2 in lining spermatogenic cells (arrow). (Bcl2 x400)

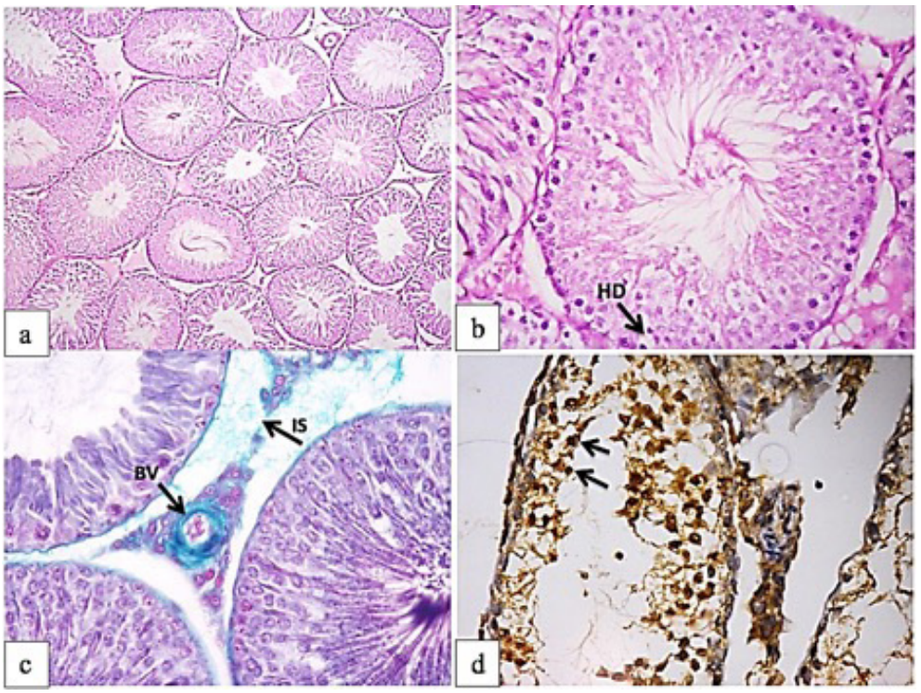

Fig. 5: a: A photomicrograph of the testicular tissue of group III showing seminiferous tubules exhibiting complete spermatogenesis. (H&E x100)
b: A photomicrograph of the testicular tissue of group III, showing complete spermatogenesis with mild focal hydropic degeneration noted in the basal spermatogonia (arrow, HD). (H&Ex400)
c: A photomicrograph of the testicular tissue of group III, showing wide interstitial spaces (IS), interstitial edema and thick-walled blood vessels (BV). (trichrome x400)
d: photomicrograph of the testicular tissue of group III, showing Positive nuclear staining for Bcl2 in lining spermatogenic cells(arrow). (Bcl2 x400)

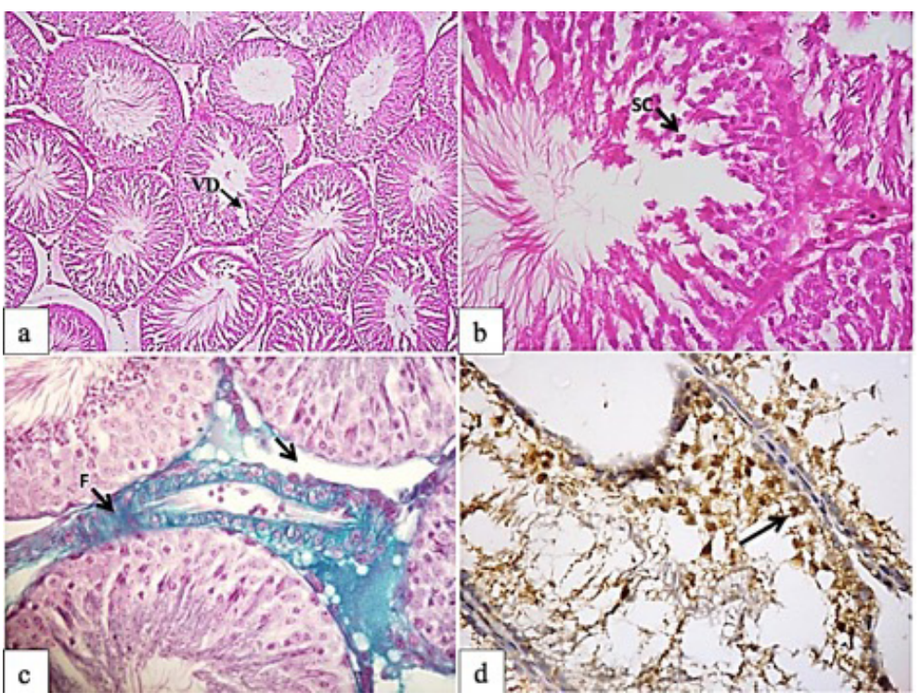

Fig. 6: a: A section of the testicular tissue of group IV showing seminiferous tubules exhibiting complete spermatogenesis with vacuolar degeneration (arrow, VD). (H&Ex100)
b: A section of the testicular tissue of group IV, showing focal detachment of the surface spermatogenic cells (arrow, SC) within the lumina. (H&E x400)
c: A section of the testicular tissue of group IV, showing wide interstitial space with interstitial fibrosis (arrow, F) and edema (arrow). (trichrome x400)
d: A photomicrograph of the testicular tissue of group IV showing focal positive staining for Bcl2 in basal spermatogenic cells (arrow). (Bcl2x400)

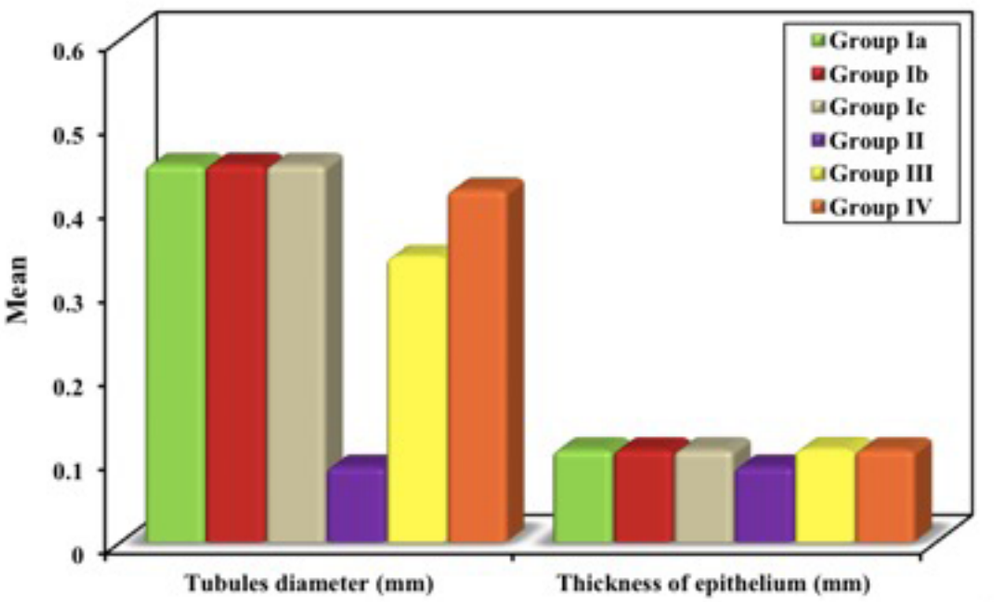

Fig. 7: Comparison between the different studied groups according to tubules diameter and thickness of epithelium

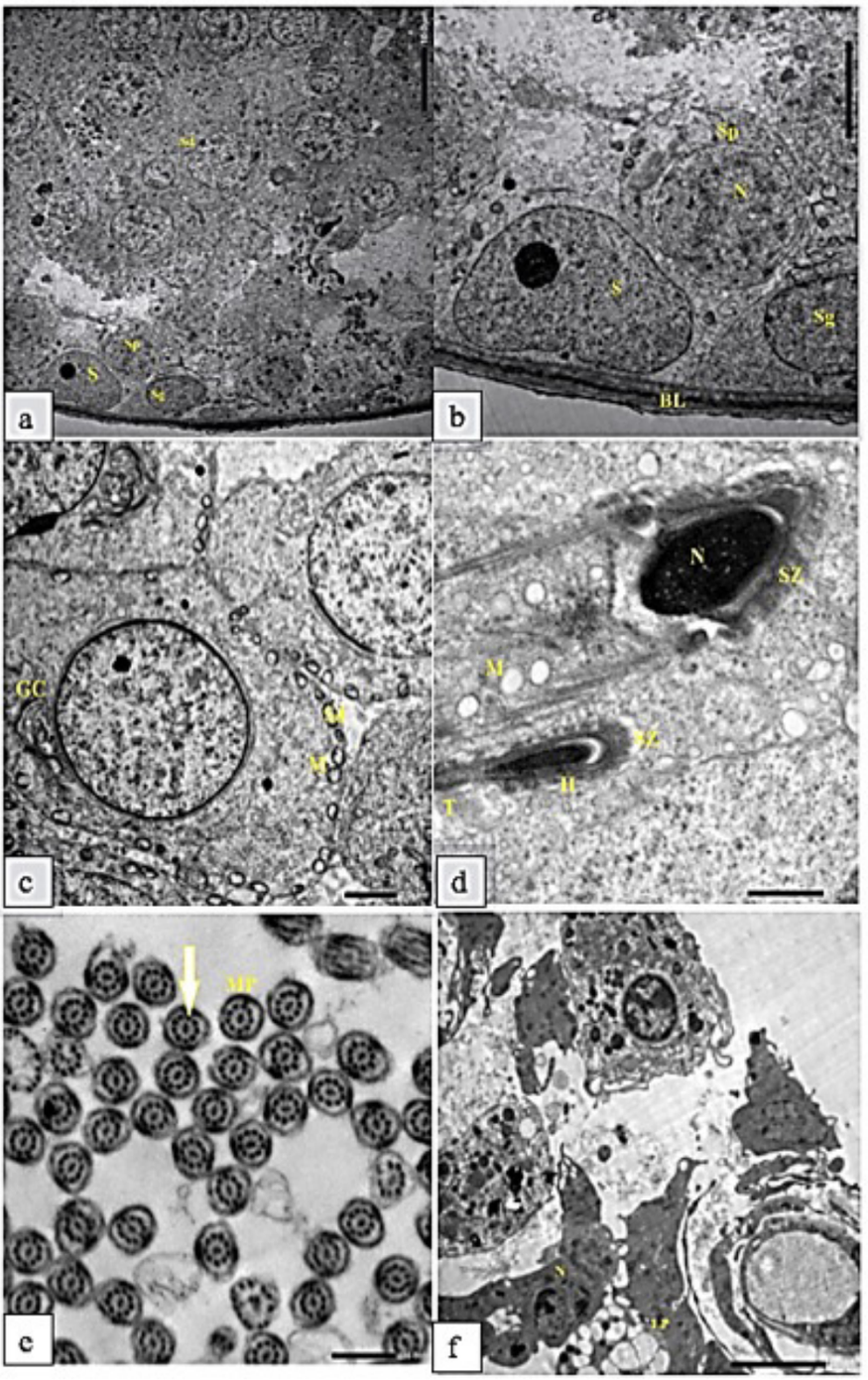

Fig. 8: a: Electron photomicrographs of control rat seminiferous tubules revealing structure of seminiferous tubules, spermatogonia (Sg) and Sertoli cell (S) are resting on basement membrane. Also it shows structure of primary spermatocyte (Sp) and spermatid (Sd). (Osmium Mic Mag X 500)
b: Electron photomicrographs of control rat seminiferous tubules revealing Sertoli cell (S) with intended nucleus, spermatogonium (Sg) resting on the basal lamina (BL) and primary spermatocytes (Sp) with its large rounded nucleus (N). (Osmium Mic Mag X 1500)
c: Electron photomicrographs of control rat seminiferous tubules revealing spermatid (Sd) with the regularly arranged mitochondria (M) at the periphery of the spermatid's cytoplasm. Notice the Golgi complex (GC) containing granules. (Osmium Mic Mag X 2000)
d: Electron photomicrographs of control rat seminiferous tubules revealing structure of mature spermatozoon (SZ) formed of head (H) that contains pyriform condensed nucleus (N); and tail (T) that contains mitochondria (M). (Osmium Mic Mag X 6000)
e: Electron photomicrographs of control rat seminiferous tubules revealing transverse section in the middle piece of tail of sperm (MP), showing nine doublets of microtubules; 2 central singlets of microtubules (yellow arrow). (Osmium Mic Mag X 10000)
f: Electron photomicrographs of control rat seminiferous tubules revealing structure of interstitial cell of Leydig normal profile of interstitial of Leydig with its characteristic irregular nucleus (N) and dark cytoplasm with lipid droplets (LP). (Osmium Mic Mag X 1500)

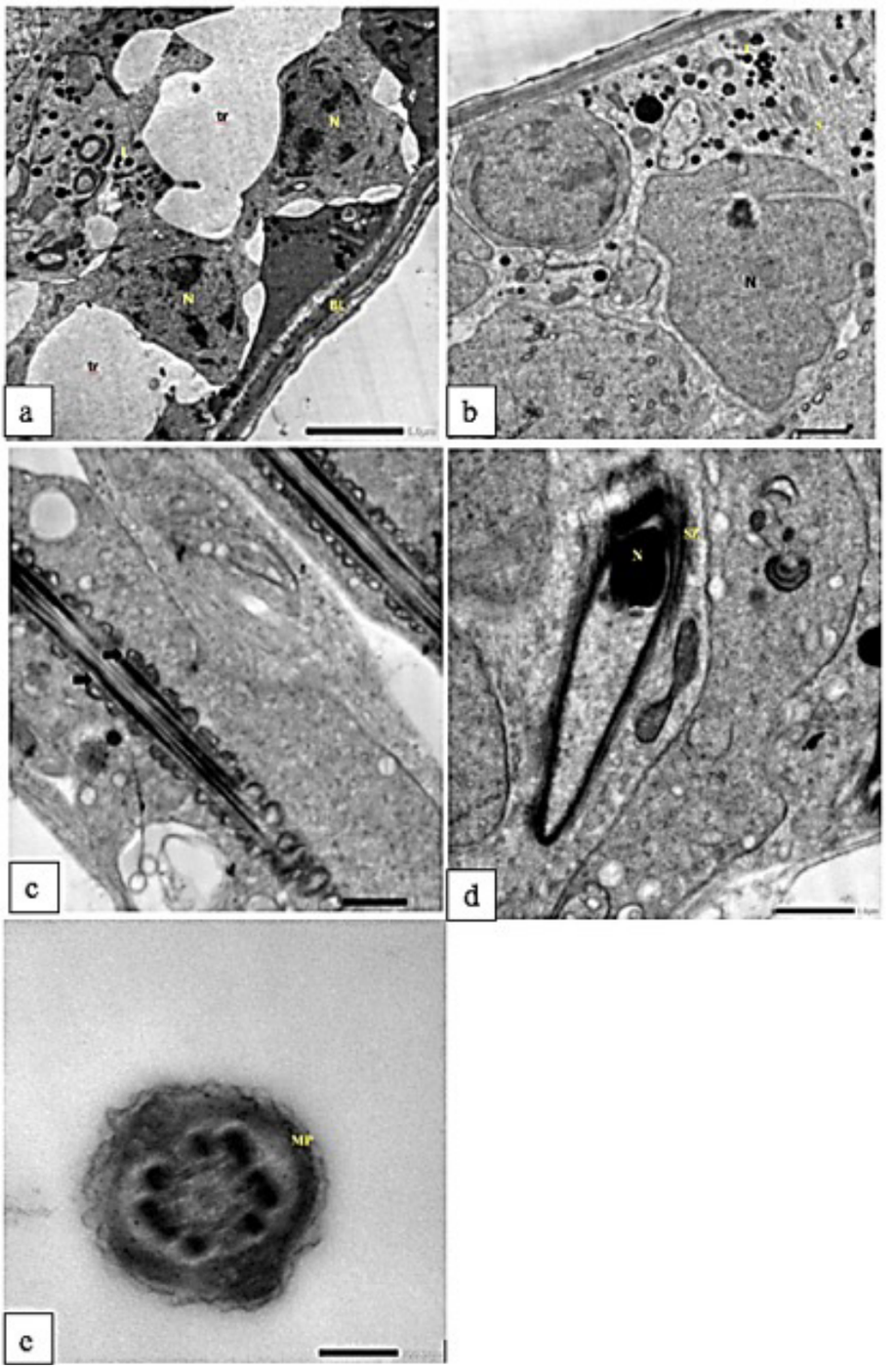

Fig. 9: a: Electron photomicrographs of group II seminiferous tubules revealing thickened basement membrane (BM), Sertoli cell with large number of lysosomes (L), annular mitochondria (m), abnormal nucleus (N). transudate present between cells making wide intercellular spaces (tr). (Osmium Mic Mag X 1500)
b: Electron photomicrographs of Group II rat seminiferous tubules revealing structure of Sertoli cell (S) with large number of lysosomes (L). (Osmium Mic Mag X 2000)
c: Electron photomicrographs of Group II rat seminiferous tubules revealing structure of middle piece of sperm with abnormal mitochondrial sheath around it (M).(Osmium Mic Mag X 5000)
d: Electron photomicrographs of Group II rat seminiferous tubules revealing shape of spermatozoa (SZ) with abnormal head containing dark nucleus (N). (Osmium Mic Mag X 6000)
e: Electron photomicrographs of Group III rat seminiferous tubules revealing structure of middle piece of sperm (MP) with abnormal number of microtubules. (Osmium Mic Mag X 30000)

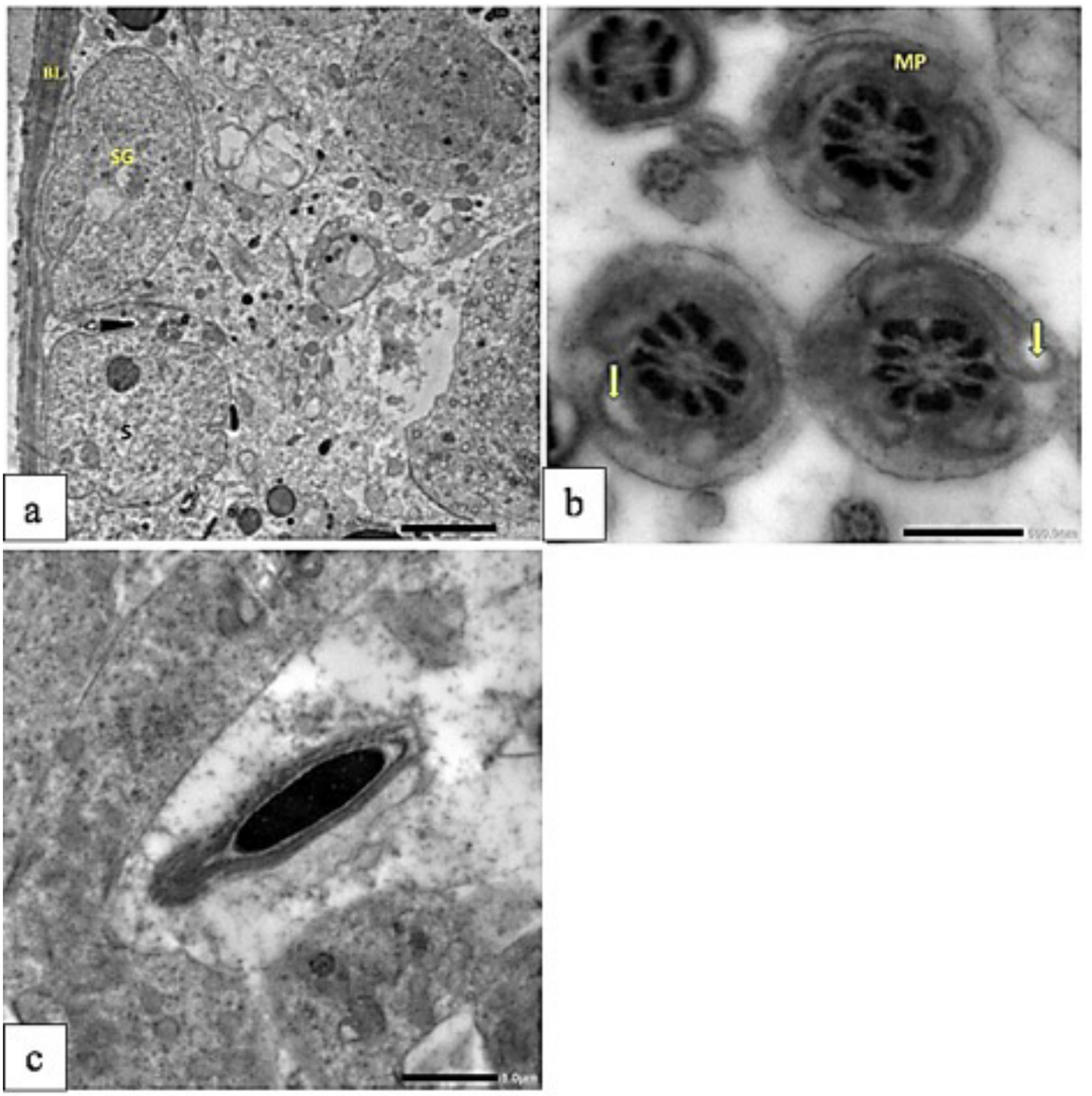

Fig. 10: a: Electron photomicrographs of Group IV rat seminiferous tubules revealing irregular basal lamina (BL) with spermatogonia (SG) and low settled Sertoli cell (S) resting on it. (Osmium Mic Mag X 1200)
b: Electron photomicrographs of Group IV rat seminiferous tubules revealing normal structure of middle pieces (MP) (nine doublets of microtubules with 2 singlets) in the middle) with some vacuoles (arrow). (Osmium Mic Mag X 10000)
c: Electron photomicrographs of Group IV rat seminiferous tubules revealing normal structure of sperm (head containing nucleus) with defective tail. (Osmium Mic Mag X 6000)

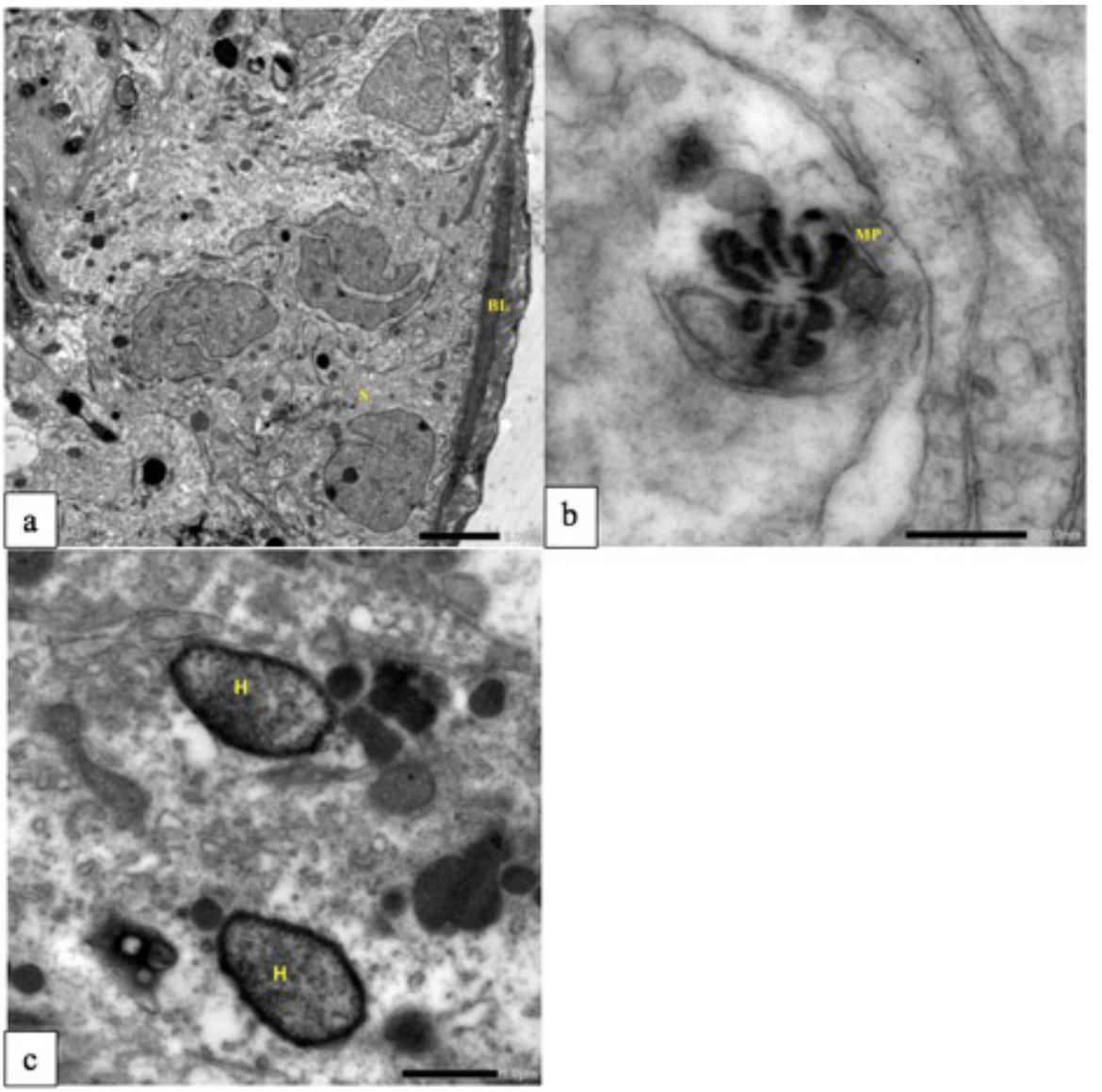

Fig. 11: a: Electron photomicrographs of Group IV rat seminiferous tubules revealing abnormal situation (low settled) multiple Sertoli cells (S) resting on abnormal thickened basal lamina (BL)(Osmium Mic Mag X 1000)
b: Electron photomicrographs of Group IV rat seminiferous tubules revealing abnormal shape of microtubules forming the middle piec (Osmium Mic Mag X 15000)
c: Electron photomicrographs of Group III rat seminiferous tubules revealing defective sperm heads. (Osmium Mic Mag X 6000)

Table 1: Comparison between the different studied groups according to body weight

Body weight (g)	Group Ia (n = 10)	Group Ib (n = 10)	Group Ic (n = 10)	Group II (n = 10)	Group III (n = 10)	Group IV (n = 10)	F	*p*
At beginning								
Min. – Max.	120 - 180	120 - 178	118 - 182	120 - 190	122 - 190	122 - 190	0.023	1.000
Mean ± SD.	150 ± 21.2	150 ± 21	150 ± 21.3	152.5 ± 24.6	151.7 ± 23.2	150.8 ± 22.4		
At sacrification								
Min. – Max.	195 – 265	195 - 265	196 - 267	115 - 185	210 - 260	165 - 225	19.576*	<0.001*
Mean ± SD.	230 ± 20	230 ± 20.1	230 ± 19.9	170 ± 19.7	240 ± 20.3	190 ± 20.5		
Sig. bet. grps.	p_1=1.000,p_2=1.000,p_3=1.000,p_4<0.001*,p_5=0.874,p_6=0.001*,p_7<0.001*,p_8=0.243,p_9<0.001*							
t (p_0)	9.600 (<0.001*)	9.921 (<0.001*)	9.511 (<0.001*)	1.997(0.077)	10.851(<0.001*)	4.267 (0.002*)		

SD: Standard deviation t: Paired t-test F: F for ANOVA test, Pairwise comparison bet. each 2 groups was done using Post Hoc Test (Tukey)
p: *p value* for comparing between the studied groups p_0: *p value* for comparing between At beginning and At sacrification in each group
p_1: *p value* for comparing between Group Ia and Group Ib p_2: *p value* for comparing between Group Ia and Group Ic
p_3: *p value* for comparing between Group Ib and Group Ic p_4: *p value* for comparing between Group Ia and Group II
p_5: *p value* for comparing between Group Ib and Group III p_6: *p value* for comparing between Group Ic and Group IV
p_7: *p value* for comparing between Group II and Group III p_8: *p value* for comparing between Group II and Group IV
p_9: *p value* for comparing between Group III and Group IV
*: Statistically significant at $p \leq 0.05$
Group Ia: Control with saline Group Ib: Control with folic acid Group Ic: Control with Ator
Group II: DOX Group III: DOX + Folic acid Group IV: DOX + Ator

Table 2: Comparison between the different studied groups according to testis weight and testis weight to body weight ratios (%)

	Group Ia (n = 10)	Group Ib (n = 10)	Group Ic (n = 10)	Group II (n = 10)	Group III (n = 10)	Group IV (n = 10)	F	*p*
Right testis weight (g)								
Min. – Max.	1.30 – 1.60	1.27 – 1.63	1.28 – 1.62	0.80 – 1.30	1.30 – 1.59	1.0 – 1.40	33.211*	<0.001*
Mean ± SD.	1.50 ± 0.11	1.50 ± 0.12	1.50 ± 0.11	1.0 ± 0.15	1.52 ± 0.08	1.17 ± 0.15		
Sig. bet. grps.	p_1=1.000,p_2=1.000,p_3=1.000,p_4<0.001*,p_5=1.000,p_6<0.001*,p_7<0.001*,p_8=0.034*,p_9<0.001*							
Left testis weight (g)								
Min. – Max.	1.30 – 1.60	1.30 – 1.61	1.30 – 1.62	0.90 – 1.10	1.30 – 1.59	1.10 – 1.30	62.796*	<0.001*
Mean ± SD.	1.48 ± 0.09	1.48 ± 0.10	1.49 ± 0.10	0.99 ± 0.07	1.52 ± 0.08	1.18 ± 0.08		
Sig. bet. grps.	p_1=1.000,p_2=1.000,p_3=1.000,p_4<0.001*,p_5=0.932,p_6<0.001*,p_7<0.001*,p_8<0.001*,p_9<0.001*							
Average testis weight (g)								
Min. – Max.	1.35 – 1.60	1.34 – 1.61	1.35 – 1.61	0.85 – 1.15	1.30 – 1.59	1.05 – 1.35	62.631*	<0.001*
Mean ± SD.	1.49 ± 0.08	1.49 ± 0.09	1.50 ± 0.08	1.0 ± 0.10	1.52 ± 0.08	1.18 ± 0.09		
Sig. bet. grps.	p_1=1.000,p_2=1.000,p_3=1.000,p_4<0.001*,p_5=0.983,p_6<0.001*,p_7<0.001*,p_8<0.001*,p_9<0.001*							
Testis weight to body weight ratios (%)								
Min. – Max.	0.58 – 0.74	0.59 – 0.74	0.58 – 0.77	0.49 – 0.96	0.51 – 0.76	0.51 – 0.79	0.722	0.610
Mean ± SD.	0.65 ± 0.05	0.65 ± 0.05	0.65 ± 0.05	0.60 ± 0.14	0.64 ± 0.08	0.62 ± 0.08		

SD: Standard deviation t: Paired t-test F: F for ANOVA test, Pairwise comparison bet. each 2 groups was done using Post Hoc Test (Tukey)
p: *p value* for comparing between the studied groups p_0: *p value* for comparing between At beginning and At sacrification in each group
p_1: *p value* for comparing between Group Ia and Group Ib p_2: *p value* for comparing between Group Ia and Group Ic
p_3: *p value* for comparing between Group Ib and Group Ic p_4: *p value* for comparing between Group Ia and Group II
p_5: *p value* for comparing between Group Ib and Group III p_6: *p value* for comparing between Group Ic and Group IV
p_7: *p value* for comparing between Group II and Group III p_8: *p value* for comparing between Group II and Group IV
p_9: *p value* for comparing between Group III and Group IV
*: Statistically significant at $p \leq 0.05$
Group Ia: Control with saline Group Ib: Control with folic acid Group Ic: Control with Ator
Group II: DOX Group III: DOX + Folic acid Group IV: DOX + Ator

Table 3: Comparison between the different studied groups according to biochemical assay

	Group Ia (n = 10)	Group Ib (n = 10)	Group Ic (n = 10)	Group II (n = 10)	Group III (n = 10)	Group IV (n = 10)	F	*p*
LH (IU/L)								
Min. – Max.	0.46 – 0.52	0.45 – 0.53	0.45 – 0.53	1.07 – 1.17	0.52 – 0.66	1.13 – 1.27	832.510*	<0.001*
Mean ± SD.	0.49 ± 0.02	0.49 ± 0.03	0.49 ± 0.03	1.12 ± 0.03	0.59 ± 0.05	1.20 ± 0.05		
Sig. bet. grps.	p_1=1.000,p_2=1.000,p_3=1.000,p_4<0.001*,p_5<0.001*,p_6<0.001*,p_7<0.001*,p_8<0.001*,p_9<0.001*							
Testosterone (ng/ml)								
Min. – Max.	3.90 – 4.50	3.90 – 4.48	3.95 – 4.52	1.08 – 1.12	3.74 – 4.30	1.68 – 1.92	716.285*	<0.001*
Mean ± SD.	4.20 ± 0.20	4.20 ± 0.20	4.20 ± 0.20	1.10 ± 0.01	4.02 ± 0.20	1.80 ± 0.10		
Sig. bet. grps.	p_1=1.000,p_2=1.000,p_3=1.000,p_4<0.001*,p_5=0.173,p_6<0.001*,p_7<0.001*,p_8<0.001*,p_9<0.001*							
Total antioxidants								
Min. – Max.	48.0 – 62.0	47.0 – 62.0	47.0 – 63.0	19.0 – 25.0	50.0 – 64.0	28.0 – 42.0	99.027*	<0.001*
Mean ± SD.	55.0 ± 5.02	55.0 ± 5.01	55.0 ± 5.03	22.0 ± 2.0	57.0 ± 4.94	35.0 ± 4.94		
Sig. bet. grps.	p_1=1.000,p_2=1.000,p_3=1.000,p_4<0.001*,p_5=0.926,p_6<0.001*,p_7<0.001*,p_8<0.001*,p_9<0.001*							

SD: Standard deviation t: Paired t-test F: F for ANOVA test, Pairwise comparison bet. each 2 groups was done using Post Hoc Test (Tukey)
p: *p value* for comparing between the studied groups p_0: *p value* for comparing between At beginning and At sacrification in each group
p_1: *p value* for comparing between Group Ia and Group Ib p_2: *p value* for comparing between Group Ia and Group Ic
p_3: *p value* for comparing between Group Ib and Group Ic p_4: *p value* for comparing between Group Ia and Group II
p_5: *p value* for comparing between Group Ib and Group III p_6: *p value* for comparing between Group Ic and Group IV
p_7: *p value* for comparing between Group II and Group III p_8: *p value* for comparing between Group II and Group IV
p_9: *p value* for comparing between Group III and Group IV
*: Statistically significant at $p \leq 0.05$
Group Ia: Control with saline Group Ib: Control with folic acid Group Ic: Control with Ator
Group II: DOX Group III: DOX + Folic acid Group IV: DOX + Ator

Table 4: Comparison between the different studied groups according to Tubules diameter and Thickness of epithelium

	Group Ia (n = 10)	Group Ib (n = 10)	Group Ic (n = 10)	Group II (n = 10)	Group III (n = 10)	Group IV (n = 10)	F	*p*
Tubules diameter (mm)								
Min. – Max.	0.30 – 0.60	0.29 – 0.59	0.28 – 0.62	0.06 – 0.10	0.20 – 0.50	0.30 – 0.55	21.411*	<0.001*
Mean ± SD.	0.45 ± 0.11	0.45 ± 0.11	0.45 ± 0.11	0.09 ± 0.02	0.34 ± 0.10	0.42 ± 0.10		
Sig. bet. grps.	p_1=1.000,p_2=1.000,p_3=1.000,p_4<0.001*,p_5=0.140,p_6=0.983,p_7<0.001*,p_8<0.001*,p_9=0.459							
Thickness of epithelium (mm)								
Min. – Max.	0.08 – 0.15	0.08 – 0.15	0.08 – 0.15	0.07 0.10 –	0.08 – 0.15	0.08 – 0.15	1.271	0.290
Mean ± SD.	0.11 ± 0.03	0.11 ± 0.03	0.11 ± 0.03	0.09 ± 0.01	0.11 ± 0.03	0.11 ± 0.03		

SD: Standard deviation t: Paired t-test F: F for ANOVA test, Pairwise comparison bet. each 2 groups was done using Post Hoc Test (Tukey)
p: *p value* for comparing between the studied groups p_0: *p value* for comparing between At beginning and At sacrification in each group
p_1: *p value* for comparing between Group Ia and Group Ib p_2: *p value* for comparing between Group Ia and Group Ic
p_3: *p value* for comparing between Group Ib and Group Ic p_4: *p value* for comparing between Group Ia and Group II
p_5: *p value* for comparing between Group Ib and Group III p_6: *p value* for comparing between Group Ic and Group IV
p_7: *p value* for comparing between Group II and Group III p_8: *p value* for comparing between Group II and Group IV
p_9: *p value* for comparing between Group III and Group IV
*: Statistically significant at $p \leq 0.05$
Group Ia: Control with saline Group Ib: Control with folic acid Group Ic: Control with Ator
Group II: DOX Group III: DOX + Folic acid Group IV: DOX + Ator

DISCUSSION

Malignancy is a worldwide rapidly increasing worldwide serious health problem with emergent synthesis and use of many anti-cancerous agents. Doxorubicin HCl was the first liposomal encapsulated anticancer drug which received clinical approval against tumors including solid tumors, blood diseases and lymphomas. The mechanism of action of doxorubicin is controversial however 2 possible mechanisms would explain its anti-cancerous activity and also are attributable to possible undesirable concomitant organs toxicity including testicular damage. One theory supports that the drug interacts with the DNA of the cells by intercalation and thus inhibition of macromolecular synthesis. Another hypothesis emphasizes that the drug generates free radicals which induces DNA and cell membrane damage with undesired side effects in remote organs other than the target one with malignancy. The unhappy incidence of organs toxicity necessitates the concomitant administration of some protective agents[25].

Folic acid and atorvastatin are among the protective agents that could be of value in ameliorating the drug induced testicular toxicity and this effect might be dose dependant, thus the current work aimed to compare their protective value at studied doses and schedule of administration in doxorubicin induced testicular damage in adult male albino rats[16,19].

The anatomical results showed that both pre-euthanization body weight and mean post- euthanization testicular weight are much lower in doxorubicin treated animals. The best results – as compared with the drug naive group – was shown in those received folic acid with Doxorubicin. Atorvastatin and doxorubicin treated animals, although their weights are much higher than those received Doxorubicin no protection, still showed lower figures than both the control and folic acid protected group. The anatomical results went hand in hand with the immunohistochemical and histological results.

S V V S Ramanjaneyulu *et al*, 2013, in their work on Atorvastatin study in hearts and testes of DOX-treated mice proved that former has an evident protective role in the hearts and testes of DOX-treated mice mostly due to the refinement of the oxidative stress reaction, DNA and cellular damage[26]. This result agrees with the work of Ramezan Ali Naeimi *et al*. in 2017, who studied the protective role of Atorvastatin in testicular damage induced by irradiation rather than chemotherapy. However, the protective role of Atorvastatin shown in bothstudies disagrees with the results of the present work that showed limited protection induced by concomitant administration of both drugs. The finding may be explained by the difference in the dose of Atorvastatin intake as in the current work Atorvastatin was taken in much lower dose, 10mg/ kg BW, comparable to the work of Ramezan Ali Naeimi *et al* who use 50 mg/ kg BW[27].

Results of the present study agree with those of Hanae Pons-Rejrajietal, 2014. Their study on human male adults showed that administration of Atorvastatin for 5 months has a significant effect on sperm parameters and semen composition for at least 3 months after withdrawal, a result that supports the current findings[28].

In 2018, Samar Sakr etal, proved the protective effect of folate against methomyl induced toxicity on the testes of male adult albino rats treated with the drug. The valuable role shown by histological and immunohistochemical study mimic the present results[29]. O Gules *et al*, in 2019, also proves that folic acid has a protective effect on testicular toxicity induced by bisphenol-A in male Wistar rats. The study mimics the findings of the present work the level of serum testosterone and number of viable sperms was significantly lower, while the head, midpiece and total sperm abnormalities were significantly higher in the BPA treated group compared to control, FA, FA + BPA groups[30].

The mechanism of action of folic acid as protective of testicular toxicity and improves fertility may be attributed to decrease elevated MDA levels. Also, it might acts as an antioxidant and has a free radical scavenging action through increase SOD and GPX levels. A finding and explanation reported by Aris Fakouri *et al*, 2017, who recommended the administration of Folic acid as an important therapeutic intervention in testicular Ischemic /Reperfusion injury[31].

For histologic results in this study, it was noted that the diameter of seminiferous tubules, the height of the spermatogenic cells were markedly decreased, while the width of the interstitial spaces and the thickness of the tubular basement membranes were markedly increased in the group received Doxorubicin than that of the control group. Those changes were markedly improved in the group received folic acid. Mild but not significant improvement was detected in the group received atorvastatin. On the spermatogenesis level mature sperms could still be retrieved in the low dose group however maturation arrest at the level of spermatogonia or spermatocytes was noted in the high dose group. On the other hand, complete spermatogenesis with many mature sperms were restored in the groups received folic acid and atorvastatin treatment. Similar results were reported by Sakai *et al*, 2018, but they found mature sperms in doxorubicin treated group may be due to the different dose or duration of the drug from that used in the present study[32]. In the study performed by Silvaa *et al*, 2018, sections of the high dose treated group exhibited massive germ cell loss, disorganized seminiferous epithelium, missing germ cell layers and, focal intraepithelial vacuolization. Similar results were attributed in the current work, that are mostly related to apoptosis of the spermatogenic cells. This was of course, confirmed by bcl2 staining done in this study[33].

For the immunostaining results revealed gradual decrease in bcl2 staining from the control to low dose then to high dose treatment, then gradual increase in atorvastatin to the folic acid group. This indicated that chemotherapy induced apoptosis has been improved in the folic acid treated group than that in the atorvastatin group. Similarly,

inhibition of the apoptosis induced chemotherapy was reported by several studies in response to numerous drugs including folic acid and atorvastatin[34,35].

Doxorubicin has a serious destructive effect on male seminiferous tubules and spermatogenesis. Folic acid intake in adequate doses has a protective effect. Atorvastatin has a limited protective role on this destructive effect however the later could be dose dependent and may show better results with administration of higher doses. It is highly recommended to include folic acid in management protocol of patients under chemotherapy especially if Doxorubicin is used. Routine administration of Folic acid would be of valuable protective effect on their testes especially in young age and actively fertile males.

CONCLUSION

Doxorubicin has a serious destructive effect on male seminiferous tubules and spermatogenesis. Folic acid intake in adequate doses has a protective effect. Atorvastatin has a limited protective role on this destructive effect however the later could be dose dependent and may show better results with administration of higher doses. It is highly recommended to include folic acid in management protocol of patients under chemotherapy especially if Doxorubicinis used. Routine administration of Folic acid would be of valuable protective effect on their testes especially in young age and actively fertile males. Protective Role of Folic Acid Versus Atorvastatin on Experimentally Induced Doxorubicin Toxicity on Testes of Adult Albino Rats: Anatomical, Histological and Immunoassay Study.

CONFLICT OF INTERESTS

There are no conflicts of interest.

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الملخص العربى

التأثير الوقائي لحمض الفوليك مقابل الاتورفاستاتين لسمية الخصية التجريبية الناتجة عن عقار الدوكسوروبوسين في الجرذان البيضاء البالغة: دراسة تشريحية ونسيجية وبالمقايسة المناعية

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الخلفية: ان العقم مشكلة صحية شائعة في جميع أنحاء العالم والتي قد تحدث بسبب العديد من العوامل. تعد مضادات السرطان من بين الأسباب الأكثر شيوعًا للعقم. تستخدم الدوكسوروبيسين والأدوية المماثلة له على نطاق واسع كعلاج للسرطان و بشكل رئيسي يستخدم عقار الدوكسوربوسين في علاج الأورام الصلبة وسرطان الغدد الليمفاوية وسرطان الدم. تُعزى إلى العقم عند الذكور بسبب تسمم الخصية، ولذا يستلزم لعلاج تلك المشكلة الاستخدام المصاحب للعوامل الوقائية خاصة مع زيادة متوسط العمر المتوقع لمرضى السرطان المرتبطين برعاية صحية أفضل.

الهدف من البحث: تم تصميم الدراسة الحالية لمقارنة التأثير الوقائي المحتمل لكل من حمض الفوليك وعقار الأتورفاستاتين في التسمم الخصوي الناجم عن دوكسوروبيسين في ذكور الجرذان البيضاء البالغة.

المواد وطرق البحث: أجريت التجربة على ستين من ذكور الجرذان البالغة مقسمة عشوائياً وبالتساوي كمجموعة ضابطة (بما في ذلك ثلاث مجموعات فرعية ، ١٠ فئران لكل منها ، لم تتلق أي علاج ، ١٠ جرذان تلقت أتورفاستاتين و ١٠ جرذان حصلت على حمض الفوليك) ، المجموعة المعالجة بالدوكسوروبيسين ومجموعتين أخريين تلقتا حمض الفوليك وعقار أتورفاستاتين في نفس الوقت كعوامل وقائية.

النتائج: تم تقييم النتائج تشريحيًا (وزن الجسم ، ووزن الخصية والجسم: نسبة وزن الخصية) ، وبالمقايسة البيوكيميائية (مستوى هرمون LH و هرمون التستوستيرون ومضادات الأكسدة بالدم) ، ونسيجيا عن طريق الفحص المجهري باليكروسكوب الضوئي والالكتروني الناقل (TEM).. هذا و قد أشارت جميع النتائج إلى أن حمض الفوليك له دور وقائي مهم في التسمم الخصوي الناجم عن دوكسوروبيسين و إن إعطاء أتورفاستاتين بالجرعة المستخدمة له دور محدود في الحماية.

الاستنتاج: أوصت النتائج باستخدام حمض الفوليك كعامل وقائي روتيني لأولئك الذين يخضعون لعلاج دوكسوروبيسين خاصة عند الذكور أثناء الطفولة والشباب.