

HISTOLOGICAL PROTECTIVE EFFECT OF WHEAT GERM OIL VERSUS ZINC ON FLUOXETINE –DAMAGED TESTES IN ALBINO RATS

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ABSTRACT:

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Background: Fluoxetine is anti-depressive drug which induces oxidative stress in different organs, Wheat germ oil and zinc have anti-inflammatory and antioxidant effects.

Aim of the Study: to assess the histological effect of wheat germ oil (WGO) versus zinc on fluoxetine induced testicular injury in albino rats.

Material & Methods: forty male albino rats were divided into four equal groups: group I (Control); group II: were received 10 mg/kg fluoxetine for 28 days; group III: administered fluoxetine + WGO at dose of 68.75 mg/kg; group IV: were received fluoxetine + zinc aspartate at dose of 30 mg/kg. samples of the testes were exposed to histological, ultrastructural and immunostaining examination.

Results: fluoxetine significantly altered testicular structure. The seminiferous tubules were widely separated with significant decrease in their diameter. Separation of basal compartment of seminiferous tubules from adluminal compartment with cellular loss. This was confirmed by significant increase in Bax immunostaining. Widening of interstitial space with collagen fiber deposition was noticed. There was improvement of all histological aspects in WGO and zinc treated groups. This improvement was more evident in WGO treated group. The results were confirmed by significant increase in tubular diameter with more or less normal epithelial lining. Significant decrease in Bax immunoreaction was detected. Interstitial tissue was normal. These data conclude that WGO augments the protection against fluoxetine induced testicular damage.

Conclusion: fluoxetine induced oxidative damage in rat testis. We concluded that both WGO and zinc have protective role in ameliorating fluoxetine -associated testicular injury. However, WGO has better effects on all aspects.

Keywords: fluoxetine, wheat germ oil, zinc, testis, Bax, ultrastructure.

INTRODUCTION:

Around 350 million individuals worldwide suffer from depression and anxiety disorders, which are major public health conditions^[1]. The World Health organization has stated that the problem of depression will increase worldwide and

expected to be the second biggest problem after cardiac ischemic disorders by 2020^[2].

At that point, it's important to stop and think because as more individuals experience anxiety & depression, the consumption of antidepressant pills will increase

significantly, and their usage will increase globally^[3].

A popularly prescribed selective serotonin reuptake inhibitor for therapeutic purposes in depression & anxiety is fluoxetine (FLX)^[4].

In rats, long-term FLX consumption resulted in a reduction in spermatogenesis, follicle stimulating hormone (FSH), and reproductive organs weights^[5].

A person's lifestyle might be significantly impacted by these sexual side effects, where this leads to decreased drug compliance with less successful treatment of the underlying psychiatric condition^[6].

The most vital component of the wheat, the wheat germ, is extracted to make wheat germ oil (WGO), a natural supplement. WGO is made up of policosanols, sterols, and other bioactive substances, such as alpha-linolenic acid (ALA), an omega-3 fatty acid, tocopherol (vitamin E), & other biologically active substances^[7].

Due to the presence of ALA, tocopherols (vitamin E), policosanols, and sterols in significant concentrations, WGO functions as anti-inflammatory and antioxidant agents^[8].

Numerous experimental research has shown that WGO functions as an additive in natural foods, health goods, and cosmetics, as well as a reproductive agent^[9].

Zinc (Zn) is one of the vital trace elements in the human body. It is commonly disseminated throughout the bodily tissues, where it plays a vital supporting role in various biochemical & physiological mechanisms^[10].

Zinc has strong antioxidant capabilities, but it is also thought to be a key regulator of the several enzymes that function in DNA replication, transcription, & protein synthesis, all of them are crucial for the growth of germinal cells^[11]. In addition, zinc was said to possess extensive anti-inflammatory effects in many bodily tissues,

maybe via controlling the expression of inflammatory mediators and pro-inflammatory cytokines^[12]. Because of all the above qualities, zinc may be thought of as the mainstay for treating testicular toxicity brought on by fluoxetine.

So, the current research was proposed to evaluate the potential protective role of WGO versus zinc in fluoxetine treated rat testis.

MATERIAL AND METHODS:

Animals: forty adult male albino Wistar rats of an average weight of 150-200 g were utilized. Animals were kept in clean plastic cages with mesh wire covers and were allowed to a free access to usual rat chow diet & tap water for the duration of the study. The animals were divided into 4 groups 10 rats each:

Group I: (control group): Rats were fed a normal chow diet for the period of the experiment (28 days).

Group II: Rats received powdered of fluoxetine (10 mg/kg) dissolved in distal water & given by intragastric feeding needle (gavages tube) for 28 days^[13].

Group III: rats co-administered wheat germ oil at dose of 68.75 mg/kg b.w with fluoxetine^[14].

Group IV: rats co-administered zinc aspartate at dose of 30 mg/kg b.w with fluoxetine^[15].

Rats were sacrificed under anaesthesia using 50 mg/kg of sodium pentobarbital.

Drugs:

1. Fluoxetine (Alzac 10mg tablets) was purchased from future pharmaceutical industries, Egypt.
2. Wheat germ oil (be active capsules, 330 mg) was purchased from Ema pharm Co., Egypt.

3. Zinc aspartate was purchased from Unizink, Koehler Pharma, Alsbach, Germany.

Histopathological analysis: The left testis, were fixed in 10% formaldehyde solution dehydrated in ethanol, embedded in paraffin wax & sectioned on 5µm thin section. They were stained with haematoxylin and eosin for light microscopic examination.

Polyclonal rabbit-anti-human (A3533 Ig fraction; Dako, Glostrup, Denmark) was used in an immunohistochemical stain of the Bcl2-associated x protein (Bax) at a dilution of 1:50^[16].

For TEM preparation, the right testis were fixed in 3% glutaraldehyde in 0.1 M Na cacodylate buffer (pH 7.0) for two hours at room temperature, bathed in the previous buffer, & post-fixed in 1% osmium tetroxide for two hours at room temperature. The specimens were dehydrated in an ethanol sequences ranging from 10% to 90% for fifteen minutes in each alcohol dilution and lastly with absolute ethanol for thirty minutes. Specimens were infiltrated with epoxy resin & acetone over a graded sequences until lastly in pure resin. Ultrathin sections were gathered on Formvar-coated Cu grids. Sections were then double stained in uranyl acetate and then lead citrate. Stained sections were detected with a JEOL JEM 1010 transmission electron microscope at 70 kV at the Regional Center for Mycology & Biotechnology (RCMB), Al-Azhar University^[17-18]. Semithin sections were stained with 1% toluidine blue/borax (pH 8.4) for two minutes and detected under microscope.

Ethical consideration:

The animal experiment was carried out at the Ain Shams University Faculty of Medicine's Research Center Institute (MASRI). It was approved by the Faculty of Medicine, Ain Shams University Research Ethics Committee (FMASU R105/2023)

organized and run under the International Council on Harmonization (ICH) and Islamic Organization for Medical Science (IOMS) guidelines, as well as the US Office for Human Research Protections and US Code of Federal Regulations and is covered by Federal Wide Assurance No. FWA 00017585

RESULTS:

Histological results:

A. Light microscopic results:

Hematoxylin and eosin staining:

Examination of H&E-stained of seminiferous tubules of group I (control group) revealed closely packed seminiferous tubules separated by slight interstitial tissue (fig. 1a). Seminefrous tubule was enclosed by regular basement membrane and was lined by spermatogenic epithelium & Sertoli cells. The spermatogenic epithelium consisted of Spermatogonia, Primary spermatocytes(basal compartment), early spermatid and sperms (adluminal compartement). The interstitium of testis(peritubular sheath) consisted of peritubular myoid cells ,blood capillaries and Leydig cells . Leydig cells had eosinophilic vacuolated cytoplasm and open face nuclei (fig. 1b). Examination of group II showed widely separated seminiferous tubules (fig. 1c). Distorted spermatogenic epithelium with pyknotic and fragmented nuclei. The tubules were resting on irregular basement membrane (fig.1e). Separation of basal compartement from adluminal compartement with sloughing of spermatogenic epithelium into the tubule's lumen were shown. Widening of peritubular sheath with abnormal cells & dilated, congested blood vessels (fig. 1 d,e). Examination of group III revealed closely packed seminiferous tubules separated by narrow interstitial tissue (fig.1f). The tubules were lined by normal spermatogenic epithelium and Sertoli cells (SC) and was surrounded by regular basement membrane. Leydig cells with eosinophilic vacuolated cytoplasm and open face nuclei were also

seen (fig. 1g). Examination of group IV showed closely packed seminiferous tubules with interstitial tissues in between (fig. 1h). Spermatogenic epithelium with fragmented nuclei was present. Sloughing and wide space of spermatogenic epithelium were still present. The basement membrane was still corrugated. Leydig cells were still seen with pyknotic and fragmented nuclei (fig. 1i).

BAX -stained sections:

Examination of group I revealed negative reaction (fig. 2a). Examination of group II revealed strong +ve brownish cytoplasmic reaction (fig. 2b). Meanwhile, examination of group III revealed weak +ve cytoplasmic reaction (fig. 2c). However, examination of group IV revealed moderate +ve cytoplasmic reaction (fig. 2d).

Semithin sections stained with toluidine blue

Examination of toluidine blue stained semithin sections of group I showed the seminiferous tubules lined with spermatogenic epithelium and Sertoli cell and was enclosed by regular basement membrane (fig. 3a). 1ry spermatocytes were the biggest cells with characteristic nucleus with condensed strands of chromatin at variable stages (fig. 3a & 3b). Early spermatid with acrosomal cap covering the anterior part of the nucleus was also seen. The interstitial tissue between the tubules containing Leydig cells and blood capillaries was seen (fig. 3b). Examination of group II revealed areas of disorganized and disrupted epithelium with wide intercellular spaces in-between within the seminiferous tubules (fig. 3c). Sertoli cell and spermatogonia resting on irregular wavy basement membrane surrounded with myoid cells (fig. 3c & 3d). Some primary spermatocytes appeared degenerated and Early spermatid with acrosomal cap was also seen, but some of them appear with abnormal location in the basal part of seminiferous tubules (fig. 3d). Many early spermatids appeared with irregular outline and without

acrosomal cap (fig. 3e). Late spermatids with diamond shaped condensed nucleus, residual bodies with vacuolations and lipofuscin brownish pigment could also be noticed (fig. 3c&3d). Group III examination showed Sertoli cell with its vesicular nucleus and spermatogonia resting on regular basement membrane (fig. 3f). 1ry spermatocytes with large nuclei with condensed strands of chromatin, Early spermatid and late spermatid appeared normal when compared to the control group (fig. 3f). There were still few intercellular spaces between the spermatogenic epithelium (fig. 3f). However, examination of group IV showed seminiferous tubules lined with spermatogenic epithelium with wide intercellular spaces (fig. 3g). Sertoli cell resting on regular basement membrane. 1ry spermatocytes were seen but some with normally appearing nucleus and others with shrunken nuclei (fig. 3g). Early spermatids with irregular outlines and late spermatids were also seen (fig. 3g).

B. Ultrastructural results:

Group I (the control group) showed spermatogonia, Primary spermatocyte and Sertoli cell with its indented euchromatic nucleus resting on regular basal lamina enclosed by myoid cells. The cytoplasm of Sertoli cell showed mitochondria, SER & fat globules of variable electron-density. Ectoplasmic specialization between Sertoli and spermatogenic epithelium was also seen (fig. 4a & 4b). Early spermatid with its large euchromatic nucleus, acrosomal vesicle spreading to cover the anterior aspect of the nucleus making acrosomal cap and peripherally organized mitochondria with clear matrix (fig. 4c). Interstitial cells of Leydig with euchromatic nucleus with thin rim of peripheral heterochromatin were seen. Their cytoplasm had elongated mitochondria, lipid droplets and smooth endoplasmic reticulum. Microvilli projecting from the surface were also seen (fig. 4d).

Group II displayed spermatogonia and Sertoli cell resting on irregular basal lamina surrounded by myoid cells. Its cytoplasm has dilated SER and some bizarre shaped mitochondria. Abnormal elongated spermatide. Loss of spermatogenic epithelium was noticed in some areas (fig. 5a). Early spermatid with abnormal dense shrunken apoptotic nucleus, and many residual bodies with vacuolations could be noticed (fig. 5b). Early spermatid with abnormal dense shrunken nucleus, electron-dense lysosomes, and areas without peripheral mitochondria. Wide intercellular spaces and many residual bodies with vacuolations could be noticed (fig. 5c). Leydig cells with shrunken nuclei, mitochondria and few dilated smooth endoplasmic reticulum were revealed (fig. 5d). Excess collagen fibers were also seen (fig. 5d).

Group III displayed spermatogonia & Sertoli cell with its identical nucleus resting on regular basal lamina bounded with myoid cells. Ectoplasmic specialization was seen between it and other spermatogenic cells (fig. 6a). 1ry spermatocyte with its characteristic nucleus was also present. Early spermatid with its euchromatic nucleus was evident. Its cytoplasm contained peripherally arranged mitochondria and some lysosomes. Acrosomal cap was covering the anterior portion of the nucleus. Few small spaces between the cells were still found (fig. 6b). Leydig cells with their identical nucleus were seen. Its cytoplasm had elongated mitochondria, SER & fat globules. Microvilli were seen protruding from the surface of the cells (fig. 6c).

Group IV displayed Sertoli cell with its identical indented nucleus resting on thickened basal lamina, surrounded by

relatively wide interstitial tissue with excess collagen fibers and ectoplasmic specialization was obvious between it and the spermatogenic epithelium. Its cytoplasm had mitochondria and slightly dilated smooth endoplasmic reticulum (fig. 7a). Early spermatid with irregular outline and its characteristic euchromatic nucleus was seen. Its cytoplasm contained peripherally arranged mitochondria in some areas and other areas without mitochondria. The acrosomal cap covering the anterior portion of the nucleus. Some irregular spaces between the cells were still found (fig. 7b). Leydig cells appeared, with their normal characteristic nuclei. The cytoplasm had elongated mitochondria, and lipid droplets. (fig. 7c).

Morphometrical and statistical results:

Mean diameter of seminiferous tubules:

Table 1 & chart 1 showing the mean diameter \pm standard deviation of seminiferous tubules in various experimental groups. Group II (diseased) exhibited a significant reduction ($P < 0.05$) in comparison to both group I and group III, meanwhile nonsignificant difference ($P > 0.05$) in comparison to group IV.

Mean optical density of BAX:

Table 2 & chart 2 showing the mean optical density of BAX \pm standard deviation in various experimental groups. Group II (diseased) exhibited a significant increase ($P < 0.05$) in comparison to all other groups. Meanwhile, group III and group IV showed a significant reduction ($P < 0.05$) in comparison to group II. However, both groups showed a significant increase in comparison to group I. Moreover, group III exhibited a significant decrease in comparison to group IV.

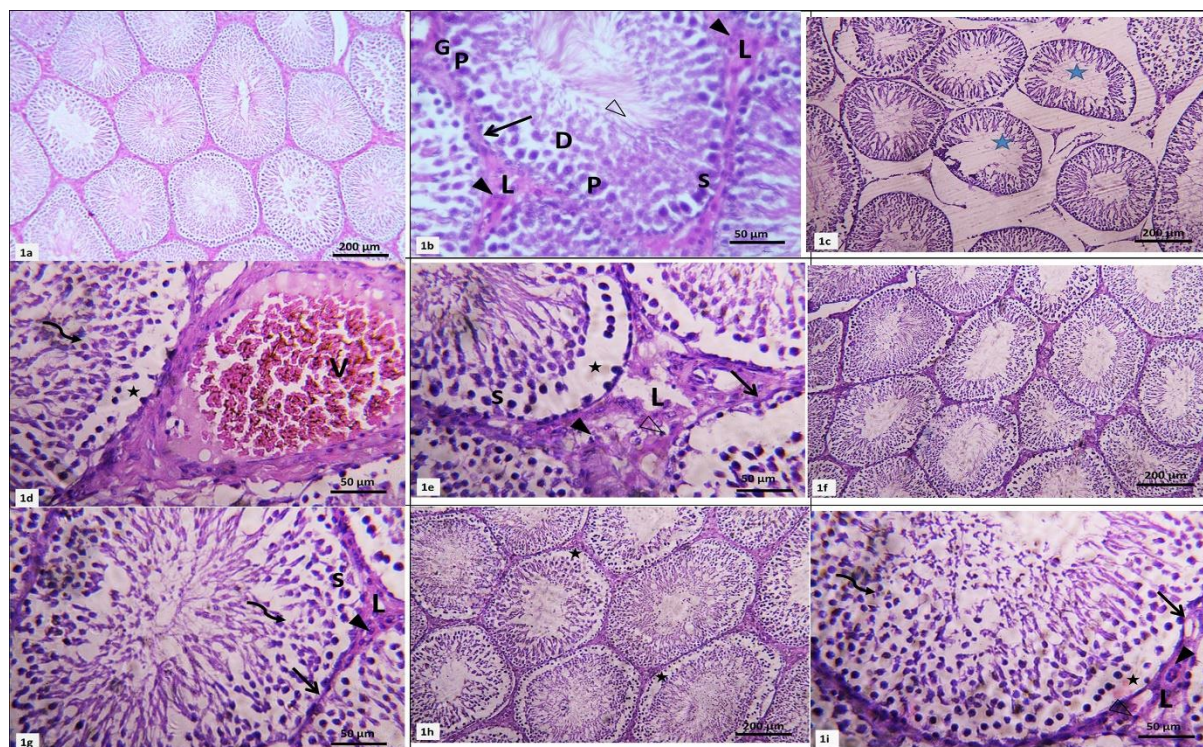


Fig. 1: photomicrographs of testis from different groups showing: (1a, 1b) group I closely packed seminiferous tubules separated by narrow interstitial tissue. Spermatogonia (G) and Sertoli cell (S) are resting on regular basement membrane (↑). Primary spermatocytes (P), early spermatid (D) and sperms in the lumen (Δ) are also seen. Notice, Leydig cells (L) with acidophilic vacuolated cytoplasm and vesicular nuclei (▲) (1c, 1d, 1e) group II widely separated seminiferous tubules. Distorted spermatogenic epithelium with pyknotic and fragmented nuclei (curved arrow) is seen. Sloughing and wide spaces between spermatogenic epithelium (black star), distorted and corrugated basement membrane (↑) are also present. Lumen of tubules are devoid of sperm (★). Leydig cells (L) are also seen with pyknotic (Δ) and fragmented nuclei (▲). Together with Congested and thickened blood vessels(v) in the interstitial tissue. (1f, 1g) group III (wheat germ oil treated) closely packed seminiferous tubules separated by narrow interstitial tissue. Sertoli cell(s) is seen resting on regular basement membrane (↑). Spermatogenic epithelium is seen with less spaces in between (curved arrow). Leydig cells (L) with acidophilic vacuolated cytoplasm and vesicular nuclei (▲) are also seen.

(1h, 1i) group IV (zinc treated) closely packed seminiferous tubules with few interstitial tissues in between. Spermatogenic epithelium with fragmented nuclei is present (curved arrow). Sloughing and wide space of spermatogenic epithelium (black star) are still seen. The basement membrane is still corrugated (↑). Leydig cells (L) are still seen with pyknotic (Δ) and fragmented nuclei (▲). H & E X100, 400.

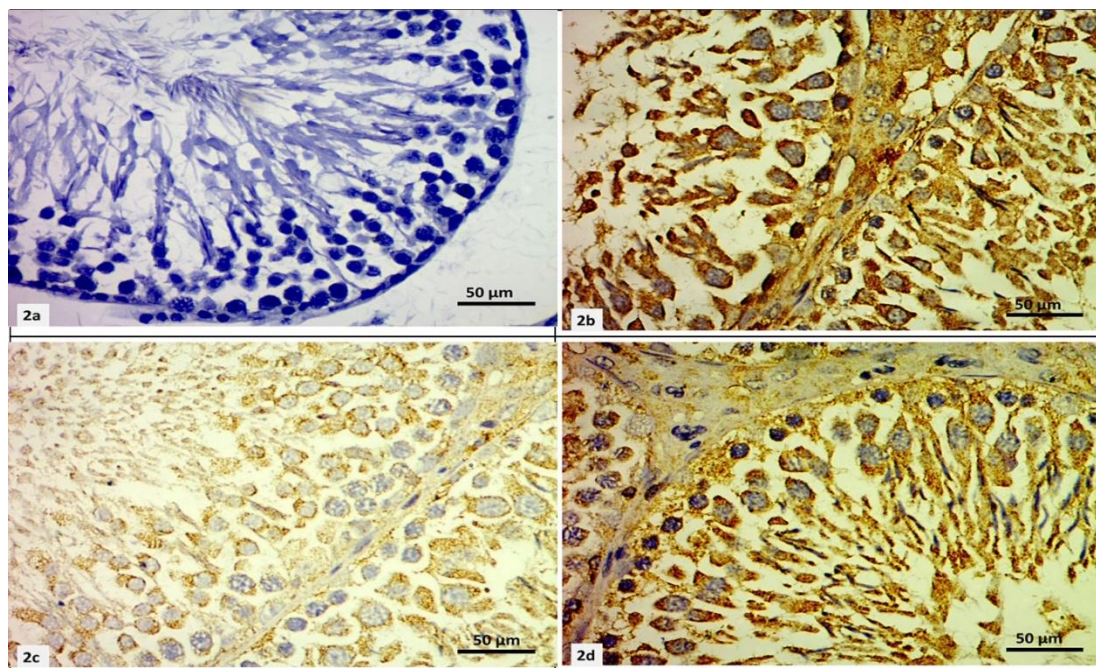


Fig. 2: photomicrographs of testis from different groups showing: (2a) group I negative BAX reaction. (2b) group II strong positive cytoplasmic brownish reaction of BAX in germinal epithelium and leydig cells. (2c) group III (WGO treated) weak positive cytoplasmic brownish reaction of BAX. (2d) group IV (zn treated) moderate to strong positive cytoplasmic brownish reaction of BAX in germinal epithelium and leydig cells. BAX X400.

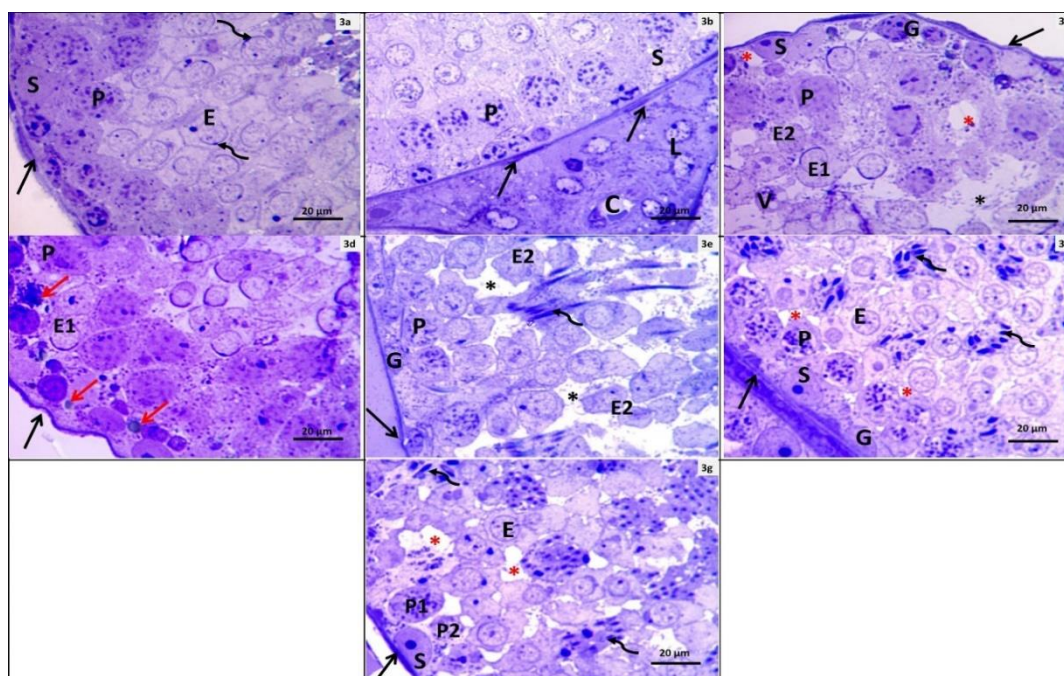


Fig. 3: photomicrographs of testis from different groups showing: (3a and 3b) group I a part of the seminiferous tubule of the control group lined with spermatogenic epithelium. Sertoli cell(S) and spermatogonia(G) are resting on regular basement membrane surrounded with myoid cells (↑). Primary

spermatocytes are seen (P), the largest cell with characteristic nucleus with condensed strands of chromatin. Early spermatid (E) with acrosomal cap (curved arrow) covering the anterior part of the nucleus is also seen. The interstitial tissue is seen between the tubules containing Leydig cells(L) and blood capillaries(c). (3c, 3d and 3e) group II parts of seminiferous tubules with areas of disorganized and disrupted epithelium (*) and wide intercellular spaces in-between(red*). Sertoli cell(S) and spermatogonia (G) are resting on irregular wavy basement membrane (↑) surrounded with myoid cells. Some primary spermatocytes (P) appear degenerated. Early spermatid(E1) with acrosomal cap is also seen, but some of them appear with abnormal location in the basal part of seminiferous tubules. Many early spermatids(E2) are with irregular outline and without acrosomal cap. Late spermatid (curved arrow) with diamond shaped condensed nucleus, residual bodies with vacuolations (V) and with lipofuscin brownish pigment (red arrow) can be noticed. (3f) group III a part of seminiferous tubule. Sertoli cell (S) with its vesicular nucleus and spermatogonia (G) are resting on regular basement membrane (↑). Primary spermatocytes (P) are seen with large nuclei with condensed strands of chromatin. Early spermatid(E) and late spermatid (curved arrow) are also seen. There are still few intercellular spaces (red*) between the spermatogenic epithelium. (3g) group IV a part of seminiferous tubule lined with spermatogenic epithelium with wide intercellular spaces (red*). Sertoli cell (S) is resting on regular basement membrane (↑). Primary spermatocytes are seen but some with normally appearing nucleus(P1) and others with shrunken nuclei(P2). Early spermatid (E) with irregular outlines and late spermatid (curved arrow) are also seen. Toluidine blue stain X1000.

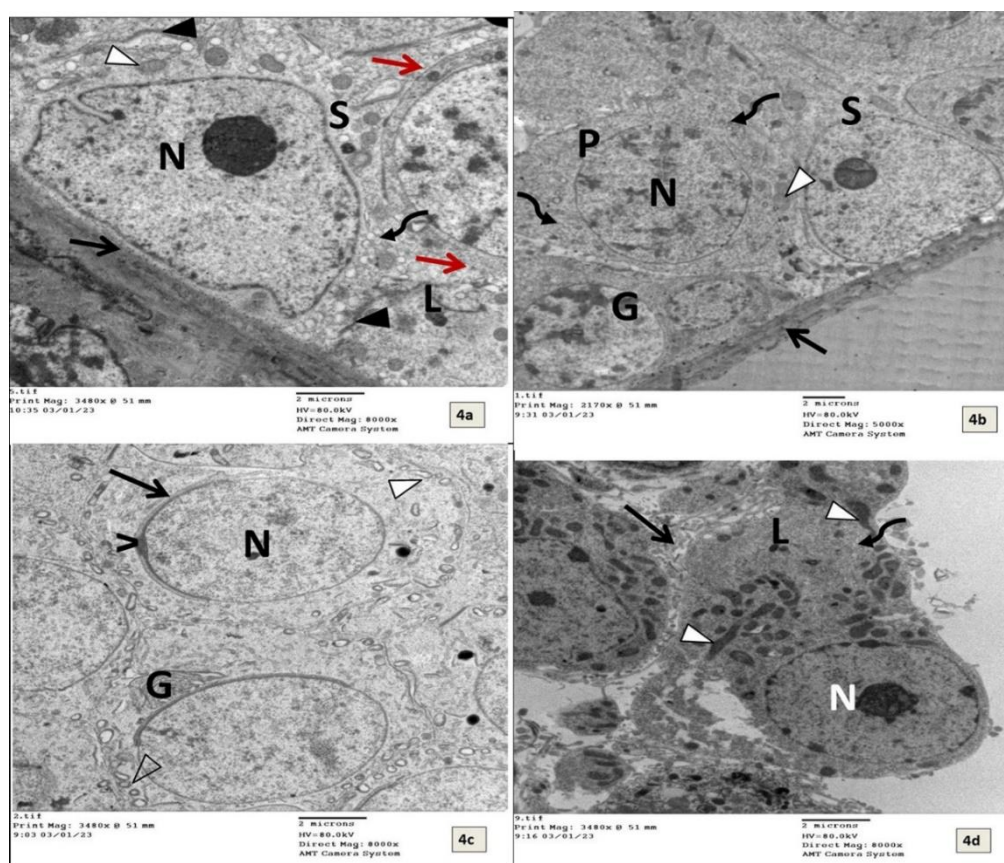


Fig. 4: Electron micrographs of control group:(4a) showing columnar Sertoli cell(s) with its indented euchromatic nucleus(N) resting on regular basement membrane (↑)with extensive lateral and apical infoldings (red arrow). The cytoplasm of Sertoli cell showing mitochondria (Δ), smooth endoplasmic reticulum (curved arrow) and lipid droplets(L) of variable electron-density. Ectoplasmic specialization between Sertoli and spermatogenic epithelium is also seen (▲). (4b): showing Sertoli cell(s), Spermatogonia(G) resting on regular basement membrane (↑). Primary spermatocyte (p) with its characteristic nucleus(N), its cytoplasm containing mitochondria (Δ) and smooth endoplasmic reticulum

(curved arrow). (4c): showing early spermatid with its large euchromatic nucleus(N), acrosomal vesicle (v) spreading to cover the anterior aspect of the nucleus making acrosomal cap (↑) and peripherally arranged mitochondria with clear matrix (Δ). (4d): showing interstitial cell of Leydig with its euchromatic nucleus with thin rim of peripheral heterochromatin(N). the cytoplasm contains elongated mitochondria (Δ), lipid droplets (L) and smooth endoplasmic reticulum (curved arrow). Microvilli (↑) projecting from its surface are also seen. TEM (a, c and d X 8000) (b X 5000).

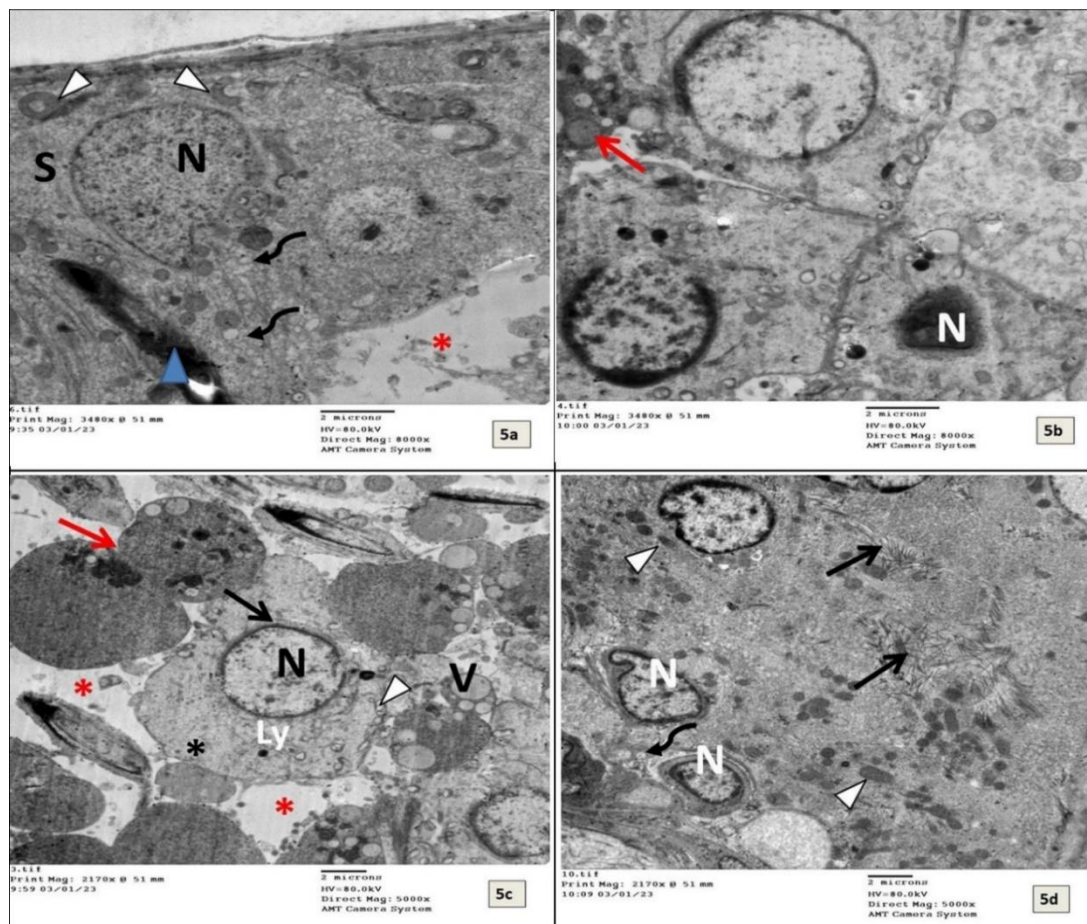


Fig. 5: Electron micrographs of group II:(5a) showing columnar Sertoli cell(s) with its indented nucleus(N) and destructed cellular process. The cytoplasm contains dilated smooth endoplasmic reticulum (curved arrow) and some bizarre shaped mitochondria (Δ). Loss of spermatogenic epithelium is noticed in some areas (red*)and elongated spermatide with heterogenous electron dense nucleus(blue Δ) (5b) showing early spermatid with abnormal dense shrunken nucleus(N), Many residual bodies (red arrow) can be noticed. 5(c): showing early spermatid with euchromatic nucleus(N) covered with acrosomal cap (↑), peripherally arranged mitochondria (Δ) and lysosomes (Ly). Wide intercellular spaces (red *) and areas without peripheral mitochondria (black *) are seen. Many residual bodies (red arrow) with vacuolations (V). 5(d): showing wide peritubular sheath , Leydig cells shows shrunken nuclei (N), mitochondria (Δ) and few dilated smooth endoplasmic reticulum (curved arrow). Excess collagen fibers (↑) are seen. TEM (a and b X 8000) (c, d X 5000).

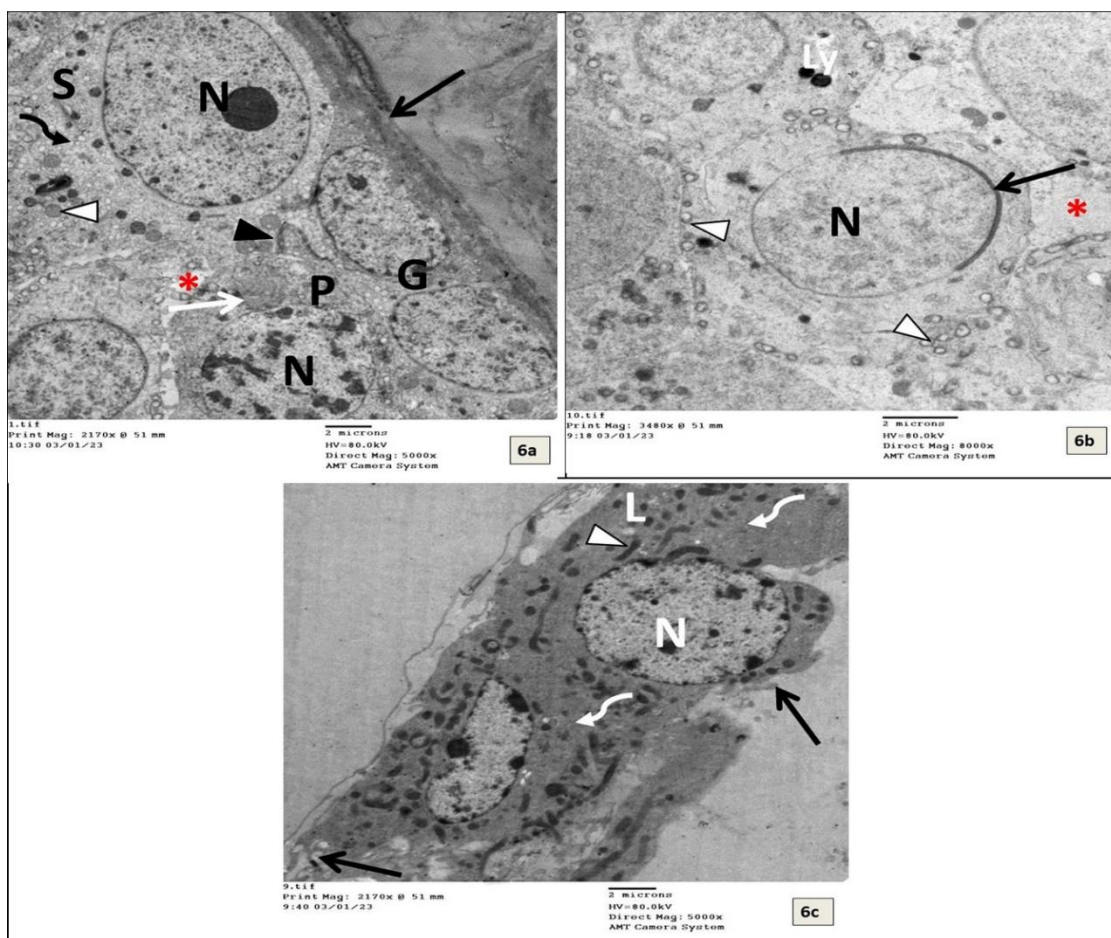


Fig. 6: Electron micrographs of group III: (6a) showing spermatogonia (G) and Sertoli cell (S) with its identical nucleus (N) are resting on regular basement membrane (↑) surrounded with myoid cells. The cytoplasm of Sertoli cell contains normally appeared mitochondria (Δ) and smooth endoplasmic reticulum (curved arrow). Ectoplasmic specialization (▲) is seen between it and other spermatogenic cells. Primary spermatocyte (P) with its characteristic nucleus (N) is also seen. (6b): showing early spermatid with its euchromatic nucleus(N). the cytoplasm contains peripherally arranged mitochondria with clear matrix (Δ) and some lysosomes (Ly). Acrosomal cap is covering the anterior portion of the nucleus (↑). Few small intercellular spaces (red*) are seen. (6c): showing interstitial cells of Leydig with their identical nucleus (N). the cytoplasm contains elongated mitochondria (Δ), smooth endoplasmic reticulum (curved arrow) and lipid droplets(L). Microvilli (↑) are seen protruding from the surface of the cells. TEM (a and c X 5000) (b X 8000).

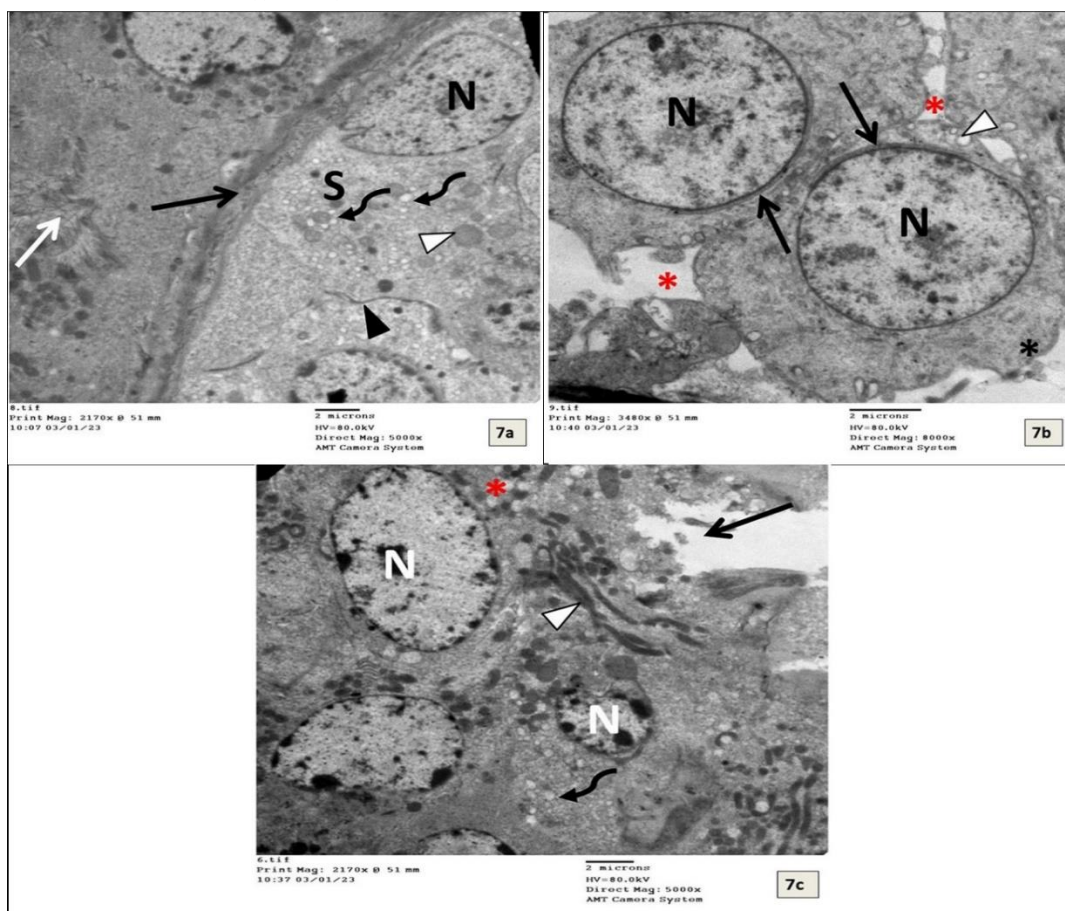


Fig. 7: Electron micrographs of group IV:(7a) :(a) Sertoli cell(S) with its identical indented nucleus(N) thin abnormal ectoplasmic specialization (▲)resting on thick basement membrane (↑) .Its cytoplasm contains numerous SER(curved arrow) and rounded mitochondria (Δ),The interstitial tissue has excess collagen fibers(↑↑)7(b): showing normal early spermatid with destructed cellular process of sertoli cell and its characteristic euchromatic nucleus(N). The cytoplasm contains peripherally arranged mitochondria (Δ) in some areas and other areas without mitochondria (*). The acrosomal cap covering the anterior portion of the nucleus (↑). Some irregular intercellular spaces (red *) are seen. 7(c): showing many interstitial cells of Leydig with euchromatic nuclei (N). The cytoplasm contains elongated mitochondria (Δ), slightly dilated smooth endoplasmic reticulum (curved arrow) and lipid droplets (red *). Few damaged cells (↑) can be noticed. TEM (a and c X 5000) (b X 8000).

Table 1. Mean diameter of seminiferous tubules in different experimental groups:

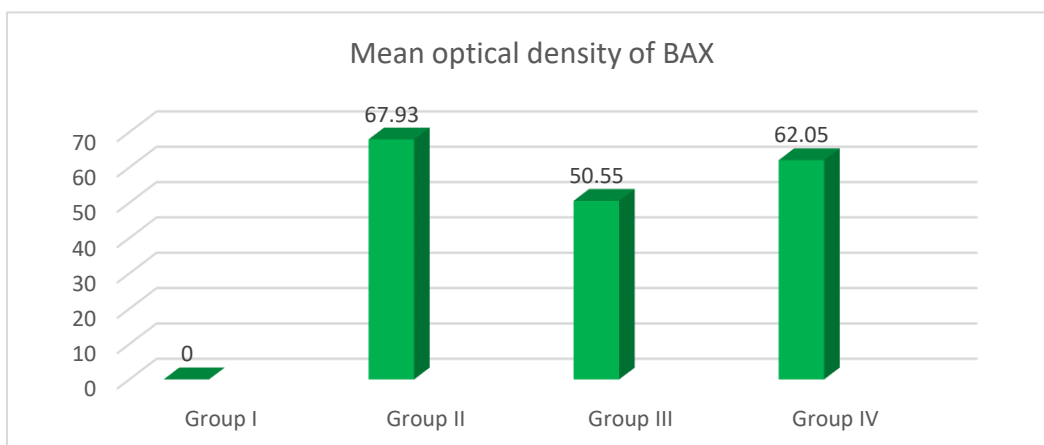
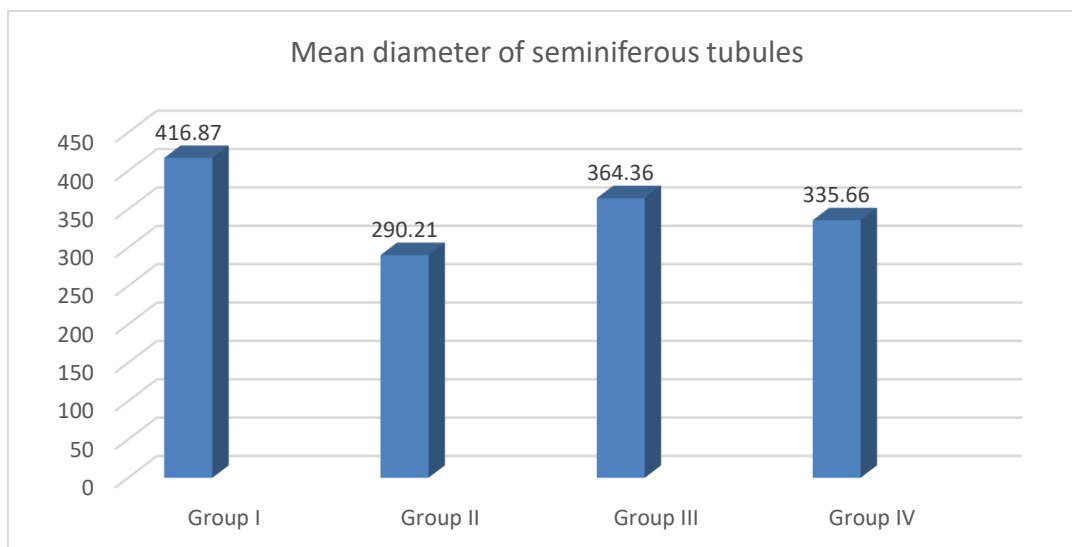
	G1 (control)	G2 (diseased)	G3 (treated with wheat germ oil)	G4 (treated with zinc)
Mean diameter of seminiferous tubules	416.87± 72.31	290.21±29.65 a *c*d ns	364.36±28.14 a ns b* d ns	335.66±28.56 a*b ns c ns

Data are presented as mean ± standard deviation (SD), ns: non-significance. Significance at * P<0.05. (a: vs. G1, b: vs. G2, c: vs G3 and d: vs G4).

Table 2. Mean optical density of BAX in different experimental groups:

	G1 (control)	G2 (diseased)	G3 (treated with wheat germ oil)	G4 (treated with zinc)
Mean optic density of BAX	0.0± 0.0	67.93±1.15 a * c*d*	50.55±4.26 a* b* d*	62.05±0.09 a* b* c*

Data are presented as mean ± standard deviation (SD), ns: non-significance. Significance at * P<0.05 (a: vs. G1, b: vs. G2, c: vs. G3 and d: vs. G4).



DISCUSSION:

More than 6% of people in the population have at some point in their lives struggled with depression [19]. According to research by Sakr et al. [20], administration of fluoxetine as antidepressant drug results in a

variety of negative effects on the body as well as on testicular tissues.

Free radicals and oxidative stress can have dangerous effects on testicular tissue. Numerous factors, including hyperactive meiosis, cell competition for oxygen, low oxygen pressure due to damaged arteries,

high amounts of unsaturated fatty acids, and inability to neutralize all free radicals, are responsible for this [21].

In the current study, the administration of adult rats with 10 mg/kg BW of fluoxetine for 28 days resulted in structural changes in the seminiferous tubules, including Small sized tubules with widening of peritubular sheath (PS). These results were proved by significant decrease in the diameter of the tubules ,Distorted spermatogenic epithelium with pyknotic and fragmented nuclei, loss of mature sperm from the lumen of some tubules, Separation of adluminal compartment of tubule from basal compartment with subsequent sloughing of germinal epithelium into tubular lumen were also detected on light microscopic examination of group II and were confirmed by electron microscope.

Abdalmuhaimn et al [22], reported considerable reduction in the thickness of its germinal epithelium and the width of its seminiferous tubules. Seminiferous tubules showed signs of deformation, as well as an almost empty lumen and decreased germinal layer. Sertoli cells were found to be decreased, Moreover, the germinal epithelium has developed numerous big vacuoles. The seminiferous tubule's border and interstitial tissue were revealed to be damaged with fluoxetine.

Elsedawi et al [23]. noticed vaculations in the germinal epithelium layers and reduced sperm concentration in some seminiferous tubules in FLX-treated rats.

According to Morrison et al [24], selective serotonin reuptake inhibitors (SSRIs) reduce the amount of nutrients obtained from food intake and intestinal efficiency as it reduces the height of intestinal villus, which may hinder nutrients absorption, which is the cause of decreased body weight as a result of fluoxetine intake. However, it is increasingly evident that the quantity of sertoli cells strongly influences the size of the testicles [25].

By preventing serotonin reuptake, FLX can limit Luteinizing hormone (LH) release from the anterior pituitary lobe and suppress steroidogenesis [26]. On the other hand, serotonin buildup in the testicles can prevent Leydig cells from being nourished; according to Erdemir et al. [27], serotonin causes vasoconstriction and reduces the blood flow to the testicles.

Testosterone withdrawal causes premature release of spermatids from SC, proving that this hormone is critical for the SC-germ cell attachment [28].

The SC microtubule cytoskeleton, whose modification results in aberrant germ cell placement, cell detachment, and death [29], is necessary for the aligning of the germ cells in the epithelium [30]. Testosterone withdrawal causes spermatids to prematurely detach from the SC, demonstrating that this hormone is necessary for the attachment of the SC to the germ cell. The α -tubulin isoform Tubb3 gene is following androgenic regulation in the testes [31]. Those findings illustrate our finding of existence of detached germ cells in the lumen, elongated spermatids abnormally placed in the epithelium which are likely induced by fluoxetine.

According to research by Inkielewicz [32], fluoxetine promotes lipid peroxidation, which releases free radicals, which disrupt membrane structure, reduce membrane fluidity, and ultimately cause severe tissue damage.

It is also known that spermatozoa and testicular tissues are extremely vulnerable to lipid peroxidation and ROS assault. Because sperm membranes consist of a lot of polyunsaturated fatty acids, testicular tissues are more prone to oxidation [33].

Aggarwal et al [26]. reported that the tubules exhibited cellular deterioration along with distortion and loss of alignment. Also the number of Leydig & Sertoli cells was shown to be reduced in rats' testis received fluoxetine for 4 weeks.

Interruption of Sertoli cell microtubule dynamics caused less basal & apical cytoplasmic processes related with germ cells^[34].

Examination of Sertoli cells of Group II of the recent study revealed destruction of their cellular processes, dilated ER, presence of ring shaped mitochondria and accumulation of residual bodies. Those findings were attributed to sertoli cell affection by flx. Donnell et al ^[35], stated that normally, The Sertoli cell captures the residual body after disengagement, and it is then presumably carried by microtubules to the base of the seminiferous epithelium. Following disengagement, Sertoli cells phagocytose those bodies. It is known that residual bodies can affect Sertoli cell activity.

Câmara et al ^[28] also reported shrunken nuclei of leydig cells in Flx treated group when compared to control group, resulting in poor steroidogenesis. Sertoli and peritubular cells were damaged by low androgenization, which interfered with spermatogenesis. Under the influence of androgens, Sertoli cell activities are modulated by a protein secreted by testicular peritubular cells. This concedes with our Em results that showed of widening of interstitial tissues with excessive deposition of collagen fibers loss of demarcation between the layers of PS. Some leydig cells revealed dilated ER indicating cell stress.

The considerable decrease in tubular cross section area seen in Flx further supports the effect of fluoxetine on SC. The epithelium basal compartment's structural integrity depends on either on the attachment of the SC to the peritubular tissue or on adhesion between the plasma membranes of adjacent SC^[36]. Hence, when the decrease of epithelial area happens without SC damage, the lumen widen as a result of damage of germinal epithelium, but the integrity of the basal compartment is still complete without alterations in the diameter of the tubules. In the current research, epithelial disarrange-

ment and a decrease in both the epithelial and overall area of the tubular sections in Flx were caused by germinal epithelium & SC degerarion linked to a decrease in the amount of SC. This similar result has also been linked in other investigations to drug-induced SC damage ^[37].

As shown in our work by significant increased Bax, A member of the Bcl-2 family that has a proapoptotic effect, the enhanced ROS production may also lead to apoptotic death of the testicular cells in the fluoxetine-administered rats^[38].

Fluoxetine-induced testicular damage was reversed by WGO, we examined closely arranged seminiferous tubules separated by slight interstitial tissue. Sertoli cell was shown resting on regular basement membrane, weak positive cytoplasmic reaction for Bax immunostaining, demonstrating potential anti-apoptotic properties.

Due to its huge content of tocopherol, or vitamin E, a potent antioxidant, wheat germ oil may possess a protective role against testicular injury^[39].

Also, antioxidant vitamin, plays a beneficial role in preserving the poly-unsaturated fatty acids present in the phospholipids of the membrane. Additionally, it is a powerful peroxy radical scavenger that protects biological plasmalemmas from free radical oxidation^[40].

It reduces DNA oxidative damage and balances the increased ROS generation in mammals. Essential fatty acids (Oleic, linoleic, and alpha-linolenic acids) are abundant in wheat germ oil, which stimulate the tocopherol-mediated redox system & prevent the production of eicosanoid, that promotes the process of lipid peroxidation^[41].

Moreover, WGO contains carotenoids, which support this anti-oxidant effect^[42]. In addition to this antioxidant impact, a prior investigation on WGO demonstrated its anti-apoptotic impact by reducing the expression

of pro-apoptotic genes in radiation-exposed rats^[43].

In our study Co-administration with Zn partially decreased testicular injury that resulted from Flx exposure, closely arranged seminiferous tubules with relatively wide interstitial tissues between them was evident. While, Spermatogenic epithelium with fragmented nuclei, sloughing and wide space of spermatogenic epithelium, the basement membrane was still corrugated and some Leydig cells were still seen with pyknotic and fragmented nuclei.

The body's second-most abundant trace element after iron is zinc. Its ability to perform metabolic tasks is largely dependent on the fact that it is a necessary part of numerous metalloenzymes^[15].

Zinc metalloenzymes are essential enzymes engaged in nucleic acid and protein synthesis, which is necessary for quickly growing tissues like gonads & tissues in renewal process. As a result, zinc is an essential element in reproduction and renewal process^[44].

Also, Zn supplementation dramatically raised the weight of the testis, suggesting that it may regulate testosterone levels and help maintain the weight of the reproductive organs. According to earlier research, Zn therapy enhances male rats' sexual power and raises blood testosterone levels^[45].

Szuster-Ciesielska et al.^[46], allocated the anti-inflammatory properties of zinc to an increase in A20, a zinc transcription factor, which ultimately suppresses the activity of NF-B, decreasing the production of the proinflammatory cytokines. Additionally, zinc supplement was found to reduce the activity of the NF-B/IkappaB and TGF-1 transduction signalling pathways.

By taking the Fe or Cu binding sites of fat, protein, and DNA and slowing the oxidative mechanisms, zinc has a direct antioxidant effect. Zn may also serve as an

antioxidant since it is a necessary part of Cu/Zn SOD^[47].

Zinc's protective benefits may be also linked to increased levels of metallothionein (MT) in a variety of organs (liver, kidney, etc.) which can prevent oxidative stress and apoptosis^[48]. Which illustrate our finding of significant decrease of BAX optical density as compared to Flx treated group.

Conclusion:

Thus, relying on our results & the previously mentioned reports, we can conclude that WGO protects against Flx-induced testicular damage essentially by antioxidant and antiapoptotic effects. Also Zn showed partial protection against Flx-induced testicular damage, but the WGO showed more significant improvement. Further studies should be conducted to better understand the protective mechanisms of Zn against Flx-induced testicular damage with different doses of Zn.

Conflicts of interest/Competing interests:

The authors have no conflicting financial or competing interests.

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Abbreviations:

FLX	fluoxetine
FSH	follicle stimulating hormone
WGO	wheat germ oil
ALA	alpha-linolenic acid
vitamin E	tocopherols
Zn	Zinc
DNA	deoxyribonucleic acid
b.w	Body weight
Bax	Bcl2-associated x protein
TEM	Transmission electron microscope
RCMB	Regional Center for Mycology & Biotechnology
H&E	Hematoxylin and eosin
SC	Sertoli cells
SER	Smooth endoplasmic reticulum
PS	peritubular sheath
SSRIs	selective serotonin reuptake inhibitors
LH	Luteinizing hormone
NF-B/ κ B	Nuclear factor kappa-light-chain-enhancer of activated B cells
TGF-1	Transforming growth factor - 1
Fe	Iron
Cu	Copper
SOD	Superoxide dismutase
MT	metallothionein

التأثير النسيجي الوقائي لزيت جنين القمح مقابل الزنك على الخصيتين التالفيتين بالفلوكستين في الفئران البيضاء

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المقدمة: فلوكستين هو دواء مضاد للاكتئاب والذي يحفز الإجهاد التأكسدي في الأعضاء المختلفة ، وزيت جنين القمح والزنك لهما تأثيرات مضادة للالتهابات ومضادة للأكسدة.

الهدف: تقييم التأثير النسيجي لزيت جنين القمح مقابل الزنك على إصابة الخصية التي يسببها فلوكستين في الفئران البيضاء.

المواد والطرق: تم تقسيم أربعين من ذكور الفئران البيضاء إلى أربع مجموعات متساوية: المجموعة الأولى (الضابطة) ؛ المجموعة الثانية: تم اعطاء ١٠ مغ/كغ من فلوكستين لمدة ٢٨ يوما؛ المجموعة الثالثة: أعطى فلوكستين + زيت جنين القمح بجرعة ٦٨,٧٥ مغ/كغ؛ المجموعة الرابعة: أعطى فلوكستين + أسبارتات الزنك بجرعة ٣٠ ملغم/كغ. تم تعريض عينات من الخصيتين للفحص النسيجي والميكروسكوب الإلكتروني والاختبارات الهستوكيميائية المناعية.

النتائج: أظهرت البيانات أن فلوكستين غير بشكل كبير بنية الخصية. تم فصل الأنابيب المنوية على نطاق واسع مع انخفاض كبير في قطرها. فصل المقصورة القاعدية للأنابيب المنوية من المقصورة اللامعة مع فقدان الخلايا. وقد تأكد ذلك من خلال زيادة كبيرة في التلوين المناعي للباكس. لوحظ اتساع المساحة الخلالية مع ترسب ألياف الكولاجين. كان هناك تحسن في جميع الجوانب النسيجية في المجموعات المعالجة بزيت جنين القمح والزنك. كان هذا التحسن أكثر وضوحا في المجموعة المعالجة بزيت جنين القمح تم تأكيد النتائج من خلال زيادة كبيرة في القطر الأنبوبي مع بطانة ظاهرية طبيعية إلى حد ما. تم الكشف عن انخفاض كبير في التفاعل المناعي باكس. كان النسيج الخلفي طبيعيا . وتخلص هذه البيانات إلى أن زيت جنين القمح يزيد من الحماية ضد تلف الخصية الناجم عن فلوكستين.

الاستنتاج: فلوكستين نجم عن الضرر التأكسدي في خصية الفئران. الا أن كلا من زيت جنين القمح والزنك لهما دور وقائي في تحسين إصابة الخصية المرتبطة بفلوكستين. ومع ذلك ، فإن زيت جنين القمح له تأثيرات أفضل على جميع الجوانب.