Original Article

# Role of Spirulina Extract in Ameliorating Histological and Immunohistochemical Changes Induced by Sertraline in the Testis of Adult Albino Rat

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## ABSTRACT

**Introduction:** Sertraline (SRT) is a selective serotonin reuptake inhibitor drug that is widely used to manage depression with several sexual side effect. Spirulina is a powerful antioxidant and anti-apoptotic as it is a rich source of proteins, polyunsaturated fatty acids, vitamins and carotenoids.

**Aim of the Work:** To assess histological and immunohistochemical changes induced by sertraline administration in testis as well as the possible role of spirulina in improving these changes in the testis of adult albino rat.

**Materials and Methods:** The current study was done on forty adult male albino rats were randomly divided into four main groups: group I represented the control group, group II was given spirulina (500 mg/kg/day) orally for consecutive 28 days, group III was given sertraline (20 mg/kg/day) orally for consecutive 28 days, and group IV was given both sertraline and spirulina in the same dose and manner as group II and group III for consecutive 28 days. The testes specimens were processed for different histological and immunohistochemical techniques. Morphometrical and statistical studies were also done.

**Results:** Sertraline induced several testicular destructive changes in the form of distortion of many seminiferous tubules, and degeneration of spermatogenic cells with cytoplasmic vacuolation and dark nuclei. Sloughing of the spermatogenic cell and absent sperms is observed. Degenerated Leydig cells, dilated congested blood vessels, and acidophilic material were observed in the interstitium. Mean area percentage of Masson trichrome showed an extremely significant increase while the means of the height of germinal epithelium, number of immunopositive cells of PCNA, and area percentage of positive immunoreaction of androgen receptors showed an extremely significant decrease. In contrast, spirulina cotreatment eliminated most of these histological alternations except for few localized areas.

**Conclusion:** Spirulina extract ameliorates the testicular destructive changes induced by sertraline.

**Received:** 17 August 2021, **Accepted:** 05 October 2021

**Key Words:** Androgen receptors, PCNA, sertraline, spirulina, testis.

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**ISSN:** 1110-0559, Vol. 46, No.1

## INTRODUCTION

Depression is the most prevalent mental illness worldwide that it is considered now as a critical health disaster. Selective serotonin reuptake inhibitor antidepressants (SSRI) are frequently used in the mainline treatment of depression[1]. In the last two decades, a significant increase in the consumption of antidepressants is documented in countries with a high standard of living. 12.7% of people aged 12 years and over in USA is reported to use antidepressants in just one month[2].

Sertraline (SRT) is one of the SSRI family that is a key drug in the treatment of major depressive disorder[3]. It inhibits the reuptake of serotonin to increase the concentration in the synaptic cleft and augment serotonergic neurotransmission in certain areas of the brain. Elevated serotonin stimulates postsynaptic terminals attenuating depression[4].

Sertraline has many side effects as diarrhea, stomachache, insomnia, ophthalmic manifestations, hyperprolactinemia, and headache[5]. Sexual disorders are terrible complications of SRT that appeared on the scene affecting 80% of both females and males. Treated patients represent a wide range from loss of sexual desire to delayed orgasm troubles[6]. Nevertheless, these sexual adverse effects compel patients to terminate its long-term administration leading to relapse of the mental illness.

Spirulina maxima is a blue-green alga that is a microscopic filamentous belongs to cyanobacterium 22. It has high levels of protein, lipids, essential amino acids, minerals, vitamins, photosynthetic pigments, biologically active substances including phycocyanin, chlorophyll, and β-carotene[7]. It has anti-oxidative, anti-hyperlipidemic, anti-bacterial, anti-viral, anti-cancer, anti-neurotoxic, and anti-inflammatory effects[8].

Considering the important role of SRT in management of various mental illness despite all its side effect, this creates an urgent need for a protective agent against its toxicity. For all previous criteria of spirulina, the present work was designed to inspect the possible ameliorative effect of spirulina extract against testicular histopathological changes induced by SRT administration.

 *DOI: 10.21608/ejh.2021.91359.1545*

## MATERIALS AND METHODS

### *Drugs*

Sertraline (SRT) is commercially available in a name of Moodapex capsules (50 mg) which is produced by Multi Pharma company, Egypt. SRT solution was prepared at a concentration of 4 mg/1ml by grinding 5 capsules (250 mg) and dissolving them in 63 ml distilled water. Spirulina powder was provided as a fine dark blue-green powder which is commercially available as a product of Imtenan, Cairo, Egypt. Dissolved spirulina solution was prepared at a concentration of 100 mg/1ml by dissolving 1000 mg spirulina powder in 10 ml in distilled water.

### *Animals*

Forty adult male albino rats were involved in this study with an average weight (180 – 200 grams). They were acclimatized to animal house conditions for 14 days. Our animal experiment was approved by the local ethical committee of the Faculty of Medicine, Tanta University, Egypt (Approval number: 34792/7/21).

### *Study Design*

The rats were randomly divided into four main groups:

**Group I (Control group):** consisted of 10 rats that were divided into 2 equal subgroups:

- Subgroup (i): 5 rats received no treatment until the end of the experiment.
- Subgroup (ii): 5 rats received 1ml of distilled water (the diluting vehicle for both spirulina and sertraline solution) for 28 consecutive days.

**Group II (Spirulina group):** 10 rats, each one received 1ml of prepared spirulina solution orally 500 mg/kg/day[9] for consecutive 28 days.

**Group III (SRT group):** 10 rats, each one received 1ml of prepared SRT solution orally (20 mg/kg/day)[10] for consecutive 28 days.

**Group IV (STR+ Spirulina group):** 10 rats, each of them received both SRT and spirulina in the same dose and manner as group II and group III for consecutive 28 days.

Lastly, 24 h after the last dose of both drugs, all animals were anesthetized by intraperitoneal injection of sodium pentobarbital at a dose of 50 mg/kg[11]. The right testes were excised and cleaned. Specimens from testes are prepared for light and immunohistochemical study.

### *Light microscopy preparation*

For histological examination, all testes of all groups were fixed in Bouin's solution and processed for paraffin blocks. 5 µm histological testicular sections were cut and exposed to the following stains:

1. Routine hematoxylin and eosin for the general examination of testicular tissue[12].
2. Masson's trichrome stain for detection of collagen fibers[13].

### *Immunohistochemical Staining*

5-µm-thick sections were utilized for immunohistochemical staining. 0.3% hydrogen peroxide was used to block endogenous peroxidase activity. Citrate buffer was used for antigen retrieval after the treatment of the sections in a microwave. The tissue sections were incubated overnight at room temperature with the following primary antibodies: proliferating cell nuclear antigen (PCNA), dilution 1: 400-800 (Lab Vision Company Clone PC 10; Dako Denmark A/S, Glostrup, Denmark[14], and androgen receptor (AR), dilution 1:500 (ab3510, Abcam, Cambridge, Massachusetts, USA)[15]. The second step for staining is using the streptavidin biotin complex detection method. Finally, the sections were stained with diaminobenzidine (DAB) solution (as chromogen) for 5-10 minutes, washed three times in PBS for two minutes each, and counterstained with Mayer hematoxylin. Negative control were done by the same previous steps without adding the primary antibody. A light microscope (Olympus, Japan) with a built-in camera was used to examine and photograph all slides in the Histology department, Faculty of Medicine, Tanta University.

### *Morphometric study*

Using image J software (National Institute of Health, Bethesda, Maryland, USA), Ten different non-overlapping randomly selected fields from each slide of each group were quantified for:

1. Mean height of spermatogenic epithelium of seminiferous tubules were measured in H&E-stained sections (× 400)[16].
2. The mean area percentage of collagen fibers in Masson's trichrome stained sections (X 400)[17].
3. The number of immune positive cells of PCNA of germ cells within the seminiferous tubule (X 400) in DAB-stained sections[18].
4. The mean area percentage of positive immunoreaction of androgen receptors (X 400) in DAB-stained sections[19].

### *Statistical analysis*

The calculated numbers were considered for comparison and statistical analyses using one-way analysis of variance (ANOVA test) followed by Turkey's test for comparison between the groups. All values were expressed as mean ± standard deviation. Differences were regarded as significant if probability *P-value* <0.05[20].

## RESULTS

In the present work, no mortality was recorded throughout the experimental period. Both subgroups of the control group showed no difference in the histological results. So, it was referred to subgroups (i& ii) as the

control group. As regard group II (spirulina group), it also showed no difference in the histological results or statistical analysis when compared with group I (control group).

## *Histological results*

**Hematoxylin & eosin- stained section**

Sections obtained from the testes of the group I (control group) showed the normal histological structure. The seminiferous tubules appeared rounded to oval and surrounded by well-defined basement membranes and spindle shaped myoid cells with their flat nuclei. They were lined by stratified epithelium showing different stages of spermatogenesis. The germinal epithelium showed two types of cells, Sertoli cells and spermatogenic cells. Spermatogenic cells were arranged in the order of; spermatogonia, primary spermatocytes, spermatids and spermatozoa from the basal compartment towards the lumen of the tubules (Figures 1,2). Moreover, spaces between the seminiferous tubules were filled with interstitial tissue containing Leydig cells with their central nuclei and prominent nucleoli (Figure 3). Examination of sections obtained from the testes of group III (SRT group) showed disturbance in the normal architecture with evidence of structural changes. Some seminiferous tubules appeared with irregular outlines, wide intercellular spaces (Figure 4) and distorted shape (Figures 4,5). Other seminiferous tubules appeared with exfoliated cells into the lumen of the tubules and absent sperms (Figure 6). The basement membrane showed areas of focal loss (Figure 5,6). The interstitial tissue contained multiple dilated congested blood vessels, homogenous acidophilic material (Figure 7) and interstitial cells of Leydig with cytoplasmic vacuoles and dark nuclei (Figure 8). Moreover, the germinal epithelium appeared with wide intercellular spaces or even areas of focal loss. Many spermatogonia showed vacuolated cytoplasm (Figure 9) and fragmented nuclei (Figure 10). On the other hand, examination of sections obtained from the testes of group IV (SRT + spirulina group) showed partial preservation of the normal structure of the seminiferous tubules. Most of them were more or less resembling the normal structure (Figures 11,12). Some interstitial cells of Leydig appeared with vacuolated cytoplasm (Figure 11). The germinal epithelium appeared normal, but some areas showed focal widening of intercellular spaces (Figures 12,13).

**Masson trichrome-stained sections**

Sections obtained from the testes of group I (control group) showed minimal deposition of collagen fibers in the connective tissue capsule, while the interstitial tissue showed absent collagen fibers (Figure 14). Examination of sections obtained from the testes of group III (SRT group) showed marked increase of collagen fibers in the connective tissue capsule. Moreover, deposition of collagen fibers around interstitial blood vessels was observed (Figure 15). On the other hand, examination of sections obtained from the testes of group IV (SRT + spirulina group) appeared more or less as control with minimal deposition of collagen fibers in the connective tissue capsule, while the interstitial tissue showed absent collagen fibers (Figure 16).

## *Immunohistochemical results*

**PCNA immuno-stained sections**

Sections obtained from the testes of group I (control group) showed positive immune stained (brown nuclear reaction) spermatogenic cells within the seminiferous tubules (Figure 17). On the other hand, group III (SRT group) showed few positive immune stained spermatogenic cells (Figure 18), while group IV (SRT + spirulina group) showed many positive immune stained spermatogenic cells within the seminiferous tubules (Figure 19).

**Androgen receptor immuno-stained sections**

Sections obtained from the testes of group I (control group) showed intense immunostaining (deep brown nuclear reaction) in myoid cells, Sertoli cells, interstitial cells of Leydig and blood vessels smooth muscle cells (Figure 20). On the other hand, group III (SRT group) showed very weak immunostaining in Sertoli cells and few immune-positive cells of both myoid & interstitial cells of Leydig (Figure 21), while group IV (SRT + spirulina group) showed intense immunostaining in myoid cells, Sertoli cells and interstitial cells of Leydig (Figure 22).

## *Morphometric results & statistical analysis*

The mean height of spermatogenic cells of group III (SRT group) showed an extremely significant decrease ($p$ *value* < 0.001) when compared with group I (control group). On the other hand, co treatment with spirulina (group IV) showed an extremely significant increase ($p$ *value* < 0.001) when compared with group III (SRT group). (Table 1, Figure 23A).

The mean area percentage of collagen fibers in Masson trichrome of group III (SRT group) showed an extremely significant increase ($p$ *value* < 0.001) when compared with group I (control group). Moreover, group III (SRT group) showed an extremely significant increase ($p$ *value* < 0.001) when compared with group IV (SRT + spirulina group). In contrast, group IV (SRT + spirulina group) showed an extremely significant decline ($p$ *value* < 0.001) when compared with group III (SRT group) (Table 1, Figure 23B).

The mean number of immune positive cells of PCNA of group III (SRT group) showed an extremely significant decrease ($p$ *value* < 0.001) when compared with group I (control group). On the other hand, there was an extremely significant increase in the mean number of immune positive cells of PCNA ($p$ *value* < 0.001) of group IV (SRT + spirulina group) compared to group III (SRT group) (Table 1, Figure 23C).

The mean area percentage of positive immunoreaction of androgen receptors of group III (SRT group) showed an extremely significant decrease ($p$ *value* < 0.001) when compared with group I (control group). On the other hand, group IV (SRT + spirulina group) showed an extremely significant increase ($p$ *value* < 0. 001) in this parameter when compared with group III (SRT group) (Table 1, Figure 23D).

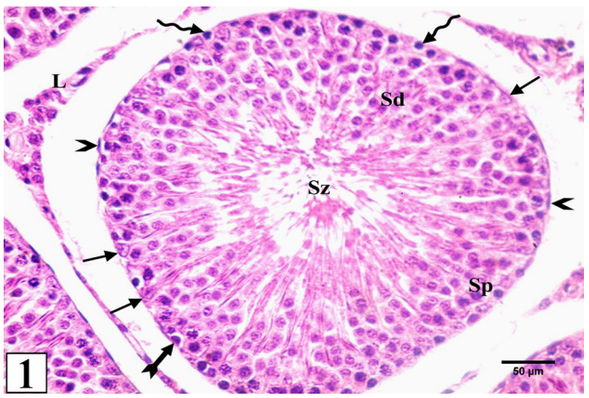


**Fig. 1:** A photomicrograph of a section in the testis of group I (control group) showing normal oval to rounded seminiferous tubule with regular basement membrane (bifid arrow) and spindle shaped myoid cells (arrow heads). Normal different stages of spermatogenesis can be seen; spermatogonia (zigzag arrows), primary spermatocytes (Sp), spermatids (Sd) & Spermatozoa (Sz). Sertoli cells (arrows) are present in-between the germ cells. Notice, clusters of Leydig cells (L) can be seen in-between the seminiferous tubules (H&E×400).

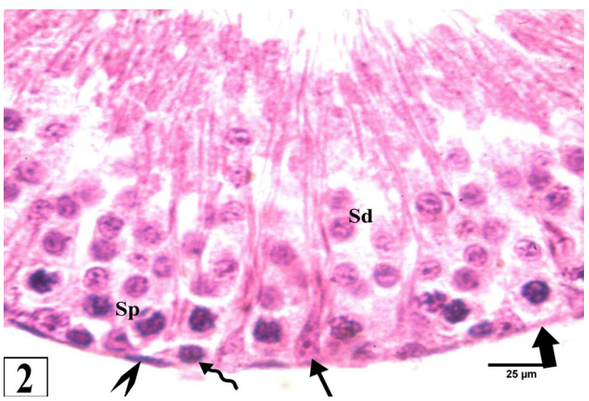


**Fig. 2:** A photomicrograph of a section in the testis of group I (control group) showing seminiferous tubule with regular basement membrane (thick arrow) and spindle shaped myoid cells (arrowhead). Spermatogonia (zigzag arrow), primary spermatocytes (Sp), spermatids (Sd) and Sertoli cells (arrow) can be seen (H&E×1000).

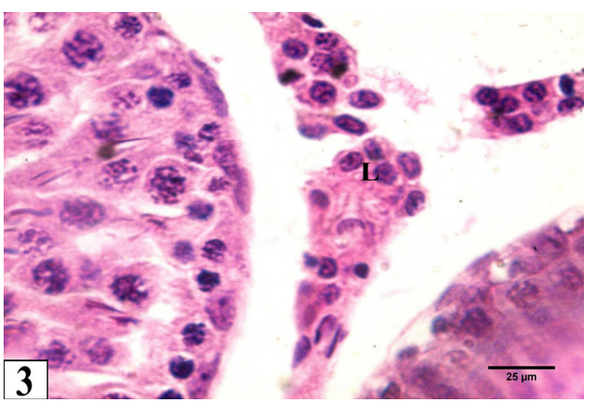


**Fig. 3:** A photomicrograph of a section in the testis of group I (control group) showing clusters of Leydig cells (L) with their central nuclei and prominent nucleoli in-between the seminiferous tubules (H&E×1000).

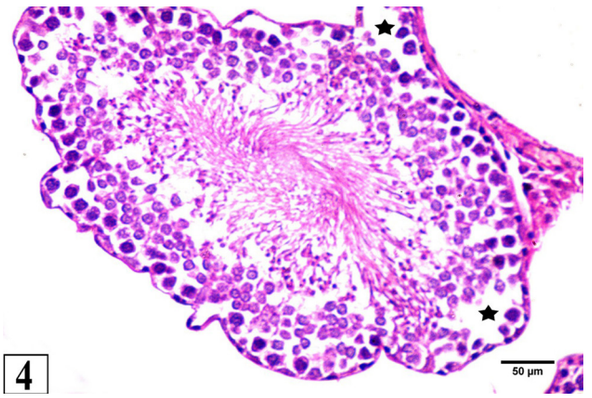


**Fig. 4:** A photomicrograph of a section in the testis of group III (SRT group) showing a distorted seminiferous tubule with irregular outlines and wide intercellular spaces (stars) (H&E ×400).

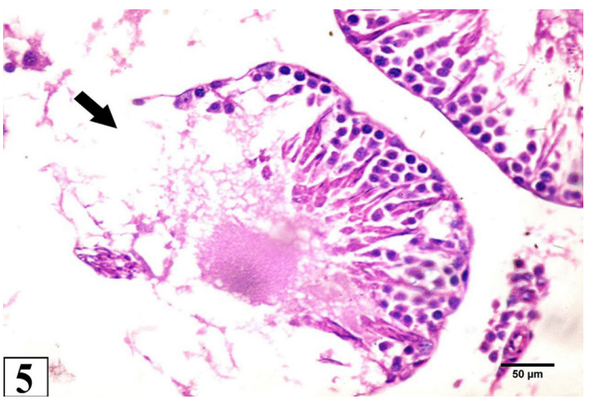


**Fig. 5:** A photomicrograph of a section in the testis of group III (SRT group) showing a distorted seminiferous tubule with area of complete loss of basement membrane and its overlying germinal epithelium (arrow) (H&E ×400).

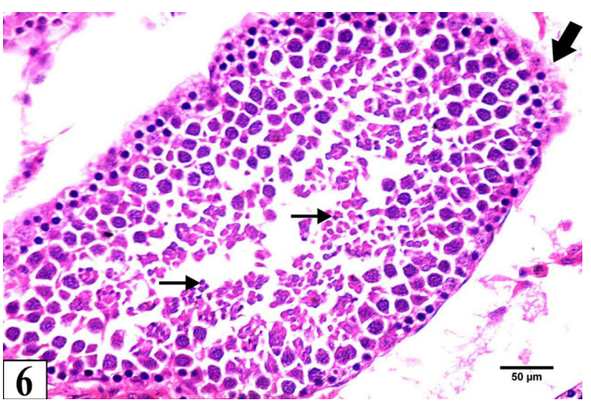


**Fig. 6:** A photomicrograph of a section in the testis of group III (SRT group) showing the lumen of the seminiferous tubule with absent sperms and exfoliated spermatogenic cells with pyknotic nuclei (arrows). Notice, focal loss of basement membrane (thick arrow) can be seen (H&E ×400).

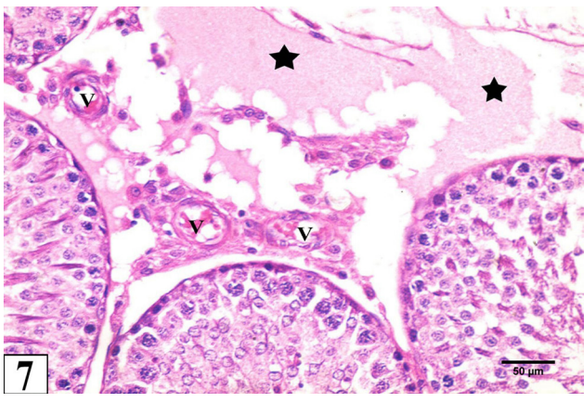


**Fig. 7:** A photomicrograph of a section in the testis of group III (SRT group) showing dilated congested blood vessels (V) and homogenous acidophilic material (stars) in the interstitial tissue in-between the seminiferous tubules (H&E ×400).

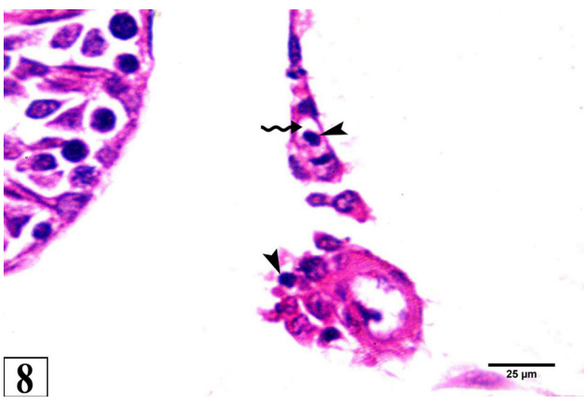


**Fig. 8:** A photomicrograph of a section in the testis of group III (SRT group) showing interstitial cells of Leydig with cytoplasmic vacuoles (zigzag arrow) and dark nuclei (arrow heads) (H&E ×1000).

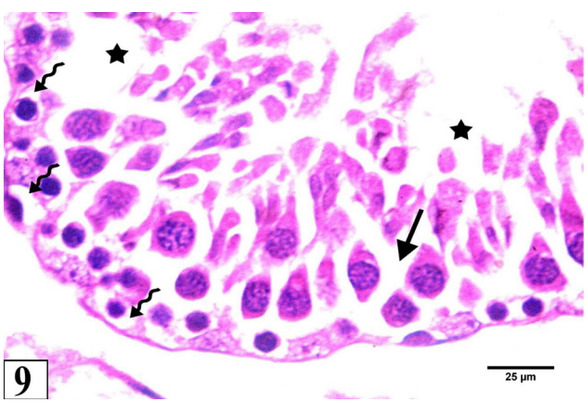


**Fig. 9:** A photomicrograph of a section in the testis of group III (SRT group) showing the germinal epithelium with wide intercellular spaces (arrow) as well as areas of focal loss (stars). Many spermatogonia with vacuolated cytoplasm (zigzag arrows) can be seen (H&E ×1000).

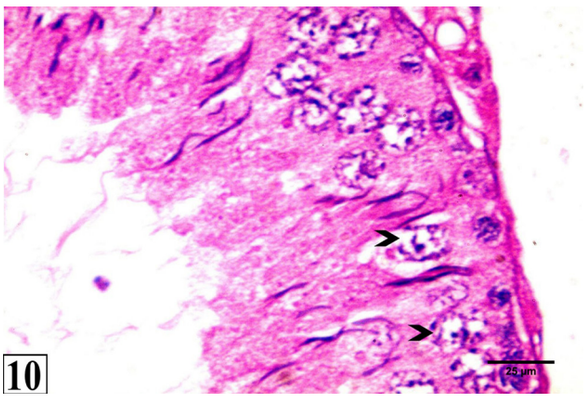


**Fig. 10:** A photomicrograph of a section in the testis of group III (SRT group) showing primary spermatocytes with fragmented nuclei (arrow heads) (H&E ×1000).

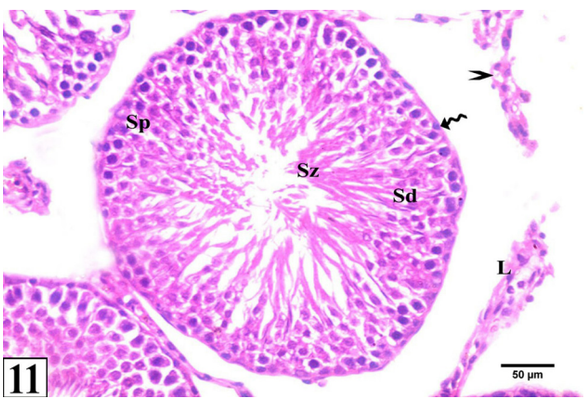


**Fig. 11:** A photomicrograph of a section in the testis of group IV (SRT + spirulina group) showing apparently normal oval to rounded seminiferous tubule with normal different stages of spermatogenesis; spermatogonium (zigzag arrow), primary spermatocytes (Sp), spermatids (Sd), Spermatozoa (Sz). Notice clusters of Leydig cells (L) can be seen in-between the seminiferous tubules while some of them appear with vacuolated cytoplasm (arrowhead) (H&E ×400).

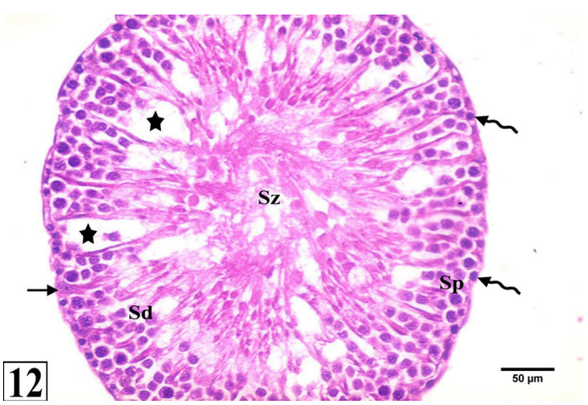


**Fig. 12:** A photomicrograph of a section in the testis from group IV (SRT + spirulina group) showing apparently normal oval to rounded seminiferous tubule with normal different stages of spermatogenesis; spermatogonia (zigzag arrows), primary spermatocytes (Sp), spermatids (Sd), Spermatozoa (Sz). Sertoli cells (arrows) are present in-between the germ cells. Notice focal widening of intercellular spaces (stars) (H&E×400).

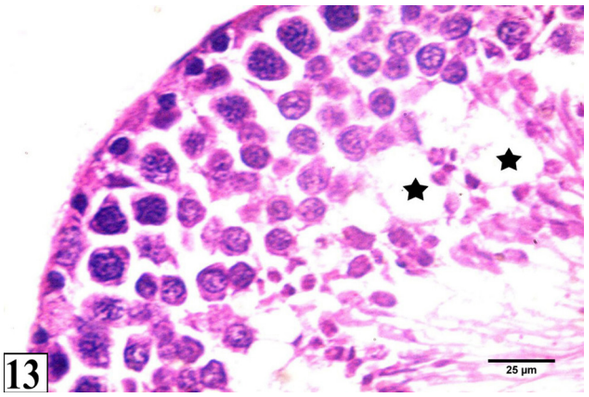


**Fig. 13:** A photomicrograph of a section in the testis of group IV (SRT+spirulina group) showing focal widening of intercellular spaces (stars) (H&E×1000).

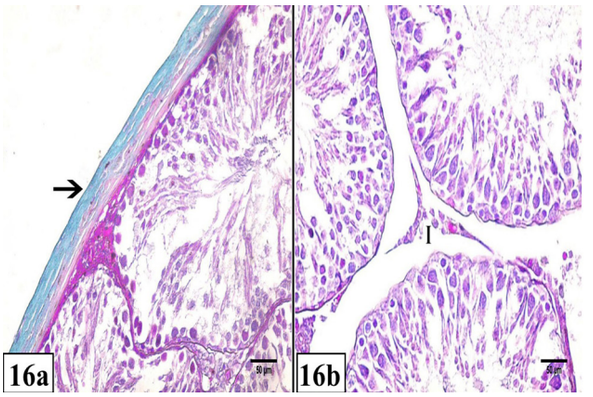


**Fig. 16:** A photomicrograph of a section in the testis from group IV (SRT + spirulina group) showing; (a) minimal collagen fibers in the connective tissue capsule (arrow) and (b) normal interstitial tissue (I) with absent collagen fibers (Masson trichrome ×400).

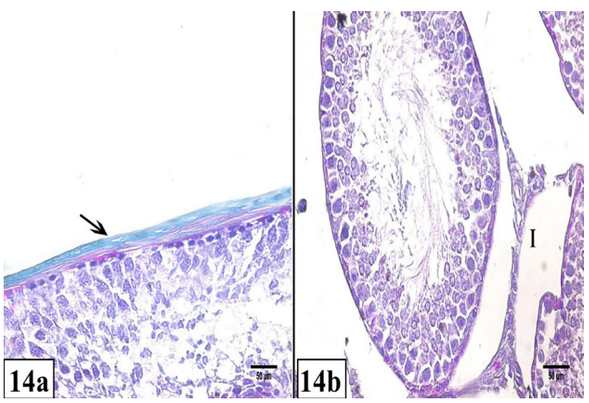


**Fig. 14:** A photomicrograph of a section in the testis of group I (control group) showing; (a) minimal collagen fibers in the connective tissue capsule (arrow) and (b) normal interstitial tissue (I) with absent collagen fibers (Masson trichrome ×400).

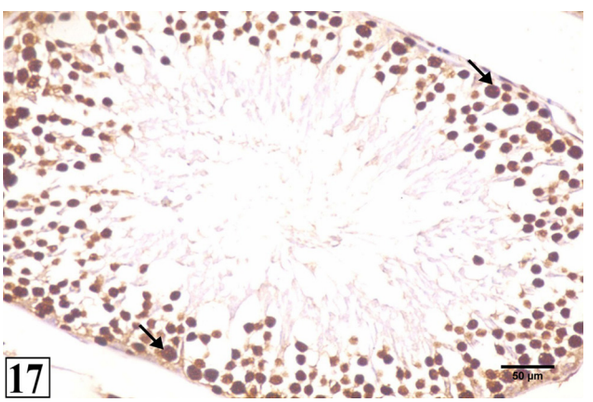


**Fig. 17:** A photomicrograph of a section in the testis from group I (control group) showing positive immune stained (brown nuclear reaction) spermatogenic cells within the seminiferous tubules (arrows) (PCNA immunostaining ×400)

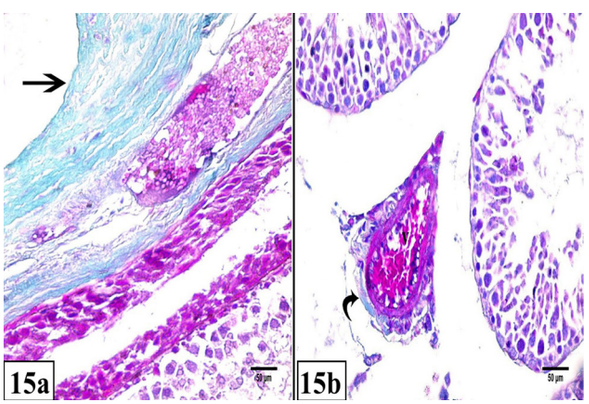


**Fig. 15:** A photomicrograph of a section in the testis of group III (SRT group) showing; (a) marked increase of collagen fibers in the connective tissue capsule (arrow) and (b) showing deposition of collagen fibers (curved arrow) around interstitial blood vessels (v) (Masson trichrome ×400).

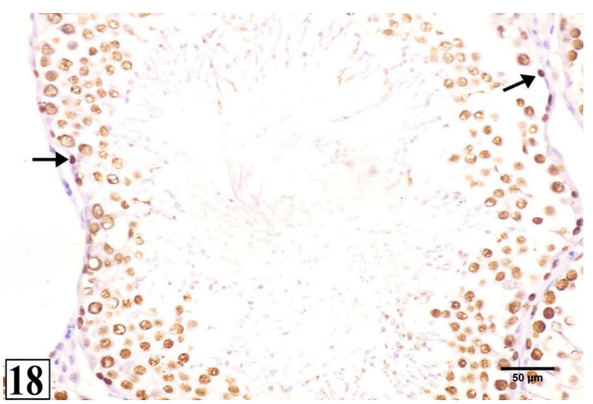


**Fig. 18:** A photomicrograph of a section in the testis from group III (SRT group) showing few positive immune stained spermatogenic cells (arrows) within the seminiferous tubules (PCNA immunostaining ×400).

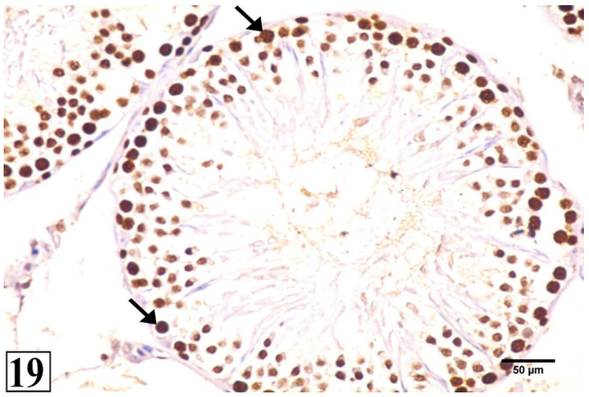


**Fig. 19:** A photomicrograph of a section in the testis from group IV (SRT + spirulina group) showing many positive immune stained spermatogenic cells in the seminiferous tubules (arrows) (PCNA immunostaining ×400).

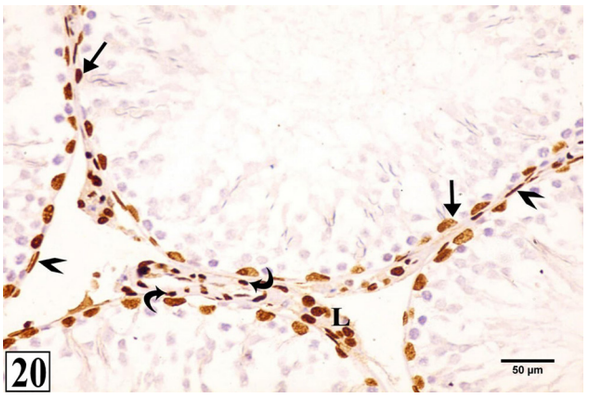


**Fig. 20:** A photomicrograph of a section in the testis from group I (control group) showing intense immuno-staining (deep nuclear reaction) in myoid (arrow heads), Sertoli (arrows), interstitial cells of Leydig (L) and smooth muscle of blood vessels (curved arrows) (AR immunostaining ×400).

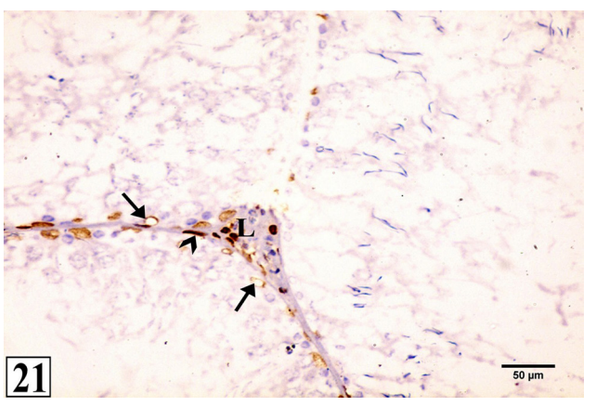


**Fig. 21:** A photomicrograph of a section in the testis from group III (SRT group) showing very weak immuno-staining in Sertoli cells (arrows) and few immuno-positive cells of both myoid (arrowhead) & interstitial cells of Leydig (L) (AR immunostaining ×400).

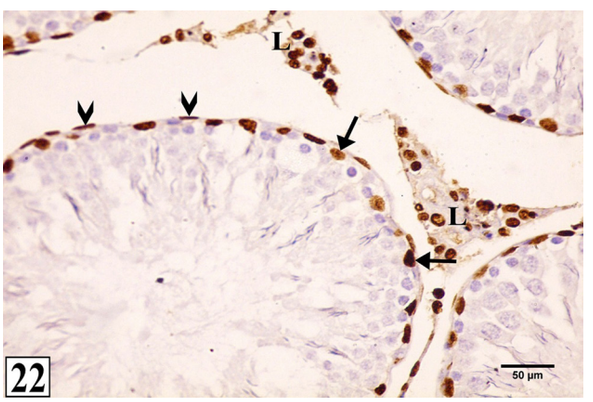


**Fig. 22:** A photomicrograph of a section in the testis from group IV (SRT + spirulina group) showing intense immuno-staining in myoid (arrow heads), Sertoli cells (arrows) and interstitial cells of Leydig (L) (AR immunostaining ×400).

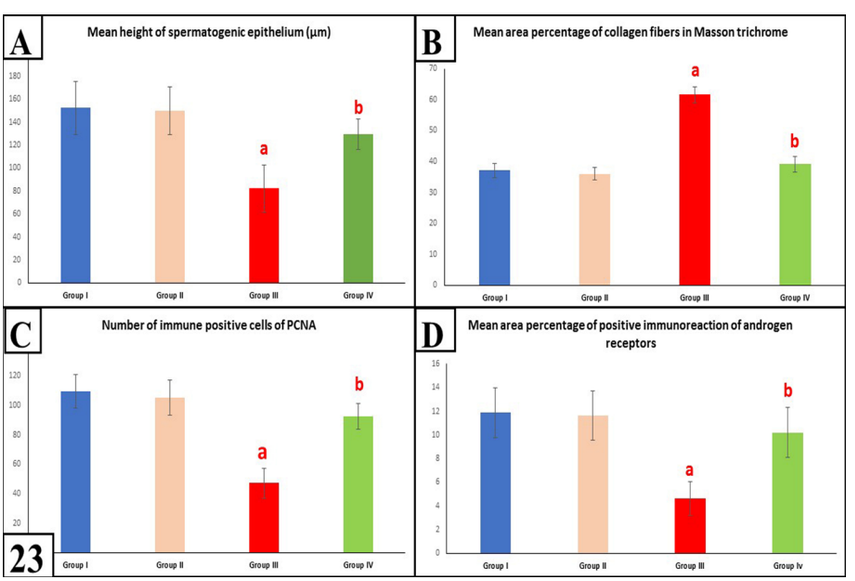


**Fig. 23:** A histogram showing: (A) Mean height of spermatogenic epithelium (µm). (B) Mean area percentage of collagen fibers in Masson trichrome. (C) Number of immune positive cells of PCNA. (D) Mean area percentage of positive immunoreaction of AR. a indicates an extremely significant vs. control group. b indicates an extremely significant vs group III.

**Table 1:** Morphometrical and statistical analysis of different studied groups

| | Group I | Group II | Group III | Group IV |
|---|---|---|---|---|
| | Mean ± SD | | | |
| Mean height of spermatogenic cells (µm) | 152.51±23.12 | 150.01±20.86 | 82.27±20.22[a] | 129.53±13.04[b] |
| The mean area percentage of collagen fibers | 37.12±7.2 | 36.05±6.5 | 61.59±8.0[a] | 39.14±8.18[b] |
| The mean number of immune positive cells of PCNA | 109.5±11.40 | 105.3±11.87 | 47.2±10.11[a] | 92.4±8.82[b] |
| Mean area percentage of positive immunoreaction of androgen receptors | 11.88±2.1 | 11.64±2.1 | 4.62±1.4[a] | 10.21±2.1[b] |

Data are shown as mean ± standard deviation. a, indicate an extremely significance versus control, b, indicates an extremely significant vs group III (SRT group) respectively.

## DISCUSSION

Sertraline (SRT) is one of the SSRI family that is widely recommended in the management of several mental illnesses as major depressive disorder, anxiety, and obsessive-compulsive disorder. It is more effective than other antidepressants as tricyclic antidepressants[21]. Unfortunately, SRT has several sexual side effects as disorders of orgasm and ejaculation[22] that force the patient to cease its long-term usage that leads to recrudescence of the psychiatric patient.

SRT induces major structural alterations in the male reproductive system by disrupting endocrine function and reducing testosterone (TS) levels with several histopathological changes[23] which is consistent with our current study's findings. We observed widespread immature degenerated germ cells with nuclear pyknosis, vacuolization of epithelium, dilated congested blood and degenerated Leydig cells. Similar findings were reported by Atli *et al*.,[24] and Hamadi[25].

SRT causes the damage of the mitochondrial membrane producing reactive oxygen species (ROS) that create an oxidative stress state in the cell. Testicular tissue and spermatozoa are very sensitive to ROS and lipid peroxidation due to the restricted antioxidant defense system and high levels of polyunsaturated fatty acids in the membrane of sperm cells[26]. Lipid peroxidation induced by ROS causes cell dysfunction due to disruption of both cell membrane and mitochondrial proteins. Additionally, SSRI drugs are reported to bind to sulfhydryl groups in the sperm membrane and impaired ATP synthesis in the sperm by interacting with phospholipids leading to loss of the basement membrane[27].

On the other hand, gonadal steroidogenesis is modulated by the brain via hypothalamic-pituitary-gonadal axis. Gonadotropin-releasing hormone (GnRH) is released from the hypothalamus to activate the pituitary gland to secrete LH (luteinizing hormone), and FSH (follicle-stimulating hormone) to the blood and transported to the gonads. In males, The LH/TS ratio is a good indicator for the pituitary-testis axis activity and gonadal function[28].

Additionally, elevated serotonin in the hypothalamus may disturb serotonergic receptors located at the hypothalamic–pituitary–testicular axis that leads to dysfunction of this axis. SRT also increases the levels of cerebral serotonin levels which in turn prevents the release of GnRH in the hypothalamus impairing FSH and LH secretion, and eventually results in spermatogenesis deficiency[24]. Several researches revealed the decline of the levels of steroidogenic enzymes, as well as Ts production (53% decrease) with SRT administration[29]. Csaba *et al*.,[30] was the first one to report that excess level of serotonin induced by SRT cause disorders in all sperm parameters proved by testicular atrophy, spermatogenesis arrest, and suppression after systemically injecting of serotonin in adult rats.

Câmara *et al*.,[31] concluded that SSRI may induce many sexual dysfunctions by disrupting Ts biosynthesis and spermatogenesis expressed in our study by irregularity of seminiferous tubules, loss of the germ cells , decrease the thickness of germinal epithelium, and decrease of spermatozoa number. A similar finding was also reported by ElMazoudy *et al*.,[32].

Moreover, SRT causes sperm deformities, impairment of sperm motility and morphology, and induction of sperm DNA damage[33]. Germinal cells are also affected due to sperm DNA damage that ends by a decrease in sperm concentration, structural abnormalities, apoptotic, and necrotic cell death[34].

Special organization of germ cells is responsible for healthy epithelial layout with a germinal cell connection. The healthy germinal epithelium is kept in place by a tight relationship between their membranes and specialized junctions of Sertoli cell membrane[35]. On the other hand, disruption of this organization is the main cause of sloughing of the germ cells leading to decrease epithelial height confirmed by our statistical analysis that declares a significant decrement in the mean of the germinal epithelium height in comparison with the control group. Hajizadeh's *et al*.,[36] work on fluoxetine toxicity on testis was in agreement with our findings.

In our study, some seminiferous tubules had no sperms at all that was in coincidence with Erdemir *et al*.,[26]. The previous finding confirming the spermicidal effect of SRT proved by the decrease in the number of spermatogenic cells and seminiferous tubule atrophy which are indicators of spermatogenesis failure[37].

Some nuclear changes were detected in some Leydig cells and spermatogonia that are attributed to irreversible

dissolution of the chromatin of the nuclei of cells undergoing necrosis due to denaturation of nuclear proteins and/or enzymatic digestion[38].

The spermatogenic cells and Leydig's cells in our research showed cytoplasmic vacuolization suggesting cellular swelling due to alternation in the cell membrane and disturbance of the energy-dependent Na+/K+ ion pumps in cell membranes induced by lipid peroxidation. Intracellular accumulation of Na+ increases the osmolarity of the tissue with the subsequent entrance of introduction of water into the cells[18].

Additionally, exposure of spermatogenic cells to ROS causes disruption of the blood-testis barrier followed by toxic agents passage between the cells and widening of intercellular spaces between cells seen in this study[39].

Regarding the basement membrane of the seminiferous tubules of SRT group, it showed focal areas of loss, and irregularity that was also noticed ultrastructurally by Pasha *et al*.,[40]. Irregularity of basement membrane may attribute to myoid cell contraction or tubular shrinkage in degenerated seminiferous tubules study[41].

Dilated congested blood vessels were seen in our work was similarly reported and explained by Madlool's *et al*.,[42] study who explained their dilatation by blocking of voltage-dependent L-type calcium channels (decreasing of intracellular calcium current) by SRT leading to relaxation of vascular smooth muscles and vasodilation[43].

Intertubular homogenous acidophilic substances found in our study were reported and explained by Yassin and Ghoneim[44] on their work on oxidative stress induced by diabetes on the testis. It may be related to the hyalinization of the degenerated germ cells of the damaged seminiferous tubules due to impaired phagocytic function of Sertoli cells. Moreover, this may also be correlated to lymphatic exudates leakage from degenerated lymphatic arteries or an increase in their vascular permeability[45].

Leydig cells and testicular interstitial macrophages are functionally related due to close physical association between them so Leydig cells are susceptible to oxidative damage due to ROS-producing macrophages[46]. Leydig cell steroidogenesis is reduced when macrophages are activated by ROS state and produce pro-inflammatory mediators that disrupt the mitochondria of Leydig cells and suppress the steroidogenesis[47]. TS is required during spermatogenesis with intact blood-testis barrier, germ meiosis, intact Sertoli-spermatid and secretion of sperms[48].

Additionally, degeneration of the Leydig cells observed in our study may be related to accumulation of serotonin in the testicles that impede the nourishment of Leydig cells and decrease testicular blood supply[49].

The marked increase in the thickness of connective tissue capsule (tunica albuginea) and perivascular collagen fibers deposition was seen in our Masson trichrome stained sections of SRT group. It was aligned with the finding of Abd El- Salam *et al*.,[50] in his work on the oxidative stress induced by lead exposure. Hydroxyl radicals and other highly reactive oxidizing molecules induced by oxidative stress cause lipid peroxidation and severe damage to proteins and nucleic acids that result in increase in the deposition of collagen fibers and ground substance formation[51].

On the other side, PCNA is regarded as a valuable intranuclear molecular marker to detect cells involved in DNA synthesis that can also evaluate the proliferation of cells and the spermatogenic function of testis in case of male infertility[52]. Significant decrement in PCNA positive immunostained cells in SRT group was observed in our current research confirming the inhibitory effect of SRT on the proliferative process in germinal cells due to DNA fragmentation. Hamdi's[25] study on SRT documented a similar finding by using comet assay to measure the breaks in DNA strands. Moreover, this finding was documented by Badr El- Din and and Abd-El Aty[51] in his work on oxidative stress induced by duloxetine hydrochloride in an animal model of depression.

TS is the main androgen generated by Leydig cell in response to LH stimulation then it diffuses into the seminiferous tubules. It is responsible for regulating spermatogenesis. Androgen activities are mediated by the androgen receptor (AR) found in the nucleus of Sertoli cells[53]. AR is also expressed in Leydig cells, myoid cells, arteriole smooth muscle, and vascular endothelial cells. There are no functioning androgen receptors in germ cells. TS diffuses into Sertoli cells, binds to AR, and activates the functional responses necessary to sustain spermatogenesis[54].

Concerning SRT group, we observed very weak immune-staining in the nuclei of Sertoli cells and few immune-positive cells of both myoid & interstitial cells of Leydig that was attributed to AR damage that occurred in response to testicular oxidative burden[55]. These findings were in accordance with the results of Soliman *et al*.,[56] in his work on fluoxetine toxicity on the testis.

To the best of our knowledge, our study is the first to show that spirulina plays a significant role in sertraline-induced testicular damage and document the protective role of co-treatment of spirulina with sertraline as it ameliorates most of the histological alterations except in few focal areas. Collagen deposition was decreased while the of the height of germinal epithelium and numbers of PCNA immunopositive cells was increased with intense immunostaining of AR in myoid cells, Sertoli cells and interstitial cells of Leydig. Our morphometrical data and statistical data confirmed our histological data.

Spirulina is one of the most common herbal medicine for curing different diseases. It has many pharmacological properties as anti-inflammatory, anti-cancer, anti-diabetic, anti-microbial, anti-histaminic, anti-infertility, hypotensive, neuroprotective, hepatoprotective, and anticarcinogenic properties[57]. It has been used in healthy

foods, animal feed, and biological products since the 1980. It is called "the food of the future" due to its cost-effective and high nutritional value with excellent components and high energy. It can be supplied as spirulina pills, capsules, pastries, blocks, and spirulina containing chocolate bars[58]. It contains multiple bioactive substances with powerful anti-oxidation properties as phycocyanin, polyunsaturated fatty acids, polysaccharide, and other bioactive compounds such as phenolic compounds, vitamins, carotenoids, trace elements, and minerals[59].

The protective effect of spirulina as a powerful antioxidant may be related to the free radical scavenging of this alga that can be exerted in different ways as preventing DNA damage and lipid peroxidation. Moreover, it enhances the activity of superoxide dismutase and catalase, as well as reducing ROS[60]. Therefore, we choose spirulina as a possible protective agent against SRT reprotoxicity.

Moreover, spirulina is a rich source of C phycocyanin which is a powerful antioxidant with a high capacity to scavenge free radicals such as superoxide and hydroxyl radicals. It provides significant protection against ROS due to its radical scavenging action and suppressive impact on lipid peroxidation chain reaction[61].

Spirulina is a rich supply of antioxidant vitamins content as tochopherol (vitamin E) or ascorbic acid (vitamin C) that can attenuate these pathological testicular alterations and help to enhance the structural and functional integrity of the testis. Vitamin E traps lipid peroxyl and many other molecules to reduce the peroxidation of cell membrane lipids and prevent oxidative damage[62]. Vitamin C influences the hypothalamo-pituitary-testicular axis, causing a rise in the testosterone level. It acts as a cytoprotective against sperm oxidative stress and attenuates testicular toxicity. It increases sperm concentration and reduces sperm motility loss[63].

Additionally, it is a great source of necessary fatty acids as gamma linoleic acid and alfa linolenic acid that ameliorate tissue regeneration. Meanwhile, it is a rich supply of flavonoids that can reduce lipid peroxidation and increase the survival of collagen fibrils by enhancing DNA synthesis[64].

Spirulina has been shown to enhance antioxidant activity in a systemic oxidative stress state and has self-regulated antioxidant activity depends on the level of oxidative stress. Moreover, it has an antioxidant role in preventing local testicular injuries as lead toxicity[65], furan toxicity[66], cadmium toxicity[67], arsenite toxicity[68] and a promising role in improving reprotoxicity and all parameters of male fertility in rats as evidenced by increasing sperm motility and sperm count[69].

Additionally, spirulina has antiapoptotic properties that improve cell injuries and enhance the oxidation process in the body[70]. Moreover, it upregulates PCNA immunoexpression reported by Khalil *et al*.,[71] in his work on oxidative stress induced by furan that was correlated to our immunohistochemical and statistical finding.

According to Ibrahim *et al*.,[72] spirulina can restore the alternations in the level of FSH and LH. It restores steroidogenesis in Leydig cells and TS-mediated spermatogenesis. Moreover, it can upregulate testicular AR which was in coincidence with our statistical findings.

## CONCLUSION

This study may therefore suggest that men suffering from depression and treated with SRT should be monitored carefully for any side-effects on the sexual dysfunction as it causes destructive histological changes mentioned in this research. Additionally, administered of spirulina extract may restore histoarchitecture of the testis that give us a push to apply it in clinical researches to assess its efficacy against the sexual side effect of SRT in humans.

## CONFLICT OF INTERESTS

There are no conflicts of interest.

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**الملخص العربى**

# دور خلاصة السبيرولينا في تحسين التغيرات الهستولوجية و الهستوكيميائية المناعية المستحثة بالسيرترالين في خصية الجرذ الأبيض البالغ

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**قسم الهستولوجيا وبيولوجيا الخلية - كلية الطب - جامعة طنطا**

**المقدمة:** السيرترالين هو دواء انتقائي مثبط لاسترداد السيروتونين يستخدم على نطاق واسع لعلاج الاكتئاب مع العديد من الآثار الجانبية الجنسية. السبيرولينا هو أحد مضادات الأكسدة القوية و مضادات موت الخلايا لأنه مصدر غني للبروتينات والأحماض الدهنية المتعددة غير المشبعة والفيتامينات والكاروتينات.

**الهدف من العمل:** هو تقييم التغيرات الهستولوجية و الهستوكيميائية المناعية المستحثة بالسيرترالين في الخصية وكذلك التأثير الوقائي المحتمل للسبيرولينا لتحسين هذه التغيرات في خصية الجرذ الأبيض البالغ.

**المواد والطرق:** أجريت هذه الدراسة الحالية على أربعين من ذكور الجرذان البيضاء البالغة ، وتم تقسيمهم بشكل عشوائي إلى أربع مجموعات رئيسية: المجموعة الأولى تمثل المجموعة الضابطة ، المجموعة الثانية أعطيت السبيرولينا (٥٠٠ مجم / كجم / يوم) بالفم على التوالي لمدة ٢٨ يوماً، وتم إعطاء المجموعة الثالثة السيرترالين ( ٢٠مجم / كجم / يوم) بالفم لمدة ٢٨ يوماً متتالية ، وتم إعطاء المجموعة الرابعة كلاً من السيرترالين والسبيرولينا بنفس الجرعات و الطريقة كالمجموعات الثانية والثالثة لمدة ٢٨ يومًا متتالية. وتم تجهيز عينات الخصيتين للدراسة الهستولوجية و الهستوكيميائية المناعية. كما تم إجراء دراسات قياسية وإحصائية.

**النتائج:** تسبب السيرترالين في العديد من التغييرات المدمرة للخصيتين متمثلة في تدمير معظم الأنابيب المنوية و موت الخلايا المنوية مع تجاويف للسيتوبلازم وظهور أنوية داكنة. لوحظ أيضا تناثر الخلايا المنوية و عدم وجود حيوانات منوية داخل تجويف الأنابيب. كما لوحظ تدمير خلايا ليدج، تمدد واحتقان الأوعية الدموية و وجود مادة ذات صبغة حمضية في النسيج الضام بين الأنابيب. تم الكشف عن زيادة ذو دلالة احصائية بالغة في متوسطات النسبة المئوية لمساحة صبغة الماسون الثلاثية بينما لوحظ انخفاض ذو دلالة احصائية بالغة في متوسط طول الخلايا المبطنة للأنابيب المنوية و عدد الخلايا المصبوغة مناعيا بمستضد تكاثر انوية الخلايا و النسبة المئوية للمساحة الإيجابية لمستقبلات الأندروجين. و في المقابل، قضى العلاج المشترك بالسبيرولينا على معظم التغيرات النسيجية باستثناء بعض المناطق الموضعية.

**الخلاصة: تعمل خلاصة السبيرولينا على تحسين تغيرات الخصية التي يسببها السيرترالين.**