Reproductive System and Sexual Disorders: Current Research

Rezk et al., Reprod Syst Sex Disord 2014, 3:4
DOI: 10.4172/2161-038X.1000146

Research Article Open Access

Effect of Green Tea Extract on the Interferon-Induced Testicular Apoptosis in the Adult Albino Rat: Immunohistochemical and Electron Microscopic Study

Hassan M Rezk[1]*, Mohamed Elsherbiny[1], Wagdi F Elkashef[2] and Medhat Taha[1]

[1]*Anatomy and Embryology Department, Faculty of Medicine - Mansoura University - Egypt)*
[2]*Pathology Department, Faculty of Medicine, Mansoura University, Egypt*

Abstract

Background: Interferons (IFNs) are widely used in the treatment of various diseases and can disrupt spermatogenesis and inhibition Leydig cell steroidogenesis through the formation of reactive oxygen species (ROSs) that cause oxidative stress.

Aim of the work: In this study we examine the effect of (IFN) on rat spermatogonia and investigate the possible protective effect of Grean tea on (IFN)-induced spermatogonia appotosis.

Material and Methods: Forty-eight adult male albino rats (240-280 g) were used in this study. They were divided into three groups. Group I in which rats were served as a control and were given sterile normal saline. The rats in Group II were given 7.500 units of interferon (INF) α-2_{β}, considered in clinical treatment dose range, 3 times weekly under inhalation anesthesia for 2 month (Group II a) and 3 month (Group II b). Group III received Green tea extract and interferon α-2_{β} in the same dose of group (II) for 2 month (Group III a) and 3 month (Group III b). 200 mg of green tea extract was dissolved in distal water and solution was administrated orally through gastric tube at dose level of (40 mg/k.g.b.w.). At the end of the 2 and 3 month respectively, bilateral orchiectomy was performed through standard ilioinguinal incisions and the rats were sacrified by pentobarbital overdose (200 mg=kg) inhalation. The testes were removed and separated. A part of one testis was fixed in Bouin fixative to be used for histopathological and immunohistochemical studies for p53 and the other part was used in a fresh state to determine the level of the Superoxide Dismutase (SOD). The other testis was fixed in 1% gluteraldehyde and 4% formaldehyde in (pH 7.4) for at least 2 hours to overnight. Ultrathin sections processed for transmission electron microscopic studies.

Results: Interferon induced testicular damage that is revealed by loss of testicular architecture through Hx. and E. stained sections, Immunohistochemical stained sections against p53 and the electron microscopic examination. These findings were accompanied by significant reduction in diameter of seminiferous tubules and thickness of the germinal epithelium with significant reduction in SOD. Otherwise, the green tea extract definitely reverse the above mentioned findings with improvement of testicular architecture and significant increase in diameter of seminiferous tubules, thickness of the germinal epithelium and elevated SOD. The mean of the diameter of the seminiferous tubules of Group IIa showed a significant decrease by nearly 34% in comparison to Group IIb showed a higher significant decrease by nearly 43%. The mean of the thickness of the seminiferous tubules of Group IIa showed a significant reduction by nearly 33% while in Group IIb showed a higher significant reduction by nearly 50%. The mean of the level of the Superoxide Dismutase in Group IIa showed significant decrease by nearly the half while in Group IIb revealed a higher significant decrease by nearly 59%. The mean of the diameter of the seminiferous tubules in Group IIIa showed a significant increase by nearly 32% and by nearly 43% in Group IIIb. The mean of the thickness of the seminiferous tubules in Group IIIa showed significant increase by nearly 11% while in Group IIIb the significant increase was by nearly 45%. The mean of the level of the Superoxide Dismutase of the seminiferous tubules in Group IIIa did not show significant increase while in Group IIIb revealed a higher significant increase by nearly 48% Group IIIb.

Conclusion: The green tea extract has a powerful antioxidant effect that can reverse the damage caused by Interferon induced oxidative stress and apoptosis.

Keywords: Green Tea; Interferon ; Spermatogonia; p53; Super Oxide Dismutase

Introduction

Interferons (IFNs) are widely used in the treatment of various diseases including acute and chronic viral illnesses, autoimmune and neoplastic diseases [1]. IFNs have direct effects such as inhibition of cell proliferation, stimulation of immunologic functions, anti-angiogenesis and production of cytokines [2]. INF-α is the most commonly used antiviral drug as it limit viral spread by increasing p53 activity, which kills virus-infected cells by promoting apoptosis [3,4], have been demonstrated that reactive oxygen species (ROS) and the resulting oxidative stress play an important role in apoptosis.

Interestingly (IFN) can severely disrupt spermatogenesis by inhibition Leydig cell steroidogenesis in vitro [5] and decreased serum testosterone and free androgen index through its effect on hypothalamicpituitary-testicular axis which leads to sterility. Sertoli cells appeared to be privileged targets of (IFN) which produce high (IFN) concentrations following viral exposure [6].

***Corresponding author:** Hassan M. Rezk, Anatomy and Embryology Department, Batterjee Medical College (BMC), Jeddah, Kingdom of Saudi Arabia (KSA), Tel: +2 01128044681 (Egypt), + 966582299573 (KSA); E-mail: hmsr_74@hotmail.com

Received October 27, 2014; **Accepted** December 24 29, 2014; **Published** January 02, 2014

Citation: Rezk HM, Elsherbiny M, Elkashef WF, Taha M (2014) Effect of Green Tea Extract on the Interferon-Induced Testicular Apoptosis in the Adult Albino Rat: Immunohistochemical and Electron Microscopic Study. Reprod Syst Sex Disord 3: 146. doi: 10.4172/2161-038X.1000146

IFNs are important triggers for the formation of reactive oxygen species (ROSs) that cause oxidative stress [7-9]. Indeed, ROS levels increased up to a two folds higher level after 48 hours in response to IFN-g in HeLa and T1 cells. Endogenous ROS formation may cause oxidant-damage to proteins. Therefore, by detection of carbonyl groups, we tested whether IFN stimulation results in increased levels of oxidized proteins in HeLa and T1 and T2 cells [10].

The apoptotic process involved in the male reproductive system regulates the ability of the male to fertilize and pass on his genes through the process of spermatogenesis. Numerous factors are involved in mediating this essential and intricate process. It is known that without programmed cell death there would be an overwhelming amount of chaos within the seminiferous tubules of the testis, which would lead to dysfunctional spermatogenesis, and problems within the male reproductive system. Many factors effecting testicular apoptosis, e.g. Nuclear Factor kappa B (NF-κB), cytokines and IFN as they appear to have a significant role in germ cell death [11]. IFN-α and γ predominate within the testis and are produced by macrophages, as well as Sertoli and Leydig cells [12]. Again, there is a decrease in testosterone production associated with the interferon family, as seen within porcine Leydig cells. IFN-γ mediates this through down regulation of the P450c17 gene, as well as inhibiting the transport of cholesterol into mitochondria [13].

Although interferon-α has been used in the treatment of mumps orchitis, the preventive role of the interferon treatment has still been controversial. Significant preventive effects of systemic treatment with interferon α-2_β for infertility from mumps orchitis has been reported by two articles [14]. In contrast to these studies, Yeniyol et al. [15] reported the role of interferon-α for the prevention of testicular atrophy in postpubertal men with unilateral mumps orchitis. They found that systemic treatment with interferon α-2_β was not completely effective in preventing testicular atrophy after mumps orchitis. Possible effects of interferon-α on sperm parameters in the treatment of male infertility have been controversial. Yamamoto and Miyake reported successful use of interferon for male infertility [16].

However, azoospermia was reported in a patient receiving interferon-α for a stage 3 melanoma Longo et al., Hibi et al. [17,18] found that daily sperm production and epididymal sperm concentrations were significantly increased after interferon-α treatment in the nude rats.

Spermatogenic cells at various stages of differentiation are prone to undergo natural apoptosis; however, excess apoptosis would cause infertility due to germ cell loss [19]. Vitamins E and C and α-lipoic acid are naturally occurring antioxidants, working in synergy with each other and against different types of free radicals [20].

In previous clinical studies, it has been shown that antioxidants are severely depleted in serum and liver tissue of patients with chronic hepatitis C infection [21]. The inverse relationship between serum antioxidants and viral disease progression has been demonstrated in several studies [22].

The antioxidant therapy may have a beneficial effect in patients with chronic hepatitis C (CHC) infection [23]. Although clinical studies with antioxidant have been conducted in patients with (CHC), green tea has not been studied alone or in combination with antiviral treatment in (CHC). Green tea contains salubrious polyphenols, in particular catechins, the most abundant of which is epigallocatechin gallate (EGCG). Green tea also contains carotenoids, tocopherols, ascorbic acid (vitamin C), minerals such as chromium, manganese, selenium or zinc, and certain phytochemical compounds. In vitro, animal, preliminary observational and clinical human studies suggest that green tea can reduce the risk of cardiovascular disease, dental cavities, kidney stones, and cancer, while improving bone density and cognitive function. However, the human studies are inconsistent [24].

Green tea extracts exhibit stronger antioxidant protection for human body than vitamin C and vitamin E [25]. scavenging effect of lipid free-radicals of green tea polyphenols (GTP) in green tea extracts can be clearly observed in experiments.

In this study we examine the effect of (IFN) on rat spermatogonia and investigate the possible protective effect of Grean tea on (IFN)-induced spermatogonia appotosis.

Materials and Methods

Animal used

Forty-eight adult male albino rats (240-280 g) obtained from Medical Research center, Faculty of Medicine, Mansoura University, Egypt were used in the present study. The animals were housed in cages at room temperature (22–25°C) and in a photoperiod of 14-h light/10-h dark/day. Rats were maintained on standard laboratory balanced commercial diet and water ad libitum. All experiments were performed in line with the ethical considerations, recommended by the Faculty of Medicine, Mansoura University, Egypt.

Experimental design

In this experimental randomized controlled trial study, the animals were divided into three groups, each group comprising of sixteen rats as detailed below:

Group I: Sixteen rats served as a control group receiving 0.5 mL of saline by injection.

Group II: Sixteen rats received 7.500 units of interferon (INF) α-2_β (Beijing Kawin Bio-Tech Co., Ltd. china), considered in clinical treatment dose range, 3 times weekly under inhalation anesthesia for 2 month (Group II a) and 3 month (Group II b).

Group III: Sixteen rats received Green tea extract and interferon α-2_β in the same dose of group (II) for 2 month (Group III a) and 3 month (Group III b). 200 mg of green tea extract was dissolved in distal water and solution was administrated orally through gastric tube at dose level of (40 mg/k.g.b.w.).

One month duration was neglected from this study due to the duration of the spermatogenic cycle. In most rodent species, the duration of the cyclic changes of the seminiferous epithelium is between 8 and 13 days [26]. Approximately four cycles of the seminiferous epithelium elapse between the initial spermatogonial division and spermiation [27].

At the end of the 2 and 3 month respectively, bilateral orchiectomy was performed through standard ilioinguinal incisions and the rats were sacrified by pentobarbital overdose (200 mg=kg) inhalation. The testes were removed and separated. A part of one testis was fixed in Bouin fixative to be used for histopathological and immunohistochemical studies for p53 and the other part was used in a fresh state to determine the level of the Superoxide Dismutase (SOD). The other testis was fixed in 1% gluteraldehyde and 4% formaldehyde in (pH 7.4) for at least 2 hours to overnight. Ultrathin sections processed for transmission electron microscopic studies.

Three slides prepared from the upper, lower and mid portions of the testes were evaluated completely for each testis and evaluated with standard light microscopy by a blinded observer in random order.

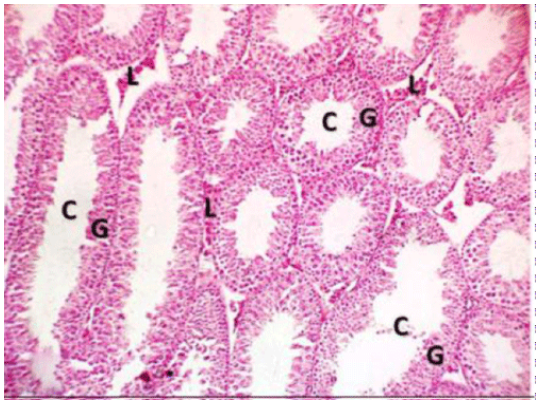

Figure 1: A photomicrograph of a section of seminiferous tubules of a control adult male albino rat group (I) showing circular to oval seminiferous tubules with central cavity (C) lined by stratified germinal epithelium (G) separated by interstitial cells of leyding (L). (Hx. & E., X100).

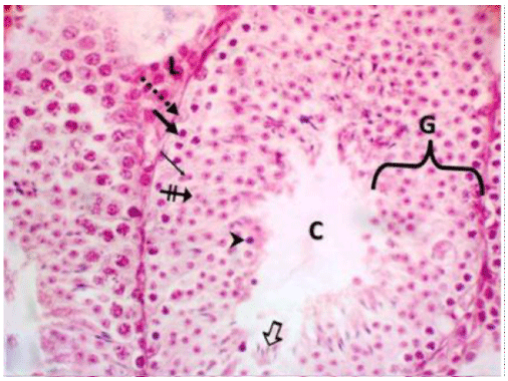

Figure 2: A photomicrograph of a section of seminiferous tubules of a control adult male albino rat group (I) showing circular to oval seminiferous tubules with central cavity (C) lined by stratified germinal epithelium (G) separated by interstitial cells of leyding (L). The normal germinal epithelium (brace) composed of spermatogonia (Large arrow), primary spermatocyte (small arrow), secondary spermatocyte (crossed arrow), spermatide (arrow head), spermatozoa (empty arrow) and sertoli cells (striped arrow). (Hx. & E., X400).

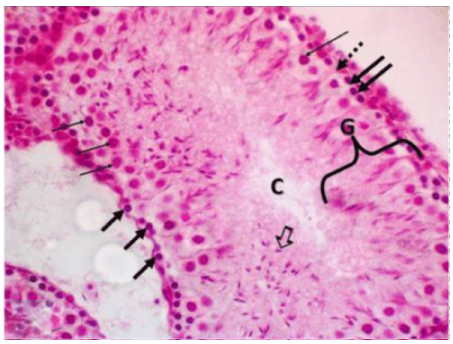

Figure 3: A photomicrograph of a section of seminiferous tubules of an adult male albino rat received interferon for 2 month group (IIa) showing abnormal circular to oval seminiferous tubules that form about 70% of the seminiferous tubules (subjective) with central cavity (C) lined by stratified germinal epithelium (G). The thickness of the germinal epithelium (brace) shows relative reduction with relative increase in number of spermatogonia (Large arrow), primary spermatocyte (small arrow) and spermatozoa (empty arrow) and sertoli cells (striped arrow) with absent secondary spermatocyte and spermatide. (Hx. & E., X400).

Histological techniques

A. **Light microscopy**

1) Hematoxylin and Eosin stain: routine staining for seminiferous tubules.

2) Immunohistochemistry for p53: for apoptosis in seminiferous tubules.

B. **Electron microscopy:** To detect ultra-structural changes in the germinal epithelium and interstitial cell of Leyding by using Transmission Electron Microscopy (TEM), JEOL, JEM-2100, Electron Microscopic Unit, Mansoura University.

Super oxide dismutase level detection (SOD) in the testicular tissue

Superoxide dismutases (SODs) are metalloenzymes that catalyze the dismutation of the superoxide anion to molecular oxygen and hydrogen peroxide and thus form a crucial part of the cellular antioxidant defense mechanism.

202”+2H++SOD ► H2 02+02

Three types of SODs have been characterized according to their metal content: copper zinc (Cu/Zn), manganese (Mn), and iron (Fe). The amount of SOD present in cellular and extracellular environments is crucial for the prevention of diseases linked to oxidative stress. This Assay of SODs relies on the ability of the enzyme to inhibit the phenazine methosulphate-mediated reduction of nitroblue tetrazolium dye [28].

Histological measures: include

a. Seminiferous Tubular Diamater (STD).

b. Germinal Epithelial Cell Thickness (GECT).

These measures were done by using Microscope Olympus BX45, Camera AmScope MU1000 and Software Toup Tek, Toup View x64. These measures were used to evaluate in 20 seminiferous tubule of each section. The mean STD and GECT in each testis were determined in mm.

Statistical analysis

All data are presented as mean ± SEM. All analyses were carried out using the SPSS software version 17. One-way analysis of variance (ANOVA) was utilized for comparison between the means of the three groups and the least significant difference test was used for comparison of each two individual means. P≤0.05 was considered statistically significant.

Results

A. **Interferon treated groups (Groups II a and b)**

The light microscopic examination of the Haematoxylin and Eosin stained sections of the seminiferous tubules of adult albino rats treated with interferon for 2 month (Group IIa) showed abnormal circular to oval seminiferous tubules that form about 70% of the seminiferous tubules with central cavity lined by stratified germinal epithelium (Figures 1-3). Some seminiferous tubules showed absent central cavity due to premature sloughing and disarrangement of the germinal epithelium layers (Figure 4).

The thickness of the germinal epithelium was relatively reduced with relative increase in number of spermatogonia, primary spermatocyte, spermatozoa and sertoli cells (Figure 3). Some seminiferous tubules

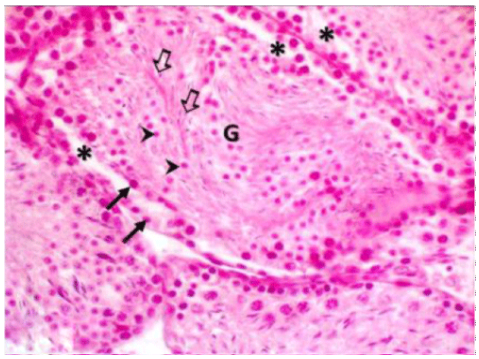

Figure 4: A photomicrograph of a section of seminiferous tubules of an adult male albino rat received interferon for 2 month group (IIa) showing oval seminiferous tubules with absent central cavity due to premature sloughing (Asterisk) of the disarrangement germinal epithelium (G) that showes spermatogonia (Large arrow), spermatide (arrow head) and spermatozoa (empty arrow) with absent primary spermatocyte and secondary spermatocyte. (Hx. & E., X400).

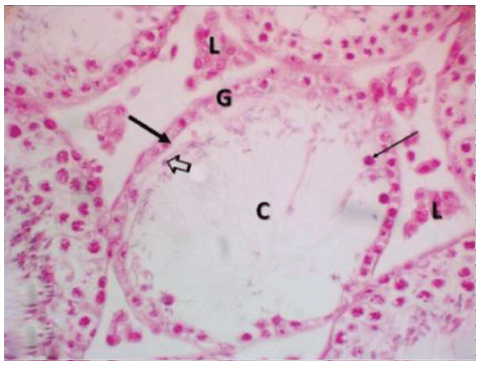

Figure 5: A photomicrograph of a section of seminiferous tubules of an adult male albino rat received interferon for 3 month group (IIb) showing abnormal oval seminiferous tubules that form about 80% of seminiferous tubules (subjectives) with central cavity (C) lined with single layer of germinal epithelium (G) that showes spermatogonia (Large arrow), primary spermatocyte (small arrow), scanty spermatozoa (empty arrow) and interstitial cells of leyding (L) with absent spermatide and secondary spermatocyte. (Hx. & E., X400).

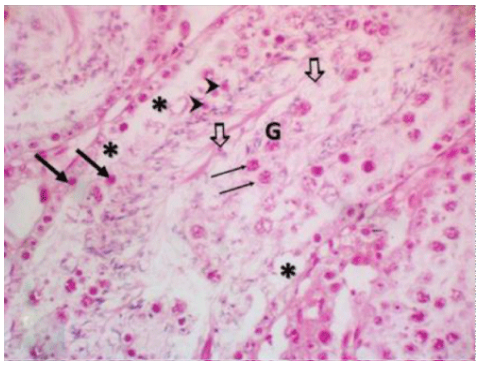

Figure 6: A photomicrograph of a section of seminiferous tubules of an adult male albino rat received interferon for 3 month group (IIb) showing oval seminiferous tubules with absent central cavity due to premature sloughing (Asterisk) of the disarrangement germinal epithelium (G) that showes spermatogonia (Large arrows), primary spermatocyte (small arrows), spermatide (arrow heads) and spermatozoa (empty arrows) with absent of secondary spermatocyte. (Hx. & E., X400).

showed absence of primary, secondary spermatocytes and spermatids (Figures 3 and 4).

The light microscopic examination of the Haematoxylin and Eosin stained sections of the seminiferous tubules of adult albino rats treated with interferon for 3 month (Group IIb) showed abnormal oval seminiferous tubules that form about 80% of seminiferous tubules. The central cavity of the seminiferous tubules lined with single layer of germinal epithelium (Figure 5) while other seminiferous tubules showed premature sloughing of the disarrangement germinal epithelium (Figure 6).

The germinal epithelium showed spermatogonia, primary spermatocyte, scanty spermatozoa and interstitial cells of leyding with absent spermatids and secondary spermatocyte (Figure 5) while other tubules showed spermatozoa with absent of secondary spermatocyte (Figure 6).

The light microscopic examination of the immunohistochemical

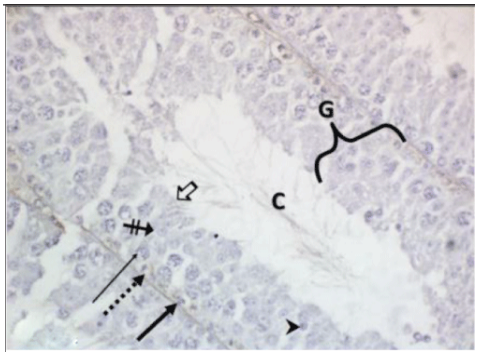

Figure 7: A photomicrograph of a section of seminiferous tubules of a control adult male albino rat group (I) showing seminiferous tubules with central cavity (C) lined by stratified germinal epithelium (G). The normal germinal epithelium (brace) composed of spermatogonia (Large arrow), primary spermatocyte (small arrow), secondary spermatocyte (crossed arrow), spermatide (arrow head), spermatozoa (empty arrow) and sertoli cells (striped arrow). All cells show negative immunohistochemical reactivity. (Immunohistochemical stain, Counterstained with Hx., X400).

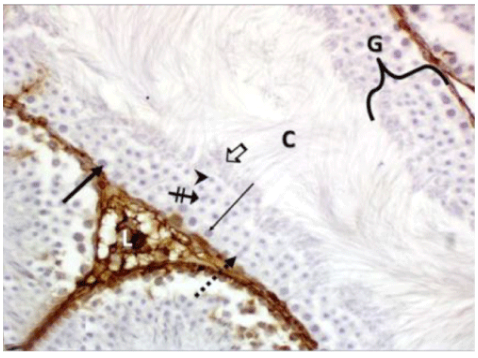

Figure 8: A photomicrograph of a section of seminiferous tubules of an adult male albino rat received interferon for 2 month group (IIa) showing seminiferous tubules with central cavity (C) filled with large amount of spermatozoa lined by stratified germinal epithelium (G). The thickness of germinal epithelium (brace) shows relative reduction with relative increase in number of spermatogonia (Large arrow), primary spermatocyte (small arrow), secondary spermatocyte (crossed arrow), spermatide (arrow head), spermatozoa (empty arrow) and sertoli cells (striped arrow). All these cells show negative immunohistochemical reactivity except interstitial cell of leyding (L) that shows strong cytoplasmic immunohistochemical reactivity . (Immunohistochemical stain, Counterstained with Hx., X400).

stained sections of the seminiferous tubules of an adult albino rats treated with interferon for 2 month (Group IIa) showed seminiferous tubules with central cavity filled with large amount of spermatozoa lined by stratified germinal epithelium. All cells of the germinal epithelium showed negative immunohistochemical reactivity except interstitial cell of leyding that showed strong cytoplasmic immunohistochemical reactivity (Figures 7 and 8).

The immunohistochemical stained sections of seminiferous tubules of an adult male albino rat received interferon for 3 month (Group IIb) showed massive reduction the thickness of germinal epithelium with decrease in the number of spermatogonia. Several spermatogonia in several seminiferous tubules and interstitial cell of leyding showed strong cytoplasmic immunohistochemical reactivity (Figure 9).

The electron microscopic examination of ultrathin section of seminiferous tubules of an adult male albino rat received interferon for

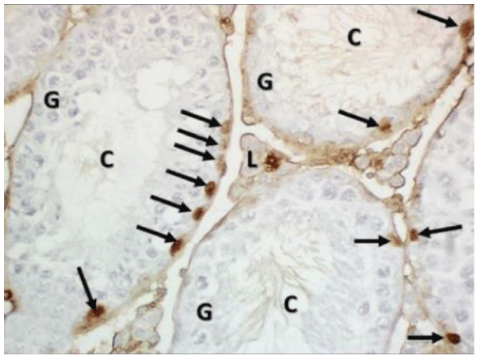

Figure 9: A photomicrograph of a section of seminiferous tubules of an adult male albino rat received interferon for 3 month group (IIb) showing seminiferous tubules with central cavity (C) lined by stratified germinal epithelium (G). The thickness of germinal epithelium (brace) shows massive reduction with decrease in the number of spermatogonia (Large arrow). Several spermatogonia in several seminiferous tubules and one interstitial cell of leyding (L) show strong cytoplasmic immunohistochemical reactivity . (Immunohistochemical stain, Counterstained with Hx., X400).

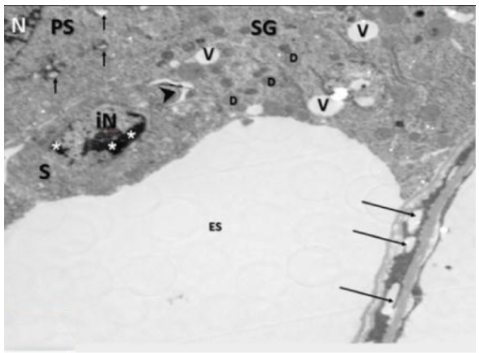

Figure 10: An electron micrograph (TEM) of ultrathin section of seminiferous tubules of an adult male albino rat received interferon for 2 month group (IIa) showing Empty Vaccules (ES), Vacuolated Basement Membrane (arrows). A Sertoli cell (S) showing irregular cell membrane (Bleb) (arrow head) with Nucleus showing irrigular nuclear envelop (iN) and the chromatin arranged against the nuclear envelop (Asterisk). A spermatogonium (SG) showing multiple Intracellular vaccule (V) and multiple electron dense bodies (Lysosomes) (D). A primary spermatocyte (PS) showing part of the Nucleus (N) and small cytoplasmic vaccules (short arrows). (Uranyl acetate and Lead citrate X 2000, Print Mag. X 11700).(Immunohistochemical stain, Counterstained with Hx., X400).

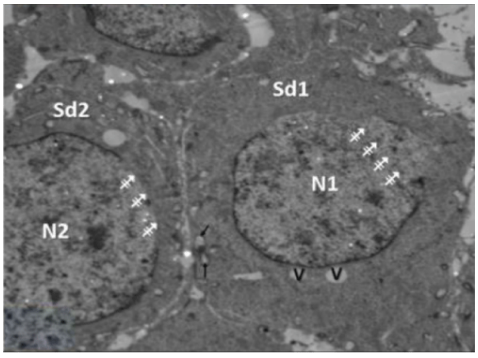

Figure 11: An electron micrograph (TEM) of ultrathin section of seminiferous tubules of an adult male albino rat received interferon for 2 month group (IIa) showing a Primary spermatocyte (Sd1) showing Nucleus (N1) with electron dense clumps of heterochromatin discontinuation of the nuclear membrane (crossed arrows). The cytoplasm showing swollen Mitochondria (short arrows) and Intracellular vaccules (V). Another Primary spermatocyte (Sd2) showing Nucleus (N2) with electron dense clumps of heterochromatin discontinuation of the nuclear membrane (crossed arrows). (Uranyl acetate and Lead citrate X 2000, Print Mag. X 11700).

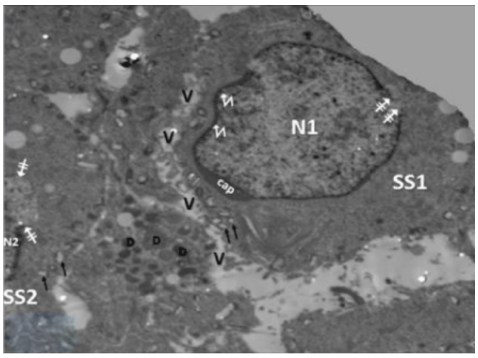

Figure 12: An electron micrograph (TEM) of ultrathin section of seminiferous tubules of an adult male albino rat received interferon for 2 month group (IIa) showing a Spermatid (Sd) showing Nucleus (N1) with electron dense clumps of heterochromatin with irrigular (twisted arrows) and discontinuation (crossed arrows) of the nuclear membrane with Acrosomal Cap (cap). The cytoplasm showing swollen Mitochondria (short arrows), Linear intracellular space (V), Multiple small electron dense bodies (Lysosomes) (D). Secondary spermatocyte (SS) showing Nucleus (N2) with discontinuation of the nuclear membrane (crossed arrows). The cytoplasm showing swollen Mitochondria (short arrows) and intracellular spaces (V). (Uranyl acetate and Lead citrate X 2000, Print Mag. X 11700).

2 month (Group IIa) showed wide variability in the degree of the tubular affection. Some seminiferous tubules showed large empty vacuoles and vacuolated basement membrane. The cell membrane a sertoli cell showed irregularity (Bleb) and the nucleus showed irrigular nuclear envelop and condensed peripheral chromatin. A spermatogonium showed multiple Intracellular vacuoles and multiple electron dense bodies (Lysosomes) (Figure 10).

The Primary spermatocyte and Secondary spermatocyte showed nucleus with electron dense clumps of heterochromatin with irregular discontinues nuclear membrane. The cytoplasm showed swollen mitochondria, multiple cytoplasmic vacuoles and multiple small electron dense bodies (Lysosomes). The Spermatids in addition showed the acrosomal cap (Figures 10-12). On the other hand, some spermatids did not show acrosomal cap while the heads of spermatozoa appeared normal (Figure 13).

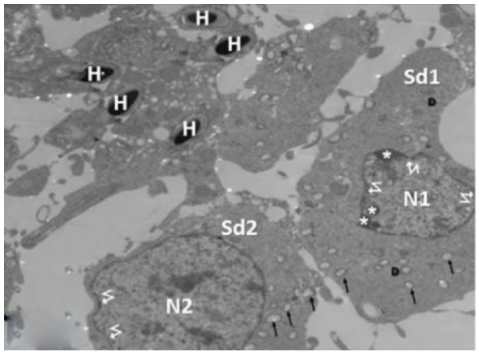

Figure 13: An electron micrograph (TEM) of ultrathin section of seminiferous tubules of an adult male albino rat received interferon for 2 month group (IIa) showing a Spermatide (Sd1) showing Nucleus (N1) with electron dense clumps of chromatine arranged against the nuclear envelop (Asterisks) with irrigular nuclear membrane (twisted arrows). The cytoplasm showing swollen Mitochondria (short arrows) and small electron dense bodies (Lysosomes) (D). Another Spermatide (Sd2) showing Nucleus (N2) irrigular nuclear membrane (twisted arrows). The cytoplasm showing swollen Mitochondria (short arrows). These spermatides do not show acrosomal cap. Heads of spermatozoa appear also in the field (H) which appear normal. (Uranyl acetate and Lead citrate X 2000, Print Mag. X 11700).

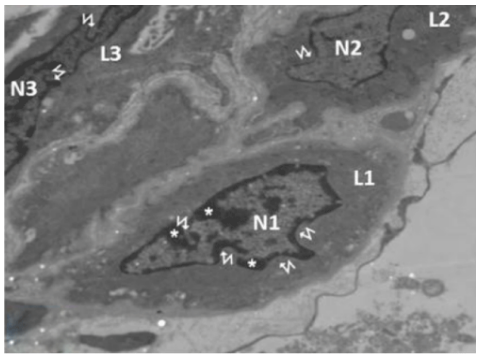

Figure 14: An electron micrograph (TEM) of ultrathin section of seminiferous tubules of an adult male albino rat received interferon for 2 month group (IIa) showing Three Interstitial cell of Lyeding (L1, L2 and L3) showing the Nucleus (N1) of the interstitial cell of lyeding (L1) with electron dense clumps of chromatine arranged against the nuclear envelop (Asterisks). The nuclei (N1, N2 and N3) of the interstitial cell of lyeding (L1, L2 and L3) showing irrigular nuclear membrane (twisted arrows). (Uranyl acetate and Lead citrate X 2000, Print Mag. X 11700).

2 month (Group IIa) showed wide variability in the degree of the tubular affection. Some seminiferous tubules showed large empty vacuoles and vacuolated basement membrane. The cell membrane a sertoli cell showed irregularity (Bleb) and the nucleus showed irrigular nuclear envelop and condensed peripheral chromatin. A spermatogonium showed multiple Intracellular vacuoles and multiple electron dense bodies (Lysosomes) (Figure 10).

The Primary spermatocyte and Secondary spermatocyte showed nucleus with electron dense clumps of heterochromatin with irregular discontinues nuclear membrane. The cytoplasm showed swollen mitochondria, multiple cytoplasmic vacuoles and multiple small electron dense bodies (Lysosomes). The Spermatids in addition showed the acrosomal cap (Figures 10-12). On the other hand, some spermatids did not show acrosomal cap while the heads of spermatozoa appeared normal (Figure 13).

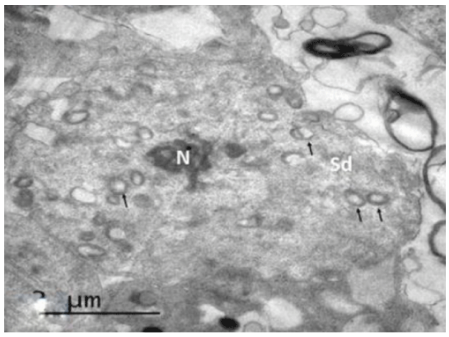

Figure 15: An electron micrograph (TEM) of ultrathin section of seminiferous tubules of an adult male albino rat received interferon for 3 month group (IIb) showing a spermatide (Sd) with irrigular shrunken Nucleus (N). The cytoplasm showing Multiple large degenerating Mitochondria (Short arrows). (Uranyl acetate and Lead citrate X 2000, Print Mag. X 11700).

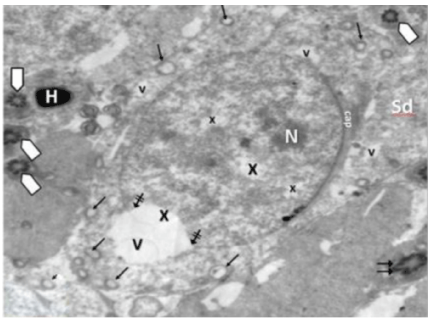

Figure 16: An electron micrograph (TEM) of ultrathin section of seminiferous tubules of an adult male albino rat received interferon for 3 month group (IIb) showing disorganization of the testicular architecture. The Spermatide (Sd) showing Nucleus (N1) intranuclear spaces (X) and discontinuation (crossed arrows) of the nuclear membrane with Acrosomal Cap (cap). The cytoplasm showing intracytoplasmic spaces (V) and excessive larger swollen degenerating Mitochondria (short arrows). The field showing Head of spermatozoan (H) and several cross sections through the middle piece of the spermatozoa (Pentagon). One cross section through the tail (double arrow) showing disorganization of the axonemes. (Uranyl acetate and Lead citrate X 2000, Print Mag. X 11700).

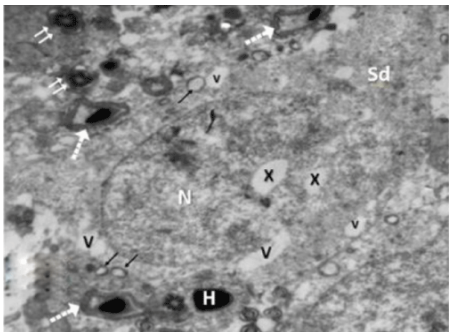

Figure 17: An electron micrograph (TEM) of ultrathin section of seminiferous tubules of an adult male albino rat received interferon for 3 month group (IIb) showing disorganization of the testicular architecture. The Spermatide (Sd) showing Nucleus (N) with intranuclear spaces (X). The cytoplasm showing intracytoplasmic spaces (V) and large swollen Mitochondria (short arrows). Normal Head of spermatozoan (H) and three abnormal heads of spermatozoa with shrunken nuclei (Interrupted arrow) appear in this field. Cross sections through the tails of the spermatozoa showing disorganization of the axonemes and the cell membrane (double arrow). (Uranyl acetate and Lead citrate X 2000, Print Mag. X 11700).

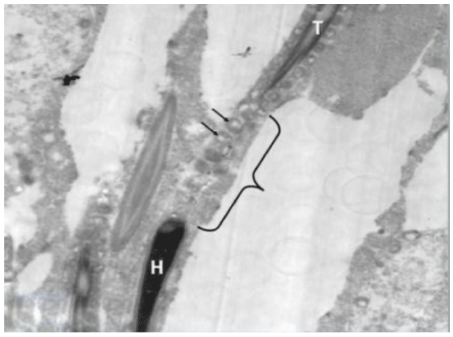

Figure 18: An electron micrograph (TEM) of ultrathin section of seminiferous tubules of an adult male albino rat received interferon for 3 month group (IIb) showing a longitudinal section through a spermatozon that shows Head (H), Middle piece (Brace) with swollen degenerating mitochondria (short arrows) and tail piece (T). (Uranyl acetate and Lead citrate X 2000, Print Mag. X 11700).

Groups	Mean ± SEM	SD	F	P	LSD
Group I (Control)	0.314 ± 0.002	0.008	209.997	<0.001***	0.029500
Group II a (Interferon 2 months)	0.208 ± 0.008	0.023			
Group II b (Interferon 3 months)	0.178 ± 0.006	0.017			
Group III a (Interferon + Green tea 2 months)	0.307 ± 0.004	0.010			
Group III b (Interferon + Green tea 3 months)	0.311 ± 0.003	0.009			

Table 1: Changes in the mean diameter of the seminiferous tubules in the adult rates of control and experimental groups:
SEM: Standard Error of the Mean.
SD: Standard Deviation.
F: F value.
P: Probability.
LSD: Least Significant Difference.
***Highly Significant.
The mean difference is significant at the 0.05 level.

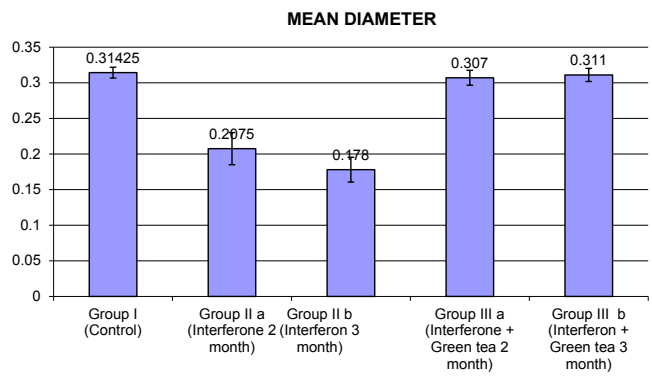

Figure 19: Changes in the mean diameter of the seminiferous tubules in the adult rates of control and experimental groups.

The nuclei of Some Interstitial cells of Leyding showed peripherally arranged electron dense clumps of chromatin. Other nuclei of the interstitial cell of leyding showed irregular nuclear membrane (Figure 14).

The electron microscopic examination of ultrathin section of seminiferous tubules of an adult male albino rat received interferon for 3 month (Group IIb) was similar to that of the 2 month (Group IIb) as mentioned above. But in Group IIb, spermatids showed typical apoptotic figure in the form of irregular shrunken nucleus. The cytoplasm showed multiple large degenerating mitochondria (Figures 15 and 16). The cross sections through the middle piece showed disorganization of the axonemes (Figure 16). The heads of spermatozoa showed shrunken nuclei (Figure 17). The longitudinal section through a spermatozon that showed that the middle piece contained swollen degenerating mitochondria (Figure 18).

Comparing the mean of the diameter of the seminiferous tubules of the experimental rats to that of the control rats revealed that the adult male albino rat received interferon for 2 month (Group IIa) had a significant decrease of the mean of the diameter of the seminiferous tubules by nearly 34% than that of the control. But, adult male albino rat received interferon for 3 month (Group IIb) revealed a higher significant decrease of the mean of the diameter of the seminiferous tubules by nearly 43% than that of the control (Table 1 and Figure 19).

The mean of the thickness of the seminiferous tubules in the experimental adult male albino rat received interferon for 2 month (Group IIa) showed significant reduction by nearly 33% than that of the control group while in adult male albino rat received interferon for

Groups	Mean ± SEM	SD	F	P	LSD
Group I (Control)	0.06862 ± 0.001877	0.007509	29.282	<0.001***	0.010500
Group II a (Interferon 2 months)	0.04625 ± 0.003172	0.008972			
Group II b (Interferon 3 months)	0.03425 ± 0.003150	0.008908			
Group III a (Interferon + Green tea 2 months)	0.05175 ± 0.003217	0.009099			
Group III b (Interferon + Green tea 3 months)	0.06225 ± 0.001980	0.005600			

Table 2: Changes in the mean thickness of the germinal epithelium of the seminiferous tubules in the adult rates of control and experimental groups:
SEM: Standard Error of the Mean.
SD: Standard Deviation.
F: F value.
P: Probability.
LSD: Least Significant Difference.
***Highly Significant.
The mean difference is significant at the 0.05 level.

GROUPS	Mean ± SEM	SD	F	P	LSD
Group I (Control)	47.3375 ± 0.24029	0.96116	1243.685	0.000***	2.65000
Group II a (Interferon 2 months)	22.7250 ± 0.25895	0.73241			
Group II b (Interferon 3 months)	19.4750 ± 0.58149	1.64469			
Group III a (Interferon + Green tea 2 months	20.0750 ± 0.61550	1.74089			
Group III b (Interferon + Green tea 3 months)	38.6375 ± 0.27706	0.78365			

Table 3: Changes in the mean of the Super Oxide Dismutase level in the µ/gm of the testis in the adult rates of control and experimental groups:
SEM: Standard Error of the Mean.
SD: Standard Deviation.
F: F value.
P: Probability.
LSD: Least Significant Difference.
***Highly Significant.
The mean difference is significant at the 0.05 level.

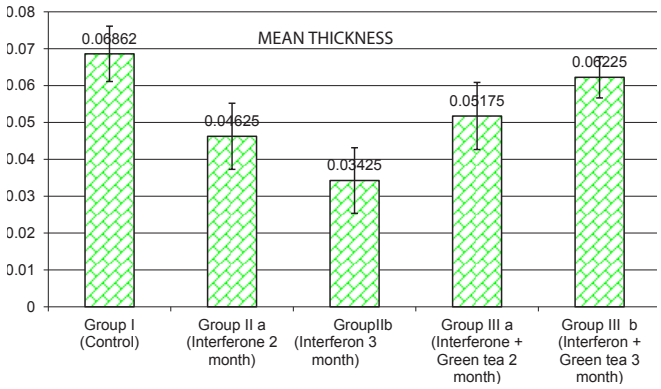

Figure 20: Changes in the mean thickness of the germinal epithelium of the seminiferous tubules in the adult rates of control and experimental groups.

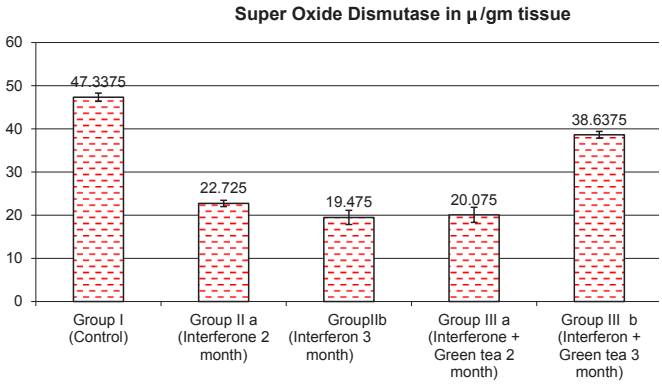

Figure 21: Changes in the mean of the Super Oxide Dismutase level in the µ/gm of the testis in the adult rates of control and experimental groups.x

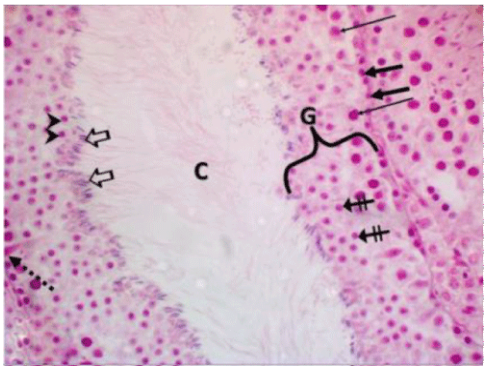

Figure 22: A photomicrograph of a section of seminiferous tubules of an adult male albino rat received green tea and interferon for 2 month group (IIIa) showing normal seminiferous tubules that form about 90% of the seminiferous tubules (subjective) with central cavity (C) lined by well arranged stratified germinal epithelium (G). The germinal epithelium (brace) showing relative increase in number of spermatogonia (Large arrows), primary spermatocyte (small arrows), secondary spermatocyte (crossed arrows), spermatide (arrow heads) and spermatozoa (empty arrows) and sertoli cells (striped arrow). (Hx. & E., X400).

3 month (Group IIb), the mean of the thickness of the seminiferous tubules revealed a higher significant reduction by nearly 50% than that of the control group (Table 2 and Figure 20).

The mean of the level of the Superoxide Dismutase of the seminiferous tubules in the experimental adult male albino rat received interferon for 2 month (Group IIa) showed significant decrease by nearly the half of that of the control group while in adult male albino rat received interferon for 3 month (Group IIb), the mean of the level of the Superoxide Dismutase of the seminiferous tubules revealed a higher significant decrease by nearly 59% than that of the control group (Table 3 and Figure 21).

B. **Interferon and Green tea extract treated groups (Groups III a and b)**

The light microscopic examination of the Haematoxylin and Eosin stained sections of the seminiferous tubules of adult albino rats treated with interferon and green tea for 2 month (Group IIIa) showed A normal seminiferous tubules that form about 90% of the seminiferous tubules with central cavity lined by well-arranged stratified germinal epithelium. The germinal epithelium showed relative increase in number of spermatogonia, primary spermatocyte, secondary spermatocyte, spermatid, spermatozoa and sertoli cells (Figure 22). Although, about 10% of the seminiferous tubules appeared abnormal

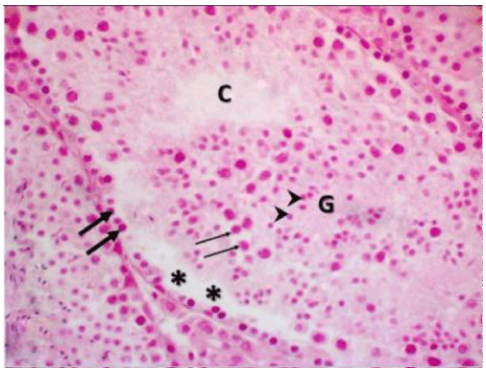

Figure 23: A photomicrograph of a section of seminiferous tubules of an adult male albino rat received grean tea and interferon for 2 month group (IIIa) showing abnormal seminiferous tubules that form about 10% of the seminiferous tubules (subjective) with a small central cavity (C) due to premature sloughing of the germinal epithelium (asterisk)lined by misarranged germinal epithelium (G) that shows spermatogonia (Large arrows), primary spermatocyte (small arrows) and spermatide (arrow heads) with absent of spermatozoa and secondary spermatocyte . (Hx. & E., X400).

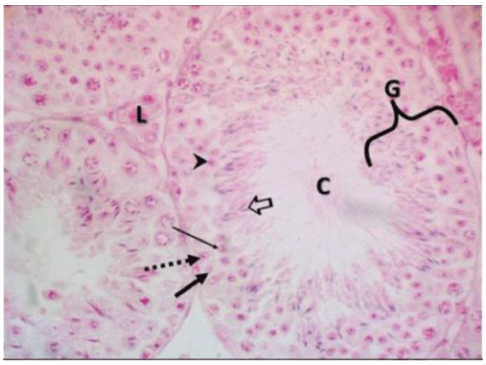

Figure 24: A photomicrograph of a section of seminiferous tubules of an adult male albino rat received grean tea and interferon for 3 month group (IIIb) showing circular to oval normal seminiferous tubules with central cavity (C) lined by stratified germinal epithelium (G) separated by interstitial cells of leyding (L). The normal germinal epithelium (brace) composed of spermatogonia (Large arrow), primary spermatocyte (small arrow), spermatide (arrow head), spermatozoa (empty arrow) and sertoli cells (striped arrow) with absent secondary spermatocyte. (Hx. & E., X400).

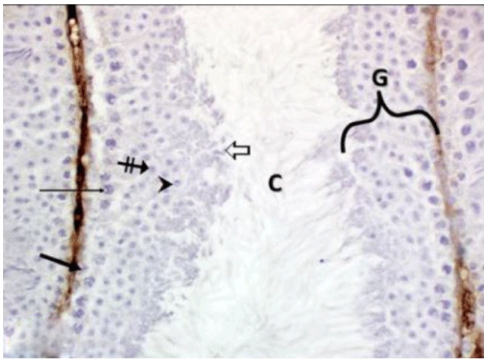

Figure 25: A photomicrograph of a section of seminiferous tubules of an adult male albino rat received green tea and interferon for 2 month group (IIIa) showing seminiferous tubules with central cavity (C) lined by stratified germinal epithelium (G). The germinal epithelium (brace) shows spermatogonia (Large arrow), primary spermatocyte (small arrow), secondary spermatocyte (crossed arrow), spermatide (arrow head), spermatozoa (empty arrow). All cells show negative immunohistochemical reactivity. (Immunohistochemical stain, Counterstained with Hx., X400).

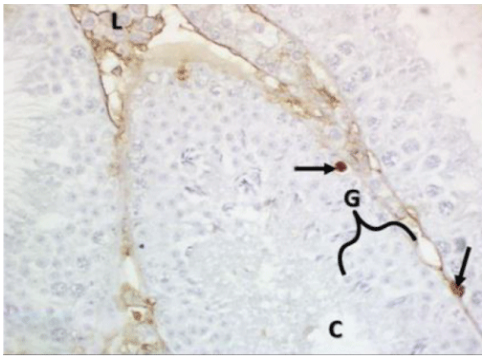

Figure 26: A photomicrograph of a section of seminiferous tubules of an adult male albino rat received green tea and interferon for 3 month group (IIIb) showing seminiferous tubules with central cavity (C) lined by stratified germinal epithelium (G). The germinal epithelium (brace) shows spermatogonia (Large arrow). Two spermatogonia in two seminiferous tubules show strong cytoplasmic immunohistochemical reactivity. The interstitial cells of leyding show negative immunohistochemical reactivity. (Immunohistochemical stain, Counterstained with Hx., X400).

with a small central cavity due to premature sloughing of the germinal epithelium lined by disarrangement germinal epithelium with absent of secondary spermatocyte and spermatozoa (Figure 23).

The seminiferous tubules of adult male albino rat received green tea and interferon for 3 month (Group IIIb) appeared typically normal with central cavity filled with spermatozoa and well-arranged stratified germinal epithelium separated by interstitial cells of leyding. The composition of the germinal epithelium was normal with absent secondary spermatocyte (Figure 24).

All cells of the germinal epithelium of adult male albino rat received green tea and interferon for 2 month (Group IIIa) showed negative immunohistochemical reactivity (Figure 25). The cells of the germinal epithelium of adult male albino rat received green tea and interferon for 3 month (Group IIIb) showed some spermatogonia in some seminiferous tubules with strong cytoplasmic immunohistochemical reactivity. The interstitial cells of leyding showed negative immunohistochemical reactivity (Figure 26).

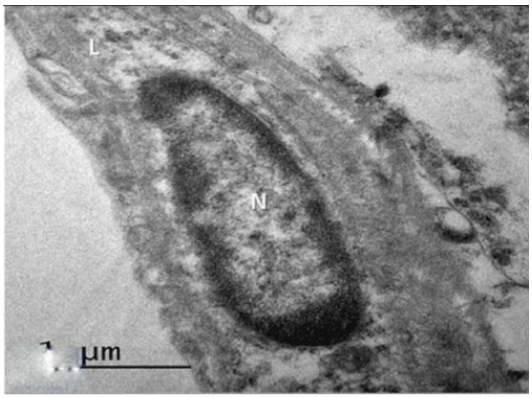

Figure 27: An electron micrograph (TEM) of ultrathin section of seminiferous tubules of an adult male albino rat received interferon and green tea for 2 and 3 month groups (III a & b) showing an interstitial cell of lyeding (L) with oval nucleus and peripherally arranged chromatine. (Uranyl acetate and Lead citrate X 2000, Print Mag. X 11700).

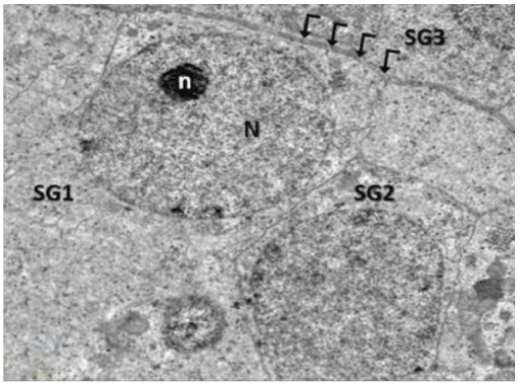

Figure 28: An electron micrograph (TEM) of ultrathin section of seminiferous tubules of an adult male albino rat received interferon and green tea for 2 and 3 month groups (III a & b) showing Spermatogonia (SG1, SG2 and SG3) with intact intercullar junctions (Right angle arrows). SG1 showing circular nucleus (N) with prominent nucleolus (n). (Uranyl acetate and Lead citrate X 2000, Print Mag. X 11700).

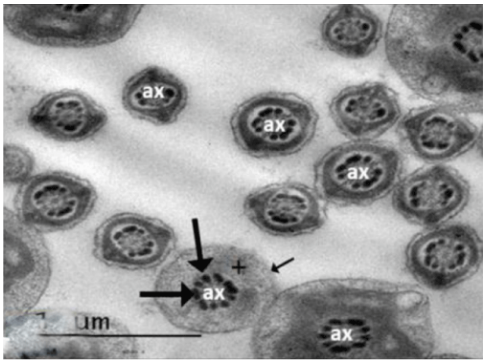

Figure 29: An electron micrograph (TEM) of ultrathin section of middle piece of spermatozoa of an adult male albino rat received interferon and green tea for 2 and 3 month groups (III a & b) showing multiple transverse sections of the middle piece of the spermatozoa. In the middle pieces, the axonemes (ax) are composed of nine peripheral doublets of microtubules (large arrows), surrounded by mitochondrial sheath (Plus) and cell membrane (small arrow). (Uranyl acetate and Lead citrate X 2000, Print Mag. X 11700).

The electron microscopic examination of ultrathin section of seminiferous tubules of adult male albino rat received interferon and green tea for 2 and 3 month groups (Group III a and b) showed normal Spermatogonia with intact intercullar junctions and prominent nucleolus. The cytoplasm did not show vacuolations (Figure 27 and 28).

The transverse sections of the middle piece of the spermatozoa showed well-arranged axonemes that composed of nine peripheral doublets of microtubules, surrounded by mitochondrial sheath and intact cell membrane (Figure 29).

The mean of the diameter of the seminiferous tubules of the adult male albino rat received interferon and green tea (Group III a and b) compared with that of the adult male albino rat received interferon only showed a significant increase by nearly 32% in 2 month and by nearly 43% in 3 month (Table 1 and Figure 19).

The mean of the thickness of the seminiferous tubules in the experimental adult male albino rat received interferon and green tea for 2 month (Group IIIa) showed significant increase by nearly 11% than that of the mean of the thickness of the seminiferous tubules in the experimental adult male albino rat received interferon only for 2 month (Group IIa)while in adult male albino rat received interferon and green for 3 (Group IIIb) the significant increase was by nearly 45% than that of the mean of the thickness of the seminiferous tubules in the experimental adult male albino rat received interferon only for 3 month (Group IIb) (Table 2 and Figure 20).

The mean of the level of the Superoxide Dismutase of the seminiferous tubules in the experimental adult male albino rat received interferon and green tea for 2 month (Group IIIa) did not show significant increase in comparison to that of the adult male albino rat received interferon only for 2 month (Group IIa). While in adult male albino rat received interferon and green tea for 3 month (Group IIIa), the mean of the level of the Superoxide Dismutase of the seminiferous tubules revealed a higher significant increase by nearly 48% than that of the adult male albino rat received interferon only for 3 month (Group IIb) (Table 3 and Figure 21).

Discussion

Our aim in this study we examine the effect of (IFN) on rat spermatogonia and investigate the possible protective effect of Grean tea on (IFN)-induced spermatogonia appotosis. The highly sensitive composition of the spermatogenic epithelium and the high rate of the mitotic activity make the testis more vulnerable to environmental and occupational hazards than other body tissues [29]. The human male fertility is even more sensitive, as the output of the human sperms is few times less than other mammals including the rats regarding the number of sperms produced per gram of tissue. So, any factor identified in laboratory studies as a reproductive hazards is also exert detrimental effects on the human reproductive performance [30].

Interferon induced testicular apoptosis that is showed by the histopathological, analytical and morphometrical changes that appeared in either seminiferous tubules and interstitial cell of leyding. The seminiferous tubules showed absent central cavity due to premature sloughing and disarrangement of the germinal epithelium layers. Similar findings were reported by El-Refaiy and Eissa [31] who stated that administration of cadmium caused extensive widening of the interstitial spaces and sloughing of all layers of seminiferous tubule. The thickness of the germinal epithelium was relatively reduced with relative increase in number of spermatogonia, primary spermatocyte, spermatozoa and sertoli cells with absence of primary, secondary spermatocytes and spermatids. Some seminiferous tubules lined with single layer of germinal epithelium. This can be explained by that, the spermatogenesis is a complex process in which there is an interaction between Sertoli, Leyding and spermatogenic cells. This interaction is tightly regulated by hormonal signal and receptor stimulation. If one specific cell type is damaged, the whole spermatogenic process will be disrupted leading to reproductive failure and infertility [32]. However, Hibi et al. [18] concluded that IFN-α may improve testicular spermatogenesis and increase the epididymal sperm concentration in the rat. These promising results with IFN-α may pave the way for a new approach to treating male infertility.

Immunohistochemically, the cells of the germinal epithelium showed negative immunohistochemical reactivity except interstitial cell of leyding that showed strong cytoplasmic immunohistochemical reactivity. But with prolonged administration of the interferon, the spermatogonia in several seminiferous tubules and interstitial cell of leyding showed strong cytoplasmic immunohistochemical reactivity. The limited immunoreactivity to spermatogonia and interstitial cell of leyding was also reported by Chen et al. and Aitken and Roman, [33,34] the spermatogenesis is an extremely active replicative process capable of generating about 1000 sperms per second. The high rates of cell division correlate correspondingly high rates of mitochondrial consumption by germinal epithelium. However, the poor vascularization of the testis means that the oxygen tension in the testis is low and the competition of the testicular tissue to consume the oxygen is extremely high. Since both Spermatogenesis and Leyding cells steroidogenesis are vulnerable to oxidative stress, the low oxygen tension may be an important mechanism by which the testis protects itself from free radical-mediated damage. In addition, the testis contain an elaborate array of antioxidant enzymes to protect the testicular tissue from oxidative stress. Anne-Pascale et al. [35] confirm these findings through their study on the immuhistochemical staining of the testicular tissue against OAS1 gene they found that Immunohistochemistry with antibodies against OAS1 demonstrated labeling at the base of seminiferous tubules.

The electron microscopic examination showed more cellular details confirming the apoptotic process. These findings range from vacuolated basement membrane, irrigular nuclear envelop and condensed peripheral chromatin, multiple Intracellular vacuoles, intercellular spaces and multiple electron dense bodies (Lysosomes).

The electron dense bodies (Lysosomes) were explained by El-Shafai et al. [36] in which the increased lysosomal contents and size is due to engulfment of apoptotic bodies results from degenerated germ cells. The vacuoles revealed in this study derived from dilatation and vesiculation of the Endoplasmic reticulum and Mitochondrial swelling. Much larger vacuoles are often phagocytic vacuoles remaining after the digestion of necrotic germ cells [37] The mitochondrial degeneration occurs due to membrane destruction by lipid peroxidation results from (ROS) and oxidative stress that increase the membrane permeabilityallowing calcium entery accompanied by uncoupled respiration and osmotic swelling which in turn breaks inner mitochondrial membrane and cause a general collapse of energy production [38,39]. Predes et al. [40] recorded an increase in the intercellular spaces mainly between sertoli cells and spermatogonia and spermatocytes and spermatids. These spaces suggest the location of the germ cells that have undergone degeneration and lost from the epithelium. Several researcher Ichihara et al. Yang et al. and Blanco et al. [41-43] was reported in cadmium toxicity a significant nuclear indentations in the nuclear envelop and some showed shrunken nuclear envelop that indicates apoptotic nucleus. Cross [44] summarized the possible function of the outer dense fibers surrounding the axonemes that provide a flexibility to the flagellum that facilitates forward progressive movements of the sperm

in male and female reproductive tracts. Any defects in the outer dense fibers can cause dyskinetic motility and may results in infertility.

Statistically, there was a significant decrease of the mean of the diameter of the seminiferous tubules by nearly 34% than that of the control. But, prolonged administration of interferon revealed a higher significant decrease of the mean of the diameter of the seminiferous tubules by nearly 43% than that of the control**.** The mean of the thickness of the seminiferous tubules showed significant reduction by nearly 33% than that of the control group while the mean of the thickness of the seminiferous tubules revealed a higher significant reduction by nearly 50% than that of the control group in 3 month administration of interferon. Ulusoy et al. [1] they stated that the mean of the diameter of the seminiferous tubules was significantly lower in the low-dose interferon and high-dose interferon groups than in the control group. The mean of the thickness of the seminiferous tubules was significantly lower in the low-dose interferon and high-dose interferon groups than in the control group. Interferon α-2_{β} may impair testicular histology even in clinical widely used treatment dose.

The findings in interferon and green tea showed a normal seminiferous tubules with central cavity lined by well-arranged stratified germinal epithelium. The germinal epithelium showed relative increase in number of spermatogonia, primary spermatocyte, secondary spermatocyte, spermatid, spermatozoa and sertoli cells**.** Although, about 10% of the seminiferous tubules appeared abnormal with a small central cavity due to premature sloughing of the germinal epithelium lined by disarrangement germinal epithelium with absent of secondary spermatocyte and spermatozoa**.** These findings are in agreement with Opuwari, [45] stated in vitro study that, only in 5% of treated cases, the green tea significantly increased testosterone level. Seminiferous tubules displayed complete spermatogenesis with abundant sperm in the lumen in all treated groups. Sperm concentration improved significantly by green tea. Furthermore, green tea was significantly improved the motility of rat sperm. In addition, green tea enhanced acrosome reaction.

All cells of the germinal epithelium of adult male albino rat received green tea and interferon for 2 month showed negative immunohistochemical reactivity**.** The ultrathin section of seminiferous tubules showed normal Spermatogonia with intact intercullar junctions and prominent nucleolus. The cytoplasm did not show vacuolations. Menegazzi et al. [46] explained how the green tea reverse the interferon induced apoptosis in which Epigallocatechin-3-gallate (EGCG), the main polyphenol component of green tea, exerts inhibitory action on urokinase, metalloproteinase, and inducible nitric oxide synthase (NOS II), accounting for its antitumor and anti-inflammatory action in which EGCG modulates the expression of a number of genes involved in inflammatory and neoplastic processes. EGCG blocks interferon γ (IFN-γ)-elicited activation of signal transducers and activators of transcription 1 (STAT1) by interfering with tyrosine phosphorylation. This also can explain the findings of well-arranged axonemes that surrounded by mitochondrial sheath and intact cell membrane. The seminiferous tubules showed high significant increase in the mean of the diameter and the thickness**.**

The mean of the level of the Superoxide Dismutase of the seminiferous tubules showed a significant increase. This finding correlate with the Gupta et al. [47] that stated oxidative stress is a common factor in about half of the infertile men examined to date. The testis expresses several antioxidant enzymes as superoxide dismutase, catalase and glutathione peroxidase to counteract the oxidative stress, their levels are greatly diminished up on Cadmium exposure. The antioxidant agents (Enzymatic and non-enzymatic) may prevent or at least reduce the cadmium toxicity to the testis [48]. The ability of GTP in green tea extracts to eliminate lipid-derived free radicals is noticeably stronger (almost 50 times) than that of ginkgo biloba extracts [49]. Further investigations indicate that the boosting level of superoxide dismutase (SOD) may account for the inhibitory effect of green tea catechins (GTC) against lipid oxidation [50].

Conclusion

It can be concluded that, the interferon produces obvious changes in testicular tissue structure and the co-administration of green tea produce significant improvement in the testicular architecture.

Acknowledgement

We thank Prof. Dr. Salwa Gawish (Professor of Histology, Faculty of Medicine, Mansoura University, Egypt) for providing us with unlimited advice during finishing our work.

References

1. Ulusoy E, Cayan S, Yilmaz N, Aktaş S, Acar D, et al. (2004) Interferon alpha-2b may impair testicular histology including spermatogenesis in a rat model. Arch Androl 50(5): 379-85.
2. Borden EC, Williams BRG (2000) Interferons In: Cancer Medicine. Toronto: B.C. Decker Inc, 815-824.
3. Moiseeva O, Mallette FA, Mukhopadhyay UK, Moores A, Ferbeyre G (2006) DNA Damage Signaling and p53-dependent Senescence after Prolonged β-Interferon Stimulation. Mol Biol Cell 17 (4): 1583-1592.
4. Mostafa MH, Sharma RK, Thornton J, Mascha E, Abdel-Hafez MA, et al. (2004) Relationship between ROS production, apoptosis and DNA denaturation in spermatozoa from patients examined for infertility. Hum. Reprod 19: 129-138
5. Lin T, Hu J (1998) Interferon-gamma inhibits the steroidogenic acute regulatory protein Messenger ribonucleic acid expression and protein levels in primary cultures of rat Leydig cells. Endocrinol 139: 2217-2222.
6. Satie AP; Mazaud-Guittot S; Seif I; Mahé D, He Z, et al. Excess type I interferon signaling in the mouse seminiferous tubules leads to germ cell loss and sterility. Biol Chem 286(26): 23280-95.
7. Pearl-Yafe M, Halperin D, Halevy A, Kalir H, Bielorai B, et al (2003) An oxidative mechanism of interferon induced priming of the Fas pathway in Fanconi anemia cells. Biochem Pharmacol 65: 833-842.
8. Sasaki M, Ikeda H, Sato Y, Nakanuma Y (2008) Proinflammatory cytokine-induced cellular senescence of biliary epithelial cells is mediated via oxidative stress and activation of ATM pathway: A culture study. Free Radic Res 4: 1-8.
9. Watanabe Y, Suzuki O, Haruyama T, Akaike T (2003) Interferon gamma induces reactive oxygen species and endoplasmic reticulum stress at the hepatic apoptosis. J Cell Biochem 89: 244-253.
10. Ulrike S, Lukasz P, Frederic E, Dawadschargal BAntje, Friederike V, et al. (2010) Immunoproteasomes Preserve Protein Homeostasis upon Interferon-Induced Oxidative Stress. Cell 142: 613-624.
11. Premendu PM, Mary F, Selvaraju V, Ashok A (2011) NF-κB in Male Reproduction: A Boon or a Bane?. The Open Repro Sci J 3: 85-91.
12. Dejucq N, Lienard M, Guillaume. E, Dorval I, Jegou B (1998) Expression of interferons-alpha and -gamma in testicular interstitial tissue and spermatogonia of the rat. Endocrinology 139: 3081-3087.
13. Orava M, Voutilainen R, Vihko R (1989) Interferon-gamma inhibits steroidogenesis and accumulation of mRNA of the steroidogenic enzymes P450scc and P450c17 in cultured porcine Leydig cells. Mol Endocrinol 3: 887-894.
14. Ku JH, Kim YH (1999) The preventive effect of systemic treatment with interferon-alpha 2B for infertility from mumps orchitis. BJU Int 84: 839-842.
15. Yeniyol CO, Sorguc S (2000) Role of interferon-alpha-2B in prevention of testicular atrophy with unilateral mumps orchitis. Urology 55: 931-933.
16. Yamamoto M, Miyake K (1994) Successful use of interferon for male infertility. Lancet 344 (8922): 614.
17. Longo I, Sanchez-Mateos P (2002) Azoospermia in a patient receiving interferon alpha for a stage 3 melanoma. Acta Derm Venereol 82: 389-390.

18. Hibi H, Yokoi K, Yamamoto M (1997) Effects of alpha-interferon on sperm production, concentration, and motility in the rat. Int J Urol 4: 603-607.

19. Shaha C (2007) Modulators of spermatogenic cell survival. Soc Reprod Fertil Suppl 63: 173-186.

20. Flora S.J (2007) Role of free radicals and antioxidants in health and disease. Cell. Mol Biol 53: 1-2.

21. Choi J, Ou JH (2006) Mechanisms of liver injury and oxidative stress in the pathogenesis of hepatitis C virus. Am J Physiol, Gastrointestinal Liver Physiol 290: 847-851.

22. Soley S, Milton M, Daryl H, Ozgur H, Yusuf B, et al. (2008) Potential Role of Lycopene in the Treatment of Hepatitis C and Prevention of Hepatocellular Carcinoma. Nutrition and Cancer 60(6): 729-735

23. Melhem A, Stern M, Shibolet O, Israeli E, Ackerman Z (2005) Treatment of chronic hepatitis C virus infection via antioxidants: results of a phase I clinical trial. J Clin Gastroenterol 39: 737-742.

24. Cabrera C, Artacho R, Giménez R (2006) Beneficial effects of green tea--a review. J Am Coll Nutr 25 (2): 79-99.

25. Murray F (2000) 100 super supplements for a longer life. Los Angeles: CA McGraw-Hill Professional pp. 181-182.

26. Peirce EJ, Breed WG (2001) A comparative study of sperm production in two species of Australian arid zone rodents (Pseudomys australis, Notomys alexis) with marked differences in testis size. Reproduction 121: 239-247.

27. Rosiepen G, Weinbauer GF, Schlatt S, Behre HM, Nieschlag E (1994) Duration of the cycle of the seminiferous epithelium estimated by the 5-bromodeoxyuridine technique, in laboratory and feral rats. J Reprod Fertil 100: 299-306.

28. Nishikimi M, Roa NA, Yogi K (1972) Biochemi Bioph Res Common 46: 849-854.

29. Queiroz K, Waissmann W (2006) Occupational exposure and effects on the male reproductive system. Cad.Saúde Públ 22: 485-493.

30. Cotran S, Kumer V, Robbins L (2005) Robbins Pathological Basis of Disease, 7th edition, Philadelphia; p. 1-34.

31. El-Rafaiy I, Eissa I (2012) Protective effects of ascorbic acid and zinc against cadmium-induced histopathological, histochemical and cytogenetic changes in rats. Comunicata Scienntiae 3(3): 162-180.

32. Bizarro P, Acevedo S, Nino-Cabrera G (2003) Ultrastructural modifications in the mitochondrion of mouse Sertoli cells after inhalation of lead, cadmium, or lead-cadmium mixure. Reprod Toxicol 17: 561-566.

33. Chen H, Liu J, Luo L (2005) Vitamin E, aging and Leyding cell steriodogenesis. Exp Gerontol 40: 728-736.

34. Aitken J, Roman D (2008) Antioxidant systems and oxidative stress in the testes. Oxid Med Cell Longev 1(1): 15-24.

35. Anne-Pascale S, Severine M, Isabelle S, Dominique M, Zhiguo H, et al. (2011) Excess Type I Interferon Signaling in the Mouse Seminiferous Tubules Leads to Germ Cell Loss and Sterility. J Biol Chem 286: 23280-23295.

36. El-Shafai A, Zohdy N, El-Mulla K, Hassan M, Morad N (2011) Light and electron microscopic study of toxic effect of prolonged lead exposure on the seminiferous tubules of albino rats and the possible protective effect of ascorbic acid. Food Chem Toxicol 49: 734-743.

37. Nolte T, Harleman H, Jahn W (1995) Histopathology of chemically induced testicular atrophy in rats. Toxic Pathol 47: 267-286.

38. Burkitt G, Stevens A, Lowe J, Young B (1996) Wheater basic histology (a color atlas and text). 3rd, Churchill Livingston, New York.

39. Nigma D, Shukla S, Agarwal K (1999) Glutathione depletion and oxidative damage in mitochondria following exposure to cadmium in rat liver and kidney. Toxicol Lett 106: 151-157.

40. Predes D, Monteiro C, Matta P, Garcia C, Dolder H (2011) Testicular histomorphometry and ultrastructure of rats treated with cadmium and ginko biloba. Biol Trace Elem Res 140: 330-341.

41. Ichihara I, Kawamura H, Nakano T (2001) Ultrastructural, morphometric and hormonal analysis of the effects of testosterone treatment on Leyding cells and others interstitial cells in young adult rats. Ann Anat 183: 413-426.

42. Yang M, Arnush M, Chen Y, Wu D, Pang B, et al. (2003) Cadmium-induced damage to primary cultures of rat Leyding cells. Reprod Toxicol 17: 553-560.

43. Blanco A, Moyano R, Molina M (2009) Quantitative study of Leyding cell population in mice exposed to low doses of cadmium. Bull Environ Contam Toxicol 82: 756-760.

44. Cross P.C (1993) Cells and tissue ultrastructure: a functional perspective, 2nd edition, Freeman and Company, New York 348.

45. Opuwari CS (2013) Effect of tea and herbal infusions on mammalian reproduction and fertility. Ph.D. thesis, Department of Medical Biosciences, Faculty of Natural Sciences, University of the Western Cape Town.

46. Menegazzi M, Tedeschi E, Dussin D, carcereri de prati A, Cavalieri E (2001) Anti-interferon γ action of epigallocatechin-3-gallate mediated by specific inhibition of STAT1 activation. FASEB Journal 15: 1309-1311.

47. Gupta RS, Gupta ES, Dhakal BK, Thakur AR, Ahnn J (2004) Vitamin C and vitamin E protect the rat testes from cadmium-induced reactive oxygen species. Mol Cells 17(1): 132-139.

48. Siu R, Mruk D, Porto S, Cheng Y (2009) Cadmium-induced testicular injury. Toxicol App Pharmaco 238: 240-249.

49. Zhen Y, Chen Z, Cheng S, Chen M (2002) Tea bioactivity and therapeutic potential, London, UK: New York Taylor and Francis 121-225.

50. Zhu Q (1999) Antioxidative activities of green tea catechins, Hong Kong: The Chinese University of Hong Kong (Hong Kong)