Impact of Finasteride Administration on Neuroactive Steroid Levels To Induce Persistent Sexual Side Effects and Anxious/Depressive Disorders and the Possible Protective Effect of Vitamin E

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Abstract: Background: With aging, abnormal benign growth of the prostate results in benign prostate hyperplasia (BPH) with concomitant lower urinary tract symptoms. Because the prostate is an androgen target tissue, and transforms testosterone into 5α-dihydrotestosterone (5α-DHT), a potent androgen, via 5α-reductase (5α-R) activity, inhibiting this key metabolic reaction was identified as a target for drug development to treat symptoms of BPH. Nowadays, Finasteride is a relatively frequently prescribed drug in the therapeutic management of BPH and male androgenic alopecia. Conflicting reports have led to two diverse and contradictory recommendations from the use of Finasteride. Histopathologic assessment of the testis is a vital component of drug safety evaluation. The reported adverse effects are notable in some patients, consisting in signs and symptoms that are encountered both during Finasteride administration and after treatment cessation. It is well known that brain and plasma levels of the neurosteroid allopregnanolone (ALLO) increase after acute environmental stress, fact that has been considered a homeostatic mechanism in restoring normal function following stress. Thus, it is of great interest to study the contribution of stress-altered plasma ALLO levels and administration of Finasteride (an ALLO synthesis inhibitor). Clinical data show that cognition and sexuality are two distinct but interrelated environmental functions, most probable due to lateralization process of the brain. Aim: This study was carried out to evaluate the changes of neuroactive steroid ALLO Level in Finasteride treated rats and its relation to induce persistent sexual side effects and anxious/depressive symptoms. The possible protective role of vitamin E was also investigated. Materials and Methods: Forty adult male rats divided into four equal groups: group I which served as the control group; group II, Finasteride group which received Finasteride daily at a dose of 5mg/kg/day; group III, Vitamin E group which received only Vitamin E at a dose of 100 mg/kg bodyweight. Group IV, Finasteride and vitamin E group received Finasteride at a dose of 5mg/kg/day alongside with Vitamin E at a dose of 100 mg/kg. Treatments were administered orally by gavage for 28 days. At the end of the experimental period, the markers of oxidative stress were investigated. The animals were submitted to swim stress test for evaluation of depressive like behavior and estimate the plasma ALLO level before and after acute swimming test. Moreover, the histological and the immunohistochemical changes occur in the rat testes were investigated. Results: Administration of Finasteride showed substantial changes in the seminiferous tubules with loss of the normal architecture. The spermatogenic cells were disorganized, degenerated, and separated from the underlying basement membranes. Some areas of interstitium were wide with congested blood vessels and extensive areas of hemorrhage can also be observed. The immunohistochemical study showed a decrease in the intensity of AR immunostaining in Sertoli, Leydig, and peritubular myoid cells and in the number of PCNA immunopositive germ cells in comparison with control. Co-administration of vitamin E with Finasteride induced improvement in testicular histological changes as well as increase in the number of AR and PCNA immunopositive cells. The immunohistochemical expression of Bax protein was high in the Finasteride group. The Bax expression was low in the control and vitamin E groups. Co-treatment with vitamin E and Finasteride displayed moderate expression on the immunoractivity of Bax. Statistically, There are significantly reduced levels of GSH, SOD and CAT (P<0.001) with increased in MDA concentration (P<0.001) in Finasteride treated group compared to control group. While, vitamin E treated group, there is significantly change compared to Finasteride group. As regards the ALLO plasma level, it was found that in Finasteride treated rats (group II); significantly decrease this level when compared with the control. In vitamin E treated rats, there was significant increase when compared with group II. However, there was no significant change in rats taken Finasteride and vitamin E (group IV) when compared with group II.
INTRODUCTION

Neurosteroids affect neurotransmission through action at the membrane ion-gated receptors and at other neurotransmitter receptors. Neurosteroids are a group of steroid hormones that have direct effects on the brain. In addition to being synthesized in the brain itself, neurosteroids are also secreted by the adrenal cortex and gonads, which are parts of the HPA axis (Pluchino et al., 2013). 5α -Hydroxy-5α -pregnan-20-one (allopregnanolone, ALLO), a representative neurosteroid, binds to the gamma-aminobutyric acid type A (GABAA) receptors with a high affinity and positively modulates the action of GABA at these receptors, and hence elicits marked anticonvulsant, antidepressant and anxiolytic effects (Belelli and Lambert, 2005). Under stressful conditions, the brain rapidly synthesizes progesterone (PROG) and subsequently converts it to ALLO to raise the threshold of brain excitability (Gonenir and Kartalci, 2015). The two-step metabolism of PROG produces ALLO through the actions of the enzymes, 5α -reductase (rate-limiting step) and 5α-hydroxysteroid dehydrogenase (3α -HSD). Two distinct 5α -reductase isozymes, types 1 (predominant form in the brain) and 2 (predominant form in the periphery), are found across mammalian species (Finn et al., 2006; Traish et al., 2011). Neuroactive steroids act as an important physiological regulators of nervous function, affecting mood, behavior, reproduction, and cognition, as well as acting as protective agents in models of injury and neurodegenerative diseases (Schumacher et al., 2012 ; Panzica et al., 2012).

Finasteride (FIN) is a selective inhibitor of 5α-reductase that has received clinical approval for the treatment of human benign prostatic hyperplasia, in the prevention and treatment of prostate cancer and androgenetic alopecia ((De-Nunzio et al., 2008; Traish et al., 2014). Both isozymes of the 5α-reductase in the rodent demonstrate comparable inhibition following FIN exposure; FIN can inhibit the 5α-reductase activity in the central nervous system as well as in the periphery (Yim et al., 2014). Based on this, FIN has also been used to manipulate the brain ALLO level for characterization of its physiological functions and the mechanism by which it affects the brain functions, and to analyze the exact mechanisms of action of psychotropic agents, whose antidepressant and anxiolytic effects are inferred to occur through an increase in the brain ALLO synthesis (Caruso et al., 2015). Conflicting reports have led to two distinct and opposite recommendations from the use of FIN. Studies have shown side effects from the use of FIN similar to the patients who used placebo (Gormley et al., 1992). Other study mentioned that most of the adversarial effects of Finasteride are alterable such as erectile dysfunction, loss of libido, a small volume of ejaculate and gynecomastia (Gлина et al., 2004; Fertig et al., 2017). Recently, there is study by Beltagy et al. (2016) suggested that sperm count in rats administered Finasteride (FIN) displayed a substantial reduction in comparison to normal sperm count in control group. Vitaly, in patients received Finasteride, reduction in semen parameters were detected (Amory et al., 2007). It has been hypothesized that long-term FIN treatment for many years, has no negative influence on androgen-dependent processes such as fertility or libido (Gupta and Charrette, 2014). Others have mentioned contradictory results (Gur et al. 2013; Ganzer et al. 2015). In studies during adulthood, George et al. (1989); Rhoden et al. (2002) suggested that, no effects of Finasteride on testicular histomorphology were found in rats. By contrast, a study accomplished in adult rats showed that administration of Finasteride suppressed the testosterone-induced restoration of spermatogenesis and eventually confirmed a role for 5-α reduced androgens in adult spermatogenesis (O’Donnell et al., 1996). Crucially, Imperato- McGinley et al. (1992) reported that if Finasteride administered in utero, external genitalia were feminized in rats or developed anomalies in rhesus monkeys (Pralhada et al., 1997). Moreover, women who had contact with FIN have given birth to babies with malformations (Lechuga et al., 2004). Some theorized that possibly Finasteride had not dramatically change the spermatogenesis process in well men as revealed by some reporters (Overstreet et al., 1999). However in patients with other problems relevant to infertility, the harmful impact of Finasteride prominent by others might be augmented (Gлина et al., 2004). Recent investigations have also indicated that patients may develop depression during FIN treatment and that this symptomatology can persist despite treatment withdrawal (Melcangi et al., 2017).

Testes produced steroid hormones such as testosterone (T), and dihydrotestosterone (DHT), which is much more active than testosterone (Pатrào et al., 2009). Testosterone is the most important androgenic hormone circulating in the blood, and is converted to dihydrotestosterone, DHT in the prostate, by 5 α-reductase (Wright et al., 1996). The current research was done through immunohistochemical studies on the following, Androgen Receptor (AR), Proliferating Cell Nuclear Antigen (PCNA), and Bax.
The Androgen Receptor (AR) acts as a vital role in androgen action. The testis and epididymis are key targets of androgen action. Definitely, androgen receptor is critical for preservation of spermatogenesis and secretory function in epididymal epithelial cells (Griswold, 1995).

Jaskulski et al. (1988) stated that Proliferating Cell Nuclear Antigen (PCNA) has been used as a marker for DNA synthesis owing to its fundamental role in the beginning of cell proliferation. Its maximum expression was around the S phase of the cell cycle (Celis and Celis, 1985). Additionally, PCNA is a nuclear matrix protein that is essential for multiple cell cycle pathways, comprising DNA replication, DNA elongation, and DNA excision repair (D’andrea et al., 2010).

According to John (2000), apoptosis is beneficial to keep the cell number in testicular tissue and it is removed unnecessary and injured cells. Importantly, excessive apoptosis could cause demolition of male reproductive function. Bax is a multi-domain proapoptotic member of the Bcl-2 family (Korsmeyer, 1999).

There has been an increased importance among investigators to use antioxidants for protection. Antioxidants have been reported that they play extensive roles to support the body’s biological systems against lipid peroxidation (Evstigneeva et al., 1998). Vitamin E is an essential constituent in human nutrition and it is the most active liposolouble antioxidant. El-Demerdash et al. (2004a) suggested that vitamin E inhibits peroxidation of membrane lipids by scavenging lipid.

Taken together, finally, Finasteride that inhibit the 5α-reductase will inhibits reduction of testosterone to dihydrotestosterone and therefore alters the androgen ratio might also be considered as one of reprotoxicants and the expected subsequent reduction in plasma ALLO levels, can modify the increasing plasma ALLO concentrations induced by acute forced swim stress and the probable occurrence of psychological changes. Histopathologic evaluation of the testis is an important component of drug safety assessment. Currently, there is no enough information regarding the protective effect of vitamin E against the Finasteride on testis. This study aimed to examine the histological and immunohistochemical and biochemical changes in the testes of rats treated with Finasteride and the probable occurrence of psychological changes. The possible protective effect of vitamin E on these parameters was also investigated.

**MATERIALS AND METHODS**

**Materials**

**Chemicals**

Finasteride (Poscar): obtained from Proscar®, MSD, Carmlington, UK.
Vitamin E (alpha tocopherol): obtained from Pharco Pharmaceuticals Co., Egypt.

**Experimental animals**

In this experiment, forty adult male rats weight ranged between 250-300 gm were used in this study. Their ages ranged from three months. This study was approved by the ethics committee on animal research in the animal house of Faculty of Medicine for Girls, Al-Azhar University, Cairo, Egypt. The experiments were directed according to the strategies of the Animal Care and Use Committee of National Research Center, Egypt. All rats were maintained on a standard pellet diet and allowed free access to water. Rats were left for two weeks before the start of experiments for acclimatization. Rats were housed in separate well-ventilated cages, under standard conditions, with free access to standard diet and water ad libitum. Rats were kept at a controlled temperature of 25±1 °C and under a 12 h light: 12 h dark schedule.

**Experimental design**

**The rats were divided into four groups of 10 rats each**

**Group I (Control group)**

The rats in this group were kept without treatment just received orally distilled water daily until the end of the experiment and served as control.

**Group II (Finasteride group)**

Rats in this group received Finasteride daily at a dose of 5mg/kg/day orally for 28 days (Kolas et al., 2015).

**Group III (Vitamin E group)**

Rats in this group received Vitamin E at 100 mg/kg body weight dissolved in 1 ml of corn oil, orally for 28 days (Yousef et al., 2006).

**Group IV (Finasteride and Vitamin E group)**

Rats in this group received vitamin E at 100 mg/kg body weight, thirty minutes before Finasteride at same dose of 5mg/kg/ day orally for 28 days.
METHODS

Histological study
The rats were sacrificed and the abdominal cavity was opened up to expose the testes which they were quickly dissected out from each animal. The right testes were fixed in 10% formal saline, dehydrated, cleared, and embedded in paraffin. 5 μm thick of paraffin sections were cut and then stained with Haematoxylin and Eosin stain which was used for study of the general structure (Bancroft and Gamble, 2013). Periodic acid Schiff’s reaction (PAS) technique was used for the study of mucopolysaccharides and polysaccharides (Bancroft and Stevens, 1996). Immunohistochemical staining for detection of Androgen Receptor (AR) (Timurkaan et al., 2012) was carried out. Proliferating cell nuclear antigen (PCNA) (Kiernan, 1999) and Bax (Mahmoud et al., 2009) was also used.

Immunohistochemical study
Androgen Receptor, Proliferating Cell Nuclear Antigen and Bax
The paraffin testicular sections were deparaffinized, rehydrated and then washed with PBS. The testicular sections were incubated with the following antibodies: Polyclonal rabbit anti-AR antibody (Thermo Scientific, Fremont, California, USA) (Pearl et al., 2011), anti-PCNA mouse (Thermo Scientific) (Tousson et al., 2011). Testicular sections were incubated with polyclonal rabbit antibody against Bax protein (DAKO Corporation Carpinteria, CA, USA) (Linderoth et al., 2003). Sections were incubated with appropriate biotinylated secondary antibody with specificity to primary antibody, followed by an avidin–biotin horseradish peroxidase complex (ABC reagent). Immunostaining was visualized using 3, 3′-diamnobenzidine (DAB) chromagen. Finally, sections were counterstained with Mayer’s haematoxylin. Negative controls were obtained by skipping the step of applying the primary antibody. The sections were evaluated by light microscopy. Examination and photography were done at the Mycology and Biotechnology Unit, Al Azhar University, Cairo, Egypt.

Biochemical study
Biochemical oxidative parameters
The testes were rinsed in ice-cold 0.175 M KCl /25 mM Tris–HCl (pH 7.4) to remove the blood, minced in the same solution, and homogenized by means of a homogenizer with a Teflon pestle. The testis homogenates were centrifuged at 10,000 rpm for 15 min. The supernatants were then used for lipid peroxidation determination, and antioxidant enzyme assays as follows:

Tissue Glutathione (GSH) Analysis
The reduced GSH content of testes tissues was estimated according to the method described by Sedlak and Lindsay (1968).

Tissue superoxide dismutase (SOD) and catalase (CAT) activity determination
The SOD activity was measured by the inhibition of nitroblue tetrazolium (NBT) reduction due to O₂ generated by the xanthine/xanthine oxidase system (Sun et al., 1988). One unit of SOD activity was defined as the amount of protein causing 50% inhibition of the NBT reduction rate. The CAT activity of tissues was determined according to the method of Sinha (1991). The enzymatic decomposition of H₂O₂ was followed directly by the decrease in absorbance at 240 nm. The difference in absorbance per unit time was used as a measure of CAT activity. The enzyme activity was given in U/mg of protein.

Determination of malondialdehyde levels
The levels of malondialdehyde (MDA) in homogenized tissue, as an index of lipid peroxidation, were determined by a thiobarbituric acid reaction using the method of Yagi (1998).

Determination of protein content
The tissue protein content was measured according to Cannon (1974) using bovine serum albumin as a standard.

Behavioral level
Forced swimming test (FST) (Tonisaar et al., 2008)
One hour after injection of Finasteride (5 mg/kg i.p.) at the end of the experiment, animals (n = 5 in each group) were placed in a transparent Plexiglas cylindrical tank (height: 40 cm, internal diameter: 19 cm), containing water (36-C) to a depth of 24 cm during 5 min. The apparatus was located in a chamber with lights off. After swim, each animal was gently dried with a cotton towel and was left in an individual cage in the dark room for 15 min. The same previous groups of rats were used as following:
I. Control group +No Swimming;
II. Control group + Swimming;
III. Finasteride treated group + No Swimming;
IV. Finasteride + Swimming;
V. Vitamin E group + No Swimming;
VI. Vitamin E group + Swimming;
VII. Finasteride + Vitamin E group + No Swimming;
VIII. Finasteride + Vitamin E group + Swimming.
Measurement of serum neurosteroids allopregnanolone (ALLO) level (Vallée et al., 2000) just after the experiment.

Statistical analyses
One-way analysis of variance (ANOVA) test followed by Student’s t test were used. The data obtained in the present study were expressed as mean ± SEM for quantitative variables and statistically analyzed by using SPSS program (version 17 for windows) (SPSS Inc. Chicago, IL, USA). P value <0.05 was considered statistically significant.

RESULTS

Histological results
H&E-stained testicular sections
Group I (Control group)
Light microscope examination of the sections of the testes of the control albino rats showed that testicular parenchyma consisted of multiple densely packed seminiferous tubules separated by interstitial tissue with regular outlines (Fig.1A). The seminiferous tubules were lined with stratified germinal epithelium formed of spermatogenic and Sertoli cells. The spermatogenic cells contained spermatogonia, spermatocytes, spermatids and spermatooza. Obviously, spermatogonia had rounded dark nuclei rested on the basal laminae and primary spermatocytes had large nuclei. Spermatids had vesicular nuclei and prominent nucleoli (Fig.2 A). The lining epithelium consisted of Sertoli cells. The sertoli cells with their large basal elongated or pyramidal pale nuclei and prominent nucleoli wedged between spermatogenic cells were detected. The interstitial tissue between the seminiferous tubules contained interstitial cells of Leydig. These cells appeared rounded in shape with rounded myoid cells and their lumina contained sperms.

Group II (Finasteride group)
Light microscope examination of sections of the testes of the Finasteride treated rats showed that variable degrees of tubular affection were detected. Some seminiferous tubules were distorted with a disorganized germinal epithelium. The testicular parynchema consisted of many distorted seminiferous with uneven outlines, disorganized epithelium, and wide lumina (Fig.1B, C & D). The disorganized epithelium showed separation and sloughing (Fig.1B). The tubules were surrounded by basal laminae and most of the tubules showed shrunken pyknotic nuclei. Some tubules showed complete or partially detachment of their basement membrane whereas others had an irregular outline (Fig.1 C). Obviously, spermatogonia appeared away from the basement membrane with small dark pyknotic nuclei (Fig.2B, C&D). Clearly, reduction in the diameter of some of seminiferous tubules can be observed (Fig.1 D). Some areas of interstitium were wide (Fig.1C&D) with congested blood vessels and extensive areas of hemorrhage can also be observed (Fig.1B & Fig.2C). The homogenous material of acidophilic vacuolated exudates can be seen (Fig.2D). Some seminiferous tubules showed the appearance of the sperms (Fig.1C), few of seminiferous tubules occupied by primary spermatogenic only with no sperm formation (Fig.2D).

Group III (Vitamin E group)
Light microscope examination of sections of the testes of the vitamin E group showed more or less normal testicular organization (Fig.1E). Closely packed seminiferous tubules with regular outlines were seen (Fig.1E). The seminiferous tubules were lined with stratified germinal epithelium and Sertoli cells. The germinal epithelium contained spermatogonia, primary spermatocytes, spermatids, and mature sperm. The interstitium contained Leydig cells (Fig.2 E).

Group IV (Finasteride and Vitamin E group)
Light microscope examination of sections of the testes of the Finasteride and vitamin E group showed that they regained nearly their normal architecture (Fig.1 F). Most of their seminiferous tubules were packed together with regular outlines and narrow interstitium. In addition, most of the tubules renovated their normal epithelial stratification. A few tubules still had incompetence in their germinal epithelium; areas of epithelial separation were detected (Fig.1 F). Apparently, acidophilic vacuolated exudates were detected in the interstitial
spaces. Most of the seminiferous tubules with abundant whorled appearance of sperm flagella filling their lumina were noticed (Fig.2 F).

**Periodic Acid-Schiff’s (PAS) stained testicular sections**

Testicular sections of the testes of the control (group I) (Fig.3 A) revealed strong PAS positive reaction confined in the spermatogenic cells, basement membrane of seminiferous tubules and in interstitial cells of Leydig. Testicular sections of the testes of Finasteride (group II) treated rats showed reduction of PAS-positive materials in the spermatogenic cells and moderate diminution of PAS positive reaction in the basement membrane of seminiferous tubules and in the interstitial cells of Leydig (Fig. 3 B, C&D). Vitamin E (group III) (Fig.3 E) revealed strong PAS positive material in the spermatogenic cells, basement membrane and interstitial cells of Leydig. On the other hand, treatment with vitamin E and Finasteride showed an obvious increase of PAS-positive materials in the basal lamina and in the interstitial cells of Leydig (Fig.3F).

**Immunohistochemical Results**

**Androgen Receptor (AR) immunostaining**

Testicular sections demonstrated strong positive immunohistochemical staining in the nuclei of Sertoli cells, Leydig cells, and peritubular myoid cells of the control group (group I) (Fig.4 A, B&C). The strongest intensity of immunostaining androgen receptor was obviously seen in the nuclei of Sertoli cells, which were known by their asymmetrical contours and localization on the basal layer of the epithelium. Immunostaining was also noticed in the nuclei of Leydig cells and peritubular myoid cells (Fig. 4 A, B&C). In Finasteride-treated rats (group II), spermatogenic cells and spermatozoa showed negative immunostaining. The immunostaining intensity was significantly decreased in Sertoli cells and peritubular myoid cells, however moderate positive nuclear AR immunoreactivity was observed in the Leydig cells (Fig.4 D). Vitamin E-treated group (group III) showed strong immunoreactivity in Sertoli cells, Leydig and myoid cells (Fig.4E). Co-administration of vitamin E with Finasteride treatment (group IV) displayed moderate intensity of positive nuclear androgen receptor in the Sertoli cells, the Peritubular myoid cells, and the Leydig cells (Fig.4 F).

**Proliferating Cell Nuclear Antigen (PCNA) immunostaining:**

Testicular section showed that PCNA labeled spermatogonia and primary spermatocytes cells. Obviously, strong positive staining in the nuclei of spermatogonia and primary spermatocytes were manifested in the control (group I) (Fig.5 A). The interstitial tissue between the seminiferous tubules showed a negative reaction for PCNA (Fig. 5A). The Testicular sections of rats treated with Finasteride (group II) showed immunohistochemical alterations. The numbers of spermatogonia and primary spermatocytes cells that have nuclear positive immunostaining for PCNA were markedly decreased (Fig.5B) as compared with the number in the control group (Fig. 5A). Vitamin E-treated rat testes displayed strong PCNA immunoreaction in the nuclei of spermatogonia and primary spermatocytes (Fig.5 C). Co-administration of vitamin E with Finasteride treatment (group IV) showed marked increase in the number of positive nuclear PCNA spermatogonia and primary spermatocytes (Fig.5 D) as compared with Finasteride treated rats (group II) (Fig.5B).

**Bax immunostaining**

Testicular sections showed that Bax labeled spermatogenic cells, spermatocytes and spermatids. Mild staining was detected in the control (group I) (Fig.6A). Testicular sections of rats treated with Finasteride (group II) showed that the intensity of Bax expression was significantly increased in the spermatogenic cells, spermatocytes and spermatids (Fig.6B). Vitamin E-treated rat testes (group III) displayed mild staining in the spermatogenic cells, spermatocytes and spermatide (Fig.6C). Co-administration of vitamin E with finasteride treatment (group IV) showed moderate Bax expression in the spermatogenic cells, spermatocytes and spermatids (Fig.6D) as compared with Finasteride treated rats (group II) (Fig.6B).

**Biochemical results**

**Biochemical oxidative parameters**

The testes contents of GSH, SOD and CAT activities were significantly decreased in Finasteride treated rats (group II) when compared to those in control (group I) (P<0.001). As regards the testes MDA content in group II showed a highly significant increase in their testes content of MDA as compared to that of control rats (Table 1 and Figs.7&8). Whereas, group III was given a significant increase in the levels of GSH, SOD and CAT and a statistically significant decrease in MDA levels were compared to those of group II (Table 1 and Figs.7&8). However, there were slightly change in group IV when compared to those of group II.
Table 1: Mean (±SEM) testes contents of MDA, GSH, SOD and CAT in the studied groups.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Parameters</th>
<th>MDA (nmol/mg) protein</th>
<th>GSH (μ/mg) protein</th>
<th>SOD (u/mg) protein</th>
<th>CAT (u/mg) protein</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control group (group I)</td>
<td></td>
<td>1.96±0.04</td>
<td>6.14±0.02</td>
<td>3.01±0.21</td>
<td>497.11±2.57</td>
</tr>
<tr>
<td>Finasteride treated group (group II)</td>
<td></td>
<td>3.89±0.07*</td>
<td>3.48±0.06*</td>
<td>1.92±0.19*</td>
<td>321.47±0.05*</td>
</tr>
<tr>
<td>Vitamin E treated group (group III)</td>
<td></td>
<td>0.59±0.41**</td>
<td>7.37±0.04**</td>
<td>4.21±0.41**</td>
<td>565.41±1.65**</td>
</tr>
<tr>
<td>Finasteride &amp; Vitamin E treated group (group IV)</td>
<td></td>
<td>2.48±0.03</td>
<td>4.98±0.22</td>
<td>2.01±0.23</td>
<td>401.62±2.47</td>
</tr>
</tbody>
</table>

Values are expressed as mean±SEM. Test of significance between control and Finasteride - treated rats at p<0.001. Test of significance between Finasteride - treated and Vitamin E treated rats at p< 0.001.

Figure 1. (A) A photomicrograph of a section in the testis of the control group (group I). (B), (C) and (D) photomicrographs of Finasteride received group (group II), (E) A photomicrograph of vitamin E received group (group III) & (F) A photomicrograph of Finasteride and vitamin E received group (group IV) showing:

(A) The testicular parenchyma with densely packed seminiferous tubules (ST) and a narrow interstitium (I) (arrows) in between. Notice the presence of many spermatozoa (Z) in the tubular lumen.

(B) The presence of a seminiferous tubule with sloughed cells (arrow) in its lumen. Severe congested blood vessels can also be seen (double arrows).

(C) Disintegration and disorder of the seminiferous tubules germinal epithelium and atrophic seminiferous tubules (curved arrow). Obviously, some tubules showed complete detachment of their basement membrane (curved double arrows). Vacuolated acidophilic exudate can be observed (straight arrow).

(D) Some seminiferous tubules that are seen resting on a corrugated basement membrane (arrows). Reduction in the diameter of some of seminiferous tubule can be noticed (curved arrows). Note, very wide interstitial spaces (stars) can be seen.

(E) Normal architecture of most seminiferous tubules (ST) and narrow interstitium(I) in between (arrows).

(F) The seminiferous tubules approximately regained its normal architecture. Most of the seminiferous tubules are packed together with even outlines and narrow interstitium (I). Acidophilic hyaline material is still noticed between some tubules (arrows). Some tubules showed partial detachment of their basement membrane in some parts (curved arrows). Abundant sperms (Z) are observed inside their lumina. A, B, C, D, E & F (H & E X 100).
Figure 2. (A) A photomicrograph of a section in the testis of the control group (group I), (B), (C) and (D) photomicrographs of Finasteride received group (group II), (E) Photomicrograph of vitamin E received group (group III) & (F) photomicrograph of Finasteride and vitamin E received group (group IV) showing:

(A) Parts of seminiferous tubules (ST) surrounded by basal lamina (curved arrow) & they lined by normal spermatogenic cells. Primary spermatogonia (G) (arrow), primary spermatocytes (SP), and spermatozoa (Z) can be seen. The interstitial (I) spaces can be clearly visualized contain Leydig cells (L) (notched arrow). Notice the presence of blood capillaries (V) (double curved arrow) in the interstitial tissue.

(B) The spermatogenic cells appear separated from the basement membrane of the seminiferous tubules (curved arrows).

(C) Areas of loss of spermatogenic cells in seminiferous tubules (curved arrows). Congestion in the testis blood vessel can be visualized (straight arrow).

(D) Parts of seminiferous tubules occupied by primary spermatogonic cells only with no sperm formation (curved arrow). The presence of acidophilic vacuolated exudates can be noted in the interstitium (straight arrow).

(E) More or less normal appearance of seminiferous tubules lined by numerous layers of spermatogenic cells surrounded by thin basement membrane. Spermatozoa (Z) can be seen in the lumen.

(F) Some spermatogenic cells separated by empty spaces (curved arrow). Most of the tubules have more or less normal epithelial stratification and sperms (Z) can be seen inside their lumina. A, B, C, D, E&F (H&E, X400).
Figure 3. (A) A photomicrograph of a transverse section of control rat testis (group I); (B), (C) and (D) Photomicrographs of rat treated with Finasteride (group II), (E) Photomicrograph of vitamin E received group (group III) & (F) Photomicrograph of Finasteride and vitamin E received group (group IV) showing:

(A) Strong PAS positive material appeared in spermatogenic cells, basement membrane of seminiferous tubules and in the interstitium (arrows). The presence of spermatozoa (Z) can be seen.

(B) Marked dilatation and congestion of blood vessels (arrow) in interstitial tissue. Notice, decreased PAS positive materials in the spermatogenic cells and moderates PAS positive in basement membrane of seminiferous tubules and in the interstitial cells of Leydig.

(C) Disturbance of spermatogenic layers and exfoliation of cells can be seen. The interstitium contains homogenous vacuolated acidophilic material (arrow) and few Leydig cells.

(D) Wide gaps in between the spermatogenic cells can be observed (straight arrow). Marked degeneration and exfoliation of cells (curved arrow) can be observed.

(E) The normal content of PAS positive material appeared in spermatogenic cells, basement membrane (straight arrow) of seminiferous tubules and in the interstitial cells of Leydig (curved arrow).

(F) Moderates PAS positive material in the spermatogenic cells, basement membrane (straight arrow) and interstitial spaces (curved arrow). (A) (PAS, X100); B, C, D, E & F (PAS, X 400).
Figure 4. (A, B & C) Photomicrographs of sections of rat testis in the control group (group I) showing strong positive nuclear androgen receptor (AR) immunoreactivity in peritubular myoid cells (M) (curved arrow) with immunopositive flat nuclei in the basal laminae around the seminiferous tubules. Notice the presence of the immunopositive nuclei of both Sertoli cells (S) (straight arrow) and Leydig cells (L) (double arrow) in the interstitial tissue. B is higher magnification of A.

(D) A photomicrograph of a section of rat testis in the Finasteride treated group (group II) showing weak positive nuclear androgen receptor (AR) immunoreactivity in Sertoli cells (S) (straight arrow), myoid cells (M) (curved arrow), and Leydig cells (L) (double arrows).

(E) A photomicrograph of a section of rat testis in the vitamin E received group (group III) showing more or less strong positive nuclear androgen receptor (AR) immunoreactivity in Sertoli cells (S) (straight arrow), myoid cells (M) (curved arrow), and Leydig cells (L) (double arrows).

(F) A photomicrograph of a section of rat testis in the Finasteride and vitamin E treated group (group IV) showing moderate positive nuclear androgen receptor (AR) immunoreactivity in Sertoli cells (S) (straight arrow), myoid cells (M) (curved arrow), and Leydig cells (L) (double arrow).

AR immunohistochemical staining, A (AR, X400); B&C (AR, X 1000); D, E&F (AR, X400).
Figure 5. (A) A photomicrograph of a section of adult albino rat testis of the control group (group I) showing the seminiferous tubule lined by several layers of strong PCNA immunopositive nuclei of spermatogonia and primary spermatocytes (arrows).
(B) A photomicrograph of rat testis in the Finasteride treated group (group II) showing few PCNA immunopositive in spermatogonia and spermatocytes (arrows).
(C) A photomicrograph of rat testis in the vitamin E received group (group III) showing many positive PCNA immunostaining in spermatogonia and spermatocytes (arrows).
(D) A photomicrograph of a section of rat testis in the vitamin E and Finasteride treated group (group IV) showing moderate to strong PCNA positive immunoreactivity in spermatogonia and spermatocytes (arrows).
( PCNA immunohistochemical staining, A, B, C&D, X400).

Figure 6. (A) A photomicrograph of a section of adult albino rat testis of the control group (group I) showing mild Bax immunostaining in spermatogonia, primary spermatocytes and spermatids (arrows).
(B) A photomicrograph of a section rat testis in the Finasteride treated group (group II) showing strong Bax immunostaining in spermatogonia, primary spermatocytes and spermatids (arrows).
(C) A photomicrograph of a section of rat testis in the vitamin E received group (group III) showing mild Bax immunostaining in the spermatogonia, primary spermatocytes and spermatids (arrows).
(D) A photomicrograph of a section of rat testis in the vitamin E and Finasteride treated group (group IV) showing moderate Bax immunostaining in the spermatogonia, primary spermatocytes and spermatids (arrows). Bax immunostaining, (A, B, C&D, X 400).
In the current study, it was found that ALLO serum level (ng/ml) in control group was 2.94±0.04, while in Finasteride treated rats (group II) significantly decrease this level to 1.18±0.07 when compared with the control (P<0.001) (Table 2; Fig.9). In vitamin E treated rats (group III), there was significant increase when compared with group II.

However, there was no significant change in rats taken Finasteride and vitamin E (group IV) when compared with group II (Table 2 and Fig. 9).

Also, the same result obtained after the animals exposed to stress swim test (Table 2 and Fig.10).

**Plasma neurosteroid ALLO**

In the current study, it was found that ALLO serum level (ng/ml) in control group was 2.94±0.04, while in Finasteride treated rats (group II) significantly decrease this level to 1.18±0.07 when compared with the control (P<0.001) (Table 2; Fig.9). In vitamin E treated rats (group III), there was significant increase when compared with group II.

However, there was no significant change in rats taken Finasteride and vitamin E (group IV) when compared with group II (Table 2 and Fig. 9).

Also, the same result obtained after the animals exposed to stress swim test (Table 2 and Fig.10).

**Table (2a): Plasma neurosteroid allopregnanolone level (ALLO) (ng/ml) in the experimental groups.**

<table>
<thead>
<tr>
<th>Groups</th>
<th>Parameter</th>
<th>ALLO (ng/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control group (group I)</td>
<td></td>
<td>2.94±0.04</td>
</tr>
<tr>
<td>Finasteride treated group (group II)</td>
<td></td>
<td>1.18±0.07**</td>
</tr>
<tr>
<td>Vitamin E treated group (group III)</td>
<td></td>
<td>2.82±0.01**</td>
</tr>
<tr>
<td>Finasteride &amp; Vitamin E treated group (group IV)</td>
<td></td>
<td>1.78±0.03</td>
</tr>
</tbody>
</table>

Values are expressed as mean±SEM. Test of significance between control and Finasteride - treated rats at p<0.001. Test of significance between Finasteride - treated and Vitamin E treated rats at p< 0.001.

**Table (2b): Plasma neurosteroid allopregnanolone level (ALLO) (ng/ml) in the experimental groups exposed to stress swim test.**

<table>
<thead>
<tr>
<th>Groups</th>
<th>Parameter</th>
<th>ALLO (ng/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control+ swim test</td>
<td></td>
<td>4.29±0.12</td>
</tr>
<tr>
<td>Finasteride treated+ swim test</td>
<td></td>
<td>1.89±0.24*</td>
</tr>
<tr>
<td>Vitamin E treated+ swim test</td>
<td></td>
<td>4.49±0.21**</td>
</tr>
<tr>
<td>Finasteride &amp; Vitamin E treated+ swim test</td>
<td></td>
<td>2.10±0.17</td>
</tr>
</tbody>
</table>

Values are expressed as mean±SEM. Test of significance between control and Finasteride - treated rats at p<0.001. Test of significance between Finasteride - treated and Vitamin E treated rats at p< 0.001.
DISCUSSION

5α-Reductases (5α-Rs) are a family of enzymes, widely distributed in many tissues. Three isoforms of 5α-reductase have been described. In humans, Type I is present in the brain, liver, muscle, and skin. Type II is present in epididymis, hair follicles, liver, prostate, and seminal vesicles. Type III is present in the brain, heart, lung, pancreas, colon, stomach, liver, muscle, prostate and testicle (Yamana et al., 2010). The steroid products of the 5α-Rs pathways undergo further metabolism by the 3α-hydroxy-steroid dehydrogenase (3α-HSD) to produce a host of active neurosteroids with important physiological function, in many tissues including the central nervous system (CNS) and reproduction (Cantagrel et al., 2010).
Finasteride (selective inhibitor of 5α–reductase) is widely used to treat benign prostatic hyperplasia and in the prevention and treatment of prostate cancer and in the male pattern baldness (Tu and Zini, 2011). The present study aimed to examine the impact effect of Finasteride on the level of neurosteroid and the outcome from this effect on the reproduction system and behavior. We are examined the histological, immunohistochemical and biochemical changes in the testes of male adult rats treated with Finasteride. Also, another aim was to assess the possible protective role of using vitamin E in combination with Finasteride treatment.

In the current work, the histological changes in the testes of rats treated with Finasteride such as distorted, atrophy of some of seminiferous tubules with uneven outlines and disorganized epithelium are observed. These changes were in agreement with those reported by some authors. 40% of animals that were administered Finasteride showed significant tubules atrophy and spermatogenesis reduction while 60% showed normal spermatogenesis compared to control group (Vidigal et al., 2008). Moreover, deleterious effects in spermatogenesis in the testis of Hamsters that received a Finasteride dose corresponding to a 5mg used in human management were also recorded (Vidigal et al., 2008). Similarly, some investigators suggested that rats were treated with Finasteride for the duration of two cycles of the seminiferous epithelium (28 days) and the total duration of spermatogenesis (56 days), they found that a 56-day DHT deficiency causes changes in the morphology of the testes (Kolasa et al., 2004).

In the current study, premature germ cell sloughing was present in some of the seminiferous tubules in the testes of rats that received Finasteride (group II). These findings were in agreement with those of Kolasa et al. (2011). They proposed that, in rats with a DHT deficiency induced by Finasteride, the structural alterations were manifested by the premature germ cells sloughing into the lumen of seminiferous tubules. The authors explained that imbalance in androgens homeostasis can lead to premature release of immature germ cells from the seminiferous epithilum into seminiferous tubules lumen, and consequently can decrease the number of spermatooza produced by the testis and thus decrease male fertility. Moffit and co-worker suggested that, the sloughing germ cells resulted from germ cells precociously losing adhesion to Sertoli cells. The germ cells exfoliated into the lumen triggering increased amounts of luminal cellular debris, blocking the efferent ducts and consequently damaging seminiferous tubule fluid passage from the testis to the epididymis (Moffit et al., 2007). DHT deficiency induced by Finasteride disturbed the integrity of the rat seminiferous epithelium by disrupting tight and adherents junctions. They suggested that the depression of N-cadherin, b-catenin and occlude in immunoeexpressions might be the cause for the release of immature germ cells from the seminiferous epithelium (Kolasa et al., 2011).

In addition, researchers suggested that chronically treated rats with Finasteride had no qualitative variations in the semen of the animals. Their fertility was reduced by 30–40%, this being predictable to a deficiency in forming the copulatory plug. The alterations triggered by Finasteride on fertility are probably correlated to a precise effect on the species, in which it is crucial that the copulatory plug is formed, but it is possibly of minor importance in species in which this stage is not vital (Cukierski et al., 1991). On the basis of the previous investigations, it could be observed that transmission electron microscopy studies revealed altered sperm morphology constant with necrosis, and FISH data displayed elevated frequencies of diploidy and sex chromosome disomy after Finasteride treatment (Collodel et al., 2007). Some reporters revealed that histological analyses of gonads of male X. laevis tadpoles exposed to Finasteride revealed a severe disruption of spermatogenesis. Emptied cavities were observed in the analyzed gonads that corresponded to former spermatocysts with secondary spermatogonia and a reduced proliferation activity in Finasteride-treated males (Urbatzka et al., 2009).

In the current work, the interstitial vaculations were observed. In agreement with the present study, some investigators reported that the chemical mediators released after tissue impairment rise the permeability of the wall of blood vessels, which causes outflow of plasma into the adjacent tissue with the formation of fluid exudate (Wheater et al., 1990).

In the present study, PAS-stained sections of the control group showed normal content of glycogen granules manifested by a strong PAS positive reaction in the germ cells, basement membrane and interstitial cells of Leydig. It is well known that the PAS reaction is an indicator of the presence of tissue carbohydrates (Bancroft and Stevens, 1996). Reduction of PAS-positive materials in the, basement membrane of seminiferous tubules and in the interstitial cells of Leydig in the Finasteride treated group were noticed. Reduction of PAS-positive cells might be due to decrease in the activity of enzymes responsible for glycogen storage. The present results demonstrated strong PAS reaction in the basement membrane of seminiferous tubules and in interstitial cells of Leydig after treatment with vitamin E (Group IV). The present data were in agreement with other researchers who described that administration of vitamin E had protective role against nicotine testicular toxicity and increased PAS reaction in rat testes (Nooh et al., 2009).

The researchers recognized that numerous of the biological actions of androgens are mediated by the androgen receptor. Androgen and the androgen receptor displayed crucial roles in male spermatogenesis and fertility through, a paracrine/autocrine system. Our immunohistochemical results of the control group showed
nuclear immunoreactivity to androgen receptors in Sertoli, myoid and Leydig cells at the basal laminae around the seminiferous tubules. Our results came in harmony with other studies. Indeed androgen receptor is expressed in Sertoli cells, peritubular myoid cells, Leydig cells and perivascular smooth muscle cells in the testis (Pearl et al., 2011). Myoid cells comprised a high percentage of androgen receptor and they can synthesize several secretory products. Definitely, Myoid cells offer a site for androgen signaling. Their capability to contract and persuade peristalsis-like waves and impulses in the seminiferous tubule was examined. Myoid cells waves help the transport of spermatozoa through the tubular lumen and into the epididymis (Romano et al., 2005).

In the current study, the intensity of immunostaining for androgen receptor was seen to be reduced in the nuclear areas of Sertoli cells, peritubular myoid cells, and Leydig cells in the Finasteride treated rat testes. These findings were in agreement with the results of some authors who suggested that myoid cells lacking androgen receptor in mice might have some imperfections in contractility, thus resulting in reduction sperm output (Zhang et al., 2006). Furthermore, Leydig cells located in the testicular interstitium are predominantly vulnerable to extracellular sources of reactive oxygen species because of their adjacent proximity to testicular interstitial macrophages (Diemer et al., 2003). The present study detected the intensity of androgen receptor-positive cells in which it was observed to be increased in the Sertoli cells, peritubular myoid cells, and Leydig cells of the Finasteride and vitamin E (group IV) in comparison with the Finasteride rat group (group II). These findings strongly suggested that vitamin E acts as an antioxidant preventing the formation of oxidative stress that could occur during metabolic processes. It acts as a free radical scavenger, scavenging superoxide, hydrogen peroxide, and hydroxyl radicals (Kartikeya et al., 2009). These potent properties of vitamin E have likely influenced the almost near to normal regenerative effects of testicular tissue.

In the current study, the immunohistochemistry was used to record the distribution of PCNA immunoreactivity in the testes of the different groups. In the present results, many positive nuclear reactions were observed in spermatogonia and proliferating spermatocytes of control testes. The staining intensity of PCNA was used to assess the proliferation of cells and the spermatogenic function of testes in case of male infertility (Xue et al., 2007). Controlled cell proliferation is of major importance during normal spermatogenesis, assuming extremely synchronized mechanisms between the mitotically inactive sertoli cells and the germ cells experiencing mitosis and meiosis. Crucially, D’andrea et al. (2010) suggested that the immunocytochemical assays, constructed on antibodies to cell proliferation-related antigens such as PCNA is effective in the assessment of cell proliferation. In the present work, the immunohistochemical investigation of Finasteride treated group showed decreased the number of PCNA immunopositive spermatogonia and spermatocytes as compared with control. Our present results indicating that Finasteride treatment badly affects spermatogenesis and the proliferating capacity of spermatogonia, in agreement with previous study, less PCNA expression in testicular epithelium was correlated to decrease DNA synthesis in the injured testes (Xue et al., 2007). PCNA is very beneficial in the pathological diagnosis of infertility, especially for the differentiation of hypospermatogenesis from partial germinal arrest. Definitely, PCNA is a useful marker of germinal arrest, because there are significantly reduced PCNA levels which they are a sign of decline of DNA synthesis (Zeng et al., 2001). The increased number of PCNA-positive cells in the spermatogonia of the Finasteride and vitamin E group in the present study as compared with the Finasteride rat group verified the role of vitamin E in improving spermatogenesis. These findings strongly supported the results of other investigators who detected the role of vitamin E, carotene, and their combination as antioxidants against the toxicity of fenvalerate insecticide (El Demerdash et al., 2004b).

In the present study, the immunohistochemical study suggested that Bax is localized in the cytoplasm of spermatogonia, spermatocytes, and spermatids during the normal seminiferous epithelial cycle. Bax immunoreactive staining in the cytoplasm of cells demonstrated that apoptosis has occurred. The results of the current work established that Bax were significantly increased in the spermatogenic cells of the Finasteride treated group (group II). Similar results have been reported that DHT acts as an apoptotic signaling modulator in immature rat Sertoli cells and treatment DHT caused in down-regulation of p53, Bax, caspase 9 and 3 mRNAs (Simões et al., 2013). Moreover, these finding coincided with the findings of some researchers who suggested the involvement of Bax in heat induced germ cell apoptosis and investigators hypothesized that redistribution of Bax may be represented an important step in the pathway by which members of this family may regulate programmed germ cell death (Cindy et al., 2000).

In addition, previous results from Rittmaster et al. (1995) demonstrated that, Finasteride caused a significant increase in the number of apoptotic cells in the prostate of rats during the first days of treatment. However, from the 14th day of treatment, there was no significant difference in the number of apoptotic cells. Glassman et al. (2001) verified that, Finasteride alone has not shown an effect on cellular proliferation, but it did increase the rate of apoptosis. Several studies have shown that apoptosis is mainly mediated by increased oxidative stress either due to increased free radical generation or due to decreased antioxidant defenses. Bakalska and his colleague demonstrated that exposure of animals to ethane 1, 2-dimetanesulfonate, a Leydig cell toxicant, results in testosterone-dependent germ cell apoptosis (Bakalska et al., 2004). Several
studies have been conducted on the Bax expression in testicular germ cells. The investigators verified that Dexamethasone can increase Bax expression in mouse testicular germ cells mainly at androgen dependent stages (Mahmoud et al., 2009). Other researchers have reported that after androgen withdrawal using Diethylstilbestrol, a strong estrogenic compound, the expression of Bax is up-regulated (Kondo et al., 2002). Similar to our results have been observed in which Bax markers were increased in the spermatogenic cells of the cadmium treated groups. (Alkhedaide et al., 2016; Shibata et al., 2003) suggested that, rat prostate involution detected under Finasteride treatment is partially due to apoptosis and to an intense deterioration of prostate vascularization. Scientifically, the mechanism of testicular cells apoptosis might be dependent on the type of the cell, androgen or endocrine disruptor. The researchers described an increase in the apoptotic index of testicular cells of male mice after cytochemical finding of DNA fragmentation in testicular apoptotic nuclei. This was attributed to the increased production of reactive oxygen species. Generation of highly ROS can damage various cellular components including proteins, membrane lipids and nucleic acids, with resultant DNA fragmentation (Adhikari et al., 2001).

The present work demonstrated that testes contents of GSH, SOD and CAT activities were significantly decreased in the Finasteride treated rats (group II) compared to those in control (group I). In agreement with the current results, the investigator described the relation between Finasteride administration in low and high doses and reactive oxygen species production. Crucially, glutathione levels were decreased with increasing dose of Finasteride which indicate statistically significant increase in the reactive oxygen levels (Serga, 2009a). Moreover, it has been demonstrated that Finasteride alters testicular function. Vitally, sperm motility and abnormal sperm forms was harmfully affected only in high dose of Finasteride group. In contrast, sperm motility and morphology were not significantly changed in low-dose Finasteride group (Serga, 2009b) . The report of the researchers Takano et al. (2002) who speculated that oxidative stress induced the triggering of multiple signaling pathways related to numerous cellular responses such as mitotic arrest, apoptosis and necrosis, depending on the H2O2 concentrations. It was shown that a reduction in ventral prostate epithelial cell proliferation under Finasteride treatment might be a result of H2O2 accumulation (Cayatte et al., 2006).

In the current work, the concomitant administration of vitamin E with Finasteride showed a significant reduction in the degenerative changes in the cells of the seminiferous tubules and the germ cell apoptosis. The protective role of vitamin E has also been described by investigators as it is involved in the elimination of reactive oxygen species and in the inhibition of oxidative stress-induced apoptosis. The protective effects of vitamin E are assumed by either reducing or stopping oxidative impairment caused by poisonous substances (Rinne et al., 2000; Altuntas et al., 2002). It prevents oxidative damage in numerous tissues by heavy metals (Acharya et al., 2008). Vitamin E diminished the harmful impact of lead on the reproductive system (Oluseyi et al., 2012). It was verified to amend aflatoxin induced alterations in the testis of mice (Verma and Nair, 2001). Furthermore, it was act as a defensive role against mercury induced reproductive toxicity in the male mice (Rao and Sharma, 2001). Many studies have explained the possible mechanism of vitamin E protection as being dependent on its antioxidative action.

With regard to the current biochemical results, vitamin E group (group III) and vitamin E and Finasteride group (group IV) were given statistically significant decreased in MDA levels which they were determined when compared to Finasteride group (group II). These results are in agreement with the investigators documented that vitamin E acts against oxidative stress by decreasing malondialdehyde level and increasing of antioxidant defense system activity in testicular cells (Yue et al., 2010). However, this protective effect of vitamin E in the present study is insufficient; as some seminiferous tubules were separated by wide interstitial spaces surrounded by partially separated basement membrane. They were lined by some dispersed and degenerated spermatogenic cells. Many investigators have explained the possible vitamin E protection as the defensive effect of vitamin E could be dose dependent; it might be that vitamin E supplementation is most useful when combined with other antioxidants or vitamins, generating a potentially synergistic effect (Azari et al., 2015). In agreement with previously mentioned results, the investigators reported the partial protective role of vitamin E during ethane dimethane sulfonate induced testicular toxicity in the rats (Sahinturk et al., 2007). Similarly, the recent study demonstrated that deltamethrin exposure had harmful effects on cell membrane and treatment with vitamin E could only partially protect fish gills (Cengiz et al., 2016).

In the current study, Finasteride administration result in significant decrease in the ALLO serum levels both in non stressed and stressed animals. These results were in line with the results of Caruso et al. (2015) and Fertig et al. (2017), who concluded that Finasteride will altered the levels of neuroactive steroids in the plasma and CNS. In addition, Römer et al. (2010) noticed significant decrease in brain 5alpha-dihydrotestosterone levels and induced a reversible reduction in the number of newborn cells and young neurons in the hippocampus. These data indicate that inhibition of 5-alpha-reductase activity by Finasteride treatment influences neuronal plasticity on a structural level. These changes might contribute to the pathophysiology of depressive episodes observed after Finasteride treatment. It has recently been shown that patients who had been treated with Finasteride have reduced or undetectable levels of neuroactive steroids in their cerebro-spinal fluid and plasma, and exhibited higher levels of precursor steroids (Melcangi and Panzica,
This observation strongly suggests that 5α-RIs have a deleterious effect on the biosynthesis and function of neurosteroids in the central nervous system. Finasteride treatment resulted in decreased levels of 5α-DHT and 3α, 5α-tetrahidroprogesterone (AP) and increased levels of testosterone supporting the hypothesis that deleterious effects of Finasteride may be persistent or irreversible. This may explain some of the noted symptoms such as anxiety, depression and suicide in patients who have been treated with Finasteride (Melcangi et al., 2013).

Neuroactive steroids elicit important neuroprotective effects during trauma and injury to the central nervous system (Melcangi and Panzica, 2014). ALLO is shown to be beneficial in the treatment of traumatic brain injury, attenuating edema, trauma, stress, inflammation, apoptosis, and reducing oxidative stress (Melcangi et al., 2013). ALLO is not only a protective agent in ischemia, but it is also in maintaining blood brain barrier integrity, and in memory and learning (Ishrat et al., 2010). Studies on CNS injury in which asphyxiation was induced in fetal sheep to stimulate neurological stressors, in the presence or absence of Finasteride, showed an increase in apoptotic cell death in the cerebellum and hippocampus in the animals treated with Finasteride (Yawno et al., 2007). Furthermore, treatment with an ALLO analogue, alfaxalone, prevented cell death. This observation suggests that ALLO exerts a neuroprotective role in the brain, which is inhibited by Finasteride (Römer et al., 2010).

The physiological basis of mood disorders caused by 5αRIs has been associated with the dysregulation of neurosteroids and androgen deficiency (Van-Broekhoven and Verkes, 2003). As 5αRIs inhibit the enzyme 5-alpha-reductase required to synthesize these neurosteroids, the resulting decrease in the neurosteroid biosynthesis could contribute to psychiatric adverse events. Reduction in allopregnanolone is associated with depressive symptoms and unipolar major depression in men (Van-Broekhoven and Verkes, 2003). A study by Barrett-Connor et al. (1999) showed that, survey scores for measuring depression were inversely associated with bioavailable DHT levels and genetic predisposition. Although Finasteride was shown to inhibit allopregnanolone in animal models, there is no information in humans. Two polymorphisms (CAG) and (GGN) in the gene encoding for the androgen receptor have been hypothesized to play a role in Finasteride sensitivity (Cecchin et al., 2014).

There is ancillary evidence supporting a potential relationship between 5ARIs and suicidality or depression. First, 5α-reductase is responsible for the production of several neuroactive steroids (Traish, 2012; Celec et al., 2015). Second, testosterone and dihydrotestosterone modulate the neuroendocrine stress response and are inversely related to depression indices (Handa et al., 2013). Third, levels of the neurosteroid allopregnanolone (produced by 5α-reductase) are lower among men with depression (Melcangi et al., 2013). Finally, patients with clinical depression have lower levels of type I 5α-reductase in the prefrontal cortex (Agis-Balboa et al., 2014).

Animal studies suggested that Finasteride could alter 5α-reductase activity in some regions of the brain, and lead to behavioral and mood changes. It has been shown that Finasteride is able to inhibit 5α-reductase in medial basal hypothalamus in pregnant rats, and induce behavioral changes (Lephart et al., 1996). Significant inhibition of hypothalamic and pituitary 5α-reductase is also noticed in adult male rats (Lephart, 1995). In addition to animal studies, although there are some case reports suggesting Finasteride induction of depressive symptoms and anxiety in humans, (Altomare and Capella, 2002) but no prospective study has been carried out, in order to investigate Finasteride behavioral effects. These changes might contribute to the pathophysiology of depressive episodes observed after Finasteride treatment.

Recently, some neurosteroids were found to be involved in the pathogenesis of severe debilitating neuropathologies; therefore it was suggested that their modulation might be a target for neuroregenerative therapies addressed to treat Alzheimer’s disease, multiple sclerosis, psychiatric disorders, post-traumatic stress disorder, epilepsy, and other neurological disorders (Irwin et al., 2014; Noorbakhsh et al., 2014). The neurosteroids actions are exerted through paracrine and/or autocrine mechanisms. They act by interacting with classic steroid receptors (i.e., progesterone receptor, PR; androgen receptor, AR; estrogen receptor, ER), as well as through non classic steroid receptors like the putative membrane receptors (mPR, mAR, mER, etc.) and some neurotransmitter receptors (Cooke et al., 2013). They modulate the gamma-aminobutyric acid (GABA), N-methyl-D-aspartate (NMDA), and 5-hydroxytryptamine type 3 (5HT3) receptors (Sedlacek et al., 2008). Allopregnanolone (ALLO), is the most important neurosteroid synthesized via a bidirectional reaction, through the action of the 5α-R-3α-HSD complex (Mellon et al., 2001). It showed potent neurogenic properties, inducing a dose-dependent proliferation of neural rat progenitor cells and human stem cells. Moreover, ALLO exerts important roles also in central and peripheral glial cells (Wang, 2014). ALLO displays rapid “nongenomic” effect, which mainly involves the potent modulation of the GABA type A (GABA-A) receptor, although, recently, some steroid membrane receptors (e.g., mPR) have been identified as target for ALLO actions in the nervous system (Cooke et al., 2013). Moreover, some ALLO’s effects on behavioural processes involve rapid actions via GABA-A and/or NMDA receptors (Frye et al., 2014).
In the animal model, these findings indicate that the loss of neurosteroid biosynthesis may be responsible for the disease state and its progression. Therefore inhibition of 5α-R by Finasteride in the course of treatment of non-life threatening conditions, such as male pattern baldness (alopecia) or BPH may have detrimental effects on the CNS.

**CONCLUSION**

Taken together, caution has been recommended concerning the use of Finasteride by male members of couples who plan to have children. The results of the current work showed harmful effects of Finasteride in spermatogenesis in the tests of rats. It must be clarified to the patient the possible effects of Finasteride on spermatogenesis. Clinicians and potential users of Finasteride should be aware of the potential risk of depressive symptoms and suicidal thoughts. Further studies should be implemented in order to elucidate the safe dose of Finasteride to be used.

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