Hormonal and Histopathological Study of Adult Albino Rats' Thyroids and Testes After Long-Term Fluoxetine Hydrochloride Administration

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Abstract

Prozac (fluoxetine hydrochloride; FLX), a selective inhibitor of serotonin reuptake by nerve terminal in the brain, is widely used in treatment of depression and other neuropsychiatric disorders since 1987. Although FLX is generally safe, effective, and well tolerated, however, several adverse effects have been reported in the literatures. This drug may be responsible for the occurrence of male's sexual dysfunction and reproductive toxicity that can alter essential sex hormones homeostasis, fertility, and seminal configurations. So, this work was designed to evaluate the toxic effects of FLX on rats' thyroid glands as well as reproductive organ (testes) functions and structures. The study was conducted on 20 adult male albino rats that divided equally into 2 groups (10 rats each). Each group had received its corresponding substance in a single dose/day via intragastric tube for 60 consecutive days as follows: Group 1 (Control group; C-gp): Each fasted rat had received 2-ml of distilled water. Group 2 (FLX treated group; FLX-gp): Each fasted rat had received 45 mg/kg of FLX per 2-ml of distilled water.

At the end of the experimental period and under anesthesia, blood samples were collected for measurement of hormonal levels, and then rats were sacrificed for harvesting thyroids, testes, and epididymides for histological examination. The obtained results of FLX-gp showed significant differences when compared with C-gp data that represented as reduction in animals' final body weights, increases in absolute and relative weights of the thyroids, decreases in absolute and relative weights of the testes and epididymides, disturbances of various seminal fluid quality parameters as evidenced by declines in sperm count, motility, and normal sperm morphology in association with increased abnormal sperm percentage and disruptions of serum hormonal analysis in the form of decreases in the follicular stimulating hormone level accompanying with decreases in the triiodothyronine, thyroxin, thyroid stimulating hormone, total testosterone, and luteinizing hormone levels. Furthermore, thyroidal and testicular tissue examination revealed several histopathological changes that supported the hormonal results. These data suggest that FLX exhibits detrimental effects on male reproductive organ functions and histomorphology by acting both centrally on the thyroid glands and peripherally on testicular tissues leading to development of hypogonadism and impairment of spermatogenesis. Hence, prolonged exposure to FLX has the potential to negatively affect the human reproductive system which reinforces the need for periodical counseling to monitor the patients' fertility indices.

Introduction

Depression is a chronic or recurrent mood disorder that affects both economic and social functions of about 121 million people worldwide. According to the World Health Organization, it will be the second leading contributor to the global burden of disease, calculated for all ages and both sexes by the year 2020 (Wille et al., 2008).

Antidepressant pharmacotherapies have been widely used in the treatment of various forms of depression for many years. Treatment options have expanded dramatically in the last 20 years to include tricyclic antidepressants, monamine oxidase inhibitors, norepinephrine and dopamine reuptake inhibitors, serotonin antagonist reuptake inhibitors, noradrenergic and specific serotonergic agents and the newer selective serotonin reuptake inhibitors (SSRI) and
serotonin norepinephrine reuptake inhibitors (Gelenberg et al., 2010).

Fluoxetine [FLX] (N-Methyl-γ-[4-(trifluoromethyl) phenoxy] benzene propanamine) is a SSRI and was approved by the US FDA in 1987. It is widely used to treat several disorders such as major depression, panic, bulimia nervosa, obsessive-compulsive behavior, anxiety and premenstrual dysphoric disorder (Sharma, 2001; Vaswani et al., 2003).

FLX induces acute increases in extracellular serotonin levels and chronic increases in the activity of the serotonergic system in different regions of the forebrain due to increased concentration of serotonin in the synaptic cleft without affecting other monoamine reuptake mechanisms or other neurotransmitter receptors. It has only weak affinity for various receptor systems, namely opiate, serotonergic 5-HT1, dopaminergic, β-adrenergic, α2-adrenergic, histaminergic, α1-adrenergic, muscarinic, and serotoninergic-5-HT2 receptors (Wille et al., 2008).

FLX is well absorbed following oral administration, and peak plasma FLX concentrations usually occur within 4–8 hours and being approximately 15–55 ng/ml (Eap and Baumann, 1996). FLX is extensively demethylated in the liver by CYP2C and 2D6 to the primary active metabolite norfluoxetine. The elimination half-life of the parent drug is 4 to 6 days, but it is increased to 4–16 days for norfluoxetine. On the other hand, FLX inhibits isoenzyme CYP2D6 and thus its own metabolism. Due to extensive tissue distribution, FLX has a high volume of distribution (Cheer and Goa, 2001). It is excreted by humans as 11% parent compound and 7% norfluoxetine, both of which are pharmacologically active, in urine, feces and breast milk (Van Harten, 1993).

The predominant adverse effects of FLX are related to cardiovascular system, central nervous system and gastrointestinal system. In addition, endocrine and metabolic disorders such as hypoglycemia, hyponatremia, hypokalemia, dehydration, hypercholesterolemia, hyperprolactinemia, and hypothyroidism were reported (Leikin and Paloucek, 2008).

On the other hand, antidepressants such as FLX is associated with a high incidence of sexual dysfunction such as alterations in libido, arousal, erectile function and orgasm as well as delayed or absent ejaculation in a large proportion of patients (Frohlich and Meston, 2005; Stimmet and Gutierrez, 2006).

Antidepressant medications can affect the hypothalamic-pituitary-thyroid-gonadal axis at many levels and may consequently influence reproductive function in men. Dopamine, serotonin, and γ-aminobutyric acid, all commonly modulated by these agents, are involved in the normal physiologic regulation of the male reproductive axes. They can also influence levels of sex steroids by altering the metabolism and protein binding of hormones. Additionally, they may influence fertility through peripheral effects as a result of their action on cholinergic and α-adrenergic receptors as well as through their effects on the production of viable spermatozoa (Hendrick et al., 2000).

Despite this, few studies have assessed the relationship between antidepressant medication use and the potential impact of these drugs on male fertility. Several early studies had reported reproductive toxicity in male patients treated with different antidepressant drugs such as trimipramine (Kurland et al., 1970), imipramine, desmethylimipramine, and nortriptyline (Levin et al., 1981), and clomipramine (Maier and Koinig, 1994). Abnormalities that were recorded in the previous studies included alterations in spermatogenesis processes as evidenced by significant reductions in sperm viability, volume and motility. Moreover, several patients have shown improved sperm counts and motility after discontinuation of antidepressant medications (Tanrikut and Schlegel, 2007). In addition, FLX may have detrimental side effects on reproductive function in both humans and animals (de Jong et al., 2006; Serretti and Chiesa, 2009).

In view of such findings, the present study was designed to further explore the effect of FLX on adult albino rats’ thyroid-testicular axis functions and morphology. The biochemical assay of male reproductive hormones (triiodothyronine, thyroxin, thyroid stimulating hormone, total testosterone,
luteinizing hormone and, follicle stimulating hormone) as well as the histological architectures of thyroids and testicular tissues after long term treatment with FLX were investigated.

Material and Methods

1- Drug:
Prozac (Fluoxetine Hydrochloride; FLX), the product of Eli Lilly and Company, Indianapolis, USA, was used in the present study. Each commercially available capsule contains 20-mg FLX. Capsules contents were gavaged to each fasted animal after being dissolved in distilled water at a single dose/day by intragaseric feeding tube.

2- Animals:
Twenty adult male albino rats, weighing between 250-280 grams were used in the present study. They were divided into 2 equal groups (10 rats each) and housed under constant environmental conditions, and were kept for 10 days of acclimatization before treatment with food and water were freely allowed. The experiments were performed in accordance with "Guide for the Care and Use of Laboratory Animals" mentioned by Cuschieri and Backer (1977). Animals were subjected to treatment regimen for 60 consecutive days as follows:

Group 1 (Control group; C-gp): Each rat had received 2-ml of distilled water orally.

Group 2 (FLX treated group; FLX-gp): Each rat had received 45 mg/kg of FLX per 2-ml solution orally. The dose was chosen according to Stark et al. (1985) and equivalent to 1/10 LD₅₀ of oral FLX in rats.

Twenty-four hours after the last dose and under ether anesthesia animals were weighted and subjected to midline incision. Blood was collected by syringe from descending aorta into clean test tubes and then animals sacrificed by cervical dislocation.

3- Methods:

A- Hormonal Study: Clotted blood samples were centrifuged and obtained sera were assayed for triiodothyronine (T₃) (Larsen, 1972), thyroxin (T₄) (Wisdom, 1976), thyroid stimulating hormone (TSH, thyrotropin) (Pekary et al., 1975), total testosterone (Blight et al., 1989), luteinizing hormone (LH), and follicular stimulating hormone (FSH) (WHO, 1973), by using ¹²⁵I-radioimmunoassay (RIA) commercial available kits, obtained from Diagnostic Products Corporation, Los Angeles, USA.

B- Seminal Characters Study: The epididymides were dissected out and weighted then processed for spermatozoal count, sperm motility, and sperm morphology according to Bearden and Fluquary (1980).

C- Histopathological Study: Finally, thyroids and testes were harvested, washed with distilled water, weighted, cut into pieces and processed for light microscopic examination. Both organs tissues were fixed in Bouin's solution, subsequently dehydrated in ascending grades of alcohol, cleaned by xylene, and embedded in paraffin wax, then sectioned at 5 µm thicknesses and stained with Hematoxylin and Eosin (H and E).

Statistical analysis
Data were analyzed by using a Student t-test and expressed as mean±SD where the level of significance of differences was accepted at p<0.05.

Results
No deaths were recorded in either control or treated animals throughout the study.

As shown in Table (1), rats treated with FLX showed significant decrease in their final body weights (changed by 16.79%) as compared to control animals. Conversely, the mean absolute (changed by 38.85%) and relative (changed by 65.51%) weights' values of thyroid glands depicted significant increase when compared with control values.

As appears in Table (2), data of hormonal analysis showed a significant decrease in the mean serum levels of T₃ (changed by 46.57%) and T₄ (changed by 34.29%) accompanied with a significant increase in serum TSH level (changed by 38.89%) in FLX gavaged rats when compared with control levels.

Normal histological pattern of the control rats' thyroid gland structures by light
microscopic examination is shown in Figure (1). The gland consists of prominent multiple follicles with regular outline. Each follicle is lined by a single layer of flattened to cuboidal epithelial cells with flattened to round nuclei and their lumina are filled with homogenous acidophilic colloidal material with interfollicular cells appears in the little connective tissue between the follicles.

On the other hand, light microscopic examination of rats' thyroid gland from FLX-gp showed different histopathological changes (Figures, 2-5). Thyroid follicles were lined with hypertrophic tall columnar cells that contained vacuolated scanty colloidal material with scalloped appearance of its edges. Few follicles showed interruption of their basement membrane. Some follicular cells appeared with marked vacuolation of their cytoplasm. Degenerative changes with increased parafollicular inflammatory cells infiltration were noticed.

On the other side, in Table (1), the mean absolute and relative weight values of testes (changed by 34.87% and 21.89%, respectively) and epididymides (changed by 40.62% and 26.56%, respectively) in FLX treated animals revealed significant reduction when compared with the control figures. In addition, results of seminal quality evaluation depicted statistically significant declines in the mean values of sperm concentration (changed by 37.74%), motility (changed by 28.81%), and normal sperm morphology (changed by 32.89%) in FLX-gp as compared to C-gp. Also, a significant elevation of abnormal sperm morphology (changed by 113%) was noticed in FLX treated rats when compared with control rats.

Table (2) reveals that, values of hormonal assay showed a significant decrease in the mean serum levels of testosterone (changed by 44.48%), LH (changed by 40.16%), and FSH (changed by 27.52%) levels in FLX-gp as compared to C-gp.

Figure (6) illustrates normal appearance of the control rats' testis structural components by light microscopic examination, which are the seminiferous tubules (SNT) and interstitial tissues in the intertubular spaces. The SNT are lined with stratified epithelium and consists of two types of cells, namely Sertoli and spermatogenic cells. Each SNT consists of well-delineated basement membrane (BM), well-preserved Sertoli cells (appears as triangular or oval cells resting on the BM), and several layers of spermatogenic cells namely spermatogonia, spermatocytes, spermatids, and mature spermatozoa. The SNT are surrounded by elongated fibroblast like myoid cells outside the BM. The interstitial tissues showed clusters of intact interstitial cells of Leydig, which appeared polygonal in shape with rounded nuclei as well as normal blood vessels that appeared patent without blood cells in the lumen.

On the contrary, light microscopic examination of FLX treated rats' testes revealed various morphological alterations (Figures, 7-10) in the form of testicular atrophy as well as total tubular degeneration, and necrosis. In addition, the SNT showed distortion with considerable decrease in the germinal cell population (spermatogonia, primary and secondary spermatocytes, spermatids, and mature sperm numbers), reduction in diameter, and irregular outlines, vacuolation of the intercellular spaces, and impairment of spermatogenesis. The space between SNT showed thick walled and congested blood vessels and edema of the intertubular space as well as considerable decrease in Leydig cells.

**Table 1.** Effect of FLX on rats' body weights, absolute and relative weights of thyroid gland, testes, and epididymides, as well as seminal quality as compared to control. Each group comprised 10 rats.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Group 1 (Control)</th>
<th>Group 2 (FLX treated)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body Weight (g)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Initial</td>
<td>Mean±SD</td>
<td>273.7±16.97</td>
</tr>
<tr>
<td>P and Sig.</td>
<td></td>
<td>&gt;0.05&lt;sup&gt;NS&lt;/sup&gt;</td>
</tr>
<tr>
<td>% of Change</td>
<td></td>
<td>+1.42% (†)</td>
</tr>
<tr>
<td>Final</td>
<td>Mean±SD</td>
<td>301.9±20.18</td>
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</tbody>
</table>

**Thyroid Gland**

<table>
<thead>
<tr>
<th>Absolute weights (mg)</th>
<th>Mean±SD</th>
<th>19.87±1.98</th>
<th>27.59±3.15</th>
<th>P and Sig.</th>
<th>&lt;0.0001***</th>
<th>% of Change</th>
<th>+38.85% (↑)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Relative weights (mg/100 g B.W.)</td>
<td>Mean±SD</td>
<td>6.64±0.37</td>
<td>10.99±1.35</td>
<td>P and Sig.</td>
<td>&lt;0.0001***</td>
<td>% of Change</td>
<td>+65.51% (↑)</td>
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</table>

**Testes (paired)**

<table>
<thead>
<tr>
<th>Absolute weights (g)</th>
<th>Mean±SD</th>
<th>4.13±0.45</th>
<th>2.69±0.29</th>
<th>P and Sig.</th>
<th>&lt;0.0001***</th>
<th>% of Change</th>
<th>-34.87% (↓)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Relative weights (g/100 g B.W.)</td>
<td>Mean±SD</td>
<td>1.37±0.11</td>
<td>1.07±0.14</td>
<td>P and Sig.</td>
<td>&lt;0.0001***</td>
<td>% of Change</td>
<td>-21.89% (↓)</td>
</tr>
</tbody>
</table>

**Epididymides**

<table>
<thead>
<tr>
<th>Absolute weights (g)</th>
<th>Mean±SD</th>
<th>1.92±0.39</th>
<th>1.14±0.19</th>
<th>P and Sig.</th>
<th>&lt;0.0001***</th>
<th>% of Change</th>
<th>-40.62% (↓)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Relative weights (g/100 g B.W.)</td>
<td>Mean±SD</td>
<td>0.64±0.13</td>
<td>0.47±0.07</td>
<td>P and Sig.</td>
<td>&lt;0.0001***</td>
<td>% of Change</td>
<td>-26.56% (↓)</td>
</tr>
</tbody>
</table>

**Seminal Quality**

<table>
<thead>
<tr>
<th>Concentration (10⁶/g epididymides)</th>
<th>Mean±SD</th>
<th>507.88±62.78</th>
<th>316.19±43.68</th>
<th>P and Sig.</th>
<th>&lt;0.0001***</th>
<th>% of Change</th>
<th>-37.74% (↓)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sperm motility (%)</td>
<td>Mean±SD</td>
<td>81.36±5.77</td>
<td>57.92±9.16</td>
<td>P and Sig.</td>
<td>&lt;0.0001***</td>
<td>% of Change</td>
<td>-28.81% (↓)</td>
</tr>
<tr>
<td>Normal Sperm morphology (%)</td>
<td>Mean±SD</td>
<td>77.45±6.38</td>
<td>51.97±8.31</td>
<td>P and Sig.</td>
<td>&lt;0.0001***</td>
<td>% of Change</td>
<td>-32.89% (↓)</td>
</tr>
<tr>
<td>Abnormal Sperm morphology (%)</td>
<td>Mean±SD</td>
<td>22.55±6.38</td>
<td>48.03±8.31</td>
<td>P and Sig.</td>
<td>&lt;0.0001***</td>
<td>% of Change</td>
<td>+113% (↑)</td>
</tr>
</tbody>
</table>

Sig = significance; NS = non-significant difference; * = significant difference; - & ↓ = decrease; + & ↑ = increase.

**Table 2.** Effect of FLX on serum levels of triiodothyronine (T₃), thyroxin (T₄), thyroid stimulating hormone (TSH), total testosterone, luteinizing hormone (LH), and follicular stimulating hormone (FSH) as compared to control rats values.
<table>
<thead>
<tr>
<th></th>
<th>P and Sig.</th>
<th>% of Change</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum testosterone level (ng/ml)</td>
<td>6.25±0.51</td>
<td>3.47±0.69</td>
</tr>
<tr>
<td>Mean±SD</td>
<td>P and Sig.</td>
<td>&lt;0.0001***</td>
</tr>
<tr>
<td>% of Change</td>
<td></td>
<td>+38.89 (%)</td>
</tr>
<tr>
<td>Serum LH level (mIU/ml)</td>
<td>2.44±0.47</td>
<td>1.46±0.17</td>
</tr>
<tr>
<td>Mean±SD</td>
<td>P and Sig.</td>
<td>&lt;0.0001***</td>
</tr>
<tr>
<td>% of Change</td>
<td></td>
<td>-44.48% (%)</td>
</tr>
<tr>
<td>Serum FSH level (mIU/ml)</td>
<td>7.85±0.67</td>
<td>5.69±0.68</td>
</tr>
<tr>
<td>Mean±SD</td>
<td>P and Sig.</td>
<td>&lt;0.0001***</td>
</tr>
<tr>
<td>% of Change</td>
<td></td>
<td>-27.52% (%)</td>
</tr>
</tbody>
</table>

Sig = significance; * = significant difference; - & ↓ = decrease; + & ↑ = increase.

**Figure (1):** Photomicrographs of normal thyroid tissue from control rat (group 1) showing thyroid follicles (F) of various size filled with colloidal thyroglobulin (C) in their lumen and lined with cuboidal epithelial cells (➔) with rounded central or basal nuclei. The follicular acini are separated by follicular spaces or septa (S). Some parafollicular cells (PC) and blood vessels (BV) are present in between thyroid follicles. H&E stain; Original magnification (OM) X 100. Small window shows follicle with high magnification OM x 400.

**Figure (2):** Photomicrograph of FLX-treated rat thyroid gland (group 2) exhibits follicular cell hypertrophy with microfollicle formation (F) and colloid depletion. The microfollicles exhibit taller epithelium with a marked

**Figure (3):** Photomicrograph of FLX-treated rat thyroid gland illustrating hypertrophic (H) tall columnar follicular cells with pyknotic nuclei (➔) and vacuolated cytoplasm (V). The colloidal material (C) inside the acini appears pale with scalloped (SC) or
reduction in lumen size and colloid content. OM x 100.

moth-eaten edges. H&E; OM x 400.

Figure (4): Photomicrograph from FLX-treated rat thyroid gland depicting the presence of follicular degeneration (FD) with desquamated epithelial cells inside the follicles that lined with high-toned epithelium and light cytoplasm (>). H&E; OM x 400.

Figure (5): Photomicrograph from FLX-treated rat thyroid gland demonstrating extensive intrafollicular vacuolization (V) and parafollicular cellular (PC) infiltration with inflammatory cells. H&E; OM x 200.

Figure (6): Photomicrograph of testicular tissue from control rat expressing normal appearance and arrangement of different germ cells. SNT = seminiferous tubules; BM = basement membrane; SG = spermatogonia; S = Sertoli cells; P1 = primary spermatocytes; P2 = secondary spermatocytes; E = early spermatids; L = late spermatids; H&E; OM x 200. Small window shows LC = Leydig cells; H&E; OM x 400.

Figure (7): Photomicrograph of FLX-treated rat testis implying widening of the intertubular space (W); congested

Figure (8): Photomicrograph of FLX-treated rat testis indicating shrinkage (S) and degenerated (D) seminiferous tubules with
thick walled blood vessel (BV); desquamated cells (DC) in the intertubular space; disorganization of germ cells and decrease of spermatogenesis in the seminiferous tubules (SNT). H&E; OM x 200.

Figure (9): Photomicrograph of FLX-treated rat testis showing atrophic (A) seminiferous tubule and edema (E) in the intertubular space. H&E; OM x 400.

Figure (10): Photomicrograph of FLX-treated rat testis announcing the presence of extensive degeneration of seminiferous tubule (SNT) with necrotic central material. OM x 400.

Discussion

The currently available selective serotonin reuptake inhibitors (SSRIs) including Fluoxetine (FLX), sertraline, paroxetine, fluvoxamine, citalopram and escitalopram represent the most widely antidepressive drugs utilized in the medical treatment of depressed patients (Costagliola et al., 2008). However, FLX is a suspected reproductive toxicant and exposure to it has the potential to negatively affect the human reproductive system. Indeed, there are now several reports on the detrimental impacts of FLX on reproduction in fish (Mennigen et al., 2008; Lister et al., 2009). While some of these effects in fish are comparable to the effects observed in pharmacological studies in mammals, particularly females (Uphouse et al., 2006), the reproductive axis in males has received less attention, in spite of the description of disruptive effects of FLX in male rats (Vega Matusczyk et al., 1998).

Hence, in this study, the effects of FLX on the male thyroid and reproductive organs were investigated by measuring different hormonal levels (T₃, T₄, TSH, testosterone, LH, and FSH) as well as thyro-testicular tissues histopathological examination.

Fluoxetine and similarly acting serotonergic drugs are being suggested as potential long-term treatments for human obesity (Lawton et al., 1995). Reduced animals' body weights in the present study is in agreement with several number of researchers who have reported that chronic intake of FLX decreases food intake and body weight in both experimental animals and human subjects (Luo and Li, 1989; McGurik et al., 1992; Heisler et al., 1997). Substantial evidence has strongly supporting a relationship between serotonin [5-hydroxytryptamine (5-HT)] and food intake. Evidence for this relationship has come from research demonstrating that pharmacological agents that increase levels of 5-HT in the central nervous system (CNS) suppress food intake (Leibowitz et al., 1993; Kitchener and Dourish, 1994). FLX significantly decreased protein and fat intakes in a dose-related manner with less pronounced effect on carbohydrate intake (Heisler et al., 1997).

Common adverse metabolic effects associated with use of many psychotropic drugs including distribution of thyroid homeostasis (Bhuvaneswar et al., 2009). In the present work, FLX treated animals
exhibited increase in thyroids’ weights. Similarly, rats administered clomipramine, a potent SSRI, showed enlargement of the thyroid gland (Beyssen et al., 1999). Acute and chronic intraperitoneal injection of 10-mg FLX in rats has been shown to cause significant toxicity to the hypothalamic–pituitary–thyroid axis resulting in higher concentration of thyrotropin-releasing hormone, which stimulates the liberation of TSH from the anterior pituitary (Golstein et al., 1983). Chronic elevations of plasma TSH induce hyperplasia and hypertrophy of thyroid follicular cells and enlargement of blood capillaries and hence contributing to augmentation of thyroid weight (Connors et al., 1988). Moreover, FLX may enhance urinary iodine excretion like clomipramine (Beyssen et al., 1999) as well as impaired iodine uptake by thyroid follicular cell like other antidepressant drugs (Rousseau et al., 1996) with subsequent formation of iodine deficiency state that leading to enlargement of thyroid volume and contributing to increased thyroid gland weight.

Apart from the well-known relationship between depression and hypothalamic-pituitary-thyroid axis, and the impact of SSRI on thyroid indices, hypothyroidism is reported in ~1% of patients receiving FLX (Sauvage et al., 1998). Wilton et al. (1998) reported congenital hypothyroidism in full term born to mother exposed to FLX during pregnancy. Long term treatment with sertraline (Harel et al., 1995) and paroxetine (Takahashi et al., 2007) had been reported to induce hypothyroidism.

Some drugs can cause alterations in the concentration of thyroid hormones in blood even without clinical signs of dysfunction or pathology of the thyroid gland (Gittoes and Franklyn, 1995). Alterations in thyroid indices have also been reported during SSRI treatment (Eker et al., 2008). In the present work, long-term administration of FLX resulted in disruption of thyroid hormones homeostasis as manifested by decreased serum thyroid hormones (T3 and T4) and increased TSH, hence developed a state of underactive thyroid in treated animals. Similarly, male rats treated chronically with FLX exhibited significant decrease in T3 and T4 serum levels in concomitant with elevation of serum TSH hormonal level as mentioned by Golstein et al. (1983). In addition, male rats injected intraperitoneally with 10 mg/kg FLX for 2 weeks displayed significant reduction in their serum T3 and T4 hormonal levels (Gutiérrez et al., 2002).

A wide variety of different agents, including therapeutic compounds, which have the capacity to inhibit synthesis of thyroid hormones, are capable of inducing thyroid hyperplasia and sometimes neoplasia following prolonged treatment of laboratory rodents. These effects occur when the treatment reduces thyroid hormones to subnormal levels and the resultant increase in circulating TSH stimulates enlargement of the thyroid gland. Agents include excess iodine, iodine deficiency, antithyroid thionamides, sulphonylureas, sulphonamides, and drugs such as phenylbutazone, iodopyrine (iodine and antipyrine), ethionamide, and lithium are responsible for development of thyroid hyperplasia (Ingbar, 1985).

In the current work, FLX exerted antithyroid activity with subsequent development of different morphological changes in thyroid glands of treated rats. Histomorphological changes similar to those seen in the present study have been reported in experimental animals exposed to antithyroid drugs or iodine disrupting agents. Takayama et al. (1986) showed that the antithyroid drugs, propylthiouracil and sulfamonomethoxine, induced reduction in T3 and T4 levels, decreased incorporation of iodine into thyroid precursors, elevation of circulating TSH, and increased thyroid weight and diffuse follicular hyperplasia in rats through inhibition of thyroid peroxidase. Furthermore, Gerber et al. (1994) reported morphological changes in female rats’ thyroids, which they referred to as microfollicular goiter with closed follicles, increased vascularity and a reduction in colloid volume, following inhibition of iodine organification by methimazole (antithyroid drug). Perchlorate (an inhibitor of iodide uptake by the thyroid) toxicity in the rat consisted of increased absolute and relative thyroid weights, decreased T3 and T4 levels, increased TSH levels, and thyroid histopathological changes characterized by
follicular cell hypertrophy with microfollicle formation, taller epithelium lining with a marked reduction in lumen size, and colloid depletion (Siglin et al., 2000). Correspondingly, Kanno et al. (1992) reported that dietary iodine deficiency in male rats produced diffuse thyroid hyperplasia, characterized by small follicles with tall epithelium and reduced colloid, together with a decrease in T₄ and an increase in TSH.

The exact mechanism of this thyroid disorder is not clear. However, these effects may arise from interaction of FLX with different steps of thyroid hormone biosynthesis. Generally, antidepressant drugs can induce a change in iodine capture by thyroid cells or can complex iodine, making it unavailable for thyroid hormone synthesis and thus decreasing thyroid hormone blood levels; they can also inhibit thyroid peroxidase activity and thus T₃ and T₄ synthesis or enhance deiodination of T₄ to T₃ by stimulation of deiodinase activity. Additionally, FLX may interfere with the hypothalamic–pituitary–thyroid axis via the serotonergic system and therefore decrease T₃ blood level (Sauvage et al., 1998). Moreover, thyroidal effects of FLX are not mediated by a local increase of 5-HT but the drug itself may exert a direct suppressive action on the thyroid T₄ output. The increase in TSH reserve is in favor of a feedback-mediated stimulation of TSH synthesis and secretion secondary to dramatic drop of T₄ levels (Golstein et al., 1983). Furthermore, this adverse effect on thyroid hormones might represent an attempt to compensate for the reduced energy intake showed by these rats, to reduce its impact on body-weight gain (Gutiérrez et al., 2002).

The SSRI drugs have been associated with male and female sexual side effects. Evidence also exists indicating a link between FLX treatment and sexual dysfunction (Shen and Hsu, 1995). Chronic administration of 200 mg/kg/day of FLX to male rats caused considerable decrease in their reproductive organs weights (testes, epididymides, ventral prostate and seminal vesicle) as reported by Bataineh and Daradka (2007). Furthermore, a 3-month oral toxicity study of FLX in mice at daily doses of up to 31 mg/kg in the diet induced decrease in absolute and relative testicular weights. Also, in a 1-year oral toxicity study of 20 mg/kg FLX in beagle dogs was associated with reduction in absolute and relative testis weights by 26 and 33%, respectively (Shelby, 2004). These findings are in line with the current work.

Testosterone, qualitatively and quantitatively, is the most important androgen secreted by the testis. It is responsible for the development of male characteristics during fetal life and during puberty and for the maintenance of virility in adulthood (Unnikrishnan et al., 2009). Hence, circulatory androgens maintain the structural and functional integrity of reproductive organs and any change or alteration in the androgen level results in general decrease in testicular and epididymal weights (Brooks, 1983; Oko and Hrudka, 1984). Decreased circulating testosterone was observed in human cases and experimental animals treated with different serotonergic substances (Ayala, 2009). Moreover, marked apoptotic cell death in the rat epididymis following androgen depletion has been observed by Cheuk et al. (2002).

Alterations in seminal quality and quantity secondary to exposure to various SSRIs antidepressant drugs were documented in human and experimental studies. However, few studies have evaluated the potential impact of antidepressant medications on male fertility (Hendrick et al., 2000). A significantly reduction in sperm motility and density from cauda epididymides and testes of FLX treated rats were documented by Bataineh and Daradka (2007). Experimentally depressed animals treated intraperitoneally with 20 mg/ kg FLX for 14 days significantly restored their sperm count almost to the control value, while in higher dose of 80 mg/kg FLX the sperm count was significantly decreased indicating dose dependent effect of the drug (Unnikrishnan et al., 2009). Additionally, citalopram and sertraline SSRI antidepressant drugs were associated with changes in semen parameters in two cases as evidenced by oligospermia, impaired motility, and increased abnormal sperm morphology (Tanrikut and Schlegel, 2007).

Although, the precise mechanism by which FLX causes spermatotoxicity has not
been fully established, generally, spermatocytes damage may occur by one of three mechanisms: physiological, cytotoxic and genetic (Lister and McLean, 1997). The SSRI can impair semen quality and induced abnormal sperm DNA fragmentation with significant increase in the amount of denatured single strand DNA in total cellular DNA was suggested by Safarinejad (2008a). This genetic alteration may in turn disrupt the process of differentiation of spermatocytes and subsequently increases sperm morphological abnormalities (Lister and McLean, 1997). Also, serotonin and its precursor 5-HT are recognized as stimuli for prolactin release by pituitary lactotrophs. In rats (Pinilla et al., 2001) and in humans (Bobo and Shelton, 2009) FLX may potentiate elevation of prolactin levels. There is an association between hyperprolactinemia and reproductive dysfunction. It is possible that elevated prolactin levels contribute to the decline of testicular function. Males may suffer hypogonadism, sexual dysfunction, decreased sperm production, and infertility (Serri et al., 2003). Moreover, seminal changes may be due to the effects of FLX on the enzymes of oxidative phosphorylation (Bataineh and Daradka, 2007).

Although the SSRI have a generally more favorable side effect profile than the older classes of antidepressants, sexual dysfunction is a frequent and disturbing adverse event that influences a patient’s desire to continue long-term antidepressant treatment (Bull et al., 2002). In the present study, serum testosterone as well as gonadotropins levels were decreased significantly in FLX treated rats. In accordance with this the study done by Bataineh and Daradka (2007) who observed depression of circulating testosterone and FSH levels in male rats injected with FLX. Also, the serum levels of FSH were lower by 15% in animals treated intraperitoneally with 5 and 10 mg/kg FLX (Silva et al., 2008). Male patients taking SSRI experienced significant reduction in their serum levels of LH, FSH, and testosterone have been demonstrated by Safarinejad (2008b).

The fall in circulating testosterone level in human (Safarinejad, 2008b) and animal (Unnikrishnan et al., 2009) males, after exposure to FLX, appears to be mediated through disturbances of their hypothalamic-pituitary-testicular axis. Changes include increased serotonin, decreased dopamine, blockade of cholinergic and alpha-1 adrenergic receptors, inhibition of nitric oxide synthase, and elevation of prolactin levels (Rosen et al., 1999; Keltner et al., 2002). The control of gonadotropin-releasing hormone (GnRH) secretion depends on several neurotransmitters, such as 5-HT, noradrenaline, dopamine, and nitric oxide. It has been previously reported that 5-HT in the medial basal hypothalamus inhibits LH secretion (Johnson et al., 1996). Hyperprolactinemia is an undesirable effect of SSRI use and can cause inhibition of GnRH release from hypothalamus with subsequent decreases in circulating testosterone levels (Misra et al., 2004; Romeo and Ybarra, 2007).

The current work flourished different histomorphological changes in rats' testicular tissues due to FLX exposure. These findings are similar to other several previous experimental studies in animals. Animals treated with 20 mg/kg and 80 mg/kg doses of FLX showed histoarchitecture alterations of testicular tissues in the form of atrophy and degeneration of SNT, lack of spermatogenesis inside the tubules, and shrinkage tubules with wide intertubular space (Unnikrishnan et al., 2009). Moreover, chronic injection of FLX in adult rats had testicular damage as demonstrated by a considerable decrease in the number of both primary and secondary spermatocystes and spermatids. Also, the fibroblast, immature and mature Leydig cells numbers were significantly decreased. However, the degenerating cells number was greatly increased (Bataineh and Daradka, 2007). Furthermore, a reduction between 30 and 32% in the number of Sertoli cells per testis was observed in the animals treated with 20 mg/kg FLX. Likewise, biometric parameters, such as volume of the testis, volume of sex cords/semiferous tubule, semiferous epithelium and total length of seminiferous tubules that possess a direct correlation with the number of Sertoli cells were reduced in FLX treated animals (Silva et al., 2008).
Various complex hormonal, central, and peripheral nervous system, and neurochemical changes occur during SSRI usage that could account for their testicular toxicity. Low serum LH, FSH, and testosterone may contribute to but unlikely to be the sole possible mechanisms of FLX-induced testicular toxicity (Safarinejad, 2008b).

In the testis, the 5-HT can reduce the steroidogenesis and the spermatogenesis by decreasing the intratesticular flow due to arterial constriction or inhibiting fundamental enzymes of the steroidogenesis (Das et al., 1986; Hedger et al., 1995). Also, Silva et al. (2008) stated that the increase of the serotonin levels provoked by the treatment with SSRI inhibited the proliferation of the testicular cells and caused retardation in testicular development. The activation of 5-HT2 receptors in the Leydig cells by serotonin provokes a cascade of autocrine events that promote the intratesticular increase of corticotrophin releasing hormone and β-endorphin. The latter, acts as paracrine modulator of the proliferative response of the Sertoli cells to FSH through the inhibition of the connection of the FSH receptor with the adenylate cyclase (Orth and Boehm, 1990; Dufau et al., 1993).

On the other hand, the reduction of testicular cellular components could be a consequence of malnutrition caused by several factors. Accumulation of serotonin at the synaptic cleft of alimentary tract decreases intestinal absorption and provoking a reduction of essential nutrients, which may adversely, affects testicular function (Simansky, 1996). Treatment of animals with FLX induced hypoglycaemia, hypercholesteremia, reduction in testicular protein, and testicular cholesterol as mentioned by Unnikrishnan et al. (2009). Glucose is an essential material for Leydig cell steroidogenesis and its reduction may contribute in development of malfunctioning testis (Chen et al., 2003). Since sugars are the main fuel of the central nervous system activity, hypoglycaemic element together with FLX itself may interfere with CNS functions and result in a stressful condition with subsequent increase in secretion of glucocorticosteroid, which affect the adrenocorticotropin (ACTH) secretion as well as the Pituitary–Adrenal–Axis (Unnikrishnan et al., 2009).

Yamamoto et al. (1999) had shown that hypercholesteremia has a detrimental effect on Leydig and sertoli cells secretory function, spermatogenesis, epididymal sperm maturation process and the overall sperm fertilizing capacity. Also, lack of cholesterol at Leydig cells is associated with decrease of steroidogenesis (Boujrad et al., 2000).

Conversely, treatment of male rats with 0.75 mg/kg body weight/day FLX by intraperitoneal injection for 4-week had no effect on relative weights of testes, epididymides, and seminal vesicles as well as testosterone levels were also unaffected (Taylor et al., 1996). In addition, there were large differences with both increases and decreases in testosterone levels in some individuals' after FLX treatment (Bell et al., 2006). These findings disagree with the present work due to difference in dosage, duration of the study, and species.

In summary, long-term administration of FLX for 60 days to adult rats was associated with detrimental effects on the thyroid and testicular functions and morphology as evidenced by disturbance of their hormonal homeostasis and seminal quality accompanied with alterations of their normal architectures. These data suggest that FLX may interfere with male reproductive function both centrally and peripherally with subsequent development of hypogonadism and impairment of fertility. Hence, prolonged exposure to FLX may have the potential to negatively affect the human reproductive system which reinforces the need for periodical counseling to monitor the patients' fertility indices.

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دراسة هرمونية وهستوباثولوجية للعدد الدرقية وخصى الجرذان البيضاء البالغة بعد التناول طويل الأمد للفلوكسيتين هيدروكلوريد

قسمى الأنسجة والخلايا والطب الشرعي والسموم الإكلينيكية

كلية طب بنها – جامعة بنها

المعطى العربي

عقار البروزاك (فلوكسيتين هيدروكلوريد) ينطوي انتقائيًا لدخول السيروتونين مرة أخرى للنهايات العصبية بالمخ، يستخدم على نطاق واسع في علاج الأكتان والاضطرابات العصبية الأخرى منذ عام 1987. وعلى الرغم من أنة منع عموماً وفعال، ويمكن التعود عليه، ومع ذلك، تم نشر العديد من الآثار السلبية في المقالات. هذا الدواء قد يكون مسؤولاً عن حدوث خلل في الوظيفية الجنسية بين الذكور وسامة الجهاز التناسلي التي يمكن أن تغير توزوان الهرمونات الجنسية الأساسية، والخصوبة، والتكوينات المنوية. لذا، تم تصميم هذا العمل لتقييم التأثيرات السامة لعقار الفلوكسيتين على وظائف وتركيبات العدد الدرقي وكذلك الأعضاء التناسليات (الخصيتين والبربخ) في الجرذان. أجريت هذه الدراسة على 20 من ذكور الجرذان البيضاء البالغة، التي قسمت بالتساوي إلى مجموعتين (10 جرذان لكل مجموعة). كل مجموعة تم إعطائها المادة المختصرة لها بجرعة واحدة/يوم بواسطة نانوب داخل المعدة لمدة 10 يوماً متتالية كالأتي:

الجموعة الأولى (الurette البداية): تم إعطاء كل فار صام 5 مل مم من الماء المغلف ( مجموعة الفلوكسيتين): تم إعطاء كل فار صام 15 مل مغلف من الفلوكسيتين في 2 مل مم من الماء المغلف في نهاية فترة التجربة وبعد تخدير الجرذان، تم أخذ عينات الدم لقياس مستويات الهرمونات، وحد ذلك تم ذبح الجرذان وجمع العدد الدرقي، الخصيتين، البربخ لفحص أشهرهم. وقد أظهرت النتائج التي تم الحصول عليها من مجموعة الفلوكسيتين وجود اختلافات ذات دلالة إحصائية عند مقارنتها بنتائج المجموعة الضابطة ممثلة على النحو التالي: انخفاض في الأوزان النهائية للحيوانات، زيادة في الأوزان الطبيعية والنسبية للعدد الدرقي، نقصان في الأوزان الطبيعية والنسبية للخصيتين والبربخ؛ اضطرابات بالمعايير المختلفة لعوامل السائل المنوي كما إلتح في خفض من انخفاض في كل من عدد الحيوانات المنوية، الحركة، والأشكال الطبيعية للحيوانات المنوية متلازمة مع زيادة نسبة الحيوانات المنوية غير طبيعية؛ وانخفاضات في تحليل الهرمونات بالأعمال في صورة زيادة بنسبة الهورمون المنفي للأجهد مصحوبة بنقصان في مستويات ثلاثي أثيوكتبين، فيتروسين، هرمونات تحرير الغدة الدرقية، هرمون النسائي الثانوي الكلي، وهرمون متواتر. علاوة على ذلك، أظهرت فحص أسماء العدد الدرقي والخصي وجود العديد من التغييرات الهستوباثولوجية مما يدعم النتائج الهرمونية. هذه البيانات تشير إلى أن الفلوكسيتين باعثر ضارة على الوظائف والتركيبات الهستوباثولوجية للجهاز التناسلي في الذكور من خلال العمل مرتكزًا على العدد الدرقي وخارجيًا على نسبة الخصية مما يؤدي إلى نشوء قصور بالعدد التناسلي وخلال في تكون الحيوانات المنوية. وبالتالي، تناول الفلوكسيتين على مدى طويل ينطوي على تأثيرات سلبية على الجهاز التناسلي في الإنسان يعزز الحاجة لتقديم مشورة دورية للمرضى بمتاعبة مؤشرات الخصوبة.