Effects of paroxetine on biochemical parameters and reproductive function in male rats

Rachid Mosbah\textsuperscript{1,2}, Aziez Chettoum\textsuperscript{*3}, Alberto Mantovani\textsuperscript{4}

\textsuperscript{1}Department of Biology, Faculty of Sciences, University of Boumerdes - 35000 Algeria
\textsuperscript{2}Department of Biology, Laboratory of Eco-biology, ENS-Kouba - 16000, Algiers, Algeria
\textsuperscript{3}Department of Biology Animal University of Mentouri- Constantine – 25002, Algeria
\textsuperscript{4}Department of Veterinary Public Health and Food Safety, Istituto Superiore di Sanita, Rome - 124, Italy

Abstract

Selective serotonin reuptake inhibitors (SSRI) are a class of molecules used in treating depression, anxiety, and mood disorders. Paroxetine (PRT) is one of the most prescribed antidepressants, which has attracted great attention regarding its side effects in recent years. This study was planned to assess the adverse effects of paroxetine on the biochemical parameters and reproductive system. Fourteen male Wistar rats were randomly allocated into two groups (7 rats for each): control and treated with paroxetine at a dose of 5mg/kg.bw for two weeks. At the end of the experiment, blood was collected from the retro-orbital plexus for measuring the biochemical parameters, whereas the reproductive organs were removed for measuring semen quality and the histological investigations. Results showed that paroxetine induced significant changes in some biochemical parameters and alteration of semen quality, including sperm count, spermatids number and sperm viability, motility and abnormalities. The histopathological examinations of testis and epididymis revealed an alteration of spermatogenesis, cellular disorganization and vacuolization, enlargement of interstitial space, shrinkage and degenerative changes in the epithelium of seminiferous and epididymal tubules with few to nil numbers of spermatozoa in their lumen. In conclusion, paroxetine treatment caused changes in some biochemical parameters and sperm profiles as well as histopathologic effects of reproductive organs.

INTRODUCTION

Mental disorders are serious health problems, which affect a person's mind and body. It has become permanent increases in the last decades over the world and requires social and medical care using different types of antipsychotics (conventional and atypical) and antidepressants drugs. These groups were classified according to their mode of action as follow: tricyclic antidepressants (TCAs), serotonin reuptake inhibitors (SRI), selective SRI (SSRIs), norepinephrine reuptake inhibitors (NRI), selective NRI (SNRI), norepinephrine and dopamine reuptake inhibitors (NDRI), monoamine oxidase inhibitors...
Selective serotonin reuptake inhibitors (SSRI) are a class of pharmacological molecules belonging to the third generation subgroup of antidepressants used worldwide since 1980 as an alternative of tricyclic agents in treating depression, anxiety, and post-traumatic stress, obsessive-compulsive, comport mental and mood disorders (Chigome et al., 2017). SSRI, including paroxetine (Paxil), sertraline (Zoloft), fluoxetine (Prozac), fluvoxamine (Luvox), escitalopram (Lexapro), Nefazodone, Trazodone, Vilazodone (Viibryd) and citalopram (Celexa), act as blockers of serotonin reuptake transporter (SERT) in the cerebral region and as a result, serotonin (5-hydroxytryptamine, 5-HT) accumulate in the synaptic cleft causing over-activation of postsynaptic receptors leading to the development of serotonin syndrome (Warner et al., 2017). In the other hand, the increase in serotonin level may induce adaptive changes in both serotonergic and noradrenergic neurotransmission, partial or complete blockade of neuromuscular transmission and subsequently alleviate depression symptoms. They also have less effect on histaminic, muscarinic, dopaminergic and noradrenergic receptors when compared with other antidepressants (Siepmann et al., 2015). Besides its benefits role in the treatment of psychotic disorders, SSRIs can cause several side effects viz. nausea, anxiety, insomnia, dry mouth, headache, somnolence, dizziness, agitation, anorexia, diarrhoea, constipation, tremor, sweating and sexual dysfunction. The main reproductive effects of SSRIs are reduced sexual desire (libido) in both sex, erectile dysfunction and increased ejaculation latency in men, genital anesthesis, anorgasmia and lack of vaginal lubrication in women (Bala et al., 2018). Previous clinical and experimental studies have been reported negative effects of SSRIs use on reproductive function. It also confirms the role of serotonin in the regulation of the spermatogenesis process and the genital tract integrity by the presence of its receptors in the reproductive organs, including vas deferens (contraction), epididymis (sperm maturation), testis (blood flow), Sertoli and Leydig cells (synthesis of testosterone), and sperm cells (spermatogenesis regulation) (Erdemir et al., 2014).

Paroxetine is one of the most prescribed second-generation SSRIs group introduced in 1992 by Glaxo Smith Kline company to treat depression and anxiety disorders. It is well absorbed by the gastrointestinal tract, distributed throughout the body, including the central nervous system and then metabolized in the liver by the enzyme cytochrome P450 D6 (CYP2D6) to paroxetine catechol intermediate (transformed to glucuro-sulfonate conjugate) and inactive polar metabolites, which excreted by the urine (64%) and faeces (36%). Concerning its side effects, to the best of our knowledge, there is a few published data on its adverse effects on various organ systems; herein, in the last decade, growing attention was focused on reproductive dysfunction and infertility. It is reported that paroxetine could decrease penile erection and libido, delay or abolishes orgasm by inhibiting nitric oxide production, diminishing dopaminergic activity and increasing norepinephrine levels, and subsequently affects fertility during and/or after treatment (Santana et al., 2019). Since paroxetine effects on spermatogenesis and semen quality are still unclear according to several clinical and experimental studies, therefore this study was planned to investigate its effects on the biochemical and reproductive parameters in male rats.

MATERIALS AND METHODS

Chemicals

Paroxetine (PRT) is a trans-4R-(4′-fluorophenyl)-3S-[3′,4′-methyleneoxyphenoxy)methyl]piperidine hydrochloride hemihydrate salt with CAS number 61869-08-7, the molecular weight of 374.8, chemical formula C19H20FNO3 and chemical structure as shown in Figure 1. It is a white crystalline powder with a melting point range of 120°C to 138°C and solubility of 5.4 mg/mL in water. Paroxetine was purchased from a local pharmacy at Boumerdes city as tablets form, dissolved in water and administered orally to animals at the dose of 5 mg/kg for two weeks of treatment.

Figure 1: Chemical structure of paroxetine

Animals and experimental design

Fourteen adult male wistar rats (260-300g) were purchased from the national institute of Pasteur Kouba- Algiers, Algeria, acclimatized for one week before the beginning of the experiment and used in

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this study. They have divided into two groups and maintained individually on a standard pellet diet and tap water ad libitum under controlled room temperature (24 ± 3°C), relative humidity (60 ± 10%) and 12h light/dark cycle. The first group served as control, and the second group received by gavage the antidepressant drug paroxetine at the dose of 5 mg/kg bw for two weeks. The animals were handled according to the standard guide for the care and use of laboratory animals.

**Body and organs weights**

The body weight of rats was measured daily during the experimental period, and the body weight gain was calculated from the difference between the final and the initial body weight. At the end of the treatment, the animals were anesthetized, sacrificed and the reproductive organs (testes, epididymis) and liver were removed, cleared from the attached tissues and weighed by analytical balance. The testes and epididymis were used for further histological investigation and semen analysis.

**Blood sampling and biochemical analysis**

Before the sacrifice of animals, light anesthesia was applied, and the blood samples were collected from the retro-orbital eye sinus, centrifuged at 3000 rpm for 15 min, and serum was taken and stored at - 20°C until use. These biochemical parameters (glucose (Glu), cholesterol (Chl), triglyceride (TG), High-Density Lipoprotein (HDL), Light Density Lipoprotein (LDL), Aspartate Aminotransferase (ASAT), Alanine Aminotransferase (ALAT), urea, creatinine (Crea), uric acid (UrAc) were measured using routine spectrophotometric methods.

**Semen analysis**

**Spermatids number and sperm count**

For counting spermatids number (StN) and sperm count (SC), the right testis and the right caudal epididymis were weighed, cut into small pieces with scissors and homogenized in 10 ml of 0.9% sodium chloride (NaCl) solution containing 0.5% Triton-X100. The homogenate of each rat was mixed gently, and a second dilution (1ml, 1:9 v/v) was done in an eppendorf tube. One drop of each suspension of testis and cauda epididymis was taken separately in the hemocytometer chamber (Malassez), then spermatids number and sperm count were counted microscopically at x 40 magnification according to the methods described by Blazak et al. (1993).

**Sperm motility, viability and morphology analysis**

The left caudal epididymis of each animal was removed as quickly as possible and cut with a scalpel in a warmed petri dish containing 2 ml of Hanks’s solution and maintained at 37°C for 15 min to obtain sperm suspension. 20 μl of the suspension was applied between a warmed microscope slide and coverslip then examined by light microscope at x40 magnification to assess the percentage of motile spermatozoa in at least 10 separate and randomly selected fields. For sperm viability and morphology measurement, the same spermatozoa suspension was allowed to dry in the air, then stained with 1% eosin Y/5% nigrosin, and at least 200 spermatozoa for each sample were examined at x40 magnification (Liobet et al., 1988).

**Histopathological investigations**

Small pieces of the left testis and epididymis were fixed in 10% buffered formaldehyde solution for one day, embedded in paraffin, sectioned to 5 μm thicknesses, mounted on a microscope slide and stained with hematoxylin-eosin (HE) according to the routine histological preparation. All of the tissue sections were examined by light microscope at x40 magnification and photographed by Sony camera 7.1 Pixel to reveal the possible histopathological changes.

**Statistical analysis**

All values were expressed as mean ± standard error of the mean (SEM) and analyzed by SPSS (version 11.0) software for Windows. Comparison between groups was made by applying student t-test, and p < 0.05 was considered statistically significant.

**RESULTS**

**Body and organ weights, food intake and water consumption**

The results for these parameters are summarized in Table 1, and it appears clearly that paroxetine treatment induced a significant reduction in the body weight gain (P < 0.05), food intake (P < 0.01) and water consumption (P < 0.05), respectively as compared with the control group, whereas, no changes were observed in the weight of vital (liver, kidney, adrenal, cardiac and cerebral) and reproductive organs (testes and epididymis).

**Biochemical parameters**

The results of the biochemical analysis are presented in Table 2. As compared to controls, Paroxetine treated rats showed a significant increase in the biomarkers of kidney function, including urea (P < 0.05), Crea (P < 0.001) and UrAc (P < 0.01). In contrast, no change has been reported in serum biochemical parameters (Glu, TG, Chl, HDL, LDL) and liver enzymes (ASAT and ALAT).
Table 1: The effects of during and after 2 weeks of treatment

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Control</th>
<th>PRT(5mg/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Initial body weight</td>
<td>289.1 ± 12.76</td>
<td>266.2 ± 3.87</td>
</tr>
<tr>
<td>Final body weight</td>
<td>302.2 ± 9.72</td>
<td>267.8 ± 8.52</td>
</tr>
<tr>
<td>BWG</td>
<td>12.14 ± 3.89</td>
<td>-3.25 ± 1.94*</td>
</tr>
<tr>
<td>LW</td>
<td>9.36 ± 1.05</td>
<td>10.16 ± 0.71</td>
</tr>
<tr>
<td>KW</td>
<td>0.91 ± 0.17</td>
<td>0.89 ± 0.13</td>
</tr>
<tr>
<td>AW</td>
<td>0.048 ± 0.01</td>
<td>0.048 ± 0.01</td>
</tr>
<tr>
<td>CW</td>
<td>0.84 ± 0.15</td>
<td>0.91 ± 0.32</td>
</tr>
<tr>
<td>CrW</td>
<td>1.89 ± 0.19</td>
<td>1.68 ± 0.19</td>
</tr>
<tr>
<td>TW</td>
<td>1.58 ± 0.33</td>
<td>1.53 ± 0.02</td>
</tr>
<tr>
<td>EW</td>
<td>0.61 ± 0.13</td>
<td>0.58 ± 0.02</td>
</tr>
<tr>
<td>FI</td>
<td>27.18 ± 0.91</td>
<td>21.42 ± 1.96**</td>
</tr>
<tr>
<td>WC</td>
<td>29.29 ± 0.80</td>
<td>25.90 ± 1.71*</td>
</tr>
</tbody>
</table>

PRT= Paroxetine, BWG=body weight gain, LW=weights of liver, KW=kidney weight, AW=adrenal weight, CW=cardiac weight, CrW= cerebral weights, TW=testes weight, EW=epididymis, FI=food intake g/day, WC=water consumption ml/day

Table 2: The effects of Paroxetine (PRT) on biochemical parameters, after 2 weeks of treatment

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Control</th>
<th>PRT(5mg/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glu (g/l)</td>
<td>1.33 ± 0.19</td>
<td>1.71 ± 0.40</td>
</tr>
<tr>
<td>TG (g/l)</td>
<td>0.44 ± 0.04</td>
<td>0.5 ± 0.06</td>
</tr>
<tr>
<td>Chl (g/l)</td>
<td>0.73 ± 0.06</td>
<td>0.64 ± 0.06</td>
</tr>
<tr>
<td>HDL (g/l)</td>
<td>1.79 ± 0.36</td>
<td>1.77 ± 0.41</td>
</tr>
<tr>
<td>LDL (g/l)</td>
<td>1.38 ± 0.31</td>
<td>1.22 ± 0.38</td>
</tr>
<tr>
<td>Urea (g/l)</td>
<td>0.43 ± 0.03</td>
<td>0.6 ± 0.07*</td>
</tr>
<tr>
<td>Crea (mg/l)</td>
<td>3.57 ± 0.37</td>
<td>6.57 ± 0.48***</td>
</tr>
<tr>
<td>UrAc (mg/dl)</td>
<td>0.5 ± 0.05</td>
<td>1.74 ± 0.30**</td>
</tr>
<tr>
<td>ASAT (UI/l)</td>
<td>182.34 ± 23.04</td>
<td>200.03 ± 28.38</td>
</tr>
<tr>
<td>ALAT (UI/l)</td>
<td>142.81 ± 15.08</td>
<td>127.91 ± 11.23</td>
</tr>
</tbody>
</table>

Glu = glucose, TG= triglycerides, Chl =cholesterol, HDL= high density lipoprotein, LDL= low density lipoprotein, urea, Crea = creatinine, UrAc = acid uric, ASAT= aspartate aminotransferase, ALAT=alanine aminotransferase

Table 3: The effect of paroxetine on the sperm characteristics and spermocytogram profile after 2 weeks of treatment

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Control</th>
<th>PRT(5mg/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>SC</td>
<td>249.43 ± 12.69</td>
<td>130.57 ± 6.39***</td>
</tr>
<tr>
<td>StN</td>
<td>159.29 ± 9.47</td>
<td>62.14 ± 3.46***</td>
</tr>
<tr>
<td>Motility</td>
<td>79.29 ± 2.47</td>
<td>51.00 ± 4.49***</td>
</tr>
<tr>
<td>Viability</td>
<td>70.00 ± 0.97</td>
<td>49.23 ± 4.40***</td>
</tr>
<tr>
<td>Abnormalities</td>
<td>4.94 ± 0.71</td>
<td>61.00 ± 2.56***</td>
</tr>
<tr>
<td>STD</td>
<td>264.23 ± 10.24</td>
<td>227.05 ± 4.03***</td>
</tr>
</tbody>
</table>

SC, 10⁶ /g = Spermatids count, StN,10⁶ /g = spermatids number, and sperm motility (%), viability (%), and abnormalities (%), STD, μm = seminiferous tube diameters
Figure 2: Photomicrographs of testis sections of control (A, x100; D, x400) and treated with 5mg/kg of paroxetine (B and C, x100; E, x400) for two weeks

Semen quality

The results of the effect of paroxetine on sperm parameters and seminiferous tube diameters are summarized in Table 3.

Semen characteristics, including sperm count (SC), motility and viability, spermatids number (StN) and seminiferous tube diameters, were significantly declined (P< 0.001) while sperm abnormalities were increased (P< 0.001) in paroxetine treated rats as compared with the control group.

Histopathological investigations

As summarized in Figure 2 and Figure 3, the histopathological examinations of testis and epididymis revealed normal architectural structure in the control group epididymis whereas, in the paroxetine group, an alteration of spermatogenesis, cellular disorganization and vacuolization enlargement of interstitial space, shrinkage and degenerative changes in the epithelium of seminiferous and epididymal tubules with few to nil numbers of spermatozoa in their lumen.

DISCUSSION

Serotonin (5-HT) is a monoamine neurotransmitter secreted from the neurons of the raphe nucleus, which connect with different regions in the brain and spinal cord by their projections. Serotonin from these projections and in outside CNS plays a central role in the regulation of several functions, including mood, anxiety, panic, memory, wake/sleep cycle, appetite, digestion and sexual behaviors, motor activity and gastrointestinal function (Berger et al., 2009). The SSRI are a group of antidepressant agents which act as blockers of reuptake serotonin into presynaptic neurons (Nash and Nutt, 2007). Paroxetine is one of the SSRIs highly prescribed due to its efficacy in the treatment of depression and anxiety symptoms, but its side-effect profiles on some organs and systems are remaining in controversy.

In this study, paroxetine treatment at a dose of 5mg/kg for two weeks caused a reduction in the body weight gain, food intake and water consumption. Consistent with our results, previous animal studies were reported that chronic paroxetine treatment caused a decrease in body weight and food intake.
This effect may be due to the loss of appetite by the antidepressant medication Amodeo et al. (2015).

In addition, in paroxetine exposed rats, the level of kidney function biomarkers (urea, uric acid and creatinine) were increased significantly (at least p < 0.05), whereas no significant differences were detected in the level of biochemical parameters (TG, Chl, HDL, LDL) and liver function biomarkers (ASAT, ALAT). Limited data were published on the effect of paroxetine on the biochemical parameters, liver and kidney functions. In accordance with our findings Yakubu and Jomih (2014) have reported increases in the levels of serum urea, uric acid and creatinine after the administration of paroxetine at the dose of 10mg/kg for 5 and 21 days. So, significant increases in serum ASAT and ALAT were observed by Elmelegy and Kamal (2013) when the rats had received paroxetine intraperitoneally (1mg/kg) and/or orally (10 mg/kg) for 21 days Yakubu and Jomih (2014) have shown in the testes a reduction in the level of proteins, glycogen, and cholesterol as well in the activities of alkaline phosphatase, acid phosphatase, lactate dehydrogenase and gamma-glutamyl transferase after the administration of paroxetine at the dose of 10mg/kg for 21 days. These parameters are biomarkers of the normal function of testes as an energy source, maturation substrate and transfer of androgen and consequently regulation of spermatogenesis.

The reproductive system is considered to be the most vulnerable system toward a huge number of chemicals and drugs (Rim, 2017). Several studies have been demonstrated that SSRIs use inhibits nitric oxide synthase (NOS) activity and reproductive hormonal profile synthesis, which plays a major role in stimulating libido, penile erection and orgasm phase and consequently could contribute to male factor infertility (Brezina et al., 2012). So, relative weak researches were done on their adverse effects on sperm quality and reproductive organs histopathology and sometimes showed inconsistent data. Hence, the obtained results in this work revealed that paroxetine altered the semen profile by a decrease in sperm count, spermatids number, sperm viability and motility whereas, sperm abnormalities are increased. In agreement with our find-
ings, (El-Sheikh et al., 2016) showed that paroxetine administration to male rats at a dose of 3.6mg/kg (for 20,30 days) and 10mg/kg (for 60 days) respectively could cause a reduction in the level of FSH, LH and testosterone accompanied by significant decreases in sperm count, motility, viability and function with an increase in sperm abnormalities. In addition, paroxetine treatment (10, 20mg/kg for five weeks) in humans (35 males aged 18-65yrs) induced abnormal sperm DNA fragmentation but no statistically significant changes in semen volume, sperm concentration, motility and morphology were noted.

Similarly, studies on different SSRIs in animals (fluoxetine, trazodone) and prospective human follow-up (escitalopram) respectively have shown a negative impact on semen quality, including sperm concentration, motility and abnormalities (Ilgin et al., 2018). Recently, related endocrine-disrupting of Hypothalamic-Pituitary – Gonadal (HPG) axis with psychological treatments drug was established. Ilgin et al. (2018) reported an increase in the serum FSH, LH, and testosterone levels by trazodone (5, 10, and 20 mg/kg/day for 28 days). Furthermore, Rasmussen et al. (1991) revealed after two months of administration of sertraline (10mg/kg/day) an increase in FSH level and a decrease in testosterone level, while, in the paroxetine group (10mg/kg/day) only testosterone level was lowered.

Testosterone has a crucial role in spermatogenesis processes and testicular activity in males, so testosterone imbalance can affect sperm quality and characteristics, as observed in paroxetine treatment in our study. The mechanism by which SSRIs underlying its effects (serotonin level) on the reproductive function is a disruption of the HPG axis by increasing serotonin levels in the CNS, which in turn increases prolactin and dopamine levels, and as a result, suppresses gonadotropin-releasing hormone GnRH, FSH, LH and consequently testosterone synthesis because serotonin inhibits LH binding to Leydig cells leading to impaired sperm production and maturation (Schenker et al., 1992).

Moreover, in the current study, the histopathological investigations of testis and epididymis sections revealed an alteration of spermatogenesis, cellular disorganization and vacuolization, enlargement of interstitial space, shrinkage and degenerative changes in the epithelium of seminiferous and epididymal tubules with few to nil numbers of spermatozoa in their lumen after two weeks of paroxetine administration. These changes may be due to an imbalance in HGP hormone profile (GnRH, FSH, LH and testosterone) as explained above (Schenker et al., 1992) and/or the oxidative stress commonly known as reactive oxygen species (ROS), cell apoptosis through extracellular Ca2+ and p38 MAPK-dependent ROS generation result in paroxetine exposure (Cho et al., 2019). Many clinical and experimental data suggest a relationship between the alteration of the reproductive tissue damage, semen quality, DNA integrity damage, cell apoptosis and generation of free radical (ROS) under paroxetine treatment (Cho et al., 2019). Support to the histological alterations in our study, Aggarwal et al. showed atrophy of seminiferous tubules and thickness of its germinal epithelium with a decrease in the number of germinal, Sertoli and Leydig cells in rats exposed to fluoxetine at the dose of 10mg/kg for two weeks. Similar findings were reported by Atli et al. (2017) using setraline at doses of 10, 20mg/kg for 28 days. Irreversible lesions occurred in the reproductive tract of male rats after dosing in toxicity studies for 2 to 52 weeks. These lesions consisted of vacuolation of the epididymal tubular epithelium at 50 mg/kg/day and atrophic changes in the seminiferous tubules of the testes with arrested spermatogenesis at 25 mg/kg/day (FDA, 2017).

CONCLUSION
By the end of this study, we conclude that paroxetine can affect renal function and impaired fertility by an alteration in kidney biomarkers, sperm parameters and histological changes in testis and epididymis; these effects maybe result in the negative impact of serotonin on HPG hormone profile.

Conflict of Interest
The authors declare that they have no conflict of interest.

Funding Support
The authors declare that they have no funding support for this study.

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