Effects of Sertraline on Spermatogenesis of Male Rats and its Reversibility after Terminating the Drug

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Purpose: The purpose of this research was to study the effects of Sertraline on spermatogenesis of male rats and whether these probable effects are constant or provisional after terminating the drug.

Materials and Methods: In this study, 32 two-month old male Wistar albino rats were equally divided into the Sertraline-treated and the control groups. The drug group was gavaged with Sertraline daily while the control group was gavaged with water at the same volume. After 80 days, half of the rats in each group were selected randomly for hormonal evaluations and bilateral orchiectomy. Histological and hormonal evaluations were performed. The remaining half of rats were kept alive for 90 more days without intervention and then underwent hormonal evaluation and bilateral orchiectomy in a similar fashion.

Results: There was no difference between the testes histology and pathology of the sertraline-treated and the control groups. There was a significant decrease in serum FSH in the Sertraline-treated group compared to the control group ($P < 0.05$). However, this decline appeared to be reversible following termination of exposure to Sertraline. FSH returned to pretreatment levels in the remaining treated rats following 90 days of treatment cessation.

Conclusion: Within the time-frame studied, Sertraline can induce transitory changes in serum FSH of male rats without concomitant spermatogenic changes within the testes. This hormonal change appears to be reversible following withholding of Sertraline. The long-term effect of Sertraline usage on hormonal status and spermatogenesis in rats needs further investigation.

Keywords: sertraline; spermatogenesis; LH; FSH; testosterone; infertility

INTRODUCTION

Infertility is a common problem in the world and in one study conducted by WHO the global rate of primary infertility in 2010 had been reported to be 1.9% and that of secondary infertility as 10.5% [(1)]. Infertility has many socio-economic consequences for infertile couples. About 27% of the causes of infertility have been reported to be attributable to men[(2)]. Side effects of drugs have an important role in infertility. Selective serotonin reuptake inhibitors (SSRIs) are the first line for the treatment of many psychological and some non-psychological disorders. Therefore, the use of these drugs is relatively common[(3)]. There have been published studies about the negative effect of SSRIs, including Sertraline, on spermatogenesis[(3,4)]. However, these studies have failed to investigate the reversibility of spermatogenesis following discontinuation of Sertraline. We conducted this study to evaluate the effect of Sertraline on male rats' spermatogenesis and its reversibility after discontinuing the administration of the drug.

MATERIALS AND METHODS

This study was conducted in the animal facility of the Mashhad Faculty of Medicine from December 2017 to June 2018.

Thirty two adult male Wistar albino rats at approximately 2 months age and 180 to 220 grams weight were selected. The exclusion criteria of our study were any sign of sickness in the rats midst the studding or obvious anomaly in external genital organs of the rats.

These rats at first were kept in 8 cages, 4 per cage. Randomly, 4 cages were labeled as the drug group and 4 as the control group. Each rat in the treatment group received (lavage) 0.5CC (2 mg) of Sertraline solution every day for 80 days. Each rat in the control group was lavaged for 80 days with 0.5 CC of water. The period of drug treatment and cessation was considered to double or triple the reported duration of rats spermatogenesis[(5)] and the treatment phase was 10 days lower than the cessation phase due to practical limitations. All rats were fed regularly with the regimen in a similar fashion.

Following 80 days of exposure, half of the rats were randomly selected and evaluated for hormone levels and the histology/pathology of the testes. Briefly, rats in each cage were numbered from 1 to 4 and 2 were randomly selected for the first phase of the study. Selected

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Table 1. Comparison of the levels of LH, FSH and Testosterone between Sertraline-exposed rats and the control group in different periods of time.

Timing	Hormones	Sertraline Group Median (IQR)[b]	Control Group Median (IQR)	*P*-Value[a]
80th day	LH (mg/dL)	3/30 (3/10-5/20)	3.40 (2/12-4/17)	0.64
	FSH (IU/L)	2/20 (2/00-3/40)	3/70 (2/95-4/45)	0.004
	Testosterone (IU/L)	5/30 (2/70-7/54)	4/99 (3/62-7/43)	0.18
170th day	LH (mg/dL)	3/70 (2.35-5/00)	2/85 (1/90-4/30)	0.34
	FSH (IU/L)	2/90 (2/20-4/07)	3/75 (3/05-4/25)	0.26
	Testosterone (IU/L)	5/40 (2/72-5/67)	3/80 (2/97-5/47)	0.83

Abbreviations: FSH, Follicular stimulating factor; LH, Luteinizing factor; IU, International Unit; L, Liter; IQR, InterQuartile range
a Mann-Whitney test
b Inter Quartile Range (percentile 25, percentile 75)
By comparing the hormonal levels in Sertraline and control group we founded that FSH, LH and testosterone levels all increased in the Sertraline group, but this increase was only significant for FSH ($P < 0.05$). There was no significant difference in LH, FSH and testosterone levels between the 80th day and 170th day in the control group ($P > 0.05$)

rats were removed one by one and exposed to ether to induce deep anesthesia. Each rat's chest was then split by scissors and blood sample was aspirated from the ventricle and poured gently into the test tubes already labeled with the information related to the sacrificed rat. Sampling of blood for all but one Sertraline-treated rat was uneventful. As a result, the number of drug rats evaluated for this phase of the study was less than the control group by one. Following the collection of blood samples, both testes were removed by pulling the spermatic cord, both were placed in a pre-labeled tube and filled with 10% formalin. Following a waiting period for the formation of a clot and serum, clot from each tube was removed and the sample was centrifuged at 1000 RPM equal to 123 g for 10 minutes. The serum was then carefully aspirated and transferred into a properly labeled microfuge tube. The tubes were then stored in a freezer at a temperature of -19 degrees of centigrade until the contents were thawed for the measurement of LH (Luteinizing Hormone), FSH (Follicular Stimulating Factor), and testosterone. Containers of testes were kept until they were delivered to a pathology laboratory for histological and pathological evaluations of the testes as soon as possible.
The remaining 16 rats were maintained safely in cages in an animal house and fed similarly for 90 more days without any intervention until they underwent the same process for similar hormonal and testicular histology/pathology evaluations.
Data analysis was done by using SPSS Version 20, with Mann–Whitney and Wilcoxon Test. A *P*-value of less than 0.05 was considered as significant.
It is worth to say the sample size is calculated based on the formula of comparing means in two independent samples and results of the article by Atli O, et al9.

RESULTS

As seen in Table 1, comparison of hormonal levels in Sertraline and control group does not show any significant difference on the 80th day after beginning the study except for the FSH that was significantly lower in the Sertraline group compared to the control group ($P = 0.004$). Also, there was not any significant difference in hormone levels between the drug and control groups on the 170th day. ($P > 0.05$, **Table 1**).
Also when comparing each hormone level in each group at two times (80th day and 170th day), we found an increase in FSH level in the treatment group from 80th day to 170th day which was returned to the baseline level.

Results of Testis Pathology

Gross pathological information about each testis, including size, weight, color, consistency, cutting surface,

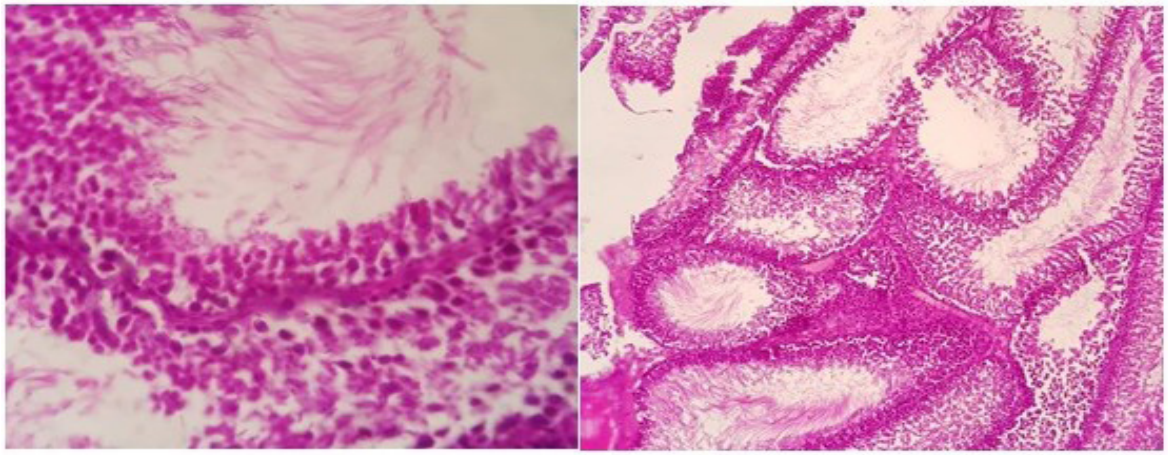

Figure 1. Microscopy of testis in a drug-treated rat in 80th day in two magnifications of 100 and 400.

Table 2. Comparison of the levels of LH, FSH and Testosterone between 80th and 170th day in Sertraline and control group.

Group	Hormones	80th day	170th day	*P*-Value[a]
		Median (IQR)[b]	Median (IQR)	
Control	LH (mg/dL)	3.40 (2/12-4/17)	2/85 (1/90-4/30)	0/34
	FSH (IU/L)	3/70 (2/95-4/45)	3/75 (3/05-4/25)	0/27
	Testosterone (IU/L)	4/99 (3/62-7/43)	3/80 (2/97-5/47	0/83
Sertraline	LH (mg/dL)	3/30 (3/10-5/20)	3/70 (2.35-5/00)	0/86
	FSH (IU/L)	2/20 (2/00-3/40)	2/90 (2/20-4/07)	0/002
	Testosterone (IU/L)	5/30 (2/70-7/54)	5/40 (2/72-5/67)	0/08

Abbreviations: FSH, Follicular stimulating factor; LH, Luteinizing factor; IU, International Unit; L, Liter; IQR, InterQuartile range
[a]Wilcoxon test
[b]Inter Quartile Range (percentile 25, percentile 75)

and any changes in the physical characteristics were recorded. The testes were then processed for, the preparation of suitable parts, tissue processing, preparation of paraffin blocks, performing thin cuts (about 4 microns), and staining with hematoxylin and eosin.

In the microscopic study of the testicular samples, examination of seminiferous tubules, interstitium, and tunica albuginea were carried out. In tubules, qualitative and semi-quantitative examinations of germ cells, including spermatogonia, primary spermatocytes, spermatids, permatozoa, and other cells, especially Sertoli cells, were performed. In addition, the thickness of the basement membrane and probable sediments were observed. In Interstitium, the status of Leydig cells and the examination for abnormal inflammation or sediment were studied.

Spermatogenesis appeared to be normal in both Sertraline- treated and control groups and no significant reduction in any of the germ cell types. In addition, no thickening of the basement membrane, atrophy or hyalinization of tubules were observed. The numbers of Leydig cells in the interstitium were in normal range and no specific pathological findings were noticed (**Figures 1-4**).

DISCUSSION

The main mechanism of SSRIs effects is primarily through the increase of serotonin activity in the brain and other organs[3]. Sertraline elevates serotonin levels in the brain through inhibition of serotonin reuptake in synaptic clefts playing an essential role in modulating nervous activity. For interpretation of the impact of each drug on organs, 2 approaches are taken. One approach is the determination of the direct effect of the drug on that organ and the other is the indirect effect of the drug on that organ through changes on the central CNS that control the peripheral organs. The complexity of the serotoninergic system is evidenced by the great variety of subtypes of receptors for the regulation of the neurotransmitter functions[3]. One of the most common and available methods for examining the functional status of reproductive system in laboratory animals is the examination of serum levels of sex hormones (including LH, FSH, and testosterone), as well as the examination of testicular pathology.

In our study, no significant differences were observed between the Sertraline-treated and the control groups of rats in regard to the testicular gross anatomy, histology, and pathology. Erdemir and his colleague also found no significant reduction in Johnson scoring on the Sertraline group compared with the control group in the results of testicular tissue pathology[6]. However, Atli, et al. reported a higher number of sperm with abnormal morphology in the treatment group and noted that changes were more significant with higher doses. He used male Wistar albino rats and categorized them into 4 groups: one control group (which was gavaged with water) and 3 treatment groups (which were gavaged

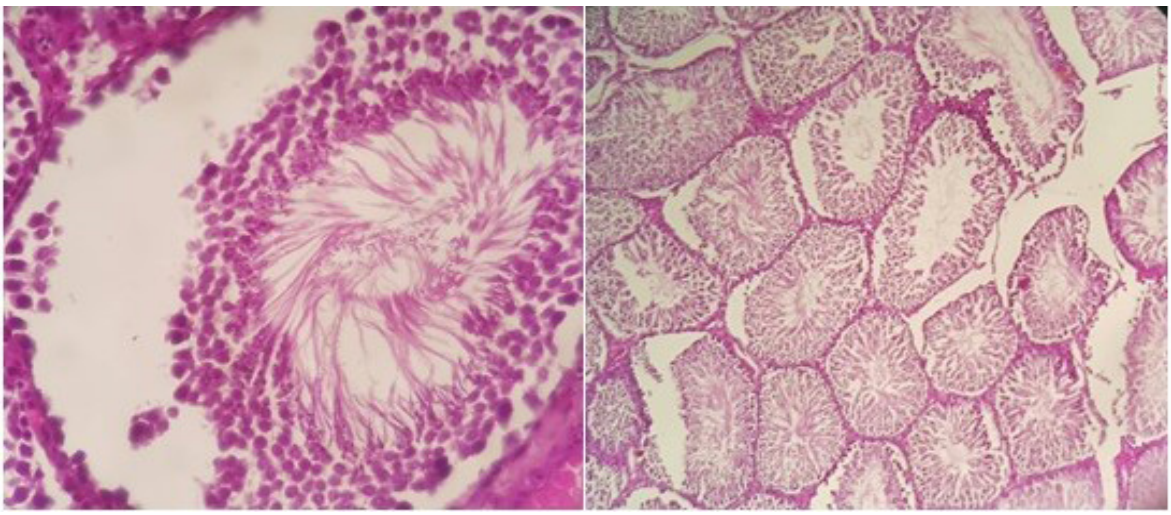

Figure 2. Microscopy of testis in a control group rat in 80th day in two magnifications of 100 and 400

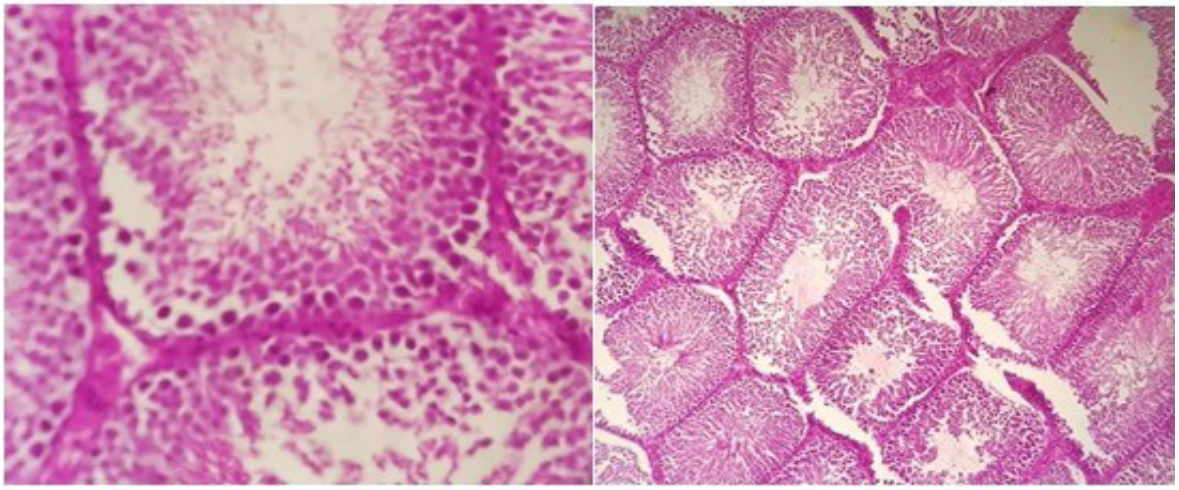

Figure 3. Microscopy of testis in a rat of drug group in 170th day in two magnifications of 100 and 400

with 3 different sertraline doses of 5m/kg.day, 10mg/kg.day and 20mg/kg.day) for 4 weeks. Madlool, et al. injected Sertraline intraperitoneally in male rats and found a significant decrease in the number of sperm and an increase in the number of deformed sperms[7].

Our study revealed a significant decrease in FSH in the drug group on the 80th day compared to the control group (p = 0.004). This significant decrease was resolved on the 170th day as there was no significant difference between the drug and control groups at that time (p = 0.26).

It is recognized that FSH leads the production and maturation of sperms. Perhaps the reason for the lack of significant changes in the spermatogenesis of the Sertraline-treated rats is the short duration of exposure for an apparent effect of reduced FSH on spermatogenesis. In other words, if the administration of Sertraline continued for a longer period of time, its negative effect on spermatogenesis may have been different.

There was no significant difference between LH and Testosterone in drug and control group in 80th day and 170th day after intervention. In male rats, LH and testosterone hormones are secreted episodically, so that LH has incremental episodes of 5 to 10 minutes, then gradually decreases over 50 to 70 minutes, and testosterone levels are within the range of 3-6 hours rise and then gradually fall[8]. This finding can be justified by the possible central effects of medication by the pituitary, hypothalamus, or even the cerebral cortex in the first 80 days which is returned to the basal state after the gavage has been stopped in the second 90 days. In one study conducted by Erdemir and his colleagues, there was no significant difference in the LH level similar to our study, but unlike to our study, there was a significant increase in FSH and a significant reduction in testosterone levels in the Sertraline group compared to the control group[6]. In another study conducted by Atli Ozlem and colleagues, contrary to the findings of our study and that of Erdemir, there were increased levels of LH, increased levels of testosterone, and no change in FSH levels in the Sertraline group compared with the control group[9]. In the study of Hadipour and colleagues on the mice of Balb/C race, there was no significant change in LH, but in contrast to our study, there was a significant

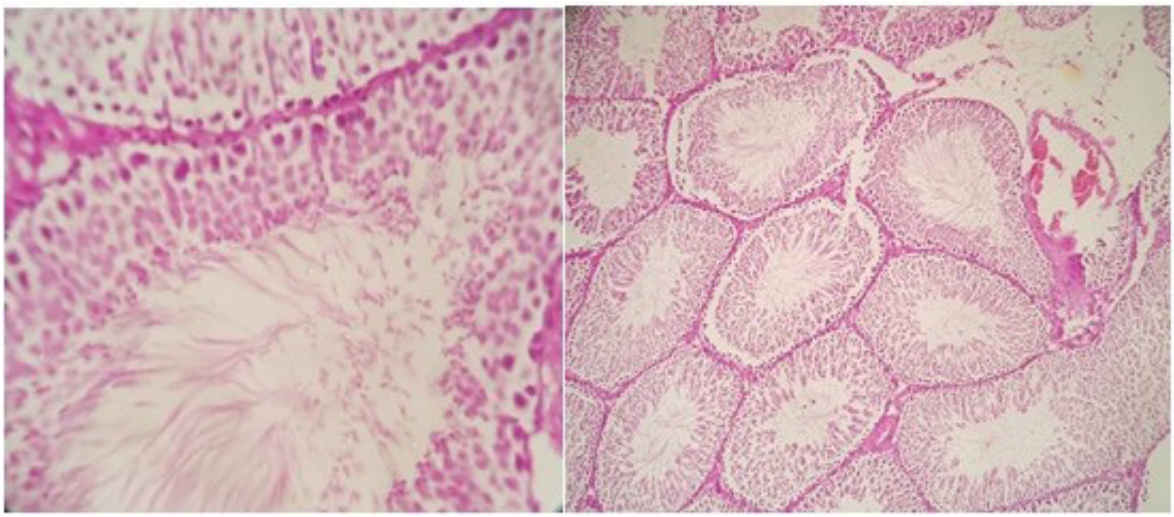

Figure 4. Microscopy of testis in a rat of the control group in 170th day in two magnifications of 100 and 400

increase in FSH and a decrease in testosterone levels[10]. In the study of Madlood, only a significant decrease in serum testosterone level was detected[7].
The possible reasons of differences we see in the findings of studies can be study design, episodic pattern of LH and Testosterone secretion in male rats[8], operator mistakes in extracting the samples, preparing the samples, way of storing the serums, period of storing, and laboratory mistakes as using expired kits or changing the samples with each other.

CONCLUSIONS

Sertraline can induce changes on sexual hormones and these changes are probably due to a central effect on the hypothalamic-pituitary-gonadal axis. These changes are reversible after drug withholding. Under the experimental conditions of our study, it appears that Sertraline does not induce spermatogenic changes in Wistar albino rats following 80 days of exposure to Sertraline. Whether or not the prolonged usage of Sertraline can negatively impact spermatogenesis in rats and if this impact is reversible needs further investigation.

ACKNOWLEDGEMENT

This study was conducted in the animal facility of the Mashhad Faculty of Medicine from December 2017 to June 2018.

CONFLICT ON INTEREST

There was no conflict of interest in this study.

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