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Effect of citalopram on sex hormones and testicular histomorphology in male albino rats: An experimental study

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Abstract---Introduction: Citalopram, a selective serotonin reuptake inhibitor (SSRI), is commonly used as a first-line treatment for depression. However, the potential effects of Citalopram on testicular histo-morphology and hormone levels have not been extensively studied. This study aimed to evaluate the impact of Citalopram on the microscopic structure of the testes and the levels of serum testosterone, luteinizing hormone (LH), and follicle-stimulating hormone (FSH) in male albino rats. Aims: The primary objective of this study was to assess the effects of Citalopram on testicular histo-morphology using the Johnson scoring system. The secondary objective was to measure the levels of serum testosterone, LH, and FSH in rats treated with varying doses of Citalopram. Methodology: A total of 25 healthy male albino rats were divided into five groups: control, group I, group II, group III, and group IV. The rats in groups I to IV were orally administered 10 mg, 20 mg, 30 mg, and 40 mg of

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Citalopram, respectively, while the control group received oral distilled water. The dosages were adjusted based on the suggested doses for humans and scaled according to the rats' weight. The rats received daily doses of Citalopram for 28 consecutive days. The testicular histo-morphology was assessed using the Johnson scoring system, and the levels of serum testosterone, LH, and FSH were measured using rat-specific ELISA kits. Results: The evaluation of testicular histo-morphology using the Johnson scoring system revealed no significant changes in the drug-treated groups compared to the control group. The testes appeared normal across all groups. The levels of serum testosterone, LH, and FSH were within the normal range for all groups. However, the high-dose groups exhibited lower hormone levels compared to the lower dose groups. Conclusion: The findings of this study indicate that Citalopram administration for a 28-day period does not negatively affect testicular histo-morphology or hormone levels in male albino rats. However, it should be noted that high doses of Citalopram resulted in lower hormone levels, suggesting a potential dose-dependent effect. Further research is warranted to investigate the long-term effects of Citalopram treatment on these parameters.

Keywords---citalopram, sex hormones, testicular histomorphology, reproductive parameters, experimental study, impact, male albino rats.

Introduction

The effect of pharmaceutical drugs on reproductive health is an important area of research, particularly concerning their impact on sex hormones and testicular histomorphology (Jurkowska et al., 2019. One such drug of interest is citalopram, a selective serotonin reuptake inhibitor (SSRI) commonly prescribed for various neuropsychiatric disorders. While citalopram has proven to be effective in treating conditions such as depression and anxiety, its potential effects on male reproductive function have raised concerns (Atmaca, 2020; Lee et al., 2010). Serotonin, a neurotransmitter involved in various physiological functions, including mood regulation, has been linked to sexual behavior and reproductive processes. Citalopram acts by blocking serotonin transporters (SERTs), resulting in increased serotonin levels in the synaptic cleft. This excess serotonin can then activate postsynaptic serotonin receptors and inhibit presynaptic SERTs, thereby reducing the reuptake of serotonin and alleviating depressive symptoms (Robichaud, 2004; Sherry, 2008).

Research has suggested that SSRIs, including citalopram, may have adverse effects on male reproductive health. Several studies have reported that the use of SSRIs is associated with a decrease in libido, delayed ejaculation, and erectile dysfunction (Rosen et al., 1999; Bala et al., 2018; & Shen, 1995). Additionally, in vitro studies using human semen samples mixed with diluted SSRIs have shown a decrease in sperm viability and motility. Increased prolactin levels, which are associated with the use of SSRIs, can also suppress follicle-stimulating hormone

(FSH) and luteinizing hormone (LH), thereby reducing testosterone production (Koyuncu et al., 2011).

The testicular biology of male albino rats provides a useful model for investigating the effects of citalopram on sex hormones and testicular histomorphology. The testes in male rats consist of seminiferous tubules where spermatogenesis occurs, and Leydig cells in the connective tissue surrounding the tubules produce testosterone. Spermatogenesis is a complex process involving different stages of germ cell development, regulated by hormones such as FSH and LH. Temperature is another crucial factor for the proper functioning and maturation of spermatozoa. Rat testes are located in the scrotum, which allows them to maintain a slightly lower temperature than the core body temperature. Any disturbance in testicular hormones can affect testicular morphology, and it has been observed that SSRIs can influence hormone levels, potentially leading to changes in testicular histomorphology (Naz & Kamal, 2017). These changes may occur directly or indirectly through altered hormone levels, such as testosterone, FSH, and LH.

Infertility is a significant concern for many couples, with more than 90% of male infertility cases attributed to low sperm count and poor sperm quality. Various factors can contribute to male infertility, including hormonal imbalances, genetic defects, anatomical anomalies, age-related decline in testosterone levels, stress, smoking, autoimmune diseases, and certain medical illnesses. Therefore, it is crucial to understand the potential effects of citalopram, as it is widely prescribed and may have implications for male reproductive health. In light of these concerns, this experimental study aims to investigate the effect of citalopram on sex hormones and testicular histomorphology in male albino rats. The rats will be administered citalopram at doses equivalent to the low and high suggested doses for humans, adjusted according to the rat's weight. The study will assess changes in sex hormone levels, including testosterone, FSH, and LH, and examine testicular histomorphology to determine any alterations induced by citalopram treatment.

Understanding the impact of citalopram on male reproductive health is essential for clinicians prescribing SSRIs and for individuals using these medications. This research will contribute to the existing body of knowledge on the potential reproductive effects of citalopram and provide valuable insights into its safety and use in clinical practice. By shedding light on the mechanisms by which citalopram affects sex hormones and testicular histomorphology, this study may pave the way for future investigations into mitigating or preventing the potential adverse effects of citalopram on male reproductive health.To conduct this experimental study, a suitable animal model is required to mimic human physiology and provide reliable results. Male albino rats are commonly used in reproductive toxicology studies due to their similarities in reproductive anatomy and endocrine regulation to humans. The rats will be divided into different groups, including a control group that receives a placebo and experimental groups that receive varying doses of citalopram.

Throughout the study, several parameters will be assessed to evaluate the impact of citalopram on sex hormones and testicular histomorphology. Blood samples

will be collected at designated time points to measure hormone levels, including testosterone, FSH, and LH. These measurements will provide insights into the hormonal changes induced by citalopram administration. Histological analysis of the testes will be performed to examine any alterations in testicular structure and morphology. Special staining techniques, such as hematoxylin and eosin staining, will be used to visualize the different cellular components and tissue architecture within the testes. This analysis will allow researchers to identify any changes in seminiferous tubules, Leydig cells, and other testicular structures that may be influenced by citalopram treatment. Additionally, other reproductive parameters, such as sperm count, sperm motility, and sperm morphology, may also be evaluated to assess the functional implications of citalopram on sperm production and quality. These parameters are crucial indicators of male fertility and can provide valuable information on the potential effects of citalopram on reproductive outcomes.

Objective

To shed light on the impact of citalopram on reproductive parameters, including sex hormone levels and testicular histomorphology.

Methodology

Study Design and Experimental Setting

An experimental study was conducted at Saidu Medical College Swat Khyber Pakhtunkhwa, Department of Bio-Technology University of Malakand Khyber Pakhtunkhwa, and Institute of Basic Medical Sciences (IBMS) Khyber Medical University (KMU) Peshawar. The duration of the experiment involved four weeks of citalopram administration, followed by sample collection in the fifth week.

Experimental Animals

Thirty mature male rats weighing between 230-270 grams and aged 8 to 10 months were procured from the University of Health Sciences (UHS), Lahore (Punjab) in March 2016. The rats were acclimatized for two weeks in the animal house with free access to food and water. The temperature and humidity in the animal house were maintained at approximately 24.5±2°C and 60-75%, respectively.

Selection Criteria

Mature healthy male rats were selected for the study.

Experimental Design and Drug Administration

The 30 rats were randomly divided into five groups: four treatment groups and one control group. Each group consisted of five rats with similar weights. Different concentrations of citalopram were prepared and administered orally once a day for four consecutive weeks. The control group received sterile water orally. The dosages of citalopram were adjusted according to the weight of the rats

and corresponded to the low and high suggested doses for humans (Table 1). Citalopram by the trade name Pramcit (A product of Nabiqasim Industries Private Limited) was used in the experiment.

Table 1
Rats Groups and Average Weight

Rats Groups	Average Weight	Drug Dose
Control	250±10gms	Distilled water
Group I	250±10gms	0.041mg
Group II	250±10gms	0.083mg
Group III	230±10gms	0.12mg
Group IV	230±10gms	0.15mg

Sample collection technique

After four weeks of drug treatment, the rats were decapitated without anesthesia and blood samples were collected from their hearts to obtain serum for hormonal analysis. The testes and epididymides from all five groups were harvested for pathological examination and sperm count. The testes were examined for changes in color, shape, size, and consistency, and their weights were measured. The specimens were washed with normal saline, cuts were made on their surface, and then transferred to containers with formalin solution. The epididymides were dissected from the testes, and semen was collected in labeled Petri dishes by applying light pressure. Sperm dilution was prepared by combining 20 µl of liquified semen with 380 µl of formal saline.

Sperm count

Sperm count was performed using a Neubauer chamber (Hemocytometer). Prior to the count, the chamber was washed and cleaned. A cover slip was placed on the counting chamber, and sperm suspension was added and examined under a light microscope with a x10 objective lens. This procedure was repeated for all 25 samples. To calculate the number of sperm per milliliter, the mean number of sperm in each chamber was determined. The mean value was then multiplied by 10,000 to obtain the number of sperm per milliliter. Finally, this number was multiplied by the dilution factor. The total number of sperm per milliliter was calculated as N (number of sperm) multiplied by 5, multiplied by 10,000, and multiplied by the dilution factor.

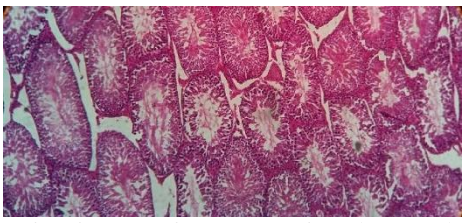

Figure 1. Photomicrograph of control group I showing active spermatogenesis and release of sperms in the tubular lumen (H&E; X= 100)

Hormone analysis

For hormone analysis, serum samples were collected and allowed to coagulate at room temperature for 10-20 minutes. Afterward, the samples were centrifuged at a speed of 2000-3000 rpm for 20 minutes to obtain clear, clot-free supernatant. The supernatant was carefully isolated in sterilized Eppendorf tubes and labeled accordingly. The serum samples were then tested for levels of testosterone, LH, and FSH. The quantitative determination of these hormones was conducted using a test kit from Glory Science (Glory Science Co., Ltd) on the UTECH UT-210RT USA instrument, ensuring regular quality control checks were performed.

Determination of rat's testosterone

For the determination of rat's testosterone, the reagents and instruments were prepared and brought to the working table at a room temperature of 25 ±2 °C. To prepare the standard dilution, five empty tubes were used. A standard diluent of 50 µl was pipetted into each tube, followed by transferring 100 µl of standard (4.5 ng/ml) into the first tube. Then, 100 µl from the first tube was transferred into the second tube, and 50 µl from the second tube was transferred to the third tube, creating a dilution series. This concentration process was repeated to obtain the mean value for each well. Additionally, a diluted wash solution was prepared according to the manufacturer's instructions by creating a 30-fold dilution with distilled water.

Determination of FSH level

Reagents and instruments were brought to the working table. The assay was performed at room temperature of 25 ± 2oC. Five empty, cleaned and dried tubes were placed and standard dilution of 50 µl was pipetted and transferred in each tube. Then 100 µl standard (13.5 IU/L) was placed in the first tube, followed by transfer of 100 µl from the first tube into the second, then transferred 50 µl from the second tube to the third tube and dilution series were produced. The concentration was repeated to get the mean value of each well. Diluted wash solution was used in this procedure as per manufacturer instructions; 30- fold dilution was prepared with distilled water.

Table 2
Standard dilution for determination

Determination of FSH level	Tubes	Standard	1	2	3	4	5
	IU/L	13.5	9	6	3	1.5	0.75
Determination of rat's testosterone	Tubes	Standard	1	2	3	4	5
	ng/ml	4.5	3	2	1	0.5	0.25

Assay procedure

A blank well was set separately, 40 µl of sample diluent was added to testing sample cuvette well, and then 10 µl of testing sample was added to each well. Each cuvette well was covered with adhesive strip and incubated for 30 minutes

at 37oC. Special care was adopted during handling of wells to minimize error in procedure. After incubation, adhesive strips were uncovered and liquid was discarded. Each well was washed with diluted wash buffer 350 µl each time. It was repeated for five times to remove unbound antibodies from the well. Next 50 µl HRP enzyme conjugate was added except blank and covered again with adhesive strips and incubated at 37oC for 30 minutes. Again the wells were washed 5 times and drained. In the next phase 50 µl of chromogen solution A and chromogen solution B were added to each well and incubated back into the dark place at 37oC for 15 minutes, finally stop solution of 50 µl was added to each well and clear color change was observed. The absorbance of each well was read at 450 nm wavelength against blank.

Tissue Processing

The tissue processing procedure consisted of several steps to prepare the tissues for analysis. First, fixation was performed using a 10% formalin solution at a 10:1 ratio. Dehydration followed, with tissues placed in increasing concentrations of ethanol (70% for 2 hours, 80% for 2 hours, 90% for 2 hours, and 100% twice for 1 hour each) to remove water from the tissue gradually. Clearing was achieved by immersing the tissue in 100% xylene twice for 1 hour each to remove alcohol. Infiltration and embedding were carried out by infiltrating the tissues with molten paraffin at around 60°C for 2 hours, replacing xylene with liquid paraffin. The tissue blocks were labeled and cooled. Next, the tissue blocks were sectioned using a microtome, producing 4-5 micron thick sections that were placed in a water bath at 35 ± 2°C to prevent wrinkles and mounted on glass slides. Staining involved removing paraffin wax by placing the slides in hot xylene for 3 minutes, followed by xylene at room temperature for 2 minutes. Rehydration was performed using a series of alcohol concentrations (2 steps of 100%, 90%, and 80% for 2 minutes each) and water for 1 minute. Hematoxylin staining was carried out for 3 to 4 minutes, followed by washing with running tap water for 1 minute. Acid alcohol was applied briefly to remove excess staining, and the sections were treated with an alkaline solution and washed with tap water. Counter staining with Eosin was performed for 1 to 2 minutes, followed by water. Dehydration was done using 80%, 90%, and two rounds of 100% alcohol for 2 minutes each. The sections were cleaned with xylene, mounted with DPX synthetic resin, covered with histological cover slips, and properly labeled.

Histomorphology

Johnson score for testicular biopsy score count system (TBS) was used for histomorphology analysis of tissue specimens in each group (table 3).

Table 3
The Johnson Criteria for histomorphology analysis of tissue specimens

Score	Histological criteria
10.	Full spermatogenesis
9.	Slightly impaired spermatogenesis, many late spermatids, disorganized epithelium
8.	Less than 5 spermatozoa per tubule, few late spermatids

7.	No spermatozoa, no late spermatids, many early spermatids
6.	No spermatozoa, no late spermatids, few early spermatids
5.	No spermatozoa or spermatids, many spermatocytes
4.	No spermatozoa or spermatids, few spermatocytes
3.	Spermatogonia only
2.	No germinal cells, Sertoli cells only
1.	No seminiferous epithelium

Statistical analysis

In this study, data interpretation was performed using the Windows program Statistical Package for the Social Sciences (SPSS) version 21. Descriptive analysis was conducted for all five groups of rats to summarize and describe the data. To assess the homogeneity of population variances, Levene's test was employed. For comparing the means among the groups, a one-way analysis of variance (ANOVA) test for independent samples was utilized. To determine significant differences in means between specific groups, the Least Significant Difference (LSD) test was applied. These statistical analyses were conducted to obtain meaningful insights and draw conclusions from the data.

Results

Neubauer chamber (Heamocytometer) was used for total sperm count of all five groups (table 4). The rats were treated with citalopram except control group. The number of sperm per milliliter of the sample was calculated. The control group was taken as a reference group for the other four groups. The average sperm count of control group was found to be 54.70 million per milliliter. Similarly, the sperm count in group-I, group-II, group-III and group-IV was observed to be 51.46, 50.32, 48.32 and 48.08 million per milliliter respectively. The Levene's statistical test was applied on the collected data which showed the homogeneity of population variances for all experimental groups. This test was applied prior to application of analysis of variance (ANOVA), which smoothen the ground for conducting ANOVA. The P-value = 0.875 >0.05 is highly insignificant, therefore the populations variances are considered to be equal (table 4).

Table 4
Analysis of sperm counts of the Albino rats

Rats Groups	Number of rates	Mean ± SD	P value	
Control Group	5	54.70 ± 6.45	Levene's Statistic	ANOVA
Group-I	5	51.40 ± 5.20	.875	.569
Group-II	5	50.32 ± 8.41		
Group-III	5	48.32 ± 5.80		
Group-IV	5	48.08 ± 8.33		

The results from the analysis (Table 4) showed that citalopram had no effect on the sperm counts of all treated rat's groups. As P-value =0.569 >0.05, showed that there is no significant difference among the average sperm count of control group as well as the four treated groups. There were no significant changes

observed in all the treated with different doses of citalopram compared with the control group. For confirmation of equality of means of all five groups, LSD test was applied (Table 5), all the P-values are greater than 0.05. This shows that all the results were insignificant.

Table 5
LSD test for sperm count of rats

Group Comparison		Mean Difference (95% CI)	Significance
Control Group	Group-I	3.24(-5.95-12.43)	.471
	Group-II	4.38(-4.81-13.57)	.332
	Group-III	6.38(-2.81-15.57)	.163
	Group–IV	6.62(2.57-15.81)	.149
	Control Group	-3.24(-12.43-5.95)	.471
Group-I	Group-II	1.14(-8.05-10.33)	.798
	Group-III	3.14(-6.05-12.33)	.484
	Group-IV	3.38(-5.81-12.57)	.452
	Control Group	-4.38(-13.57-4.81)	.332
Group-II	Group-I	-1.14(-10.33-8.05)	.798
	Group-III	2.00(-7.19-11.19)	.655
	Group-IV	2.24(-6.95-11.43)	.617
	Control Group	-6.38(-15.57-2.81)	.163
Group-III	Group-I	-3.14(-12.33-6.05)	.484
	Group-II	-2.00(-11.19-7.19)	.655
	Group-IV	.24 (-8.95-9.43)	.957
	Control Group	-6.62(-15.81-2.57)	.149
Group-IV	Group-I	-3.38(-12.57-5.81)	.452
	Group-II	-2.24(-11.43-6.95)	.617
	Group-III	-.24(-9.43-8.95)	.957

All samples were collected and assays were performed for all five groups (including control group). All obtained results were given in the following tables. Detection range of testosterone kit: 0.1 ng/ml-3.8ng/ml (nanogram per milliliter), detection range of LH kit: 2 ng/L - 40 ng/L (nanogram per liter) and detection range of FSH kit: 0.5I U/L - 10I U/L (International unit per liter). The testosterone level was determined through rat testosterone ELISA kit and UTECH UT-210RT USA, the assay was performed at room temperature (25 ±2oC). The average testosterone level of control group was found to be 2.02 ng/ml. Similarly, the testosterone levels in group-I, group II, group-III and group IV observed to be 1.98, 1.94, 1.53, 1.2 ng/ml respectively (Table 6). The Levene's statistical test was applied on the collected data which showed the homogeneity of population variances for all experimental groups (10). This test was applied prior to application of ANOVA. Here the P-value = 0.841 > 0 .05 is highly insignificant, therefore the population variances are considered to be equal.

Table 6
Analysis of Testosterone level of rats

Rats Groups	Number of rats	Mean ± SD	P value	
Control Group	5	2.02 ± .39	Levene's Statistic	ANOVA
Group-I	5	1.98 ± .46	.841	.398
Group-II	5	1.94 ± .21		
Group-III	5	1.53 ± .22		
Group-IV	5	1.21 ± .35		

The results from the analysis showed that citalopram had no effect on the testosterone level of all treated rats groups. As P-value =0.398> 0.05, showed that there is no significant difference among the average sperm count of control group as well as the four treated groups. There was no significant changes observed in all the treated with different doses of citalopram compared with the control group. For confirmation of equality of means of all five groups, LSD test was applied (Table 7), all the P-values are greater than 0.05. This shows that all the results were insignificant.

Table 7
LSD test for testosterone level

Group Comparison		Mean Difference (95% CI)	Significance
Control Group	Group-I	.048(-.96-1.05)	.92
	Group-II	.084(-.92-1.09)	.86
	Group-III	.490(-.52-1.49)	.32
	Group-IV	.812(-.19-1.82)	.11
	Control Group	-.048(-1.05-.96)	.92
	Group-II	.036(-.97-1.04)	.94
Group-I	Group-III	.442(-.56-1.45)	.37
	Group-IV	.764(-.24-1.77)	.13
	Control Group	-.084(-1.09-.92)	.86
Group-II	Group-I	-.036(-1.04-.97)	.94
	Group-III	.406(-.60-1.41)	.41
	Group-IV	.728(-.28-1.73)	.15
Group-III	Control Group Group-I Group-II Group-IV	-.490(-1.49-.52)	.32
		-.442(-1.45-.56)	.37
		-.406(-1.41-.60)	.41
		.322(-.685-1.33)	.51
	Control Group	-.812(-1.82-.19)	.11
Group-IV	Group-I	-.764(-1.77-.24)	.13
	Group-II	-.728(-1.73-.28)	.15
	Group-III	-.322(-1.33-.68)	.51

The Luteinizing hormone level was determined through LH ELISA kit and UTECH UT-210 RT USA; the assay was performed at room temperature (25 ±2oC). The average LH level of control group was found to be 3.54 ng/ml (table 8). Similarly, the testosterone levels in group-I, group II, group-III and group IV observed to be 3.49, 3.42, 3.3, 3.30 nanograms per liter respectively.

Table 8
Analysis of LH level of rats

Rats Groups	Number of rats	Mean ± SD	P value	
Control Group	5	3.54 ± .255	Levene's Statistic	ANOVA
Group-I	5	3.49 ± .431	0.29	0.997
Group-II	5	3.42 ± .703		
Group-III	5	3.33 ± .691		
Group-IV	5	3.30 ± .412		

The Levene's statistical test was applied on the collected data which showed the homogeneity of population variances for all experimental groups. Here the P-value = 0.29 > 0 .05 is highly insignificant (table 8), therefore the population variances are considered to be equal. The results from the analysis shows that there is no effect of citalopram on the LH level of all treated Albino rats groups. As P=0.997, which is >0.05 shows that there is no significant difference among the average LH Level of control group as well as the four treated groups. The changes in LH Level determined in each group treated with different doses of citalopram were not significant in all the four groups as compared to the control group. For confirmation of equality of means of all five groups, LSD test was applied (Table 10), all the P-values are greater than 0.05. This shows that all the results were non-significant.

Table 10
LSD test for LH level

Group Comparison		Mean Difference (95% CI)	Significance
Control Group	Group-I	.050(-1.51-1.61)	.947
	Group-II	.124(-1.43-1.69)	.870
	Group-III	.212(-1.34-1.77)	.779
	Group-IV	.244(-1.31-1.80)	.747
Group-I	Control Group	-.050(-1.61-1.51)	.947
	Group-II	.074(-1.48-1.63)	.922
	Group-III	.162(-1.39-1.72)	.830
	Group-IV	.194(-1.36-1.75)	.797
	Control Group	-.124(-1.68-1.43)	.870
Group-II	Group-I	-.074(-1.63-1.48)	.922
	Group-III	.088(-1.47-1.64)	.907
	Group-IV	.120(-1.44-1.68)	.874
	Control Group	-.212(-1.77-1.34)	.779
Group-III	Group-I	-.162(-1.72-1.39)	.830
	Group-II	-.088(-1.64-1.47)	.907
	Group-IV	.032(-1.52-1.59)	.966
	Control Group	-.244(-1.80-1.31)	.747
Group-IV	Group-I	-.194(-1.75-1.36)	.797
	Group-II	-.120(-1.68-1.44)	.874
	Group-III	-.032(-1.59-1.52)	.966

Table 11 shows the descriptive Analysis of FSH level of rats. The Homogeneity of Variances was found by Levene's test for FSH (table 18). The results from the analysis shows that there is no effect of citalopram on the FSH Level of all treated Albino rats groups. As P=1.997, which is>0.05 shows that there is no significant difference among the average FSH Level of control group as well as the four treated groups. The changes in FSH Level determined in each group treated with different doses of citalopram were not significant in all the four groups as compared to the control group. For confirmation of equality of means of all five groups, LSD test was applied, all the P-values are greater than 0.05. This shows that all the results were insignificant. Here the P-value = 0.43 > 0 .05 is highly insignificant, therefore the population variances are considered to be equal.

Table 11
Descriptive Analysis of FSH level of rats

Rats Groups	Number of Rats	Mean ± SD	P value	
Control Group	5	2.48 ± .179	Levene's Statistic	ANOVA
Group-I	5	2.48 ± .425	.430	1.000
Group-II	5	2.47 ± .364		
Group-III	5	2.47 ± .255		
Group-IV	5	2.46 ± .342		

The results from the analysis shows that there is no effect of citalopram on the FSH Level of all treated Albino rats groups. As P=1.997, which is>0.05 shows that there is no significant difference among the average FSH Level of control group as well as the four treated groups. The changes in FSH Level determined in each group treated with different doses of citalopram were not significant in all the four groups as compared to the control group. For confirmation of equality of means of all five groups, LSD test was applied (Table 12), all the P-values are greater than 0.05. This shows that all the results were insignificant.

Table 12
LSD test for follicle stimulating hormone level

Group Comparison		Mean Difference (95% CI)	Significance
Control Group	Group-I	.006(-.952-.964)	.990
	Group-II	.010(-.948-.968)	.983
	Group-III	.014(-.944-.972)	.976
	Group-IV	.024(-.934-.982)	.959
	Control Group	-.006(-.964-.952)	.990
Group-I	Group-II	.004(-.954-.962)	.993
	Group-III	.008(-.950-.966)	.986
	Group-IV	.018(-.940-.976)	.969
	Control Group	-.010(-.968-.948)	.983
Group-II	Group-I	-.004(-.962-.954)	.993
	Group-III	.004(-.954-.962)	.993
	Group-IV	.014(-.944-.972)	.976
	Control Group	-.014(-.972-.944)	.976
Group-III	Group-I	-.008(-.966-.950)	.986

	Group-II	-.004(-.962-.954)	.993
	Group-IV	.010(-.948-.968)	.983
	Control Group	-.024(-.982-.934)	.959
Group-IV	Group-I	-.018(-.976-.940)	.969
	Group-II	-.014(-.972-.944)	.976
	Group-III	-.010(-.968-.948)	.983

Discussion

Many studies conducted in humans and animal models have reported that citalopram and other selective serotonin reuptake inhibitors (SSRIs) may play a possible role in male infertility and the alteration of normal semen parameters (Nørr et al., 2016; Erdemir et al., 2014; Moradi et al., 2023; Prasad et al., 2015). Depression itself is a cause of sexual dysfunction in many individuals. In 1966, Simpson identified the relationship between antidepressants and male infertility when he incidentally found a disorder in spermatogenesis in a patient taking trimipramine, a tricyclic antidepressant (Simpson et al., 1966). Subsequent studies have confirmed this observation (Kurland et al., 1970). Therefore, to establish a relationship between infertility, sex hormones, and testicular histomorphology with the use of SSRIs, further work is needed to investigate the relationship between serotonin and infertility. This was the aim of our study. Limited research exists regarding the effects of SSRIs on testicular histomorphology. In one study, different SSRIs were administered to male adult rats for two months, resulting in negative impacts on testicular histomorphology. Another study administered an SSRI (fluoxetine) to neonatal rats, which led to some adverse changes in testes morphology upon maturity. In our study, gross morphology examination of harvested testes did not reveal any noticeable changes, and microscopic histological analysis showed normal spermatogenesis without any abnormal pathology. Therefore, the specific effect of citalopram on testicular morphology remains unclear based on our findings. To establish a clearer understanding of the relationship between serotonin, SSRIs, and infertility, future research is warranted.

In comparison to the study by Ilgin et al. (2017) titled "Citalopram Induces Reproductive Toxicity in Male Rats," the current study also examined the effects of citalopram on male reproductive parameters. Both studies revealed negative effects on sperm parameters, including decreased sperm concentration and increased abnormal sperm morphology, indicating potential reproductive toxicity. Additionally, histopathological changes in the testes were observed in both studies, suggesting structural alterations in testicular tissues. Both studies also detected hormonal alterations, with increased luteinizing hormone levels found in rats treated with citalopram. However, there were some differences in the findings. The Ilgin et al. study reported increased testosterone levels in certain dosage groups of citalopram, whereas the current study did not find a significant difference in testosterone levels. Furthermore, the Ilgin et al. study specifically investigated the oxidative status of the testes and highlighted the presence of oxidative stress, while the current study did not assess this aspect. In conclusion, both studies provide evidence that citalopram has detrimental effects on male reproductive function, leading to impaired sperm parameters and histological changes in the testes. The studies also suggest the involvement of hormonal

changes, particularly increased luteinizing hormone levels, in the development of reproductive disorders. While the Ilgin et al. study additionally emphasizes the role of oxidative stress, this aspect was not explored in the current study. Further research is needed to fully understand the mechanisms underlying citalopram-induced reproductive toxicity and the potential implications for human reproductive health.

Several studies, including one conducted by Fikret Erdemir et al. (2014), have investigated the effects of selective serotonin reuptake inhibitors (SSRIs) on testicular tissue and serum malondialdehyde (MDA) levels in rats. While the specific SSRIs evaluated in their study were sertraline, fluoxetine, escitalopram, and paroxetine, and your data focused on citalopram, there are similarities and differences worth noting. Both studies found negative effects of SSRIs on testicular tissue, with Erdemir et al. reporting a decrease in the Johnsen score in the paroxetine group compared to the control group, indicating impaired histological quality, and your data indicating a decline in average sperm count among the groups treated with citalopram and other SSRIs. However, there are discrepancies regarding hormone levels, with Erdemir et al. observing an increase in follicle-stimulating hormone (FSH) levels only in the sertraline group, while your data mentioned a decline, although not statistically significant, in testosterone, luteinizing hormone (LH), and FSH levels in the treated groups. Both studies did not find significant differences in malondialdehyde (MDA) and LH levels between the treated groups and the control group. Further well-designed studies, including randomized controlled clinical trials with larger populations, are needed to better understand the specific effects and mechanisms of SSRIs on testicular tissue and male fertility.

The study by Inbar Zohar et al. (2014) focused on the effects of prenatal stress on the expression of serotonin receptors and corticotropin-releasing factor receptors in rats, whereas your data examined the impact of selective serotonin reuptake inhibitors (SSRIs) on testicular tissue and hormone levels in rats. Both studies contribute to our understanding of the effects of serotonin-related factors on physiological systems. In terms of similarities, both studies highlight the involvement of serotonin signaling in the examined systems. In your data, the use of SSRIs, such as citalopram, was associated with changes in testicular histomorphology and hormone levels. Similarly, the study by Inbar Zohar et al. explored the expression of serotonin receptors (5HT1AR) in the prefrontal cortex and corticotropin-releasing factor receptors (CRFR2) in the dorsal raphe nuclei, indicating the role of serotonin-related receptors in anxiety and depressive disorders. Regarding differences, your data focused on male infertility and semen parameters, while the study by Inbar Zohar et al. primarily examined anxiety-related behaviors and receptor expression in different brain regions. Moreover, your data specifically investigated the effects of SSRIs on testicular tissue, whereas the other study investigated the impact of prenatal stress on receptor expression and the subsequent effects of citalopram treatment. Despite these differences, it is interesting to note that both studies point to the involvement of serotonin-related factors in the examined systems. Your data highlighted potential negative effects of SSRIs on testicular histomorphology and hormone levels, while the study by Inbar Zohar et al. demonstrated alterations in receptor expression under conditions of prenatal stress and their modulation by

citalopram treatment. Overall, these studies collectively contribute to our understanding of the effects of serotonin-related factors in different physiological systems. While your data specifically focused on male infertility and the impact of SSRIs on testicular tissue, the study by Inbar Zohar et al. shed light on the involvement of serotonin receptors in anxiety and depressive disorders.

The study by Bryan K Sanders (2013) discusses the potential links between steroid dysgenesis, epigenetics, and brain disorders, with a particular focus on the effects of selective serotonin reuptake inhibitors (SSRIs) and sex steroids. While the study does not directly relate to your data on the effects of SSRIs on testicular tissue and hormone levels, it provides insights into the broader impacts of serotonin-related factors on brain function and development. The study highlights that certain SSRIs can alter steroidogenesis, specifically the activity of 3α-hydroxysteroid dehydrogenase, which is involved in steroid synthesis. It suggests that these alterations in steroidogenesis may have implications for brain disorders, including autism spectrum disorder (ASD). The study cites research showing that repeated administration of citalopram, an SSRI, can increase testosterone levels in serum. Additionally, there is evidence from in vitro studies suggesting that sex steroids can influence serotonin homeostasis.

The study also introduces the concept of epitestosterone (epiT), a naturally occurring steroid, as a potential central mediator in the epigenetic regulation of gene expression. The production of epiT in the rat brain has been found to be higher in females than in males, which may contribute to sex differences in the incidence of autism and other brain disorders. However, the role of epiT in brain development has been relatively understudied. While this study does not directly compare with your data, it offers valuable insights into the broader effects of serotonin-related factors on brain function and the potential interplay between sex steroids, serotonin, and brain disorders. It underscores the complexity of these interactions and highlights the need for further research in understanding the role of epiT in brain development and its potential implications for brain disorders.

The current study on the effects of citalopram on sperm count, testosterone levels, LH levels, FSH levels, and testicular morphology in rats can be compared to the study titled "Increased dopamine transporter density in the male rat brain following chronic nandrolone decanoate administration" by Kindlundh et al. (2004). While the current study focused on the reproductive parameters and testicular histology, the study by Kindlundh et al. explored the impact of chronic nandrolone decanoate administration on the dopamine transporter (DAT) density in the male rat brain. The findings of the current study did not show any significant effects of citalopram on the reproductive parameters measured, including sperm count, testosterone levels, LH levels, and FSH levels, as well as testicular morphology. In contrast, the study by Kindlundh et al. demonstrated that chronic nandrolone decanoate administration increased DAT density in the caudate putamen of male rats. Both studies contribute to our understanding of the effects of pharmacological interventions on physiological systems, with the current study emphasizing reproductive parameters and the study by Kindlundh et al. shedding light on the impact of anabolic-androgenic steroids on the dopamine system in the brain.

The current study on the effects of citalopram on sperm count, testosterone levels, LH levels, FSH levels, and testicular morphology in rats can be compared to the study titled "The effect of sertraline, paroxetine, fluoxetine and escitalopram on testicular tissue and oxidative stress parameters in rats" by Erdemir et al. (2014). Both studies aimed to investigate the impact of selective serotonin reuptake inhibitors (SSRIs) on reproductive parameters and testicular tissue in rats.

In the current study, citalopram treatment did not significantly affect sperm count, testosterone levels, LH levels, FSH levels, or testicular morphology. In contrast, the study by Erdemir et al. found that SSRIs, particularly paroxetine, had a negative effect on testicular tissues in rats. The Johnsen scores, which indicate the histological quality of spermatogenesis, were significantly lower in the paroxetine group compared to the control group. Furthermore, testosterone levels were lower in all the SSRIs groups except for the fluoxetine group. While the current study focused on citalopram, the study by Erdemir et al. investigated the effects of different SSRIs including sertraline, fluoxetine, escitalopram, and paroxetine. The findings of both studies suggest that SSRIs can have varying effects on reproductive parameters and testicular tissue in rats. However, it is important to note that these studies were conducted in animal models, and further research, including well-designed randomized controlled clinical studies on a larger population, is necessary to determine the exact mechanisms and clinical implications of SSRIs on testicular function in humans.

The current study on the effects of citalopram on reproductive parameters in rats can be compared to the study titled "Prenatal methamphetamine attenuates serotonin-mediated renin secretion in male and female rat progeny: evidence for selective long-term dysfunction of serotonin pathways in brain" by Cabrera et al. (1994). Both studies investigated the long-term impact of drug exposure during pregnancy on serotonin (5-HT) pathways in the brain. In the current study, citalopram did not significantly affect serotonin levels or reproductive parameters in rats. In contrast, the study by Cabrera et al. focused on prenatal exposure to methamphetamine and its effects on postnatal serotonin systems. The researchers found that prenatal methamphetamine exposure resulted in long-term functional alterations in the 5-HT system, specifically marked attenuation of plasma renin responses to a 5-HT releaser in both male and female progenies. While the current study examined the effects of citalopram, Cabrera et al. investigated the impact of prenatal methamphetamine exposure. Both studies demonstrate that drug exposure during pregnancy can have long-term effects on serotonin pathways in the brain. However, the specific mechanisms and outcomes differ between the studies due to the different drugs being investigated. Further research is needed to understand the implications of these findings on neurodevelopment and long-term physiological functioning in humans.

In conclusion, the reviewed studies have revealed potential negative effects of citalopram and other SSRIs on male reproductive parameters and testicular tissue. These effects include changes in sperm count, abnormal sperm morphology, and histological alterations in the testes. Hormonal changes, particularly increased luteinizing hormone levels, appear to play a role in the development of reproductive disorders. While there is agreement among the

studies regarding the detrimental effects of SSRIs on male fertility, there are variations in specific outcomes such as hormone levels and oxidative stress. Therefore, further research is necessary to understand the underlying mechanisms of citalopram-induced reproductive toxicity and its implications for human reproductive health.

Recommendations

- Enhanced Monitoring: Healthcare professionals should consider closely monitoring male patients who are prescribed citalopram or other SSRIs for any potential adverse effects on reproductive parameters. Regular assessments of sperm count, morphology, and testicular health could help identify any early signs of reproductive dysfunction.
- Individualized Treatment Approach: In cases where SSRIs are necessary for male patients, healthcare providers should carefully evaluate the risks and benefits and consider alternative treatment options if possible. Personalized treatment plans can help minimize the potential negative impact on male reproductive function while still addressing the underlying mental health condition.
- Comprehensive Research: Further research is needed to gain a more comprehensive understanding of the effects of SSRIs on male reproductive function. This includes investigating the mechanisms through which these medications affect sperm count, morphology, and testicular tissue. Additionally, exploring the broader interactions between serotonin, sex hormones, brain disorders, and other factors can provide valuable insights into the complexities of male reproductive health and guide future treatment strategies.

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