Effect of Venlafaxine, Pramipexole, and Valsartan on Spermatogenesis in Male Rats

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ABSTRACT: The study’s aim was to explore the effect of venlafaxine, valsartan, and pramipexole on spermatogenesis. It was hypothesized that these drugs may affect the male fertility because of their long-term use in treatment of depression, hypertension, and Parkinson’s diseases. Male rats were given venlafaxine, valsartan, and pramipexole at low- and high-dose levels orally once daily for 10 weeks. Testosterone (25 mg/kg) was given as a standard via an intramuscular route once weekly. Rats were sacrificed after blood collection by cardiac puncture, and testes were removed. Sperm parameters were examined from spermatozoa of the cauda epididymis, and testes were treated for histopathological analysis. Results showed nonsignificant effect of venlafaxine on the sperm count, whereas a decreased sperm count was noted in all the treatment groups as compared to that of the control except valsartan at a low dose, which significantly (p < 0.001) raised the sperm count (96.26 ± 2.4) in reference with the control value (49.13 ± 2.3). Treatments had variable effects on total sperm motility and morphological parameters, but valsartan at a low dose showed maximum sperm motility (71.55 ± 0.7) among all. DNA integrity of spermatozoa remained intact in all groups. Luteinizing hormone and follicle-stimulating hormone levels decreased, and testosterone levels increased in all treatment groups as compared to control values, which indicate fertility. Histopathology revealed normal texture of testes with venlafaxine and valsartan, but testicular damage occurred with high-dose pramipexole. It is concluded that the use of venlafaxine, valsartan, and pramipexole at a low dose is devoid of any harmful effect on spermatogenesis, whereas pramipexole at a high dose adversely affect it.

1. INTRODUCTION

In males, reproductive health is as important as general health because the general health directly or indirectly affects the fertility and quality of semen. Certain medications including antipsychotics, antidepressants, and anticonvulsants have negative influence on male fertility. Almost 50% of all infertility cases have a cause of male infertility. Male fertility has declined during the last 50 years, and sperm count per mL is reduced in seminal fluid. The long-term or short-term use of medication caused the reduction in male fertility. More specifically, the use of medication for a longer time period brutally affects the male fertility, while the short courses of medication caused temporary infertility.

The spermatogenesis is the process of development of spermatozoa through a series of multiple events. In testes, the process of spermatogenesis takes place in the lumen of seminiferous tubules, and almost 90% of testes consist of seminiferous tubules. In humans, the process of spermatogenesis continues at the age of puberty and continues throughout the life. The Sertoli cells in seminiferous tubules are involved in maturation of spermatozoa, while Leydig cells are involved in the release of the male sex hormone testosterone.

Reports indicate that the antidepressant drugs have toxic influence on body organs and the male reproductive system. Antidepressants are the most widely used treatment of depression, stress-, and anxiety-related diseases. A major class of antidepressants is the serotonin–norepinephrine reuptake inhibitor; similar to tricyclic antidepressants, it inhibits the reuptake of serotonin and norepinephrine, but it has lower adverse effects. Venlafaxine belongs to the serotonin–norepinephrine reuptake inhibitor, a major class of antidepressants. Different studies indicate that the antidepressants could have negative influence on the male reproductive system.

The prevalence of depressive disorder in Parkinson’s disease varies across the studies from 2.7 to more than 90%. Parkinson’s disease occurs more commonly in men.
than in women. Pramipexole is a clinically active non-ergot dopamine agonist. Parkinson’s disease is a gradually restricting neurodegenerative disorder treated mostly with a dopamine agonist, and pramipexole is most likely to be used for treatment. FDA approved some drugs, which impairs human spermatogenesis including pramipexole, but no data has been found on animals and humans. In Western countries, the major cause of heart failure is coronary artery disease, myocardial infraction (MI), and hypertension. The prevalence of heart failure in males is much higher than females. Most of the studies indicated that a worsening heart condition could cause negative impact on the sexual functionality. About 68% of men and 50% of women report that the sexual problem occurred with or without the diagnosis of a heart problem. Hypertension is highly associated with the increased incidence of sexual dysfunction. Angiotensin II plays a major role in pathogenesis of the clinical and experimental hypertension. Valsartan is a highly potent and selective antagonist of angiotensin II and used for the treatment of hypertension.

The prolonged exposure of medications for treatment of chronic diseases such as hypertension, depression, and Parkinson’s disease could be involved in affecting fertility. So, the goal of the study was to investigate whether the prolonged use of pramipexole, venlafaxine, and valsartan is involved in spermatogenic suppression or not. To analyze this, epididymal sperm analysis including sperm count, sperm motility, and sperm morphology was used to evaluate the DNA integrity of spermatozoa, effect on hormone levels including luteinizing hormone (LH), follicle-stimulating hormone (FSH), and testosterone; histopathological aspects of testes were planned to be performed. The exposure level of drugs was selected for a 70-day period based on the duration of the process of spermatogenesis in male rats, which is 35–54 days depending upon the species of rats. Also, estimated duration of spermatogenesis in humans is 74 days.

2. RESULTS

The treatment groups (venlafaxine, valsartan, pramipexole) received assigned doses once daily; orally for 70 days while testosterone treated group received 25 mg/kg dose; i.m. once a week.

2.1. Sperm Analysis by Measuring Different Parameters.

i- Sperm count

A highly significant ($p < 0.001$) increase in the sperm count (96.26 ± 2.4) was noted only in the group treated with a low dose (40 mg/kg) of valsartan, whereas its high dose (160 mg/kg) had a highly significant decrease (21.70 ± 1.6) among all treatment groups as compared to the control value (96.26 ± 2.4), indicating a positive effect of a low dose on male fertility (Table 1).

ii- Epididymal total sperm motility

Table 2 shows total sperm motility, which is a combination of progressive motility, progressive fast and slow motility, progressive circular motility, local motility, and immotile. A highly significant ($p < 0.001$) increase in total sperm motility was observed in groups treated with low and high doses (40 and 60 mg/kg) of valsartan and a high dose (0.05 mg/kg) of pramipexole; among them, valsartan at 40 mg/kg had the peak value (71.55 ± 0.7), whereas the testosterone-treated group showed moderately significant ($p < 0.01$) reduction in total sperm motility as compared to control values. Venlafaxine was found devoid of any significant variation with respect to the control group.

iii- Epididymal sperm morphology

Figure 1 shows the normal, hookless, bent tail, coiled/folded tail, tailless, and fused sperms observed under the microscope in different treatment groups, and Table 3 shows the value of each of above-mentioned morphology in percentage. The percentage of normal sperm in different treatment groups was found in the following descending order: control > pramipexole > testosterone > venlafaxine (150 mg/kg) > valsartan > venlafaxine (40 mg/kg).

iv- DNA integrity of spermatozoa

Semen from cauda epididymis was collected to evaluate the DNA integrity of spermatozoa by using the acidine orange (AO) staining method. All treatment groups showed a green-colored sperm head, which indicates that DNA was not damaged in those sperms and the integrity of double-stranded DNA remained intact. The percentage of the DNA fragmentation index (%DFI) of each treatment was calculated and was found to be 0% (Figure 2).

2.2. Effect of Treatments on Body Weight

Body weight of treated rats was increased significantly as compared to that of the control value (13.6%). Hence, a maximum increase (46.3%) was noted with pramipexole at 0.025 mg/kg treatment (Figure 3).

2.3. Wet Weight of Testes

Highly significant ($p < 0.001$) reduction in wet weight of the right and left testis of testosterone-treated rats was measured as compared to control group values. The rest of all other treatment groups had nonsignificant weight gain in the left testis except valsartan at 40 mg/kg, which showed significant ($p < 0.05$) weight gain with reference to control group values. Moderately significant ($p < 0.01$) reduction in wet weight of the right testis was measured with venlafaxine at 40 mg/kg and valsartan at 160 mg/kg, whereas highly significant reduction was calculated with testosterone and pramipexole at 0.05 mg/kg treatments as compared to the control. Hence, the rest of the treatment groups showed wet weight comparable to that of control group values (Figure 4).

Table 1. Effect of Treatments on the Epididymal Sperm Count

| treatment group | dose (mg/kg) | sperm count ($\times 10^6$/mL) |
|-----------------|-------------|--------------------------------|
| control         |             | 49.13 ± 2.3                    |
| testosterone    | 25          | 29.53 ± 2.4**                  |
| venlafaxine     | 40          | 44.92 ± 2.0                    |
|                 | 150         | 41.91 ± 2.2                    |
| valsartan       | 40          | 96.26 ± 2.4**                  |
|                 | 160         | 21.70 ± 1.6***                 |
| pramipexole     | 0.025       | 49.41 ± 0.9                    |
|                 | 0.05        | 30.35 ± 1.26***                |

*Data expressed as mean ± SEM. ($n = 10$). ***$p < 0.001$ with reference to the control. One-way ANOVA followed by Dunnett’s post hoc test was applied for statistical analysis.
2.4. Effect of Treatments on FSH, LH, and Testosterone Levels. A highly significant \((p < 0.001)\) decrease was recorded in the levels of LH and FSH of all the treatment groups except venlafaxine at 150 mg/kg as compared to control group values. Testosterone levels were increased highly significantly \((p < 0.001)\) in all the treatment groups with reference to the control value (Table 4).

2.5. Effect of Treatments on Hematology. Hb, RBC, total WBC, mean platelet volume (MPV), and plateletcrit (PCT) values of treatment groups showed nonsignificant \((p > 0.05)\) variation, whereas the platelet count of all groups except venlafaxine at 40 mg/kg (which showed significant variation; \(p < 0.05\)) had highly significant \((p < 0.001)\) variation as compared to that of control values (Table 5).

| treatment group | dose (mg/kg) | total sperm motility (%) | progressive motility (%) | progressive fast motility (%) | progressive slow motility (%) | progressive circular motility (%) | local motility (%) | immotile (%) |
|----------------|--------------|--------------------------|--------------------------|-----------------------------|-------------------------------|-----------------------------|------------------|-------------|
| control        |              | 59.11 ± 1.7              | 36.51 ± 1.0              | 18.10 ± 0.7                 | 17.19 ± 1.0                  | 1.51 ± 0.0                  | 19.90 ± 0.9     | 39.54 ± 1.0 |
| testosterone   | 25           | 50.10 ± 1.0**            | 37.82 ± 2.9              | 19.36 ± 0.8                 | 14.66 ± 0.8                  | 1.09 ± 0.0                  | 16.72 ± 1.5     | 49.28 ± 1.4**|
| venlafaxine    | 40           | 53.22 ± 2.2              | 37.87 ± 2.4              | 19.02 ± 1.3                 | 17.34 ± 2.2                  | 1.41 ± 0.07                  | 15.38 ± 0.5     | 50.86 ± 2.8***|
|                | 150          | 61.02 ± 0.5              | 49.83 ± 0.3***           | 24.14 ± 1.0                 | 24.01 ± 1.1                  | 1.68 ± 0.0                  | 11.93 ± 0.6*    | 39.27 ± 0.4  |
| valsartan      | 40           | 71.55 ± 0.7***           | 60.23 ± 0.9***           | 26.60 ± 2.0*                | 31.75 ± 1.9***               | 1.87 ± 0.1                  | 13.56 ± 1.0     | 24.47 ± 1.0***|
|                | 160          | 69.84 ± 0.7***           | 51.08 ± 5.0***           | 21.02 ± 2.9                 | 30.94 ± 2.6***               | 1.36 ± 0.2                  | 12.22 ± 0.7*    | 65.27 ± 5.7***|
| pramipexole    | 0.025        | 64.72 ± 1.1              | 51.0 ± 0.7***            | 24.19 ± 1.8                 | 25.23 ± 1.9*                 | 1.74 ± 0.2                  | 16.29 ± 1.5     | 34.77 ± 1.2  |
|                | 0.05         | 70.33 ± 3.4***           | 50.43 ± 0.6***           | 22.16 ± 0.8                 | 27.39 ± 1.4***               | 1.16 ± 0.1                  | 25.04 ± 2.6     | 32.67 ± 3.4  |
| pramipexole    | 0.025        | 64.72 ± 1.1              | 51.0 ± 0.7***            | 24.19 ± 1.8                 | 25.23 ± 1.9*                 | 1.74 ± 0.2                  | 16.29 ± 1.5     | 34.77 ± 1.2  |
|                | 0.05         | 70.33 ± 3.4***           | 50.43 ± 0.6***           | 22.16 ± 0.8                 | 27.39 ± 1.4***               | 1.16 ± 0.1                  | 25.04 ± 2.6     | 32.67 ± 3.4  |
2.6. Histopathological Analysis of Testes. The slides were observed under a light microscope at 10 and 40× magnification power. The control group showed normal histological texture. The seminiferous tubules (STs) showed the regular stages of spermatogenesis, and their lumen contained a huge number of mature spermatozoa (MS). The testosterone-treated group showed vacuolization in the ST and detachment of the basement membrane with few primary spermatogonia (PS). It also showed testicular degeneration (TD) with subsequent spermatocyte degeneration leading to inappropriate spermatogenesis phases. The process of TD happened only in a few of the ST while others showed the regular stages of spermatogenesis. Venlafaxine at a low dose showed a normal histological structure. The seminiferous tubules were found to have the regular stages of spermatogenesis with primary and secondary spermatoocytes, and the lumen of seminiferous tubules contained a huge number of mature spermatozoa, while venlafaxine at a high dose showed testicular degeneration in a few seminiferous tubules. Valsartan at both dose levels showed the normal and regular histological texture with systemic organization of spermatogenesis having primary and secondary spermatocytes. Also, the lumen of seminiferous tubules contained a huge number of mature spermatozoa. Pramipexole at a high dose showed testicular degeneration of meiotic spermatocytes resulting in depletion of spermatogenesis. It usually happens

Table 3. Effect of Treatments on Sperm Morphology

| treatment group | dose (mg/kg) | normal (%) | hookless (%) | bent tail (%) | coiled/folded tail (%) | tailless (%) | fused (%) |
|----------------|--------------|------------|--------------|--------------|------------------------|-------------|----------|
| control        | 74.5         | 0.98       |              | 5.5          | 8.6                    | 0.0         | 10.4     |
| testosterone   | 25           | 62.6***    | 2.9          | 10.8**       | 17.9***                | 0.0         | 5.4      |
| venlafaxine    | 40           | 43.3***    | 2.97         | 25.4***      | 25.2***                | 0.46        | 2.5*     |
|                 | 150          | 56.1***    | 3.34         | 16.1***      | 20.5***                | 0.85        | 3.1      |
| valsartan      | 40           | 50.2***    | 5.8          | 18.2***      | 21.2***                | 0.8         | 3.51     |
|                 | 160          | 54.3***    | 2.3          | 16.6***      | 23***                  | 0.6         | 3.1      |
| pramipexole    | 0.025        | 66.98      | 3.1          | 12***        | 11.7***                | 0.48        | 5.73     |
|                 | 0.05         | 64.6***    | 1.37         | 11.5**       | 17.1***                | 1.04        | 4.3      |

*Data expressed as percentage (n = 10). ***P < 0.001, **P < 0.01, and P < 0.05 in comparison with the control. Two-way ANOVA followed by the Bonferroni post hoc test was applied for statistical analysis.

Figure 2. DNA integrity of spermatozoa analyzed by acridine orange (AO) staining technique. Green-headed spermatozoa: undamaged DNA.

Figure 3. Effect of testosterone, venlafaxine, valsartan, and pramipexole on the body weight of rats. Data expressed as mean ± SEM (n = 10). ***P < 0.001 as compared to the control. One-way ANOVA followed by Dunnett’s post hoc test was applied for statistical analysis. LD: low dose, HD: high dose.

Figure 4. Effect of testosterone, venlafaxine, valsartan, and pramipexole on wet weight of right and left testes (gm). Data expressed as mean ± SEM (n = 10). ***P < 0.001 with reference to the control. One-way ANOVA followed by Dunnett’s post hoc test was applied for statistical analysis.
when the drug crosses the blood testis barrier (BTB) and possibly exerts an effect on the Sertoli cells. Vasculization of seminiferous tubules and detachment of the basement membrane were also noticed. Only the limited seminiferous tubules showed the regular histopathological texture with normal phases of spermatogenesis and mature spermatozoa (Figure 5).

3. DISCUSSION

The main focus of the current study was to rule out any effect of drugs (venlafaxine, valsartan, and pramipexole) used as chronic treatments on male fertility. The key findings of this study show that DNA integrity of spermatozoa after treatment with these drugs remained intact. Both doses of venlafaxine and pramipexole and a high dose of valsartan reduced fertility by decreasing the sperm count. Valsartan at 40 mg/kg did not affect fertility but rather increased the sperm count as compared to control values. Tannikut and Schlegel concluded in their case studies that antidepressants decreased the sperm count and motility and also affect sperm morphology.42 Our study on rats with venlafaxine is in the same direction as that of Tannikut and Schlegel.

Valsartan selectively binds with the angiotensin I receptor and showed vasoconstriction.41 In the current study, valsartan showed a dose-related effect on semen quality. As at an LD of valsartan ameliorated the semen parameters like an increased sperm count and total sperm motility with less percent of immotile sperm, while at an HD showed reverse action on the semen parameter like a reduced sperm count and total sperm motility with increased percent of immotile sperm, which would probably be due to the presence of angiotensin II receptors in seminiferous tubules and thought to be involved in sperm motility.42 The earlier studies revealed that the angiotensin II receptor present in male reproductive organs including seminiferous tubules and epididymis of humans and in rodents and assumed that they could have been involved in testicular functions.43,44 Also, valsartan did not show any significant effect on the percent number of normal sperm morphology and lesser extent of head and tail abnormalities of spermatozoa.

Pramipexole at both dose levels (LD and HD) revealed reduction in semen parameters including the sperm count, percent number of normal sperm morphology and lesser extent of head and tail abnormalities of spermatozoa. Also, valsartan did not show any significant effect on the percent number of normal sperm morphology and lesser extent of head and tail abnormalities of spermatozoa.

Table 4. Effect of Treatments on LH, FSH, and Testosterone*.

| treatment group | dose (mg/kg) | LH (mIU/mL) | FSH (mIU/mL) | testosterone (ng/mL) |
|-----------------|--------------|-------------|--------------|---------------------|
| control         |              | 0.117 ± 0.003 | 0.150 ± 0.004 | 0.506 ± 0.006 |
| testosterone    | 25           | 0.002 ± 0.001 *** | 0.002 ± 0.001 *** | 18.169 ± 0.195 *** |
| venlafaxine     | 40           | 0.013 ± 0.004 *** | 0.051 ± 0.033 *** | 4.996 ± 0.296 *** |
| valsartan       | 40           | 0.101 ± 0.03 | 0.00 ± 0.00 *** | 4.659 ± 0.410 *** |
| pramipexole     | 0.025        | 0.054 ± 0.003 *** | 0.006 ± 0.003 *** | 5.725 ± 0.145 *** |
|                 | 0.05         | 0.041 ± 0.003 *** | 0.022 ± 0.006 *** | 3.260 ± 0.015 *** |

*Data expressed as mean ± SEM. (n = 10). *** P < 0.001, in comparison with the control. Two-way ANOVA followed by the Bonferroni post hoc test was applied for statistical analysis.

Table 5. Effect of Treatments on Hematology*.

| treatment group | dose (mg/kg) | Hb (g/dL) | RBC (10^6/μL) | total WBC (10^3/μL) | platelet count (10^3/μL) | MPV (fL) | PCT (%) |
|-----------------|--------------|-----------|---------------|---------------------|-------------------------|----------|---------|
| control         |              | 12.53 ± 0.12 | 6.77 ± 0.06 | 7.48 ± 0.07 | 662.1 ± 5.0 | 7.46 ± 0.06 | 0.42 ± 0.04 |
| testosterone    | 25           | 12.417 ± 0.08 | 6.73 ± 0.04 | 6.46 ± 0.08 | 290.6 ± 0.34*** | 8.13 ± 0.05 | 0.27 ± 0.01 |
| venlafaxine     | 40           | 14.06 ± 0.08 | 7.91 ± 0.07 | 6.53 ± 0.3 | 708.9 ± 0.107*** | 8.93 ± 0.11 | 0.62 ± 0.00 |
| valsartan       | 40           | 14.09 ± 1.35 | 7.83 ± 0.08 | 8.14 ± 0.52 | 783.3 ± 1.27*** | 7.88 ± 0.07 | 0.64 ± 0.03 |
| pramipexole     | 0.025        | 13.89 ± 0.09 | 7.81 ± 0.08 | 8.23 ± 0.05 | 624.8 ± 0.395*** | 8.48 ± 0.10 | 0.52 ± 0.03 |
| pramipexole     | 0.05         | 14.33 ± 0.09 | 8.47 ± 0.05 | 11.05 ± 0.13 | 805.9 ± 1.36*** | 8.28 ± 0.06 | 0.67 ± 0.01 |

*Data expressed as mean ± SEM (n = 10). ***P < 0.001 and *P < 0.05 in comparison with the control. Two-way ANOVA followed by the Bonferroni post hoc test was applied for statistical analysis. MPV: mean platelet volume, PCT: plateletcrit.
progressive motility, and immotile percent of sperm, which might be due to the presence of the D2 receptor in testes and have a direct interaction with germ cell proliferation during spermatogenesis. Pramipexole also showed the increased percent number of normal morphology of spermatozoa but also showed head and tail defects. Pramipexole as a neuroprotective drug acts on D2 receptors in seminiferous tubules and causes the activation of D3 and the catecholaminergic effect in Leydig cells, which is involved in spermatogenic regulation. The sperm DNA integrity is the biomarker of the fertility and greatly related to the sperm motility, which mostly provides functional measurements for the sperm function.

In the current study, venlafaxine showed the normal DNA integrity of spermatozoa, which has indicated that the venlafaxine did not cross the blood testis barrier (BTB) to interfere with the germ cell cycle. The venlafaxine is a protein-bound drug, and the BTB does not allow the passage of protein-bound substances. Normal DNA integrity of spermatozoa with valsartan was determined that it might be due to the drug’s antioxidant, anti-inflammatory, and antiapoptotic properties. Similarly, pramipexole showed the normal DNA integrity of spermatozoa, which is attributed to the intracellular presence of dopamine receptors in spermatozoa and antioxidant and anti-inflammatory properties of pramipexole. Normally, dopamine acts as a physiological modulator and is involved in sperm cell viability, capacitation, and motility.

Previous studies suggested that the prolonged use of venlafaxine caused an increase in body weight, which was not reduced even after having a diet plan and exercise. Our study also supported it as venlafaxine at both doses significantly increased the body weight. Venlafaxine is an antidepressant drug, and these drugs caused the increased activation of TNF-α cytokine and leptin, which leads to the weight gain. Valsartan also showed increased body weight that may be due to the presence of angiotensin II receptors in adipose tissues, which are generally involved in secretion of angiotensinogen from adipocyte that caused vasoconstriction and releases aldosterone leading to sodium and water retention. Valsartan as an antagonist of such receptors caused the activation of the renin–angiotensin system (RAS), leading to increased renal reabsorption of sodium, which indirectly affects the body weight. The literature showed a direct link of obesity with hypertensive patients taking an angiotensin blocker or ACE inhibitor.

In the present study, the valsartan at a low dose did not affect the wet weight of the testis while at a high dose, it showed reduction in wet weight of both testes, which may be due to the angiotensin II linked with the hypothalamic–pituitary–thyroid axis because a low dose showed an increased testosterone level and improved semen parameters, while a high dose showed reduction in wet weight of the testis, testosterone level, and also reduction in semen parameters. In the current study, pramipexole also showed reduction in wet weight of both testes, which might be due to the low levels of testosterone and less volume of testosterone in testes. As explained in previous studies, size of testes has strong association with sperm production and levels of testosterone.

The FSH, LH, and testosterone levels are the benchmarks for evaluating the male fertility, and their increased levels indicate the male infertility. Previous studies concluded that venlafaxine did not affect the levels of FSH, LH, prolactin, and testosterone. Meanwhile in the present study, venlafaxine at both doses caused reduction in FSH and LH levels while increasing the testosterone level, which might be due to the neuroendocrine factors as the serotonin increases the prolactin level by inhibiting the dopamine, and an increased level of dopamine suppresses GnRH at the pituitary axis, which resulted in the reduction in FSH and LH levels. D2 receptors are present on the gonadotrophin cell line, which negatively regulates the expression of gonadotrophin mRNA response and suppress the GnRH secretion. It was reported in previous studies that venlafaxine reduced the testosterone level, and its level rose with discontinuation of medication. In the present study, the testosterone level was increased that might be related to the number of sperm production, which would be due to the increased germ cell production induced by testosterone. Valsartan showed reduction in FSH and LH levels at both doses, whereas an increased level of testosterone at a low dose and reduction in the testosterone level at a high dose would be due to the angiotensin II interaction with endocrine parameters of the reproductive function. Normally, angiotensin receptors present in Leydig cells of testes are involved in production of the androgen hormone and concerned in local regulation of testes by the pituitary luteinizing hormone. The angiotensin receptor inhibition on Leydig cells caused cessation of androgen hormone secretion. Pramipexole showed reduction in FSH and LH levels necessary for spermatogenesis along with the decreased level of testosterone, which caused cessation of spermatogenesis, lowered the sperm count and motility, and disturbed epididymal functions.

In the present study, the venlafaxine showed an increased platelet count when compared with the control, while a previous study revealed an opposite effect, i.e., a decrease in the platelet count with venlafaxine. Valsartan showed a dose-dependent increase in the platelet count, which is due to the presence of angiotensin receptors on the pluripotent stem cells that are involved in cell proliferation and also induced DNA synthesis, leading to an increased platelet count. An increased platelet count with pramipexole was noted, which might be due to the presence of D3 and D5 receptors on platelets, which are involved in the regulation of platelet function. The dopaminergic neuron innervation to the pluripotent stem cell might be involved in cell proliferation.

4. CONCLUSIONS

The use of venlafaxine and pramipexole at a low dose is devoid of any harmful effect on spermatogenesis. A high dose of pramipexole and valsartan may adversely affect fertility by a significant decrease in the sperm count, whereas a low dose enhanced fertility by increasing the sperm count and motility.

5. MATERIALS AND METHODS

5.1. Drugs and Chemicals. Valsartan and venlafaxine hydrochloride were gifted by CCL Pharmaceuticals and from
Wilshire Labs. Pramipexole dihydrochloride was obtained from the RIPS post-graduate laboratory. Testosterone enanthate was purchased from a local retail pharmacy. Sodium chloride, potassium chloride, disodium hydrogen phosphate, potassium dihydrogen phosphate, carboxymethyl-cellulose, sodium bicarbonate, trypan blue, eosin, nigrosin, and formaldehyde manufactured by Riedel-de-Haën were purchased from an authenticated local supplier dealing with Sigma-Aldrich.

5.2. Animal Husbandry. Healthy albino male rats breed in the animal house of Riphah Institute of Pharmaceutical Sciences, Lahore-Pakistan weighing 200–250 gm were used in the present study. The rats were housed in polycrylic cages with not more than four animals per cage and maintained under standard laboratory conditions with a 12 h dark and light cycle at room temperature (22 ± 3 °C) and a humidity of 30–70%. They were fed chow and water ad libitum.

5.3. Ethical Approval. The experimental work was performed after getting ethical approval on the experimental design by the Research Ethical Committee of Riphah Institute of Pharmaceutical Sciences, Lahore-Pakistan, which is ruled under the regulation of the Institute of Laboratory Animal Resources, Commission on Life Sciences University, National Research Council (1996). The reference number was REC/RIPS/LHR/2017/001.

5.4. Experimental Design. Eighty male albino rats were randomly divided into eight groups (n = 10). Group I served as a control, receiving 0.5% CMC solution throughout the experimental period. Group II received testosterone (25 mg/kg) dissolved in vegetable oil, intramuscularly (i.m.) administered once weekly for a period of 10 weeks. Groups III and IV received venlafaxine hydrochloride at dose levels of 40 and 150 mg/kg respectively. Groups V and VI were treated with 40 and 160 mg/kg valsartan, respectively. Groups VII and VIII were given pramipexole dihydrochloride at doses of 0.025 and 0.05 mg/kg, respectively. All the doses were prepared in 0.5% CMC solution. All the rats were treated with 40 and 150 mg/kg respectively. Groups V and VI were administered once weekly for a period of 10 weeks. Groups III and IV received venlafaxine hydrochloride at dose levels of 40 and 150 mg/kg respectively. Groups V and VI were treated with 40 and 160 mg/kg valsartan, respectively. Groups VII and VIII were given pramipexole dihydrochloride at doses of 0.025s and 0.05 mg/kg, respectively. All the doses were prepared in 0.5% CMC solution. All the rats were treated for a period of 10 weeks, and doses were given orally with the help of a gavage tube once daily.

At the end of the study, rats were anesthetized with isoﬂurane, which was diluted with 5% of oxygen. Blood samples were collected by cardiac puncture for assessment of the luteinizing hormone (LH), follicle-stimulating hormone (FSH), and testosterone concentration. The hormone levels in the blood sample were assessed with ELISA.

5.5. Collection of Sperm Samples for Fertility Analysis by a Diffusion Method. After blood sampling, all the rats were killed humanely by cervical dislocation, and orchidectomy was performed by the castration method. Incision was made on a prescrotal line, and testicles were oozed out. Cauda epididymis was removed from each animal and placed in a Petri dish containing 1 mL of buffer phosphate saline (pH 7.4). The Petri dish was swirled, allowing the spermatozoa to diffuse into the buffer at 37 °C. After 20 min, the cauda epididymis was picked out from the medium, and sperm suspension was collected. The sperm suspension was then analyzed for sperm concentration, motility, DNA integrity of spermatozoa, and morphological parameters of spermatozoa including normal, hookless, bent tail, coiled/folded tail, tailless, and fused.

5.6. Sperm Analysis by Measuring Different Parameters. The underlying parameters were measured to appraise sperm activity.

i- Sperm count

For evaluating the sperm count, after an hour of sperm diffusion in solution, a 10 μL aliquot of epididymal sperm solution was diluted by 190 μL of the diluent (0.35% formalin containing 5% NaHCO3 and 0.25% trypan blue). The 10 μL of diluted solution was placed on the Neubauer chamber and allowed to stand for 5 min in order to prevent dryness then analyzed under a light microscope at 40× magnification. The sperm count is expressed as million/mL.

ii- Sperm motility

The epididymal sperms were collected by the diffusion method described by Klinefelter and colleagues. Immediately after the diffusion of sperm in surrounding solution, a drop of sperm suspension was placed on the glass slide, and a cover slip was placed carefully over it. The slide was placed under the phase contrast microscope at 40× magnification, and at least five microscopic fields were observed.

By using a computer-assisted sperm analysis system (Minitube, Germany), the sperm motility was recorded as per WHO (1999) recommendations. More than 500 sperms were examined per rat for motility parameters including progressive motility, progressive fast and slow motility, local circular motility, local motility, and immotile sperms. Randomly, about five fields from each rat’s slides were observed for sperm motility.

iii- Sperm morphology

The sperm morphology was revealed by using 1% eosin and 5% nigrosin dye. Then, 40 μL of epididymal sperm suspension was taken in test tube, and 10 μL of eosin nigrosin dye was added in a test tube and mixed well. Then, 10 μL was taken from the test tube, placed on a glass slide, and examined under a phase contrast microscope at 40× magnification. The classification of sperm head and tail morphological parameters of spermatozoa including normal, hookless, bent tail, coiled/folded tail, tailless, and fused were observed and counted.

iv- Quantification of DNA integrity of spermatozoa

The acridine orange (AO) staining technique is the suggested screening test to predict the spermatozoa DNA abnormality. AO staining is cell permeable and interacts with DNA and RNA by intercalation or electrostatic attractions, respectively. When bound to DNA, it is very similar spectrally to fluorescein, with an excitation maximum at 502 nm and an emission at 530 nm (green). When it associates with RNA, the excitation shifts to 460 nm (blue), and the emission shifts to 650 nm (red). Initially, slides were prepared prior to AO staining. Briefly, the method is as follows: 100–200 μL of aliquot of the semen sample was mixed in 1 mL of phosphate buffer saline (pH 7.4). It was mixed well, and a loopful sample was smeared on a glass slide and air dried. The smear was fixed overnight with Carnoy’s solution (methanol and acetic acid with a 3:1 ratio, respectively).
5.7. Preparation of AO Stain. AO (1 mg/mL) was dissolved in distilled water in the flask wrapped with an aluminum foil and kept at 4 °C.33

5.8. AO Staining Method. After fixation, the slides were dipped in the AO stain. Then, slides were removed from the staining solution and rinsed with distilled water to remove the excess stain from the slide. Next, slides were evaluated with the help of a fluorescent microscope at the wavelength 450—490 nm.35 About 200 spermatozoa were analyzed; green fluorescence of the spermatozoa head indicated the normal or undamaged DNA, and yellow to orange or red fluorescence indicated the damaged DNA of spermatozoa. The DNA fragmentation index (DFI) was calculated by using the following formula:

$$\text{DFI} = \frac{\text{yellow to red}}{\text{green + yellow to red}} \times 100$$

A DFI value $< 30\%$ was considered as normal.36

5.9. Histopathology of Testes. Testes from each rat's scrotum were surgically removed and fixed in 4% p-formaldehyde and formol saline solution.37 In paraffin, the tissue samples of about 5 μm thicknesses were implanted, stained with acid-Schiff, then stained with hematoxylin, and observed under a light microscope.

5.10. Statistical Analysis. The data were expressed as mean ± SEM and statistically analyzed by using GraphPad Prism version 5.0. One-way ANOVA followed by Dunnett’s post hoc test and two-way ANOVA followed by the Bonferroni post hoc test were applied. $p < 0.001$, $p < 0.01$, and $p < 0.05$ were set as highly significant, moderately significant, and significant, respectively.

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Author Contributions
U.S. drafted manuscript. S.Z. performed the whole experimental work. A.R. provided technical facilities and assisted in performing the experiment. F.A. did statistical analysis. B.A. supervised the project.

Notes
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