

Original Research Article

CHRONIC EFFECTS OF ATYPIC ANTIPSYCHOTICS ON MICE ISOLATED VAS DEFERENS CONTRACTILITY; AN IN VITRO STUDY

ABSTRACT

Aims: Antipsychotic drugs are known to be commonly associated with sexual dysfunction. The aim of this study to investigate the effects of sertindole, asenapine and ziprasidone on serotonin (5-HT), noradrenaline (NA), adenosine triphosphate (ATP) and potassium chloride (KCl)-induced contractions of the **isolated** vas deferens in mice.

Study design: All the drugs were administered intraperitoneally (i.p.) in a volume of 0.1 ml per 10 g body weight of mice.

Place and Duration of Study: Department of Pharmacology, Kocaeli University, Animal Research Center, between May 2018 and August 2019.

Methodology: The mice were randomly divided into groups (n=7) as follows: saline; sertindole 1.3 mg/kg; sertindole 2.5 mg/kg; asenapine 0.05 mg/kg; asenapine 0.075 mg/kg; ziprasidone 1 mg/kg; ziprasidone 2 mg/kg **once daily for 21 days**. Mice receiving only the vehicle (0.9% saline, i.p.) served as control group. After 21 days of treatment, the effects of drugs were investigated on 5-HT, NA, ATP and KCl-**induced contractions of isolated vas deferens**.

Results: The results showed that serotonin-induced contractions of vas deferens were affected by the chronic treatments of sertindole, asenapine and ziprasidone, however these drugs had no significant effect on NA, ATP, and KCl-induced contractions of mice vas deferens.

Conclusion: Serotonergic receptors may contribute to changes in vas deferens contractions in mice with chronic treatment of sertindole, asenapine and ziprasidone. Thus, our results may explain one of the causes of sexual dysfunction of sertindole, asenapine and ziprasidone.

Keywords: Asenapine; sertindole; ziprasidone; in vitro; vas deferens ;**erectile dysfunction;**

1. INTRODUCTION

Neurobiological, physiological, and psychological factors play role in the etiology of erectile dysfunction [1]. Sexual function is controlled by different sections of the brain, including the medial preoptic area, para-gigantocellular reticular nucleus, cerebellum, stria terminalis, amygdala, and thalamus. Sympathetic nerves originating from T10-L2 and parasympathetic

nerves originating from S2-4 play a role in peripheral control of ejaculation. Serotonin (5-HT), endothelin, oxytocin, gamma aminobutyric acid (GABA), and nitric oxide (NO) are neurotransmitters that play an active role in this process. While the medial preoptic area stimulates ejaculation via 5-HT1a, the para-gigantocellular reticular nucleus inhibits ejaculation through 5-HT1b and 5-HT2c receptors. Because of this process, selective serotonin re-uptake inhibitors (SSRIs) are used in the treatment of premature ejaculation [2].

Inhibition of testosterone secretion due to high prolactin level affects sexual function. Many antipsychotics can increase prolactin release, that can cause many side effects as sexual dysfunction, anovulation, galactorrhea and gynecomastia. All first-generation antipsychotics like risperidone, paliperidone, and amisulpride are prolactin-inducing group; clozapine, quetiapine, ziprasidone, and aripiprazole are prolactin-sparing group [3]. These antipsychotics cause an increase in prolactin levels and consequently, lead to a decrease in testosterone levels, which causes a reduction in sexual desire [4]. Otherwise, hypothalamic dopamine has an inhibitory effect on prolactin release. Antipsychotic drugs that reduce dopaminergic activity lead to increase in prolactin levels. Contrary to the suppressive effect of dopamine on prolactin, an increase in 5-HT activity causes prolactin release [5].

Ejaculation consists of two phases: (1) emission and (2) expulsion. Their control is provided by both the autonomic and somatic nervous systems. The emission phase is generally under the control of sympathetic nervous system through noradrenaline (NA), ATP, NO, vasoactive intestinal peptide (VIP), and neuropeptide-Y neuromediators. The expulsion phase is under the influence of autonomic and somatic nervous system through the NA, acetylcholine, ATP, NO, and VIP neurotransmitters [6]. The reflex center, also called the spinal ejaculation generator center is located at the thoracolumbar level and provides coordination of sympathetic and parasympathetic neurons [7]. Moreover, the presence of 5-HT nerve fibers have been demonstrated in vas deferens, seminal vesicles, prostate gland, and urethra [8].

Hyperprolactinemia and sedation are observed due to various mechanisms. Especially antipsychotics used for the treatment of schizophrenia cause hyperprolactinemia and sedation. Erectile dysfunction is associated with antipsychotic agents and changes in many neurotransmitter receptors (alpha adrenergic, dopaminergic, histaminergic, and muscarinic) [9].

The mechanism of antipsychotics that triggers erectile dysfunction has not been clearly revealed. Also, psychological factors should be taken into account. It is shown that the ejaculation occurs because of the rhythmic contractions in the vas deferens. Hence, reduced motility of the vas deferens can cause delayed ejaculation, whereas increased motility causes premature ejaculation [10]. The studies on the effects of atypical antipsychotic drugs are very limited. In the direction of all these findings, the aim of this work was to find out the effects of chronic administration of aripiprazole, risperidone, and ziprasidone use on contractions of the vas deferens in mice.

2. METHODOLOGY

2.1 Animals

Male inbred BALB/c ByJ mice (Animal Research Center, Bursa-Turkey) aged seven weeks upon arrival at the laboratory were used in this study in Kocaeli University laboratory in 2019. Animals (4–5 per cage) were kept in the laboratory at 21 ± 1.5 °C with 60% relative humidity under a 12 h light/dark cycle (light on at 8:00 p.m.) for two weeks before starting the experiments. Tap water and food pellets were available ad libitum. All authors hereby

declare that "Principles of laboratory animal care" (NIH publication No. 85-23, revised 1985) were followed, as well as specific national laws where applicable. All experiments have been examined and approved by the appropriate ethics committee. Ethical approval was granted by the Kocaeli University Ethics Committee (Date: 22.07.2014 and Number: KOÜ HADYEK 7/4-2014 Kocaeli, Turkey).

2.2. Drugs

Sertindole, asenapine, ziprasidone, serotonin, noradrenaline, ATP, and potassium chloride (KCl) were obtained from Sigma Chemicals (St Louis, Mo, USA). All drugs were dissolved in 0.9 % physiological saline. Saline was used as the vehicle controls Sertindole, asenapine, and ziprasidone were administered intraperitoneally (i.p.) in a volume of 0.1 ml per 10 g body weight of mice. Drugs were prepared fresh on the day of experiment.

2.3. Experimental Design

The mice were randomly divided into experimental groups (n = 7): (1) saline, (2) sertindole 1.3 mg/kg, (3) sertindole 2.5 mg/kg, (4) asenapine 0.05 mg/kg, (5) asenapine 0.075 mg/kg, (6) ziprasidone 1 mg/kg, and (7) ziprasidone 2 mg/kg. Chronic treatment was carried out by intraperitoneal drug injection (i.p.) for 21 days. The control group mice received i.p. (0.9% saline) during the course of the experiment.

Mice were sacrificed after 21 days of treatment followed by the removal of vas deferens from each side. Later, each vas deferens was separated into prostatic and epididymal portions of 1 to 2 cm in length.

The epididymal and prostatic portions of vas deferens were surgically dissected free and soaked in 20 mL organ baths containing Krebs's Ringer solution with a composition (mM) of 113 NaCl, 4.8 KCl, 2.5 CaCl₂, 1.2 KH₂PO₄, 1.2 MgSO₄, 25 NaHCO₃, and 11.7 glucose and then equilibrated with 95%O₂/5%CO₂ at 37 °C during the study. The tissues were connected to an isometric force transducer (FDT 10 A Commat Iletisim, Ankara, Turkey) for the measurement of isometric force, which was continuously recorded on a computer via a four-channel transducer data acquisition system (MP150 Biopac Systems Inc. Goleta) using software (ACQ4.0 Biopac Systems Inc. Goleta) that also could analyze the data. The upper end of the tissue was attached to an isometric force transducer (FDT 10 A Commat Iletisim, Ankara, Turkey) to measure the isometric force, and the lower end was fixed.

Equilibration was done so that each strip was exposed to a basal tension of 1 g for 1h after the tissue was assembled. At the end of the equilibration, the strips were depolarized in Krebs solution with 80 mM KCl and left to equilibrate for 30 min. The Krebs Henseleit solution was replaced with a new solution every 15 min. After equilibration, the dose-response curves to serotonin (ranging from 10⁻⁸ to 10⁻⁴ M), NA (10⁻⁸ to 10⁻⁴ M), and ATP (10⁻⁸ to 10⁻⁴ M) were obtained cumulatively. Each response was expected to plateau, after which the next drug bolus was added. After the dose-response graphs of the drugs were complete, the tissues were washed for another 30 min.

2.4. Statistics

Results were expressed as the mean ± standard error of the mean (S.E.M.) of different experiments. Contractile responses to 5-HT, NA, and ATP were calculated as a percentage of the maximal contraction caused by KCl (80 mM). Statistical comparison between the

groups was performed using analysis of variance (ANOVA) supported by Dunnett's post-hoc test. Results were considered to be significantly different at a **P-value of < 0.05**.

3. RESULTS

Results of isolated organ bath experiments demonstrated that 5-HT-induced contractions had significantly increased in both prostatic and epididymal portion of the mice vas deferens obtained from the sertindole (1.3 and 2.5 mg/kg) groups; epididymal data are shown in Figure 1 ($p < 0.05$). The Emax value for 5-HT was significantly higher in prostatic and epididymal portions of the mice vas deferens obtained from the sertindole-treated groups than in the control group ($P < 0.05$; Table 1).

Table. 1. Emax (% of 80 mM KCl) values for serotonin, noradrenaline, ATP, and Emax value (mg) for 80 mM KCl in vas deferens obtained from sert (sertindole), asen (asenapine), zipr (ziprasidone) treatment and control mice (n=7).

	Control	Zipr 1	Zipr 2	Asen 0,05	Asen 0,075	Sert 1,3	Sert 2,5
Epididimal							
KCl	1496±196	1366±124	1294±123	1356±178	1382±192	1284±226	1382±194
Serotonine	23±2	42±4*	43±4*	46±5*	48±5*	54±5*	65±6*
NA	43±4	42±4	41±4	40±4	39±4	42±4	41±4
ATP	39±4	39±4	38±4	38±4	36±4	37±4	35±3
Prostatic							
KCl	1946±156	1986±263	2118±274	1958±196	1896±176	1872±181	1846±192
Serotonine	15±2	19±2*	21±2*	24±3*	28±3*	30±3*	35±4*
NA	17±2	17±2	16±2	15±2	14±2	16±2	15±2
ATP	26±3	26±3	25±3	24±2	23±2	25±3	24±3

Data are presented as mean ± SEM. Significance differences were found at * $p < 0.05$.

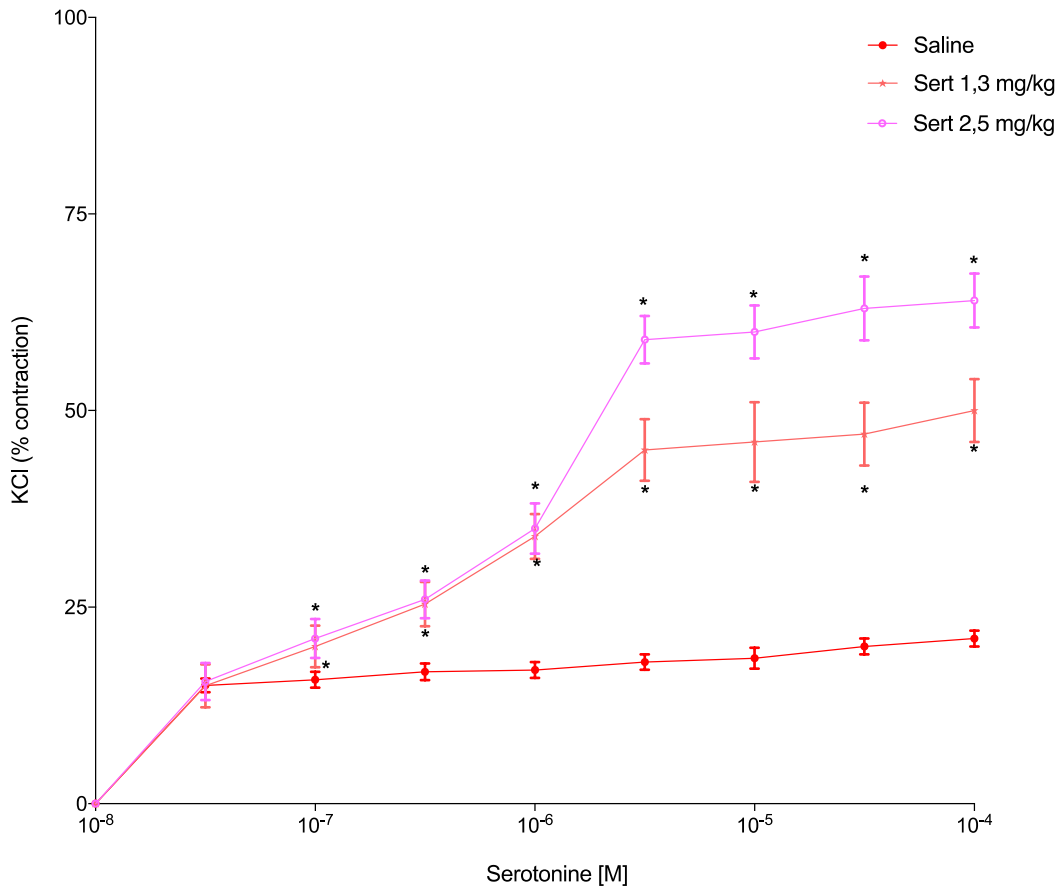


Fig. 1. Serotonin concentration-responses curves of sert (sertindole) in isolated epididymal segment of mice vas deferens smooth muscle.

*Each point is expressed as a percentage of the contraction induced by 80 mM KCl is given as the mean \pm standard error of the mean (SEM). Significance differences were found at * $p < 0.05$.*

The findings of the study clearly showed that 5-HT-induced contractions had significantly increased in both prostatic and epididymal portion of the mice vas deferens obtained from the asenapine (0.05 and 0.075 mg/kg) groups when compared with the control group. Epididymal data are shown in Figure 2 ($p < 0.05$). The E_{max} value for 5-HT was significantly higher in prostatic and epididymal portions of the mice vas deferens obtained from asenapine-treated groups than in the control group ($P < 0.05$; Table 1).

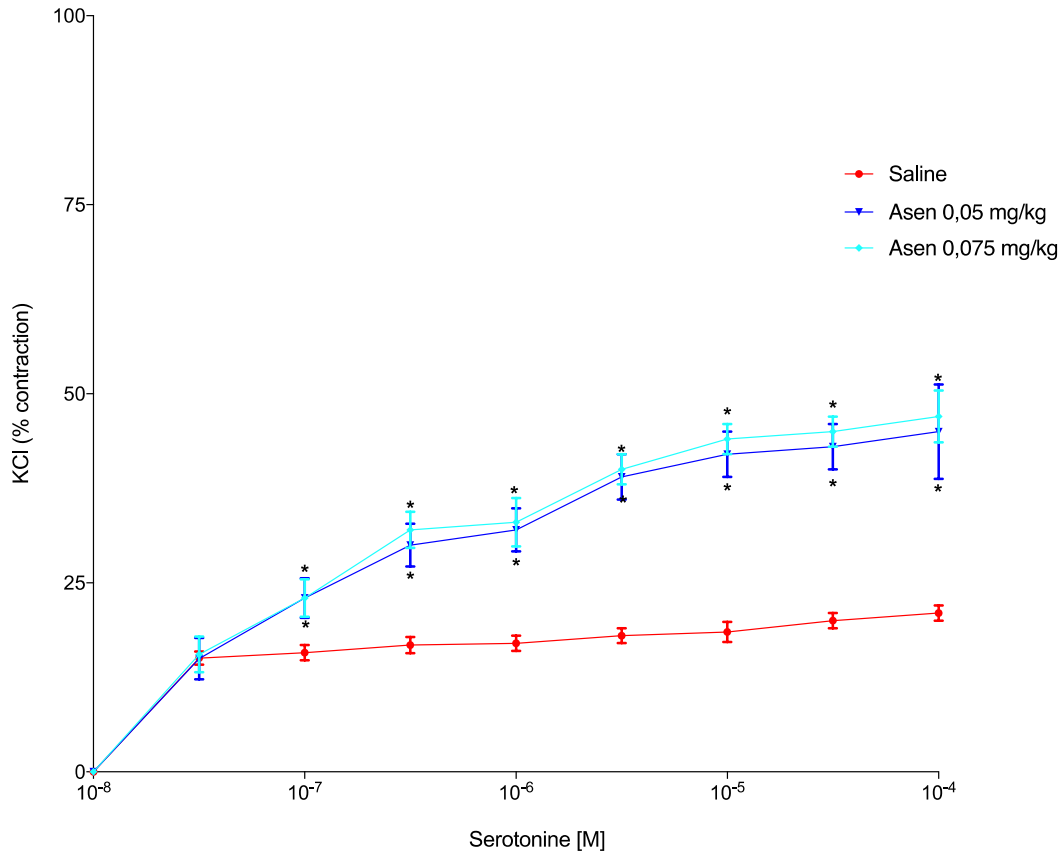


Fig. 2. Serotonin concentration-responses curves of asen (asenapine) in isolated epididymal segment of mice vas deferens smooth muscle.

*Each point is expressed as a percentage of the contraction induced by 80 mM KCl is given as the mean \pm standard error of the mean (SEM). Significance differences were found at * $p < 0.05$.*

In addition, 5-HT-induced contractions had significantly increased in both the prostatic and epididymal portion of the mice vas deferens obtained from ziprasidone (1 and 2 mg/kg) groups when compared with control group. Epididymal data are shown in Figure 3 ($p < 0.05$). The E_{max} value for 5-HT was significantly higher in the prostatic and epididymal portions of the mice vas deferens obtained from the ziprasidone-treated groups than from the control group ($P < 0.05$; Table 1).

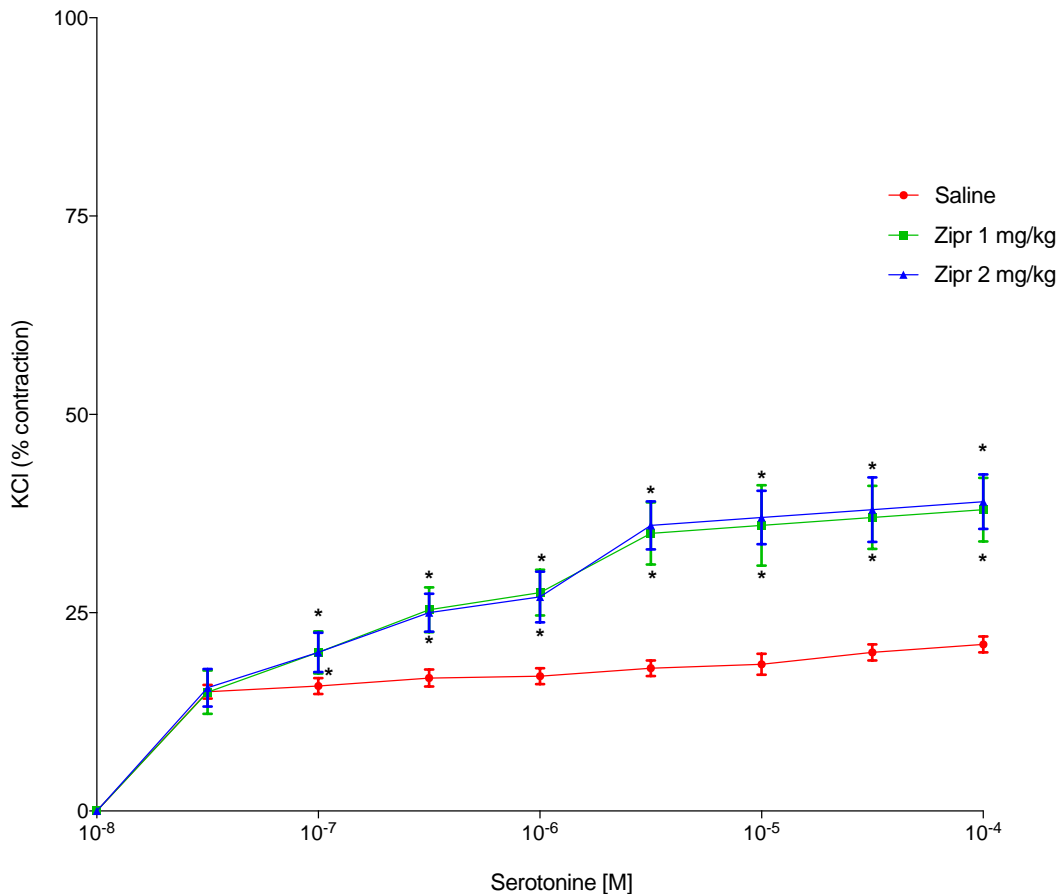


Fig. 3. Serotonin concentration-responses curves of zipr (ziprasidone) in isolated epididymal segment of mice vas deferens smooth muscle.

*Each point is expressed as a percentage of the contraction induced by 80 mM KCl is given as the mean \pm standard error of the mean (SEM). Significance differences were found at * $p < 0.05$.*

Sertindole (1.3 and 2.5 mg/kg), asenapine (0.05 and 0.075 mg/kg), and ziprasidone (1 and 2 mg/kg) treatments had no effects on the NA-induced contractile responses in either portion of the vas deferens. The E_{max} value for noradrenaline was not affected by sertindole, asenapine, and ziprasidone treatment and was not significantly different from the control group as shown in Table 1 ($p > 0.05$).

ATP had no significant effects on either the prostatic or epididymal portion of the mice vas deferens after treatment with sertindole (1.3 and 2.5 mg/kg), asenapine (0.05 and 0.075 mg/kg), or ziprasidone (1 and 2 mg/kg) groups when compared with control group ($p > 0.05$). The E_{max} values for **ATP** was not affected by sertindole, asenapine, or ziprasidone treatment and were not significantly different from the control group ($p > 0.05$) (Table 1)

There were no significant differences in KCl-induced contractile responses between the groups.

4. DISCUSSION

Erectile dysfunction includes sexual reluctance, erectile dysfunction, orgasmic disorders, premature ejaculation, and/or painful ejaculation. These conditions can be congenital or acquired, diffuse or situational, psychological, organic, or combined. Ejaculation disorders can be due to neurological (autonomic neuropathy, Parkinson's disease, spinal cord trauma, after surgery), pharmacological (antihypertensive, antipsychotic, antidepressant, and alpha blockers), bladder, neck, and prostate diseases [11]. Premature ejaculation can be of psychological or organic origin. Psychosexual and behavioral therapy, topical applications, antidepressants, alpha blockers, and/or sildenafil are used in for its treatment. Delayed ejaculation is generally psychological, and it can be seen after SSRI usage, neuropathy, and spinal trauma. Buspirone has been found to be the most effective drug for treatment of premature ejaculation due to SSRIs.

Premature ejaculation is the most common sexual dysfunction affecting 25%–35% of men. Its pathogenesis has still not been explained. Delayed ejaculation is a rare cause of ejaculation disorders (approximately 3% of all sexual disorders). Although its etiopathogenesis is not fully understood, psychological, pharmacological, traumatic, and hormonal factors are thought to play a role. Yohimbine, bupropion, buspirone, cabergoline, cyproheptadine, amantadine as well as pramipexole, ropirinol, oxytocin, and/or flibanserin are used in the treatment of delayed ejaculation [12].

In recent years, there are many predictions about the value of 5-HT pathways in the control of male sexual function. However only a few in-vivo studies are about the peripheral serotonergic transmission. In a recent study, 5-HT is believed to be an inhibitory transmitter in the control of sexual drive [13]. High central 5-HT levels are associated with ejaculation inhibition [14]. In our study, 5-HT-induced contractions significantly increased in both prostatic and epididymal portion of the mice vas deferens that were obtained from sertindole (1.3 and 2.5 mg/kg), asenapine (0.05 and 0.075 mg/kg), and ziprasidone (1 and 2 mg/kg) treated groups when compared with the control group; this condition causes premature ejaculation. In a previous study, SSRT inhibitors have been found to impair sexual functions. The reason for this impairment is disruption of erectile pathways in the spinal cord with an increase in 5-HT receptors [15].

There are no significant difference of the NA concentration-response curve in the epididymal and prostatic portions of the vas deferens between experimental groups. Also, no change in E_{max} values was noted. Ineffectiveness of sertindole, asenapine, and ziprasidone treatment on NA-evoked contractions may be explained by post-receptor mechanisms.

The concentration-response curve for the contractile response to ATP (10^{-8} to 10^{-4} M) in mice did not vary significantly among any of the prostatic and epididymal portions of the mice vas deferens. Also, E_{max} values for ATP-induced contractions were not statistically different between the groups.

Studies have shown that 5-HT has an inhibiting effect on ejaculation [16]. Therefore, SSRIs can be the therapy of choice for premature ejaculation because they can cause a slowing of ejaculation. Given that the spine-derived 5-HT effect is possibly stimulatory to ejaculation, it can be considered that this inhibitory effect is of brain origin [17,18].

In rats with spinal cord injury, the use of amphetamine-derived p-chloroamphetamine caused a sudden release of serotonin in synaptic clefts, leading to ejaculation [18]. Administration of intrathecal 5-HT or SSRI can cause an increase in the expulsion phase of ejaculation [17]. 5HT1A, 5HT1B, and 5HT2C receptor subtypes are associated with ejaculation; however,

nearly 14 5-HT receptor subtypes play role in ejaculation via inhibition or activation according to their place in the central nervous system, and it is hard to determine the effects of each receptor subtype [17,19].

5. CONCLUSION

In conclusion, according to our results, chronic treatment with sertindole, asenapine, and ziprasidone affects vas deferens motility, which causes an increase in 5-HT response. Also, the present results might indicate the possible clinical implications of male reproductive function in patients with anti-psychotic medication. On the other hand, these drugs can be used in delayed ejaculation treatment because these drugs stimulate ejaculation by stimulating 5-HT neurons. Detailed studies are needed on this subject.

UNDER PEER REVIEW

COMPETING INTERESTS DISCLAIMER:

Authors have declared that no competing interests exist. The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

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