

# Effects of Sildenafil Citrate Administration on Cardiovascular System of Subchronic Fluoxetine HCl Treated Male Rats

## SUBKRONİK OLARAK FLUOKSETİN HCl UYGULANAN ERKEK RATLARDA SILDENAFİL SİTRATIN KARDİYOVASKÜLER SİSTEM ÜZERİNE ETKİLERİ

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### Abstract

**Objective:** Sildenafil citrate is a commonly used drug for the treatment of erectile dysfunction accompanied with fluoxetine HCl treatment. Fluoxetine HCl inhibits the cytochrome P450 CYP3A4 and CYP2C enzymes which are the main metabolizing isozymes of sildenafil citrate. Since sildenafil citrate, activating mainly NO pathway, can cause vasodilation and hypotension. The prolonged metabolisation of this compound may aggravate the severity of hypotension. In the present study, cardiovascular changes upon sildenafil citrate administration alone and after fluoxetine HCl treatment for two months were investigated in anesthetized male Sprague-Dawley rats.

**Material and Methods:** A single dose of sildenafil citrate was administered i.p. into control (n= 18) and two months of fluoxetine HCl (0.285 mg/kg/day) treated experimental (n= 18) group rats. The operated rats were monitored to record the changes of systolic, diastolic, mean and pulse pressures and heart rate for six hours under ketamine and xylazine induced anesthesia.

**Results:** Our results indicate that there was a gradual and significant decrease in blood pressure by the time within the groups (ranging from 93.8 ± 4.9 mmHg basal MAP to 56.0 ± 7.9 mmHg of 360 minute MAP value in control and from 88.5 ± 7.6 mmHg to 52.2 ± 14.0 mmHg for experimental group). But decrease pattern in blood pressure was not significantly different between the groups. No statistical differences were found between control and experimental groups for each cardiovascular parameter.

**Conclusion:** In conclusion, sildenafil citrate injection caused a gradual hypotension, and there was no significant additive effect on the hypotension and other examined cardiovascular parameters at 0.357 mg/kg dose in the subchronically fluoxetine HCl treated male rats. However when the higher doses of sildenafil was treated, it has interacted with anesthesia and all the animals have died.

**Key Words:** Sildenafil, fluoxetine, cardiology, physiological effects of drugs

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### Özet

**Amaç:** Sildenafil sitrat fluoksetin HCl tedavisine bağlı olarak gelişen erektil disfonksiyon tedavisinde sıklıkla kullanılan bir ilaçtır. Fluoksetin HCl sildenafil metabolizmasında rol oynayan P450 CYP3A4 ve CYP2C enzimlerini inhibe eder. Sildenafil NO yolağını aktive ederek etki gösterdiğinden, vazodilatasyon ve hipotansiyon gelişimine neden olabilir. Bu bileşiğin metabolizmasının yavaşlaması, bileşiğe bağlı olarak gelişen hipotansiyonu şiddetlendirebilir. Bu çalışmada, 2 ay boyunca fluoksetin HCl tedavisi yapılan ve herhangi bir ilaç tedavisi uygulanmayan erkek Sprague-Dawley ratlarında, anestezi altında, sildenafilin oluşturduğu kardiyovasküler değişimler incelenmiştir.

**Gereç ve Yöntemler:** Kontrol grubundaki (n= 18) ve 2 ay fluoksetin HCl (0.285 mg/kg/gün) uygulanan deney grubundaki (n= 18) ratlara tek doz sildenafil sitrat intraperitoneal yoldan verilmiştir. Ketamin ve ksilazin anestezisi altında opere edilen hayvanların sistolik, diyastolik, ortalama kan basınçları (OKB), nabız basınçları ve kalp hızları 6 saat boyunca izlenmiştir.

**Bulgular:** Elde edilen sonuçlar, her 2 gruptaki ratların kan basınçlarının zamanla, şiddetli bir şekilde ve önemli oranda düştüğü (Kontrol grubunda 93.8 ± 4.9 mmHg bazal OKB seviyesinden 360 dk.da 56.0 ± 7.9 mmHg düzeyine, deney grubunda ise 88.5 ± 7.6 mmHg seviyesinden 52.2 ± 14.0 mmHg düzeyine) gözlenmiştir. Ancak, gruplar arasında kan basıncındaki bu düşmenin istatistiksel olarak önemli olmadığı bulunmuştur. Kontrol ve deney grubundaki hayvanların tüm kardiyovasküler parametrelerinin istatistiksel olarak birbirinden farklı olmadığı gözlenmiştir.

**Sonuç:** Sonuç olarak 0.357 mg/kg dozda enjekte edilen sildenafilin şiddetli hipotansiyon gelişimine neden olduğu, ancak bu uygulamanın subkronik olarak fluoksetin tedavisi yapılan hayvanlarda gelişen hipotansiyon ve diğer kardiyovasküler parametreler üzerinde önemli bir additif etkisi olmadığı gözlenmiştir. Ancak, sildenafil dozu yükseltildiğinde, madde anestezi ile etkileşmiş ve tüm hayvanlar ölmüştür.

**Anahtar Kelimeler:** Sildenafil sitrat, fluoksetin HCl, kardiyoloji, ilaç etkileri

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**M**ale erectile dysfunction (ED), inability to attain and/or maintain penile erection sufficient for satisfactory sexual performance, is a common disorder in male with an estimated prevalence across all ages of 10%.<sup>1</sup> Several physiological disorders such as cardiovascular

diseases, diabetes, endocrinological disorders and depression induce ED development in the patients. On the other hand, antidepressant medications may also induce impotence as an adverse effect.<sup>2</sup> Fluoxetine and other selective serotonin reuptake inhibitors (SSRI), commonly prescribed drugs for the treatment of depression, have inhibitory effect on sexual function in a wide range, from 1.9 to greater than 30% of treated women and greater than 40% in men.<sup>3</sup> Sildenafil is effectively used to improve the sexual dysfunction accompanied with SSRI therapy.<sup>4,5</sup>

Sildenafil is metabolized by the cytochrome P450 CYP3A4 (major route) and CYP2C9 (minor route) hepatic microsomal enzymes.<sup>6,7</sup> Potent inhibitors of these isozymes, such as the specific inhibitor erythromycin and the nonspecific inhibitor cimetidine, will limit first-pass metabolism and increase the plasma concentration of sildenafil.<sup>8</sup> Fluoxetine also has inhibitory effect on the cytochrome P450 CYP 2C and CYP3A4 enzymes.<sup>3</sup> An interaction between fluoxetine and sildenafil may arise due to the inhibition of this enzymatic system. Fluoxetine may potentiate the effects of sildenafil via protecting the biotransformation of the compound and increasing its plasma concentration which in turn would aggravate side effects of sildenafil, such as headache, flushing, dyspepsia, abnormal vision and hypotension.<sup>9-11</sup>

Sildenafil exerts its pharmacological effect via inhibiting cyclic guanosine monophosphate (cGMP)-specific phosphodiesterase type V (PDE5) enzymes. As a result of this inhibition, breakdown of cGMP, a second messenger of NO, is exhausted and relaxation occurs in the smooth muscle cells of arteries, arterioles and sinusoids at the corpus cavernosum.<sup>6,12</sup> Since PDE5 is found in other tissues besides the genitalia, including vascular and visceral smooth muscles, platelets and skeletal muscle in small amounts, sildenafil has a mild systemic hypotensive effect, as well.<sup>12</sup> Small, but statistically significant reductions in systolic blood pressure (SBP), diastolic blood pressure (DBP) and MAP after sildenafil administration have been reported.<sup>13</sup> The mean SBP and DBP reductions have been observed after intravenous administra-

tion of 40 and 80 mg of sildenafil as 7/7 and 9/7 mmHg, respectively and the mean maximum decrease of SBP/DBP was 10/7 mmHg after 3 hours of oral sildenafil administration.<sup>14</sup>

Since fluoxetine is a cytochrome P450 CYP2C and CYP3A4 inhibitory drug, it can prevent the metabolism of sildenafil citrate and prolong and/or increase its cardiovascular side effects via increasing the plasma concentration and half life of the substance. In the present study, we aimed to investigate the effects of sildenafil citrate administration on cardiovascular parameters of subchronic fluoxetine HCl treated male rats, to put forward any cardiovascular side effects as a result of possible interaction between these two drugs.

## Material and Methods

### Animals

3 months of age, 36 (n= 18 for control and experimental groups) male Sprague Dawley rats, weighing 250-300 g, were used in present study. The animals, fed with standard diet and tap water, *ad libitum*, were provided and housed in the university medical and surgical experimental research center. All animals were maintained in accordance with the recommendations of the Guide for the Care and Use of Laboratory Animals and the experiments were approved by the Osmangazi University Medical Faculty Animal Care Committee. Eighteen control animals were divided into 3 subgroups for 0.357, 0.714 and 1.428 mg/kg doses of sildenafil citrate (n= 6 for each dose level) administration which are equivalent to 25, 50 and 100 mg/70 kg human oral doses respectively, and treated with sterile distilled water (1 mL/kg/day) for two months. Eighteen experimental animals were also divided into 3 subgroups and 0.285 mg/kg of fluoxetine HCl was injected daily, which is equivalent to 20 mg/70 kg human oral dose, for two months, because the depression treatment with fluoxetine may require such a long period and the sexual dysfunctions become clear in the first 2 months of the treatment.<sup>3</sup> The last dose of fluoxetine was injected into the animals 1 hour prior to sildenafil administration. After this period, the same doses of sildenafil, used in the control

groups, were also applied to the experimental animals ( $n= 6$  for each sildenafil dose level). Sildenafil and fluoxetine doses were calculated as mg/kg based on the therapeutic oral dose used for 70 kg human body weight. Because the oral bioavailability of sildenafil is 23% in male rats and 38% in man, we adjusted the doses given intraperitoneally to reach the pharmacologically effective plasma concentration limits.<sup>15</sup> Drug solutions were prepared as 0.285 mg/mL for fluoxetine in distilled water and, 0.357, 0.714 and 1.428 mg/mL for sildenafil in 0.1 N HCl.<sup>15</sup>

### Chemicals

Sildenafil citrate was obtained from “Fako İlaçları A.Ş., İstanbul”. Prozac liquid® (Lilly, İstanbul) was used for the fluoxetine treatment. Ketamine (Ketalar® Parke-Davis, İstanbul) and xylazine HCl (Rompun® Bayer, İstanbul) were administered to induce anesthesia. Neuparin® 25000 IU/5 mL (Mustafa Nevzat İlaç A.Ş. İstanbul) was used for heparinization.

### Hemodynamic Data

For the investigation of cardiovascular parameters after sildenafil administration, animals were anesthetized with ketamine HCl (60 mg/kg) and xylazine HCl (25 mg/kg) combination and placed prone position on a warmed surgical plate to maintain body temperature at  $37 \pm 0.5^\circ\text{C}$  throughout the experimental procedure. Surgical area was disinfected with betadine solution. A single dose of sildenafil citrate was given i.p. into the rats following the stabilization period (30 minutes) of the surgery. In surgery, left femoral artery for cardiovascular monitoring and left femoral vein for fluid replacement (25 UL/min infusion of 0.9% NaCl throughout the experiment) were cannulated with PE50 cannula and 0.2 mL heparinized saline solution (100 mU/mL heparine in serum physiologic) was given following the cannulation to prevent the coagulation. The anesthesia was maintained by injecting 0.05 mL of ketamin and 0.05 mL of xylazine HCl combination intraperitoneally at each 2.5 hours after the first dose. Cardiovascular parameters were monitorized via a pressure transducer connected to a data acquisition system

(Biopac, USA). Changes of systolic (SP), diastolic (DP), MAP, pulse pressures and heart rate (HR) were recorded at 1, 15, 30, 60, 90, 120, 180, 240 and 360 minutes.

### Statistical analysis

All parametric data (SBP, DBP, MAP, HR, and Pulse Pressure) obtained from 0.357 mg/kg sildenafil administered control and experimental groups were analysed. Student's t-test was performed to evaluate the difference of the data data between the groups. Statistical analysis was done using one way ANOVA with Tukey's post-hoc test. Results were expressed as mean  $\pm$  standard deviation and  $p < 0.05$  was accepted as statistically significant.

### Results

The recommended dose for sildenafil is 25 mg for 70 kg (0.357 mg/kg) human when it is used in combination with CYP3A4 inhibitory drugs.<sup>8</sup> SP, DP, MAP and pulse pressures and HR changes recorded after i.p. sildenafil citrate administration at 0.357 mg/kg dose level alone and after fluoxetine HCl treatment were given in Table 1.

As seen in Figure 1A, a significant reduction took place in systolic pressure during the first 15 minutes period of the experiment ( $p < 0.05$ ) in both control and experimental groups. The reduction percentages at minute 15 were 22.5% and 15.88% for control and experiment animals, respectively. Following the first 15 minutes, the reduced systolic pressures were maintained and slight but not significant blood pressure elevations were observed until the end of the experiment. There was no statistical difference in the systolic blood pressure of the two groups throughout the experiment.

Decrease of diastolic pressure was significant in 0.375 mg/kg sildenafil treated control group at minute 15 and in experimental group at minute 30 ( $p < 0.05$ ) Reduction percentages in diastolic pressures from the baseline were approximately 22.3% and 21.2% for control and experimental (0.375 mg/kg sildenafil + 0.285 mg/kg fluoxetine) groups, respectively. Moreover, these reductions are greater than those reported in the literature.<sup>13</sup> After

**Table 1.** Mean systolic (SBP), diastolic (DBP), mean arterial pressure (MAP), pulse pressure (PP) and hearth rate (HR) values obtained after 0.357 mg/kg sildenafil administration.

		Time (minute)									
		-1	0	15	30	60	90	120	180	240	360
SBP	Control	114.3 ± 6.0	99.3 ± 5.0	88.5 ± 7.4	84.8 ± 3.3	86.8 ± 4.6	93.3 ± 7.1	95.0 ± 11.2	75.5 ± 7.4	75.8 ± 13.3	70.3 ± 10.7
	Experiment	110.1 ± 7.1	105.1 ± 10.2	92.7 ± 3.3	87.3 ± 2.3	90.8 ± 5.4	90.0 ± 6.4	91.5 ± 5.5	83.3 ± 9.1	71.8 ± 10.0	65.0 ± 17.8
DBP	Control	79.3 ± 3.4	67.5 ± 4.4	61.5 ± 6.2	58.5 ± 1.7	59.5 ± 3.1	65.8 ± 8.7	68.3 ± 12.8	53.8 ± 4.3	50.0 ± 3.1	47.0 ± 5.1
	Experiment	75.3 ± 6.7	71.8 ± 10.5	63.6 ± 2.0	59.3 ± 3.2	62.5 ± 4.0	63.8 ± 2.0	67.0 ± 5.5	58.0 ± 7.4	50.3 ± 6.6	44.3 ± 13.0
MAP	Control	93.8 ± 4.9	79.3 ± 4.5	71.5 ± 7.0	68.5 ± 2.0	69.5 ± 3.3	75.8 ± 8.7	78.0 ± 12.2	61.3 ± 4.2	59.3 ± 5.3	56.0 ± 7.9
	Experiment	88.5 ± 7.6	83.8 ± 10.3	74.1 ± 2.4	69.3 ± 2.4	72.6 ± 4.3	73.3 ± 3.5	75.3 ± 5.2	66.8 ± 8.3	58.0 ± 7.8	52.2 ± 14.0
PP	Control	35.0 ± 3.2	31.8 ± 1.7	27.0 ± 2.2	26.3 ± 2.5	27.3 ± 3.8	27.5 ± 1.7	26.8 ± 3.1	21.8 ± 6.1	25.8 ± 12.1	23.3 ± 6.8
	Experiment	34.8 ± 1.7	33.3 ± 2.3	29.0 ± 3.0	28.0 ± 2.7	28.3 ± 4.3	26.2 ± 5.1	25.3 ± 4.9	25.3 ± 3.5	21.5 ± 4.8	20.7 ± 6.0
HR	Control	252.5 ± 31.2	252.5 ± 31.2	258.8 ± 14.4	247.5 ± 26.0	247.5 ± 35.7	230.0 ± 71.4	228.8 ± 57.9	236.3 ± 52.5	228.8 ± 61.7	281.3 ± 64.1
	Experiment	242.5 ± 17.5	240.0 ± 16.4	240.0 ± 21.2	245.0 ± 22.6	252.5 ± 19.9	255.0 ± 25.1	242.5 ± 37.2	220.0 ± 39.9	242.5 ± 45.9	272.5 ± 81.8

minute 180, diastolic pressure levels were significantly lower than those of baseline until the end of the experiment. There was no significant difference between the diastolic blood pressures of control and experimental groups during the experimental period (Figure 1B).

MAP significantly reduced at minute 15 within the groups due to decreasing of systolic and diastolic pressures, but there was no difference between control and experimental groups (Figure 1C).

There was no difference between the pulse pressure levels of the groups throughout the experiment (Figure 1D). Drug administration slightly increased the HR in each groups (Figure 1E).

The experiment was also carried out for the 0.714 and 1.428 mg/kg doses of sildenafil which represent 50 and 100 mg/70 kg human doses. In both doses, all the animals in control and experimental groups died with severe cardiovascular and pulmonary failure, after 1.5-4 hours of the sildenafil administration. Because of this reason, the 6 hours experiment protocol could not be completed and the recorded cardiovascular parameters have not been given. The characteristic symptoms were bradypnea, hyperpnea, acute severe hypotension, apnea and death. Deaths have generally occurred after maintaining doses of anesthetic administration.

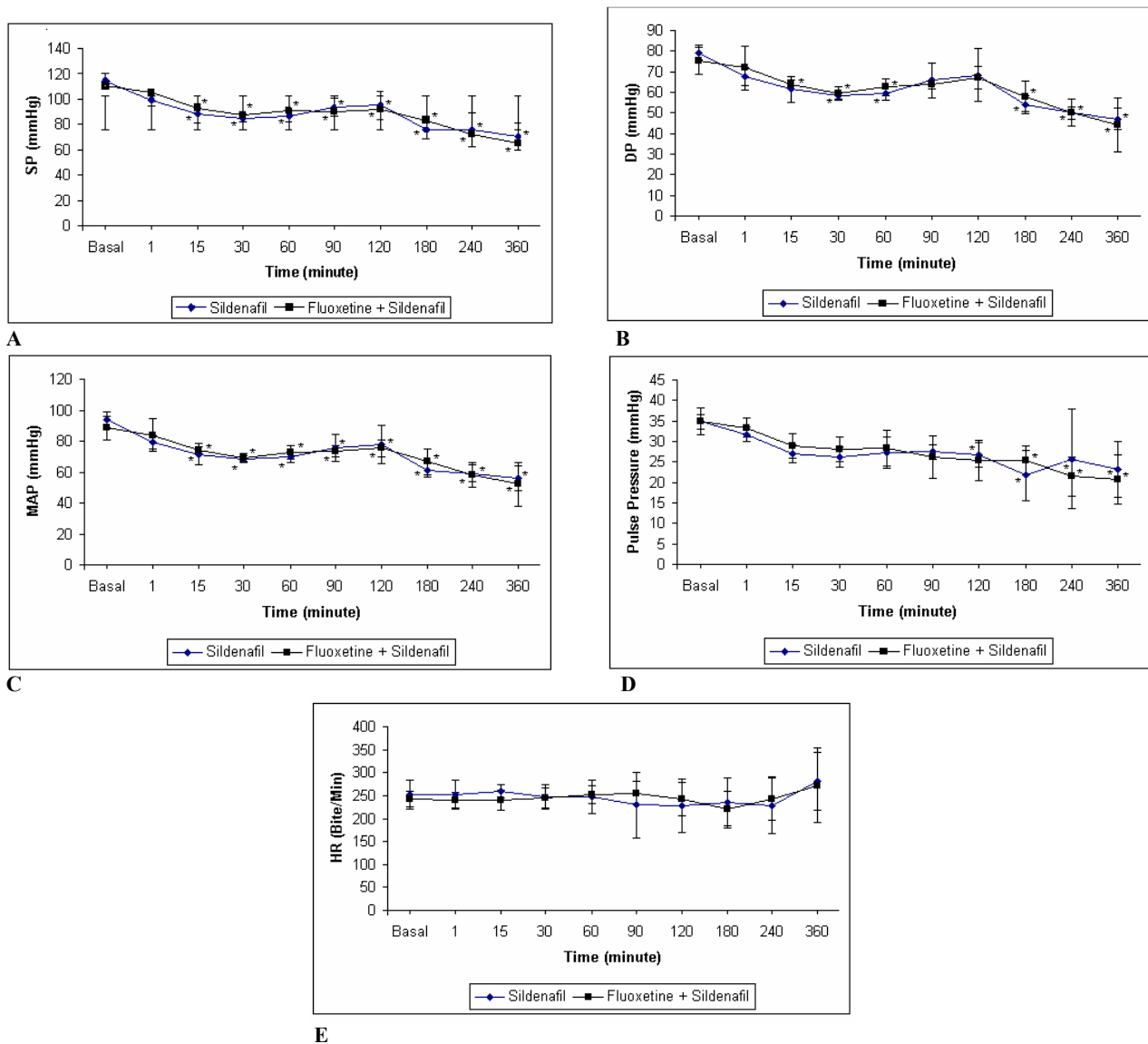
## Discussion

In this study, sildenafil and fluoxetine administrations were carried out via i.p. injection. This is, of course, different from the normal oral route of administration of the drug in humans. However,

the applied doses were similar to those in adult human therapeutic range.<sup>14</sup>

Our findings showed that, sildenafil, at 0.357 mg/kg dose, did not affect the cardiovascular parameters in both animal groups. Thus, the expected increase in plasma concentration of sildenafil in the presence of fluoxetine which inhibits CYP3A4 isozymes, did not change the SP, DP, MAP and pulse pressures markedly in the experimental rats compared to the control rats. However, the decrease of SP and DP were higher than that of reported in the literature.<sup>14</sup> These findings support the view that, usage of the low effective dose of sildenafil may be reliable in the patients with erectile dysfunction accompanied with fluoxetine treatment.<sup>2,4</sup> It should be noted that the results of the present study are valid for only the low dose of fluoxetine HCl treatment. Because it is the common used and generally effective dose for depression therapy. We do not know whether the higher doses of fluoxetine may cause more severe microsomal enzyme inhibition and dramatic changes in sildenafil side effects. Moreover, developing and severity of the other side effects such as vision disturbances and dyspepsia were not investigated, as well.

We have considered that the deaths of the animals administered higher doses of sildenafil, occurred related to the interaction between sildenafil and anesthesia, because all the animals in control and experimental groups had died. To justify this conclusion, the same doses of sildenafil and fluoxetine, alone and in combination, applied



**Figure 1.** Changes of SP (A), DP (B), MAP (C), Pulse pressure (D) and HR (E) during the 360 minutes of experimental period. (Data expressed as  $X \pm SD$ ) \*:  $p < 0.05$  compared to basal value.

to the unanesthetized animals and no death occurred in any dose level and any groups. We suggest that high decreases of SBP and DBP, seen in the higher sildenafil doses, may be attributed to the interaction between sildenafil and anesthetic agents.

In conclusion, sildenafil citrate is tolerable at 0.357 mg/kg dose, which is recommended dose when used in combination with CYP3A4 inhibitors, without any additive effect on the examined cardiovascular parameters in subchronic fluoxetine

HCl treated rats. However, if the dose of sildenafil needs to be increased, the microsomal enzyme inhibitory effect of fluoxetine should be carefully considered.

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