



# Fluoxetine enhances the anticonvulsant effects of conventional antiepileptic drugs in maximal electroshock seizures in mice

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## Abstract:

The present study was designed to investigate the effects of fluoxetine (FXT), a selective serotonin reuptake inhibitor, on the effect of antiepileptic drugs (AEDs) in the maximal electroshock seizure (MES) model in mice. FXT at the doses of 25, 20 and 15 mg/kg significantly increased the electroconvulsive threshold. The antidepressant applied at the lower doses (10, 5 and 2.5 mg/kg) did not influence the threshold. Moreover, FXT (at the highest subprotective dose of 10 mg/kg) increased the anticonvulsive potential of carbamazepine (CBZ), diphenylhydantoin (DPH), valproate (VPA) and phenobarbital (PB), producing a dose-related decrease in their ED<sub>50</sub> values against MES. Nevertheless, pharmacokinetic events may be involved in the interaction between FXT and PB or CBZ, since the antidepressant raised the total brain concentration of the two antiepileptics. FXT in combination with AEDs did not influence the motor performance in the chimney test and long-term memory. In conclusion, the data suggest that FXT modulates seizure processes in the brain and may be advantageous in the treatment of epilepsy in depressed patients, improving the seizure control in epilepsy.

## Key words:

fluoxetine, antiepileptic drugs, epilepsy, depression, antidepressant, 5-HT reuptake inhibition

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**Abbreviations:** ADs – antidepressants, AEDs – antiepileptic drugs, CBZ – carbamazepine, DPH – diphenylhydantoin, FXT – fluoxetine, MES – maximal electroshock, PB – phenobarbital, VPA – valproate, 5-HT – 5-hydroxytryptamine

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## Introduction

Depression is one of the most frequent psychiatric disorders with growing importance in the present social and civilizational conditions. Signs and symptoms of depression may be manifested in about 66% of epileptic patients [8–10, 18, 24]. Fluoxetine (FXT)

is a selective serotonin reuptake inhibitor (SSRI), that belongs to antidepressant drugs of newer generation. It proved its mood-enhancing properties in a variety of clinical trials [10, 15, 26, 32]. Several years ago, some anticonvulsant effects of FXT were also discovered. The drug was also reported [18] to attenuate pentetrazole-induced convulsions in mice, maximal electroshock (MES)-induced seizures in rats [26], audiogenic seizures in both mice and rats, and focal limbic motor seizures in rats. Particularly, FXT exerts anticonvulsant action against MES convulsions in genetically seizure-prone rodents (GEPRs). Some authors have suggested that enhancement of serotonin transmission may selectively reduce the sensitivity of brainstem circuits to seizure initiation [26]. This phe-

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nomenon explains, at least partially, the anticonvulsant activity of FXT.

Several clinical observations and animal studies showed that FXT enhanced the anticonvulsant potency of various antiepileptic drugs (AEDs) like diphenylhydantoin (DPH) [14, 18, 27] and carbamazepine (CBZ) [15, 19]. According to Mamiya et al. [19], some clinical studies have shown that co-administration of FXT or sertraline may increase serum concentrations of DPH. Besides, it has been proven that most AEDs and antidepressants (ADs) cause mutual pharmacokinetic interactions, with possible marked changes in blood levels of both agents and consequent modification of the expected clinical response [25]. Furthermore, Pasini et al. [22] have found the antidepressant protective when administered either systematically or focally into the substantia nigra. In this case, bilateral microinjection of FXT into the substantia nigra protected against seizures evoked focally from area tempestas, an epileptogenic site within the deep prepiriform cortex. Moreover, intranigral injections of FXT increased the latency to audiogenic seizures in GERPs treated with 5-hydroxytryptamine (5-HT) [22]. Dailey et al. [6] have also evaluated FXT as an anticonvulsant in GEPRs, because seizure predisposition in GEPRs is partially dependent on deficits in brain serotonin. Pericic et al. [24] have demonstrated that the antidepressant drug, given acutely or repeatedly, exerts anticonvulsant properties against convulsions induced in unstressed and swim-stressed mice by an antagonist of GABA<sub>A</sub> receptors, picrotoxin. Swim stress does not appear to modify the anticonvulsant properties of FXT, however the exact mechanism of FXT action in this model remains unknown [24].

Pharmacological treatments that enhance 5-HT neurotransmission and can suppress experimentally induced seizures have been shown to possess antagonistic properties at presynaptic 5-HT<sub>1A</sub> autoreceptors [3]. Researchers have suspected that the mechanism of anticonvulsant action of FXT was due to an enhancement of endogenous 5-HT transmission. Moreover, the anticonvulsant effect of FXT has been shown to be dependent on primary brain levels of serotonin [3].

However, according to Dailey and Naritoku [5], ADs are similar in their action to AEDs in their ability to both prevent and induce seizures. There are some data confirming [14] that the use of ADs in epileptic patients has been a concern to clinicians because of reports that these drugs have proconvulsant or convul-

sant effect [14]. The activity derives from the drugs' capacity to block norepinephrine [5, 8, 23] and/or serotonin reuptake [5, 26]. Authors refer also to Goodman and Gilman's *The Pharmacological Basis of Therapeutics* data about ADs with a seizure liability. FXT was one of those described as the drugs with a non-significant seizure liability. Besides, they assume that it is not reasonable to think it can bring a seizure risk because it blocks serotonin reuptake.

Depletion of serotonin greatly reduces the anticonvulsant effect of FXT [5]. Enhancing serotonin release by combining 5-HT and FXT synergistically increases extracellular brain serotonin and the anticonvulsant effect of these two drugs. It was also found that some established AEDs, like CBZ and valproate (VPA), increase extracellular serotonin level as a part of their anticonvulsant mechanism of action [5]. According to Ugale et al. [31], *in vitro* studies revealed that among ADs that inhibit GABA-stimulated Cl<sup>-</sup> influx through the anion channel in the GABA<sub>A</sub> receptor complex, FXT in particular acted by increasing GABA<sub>A</sub> receptor activity, which mediates most of fast inhibitory transmissions in the brain. Recent studies indicated [18], however, that FXT had several additional effects. Many of them involve inhibition of various types of ion channels, such as muscular and neuronal nicotinic receptors, volume-regulated anion channels, delayed rectifier K<sup>+</sup> channels in smooth muscle cells, voltage-gated Na<sup>+</sup> and K<sup>+</sup> channels in neurons and epithelial cells, voltage-gated Ca<sup>2+</sup> channels in cardiac myocytes, nerve terminals, and hippocampal pyramidal cells. Thus, the other possible explanation for the anticonvulsant action of FXT may be related to its inhibitory effect on ion channels [18].

In the present study, we wanted to evaluate the effects of the administration of FXT on the anticonvulsant activity of CBZ, VPA, phenobarbital (PB) and DPH to determine whether FXT's anticonvulsant action could be confirmed in our MES study on mice.

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## Materials and Methods

### Animals

The experiments were carried out on male Swiss mice weighing 20–25 g. The animals were housed in colony cages with free access to food (chow pellets) and

tap water. The experimental temperature was  $21 \pm 1^\circ\text{C}$  and mice were kept under a natural light-dark cycle. The experimental groups, consisting of 8–10 animals, were chosen by means of a randomized schedule. The Lublin Bioethical Committee approved all experimental procedures.

### Drugs

The following antiepileptic drugs were used: DPH (Polfa, Warszawa, Poland), VPA magnesium, CBZ (both drugs from Polfa, Rzeszów and Starogard, Poland) and PB sodium (Polfa, Kraków, Poland). The AD used was FXT (from Anpharm, Poland). CBZ, DPH, PB and FXT were suspended in 1% solution of Tween 80 (Sigma, St. Louis, MO, USA). VPA was dissolved in a sterile saline. All agents were given *ip* in a volume of 10 ml/kg. PB was administered 60 min, VPA, CBZ and FXT 30 min, DPH 120 min before respective tests.

### Electroconvulsions

Electroconvulsions were evoked with the use of alternating current (50 Hz) produced by a Rodent Shocker Type 221 (Hugo Sachs, Freiburg, Germany) generator, and delivered by ear-clip electrodes. The stimulus duration was 0.2 s. Tonic hindlimb extension was taken as the endpoint. The electroconvulsive threshold was evaluated as  $CS_{50}$ , which is the current strength (in mA) necessary to produce tonic hindlimb extension in 50% of the animals tested. To estimate the electroconvulsive threshold, at least four groups of mice (8–10 animals per group) were challenged with electroshocks of various intensities. Subsequently, an intensity-response curve was calculated on the basis of percentage of mice convulsing. In order to evaluate the respective  $ED_{50}$  values (in mg/kg), mice pretreated with different doses of AED were challenged with maximal electroshock (25 mA). Again, at least four groups of mice, consisting of 8–10 animals, were used to estimate each  $ED_{50}$  value. A dose-effect curve was constructed, based on the percentage of mice protected.

### Chimney test

The effects of AEDs on motor impairment were quantified with the chimney test of Boissier et al. [1]. In this test, animals had to climb backward up the plastic

tube (3 cm inner diameter, 25 cm length). Motor impairment was indicated by the inability of mice to climb backward up the tube within 60 s.

### Passive avoidance task

The mice were placed in an illuminated box ( $10 \times 13 \times 15$  cm) connected to a dark box ( $25 \times 20 \times 15$  cm), which was equipped with an electric grid floor. Entrance to the dark box was punished by an electric footshock (0.6 mA for 2 s). The mice that did not enter the dark compartment within 60 s were excluded from the experiment. On the next day (24 h later), the same animals were put into the illuminated box and observed for up to 180 s. The median time with 25 and 75 percentiles to enter the dark box was subsequently calculated. According to Venault et al. [33], the step through passive avoidance task may be recognized as a measure of long-term memory.

### Measurement of total brain AED concentrations

The animals were administered with an AED plus vehicle or a combination of FXT with the respective AED. The combination for estimating the total brain concentrations of AEDs was chosen on the basis of interactions for FXT and a conventional AED. Mice were killed by decapitation at times coinciding with that scheduled for the MES test and the whole brains of mice were removed from skulls, weighed, and homogenized using an Abbott buffer (2:1 v/w) in a Ultra-Turrax T8 homogenizer (Staufen, Germany). The homogenates were centrifuged at  $10\,000 \times g$  (MPW-360 centrifuge; Mechanika Precyzyjna, Warszawa, Poland) for 10 min. The supernatants of 75 ml were put into Abbott system cartridges, which were subsequently put into a carousel for up to 20 samples. Control samples of a conventional AED were placed at the beginning and end of each carousel for verification of the calibration. The total brain AED concentrations were analyzed by fluorescence polarization immunoassay using a TDx analyzer and reagents exactly as described by the manufacturer (Abbott Laboratories, North Chicago, IL, USA). In the case of the estimation of AEDs concentrations, original Abbott reagents were used. The AEDs analyzed were CBZ, VPA, PB, and DPH, and total brain concentrations were expressed in mg/ml of brain supernatants as means  $\pm$  SD of at least eight determinations.

## Statistics

CD<sub>50</sub> or ED<sub>50</sub> values and statistical analysis of the data obtained in the electroconvulsive tests were calculated by computer probit analysis, according to Litchfield and Wilcoxon [16]. Qualitative variables from the chimney test were compared with Fisher's exact probability test. The results from the passive avoidance task were compared by Kruskal-Wallis test, followed by Dunn's *post-hoc* test. Plasma levels and brain levels of AEDs were evaluated with the unpaired Student's *t*-test.

## Results

### Effects of fluoxetine (FXT) on the electroconvulsive threshold

FXT administered 30 min before the test at the dose of 25, 20 and 15 mg/kg significantly increased the electroconvulsive threshold from 4.9 mA to 6.2, 5.7 and 5.8 mA, respectively. No effect was observed when FXT was given at the dose of 10 mg/kg (Tab. 1).

**Tab. 1.** Effect of fluoxetine (FXT) on the electroconvulsive threshold in mice

Treatment (mg/kg)	CS <sub>50</sub> (mA)
Vehicle	4.9 (4.5–5.3)
FXT (25) + vehicle	6.2 (5.6–6.8)***
FXT (20) + vehicle	5.7 (5.1–6.3)*
FXT (15) + vehicle	5.8 (5.4–6.3)*
FXT (10) + vehicle	5.2 (4.7–5.8)

CS<sub>50</sub> (in mA) is a current strength, which produces convulsions in 50% of animals tested; FXT was administered 30 min before the electroconvulsions; \* *p* < 0.05, \*\*\* *p* < 0.001 vs. vehicle

### Influence of FXT on the protective activity of antiepileptic drugs against maximal electroshock-induced seizures in mice

FXT administered at the subprotective dose of 10 mg/kg significantly increased the antielectroshock activity of CBZ, DPH and PB reducing their ED<sub>50</sub> values from 11.8 to 3.1 mg/kg, from 10.5 to 4.8 mg/kg and from 23.5 to 3.5 mg/kg, respectively. Additionally, lower

**Tab. 2a.** Effect of fluoxetine (FXT) on the protective action of carbamazepine (CBZ) against maximal electroshock in mice

Treatment (mg/kg)	ED <sub>50</sub> (mg/kg)
CBZ + vehicle	11.8 (10.3–13.7)
CBZ + FXT (25)	5.6 (4.5–7)***
CBZ + FXT (20)	9.7 (8.6–11)*
CBZ + FXT (15)	2.8 (1.6–4.8)***
CBZ + FXT (10)	3.1 (2.7–5.4)***
CBZ + FXT (5)	7.5 (6.1–9.3)*
CBZ + FXT (2.5)	6.9 (5.2–9.3)*
CBZ + FXT (1)	6.8 (5.4–8.7)**
CBZ + FXT (0.5)	8.8 (7.3–10.7)

Table data are presented as ED<sub>50</sub> values (in mg/kg) with 95% confidence limits in parentheses. ED<sub>50</sub> values and statistical comparisons were calculated according to Litchfield and Wilcoxon [16]. For more details see legend to Table 1; \* *p* < 0.05, \*\* *p* < 0.01, \*\*\* *p* < 0.001 vs. the ED<sub>50</sub> value of respective control

**Tab. 2b.** Effect of fluoxetine (FXT) on the protective action of valproate (VPA) against maximal electroshock in mice

Treatment (mg/kg)	ED <sub>50</sub> (mg/kg)
VPA + vehicle	212.5 (189.3–238.6)
VPA + FXT (25)	110.3 (88.3–137.5)***
VPA + FXT (20)	148.1 (119.8–183.1)**
VPA + FXT (15)	151.1 (112.7–205.2)

Table data are presented as ED<sub>50</sub> values (in mg/kg) with 95% confidence limits in parentheses. ED<sub>50</sub> values and statistical comparisons were calculated according to Litchfield and Wilcoxon [16]. For more details see legend to Table 1; \*\* *p* < 0.01, \*\*\* *p* < 0.001 vs. the ED<sub>50</sub> value of respective control

**Tab. 2c.** Effect of fluoxetine (FXT) on the protective action of diphenhydantoin (DPH) against maximal electroshock in mice

Treatment (mg/kg)	ED <sub>50</sub> (mg/kg)
DPH + vehicle	10.5 (8.8–12.5)
DPH + FXT (25)	5.3 (3.5–7.9)**
DPH + FXT (20)	6.9 (5.2–9.3)*
DPH + FXT (15)	5.2 (4–6.7)***
DPH + FXT (10)	4.8 (3.4–6.6)***
DPH + FXT (5)	5.0 (3.8–6.6)***
DPH + FXT (2.5)	5.3 (3.5–7.9)**
DPH + FXT (1)	4.9 (3.3–7.3)**
DPH + FXT (0.5)	8.7 (7–10.7)

Table data are presented as ED<sub>50</sub> values (in mg/kg) with 95% confidence limits in parentheses. ED<sub>50</sub> values and statistical comparisons were calculated according to Litchfield and Wilcoxon [16]. For more details see legend to Table 1; \* *p* < 0.05, \*\* *p* < 0.01, \*\*\* *p* < 0.001 vs. the ED<sub>50</sub> value of respective control

**Tab. 2d.** Effect of fluoxetine (FXT) on the protective action of phenobarbital (PB) against maximal electroshock in mice

Treatment (mg/kg)	ED <sub>50</sub> (mg/kg)
PB + vehicle	23.5 (18.7–29.5)
PB + FXT (25)	4.6 (2.6–8.3)***
PB + FXT (20)	4.8 (2.8–8)***
PB + FXT (15)	3.3 (2–5.7)**
PB + FXT (10)	3.5 (1.9–6.5)*
PB + FXT (5)	6.1 (4.1–9.1)

Table data are presented as ED<sub>50</sub> values (in mg/kg) with 95% confidence limits in parentheses. ED<sub>50</sub> values and statistical comparisons were calculated according to Litchfield and Wilcoxon [16]. For more details see legend to Table 1; \*  $p < 0.05$ , \*\*  $p < 0.01$ , \*\*\*  $p < 0.001$  vs. the ED<sub>50</sub> value of respective control

**Tab. 3.** Effect of fluoxetine alone or combined with conventional antiepileptic drugs on long-term memory in the conventional variant of step-through passive avoidance task in mice

Treatment (mg/kg)	Retention (s)
Control	180 [180–180]
FXT (20)	180 [180–180]
CBZ (11.8)	180 [180–180]
CBZ (6.8)	180 [180–180]
CBZ (6.8) + FXT (1)	180 [180–180]
DPH (10.5)	180 [180–180]
DPH (5.3)	180 [104–180]
DPH (5.3) + FXT (2.5)	180 [180–180]
VPA (212.5)	180 [154–180]
VPA (148.1)	180 [180–180]
VPA (148.1) + FXT (20)	180 [180–180]
PB (23.5)	180 [180–180]
PB (3.5)	180 [86–180]
PB (3.5) + FXT (10)	180 [180–180]

Results are presented as median retention time (in seconds; 25 and 75 percentiles in parentheses) of ten determinations. Each group of animals was exposed to an electric stimulus, the duration of which was previously established in the pain threshold test. Statistical evaluation of the data was performed with Kruskal-Wallis nonparametric ANOVA test followed by Dunn's *post-hoc* test. CBZ – carbamazepine, PB – phenobarbital, VPA – valproate, DPH – diphenylhydantoin, FXT – fluoxetine

doses of FXT (5, 2.5 and 1 mg/kg) diminished the ED<sub>50</sub> value of CBZ to 7.5, 6.9 and 6.8, respectively. Similar results were obtained in the case of lower subthreshold doses of FXT (5, 2.5 and 1 mg/kg) in combination with DPH reducing the ED<sub>50</sub> value from 10.5 to 5.0, 5.3 and 4.9, respectively. In combination with PB, FXT at the lower subthreshold dose of 5 mg/kg increased the antielectroshock activity of the drug, reducing the ED<sub>50</sub> value from 23.5 to 6.1 mg/kg simultaneously. In contrast, FXT (10 mg/kg) remained without effect upon the protective action of VPA (Tab. 2a–d).

### Influence of FXT alone and in combinations with conventional AEDs on motor performance in the chimney test in mice

FXT up to the dose of 20 mg/kg (single, *ip* injection, 30 min prior to the test) did not impair the motor coordination in the animals.

It was observed that out of 10 animals in the experimental group, one mouse injected with CBZ (6.8 mg/kg), and one mouse injected with combination of VPA (148.1 mg/kg) and FXT (20 mg/kg), did not correctly perform the chimney test. Moreover,

**Tab. 4.** Effects of fluoxetine alone or in combinations with conventional antiepileptic drugs on the motor performance of mice in the chimney test

Treatment (mg/kg)	Mice impaired (%)
Control	0
FXT (20)	0
CBZ (11.8)	0
CBZ (6.8)	10
CBZ (6.8) + FXT (1)	0
DPH (10.5)	0
DPH (5.3)	0
DPH (5.3) + FXT	0
VPA (212.5)	0
VPA (148.1)	0
VPA (148.1) + FXT (20)	10
PB (23.5)	0
PB (3.5)	0
PB (3.5) + FLX (10)	0

The results of the chimney test are expressed as a percentage of animals showing motor impairment. Each experiment group consisted of 10 animals. The Fisher's exact probability test was used for statistical comparisons. CBZ – carbamazepine, PB – phenobarbital, VPA – valproate, DPH – diphenylhydantoin, FXT – fluoxetine

none of the examined combinations of FXT with CBZ, PB, VPA, and DPH, applied at the doses corresponding to their ED<sub>50</sub>, produced motor deficits in the chimney test in mice (Tab. 4).

#### Dark avoidance acquisition and retention testing

The saline-treated animals did not enter the dark box within the observation time limit (180 s). PB, CBZ, and DPH administered at doses equal to their ED<sub>50</sub> values did not impair retention in mice. Also, VPA applied at its ED<sub>50</sub> value did not cause significant impairment of long-term memory (Tab. 3).

#### Effect of FXT on the total brain concentrations of conventional AEDs

FXT at a dose of 20 mg/kg did not affect the total brain concentrations of VPA and DPH (Tab. 5). In contrast, FXT at 20 mg/kg raised the total brain concentrations of PB (2.8 mg/kg) by 4.26% from 2.58 ± 0.10 to 2.69 ± 0.10 mg/ml (*p* < 0.05; Tab. 5). The same dose of FXT increased the level of CBZ in the mouse brain from 1.434 ± 0.174 to 1.895 ± 0.339 (by 32% higher), which was a very significant result (*p* < 0.01, Tab. 5).

**Tab. 5.** Influence of fluoxetine upon the total brain concentrations of conventional antiepileptic drugs in mice

Treatment (mg/kg)	Brain concentrations (µg/ml)
CBZ + vehicle	1.43 ± 0.17
CBZ + FXT (20)	1.90 ± 0.34**
DPH + vehicle	0.78 ± 0.37
DPH + FXT (20)	1.00 ± 0.22
PB + vehicle	2.58 ± 0.10
PB + FXT (20)	2.69 ± 0.10*
VPA + vehicle	9.96 ± 1.20
VPA + FXT (20)	10.57 ± 1.11

Results are presented as the means ± SD of at least eight determinants and expressed as µg/ml of brain supernatants. Statistical evaluation of data was performed with unpaired Student's *t*-test; CBZ – carbamazepine, PB – phenobarbital, VPA – valproate, DPH – diphenylhydantoin, FXT – fluoxetine, \* *p* < 0.05 vs. PB-alone-treated group, \*\* *p* < 0.01 vs. CBZ-alone-treated group

## Discussion

The results of the present study demonstrated the anti-convulsant properties of acute FXT treatment against convulsions induced in mice by MES. FXT have not only potentiated the anticonvulsant effect of all applied AEDs, but also elevated the brain concentration level of two of them. As for motor performance the AD did not influence it significantly. The results of our research require further discussion and comparison with experiments and clinical studies that have been performed so far.

FXT, the prototype SSRI, is widely used as an AD that counteracts anxiety, panic state, premenstrual dysphoria, posttraumatic stress, and obsessive-compulsive disorder in clinical studies and exhibits anticonvulsant activity in several animal models of seizures [21, 31].

However, the mechanism underlying the anticonvulsant activity of FXT is far from clear. Many investigators now believe that the effectiveness of FXT may be due to the modulation of neurotransmitter systems beyond its direct effect on serotonin signaling [31]. In particular, GABAergic neurotransmitter system is a major molecular target for many clinically used AEDs [28]. Positive modulators of inhibitory GABA<sub>A</sub> receptors exhibit anticonvulsant properties in most experimental seizures, with an exception of absence epilepsy models. Nevertheless, it was also reported that trans-4-aminocrotonic acid, a potent agonist of GABA<sub>A</sub> and GABA<sub>C</sub> receptors, showed a significant proconvulsive action, significantly decreasing the electroconvulsive threshold in mice [2]. Regarding the effective anticonvulsant dose of the drug, FXT exerted protective effect in 50% of animals at 5 mg/kg in the limbic motor seizure model, whereas in the genetically epilepsy-prone rats (GEPR-9) the ED<sub>50</sub> was 16 mg/kg [17]. In the Magyar's study [18], FXT showed a significant anticonvulsant action at the dose of 10 mg/kg. However, in our study results were dependent on the anticonvulsive drug that was applied in combination with FXT. The lowest effective dose of FXT, that decreased the ED<sub>50</sub> value of CBZ and DPH was 1 mg/kg, whereas in the case of VPA, FXT at the dose of 15 mg/kg diminished the above-mentioned value. In case of PB, FXT at the dose of 10 mg/kg appeared to change the ED<sub>50</sub> value.

Hernandez et al. [11] have shown that FXT decreased the frequency of spontaneous motor seizures in the pilocarpine model of temporal lobe epilepsy,

which has suggested that one or more subtypes of the 5-HT receptor may be a significant turning point in the development of new AEDs. Also, Wada et al. [34] have highlighted that FXT is known to enhance 5-HT transmission by inhibiting 5-HT uptake into presynaptic nerve terminals, and when administered acutely, it decreased the firing rate of raphe cells, suggesting that they can also reduce the amount of 5-HT released.

Another important issue is that this study examined some doses of FXT and AEDs and, therefore, the range of reported lower doses referred to those indicated by other researches [11]. Our results were optimally effective both at lower and higher doses of FXT. The question of which doses of FXT exert a therapeutic anticonvulsant effect versus a possible proconvulsant effect also will require further investigation. It is noteworthy that seizures are a serious but less common adverse reaction associated with the use of ADs including SSRI [18]. The incidence of AD-related seizure ranges from 0.1–4%. Less commonly, seizures can occur at therapeutic doses and according to Oke et al. [21] there are two case reports of seizure associated with FXT at the dose of 20 mg. Some cases of interaction between DPH and FXT were described in psychiatric literature. Jalil [12] has described two patients who developed symptoms and signs of intoxication with DPH a few days after initiating the use of FXT. In animal studies, it was found that FXT was a potent inhibitor of hepatic microsomal metabolism, which was probably responsible for the increase in the plasma DPH level in these two cases [12]. In contrast, our results did not reveal such interaction, especially because we examined FXT in acute experiment (single administration of the drug). FXT with little effect on other neurotransmitters than serotonin generates a variety of adverse effects [4, 35]. The occurrence of convulsions at high doses (*iv* 36.5–56 mg/kg and oral doses of 160–700 mg/kg) during animal toxicity studies can be attributed to the effects of FXT on the central nervous system [35]. However, another clinical study has shown that most patients will not have an increased frequency of seizures when psychotropic medication is used at low to moderate doses and is introduced slowly [20]. Rosenstein et al. [28] have also confirmed that seizures associated with AD pharmacotherapy are seen most frequently after acute overdose. It is also noteworthy that apart from FXT, sertraline, fluvoxamine, trazodone, nomifensine, and monoamine oxidase inhibitors appear to have a lower incidence of seizures than the other ADs [28].

According to Lucena et al. [17] the combination of FXT and VPA is frequently used in psychiatric patients particularly with bipolar depression. However, there is a growing recognition that the inhibitory effect of FXT on cytochrome P450 may lead to serious adverse reactions resulting from a drug interaction between FXT and VPA [17]. Also Droulers et al. [7] have described two cases of VPA blood concentration increase when co-administered with FXT. The authors suggested that FXT reduced the activity of cytochrome P450CYP2D6, whereas metabolism of VPA is complex and involves both enzymatic and oxidative enzymes, that can be a target of induction by FXT [7].

In our study, plasma FXT concentration has not been measured, however, in other experimental models [13], it ranged from approximately 0.3 to 1.5  $\mu\text{M}$ . According to those authors, the brain concentration of FXT has been approximately 10 or 20 times higher than the corresponding blood levels. Kobayashi et al. [13] have suggested that G protein-activated inwardly rectifying  $\text{K}^+$ (GIRK) channels in the brain may be inhibited by FXT at clinically relevant concentrations. Additionally, the inhibition of neuronal GIRK channels by FXT may contribute to additive therapeutic effects in depression and other related psychiatric disorders. The plasma concentrations of FXT in several patients who experienced seizures were reported to be approximately 3.3–7.1  $\mu\text{M}$  [13]. Therefore, FXT at the corresponding brain levels may potentially inhibit neuronal GIRK channels, which play an important role in the inhibitory regulation of neuronal excitability in most brain regions.

Another clinical study of that interaction was described by Spina et al. [30]. They have proposed the mechanism of antidepressant metabolism inhibition due to the similarity between CBZ and FXT structure. That resulted in a 30% increase in CBZ concentration. Similarly, in our study we have noted a very significant elevation in CBZ brain concentration, which reached 32%.

As for CBZ and DPH blood concentration, their estimated clearance was decreased after the addition of FXT [29], however, in another report [14] there were some evidences supporting the suggestion, that co-administration of FXT enhanced the anticonvulsant effects of DPH and CBZ, probably by increasing the plasma levels of these anticonvulsants.

In conclusion, the data suggest that FXT modulates seizure processes in the brain and may be advantageous in the treatment of epilepsy in depressed patients, improving the seizure control of epilepsy.

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