Original article

An experimental study to evaluate the anticonvulsant activity of SSRI (Fluoxetine, Citalopram) and SNRI (Venlafaxine) in albino mice

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Abstract

Background: One of the challenges faced today by physicians is to understand the exact etiology and pathophysiology of epilepsy and its successful treatment. In recent years there has been increasing evidence that serotonergic neurotransmission modulates a wide variety of experimentally induced seizures. The 5-HT can cause a significant shift of excitability in most networks involved in epilepsy and thus drugs that alter the concentration of 5-HT or exert serotonin receptor agonist and/or antagonist properties can be considered as important factors for the pathogenesis of epilepsies. The association between epilepsy and depression has been known since antiquity. Noradrenergic and/or serotonergic deficits, as well as other abnormalities, may contribute to a predisposition to some epilepsy and depression. Our objectives were to study anticonvulsant activity of SSRIs (Fluoxetine, Citalopram) and SNRI (Venlafaxine) by maximal electroshock method (MES) and pentylentetrazol (PTZ) induced seizure model.

Introduction

Epilepsy is one of the oldest known diseases. Despite remarkable advances in neuropharmacology; the etiology, pathophysiology and manifestations of epilepsy are poorly understood. A recent meta-analysis has estimated that the prevalence of median lifetime epilepsy for developed countries was 5.8 per 1,000 compared to 15.4 per 1,000 for rural and 10.3 for urban studies in developing countries. Up to 10% of individuals experience at least one epileptic seizure in their lifetime, and about one-third of them will go on to develop epilepsy. An epileptic seizure is a transient occurrence of signs and/or symptoms due to abnormal excessive or synchronous neuronal activity of the brain. The International League Against Epilepsy (ILAE) and International Bureau for Epilepsy (IBE) has defined epilepsy as “a disorder of brain characterised by an enduring predisposition to generate epileptic seizures and by the neurobiologic, cognitive, psychological, and social consequences of this condition.”
definition of epilepsy requires the occurrence of at least one epileptic seizure. In recent years there has been increasing evidence that serotonergic neurotransmission modulates a wide variety of experimentally induced seizures. Various studies have shown that the agents that elevate extracellular serotonin (5-HT) levels, such as 5-hydroxytryptophan and serotonin reuptake blockers, inhibit both focal and generalized seizures. Conversely, depletion of brain 5-HT lowers the threshold of audiogenically, chemically and electrically evoked convulsions. Furthermore, it has been shown that several antiepileptic drugs increase endogenous extracellular 5-HT concentration. 5-HT receptors are expressed in almost all networks involved in epilepsies. The 5-HT can cause a significant shift of excitability in most networks involved in epilepsy and thus drugs that alter the concentration of 5-HT or exert serotonin receptor agonist and/or antagonist properties can be considered as important factors for the pathogenesis of epilepsies.

The association between epilepsy and depression has been known since antiquity. Noradrenergic and/or serotonergic deficits, as well as other abnormalities, may contribute to a predisposition to some epilepsy and depression. A history of depression or depressive symptoms has been reported in up to two-thirds of patients with medically intractable epilepsy, therefore in the treatment of epilepsy it is often necessary to treat depression. The literature has shown both, the anticonvulsant and proconvulsant activities of antidepressant drugs. Fluoxetine and citalopram, the selective serotonin reuptake inhibitors (SSRI); are one of the most prescribed antidepressant drugs. They are widely used for the treatment of endogenous depression, panic disorders, and obsessive compulsive disorders. They selectively increase the level of serotonin in the synaptic cleft by blocking its uptake in serotonergic neurons. Whereas venlafaxine, the serotonin norepinephrine reuptake inhibitor (SNRI), increase the levels of 5-HT, norepinephrine and to some extent dopamine levels in the synaptic cleft. Hence the present study was planned to see whether selective serotonin reuptake inhibitors (SSRIs) i.e. fluoxetine, citalopram and serotonin norepinephrine reuptake inhibitor (SNRI) i.e. venlafaxine possess anticonvulsant activity in conventional models of grand mal (maximal electroshock induced seizures) and petit mal epilepsy (pentylentetrazol-induced seizures) in albino mice.

**Material and Methods**

The study was conducted at S R T R Government Medical College, Ambejogai, India after approval from the Institutional Animal Ethics Committee (Approval letter number SRTRGMC/-IAEC/517/2010), which is an approved body by Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA).

**Animals:**

Swiss albino mice of either sex weighing 20-25 g were used in this study. They were randomly allocated to different experimental groups and placed in polypropylene cages on husk bedding in groups of six at a controlled temperature of 25 ± 2°C and 12h: 12h light dark cycle. They had free access to food and water. The animals were allowed to adjust to the laboratory conditions such as light, temperature and noise before being subjected to the experiment. All the experiments were carried out at the same time of the day i.e. between 10.00 a.m. to 5 p.m. to minimize circadian influences on seizure susceptibility.
Drugs:
Fluoxetine, Venlafaxine (Sun Pharma) and Citalopram (Cipla Pharmaceuticals) were obtained as a gift samples. Pentylenetetrazol, phenytoin and diazepam were obtained from commercial sources. All the drugs were dissolved in normal saline.

Experimental Design
A) Evaluation of anticonvulsant activity:
   a. Electroconvulsion:
      For the evaluation of anticonvulsant activity of drugs by electroconvulsion, the maximal electroshock seizure test (MES) described by Toman, Swinyard, and Goodman (1946)\textsuperscript{17} was followed. Accordingly, alternating current of 50mA was delivered for duration of 0.2 sec through ear electrodes, from an electroconvulsiometer constructed according to the design of Woodbury and Davenport (1952).\textsuperscript{18} The maximal seizure shown by normal mice typically consists of preliminary phase of tonic limb flexion, and then full extension of limbs, a short clonic interval and asphyxial death in some animals. (Photograph 1) The latency to convulsion, duration of tonic convulsion (a tonic extension of the hind limb), the percentage seizure protection and the mortality were recorded.\textsuperscript{19} Failure to extend the hind limbs to an angle with the trunk greater than 90 degree was defined as protection.\textsuperscript{20} Effect of the SSRI and SNRI was studied on MES induced seizures after administering drug intraperitoneally 30 minutes prior to electroshock.
   b. Chemoconvulsion:
      For the evaluation of anticonvulsant activity of drugs by chemoconvulsion, the pentylenetetrazol (PTZ) induced seizure method described by Goodman et al (1953)\textsuperscript{21} was followed. PTZ dissolved in normal saline was given intraperitoneally in a dose of 75 mg/kg in volume not exceeding 0.01 ml/gm of body weight.\textsuperscript{22} The control mice within 30 minutes develop a sequence of excitement, myoclonic jerks, clonic seizures, one or more maximal tonic seizures and death in some animals. Animals were tested for PTZ induced convulsions 30 minutes after the intraperitoneal administration of the test drug, diazepam and were observed for 30 minutes. The time taken before the onset of clonic convulsions, the duration of clonic convulsions, and the percentage of seizure protection and mortality were recorded.\textsuperscript{19} Abolition of clonic phase was considered as protection.
B) Tests for neurological deficit
   i. Inverted screen test:
      The inverted screen test was used to assess one form of behavioral toxicity induced by test compounds. The test was an adaptation (Ginski and Witkin, 1994) of that initially described by Coughenour et al (1977).\textsuperscript{23} In this test, compounds with sedative and/or ataxic properties produce dose dependent increase in the screen test failures whereas other classes of drugs do not. Six mice per group were pre-treated intraperitoneally with either vehicle or test compounds and returned to their cages. After 30 minutes, they were individually placed on a 14x14 cm wire screen (0.8 cm mesh) elevated 30 cm above the ground. After slowly inverting the screen, the mice were tested during a two-minute trial from their ability to climb the top. (Photograph 2) Mice not climbing to the top (all four paws on the upper surface) were counted as failure.
   ii. Spontaneous motor activity:
      Spontaneous motor activity was measured using digital actophotometer, equipped with six infrared light sources and photocells. The digital counter connected to the photocells recorded the locomotor activity counts when mice interrupted the beam of light falling on photocells. Each mouse was
placed individually in the actophotometer for 5 minutes for acclimatization and after that a baseline (0 hr) locomotor activity was recorded during 5-minute period. These mice were then treated with test drugs, phenytoin, diazepam, vehicle and activity counts were again recorded individually for each mouse at 30, 60 and 90 minutes of treatment.

**Statistical analysis:**

The data was analysed by using “GraphPad Prism, version 5.00 for Windows, GraphPad Software, San Diego California USA, www.graphpad.com”. The latency to convulsion, duration of convulsions and number of convulsions were analyzed by one way analysis of variance (ANOVA) followed by post hoc tukey test for comparison between multiple groups. The seizure protection and percentage mortality were analyzed by Fisher’s exact test. The ‘p’ value less than 0.05 was considered as statistically significant.

**Results**

**Table 1. Effect of drugs (i.p.) on maximal electroshock induced seizures in albino mice**

<table>
<thead>
<tr>
<th>Drugs and Dose (n=6)</th>
<th>Latency to convulse (sec) Mean ±S.E.M.</th>
<th>Duration of T.H.E. (sec) Mean ±S.E.M.</th>
<th>% Seizure Protection</th>
<th>% Mortality</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (Normal saline) 0.2 ml</td>
<td>1.652 ±0.089</td>
<td>14.86 ±1.319</td>
<td>0</td>
<td>33.33</td>
</tr>
<tr>
<td>Phenytoin 100 mg/kg</td>
<td>---</td>
<td>---</td>
<td>100**</td>
<td>0</td>
</tr>
<tr>
<td>Fluoxetine 20 mg/kg</td>
<td>1.965 ±0.388</td>
<td>11.69 ±1.115</td>
<td>0</td>
<td>33.33</td>
</tr>
<tr>
<td>Citalopram 1 mg/kg</td>
<td>2.093 ±0.059</td>
<td>11.45 ±0.649</td>
<td>0</td>
<td>50</td>
</tr>
<tr>
<td>Venlafaxine 25 mg/kg</td>
<td>2.65 ±0.149*</td>
<td>10.51 ±0.516*</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

* p < 0.05 when compared to control group by post hoc tukey test.
## p < 0.01 when compared to control group by Fisher’s exact test.

i.p. intraperitoneal.

T.H.E. Tonic Hind Limb Extension  S.E.M. Standard Error of Mean

Table 1 show that the anticonvulsant effect was observed in venlafaxine group in maximal electroshock induced seizures method. The latency for convulsion was significantly more when compared statistically with control group (p<0.05). The duration of tonic hind limb extension phase was significantly lower in venlafaxine group as compared statistically with control group (p<0.05). The fluoxetine and citalopram group did not show statistically significant difference when compared to control group in latency to convulsion and duration of tonic hind limb extension. The study drugs did not show statistically significant seizure protection when compared to control group. Venlafaxine group showed no mortality when compared to the control group, but was statistically not significant. Fluoxetine and citalopram groups did not show any reduction in mortality as compared to control group.
Table 2: Effect of drugs on pentylenetetrazol induced convulsions in mice.

<table>
<thead>
<tr>
<th>Drugs &amp; Dose (n=6)</th>
<th>Latency to convulse (sec)</th>
<th>Duration of clonic convulsions</th>
<th>Number of clonic convulsions</th>
<th>% Seizure protection</th>
<th>% Mortality</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (Normal saline) 0.2 ml</td>
<td>63.07 ±6.427</td>
<td>23.83 ±3.554</td>
<td>3.500±0.428</td>
<td>0</td>
<td>100</td>
</tr>
<tr>
<td>Diazepam 2.5 mg/kg</td>
<td>---</td>
<td>---</td>
<td>0</td>
<td>100##</td>
<td>0##</td>
</tr>
<tr>
<td>Fluoxetine 20 mg/kg</td>
<td>136.5 ±15.34*</td>
<td>12.65 ±3.896</td>
<td>1.167 ±0.477***</td>
<td>33.33</td>
<td>50</td>
</tr>
<tr>
<td>Citalopram 1 mg/kg</td>
<td>155.1 ±17.81**</td>
<td>14.90 ±2.878</td>
<td>1.500 ±0.224**</td>
<td>0</td>
<td>83.33</td>
</tr>
<tr>
<td>Venlafaxine 25 mg/kg</td>
<td>117.4 ±17.23</td>
<td>14.36 ±1.084</td>
<td>2.500 ±0.224</td>
<td>0</td>
<td>66.67</td>
</tr>
</tbody>
</table>

Values are in Mean ±S.E.M.

* p<0.05 when compared to control group by post hoc tukey test
** p<0.01 when compared to control group by post hoc tukey test
*** p<0.001 when compared to control group by post hoc tukey test
## p<0.01 when compared to control group by Fisher’s exact test.

Table 2 shows that anticonvulsant effect in pentylenetetrazol induced seizure method was observed in fluoxetine and citalopram groups. Fluoxetine and citalopram groups prolonged the latency to convulsions when compared statistically with the control group (p<0.05 and p< 0.01, respectively). Venlafaxine group also prolonged the latency to convulsion as compared to the control group, but was not statistically significant. Fluoxetine and citalopram group significantly reduced the total number of clonic convulsions when compared to control group (p<0.001 and p<0.01, respectively). There was no statistically significant reduction in the number of clonic convulsions in venlafaxine group. There was no statistically significant reduction in duration of clonic convulsions in all the three study drugs when compared to control group. Fluoxetine prevented the occurrence of seizures in two out of six mice in the group, offering a seizure protection of 33.33%, but it was statistically not significant when compared to control group. Citalopram and venlafaxine did not show any seizure protection. There was no any statistically significant reduction in mortality in any of the three study drugs when compared with control group.
Table 3: Effect of different drugs (i.p.) on Inverted screen test in albino mice

<table>
<thead>
<tr>
<th>Drug (n=6)</th>
<th>Dose mg/kg</th>
<th>Number of mice falling</th>
<th>% Mice falling</th>
<th>% Mice climbing</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (Normal Saline)</td>
<td>0.2 ml</td>
<td>0/6</td>
<td>0</td>
<td>100</td>
</tr>
<tr>
<td>Phenytoin</td>
<td>100</td>
<td>6/6</td>
<td>100**</td>
<td>0</td>
</tr>
<tr>
<td>Diazepam</td>
<td>2.5</td>
<td>6/6</td>
<td>100**</td>
<td>0</td>
</tr>
<tr>
<td>Fluoxetine</td>
<td>20</td>
<td>0/6</td>
<td>0</td>
<td>100</td>
</tr>
<tr>
<td>Citalopram</td>
<td>1</td>
<td>2/6</td>
<td>33.33</td>
<td>66.67</td>
</tr>
<tr>
<td>Venlafaxine</td>
<td>25</td>
<td>2/6</td>
<td>33.33</td>
<td>66.67</td>
</tr>
</tbody>
</table>

** p < 0.01 when compared to control group by Fisher’s exact test.

The tendency to cause neurodeficit by the drugs is evaluated in Table 3. Six out of six mice failed to climb to the top with inverted screen test in both phenytoin and diazepam group. It was statistically significant (p<0.01) when compared to control group. All the mice were able to climb the inverted screen in fluoxetine group showing no neurological deficit. Two out of six mice failed to climb to the top in both citalopram and venlafaxine groups, but it was not statistically significant when compared to control group.

Table 4: Effect of drugs (i.p.) on Locomotor activity of albino mice at different time interval.

<table>
<thead>
<tr>
<th>Drug (n=6)</th>
<th>0 min</th>
<th>30 min</th>
<th>60 min</th>
<th>90 min</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (Normal saline) 0.2 ml</td>
<td>171.5±8.225</td>
<td>150.3±11.64</td>
<td>135.0±4.374</td>
<td>101.2±4.512</td>
</tr>
<tr>
<td>Phenytoin 100 mg/kg</td>
<td>170.8±12.99</td>
<td>69.67±11.22 ***</td>
<td>75.67±3.263 **</td>
<td>85.17±9.332</td>
</tr>
<tr>
<td>Diazepam 2.5 mg/kg</td>
<td>152.7±3.964</td>
<td>51.50±5.578 ***</td>
<td>72.33±3.353 **</td>
<td>75.83±3.331</td>
</tr>
<tr>
<td>Fluoxetine 20 mg/kg</td>
<td>173.0±6.899</td>
<td>74.83±5.218 **</td>
<td>89.67±3.373 *</td>
<td>90.50±3.640</td>
</tr>
<tr>
<td>Citalopram 1 mg/kg</td>
<td>159.0±9.216</td>
<td>133.3±10.57</td>
<td>105.7±16.08</td>
<td>89.50±9.319</td>
</tr>
<tr>
<td>Venlafaxine 25 mg/kg</td>
<td>155.3±5.321</td>
<td>140.2±20.09</td>
<td>117.5±16.18</td>
<td>107.2±5.237</td>
</tr>
</tbody>
</table>

Values are in Mean ±S.E.M.
*p <0.05 when compared to control by post hoc tukey test
**p <0.01 when compared to control by post hoc tukey test
*** p<0.001 when compared to control by post hoc tukey test

Table 4 shows that the basal counts of spontaneous activities were comparable in all the six groups in spontaneous motor activity test by using digital actophotometer. There was a significant reduction in motor activities in phenytoin and diazepam groups at 30 minutes (p<0.001) and 60 minutes (p<0.01) when compared to the control group. Fluoxetine group showed a significant reduction in spontaneous activities at 30 minute and 60 minute interval (p<0.01 and p<0.05 respectively) when compared to the control group. The activities of citalopram and venlafaxine groups were comparable with control group at 30 and 60 minute interval. At 90 minutes, the spontaneous activities were comparable in all the six groups.

Discussion
Antiepileptic drug therapy remains far from optimal. The presently available AEDs i.e. phenytoin, phenobarbitone, carbamazepine, benzodiazepines, valproate, ethosuximide are unable to control seizures effectively in about 30% patients. Furthermore, the dose related neurotoxicity and other side effects associated with established AEDs are major limitations in their clinical use. The newer AEDs vigabatrin, lamotrigine, gabapentin, topiramate, felbamate have turned out to be a real progress in the treatment of non-responders or refractory patients. However, it is apparent that they are neither panaceas nor magic bullets for epilepsy. Furthermore they have been mainly tried as add on therapy and with these drugs also only a small percentage of patients become seizure free. The problem of adverse effects has also not been circumvented completely. Hence,
the search continues to develop newer, more effective, safe, neuroprotective agents for the treatment of epilepsy.

In the present study the SSRIs (Fluoxetine and Citalopram) and SNRI (Venlafaxine) were evaluated for anticonvulsant activity in experimental models using albino mice i.e. maximal electroshock and pentyleneetetrazol-induced seizures, known validated models for grand mal epilepsy and petit mal epilepsy respectively. Acute toxicity from anticonvulsant drugs almost invariably exhibits itself in some type of neurological abnormality. Each animal was therefore examined for its neurological status before the administration of the test drug and at a suitable interval thereafter. The inverted screen test and spontaneous motor activity with digital actophotometer were used to detect any neurological deficit. The tendency to cause neurodeficit was studied by observing the ability of mice to climb the inverted screen. In this test, drugs with sedative and/or ataxic properties produce dose dependent failure to climb the inverted screen. In the present study, none of the study drugs showed significant neurological deficit in the inverted screen test. There was a significant reduction in the spontaneous motor activity in fluoxetine treated group at 30 and 60 minute interval.

The maximal electroshock induced seizures is a test that predicts drugs effective in generalized tonic-clonic (grand mal) seizures. Evaluation of the anticonvulsant effects of venlafaxine demonstrated the anticonvulsant efficacy in MES induced seizures, but did not show protection against pentylenetetrazol induced seizures. It significantly prolonged (p<0.05) the mean latency to convulsion. The mean duration of tonic hind limb extension was also significantly reduced (p<0.05) in comparison with the control group. There was no significant seizure protection in venlafaxine treated mice, and there was no mortality in comparison to control group.

PTZ test is widely used as a standard model for absence (petit mal) epilepsy. Our study showed that fluoxetine and citalopram have clear-cut anti-petitmal activity in albino mice, but no anticonvulsant efficacy in MES induced seizures. Fluoxetine at 20 mg/kg significantly (p<0.05) prolonged the mean latency to convulsions when given intraperitoneally. It also significantly reduced (p<0.001) the mean number of convulsions in the mice. Citalopram also significantly prolonged the mean latency to convulsion and reduced the total number of convulsions (p<0.01). However, both the drugs were unable to show significant reduction in duration of convulsions and mortality. The probable mechanism of anticonvulsant action of fluoxetine and citalopram in PTZ seizures in albino mice in our study is mainly attributable to their ability to increase extracellular 5-HT. Serotonergic neurotransmission modulates a wide variety of experimentally induced seizures and is involved in the enhanced seizure susceptibility observed in rodents genetically prone to epilepsy. Elevation of extracellular 5-HT leads to inhibition of both focal (limbic) and generalized seizures. Serotonin causes a significant shift in the excitability in most networks involved in epilepsy. The anticonvulsant action of fluoxetine may also be related to its inhibitory effect on ion channels. Deak et al (2000) demonstrated that fluoxetine inhibited L-type more potently than T-type Ca** channels or Na+ channels and inhibition of L-type Ca** channels may contribute to its anticonvulsant action. The anticonvulsant activity of fluoxetine may also be attributed to its ability to increase the concentration
of allopregnanolone, a GABAergic neuroactive steroid.\textsuperscript{11}

The anticonvulsant action of venlafaxine seems to result from the enhanced serotonergic and noradrenergic neurotransmission. Venlafaxine blocks the reuptake of noradrenaline, serotonin and, to a lesser extent, dopamine, enhancing binding of these neurotransmitters to pre- and postsynaptic receptors. Fluoxetine and citalopram were totally devoid of anticonvulsant activity in maximal electroshock induced seizures. Since the MES test is thought to indicate a drug’s ability to prevent seizure spread,\textsuperscript{30} it can be concluded that both the drugs are incapable of preventing spread of seizure discharge. In the MES test usually a supramaximal current strength (50 mA in mice and 150 mA in rats) is given. The disadvantage is that anticonvulsant which increases the seizure threshold but is not potent enough to raise the threshold above 50 mA or 150 mA are missed by this test, although such drugs could be of clinical value.\textsuperscript{31}

In our study also, it is quite possible that the drugs may have an anticonvulsant effect but it was missed because of the higher intensity of current. Furthermore, inactivity of a drug in the supramaximal MES test does not necessarily mean that the drug is not effective against generalized tonic clonic seizures in humans.

**Conclusion**

Most of the epilepsies are idiopathic. Presently available drugs are not antiepileptic but are anti-ictal in true sense, since they do not attack to the cause. Most of the epilepsies are refractory to the existing drugs.

Venlafaxine showed statistically significant anticonvulsant activity against maximal electroshock induced seizures which can be mainly attributed to enhancement of the serotonin and norepinephrine neurotransmission in the brain. Fluoxetine and citalopram have shown statistically significant anticonvulsant action against pentylenetetrazol induced seizures, mainly due to their ability to increase in extracellular levels of serotonin in the brain. Elevated serotonin limits the spread of focal and generalized seizures.

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**References**


