

## Studies on the role of 5-HT<sub>2A</sub> and 5-HT<sub>2C</sub> receptor antagonist and effects of co-administration of Fluoxetine in regulating generalized seizures in albino rats

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**Abstract:** *Introduction:* Epilepsy is due to imbalance between inhibitory & excitatory neurotransmitter release at synaptic level in brain such as GABA, Serotonin, Glutamate and nor epinephrine. Recently there are few reports suggesting that, 5-HT<sub>1A</sub> receptor antagonist with co-administration of fluoxetine has shown anticonvulsant activity. The present study is undertaken to evaluate the action of 5-HT<sub>2A/2C</sub> mediated anticonvulsant action of Trazodone in MES (Maximum Electro Shock) model in albino rats. *Materials & Methods:* Fifty albino rats of 200-250 gms of either sex were divided into five groups each of 10 rats (n=10), Group-I received distil water 0.5ml oral, Group -II- received sodium valproate - 200mg/kg bw intra peritoneal(i.p.)acted as positive control, Group -III- received Trazodone 54mg/bw, orally Group- IV- received sub-anticonvulsant dose of Fluoxetine 6mg/kg/bw i.p. Group- V- received Trazodone 54mg/kg/bw and Fluoxetine 6mg/kg bw. Subsequently all groups were subjected for MES. The results were analyzed by calculating the mean duration of convulsions & absence of HLE and comparison was done by student't' test. *Results:* The present study revealed that sodium valproate showed 100% protection against MES as compared to negative control,(P<0.05). Trazodone showed 40% protection against MES& decrease in the duration of convulsions by 60%, and Fluoxetine sub-anticonvulsive dose combined with Trazodone 54 mg /kg b.w. has shown 90% protection against MES. The results are parallel to standard drug sodium valproate. *Conclusion:* Trazodone has exerted anticonvulsant activity, by enhancing 5-HT&NE extra cellular level in brain, and probably potentiated the action of sub anticonvulsive dose of fluoxetine in combination. However, further investigative studies are needed to confirm the potentiation of trazodone action.

**Key words:** Epilepsy, Serotonin, Fluoxetine, Trazodone

### Introduction

Bioamine theory of depression implicates nor-epinephrine and serotonin. Most of the clinically used drugs are believed to act by influencing re-uptake of nor-epinephrine or 5-HT [1]. Though paucity of information regarding subtypes of 5-HT receptor implicated in depression has not been elucidated, antidepressants belonging to SSRI group (fluoxetine etc....) act by enhancing 5-HT level. Some atypical antidepressants like Trazodone are believed to act by influencing serotonin receptor subtype 5-HT 2A / 2C [2]. It is of interest to note that 5-HT 2A subtype receptors are also located on GABAergic interneurons in brain and their activation enhances GABA release leading to decreased firing from involved neurons [3]. In view of this Trazodone like agents might have anti convulsant action. In clinical practice it is more often used as sedative than anti-depressant [4]. Trazodone's anti convulsant activity, as hypothesized does not seem to have been explored. On other hand fluoxetine, a SSRI has been reported to exert anti-seizure action in animal model [5]

and also clinically [6]. Hence, it is worthwhile to screen Trazodone for its anti-seizure activity and see if fluoxetine, despite being shown as anti-seizure has, according to some authors<sup>5</sup> seizure precipitating potential. Trazodone does not behave like wise.

### Materials and Methods

The present study was under taken to evaluate anti-convulsant activity of Trazodone and Fluoxetine, with known antiepileptic drug Sodium valproate for comparison. Drugs for the experiments were obtained as follows:

I} (1) Trazodone – (Tablet-Trazonil 100mg) purchased. (2) Fluoxetine – provided by Natco pharma limited, Hyderabad in pure form. (3) Sodium valproate provided by Sun Pharma limited Ahemdabad.

II} The instruments used for the experiments are: (1) Electroconvulsimeter (Techno). (2) Syringes including tuberculin syringe, beaker, measuring jars, weighing balance and stop watch.

III} Animal: Healthy adult Albino rats of either sex weighing 200 to 250 gms were housed in group of 5 per cage and provided with a free access to food and water. Only animals showing Hind Limb Extensor tone (HLE +ve) were chosen. The experiment was performed in freely moving animals adjusted to the laboratory condition prior to experiments. The animals were procured from central animal house facility at BLDEU's Shri B.M.Patil Medical College and Research Centre Bijapur. Animals were divided into 5 groups; each group consisting 10 animals (n=10). The grouping was done as shown in table No.1 Maximum electroshock seizure (MES) [7] was produced with an alternating current of 150 mA, delivered through the ear clip electrodes for 0.2 seconds. Initially all the animals were given MES, only those animals showing HLE tone were selected for subsequent testing.

Group 1: Distilled water 0.5ml treated group served as negative control,

Group 2: Sodium valproate (200mg/kg bw), i.p. given 1 hour before induction of MES as positive control.

Group 3: Test drug Trazodone 54 mg/kg bw administered orally, 4 hours before induction of MES.

Group 4: Test drug sub anticonvulsive dose of Fluoxetine 6 mg/kg bw i.p. given 1 hour prior to MES.

Group 5: Test drug Trazodone 54mg/kg bw given orally, after 4 hours the Fluoxetine 6mg/kg bw i.p. given, one hour before induction of MES

Table 1: Study design: parallel group study showed as below:

Sl. No	Group	No. of animal	Drug with dose
1	Negative control	10	D.W.0.5ml i.p.
2	Positive control	10	Sodium valproate 200 mg/kg bw i.p.
3	Test drug	10	Trazodone 54 mg/kg bw oral
4	Test drug	10	Fluoxetine 6mg kg/bw i.p.
5	Test drug combination	10	Trazodone 54 mg/kg bw (oral) + Fluoxetine 6 mg/kg bw i.p.

*Note:* All doses of drugs were selected by converting from human dose to animal dose using Paget & Barnes [8-9] table & drugs were administered prior to induction of MES as shown above. All Animals were tested for MES at the time of peak concentration of drugs and the parameters observed.

*Parameters:* (1) Duration of HLE (in sec.) (2) Hind limb extension tone present/ Absent (3) Percentage of animals showing abolition of HLE (4) Mortality observed up to 48 hrs. Complete absence of HLE and decrease in duration of HLE were taken as protection against convulsions and significant protection in various treated group in contrast to control was assessed by student 't' test [8].

### Results

As stated earlier this study has been undertaken to evaluate the anticonvulsant activity of Trazodone and fluoxetine given alone and in combination in albino rats in Maximum electroshock seizure (MES). Control animal showed duration of HLE  $11.77 \pm 3.84$  in seconds and 0% protection against HLE, No mortality were observed for 48 hours. Sodium valproate 200mg/kg bw i.p., showed complete abolition of HLE (100 %), No animal mortality up to 48 hours. Trazodone 54mg/kg bw has decreased the duration of HLE in 6 animals ( $4.7 \pm 1.85$  sec) and complete abolition of HLE in 4 animals i.e. (40%) protection, against MES, No mortality. Pilot study done showed 6mg/kg bw of Fluoxetine as anticonvulsant dose. In this dose duration of HLE was  $9.78 \pm 0.63$  in 8 animals and 20% protection against HLE. The sub anticonvulsive dose of Fluoxetine (6mg/kg bw) when combined with Trazodone 54mg/kg bw has shown 90 % protection against HLE, one rat was not protected but showed decrease in HLE duration (4.0sec). No mortality was noted.

Table 2: Anticonvulsant activity of Drugs (Mono & Combined therapy) in MES Model, N= 10, Mean  $\pm$  S.D

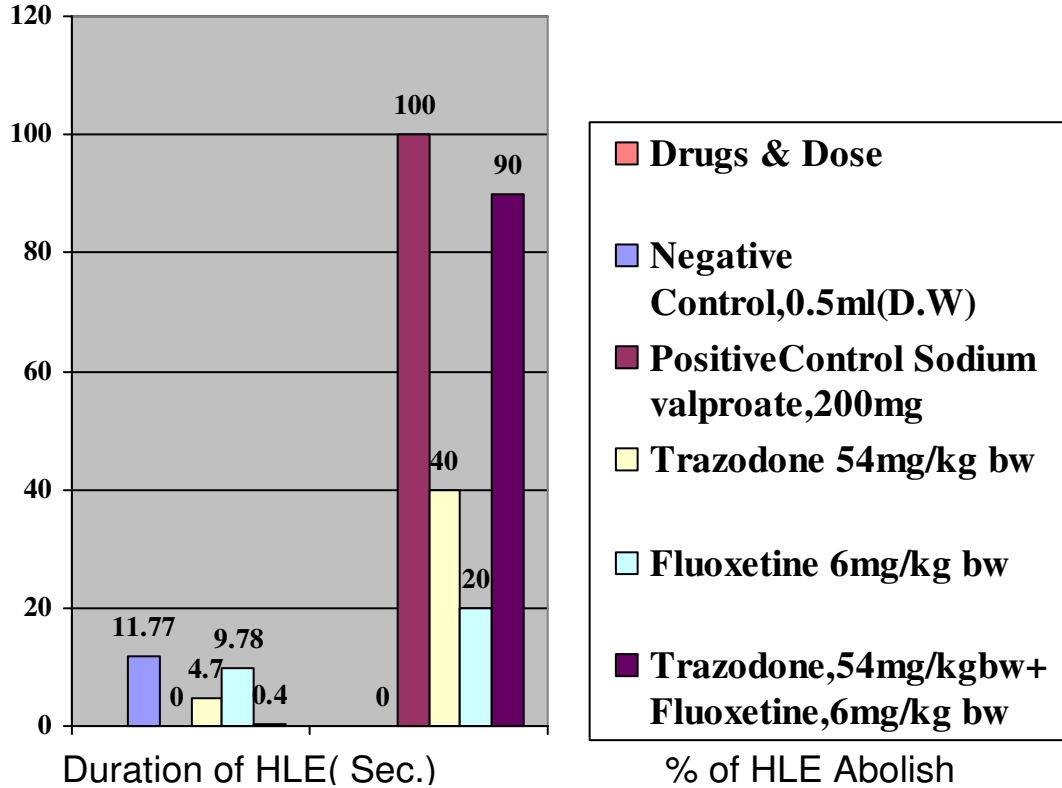
Groups	Drugs & Dose	Duration of HLE (sec)	% of HLE abolished	Mortality observed up to 48 hrs.
1	Negative control(Distil Water 0.5ml,oral)	$11.77 \pm 3.84$	0%	Nil
2	Positive control sodium valproate 200 mg(i.p)	Nil	100%	Nil
3	Trazodone (54mg/kg bw, oral)	$4.7 \pm 1.85$	40%	Nil
4	Fluoxetine(6mg/kg/BW.i.p.)	$9.78 \pm 0.63$	20%	Nil
5	Trazodone(54mg/kgbw oral + Fluoxetin 6 mg/kg/bw,i.p.)	$4.0 \pm 0.0$ Nil	90% *	Nil

\*p<0.05

**Note:**

- \* Optimum Dose of Trazodone has shown 40% protection against HLE.
- \* Sub anticonvulsive dose of fluoxetine has shown 20% protection against HLE.
- \* Two drug combinations (Trazodone 54mg+ Fluoxetine 6mg, sub anticonvulsive dose) has shown 90% protection against seizure.

**Chart:**



**Discussion**

The bioamine theory of depression implicates decreased level of 5-HT and norepinephrine, most of the clinically used drugs in depression act by inhibiting reuptake of 5-HT or norepinephrine<sup>1</sup> and evidence suggested that reduced brain serotonin and nor epinephrine content have a proconvulsant effect in animal models[2]. Experimental studies revealed that brain 5 HT plays a important role in various model of generalized seizures, increase in 5-HT level in brain can be achieved by

- a) Electrical stimulation of the medial raphe nucleus (MRD) [3].
- b) 5-HT uptake blockers [4]
- c) By administration of 5 Hydroxytryptophan.
- d) Co-administration of mono amino oxidase inhibitors with SSRI's [10].
- e) By modulating sub type of 5-HT receptor, for enhancing serotonin release i.e. 5-HT<sub>1A</sub> blockade [10].

With above background 5-HT up take blockers reduced the severity of generalized seizure and delayed their onset and duration in electroshock seizure in rats and this study is supported by enhancement of 5-hydroxytryptaminergic neurotransmission which has an anticonvulsant effect in genetically epilepsy prone rats [5]. Another study shows that the possible relationship between brain biogenic amines i.e. endogenous norepinephrine (NE) and serotonin, but not dopamine depletion attenuated audiogenic seizures [11]. It was suggested that both 5 HT and NE play a major role in inhibiting seizure activity in the brain<sup>17</sup> and such type of depletion of biogenic amines results in “release” of inhibitory influence on nerve transmission [7]. Ronald A Brown (1997) has shown in his study that enhancement of anticonvulsant effect of fluoxetine following blockade of 5HT<sub>1A</sub> receptor [9] are located at hippocampus & limbic system etc [12]. But, it is of interest that, no literature reveals about 5HT<sub>2A/2C</sub> receptor role involvement in seizures. The 5HT<sub>2A/2C</sub> receptor are predominantly located in cortex, neocortex, hippocampus and caudate nucleus. The precise roles in the CNS remain unclear, but in rodent agonist’s activity over 5HT<sub>2</sub> receptor evoked in motor behaviors [12]. In this context we wished to determine whether acute administration of Trazodone could exert anticonvulsant action against maximum electroshock induced seizures in otherwise normal rats. Particularly this study has focused on 5HT<sub>2A/2C</sub> receptor mediated anticonvulsive activity of Trazodone. Trazodone is a metabolite of m-chlorophenylpiperazine (mcpp). Reflex activation of 5-HT<sub>2A/2C</sub> receptor exerts antidepressant action and is approved clinically [2]. In our study, Trazodone alone has shown anticonvulsant activity by inhibiting MES induced convulsion (40% HLE) and decreased duration of seizure. Fluoxetine 6 mg/kg bw has shown 20% protection against MES in our study but Fluoxetine 10mg/kg bw has show complete protection against- seizure [13]. Trazodone 54mg/kg bw and sub anticonvulsant dose of Fluoxetine 6mg/kg bw has shown significantly protection against- MES (90%), and duration of convulsions as compared to sodium valproate which has show 100% protection as shown in table No1. The anticonvulsant action of Trazodone could be due to activation of 5HT<sub>2A/2C</sub> receptor in the cortex of brain, which enhanced the 5-HT and nor epinephrine by blocking its reuptake. Another possibility could be, stimulation of 5HT<sub>2A/2C</sub> sub type receptor which are located on piriform cortex GABAergic interneuron’s in brain, and their activation enhances GABA release which decreases firing of involved neurons[5]. Trazodone also has sedative-hypnotic action similar to benzodiazepine [4] and also by activation of 5HT<sub>2A/2C</sub> receptor mediated release of GABA could be responsible for added anti-seizure effect. Fluoxetine (SSRI) appears to be selectively correlated with an enhanced synaptic availability of serotonin [5] and Augusto Pasin et al mentioned in his study that fluoxetine may act to reduce seizure susceptibility in concert with GABA. It is possible that other site at which GABA exerts an anticonvulsant action may also be sites at which fluoxetine- induced enhancement of serotonin transmission may result as anticonvulsant [14-15]. Our study reveals that the Trazodone and sub anticonvulsive dose of fluoxetine could exert anticonvulsive action by above mechanism which have shown synergetic action, or Trazodone has potentiated action of fluoxetine or vice-versa. Thus, Trazodone and fluoxetine may have major indication in treating depressed patients who might be at

risk for seizure, as suggested by Simon Fleminger that in post traumatic epilepsy with depression, prophylactic anti-convulsants have no role in reducing seizures [16]. Such cases can be managed by antidepressants with Fluoxetine (selective serotonin-reuptake inhibitor) and by combined with a Trazodone, (tricyclic antidepressant) being a sedative may be particularly useful [17].

### Conclusion

Trazodone has exerted anticonvulsant activity, probably by enhancing 5-HT, NE level & release of GABA from GABAergic Neurons in brain and potentiate the action of sub anticonvulsive dose of Fluoxetine. However, further investigative studies are needed to confirm these findings.

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