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Evaluation of antiepileptic activity of Ondansetron in Albino Rats

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Abstract
Receptors like $5HT_3$, $GABA_A$, glycine, and nicotinic acetylcholine (nACh) belong to a superfamily of ligand gated ion channels and subunits of these receptors exhibit extensive amino acid sequence homology receptors. May be due to these facts drugs acting on the 5HT3 receptor can also act on other. For example, some 5HT3 receptor antagonists can act on the $GABA_A$ receptor complex in addition to their effects on 5HT3 receptors**.**
This particular study was conducted to evaluate the anticonvulsant action of Ondansetron and compare it with Phenytoin sodium.
30 albino rats of male sex weighing 150-200g each are selected and randomly divided into 5 equal groups. Maximal Electroshock (MES) seizures were induced in albino rats via transauricular electrodes (150mA, 0.2 seconds). Each rat were pretreated with intraperitoneal drugs i.,e, 30 minutes before MES test. The group 1 is given normal saline 1ml/kg, Group 2 is given phenytoin Sodium (25 mg/kg), group 3 ondansetron (0.5 mg/kg), group 4 ondansetron (1 mg/kg) and group 5 ondansetron (2 mg/kg). ANOVA test was used for statistical analysis of the data.
In present study only 33.33% of the rats were protected from MES induced seizures in Ondansetron 0.5 mg/kg group but protection was not comparable to standard Phenytoin sodium. Ondansetron in 1 mg and 2 mg/kg did not give any protection against MES induced seizures. Though the previously conducted studies on the antiepileptic activity of ondansetron provide sufficient evidences to support its antiepileptic activity, present study failed to reproduce similar protection in MES induced seizures.

Keywords: HT3 antagonist, Ondansetron, Maximal Electroshock (MES) Seizures, Phenytoin sodium

Introduction
Epilepsy is a common neurological disorder having important medical, social and psychological consequences [1]. It is characterized by an enduring predisposition to generate epileptic seizure and by the neurobiologic, cognitive, psychological and social consequences of the condition [2]. Epilepsy is a heterogeneous symptom complex characterized by recurrent seizures. Seizures are finite episodes of brain dysfunction resulting from abnormal discharge of cerebral neurons [3]. An epileptic seizure is a transient occurrence of signs and/or symptoms due to abnormal, excessive and synchronous neuronal activity in the brain [4].
Before the antiepileptic drugs were discovered and developed, treatment of epilepsy consisted of trephining, cupping and administration of herbal medicines [5].
The first antiepileptic drug was *bromide*, which was used in the late nineteenth century. *Phenobarbital* was the first synthetic organic agent recognized as having antiseizure activity. Its usefulness however, was limited to generalized tonic clonic seizures, and to a lesser degree, simple and complex partial seizures. It had no effect on absence seizures [6].
The chemical structures of most of the drugs like hydantoins, succinimides introduced before 1965 were closely related to phenobarbital. Later chemically distinct structures of the benzodiazepines, an iminostilbene (carbamazepine), and a branched-chain carboxylic acid (valproic acid) were introduced, followed by a phenyltriazine (lamotrigine), a cyclic analog of GABA (*gabapentin*), a sulfamate-substituted monosaccharide (topiramate), a nipecotic acid derivative (tiagabine), and a pyrrolidine derivative (*levetiracetam*) [6].
Despite such a wide therapeutic armamentarium, it is currently estimated that about 30% of epileptic patients do not receive satisfactory treatment. Thus, to overcome limitations of current anticonvulsant treatment, one of the most ambitious goals in today's antiepileptic research is the identification of additional molecules targeting novel molecular mechanisms involved in neuronal excitability control [7].

Receptors like $5HT_3$, $GABA_A$, glycine, and nicotinic acetylcholine (nACh) belong to a superfamily of ligand gated ion channels and subunits of these receptors exhibit extensive amino acid sequence homology receptors. May be due to these facts, drugs acting on the 5HT3 receptor can also act on other receptors [8]. The $5HT_3$ receptor subtype is found in the peripheral and central nervous system [9]. Hence $5HT_3$ antagonists, in addition to their antiemetic effects, could have other potential clinical applications in anxiety disorders, drug abuse, appetite disorders, and enhancement of cognitive functions [10].

5-HT_3 receptor being ion gated receptor induces movement of cations (Na^+, k^+) into the cells leading to depolarization. 5-HT_3 antagonist by interfering with the movement of cations, may decrease the depolarization of the cell [11].

Therefore Ondansetron, a potent antiemetic and 5-HT_3 receptor antagonist was investigated for its possible antiepileptic action. So this study is undertaken to evaluate the anticonvulsant effect of Ondansetron in albino rats.

Phenytoin being one of the most widely used anti-seizure agent has been taken as standard drug

Materials & Methods

For the present study, Albino rats of male sex weighing 150-200g were bred in the central animal house J.J.M. Medical College, Davangere.

The above test animals were subjected to electroshock of 150mA intensity for 0.2 seconds, through auricular electrodes, (covered in cotton wool and saline moistened). A majority of rats showed tonic flexion and extension of fore and hind limbs, clonus, stupor followed by postictal depression and recovery. Only those rats showing the convulsive responses were used for the experiment.

Drugs used:

Phenytoin sodium: Eptoin 50mg/2ml, from Abott Pharmaceuticals, was used at a dose of 10mg/kg and was administered intraperitoneally.

Ondansetron: Periset 4mg/2ml, from IPCA Pharmaceuticals was used in the dose of 0.5mg/kg, 1 mg/kg, 2 mg/kg and was administered intraperitoneally.

Distilled Water: As control and vehicle to mix the test drugs.

Inclusion criteria

- Male rats weighing 150-200g.
- Healthy rats with normal behavior and activity.

Exclusion Criteria

- Animals weighing more than 200g and less than 150g.
- Pregnant females and those which have delivered once.
- Animals previously used for any other experiment.

A total of 30 animals (N=30) were used which were divided into 5 groups of 6 (n=6) animals each. Animals were housed randomly containing with 6 animals per cage at a controlled temperature of 21°± 3 °C, with a 12 hour light: dark cycle. Free access to standard pellet and water was provided. All the test animals were subjected to further experiment of this study after 24hrs (to avoid any possible "Kindling" effect). All the preparations were administered intraperitoneally (ip).

Group I – Control, distilled water 1 ml/kg i.p.
Group II – Standard, Phenytoin sodium 25mg/kg i.p.
Group III – Test drug Ondansetron 0.5 mg/kg i.p
Group IV— Test drug Ondansetron 1 mg/kg i.p
Group V—Test drug Ondansetron 2 mg/kg i.p

They were evaluated for antiepileptic activity using MES model. The experiment was conducted in Post Graduate Experiment Laboratory of the Department of Pharmacology, J.J.M. Medical College, Davangere between 8:00 A.M. to 2:00 P.M. The laboratory was equipped with standard fluorescent lighting. Animals were brought to the experiment room 1 hr prior to the beginning of experiment and identification mark was placed on the animal tail with indelible ink. The food and water was removed for the duration of test. Animals were weighed and appropriate dose of drug was injected intraperitoneally to different groups. After an interval of 30 minutes they were subjected to Maximal electroshock (MES) stimulation of 150mA for 0.2 seconds through transauricular electrodes by using technoelectroconvulsiometer.

The Parameters studied are

1. Hind limb tonic extension (HLTE).
2. Duration of tonic hind limb extension (HLTE)
3. Duration of time to regain righting reflex (from the end of HLTE till the animal could stand on 4 legs, RR)
4. Duration of postictal depression.(from the end of regaining righting reflex till the animal walks away, PID)

Statistical Analysis

Results are presented as Mean ± SEM. One way ANOVA was used for multiple comparisons followed by Tukey's post hoc test for comparison between groups. For all the tests a 'P' value of 0.05 or less was considered for statistical significance.

Results

Table 1: Group 1, Control (C) Distilled Water 1ml/Kg

Parameters		Subgroups								
Duration In Secs	1	2	3	4	5	6	Mean	SD	SE
HLTE (abolition)	NO	NO	NO	NO	NO	NO	-	-	-
Duration of HLTE	18	11	12	14	19	10	14.8	3.639	1.486
Duration for righting reflex	134	224	324	180	200	210	212	63.16	25.78
Duration of postictal depression	32	30	24	29	21	35	28.5	5.16	2.11

Table 2: Group 2, Standard (S) Phenytoin Sodium 25mg/kg BODY WT (I.P)

Parameters Duration In Secs	Subgroups						
	1	2	3	4	5	6	Mean
HLTE (abolition)	YES	YES	YES	YES	YES	YES	-
Duration of HLTE	-	-	-	-	-	-	-
Duration for righting reflex	-	-	-	-	-	-	-
Duration of postictal depression	-	-	-	-	-	-	-

Table 3: Group 3, Test Group (T1) Ondansetron 0.5 mg/kg BODY WT (I.P)

Parameters Duration In Secs		Subgroups					Mean	SD	SE
	1	2	3	4	5	6			
HLTE (abolition)	NO	NO	YES	NO	NO	YES	-	-	-
Duration of HLTE	12	11	-	10	10	-	7.167	5.60	2.286
Duration for righting reflex	136	120	100	180	206	60	133.	53.16	21.7
Duration of postictal depression	18	17	24	21	20	21	20.17	2.48	1.01

Table 4: Group 4, Test Group (T2) Ondansetron 1 mg/kg Body Wt (I.P)

Parameters Duration In Secs		Subgroups							
	1	2	3	4	5	6	Mean	SD	SE
HLTE (abolition)	NO	NO	NO	NO	NO	NO	-	-	-
Duration of HLTE	13	14	20	20	17	15.5	16.58	2.97	1.21
Duration for righting reflex	146	186	134	152	140	130	148	20.24	8.26
Duration of postictal depression	24	20	26	21	21	19	21.83	2.639	1.07

Table 5: Group 5, Test Group (T3) Ondansetron 2 mg/kg BODY WT (I.P)

Parameters Duration In Secs		Subgroups							
	1	2	3	4	5	6	Mean	SD	SE
HLTE (abolition)	NO	NO	NO	NO	NO	NO	-	-	-
Duration of HLTE	17	16	11.4	13	11	10.2	13.10	2.8	1.14
Duration for righting reflex	107	180	174	162	178	182	163.8	28.74	11.73
Duration of postictal depression	19	21	27	22	18	27	22.33	3.88	1.58

Table 6: Group Wise Comparison of Percentage of Protection for Mes Seizures (Abolition of Hlte)

Drug	% of protection
Normal saline	0
Phenytoin sodium 25mg/kg	100%
Ondansetron 0.5mg/kg	33.33%
Ondansetron 1 mg/kg	0
Ondansetron 2 mg/kg	0

Table 7: Tukey's Multiple Comparison Test Showing Difference between Groups in Duration of Hlte in Mes

difference between groups		
Groups Compared	**Mean Difference**	**(P<0.05)stastical significance**
Group 1&2	14.08	S
Group1&3	6.19	S
Group1&4	-2.5	NS
Group1&5	0.9833	NS
Group 2&3	-7.16	S
Group2&4	-16.58	S
Group2&5	-13.10	S
Group3&4	-9.41	S
Group3&5	-5.93	NS
Group 4&5	3.48	NS

S – Significant, NS – Not significant F=21.71, P<0.0001

Table 8: Tukey's Multiple Comparison Test Showing Difference between Groups in Duration for Regaining Righting Reflex

Difference Between Groups		
Groups Compared	**Mean Difference**	**(P<0.05)stastical significance**
Group 1&2	212.0	S
Group1&3	78.33	S
Group1&4	64.00	NS
Group1&5	48.17	NS
Group 2&3	-133.7	S
Group2&4	-148.0	S
Group2&5	-163.8	S
Group3&4	-14.33	NS
Group3&5	-30.17	NS
Group 4&5	-15.83	NS

Table 9: Tukey's Multiple Comparison Test Showing Difference between Groups in Duration of Postictal Depression

Difference Between Groups		
Groups Compared	**Mean Difference**	**(P<0.05)stastical significance**
Group 1&2	28.50	S
Group1&3	8.33	S
Group1&4	6.66	S
Group1&5	6.16	S
Group 2&3	-20.17	S
Group2&4	-21.83	S
Group2&5	-22.33	S
Group3&4	-1.66	NS
Group3&5	-2.16	NS
Group 4&5	-0.50	NS

S – Significant, NS – Not significant F=64.32, P< 0.0001

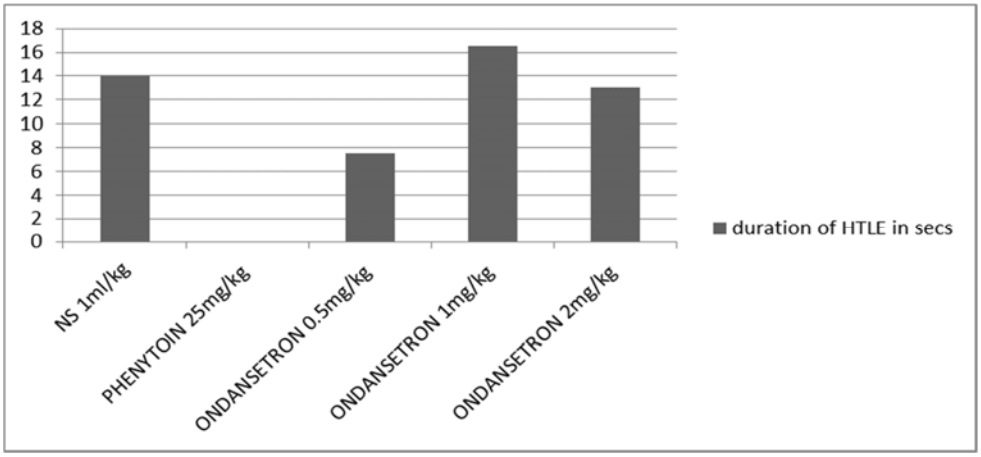

Fig 1: Duration of Htle in Seconds

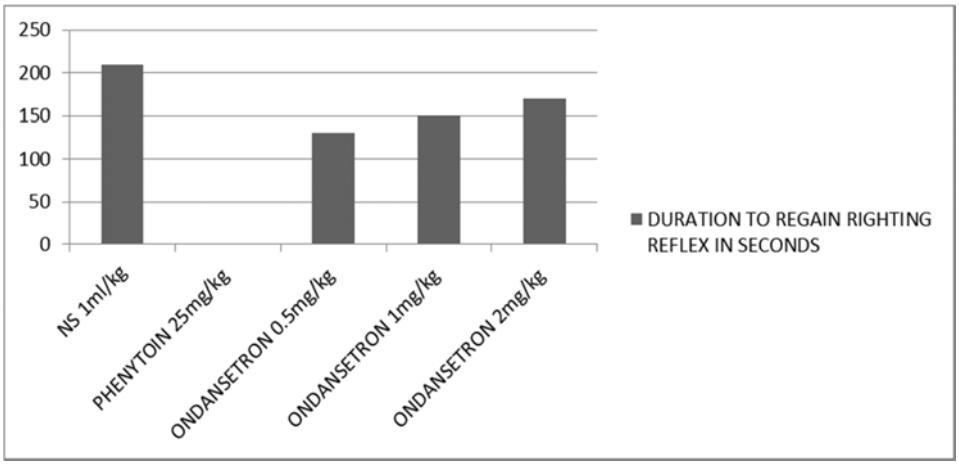

Fig 2: Duration to Regain Righting Reflex in Seconds

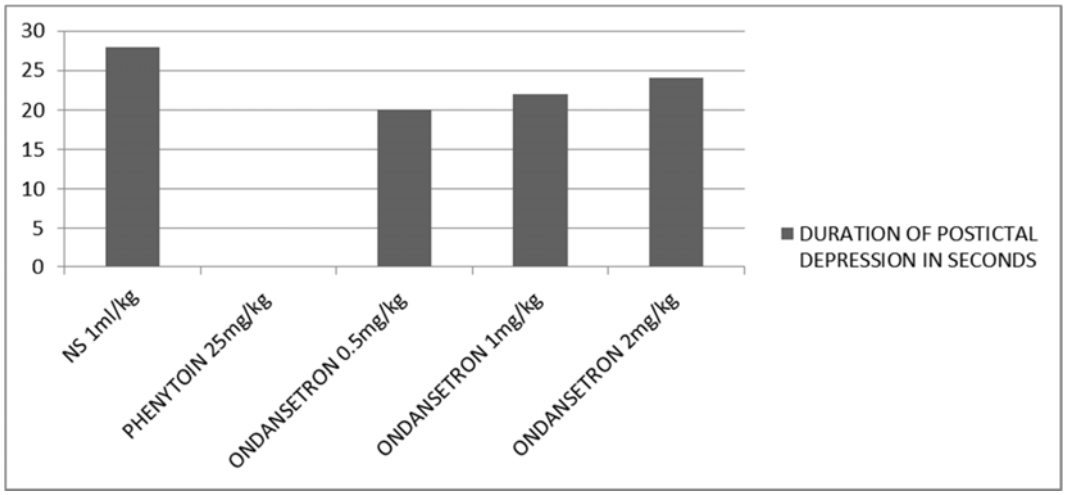

Fig 3: Duration of Postictal Depression in Seconds

Results

Duration of HTLE

Table 1-9 and Fig 1shows the results of duration of HLTE, The mean duration of HLTE in Ondansetron 0.5 mg/kg group was 7.167±5.601 which was statistically significant when compared standard Phenytoin group (7.167±5.601 vs 0) and control group (7.167±5.601 vs 14.05±3.639) but there was no significant difference observed between the control (14.05±3.639) and other Ondansteron groups (1 mg/kg 16.58±2.973, & 2 mg/kg 13.1±2.805). There was no significant difference in mean duration of HLTE between the ondansetron 1 mg/kg and 2 mg/kg (16.58±2.973 vs 13.1±2.805). In phenytoin group all the animals were protected from MES induced seizures but in Ondansetron 0.5 mg/kg group only 33% of the animals were protected and in other two test groups no animals were protected from seizures.

Duration to Regain Righting Reflex

Table 1-9 and Fig 2 shows the results of duration to regain righting reflex, The mean duration to regain righting reflex in Ondansetron 0.5 mg/kg group was 133±53.16 which was statistically significant when compared standard Phenytoin group (133±53.16 vs 0) and control group (133±53.16 vs 212±63.16) but there was no significant difference observed between the control (212±63.16) and other Ondansteron groups (1 mg/kg 148±20. 24, & 2 mg/kg 163.8±28.74). There was no significant difference in mean duration to regain righting reflex between the ondansetron 0.5 mg/kg, 1 mg/kg and 2 mg/kg (133±53.16 vs 148±20.24 vs 163.8±28.74).

Duration of Postictal Depression

Table 1-9 and Fig 3 shows the results of duration of postictal depression, The mean duration of postictal depression in Ondansetron 0.5 mg/kg group was 20.17±2.48 which was statistically significant when compared standard Phenytoin group (20.17±2.48 vs 0) and control group (20.17±2.48 vs 28.5±5.162) but there was no significant difference observed between the control (28.5±5.162) and other Ondansteron groups (1 mg/kg 21.83±2.639, & 2 mg/kg 22.33±3.882). There was no significant difference in mean duration of postictal depression between the ondansetron 0.5 mg/kg, 1 mg/kg and 2 mg/kg (20.17±2.48 vs 21.83±2. 639 vs 22.33±3.882).

Discussion

Though the previously conducted studies on the antiepileptic activity of ondansetron provide sufficient evidences to support its antiepileptic activity, present study failed to reproduce similar protection in MES induced seizures. In this study only 33.33% of the rats were protected from MES induced seizures in Ondansetron 0.5 mg/kg group but in other two test groups, animals were not protected from seizures which was contrary to the previously conducted studies. This present study did not show any dose dependent protection against MES induced seizures.

Analysis of the group 3 that received Ondansetron 0.5 mg/kg showed that mean duration of HLTE is 7.167±5.601, mean duration to regain righting reflex is 133±53.16 and mean duration of postictal depression is 20.17±2.48. The mean durations of these various parameters were significantly reduced compared to control group ($p<0.05$). But there were no significant differences when compared to standard group Phenytoin and other two Ondansetron groups (1 mg/kg, 2 mg/kg). This implies that Ondansetron at 0.5 mg/kg had some protection in MES induced seizures.

Analysis of the group 4 that received Ondansetron 1 mg/kg, mean values of various parameters were: duration of HTLE (16.58±2.973), duration to regain righting reflex (148±20.24) and duration of postictal depression (21.83±2.639). Mean duration of these parameters were almost same as compared to control group ($p>0.05$), and also there was no significant difference among other two test groups (Ondansetron 0.5 mg/kg, 2mg/kg). This implies that Ondansetron at 1 mg/kg did not give protection against MES induced seizures.

Analysis of group 5 that received Ondansetron 2 mg/kg, mean duration of various parameters, duration of HTLE (13.1±2.805), duration to regain righting reflex (163.8±28.74) and duration of postictal depresson (22.33±3.882) was same as that of control group. Mean duration of these parameters were also comparable with the other two test groups (Ondansetron 0.5 mg/kg, 2 mg/kg). Ondansetron at 2 mg/kg did not show any significant protection against MES induced seizures.

In Ondansetron at 0.5 mg/kg only 33.33% animals were protected for seizures but there was no protection in other two test groups. Mean duration of HLTE, duration to regain righting reflex and duration of postictal depression were significantly lower in this group, but the other two test groups had mean values similar to control group. This implies that

Ondansetron 0.5 mg/kg had antiepileptic activity but the animals in other two doses of test group were not protected and these doses had no antiepileptic effect.

Drugs acting on the $5HT_3$ receptor can act on other receptors because $5HT_3$, $GABA_A$, glycine, and nicotinic acetylcholine (nACh) receptors all belong to a superfamily of ligand-gated ion channels. The subunits of these receptors exhibit extensive amino acid sequence homology. Because of these similarities, many drugs that act on one type of receptor often act on other receptors in this group. $5HT_3$ receptor antagonists act on the $GABA_A$ receptor complex in addition to their effects on $5HT_3$ receptors. The anticonvulsant activity of Ondansetron may be attributable to action on GABAergic system. But mechanism of how the Ondansetron gives protection in MES induced seizures is less clear. Complex changes in neuronal excitability may take place at low doses of ondansetron that may give protection against the MES seizures [8].

The reasons for the loss of antiepileptic activity at higher doses of Ondansetron may be manifold: Studies have shown that $5HT_3$ receptor antagonists inhibit GABA actions because they act as inverse agonists at the benzodiazepine site on the $GABA_A$ receptor [8]. Ondansetron also inhibits the GABA current of rat central neurons [12]. It has been demonstrated that Ondansetron reverses the inhibitory effect of GABA on whole rat forebrain membranes [8]. Recently it was observed that glycine depolarizes and hyperpolarizes neurons of neonatal and mature rats, respectively. Ondansetron suppresses both the effects of glycine. By suppressing the glycine and GABA responses, Ondansetron decreases and increases the excitability of the central nervous system (CNS) of neonates and adult rats, respectively. At higher doses Ondansetron can produce CNS disinhibition, leading to hyperexcitation and convulsions [13]. This could possibly explain the zero percent protection afforded by 1 and 2 mg/kg Ondansetron in this study.

A study was conducted by Trupti *et al* to evaluate the antiepileptic action of Ondansetron in Rat model of Maximal electroshock (MES) induced seizure, revealed a significant increase in the Minimal electroshock seizure threshold (MET) obtained after administration of 1, 2, 4 mg/kg body weight dose of Ondansetron intraperitoneally where Ethosuximide was taken as standard drug. All the doses gave similar results. Results were noted in the form of elevation of MET and percentage of protection at different time intervals [14].

In another study conducted by S. Balakrishnan *et al* to evaluate the anticonvulsant action of Ondansetron and its combination with Phenytoin and its effects on the cognitive deficits induced by Phenytoin were studied: Body weight Combination of Ondansetron with Phenytoin not only had potentiating effect against the MES but also attenuated the cognitive dysfunction induced by Phenytoin.

The results of the present study demonstrate that Ondansetron 0.5 mg/kg body wt elicited an effective protection against MES seizures in albino rats. This anticonvulsant effect was very much significant when compared to control. But the other two doses did not show any protection against MES induced seizures.

The limitations of this study in which the consideration was not given to the statistical analysis of seizure latency, as the onset of convulsions after the shock was within a fraction of a second, which was very difficult to observe in any group and higher doses of Ondansetron did not show any protection against the MES induced seizures which was contrary to above mentioned study results.

Summary and Conclusion

1. The potential antiepileptic effect of Ondansetron was evaluated in Swiss albino rats using Maximal electro shock model
2. Distilled water 1 ml/kg, Phenytoin sodium 25mg/kg and Ondansetron 0.5mg/kg, 1 mg/kg and 2 mg/kg were injected intraperitoneally 30 min before the induction of seizures.
3. In MES, HLTE, duration of HLTE, duration to regain righting reflex and postictal depression were taken as parameters.
4. In Ondansetron (0.5 mg/kg) group 33.33% of animals were protected from MES seizures. On the contrary, in other two Ondansetron groups, animals were not protected from seizures.
5. The mean duration of HLTE, duration to regain righting reflex and duration of post ictal depression were significantly reduced in Ondansetron 0.5 mg group when compared to control group, but it was not comparable to Phenytoin group.
6. Considering the above results, Ondansetron at 0.5 mg/kg has antiepileptic activity but it is not comparable to standard Phenytoin sodium.
7. This study proves that Serotonergic system may be used to develop newer drugs for the treatment of epilepsy.

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