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Original Research Article

Nociceptive Analgesic and Anxiolytic Effects of Ondansetron in Wistar Rats: A Prospective Observational Study

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Abstract

Introduction: Serotonin plays an important role in pathogenesis of many diseases. Ondansetron is a selective 5HT3 antagonist used in patients with nausea and vomiting. It has also shown effectiveness in treatment of epilepsy, severe neuropathic pain, anxiety attacks & CNS-related disorders. The aim of this study was to evaluate the anticonvulsant, anxiolytic & antinociceptive effect of ondansetron in rats.

Materials: Rats were treated with different doses of ondansetron intraperitoneally (i.p. - 0.5mg/kg, 1mg/kg and 2mg/kg) , phenytoin 5mg/kg and control group received normal saline 0.1 mg/kg for 3 days. MES induced convulsion model is used to study the anticonvulsant effect. To study the anxiolytic effect of drugs ondansetron(0.16mg/kg), diazepam (1 mg/kg) & distilled water, we used an elevated plus maze test model, in which we recorded number of entries and time spent in open & close arm. Similarly, the nociceptive effect was evaluated by Tail flick test using radiant heat. Ondansetron as 0.5 mg/ kg and 1 mg/ kg; tramadol as 5 & 10 mg/ kg & normal saline were given i.p 30 mins prior to experiment. The efficacy of drugs were tested on basis of increase in pain threshold and suppression of symptoms depicting pain.

Results: The percentage protection from seizures in ondansetron 2mg/kg group is 16.7% as compared to control group. The onset and duration of tonic hind limb extension was less in ondansetron 1mg/kg (9.3 sec & 6.7 sec) as compared to other groups. Ondansetron showed dose dependant anti-nociceptive action similar to but less than that of tramadol. Similarly animals receiving ondansetron at dose 0.16 mg/kg showed anxiolytic effects in compared to control group and spent more time in open arms as compared to close arms.

Conclusion: Being a potent 5HT3 antagonist, Ondansetron has shown its potential role in modulating various CNS actions at higher dose.

Keywords: Ondansetron, Anticonvulsant, Anti-nociceptive, Anxiolytic.

Introduction

The central serotoninergic system plays an important role in the pathogenesis of numerous neurological disorders like migraine, panic attacks and depression and seizure [1]. Serotonin (5-hydroxytriptamine, 5-HT) is an amide derived neurotransmitter acting on 5HT receptors in this serotoninergic system. There are 7 different types of serotonergic receptors documented of which the 5-HT3 receptors are widely distributed in the body and the role of these receptors in various pathologies have provided the rationale for observing the effect of ondansetron for applications [2].

The central serotoninergic system takes part in the regulation of many life functions, such as sleep, wakefulness, blood pressure, pain perception or sexual behaviours. Moreover, it is involved in the pathogenesis of depression, anxiety, addictions, migraine and other headaches. Serotonin (5-hydroxytriptamine, 5-HT) is thought to be the major mood-regulating factor. Even trace amounts of this neurotransmitter affect mood, appetite, sleep and pain tolerance. A drop in its level may cause 'addictive' binge eating, sleeplessness, depression, aggression, low pain tolerance, and impair thermoregulatory mechanisms [1,2].

Epilepsy is a major neurological disorder encountered in all age group. (Hauser WA*et al.,* 1991, 1993) It is a collective term used for a group of chronic seizure disorders associated with sudden and transient episodes (seizures) of loss or disturbance of consciousness, usually but not always with characteristic body movements (convulsions) and sometimes with autonomic hyperactivity [3]. Seizure is due to abnormal discharge of some neurons within the brain [4].

The current therapy of epilepsy is associated with various side effects, dose-related and chronic toxicity, as well as teratogenic effects [5].

5-HT3 receptor, being ion gated receptor, induces movement of cations (Na+, k+) into the cells leading to depolarization. [4]. They are located both centrally and peripherally (within nerve endings of afferent nociceptive fibers) and are involved in the process of generation and perception of pain by an inflammatory process.

Ondansetron, a selective serotonin 5-HT3 receptor antagonist, is most commonly used to treat patients with nausea and vomiting associated with cancer chemotherapy or radiotherapy or anesthesia and post-op surgery [6]. In contrast to conventional antiemetics, ondansetron is generally well tolerated with a lower incidence of sedation and only isolated case reports of extrapyramidal reactions. It has also shown effectiveness in treatment of severe neuropathic pain, anxiety attacks & CNS-related disorders [e.g. - epilepsy, alcohol (ethanol) dependence, vertigo, cerebellar tremor and Drugs-related psychosis] [7].

So this study was conducted to evaluate the various effects of Ondansetron.

Elevated plus maze:

The elevated plus maze test is used to measure anxiety-like behavior in rodents. It can be used to gain insight into conditions such as posttraumatic stress disorder (PTSD) and other conditions marked by anxious behavior. It can also be used as a component in screening of novel compounds for anxiolytic properties. This model is based on aversion to open spaces, which is seen as the animal spending more time in the enclosed arms of the maze.

The scope of this study also includes the discussion of the implications of these findings for future research in patients who suffer from pain seizure disorder or anxiety.

Materials & Methods

Albino Wistar rats of around 160 gm - 200 gm in weight were used in this study to evaluate anticonvulsant and anxiolytic effects of Ondansetron. The rats were housed in polypropylene cages with 6/3 rats of same group in each cage. Animals were fed with standard pellet diet and water ad libitum and observed for few days before starting the experiment. Rats with normal behavior and activity were considered to be healthy and included in the study. Female animals who were pregnant or weighed more than 200 gm were excluded. All the procedures were carried out in accordance to standard guidelines of CPCSEA after obtaining prior permission from the Institutional animal ethics committee.

Anticonvulsant action

In this study, the maximum electroshock (MES) induced convulsion model is used. It represents tonic-clonic type of epilepsy.30 rats were taken for this experiment and were divided into 5 groups each containing 6 animals. Animals were housed randomly in cages at controlled temperature of 21±3 C with 12hour light: dark cycle. They were brought to the experiment room 1 hour prior to experiment and each animal were marked with marker on their tail and they were placed in marked cages to avoid confusion.

Each animal was given 150mA current for 0.2 seconds through ear electrode to induce convulsions.

Drugs used:

1. Phenytoin sodium(50mg/2ml) - standard drug
2. Diazepam 1 mg/kg body weight/day
3. Ondansetron(4mg/2ml) -test drug was purchased from local pharmacy.
4. Distilled water- As control and vehicle to mix the test drugs.

Appropriate dose was calculated for each group of rats based on their mean weight. All the drugs were given intraperitoneal (i.p) up to 3 days.

Group I - Control, distilled water 1m/kg i.p.

Group II - Standard, Phenytoin sodium 5 mg/kg

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.

On the third day, half hour after drug administration the animals were subjected to MES induced convulsion. After inducing convulsions, the animals were observed, and findings were recorded. Animals were observed for 21 days for any chronic effects.

The parameters measured were

1. Incidence of Convulsions
2. Onset of Tonic Hind Limb extension (HLTE)
3. Duration of HLTE
4. Duration of Righting Reflex (RR) [from the end of HLTE till the animal could stand on 4 legs]
5. Duration of Post-Ictal Depression (PID) [from the end of regaining righting reflex till the animal walks away, PID]
6. Whether convulsion was followed by recovery or death.

The animals were reused in the study after giving adequate washout period of 15 days.

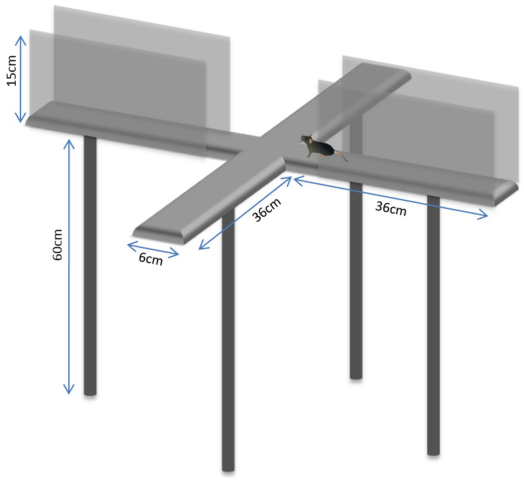

Figure 1: Elevated Plus Maje

Results

Table 1: Anti-Convulsant Effect

Groups	Groups	No. of Animals Protected from Seizure	Percentage Protection (%)
1	Normal Saline (n=6)	0	0
II	Ondansetron 0.5 mg/kg (n=6)	0	0
III	Ondansetron 1 mg/kg (n=6)	0	0
IV	Ondansetron 2 mg/kg (n=6)	1	16.7
V	Phenytoin 2.5 mg/kg (n=6)	6	100

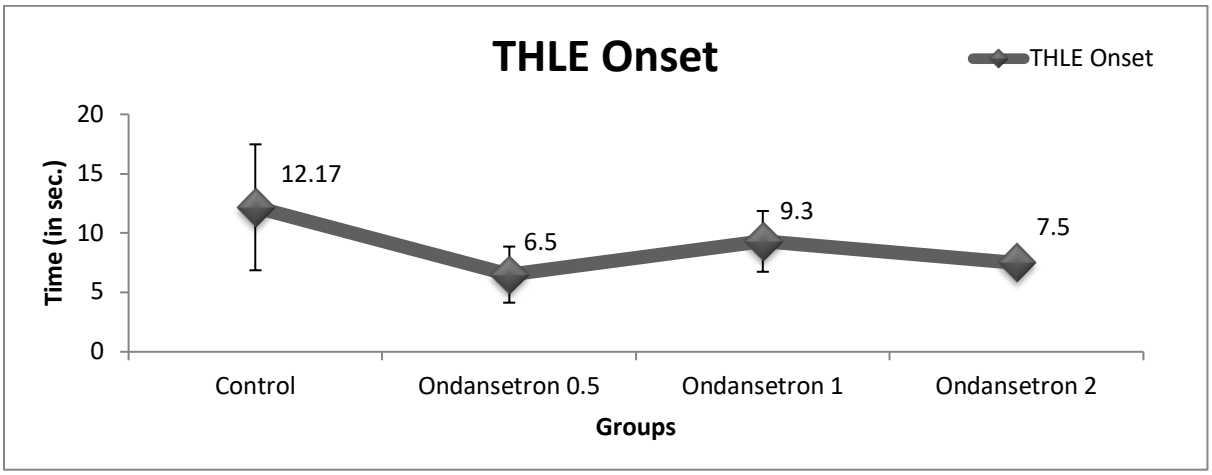

Figure 1: Onset of tonic hind limb extension in different doses of ondansetron

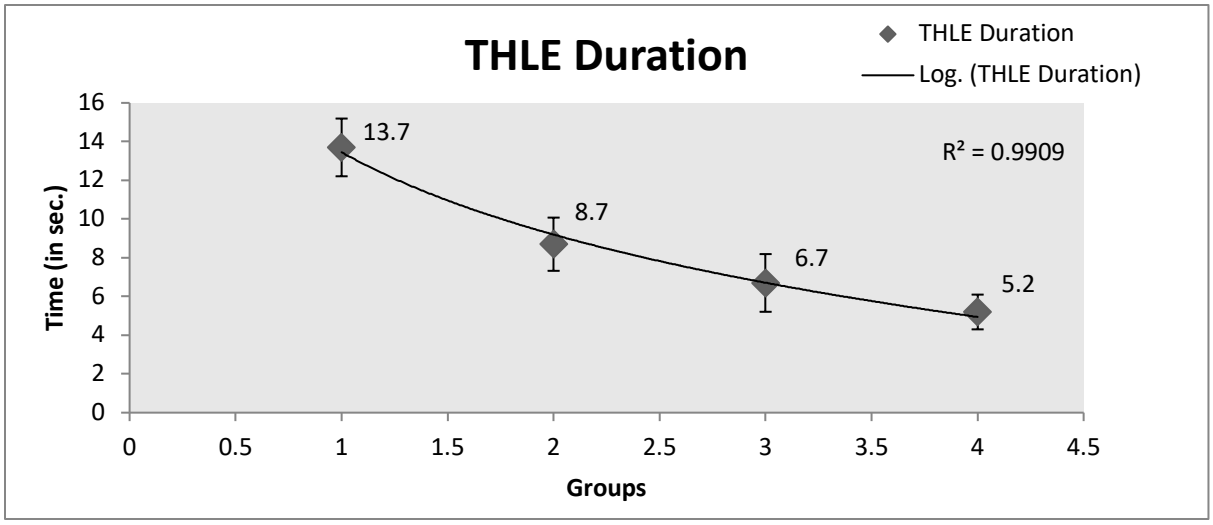

Figure 2: Tonic hind limb extension duration

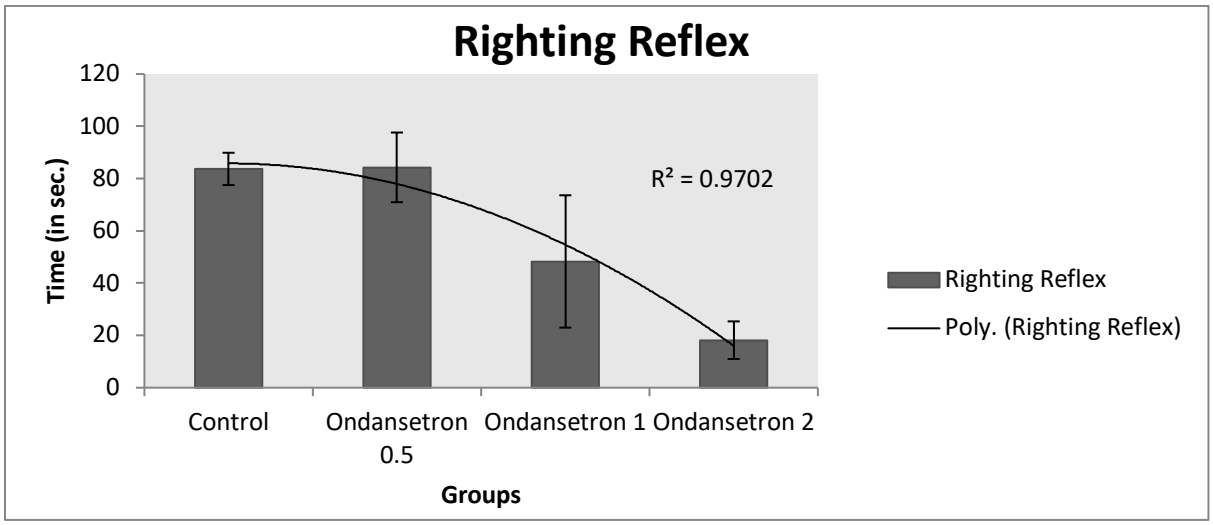

Figure 3: Righting reflex in different groups

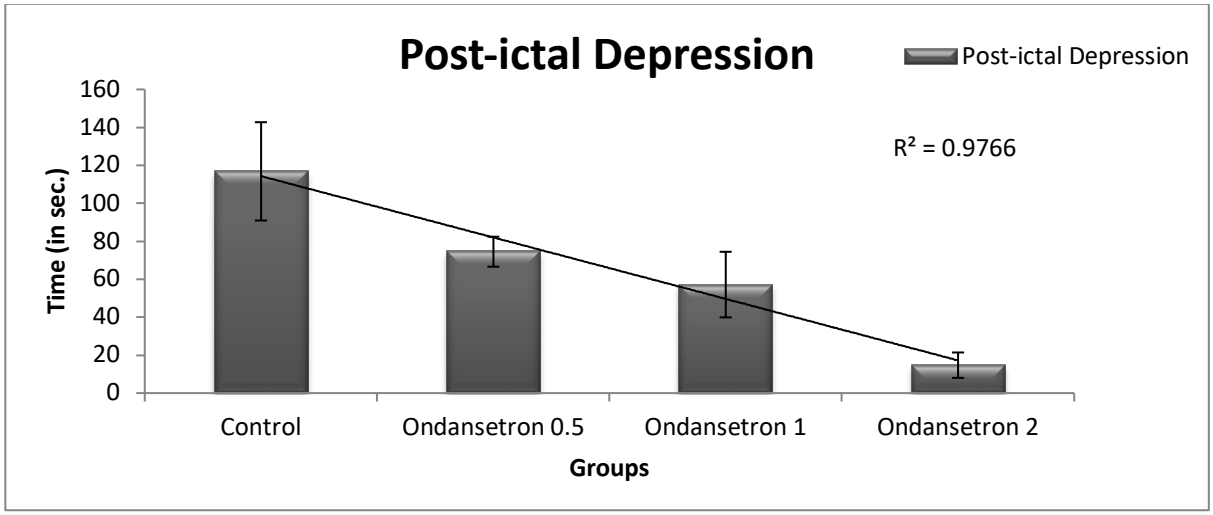

Figure 4: Post-ICTAL depression

Table 2: Effect of combination of drugs on Tail flick latency

Drug	0 min	30 min	60 min	120 min
Ondan 0.5 mg/kg	5.23 ± 0.32	5.56 ± 0.45	5.81 ± 0.63	5.33 ± 0.38
Ondan 1 mg/kg	5.51 ± 0.49	7.6 ± 1	6.93 ± 0.67	6.96 ± 0.43
Trama 5 mg/kg	5.71 ± 0.76	7.65 ± 1.12	7.95 ± 0.58	6.28 ± 0.71
Ondan 0.5 mg/kg ± Trama 5 mg/kg	5.6 ± 0.48	8.88 ± 0.45*	7.96 ± 1.28	7.26 ± 0.74
Ondan 1 mg/kg ± Trama 5 mg/kg	5.6 ± 0.56	8.13 ± 0.52	9.45 ± 0.7**	9.21 ± 0.77**

- Repeated measure ANOVA with post-hoc Dunnett test (* p< 0.05, **p< 0.01)

Table 3: Anxiolytic effects in different groups of rat

	CONTROL (Mean+ SD)	DIAZEPAM(Mean+SD)	ONDEM 0.16 mg/kg(Mean+SD)
Animals	6	6	6
No of entries in open arm	1.5+0.5	5.4+0.8	6.5+0.5
No of entries in close arm	1.8+0.8	4.3+1.7	4.8+1.4
Time spent in open arm	26.3+3.5	74.4+4.4	76.3+3.5
Time spent in close arm	86.3+11	92.5+12	91.3+11

Anxiolytic effect

30 rats were taken dividing into 5 groups, each containing 6 animals. Pregnant animals and animals with more weight 250 gm were excluded as it can alter mobility of the animal as well as distribution of the drugs. Twelve-hour light-dark cycle was maintained, and experiments were carried out in the light phase.

Drugs

Diazepam was diluted to obtain a solution of concentration 0.01mg/ml and was administered intraperitoneally at dose of 1mg/kg. Ondansetron was administered at 0.16 mg/kg body weight. Distilled water was used as control.

Group I - Distilled water

Group II - Standard drug Diazepam 1 mg/kg body weight

Group III- Ondansetron 0.16 mg/kg body weight

Drugs were given intraperitoneally maintaining aseptic conditions following standard care and precautions with pellet diet and water. After 30 mins of drug administration rats were observed for results

Elevated Plus Maze

The elevated plus maze apparatus consisted of two open (36 cm× 5cm ×1cm) and two closed (36 cm ×5 cm×15 cm) arms, extending from a central platform (5cm×5 cm) and elevated to height of 60 cm above the floor. The entire wooden maze was kept in one place during whole experiment and the closed arms were surrounded by similar looking walls(8).

Evaluation of Anti-anxiety effect

The animals were allowed to adapt to environment for at least one hour prior to

experiment. The animals were placed in center of the elevated plus maze. The number of entries and the time spent in closed and open arms were recorded during a 5 min observation period. If a rat enters in any arm with all four paws, then it is considered as entry.

Animals with 0% time spent on open arms or showing any type of abnormal movements, i.e. no movement at all in home cage or staying in one corner were excluded from experiment and were replaced by new, randomly chosen animal. They were not included in experiment or statistical evaluation. All their movement were observed, tabulated and analyzed statistically.

Antinociceptive effect

To analyze the effect of nociception, we took albino mice weighing 20-30 gm. All the protocol was followed for mice as it was followed in case of rats.

Ondansetron as 0.5 mg/ kg and 1 mg/ kg; tramadol as 5 & 10 mg/ kg were used to see this effect. The drugs and normal saline were given intraperitoneally 30 minutes prior to the experiment. The efficacy of drugs were tested on basis of increase in pain threshold and suppression of symptoms depicting pain.

The nociceptive effect was evaluated by Tail flick test using radiant heat. In this model radiant heat is applied to small surface of tail. Mice were introduced in open end cylindrical space with tail lying outside. The application of thermal radiation to tail provokes the withdrawal of tail by brief vigorous movement. The withdrawal of tail from heat source is referred as tail-flick latency. A timer is started at the time of application of heat source and time taken by the rat to withdraw its tail is recorded.

The lengthening of this reaction time by the animal seen after administration of a drug is interpreted as time latency showing analgesic action. Baseline latency time was 2-4 sec. The test was repeated at 0,30,60 & 120 min. Post experimental care was taken by applying anti septic to tail, if there is any type of injury.

Statistical Analysis

Results are presented as Mean $\pm$ SEM. A p value of 0.05 or less was considered for statistical significance in all parameters. Data were analyzed using Microsoft excel.

Discussion

The Elevated Plus Maze model introduced around 20 years ago is popularly used to evaluate natural and synthetic compounds to be used as anxiolytics as it uses natural stimuli (fear of a novel open space and fear of balancing on a relatively narrow, raised platform) that can induce anxiety in humans. The anti-anxiety effect was evaluated by number of entries in closed and open arm and the time spent there by rodents in each visit.

The number of entries into open arm for control group was 1.5± 0.5, for standard group (diazepam) was 5.4±0.8 for test group (ondansetron) was 6.5±0.5. The number of entries into open arm was highest in the ondansetron group where as in a study conducted by Jafrin A *et al*. it was highest in the standard drug group where similar strength of drug was used as standard and test drug [9].

The time spent by rodents in open arm was 26.3±3.5 mins for control group, 74.4±4.4 mins for standard group and 76.3±3.5 mins for group 3 ondansetron group. The time spent in open arm was highest in our study for ondansetron group where as it was highest for standard group in a study by Jafrin A *et al* [9].

The number of entries into close arm for control group was 1.8±1.4 followed by standard group was 4.3±1.7 and for the test group was 4.8±1.4. The time spent in close arm for control group was 86.3±11mins, for

standard group was 92.5±12mins, for ondansetron group was 91.3±11 mins.

In this study the rodents in each group have spent more time in the close arm as compared to open arms but was not statistically significant. Rodents usually freeze, defecate or have reduced mobility when they enter open arms because they are naturally aversed to high and open space [9].

In the tail flick test, a thermal stimulus is focused on the skin of the animal's tail, activating nociceptors in the surface layers of the skin [10]. In our study the tail flick latency was increased for animals in all groups at 30 minutes interval. In group 4 and group 5 when tramadol at 5 mg/kg was added to ondansetron at o.5 mg/kg and ondansetron at 1mg/kg the tail flick latency increased significantly as compared to the group of animals receiving only ondansetron at 0.5 mg/kg. The tail flick latency was significantly increased by ondansetron at a dose of 0.5 mg/kg, 1 mg/kg and 2mg/kg i.p. in a study done by Mahesh *et al* which is similar to the findings in our study.) [11].

In a study by Purohit *et al*, ondansetron demonstrated moderate anti-nociceptive effect at both 0.5 mg/kg and 1mg/ kg dose which is similar to the findings in our study [12].

The analgesic activity of ondansetron has also been seen in patients with chronic benign neuropathic pain [13].

Additive effect of ondansetron coadministration on paracetamol analgesia was seen in another study along with reduction in the postoperative analgesic requirement, and improvement in the postoperative comfort level [14].

Following few studies on ondansetron have reported conflicting results. Some studies have reported ondansetron to possess antinociceptive [11,13-15].

In some studies ondansetron was seen to block antinociceptive effect of some drugs given [16-19].

while in one study ondansetron did not antagonize the antinociceptive effect of alfentanil, short acting synthetic opioid [20].

OND exhibits anti-nociceptive activity consistent with previous reporting, that activation of spinal 5-HT receptors produces a 3 nociceptive effect that is reversed by specific 5-HT 3 receptor blockade [21].

In a study conducted by Purohit *et al* the authors could conclude that damage to the central noradrenergic system at an early stage of individual development has no effect on the antinociceptive effects of the serotonin (5- HT3) receptor antagonist, ondansetron, in the persistent pain model, suggesting the site of action of antinociceptive effect of ondansetron other than the serotonergic system [12].

THLE: In this study the onset of THLE was highest in control group (12.17 sec), it was 6.5 sec in II group (ondansetron 0.5 mg), 9.3 sec in IIIrd group and in the IVth group it was 7.5 sec. Also, the THLE duration was highest in control group (13.7 sec), it was 8.7 sec in IInd group (Ondansetron 0.5 mg), in IIIrd group it was 6.7 sec and in IVth group it was 5.2 sec.

In a study by Gokul *et al* where the anticonvulsant action of Ondansetron was compared with Phenytoin sodium. it was seen that Ondansetron at 2 mg/kg did not show any significant protection against MES induced seizures [4].

In this study the THLE duration was lowest in animals receiving ondansetron 1mg/kg and highest in the control group. However, in a study by Gokul *et al* the THLE duration was lowest in animals receiving ondansetron at 0.5 mg/kg and highest in the group of animals receiving ondansetron at 1 mg/kg which was

even more in comparison to their control group.

Righting reflex: 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). (Gokul *et al*) In this study the mean duration to regain Righting reflex was highest in the IInd (ondansetron 0.5 mg/kg) group where as it was highest in the ondansetron 2 mg/kg group in a study by Gokul *et al*. In this study the mean duration to regain Righting reflex was lowest in Ondansetron 2 mg/kg group where as it was lowest in 0.5 mg/kg ondansetron group in the study conducted by Gokul *et al* [4].

Post ictal depression period: In this study, the post ictal depression is highest in 0.5 mg/kg ondansetron group but in Gokul *et al* it is highest in 2 mg /kg. It was lowest in IVth group when ondansetron 2mg/kg was given where as in other study by Gokul *et al* it was lowest in 0.5mg/kg group. However, the duration was maximum in the animals of the control group in both the studies mentioned above respectively.

In a study by Gokul *et al* it was found 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. (Gokul *et al*) In this study the percentage protection was 16.7% when ondansetron was given 2 mg/kg [4].

Conclusion: Being a potent 5HT3 antagonist, Ondansetron has shown its potential role in modulating various CNS actions at higher dose.

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