ISSN: 0975-8585

Research Journal of Pharmaceutical, Biological and Chemical Sciences

Anxiolytic Effect of Ondansetron, a 5-HT_3 Antagonist on male *albino mice* in the Elevated Plus Maze

Lourdu Jafrin A[*1], Shanthi M[2], and Meher Ali R[1]

[1]Department of Pharmacology, Sri Manakula Vinayagar Medical College, Puducherry, India.
[2]Institute of Pharmacology, Madurai Medical College, Tamil Nadu, India.

ABSTRACT

Anxiety affects one-eighth of the total population world-wide and has become an important area of psychopharmacological research during this decade. Ondansetron a selective 5HT-3 antagonist commonly used as an antiemetic has been claimed to have psychotropic effects. The aim of this study is to evaluate the anxiolytic and sedative properties of ondansetron. Male albino mice were treated with ondasetron (0.04, 0.08, 0.16 mg/kg), distilled water and diazepam intraperitoneally and the anxiolytic effect was evaluated using elevated plus maze. The spontaneous locomotor activity of mice after administering ondansetron (0.08, 0.16 mg/kg) was also tested using the actophotometer. Ondansetron showed significant anxiolysis at doses of 0.08mg/kg and 0.16mg/kg with increase in time spent in open arm($p<0.001$)and increase in open arm entries ($p<0.01$). Ondansetron showed no significant($p > 0.05$) decrease in counts in the actophotometer when analysed ($p > .05$). Hence ondansetron showed anxiolytic effects and had no sedative properties.

Keywords: anxiety disorders, serotonin antagonist, elevated plus maze , ondansetron

Corresponding author

ISSN: 0975-8585

INTRODUCTION

Anxiety disorders are among the most prevalent psychiatric disorders in the general population. These disorders are associated with significant morbidity and affect their routine day to day activities. Generalized anxiety disorders occur in 4-6 % of the population and are more common in women [1]. Anxiety disorders are also more common in children and adolescents occurring in 13% of young people [2] .

Anxiety is a feeling of apprehension, uncertainty and fear without apparent stimulus, associated with physiological changes like tachycardia, sweating and tremor. There is no identifiable triggering stimulus in anxiety. Anxiety is a normal reaction to stress. When anxiety becomes excessive, it falls under the classification of anxiety disorder.

Three major neurotransmitters are associated with anxiety namely, norepinephrine, serotonin and gamma aminobutyric acid (GABA). Most of the available drugs to date reduce anxiety by modulating one of the above neuro transmitter mechanisms. There are also minor neurotransmitters which are under research to help patients with anxiety[3].

Some of the available drugs are selective serotonin reuptake inhibitors, selective norepinephrine reuptake inhibitors, benzodiazepines, beta adrenergic antagonists and barbiturates. Newer drugs like melatonin and hydroxyzine are also used. As these drugs have to be used on a prolonged basis chances of adverse effects like impotence, weight gain and psychomotor impairment are inevitable.

Some of the herbal preparations which are used to reduce the ill effects of stress are Ocimum sanctum (Tulsi) , Withania somnifera (Ashwagandha) , Altingia excelsa , Diospyros peregrina , Seleginella bryopteris(Sanjeevani) , Panax ginseng [4-6].

Given the above difficulties in administering anxiolytics, untiring efforts have been put into the discovery of newer anxiolytics with fewer adverse effects and without psychomotor impairment.

This study is an effort in the above direction in the interest of the society at large. Ondansetron a well known $5HT_3$ antagonist, widely used as an antiemetic has been claimed with a number of other uses in the realm of psychopharmacology.

Evidence has been mounting over the fact that 5-HT (serotonin) acting through $5HT_3$ receptor can influence behavior relevant to anxiety, schizophrenia and cognitive disorders. Ondansetron has also been found to be beneficial in the management of postcardiotomy delirium, morphine discontinuation phenomena, vertigo and intentional tremor.

Serotonin plays a key role in the pathophysiology of anxiety and ondansetron being a serotonin antagonist was selected to study its anxiolytic and sedative properties in comparison with diazepam, a well known anxiolytic.

MATERIALS AND METHODS

The study was conducted for a period of eight months after obtaining ethical clearance from the institutional animal ethical committee, Madurai Medical College, Madurai. CPSCEA guidelines were adhered to throughout the study.

Animals

Inbred male albino mice from central animal house 14-20 weeks old and weighing 24(±2) g were included in the study. Animals were fed with standard pellet diet and water *ad libitum*. Twelve hour light-dark cycle was maintained and experiments carried out in the light phase. The mice were housed in polypropylene cages with six animals in each cage.

Drugs

Diazepam (Ranbaxy) was diluted to obtain a solution of concentration 0.01mg/ml and was administered intraperitoneally at the dose of 1mg/kg. Ondansetron was administered intraperitoneally at the graded doses of 0.04 mg / kg ,0.08 mg / kg and 0.16 mg / kg . Distilled water was used as vehicle control.

Elevated Plus Maze

The elevated plus maze apparatus consisted of two open (30 cm× 5 cm× 1 cm) and two closed (30 cm× 5 cm× 15 cm) arms, extending from a central platform (5 cm × 5cm) and elevated to a height of 50 cm above the floor. The entire wooden maze was painted black and surrounded by similar looking walls. Lighting was kept constant and to a minimum by a 15 watt bulb hung 1.5 m from above.

Actophotometer

The Digital actophotometer is designed to study the spontaneous locomotor activity in small animals like mice or rats. This apparatus uses optical sensors and emitters to record the horizontal movement of the animals on a four digit electronic counter display.

METHODOLOGY

Elevated Plus Maze for anti anxiety effect

30 male albino mice were divided into 5 groups of 6 animals in each group namely control, standard, test 1, test 2 and test 3 groups.

Animals were assigned randomly to the control or treatment groups and only naive mice were used. All animals used were weighing between 24 and 26 g. Animals with a greater weight were excluded since fat distribution might change the distribution of compounds with a high volume of distribution (e.g. diazepam) and therefore influence the pharmacological response.

Animals with 0% time spent on open arms or presenting with clear symptoms of abnormal behavior (e.g. no movement at all in home cage) were excluded from the experiment prior to statistical evaluation and replaced by a new, randomly chosen animal.

The animals were allowed to adapt to the environment at least for one hour prior to the experiment. Lighting was kept constant.The control group animals were given distilled water i.p, the standard group of animals received inj.Diazepam 1 mg/kg i.p. The test 1, test 2 and test 3 group of animals received inj.Ondansetron in the doses of 0.04 mg / kg i.p, 0.08 mg / kg i.p and 0.16 mg / kg i.p respectively.

After 30 minutes of drug administration mice were individually placed on the center of the elevated plus maze facing a closed arm, and the number of entries and the time spent in closed and open arms were recorded during a 5 min observation period. Arm entries were considered as entry only if all four paws enter into an arm.

The observations were tabulated and analysed statistically using unpaired‘t’ test.

Spontaneous locomotor activity by Actophotometer

The same animals used previously for anxiolytic effect were used after a wash out period of 15 days. These 30 male albino mice were grouped into five groups with six animals in each. The total number of counts made by each animal in the actophotometer for a period of 10 min was calculated.

The control group of animals were administered inj.Distilled water i.p, the standard group of animals were given inj.Diazepam 1 mg /kg i.p, the test groups 1, 2 and 3 were given inj.Ondansetron in the dose of 0.04 mg / kg i.p , 0.08 mg / kg i.p and 0.16 mg / kg i.p respectively.

After 30 min of drug administration, spontaneous locomotor activity for each animal for a period of 10 min was calculated and the observations were tabulated and analyzed statistically by using unpaired “t” test.

RESULTS

In the present study, 30 male albino mice were selected and were evaluated for anti anxiety and sedative effects. Anti anxiety effect was evaluated by elevated plus maze method and sedative effect was evaluated by spontaneous locomotor activity in actophotometer.

ISSN: 0975-8585

Anti anxiety effect

Anti anxiety effect was evaluated using elevated plus maze. The time spent in the open arm and also the number of entries into the open arm was noted in the control, standard and test groups.

Time spent in open arm

The time spent in open arm for control group of mice was 11± 3.17 seconds. The time spent in open arm for standard group was 98 ± 7.97 seconds. The time spent in open arm for test groups (1, 2, 3) was 18.83 ± 3.24, 53.83 ± 1.42 and 54.83 ± 2.74 seconds respectively (table & chart 1).

The results were tabulated and analysed using unpaired student's "t" test. The anti anxiety effect was not statistically significant for the test group 1 ($P = 0.115$) in comparison with control group but was statistically significant for the test group 2 and group 3($P < 0.001$) in comparison with control group. The anti anxiety effect was statistically significant for the standard group ($P < 0.001$) in comparison with control group.

Number of entries into open arm

The number of entries into open arm for control group was 2.16 ±0.60, for standard group was 14.5 ± 2.05, for test groups (1, 2, 3) were 3.83 ± 0.60, 6.67 ± 1.05 and 7.67 ± 1.23 (table & chart 2) respectively (fig 2).

The results were tabulated and analysed using unpaired student's "t" test. The anti anxiety effect was not statistically significant for the test group 1 ($P >0.05$) in comparison with control group. The anti anxiety effect was statistically significant for the test group 2 ($P < 0.01$) and test group 3 ($P < 0.01$) in comparison with control group. The anti anxiety effect was statistically significant for the standard group ($P < 0.001$) in comparison with control group.

Spontaneous locomotor activity

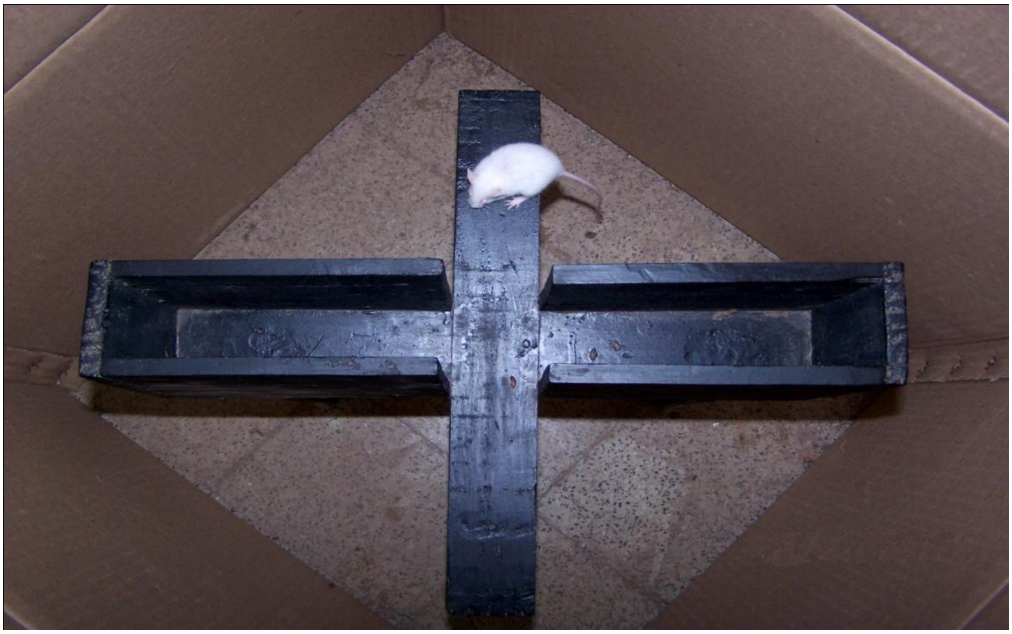
Sedative activity was evaluated by using Actophotometer. The spontaneous locomotor activity made by a mouse was noted in control, standard and test group before and 30 min after the administration of control, standard and test drugs. The average number of counts before and after 30 min for control group of mice was 623.17 ± 23.75 and 626.17 ± 34.70. The average number of counts before and after 30 min for standard group of mice was 630.83 ± 18.43 and 346 ± 13.93. The average number of counts before and after 30 min for test groups (1, 2, 3) was 603.33 ± 11.83 and 609.67 ± 19.78, 606.67 ± 24.91 and 612.33± 31.22, 615.17 ± 16.48 and 623± 24.18 (table & chart 3) respectively .

The results were tabulated and analysed using unpaired student's "t" test. The sedative effect was not statistically significant for the test groups (1, 2, 3) after 30 min of drug administration in

comparison with control group. The sedative effect was statistically significant for the standard group ($P < 0.001$) in comparison with control group.

Fig 1: Mice In Closed Arm

Fig 2: Mice In Open Arm



ISSN: 0975-8585

Table 1: Time spent in open arm

S.No.	Groups	Treatment	Time spent in open arm (in seconds) (MEAN± SEM)
1.	Control	Distilled water	11.0 ± 3.17
2.	Standard	Diazepam (1mg/kg)	98 ± 7.47***
3.	Test 1	Ondansetron(0.04 mg/kg)	18.83 ± 3.24
4.	Test 2	Ondansetron(0.08mg/kg)	53.83 ± 1.42***
5.	Test 3	Ondansetron (0.16mg/kg)	54.83 ± 2.74***

n=6, *** p < 0.001 *(in comparison with control)*
SEM – standard error of mean

Table 2: Number of entries in Open Arm

S.No.	Groups	Treatment	No.of entries in open arm (MEAN± SEM)
1.	Control	Distilled water	2.17 ± 0.60
2.	Standard	Diazepam (1mg/kg)	14.50 ± 2.05 ***
3.	Test 1	Ondansetron(0.04 mg/kg)	3.83 ± 0.60
4.	Test 2	Ondansetron(0.08mg/kg)	7.67 ± 1.23 *
5.	Test 3	Ondansetron (0.16mg/kg)	7.50 ± 1.34 *

n=6, *** p < 0.001 * p < 0.01 *(in comparison with control)*

Table 3: Comparison Of Total Counts In 10 Min In Actophotometer

S.No.	Treatment	Baseline	Counts 30 min after treatment (MEAN± SEM)
1.	Distilled water	623.17 ± 23.75	626.17 ± 34.7
2.	Diazepam (1mg/kg)	630.83 ± 18.43	346 ± 13.93 ***
3.	Ondansetron (0.04 mg/kg)	606.67 ± 24.91	612.33 ± 31.22
4.	Ondansetron(0.08mg/kg)	615.17 ± 16.48	623 ± 24.18
5.	Ondansetron (0.16mg/kg)	603.33 ± 11.83	609.67 ± 19.78

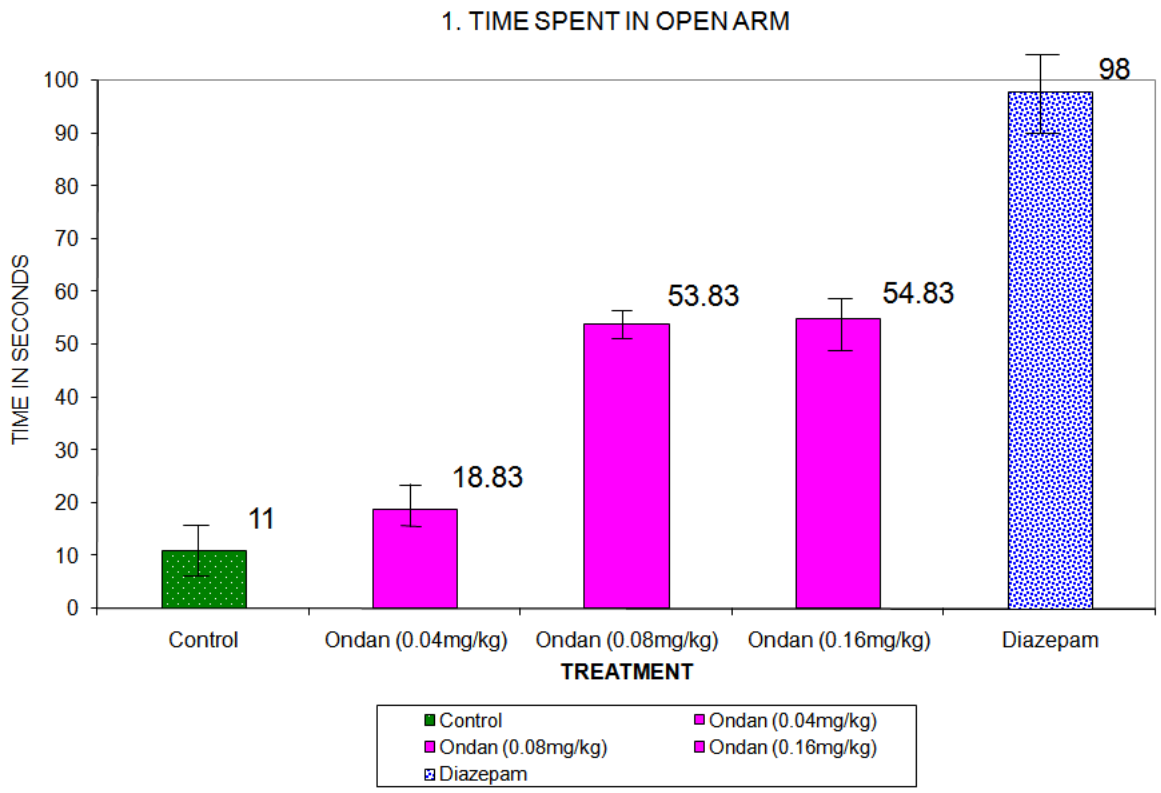
n=6, *(P<0.001)** *(in comparison with control)*

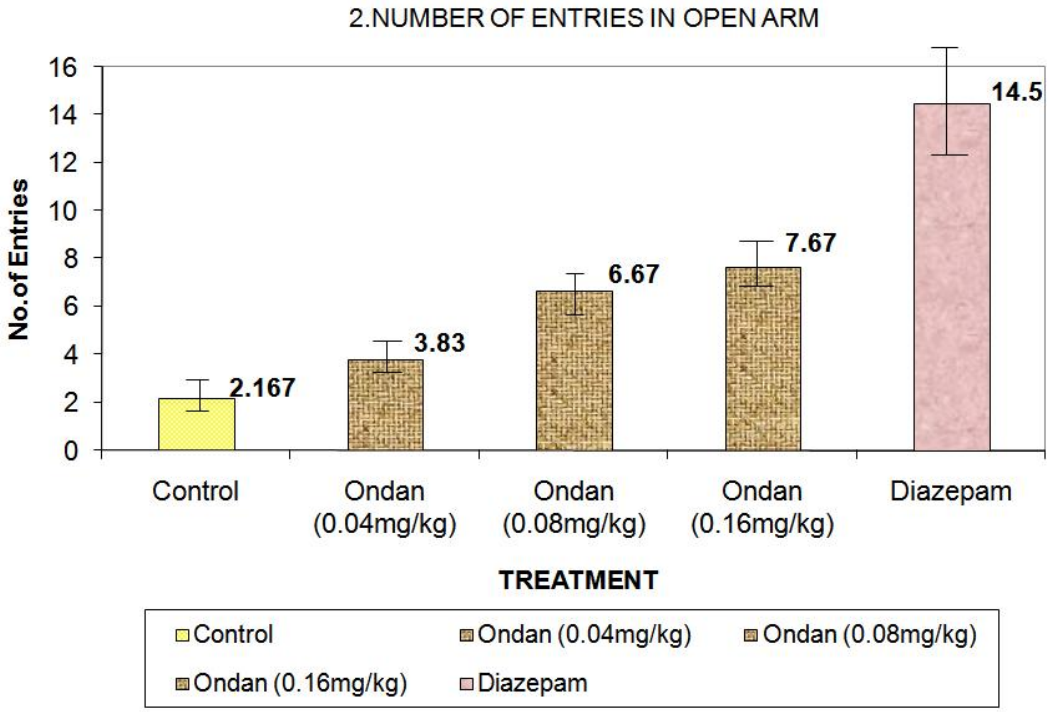
DISCUSSION

The elevated plus maze (EPM) is considered to be an etiologically valid animal model of anxiety because 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 model was introduced about 20 years ago and has been used extensively for the evaluation of natural products as well as synthetic compounds for their potential use as anxiolytics.

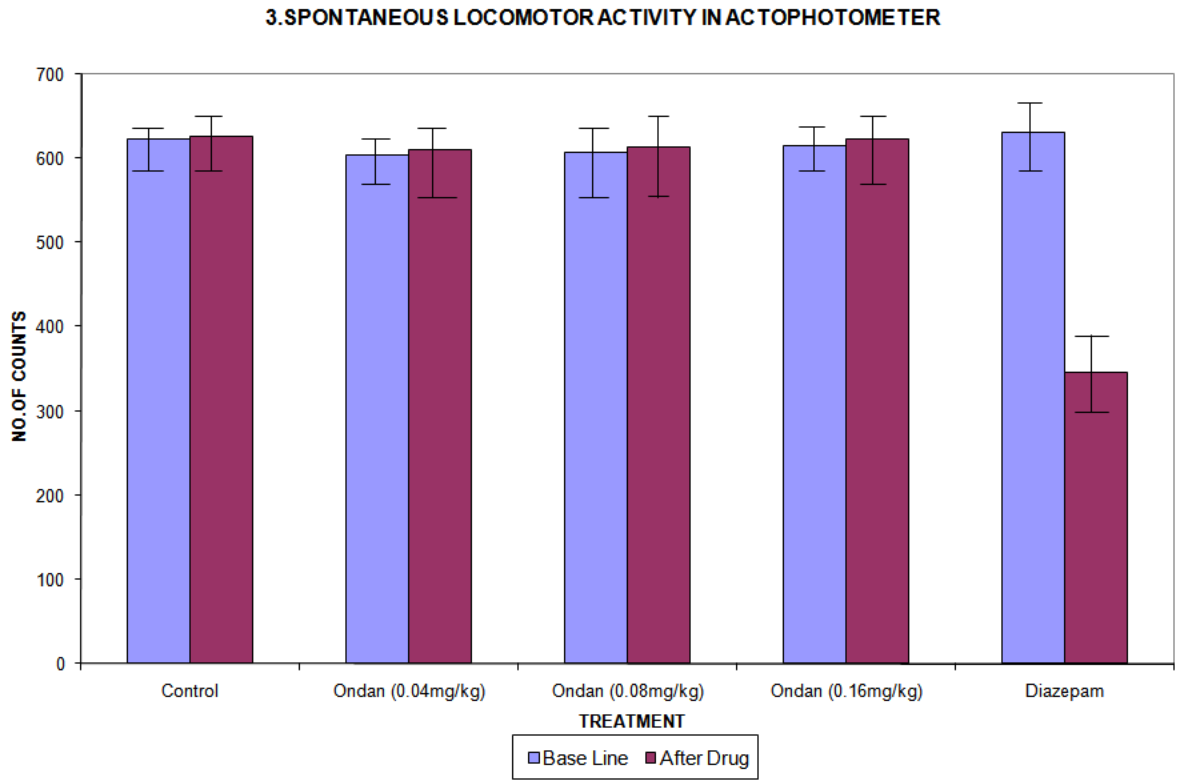
Rodents have aversion for high and open space and prefer enclosed arm and, therefore, spend greater amount of time in enclosed arm. When animals enter open arm, they freeze, become immobile, defecate and show fear-like movements [7].

ISSN: 0975-8585





ISSN: 0975-8585



An anxiolytic agent increases the frequency of entries into the open arms and increases the time spent in open arms of the EPM . Known anxiolytic agents such as the benzodiazepine diazepam and the azapirone buspirone hydrochloride, which are used clinically for the treatment of anxiety disorders, show reliable anxiolytic effects in the EPM.

From the results of the **time spent** in open arm, it was observed that ondansetron at the lower dose of 0.04 mg/kg had no anxiolytic properties. At the same time Ondansetron at the dose of 0.08mg/kg and 0.16mg/kg showed significant ($P < 0.001$) anxiolytic effects in comparison with control.

From the results of the **number of entries** into open arm, it was observed that ondansetron at the lower dose of 0.04 mg/kg has no anxiolytic properties but Ondansetron at the dose of 0.08mg/kg and 0.16mg/kg showed significant ($P < 0.01$)anxiolytic effects in comparison with control.

The results of the present study were in agreement with the study by B.J.Jones et al where a highly selective 5-HT_3 antagonist showed anxiolytic properties in different animal models namely social interaction test in rat, light/dark exploration test in mice, behavioral observation of marmosets and cynomolgus monkeys [8].

Sedative effect was evaluated with spontaneous locomotor activity in Actophotometer. The sedative effect was not statistically significant for ondansetron at all the three doses in comparison with control group.

Benzodiazepines cause a number of side effects like ***sedation***, light-headedness, psychomotor and cognitive impairment, vertigo, confusional state (especially in elderly), increased appetite and weight gain, alterations in sexual function. Some women fail to ovulate while on regular use of BZDs. The major constraint in their long term use for anxiety disorders is their potential to produce dependence [9].

The above mentioned side effects are not seen in ondansetron. Hence it could be safely administered in elderly, women and also children who require treatment for anxiety disorders. Also in people handling heavy machinery and drivers, where sedation is undesirable, ondansetron can be given safely. Given the chronicity of treatment of anxiety disorders, ondansetron is a better drug as there is no risk of abuse potential or dependence.

Hence ondansetron having a better safety profile with fewer side effects, could score better than the currently available anxiolytics with further evaluation and clinical studies.

CONCLUSION

Past attempts to treat anxiety disorders have only been partially successful. Several converging lines of evidence from molecular, animal and clinical studies have demonstrated that the $GABA_A$ – Benzodiazepine receptor complex plays a central role in modulation of anxiety. Benzodiazepines, which act at this receptor, have anxiolytic properties, but are limited by side effects like sedation, tolerance and concerns of potential abuse/dependence.

Ondansetron, a selective $5HT_3$ antagonist, used as antiemetic in post operative nausea and vomiting (PONV) and cancer chemotherapy induced emesis, produces significant anxiolysis at the antiemetic dose itself. Hence ondansetron could reduce the stress which frequently accompanies the above conditions and also reduce the need for additional anxiolysis.

From the present study a significant anxiolytic effect without the sedative side effect of benzodiazepines was found to be present for ondansetron in mice. Therefore ondansetron could become an alternative anxiolytic with better patient compliance.

Further studies are warranted to explore the long term effects in treating anxiety disorders in humans as well as for the development of tolerance. Also whether other $5HT_3$ antagonists also exhibit anxiolytic effects is still to be determined.

REFERENCES

[1] PD White and AW Clare. Psychological medicine. In : M. Parveen Kumar, Michael Clarke. Kumar & Clarke's Clinical Medicine, 7th ed, Saunders Elsevier; 2006 ; 1206.

[2] Helen Pratt, Anuradha Sorani. Anxiety disorders in Adolecence. In: Swati Y Bhave, Bhave's Text book of Adolescent Medicine, Jaypee ; 2006 ; 757.
[3] Niraj Ahuja. A short text book of Psychiatry, 5th ed. Jaypee ; 2002 ; 93.
[4] Rakesh K. Sharma, Rajesh Arora. Herbal drugs, A twenty first century perspective, Jaypee ; 1997 ; 582.
[5] Arulmozhi S. et al. Pharmacologyonline ; 3 : 761 – 775 (2008).
[6] AV Yadav, LA Kawale, VS Nade. Indian J Pharmacol 2008;40(1).
[7] S.K. Kulkarni. Practical Pharmacology and clinical pharmacy, 1st ed. Delhi Vallabh Publication; 2008 ; 146-147.
[8] BJ Jones, B Costall et al. British J Pharmacol 1988; 93: 985-993.
[9] KD Tripathi M.D. Essentials of Medical Pharmacology, 6th ed., Jaypee Brothers Medical Publishers ; 2008 ; 397