A STUDY ON THE ANXIOLYTIC AND SEDATIVE EFFECTS OF ONDANSETRON IN MICE

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BONAFIDE CERTIFICATE

This is to certify that the dissertation entitled **"A STUDY ON THE ANXIOLYTIC AND SEDATIVE EFFECTS OF ONDANSETRON IN MICE"** is a bonafide record work done by **Dr.LOURDU JAFRIN.A** under my direct supervision and guidance in the Institute of Pharmacology, Madurai Medical College, Madurai during the period of his post graduate study for MD, Branch VI –Pharmacology and appearing in March 2010.

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DECLARATION

I, **Dr. LOURDU JAFRIN.A** solemnly declare that the dissertation titled **"A STUDY ON THE ANXIOLYTIC AND SEDATIVE EFFECTS OF ONDANSETRON IN MICE"** has been prepared by me under the able guidance and supervision of my guide **Dr.S.THAMILARASI. M.D.,** Director in charge, Institute of Pharmacology, Madurai Medical College, Madurai, in partial fulfillment of the regulation for the award of MD (Pharmacology) degree examination of The Tamilnadu Dr. M.G.R. Medical University, Chennai to be held in March 2010.

This work has not formed the basis for the award of any degree, or diploma or any other to one previously from any other university.

Place : Madurai **Dr.LOURDU JAFRIN.A**

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CONTENTS

INTRODUCTION

Anxiety disorders are among the most prevalent mental disorders in the general population. These disorders are associated with significant morbidity and are often of long duration. These disorders cause significant embarrassment to the person involved and affect their routine day to day activities.

Anxiety is a feeling of apprehension, uncertainty and fear without apparent stimulus, associated with physiological changes like tachycardia, sweating, tremor etc. 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 transmitters. There are also minor neurotransmitters which are under research to improve the condition of patients with anxiety [1].

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 are inevitable. Sedation is another acute adverse effect which leads to reduced performance and has to be used cautiously in drivers and in persons handling heavy machinery.

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.

The present dissertation work 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. $5HT_3$ receptor antagonist like Ondansetron is claimed to have a broad range of action in animal models of anxiety. It also inhibits certain symptoms of withdrawal from drugs of abuse, alcohol, nicotine, diazepam and cocaine. It antagonises increased locomotor activity caused by mesolimbic dopamine excess. But more research is needed in these areas for more comprehensive and conclusive evidence.

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.

AIM AND OBJECTIVES

The aim of the present dissertation is to evaluate the anxiolytic and sedative properties of ondansetron in comparison with diazepam.

REVIEW OF LITERATURE

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REVIEW OF LITERATURE

From time immemorial, in every society, it has been realized that there are many troubled individuals who are neither insane nor mentally retarded. They differ from other people in being plagued by feelings of inferiority or self doubt, suspicion about the motives of others, low energy, inexplicable fatigue, shyness, irritability, moodiness, sense of guilt, and unreasonable worries and fears. They suffer as a result of these feelings or they behave in ways that are upsetting to those around them. Yet none of these conditions precludes partaking in the everyday affairs of life, such as attending school, working, marrying and rearing a family[2].

Beard in 1869 introduced the term neurasthenia, when lesser degrees of anxiety were grouped with minor depressive disorders. The first step in identifying anxiety disorder was taken by Westphal in 1871 who described the syndrome of agoraphobia. At that time, it appeared that the word 'anxiety' was derived from the mistranslation of Freud's word for fear (angst). In the year 1895, Freud separated anxiety disorders from neurasthenia and suggested the name anxiety neurosis [3].

As these conditions were more carefully documented, the ones that caused an individual much personal distress came to be called psychoneuroses and later, neuroses, and those that created difficulties in the society were called psychopathies and more recently, sociopathies[2].

Recent studies suggest that chronic anxiety disorder may increase the rate of cardiovascular related mortality. Hence, clinicians in psychiatry and other specialties must make the proper diagnosis of anxiety disorders rapidly and initiate treatment.

Yerkes Dodson Law [3]

Anxiety has an 'inverted U-shaped relationship' with performance, as demonstrated by well known Yerkes-Dodson law. In Yerkes Dodson law, it is seen that at very low level of anxiety, the performance is poor. Each increment of anxiety produces equivalent increment in performance. This can be regarded as normal or **healthy anxiety**.

Then, there is a phase where performance has reached its maximum and any increase in anxiety does not improve the performance any further. At this stage, the subject may in fact begin having the symptoms of anxiety, although symptoms produced at this stage do not affect performance.

Later on, any minor increase in anxiety causes deterioration in performance, which may in turn produce more anxiety.

Epidemiology

Ewald Horwath and Myrna Weissman reviewed epidemiological data and studies on the anxiety disorders. The data presented demonstrate that anxiety disorders are highly prevalent and that rates of illness are fairly uniform across cultures. In most cases, women are more likely to have anxiety disorders than men, a phenomenon that still begs for adequate explanation. Of particular interest is the finding that social phobia is more common in women than men [4].

Anxiety disorders are also more common in children and adolescents occurring in 13% of young people [5] .

General Adaptation Syndrome[6]

Hans Selye termed the **body's response to stressors** the general adaptation syndrome. The general adaptation syndrome consists of three stages :

- ♣ Alarm reaction
- ♣ Stage of resistance
- ♣ Stage of exhaustion.

Alarm Reaction :

In this stage, the body responds promptly, and many of these responses are mediated by the sympathetic nervous system, which prepares us to cope with the stressor.

Stage of Resistance:

If the stressor continues to be present, the stage of resistance begins, wherein the body resists the effects of the continuous stressor.

During this stage, certain hormonal responses of the body are an important line of defense in resisting the effects of stressors. Especially important among these hormonal responses is increased activity in the Adreno Cortico Trophic (ACTH) axis.

Stage of Exhaustion :

The final stage of the general adaptation syndrome is the stage of exhaustion. In this stage, the body's capacity to respond to both continuous and new stressors has been seriously compromised.

Because of stressor-induced hormonal effects, stomach ulcers, diabetes, skin disorders, asthma, hypertension and increased susceptibility to cancer may occur at this stage.

Etiology[4]

Another fascinating aspect of anxiety disorders is the exquisite interplay of genetics and experience. While there is little doubt that abnormal genes predispose to pathological anxiety states, evidence clearly indicates that traumatic life events and stress are also etiologically important. Study of the anxiety disorders thus presents a unique opportunity to understand the relation between nature and nurture. Medical illness, drugs, toxins and substance abuse also play a role in the etiology of anxiety disorders.

Genetics

Many studies have shown that anxiety disorders tend to run in families. It is not difficult to conceive that growing up with anxious parents or siblings might influence the development of anxiety in any individual. Therefore, family studies are only leads that prompt genetic investigators to attempt to determine whether any anxiety disorders are indeed inherited.

One compelling hypothesis is that individuals inherit a temperament like shyness, hyperactive autonomic nervous system responses, or behavioral inhibition. Then, depending on a variety of life

circumstances, these genotypes are expressed as specific phenotypes - one or more of the anxiety disorders themselves.

It may also be seen that more powerful "***anxiety genes***" require less environmental stress to be expressed. Clearly a variety of environmental influences activate latent genes through complex biochemical process, and these operate in the central nervous system. Hence, it is likely that genetic susceptibility to an anxiety disorder becomes an actual anxiety disorder when some set of environmental influences causes anxiety proneness genes to become active.

Drugs Associated with Anxiety Symptoms[7]

Anticonvulsants	:	Carbamazepine
Anti depressants	:	Selective serotonin reuptake inhibitors, Tricyclic Antidepressants
Anti hypertensive	:	Felodipine
Antibiotics	:	Quinolones, Isoniazid, Penicillin
Bronchodilators	:	Albuterol, Theophylline
Corticosteroids	:	Prednisone
Dopa agonists	:	Levodopa
Nonsteroidal anti-inflammatory drugs	:	Ibuprofen
Stimulants	:	Amphetamines, Methylphenidate, Caffeine, Cocaine

Sympathomimetics	:	Pseudoephedrine
Thyroid hormones	:	Levothyroxine
Toxicity	:	Anticholinergics,Antihistamines, Digoxin
Withdrawal	:	Alcohol, Sedatives

Common Medical illnesses associated with Anxiety symptoms[7,8,9]

Cardiovascular

- Angina
- Arrhythmias
- Congestive heart failure
- Hypertension
- Ischemic Heart Disease
- Myocardial Infarction

Endocrine and metabolic

- ✓ Cushing's disease
- ✓ Hyperparathyroidism
- ✓ Hyperthyroidism
- ✓ Hypoglycemia
- ✓ Hyponatremia
- ✓ Hyperkalemia
- ✓ Pheochromocytoma
- ✓ Vitamin B_{12} or Folate Deficiencies.

Gastrointestinal disorders

- Hiatus hernia
- Peptic Ulcer Disease
- Ulcerative Colitis
- Crohn's Disease.

Neurologic

- ❖ Dementia
- ❖ Migraine
- ❖ Parkinson's Disease
- ❖ Seizures
- ❖ Stroke
- ❖ Encephalitis
- ❖ Neurosyphilis
- ❖ Multiple Sclerosis
- ❖ Wilson's Disease
- ❖ Huntington's Chorea
- ❖ Neoplasm
- ❖ Poor Pain Control.

Respiratory system

- ☞ Asthma
- ☞ Chronic Obstructive Pulmonary Disease

- ☞ Pulmonary Embolism
- ☞ Pneumonia

Others

- ✞ Anemias
- ✞ Systemic Lupus Erythematosus
- ✞ Vestibular dysfunction
- ✞ Aspirin Intolerance
- ✞ Brucellosis
- ✞ Infectious Mononucleosis
- ✞ Carcinoid Syndrome
- ✞ Porphyria
- ✞ Uremia
- ✞ Premenstrual Tension.

Anxiety Disorders: Neuro Biology

Research on anxiety disorders is undergoing a revolutionary paradigmatic transformation as disparate areas of psychiatric nosology, pharmacologic dissection, and cognitive neuroscience converge towards an integrated neurobiological understanding of the pathologies. The anxiety disorders are separated as per the fourth edition of Diagnostic and Statistical Manual of Mental Disorders (DSM-IV) although it is assumed

that many aspects of the functional- neuroanatomical pathways cross diagnostic boundaries.

Amygdala, Corticotropin-Releasing Factor, And Monoamines

An abundance of preclinical evidence now points to the **amygdala** as the major mediator of the stress response, fear, and possibly also anxiety. The amygdala receives excitatory glutamatergic thalamic and cortical sensory input that it appears to process in series and in parallel through an organized array of intraamygdaloid circuitries nuclei. Such a pathway allows a stimulus representation to be modulated by different functional systems such as those mediating memories from past experience and information about the hormonal and homeostatic milieu of the organism.

The amygdala also has caudal projections to monoaminergic loci such as noradrenergic neurons of the locus ceruleus, dopaminergic neurons of the ventral tegmental area, and serotonergic neurons of the raphe nuclei. The amygdaloid nuclei appear to have the appropriate connections to orchestrate the simultaneous elaboration of individual anxiety disorder correlates such as cognitive misappraisal and fear (cortical areas), escape and freezing behavior (periaqueductal gray region), hyperventilation (parabrachial nucleus), sympathetic activation

(lateral hypothalamus), endocrine activation (paraventricular nucleus of the hypothalamus), gastrointestinal distress (dorsal motor nucleus of the vagus), increased startle (nucleus caudalis pontis), and motor activation (striatum).

Lesions of the amygdala in ***animal models*** lead to attenuated fear and emotional responsiveness as well as blockade of autonomic and neuroendocrine responses to learned stress. Direct electrical stimulation of the amygdala in animal models elicits both fear-like behavior and autonomic arousal. Stereotactic electrical stimulation of the amygdala in conscious humans has been reported to elicit symptoms of anxiety including fear, anxiety, depersonalization, visceral sensations in the chest and epigastric region, and changes in autonomic function.

Many studies have shown that local infusion of benzodiazepines into the amygdala has anxiolytic effects as measured in the operant conflict test, the light dark box, and the elevated plus maze [10].

Also emerging is the recognition of a wide extrahypothalamic network of corticotropin releasing factor (CRF) secreting neurons originating in the central amygdaloid nuclei. Intracerebroventricular injection of CRF in animals produces physiological changes similar to those produced by stimulation of the amygdala and a range of behaviors analogous to components of human anxiety and affective disorders; these

include anorexia, insomnia, decreased libido, hyperactivity, reduced exploratory activity, and increased startle response [4].

A transgenic strain of interest is a mouse strain created to over express corticotropin releasing factor (CRF). As expected, these animals had constitutionally high ACTH and glucocorticoid production (Stenzel Poore et al., 1994). Since the hypothalamic pituitary adrenal axis is often abnormal in affective, anxiety, and eating disorders in humans, the model is relevant to these conditions. With regard to anxiety, the transgenic mice had decreased spontaneous exploratory behaviour in novel environments (open field activity) and were more avoidant of the open arms of elevated mazes compared with unaltered animals [10].

One model relevant to anxiety and panic puts forth that the ***serotonin pathway*** originating in the dorsal raphe nucleus and running along the medial forebrain bundle innervates the amygdala and frontal cortex, thus facilitating active escape or avoidance behaviors in response to distal threat. This pathway likely involves ***postsynaptic*** serotonin (5-hydroxytryptamine [5-HT]) type 5-$HT_{2A/2C}$ and **5-HT_3** receptor activation. It is assumed that such resultant behavior relies on learning and relates to conditioned or anticipatory anxiety. Such a pathway therefore may be relevant to ***generalized anxiety disorder***.

A separate pathway originating in the dorsal raphe nucleus involves innervation of the periventricular and periaqueductal gray region such that serotonin neurons inhibit inborn fight or flight reactions in response to proximal danger, acute pain, or asphyxia. This pathway may be relevant to panic attacks and is likely to be mediated by 5-$HT_{2A/2C}$ and 5-HT_{1A} postsynaptic receptors.

A further suggestion is that with chronic stress, the serotonin pathway connecting the median raphe nucleus to the hippocampus, likely mediated by postsynaptic 5-HT_{1A} receptors, promotes resistance to such stress by disconnecting the aversive events from processes underlying appetitive and social behaviors. It has been suggested that this pathway may be relevant to avoidance and numbing found in post traumatic stress disorder.

The locus ceruleus noradrenergic and dopaminergic systems are believed to increase autonomic arousal and vigilance in response to threat. ***Noradrenergic neurons*** of the locus ceruleus appear to be **excitatory to serotonin** neurons of the dorsal raphe nucleus, while 5-HT neurons of the dorsal raphe nucleus inhibit locus ceruleus firing. The locus ceruleus may also stimulate the periaqueductal gray via an indirect pathway through the amygdala.

So again, the amygdala may mediate in a complex orchestration of coping behaviors and adaptive responses to future stressors. Over stimulation of such adaptive mechanisms by repeated or chronic stressors as well as deficits in function of components of these systems could theoretically lead to the pathological responses observed in anxiety disorders [4].

Classification of anxiety disorders [11,12] :

The category of anxiety disorders has been further subdivided in the fourth edition of DSM (DSM-IV) into the following entities:

- Panic disorder with or without agoraphobia
- Agoraphobia with or without panic disorder
- Specific phobia
- Social phobia
- Obsessive compulsive disorder (OCD)
- Post traumatic stress disorder (PTSD)
- Acute stress disorder
- Generalized anxiety disorder (GAD)
- Anxiety disorder due to general medical condition
- Substance induced anxiety disorder
- Anxiety disorder not otherwise specified

Clinical Presentation And Biochemical Determinants

Panic Disorder

Panic attacks, the hallmark of panic disorder, are unexpected and rapidly progressing bursts of anxiety accompanied by an array of cognitive and autonomic symptoms such as immense fear, palpitations, hyperventilation, lightheadedness, and sweating. By definition, the intensity of symptoms should reach a peak within 10 minutes, and most attacks have a relatively short time course of about 5 to 30 minutes.

According to DSM-IV, the sequelae of such attacks are necessary for a diagnosis of panic disorder. These can include anticipatory anxiety ("persistent concern about having additional attacks"), worry about the mental and physical implications of the attacks, and phobic avoidance of situations associated with the attacks.

Panic disorders initially present in the early twenties and are rare after the age of forty[13].

A variety of agents have been successfully used to induce panic-like reactions in efforts to elucidate the underlying pathophysiology. A distinction has been proposed between pharmacological challenge agents that induce significant respiratory symptoms and those that have few, if any, respiratory symptoms but significantly activate the hypothalamic-pituitary-adrenal (HPA) axis and result in significant anxiety.

The former group of "**respiratory panicogens**" includes sodium lactate, CO_2, sodium bicarbonate, isoproterenol and doxapram; these agents induce a state more closely resembling spontaneous panic attacks.

The latter group of "**hypothalamic-pituitary-adrenal"–activating anxiogens**" includes yohimbine, m-chlorophenylpiperazine (mCPP), fenfluramine, and β-carboline. These agents are more notable for inducing a more generalized or anticipatory anxiety.

Cholecystokinin (CCK) agonists appear to induce both a prominent respiratory component and robust hypothalamic-pituitary-adrenal activation and thus have qualities of both classes of agents.

Generalized Anxiety Disorder

According to DSM-IV, generalized anxiety disorder is characterized by excessive and uncontrollable anxiety or worry persisting for at least 6 months, combined with three of six additional symptoms ("restlessness or feeling keyed up or on edge," "being easily fatigued," "difficulty in concentrating or mind going blank," "irritability," "muscle tension," and "sleep disturbance").

Recent epidemiological studies indicate that generalized anxiety disorder is one of the most common anxiety disorders; despite such

prevalence, a paucity of research on its neurobiology exists [4]. They occur in 4-6 % of the population and are more common in women[14].

The anxiolytic effects of 5-HT_{1A} partial agonists (buspirone and ipsapirone) and 5-HT_2 antagonists (ritanserin) in generalized anxiety disorder patients has suggested serotonin involvement. These pharmacological dissection results in generalized anxiety disorder patients are consistent with the model of excessive limbic forebrain 5-HT_2 and 5-HT_3 activity leading to anxiety by overstimulation and hippocampal 5-HT_{1A} stimulation leading to anxiolysis by adaptation.

A contrasting hypothesis suggests that 5-HT_{1A} agonists are efficacious due to stimulation of presynaptic 5-HT_{1A} autoreceptors, thus reducing the elevated serotonin activity responsible for activating the hypothalamus, basal ganglia, and limbic system.

Social Phobia

According to DSM-IV, social phobia is characterized by fear of social or performance situations involving "exposure to unfamiliar people or possible scrutiny by others," combined with fear of acting in a way that "will be humiliating or embarrassing." The exposure also almost invariably provokes an anxiety reaction, which may have prominent cognitive and autonomic components similar to a situationally bound or predisposed panic attack. The situations are "avoided or else are endured

with intense anxiety or distress," and the fears are recognized as "excessive or unreasonable."

Specific Phobia

According to DSM-IV, specific phobia is characterized by a "marked and persistent fear that is excessive or unreasonable" and is brought on "by the presence or anticipation of a specific object or situation (e.g., flying, heights, animals, receiving an injection, seeing blood)." The response may take the form of a situationally bound or predisposed panic attack, and the phobia causes marked distress or interferes with role functioning. A prominent vasovagal response was observed in a subgroup of patients with specific phobia.

Post Traumatic Stress Disorder

DSM-IV defines post traumatic stress disorder (PTSD) as a disorder in which a person has been exposed to a traumatic event or events that included "actual or threatened death or serious injury, or threat to the physical integrity of self or others," and "the person's response involved intense fear, helplessness or horror." Symptoms of increased arousal are also present and includes sleep disturbance, irritability, poor concentration, and exaggerated startle reflex.

Obsessive-Compulsive Disorder [4,15]

According to DSM-IV, obsessive-compulsive disorder (OCD) is characterized by repetitive thoughts, impulses, or images that are intrusive and inappropriate and cause anxiety or distress, or repetitive behaviors that the person feels driven to perform in response to an obsession or rigid rules that must be applied. These persons also recognize that the obsessions are a product of their own mind. The obsessions or compulsions are time consuming or interfere with role functioning.

OCD is unique among the anxiety disorders by appearing to be much more dominated by cognitive and related complex behavioral symptomatology, with autonomic dysregulation playing little role.

Recent findings suggest that the serotonin (5-HT) transporter might be linked to both neuroticism and sexual behaviour as well as to obsessive-compulsive disorder (OCD)[16].

The subjects who were in the early romantic phase of a love relationship were not different from OCD patients in terms of the density of the platelet 5-HT transporter, which proved to be significantly lower than in the normal controls [16].

Pharmacotherapy of Anxiety Disorders :

Medication to treat anxiety is centuries old if one includes alcohol. Until recently, most antianxiety medications were developed empirically rather than on the basis of known organic pathology. Benzodiazepines and SSRIs are the commonly prescribed class of drugs for clinical anxiety [17].

Evidence clearly shows that anxiety disorders are, in general, chronic illnesses that often require long-term therapy. Thus the medication selected must be well tolerated and safe even if the patient must continue to take it for a prolonged period to prevent relapse [4].

Adverse effects of benzodiazepines include drowsiness, dizziness, ataxia, CNS depression, psychomotor impairment, confusion, cognitive impairment, aggression, increased risk of fall or fracture (especially in elderly), and anterograde amnesia.

Tolerance and dependence to benzodiazepine can occur if used for a longer period of time. On sudden discontinuation, benzodiazepine withdrawal can occur which is characterized by insomnia, nausea and vomiting, twitching, irritability, anxiety, paresthesias, tinnitus, delirium and seizures. Excessive sedation and overdose can be reversed with

flumazenil, a benzodiazepine antagonist. A total dose of 3 mg can be administered depending on the circumstances[18].

History of anxiolytic drugs: [4]

The oldest antianxiety drug is alcohol, and it remains the most frequently used and most easily accessible tranquilizer. Modern medical anxiolysis began with the introduction of paraldehyde and bromides around the turn of the century, followed by the first medical use of barbiturates in 1903.

The development of the so called non-barbiturate non-benzodiazepine hypnotic drugs in the 1930s namely meprobamate, tybamate, methaqualone , methyprylone and glutethimide did not address any of the deficiencies of the barbiturates. These drugs proved more problematic, possessed very low therapeutic index, were highly addicting and could be fatal in overdose.

The synthesis of the first benzodiazepine, chlordiazepoxide in 1957 heralded a new era of safe and effective medical management of anxiety. Because of their safety, efficacy, and high therapeutic index, benzodiazepines have for the most part replaced barbiturates and the nonbarbiturate, nonbenzodiazepine type drugs.

The demonstration in the early 1960s that imipramine controls panic attacks was the first evidence that antidepressant drugs may

alleviate anxiety and that this effect may be independent of their antidepressant property.

The historic observation that panic attacks were specifically responsive to antidepressants also marked the beginning of a new diagnostic system that differentiates the subtypes of anxiety neuroses on the basis of medication response.

Parallel to the work with imipramine in the United States, British investigators found that another class of antidepressants, the monoamine oxidase inhibitors, specifically benefits hysterical patients with phobic symptoms. Since these patients show many features of panic disorder and agoraphobia, tricyclic drugs and MAOIs quickly became the first-line treatment choice in panic disorder.

Of the many subsequently introduced antidepressants with anxiolytic properties, fluoxetine was the next milestone in the pharmacology of anxiety. This first drug in a series of ***serotonergic agents*** became the best-selling antidepressant by 1990. The efficacy of serotonergic drugs in the treatment of panic disorder and obsessive-compulsive disorder significantly advanced the treatment of these anxiety disorders and gave rise to new theories implicating the serotonergic system in the neurobiology of anxiety.

In addition to benzodiazepines and several classes of antidepressants currently available anxiolytics agents include Beta-adrenergic receptor antagonists and the azapyrone, buspirone.

New drug development targets neurotransmitter systems identified primarily by pharmacological challenges as pertinent to the neurobiology of anxiety. Candidates include partial benzodiazepine agonists and reverse benzodiazepine antagonists, neurosteroids, neuropeptide agonists and antagonists such as cholecystokinin B antagonists, corticotropin releasing factor antagonists, neuropeptide Y agonists and serotonergic agents acting on specific serotonin receptor subtypes, 5-HT_{1A} agonists, and 5-HT_2 and 5-HT_3 antagonists. The accelerated drug development process promises highly effective anxiolytic agents with minimal adverse effects in the near future .

In essence, anxiolytics drugs can be classified into those which work immediately or at least very fast (in the order of less than an hour) and those which have a delayed action (generally 2-6 weeks).

Current Trends In Anxiety Management

Antidepressant medication is increasingly seen as the medication treatment of choice for the anxiety disorders. More specifically, drugs with primary effects on the serotonin neurotransmission system have

become first-line recommendations for panic disorder, social phobia, obsessive-compulsive disorder, and post traumatic stress disorder.

Although they generally take longer to work than benzodiazepines, the selective serotonin reuptake inhibitors such as fluoxetine, sertraline, paroxetine, fluvoxamine, and citalopram, as well as venlafaxine and nefazodone are probably more effective than benzodiazepines and easier to discontinue.

Increasingly, benzodiazepines are used only for the temporary relief of extreme anxiety, as clinician and patient wait for the effects of antidepressants to take hold. Long term administration of benzodiazepines is reserved for patients who do not respond to, or cannot tolerate antidepressants.

Monoamine oxidase inhibitors are given only to patients with anxiety disorders who do not respond to trials with several medications.

However, most clinicians believe that the best result for anxiety disorder patients have come with combination of medication with one or more types of psychotherapy.

Immediate anxiolytics	**Delayed anxiolytics**
Benzodiazepines	Tricyclic antidepressants
Barbituratates , Alcohols	MAOIs, SSRIs/SNRIs
Beta blockers	All psychotherapies

Preclinical and clinical studies suggest that there exists a neurobiological link between emotional and cognitive processes. Clinically effective anxiolytics may reduce anxiety through a disruption of the association between emotion and cognition.

Classification of Anxiolytics [19,20,21]

SSRIs : Fluoxetine Citalopram
Escitalopram Paroxetine
Sertraline Fluvoxamine

SNRI : Venlafaxine

Benzodiazepines :

- Chlordiazepoxide Diazepam
- Alprazolam Prazepam
- Medazepam Chlorazepate
- Oxazepam Lorazepam
- Flurazepam Bromazepam

Diphenylmethane : Hydroxyzine, Captodiame

Azaspirodeconediones : Buspirone, Gepirone
Ipsapirone, Tandospirone

Beta adrenoceptor antagonist : Propranolol

Monoamine Oxidase Inhibitors[22] : Phenelzine, moclobemide.

Tricyclic Antidepressants (TCA) [22]**:** Amitryptyline, Imipramine, Clomipramine

Imidazopyridines[23] **:** Zolpidem , Alpidem.

Beta carbolines[23] **:** Abecarnil

Drug choices for Anxiety Disorders [9,24]

Anxiety disorder	First Line Drugs	Second line drugs	Alternatives
Generalised anxiety	Duloxetine Escitalopram Paroxetine Venlafaxine	Benzodiazepines Buspirone Imipramine Sertraline	Hydroxyzine Pregabalin
Panic Disorder	SSRIs Venlafaxine	Alprazolam Clomipramine Clonazepam	Phenelzine
Social anxiety disorder	Escitalopram Fluvoxamine Paroxetine Sertraline Venlafaxine	Citalopram Clonazepam	Buspirone Gabapentin Mirtazapine Phenelzine Pregabalin
OCD	CBT (cognitive behavioral therapy)	High dose SSRI	Finally change to another SSRI or clomipramine

Treatment of Generalized Anxiety Disorder :

Because of the lack of dependence and tolerable adverse effect profile, antidepressants have emerged as the treatment of choice for the long term management of chronic anxiety, especially in the presence of co morbid depressive symptoms.

Among the antidepressants, Venlafaxine, Paroxetine and Escitalopram are FDA-approved antidepressants for generalized anxiety disorder (GAD). Imipramine is considered when patients fail to respond to SSRIs or Venlafaxine .

The benzodiazepines are more effective in treating the somatic and autonomic symptoms of GAD as well as the acute symptoms of anxiety as opposed to the psychic symptoms (e.g., apprehension and worry), which are reduced by antidepressants [24]. Beta blockers are not the first line drugs in treating GAD[25]

Treatment of Panic Disorder :

Panic disorder is treated effectively with several drugs including the SSRIs, the TCA imipramine, and the benzodiazepines alprazolam and clonazepam. Alprazolam, clonazepam, sertraline, paroxetine, and venlafaxine are approved for this indication. SSRIs are the first-line agents because of their tolerability and efficacy in acute and long-term

studies however, the benzodiazepines are the most commonly used drug for panic disorder [24]

Propranolol (40-160 mg/day orally) can mute the peripheral symptoms of anxiety without significantly affecting motor and cognitive performance.

Valproate has been found to be as effective as the antidepressants in panic disorder and is hence another useful alternative [26].

Treatment of social anxiety disorders (SAD):

SAD can present in children of preschool to elementary school age. If the disorder is not treated, it can persist into adulthood and increase the risk of depression and substance abuse. Placebo-controlled and open-label trials have provided evidence of efficacy of pharmacotherapy with an SSRI or SNRI in children 6 to 17years of age.

Benzodiazepines should be reserved as the last-line agents in children with SAD. Approximately one-fifth of patients with SAD also suffer from an alcohol use disorder. Paroxetine significantly reduced social anxiety and decreased the frequency and severity of alcohol use in patients with SAD and an alcohol use disorder.

People who abuse alcohol are at risk for abusing or becoming dependent on benzodiazepines. SSRI therapy is the treatment of choice in this patient population [24].

Treatment of Phobic Disorders:

Specific phobia is considered unresponsive to drug therapy, although highly responsive to CBT. The use of benzodiazepines or paroxetine in patients who failed CBT is supported by limited data. Benzodiazepines can be detrimental in patients with specific phobias treated with cognitive behavioural therapy (CBT) [24] .

Global social phobias may be treated with SSRIs, such as Paroxetine, Sertraline and Fluvoxamine, MAOIs in the same dosage as used for depression.

Gabapentin may be an alternative to antidepressants in the treatment of social phobia in a dosage of 300-3600 mg / day.

Specific phobias such as performance anxiety may respond to propranolol, 20-40 mg 1 hour prior to exposure [26] .

Treatment of Obsessive-Compulsive Disorder (OCD):

OCD responds to serotonergic drugs in about 60% of cases and usually requires a longer response time than for depression (up to 12 weeks) [26.]

Fluoxetine is used at the dose of 60-80mg/day. Alternatives are Buspirone at 15-60 mg/day used as augmenting agent along with SSRIs and antipsychotics such as Pimozide at the dose of 1-3 mg/day .

Individuals with intractable obsessive symptoms can be benefited from surgical procedures like anterior capsulotomy, cingulotomy and limbic leucotomy – all aim to interrupt the connection between basal ganglia and cortex [27].

Treatment of Post Traumatic Stress Disorder (PTSD):

Psychotherapy should be initiated as soon as possible after the traumatic event, and it should be brief and simple. Early treatment of anxious arousal with beta-blockers (eg, propranolol, 80–160 mg orally daily) may lessen the peripheral symptoms of anxiety (eg, tremors, palpitations) and help prevent development of the disorder.

Antidepressant drugs, particularly selective serotonin reuptake inhibitors (SSRIs), in full dosage are helpful in ameliorating depression, panic attacks, sleep disruption, and startle responses in chronic PTSD.

Sertraline and paroxetine are approved by the US Food and Drug Administration (FDA) for this purpose.

The alpha-blocking agent prazosin (2–10 mg orally at bedtime) has been successfully used to decrease nightmares and improve quality of sleep in PTSD [26].

Non pharmacological therapies of Anxiety Disorder:

These treatments may be given as first-line approaches to patients who refuse or cannot tolerate medication or in combination with medication. Indeed, some imaging studies already suggest that psychotherapy may alter abnormal patterns of brain activation, but much more work is required in this area [4].

1. **Behavioral therapy :**

Behavioral approaches are widely used in various anxiety disorders, often in conjunction with medication techniques like muscle relaxation, control of breathing and diaphragmatic breathing, communication skills, guided self-dialogue, and Stress Inoculation Training. Desensitization is the technique of exposing the patient to graded doses of a phobic object or situation and is used in treating phobias. Physiologic symptoms in panic attacks respond well to relaxation training. Exposure techniques with response prevention are useful for OCD [26].

2. **Psychodynamic therapy:**

This is based mostly on Sigmund Freud's work. It relies on the concept that symptoms result from mental processes that may be outside

the patient's conscious awareness and elucidating these processes can lead to remission of symptoms.

3. **Cognitive therapy [23].**

   It involves treating the anxiety by teaching patients to identify, evaluate, and modify the chronically worrisome danger related thoughts and associated behaviors.

4. **Social**

Peer support groups for panic disorder and agoraphobia have been particularly helpful. Social modification may require measures such as family counseling to aid acceptance of the patient's symptoms and avoid counterproductive behavior in behavioral training [26.]

Novel approaches in the treatment of Anxiety Disorder: [17,23,28]

- Inhibitor of neuronal transport of one or more monoamines, including nor epinephrine or dopamine, as well as serotonin (eg. Milnacipran, MCI-225)
- Sunepitron – serotonin agonists
- MEM-1414 – inhibitor of Phosphodiesterase 4
- Ampakines – glutamate AMPA receptor modulators.
- Ocinaplon, Pagoclone – $GABA_A$ receptor agonists
- CP-122721, GB-823296 – inhibitor of Neurokinin-1 receptors
- SR48968,GR159897 - inhibitor of Neurokinin-2 receptors

- Osnetant, Talnetant - inhibitor of Neurokinin-3 receptors
- AG-561,AVE-4579,DPC-368 – CRF-1 receptor antagonists
- MK-801 – NMDA receptor antagonist
- Partial benzodiazepine receptor agonists-
    - Alpidem,
    - Bretazenil,
    - Imidazenil,
- Neuroactive steroids – neuroactive steroids are molecules based on a steroid chemical structure, which interact with the GABA-benzodiazepine receptor complex. Such agents are in early development.
- Flesinoxan : highly selective 5-HT1A receptor agonist. It is under clinical development for GAD.
- 5-HT_{2A} antagonists – Pirenperone, Ritanserin, Ketanserin.
- Pentagastrin (CCK – 5) antagonists
- Neuropeptide Y analogue – galanin
- Inositol – for panic and OCD.
- Antiandrogens and opioids may be of use in OCD.
- CCKB Antagonists[29]

Non psychiatric uses of anxiolytics include facilitation of cooperation during painful procedures, controlling seizures, treating alcohol,sedative, or hypnotic withdrawal[18].

Plants with anti stress properties [30, 31 ,32] :

As long as humans have been around, there has been stress. Stress plays an important role in the genesis of different diseases and can cause gastric ulcers, neurohumoral and hormonal changes.

Herbal drugs or medicinal plants, their extracts and their isolated compounds have demonstrated spectrum of biological activities. They continued to be used as medicine in folklore or food supplement for various disorders.

Some of the plants 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 ,
- Alstonia schlolaris ,
- Morus alba (Mulberry

Serotonin – *an overview:*

Serotonin is present in highest concentration in blood platelets and in the GIT, where it is found in the enterochromaffin cells and the myentric plexus. Lesser amounts are found in brain and retina [33,34.] 5-HT is inactivated by mono-amine-oxidase (MAO) and aldehyde-dehydrogenase into 5-hydroxy-indole-acetic acid (5-HIAA), which is excreted in the urine. N – Acetylation of serotonin followed by its 0- methylation in the pineal body forms melatonin[35].

5-HT plays a physiological role in sleep, aggression, thermoregulation, cardiovascular system, sexual activity, neuroendocrine system, appetite, motor drive, learning and memory. 5-HT is implicated in psychiatric syndromes as major depression, anorexia nervosa, bulimia, anxiety disorders, schizophrenia and obsessive-compulsive disorder[36].

The 5-HT receptor types

Until now the 5-HT receptors are divided into 7 subfamilies; 5-HT_1 up to 5-HT_7 receptors. All 5-HT receptors are G-protein coupled receptors, with the *exception of the 5-HT_3 receptor which is a ligand-gated ion-channel*.

5-HT_{1A} receptors are located both presynaptically on the cell body and postsynaptically, and mediate neuronal inhibition. 5-HT_{1D} has

both autoreceptor function on cortical neurons and postsynaptic function[36].

5-HT_3 receptors :

These occur mainly in the peripheral nervous system, particularly on nociceptive sensory neurons and on autonomic and enteric neurons, where 5-HT exerts a strong excitatory effect. 5-HT_3 receptors also occur in the brain, particularly in the *area postrema*, a region of the medulla involved in the vomiting reflex, and selective 5-HT_3 antagonists are used as antiemetic drugs. 5-HT_3 receptors are exceptional in being directly linked to membrane ion channels and cause excitation directly, without involvement of any second messenger [37] .

5-HT_3 antagonists :

With the notable exception of alosetron and cilansetron, which are used in the treatment of irritable bowel syndrome, most 5-HT_3 antagonists are antiemetics, used in the prevention and treatment of nausea and vomiting.

Bemesetron was the first selective 5-HT 3 antagonist to be synthesised. Other 5-HT3 antagonists are Ondansetron, Granisetron , Tropisetron , Dolasetron , Palonosetron , Ramosetron, Itasetron, Fabesetron, Ricasetron and Zatosetron [36].

Ondansetron belongs to the group of 5-HT_3 receptor antagonist that possess an imidazole or related heterocyclic terminal amine which also include Alosetron, Fabesetron and Ramosetron[36].

Mechanism of action

Its effects are thought to be on both peripheral and central nerves. As the name implies, 5-HT_3 antagonist ondansetron prevents serotonin from binding to 5-HT_3 receptors. Such receptors are present mostly on the ends of afferent branches of the vagus nerve, which send signals directly to the brain's vomiting center in the medulla oblongata, and in the chemoreceptor trigger zone of the area postrema of brain, which receives "input" from nausea-inducing agents in the bloodstream and communicates with the vomiting center. By preventing activation of these receptors, 5-HT_3 antagonists interrupt one of the pathways that lead to vomiting [38].

Adverse effects

Ondansetron is a well-tolerated drug with few side effects. Headache, constipation, and dizziness are the most commonly reported side effects associated with its use. Transient rise in liver enzymes is seen rarely.

There have been no significant drug interactions reported with the use of this drug. It is broken down by the hepatic cytochrome P450

isoenzyme CYP3A4 and it has little effect on the metabolism of other drugs (eg. Rifampicin) broken down by this system [39].

5 - HT_3 antagonists ongoing research:

1. Since 5-HT_3 receptors not only have a high density in the area postrema but also in the hippocampal and amygdala region of the limbic system, it has been suspected that 5-HT_3 selective agents have psychotropic effects[40].
2. 5-HT3 antagonists may have an anxiolytic profile, but clinical results are still prelimnary and need more validation[23].
3. In different models of memory and learning a positive effect on basal learning behavior was seen [40].
4. There is no single best treatment for postcardiotomy delirium but ondansetron may be used because it is safe, effective, and without side effects [41].
5. The persistent erythema and flushing in rosacea responded well to 5- HT_3 antagonists [42].
6. 5-HT_3 receptors play an important role in morphine discontinuation phenomena[43].
7. Ondansetron may also find use in the treatment of psychiatric diseases like schizophrenia[44].

8. There are reports that ondansetron may be useful in Pruritis, opioid withdrawal syndrome, gastrointestinal motility disorders and Tourette's syndrome[45] .
9. May be useful in vertigo and cerebellar tremor[46].
10. CNS effects comprise attenuation of age-associated memory impairment, reduction of alcohol consumption in moderate alcohol abuse and an antipsychotic effect in patients with Parkinson's psychosis[47].

Animal models for antianxiety effects:[48]

- ❖ Foot shock induced aggression
- ❖ Isolation – induced aggression
- ❖ Anticipatory anxiety in mice
- ❖ Social interaction in rats
- ❖ Elevated plus maze test
- ❖ Water maze
- ❖ Staircase test
- ❖ Cork gnawing test
- ❖ Distress vocalization in rat pups
- ❖ Light-dark model

Animal models for evaluation of Sedative activity:

- Open field test
- Hole-board test
- EEG analysis from rat brain by telemetry.
- Spontaneous locomotor activity testing with Photoactometer.

MATERIALS AND METHODS

Study centre :

This study was carried out in the Institute of Pharmacology and Central animal house, Madurai Medical College, Madurai.

Period of Study :

The study was conducted for a period of eight months from 08.01.09 to 01.09.09 after obtaining ethical clearance from the Institutional Animal ethical committee, Madurai Medical College, Madurai.

Materials required for the study

1.Animals :

Inbred male albino mice from central animal house, Madurai Medical College were utilized in this study. Thirty male albino mice each weighing 24 to 26 grams were included in the study. Animals were allowed standard diet and tap water ad libitum.

2. Standard drug

Inj. Diazepam was mixed with distilled water to obtain a solution of concentration 0.01mg/ml and was administered intraperitoneally at the dose of 1mg/kg.

3. **Test Drug:**

Inj. Ondansetron was mixed with distilled water to obtain a homogenous solution and was administered intraperitoneally at the graded doses of 0.04 mg / kg ,0.08 mg / kg and 0.16 mg / kg respectively.

4. Distilled water :

Inj.distilled water was used as vehicle for control group of animals.

5.Elevated Plus Maze :

The elevated plus maze apparatus was indigenously designed in our institute using wood as the raw material. The maze 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 whole apparatus was painted dull black. Lighting was kept constant and to a minimum by a 15 watt bulb hung from above.

6. 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:

(I) 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.

GROUPS	TREATMENT
CONTROL	Normal feed + water Distilled water i.p
STANDARD	Normal feed + water Diazepam 1 mg/kg i.p
TEST 1	Normal feed + water Ondansetron 0.04 mg / kg i.p
TEST 2	Normal feed + water Ondansetron 0.08 mg / kg i.p
TEST 3	Normal feed + water Ondansetron 0.16 mg / kg i.p

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 5min 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.

(II) Actophotometer for sedative effect:

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.

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$\pm$ 3.17 seconds. The time spent in open arm for standard group was 98 $\pm$ 7.97 seconds. The time spent in open arm for test groups (1, 2, 3) was 18.83 $\pm$ 3.24, 53.83 $\pm$ 1.42 and 54.83 $\pm$ 2.74 seconds respectively.

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 $\pm$0.60, for standard group was 14.5 $\pm$ 2.05, for test groups (1, 2, 3) were 3.83 $\pm$ 0.60, 6.67 $\pm$ 1.05 and 7.67 $\pm$ 1.23 respectively .

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.

Sedative effect:

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 $\pm$ 23.75 and 626.17 $\pm$ 34.70. The average number of counts before and after 30 min for standard group of mice was 630.83 $\pm$ 18.43 and 346 $\pm$ 13.93. The average number of counts before and after 30 min for test groups (1, 2, 3) was 603.33 $\pm$ 11.83 and 609.67 $\pm$ 19.78, 606.67 $\pm$ 24.91 and 612.33$\pm$ 31.22, 615.17 $\pm$ 16.48 and 623$\pm$ 24.18 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.

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[49].

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[50].

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[51].

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.

SUMMARY AND 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.

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**

Control Vs Standard : t-value = -12.11 (P<0.001)

Control Vs Test 1 : t-value = -1.73 (P=0.12) not significant

Control Vs Test 2 : t-value = -12.32 (P<0.001)

Control Vs Test 3 : t-value = -10.46 (P<0.001)

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

Control Vs Standard: t-value = -4.29 (P<0.001)

Control Vs Test 1 : t-value = -1.96 (P=0.08) not significant

Control Vs Test 2 : t-value = -3.71 (P<0.01)

Control Vs Test 3 : t-value = -3.64 (P<0.01)

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)**

Control Vs Standard : t-value = 7.49 (P<0.001)

Control Vs Test 1 : t-value =0.413 (P=0.69) not significant

Control Vs Test 2 : t-value =0.296 (P=0.77) not significant

Control Vs Test 3 : t-value =0.07 (P=0.94) not significant

MICE IN CLOSED ARM

MICE IN OPEN ARM

ELEVATED PLUS MAZE

ACTOPHOTOMETER

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