A STUDY ON ANXIOLYTIC, SEDATIVE AND HYPNOTIC ACTIVITIES OF AQUEOUS EXTRACT OF MORINDA CITRIFOLIA FRUIT IN MICE

DISSERTATION SUBMITTED FOR THE DEGREE OF

M.D. BRANCH – VI

PHARMACOLOGY

MARCH - 2009

THE TAMILNADU

DR. M.G.R. MEDICAL UNIVERSITY

CHENNAI, TAMILNADU

BONAFIDE CERTIFICATE

This is to certify that the dissertation entitled "**A STUDY ON ANXIOLYTIC, SEDATIVE AND HYPNOTIC ACTIVITIES OF AQUEOUS EXTRACT OF MORINDA CITRIFOLIA FRUIT IN MICE "** is a bonafide record work done by **Dr. S.KANNAN** 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 2009.

Place :

Date :

Director & Professor,
Instituteof Pharmacology,
Madurai Medical College
Madurai 625 020.

DECLARATION

I, **Dr. S.KANNAN** solemnly declare that the dissertation titled **"A STUDY ON ANXIOLYTIC, SEDATIVE AND HYPNOTIC ACTIVITIES OF AQUEOUS EXTRACT OF MORINDA CITRIFOLIA FRUIT IN MICE "** has been prepared by me under the able guidance and supervision of my guide **Dr. R. MEHER ALI. M.D.,** Former Director and Professor of Pharmacology, 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 2009.

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.S.KANNAN**

Date :

ACKNOWLEDGEMENT

At the outset, I thank the **DEAN,** Madurai Medical College, Madurai for permitting me to carry out the study in the Institute of Pharmacology, Madurai Medical College, Madurai.

I express my sincere gratitude to my respective teacher and guide **Dr. R. MEHER ALI. M.D.,** Former Director and Professor, Institute of Pharmacology for his constant encouragement and invaluable guidance at every stage of this study. I have gained much from his immense wealth of knowledge and deep understanding of research principles that I could complete the study with little difficulty is a testimony to his vast experience and qualities as a teacher and guide.

I am extremely thankful to my co-guides **Dr.S.TAMILARASI, M.D.,** Additional Professor and director in charge of Institute of Pharmacology,Madurai medical college and **Dr. M. SHANTHI, M.D.,** Additional Professor for their critical review, valuable suggestion at every stage for the successful completion of the study with better precision.

I express my heartful thanks to **Dr. S. VIJAYALAKSHMI. M.D.,** Reader for her genuine concern and interest in my work and for her helpful suggestion during the course of the work.

I recollect with pleasure the invaluable support and encouragement extended by Assistant Professors DR.R. SAROJINI MD, DR.K.M.S. SUSILA, M.D., DR.R.RENUKA DEVI M.D., DR. R. NAVAJOTHI. M.D., and DR. R. SUDHA.M.D.

I am indebted to Dr. A. MAHESWARAN. M.VSC., Veterinary Surgeon and all staff of the Institute of pharmacology and central animal house, Madurai Medical College for their kind support. I also express my appreciation to Mr. K.Periyanayagam, Assistant Reader of Pharmacognosy for his great help.

It is my duty to express deep appreciation to my collegues Dr. S. Siddharthan, Dr. K. Radhika, Dr.V. Theivanai, Dr. R.Hema, Dr.K. Geetha, Dr.A. Mohamed Gani, Dr. B.Maharani, Dr. M. Malathi, Dr. A. Lourdu Jafrin, Dr.M.Sheik Davooth Dr.S. Deepak, Dr.B.Jeyapriya, Dr.P.B.Arulmohan and Dr.Jeyaponmari for their assistance.

I thank Mr. P. Shenrayan. M.Sc., M.A., B.Ed., Head, Department of Statistics, Madurai Medical College for his valuable help.

Finally, I thank my family members for their kind support and encouragement throughout my study.

CONTENTS

INTRODUCTION

Anxiety disorders are the most common mental disorders in the community [1]. About 25% of the population will experience an anxiety disorder at some time in their life [2]. Anxiety is an important adaptive mechanism vital to an organism's survival, but excessive anxiety can be very disabling [3] and has been called either morbid or pathological anxiety .

Anxiety disorders themselves cause profound individual distress, suffering and reduced work and social achievement. There is also a major risk factor for other types of psychiatric disorders, particularly depression and alcohol or drug abuse [4.] Furthermore, anxiety disorders may contribute to morbidity and mortality through neuroendocrine and neuroimmune mechanisms or by direct neural stimulation e.g.: Hypertension , cardiac arrhythmias,coronary heart disease and functional gastrointestinal disorders [5]. Severe anxiety may also be complicated by suicidal tendencies. [6].

The neurobiology of anxiety is better understood with convincing evidence for association of several neurotransmitter systems, including glutamate, GammaAminoButyricAcid and other neurotransmitters and

neurochemical compounds such as catecholamine, benzodiazepines, serotonin, cholecystokinin, Corticotrophin releasing factor and Somatostatin [7]. A broad range of pharmacologic agents are available to treat anxiety disorders namely Selective Serotonin Reuptake Inhibitors, Selective Norepinephrine Reuptake Inhibitors, Tricyclic Antidepressants, MonoAmineOxidase Inhibitors, Buspirone, Benzodiazepines,Hydroxyzine, Antipsychotic, Anticonvulsants and Adrenergic agents [8]. For the past 40 years, Benzodiazepines such as Diazepam and Lorazepam, have dominated the field. They are the most effective anxiolytics in the short term but their long term efficacy remains in dispute. Their drawbacks include cognitive and psychomotor impairment, paradoxical reactions and tolerance [9].

Sleep is very essential for a normal life. Inadequate sleep leads to daytime tiredness, lack of energy, irritability, poor concentration, anticipatory anxiety and psychiatric problems like anxiety and depression can be encountered .

In recent years there has been growing concern among members of the public and the medical profession regarding the problem of dependence and possible abuse of the benzodiazepines [10]. It is because

of the adverse effects that a search for more selective compound with improved risk- benefit ratio is always there. Many studies were conducted to find an alternative medicine or plant – derived medications with more specific anxiolytic effects. Some of these plants that have been tested had shown to 'calm down', tranquilize include Valeriana officeinalis, Matricaria recutita, Passiflora caeulea, Salvia guaranifica, Tilia tomentova, Tilia europaea, Stachys lavanaulifolia, Echium amoenum and Salvia reterana and Nepeta cataria [11].

Morinda citrifolia, commonly called as "Noni" or "Indian Mulberry" is a traditional plant with wound healing, analgesic, immunomodulatory, anti aging and antibacterial properties. A principle substance isolated from the Morinda citrifolia fruit is similar in structure to Benzodiazepines and hence the present study is undertaken to find out the anxiolytic, sedative and hypnotic effects of aqueous extract of Morinda citrifolia fruit in comparison with Diazepam in mice.

AIM

The aim of the present study is to evaluate the anti anxiety, sedative and hypnotic activities of aqueous extract of Morinda citrifolia fruit extract in comparison with diazepam in mice

REVIEW OF LITERATURE:

Anxiety refers to a vague, unpleasant, emotion associated with a sense of apprehension, arousal or tension . Anxiety is commonly divided into state and trait anxiety. State anxiety refers to anxiety in the setting of a specific stressor, whereas strait anxiety refers to an enduring pattern of high anxiety at a baseline or a tendency to remain with an exaggerated anxiety in mildly stressful situations [12]. It is important to distinguish among the various anxiety disorders and identify possible co morbidities because of difference in treatment, Complications and prognosis.

Anxiety disorders are usually of longstanding and may be quite difficult to treat [13]. Most familiar is a pattern including clammy palms, butterflies in the stomach, racing pulse and pounding in the chest. Another common presentation is the chronic warmer who looks tense and pale and whose brow is furrowed from the constant strain. Other typical complaints include intrusive thoughts (e.g.: images, ruminations, frightening dreams), vigilance or trouble concentrating, or altered awareness of one's self or one's environment (e.g., depersonalization, derealization) [14]. Anxiety can be situational,

intermittent or attack-line, or persistent; most often it is short-lived, When it reaches distressing levels and interferes with functioning; a clinical diagnosis of an anxiety disorder is made, from the DSM-IV criteria for anxiety disorders.

Diagnostic and Statistical Manual of Mental Disorders,4th edition(DSM-IV) criteria for Generalized Anxiety Disorder [15]

A. The anxiety is difficult to control

B. At least 3 of the following :

1. Restlessness or feeling on edge.
2. Easy fatigability
3. Difficulty in concentrating
4. Irritability
5. Muscle tension
6. Sleep disturbance.

C. The focus of anxiety is not anticipatory anxiety about having a panic, as in panic Disorder.

D. The anxiety or physical symptoms cause significant distress or impairment in functioning

E. Symptoms are not caused by substance use or a medical condition and symptoms are not related to a mood or psychotic disorder.

Classification of Anxiety Disorders: [6, 14]

- Anxiety due to a general medical condition
- Generalized Anxiety Disorder
- Panic Disorder
- Post Traumatic Stress Disorder
- Adjustment disorder with anxious features
- Social Phobia
- Specific Phobia
- Obsessive Compulsive Disorder
- Psychotic terror
- Anxious Depression
- Situational Anxiety
- Substance induced anxiety disorder

Anxiety due to a general medical condition:

Pathologic anxiety secondary to a general medical condition may occur in the form of well-circumscribed and transient panic attacks or in a generalized, more chronic form.

Causes of anxiety disorder secondary to a general medical condition:[16]

- partial seizures
- paroxysmal atrial tachycardia
- hypoglycemia
- angina or acute myocardial infarction
- pulmonary embolus
- acute asthmatic attack
- pheochromocytoma
- parkinson's disease
- hyperthyroidism
- cushing's syndrome
- hypocalcemia
- chronic obstructive pulmonary disease

- post stroke
- post-head trauma

Generalized Anxiety Disorder:

Generalized Anxiety Disorder is characterized by a pattern of frequent, persistent worry and anxiety that is out of proportion to the impact of the event or circumstance that is the focus of the worry [17]. Generalized Anxiety Disorder is the most common of the anxiety disorders [15].

Panic Disorder:

Panic Disorder is defined by the presence of spontaneous, recurrent panic attacks followed by a fear of repeated attacks for at least one month. The panic attack is classically sudden in onset and typically reaches maximum intensity within ten minutes, the duration typically last ten to twenty minutes. In addition to intense fear or anxiety, patients may experience a rapid even pounding heart rate, sweating, shaking, tremor, shortness of breath, hyperventilation, a choking sensation, chest discomfort, nausea, dyspepsia, lightheadedness, unsteadiness, paresthesia , chills, derealisation,

depersonalization, sense of losing control, sense of impending doom or death [12].

Post Traumatic Stress Disorder and Acute Stress Disorder:

Post Traumatic Stress Disorder follows the experiencing or witnessing of a traumatic event which threatened death, serious injury or the physical integrity of self or others evoked fear, unpleasantness or horror. The symptoms must result in significant distress or impairment and must last for more than one month [17].

Substance-induced anxiety disorder :

Symptoms resembling an anxiety disorder can be triggered by use of or intoxication from over-the-counter cold preparations, caffeine, cocaine, theophylline preparations, amphetamines, and marijuana or withdrawal from alcohol, benzodiazepines, barbiturates, sedative-hypnotic agents and other CNS depressants.[2]

Social Phobia:

Individuals with Social Phobia (also called Social Anxiety Disorder) have a persistent and recognizably irrational fear of performing in social situations, believing that their performance will be

found wanting in some way and lead to humiliation or embarrassment [18].

Specific Phobia:

Specific Phobia is characterized by marked and persistent fear that is excessive or unreasonable, that is caused by the presence or anticipation of a specific object or situation, which may be any of the following:

Animal (e.g.: Dogs)

Natural Environment (e.g.: heights, storms, water)

Blood- injection injury

Situational (e.g.: airplanes, elevators, enclosed places) [15].

Obsessive Compulsive Disorder:

Obsessions and Compulsions are the essential features of Obsessive Compulsive Disorder. Obsessions are "persistent ideas, thoughts, impulses, or images that are experienced as intrusive and inappropriate" causing distress. Compulsions are defined as repetitive acts, behaviors or thoughts that are designed to counteract the anxiety associated with an obsession. Obsessions are anxiety provoking and compulsions reduced the anxiety associated with the obsessions [17].

Psychotic terror:

It's an acutely disorganized, frightened state where paranoia and hallucinations are prominent symptoms. Visual hallucinations in a patient indicates toxic psychosis due to amphetamines, cocaine or anticholinergic substance.

Anxious Depression:

In anxious depression, anxiety, tension or agitation accompanies overt depressive affect. Over 60% of anxious patients eventually have symptoms of depression. Other patients are chronically depressed with intermittent exacerbations of anxiety symptoms [14].

Somatosthenic anxiety:

Anxiety associated with somatic orientation, in which patient gives a special emphasis to somatic symptoms [19].

Situational anxiety: [14]

Situational anxiety also known as context specific anxiety, describes reactions to a variety of stressful stimuli, such as interviews, tests, public speaking, planned presentations, auditions and surgery. Such anxiety is short-lived and it ends once the expectation is started or completed.

Pathophysiology of Anxiety Disorder:

Several preclinical evidence now point to the amygdala as the major mediator of stress response, fear and anxiety. The major mediators of anxiety disorder appears to be Norepinephrine, Serotonin and GABA [5] .

GABA is one of the most widely distributed neurotransmitters in the mammalian brain, as it is expressed in about 30% of all synapse [20]. GABA is an inhibitory transmitter and therefore reduced the firing rate of excitatory neurons with which it is in contact. In various animal models of anxiety, the facilitation of GABAergic activity is associated with a reduction in anxiety.

Noradrenaline is the neurotransmitter most closely associated with peripheral and central stress response. Drugs that stimulate alpha$_2$ receptors such as clonidine diminishes the anxiety state by reducing the release of noradrenaline. Patients with panic disorders have increased sensitivity to challenge with isoproterenol because of increased peripheral beta receptor sensitivity [10].

Several experimental studies have suggested that a reduction in serotonin in the brain results in anxiolysis [10]. Serotonin pathway

originating in the dorsal raphe nucleus and innervating the amygdala and frontal cortex facilitate avoidance behaviour in response to distal threat. This pathway involves $5HT_{2A/2C}$ and $5HT_3$ post synaptic receptors and may be relevant to Generalised anxiety disorder. A separate pathway from the dorsal raphe nucleus and innervating the periventricular and periaqueductal grey region inhibit inborn fight or flight reactions in $5HT_{1A}$ receptors and may be relevant to panic attacks. With chronic stress, the serotonin pathway connecting the median raphe nucleus to the hippocampus, likely mediated by postsynaptic $5HT_{1A}$ receptor may be relevant to avoiding and numbing found in post traumatic stress disorders [17].

Several neuropeptides have been shown to play a role in anxiety but so far none has been developed as a drug largely because of their poor pharmacokinetic properties and difficulty in penetrating the blood brain barrier.

Angiotensin peptides – Angiotensin Converting Enzyme inhibitors like captopril, has anxiolytic activity in both experimental and clinical studies. It has recently been shown that the angiotensin 1

receptor antagonist, Losartan has anxiolytic properties whereas the angiotensin 2 antagonists are inactive.

Cholecystokinin ligands – agonists of the central cholecystokinin receptors cause anxiety and precipitate panic attacks in predisposed individuals. Two types of Cholecystokinin receptors have been identified, CCK-A and CCK-B , both of which occur in mammalian brain. CCK-B agonists initiate anxiety while the antagonists are anxiolytic in both experimental and clinical situations [10].

Neurokinin receptor ligands – There are two types of Neurokinin receptors in the brain namely NK1 and NK2. NK 2 agonists have been found to be anxiogenic while the antagonists are anxiolytic at least in animal studies. Some NK 1 antagonists have also been shown to be anxiolytic in experimental studies.

Corticotrophin releasing factor ligands – alpha helical CRF has been shown to block the anxiogenic effects of alcohol withdrawal in rats. It is possible that CRF interacts with neuropeptide Y receptors; NPY1 receptor agonists to have anticonflict effects in animal studies.

NMDA – glutamate ligands , the glycine antagonist- HA-966 have anxiolytic effects in animal studies.

Adenosine receptor ligands – the adenosine receptor antagonist, caffeine, induces anxiety in both animals and humans while agonists have anxiolytic effects [10].

The results of studies investigating neuroactive steroid levels in patients with anxiety disorders are conflicting [21]. Brain-derived pregnane steroids can potently and specifically enhance $GABA_A$ receptor functions . In addition, further studies are needed to determine the precise role of neuroactive steroids in the treatment of anxiety symptoms; pharmacological agents used to treat the symptoms of anxiety disorders often alter brain steroid levels, and understanding the role of these changes in steroid levels in the future lead to more specific and effective drug treatments [21].

Several lines of investigators support the involvement of the opioid receptor system in the regulation of anxiety. The most compelling evidence for the involvement of the delta opioid receptor system in anxiety comes from a study on delta opioid receptor knockout mice. Specifically, delta opioid receptor deficient mice exhibit anxiogenic – like phenotype. The modulation of anxiety-like behavior by delta opioid receptor agonists may prove to be a useful

clinical alternative to treat anxiety disorders that are resistant to typical anxiolytics [22].

Bombesin (BB), an amphibian peptide and its mammalian counterparts [various forms of neuromedin B(NMB)] and Gastrin Releasing Peptide(GRP),elicit their effects through various BB receptor subtypes. Neuromedin B binds preferentially to BB 1 subtype and GRP binds to BB2 receptor. BB and NMB increased the firing rate of serotonin cells in the dorsal raphe nucleus. Because reduced Serotonin release has been linked to reduced anxiety. Antagonists of the excitatory actions of BB like peptides on dorsal raphe nucleus serotonin neurons might be expected to decrease anxiety [23].

Sleep disorders:

The sleep disorders are categorized into primary disorders (i.e. Dyssomnias and Parasomnias), those related to another mental disorder or to a general medical disorder, and those that are substance induced.

Primary Insomnia Disorders:

Primary insomnia is characterized by difficulty in initiating or maintaining sleep or by not feeling rested after an apparently adequate amount of sleep for at least 1 month. It is characterized by excessive

daytime worry about being able to fall or stay asleep. Anxiety tends to perpetuate a vicious cycle of sleeplessness that is aggravated by worry about sleeplessness [6]. primary insomnia disorders includes dyssomnias and parasomnias. The dyssomnias may occur due to intrinsic sleep disorders, extrinsic sleep disorders and disturbance of the circadian rhythm.

(i) Intrinsic sleep disorders:

(a) Psychogenic insomnia:

psychogenic insomnia is characterized by increased mental tension (inability to relax, anxiety, brooding) and excessive concern about sleep itself. Sleep often improves in a new environment (e.g. on vacation).

(b) Pseudo insomnia :

pseudo insomnia is a subjective feeling of disturbed sleep in the absence of objective evidence (i.e. normal polysomnography).

(c) Restless legs syndrome:

Restless legs syndrome is characterized by ascending abnormal sensations in the legs when they are at rest (e.g. when the patient watches television, or before falling asleep) accompanied by an

urge to move the legs. It is sometimes present as a genetic disorder with autosomal dominant inheritance. Periodic leg movements during sleep are repeated, abrupt twitching movements of the legs that may persist for minutes to hours.

(d) Narcolepsy:

Daytime somnolence and frequent, sudden, uncontrollable episodes of sleep (imperative sleep), which tend to occur in restful situations (e.g. reading, hearing a lecture, watching television, long automobile ride. It may be associated with cataplexy (sudden, episodic loss of muscle tone without unconsciousness), sleep paralysis (inability to move or speak when awaking from sleep) and hypnagogic hallucinations (visual or acoustic hallucinations while falling asleep) [6].

(e) Obstructive sleep apnea:

Obstructive sleep apnea is characterized by daytime somnolence with frequent nocturnal respiratory pauses and loud snoring. Impaired concentration, decreased performance and headaches are also common.

(ii) Extrinsic sleep disorders:

Sleep may be disturbed by external factors such as noise, light, mental stress and medication use.

(iii) Disturbance of the circadian rhythm:

Sleep may be disturbed by shift work at night or by intercontinental travel (jet lag) [6].

(iv) Parasomnias:

These disorders include confusion on awakening (sleep drunkenness), sleepwalking (somnambulism), nightmares, Sleep myoclonus, bedwetting (enuresis), and nocturnal grinding of the teeth (bruxism).

Secondary Sleep Disorders:

(i)Psychogenic Sleep Disorders:

Depression (of various types) can impair sleep, though paradoxically sleep deprivations can ameliorate depression. Depressed persons typically complain of early morning awakening, nocturnal restlessness, and difficulty in starting the day. Sleep disturbances are also common in patients suffering from psychosis, mania, anxiety disorders, and alcoholism and drug abuse.

(ii) Neurogenic sleep disorders:

Sleep can be impaired by dementia, parkinson disease, dystonia, respiratory disturbances secondary to neuromuscular disease (muscular dystrophy, acute coronary syndrome), epilepsy(nocturnal attacks), and headache syndromes (cluster headaches, migraine). Fatal familial insomnia is a genetic disorder of autosomal dominant inheritance.

(iii) Sleep disorders due to systemic disease:

Sleep can be impaired by pulmonary diseases (asthma, COPD, angina pectoris, nocturia, fibromyalgia and chronic fatigue syndrome) [24].

Pathophysiology of sleep disorders: [25]

The physiological mechanisms regulating the sleep-wake rhythm are not completely known. There is evidence that histaminergic, cholinergic, glutamatergic, and adrenergic neurons are more active during waking than during the NREM sleep stage. Via their ascending thalamopetal projections, these neurons excite thalamocortical pathways and inhibit GABA-ergic neurons. During sleep, input from the brainstem decreases, giving rise to diminished thalamocortical activity and disinhibition of the GABA neurons. The shift in balance

between excitatory and inhibitory neuron groups underlies a circadian change in sleep propensity, causing it to remain low in the morning, to increase towards early afternoon (middle siesta), then to decline again, and finally to reach its peak before midnight.

As the margin between excitatory and inhibitory activity decreases with age, there is an increasing tendency towards shortened daytime sleep periods and more frequent interruption of nocturnal sleep. Imbalance between the excitatory and inhibitory neurotransmission with more shift toward excitatory system underlies many of the sleeping disorders.

Pharmacotherapy of Anxiety Disorders and Sleep Disorders:

After decades of neglect and controversy, anxiety disorders are increasingly recognized as legitimate medical conditions requiring specific treatment. The unproductive debate over the primary biological or psychological factors in the pathophysiology of anxiety is gradually being replaced by a pragmatic approach based on research on the relative contributions of both [17.]

A **Sedative** is a drug that produces a relaxing, calming effect.

A **Hypnotic** is a drug that induces sleep similar to normal arousable sleep and is also called as **Soporifics** [26.]

History of anxiolytics drugs: [17]

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 nonbarbiturate nonbenzodiazepine hypnotic drugs in the 1930s namely meprobamate , tybamate, methaqualone , methyprylone and glutethimide did not address any of the deficiencies of the barbiturates, and in many cases these drugs proved more problematic, possess very low therapeutic index, are highly addicting and can be fatal in overdose, and glutethimide overdose can result in convulsion and fluctuating coma. The synthesis in 1957 of the first benzodiazepine, chlordiazepoxide, 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 and a tetra cyclic drugs and MAOIs quickly became the first-line treatment choice in panic disorder [17].

Of the many subsequently introduced antidepressants with anxiolytics 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, corticotrophin-releasing factor antagonists, neuropeptide Y agonists and serotonergic agents acting on specific serotonin receptor subtypes agonists, 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 [17].

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).

Immediate anxiolytics	Delayed anxiolytics
Benzodiazepines	Tricyclic antidepressants
Barbituratates	MAOIs
Alcohols (Chlormethiazole)	SSRIs / SNRIs
Beta blockers	all psychotherapies [4]

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 [27].

Classification of Anxiolytics and Hypnotic drugs [28,29,30]

(i) Benzodiazepines :

Long acting (half life >24 hr)	Ultra short acting (half life <5hr)
Chlordiazepoxide	Triazolam
Diazepam	Midazolam
Chlorazepate	
Prazepam	
Medazepam	

short acting (half life 5-24 hr)

Nitrazepam

Flunitrazepam

Estazolam

Bromazepam

Alprazolam

Lorazepam

(ii) Non benzodiazepines :

Imidazolopyrimidines : Zolpidem, Alpidem, Saripidem

Pyrazolopyrimidines : Zaleplon, Indiplon, Ocinaplon

Cyclopyrrolones : Eszopiclone, Zopiclone, Pagoclone, Suriclone , Pazinaclone, Suproclone

(iii Diphenylmethane : Hydroxyzine

Captodiame

(iv) Azaspirodeconediones : Buspirone , Gepirone

Ipsapirone, Tandospirone

(V) Barbiturates :

Long acting	Short acting	ultra short acting
Phenobarbital	Pentobarbital	Thiopental
Mephobarbital	Secobarbital	Methohexital
	Amobarbital	

(vi) Beta adrenoceptor antagonist : Propranolol

(vii) Carbamates : Ethylcamate , Meprobamate , Carisoprodol , Phenprobamate , Mebutamate , Tybamate

(viii) Beta Carbolines : Abecarnil , Gedocarnil

(ix) Dibenzo – bicycle – octadiene : Benzoctamine

(x) MonoAmine Oxidase Inhibitors : Phenelzine

(xi) Others : Mephenoxalone

Benzodiazepines:

The benzodiazepines are the most frequently prescribed drugs for treating anxiety [31]. Schmidt et at (1967) were the first to observe the potentiation by diazepam of presynaptic inhibition in the cat Spinal cord [32]. The Benzodiazepines bind with high affinity to the benzodiazepine receptor and, as a result, change the structural

conformation of the GABA receptor so that the action of GABA on its receptor is enhanced [10]. Benzodiazepines bind to the $GABA_A$ receptor at the α1, α2, α3, α5 sites [31]. Benzodiazepine with high activity at α1 is associated with sedation, whereas those with higher affinity for $GABA_A$ receptors containing α2 and / or α3 subunits have good antianxiety activity [30]. When benzodiazepine bind to the $GABA_A$ receptor, the frequency of the chloride ion channels opening and the influx of chloride ions into the neuronal cell are increased. The resulting negatively charged hyperpolarized membrane prevents further depolarization by excitatory neurotransmitters [31]. Benzodiazepine also affects other monoamines, like serotonin, noradrenaline, dopamine and acetylcholine [33].

The sedative action of benzodiazepines has two aspects: the normalization of excessive alertness and responsiveness to normal stimuli and the damping of normal responses to excessive stressful factors result in the therapeutically desired calming effect, while depression of a normal behavioral responsiveness in physiological conditions is the basis of sedative side effects [32.]

In psychiatric disorders, the benzodiazepines are usually given orally; in controlled medical environments (e.g., the ICU), where the rapid onset of respiratory depression can be assessed, they are often given intravenously.

The longer acting benzodiazepine(e.g diazepam,chlordiazepoxide) are used for the treatment of alcohol withdrawal and anxiety symptoms; the intermediate drugs are useful as sedatives for insomnia (e.g., lorazepam), while short-acting agents (e.g., midazolam) are used for medical procedures such as endoscopy [13].

Drug Interactions: [34]

Antacids - decrease absorption of benzodiazepines

Cimetidine – increase half lives of diazepam and triazolam

Contraceptives – increase serum levels of diazepam and triazolam

Disulfiram – increase duration of action of benzodiazepines

Isoniazid – increase plasma levels of diazepam

Propoxyphene – impaired clearance of diazepam

Adverse effects:

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 if used for a longer period of time. On sudden discontinuation, benzodiazepine withdrawal can occur characterized by insomnia, nausea and vomiting, twitching, irritability, anxiety, paresthesias, tinnitus, delirium and seizures [27].

Contra indications:

Acute narrow angle glaucoma (Diazepam emulsion injection)

Hypersensitivity to Soy protein [35]

Non Benzodiazepines : [29]

Non benzodiazepines act on BZ_1 receptor only. Hence, has anxiolytic, sedative and hypnotic actions but devoid of muscle relaxant, amnesic and anticonvulsant actions. Non benzodiazepines are mainly used as hypnotics and they have faster onset of action with shorter duration. Another advantage of them as hypnotics includes minimal alteration of REM sleep pattern and also minimal daytime sedation.

Zolpidem and Zaleplon are used to treat transient insomnia while Zopiclone is used to treat short term insomnia.

Azaspirodecanediones :

These drugs act through non-GABA ergic system and have low propensity to side effects compared to benzodiazepines. These drugs exert anxiolytic effects by acting as a partial agonist primarily at brain 5-HT_{1A} receptors but also on brain dopamine D_2 receptors. Hence by selective activation of the inhibitory pre-synaptic 5-HT_{1A} receptor, they suppress 5-HT neurotransmission through neuronal system. Buspirone is used in anxiety states except in Panic disorder [29]. But the onset of anxiolytic action takes 2-4 weeks. Effects are mainly for the cognitive rather than the behavioral manifestations of anxiety. Patients who have responded well to benzodiazepines may not respond well to buspirone. Buspirone has a relatively low efficacy in long-term treatment [10].

Beta blockers: [29]

Propranolol helps to suppress such performance anxiety by breaking this vicious cycle. Propranolol through its beta blocking action decreases palpitation, tremors, GIT upset, hypertension and blood lactic acid levels.

Initial management of the anxiety disorder begins with providing the patient with a clear understanding of the problem. Appropriate treatment may also reduce substance abuse among patients who self-medicate with alcohol, benzodiazepine, or other substances in an effort to ameliorate their symptoms [2].

Treatment of Generalized Anxiety Disorder :

Benzodiazepines are the most effective, safe, and commonly prescribed drugs for the rapid relief of acute anxiety symptoms. They are also used intermittently or adjunctively for acute Generalized Anxiety Disorder exacerbations and for sleep disturbances at the outset of antidepressant therapy [31]. Since clonazepam and diazepam have long half-lives, they are less likely to result in interdose anxiety and are easier to taper [15].

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. Imipramine is considered when patients fail to respond to SSRIs or Venlafaxine [31]. At the initiation of treatment, antidepressants can themselves be anxiogenic- thus, an initial dose, in conjunction with short-term treatment with a benzodiazepine, is often indicated [13]. Venlafaxine (dosed once daily) is effective at doses of 150 mg and 225 mg for treating a patient with GAD.

Treatment of Panic Disorder :

Benzodiazepines are the most commonly used drug for panic disorder especially in instances in which rapid response is required. Alprazolam is dosed between 4 – 10 mg and Clonazepam 1- 4 mg. Among SSRIs, Fluoxetine is begun at 5 mg/day with dosage increase every 2or3 days to a dosage range of 10-20 mg/day. The most refractory or difficult patients of PD are started Phenelzine at 15 mg/day after the evening meal, increasing by 15 mg/day every 3 to 4 days until a minimum dose of 45 mg/day is reached [31]. 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 [13].

Treatment of Phobic Disorders:

Global social phobias may be treated with SSRIs, such as Paroxetine, Sertraline and Fluvoxamine, MAOI 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.

Treatment of Obsessive-Compulsive Disorder:

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 [13].

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 [16].

Treatment of Post Traumatic Stress Disorder:

Antidepressants, such as SSRIs, are the first line treatment for patients with PTSD. Up to 6-8 weeks may be needed for patients to

notice a therapeutic benefit. If patients show no improvement in symptoms with SSRIs, other medications should be considered. Augmentation should be considered for patients who respond partially to SSRIs. TCAs and MAOIs also may be beneficial. Benzodiazepines may be useful in reducing anxiety and improving sleep. Atypical antipsychotics may be efficacious as adjunctive treatment. Patients with aggressiveness, impulsiveness or lability as prominent symptoms of PTSD may benefit from a mood-stablilizing anticonvulsant [8].

Treatment of anxiety secondary to medical conditions : [14]

Treating anxiety secondary to medical conditions always be directed at the underlying medical conditions. The anxiety associated with angina, is best treated with nitroglycerin. Low doses of antipsychotic agents (e.g.: Risperidone, Haloperidol) may be beneficial in some patients who show anxiety and agitations associated with delirium or dementia. Hypoxic states that produce anxiety or agitation are best treated with oxygen, rather than with sedative-hypnotics, anxiolytic or antipsychotic agents, some of which could actually produce a further degree of respiratory depression.

Non pharmacological therapies of Anxiety Disorder: [2, 13, 16, 36]

(1) Behavioral therapy :

Techniques like muscle relaxation, control of breathing and diaphragmatic breathing, communication skills, guided self-dialogue, and Stress Inoculation Training.

(2) Psychodynamic therapy

(3) Cognitive therapy

(4) Bibliotherapy

Treatment of insomnia: [31]

Management of insomnia is initially based on whether the individual has experienced a short-term, transient, or chronic sleep disturbance. Transient insomnia resolves quickly and should be treated with good sleep hygiene and careful use of sedative hypnotics.

For treating short term insomnia that is lasting up to 3 weeks, non pharmacologic treatment is important and if sedative hypnotics are used, care must be taken to prevent the development of tolerance or dependence. Chronic insomnia requires careful assessment for the medical reason for the insomnia, as well as

nonpharmacologic techniques and careful and less frequent use of sedative-hypnotics to prevent tolerance and dependence.

Non pharmacologic therapy:

- Stimulus control therapy
- Sleep restriction
- Relaxation therapy
- Cognitive therapy
- Paradoxical intention
- Sleep hygiene

Pharmacologic therapy:[30]

Benzodiazepines:

The most commonly used treatment for insomnia has been the benzodiazepines. Benzodiazepines reduce sleep latency and increase total sleep time. Benzodiazepines increase stage 2 sleep while decrease REM, stage 3, stage 4 sleep. As REM sleep is interfered with, increased incidence of rebound insomnia and night mares occurs. Prolonged sedation and cognitive and psychomotor impairment are concerns in the elderly. There is an association between falls and hip fractures and use of benzodiazepines with long

elimination half-lives. Drug dependency and abuse may pose a problem if used for a longer period. Commonly used benzodiazepines as hypnotic agents include Estazolam, Flurazepam Quazepam, Temazepam, Triazolam, Nitrazepam, Alprazolam.

Zolpidem :

Zolpidem is a selective GABA Benzodiazepines – 1 receptor agonist. It reduces sleep latency, nocturnal awakenings increases total sleep time and does not appear to have significant effects on next-day psychomotor performance. Treatment is initiated with 5 mg and can be increased to 10 mg as a daily dose and optimally should not exceed 4 weeks to minimize tolerance and dependence.

Zaleplon:[30]

Zaleplon has rapid onset of action, short half-life of 1 hour. Effective in decreasing time of onset to sleep onset but not for reducing nighttime awakening or for increasing the total sleep time. It does not interfere with stages of sleep and so rebound insomnia and nightmares are in fewer incidences when compared to Benzodiazepines.

Antihistamines:

Antihistamines are effective in the treatment of mild insomnia and are generally safe. diphenhydramine and doxylamine are preferred agents.

Amino acid L-Tryptophan:

Tryptophan is a precursor of serotonin and was once a popular natural sedative. Cases of Eosinophilia-myalgia syndrome removed this product from the market.

Antidepressants:[30]

Antidepressants are alternatives for patient with non restorative sleep who should not receive benzodiazepines, especially those who have depression, pain or a risk of substance abuse. Sedative antidepressants such as amitriptyline, doxepin and nortriptyline are effective for inducing sleep continuity. Trazodone is used for insomnia patients who are prone to substance abuse. It is frequently used in patients with SSRIs and bupropion-induced insomnia in doses of 25 to 75 mg.

Melatonin:

Melatonin is a hormone released by the pineal gland during the night. It is promoted as a sleep aid. Most studies with melatonin are in children with neurological impairment and in individuals with jet lag. Ramelteon was designed to be a chemical mimic of the endogenous hormone melatonin and is more potent than melatonin and was recently approved for treatment of insomnia characterized by difficulty with sleep onset. It has the distinction of being the only hypnotic prescription that is not a controlled substance .

Valerian:

Valerian is a herbal sleep remedy. Its mechanism may involve inhibition of an enzyme that breaks down GABA.

Novel approaches in the treatment of Anxiety Disorder: [26, 37, 38, 39, 40]

(i) Milnacipran – inhibitor of neuronal transport of one or more monoamines, including nor epinephrine or dopamine, as well as serotonin.

(ii) Sunepitron – serotonin agonists

(iii) MEM-1414 – inhibitor of Phosphodiesterase 4

(iv) Ampalcines – glutamate AMPA receptor modulators.

(v) Ocinaplon, Pagoclone – $GABA_A$ receptor agonists

(vi) CP-122721, GB-823296 – inhibitor of Neurokinin-1 receptors

(vii) AG-561,AVE-4579,DPC-368 – CRF-1 receptor antagonists

(viii) MK-801 – NMDA receptor antagonist

(ix) Aniracetam

(x) Neuroactive steroids – neuroactive steroids are molecules based on a steroid chemical structure, which interact with the GABA- benzodiazepine receptor complex. As some of them are naturally occurring, it is hoped that analogues may be effective anxiolytic, with perhaps more "natural" actions that the marketed Benzodiazepines anxiolytic. Such agents are in early development.

(xi) Flesinoxan : highly selective 5-HT1A receptor agonist. It is under clinical development for GAD.

Role of Natural herbal treatment in anxiety disorders:[41]

Plants have played a significant role in maintaining human health and improving the quality of human life for thousands of years and have served humans well as valuable components of

medicines, seasonings, beverages, cosmetics and dyes. Herbal medicine is based on the premise that plants contain natural substances that can promote health and alleviate illness. Herbal drugs or medicinal plants, their extracts and their isolated compounds have demonstrated spectrum of biological activities. Such have been used and continued to be used as medicine in folklore or food supplement for various disorders. Ethnopharmacological studies on such herbs or medicinally important plants continue to interest investigators throughout the world [41].

Among the **herbal remedies for anxiety disorders**, notable are Passion flower, Valerian, Chamomile, Hops, Gingko Biloba, Siberian Ginseng [42].

Morinda citrifolia is one of the important traditional folk medicinal plants that have been used for over 2000 years in Polynesia. It has been reported to have a broad range of therapeutic and nutritional value. The ancestors of Polynesians are believed to have brought many plants with them, as they migrated from Southeast Asia 2000 years ago. Of the 12 most common plants they

brought, Morinda citrifolia was the second most popular plant used in herbal remedies to treat various common diseases and to maintain overall good health.

Morinda citrifolia is also called as Indian Mulberry. It is also known in different names locally as Cheese Fruit, Forbidden Fruit, Headache Tree, Hog Apple, Mona, Mora de la India, Nino, Nona, Nono, Nonu, Nuna, Pain Bush, Pain Killer Tree, Pinuela, Wild Pine, etc. in various parts of the world.

Morinda citrifolia is an evergreen tree found growing in open coastal regions at sea level and in forest areas up to about 1300 feet above sea level. It is often found growing along lava flows. Morinda is identifiable by its straight trunk, large, bright green and elliptical leaves, white tubular flowers and its distinctive, ovoid, "grenade-like" yellow fruit. The fruit can grow in size up to 12 cm or more and has a lumpy surface covered by polygonal-shaped sections. The seeds, which are triangular shaped and reddish brown, have an air sac attached at one end, which makes the seeds buoyant. The mature Morinda citrifolia fruit has a foul taste and odour [43].

Chemical properties of Morinda citrifolia fruit:

According to bio scientific investigations of Morinda citrifolia fruit conducted over the past fifty years, constituents found in ripe Morinda citrifolia fruit demonstrate a plethora of biological activities. The following is a partial list of the phyto chemical constituents in ripe Morinda citrifolia fruit, and some of their biological activities.

1- Hexanol – antiseptic
2- Acetic acid – bactericide, fungicide
3- Asperuloside – anti inflammatory, laxative
4- Aucubin - antioxidant, bactericide, laxative
5- Benzoic acid – antiseptic, bactericide, fungicide
6- Benzyl alcohol – anesthetic, antiseptic
7- Caprylic acid – candidacide, fungicide cathartic
8- Eugenol – analgesic, anesthetic, anti inflammatory, antiseptic, cancer-preventive
9- Glucuronic acid – detoxicant
10. Limonene – anticancer, antitumor, hypercholesterolemic

11. Linoleic acid – anti arteriosclerotic, cancer-preventive, hepatoprotective

12. Myristic acid – cancer-preventive

13. Noni-ppt – anti tumor, immunomodulatory

14. Oleic acid – cancer-preventive

15. Palmitic acid – anti fibrinolytic

16. Scopoletin – analgesic,anti edemic,anti inflammatory [45]

Morinda citrifolia is typically taken to enhance bodily function and support overall good health and is widely known as the "Aspirin of the Ancient" [45]. The fruit juice is in high demand in alternative medicine for different kinds of illnesses such as arthritis, diabetes, high blood pressure, muscle aches and pains, menstrual difficulties, headaches, heart disease, AIDS, cancers, gastric ulcer, sprains, mental depression, senility, poor digestion, arteriosclerosis, blood vessel problems, and drug addiction. Morinda citrifolia fruit was a traditional remedy used to treat broken bones, deep cuts, bruises, sores and wounds . The safety of the Morinda citrifolia fruit juice is

evaluated in animals. The LD_{50}s of intraperitoneally injected aqueous extract of noni fruit was determined to be 7500 mg/kg body weight in mice [46].

Animal models for antianxiety effects:

1. Foot shock induced aggression
2. isolation – induced aggression
3. anti anxiety test in mice
4. anticipatory anxiety in mice
5. social interaction in rats
6. elevated plus maze test
7. Water maze
8. Staircase test
9. Cork gnawing test
10. Distress vocalization in rat pups
11. Light-dark model
12. Schedule induced polydipsia in rats
13. Four plate test in mice
14. Foot shock induced freezing behavior in rats
15. Acoustic startle response in rats

16. Unconditioned conflict procedure
17. Conditioned behavioral response
18. Sidman avoidance paradigm
19. Geller conflict paradigm
20. Conditioned defensive burying in rats
21. Plasma catecholamine level measurements during and after stress.

Animal models for evaluation of Hypnotic activity:

1. Potentiation of hexobarbital sleeping time
2. Experimental insomnia in rats
3. EEG registration in conscious cats
4. Prolongation of Ketamine induced sleeping time

Animal models for evaluation of Sedative activity:

1. Open field test
2. Hole-board test
3. Combined open field test
4. EEG analysis from rat brain by telemetry.
5. Method of intermittent observations

6. Spontaneous loco motor activity testing with Photoactometer.

Experimental results with Morinda citrifolia fruit indicate the presence of competitive ligands, which may bind to the $GABA_A$ receptor as an agonist, and thus induce its anxiolytic and sedative effects [47]. Hence, in present study evaluation of the anxiolytic, sedative and hypnotic properties of Morinda citrifolia fruit extract which are inherent property of a compound that has got $GABA_A$ agonistic property is done.

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 seven months from February 2008 to September 2008, 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. Fifty four male albino mice each weighing 18 to 25 grams were included in the study. Animals were allowed standard diet-(Pellet feed from Hindustan lever limited, Mumbai) and tap water ad libitum.

2. Standard drug

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

3. **Ketamine:**

Injection Ketamine is mixed with distilled water to obtain a solution of concentration 10mg/ml and is administered intraperitoneally at the dose of 100mg/kg.

4. **Test Drug:**

The aqueous extract of Morinda citrifolia fruit extract is mixed with distilled water to obtain a solution of concentration 50mg/ml and is administered intraperitoneally at the dose of 500mg/kg. The Morinda citrifolia fruit was dried at room temperature for a period of 7 days and then powdered. Then aqueous extract of the fruit powder is prepared.

5. Distilled water :

The distilled water is used as vehicle for control group of animals.

6. Photoactometer :

The Digital photoactometer is designed to study the spontaneous or induced 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) Isolation induced aggression:

Male mice with an initial weight of 12 gram were kept isolated in small cages made of polypropylene of dimensions 290 x 220 x 140 mm for a period of 6 weeks. Prior to the administration of the test drug, the aggressive behavior of the animals was tested. A male mouse being accustomed to live together with other animals was placed into the cage of an isolated mouse for 5 minutes. Immediately, the isolated mouse started to attack the "intruder". The aggressive behavior of the isolated mouse was characterized by hitting the tail on the bottom of the cage, screaming and biting. The reaction time for any of these aggressive behaviors was calculated. After these initial tests, control group of isolated mice received distilled water i.p, standard group of

mice received inj. Diazepam 1 mg/kg i.p and test group of isolated mice received aqueous extract of Morinda citrifolia 500 mg/kg i.p..

Control group	Distilled water i.p
Standard group	Inj. Diazepam 1mg/kg i.p
Test group	Aqueous extract of Morinda citrifolia fruit 500mg/kg i.p

After 30 min of administration of compounds, reaction time for the development of aggression in isolated mice after the placement of intruder was noted in each group. The number of animals with complete suppression of the fighting behavior was calculated. The results were tabulated and analysed statistically.

(II) Testing of Spontaneous Locomotor Activity (SMA) using Photoactometer:

18 male mice of 18 g – 25 g in weight were grouped into three with six animals in each. The total number of counts made by each animal in the Photoactometer for a period of 10 min was calculated. The control group of mice received distilled water i.p, standard group of mice received inj. Diazepam 1 mg/kg i.p and the test

group of mice received aqueous extract of Morinda citrifolia fruit 500 mg/kg i.p.

Control group	Distilled water i.p
Standard group	Inj. Diazepam 1mg/kg i.p
Test group	Aqueous extract of Morinda citrifolia fruit 500mg/kg i.p

After 30 min and 60 min of drug administration, SMA for each animal for a period of 10 min was calculated and the observations were tabulated and analyzed statistically by using unpaired Students "t" test.

(III) Testing of Hypnotic activity by Prolongation of Ketamine induced sleeping time:

18 male albino mice were divided into 3 groups of 6 animals in each. Prior to 30 minutes of administration of Ketamine at 100 mg/kg i.p, the control group of animals were pretreated with distilled water i.p, standard group of animals were pretreated with Inj. Diazepam 1 mg/kg i.p and test group of animals were pretreated with aqueous extract of Morinda citrifolia fruit extract of 500 mg/kg.

Control group	Distilled water i.p + Inj.Ketamine 100mg/kg
Standard group	Inj. Diazepam 1mg/kg + Inj.Ketamine 100mg/kg i.p
Test group	Aqueous extract of Morinda citrifolia fruit 500mg/kg + Inj.Ketamine 100mg/kg i.p

The time at which the righting reflex is lost was taken as onset of anesthesia and the duration between the time at which the righting reflex is lost and is regained was taken as duration of anesthesia. The onset and duration of anesthesia were compared between test, control and standard groups. The results were tabulated and analyzed statistically by using unpaired Students “t” test.

RESULTS

In the present study, 54 male albino mice were selected and were evaluated for Anti anxiety, Sedative and Hypnotic activities. Anti anxiety effect was evaluated by isolation induced aggression method, Sedative effect was evaluated by Spontaneous Loco motor Activity in Actophotometer and Hypnotic effect by Prolongation of Ketamine induced sleeping time by selecting 18 animals in each group.

Anti anxiety effect:

18 male albino mice isolated for a period of 6 weeks were assessed for the development of aggression. The reaction time for the development of aggression in an isolated male mouse was noted in conrol, standard and test group of animals after introducing another male mouse which had stayed with its counterparts. Anti anxiety effect was assessed by complete suppression of reaction time and the total number of animals that have shown complete suppression was calculated in each groups and compared. In the control group of mice, in all the 6 animals that had shown aggression before the administration of distilled water none of them showed the inhibition of aggression

after the administration. In the Standard group of animals, all the 6 animals that had aggression before the administration of Diazepam, showed inhibition of aggression after the drug administration that accounts for 100% of anxiolytic activity. In the test group of animals 5 out of 6 had shown inhibition of aggression after the administration of test compound that accounts for 83% of anxiolytic activity. The results were tabulated in table I.

Sedative effect:

Sedative activity is evaluated by using Actophotometer. The spontaneous locomotor activity made by a mouse was noted in control, standard and test group before and 30 and 60 min after the administration of control, standard and test drugs. The average number of counts at 30 min and 60 min for control group of mice was 364.67 $\pm$ 10.74 and 209 $\pm$ 12.98. The average number of counts at 30 min and 60 min for standard group of mice was 123.16 $\pm$ 8.33 and 49 $\pm$ 5.78. The average number of counts at 30 min and 60 min for test group of mice was 196.67 $\pm$ 3.7 and 92 $\pm$ 2.5. The results were tabulated in table II and analysed using unpaired students “t” test. The sedative effect was statistically significant ($P < 0.001$) for both the test and the standard

groups after 30 min and 60 min of drug administration in comparison with control group.

Hypnotic effect:

Hypnotic activity is measured by prolongation of Ketamine induced sleeping time. The onset and duration of loss of righting reflex is compared between each group of animals. The average onset and duration in control group of mice is 4.01 $\pm$ 0.22 and 44.23$\pm$ 0.59 minutes respectively. The average onset and duration in standard group of mice is 1.23 $\pm$ 0.05 and 56.03 $\pm$ 1.34 minutes respectively. The average onset and duration in test group of mice is 2.23 $\pm$ 0.07 and 50.57 $\pm$ 0.36 respectively. The results are tabulated in Table III. On comparison with control group of mice, the hypnotic activity is statistically significant for standard and test group of mice with $P < 0.001$.

TABLE – I

COMPARISON OF PERCENTAGE OF INHIBITION OF AGGRESSION IN "ISOLATION INDUCED AGGRESSION" METHOD

GROUP	NUMBER OF ANIMALS WITH AGGRESSION BEFORE DRUG ADMINISTRATION	NUMBER OF ANIMALS WITH AGGRESSION AFTER DRUG ADMINISTRATION	PERCENTAGE OF INHIBITION OF AGGRESSION
CONTROL	6	6	0%
STANDARD	6	0	100%
TEST	6	1	83%

comparison of anxiolytic activity in isolation induced aggression

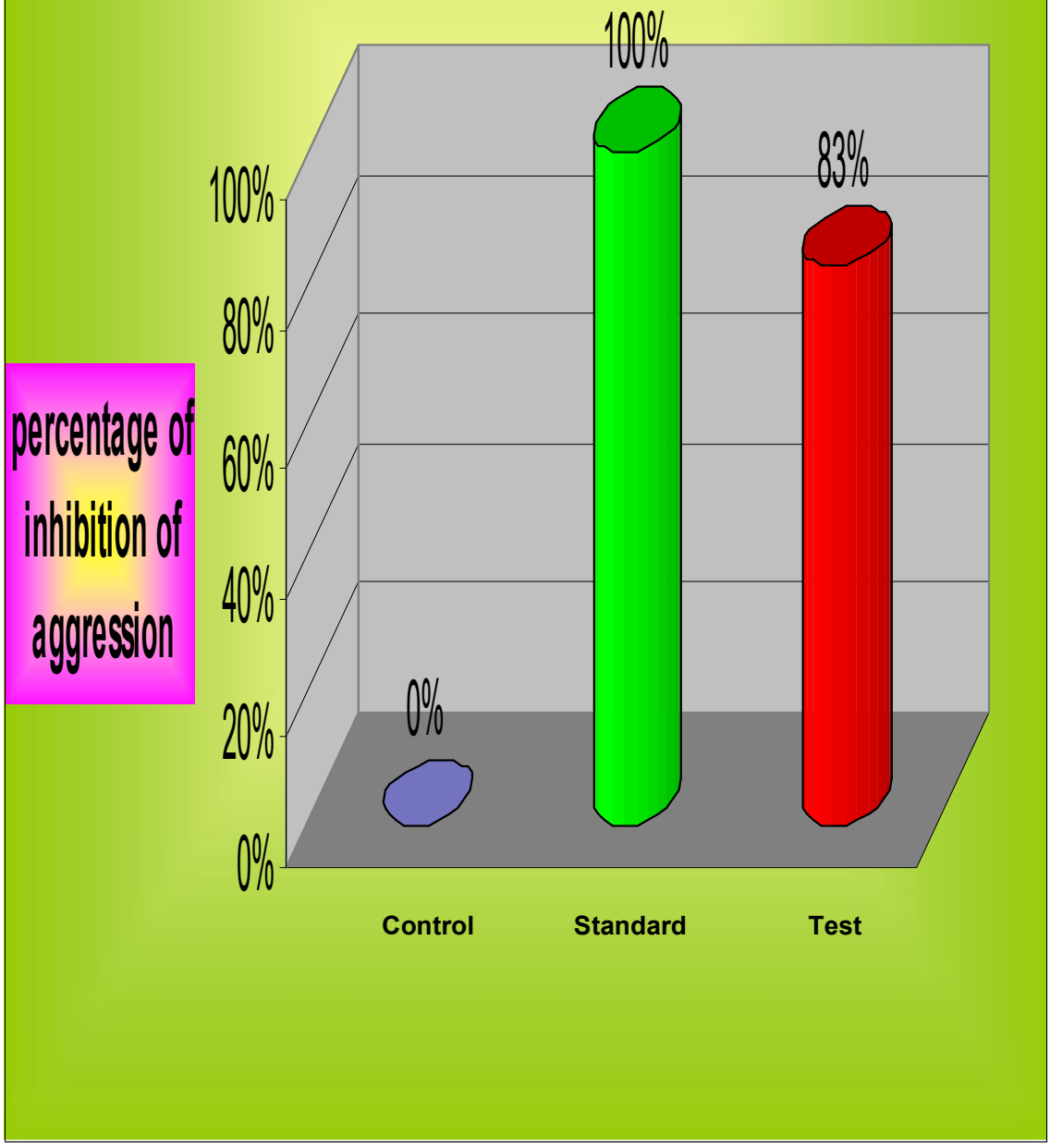


TABLE - II

COMPARISON OF TOTAL COUNTS IN 10 MIN IN ACTOPHOTOMETER

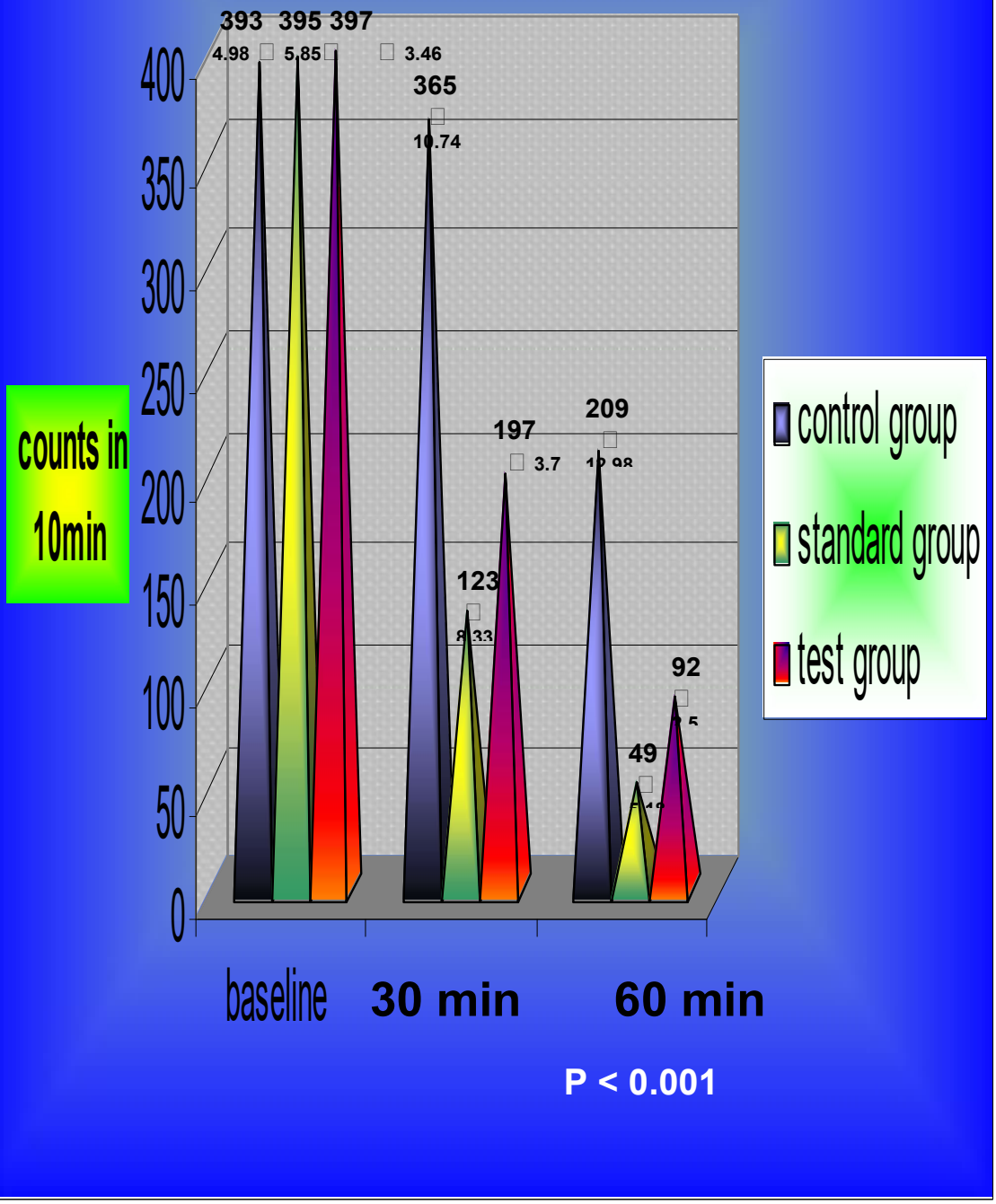
GROUP	BASELINE (MEAN± SD)	AFTER 30 MIN (MEAN± SD)	AFTER 60 MIN (MEAN± SD)
CONTROL	393 ± 4.98	364.67 ± 10.74	209 ± 12.98
STANDARD	395 ± 5.85	123.16 ± 8.33***	49 ± 5.18***
TEST	397 ± 3.46	196.67 ± 3.7***	92 ± 2.5 ***

*** P < 0.001

Control Vs Standard : t-value= 17.8 (P<0.001)

Conrol Vs Test : t-value = 11.5 (P<0.001)

spontaneous locomotor activity in actophotometer



P < 0.001

TABLE - III

KETAMINE INDUCED SLEEPING TIME PROLONGATION

GROUP	AVERAGE ONSET (MIN) (MEAN± SD)	MEAN DURATION (MIN) (MEAN± SD)
CONTROL	4.01 ± 0.22	44.23 ± 0.59
STANDARD	1.23 ± 0.05 ***	56.03 ± 1.34 ***
TEST	2.23 ± 0.07 ***	50.57 ± 0.30 ***

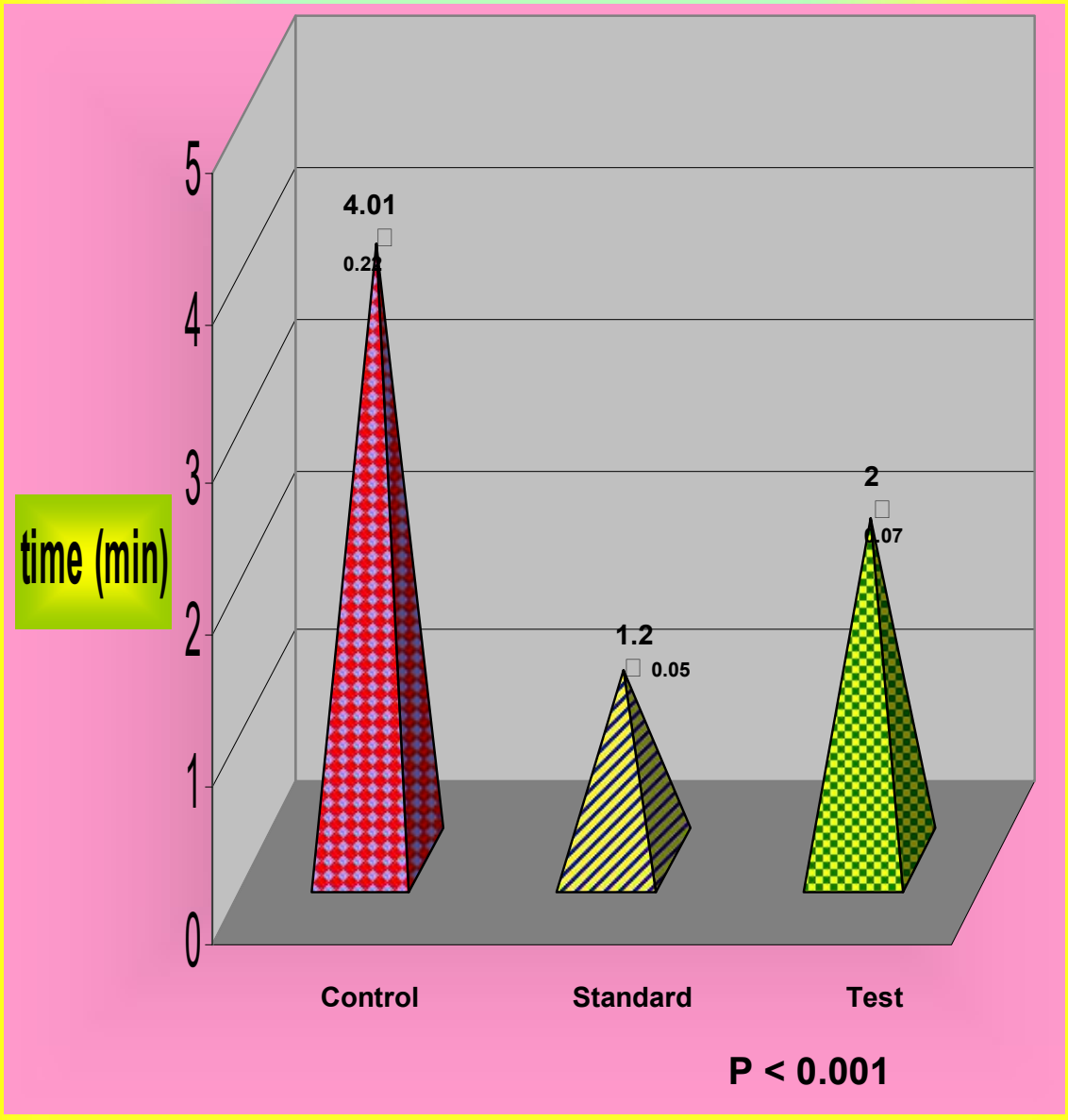
*** p < 0.001

onset : Control Vs Standard : t-value =12.9 (P<0.001)
Control Vs Test : t-value = 8.6 (P<0.001)

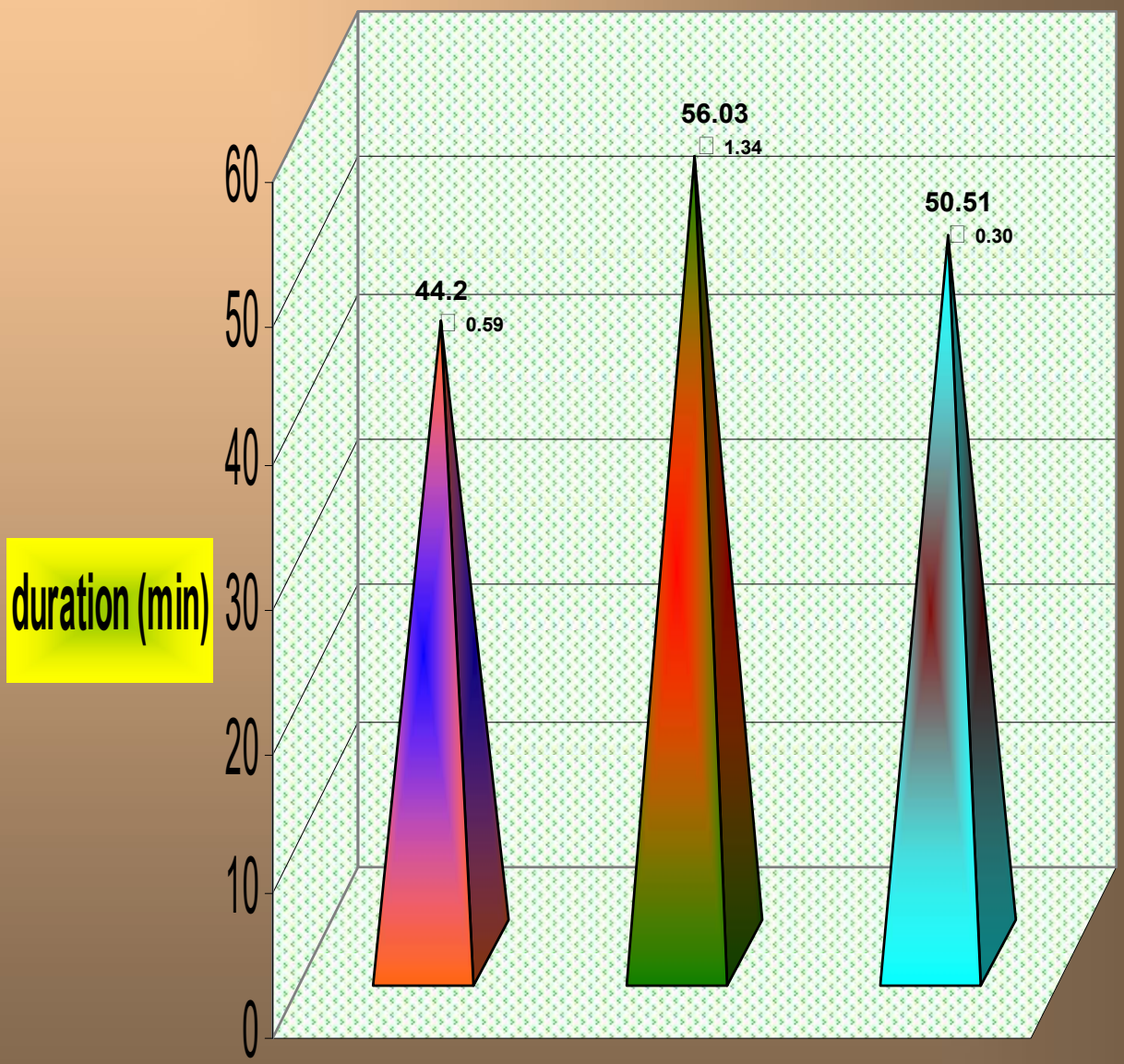
Duration :

Control Vs Standard: t-value =8.03 (P<0.001)
Control Vs Test : t-value = 9.6 (P<0.001)

comparison of onset of loss of righting reflex after ketamine administration



comparison of duration of anaesthesia with ketamine



P < 0.001

DISCUSSION

In the present study, antianxiety, sedative and hypnotic activities of the aqueous extract of Morinda citrifolia fruit extract were evaluated in comparison with Diazepam in 54 male albino mice. The anxiolytic activity was evaluated by " isolation induced aggression" method, where 18 male albino mice were isolated for a period of six weeks and aggression was induced by placing an intruder into the cage of the isolated mouse and the percentage of animals showing complete suppression of aggression was compared in control, standard(diazepam) and test groups of animals. The development of aggression was inhibited in 84% of animals in test group and 100% in standard group of animals when compared to control group.

Since, an anxiolytic also produces sedation and hypnosis, these activities were evaluated with spontaneous locomotor activity in Actophotometer and Ketamine induced sleeping time test. A statistically significant($P<0.001$) reduction in spontaneous locomotor activity in Actophotometer after 30 and 60 minutes of administration of standard and test compounds were noted in comparison with control group of animals

that signifies sedative activity . The shortened latency of onset as well as prolonged duration of action of anaesthesia in test and standard group of animals in comparison with control group of animals showed statistically significant ($P<0.001$) hypnotic activity.

The results of the present study were in agreement with the study by Deng S et al where a principle was isolated from the Morinda citrifolia fruit extract that served as a competitive ligand with GABA at $GABA_A$ receptor. Further fractionisation and isolation of the active principle from the fruit is warranted. Had the genetic models for anxiety been used for the study, dose for antianxiety and sedative effects may be lowered than for hypnotic activity.

SUMMARY AND CONCLUSION

Anxiety disorders constitute highly disabling and prevalent conditions. However, past attempts to treat these 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. Benzodiazepine, which act at this receptor, have anxiolytic properties, but are limited by side effects like tolerance and concerns of potential abuse/dependence. Noni, Morinda citrifolia, is a highly regarded folk remedy which appears to be beneficial to health in numerous ways. Since Parkinson's description of noni fruit as a food in 1769, many studies were done and its safety was proved over a wider range of doses and it is practically nontoxic. From the present study a significant anxiolytic, seadative and hypnotic effects were proved to be present with aqueous extract of Morinda citrifolia fruit. Further studies are warranted to explore the long term effects in treating anxiety disorders as well as for the development of tolerance and potential for drug abuse.

BIBLIOGRAPHY

1. **M.Katherine Shear. Anxiety Disorders. In : Dale David C. Federman, Daniel D. American College of Physicians Medicine, 2007.USA : WebMD.Inc.**
2. **Jason M.Satterfield ,Bruce L.Rollman. Anxiety. In : Mitchell D.Feldman ,John F Christensen. Behavioral Medicine- A guide for clinical practice, 3rd ed.USA: McGraw-Hill; 2008;227-239.**
3. **Israel Liberzon ,K.Luan Phan ,Samir Khan ,James L.Aabelson. Role of the $GABA_A$ Receptor in anxiety. Evidence from animal models, molecular and clinical psychopharmacology and brain imaging studies: Current Neuropharmacology 2003;1:267-283.**
4. **D.J.Nutt. The pharmacology of Human Anxiety. In: Eric J.L.Griez ,Carlo Faravelli ,David Nutt ,Joseph Zohar. Anxiety Disorders An introduction to clinical management and Research, 1st ed. UK: John Wiley and Sons; 2001; 309-324.**

5. **Janicak Philip G et al. Principles and practice of Psychopharmacotherapy, 4th ed. USA : Lippincott Williams and Wilkins;2006;455-470.**
6. **www.emedicine.com/anxiety disorders/william yates.**
7. **Richard I.Shader ,Daviod J.Greenblatt. Approaches to the treatment of Anxiety states. In: Shader Richard I. Manual of psychiatric therapeutics, 3rd ed . USA:Lippincott Williams and Wilkins; 2003;184-209.**
8. **Raj K Kalapatapu. Pharmacologic Treatment of Anxiety Disorders, Depression and Anxiety newsletter – Series 2, Issue 11, 2008.**
9. **Michael G.Gelder ,Juan J.Lopez-Ibor ,Nancy Anderson. New Oxford Textbook of Psychiatry, 4th ed. USA:Oxford University Press; 2003.**
10. **Brian E.Leonard. Anxiolytics and the treatment of Anxiety Disorders. In: Brian E.Leonard. Fundamentals of Psychopharmacology, 3rd ed. USA : John Wiley and Sons, Ltd; 2003; 211- 254.**

11. **Rabbani M S.E.Sajjadi ,A.Mohammadi. Evaluation of the anxiolytic effect of Nepeta Persica Boiss. in mice. Oxford Journals Access 2007.**
12. **Robert G.Robinson, Jess G.Fiedorowicz. Anxiety. In: Coffey C.Edward; McAllister Thomas W.;Silver Jonathan M. Guide to Neuropsychiatric Therapeutics, 1st ed. USA: Lippincott Williams and Wilkins ; 2007; 138-170.**
13. **Stephen J.Mcphee ,Maxien A.Papadakis ,Lawrence M.Tierney. Lange 2008 Current Medical Diagnosis and Treatment,47th ed.USA:McGraw-Hill;2008;1897-1948.**
14. **Richard I.Shader ,Daviod J.Greenblatt. Approaches to the treatment of Anxiety states. In: Shader Richard I. Manual of psychiatric therapeutics, 3rd ed . USA:Lippincott Williams and Wilkins; 2003;184-209.**
15. **Rhoda K Hahn ,Laurence J.Albers ,Christopher Reist. Current clinical strategies.USA: Current clinical strategies publishing.2002; 39-50.**

16. Michael B.First Allan Tasman. Clinical guide to the diagnosis and treatment of mental disorders, 1st ed.USA: John Wiley and sons; 2006;321-344.
17. Laszlo A.Papp. Anxiety disorders: somatic treatment. In: Benjamin J.Sadock. Kaplan and Sadock's comprehensive textbook of psychiatry, 7th ed. USA : Lippincott William and Wilkins; 2000; 2976-3078.
18. John H.Griest, James W.Jefferson. Anxiety Disorders. In : Howard H.Goldman. Review of General Psychiatry, 5th editon. USA: McGraw-Hill; 2000; 284-300.
19. Basant K.Puri Peter J.Tyrer. Antianxiety drugs. In : Basant K.Puri. Sciences Basic to Psychiatry, 2nd ed. India : Elsevier; 155-158.
20. Oliver Von Bohlen ,Halb ,Rolt Dermietzel. Neurotransmitters and Neuromodulators. Handbook of receptors and Biological effects, 2nd ed. Germany: Wiley Vch; 2006; 75-89.
21. Erim M.Mackenzie ,Glen B.Baker ,Jean-Michel Le melledo. The Role of neuroactive steroids in Anxiety disorders. In :Michael S.Ritsner Abraham Weizman.Neuroactive steroids

in Brain Function, Behaviour and Neuropsychiatric Disorders, 1st ed.USA: Springer; 2008; 434-443.

22. Shane A Perrine ,Brian A Hoshaw ,Ellen M Unterward. Delta opioid receptor ligands modulate anxiety-like behaviours in the rat: British Journal of Pharmacology 2006; 147,864-872.

23. Zul Merali et al. Bombesin receptors as a novel anti-anxiety therapeutic target : BB1 receptor actions on anxiety through alterations fo Serotonin activity.The Journal of Neuroscience, 2006; 26(41):10387-10396.

24. Reinhard Rohkamm. Color Atlas of Pharmacology, 2nd edition. Germany: Thieme ; 2004; 112- 115.

25. Heinz Lullmann, Llaus Mohr ,Albrecht Ziegler, Dehlef Bieger. Color Atlas of Pharamcology, 2nd ed. USA: Thieme Publications; 2000;222-229.

26. Sally S.Roach.Pharmacology for Health professionals, 1st ed. USA:Lippincott Williams and Wilkins; 2005;64-75.

27. Peter Hamilton ,David Hui. Drugs and Drugs: A practical guide to the safe use of common drugs in Adults, 2nd ed. Canada:2006;30-33.

28.D.J.Greenblatt, R.L. Shader, M.Divoll ,J.S.Harmatz. Benzodiazepines: A summary of Pharmacokinetic properties. British Journal of Clinical Pharmacology 1981; 11: 115-165.

29.HL Sharma ,KK Sharma. Principles of Pharmacology, 1st ed. India: Paras Medical Publisher; 2007;449-458.

30.http://en.wikipedia.org/wiki/benzodiazepines

31.Cynthia K.Kirkwood , Sarah T.Melton. Anxiety Disorders. In : Joseph T.Dipiro. Pharmacotherapy: A pathophysiologic approach, 2nd ed.UK: McGraw-Hill publishers;1999;1285-1332.

32. W.E.Haefely. Central actions of Benzodiazepines : General Introduction. British Journal of Psychiatry 1978, 133; 231-238.

33. T.Mennini ,S.Caccia ,S.Garattiri. Anxiolytics. In : Satyavan Sharma, Eric J Lien ,Anil K. Saxena. Progress in Drug Research, 1st ed. USA:Springer;1987; 316-325.

34. David E.Moody. Drug interactions with Benzodiazepines In : Ashraf Mjozayani, Lionel P.Raymon. Handbook of Drug Interactions A clinical and forensic guide. USA.Humana

press; 2004;3-65.

35. Philip O.Anderson ,James E.Knoben ,William G.Toutman. Handbook of clinical drug data,10th ed.USA:McGraw-Hill;2002;470-479.
36. www.eTG.com.gu/complete/Therapeutic guidelines-2007
37. Laurence L.Brunton. Pharmacotherapy of anxiety. In: Laurence L.Brunton Goodman and Gilman's The pharmacological basis of Therapeutics, 11th ed. USA : McGraw-Hill; 2005. 429-460.
38. Avadhesh C.Sharma ,S.K.Kulkarni. MK-801 produces antianxiety effect in elevated plus-maze in mice: Drug Development Research 1991; 22: 251-258.
39. Kazuo Nakamura ,Mitsue Kurasawa. European Journal of Pharmacology 2001; 420:33-43.
40. Daniel S.Pine ,Jeremy D.Coplan ,Laszlo A.PApp Jack M.Gorman. Determinants: The Anxiety model. In : Stuart A.Montgomery Uriel Halbelch. Pharmacotherapy for Mood,anxiety and cognitive Disorders, 1st ed. USA:American Psychiatric Publications,Inc.;2000;341-411.

41. Arulmozhi S ,Papiya Mitra Mazumdar ,Purnima Ashok L.Sathiya Narayanan. Pharmacological activitiies of Alstonia scholaris linn (Apocynaceae). Pharmacognosy Reviews 2007;1.

42. www.nutritional-supplement-truth.com/natural-herbs-for-anxiety.html.

43. P.I.Peter R.Manimala. International Journal of Noni Research; 2005;1:1.

44. www.http//-probe. Nalusda.gov.8300- cgi-bin-browse-phytochemicals.

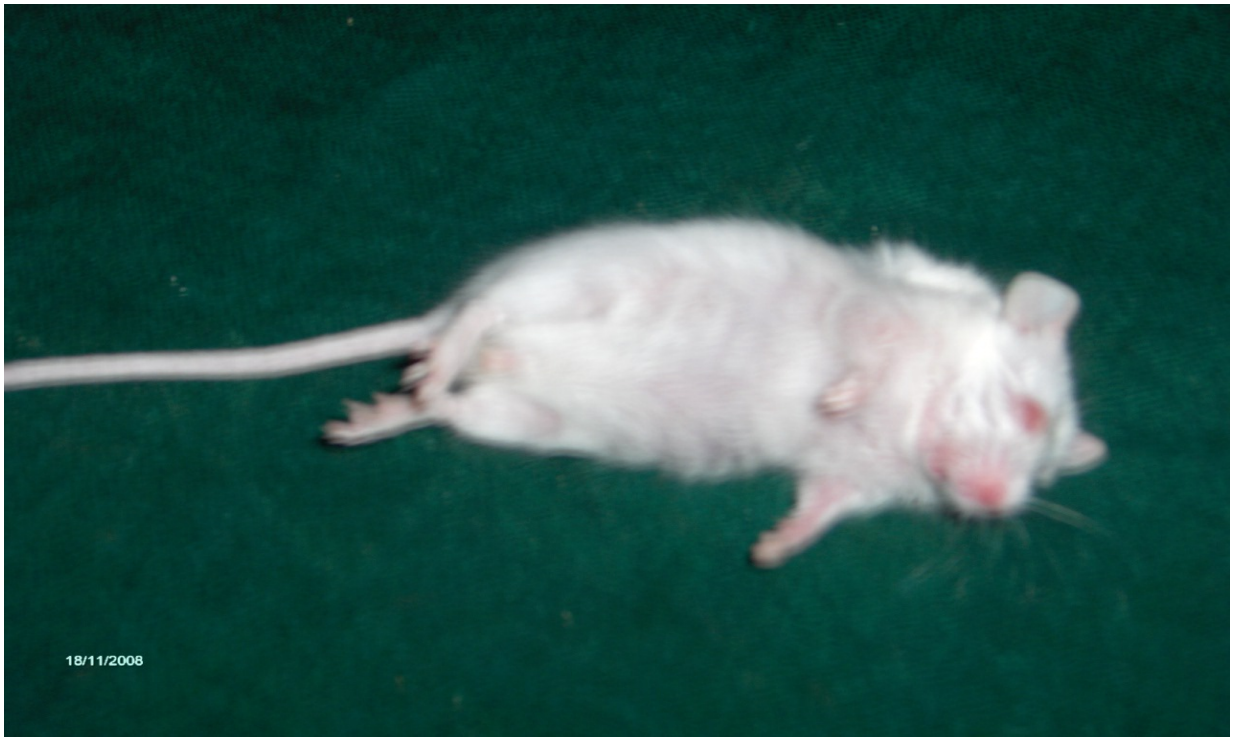
45. Etkin and McMillen.Medically and clinically Therapeutically eRegenerate Crinux Domain Names management;2003.

46. B.J.West ,C.J.Jensen ,L.Westendorf, L.D.White. A safety review of Noni fruit juice: Journal of Food science 2006; 71.

47. Deng S et al. Noni as an anxiolytics and sedative : Mechanism involving its gamma-amino butyric acidergic effects. Phytomedicine 2007; 14(7-8): 517-522.

MORINDA CITRIFOLIA FRUIT

MICE UNDER KETAMINE ANAESTHESIA



ACTOPHOTOMETER