A STUDY ON THE SEDATIVE, HYPNOTIC AND ANXIOLYTIC EFFECTS OF CLONIDINE IN MICE.

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BONAFIDE CERTIFICATE

This is to certify that the dissertation entitled "**A STUDY ON THE SEDATIVE, HYPNOTIC AND ANXIOLYTIC EFFECTS OF CLONIDINE IN MICE"** is a bonafide record work done by **Dr.S.JEYA PONMARI** under my direct supervision and guidance in the Institute of Pharmacology, Madurai Medical College, Madurai during the period of her post graduate study for MD, Branch VI –Pharmacology and appearing in April 2011.

Place: Madurai

Date:

Director & Professor,

Institute of Pharmacology,

Madurai Medical College

Madurai - 625 020.

DECLARATION

I, **Dr. S.JEYA PONMARI** solemnly declare that the dissertation titled **"A STUDY ON THE SEDATIVE, HYPNOTIC AND ANXIOLYTIC EFFECTS OF CLONIDINE IN MICE"** has been prepared by me under the able guidance and supervision of my guide **Dr.R.PARAMESWARI, M.D.,** Director, 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 April 2011.

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.JEYA PONMARI**

Date :

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CONTENTS

INTRODUCTION

INTRODUCTION

Sleep can be conceptualized as a motivated behaviour, something the organism "needs" to do in order to survive and for which there is a pressure to perform.[1]

Sleep is a process the brain requires for proper functioning.[2] Humans spend about a third of their lives in sleep. Sleep is a state of inactivity accompanied by loss of awareness and a markedly reduced responsiveness to environmental stimuli.[3]

Sleep disorders are frequent in the general population. Up to 30% of adults complain of insomnia.[4] Primary sleep disorders include dyssomnias and parasomnias. Dyssomnias can be characterized by disturbance in amount, quality, or timing of sleep. Parasomnias are characterized by abnormal behaviours or physiological events associated with sleep.[2] There are number of interesting and common irregularities of sleep, some of which approach serious extremes. Inadequate sleep leads to daytime tiredness, lack of energy, irritability, poor concentration, anticipatory anxiety and psychiatric problems like anxiety & depression. The psychologic and physiologic benefits of sleep are of paramount importance and it is increasingly recognized that disruption of sleep increases the

risks for a number of medical diseases including stroke, hypertension and coronary disease.[5]

The neurotransmitters and neuromodulators that may regulate sleep-wake cycle are serotonin, norepinephrine, acetylcholine, dopamine, γ aminobutyric acid (GABA), adenosine, interleukins, prostaglandins and certain endogenous sleep factors like peptides and uridine.[2]

Anxiety is a feeling of apprehension, uncertainty or tension stemming from the anticipation of an imagined or unreal threat. Approximately 4-6% of population suffers from anxiety. It is so severe that it disrupts routine life functions. Hence anxiolytic drugs are extensively prescribed. [6]

Any person facing a challenging or threatening task for which he may feel unprepared, experiences some degree of nervousness and anxiety. Anxiety is then not abnormal, and the alertness and attentiveness that accompany it may actually improve performance up to a point, after which increasing anxiety causes a rapid decline in performance.[5] Indeed it is a universal human emotion, closely allied with appropriate fear and presumably serving psychobiologically adaptive purposes.

Anxiety is a cardinal symptom of many psychiatric disorders. Anxiety is an inevitable component of many medical and surgical conditions. Sometimes no

treatable primary illness is found.[7]

The disability and health costs caused by anxiety are high and comparable with those of other common medical conditions such as diabetes, arthritis or hypertension.[3]

Anxiety states & sleep disorders are common problems and hence sedative hypnotics & anxiolytics are among the most widely prescribed drugs today.

A broad range of pharmacologic agents are available to treat anxiety and sleep disorders namely Benzodiazepines, Tricyclic antidepressants, Monoamino oxidase inhibitors, Selective Serotonin Reuptake Inhibitors (SSRIs), Serotonin and Noradrenaline Reuptake Inhibitors (SNRIs), Antiadrenergic agents, Antipsychotic drugs, Hydroxyzines and Buspirone.

Benzodiazepines are the most commonly prescribed agents. They are primarily introduced for their sedative, hypnotic and anxiolytic effects. They are also preferred for preanaesthetic medication.[8] They can produce anaesthesia similar to barbiturate but not used in general anaesthesia because of prolonged amnesia and sedation. Nowadays it is also indicated for other diseases like panic disorders, phobia, epilepsy, bipolar disorders and muscle spasms. Their drawbacks include excessive sedation, cognitive & psychomotor impairment, paradoxical

effects, tolerance and dependence.[9]

Hence there is always a search for drugs with lesser side effects.

'Clonidine', an α_2 agonist known for its antihypertensive effect also possesses sedative, hypnotic, anxiolytic, analgesic and anti-inflammatory properties. Clonidine is also useful in selected patients receiving anaesthesia because it increases hemodynamic stability and decreases the anaesthetic requirement.[7]

Hence the present study is undertaken to find out the sedative, hypnotic and anxiolytic effects of clonidine in comparison with the standard drug diazepam in mice.

AIM

AIM

The aim of present study is to evaluate the sedative, hypnotic and anxiolytic effects of clonidine in comparison with diazepam in mice.

REVIEW OF

LITERATURE

REVIEW OF LITERATURE

Sleep, an elemental phenomenon of life and an indispensable phase of human existence represents one of the basic 24-hr (circadian) rhythms traceable through all mammalian, avian, and reptilian species.

The neural control of circadian rhythms is thought to reside in the anterior region of the hypothalamus, more specifically in the suprachiasmatic nuclei. Lesions in these nuclei result in disorganization of the sleep-wake cycles as well as of the rest-activity, temperature and feeding rhythms.[5]

Essential relationships between a troubled mind and disturbances in sleep have been recognized since antiquity.[1] Sleep disturbances are particularly pertinent to psychiatrists and other mental health professionals because these complaints are frequently associated with depression, anxiety, substance abuse, obsessive-compulsive disorders, psychosis, acute & chronic stress, and many other disorders. The neuroscience of sleep regulation, in addition is important to understand the pathophysiology of psychiatric disorders and the mechanism of action of psychoactive drugs.

Many sleep disorders are life threatening either directly (e.g. fatal familial insomnia and obstructive sleep apnea) or indirectly as result of sleep related accidents.

The 24 hour sleep-wake rhythm is driven by the suprachiasmatic nuclei synchronized to the environmental light-dark cycle by Zeitgebers. A number of sleep disorders specifically involve disturbances of the circadian sleep-wake system (jetlag, shift work schedules). The flexibility of the circadian system diminishes with age, so the elderly suffer more from jetlag and shift work schedules.

The frequency and severity of insomnia increase with age in both sexes. Total sleep time decreases from an average of 8 hours daily in young adulthood to about 6 hrs daily at the age of 90. This is accompanied by more nocturnal arousals.[5]

Classification of primary sleep disorders in DSM IV [4]

Dyssomnias

Primary insomnia

Primary hypersomnia

Narcolepsy

Breathing related sleep disorder

Circadian rhythm sleep disorder

Dyssomnia not otherwise specified

Parasomnias

Nightmare disorder (dream anxiety disorder)

Sleep terror disorder

Sleep-walking disorder

Parasomnia not otherwise specified

Sleep disorder related to another mental disorder

Insomnia

Hypersomnia

Other sleep disorders

Secondary sleep disorder due to a general medical condition

Substance-induced sleep disorder

Primary insomnia

Primary insomnia is diagnosed only after a patient complains of sleeplessness or poor quality of sleep for one month or more. The insomnia, or consequent sleepiness must produce significant distress or impair activities of daily life.[2]

Insomnia in clinical practice is usually *secondary* to other disorders, notably painful physical conditions, depressive disorders and anxiety disorders, and is often clinically overlooked.

In about 15% of cases of insomnia, no cause can be found (*Primary insomnia*).

Primary hypersomnia

Patients complain that they are unable to wake completely until several hours after getting up. During this time they feel confused and possibly disoriented ('Sleep drunkenness').[4]

Narcolepsy

Narcolepsy is a syndrome of unknown origin characterized by irresistible urges to sleep. These sleep attacks typically occur two to six times a day and last for 10-20 minutes. They may occur at inappropriate times (e.g.) while eating, talking, or driving. Symptoms typically appear in the second decade of life. It is usually associated with one or more of the following symptoms: Cataplexy (brief episodes of sudden bilateral loss of muscle tone in association with intense emotion), sleep paralysis (inability to move or speak when awaking from sleep) and hypnagogic hallucinations (frightening hallucinations experienced during sleep onset or waking).[10]

Breathing related sleep disorders

This syndrome consists of daytime drowsiness together with periodic respiration and excessive snoring at night. It is usually associated with upper airway obstruction and typically occurs in middle aged overweight patients.

Circadian rhythm sleep disorder

(Sleep-wake schedule disorders)

Shift work type and jetlag are familiar forms of circadian rhythm sleep disorder.[4]

Parasomnias

Parasomnias can simply be defined as intermittent undesirable events arising from sleep that are not epileptic in nature. The spectrum ranges from visual imagery at sleep onset to complex motor behaviours, occasionally with violent components.[11]

A *nightmare* is an awakening from REM sleep to full consciousness with detailed dream recall. Peak frequency occurs around the ages of five or six years. *Night terrors* are less common and, begin and end in childhood. There is no dream recall.

Sleep walking is an automatism occurring during deep non-REM sleep, usually in the early part of the night. It is most common between the ages of 5 and 12 years.[4]

Insomnia related to another mental disorder

Approximately 80% of patients with psychiatric disorders describe sleep complaints. Sleep disturbances are common in patients suffering from depression, alcoholism, mania, anxiety disorders and schizophrenia.

Neurogenic sleep disorders

Sleep disruption can occur in dementia, epilepsy, Parkinson's disease, Huntington's chorea, Tourette syndrome, headache syndromes (migraine or cluster headache).

Insomnia due to other medical disorders

Sleep may be impaired due to chronic pain from rheumatologic disorders, pulmonary diseases (asthma, COPD), cardiac ischemia, hyperthyroidism, chronic renal failure, and liver failure.[12]

Medication, drug or alcohol – dependent insomnia

Alcohol, anxiolytics, opioids and sedative hypnotics promote sleep by sedation. But the sleep is of poorer quality though of greater quantity. Psycho stimulants like cocaine, amphetamine, caffeine-containing beverages, sympathomimetics, theophylline and antidepressants cause CNS arousal and produce insomnia.

Pathophysiology of sleep disorders: [13]

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. Through the 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 (midday 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 towards excitatory system underlies many of the sleeping disorders.

Anxiety disorders

Anxiety disorders are the most common psychiatric disorders in the world among both children and adults. Much of the emotional and economic burden caused by these disorders could be alleviated by improving the diagnosis and treatment.[14] In most cases, women are more likely to have anxiety disorders than men, a phenomenon that still begs for adequate explanation. Of particular interest in the finding that social phobia is more common in women than men.

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 mistranslation of Freud's word for fear (angst). In 1895, Freud separated anxiety disorders from neurasthenia and suggested the name anxiety neurosis.[9]

Everyone experiences anxiety. It is characterized most commonly as a diffuse, unpleasant, vague sense of apprehension often accompanied by autonomic symptoms such as headache, perspiration, palpitations, tightness in the chest, mild stomach discomfort and restlessness, indicated by an inability to sit or stand still for long.[15]

Trait anxiety refers to a lifelong pattern of anxiety as a feature of temperament and state anxiety refers to episodes of anxiety that are tightly bound to specific situations and that do not persist after the provoking situation has abated. Free floating anxiety is characterized by a persistently anxious mood in which the cause is unknown. But situational anxiety occurs only in relation to specific occasions or external stimuli.

Recent studies also suggest that chronic anxiety disorder may increase the rate of cardiovascular-related mortality. Another fascinating aspect of anxiety disorders is the exquisite interplay of genetic and experiential factors.

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. Genetic susceptibility to an anxiety disorder becomes an actual anxiety disorder when some set of environment influences

causes anxiety proneness genes to become active. Medical illness, drugs, toxins and substance abuse also play a role in the etiology of anxiety disorder.[2]

It is important to distinguish among the various anxiety disorders and identify possible comorbidities because of difference in treatments, complications and prognosis.

Classification of Anxiety disorders [9]

According to DSM IV, anxiety disorders are classified as follows:

1. Panic disorder without agoraphobia
2. Panic disorder with agoraphobia
3. Agoraphobia without history of panic disorder
4. Specific phobia
5. Social phobia
6. Obsessive compulsive disorder
7. Post traumatic stress disorder
8. Acute stress disorder
9. Generalized anxiety disorder
10. Anxiety disorder due to a general medical condition
11. Substance induced anxiety disorder
12. Anxiety disorder not otherwise specified

Panic disorder

Panic disorder, the most commonly encountered anxiety disorder in medical settings, is also the most disabling, with financial, social, and occupational impairment.[16] The main feature of panic disorder is recurrent, unexpected panic attacks accompanied by characteristic physical symptoms such as pounding of heart, sweating, hot flushes or chills, trembling/shaking, breathing difficulties, chest pain, nausea, diarrhoea.

Usually the attack lasts no more than 20 – 30 minutes, reaches peak within 10 minutes.

Secondary to panic attacks, many patients develop agoraphobia which is anxiety about being in places or situations in which escape might be difficult.[17]

Generalized anxiety disorder

According to DSM-IV, generalized anxiety disorder is characterized by excessive and uncontrollable anxiety or worry persisting for at least six months, combined with three of six additional symptoms ("restlessness or feeling keyed up or on edge," "being easily fatigued," "difficulty 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.

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, similar to a situationally bound or predisposed panic attack. The situations are avoided or else are endured with intense anxiety or distress.

Specific phobia

According to DSM – IV, specific phobia is characterized by a "marked and persistent fear that is excessive or unreasonable" 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. A prominent vasovagal response was observed in a subgroup of patients.

Post traumatic stress disorder

DSM-IV defines posttraumatic 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 can include sleep disturbance, irritability, poor concentration, and exaggerated startle reflex.

Obsessive compulsive disorder

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 behaviours 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 behavioural symptomatology, with autonomic dysregulation playing little role.

Acute stress disorder

Acute stress disorder is anxiety in response to a recent extreme stress. Although in some respects it is normal and understandable reaction to an event, the problems associated with it are not only the severe distress the anxiety causes but also the risk that it may evolve into a more persistent state.[3]

Anxiety disorders due to general medical condition

Anxiety symptoms are an inherent part of the initial clinical presentation of several diseases, thus complicating the distinction between anxiety disorders and medical disorders. They usually will subside as the medical situation stabilises.

Causes of anxiety disorder secondary to a general disorder [17]

Angina, Arrhythmias, Myocardial infarction

Cushing's disease

Hyperparathyroidism

Hyperthyroidism

Dementia

Migraine

Parkinson’s disease

Neoplasm

Asthma

Chronic obstructive pulmonary disease

Anemia

Systemic lupus erythematosus

Drug induced anxiety

CNS stimulants can cause anxiety in a dose dependent manner.

Pathophysiology of Anxiety disorder [9]

Several preclinical evidences now indicate that the amygdala plays a crucial role in the development of stress response, fear and anxiety.

The central nucleus of amygdala has direct projections to a variety of anatomical areas that might mediate many of the symptoms of fear and anxiety.

At least three neurotransmitter systems appear to modulate the symptoms of many anxiety disorders. These are the norepinephrine, serotonin, and GABAergic systems.

Norepinephrine

Anxiety disorder patients have poorly regulated noradrenergic system that has occasional bursts of locus ceruleus. Stimulation of local ceruleus produces a fear response in animals and ablation of that area inhibits the ability of the animals to form a fear response.

Human studies have found that, in patients with panic disorder, α-adrenergic agonists (e.g. isoproterenol) and α_2 adrenergic antagonists (e.g. yohimbine) can produce frequent and severe panic attacks. Conversely clonidine, an adrenergic agonist reduces anxiety symptoms in some experimental and therapeutic situations.

Serotonin

Observations regarding the role of serotonin are contradictory. On the one hand, clomipramine (serotonergic antidepressant) and buspirone ($5HT_{1A}$ receptor agonist), are beneficial in the treatment of anxiety disorders. On the other hand serotonergic hallucinogens (e.g. Lysergic acid diethylamide or LSD), and fenfluramine (which causes release of serotonin) are associated with development of both acute and chronic anxiety disorders.

GABA

There is inconclusive evidence regarding the role of GABA in anxiety disorders. The effectiveness of benzodiazepines, which enhance activity of GABA at the $GABA_A$ receptor in the treatment of some anxiety disorders and occurrence of panic attacks in panic disorder patients by flumazenil (a benzodiazepine antagonist) are suggestive of the role of GABA in the genesis of anxiety disorder.

Studies of patients with post traumatic stress disorder have revealed a down regulated hypothalamic-pituitary-adrenal axis with reduced secretion of cortisol, presumably as a result of chronically increased production of corticotropin releasing factor.

One model relevant to anxiety and panic puts forth that the serotonin pathway is originating in the dorsal raphe nucleus. This pathway involves post synaptic serotonin $5HT_{2A/2C}$ and $5HT_3$ receptor activation. Such a pathway is relevant to generalized anxiety disorder. A separate pathway originating in dorsal raphe nucleus involves innervation of the periventricular and periaqueductal gray region. This pathway is relevant to panic attacks mediated by $5HT_{2A/2C}$ and $5HT_{1A}$ postsynaptic receptors. In chronic stress activation of $5HT_{1A}$ receptor promotes resistance to such stress by disconnecting the aversive events from processes underlying appetitive and social behaviours.

The locus ceruleus noradrenergic and dopaminergic systems are believed to increase autonomic arousal and vigilance in response to threat. Overstimulation of such adaptive mechanisms by repeated or chronic stressors as well as deficits in function of components of these systems could theoretically lead to pathological responses observed in anxiety disorders.

Pharmacotherapy of sleep disorders and anxiety disorders

The primary use of drugs, classified as sedative, hypnotic and anxiolytic is to produce calmness and sleep. A sedative drug reduces daytime activity and calms the recipient; an anxiolytic drug reduces pathological anxiety; and a hypnotic drug produces drowsiness and facilitates the onset and maintenance of sleep. In addition to inducing sleep and reducing pathological anxiety, the various sedative and hypnotics are used as antiepileptic agents, muscle relaxants, antipanic agents, in the treatment of alcohol withdrawal states and as anaesthetic agents.

History of Sedative and Hypnotic drugs [7]

The first agent used as sedative and hypnotic was bromide (1853). Other sedative hypnotic drugs used before 1900 were chloral hydrates, paraldehyde, urethane and sulfonal. Barbital was introduced in 1903 and phenobarbital in 1912. Barbiturates were most commonly used sedative-hypnotics till 1961 when

chlordiazepoxide was introduced. Since then many benzodiazepines were synthesized and marketed.

Most of the benzodiazepines that have reached the market place were selected for high anxiolytic potency in relation to their depression of CNS function. However all benzodiazepines possess sedative, hypnotic properties to varying degrees. These properties are exploited extensively clinically, especially to facilitate sleep.

Classification of Anxiolytic and Hypnotic drugs [18]

(i) Benzodiazepines:

Hypnotics	Anxiolytics	Anticonvulsants
Diazepam	Chlordiazepoxide	Diazepam
Nitrazepam	Diazepam	Clonazepam
Lorazepam	Alprazolam	Clobazam
Temazepam	Oxazepam	
Triazolam	Lorazepam	
Midazolam	Flurazepam	
Flurazepam		

(ii) Non benzodiazepine Hypnotics:

Zopiclone, Zolpidem, Zaleplon

(iii) Atypical Anxiolytics:

Buspirone, Ipsapirone, Gepirone

(iv) Beta adrenoceptor antagonist: Propranolol

(v) Barbiturates:

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

(vi) Miscellaneous group:

Melatonin, Triclophos, Hydroxyzine, Promethazine

Management of insomnia

Management of insomnia includes pharmacological and non-pharmacological treatment.[19] The side effects of hypnotic medications must be weighed against the sequelae of chronic insomnia which includes a fourfold increase in serious accidents.

Categories of insomnia [7]

National Institute of Mental Health Consensus Development Conference (1984) divided insomnia into 3 categories.

1. Transient insomnia:

Transient insomnia lasts less than 3 days and usually is caused by a brief environmental or situational stressor. It may respond to sleep hygiene rules. If hypnotics are prescribed they should be used at the lowest dose and for only 2 to 3 nights.

2. Short term insomnia:

Lasts from 3 days to 3 weeks and usually is caused by a personal stressor such as illness, grief or job problems. Sleep hygiene education is the first step. Hypnotics may be used adjunctively for 7 to 10 nights.

3. Long term insomnia:

Long term insomnia is insomnia that has lasted for more than 3 weeks. No specific stressor may be identified. A more complete medical examination is necessary. Insomnia caused by major psychiatric illness often responds to specific pharmacological treatment for that illness.

Adequate control of anxiety in patients with anxiety disorders often produces adequate resolution of the accompanying insomnia.

The profound insomnia of patients with acute psychosis owing to schizophrenia or trauma usually responds to dopamine receptor antagonists.

Non pharmacological treatments for insomnia [2]

Sleep hygiene refers to basic rules designed to provide circumstances and conditions conducive to sleep. They include the following list of things:

Universal sleep hygiene:

This includes maintaining a regular sleep-wake schedule, daily exercise, insulating the bedroom against excessive noise, light, cold and heat; eating a light snack before retiring if hungry and setting time aside to relax before getting into bed.

Stimulus control therapy:

The first rule is to go to bed only when sleepy. Second, use bed only for sleeping. Rule three instructs the patient not to lie in bed and become frustrated if unable to sleep. Go to another room and do something nonarousing until sleepiness returns.

Rule four is to awaken at the same time every morning, regardless of bedtime. The final rule is to totally avoid napping.

Sleep restriction therapy:

Restricting time in bed can help consolidate sleep for patients who find themselves lying awake in bed unable to sleep.

Relaxation therapy:

Self-hypnosis, progressive relaxation and deep breathing exercise are all effective if they produce relaxation.

Pharmacological Treatment of insomnia

Commonly used compounds are benzodiazepines and nonbenzodiazepines. Selecting an appropriate drug depends on the duration of action.[19]

Benzodiazepines

Benzodiazepines are the most frequently prescribed drugs for treating insomnia and are Food and Drug Administration (FDA) labeled for the treatment of insomnia. All benzodiazepine receptor agonists are effective as sedative hypnotics. Benzodiazepines relieve insomnia by reducing sleep latency and increasing total sleep time. They increase stage 2 sleep while decreasing REM, stage 3 and stage 4 sleep.[17]

Mechanism of action

Specific cellular membrane binding sites were determined by Young in 1981. These sites are confined to the CNS and are predominately localized in neuronal surface membranes. The distribution within the CNS is extensive, with concentrations in every brain region. The action of benzodiazepines is mediated through a facilitation of the GABAergic transmission at specific receptor sites.[20]

Benzodiazepines bind to the $GABA_A$ receptor at the α_1, α_2, α_3, α_5 subunits. Benzodiazepines with high affinity at α_1 subunit is associated with sedation, hypnosis and anticonvulsant activity and those with affinity for α_2 subunits mediate anxiolytic and muscle relaxant actions.[21]

When benzodiazepines bind to the $GABA_A$ receptor, it opens the chloride ion channels. A widening of the channels, with an increase in ion conductance, occurs to alter the membrane potential and to prevent an action potential. Benzodiazepines act by increasing the frequency of openings of the GABA-modulated chloride channels.[20]

Pharmacokinetics

Benzodiazepines are effective orally. There are marked pharmacokinetic differences among benzodiazepines because they differ in lipid soluability by more than 50 fold. Absorption from intramuscular sites is irregular except for lorazepam. Benzodiazepines are metabolized by liver and excreted as glucuronide conjugates in urine. Many of them are converted to active metabolites such as N-desmethyldiazepam, which has a half-life of about 60 hours, and which accounts for the tendency of many benzodiazepines to produce cumulative effects and long hangovers when they are given repeatedly.[22]

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

The longer acting benzodiazepines 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.[23]

Side effects

Side effects are dose dependant and vary according to the pharmacokinetics of the individual benzodiazepine. High doses of benzodiazepines with long or intermediate elimination half-lives have a greater potential for producing day time sedation and performance impairment. They cause excessive drowsiness, psychomotor in-coordination and cognitive deficits. Tolerance to hypnotic effect may develop with time. Rapidly eliminated benzodiazepines have less potential for daytime sedation.[17]

Drug interactions [23]

Antacids - Decreased absorption of benzodiazepines

Cimetidine - Increased half-life of diazepam and triazolam

Contraceptives – Increased levels of diazepam and triazolam

Disulfiram - Increased duration of action of benzodiazepines

Rifampin - Decreased plasma diazepam

Non-Benzodiazepine $GABA_A$ Agonists

Zolpidem, zaleplon and eszopiclone are non benzodiazepine hypnotics that selectively bind to $GABA_A$ receptors and effectively induce sleepiness.

Zolpidem:

It is an imidazopyridine, comparable in efficacy to benzodiazepine hypnosis and is effective for reducing sleep latency, nocturnal awakenings and increasing total sleep time. It does not appear to have significant effects on next day psychomotor performance. Recommended daily dose is 10mg and can be increased to 20mg per night. It should be taken on an empty stomach and treatment optimally should not exceed four weeks to minimize tolerance and dependence.

Zaleplon:

Zaleplon is a pyrazolopyrimidine. It has a rapid onset of action, and a half life of one hour. It is effective for decreasing the time of sleep onset, but not for reducing night time awakening or for increasing total sleep time. Because of its short half life zaleplon has no effect on next day performance.[17]

Eszopiclone:

Eszopiclone is an oral nonbenzodiazepine hypnotic utilizing BZ_1 receptor similar to zolpidem and zaleplon and used for treating insomnia. It is rapidly absorbed and mainly excreted in urine. It is effective for up to 6 months compared to a placebo.[24]

Antihistamines:

Antihistamines are included in many over the counter sleep agents. They are effective in the treatment of mild insomnia and are generally safe. Diphenhydramine and doxylamine are more sedating. They have the disadvantage of anticholinergic side effects.

Antidepressants:

They are alternatives for patients with non-restorative sleep who should not receive benzodiazepines, especially those who have depression, pain or a risk of substance abuse.

Sedating antidepressants such as amitriptyline and nortriptyline are effective for inducing sleep continuity.

Trazodone is popular for the treatment of insomnia in patients prone to substance abuse, as dependence is not a problem. It is used in patients with Selective Serotonin Reuptake Inhibitor (SSRI) and bupropion induced insomnia. Caution should be exercised to avoid serotonin syndrome when used in these combinations.[17]

Melatonin:

Melatonin is a hormone produced in the pineal gland from the amino acid tryptophan. The secretion of melatonin follows a diurnal rhythm. It acts as a chronobiotic which therapeutically adjusts the timing of circadian rhythm. It is primarily used to alleviate symptoms of jetlag and other disorders resulting from delay of sleep in the dose of 3 mg two hours before bedtime.[18]

Ramelteon:

Ramelteon is a melatonin receptor agonist that has recently been approved for the treatment of sleep onset insomnia.[17] It is a novel hypnotic drug. It is selective for the MT1 and MT2 melatonin receptors. Ramelteon reduces the latency of persistent sleep with no effects on sleep architecture and no rebound insomnia. Ramelteon is not a controlled substance and can be a viable option for patients with a history of substance abuse.[25]

Valerian:

Valerian is an herbal sleep remedy. The mechanism of action may involve increasing concentrations of γ aminobutyric acid [GABA].[17]

History of Anxiolytic drugs [26]

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 non-barbiturate, non-benzodiazepine hypnotic drugs in the 1930s did not address any of the deficiencies of barbiturates and proved more problematic in many cases. Meprobamate, and tybamate possess very low therapeutic index, methaqualone is highly addicting and fatal in over dose, glutethimide overdose can result in convulsions and 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 non barbiturate, non benzodiazepine 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. Parallel to the work with imipramine in the United States, British investigators found that another class of antidepressants, monoamine oxidase inhibitors (MAOIs) specifically benefit hysterical patients with phobic symptoms. Since these patients show many features of panic disorder and agoraphobia, tricyclic and tetracyclic 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. 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 anxiolytic agents include β-adrenergic receptor antagonists and the azapyrone buspirone. New drug development targets 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.

Treatment of anxiety disorders

Nonpharmacological treatment:

Nonpharmacological methods for the treatment of generalized anxiety include behavioural and cognitive psychotherapy, meditation techniques and biofeedback.[27] Psychotherapeutic interventions are often considered the first line intervention in anxiety disorders, particularly for patients with mild symptoms and for children. Cognitive behaviour therapy (CBT) is a specific type of psychotherapy and has the best evidence-based research supporting its use in anxiety disorders.[14] Cognitive behaviour therapy (CBT) is the treatment of choice for panic disorder and general anxiety disorder.[28]

Pharmacological treatment:

Benzodiazepines:

Benzodiazepines are the most important class of drugs used for treating both anxiety states and insomnia. Benzodiazepines achieve an anxiolytic effect by inhibiting synapses in the limbic system, a CNS region that controls emotional behaviour and is characterized by a high density of $GABA_A$ receptors. Benzodiazepines such as diazepam and alprazolam are used to mitigate chronic, severe anxiety, and the anxiety associated with some forms of depression and schizophrenia.[29]

Buspirone:

These drugs act through non-GABAergic 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 inhibitory pre-synaptic 5-HT_{1A} receptor, they suppress 5-HT neurotransmission through neuronal system.

The antianxiety effect of buspirone appears after a week of administration of the drug; therefore, the drug is indicated in anxiety states and not in panic

disorders. As compared to diazepam, psychomotor impairment produced by buspirone is less. Buspirone is given orally in the dose of 5 – 15 mg, 2 -3 times a day.

β-adrenoceptor blockers:

Worrying situations and apprehensions associated with examinations, public address or job interviews are conducive to palpitation, tremors, gastrointestinal tract upset or even hypertension due to sympathetic overactivity. These symptoms in turn reinforce anxiety. Propranolol helps to suppress such performance anxiety through its beta blocking action.[18]

Treatment of panic disorders

Panic attacks may be treated in several ways. A sublingual dose of lorazepam (0.5-2 mg) or alprazolam (0.5-1 mg) is effective for urgent treatment. For sustained treatment SSRIs are the initial drugs of choice. Sertraline starting at 25 mg/day and increased after one week to 50 mg/day may be effective. High potency benzodiazepines may be used for symptomatic treatment as the antidepressant dose is titrated upward. Clonazepam (1-6 mg/day) orally and alprazolam (0.5-6 mg/day) orally are effective alternatives to antidepressants. Both drugs should be tapered to avoid withdrawal symptoms. Propranolol (40-160

mg/day orally) can mute the peripheral symptoms of anxiety without affecting motor and cognitive performance. Valproate has been found to be as effective as the antidepressants in panic disorder and is another useful alternative.

Treatment of Phobic disorder

Global social phobias may be treated with SSRIs such as paroxetine, sertraline and fluvoxamine or monoamine oxidase (MAO) inhibitors 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 phobia such as performance anxiety may respond to propranolol in the dose of 20-40 mg one hour prior to exposure.[23]

Obsessive compulsive disorder (OCD)

Selective serotonin reuptake inhibitors are considered the first line agents in the treatment of obsessive compulsive disorder in children and adults as they are better tolerated. Among tricyclic antidepressants only clomipramine has specific antiobsessive effect, others are ineffective. Venlafaxine was shown to be equally effective as paroxetine. Clonazepam, a benzodiazepine that also affects

serotonergic neurotransmission, may be of some value in OCD, in treatment-resistant cases. Few intractable cases of OCD may respond to electroconvulsive therapy (ECT).[30]

Post-traumatic stress disorder (PTSD)

Psychotherapy should be initiated as soon as possible. Early treatment of anxious arousal with Beta-blockers (e.g. propranolol 80-160 mg orally daily) may lessen peripheral symptoms of anxiety (e.g. tremors, palpitations) and help prevent development of the disorder.

Antidepressant drugs, particularly selective serotonin reuptake inhibitors – in full dosage are helpful in ameliorating depression, and panic attacks in chronic PTSD. Sertraline and paroxetine are approved by the US Food and Drug administration (FDA) for this purpose.

The α-blocking agent prazosin (2-10 mg orally at bed time) has been successfully used to decrease nightmares and improve quality of sleep in PTSD.

Treatment of Generalized anxiety disorder

Benzodiazepines are the anxiolytics of choice in the acute management of generalized anxiety disorder. Antidepressants can be efficacious for the long term

management. In psychiatric disorders the benzodiazepines are usually given orally. The longer acting benzodiazepines are used for the treatment of alcohol withdrawal and anxiety symptoms.

Antidepressants are the first line medications for sustained treatment of generalized anxiety disorder, having the advantage of not causing serious physiologic dependency problems. Venlafaxine and duloxetine (serotonin and norepinephrine reuptake inhibitors) are FDA approved for generalized anxiety disorder. Buspirone is also effective in generalized anxiety disorder.

β-blockers such as propranolol reduce the peripheral somatic symptoms. Phenobarbital, an anticonvulsant is a reasonably safe and inexpensive sedative.[23]

Several newer drugs are emerging as sedative, hypnotic, anxiolytics to overcome the adverse effects associated with the older drugs. Some drugs with anxiolytic effects exert their actions directly on adrenergic receptors. Although marketed for other purposes, β-adrenergic antagonist – propranolol is in use for years in the management of several anxiety conditions.

Clonidine, an α_2 agonist, originally introduced as an antihypertensive agent, widely used in several psychiatric disorders, is recently emerging as a sedative drug, and possess anxiolytic effects also.[31]

Clonidine – an overview

Clonidine is a presynaptic α_2-adrenergic receptor agonist that is approved for use as an antihypertensive agent. Stimulation of α_2-adrenergic receptors reduces the firing rate of noradrenergic neurons and reduces the plasma concentrations of norepinephrine. Because of widespread actions of the noradrenergic system, Clonidine has also been adopted for use as a psychopharmacological agent. It is also used as a pharmacological probe to assess central α_2-receptor sensitivity in psychiatric disorders.

Clonidine, an imidazoline derivative was synthesized in the early 1960s and found to produce vasoconstriction that was mediated by α receptors. The drug was initially developed as a topical nasal decongestant. During clinical testing, it was found to cause hypotension, sedation and bradycardia.[32] Clonidine is a selective α_2-adrenoceptor agonist with a α_2: α_1 ratio of 200:1. It is currently the only drug in this group available for use in anaesthetic practice.[33]

Chemistry:

It is an imidazoline derivative and exists as a mesomeric compound. Its chemical name is 2-(2.6 - dichloroanilino) - 2 - imidazoline hydrochloride. Clonidine hydrochloride is an odourless, white crystalline powder soluble in water

and in dehydrated alcohol. Its molecular weight is 266.6. [34]

Pharmacokinetics:

Clonidine is well absorbed after oral administration. Since it is subjected to little or no presystemic elimination, its bioavailability is nearly 100%. Peak plasma concentrations are achieved 90 minutes after an oral dose. Clonidine is widely distributed throughout most tissues. Clonidine is 20 to 40% bound to plasma proteins. Good correlation exists between plasma concentration and its pharmacological effects. Although clonidine may cross the placenta it does not reach concentrations sufficient to affect the fetus. A transdermal delivery patch releases the drug at a constant rate for a week.

Clonidine is approximately forty percent cleared by metabolism, predominantly in the liver to inactive metabolites and approximately sixty percent is excreted unchanged in urine.[35]

Mechanism of action:

Clonidine is the best characterized α_2 adrenoceptor agonist. α_2 receptors are located centrally and peripherally. Clonidine produces its effects by binding to α_2 receptors of which there are three types (A, B, C), each with its own area of anatomical predominance.

α_{2A} Receptors

- Most predominant and broadly distributed subtype
- Concentrated in the locus ceruleus, cortex, amygdala, septum, hippocampus and hypothalamus.
- Mediate sedation, analgesia, and sympatholysis.

α_{2B} Receptors

- Mainly restricted to thalamus.
- Mediate sedation, vasoconstriction and possibly antishivering mechanism.

α_{2C} Receptors

- High densities in the hippocampus, the olfactory system, the striatum and the cerebral cortex

Less is known about the effects of Clonidine on α_{2B} and α_{2C} adrenoceptors.

Each of these subtype is a G_i/G_o protein coupled receptor, when activated by agonists leads to suppression of voltage gated calcium channels and activation of potassium channels. This interaction suppresses adenyl cyclase activity and phospholipase C activity.

Clonidine, an imidazoline derivative also binds to imidazoline binding site (IBS) located throughout the body and in the CNS. There are 3 subtypes of IBS: I_1,

I_2 and I_3. Clonidine has greater affinity for I_1 IBS which is involved in the control of blood pressure.[32]

The central hypotensive action of Clonidine needs both I_1 IBS and α_2 adrenoceptors to produce their central sympatholytic response. Clonidine appears to be more selective for α_2 adrenoceptors than for I_1 IBS.[36]

Pharmacological effects:

The major pharmacological effects of Clonidine involve changes in blood pressure and heart rate.

Stimulation of these α_2 receptors brings about a decrease in sympathetic outflow from the CNS, which in turn leads to decreases in peripheral vascular resistance and blood pressure. Clonidine also produces bradycardia as a result of a centrally induced facilitation of the vagus nerve and stimulation of cardiac prejunctional α_2-adrenergic receptors. The ability of Clonidine to produce an antihypertensive effect depends not only to interact with α_2-receptor, but also to gain entry into the CNS.[37]

Characteristic central effects of Clonidine are sedation, anxiolysis and hypnosis. Stimulation of α_2 adrenoceptors in locus ceruleus inhibits

neurotransmitter release which causes sedation.[32] The central action also reduces salivary flow, intestinal motor activity and gastric acid secretion. When administered by mouth preoperatively, it reduces the Minimum Alveolar Concentration (MAC) of inhalational anaesthetic agents.[38]

Clonidine mediates its analgesic effects primarily through the spinal dorsal horn α_2 adrenoceptors on primary afferents and interneurons, as well as the descending noradrenergic pathway.[39] It produces analgesia without respiratory depression and it is associated with dose dependant sedation, hypotension and bradycardia. These factors prolong local anaesthetic neuraxial blockade and provide immediate postoperative analgesia. Currently, clonidine is approved for epidural use for treatment of chronic pain.[40]

Administered as preoperative medication clonidine produces sedation and attenuation of the autonomic nervous system reflex responses [hypertension, tachycardia, catecholamine release] associated with preoperative anxiety and surgical stimulation. It also reduces the incidence of suspected or documented myocardial ischemia in patients with coronary artery disease.[41]

Adverse Effects:

Initially, sedation and dry mouth are encountered and usually subside in two to three weeks. It may aggravate depressive illness. In the early stage of treatment there may be a little fluid retention and weight gain which are usually transient. It commonly causes skin rashes and constipation. Other occasional side effects include dizziness, headache, nausea, euphoria and rarely, impotence.[42]

Therapeutic Indications:

- Hypertension

Major therapeutic use of clonidine is in the treatment of hypertension. Clonidine is especially useful in patients with renal failure since its duration of action is not appreciably altered by renal disease and it does not compromise renal blood flow.[43]

- Prophylaxis of migraine or recurrent vascular headache
- Treatment of menopausal hot flushes
- In management of severe cancer pain
- Anxiety disorders. Useful as a last line anxiolytic in patients unresponsive to standard treatment
- Atrial fibrillation

- ❖ Diarrhoea, especially in diabetic neuropathy
- ❖ Antipsychotic induced akathisia, tardive dyskinesia
- ❖ Growth retardation as it stimulates growth hormone secretion
- ❖ Attention deficit hyperactivity disorder as an adjunct to stimulant therapy
- ❖ Tourette's syndrome
- ❖ Symptomatic treatment of opioid and alcohol withdrawal
- ❖ Diagnosis of pheochromocytoma
- ❖ Clonidine is useful in selected patients receiving anaesthesia because it may decrease the requirement for anaesthetic and increase hemodynamic stability

Precautions:

Clonidine should be used with caution in patients with cerebrovascular disease, ischemic heart disease, peripheral vascular disorders, renal impairment, occlusive peripheral vascular disorders such as Raynaud's disease or those with a history of depression. Intravenous injections of clonidine should be given slowly to avoid a possible transient pressor effect.

Interactions:

Hypotensive effect of Clonidine may be enhanced by diuretics, other antihypertensives, and drugs that cause hypotension. Tricyclic antidepressants may antagonize the hypotensive effect. The sedative effect of Clonidine may be enhanced by CNS depressants.[35]

Animal models for evaluation of sedative activity [44]

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 locomotor activity testing with Actophotometer

Animal models for evaluation of hypnotic activity [44]

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 antianxiety activity [44]

1. Foot shock induced aggression
2. Isolation – induced aggression
3. Antianxiety test in mice
4. Anticipatory anxiety in mice
5. Social interaction in rats
6. Elevated plus maze test
7. Water maze test
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 behaviour in rats
15. Acoustic startle response in rats
16. Unconditioned conflict procedure
17. Plasma catecholamine level measurement during and after stress
18. Electrical stimulation of the brain

19. Pharmacologic manipulations (drug discrimination tests)[6]

- FG- 7142 induced anxiety
- Caffeine induced anxiety
- Yohimbine induced anxiety
- Flumazenil induced anxiety
- Pentylenetetrazole induced anxiety
- Piperoxane induced anxiety
- Cocaine induced anxiety

MATERIALS AND METHODS

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 15-09-2009 to 11-05-2010, after obtaining ethical clearance from the Institutional Animal Ethical committee, Madurai Medical College, Madurai.

Materials required for the study

1. Animals : Sixty inbred male albino mice

2. Drugs : Injection Diazepam

Injection Ketamine

Tablet Clonidine

Pentylenetetrazole dry powder

Water for injection

Normal saline

3. Instruments : Actophotometer

1. Animals:

Inbred male albino mice from Central animal house, Madurai Medical College were utilized in this study. Sixty male albino mice each weighing 18 to 25 grams were included in the study. Animals were allowed standard diet and tap water ad libitum.

2. Standard drug:

Injection Diazepam is mixed with normal saline to obtain a solution of concentration 0.01 mg/ml and is administered intraperitoneally at the dose of 1.5 mg/kg.[45]

3. Ketamine:

Injection Ketamine is mixed with water for injection to obtain a solution of concentration 10 mg/ml and is administered intraperitoneally at the dose of 100 mg/kg. [45]

4. Test drug:

Tablet Clonidine was dissolved in water for injection and administered intraperitoneally at graded doses of 0.05 mg/kg and 0.1 mg/kg. [6]

5. Pentylenetetrazole

Pentylenetetrazole was dissolved in cold saline and administered intraperitoneally at the dose of 5 mg/kg to induce anxiety.[46]

6. Water for injection:

Water for injection was administered intraperitoneally to control group of animals.

7. Normal saline:

Normal saline was used as a vehicle for diazepam and pentylenetetrazole.

8. Actophotometer:

The Digital Actophotometer 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) Testing of Spontaneous Locomotor Activity (SMA) using Actophotometer:

24 male mice each weighing 18-25 grams were grouped into four with six animals in each group. The total number of counts made by each animal in the actophotometer for a period of 10 minutes was observed. The control group of mice received water for injection intraperitoneally, standard group of mice received injection Diazepam 1.5 mg/kg intraperitoneally and the test groups of mice received Clonidine aqueous preparation 0.05 mg/kg and 0.1 mg/kg intraperitoneally.

Control group	Water for injection i.p.
Standard group	Inj. Diazepam 1.5 mg/kg i.p.
Test group I	Aqueous preparation of Clonidine 0.05 mg/kg i.p.
Test group II	Aqueous preparation of Clonidine 0.1 mg/kg i.p.

After 30 minutes of drug administration Spontaneous Motor Activity (SMA) for each animal for a period of 10 minutes was observed and the observations were tabulated and analyzed statistically using unpaired Student's t-test.

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

18 male albino mice were divided into 3 groups with 6 animals in each group. Prior to 30 minutes of administration of Ketamine 100 mg/kg intraperitoneally, the control group of animals received water for injection intraperitoneally, standard group of animals received injection Diazepam 1.5 mg/kg intraperitoneally and test group of animals received 0.1 mg/kg of aqueous preparation of Clonidine intraperitoneally which was the optimum dose that produced sedative effect.

Control group	Water for injection i.p. + Inj. Ketamine 100 mg/kg i.p.
Standard group	Inj. Diazepam 1.5 mg/kg i.p. + Inj. Ketamine 100 mg/kg i.p.
Test group	Aqueous preparation of Clonidine 0.1 mg/kg i.p.+ Inj. Ketamine 100 mg/kg i.p.

The time at which the righting reflex is lost was taken as onset of sleep and the duration between the time at which the righting reflex is lost and is regained was taken as duration of sleep.[45] The onset and duration of sleep were compared between test, control and standard groups. The results were tabulated and analyzed statistically using unpaired Student's t-test.

(III) Pentylenetetrazole induced Anxiety:

18 adult male naive mice weighing 18-25 grams were divided into three groups of 6 animals in each group namely control, standard, and test groups. 30 minutes prior to administration of pentylenetetrazole 5 mg/kg intraperitoneally, Control group of animals received water for injection intraperitoneally, Standard group of animals received injection Diazepam 1.5 mg/kg intraperitoneally and Test

group of animals received 0.1 mg/kg of aqueous preparation of Clonidine intraperitoneally which was the optimum dose that produced sedative effect.

Control group	Water for injection i.p. + Inj. Pentylenetetrazole 5 mg/kg i.p.
Standard group	Inj. Diazepam 1.5 mg/kg i.p. + Inj. Pentylenetetrazole 5 mg/kg i.p.
Test group	Aqueous preparation of Clonidine 0.1 mg/kg i.p. + Inj. Pentylenetetrazole 5 mg/kg i.p.

After pentylenetetrazole administration the animals were placed in isolation and observed individually for grooming activity. The number of bouts of grooming and the duration of grooming activity were observed for 10 minutes for each animal. The results were tabulated and analyzed statistically using Student's t-test.

RESULTS

RESULTS

In the present study, 60 naive male adult albino mice were selected and evaluated for sedative, hypnotic and anxiolytic effects. Sedative effect was evaluated by spontaneous locomotor activity in Actophotometer, Hypnotic effect by prolongation of Ketamine induced sleeping time and Anxiolytic effect was evaluated by pentylenetetrazole induced grooming.

Sedative effect:

Sedative effect was evaluated by using actophotometer. The spontaneous locomotor activity made by each mouse was noted in control, standard and test groups, 30 minutes after the administration of water for injection, diazepam and clonidine respectively. The average number of counts for control group of mice was 615.83 ± 10.25. The average number of counts for standard group of mice was 258.83 ± 94.36. The average number of counts for test group I was 564.67 ± 70.73.The average number of counts for test group II was 240 ± 57.23. The results were tabulated in Table I and analyzed using unpaired student's "t" test. The sedative effect was not statistically significant ($P > 0.05$) for the test group I in comparison with control group. The sedative effect was highly significant ($P < 0.001$) for both the standard and test group II in comparison with control group

TABLE I

SEDATIVE EFFECT

GROUP	TREATMENT	COUNTS AFTER 30 MIN (MEAN ± SD)
CONTROL	Water for Injection	615.83 ± 10.25
STANDARD	Diazepam	258.83 ± 94.36 ***
TEST I	Clonidine	564.67 ± 70.73 *
TEST II	Clonidine	240 ± 57.23 ***

Control Vs Test I - * P>0.05

Control Vs Standard & Test II - *** P<0.001

statistically. Hence the dose of aqueous preparation of clonidine 0.1 mg/kg was used in the test group for hypnotic effect and anxiolytic effect.

Hypnotic effect:

Hypnotic effect is measured by prolongation of Ketamine induced sleeping time. The onset and duration of sleep is compared between each group of animals. The average onset and duration in control group of mice is 115 ± 33.96 and 588.33 ± 103.28 seconds respectively. The average onset and duration in standard group of mice is 57.16 ± 9.54 and 1925.83 ± 286.3 seconds respectively. The average onset and duration in test group of mice is 61.5 ± 6.02 and 1103 ± 99.56 seconds respectively. The results were tabulated in Table II and analyzed using unpaired student's t-test. In comparison with control group of mice, the hypnotic effect is highly significant ($P<0.001$) for standard and test group of mice statistically.

TABLE II

HYPNOTIC EFFECT

GROUP	TREATMENT	ONSET (MEAN ± SD)	DURATION (MEAN ± SD)
CONTROL	Water for Injection + Ketamine	115 ± 33.96	588.33 ± 103.28
STANDARD	Diazepam + Ketamine	57.17 ± 9.54 ***	1925.83 ± 286.3 ***
TEST	Clonidine + Ketamine	61.5 ± 6.02 ***	1103 ± 99.56 ***

Onset : Control Vs Standard & Test - *** P<0.001

Duration: Control Vs Standard & Test - *** P<0.001

Anxiolytic effect:

The animals were observed individually for grooming activity after pentylenetetrazole administration. Anxiolytic effect was measured by reduction in the number of bouts and duration of grooming induced by pentylenetetrazole. Both the standard and the test group of animals showed reduction in the number of grooming bouts as well as duration of grooming. The average number and duration of bouts in control group of mice is 28.83 ± 9.13 and 51.33 ± 7.39 seconds respectively. The average number and duration of bouts in standard group of mice is 9.67 ± 4.03 and 24.67 ± 6.5 seconds respectively. The average number and duration of bouts in test group of mice is 11.83 ± 3.87 and 21 ± 2.83 seconds respectively. The results were tabulated in Table III and analyzed statistically using unpaired student's t-test. The results were highly significant ($P < 0.001$) for both test and standard groups in comparison with control group statistically.

TABLE III

ANXIOLYTIC EFFECT

GROUP	TREATMENT	NUMBER OF BOUTS (MEAN ± SD)	DURATION OF BOUTS (MEAN ± SD)
CONTROL	Water for Injection + Pentylenetetrazole	28.83 ± 9.13	51.33 ± 7.39
STANDARD	Diazepam + Pentylenetetrazole	9.67 ± 4.03 ***	24.67 ± 6.5 ***
TEST	Clonidine + Pentylenetetrazole	11.83 ± 3.87 ***	21 ± 2.83 ***

Bouts : Control Vs Standard & Test - *** P<0.001

Duration: Control Vs Standard & Test - *** P<0.001

DISCUSSION

DISCUSSION

Sedative, hypnotic, and anxiolytic drugs are commonly prescribed for the treatment of sleep and anxiety disorders. Their sedative, hypnotic and anxiolytic effects are also useful in anaesthetic practice as preanaesthetic medication. Benzodiazepines are commonly used drugs in preanaesthetic medication.

In the present study sedative, hypnotic and anxiolytic effects of clonidine is evaluated in comparison with diazepam in mice. From the results, it was observed that clonidine in the dose of 0.05 mg/kg had no significant sedative effect but showed significant sedative effect at the dose of 0.1 mg/kg in comparison with the control. Hence the same sedative dose of clonidine (0.1 mg/kg) was used in the test group for the evaluation of hypnotic and anxiolytic effects.

Hypnotic activity was evaluated by prolongation of ketamine induced sleeping time.

Clonidine exerts its sedative, hypnotic and anxiolytic effects by acting on the α_{2A} receptor subtype of adrenergic receptors which are present abundantly in the locus ceruleus nucleus.

α_{2A} receptor subtype is pivotal for both sedative and analgesic effects.

The quality of sedation produced by α_2 agonists differs from sedation produced by drugs that act on GABA receptors such as midazolam. Clonidine produces sedation by decreasing the sympathetic nervous system activity and the level of arousal. Clonidine lacks the psychotropic quality of benzodiazepines and causes a state of sedation more similar to normal sleepiness, where a patient can be easily aroused. Drugs that activate GABA receptors produce a clouding of consciousness and can cause paradoxical agitation as well as tolerance or dependence. Clonidine is devoid of respiratory depressant action and lacks the negative effects on cognition, memory and behaviour as seen with midazolam.[47]

In our study clonidine showed a significant prolongation of ketamine induced sleeping time. Several lines of evidence suggest that α_2 adrenergic activity is involved in the mechanism of anaesthesia induced sleeping time. So α_{2A} receptor is a possible anaesthetic target.

Clonidine also exerts additional beneficial effects during general anaesthesia. Norepinephrine release is increased in the posterior hypothalamus during emergence from sevofluorane or halothane anaesthesia. Norepinephrine release is modulated via presynaptic auto receptors. Activation of these receptors by norepinephrine or exogenously administered drugs like clonidine, inhibit the

release of norepinephrine and reduce the adverse effects due to sympathetic stimulation.

The results of the present study were in agreement with the study by Bernard Delbarre and Henri Shmitt, where clonidine induced a loss of righting reflex in chickens and prolonged the sleeping time induced by chloral hydrate in mice.[48]

Anxiolytic effect was evaluated by observing the grooming effect induced by pentylenetetrazole in the dose of 5 mg/kg intraperitoneally. Self grooming is an important part of animal behavioural repertoire, which in rodents has long been considered as a complex ethologically "rich" response, particularly sensitive to various endogenous or exogenous factors.

Grooming normally proceeds in a cephalocaudal direction and consists of licking the paws, washing movements over head, fur licking and tail/genitals cleaning. Pentylenetetrazole is a powerful CNS stimulant which causes convulsions in high doses and produces anxiety in low doses. Anxiety induces increased grooming activity in rodents.[49]

In this study there was a highly significant reduction in number and duration of grooming bouts with clonidine in comparison with the control statistically.

The results of this study were in agreement with the study by Tornatzky W, Miczek KA, where clonidine showed significant anxiolytic effect in rodents during acute social stress situation.[50]

In the present study clonidine showed significant sedative, hypnotic and anxiolytic effects. Several other studies have demonstrated the analgesic effect of clonidine which is due to its actions on α_2 adrenoceptors of dorsal horn. Hence combining clonidine with opioids will enable lower doses to be used while enhancing sedation and analgesia.[39]

All these properties make clonidine a very useful drug in premedication.

CONCLUSION

CONCLUSION

Sleep & anxiety disorders are common nowadays and hence several classes of sedative, hypnotic and anxiolytics are widely prescribed. Sedative, hypnotic and anxiolytic effects of these drugs are used in anaesthetic practice, mainly as preanaesthetic medication.

Benzodiazepines are the most commonly used sedative, hypnotic, and anxiolytic drugs. They are used as a slow inducing agent in general anaesthesia and also as a preanaesthetic agent. Benzodiazepines can cause several unwanted side-effects like tolerance, dependence and withdrawal symptoms. Acute overdose may result in respiratory depression.

α_2-adrenoceptor agonists in anaesthetic practice is limited despite an increasing evidence promoting their usefulness. The α_2-adrenoceptor agonist clonidine has been used for 30 years as a centrally acting antihypertensive agent, and also found to be useful in psychiatric disorders. It is gaining popularity in anaesthetic practice for the beneficial effects it exerts over a number of organ systems.

From the present study a significant sedative, hypnotic and anxiolytic effects of clonidine were proved to be present. Clonidine was found to have beneficial

effects like reduced bronchospasm in asthmatics, analgesia and a greater degree of cardiovascular stability by reducing the catecholamine levels and these properties are useful in anaesthesia. It also reduces the anaesthetic requirement by reducing Minimum Alveolar Concentration (MAC). Other possible benefits include decreased post operative shivering, inhibition of opioid induced muscle rigidity, and attenuation of opioid withdrawal symptoms.

Further clinical studies to evaluate the safety, will render clonidine a popular preanaesthetic agent, especially in situations causing adverse effects due to sympathetic release.

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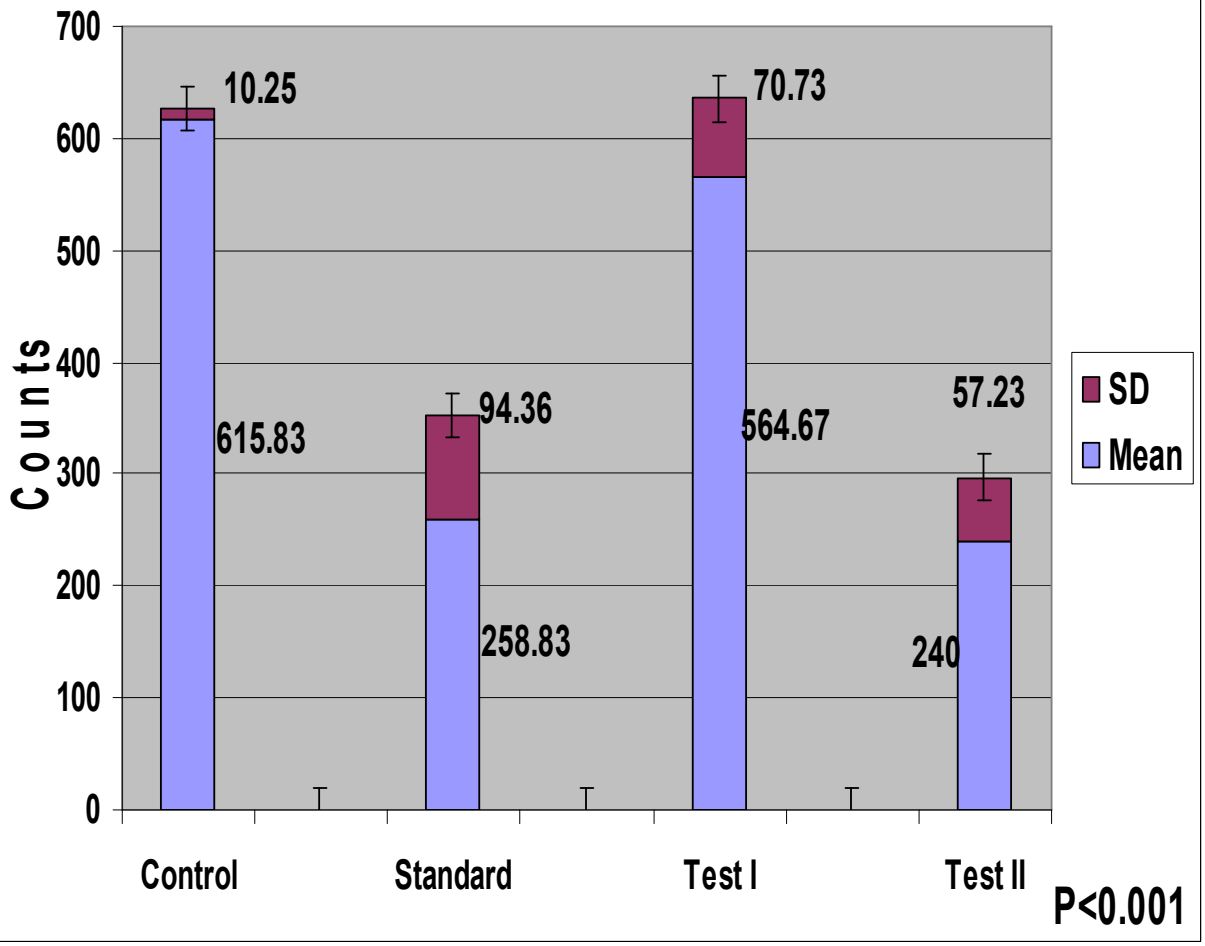
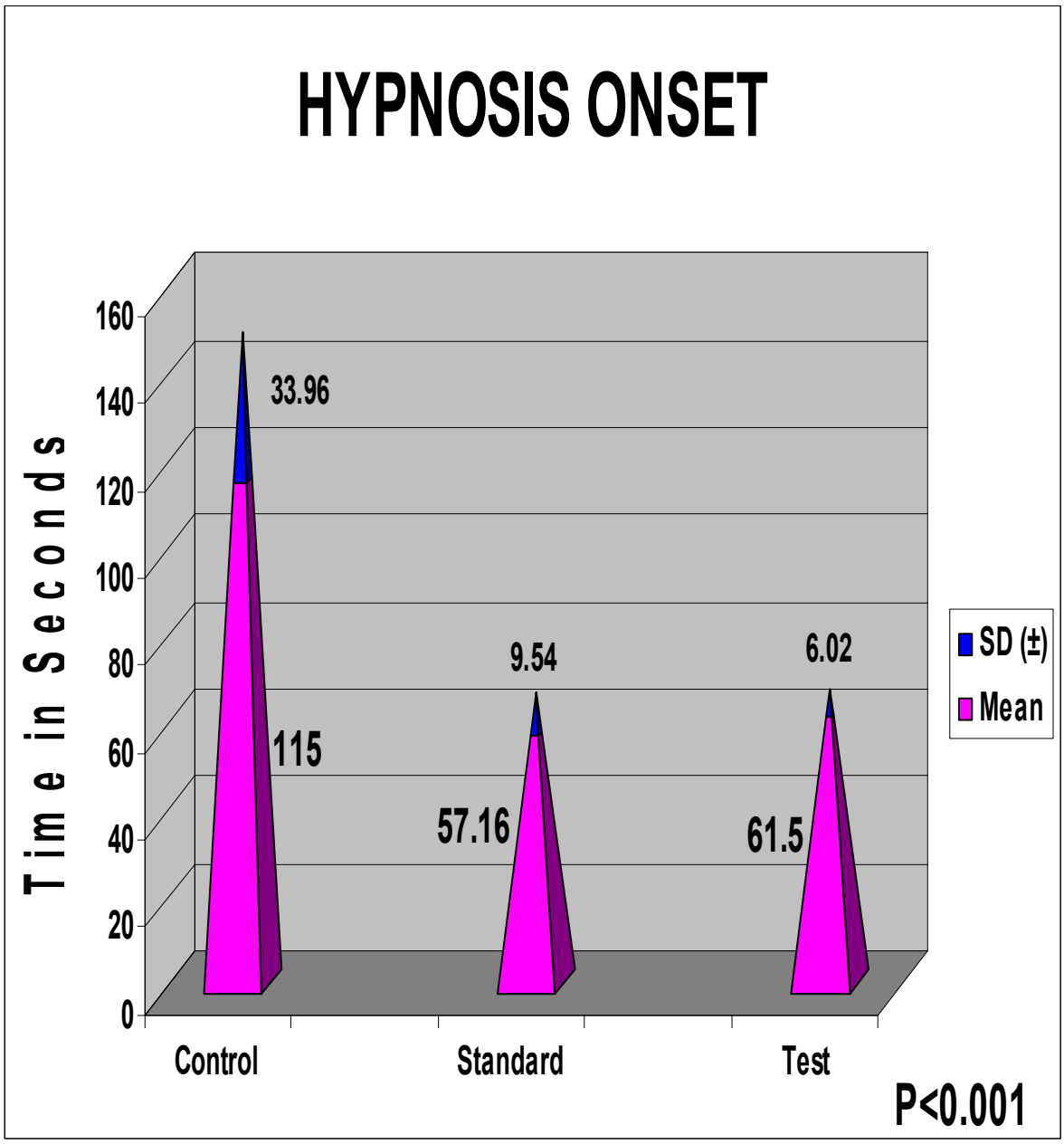
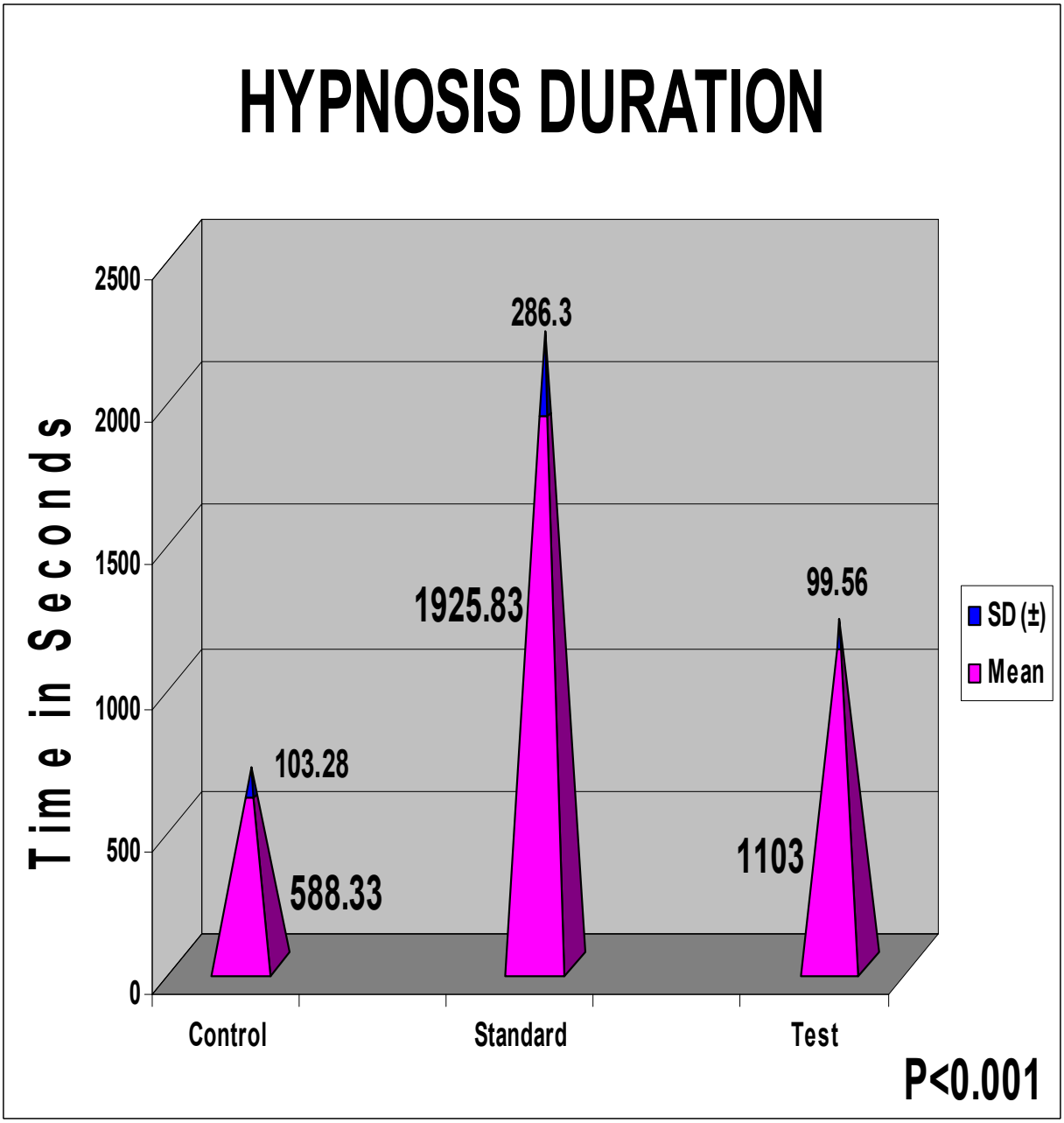
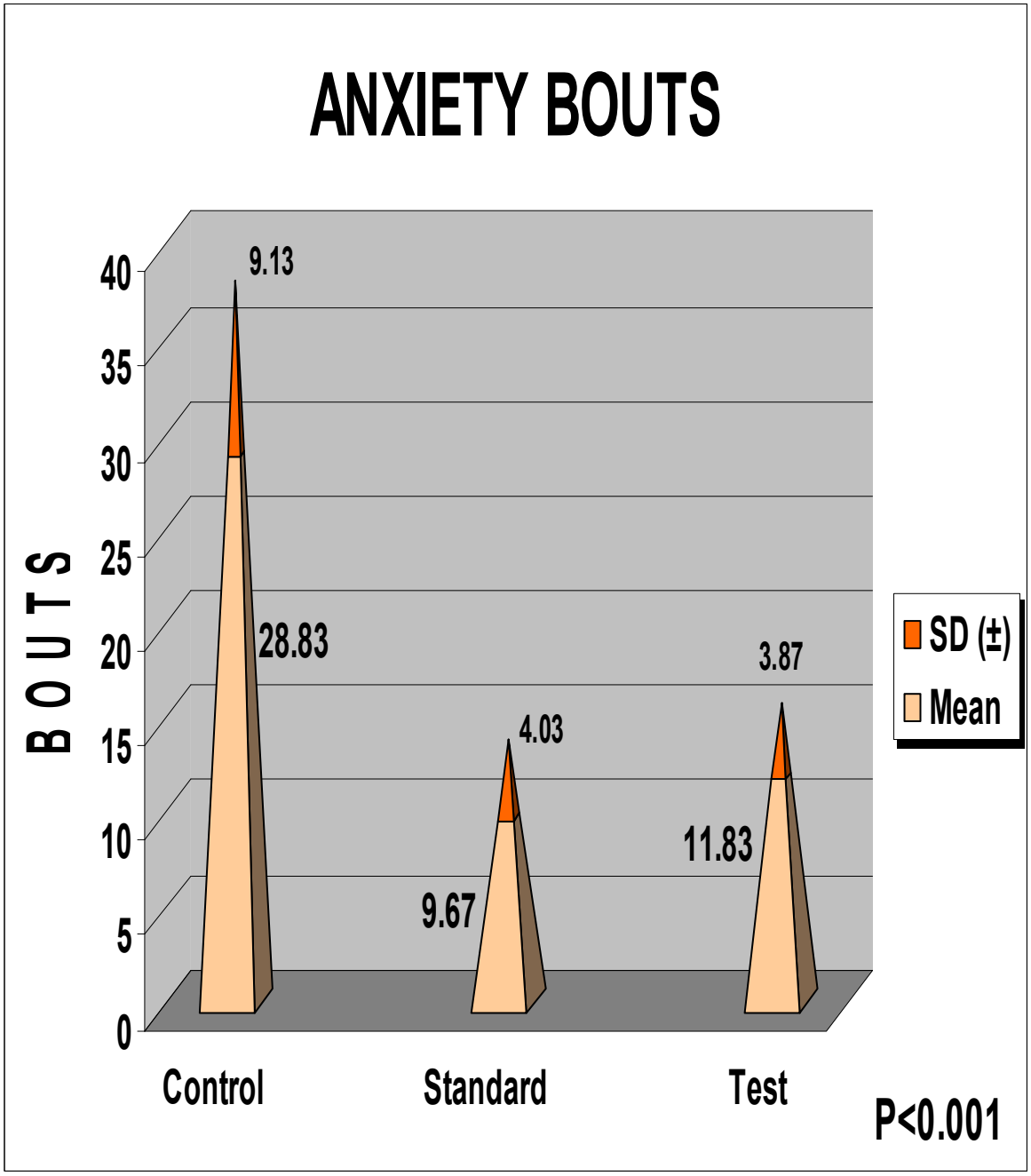
ANNEXURES

Spontaneous locomotor acivity in actophotometer

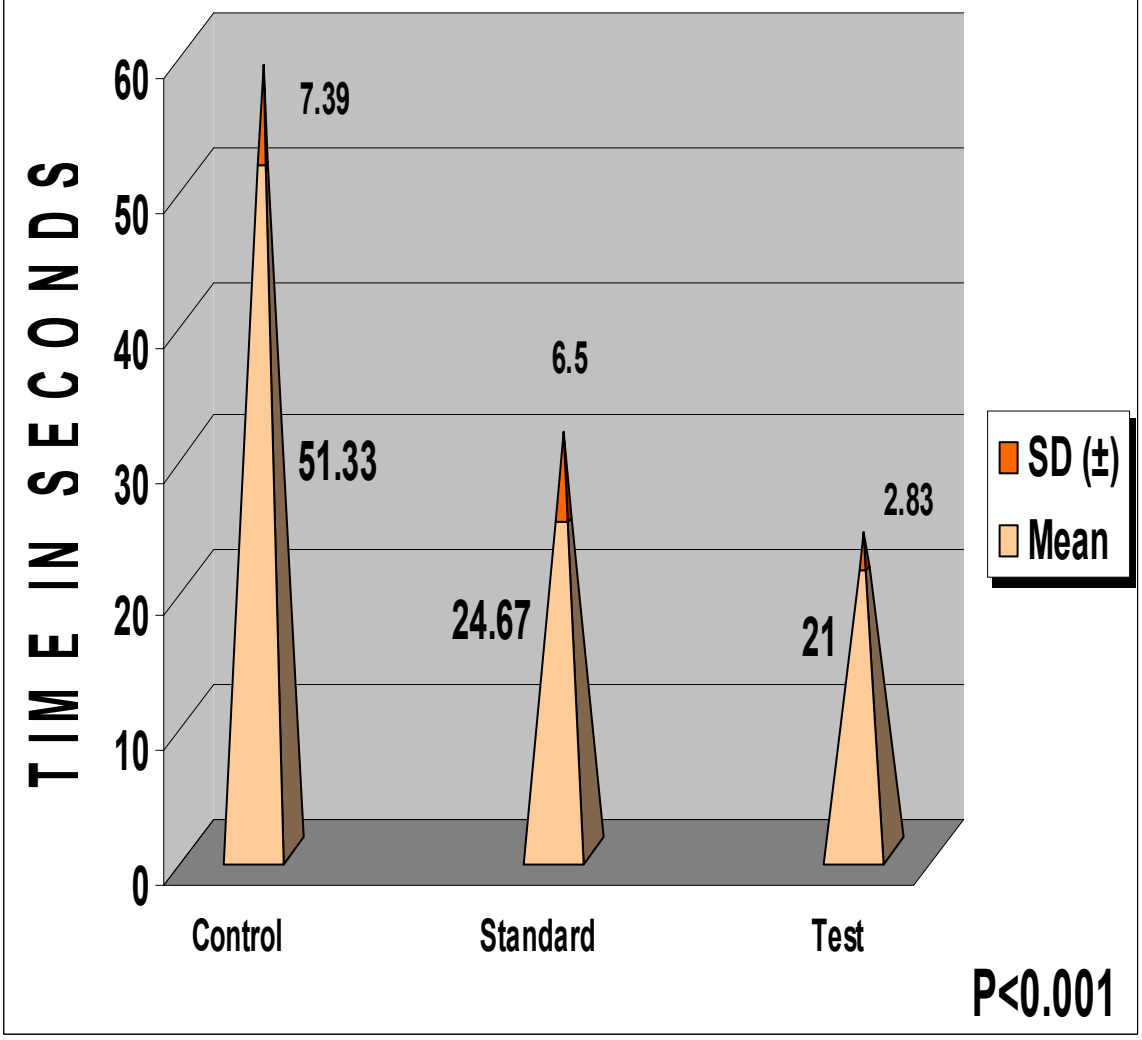



HYPNOSIS ONSET
Time in Seconds
160
140
120
100
80
60
40
20
0
33.96
115
9.54
57.16
6.02
61.5
SD (±)
Mean
Control
Standard
Test
P<0.001


HYPNOSIS DURATION
Time in Seconds
2500
2000
1500
1000
500
0
286.3
1925.83
99.56
103.28
588.33
1103
Control
Standard
Test
SD (±)
Mean
P<0.001


ANXIETY BOUTS
BOUTS
40
35
30
25
20
15
10
5
0
9.13
28.83
4.03
9.67
3.87
11.83
Control
Standard
Test
SD (±)
Mean
P<0.001

ANXIETY DURATION



ACTOPHOTOMETER

MICE UNDER KETAMINE ANAESTHESIA