NEUROPHARMACOLOGICAL PROPERTIES OF ETHANOL EXTRACT OF THE LEAVES OF *Olax subscorpioidea* OLIV. (OLACACEAE) IN MICE

BY

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CERTIFICATION

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DEDICATION

This work is dedicated to Almighty God

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ABSTRACT

Olax subscorpioidea is used in the management of mental illness, fever and pain in ethnomedicine. However, there is scanty information on the neuropharmacological activities that supports its use. The study was designed to investigate the neuropharmacological properties of Ethanol Extract of *Olax subscorpioidea* Leaves (EEOSL) in male mice.

Air-dried leaves (150 g) were pulverized and soaked in 50% ethanol (1.5 L) for 48 hours. The filtrate was concentrated and evaporated to dryness (8.7 g). Twenty-five Swiss male albino mice (20-22 g) were allotted into five treatment groups viz: control (distilled water), and EEOSL (3.1, 6.3, 12.5, 25 mg/kg) with five animals in each group. They were pretreated thirty minutes intraperitoneally (i.p.), before neurobehavioural effects of EEOSL on novelty-induced behaviours (rearing and grooming) and frequency of head dips were investigated using open-field and hole-board tests respectively. Another twenty male mice (22-25 g) divided into four treatment groups: control (distilled water) and EEOSL (12.5, 25, 50 mg/kg). Thirty minutes after i.p. treatment, pentobarbitone-induced sleeping time was investigated. For analgesic study, eighty male mice (22-25 g) were allocated into four treatment groups: control (distilled water), and EEOSL (12.5, 25, 50 mg/kg) with five animals in each group. Thirty minutes after i.p. treatment they were subjected to acetic-acid induced writhing, formalin, tail immersion and hot plate tests. Similarly, for antidepressant study, another set of eighty male mice (22-25 g) were randomly allotted into four treatment groups: control (distilled water), and EEOSL (6.3, 12.5, 25 mg/kg) with five animals in each group. Thirty minutes after i.p. treatment, animals were subjected to despair, tail suspension, reserpine-induced depression, and yohimbine lethality tests. Data were analysed using descriptive statistics and ANOVA at $p = 0.05$.

The EEOSL (3.1, 6.3, 12.5, 25 mg/kg) significantly inhibited rearing (99.8±2.8, 76.2±2.9, 37.4±1.2, 5.8±0.8) and grooming (48.0±3.6, 33.8±2.9, 25.4±1.6, 7.6±0.8) compared with controls (185.8±5.1; 63.8±4.3) respectively. Treatment with EEOSL (3.125, 6.25, 12.5, 25 mg/kg) significantly decreased the frequency of head dips on hole-board (10.6±1.9, 8.8±1.2, 7.2±0.9, 6.0±1.1) compared with control (27.8±1.5). The EEOSL (12.5, 25, 50 mg/kg) significantly prolonged pentobarbitone-induced sleeping time (43.0±1.4, 51.0±1.2, 61.0±1.8) compared with

control (31.0±0.7). The EEOSL (12.5, 25, 50 mg/kg) significantly inhibited acetic acid-induced pain by 66.9%, 72.7% and 81.5% respectively. In formalin test, EESOL (12.5, 25, 50 mg/kg) inhibited neurogenic (41.1%, 63.1%, 66.0%) and inflammatory (57.1%, 75.3%, 79.4%) pains. Reaction times were prolonged only by EEOSL (50 mg/kg) in hot plate (0.9±01) and tail immersion (2.1±0.2) compared with controls (0.6±0.1; 0.5±0.0) respectively. The EEOSL (6.3, 12.5 mg/kg) significantly reduced immobility periods in despair (124.2±4.5, 85.2±6.0) and tail suspension tests (110.4±6.8, 68.0±15.9) compared with controls (190.2±15.3; 155.6±8.9) respectively. In reserpine-induced depression, EEOSL (6.3, 12.5, 25 mg/kg) reduced significantly the feacal matter (2.4±0.9, 1.2±0.6, 1.2±0.4) compared with control (8.2±0.9). However, EEOSL did not potentiate yohimbine-induced lethality.

Olax subscorpioidea possessed sedative, antidepressant and analgesic properties. These therefore support its traditional claims in the management of mental illness and pain.

Keywords: *Olax subscorpioidea*, Neurobehavioural, Analgesic, Antidepressant.

Word count: 479

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CHAPTER ONE

1.0 INTRODUCTION

For centuries, plants have provided man with an array of products crucial to social-economic life. Medicinal plants in particular have been highly valued and used regularly for thousands of years by the people of the world as the medicine of the masses. Man has always searched for that herb that heals the body and soothes the mind and there has never been a shortage of vegetation to investigate with some 20,000 species that have been used by various cultures. Medicinal plants have been used to treat psychiatric and behavioral conditions such as anxiety, depression, seizures, dementia and insomnia (Klemens, 2006).

However, as western medicine evolved through the twentieth century, people wanted to swallow pill of a concentrated "active ingredient," or, perhaps a synthetic pharmaceutical equivalent. As a result, potentially valuable herbal formulation may not have been investigated and are forever lost owing to a dramatic substitution of herbs for orthodox drugs. The stigma attached to substance use and abuse also contributes to insufficient documentation and scientific investigation of African psychotropic plants, thus resulting to low priority ascribed to medicinal plants and hence silence and loss of research interest in psychoactive plants (Carlini, 2003). Carlini (2003) reported that "most psychoactive plants first used by the so-called primitive cultures were relegated by European occidental culture to a second plan and considered them as sorcerer's therapeutics and often viewed in a negative light". This impression has led to the abandonment of the endowment of nature for therapeutic remedies.

Although current drugs used for the treatment of these disorders have gained popularity among the users, there is still a serious and growing concern on their therapeutic effectiveness. The prevalence of mental illness is on the rise with the quality of life of the victims perpetually declining. The drugs and synthetic equivalents have been said to be symptomatic in action without affecting or addressing the pathological basis of the conditions (Klemens, 2006).

This is a serious threat to the quality of life and wellness as the World Health Organization in 2003, predicted that one in four people in the world will be affected by mental disorders at some point in their lives and that 10% of the world's population suffers from depression. Studies have

shown that about 7% of Americans will suffer from schizophrenia at some point in their lifetimes and about 2.5 million will suffer from bipolar disorder (Klemens, 2006).

Interestingly, some psychoactive plants have provided valuable insight into the neurochemistry of many central nervous system disorders (Lewin, 1924; Nichols, 2004). Lysergic acid diethylamide (LSD) is the analogue of ergot alkaloids produced by *Claviceps purpurea*. The observation that serotonin and lysergic acid diethylamide (LSD) share structural and pharmacological properties has led to the suggestion that biogenic amines, like serotonin, are involved in mental disorders such as schizophrenia (Gaddum and Hameed, 1954; Woolcy and Shaw, 1954). Also, the discovery that the depletion of biogenic amines by the active ingredient in *Rauwolfia serpentina* (reserpine) induce depression, which suggests that lack of serotonin and/or noradrenalin as the underlying factor(s) in the pathophysiology of depression (Vertulani and Sulser, 1975), has caused rebirth of interest in investigating psychoactive plants. Current basic understanding of mental illness as neurochemical diseases, as well as science's ability to, treat these disorders has been greatly enhanced through the study of psychoactive plants (Gary 2009). These observations and shortcomings of the current drugs have provided basis for exploring the nature for more promising and novel drugs.

As orthodox medicine does not offer total therapeutic solution to various health challenges, search for alternative to the treatment of some psychiatric conditions becomes inevitable. Presently there is a resurgence of herbal medicine as people want more control in their personal healthcare.

The World Health Organization in 2001 estimated that eighty percent of the world population use medicinal plants in the treatment of diseases. In African countries, this rate is said to be much higher. It was also estimated that up to 90% of the population in developing countries rely on the use of medicinal plants to help meet their primary health care needs (WHO, 2002). As noted by Okafor and Ham (1999), dependence on indigenous plants as medicine is due to unavailability of western medicine or high cost of it. Available report showed that more than 300 distinct ethnic groups making up the Nigerian society has its own unique indigenous healing heritage, which has evolved in response to the specific experiences and needs of its people. Currently, it is estimated that traditional medicine is the only healthcare resource accessible to a third of all Nigerians (Ogunbodede, 1997)

The United State herbal market is growing tremendously with consumer demand way ahead of regulatory agencies. It is interesting to note that four (Ginkgo, St. John's Wort, Valerian, Kava) of the top ten herbs purchased in the U.S. (according to 1999 Whole Foods Survey) have psychotropic activity. Nature has blessed Africa continent with herbs to meet their primary health care. According to Klemens (2006), medicinal plants have been used to treat behavioural conditions like anxiety, depression, seizures, dementia, insomnia, and drug intoxication and may well provide an alternative to the treatment of some psychiatric conditions. Among the notable ones (herbs) used in psychiatric condition in African folkloric medicine is *O. subscorpioidea*.

O. subscorpioidea locally called 'ifon' belongs to family olaceaceae. It is a shrub distributed in Nigeria and some parts of Africa. *Olax subscorpioidea* is used in the management of mental illness, fever, convulsion and pain in traditional medicine. However, there is scanty information on the neuropharmacological activities that supports its use. The study was designed to investigate the neuropharmacological properties of ethanol extract of *O. subscorpioidea* leaves (EEOSL) in mice.

1.1 OBJECTIVES

The specific objectives of the study are to:

- determine the acute toxicity profile of the extract;
- evaluate neurobehavioural properties of *Olax subscorpioidea*;
- examine analgesic property of *Olax subscorpioidea*;
- evaluate antidepressant property of *Olax subscorpioidea*, and
- assess anticonvulsant property of *Olax subscorpioidea*.

CHAPTER TWO

LITERATURE REVIEW

2.0 MENTAL ILLNESS

Mental illness is a psychological, neurological or behavioural disorder pattern that occurs in an individual that is not expected as part of normal development. Although, the definition, assessment and classification can vary and there is no single accepted or consistent cause of mental disorders. However, they are explained in terms of a Diathesis-stress model an Biopsychosocial model. Services for mental disorders may be based in hospitals or in community.

Mental health professionals diagnose individuals using different methodologies: often relying on case history and interview. Any of these disorders can arise from a combination of environmental, biological and psychological sources. Some biological factors include genetics: it is often found that people who have or had a mental disorder within their family are more susceptible to developing a mental disorder. Other factors include, infections, prenatal damage lack of oxygen to the brain. Substance abuse can lead to addiction or dependence which is the disorder characterized by a pattern of continued pathological use of a medication. Non-medically indicated drug or toxin results in repeated adverse social consequences related to drug use, such as failure to meet work, family or school obligations, interpersonal conflicts or legal problems There are ongoing debates as to the exact distinction between substance abuse and substance dependence, but current practice standard distinguishes the two by defining substance dependence in terms of physiological and behavioural symptoms of substance use, and substance abuse in terms of the social consequences (Pham-Kanteret et al., 2001). Medically, physiologic dependence requires the development of tolerance leading to withdrawal symptoms. Both abuse and dependence are distinct from addiction which involves a compulsion to continue using the substance despite the negative consequences, and may or may not involve chemical dependency. Dependence almost always implies abuse, but abuse frequently occurs without dependence, particularly when an individual first begins to abuse a substance. Dependence involves physiological processes while substance abuse reflects a complex interaction between individuals, the abused substance and society (Blakemore et al., 2007). Psychological and

environmental causes are found when the person concerned has experienced a trauma or abuse. Psychotherapy and psychiatric medication are two major treatment options, as well as supportive intervention and self help (Blakemore et al., 2007).

2.1 TYPES OF MENTAL DISORDERS

There are many different categories of mental disorder and many different facets of human behaviour and personality that can become disordered. Some of these categories include;

Obsessive Compulsive Disorders: Constant stressful thoughts (obsession) and a powerful urge to perform repetitive acts, such as hand washing (compulsion).

Depression: The person concerned is no longer interested in and does not enjoy previous activities that he got pleasure from. There are extreme or prolonged periods of sadness.

Anxiety: The sufferer has a severe fear which is linked to certain objects or situation. Most people with this disorder will try to avoid exposure to whatever triggers their anxiety.

Post Traumatic Stress: This can occur after somebody has been a traumatic event: something horrible and scary that the person sees or that happens. During this type of event, the person thinks that he or other people's lives are in danger. The sufferer may feel afraid or feel that there is control over what is happening.

Dysthemia: The patient has a mild chronic depression. A chronic feeling of ill being and/or lack of interest in activities that the patient once enjoyed.

Bipolar Disorder: Also known as maniac-depressive illness. The sufferer oscillates from episodes of euphoria (mania) and depression (despair).

Epilepsy: It is a major neurological disorder characterized by recurrent, spontaneous brain seizures

2.2 DEPRESSION

Depression is an affective disorder characterized by change in mood, loss of interest or pleasure, feelings of guilt or low self-worth, disturbed sleep or appetite, low energy, psychomotor retardation, melancholia, intense sadness and despair, mental slowing and loss of concentration, pessimistic worry, and variable agitation or hostility. Physical changes also occur, particularly in severe, vital, or melancholic depression. These include insomnia or hypersomnia; altered eating patterns, with anorexia and weight loss or sometimes overeating; decreased energy and libido; and disruption of the normal circadian and ultradian rhythms of activity, body temperature, and many endocrine functions (Tondo et al., 2003). Dysthymic disorder, also called dysthymia, psychotic depression, postpartum depression (Altshuler et al., 1998) and seasonal affective disorder are also kinds of depression (Rohan et al., 2004). It is a major health condition with life time prevalence in the range of 10-15% (Lépine and Briley, 2011). World Health Organization (1998) and Richelson et al., (2001) estimated that 5.8% of men and 9.5% of women undergoing a depressive episode in their life time mostly end up committing suicide. Its negative social impact and impairing effect on daily activities and wellbeing has led to incapability and loss of productivity (Ebmeier et al., 2006).

Depression is an heterogeneous disorder with a highly variable course, an inconsistent response to treatment, and no established mechanism. Some features of depressive disorder overlap those of the anxiety disorders, including severe phobias, generalized anxiety disorder, social anxiety disorder, post traumatic stress disorder, and obsessive-compulsive disorder (America Psychiatric Association, 2000). The major disorders of mood or affect include the syndromes of major depression (formerly termed melancholia) and bipolar disorder (formerly termed manic-depressive disorder). There is no single known cause of depression. Rather, it likely results from a combination of genetic, biochemical, environmental, and psychological factors. Some types of depression tend to run in families, suggesting a genetic link. However, depression can occur in people without family histories of depression as well (Tsuang and Faraone, 1990).

Studies comparing concordance rates for major depression between monozygotic and dizygotic twins suggest a heritability of about 37%, which is much lower than the heritability of bipolar disorder or schizophrenia. Some aspects of the normal personality, such as avoidance of harm, anxiousness, and pessimism, are also partly heritable. Kendler et al., (2006) showed that

although depression is due in part to heritable depression prone personality traits, it is also the result of heritable factors that are independent of personality. Early-onset, severe, and recurrent depression may have a higher heritability than other forms of depression (Kendler et al., 2006). It is clear from studies of families that major depression is not caused by any single gene but is a disease with complex genetic features.

Studies of pedigrees with multiple cases of major depression have identified chromosomal regions with linkage to the disorder. and some of these loci have been replicated in more than one study, although no single chromosomal region has been replicated in every family study of genetic linkage in depression.

2.2.1 MONOAMINES AND THEIR ROLE IN DEPRESSION

The monoamine deficiency hypothesis posits that depressive symptoms arise from insufficient levels of monoamine neurotransmitters 5-HT, NE, and/or DA. This hypothesis grew out of observations that antidepressant therapies raise neurotransmission tone depending on one or more of these neurotransmitters. Considerable experimental and clinical evidence support the fundamental role of NA and 5-HT in the etiology of depression (Elhwuegi, 2004). There are four classes of standard antidepressants, monoamine oxidase (subtype-A) inhibitors (MAOIs), tricyclic antidepressants (TCAs), serotonin reuptake inhibitors (SSRIs) and noradrenaline (NA)-serotonin reuptake inhibitors. All these antidepressant drugs increase acutely the availability of these monoamines at the synapse (Baldessarini, 1989). It is well established that neurotransmitter transporters have roles in several neurological and psychiatric diseases. This is directly supported by several examples of naturally occurring mutations in the transporter genes that cause or increase the risk of developing certain diseases. Examples include mutations in the gene encoding SERT that are associated with symptoms of obsessive-compulsive disorder, Asperger's syndrome, anorexia and autism (Kilic et al., 2003).

2.2.2 MONOAMINE TRANSPORTERS

The trans-membrane transport of neurotransmitters is of primary importance for proper signaling between neurons. The transport processes are mediated by distinct classes of membrane transport protein that have key roles in controlling the neurotransmitter concentration in the synaptic cleft (Gether et al., 2006). These transporters can be classed as intracellular vesicular transporters that are responsible for sequestering transmitters from the cytoplasm into synaptic vesicles, and

plasma membrane transporters that are responsible for sequestering released transmitter from the extracellular space. There are three subclasses of intracellular transporter the vesicular amine transporters (SLC18) gene family. the vesicular inhibitory amino acid transporter family (SLC32) and the vesicular glutamate transporters (SLC17) gene family.

There are two major subclasses of plasma membrane transporter: the high-affinity glutamate transporters (SLC1) gene family and the Na+/Cl--coupled transporters (SLC6) gene family. The latter subclass is the largest and includes transporters of dopamine. serotonin. noradrenaline. glycine and GABA.

As mentioned previously the monoamine transporters are of therapeutic significance as targets of antidepressants. Classical tricyclic antidepressants target primarily NAT and/or SERT; selective 5-HTreuptake inhibitors (SSRIs) are specific inhibitors of SERT. and 5-HT–norodrenaline-reuptake inhibitors (SNRIs) are active at both SERT and NAT (Leonard. 1997)

2.2.3 LINK BETWEEN THE SEROTONERGIC AND NORADRENERGIC SYSTEMS

Chronic administration of antidepressants enhances the inhibitory response of forebrain neurons to microiontophoretically applied 5-HT. This enhanced response is blocked by lesions of the noradrenergic projections to the cortex. This dual effect could help to explain enhanced serotonergic function that arises after chronic administration of antidepressants. Conversely, impairment of serotonergic function by means of selective neurotoxins (e.g. 5, 7-dihydroxytryptamine) or a 5-HT synthesis inhibitor (e.g. parachlorophenylalanine) largely prevents the decrease in functional activity of cortical β-adrenoceptors that usually arises following chronic antidepressant treatment. 5-HT_{1b} receptors are located on serotonergic nerve terminals and act as autoreceptors; stimulation by serotonin decreases the further release of this amine. Chronic administration of selective SSRIs slowly desensitizes the inhibitory 5-HT_{1b} autoreceptors and thereby enhances serotonin release (Tome et al., 1997).

Likewise, the 5-HT_{1a} somatodendritic receptors inhibit the release of serotonin and it is postulated that the enhanced release of the transmitter following chronic administration of SSRIs is a consequence of adaptive down-regulation of the inhibitory 5-HT_{1a} receptors (Blier et al., 1990). The validity of this hypothesis is supported by the pharmacological effect of 5-HT_{1a}

antagonists. Thus, the beta-blocker and 5-HT_{1a} antagonist pindolol, in combination with fluoxetine or paroxetine, may enhance the therapeutic efficacy of the SSRI and, in some studies, reduce the time of onset of the peak therapeutic effect (Tome et al., 1997). However, several investigators have not been able to replicate such findings (McAskill et al., 1998).

Serotonin can also regulate dopamine turnover. Stimulation of the 5-HT cell bodies in the median raphe causes reduced firing of the substantia nigra, where dopamine is the main neurotransmitter. Serotonin plays an important role in modulating dopaminergic function in many regions of the brain, including the mesolimbic system. The effects of some antidepressants that show an apparent selectivity for the serotonergic system could be ascribed to secondary changes in dopaminergic pathways (McAskill et al., 1998).

2.2.4. ROLE OF THE GLUTAMATERGIC SYSTEM IN THE ACTION OF ANTIDEPRESSANTS

The glutamatergic system is the main excitatory neurotransmitter pathway in the brain. Tricyclic antidepresssants (TCAs) inhibit the binding of dizolcipine to the ion channel of the main glutamate receptor, the NMDA-receptor in the brain (Reynolds and Miller, 1988). Newer antidepressants have a qualitatively similar effect (Kitayama et al., 1997). Whether this is due to direct action on glutamate receptor sites or indirect action via the glycine receptor site is uncertain. Glycine and drugs modulating the glycine site show antidepressant-like activity in animal models of depression. Thus, antidepressants may act as functional NMDA receptor antagonists.

2.2.5. INTRACELLULAR CHANGES THAT OCCUR FOLLOWING CHRONIC ANTIDEPRESSANT TREATMENT

The coupling of the NDMA-receptor on the cell surface to the intracellular second messenger is brought about by a member of the G-protein family. Beta-adrenoceptors are linked to adenylate cyclase and, depending on the subtype of receptors, 5-HT is linked to either adenylate cyclase (5-HT_{1a}, 5-HT_{1b}) or phospholipase (5-HT_{2a}, 5-HT_{2c}). Activation of phospholipase results in an intracellular increase in the secondary messengers diacylglycerol and inositol triphosphate (IP3).

the IP3 then mobilising intraneuronal calcium. The net result of the activation of the secondary messenger systems is to increase the activity of the various intracellular protein kinases (termed third messengers) that phosphorylate cell proteins to produce a physiological response. Racagni et al (1991) have investigated the effect of chronic antidepressant treatment on the phosphorylation of proteins associated with the cytoskeletal structure of the nerve cell. Antidepressants could affect the function of the cytoskeleton by changing the component of the associated protein phosphorylation system. Thus, both typical (e.g. desipramine) and atypical antidepressants (e.g. (+) oxaprotiline, a specific noradrenaline reuptake inhibitor, and fluoxetine, an SSRI) increased the synthesis of a microtubule fraction. These changes occurred only after chronic antidepressant treatments. Antidepressants might thereby change neuronal signal transduction processes distal to the receptor (Racagni et al., 1991).

2.2.6. GLUCOCORTICOID RECEPTORS: ADAPTIVE CHANGES FOLLOWING ANTIDEPRESSANT TREATMENT

Glucocorticoid receptors have been identified in the nuclei of catecholamine and 5-HT-containing cell bodies in the brain. Glucocorticoid receptors activate deoxyribonucleic acid-binding proteins, which can modify the transcription of genes. Chronic administration of imipramine increases glucocorticoid receptors in rat brain, particularly the noradrenergic and serotonergic cell body regions (Mansbach et al., 1997).

Lymphocyte glucocorticoid receptors are subsensitive in patients with depression. The failure of the negative feedback mechanism that regulates the secretion of adrenal glucocorticoids further suggests that the central glucocorticoid receptors are subsensitive. Hypersecretion of cortisol is a characteristic feature of many patients with major depression (Dinan, 1994). Central neurotransmission occurring in depression may, in part, result from the effects of chronic glucocorticoids. Glucorticoid synthesis inhibitors (e.g. metyrapone) may be able to reduce the abnormality in neurotransmitter function by decreasing the cortisol concentration.

Typical antidepressants increase the density of glucocorticoid receptors in rats. Such an effect could increase the negative feedback mechanism and thereby reduce the release of cortisol. In support of this hypothesis, there is preliminary clinical evidence that metyrapone (and the steroid synthesis inhibitor ketoconazole) may have antidepressant effects. Recently, several lipophilic antagonists of corticorophin-releasing factor (CRF) type 1 receptor, which appears to be hyperactive in the brain of patients with depression, have been shown to be active in animal models of depression. Clearly, this is a potentially important area for antidepressant development (Mansbach et al., 1997).

Glucocorticoid receptors are present in high density in the amygdala, and neuroimaging studies have shown that the amygdala is the only structure in which the regional blood flow and glucose metabolism consistently correlate positively with the severity of depression (Lesser et al., 1994). This hypermetabolism appears to reflect an underlying pathological process as it also occurs in asymptomatic patients and in the close relatives of patients.

2.2.7 EFFECT OF ANTIDEPRESSANTS ON ENDOCRINE-IMMUNE FUNCTIONS

Stress is frequently a trigger factor for depression in vulnerable patients. Corticotrophin-releasing factor is elevated in the CSF of untreated patients with depression, which presumably leads to the hypercortisolaemia that usually accompanies the condition. One of the consequences of elevated plasma glucocorticoids is a suppression of some aspects of cellular immunity. Many aspects of both cellular (for example, natural killer cell activity, T-cell replication) and non-cellular (for example, raised acute phase proteins) immunity are abnormal in untreated patients with depression (Song and Leonard, 1995). This may contribute to the susceptibility of patients with depression to physical ill health.

A link between CRF, the cytokines that orchestrate many aspects of cellular immunity and the prostaglandins of the E series has been the subject of considerable research in recent years. Prostaglandin E2 (PGE2) concentrations are raised in the plasma of untreated patients with depression and are normalised following effective treatment with TCAs. Raised PGE2

concentrations reflect increased proinflammatory cytokines (particularly interleukins 1 and 6 and tumour necrosis factor), which occur as a consequence of increased macrophage activity in the blood and brain. In the brain, the microglia function as macrophages and produce such cytokines locally. Thus, the increased synthesis of PGE2 may contribute to the reduction in amine release in the brain that appears to underlie the pathology of depression. It has recently been postulated that antidepressants normalise central neurotransmission by reducing brain concentrations of both the cytokines and PGE2 by inhibiting central and peripheral macrophage activity together with cyclooxygenase type 2 activity in the brain. Cyclooxygenase is the key enzyme in the synthesis of the prostaglandins. The usefulness of TCAs in severe rheumatoid arthritis may reflect the inhibitory action of such drugs on cyclooxygenase activity. These effects of TCAs, together with those in glucocorticoid receptor function, may thus normalise defective central neurotransmission (Song and Leonard, 1995).

2.2.8 ANTIDEPRESSANTS AND CHANGES IN NEURONAL STRUCTURE

Another possible mechanism whereby antidepressants may change the physical relationship between neurons in the brain is by inhibiting neurite outgrowth from nerve cells. Amitriptyline inhibits neurite outgrowth from chick embryonic cerebral explants *in vivo* (Wong et al., 1991). A common mode of action of all antidepressants could be to modify the actual structure of nerve cells and possibly eliminate inappropriate synaptic contacts that are responsible for behavioural and psychological changes associated with depression.

It is also possible that chronic antidepressant treatment may affect pathways that involve receptor interactions with protein tyrosine kinases, by increasing specific growth factor synthesis or by regulating the activity of proinflammatory cytokines (Duman et al., 1997). These pathways control many aspects of neuronal function that ultimately underlie the ability of the brain to adapt and respond to environmental stimuli. The infusion of one of these transcription factors (brain-derived neutrophic factor) into the mid-brain of rats results in antidepressant-like activity (Siuciak et al., 1996), an action associated with an increase in the synthesis of tryptophan hydroxylase, the rate limiting enzyme in the synthesis of serotonin.

2.3 PAIN

Pain is one of the common symptoms in medicine and is said to be the prime cause of one third of all first consultations. While curing the causative condition usually relieves the pain, it may on the other hand continue beyond its diagnostic usefulness, either because the disease is itself incurable, or because irreversible anatomical changes lead to continuing noxious stimulation (Bowsher, 1987). Acute and chronic pain control is now a major concern especially with population aging and associated pain of the chronic degenerative conditions of the elderly such as osteoarthritis, post-herpetic neuralgias, trigeminal neuralgia, reflex sympathetic dystrophy, 'thalamic pain syndrome' and malignant diseases. Thus in an aging population the medical, social, and economic consequences of chronic pain may be expected to increase (Bowsher, 1987).

2.3.1 PAIN AND THRESHOLDS

Pain is not a simple, straightforward sensory experience, in the manner of seeing or hearing, as it has both emotional and physical components (Baldry, 1993). The definition of pain recommended by the International Association for the Study of Pain is that it is an unpleasant sensory and emotional experience associated with actual or potential tissue damage (Merskey 1979). For a given noxious stimulus the intensity with which pain is felt varies from person to person, and with regard to this a distinction has to be made between an individual's pain threshold and pain tolerance (Baldry, 1993). The pain threshold, like other sensory thresholds, is fairly constant, but pain tolerance level defined as the amount of pain a subject is prepared to put up with, varies enormously and clinically patients do not usually seek medical advice until they are beyond pain tolerance level, that is the degree of pain within which an individual can usefully be measured by using a visual analogue pain scale (Bowsher, 1987). There are, however, several methods used to measure pain including the McGill Pain Questionnaire - a verbal selection method; the Submaximal Effort Tourniquet Test - a comparative physical test method; the Visual Analogue Scale - a progressive method using a 10 cm line anchored by 2 extremes of pain; the 101-point Numerical Rating Scale (NRS-101) - a progressive numerical scaling method from 1-100; and several behavioral and verbal rating scales. A comparison of methods of measuring clinical pain intensity favoured the NRS-101 numerical rating scale as the most practical index, to the degree that a standard measure of pain intensity is needed to facilitate comparisons of

treatment outcome, and to index chronic patient's pain intensity levels at different times in their lives (Jensen, 1986).

2.3.2 TYPES OF PAIN

Pain is occasionally purely psychogenic, though this is somewhat rare, but more often (when seen from a western neurophysiologic viewpoint) it is an organic physio-emotional experience occurring either as a result of the primary activation of visceral or somatic nociceptors, by disease, trauma or from potentially damaging stimuli, (nocigenic or nociceptive pain), or as a result of actual damage to the peripheral or central nervous system (neurogenic or neuropathic pain) (Baldry, 1993). Referred pain is pain felt in a site or zone some distance from the primary site. There is much evidence to support several explanatory mechanisms for this phenomenon, and there are variations by case too, but it remains unclear which of these mechanisms are significant at this time. The structures identified, so far, in the complex processes of pain and pain relief include the sensory receptors, their associated afferent nerve fibers, the dorsal horns, ascending and descending pathways, the reticular formation in the midbrain and medulla, the thalamus, the limbic system and the cerebral cortex (Baldry, 1993).

2.3.3 NOCIGENIC PAIN, PAIN RECEPTORS, AND THEIR AFFERENT NERVE FIBERS

Although the experience of nocigenic pain ultimately depends on interpretative processes in the neurons of the cerebral cortex, it occurs primarily as a result of a noxious stimulus activating myelinated and unmyelinated nociceptors (Baldry, 1993). Two distinct types of receptor and peripheral nerve fibers subserve two distinct sensory experiences; A-d nociceptors, with a multipunctate receptive field, transduce pricking or stabbing sensations (fast or first pain) which cause organisms to withdraw, whilst C-polymodal nociceptors, usually in a single receptive area, convey messages generated by tissue damage, (slow or second pain), which cause the organism to immobilize. A-d nociceptors are connected to the spinal cord's dorsal horns via medium diameter myelinated A-d nerve fibers, and are found mainly in and just under the skin. They are activated by noxious stimuli such as pressure, surgery, ischemia, sharps and are known as high-threshold mechanoreceptors. Some also respond to heat and are known as mechanothermal nociceptors (Baldry, 1993).

The pain impulses, as afferent information, pass along the A-d libers and C fibers to the central nervous system. A-b mechanoreceptors are also present in the skin, muscles, tendons and joints and are not responsive to noxious stimuli but are activated by innocuous ones such as light touch and hair movement. A-b proprioceptors in muscle are present in the form of Type I muscle spindles, and in tendons as tendon organs. Thcy are connected to the spinal cord's dorsal horn via large diameter A-b myelinated nerve fibers.

C-polymodal nociceptors: are connected to the spinal cord's dorsal horns via small diameter unmyelinated C afferent nerve fibers. They are called polymodal because of their ability to respond to a mechanical, thermal or chemical stimulus. However, such activation is invariably only produced by chemicals released as a result of the ensuing tissue damnge. The C nerve libers connected to those present in muscle are called Group IV fibers. It is the stimulation of C polymodal nociceptors in any deeply situated tissue such as muscle that leads to the development of slow onset pain, characterized by a widespread, ill-defined, deep seated and dull aching sensation. This activation is due to the effects of substances released and triggered by the damaged cells, which include bradykinin, histamine, leukotrienes, prostaglandins, platelet activating factor and subsequently substance P released from sensitized C-sensory afferents There are also a certain number of A-d (Groups II and III) nerve libers in muscle. (Nerve fibers are classified by size and according to whether they originate in skin or muscle: large diameter myelinated nerves A b [skin] or type I [muscle] carry 'touch' and proprioception, respectively. Small diameter myelinated A d [skin] or types II and III [muscle] carry 'pain'; the smallest unmyelinated C [skin] and type IV [muscle] also carry 'pain'. Types II, III, IV, and C also carry nonpainful messages (Stux and Pomeranz, 1991).

2.3.4 NEUROGENIC PAIN

Burning and/or stabbing neurogenic pain is caused by lesions of the nervous system, resulting in structural damage to the peripheral or central nervous units, rather than by receptor stimulation as described above. Neurogenic pain is much less responsive than nocigenic pain to the electroanalgesia techniques of evoking activity in endogenous opioid peptide and non-opioid peptide mediated pain modulating mechanisms. It is also mostly resistant to narcotic analgesics. as well as the endogenous opioid peptides, but can sometimes be relieved by sympathetic blockade, tricyclics (which facilitate noradrenergic inhibition) and anticonvulsants (Bowsher,

1987). However, some elderly patients with neurogenic pain respond very well clinically to electrical stimulation.

2.3.5 THE DORSAL HORN AND SEGMENTAL MECHANISMS

The cells of the spinal cord are arranged in layers or laminae, six in the dorsal horn (I-VI), three in the ventral horn (VII-IX) and an additional column of cells clustered around the central canal as Lamina X (Baldry, 1993). The thin unmyelinated C nociceptive afferents terminate mainly in Laminae I and II where their axons secrete Substance P (SP) or Vasoactive Intestinal Polypeptide (VIP), according to whether they arise from somatic structures or visceral ones respectively. The medium size myelinated A-d afferents terminate chiefly in Laminae I II and V. The A-b afferents on entering the spinal cord, give off branches which make contact with gamma-aminobutyric acid (GABA) mediated interneurons but most pass directly up the dorsal column to the medulla oblongata's gracile and cuneate nuclei. Axons from these nuclei form the medial lemniscus which terminates in the thalamus. The medial lemniscus is connected, via the anterior pretectal nucleus, to the periaqueductal grey area in the midbrain at the upper end of the opioid peptide mediated serotinergic descending inhibitory system (Baldry, 1993). As a result of these connections, A-beta afferent activity is enabled to block the C afferent input to the spinal cord by promoting activity in this descending system (Bowsher, 1991).

It therefore follows that the high-frequency Transcutaneous Electrical Nerve Stimulation (TENS), which exerts its pain modulating effect by recruiting A-b nerve fibers, could be seen to achieve this effect partly by these fibers when stimulated evoking activity in the opioid peptide mediated descending inhibitory system and partly by them evoking activity in dorsal horn GABA-ergic interneurons (Baldry, 1993).

There are three main types of dorsal horn transmission neurons - low-threshold mechanoreceptor cells, nociceptive-specific cells, and wide dynamic range cells which are responsible for transmitting sensory afferent information to the brain. The dorsal horn excitatory and inhibitory neurons modify the C afferent nociceptive information before reception and projection by the dorsal horn transmission cells.

2.3.6 PHYSIOLOGY OF PAIN: PATHWAY OF PAIN

The sense organs for pain are the naked nerve endings found in almost every tissue of the body. Pain impulses are transmitted to the central nervous system by two fibre systems. One nociceptor system is made up of small myelinated Aδ fibres 2-5μm in diameter, which conducts at rates of 12-30m/s. The other consists of unmyelinated C fibres 0.4-1.2 μm. these latter fibres are found in the lateral division of the dorsal roots and are often called dorsal root C fibres. They conduct at the low rate of 0.5-2 m/s. Both fibre groups end in the dorsal horn. Aδ fibres terminate primarily on neurons in laminas I and V, whereas the dorsal root C fibres terminate on neurons in laminas I and II. There is abundant evidence that the synaptic transmitter secreted by primary afferent fibres subserving pain in substance P (Melzack and Wall. 1965).

Some of the axons of the dorsal horn neurons end in the spinal cord and brain stem. Others enter the anterolateral system, including the lateral spinothalamic tract. A few ascend in the posterolateral portion of the cord. Some of the ascending fibres project to the specific sensory relay nuclei of the thalamus and from there to the cerebral cortex. Positron Emission Tomography and Magnetic Resonance Imaging (MRI) studies in normal humans indicate that pain activates three cortical areas: SI, SII and the cingulated gyrus on the side opposite the stimulus. The cingulated gyrus is involved in emotion, and cingulated gyrectomy has been reported to lessen the distress associated with chronic pain.

Pain is produced by excitation of nociceptor. Sensation is carried by afferent fibres of the dorsal root ganglia to the substantial gelatinosa of the spinal cord or by the cranial nerve V to the brain stem trigeminal nuclear complex. From these sites, the spinothalamic neurons carry sensory impulses to the thalamus and then to the sensory-discriminative impulses to higher centres of pain perception. Another system is concerned with the unpleasant and aversive aspects of pain sensations and involves the ascending reticular-activating system that projects to the cortex via medial thalamic nuclei and to the limbic areas. This system thus participates in the emotional aspect of pain and is concerned with the suffering aspect arousing the desire to escape pain

2.3.7 THE GATE CONTROL THEORY

Melzack and Wall (1965), developed their now-famous theory on pain mechanisms, which postulated that in each dorsal horn of the spinal cord there is a gate-like mechanism which inhibits or facilitates the flow of afferent impulses into the spinal cord before it evokes pain

perception and response. Their theory was proposed as an alternative to the specificity theory of pain, which holds that pain is a specific modality with its own specialized sensors, neuronal pathways and centers and the pattern theory which maintains that stimulus intensity of nonspecific receptors and central summation were the critical determinants of pain. The theory, as originally propounded, stated that the opening or closing of the 'gate' is dependent on the relative activity in the large diameter (A-b) and small diameter fibers (A-d and C), with activity in the large diameter fibers tending to close the 'gate', and activity in the small diameter fibers tending to open it (Baldry, 1993). Research by Garrison and Foreman (1994) supports this theory insofar as their study shows that dorsal horn neurons which can potentially transmit noxious information to supraspinal levels, can have their cell activity decreased during TENS application to somatic receptive fields. These findings are consistent with the concept of the 'gate control theory of pain' in that less noxious information would be involved in the pain perception process (Garrison and Foreman, 1994). They also showed that there is a differential effect in that more cells respond to conventional high frequency, low intensity (TENS) variables than they do to low frequency, high intensity (TENS) variables.

The gate control theory proposes that the substantia gelatinosa, which caps the grey matter of the spinal horn in the spinal cord, is the essential site of control. The control mechanism is referred to as a 'gate' and is operated by external and internal influences. Pain impulses can only pass through when the gate is open, and not when it is closed (Davis, 1993). So if nociceptive input exceeds A-b fiber input, then the gate is open and the pain impulse ascends the spinal cord to the brain. If A-b fiber input exceeds nociceptive input then the gate is closed and the pain impulse is stopped or diminished due to the action of the inhibitory neurotransmitters and, therefore, does not pass up the spinal cord (Davis, 1993). An essential part of the theory ever since the time it was first put forwaid is that the position of the 'gate' is in addition influenced by the brain's descending inhibitory system (Baldry, 1993).

2.3.8 NON-OPIOID PEPTIDE MEDIATED DESCENDING SYSTEMS

It is now accepted that there are several descending control systems, and that, whereas one of these is opioid peptide mediated, others must be mediated by various other transmitters. Most of these have yet to be discovered and their transmitters identified. However, Melzack and Wall in 1988, describe one such system that is known to have its origin in the dorsolateral pons where

noradrenalin-containing cells project into the spinal cord (Baldry, 1993). It is also possible that there is more than one system active at any given time.

2.3.9 CHEMICAL MEDIATORS OF PAIN

In most cases, acute pain resulting from excessive mechanical or thermal stimuli persists even after the removal of the stimuli. This continuous feeling of pain in the absence of stimulus indicates the role of inflammatory agents at the site of injury, acting as pain mediators. This therefore confirms the fact that apart from mechanical or thermal origin; pain can be of chemical origin. Chemical (inflammatory substances) that mediate pain are: prostaglandin, substance P, kinins, histamine and serotonin.

2.3.9.1 Kinins

The kinins are powerful algesic agents that cause an intense, burning pain when applied to the exposed base of a blister. They are of two types: bradykinin and kallidin. Bradykinin excites primary sensory neurons and provokes the release of neuropeptides such as substance P, neurokinin A and calcitonin gene-related peptide (Geppetti, 1993). In acute pain, B_2 receptors mediate bradykinin algesia. This pain is significantly reducted by B_2 antagonist but not by B_1 antagonists (Nancy et al., 2001). B_2 receptor is a G- protein-coupled-7-transmembraine-domain receptor that activates phospholipase A_2 and phospholipase C, apparently via interaction with distinct G proteins. Bradykinin complexes with it and produces its cellular effects through production of various intracellular messengers.

2.3.9.2 Prostaglandins

Prostaglandins are products of cyclooxygenase pathway. They are formed from polyunsaturated fatty acid; arachidonic acids that are released in response to physiological insults and play an important role as pain mediator. Prostaglandins are associated particularly with the development of pain that accompanies injury or inflammation. Prostaglandins cause pain when injected intradermally; these effects are generally not as immediate or intense as those caused by bradykinin or histamine, but they outlast those caused by the other autacoids (Nancy et al., 2001).

Prostaglandings themselves do not induced pain except at very high doses, but they can potentiate pain induced by other algesic substances. They are thought to sensitize the

nociceptors. The capacity of prostaglandins to sensitize pain receptors to mechanical and chemical stimulation has been confirmed by electrophysiological measurements and appears to result from a lowering of the threshold of the polymodal nociceptors of C fibres. This was further demonstrated by Nancy et al. (2001), who reported that PGEs and PGI sensitize the afferent nerve endings to the effects of chemical or mechanical stimuli by lowering the threshold of the nociceptors. Hyperalgesia also is produced by Leukotriene B4 (LTB_4). The release of these prostaglandins and LTB_4 during the inflammatory process thus serves as an amplification system for the pain mechanism (Moneada et al., 1978).

2.3.9.3 Substance P

Substance P is present predominantly in the neurons composing the dorsal horn of the spinal cord. Substance P (SP) an undecapeptide is present in the sensory neurons. This peptide is found in association with pain fibres as in the skin or dental pulp. The central branch of the sensory fibres containing substance P terminates mainly in the dorsal horn of the spinal cord. Strong afferent stimulation releases substance P from the perfused cord, indicating that substance P is an excitatory neurotransmitter of the first afferent synapse of the pain pathway (Baldry, 1993). However, since excitation produced by substance P is markedly slower than that induced by natural stimulation, substance P has been proposed instead to act as a neuromodulator that modifies neuronal excitability. Substance P is also widely distributed in the central nervous system and has been shown to produce algesic and analgesic action depending on the species, the dose and the route of administration (Baldry, 1993).

2.3.10 RELIEF OF PAIN WITH ANALGESICS

Analgesics are the drugs of choice in pain management. They act on nervous system to relieve pain and distress without loss of consciousness. Analgesics constitute the most widely used medication, since pain accompanies injury and most diseases.

Pain may be relieved by:

1. Eliminating painful stimuli at their point of origin;
2. The application of physical measures or anesthetics to the point to which the pain is referred or to the hyperirritable segment of the cord; and
3. Interrupting the impulses which convey the pain by anaesthetizing or destroying the nerve tracks.

2.3.11 ANALGESIC AGENTS

These are the drugs that produce analgesia without rendering the patient unconscious. They are grouped into two different classes based on their mechanism of actions.

a. Narcotic analgesics: they are also called opiate and opioids. Examples are morphine, heroin etc. They act centrally and hence called centrally acting analgesics.

b. Non-narcotic analgesics: They are also known as non-steroidal anti-inflammatory agents. Examples are aspirin, indomethacin, ibuprofen etc. They have antipyretic and in some instances antinflammatory activities. Unlike narcotic analgesics, their actions are predominantly peripherally.

2.3.12 MECHANISM OF ANALGESIC ACTIONS

Morphine depresses polysynaptic reflexes in the nociceptive system; this includes the psychic correlates which are also activated by the nociceptive system (Rang et al., 2005). Due to the large number of opiate receptors in the central nervous system, a comprehensive understanding of the mechanism of action of morphine in the various regions of the central nervous system has so far eluded us. Morphine exerts its effects not only in the brain (supraspinal sites of action), but also in the spinal cord (spinal sites of action). It is evidently by it action on opiate receptors in the substantia gelatinosa of the spinal cord that the latency of polysynaptic reflexes in this area (flight reflex) is prolonged (Davis, 1993). This holds good even in spinalized animals, a fact which suggests that the ascending axons in the spinothalamic tract possess opiate receptors which must be regarded as possible sites of action for peptides with opiate-like effects and may be connected with descending pathways from the reticular formation. The analgesic action of morphine in the spinal cord is utilized in the procedure known as spinal analgesia. An intrathecal or epidural injection of morphine produces analgesia which differs its effect is confined to pain sensations and fight and flight reflexes. these and only these are abolished.

2.4 EPILEPSY

Epilepsy is a major neurological disorder characterized by recurrent, spontaneous brain seizures or convulsions and its prevalence in developing countries is generally higher than in developed countries (Sander et al., 1996). Epilepsy is the second most common neurological disorder after stroke and it is estimated that approximately 0.8% of the population is affected by some form of epilepsy (Pitkanen et al., 2009). Recent studies suggest an increased risk of dying and a greater

proportion of deaths that are epilepsy-related in Africa as high as a six-fold increase in mortality in people with epilepsy. This is higher than the two-to-three fold increase reported in developed countries (Christianson et al., 2000; Diop et al., 2005). Though not clear, the reasons for this gap might be due to social deprivation (Sander, 2003). Recent data suggest that people from socio-economically deprived backgrounds in developed countries are more likely to develop epilepsy (Heaney et al., 2002). This neurological disorder is viewed as a shameful disorder and has severe social implications in African communities as it carries a stigma. Sufferers are often shunned and discriminated against with respect to education, employment and marriage (Baskind et al., 2005).

Drug therapy of epilepsy with currently available Antiepileptic Drugs (AEDs) is associated with side effects, dose-related and chronic toxicity that involve virtually every organ system. Moreover, all the currently available AEDs have potential for adverse effects on cognition and behaviour (Samren et al., 1997; Duncan, 2002). The practice of polypharmacy in the therapy of epilepsy has increased the risk of side effects and drug interactions. It can be said that all problems with the current AED therapy of epilepsy are more prevalent in underdeveloped countries due to lack of facilities for proper diagnosis, treatment and monitoring of serum levels of AEDs. Another critical issue associated with currently available AEDs is recent clinical and experimental data that strongly suggest that AED therapy does not alter the course or natural history of epilepsy and though AEDs suppress the seizures, they may not affect the underlying disorder (Chadwick, 1995; Shinnar and Berg., 1996; Loscher, 2002). Only a few AEDs have been shown to be antiepileptogenic including valproate and phenobarbitone (Silver et al., 1991; Duncan, 2002) and levetiracetam (Loscher et al., 1998; Duncan, 2002) but these are not well substantiated. There is pressing need for further research especially in the field of pharmacotherapy of epilepsy to find drugs which are not only anticonvulsant but also antiepileptogenics that either prevent epilepsy or alter its natural course. Natural products and plants for that matter, used in traditional medicine can be an invaluable source for search for novel antiepileptic compounds (Meldrum, 1997).

2.4.1 PATHOPHYSIOLOGY OF EPILEPSY

A variety of different electrical or chemical stimuli can easily give rise to a seizure in any normal brain. The epileptic seizure always reflects abnormal hypersynchronous electrical activity of neurones caused by an imbalance between excitation and inhibition in the brain. Neurones are

interconnected in a complex network in which each individual neurone is linked through synapses with hundreds of others. A small electrical current is discharged by neurones to release neurotransmitters of synaptic levels to permit communication with each other. More than hundred neurotransmitters or neuromodulators have been shown to play a role in neuronal excitation. However, the major excitatory neurotransmitter in the brain is L-glutamate and the major inhibitory neurotransmitter in the brain is gamma-amino butyric acid (GABA). An nbnormal function of either of these could result in a seizure. An excited neurone will activate the next neurone whereas an inhibitory neurone will not. A normal neurone discharges repetitively at a low baseline frequency, and it is the integrated electrical activity generated by the neurones of the superficial layers of the cortex that is recorded in a normal electroencephalogram. If neurones are damaged, injured or suffer electrical or metabolic insult, a change in the discharge pattern may develop. In the case of epilepsy, regular low-frequency discharges are replaced by bursts of high-frequency discharges usually followed by periods of inactivity. An epileptic seizure is triggered when a whole population of neurons discharges synchronously in an abnormal way. This abnormal discharge may remain localized or it may spread to adjacent areas, recruiting more neurons as it spreads.

2.4.2 ROLE OF GABA AND GLUTAMATE IN THE PATHOGENESIS OF EPILEPSY

It is important to emphasize the role of neurotransmitters especially, γ-amino butyric acid (GABA) and glutamate in epileptogenesis, since they are the major inhibitory and excitatory transmitters in the central nervous system, respectively, and the fact that generation of seizures has been attributed to imbalance between excitatory and inhibitory neurotransmission in epileptic brains.

The GABA plays an important role in regulation of neuronal excitability and impairment of GABA function produces seizures (Olsen et al., 1997). Compounds that enhance GABA-mediated inhibition are anticonvulsants (Sieghart, 1992; Scholze et al., 1996). GABA exerts its major inhibitory effect via GABAA receptor (which is a ligand-gated ion channel) by increasing neuronal membrane conductance for chloride ions causing membrane hyperpolarization resulting in reduced neuronal excitability and most rapid inhibition in brain (Sieghart, 1992). GABAA receptor is target for many important neuroactive drugs including antiepileptic drugs benzodiazepines and barbiturates (Sieghart, 1992; Scholze et al., 1996). GABAA receptor

consists of five subunits that form a chloride ion channel (Macdonald and Olsen, 1994). The subunits consist of various subtypes and pharmacological studies have shown that individual subunits and subtypes confer different sensitivities to agents acting on GABAA receptors (Neelands et al., 1998). It is postulated that exposure of GABA to postsynaptic receptors for a brief period of time results in generation of Inhibitory Post-Synaptic Currents (IPSCs) (Hill et al., 1998). GABAA receptor-mediated miniature IPSCs play important physiological role in preventing the development of neuronal hyperexcitability (Salin et al., 1996). Decrease in GABAA from receptor-mediated IPSCs is observed in cells from hippocampi of animals with chronic experimental epileptic seizures and humans with chronic intractable temporal lope epilepsy (Isokawa, 1996).

Glutamate is the most important excitatory neurotransmitter in all rapidly conducting relay pathways of the motor and sensory systems of the outer tube of the central nervous system. It produces fast or prolonged synaptic excitation and triggers various calcium dependent processes in the target cells, including production of nitric oxide (Bienvenu et al., 2002). Glutamate is a transmitter in the corticospinal, corticostriatal pathways, intrahemispheric and interhemispheric association pathways, hippocampal circuits, primary afferents, and somatosensory and special sensory pathways, cerebellar afferents and excitatory inter-neurones. Glutamate acts via two types of receptors, ionotropic glutamate receptors (iGluR) which are ligand-gated cation specific channels and metabotropic glutamate receptors (mGluR) which are G-protein-coupled receptors. Ionotropic glutamate receptors are classified according to their prototype agonists: NMDA (N-methyl-D-aspartate), kainite and AMPA (alpha-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid). Practically all agonists are able to induce epileptic seizures and brain damage whereas antagonists have been shown to be anticonvulsant (Mares et al., 2004a; Mares et al., 2004b). The role played by metabotropic glutamate receptors depends on the type of receptors: activation of type I is proconvulsant and convulsants, whereas activation of type II and III is anticonvulsant (Moldrich et al., 2003).

Epilepsy may arise as a consequence of a dramatic release of glutamate from central nerve terminals. Sustained seizures of the limbic system in experimental animals result in brain damage that resembles glutamate toxicity. Similar changes are seen at autopsy in patients with intractable epilepsy. In animals such seizure-related brain damage may be reduced by the administration of

non-competitive NMDA receptor antagonists, but it would appear that not all seizure activity is suppressed by drugs (Leonard, 2003). The precise mechanism whereby persistent seizure activity results in neuronal degeneration is not completely understood. It seems possible that repetitive depolarization and repolarization of the nerve membrane eventually leads to an energy-deprived state within the cell, thereby preventing the restoration of the cell membrane potential. Each depolarization will also lead to an influx of calcium ions and efflux of potassium ions, which if prolonged, can result in cell death. The reduced efficiency of glial cells to remove potassium ions, and the ability of high extracellular concentration of potassium ions to depolarize neurons and cause neurodegenerative changes also play a critical role in causing the degenerative changes that are a feature of status epilepticus and intractable epilepsy (Leonard, 2003). Recent advances have indicated that GABAA receptors work synergistically with NMDA receptors to increase the influx of calcium ions into neuroblasts and immature neurons. This is essential for the modulation of early CNS development (DeLorey et al., 1999). It is evident that GABA is a critical inhibitory transmitter and seizures can rapidly be elicited by pharmacological disruption of GABAergic mechanism (Feldman and Felder, 1991). Drugs have also been developed to modulate glutamic acid function. Reduction of excitatory glutaminergic neurotransmission is potentially important; AMPA receptor blockade probably contributes to the anticpileptic effect of drugs such as lamotrigine (Lee et al., 2008).

2.4.3 TYPES OF EPILEPSY

The clinical classification of epilepsy recognizes two categories, namely; partial seizures and generalized seizures, although there are some overlaps and many varieties of each. A seizure is said to be partial if it is restricted to a regional disturbance. Partial seizures are those in which the discharge begins locally and often remains localized. These may produce relatively simple symptoms without loss of consciousnesses, such as involuntary muscle contractions, abnormal sensory experiences or autonomic discharge or they may cause more complex effects on consciousness, mood and behaviour, often termed psychomotor epilepsy (Rang et al., 2003). Generalized seizures involve the whole brain, including the reticular system, thus producing abnormal electrical activity throughout both hemispheres. Immediate loss of consciousnesses is characteristic of generalized seizures (Bienvenu et al., 2002). The main categories are generalized tonic-clonic seizures (*grand mal*) and absences seizures (*petit mal*). A generalized tonic-clonic seizure consists of an initial strong contraction of the whole musculature, causing a

rigid extensor spasm. Respiration stops and defaecation, micturition and salivation often occur. This tonic phase lasts for about 1 minute and is followed by a series of violent, synchronous jerks, which gradually dies out in 2-4 minutes. Most types of epilepsy are characterized by more than one type of seizure. Patients with focal (or partial) epilepsy may have simple partial, complex partial and secondarily generalized tonic-clonic seizures (e.g. partial seizures with secondary generalization). Patients with generalized epilepsy may have one or more of the following seizure types: absence, myoclonic, and tonic, clonic, tonic-clonic and atonic. Thus, no seizure type is specific for a single type of epilepsy. Seizures are symptoms, and patients should be treated for a type of epilepsy, not for a type of seizure (Benbadis et al., 2001).

2.4.4 CAUSES OF EPILEPSY

The cause of convulsions must be clearly understood through some precise observations. The type of seizure depends on the site of the focus in the brain. Epileptic attack can be caused by biochemical insults to the brain, such as hypoglycaemia, anoxia, hypocalcaemia, hyperventilation, water intoxication and sudden withdrawal of certain drugs such as barbiturates or alcohol (Bienvenu et al., 2002). Epilepsy can also be caused by previous active pathology, such as birth trauma to the brain, during or following meningitis, trauma to the skull and brain later in life, cerebral abscesses, cerebral infarction, cerebral haemorrhage or subarachnoid haemorrhage (Bienvenu et al., 2002). Further analysis shows that the blockade of post-synaptic gamma-amino butyric acid receptors or an inhibition of GABA synthesis is the principal origin of brain discharge. According to Bienvenu et al. (2002), an epileptic attack can be triggered by a sensory stimulus, which is specific for individuals. To date, there is no single unifying explanation as to how these diverse factors cause seizures. Hence, it is difficult to determine the exact cause of epilepsy, even though it has been possible to investigate the physiological events which participate in the genesis of epilepsy.

2.4.5 MECHANISM OF ACTION OF ANTIEPILEPTIC DRUGS

With the exception of valproate, the established AEDs tend to have clearly defined, single mechanisms which facilitates the prediction of effectiveness of treatment on the basis of pharmacology. At the cellular level, three major mechanisms of action of anticpileptic drugs are recognized; modulation of ion channels, enhancement of GABA inhibitory neurotmnsmission, and attenuation of glutamate mediated excitatory transmission (Kwan et al., 2001).

2.4.5.1 MODULATION OF ION CHANNELS

The intrinsic excitability of the nervous system is ultimately controlled by voltage-gated ion channels which regulate the flow of cations across surface and internal cell membranes. The sodium channel is arguably the most important and responsible for depolarization of the cell membranes and the characteristic upstroke of the neuronal action potential. Blockade of voltage-gated sodium channels is the most common mechanism of action amongst currently available AEDs (Deckers et al., 2003). Well established AEDs, phenytoin and carbamazepine are prototype sodium channel blockers and this mechanism is shared by the newer drugs lamotrigine, felbamate, topiramate and oxcarbazepine (Deckers et al., 2003). These drugs mainly bind to the inactivated state of the sodium channel and produce a voltage- and frequency-dependent reduction in channel conductance, resulting in a limitation of repetitive neuronal firing with little or no effect on the generation of single action potentials (Kwan et al., 2001). Voltage-gated calcium channels, likewise sodium channels, are involved in depolarization, often recruited in response to initial sodium-dependent action potential generation. Calcium channels are distributed throughout the nervous system on dendrites, cell bodies and nerve terminals. The N-, P- and Q-type calcium channels have been implicated in the control of neurotransmitter release at the synapse, whereas the T-type channel, expressed predominantly in the thalamocortical relay neurones, is believed to play a role in the distinctive rhythmic discharges of generalised absence seizures (Kwan et al., 2001). These channels represent a major target for AEDs. Ethosuximide efficacy against generalised absence seizures is believed to be mediated by blockade of the T-type calcium channel (Deckers et al., 2003). Evidence suggests that valproate may have similar effects (Deckers et al., 2003). Lamotrigine has also been reported to limit neurotransmitter release by blockade of the N- and P- subtypes of voltage-sensitive calcium channel while gabapentin binds to the α2δ-subunit of the L-type channel (Kwan et al., 2001).

2.4.5.2 ENHANCEMENT OF INHIBITORY NEUROTRANSMISSION

GABA is the predominant inhibitory neurotransmitter in the mammalian central nervous system. Following synaptic release, GABA acts at three specific receptors, GABAA, GABAB, and GABAC (Deckers et al., 2003). The GABA belongs to the ligand-gated ion channel superfamily and responds to GABA binding by increasing chloride conductance, resulting in neuronal hyperpolarization. GABA is removed from the synaptic cleft into localised nerve terminals and glial cells by specific transport molecules. Thereafter, GABA is either recycled to the readily

releasable neurotransmitter pool or metabolized by the action of the mitochondrial enzyme GABA-transaminase, thereby completing the cycle (Kwan et al., 2001). Phenobarbital and the benzodiazepines bind to distinct sites on the GABAA receptor complex and exert an allosteric influence on the opening of the chloride ion channel in response to GABA. Phenobarbital increases the duration of channel opening, while the benzodiazepines increase the frequency of opening (Deckers et al., 2003). Vigabatrin and tiagabin exert their antiepileptic actions by selective effects at the GABA synapse. Vigabatrin is an irreversible inhibitor of the enzyme GABA-transaminase, while tiagabin prevents the uptake of GABA from the synaptic cleft by blockade of the GAT-1 transporter.

2.4.5.3 ATTENUATION OF EXCITATORY NEUROTRANSMISSION

Glutamate is the principal excitatory neurotransmitter in the mammalian brain. Following synaptic release, it exerts its effects on both ionotropic and metabotropic receptor types. The ionotropic glutamate receptors are arguably the best characterized and are classified into three subtypes, AMPA, kainite and NMDA, which form ligand-gated ion channels permeable to sodium and depending on subtype and subunit 15 composition, calcium ions. The AMPA and kainite subtypes are implicated in fast excitatory neurotransmission, whereas the NMDA receptor, quiescent at resting membrane potential, is recruited during periods of prolonged depolarization (Kwan et al., 2001). None of the current AEDs available exerts its pharmacological effects solely by an action on the glutamatergic system (Deckers et al., 2003). However, blockade of the NMDA subtype of glutamate receptor has been reported to contribute to the antiepileptic effects of felbamate (Deckers et al., 2003). Topiramate is similarly distinguished by an inhibitory action on AMPA receptors. Furthermore, several AEDs have been reported to reduce glutamate release, although this effect may be more indicative of their actions on calcium channels than a direct effect on the glutamate system (Kwan et al., 2001).

2.5 MEDICINAL PLANTS AND HERBAL MEDICINE

The term "herbs" refers to plants or parts of them, including grasses, flowers, berries, seeds, leaves, nuts, stems, stalks and roots, which are used for their therapeutic and health- enhancing properties. Generations of skilled herbal practitioners, researchers and scholars have refined and tested the vast science of herbology, producing thousands of plant-based remedies that are safe and effective. The proper and judicious use of herbs is often successful in the treatment of illness

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where other, more conventional medicines and methods fail. Topically, herbs can repair damaged skin, soothe a wound, improve complexion, heal bruises and relieve aching muscles. Herbs demonstrate great versatility for the treatment of a broad variety of health needs. People world-over long applied poultices and imbibed infusions of thousands of indigenous plants dating to prehistory. Human disease management in Nigerian history also provides evidence of the relationship of plants and medicine (Ayandele and Adebiyi, 2007). The medicinal flora in the tropical eco-region has a preponderance of plants that provide raw material for addressing a range of medical disorders and pharmaceutical requirements. Collectively, plants produce a remarkably diverse array of over 500,000 low molecular mass natural products also known as secondary metabolites (Fatope et al., 2001). The medicinal value of these secondary metabolites is due to the presence of chemical substances that produce a definite physiological action on the human body. The most important of these include: alkaloids, glucosides, glycosides, steroids, flavanoids, fatty oils, phenols, resins, phosphorus and calcium for cell growth, replacement, and body building (Chidumbaro et al., 2003).

While medicinal plants are the actual plants themselves, plant/ herbal medicines are preparations made from those plants. Plant medicines are the most widely used medicines in the world today. An estimated ninety percent (90%) of the world's population employs herbs as primary medicines (WHO, 2002). Drugstore shelves in the United State are stocked mostly with synthetic remedies, in other parts of the world the situation is quite different. In parts of Europe, for example, pharmacies dispense herbs prescribed by physicians. Natural plant-based remedies are used for both acute and chronic health problems, from treating common colds to controlling blood pressure and cholesterol. Not so long ago, this was true in the United State as well.

Plants are the original source materials for as many as 40% of the pharmaceuticals in use in the United States today. This is to say that either the drugs currently contain plant-derived materials, or synthesized materials from agents originally derived from plants. Some medicines, such as the cancer drug Taxol (from *Taxus brevifolia*) and the anti-malarial quinine from *Cinchona pubescens* are manufactured from plants.

2.6 PSYCHOACTIVE HERBAL MEDICINES

St. John's Wort (*Hypericum perforatum*):	Promoted for the treatment of mild to moderate depression (Hicks, 2011).
Kava-kava (*Piper methysticum*):	Anxiety, insomnia (Hicks, 2011).
Valerian (*Valeriana officinalis* and *edulis*):	Anxiety, depression, insomnia, cardiac arrhythmias, spasmolytic (Hicks, 2011).

Maidenhair *(Ginkgo biloba)*: Used mainly for memory deficits and tinnitus (Hicks, 2011)

Asian ginseng *(Panax ginseng)*: Primarily marketed in the U.S. to improve energy and physical or cognitive performance (Hicks, 2011).

Chamomile: Commonly used for insomnia and gastrointestinal problems, however, no good data support either use. Receptor-binding studies have found components of chamomile extract to bind to GABA receptors, however, there is no evidence confirming its effectiveness as a sleep aid (Hicks, 2011).

Free and Easy Wanderer Plus (FEWP) Chinese name: Jia-Wei-Xiao-Yao-San): Used for depression and anxiety. Case studies have reported beneficial effects in panic disorder. Li et. al. (2008) report efficacy, safety and tolerability in post-stroke depression, in a fluoxetine and placebo-controlled study (Hicks, 2011).

Yokukansan: A traditional Japanese herbal medicine reported to improve behavioral and psychological symptoms of dementia

- Originally used for treatment of insomnia, night terrors;
- Case reports suggest that it helps control aggressive and impulsive behavior in patients with borderline personality disorder;
- Possible neuroprotective effects;
- Used in some hospitals in Japan for prevention of post-operative delirium; and
- Mechanism of action: partial agonist at 5-HT $_{1A}$ receptors, 5-HT $_{2A}$ antagonist, protects against glutamate-induced excitatory neurotoxicity by amelioration of astrocytic dysfunction. (Hicks, 2011).

Papaver somniferum: the opium poppy yields a sap of narcotic opium, from which the potent pain killer morphine is made. In the eighth century Persian caravans bore both opium and its methods of euphoric use to India and China. Simultaneously, opium and its products heroin and morphine established themselves among drug users and in the field of medicine. In modern medicine, morphine and its analogues remain unsurpassed pain killers.

2.7 LESS COMMONLY USED HERBAL MEDICINES

- Ashwagandha (*Withania somnifera*)
- *Banxia houpu*
- Brahmi (*Bacopa monniera*)
- California poppy (*Eschzoltzia californica*)
- *Dan zhi xiao yao*
- Gotu kola (*Centella asiatica*)
- Hange koboku-to
- Hawthorn berry/leaf (Crataegus spp.)
- Lavender(*Lavandula spp.*)
- Lemon balm (*Melissa officinalis*)
- Lemongrass (*Cymbopogon citratus*)
- Nelumbinus semen
- Passion flower (*Passiflora incarnata*)
- Rose root (*Rhodiola rosea*)
- Saffron (*Crocus sativus*)
- Scullcap (*Scutellaria lateriflora*)
- Sour date nut (*Ziziphus jujuba*)

Herbal medicines remain indispensable to modern pharmacology and clinical practice. Much of the current drug discovery and development process is plant-based, and new medicines derived from plants are inevitable.

2.8 DESCRIPTION OF *OLAX SUBSCORPIOIDEA* PLANT

O. subscorpioidea locally called ifon is a shrub tree of 10 m high, bole to 60 cm girth with long thin, often drooping branches, but sometimes a many-stemmed shrub; of deciduous forest and jungle as undergrowth, and as thickets in savanna; across the Region from Senegal to West Cameroons. It is also widely distributed in Nigeria.

2.9 ETHNOMEDICINAL USES OF *OLAX SUBSCORPIOIDEA*

Ethnomedicinal use of *O. subscorpioidea* varies with people and traditions. In Congo Republic ethnomedicine, decoction of stem and leaves of *O. subscorpioidea*, is used to treat rheumatism and articular pains (Bouquet, 1969). Ethno-botanical survey showed that *Olax subscorpioidea* has been indicated in the management of asthma in South West Nigeria and cancer (Sonibare and Gbile, 2008; Mike et al., 2010). Its use in combination with another plant in the treatment of anxiety and mental illness disorders has been reported (Ibrahim et al., 2007). Adjanohoun et al. (1986) listed the leaves as one of recipes used in the management of convulsion. In some parts of Africa, it used as antidotes, febrifuges and genital stimulants/depressants. It has also been indicated in the management of venereal diseases, cutaneous, subcutaneous parasitic infection.

2.10 PHARMACOLOGICAL PROPERTIES OF *OLAX SUBSCORPIOIDEA*

Ayandele and Adebiyi (2007) demonstrated its antimicrobial activity. Previous study has shown that stem of *O. subscorpioidea* contains alkaloids, steroids, and flavonoids together with other active ingredients in the ethanoic extract with the exception of saponins which is present in the aqueous extract alone (Ayandele and Adebiyi, 2007). Roots of *O. subscorpioidea* has also been shown to possess antiulcer activity in experimental rats as claimed by traditional users (Ukwe et al., 2010).

2.11 PHYTOCHEMISTRY

Phytochemistry revealed presence of alkaloids, steroids, glycosides, saponins, flavonoids and terpenoids (Ayandele and Adebiyi, 2007; Ukwe et al., 2010)

2.12 TAXONOMICAL CLASSIFICATION OF *OLAX SUBSCORPIOIDEA*

Domain: *Eukaryota*

Kingdom: *Plantae*

Subkingdom: *Viridaeplantae*

Phylum: *Tracheophyta*

Subphylum: *Euphyllophytina*

Infraphylum: *Radiotopses*

Class: *Magnoliopsida*

Subclass: *Rosidae*

Superorder: *Santalanae*

Order: *Santalales*

Family: *Olacaceae*

Subfamily: *Olacoideae*

Genus: *Olax*

Specific epithet: *subscorpioidea*

Botanical name: - *Olax subscorpioidea*

http://zipcodezoo.com/Plants/O/Olax_subscorpioidea/11/04/2014

Figure 1: LEAVES OF *OLAX SUBSCORPIOIDEA*

http://zipcodezoo.com/Plants/O/Olax_subscorpioidea/11/04/2014

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 PLANT MATERIALS

The leaves of *O. subscorpioidea* were collected in February 2012 at the Gambari Forest Reserve, Ibadan, the Oyo state capital, Nigeria. The taxonomical identification and authentication of the plant was done at the herbarium section of the Forestry Research Institute of Nigeria (FRIN), Ibadan, Nigeria. A voucher specimen with identification number 109924 was deposited and compared with the reference specimen.

3.2 PREPARATION OF PLANT MATERIAL AND DRUGS

Air-dried leaves (150 g) were pulverized and soaked in 50% ethanol (1.5 L) for 48 hours. The filtrate was concentrated with a rotary evaporator to give a semisolid residue and evaporated to dryness to form solid residue (8.7 g). It was kept in the desiccator for further use. The dried extract was then subsequently reconstituted in distilled water at appropriate concentrations for the various experiments. All drugs and the extract were dissolved in distilled water and administered by intraperitoneal (i.p.) route.

3.3 LABORATORY ANIMAL

Swiss Albino mice (20 – 25 g) of either sex used in this study were obtained from the Laboratory Animal Centre of the College of Medicine, University of Ibadan, Nigeria. The animals were kept in well-ventilated and hygienic compartments, maintained under standard environmental conditions and fed with standard rodent pellet (Livestock Feed PLC. Lagos, Nigeria) and water ad libitum. The experimental procedures adopted in this study were in accordance with the United States National Institutes of Health Guidelines for Care and Use of Laboratory Animals in Biomedical Research (NIH, 1985).

3.4 DRUGS AND CHEMICALS

The chemicals used were: Acetic acid (May & Baker Ltd., Dagenham, England), Formaldehyde (Griffin & George, Leics, England), Acetylsalicylic acid, (ASA) (Dispirin®; Reckitt & Coleman Ltd., Pakistan), Morphine (Martindale Pharma®, Essex, United Kingdom), Naloxone (Sigma,

USA), Yohimbine (Sigma- Aldrich (St.Louis, MO, USA), reserpine (Pfizer Inc., New York, NY, USA)., imipramine (Shanghai Zhongxi Pharmaceutical Co., Ltd. Shanghai, China), Phenobarbitone (Sigma, USA), picrotoxin (Sigma-Aldrich, St. Louis, USA), strychnine (Sigma, USA) and pentylenetetrazole (Sigma-Aldrich, St. Louis, USA) were used in the current investigation, ciproheptadine, propranolol, haloperidol and atropine

3.5 ACUTE TOXICITY TEST

The method described by Lorke (1983) was used to determine the LD_{50}, which is the index of acute toxicity. Albino mice (20 – 25 g) of either sex were used. This method involved an initial dose finding procedure, in which the animals were divided into three groups of three animals. Doses of 10, 100 and 1000 mg/kg were administered intraperitonealy (i.p.), one dose for each group. The treated animals were monitored for 24 hours mortality and general behavior. From the results of the above step, four different doses of (500, 800, 2000 and 3000 mg/kg) were chosen and administered i.p. respectively to four groups of one mouse per group. The treated animals were monitored for 24 hours. The LD_{50} was then calculated as the geometric mean of the highest dose showing no death and the lowest dose showing death.

3.6 NEUROBEHAVIOURAL ASSAYS

3.6.1 *Novelty-induced rearing (NR) and grooming (NG) in mice*

The behavioral profiles of albino mice under the influence of the extract were assessed singly in a white plexiglas cage measuring (45 cm × 25 cm × 25 cm). Behavioural measurements were carried out after i.p. administration of vehicle (0.2 ml/20 g distilled water) to group (1) and different doses of the extract (3.125, 6.25, 12.5 and 25 mg/kg, i.p) to group (2-5) into mice (n = 5). The animals were placed directly from home cage into an opaque Plexiglas observation cage with only one side transparent for observation. Each animal was used only once, with the observation cage cleaned with 70% ethanol after each assessment to remove olfactory cue from previous animal to the other. The time of the experiment was kept constant (9.00 am-1.00 pm) daily to avoid changes in biological rhythm. The behavioural components employed in this observational analysis were rearing and grooming (Ajayi et al., 1994; Onigbogi et al., 2000). Diazepam (2 mg/kg, i.p.) administered to group (6) served as reference drug. The frequency of rearing episode was quantified by using a manual counter and a stop watch. The total frequency was summed up for each animal for the 30 min of observation time. Rearing was taken as the

number of times the mouse was standing on its hind limbs or with its forelimbs against the wall of the observation cage or in the free air. Grooming was taken as the number of body cleaning with paws, picking of the body and pubis with mouth and face washing actions. In another set of experiments, mice were pretreated 15 minutes prior with neurotransmitter blockers to evaluate the mode of actions of the extracts on novelty-induced behaviour in mice. The following receptor blockers were used: atropine (0.5 mg/kg), naloxone (0.25), propanolol (0.2 mg/kg), haloperidol (0.2 mg/kg), ciproheptadine (0.5 mg/kg) and yohimbine (1 mg/kg) (Adenibigbe et al., 2010).

3.6.2 *Exploratory behaviour on hole-board apparatus*

The effect of the extract on the frequency of head dipping was determined in the hole-board with a number of holes (usually 16) in the floor through which the animal can poke its head. The test is a measure of exploratory behavior that reveals sedative activity of agents. The animals were divided into six groups (n = 5). Group (1) was given distilled water (0.2 mL/20 g, i.p), while group (2-5) received *O. subscorpioidea* (3.125, 6.25, 12.5 and 25 mg/kg, i.p) respectively and group (6) received diazepam (2 mg/kg). The animals were placed on top of the wooden board 30 minutes after administration of the extract at different doses. The number of times that each animal dipped its head into the holes was counted for the period of 5 min (Dorr et al., 1971).

3.6.3 *Locomotor activity in the open field*

Motor activity was measured in an open field apparatus consisting a white plexiglas box (28 cm × 28 cm × 25 cm) with a painted black grid dividing the floor into 16 (7 × 7 cm) equal squares. The animals were divided into six groups (n = 5). Group (1) was given the vehicle (0.2 mL/20 g distilled water), while group (2-5) were given crude extract (3.125, 6.25, 12.5 and 25 mg/kg, i.p) respectively and group (6) received diazepam (2 mg/kg). Thirty minutes after a single i.p. injection of extract and control, the animals were placed singly in one of the corners of the box; the number of squares crossed with all four paws was counted for 5 min. The cages were cleaned with 70% ethanol at intervals when the animal is removed (Akanmu et al., 2011). Diazepam (2 mg/kg, i.p.) administered to group (6), served as reference drug.

3.6.4 *The Y maze test*

The Y-maze can be used as a measure for short term working memory. Spontaneous alternation is a measure of spatial working memory. To alternate among spatial location, a mouse must remember its previous location. Spontaneous alternation performance was assessed using a Y-

maze composed of three equally spaced arms (120°, 41 cm long x 15 cm high). The floors of each arm consisted of wood (5 cm wide). The test was carried out using this apparatus to obtain results for spontaneous alternation performance (memory). The animals were divided into six groups. Group (1) was given distilled water (0.2 ml/20 g, i.p), group (2-5) received *O. subscorpioidea* (3.125, 6.25, 12.5 and 25 mg/kg, i.p) respectively and group (6) received diazepam (2 mg/kg) 30 minutes prior the observation. Each mouse was placed in one of the arm compartments usually arm C for consistency and was allowed to move freely for 5 min without reinforcers. An arm entry is defined as the body of a mouse except for its tail completely entering into an arm compartment. The sequence of arm entries is manually recorded. An alternation is defined as an entry into all three arms on consecutive devices. The percentage alternation was expressed as the ratio of actual alternations to possible alternations (defined as the total number of arm entries minus two) multiplied by 100. Seventy percent (70%) ethanol was used to clean the Y-maze at interval (Akanmu et al., 2007).

3.6.5 *Elevated plus maze test*

The elevated plus maze test was carried out to assess for possible anxiolytic effect of the extract. It is a modification of the apparatus validated for mice by Lister (1987) consisting of two open arms (30 x 5 x 0.25 cm) and two closed arms (30 x 5 x 15 cm) emanating from a common central platform (5 x 5 cm). The apparatus is made of wood, with the two pairs of identical arms opposite each other. The entire apparatus is elevated to a height of 50 cm above floor level. The animals were divided into six groups of five animals each. Group (1) received distilled water (0.2 mL/ 20 g, i.p), group (2-5) received *O. subscorpioidea* (3.125, 6.25, 12.5 and 25 mg/kg, i.p) and group (6) received diazepam (1 mg/kg, i.p.) thirty minutes before observation. At the start of the session the mouse was placed at the edge of an open arm, with its head facing the center and allowed to explore the maze for 5 min. During this test period, the following measurements were recorded: the total number of arm entries and the time spent in open and closed arms. An entry with all feet put into one arm is defined as an arm entry in this experiment. Seventy percent (70%) ethanol was used to clean the plus maze after each animal to prevent odor bias. The Index open arms avoidance [IOAA] was determined i.e. IOAA= 100 - (% time spent in open arms + % entries into open arms)/2.

3.6.6 *Pentobarbitone induced hypnosis*

The experiment was conducted following the method described by Ferrini et al. (1974). Swiss Albino mice (20–25 g) were divided into five groups (n = 5) in each group. Group (1) received the vehicle (distilled water, 0.2 mL/ 20 g, i.p), groups (2-4) received extract (6.25, 12.5 and 25 mg/kg, i.p.). Group (5) received Diazepam (2 mg/kg, i.p.) as positive control. All the treatments were administered 30 min prior to the administration of pentobarbitone (40 mg/kg, i.p.). The animals were observed for the latent period (time between pentobarbitone administrations to loss of righting reflex) and duration of sleep *i.e.* time between the loss and recovery of righting reflex.

3.6.7 *Monitoring of rectal body temperature*

The recording of the rectal body temperature was carried out using a thermoprobe. The effect of the crude extract on the body temperature was performed in five groups of male mice. Group (1) received the vehicle (distilled water, 0.2 mL/ 20 g, i.p), groups (2-4) received extract (6.25, 12.5 and 25 mg/kg i.p.). Group (5) received Diazepam (2 mg/kg, i.p.) as positive control. The probe of the thermometer was inserted 1.5 cm into the rectum. The temperature of the animals was recorded immediately before the test and 30, 60, 90, 120 and 180 min after the administration of control and extract. The pre drug recording served as the reference point for the determination of temperature change.

3.7 ANALGESIC ASSAYS

3.7.1 *Acetic acid-induced writhing test*

Acetic acid-induced writhing in mice was carried out according to the method described by Koster *et al* (1959). Animals were divided into five groups of five mice each. Group (1) received distilled water (10 ml/kg), group (2-4) received extract (12.5, 25, 50 mg/kg) respectively while group (5) received acetylsalicylic acid (150 mg/kg). Both the standard drug and the extract were administered 30 min before induction of nociception with 10 ml/kg of 0.6% acetic acid (i.p.). Five minutes after the administration of acetic acid, numbers of writhes were recorded for duration of 15 min.

3.7.2 *Formalin-induced paw licking test.*

Formalin test was carried out using the method of Santos and Calixto (1997). Animals were divided into five groups of five mice each. Group (1) received distilled water (10 mL/kg), group (2-4) received extract (12.5, 25, 50 mg/kg) respectively while group (5) received morphine (5 mg/kg). Both the standard drug and the extract were administered 30 min before induction of nociception with 20 µL of 1% formalin in the sub-planter space of the right-hind paw. The duration of paw licking as an index of painful response was determined at 0–5 min (early phase, neurogenic) and 20-30 min (late phase, inflammatory) after the formalin injection (Oyemitan et al., 2008).

3.7.3 *Tail immersion test*

Hot water-induced tail withdrawal reflex as a model of nociception was carried out according to the method of Janssen *et al* (1963). Animals were divided into five groups of five mice each. Group (1) received distilled water (10 mL/kg), group (2-4) received extract (12.5, 25, 50 mg/kg) respectively while group (5) received morphine (5 mg/kg). Thirty minutes later, the tail of each animal (up to 5 cm) was dipped in water at 55.0 ± 0.2°C. The time (in seconds) it took the animal to withdraw the tail clearly out of the water was taken as the reaction time to pain. The cut-off time of 10 s was used to avoid tissue damage. In order to assess possible involvement of opioid receptors, another 2 groups containing 5 mice each were randomly selected, a dose of extract (50 mg/kg) and morphine (5 mg/kg) were interacted with naloxone (1 mg/kg, i.p.). Naloxone was administered 15 min prior to administration of the extract (50 mg/kg) and morphine (5 mg/kg, i.p.) respectively. Thirty minutes later, the mice were subjected to the test as described (Janssen et al., 1963).

3.7.4 *Hot plate test*

The hot plate test was carried out in groups of male mice according to Franzotti et al. (2000) using a hot plate apparatus maintained at 55 ± 0.5 °C. Only mice that showed initial nociceptive responses within 1s were selected for the experiment. The animals were divided into five groups of five mice each. Group (1) received distilled water (10 mL/kg), group (2-4) received extract (12.5, 25, 50 mg/kg) respectively while group (5) received morphine (5 mg/kg). Thirty minutes after the treatment, the reaction time of animals was recorded. A post-treatment cut-off time of 10 s was used.

3.8 ANTIDEPRESSANT ASSAYS

3.8.1 *Forced swimming test (FST)*

Male mice (20-24 g) were used in the forced swimming test (Porsolt et al., 1977). Mice were assigned to six different groups (n = 5 for each group). Group (1) received distilled water (10 mL/kg), group (2-5) received extract (6.25 12.5, 25, 50 mg/kg) respectively while group (6) received Imipramine (25 mg/kg). Thirty minutes later, mice were dropped one at a time into a Plexiglas cylinder (25 cm height, diameter 10 cm containing water to a height of 10 cm at 23-25°C) and observed for 6 min. After the first 2 min of the initial vigorous struggling, the animals were immobile. A mouse was judged immobile if it floated in the water in an upright position and made only slight movements to prevent sinking. The total duration of immobility was recorded during the last 4 min of the 6 min test.

3.8.2 *Tail Suspension Test*

The total duration of immobility following tail suspension was measured according to the method described for evaluating potential antidepressants (Rodrigues, 2002). Mice were assigned to six different groups (n = 5 for each group). Group (1) received distilled water (10 mL/kg), group (2-5) received extract (6.25 12.5, 25, 50 mg/kg) respectively while group (6) received imipramine (25 mg/kg). Thirty minutes later mice were suspended on the edge of a table, 50 cm above the floor with the help of an adhesive tape placed approximately 1 cm from the tip of the tail. Immobility time was recorded during 6 minutes period in different groups. The animal was considered to be immobile when it did not show any movement of the body and hanged passively.

3.8.3 *Open field Test (OFT)*

In order to rule out any unspecific locomotor effect of *O. subscorpioidea* on antidepressant-like effect of these compounds, mice were administered with the same regimen as in the FST or TST. Their locomotor activities (crossing activity) were evaluated in the open field paradigm. Before each test, animals were kept in the test room at least 1 hour before the open-field test (OFT) for habituation. The ambulatory behavior was assessed in open-field test described by Rodrigues et al. (1996). The main apparatus consisted of square arena (50 cm × 50 cm × 40 cm) high with grey surface covering every wall. The floor of the arena was divided equally into twenty-five squares (10 cm × 10 cm) marked by black lines. All animals were used only once in this test.

These animals were different from those used in the FST and TST. Group (1) received distilled water (10 ml/kg), group (2-5) received extract (6.25 12.5, 25, 50 mg/kg) respectively while group (6) received imipramine (25 mg/kg). Thirty minutes after, each mouse was placed individually into the center of the arena and allowed to explore freely. The number of squares crossed with all paws (crossing) were observed and counted in 5 minutes. The square arena was cleaned with a solution of 70% alcohol between tests and dried after occupancy by each mouse in order to hide animal clues and to prevent each mouse from being influenced by the odors present in the urine and feces of the previous mouse.

3.8.4 *Yohimbine induced lethality test*

To reveal whether the noradrenergic system is involved in the antidepressant-like effect of the extract, the yohimbine induced lethality test was performed (Voger and Voger, 1997). Mice were assigned to six different groups (n = 5 for each group). Group (1) received distilled water (10 mL/kg), group (2-5) received extract (6.25 12.5, 25, 50 mg/kg) respectively while group (6) received imipramine (25 mg/kg) 30 minutes prior to yohimbine administration (35 mg/kg, i.p.). The number of dead mice was calculated during a 24 h period after the injection of yohimbine.

3.8.5 *Reserpine indueal Depression*

Six groups of animal (Group 1 to 6) were reserpinised by administration of reserpine (2.5 mg/kg, i.p.) one hour after the respective drug administration. Group (1) received distilled water (10 mL/kg), group (2-5) received extract (6.25 12.5, 25, 50 mg/kg) respectively while group (6) received imipramine (25 mg/kg). The acute effects of *Olax subscorpioidea* and Imipramine on reserpine induced diarrhea were observed. Mice were observed for the presence of diarrhea at 1, 2, 3 and 4 hours after reserpine injection.

3.9 ANTICONVULSANT ASSAYS

3.9.1 *Pentelenetetrazole (PTZ)- induced convulsions in mice*

Swiss Albino mice (20–25 g) were divided into live groups (n = 5) in each group. Group (1) received distilled water (10 mL/kg). Groups (2 - 4) received extract (12.5, 25, 50 mg/kg, i.p.) respectively. Group (5) received phenobabitone (40 mg/kg). All the treatments were administered intraperitoneally 30 min prior to administration of PTZ (85 mg/kg). Each animal was observed for 30 min by placing in a separate cage. The onset of action and the time taken for

death/recovery as well as the percentage of protection against mortality (Hosseinzadeh and Parvardeh, 2004) were recorded.

3.9.2 *Strychnine -induced convulsions in mice*

Swiss Albino mice (20–25 g) were divided into five groups (n = 5) in each group. Group (1) received distilled water (10 mL/kg). Groups (2 - 4) received extract (12.5, 25, 50 mg/kg, i.p.) respectively. Group (5) received phenobabitone (40 mg/kg). All treatments were administered intraperitoneally 30 min prior to the administration of strychnine (2 mg/kg). Each animal was observed for 30 min by placing in a separate cage. The onset of action and the time taken for death/recovery as well as the percentage of protection against mortality (Hosseinzadeh and Parvardeh, 2004) were recorded.

3.9.3 *Picrotoxin-induced convulsions in mice*

Swiss albino mice (20–25 g) were divided into five groups (n = 5) in each group. Group (1) received distilled water (10 mL/kg). Groups (2-4) received extract (12.5, 25, 50 mg/kg, i.p.) respectively. Group (5) received phenobabitone (40 mg/kg). All treatments were administered intraperitoneally 30 min prior to the administration of picrotoxin (7 mg/kg). Each animal was observed for 30 min by placing in a separate cage. The onset of action and the time taken for death/recovery as well as the percentage of protection against mortality (Hosseinzadeh and Parvardeh, 2004) were recorded.

3.10 STATISTICAL ANALYSIS

All data are presented as Mean ± SEM. The results were analyzed by One way analysis of variance (ANOVA) and post hoc tests (Student's-Newman-Keuls) were carried out to determine the source of significant main effect using GraphPad InStat® Biostatistics software. The level of significance for all tests was set at $p < 0.05$.

CHAPTER FOUR

4.0 RESULTS

4.1 TOXICITY TEST

The LD_{50} of *Olax subscorpioidea* crude extract in mice was found to be 300 mg/kg i.p body weight.

4.2 EFFECT OF THE *OLAX SUBSCORPIOIDEA* ON NOVELTY-INDUCED REARING (NIR) AND GROOMING (NIG) BEHAVIOUR IN MICE.

Intraperitoneal administration of the extract (3.125 - 25.0 mg/kg) induced a dose dependent decrease in the novelty-induced rearing and grooming activity in mice compared with the control. A significant dose dependent reduction in the frequency of rearing ($P < 0.05$) and grooming ($P < 0.05$) episodes was observed when compared with the control. Maximal inhibition of NIR and NIG was observed at 25 mg/kg (Fig. 2a & 2b).

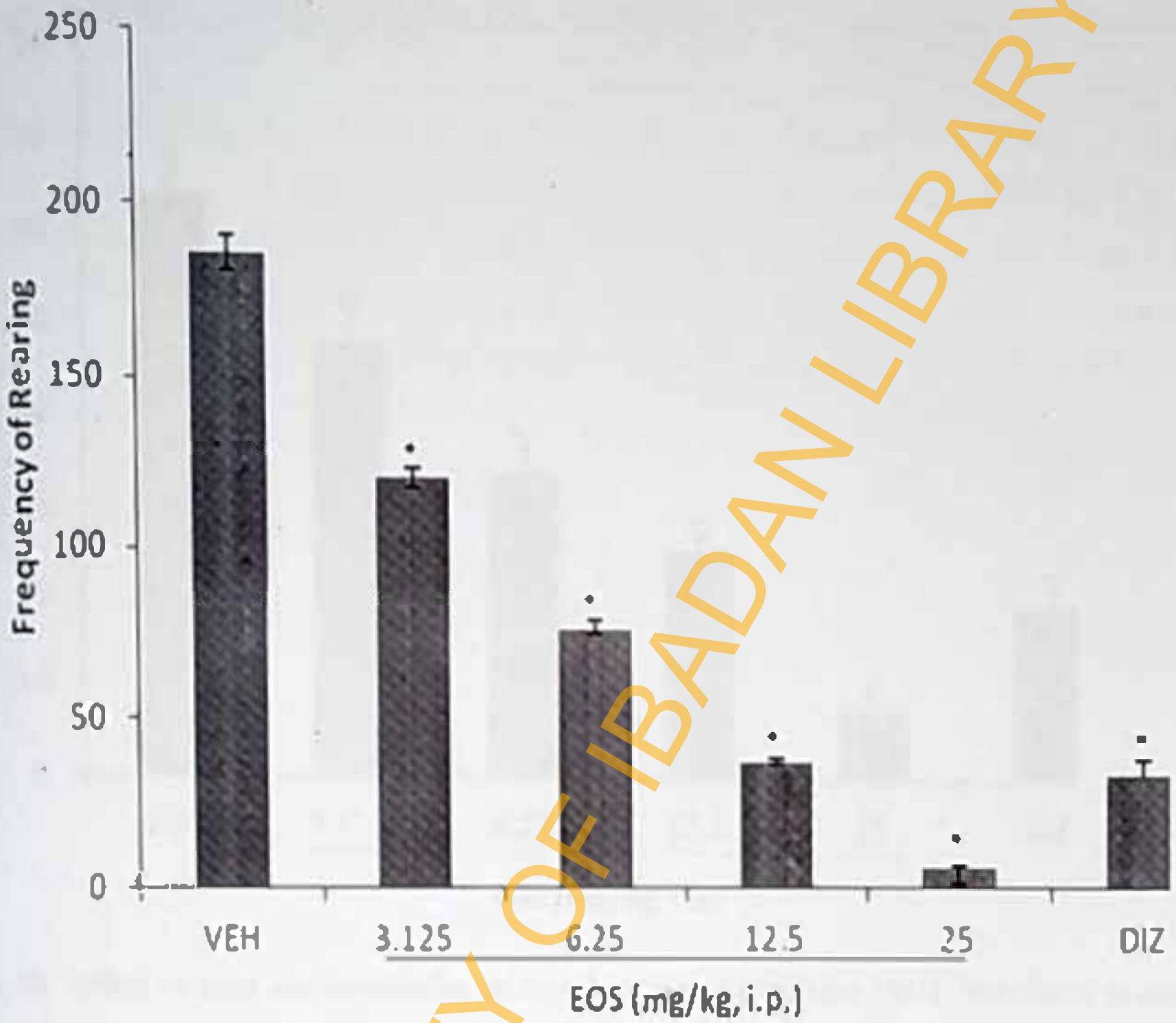

Fig. 2a: Effect of *Olax subscorpioidea* on novelty-induced rearing (NIR) behaviour in mice. The results are expressed as Mean ± SEM (n= 5). One way ANOVA revealed that there is significant [F (5, 24) = 387.8, p< 0.0001 (Rearing) difference between various treatment groups.

• indicates significant difference from the control P < 0.05

VEH: Vehicle; **EOS**: Ethanol Extract of *Olax subscorpioidea;* **DIZ**: Diazepam (2 mg/kg, i.p.)

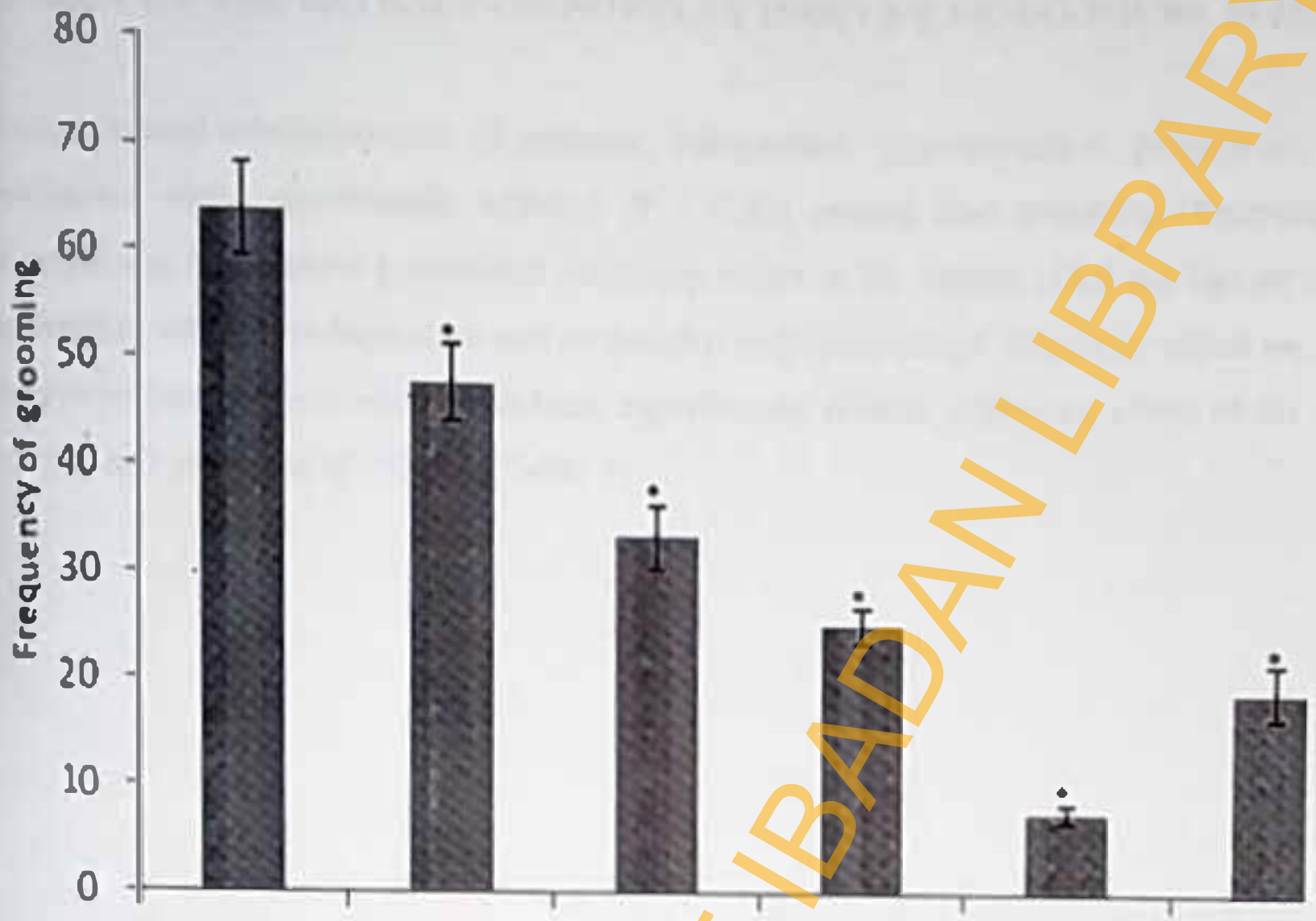

Fig. 2b: Effect of *Olax subscorpioidea* on novelty-induced grooming (NIG) behaviour in mice. The results are expressed as Mean ± SEM (n= 5). One way ANOVA revealed that there is significant F (5, 24) = 49.78, p< 0.0001 (Grooming)] difference between various treatment groups.

* indicates significant difference from the control P < 0.05

VEH: Vehicle; **EOS**: Ethanol Extract of *Olax subscorpioidea*; **DIZ**: Diazepam (2 mg/kg, i.p.)

4.3 EFFECT OF PRE-TREATMENT WITH ANTAGONISTS ON THE INHIBITORY EFFECT OF THE EXTRACT ON NOVELTY INDUCED BEHAVIOURS IN MICE

Intraperitoneal administration of atropine, haloperidol, ciproheptadine, propanolol, naloxone, yohimbine alone significantly reduced ($P < 0.05$) rearing and grooming. Pretreatment with atropine and haloperidol potentiated inhibitory effect of the extract (12.5 mg/kg) on rearing and grooming, while ciproheptadine and propanolol only potentiated inhibitory effect on grooming. However, pretreatment with yohimbine, significantly reverse inhibitory effect of the extract on rearing and grooming ($P < 0.05$). (Table 1)

Table 1: Effect of pre-treatment with antagonists on the inhibitory effect of the extract on novelty induced behaviours in mice.

Group	Dose (mg/kg)	Rearing	Grooming
VEH	10 mL/kg	185.80 ± 5.11	63.80 ± 4.27
EOS	12.5	37.40 ± 1.20	25.40 ± 1.63
Atropine	0.5	128.80 ± 8.42*	40.60 ± 2.56*
Atropine+ EOS		16.40 ± 1.03**	16.40 ± 1.03**
Haloperidol	0.2	107.06 ± 11.64*	46.00 ± 0.70
Haloperidol + EOS		15.40 ± 1.96**	7.80 ± 0.48**
Cyproheptadine	0.5	103.00 ± 3.97*	17.20 ± 1.39*
Cyproheptadine+ EOS		30.80 ± 5.79	16.20 ± 1.56**
Propanolol	0.2	96.00 ± 4.03*	42.60 ± 5.41*
Propanolol + EOS		34.00 ± 6.16	12.00 ± 2.38**
Naloxone	0.25	81.12 ± 2.12*	29.5 ± 2.33*
Naloxone+ EOS		44.00 ± 1.23	36.60 ± 5.23
Yohimbine	1	127.20 ± 14.39*	38.20 ± 7.94*
Yohimbine + EOS		100.40 ± 9.10**	50.20 ± 6.20**

The results are expressed as mean ± SEM (n= 5). One way ANOVA revealed that there is significant [F (3, 16) = 16.86, p< 0.0001; F (3, 16) = 29.96, p< 0.0001] difference between various treatment groups.

* indicates significant difference from the control P < 0.05.

** indicates significant difference from the extract at P< 0.05

VEH: Vehicle; **EOS:** Ethanol Extract of *Olax subscorpioidea;*

1.4 EFFECT OF *OLAX SUBSCORPIOIDEA* OF ON THE FREQUENCY OF HEAD DIP ON HOLE-BOARD

Intraperitoneal administration of the crude extract (3.125 - 25.0 mg/kg) induced significant dose dependent reduction ($p < 0.05$) in the frequency of head-dip in mice. Maximal reduction was observed at 25 mg/kg. Similarly, diazepam caused a significant ($p < 0.05$) decrease in the frequency of head-dips (Fig. 3).

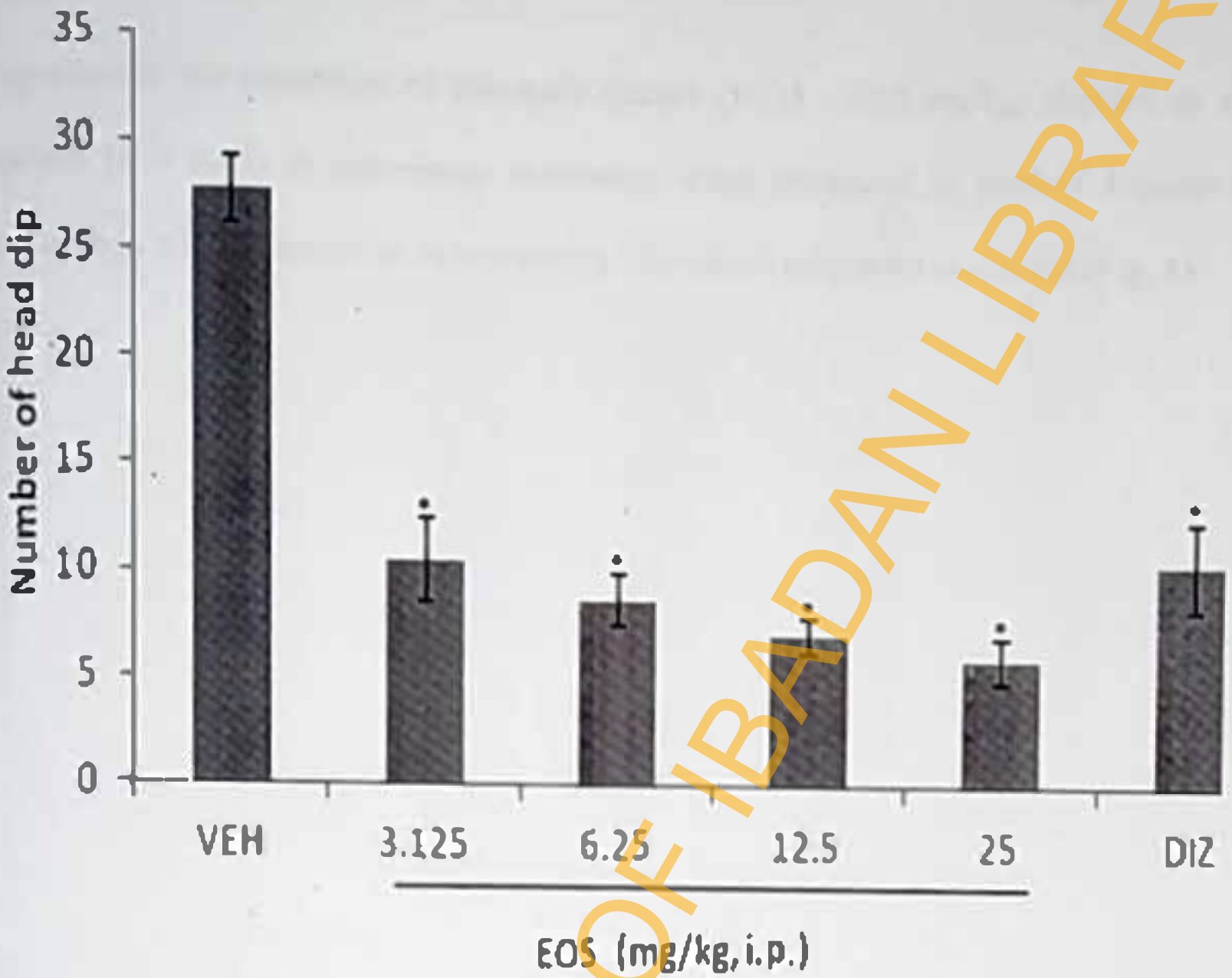

Fig. 3: Effect of *Olax subscorpioidea* oil on the frequency of Head dips

The results are expressed as Mean ± SEM (n= 5). One way ANOVA revealed that there is significant [F (5, 24) = 29.86, p< 0.0001] difference between various treatment groups.

• indicates significant difference from the control P < 0.05

VEH: Vehicle; **EOS**: Ethanol Extract of *Olax subscorpioidea*; **DIZ**: Diazepam (2 mg/kg, i.p.)

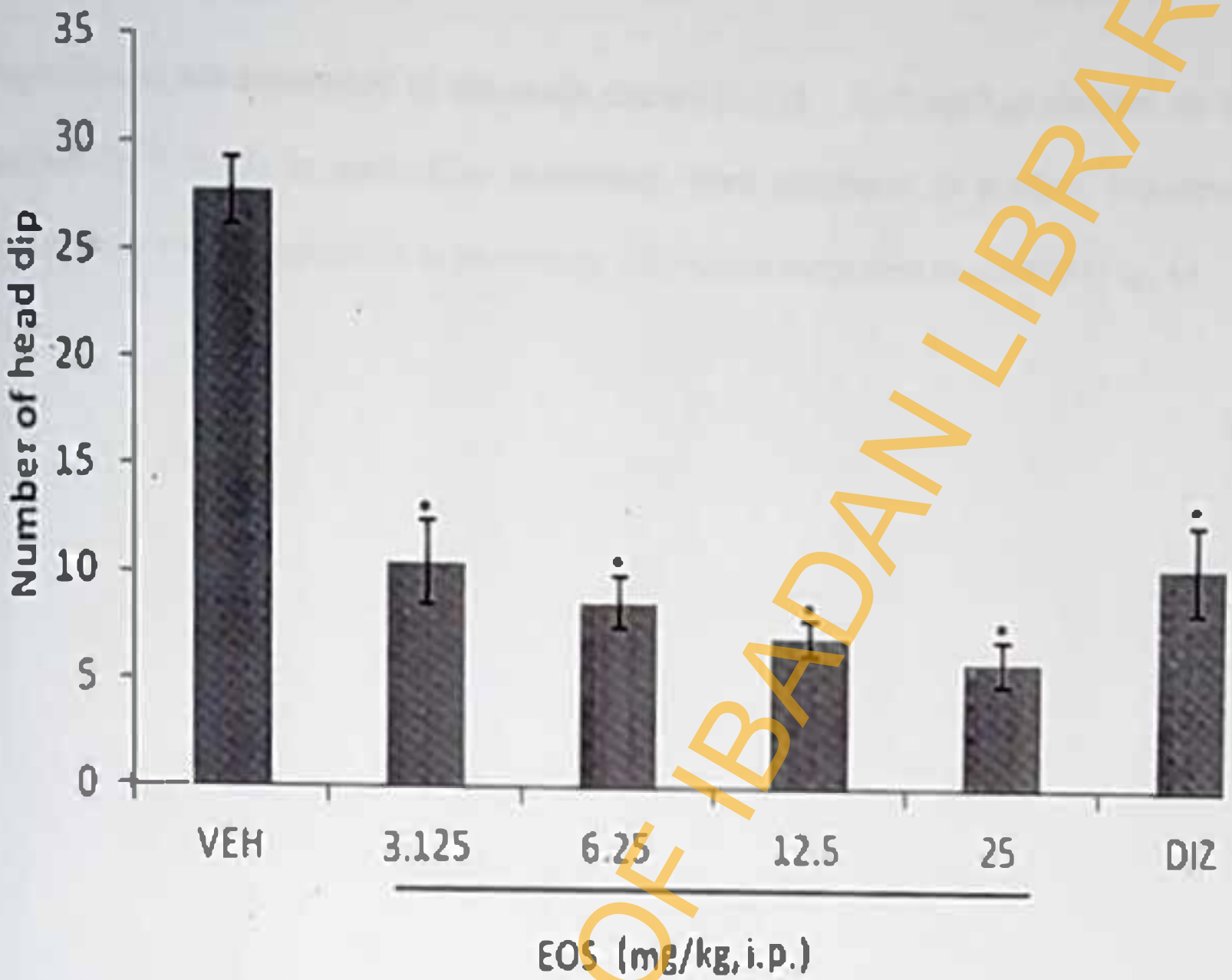

Fig. 3: Effect of *Olax subscorpioidea* of on the frequency of Head dips

The results are expressed as Mean ± SEM (n= 5). One way ANOVA revealed that there is significant [F (5, 24) = 29.86, p< 0.0001] difference between various treatment groups.

* indicates significant difference from the control P < 0.05

VEH: Vehicle; **EOS**: Ethanol Extract of *Olax subscorpioidea*; **DIZ**: Diazepam (2 mg/kg, i.p.)

.5 EFFECT OF EXTRACT OF *OLAX SUBSCORPIOIDEA* ON LEARNING AND IEMORY IN THE Y-MAZE

ntraperitoneal administration of the crude extract (3.125 - 25.0 mg/kg) showed no significant reduction ($p > 0.05$) in percentage alternation when compared to control. Diazepam caused significant ($p < 0.05$) reduction in percentage alternation compared to control (Fig. 4).

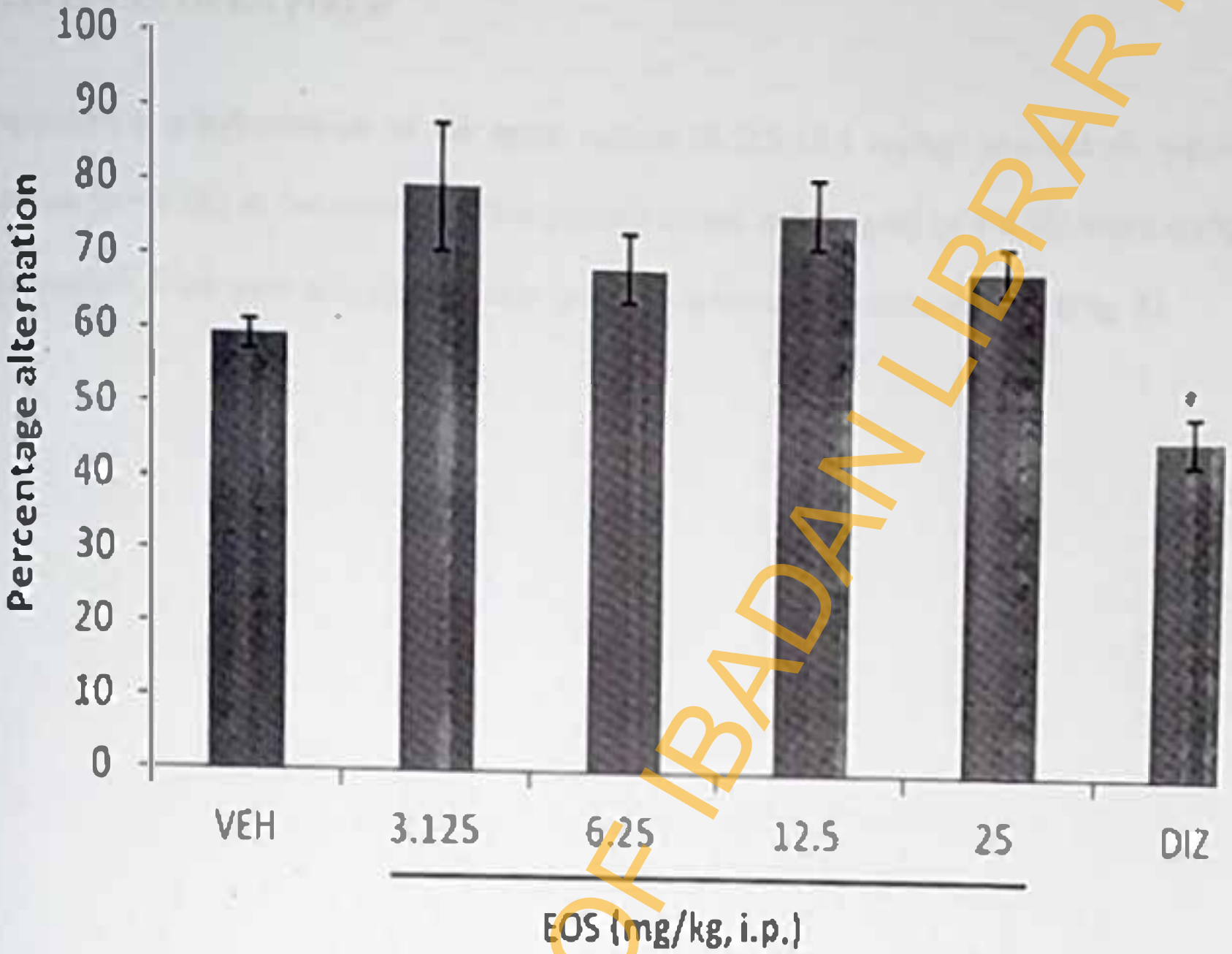

Fig. 4: Effect of extract of *Olax subscorpioidea* on learning and memory in the Y-maze

The results are expressed as Mean ± SEM (n= 5). One way ANOVA revealed that there is significant [F (5, 24) = 7.596, p = 0.0002] difference between diazepam and control groups.

• indicates significant difference from the control $P < 0.05$

VEH: Vehicle; **EOS**: Ethanol Extract of *Olax susbscorpioidea*; **DIZ**: Diazepam (2 mg/kg, i.p.)

4.6 EFFECT OF THE EXTRACT OF *OLAX SUBSCORPIOIDEA* ON LOCOMOTOR ACTIVITY IN OPEN FIELD

Intraperitoneal administration of the crude extract (3.125-12.5 mg/kg) showed no significant reduction ($p > 0.05$) in the number of line crossed except at 25 mg/kg ($p < 0.05$) when compared to the control. Diazepam also significantly ($p<0.05$) reduced locomotor activity (Fig. 5).

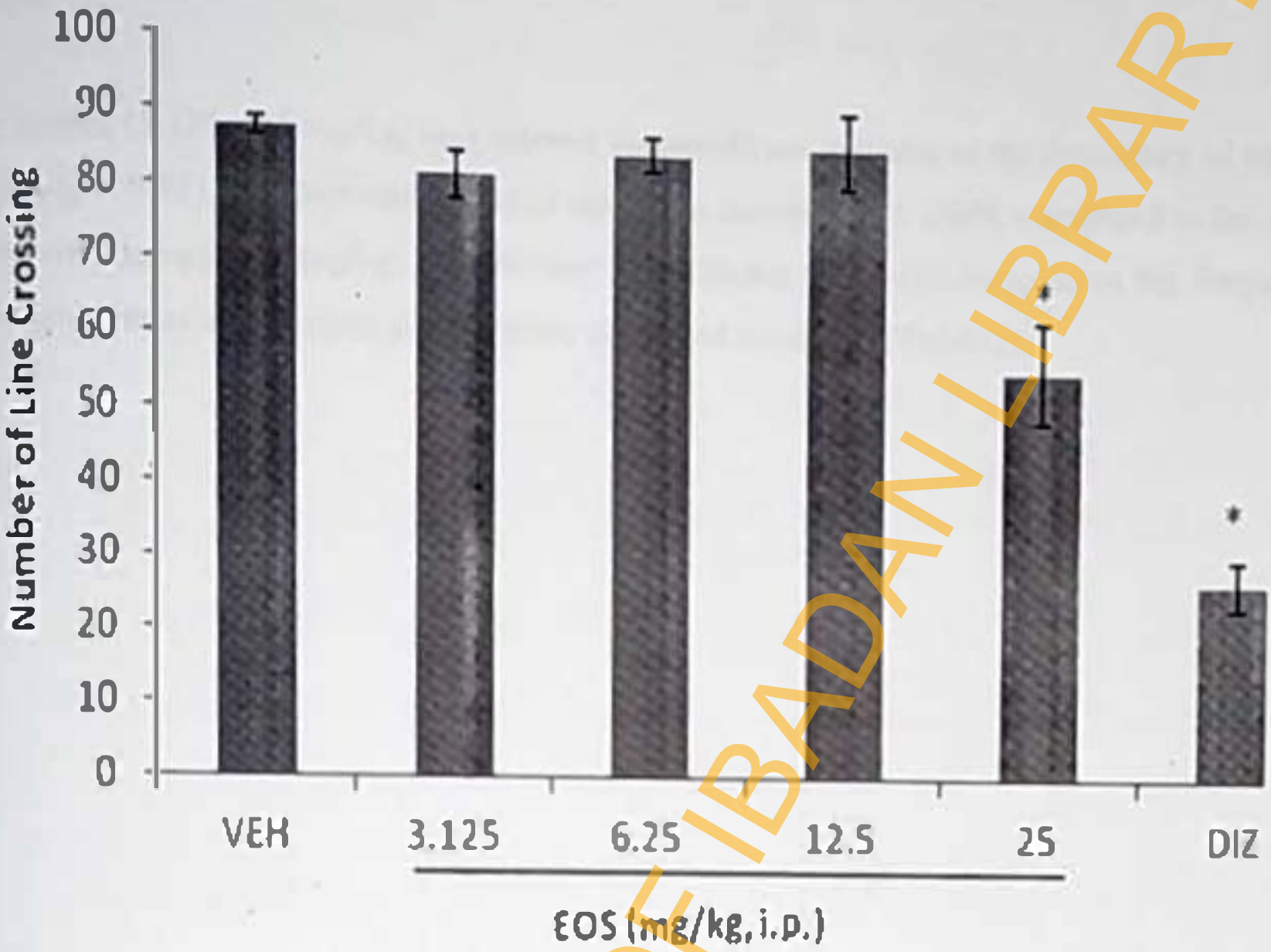

Fig. 5: Effect of the extract of *Olax subscorpioidea* on locomotor activity in open field

The results are expressed as Mean ± SEM (n= 5). One way ANOVA revealed that there is significant [$F(5, 24) = 35.98$, $p < 0.0001$] difference between various treatment groups.

* indicates significant difference from the control $P < 0.05$

VEH: Vehicle; **EOS:** Ethanol Extract of *Olax subscorpioidea*; **DIZ:** Diazepam (2 mg/kg, i.p.)

.7 EFFECT OF THE EXTRACT OF ON THE ELEVATED-PLUS MAZE (EPM) IN MICE

The extract (3.125 - 25 mg/kg, i.p.) showed no significant increase in the frequency of open arm entries ($p > 0.05$), and percentage (%) of open arm duration ($p > 0.05$), compared to the control. However, diazepam (1 mg/kg, i.p.) showed a significant ($p < 0.05$) increase in the frequency of open arm entries and % open arm duration compared to control (Table 2).

Table 2: Effect of the extract of on anxiety in mice using the Elevated-Plus Maze (EPM)

Group	Dose (mg/kg)	OPEN ARM ENTRY	CLOSED ARM ENTRY	% OPEN ARM DURATION	INDEX OF OPEN ARM AVOIDANCE
VEH	10 mL/kg	1.40 ± 0.40	7.80 ± 0.73	17.78 ± 3.23	88.28 ± 1.66
EOS	3.125	2.20 ± 0.48	6.40 ± 0.67	39.41 ± 1.34	65.11 ±2.43
	6.25	3.60 ± 0.24	7.80 ± 0.58	31.97 ± 1.81	68.99 ± 1.10
	12.5	3.80 ± 1.02	10.20 ± 1.11	34.98 ± 3.12	72.81 ± 2.57
	25	3.20 ± 1.15	7.80 ± 1.11	34.96 ± 5.36	70.48 ± 5.39
DIZ	1	8.20 ±1.39*	1.80 ± 0.37*	80.40 ± 4.57*	18.23 ± 2.59*

The results are expressed as Mean ± SEM (n= 5). One way ANOVA revealed that there is significant [F (5, 24) = 35.98, $p < 0.0001$] difference between various treatment groups.

* indicates significant difference from the control $P < 0.05$

VEH: Vehicle; **EOS**: Ethanol Extract of *Olax subscorpioidea*; **DIZ**: Diazepam (1 mg/kg, i.p.)

.8 EFFECT OF *OLAX SUBSCORPIOIDEA* ON PENTOBARBITONE INDUCED HYPNOSIS

The extract (12.5-50 mg/kg, i.p.) showed no significant ($p<0.05$) increase in the onset of sleep (Fig. 6a). However, there was a significant and dose dependent increase in the duration of sleep in mice compared to the control (Fig.6b).

4.8 EFFECT OF *OLAX SUBSCORPIOIDEA* ON PENTOBARBITONE INDUCED HYPNOSIS

The extract (12.5-50 mg/kg, i.p.) showed no significant ($p<0.05$) increase in the onset of sleep (Fig. 6a). However, there was a significant and dose dependent increase in the duration of sleep in mice compared to the control (Fig.6b).

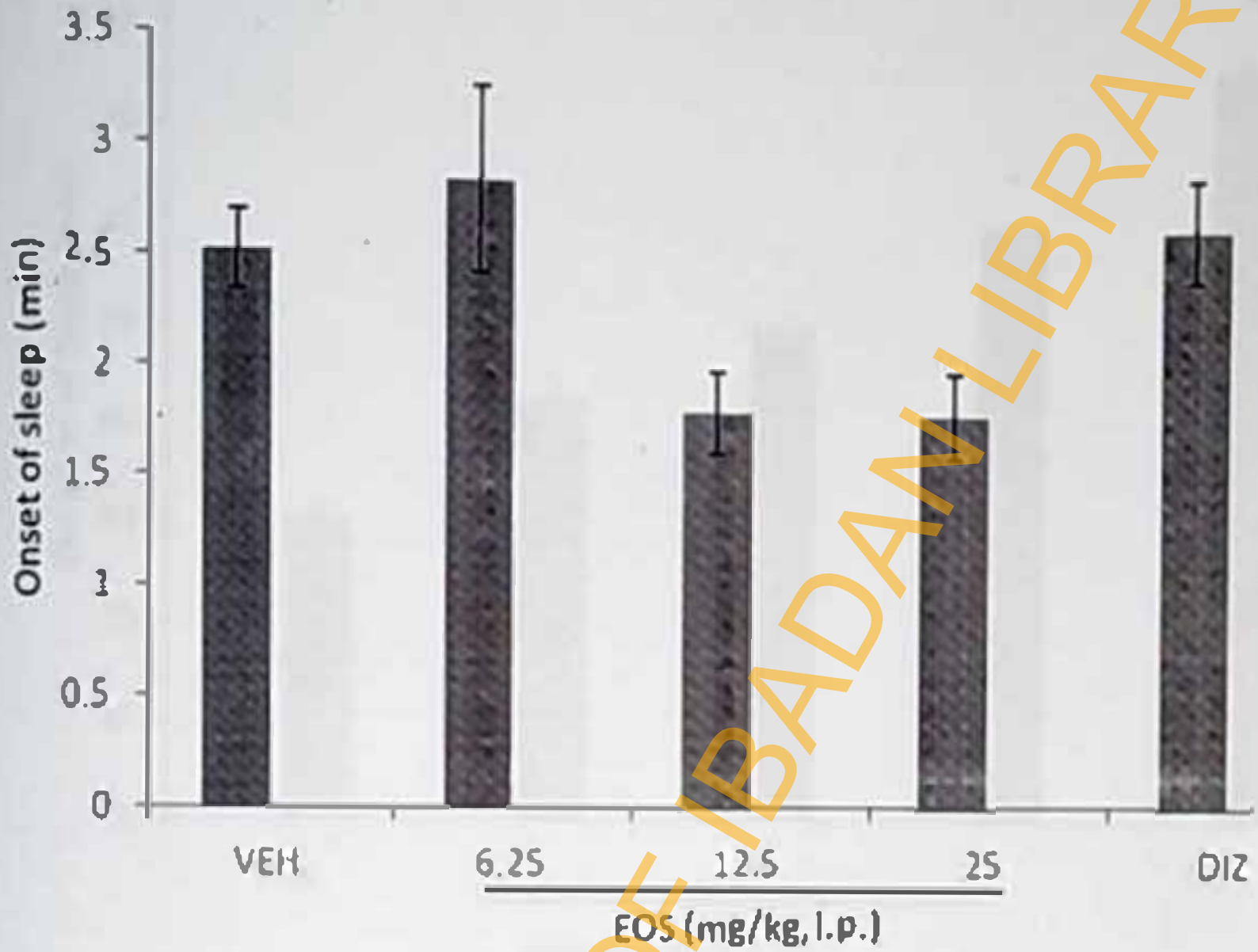

Fig. 6a: Effect of *Olax subscorpioidea* on pentobarbitone induced Sleeping time

The results are expressed as Mean ± SEM (n= 5). One way ANOVA revealed that there is significant F (4, 20) = 187.0 $p<0.0001$ (Duration)] difference between various treatment groups.

• indicates significant difference from the control $P < 0.05$

VEH: Vehicle; **EOS**: Ethanol Extract of *Olax susbscorpioidea*, **DIZ**: Diazepam (2 mg/kg, i.p.)

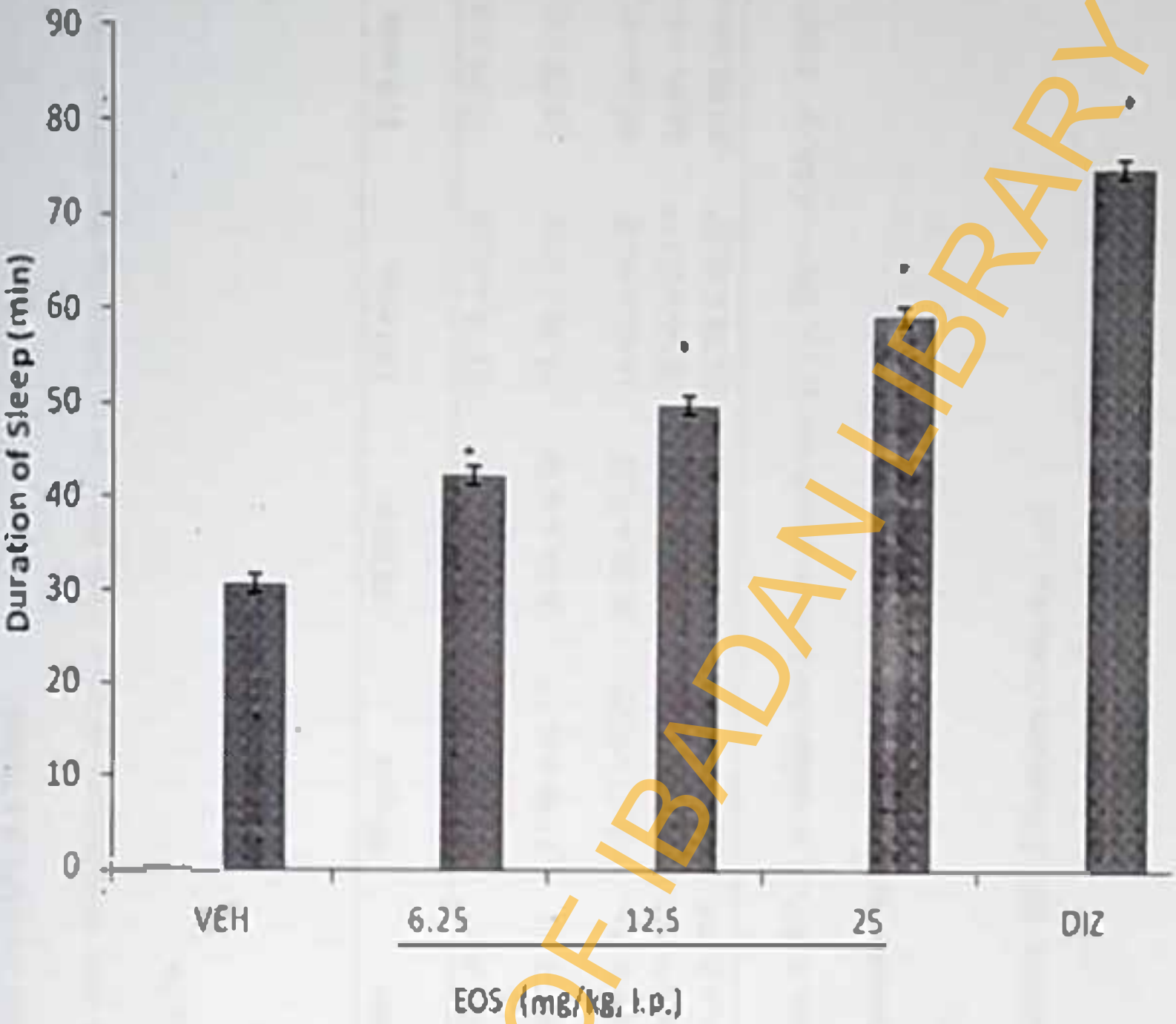

Fig. 6b: Effect of *Olax subscorpioidea* on pentobarbitone induced Sleeping time

The results are expressed as Mean ± SEM (n= 5). One way ANOVA revealed that there is significant F (4, 20) = 187.0 p<0.0001 (Duration)] difference between various treatment groups.

* indicates significant difference from the control P < 0.05

VEH: Vehicle; **EOS**: Ethanol Extract of *Olax subscorpioidea;* **DIZ**: Diazepam (2 mg/kg, i.p.)

4.9 EFFECT OF THE EXTRACT ON RECTAL BODY TEMPERATURE

The extract (12.5-25 mg/kg i.p) showed no effect on the body temperature of mice while the dose of 50mg/kg significantly ($p< 0.05$) reduced mice body temperature compare to control (Table 3).

Table 3: Effect of the extract on rectal body temperature

Group	Dose mg/kg	0 min	30 min	60 min	90 min	120 min	180 min	210 min
VEH	10 mL/kg	38.30 ± 0.19	37.76 ±0.18	38.00 ± 0.05	37.94 ± 0.12	37.94 ± 0.17	37.78 ± 0.19	37.98 ± 0.05
EOS	12.5	38.08 ± 0.18	38.00 ± 0.34	37.90 ± 0.23	37.86 ± 0.19	38.00 ± 0.34	37.90 ± 0.23	37.78 ± 0.20
	25	38.62 ± 0.13	38.02 ± 0.27	38.14 ± 0.20	38.22 ± 0.25	38.38 ± 0.22	38.00 ± 0.12	38.56 ± 0.20
	50	38.32 ± 0.25	36.08 ± 0.19*	35.78 ± 0.11*	36.02 ± 0.20*	36.30 ± 0.16*	36.56 ± 0.11*	36.88 ± 0.12*
DIZ	2	38.12 ± 0.15	35.08 ± 0.29*	35.01 ± 0.20*	35.00 ± 0.10*	35.63 ± 0.36*	36.78 ± 0.53*	36.98 ± 0.01*

The results are expressed as mean ± SEM (n= 5). One way ANOVA revealed that there is significant [$F(3, 16) = 16.86$, $p< 0.0001$; $F(3, 16) = 29.96$, $p< 0.0001$] difference between various treatment groups.

* indicates significant difference from the control $P < 0.05$.

VEH: Vehicle; **EOS**: Ethanol Extract of *Olax subscorpioidea*; **DIZ**: Diazepam (2 mg/kg, i.p.)

4.10 EFFECT OF *OLAX SUBSCORPIOIDEA* ON ACETIC ACID INDUCED ABDOMINAL CONSTRICTION/ WRITHING

Olax subscorpioidea produced a significant ($P< 0.05$) and dose-dependent reduction in the number of writhes compared to control. The effect of the extract at 12.5, 25 and 50 mg/kg (66.92%, 72.69% and 81.54% inhibition) and that of acetylsalicylate (150 mg/kg) (70% inhibition) are comparable (Fig. 7).

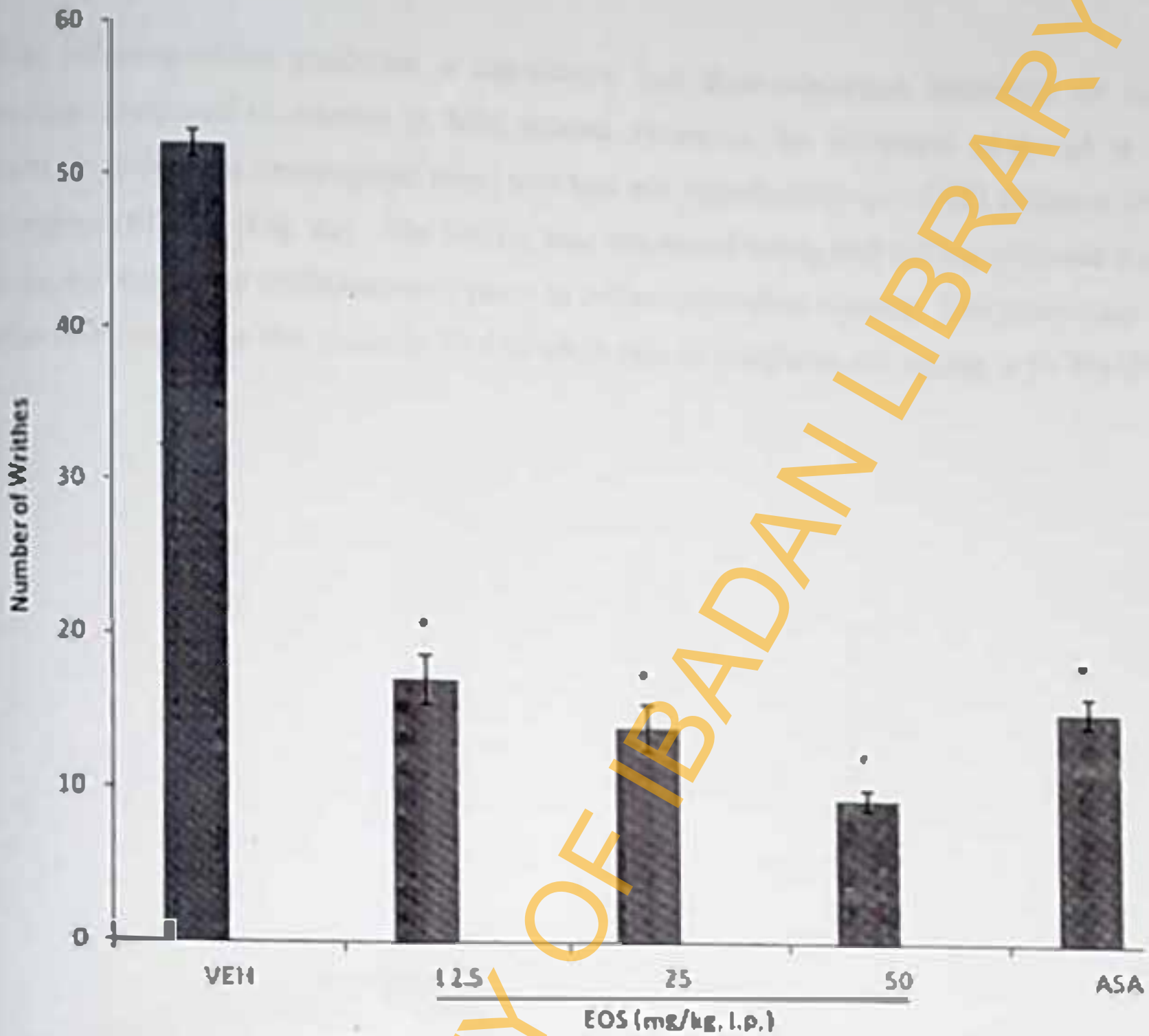

Fig. 7: Effect of the *Olax subscorpioidea* and Acetyl Salicylic Acid (ASA) on acetic acid-induced writhing in mice

The results are expressed as Mean ± SEM (n= 5). One way ANOVA revealed that there is significant [F (4, 20) = 155.9, p< 0.0001] difference between various treatment groups.

• indicates significant difference from the control P < 0.05.

VEH: Vehicle; **EOS**: Ethanol Extract of *Olax susbscorpioidea*; **ASA**:acetylsalicylic acid (150 mg/kg)

4.11 EFFECT OF *OLAX SUBSCORPIOIDEA* ON FORMALIN PAW-LICKING TEST IN MICE

Olax subscorpioidea produced a significant and dose-dependent inhibition of nociceptive reaction compared to control in both phases. However, the inhibition produced at 50 mg/kg (66%) in first phase (neurogenic pain) was less and significantly ($p < 0.05$) different from that of morphine (87.6%) (Fig. 8a). The extract also attenuated biting and licking response significantly in the second phase (inflammatory pain) in a dose dependent manner. The percentage inhibition offer at 50 mg/kg in this phase is 79.4 % while that of morphine at 5 mg/kg is 91.3% (Fig. 8b).

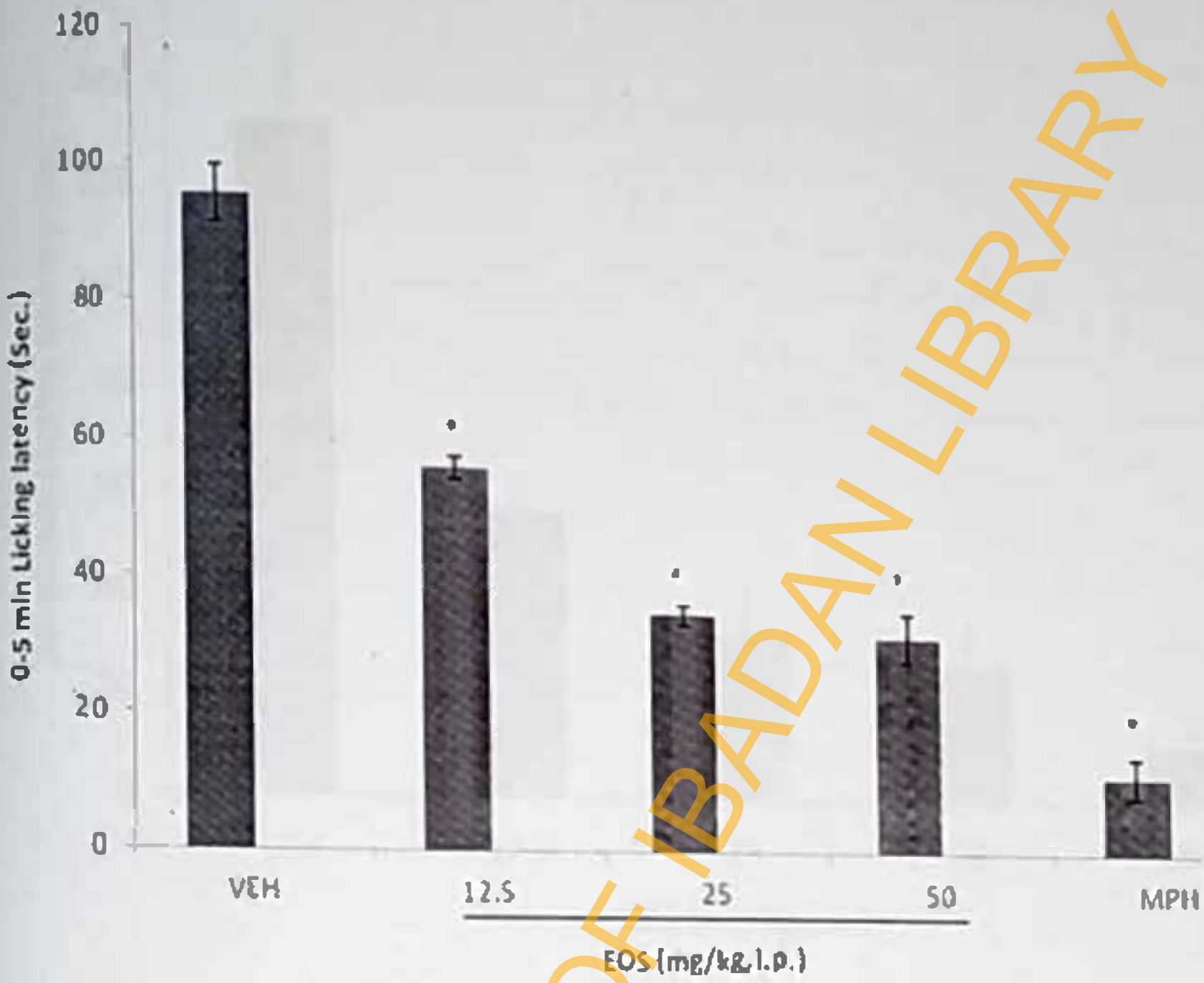

Fig. 8a: Effect of *Olax subscorpioidea* and morphine on formalin induced nociception in mice. The results are expressed as Mean ± SEM (n= 5). One way ANOVA revealed that there is significant [F (4, 20) = 116.7($p < 0.0001$)] difference between various treatment groups.

• indicates significant difference from the control $P < 0.05$.

VEH: Vehicle; **EOS:** Ethanol Extract of *Olax susbscorpioidea;* **MPH:** Morphine (5 mg/kg, i.p.)

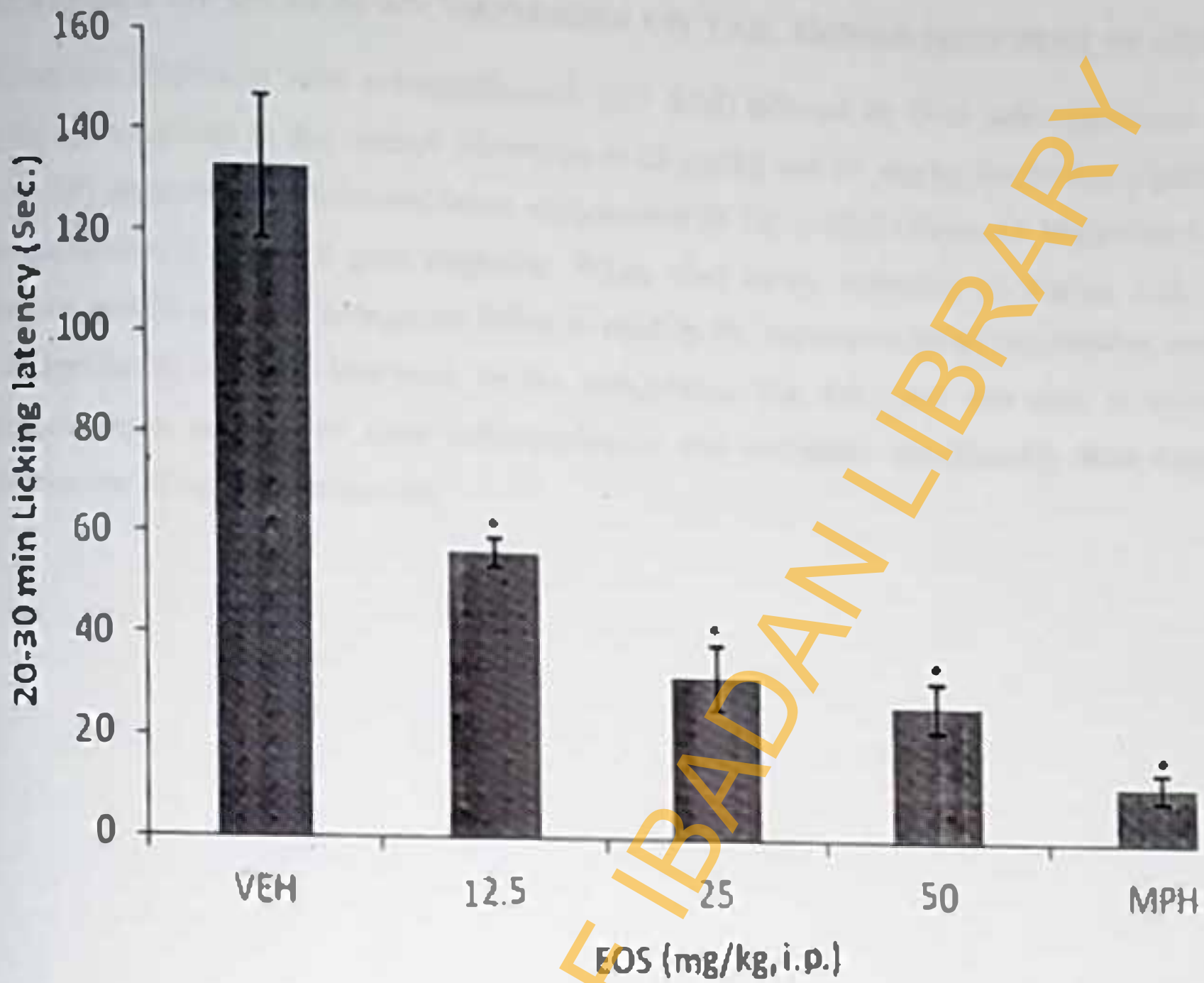

Fig. 8b: Effect of *Olax subscorpioidea* and morphine on formalin induced nociception in mice. The results are expressed as Mean ± SEM (n= 5). One way ANOVA revealed that there is significant [$F(4, 20) = 40.05$ ($p < 0.0001$)] difference between various treatment groups.

• indicates significant difference from the control $P < 0.05$.

VEH: Vehicle; **EOS:** Ethanol Extract of *Olax subscorpioidea*; **MPH:** Morphine (5 mg/kg, i.p.)

4.12 EFFECT OF *OLAX SUBSCORPIOIDEA* ON TAIL IMMERSION TEST IN MICE

Nociceptive responses were not significantly ($p > 0.05$) affected by *Olax subscorpioidea* at 12.5 mg/kg i.p compared to the control. However, at 25 mg/kg and 50 mg/kg the extract significantly ($p < 0.05$) reduced pain threshold when compared with the control (Table 4). Morphine 5 mg/kg also significantly reduced pain response. When used alone, naloxone (1 mg/kg, i.p), a non-selective opioid receptor antagonist failed to modify the thermal-induced nociceptive responses in a significant manner. However, in the antagonism test, naloxone was able to reverse the antinociceptive activity of *Olax subscorpioidea* and morphine significantly thus suggesting involvement of opiate mechanism.

Table 4. Effect of *Olax subscorpioidea* on the tail immersion test in mice

Group	Dose (mg/kg)	Reaction Time (sec.) (Mean ± S.E.M)			
		30 min	60 min	90 min	120 min
VEH	10 ml/kg	0.55±0.08	0.61± 0.06	0.61±0.06	0.63± 0.04
EOS	12.5	0.56± 0.04	1.03± 0.19	0.87± 0.22	1.06± 0.15
	25.0	1.39± 0.25*	1.55± 0.30*	1.43± 0.22*	1.60± 0.18*
	50.0	2.05± 0.23*	2.04± 0.21*	1.65± 0.27*	1.89± 0.26*
MPH	5.0	3.28± 0.17*	2.99± 0.22*	3.01± 0.11*	2.37± 0.14*
NAL	1.0	0.67± 0.04	0.54±0.35	0.47±0.23	0.58±0.05
NAL + EOS (50 mg/kg)		0.48±0.03**	0.81±0.11**	0.79±0.05**	0.89±0.21**
NAL + MPH		0.51±0.10***	0.51±0.10***	0.52±0.10***	0.50±0.10***

The results are expressed as Mean ± SEM (n= 5). One way ANOVA revealed that there is significant [F (6, 28) =43.2 ($p < 0.0001$) (30 min); F (6, 28) =19.49 ($p < 0.0001$) (60 min); F (6, 28) =22.7 ($p < 0.0001$) (90 min); F (6, 28) =15.83 ($p < 0.0001$) (120 min)] difference between various treatment groups.

* indicates significant difference from the control $P < 0.05$: ** indicates significant difference from the OS (50 mg/kg) $P < 0.05$; *** indicates significant difference from the morphine (5 mg/kg) $P < 0.05$

VEH: Vehicle; **EOS:** Ethanol Extract of *Olax subscorpioidea*; **MPH:** Morphine (5 mg/kg); **NAL:** naloxone (1 mg/kg)

4.13 EFFECT OF *OLAX SUBSCORPIOIDEA* ON THE HOT PLATE TEST IN MICE

Olax subscorpioidea (12.5 and 25 mg/kg) did not prolong latency period significantly ($p > 0.05$) compared to control. However, the effect of EOS (50 mg/kg) on latency was statistically significant at 90 and 120 minutes compared to the control (Table 5). Moreover, morphine (5 mg/kg) also exhibited significant ($P < 0.05$) antinociceptive activity compared to control (Table 5).

Table 5. Effect of *Olax subscorpioidea* on the hot plate test in mice

Group	Dose (mg/kg)	Reaction Time (sec.) (Mean ± S.E.M)			
		30 min	60 min	90 min	120 min
VEH	10 mL/kg	0.56 ±0.03	0.44 ± 0.06	0.46 ± 0.04	0.46 ± 0.04
EOS	12.5	0.43 ± 0.02	0.54 ± 0.06	0.45 ± 0.02	0.65 ± 0.04
	25	0.64 ± 0.05	0.62 ± 0.03	0.49 ± 0.02	0.72 ± 0.04
	50	0.77 ± 0.07	0.77 ± 0.04	0.93 ± 0.05*	0.94 ± 0.05*
MPH	5	2.10 ± 0.14*	2.75 ± 0.30*	3.02 ± 0.24*	3.05 ± 0.22*

The results are expressed as Mean ± SEM (n= 5). One way ANOVA revealed that there is significant [F (4, 20) = 97.95 (p< 0.0001) (90 min); F (4, 20) = 104.7 (p < 0.0001) (120 min)] difference between various treatment groups.

* indicates significant difference from the control P < 0.05.

VEH: Vehicle; **EOS**: Ethanol Extract of *Olax subscorpioidea*; **MPH**: Morphine (5 mg/kg)

4.14 EFFECT OF *OLAX SUBSCORPIOIDEA* ON IMMOBILITY TIME IN FORCED SWIMMING TEST (FST)

Olax subscorpioidea at 6.25 mg/kg and 12.5 mg/kg significantly reduced ($P < 0.05$) immobility time of mice in FST compared to the control (vehicle) while doses at 25 mg/kg and 50mg/kg did not reduce immobility time in mice. The clinically effective antidepressant imipramine at 25 mg/kg produced a marked significant reduction ($P < 0.05$) in the duration of immobility as compared with control. This is comparable to the 12.5 mg/kg dose of the extract (Fig 9).

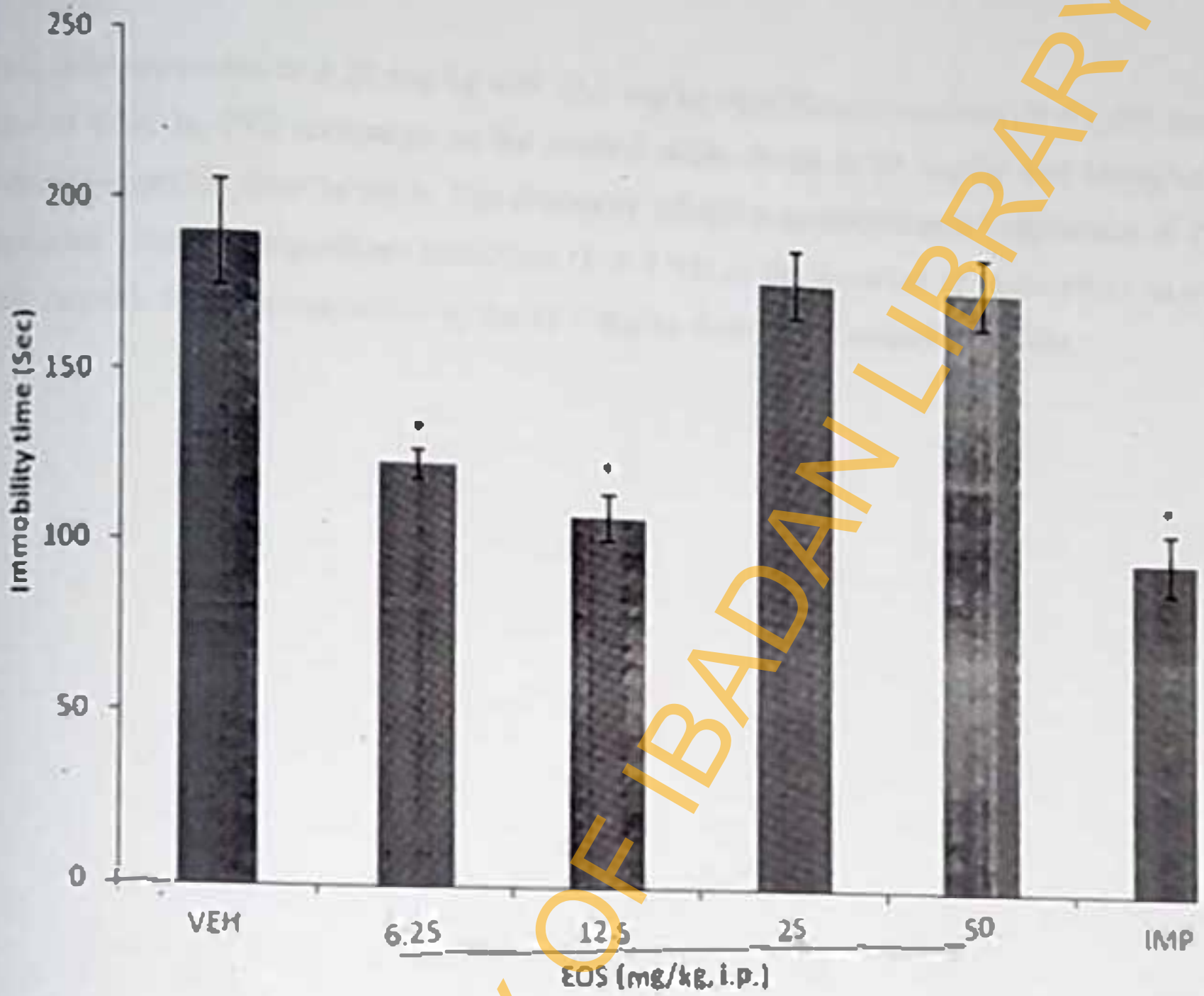

Fig. 9: Effect of *Olax subscorpioidea* on immobility time of Forced Swimming Test (FST)

The results are expressed as Mean ± SEM (n= 5). One way ANOVA revealed that there is significant [F (5, 24) = 17.22, p< 0.0001] difference between various treatment groups

* indicates significant difference from the control P < 0.05.

VEH: Vehicle; **EOS**: Ethanol Extract of *Olax subscorpioidea*; **IMP**: imipramine (25 mg/kg. i.p.)

4.15 EFFECT OF *OLAX SUBSCORPIOIDEA* ON IMMOBILITY TIME IN TAIL SUSPENSION TEST (TST)

Olax subscorpioidea at 6.25 mg/kg and 12.5 mg/kg significantly reduced ($P < 0.05$) immobility time of mice in TST compared to the control while doses at 25 mg/kg and 50mg/kg did not reduce immobility time in mice. The clinically effective antidepressant imipramine at 25 mg/kg produced a marked significant reduction ($P < 0.05$) in the duration of immobility as compared with control. This is comparable to the 12.5 mg/kg dose of the extract (Fig. 10).

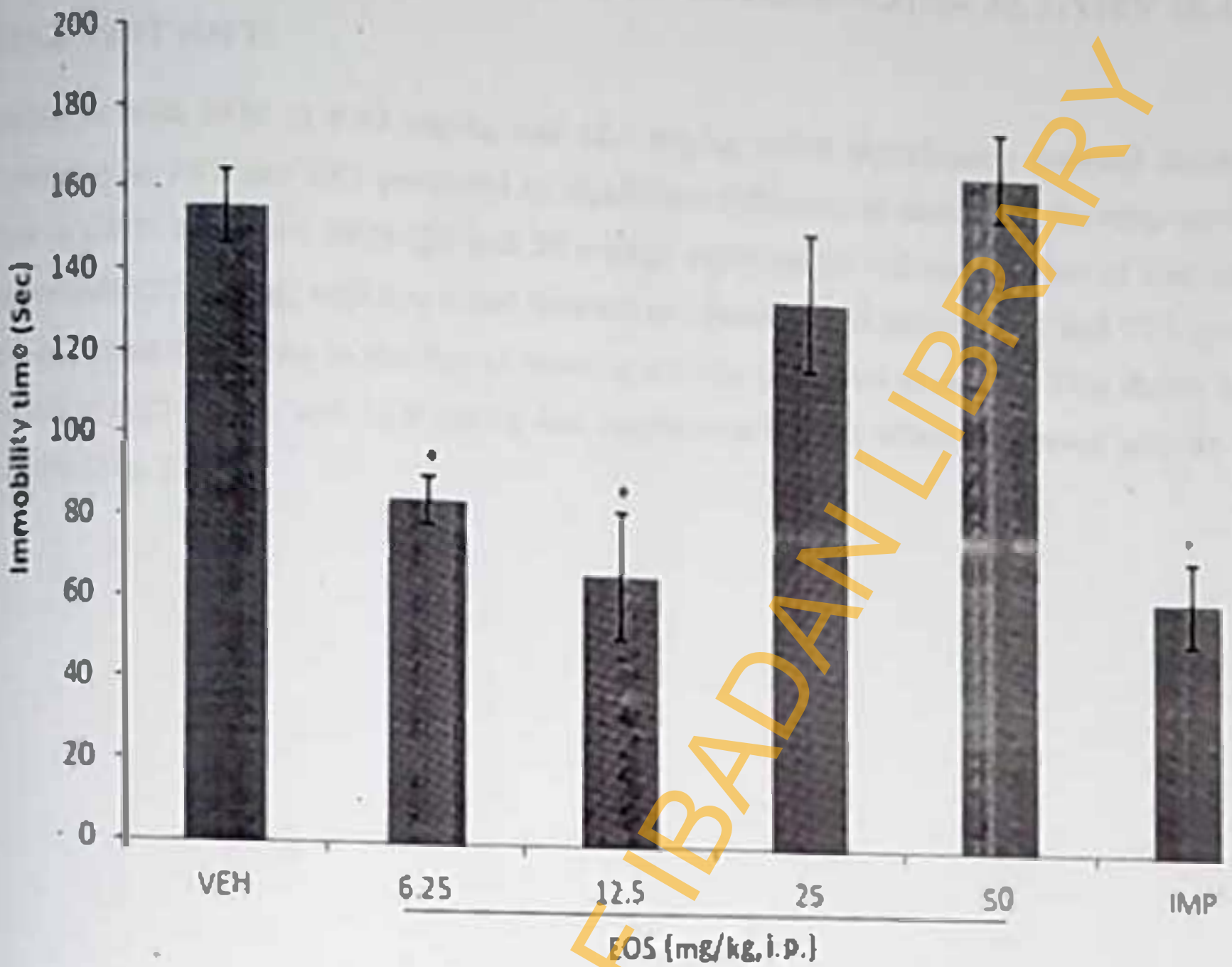

Fig. 10: Effect of OS on immobility time in Tail Suspension Test (TST)

The results are expressed as mean ± SEM (n= 5). One way ANOVA revealed that there is significant [F (5, 24) = 14.94, p< 0.0001] difference between various treatment groups.

* indicates significant difference from the control P < 0.05.

VEH: Vehicle; **EOS**: Ethanol Extract of *Olax subscorpioidea*; **IMP**: imipramine (25 mg/kg, i.p.)

4.16 EFFECT OF *OLAX SUBSCORPIOIDEA* ON LOCOMOTOR ACTIVITY IN OPEN FIELD TEST (OFT)

Treatment with EOS at 6.25 mg/kg and 12.5 mg/kg which significantly reduced duration of immobility in FST and TST produced no significant difference in number of crossing activity of mice in OFT. However, EOS (25 and 50 mg/kg) significantly reduced number of line crossed. Imipramine (25 mg/kg) which reduced duration of immobility of mice in FST and TST produced no significant difference in number of crossing activity compared to control. This shows that the extract at 6.25 mg/kg and 12.5 mg/kg and imipramine did not affect locomotor activity of the animals (Fig. 11).

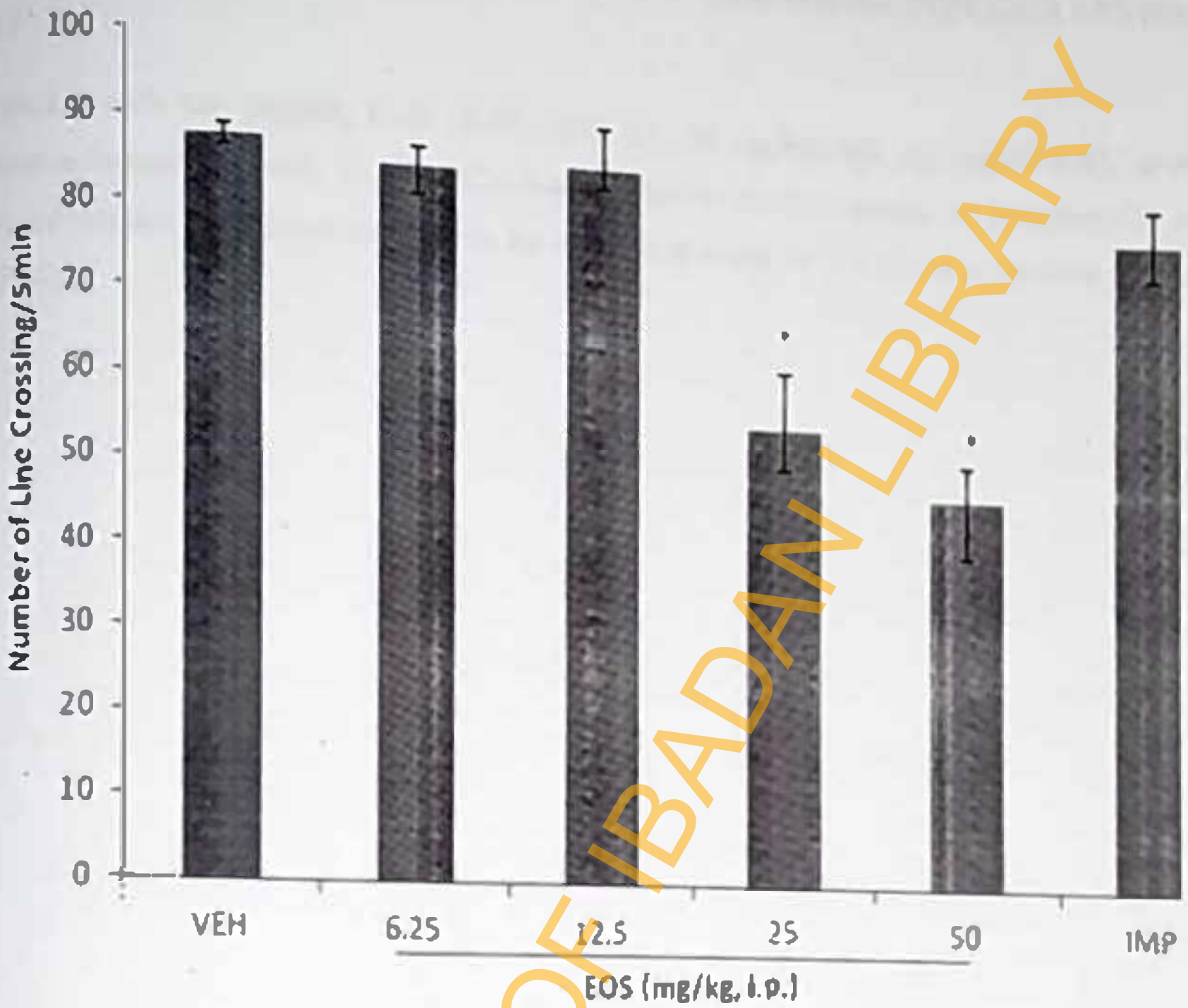

Fig. 12 Effect of EOS on the locomotor activity of mice in OFT

The results are expressed as mean ± SEM (n= 5). One way ANOVA revealed that there is significant [F (5, 24) = 35.98, p< 0.0001] difference between various treatment groups.

* indicates significant difference from the control P < 0.05.

VEH: Vehicle; **EOS**: Ethanol Extract of *Olax subscorpioidea*; **IMP**: imipramine (25 mg/kg, i.p.)

4.17 EFFECT OF *OLAX SUBSCORPIOIDEA* ON YOHIMBINE INDUCED LETHALITY TEST

Comparing with the control, EOS (6.25, 12.5, 25, 50 mg/kg) did not significantly potentiate yobimbine induced toxicity in mice. Clinically effective antidepressant, imipramine (25 mg/kg) produced marked significant increase in the number of death ($P < 0.05$) as compared with control (Table 6).

Table 6: Effect of *Olax subscorpioidea* on Yohimbine-induced lethality Test

Group	Dose (mg/kg)	No of death	% mortality
VEH	10 ml/kg	2/10	20
EOS	6.25	2/10	20
	12.5	0/10	0
	25	0/10	0
	50	2/10	20
IMP	25	7/10	70 *

The results are expressed as mean ± SEM (n= 5). One way ANOVA revealed that there is significant [F (5, 24) = 21.73, p< 0.0001] difference between imipramine and the control.

* indicates significant difference from the control P < 0.05.

VEH: Vehicle; **EOS**: Ethanol Extract of *Olax subscorpioidea*, **IMP**: imipramine (25 mg/kg, i.p.).

4.18 EFFECT OF CRUDE EXTRACT OF *OLAX SUBSCORPIOIDEA* IN RESERPINE INDUCED DEPRESSION

In reserpine induced depression test in mice, EOS (6.25, 12.5, 25, 50 mg/kg) produced significant ($p< 0.05$) decrease in the mean faecal droppings in all the groups compared to control. Anti-diarrhea effect of *Olax subscorpioidea* was comparable to that of imipramine (Table 7).

Table 7: Effect of crude extract of *Olax subscorpioidea* in Reserpine induced diarrhoea

Group	Dose mg/kg	60 min	120 min	180 min
VEH	10 mL/kg	4.80± 0.80	7.60± 0.92	8.20± 0.86
EOS	6.25	1.60± 0.50*	1.80± 0.66*	2.40± 0.92*
	12.5	1.20± 0.58*	1.20± 0.58*	1.20± 0.58*
	25	0.40± 0.24*	0.60± 0.40*	1.20± 0.37*
	50	1.00± 0.44*	1.20± 0.37*	2.80± 0.58*
IMP	25	0.71± 0.24*	0.82± 0.28*	1.56± 0.34*

The results are expressed as mean ± SEM (n= 5). One way ANOVA revealed that there is significant [F (5, 24) = 10 p< 0.0001; F (5, 24) = 21.58 p< 0.0001; F (5, 24) = 17.45 p< 0.0001] difference between various treatment groups.

* indicates significant difference from the control P < 0.05.

VEH: Vehicle, **EOS**: Ethanol Extract of *Olax subscorpioidea*: **IMP**: imipramine (25 mg/kg, i.p.):

4.19 EFFECT OF *OLAX SUBSCORPIOIDEA* ON PENTELENETETRAZOLE INDUCED CONVULSIONS

Penteleneletrazole (85 mg/kg) produced tonic seizures in all the animals. The EOS (12.5 & 25 mg/kg i.p.) did not alter onset and duration of seizures. However, at 50 mg/kg there was a significant prolongation of the onset and Latency to death. The standard drug, phenobarbitone (40 mg/kg) showed anticonvulsant effect by offering full protection without seizure (Table 8).

Table 8: Effect of *Olax subscorpioidea* on PTZ -Induced Convulsions

Group	Dose (mg/kg)	Onset of Seizure (min)	Latency to Death (min)
VEH	10 mL/kg	0.54 ± 0.02	2.00 ± 0.44
EOS	12.5	0.49 ± 0.05	4.00 ± 0.44
	25	0.68 ± 0.20	3.40 ± 1.28
	50	2.72 ± 2.07*	9.20 ± 1.24*
Phenobarbitone	40	NC	NC

The results are expressed as Mean ± SEM (n= 5). One way ANOVA revealed that there is significant [F (3, 16) = 1.064, p< 0.0001 (Onset); F (3, 16) = 11.00, p = 0.0004 (Latency)] difference between various treatment groups.

* indicates significant difference from the control P < 0.05.

VEH: Vehicle; EOS: Ethanol Extract of *Olax subscorpioidea*; NC: No convulsion

4.20 EFFECT OF *OLAX SUBSCORPIOIDEA* ON STRYCHNINE-INDUCED CONVULSIONS

Strychnine produced tonic seizures in all the animals. The extract (12.5-50 mg/kg i.p.) did not affect the onset of seizures and latency to death. The standard antiepileptic drug, phenobarbitone (40 mg/kg i.p.) significantly ($p < 0.05$) prolonged both the onset of convulsions and latency to death in the animals (Table 9).

Table 9: Effect of *Olax subscorpioidea* on Strychnine-Induced Convulsion

Group	Dose (mg/kg)	Onset of Seizure (min)	Latency to Death (min)
VEH	10 mL/kg	2.17 ± 0.33	1.00 ± 0.00
EOS	12.5	2.14 ± 0.19	0.99 ± 0.35
	25	2.96 ± 0.80	0.77 ± 0.13
	50	1.9 ± 0.10	1.00 ± 0.00
Phenobarbitone	40	4.04 ± 1.12*	4.23 ± 1.32*

The results are expressed as Mean ± SEM (n= 5). One way ANOVA revealed that there is significant F (4, 20) = 29.97, p< 0.0001 (Latency)] difference between various treatment groups.

* indicates significant difference from the control P < 0.05.

VEH: Vehicle; EOS: Ethanol Extract of *Olax subscorpioidea*, NC: No convulsion

421 EFFECT OF *OLAX SUBSCORPIOIDEA* ON PICROTOXIN-INDUCED CONVULSION

Picrotoxin produced tonic seizures in all the animals. The EOS (12.5 - 50 mg/kg) neither affect the onset of seizures, nor latency to death. The standard drug, phenobarbitone (40 mg/kg) showed anticonvulsant effect by offering full protection without seizure (Table 10).

421 EFFECT OF *OLAX SUBSCORPIOIDEA* ON PICROTOXIN-INDUCED CONVULSION

Picrotoxin produced tonic seizures in all the animals. The EOS (12.5 - 50 mg/kg) neither affect the onset of seizures, nor latency to death. The standard drug, phenobarbitone (40 mg/kg) showed anticonvulsant effect by offering full protection without seizure (Table 10).

Table 10: Effect of *Olax subscorpioidea* on Picrotoxin-induced Convulsions

Group	Dose (mg/kg)	Onset of Seizure (min)	Latency to Death (min)
VEH	10 ml/kg	3.49 ± 0.15	2.80 ± 0.20
EOS	12.5	3.79 ± 0.19	3.40 ± 0.24
	25	3.56 ± 0.26	4.60 ± 0.87
	50	3.96 ± 0.27	3.40 ± 0.60
Phenobarbitone	40	NC	NC

The results are expressed as Mean ± SEM (n= 5). One way ANOVA revealed that there is significant [F (3, 16) = 0.9438, p = 0.4427 (Onset); F (3, 16) = 1.869, p = 0.1756 (Latency)] difference between various treatment groups.

• indicates significant difference from the control P < 0.05.

VEH: Vehicle; EOS: Ethanol Extract of *Olax subscorpioidea*. NC: No convulsion

CHAPTER FIVE

5.0 DISCUSSION

Toxicological study for medicinal plants is desirable and necessary, in order to appropriately evaluate the safety of natural products. The acute lethal dose (LD_{50}) of ethanol leaf extract of *Olax subscorpioidea* for intraperitoneal administration is 300 mg/kg.

Behavioural effects of *Olax subscorpioidea* on novelty induced behaviours (NIB) (rearing, grooming and locomotor activity) in mice were observed. Rearing a vertical locomotor activity involves an animal standing on its hind limbs while raising up with its forearms in the air or placed on the wall of the cage (Onigbogi *et al.*, 2000). It is an indication of explorative behaviour which measures central nervous system excitation (Labella et al., 1979). This behaviour has been used to classify test drugs/substances as sedatives or stimulants (Blanchard et al., 2001). Aderibigbe and Agboola (2011) reported that CNS stimulants increase rearing while Hellion-Ibarrola et al., (1999) submitted that CNS depressants inhibit this behaviour. The ability of the extract to inhibit rearing suggests its sedative effect. The extract also produced attenuation of novelty-induced grooming (NIG).

Grooming is a "maintenance" behaviour that is specifically elicited in situation in which an animal is in stress-induced frustration (Akanmu et al., 2007). It is described as face or head washing with forearms or body cleaning with mouth (Ukponmwan et al., 1985). It is an important behavioural component in animals that plays a deactivating role in restoring homeostasis under stressful situation (Gispen, 1981). Inhibitory effect of the extract on NIG suggests its stress attenuating role in novel environment.

Studies have shown that rearing and grooming are regulated by multiple neurotransmitter systems. Among the implicating systems are GABAnergic, dopaminergic, adrenergic, cholinergic and opioids systems (Walling and Keith, 1998). Reduction or inhibition of rearing may not be unconnected with inhibitory action on such excitatory neural systems as adrenergic, glutaminergic and dopaminergic systems among others or possible potentiation of the central inhibitory systems such as γ- amino butyric acid (GABA) (Akanmu et al., 2011). Grooming behaviour in rodents has long been associated with dopamine receptors in the brain and an

involvement of mesolimbic doparnine system has been suggested (Gricbel et al., 1994). Accordingly, D1 like receptors (D1 and D5 receptor) agonists elicit an intense grooming behaviour but not D2-like (D2-4) receptors agonists. Drugs that block D1 and D5 receptors attenuate grooming behaviour. In this study, neural mechanism of action of the extract was investigated by interacting the extract with the antagonists of the systems that regulate neurobehaviours in rodents. Atropine, ciproheptadine, haloperidol and propanolol did not reverse inhibitory effect the extract on rearing and grooming, but rather potentiated it, thus precluding involvement of cholinergic, histaminergic, and dopaminergic systems in the inhibition of NIB by the extract. However, pretreatment with yohimbine reversed inhibitory effect of the extract on rearing and grooming. This shows that the extract may contain compound (s) that has affinity for α_2-adrenergic receptor, thus suggesting participation of noradrenergic system in the inhibitory effect of the extracts on NIB.

The effect of the extract on spontaneous motor activity was assessed by considering number of lines crossed by animals in open field. Spontaneous motor activity is a parameter used to measure of central excitability of animals. However, this behaviour (motor activity) has been shown to be mainly governed by motor area of frontal cortex, corpus striatum and brainstem. Any morphological changes or change in the level of brain amines in these areas is expected to cause neurotoxicity which may be in the form of motor deficit (Richard, 1983). Dopamine is one of the main amines found in these areas, and has been implicated in locomotor and exploratory activity. Studies have shown that decreased activity in central dopaminergic systems of adult animals produced hypoactivity (Dulawa *et al.*, 1999). While dopamine receptor blocking drugs such as haloperidol has impaired locomotor activity, the electrolytic lesions of ascending dopamine systems has also been found to cause a similar effect on locomotor activity (Carlsson, 1958). Drugs that enhance dopaminergic transmission produced increased locomotor activity (Fray, 1980; Akaluru *et al.*, 2007) and are said to be stimulants, while agents that reduce dopaminergic transmission suppress locomotor activity. The extract at the highest dose decreased locomotor activity while the other doses did not have any effect on the locomotor activity of the animals. The decrease in locomotor activity with the highest dose of the extract may be indicative of its CNS depressant effect

Also exploratory activity of animals treated with the extract was found to decline on hole board apparatus. The test is a measure of exploratory behaviour in rodents (File and Wardill 197; Crawley 1985) and has been employed to screen sedative agents. File and Wardill (1975) submitted that reduced frequency of head dips reflects CNS depression. This method can also be used to measure anxiety and test anxiolytic agents. In this regards, the test is based on assumption that head-dipping of animals is inversely proportional to their anxiety state in moderately aversive environment. Therefore increased number of head dips on the board means reduced anxiety state (Bilkei-Gorzo and Gyertyan, 1996). In more aversive situations, when the anxiety level of the animals is high, the holes may represent a possible way to escape from the aversive environment instead of an explorable object. In this case the relationship between anxiety state and head-dipping activity is directly and not inversely proportional (Bilkei-Gorzo and Gyertyan, 1996). In a moderate condition, anxiolytics increase the frequency of head poking, while sedative agents decrease the frequency of head poking. The reduction in the frequency of head dips in hole board by the extract thus suggests its sedative effect.

Furthermore, anxiolytic potential of the extract was evaluated in elevated plus maze. This test has been widely used for modeling anxiety and it has been developed for predicting the efficacy of clinically used compounds for treating anxiety. Anxiety, a state of excessive fear, is characterized by motor tension, sympathetic hyperactivity, apprehension and vigilance syndromes (Sadock and Sadock, 2003). Anxiety may interfere with intelligence, psychomotor function and memory (Pine et al., 1999). The EPM situation rests on the conflict between the innate tendencies of the rodents to explore novel environments and avoid open and brightly lit areas. Generally, anxious mice or rats tend to spend little time in the open arms. Anxiolytic drugs increase the time spent in the open arms and increase the number of open arm entries during the test. The extract at all doses did alter activity of mice in the open arms thus suggesting that the extract is neither anxiolytic nor anxiogenic.

Spontaneous alternation is a behavioural test for measuring the willingness of rodents to explore new environments. Rodents typically prefer to explore a new arm of Y-maze rather than returning to one that was previously visited. This test is used to quantify cognitive deficits in transgenic strains of mice and evaluate novel chemical entities for their effects on cognition. The extract did not significantly affect percentage alternation in mice.

The extract potentiated hypnotic effect induced by pentobabitone in a dose dependent manner, thus suggesting its sedative property. This assay is very sensitive and has been routinely used to screen agents with CNS depressant effect. Gamma-amino-butyric acid (GABA) is the major inhibitory neurotransmitter in the central nervous system. CNS depressant drugs mainly exert their action through GABAA receptor. Therefore, the extract may acts by hyperpolarization of the CNS via GABA receptor or benzodiazepine receptor located adjacent to the GABA receptor.

Furthermore in this study, ethanol leaf extract of *O. subscorpioidea* demonstrated antinociceptive action in chemical and thermal models of nociception in mice. In acetic acid induced writhing test, abdominal contractions which indicate viscerosomatic pain were attenuated by *O. subscorpioidea* in a dose dependent manner. Pain process in this assay is associated with the release of prostaglandins, leukotrienes, 5-HT, histamine and kinnes in the peripheral tissues (Hajare et al., 2000) and the abdominal writhes which characterize this assay are thought to be mediated by peritoneal mast cells (Ribeiro et al., 2000), acid sensing ion channels, (Voilley, 2004) and prostaglandin pathways i.e. sensation of the chemosensitive nociceptors by prostaglandins (Sutradhar et al., 2007). Inhibition of abdominal constrictions by the extract is thus suggestive of its antinociceptive action which may be due to inhibitory action against the synthesis and release of inflammatory mediators. Although, this test of nociception has been successfully employed to screen peripherally acting analgesics, centrally acting analgesic such as opioid agonists without peripheral action have effectively attenuated nociception in this model, thus showing its lack of specificity between central and peripheral effects (Sanchez-Mateo et al., 2006). Hence the need for a model that discriminates pains in the central and peripheral components.

Formalin model of nociception described by Tjolsen *et al.* (1992) consists of two distinct phases describing two types of pain. An early phase describing the central component of the pain (neurogenic pain) seems to be due to direct chemical stimulation of nociceptors. The late phase that describes the peripheral component (inflammatory pain) has been shown to involve pro-inflammatory mediators such as histamine, prostaglandins, nitric oxide and bradykinin (Hunskar et al., 1985; Tjolsen et al., 1992). Because of the characteristic biphasic nature of this model, it has therefore been employed to discriminate pain in its central and /or peripheral components. Centrally acting analgesics such as morphine, inhibit equally both phases, while

peripherally acting drugs, such as steroids (dexamethasone) and non-steroidal anti-inflammatory drugs (acetylsalicylate) suppress mainly the late phase (Trongsakul et al., 2003). In this study, *O. subscorpioidea* dose-dependently attenuated hind paw licking responses elicited in both phases, thus indicating its antinociceptive activity. Inhibition of the two phases is indicative of central and peripheral mechanism of actions. Pronounced effect of *O. subscorpioidea* in the inflammatory phase (second phase) suggests greater involvement of peripheral mechanism in the anti-nociceptive action and thus gives credence to its anti-inflammatory potential.

In the behavioural responses in tail immersion and hot plate tests, which are considered to be supraspinally integrated, *O. subscorpioidea* altered reactions to pain stimuli at 12.5 mg/kg and 50 mg/kg in tail immersion and 50 mg/kg in hot plate tests. These results indicate participation of thermal stimulation associated with central neurotransmission when the heat activates nociceptors (Aδ and C fibers) by driving the impulse of the dorsal horn of the spinal cord and subsequently to cortical centers (Chapman et al., 1985). The result further confirms central analgesic effect since both tests are predominantly a spinal reflex and are considered to be selective for opioids like and centrally acting analgesics, while peripheral analgesic are known to be inactive on thermal stimulus (Janssen et al., 1963; Srinivasan et al., 2003). Antagonism test with naloxone was carried to confirm involvement of central opiate mechanism. The dose of naloxone (1 mg/kg) used in the experiments was high enough to block opiate receptors, as demonstrated previously in the pain induced-functional impairment model (Yaksh, 1997). In the study, naloxone alone did not affect nociception, but abolished antinociceptive effect of the extract and thus indicates participation of endogenous opioid peptides in mediation of antinociceptive response of the extract.

Behavioral studies have been shown to play an important part in the evaluation and development of antidepressant drugs (Xu, 2008). Forced swimming test (FST) and tail suspension test (TST) are two important behavioral models widely and routinely used for screening new antidepressant compounds (Cryan, 2005). According to Porsolt (1981) and Steru *et al.* (1987) existence of significant correlation between clinical potency and potency of antidepressants has been established. The immobility displayed by animals subjected to an unavoidable and inescapable stress that characterizes these models has been hypothesized to reflect behavioral despair which in turn may reflect depressive disorders in humans. This immobile position is reduced by variety

of therapeutically active antidepressants e.g. tricyclics, monoamine oxidase-inhibitors, and newer antidepressants (Cryan and Lucki, 2000; Cryan et al., 2002).

Forced swimming test is based on the assumption that animals forced to swim in a restricted space will ultimately cease to apparent attempts to escape and become immobile making only small movements necessary to keep their heads above the surface of water (Porsolt et al., 1978). In this present study. *O. susbscorpioidea* (6.25 and 12.5 mg/kg) produced a significant reduction in immobility time in forced swimming test, while *O. susbscorpioidea* (25 and 50 mg/kg) produced no effect on immobility time. Positive control antidepressant drug imipramine produced significant reduction in immobility time. Therefore the ability of *O. susbscorpioidea* to reduce the immobility time in animals subjected to this stressful situation indicates its antidepressant like activity.

Similarly, in TST *O. susbscorpioidea* (6.25 and 12.5 mg/kg) and imipramine produced significant reduction in immobility time while *O. susbscorpioidea* (25 and 50 mg/kg) did not affect immobility in these animals. The test having got an advantage of being able to detect broad spectrum of antidepressants irrespective of their mechanism of action, is however based on the observations that rodents mostly mice after initial escape behavior, develop an immobile position when subjected to inescapable stressful situation (Varty, 2003). Animals are considered as immobile when they hang passively and completely motionless. The development of immobile posture by the animals which disengages them from active form of coping with stressful stimuli (Lucki, 2001) represents behavioral despair which in turn may reflect depressive disorders in humans. Clinically effective antidepressants decrease the immobility time in TST. The ability of the extract to reduce immobility time suggests that it may possess antidepressant activity.

While interpreting antidepressant-like effect of any test substance based on swimming performance and exploratory behaviour of rodents in either FST or TST, it is noteworthy that false positive results can be obtained for agents that stimulate locomotor activity (Bourin, 2001). Therefore the influence of the test substance in baseline locomotion in animal is of prime concern (Boissier and Simon, 1965). Agents like amphetamine, convulsants and anticholinergic which enhance locomotor activity or cause hyperkinesias in open field test (OFT) produce false positive results in FST and TST (Bourin, 2001; Butterweck et al, 2003; Takahashi et al., 2008).

Hence the need of OFT as a paradigm to eliminate the bias that anti-immobility effect could be associated with hyperkinesia (Kwon et al., 2009). As psycho-stimulant will always give false positive result in FST and TST, one way to discern or discriminate between antidepressants and psycho-stimulants is that antidepressants would not cause general increase in motor activity (Borsini and Meli, 1988). Potential antidepressant activities of a selective serotonin1A agonist based on anti-immobility activity in the forced swimming test in rats without effect on open-field activity have been suggested (Cervo and Samanin, 1987). The observation that *Olax subscorpioidea* (6.25 and 12.5 mg/kg) did not increase the number of line crossed in the open field test eliminates exertion of psycho-stimulant-like action and confirms the assumption that antidepressant-like effect of the extract in the TST and FST is specific (Sánchez-Mateo et al., 2005). Conversely, *O. susbscorpioidea* (25 and 50 mg/kg) produced a significant reduction in the number of line crossed indicating gross sedation at these doses which masks its antidepressant effect. This probably explains why the extract at higher doses could not exhibit anti-immobility effect seen at lower doses.

Yohimbine induced lethality test is another animal model used to further screen antidepressant potential of *O. subscorpioidea*. This model has been developed not only to detect potential antidepressant properties of new substances, but also to identify neurotransmitter systems that may be involved in the mechanisms of actions of antidepressant drugs (Leonard, 1986). Yohimbine, an alpha 2 adrenergic antagonist, causes increased sympathetic discharge both in the peripheral and central nervous system. The antagonism of alpha 2 receptors also causes an increase in the level of serotonin (Blier and Montigny, 1994). Antidepressant drugs by enabling more amines to reach the receptors potentiate yohimbine induced lethality. This occurs either by their reuptake inhibition or reduced inactivation by monoamine oxidase inhibitors. Yohimbine induced lethality test reveals an adrenergic component of pharmacological activity of antidepressants and is sensitive to detect MAO inhibitors, tricyclic antidepressants, noradrenaline (NA), and selective serotonin reuptake inhibitors (Malick, 1983). The present study showed that the extract did not potentiate yohimbine induced lethality at all doses thus precluding involvement of the central adrenergic mechanism in its antidepressant action.

Studies have shown that reserpine can deplete amine stores and irreversibly inhibit the vesicular uptake of monoamines. This reduced level of monoamines in the brain has been implicated to be

an underling factor in the pathophysiology of depression. In this paradigm, physiological effects such as diarrhoea, ptosis and hypothermia are observed and these have been associated with the effect of reserpine as signs of depression (Bourin et al., 1983). These syndromes have been inhibited or reversed by major classes of antidepressant drugs. In this study, like imipramine significant reversal of diarrhoea by the extract at all four dose levels used was noticed and thus indicating its antidepressant property.

Pentelenetetrazole induced convulsion represents a valid model for human generalized myoclonic and also absence seizures. The PTZ assay has been used primarily to evaluate antiepileptic drugs. It exerts its convulsive effect by inhibiting activity of gamma amino butyric acid (GABA) at $GABA_A$ receptors (De Sarro et al., 1999). Enhancement and inhibition of neurotransmission of GABAergic system will attenuate and enhance convulsion respectively (Gale, 1992; Westmorland et al., 1994). Anticonvulsant activity of a novel compound is not only measured by its ability to prevent convulsions but also to delay onset of seizures or to reduce death rate and/or to decrease frequency of the episodes (Kendall et al., 1981). Compounds whose actions are just to delay onset of seizures or reduce frequency of the episodes in experimental animals have been shown to arrest the spread of seizures in epileptic brains (Corda et al., 1982). Although *O. subscopioidea* did not prevent death, but significant delay in the onset of seizures and latency to death seen in mice treated with the highest dose portends its anti-seizure potential. This action may be due to activation of GABAergic neurotransmission. This mild anticonvulsant activity may justify the decision of the natives to combine it with other leaves in managing convulsion (Adjanohoun et al., 1986). Phenobarbitone antagonism on the pentelenetetrazole-induced convulsions is attributed to its ability to enhance GABA mediated inhibition in the brain (Rang et al., 2005)

Glycine is an amino acid which acts as an inhibitory neurotransmitter in the central nervous system, the inhibition of which has been implicated in convulsions. Strychnine, a potent spinal cord convulsant, induces seizure by blocking glycine receptors selectively and antagonizing its inhibitory spinal reflexes to provoke excitatory response in the central nervous system (Sabin et al., 1993). In this study, the extract did the alter onset of seizure and latency to death thus precluding its interaction with glycine inhibitory system.

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Picrotoxin antagonizes GABA inhibitory system by blocking $GABA_A$ receptors to induce generalized seizures. It produces a strong clonic/tonic convulsions with a high mortality rate and a significant reduction in GABA content of brain at the onset of convulsion (Loscher and Frey, 1977; Abdul-Ghani et al., 1987). This antagonism on GABAergic system has been associated with its potential to cause closure of chloride channels linked to $GABA_A$ receptor (Rang et al., 2005). Agents that reverse the closure of, or reopen this chloride channels will affect episode of seizure in this model. Failure of the extract to affect picrotoxin-induced seizures shows that it does not affect chloride ion entry at $GABA_A$ receptor sites. Phenobarbitone delayed onset of action of convulsion and time of death thus suggesting interaction with chloride ion channels (Corda et al., 1982; Zetler, 1981).

5.1 CONCLUSION

The study shows that the ethanol leaf extract of *O. subscorpioidea* possesses bioactive principles that have sedative, antinociceptive, antidepressant and anticonvulsant activities. Its inhibitory effect on rearing and grooming may be due to involvement of noradrenergic system. The central mechanisms involving the opioid systems may be responsible for its antinociceptive activity. Enhancement of GABAergic neurotransmission may be involved in the anticonvulsant activity of the extract. Also, this GABAergic mechanism may account for its effect on pentobarbitone induced hypnosis in mice. These findings therefore established scientific basis for its uses in the management of neurological disorder and pain.

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