ANTICONVULSANT, ANTIDEPRESSANT AND ANXIOLYTIC EFFECTS OF *MALLOTUS OPPOSITIFOLIUS* (GEISELER) MÜLL. ARG. (EUPHORBIACEAE)

A THESIS SUBMITTED IN FULFILLMENT OF THE
REQUIREMENTS FOR THE DEGREE OF

DOCTOR OF PHILOSOPHY

In the

Department of Pharmacology,
Faculty of Pharmacy and Pharmaceutical Sciences

by

KENNEDY KWAMI EDEM KUKUIA

KWAME NKRUMAH UNIVERSITY OF SCIENCE & TECHNOLOGY,

KUMASI

AUGUST, 2012

DECLARATION

I hereby declare that I am the sole author of this thesis. The experimental work described in this thesis was carried out at the Department of Pharmacology, KNUST. This work has not been submitted for any other degree. This is a true copy of the thesis, including any required final revisions, as accepted by my examiners.

I understand that my thesis may be made electronically available to the public.

……..

Kennedy Kwami Edem Kukuia

……

Prof. Eric Woode

(Supervisor/ Head of Department)

ABSTRACT

Mallotus oppositifolius is used in Ghana for CNS disorders but very little scientific evidence exists to support its use. Thus central effects of 70% v/v hydroalcoholic extract of the leaves of *Mallotus oppositifolius* (MOE*)* was assessed. Anticonvulsant effects of the extract in acute and chronic seizure models were evaluated. The study also investigated the effect of the extract on animal models of depression and anxiety.

In a preliminary screening of the central effects of the extract, oral dose of MOE induced sedation (1000 – 3000 mg kg^{-1}); caused neuromuscular deficits in the rotarod test (300 – 3000 mg kg^{-1}); reduced spontaneous locomotor activity in the activity cage; exhibited anticonvulsant effect (30 – 3000 mg kg^{-1}) and central analgesic effect in the tail immersion test (100 – 3000 mg kg^{-1}). The LD_{50} was approximately 6000 mg kg^{-1} in mice.

M. oppositifolius (10 - 100 mg kg^{-1}, *p.o.*) exhibited anticonvulsant effect in the picrotoxin and strychnine induced seizure tests. The extract significantly delayed onset of myoclonic jerks and clonic convulsions; decreased the frequency and duration of clonic convulsions in these models. In the MES test, the extract caused a significant and dose dependent decrease in the duration of tonic limb extensions. In the pilocarpine induced *status epilepticus*, MOE delayed the onset of clonic convulsions and decreased the duration of these seizures. Furthermore, the extract protected mice against mortality induced by 4-aminopyridine and delayed the onset of both clonic and tonic convulsions. Flumazenil, a $GABA_A$/benzodiazepine antagonist, reversed the anticonvulsant effect of the extract in the PTZ-induced seizure test suggesting enhancement of $GABA_A$ neurotransmission is involved in the anticonvulsant effect of the extract. Isobolographic analysis of the combination of diazepam and extract showed a synergistic effect but the mode of action of this effect may not be dependent on enhancement of $GABA_A$ neurotransmission since flumazenil failed to reverse their anticonvulsant effect.

Oral doses of MOE (10 - 100 mg kg^{-1}), fluoxetine (3 - 30 mg kg^{-1}) and imipramine (3 - 30 mg kg^{-1}) decreased the frequency of immobility and immobility periods of mice in both the FST and TST when compared to control group, indicating significant antidepressant activity. In the open space swim test, a chronic depression model, MOE demonstrated antidepressant-like effect on the first day of treatment and sustained it throughout the period of drug treatment. MOE decreased immobility time while increasing the distanced travelled by the mice. The depression induced in this model induced significant impairment in spatial learning and memory in the Morris water maze—this was reversed by the extract and fluoxetine but not imipramine. Extract, fluoxetine and imipramine treatments did not have significant effects on weight variation. A day after the 14th day of drug treatment, the antidepressant effect was still

significant. A 3-day subcutaneous pretreatment with 200 mg kg^{-1} para-chlorophenylalanine (*p*CPA), reversed the antidepressant effect of MOE and fluoxetine but not imipramine, suggesting that serotoninergic enhancement may be involved in the behavioural effect of the extract. This was confirmed by the ability of the extract to potentiate the head twitch responses induced by 5-hydroxytryptophan in mice, a model sensitive to 5-HT_{2A} receptor activation. Pretreatment with α-methyldopa (400 mg kg^{-1}) however, failed to reverse the behavioural effect of the extract and fluoxetine treatments in the forced swim test. The same result as above was observed for extract and fluoxetine treatments when mice were pretreated with reserpine (1 mg kg^{-1}) or a combination of α-methyldopa (200 mg kg^{-1}) and reserpine (1 mg kg^{-1}). This suggests that the antidepressant effect of the extract may not be dependent on central noradrenergic mechanisms. Administration of D-serine (600 mg kg^{-1}), a full agonist on the glycine site of the NMDA receptors, reversed the antidepressant effect of the extract, fluoxetine and desipramine in both the TST and FST. D-cycloserine (2.5 mg kg^{-1}), a partial agonist potentiated this behavioural effect in both extract and fluoxetine treated mice but not desipramine in both the TST and FST. This suggests possible involvement of glycine/NMDA receptor or pathway antagonism in the antidepressant effect of the extract. MOE slightly increased curling score in the tail suspension test and this was significantly potentiated by D-cycloserine, suggestive of possible opioidergic activity.

MOE (10 - 100 mg kg^{-1}, *p.o*.) showed anxiolytic effect in the three anxiety models used namely; elevated plus maze, light-dark box and open field tests. *M. oppositifolius* treatment significantly increased the percentage of centre entries and the percentage time spent in the centre of the open field. *M. oppositifolius* also increased the time spent in the lit area and the latency to leave the lit area in light/dark box. In the EPM, it significantly increased open arm activities by increasing percentage open arm entries and duration. MOE also decreased risk assessment behaviours such as the head dips, stretch-attend postures and rearing.

Acute and subacute toxicity in rats did show deaths after 14 day treatment with the extract (30 – 3000 mg kg^{-1}). Extract treatment did not affect weight of rats or the relative organ weights. Haematological or serum biochemical parameters were not affected except increases in serum bilirubin (300 and 3000 mg kg^{-1}), urea and creatinine (30 and 100 mg kg^{-1}). Histopathological examination did not reveal toxic effect on the stomach, heart, liver and spleen. There were however some morphological changes of the kidney at 30 mg $kg^{-1.}$

These results suggest that the extract has anticonvulsant effect possibly through enhancement of GABAergic, glycinergic and potassium channel activation or increased potassium conductance. Possible inhibition of muscarinic and glutamatergic transmission may also be

involved. The antidepressant-like effects of the extract may be due to the interplay of serotoninergic, glycine/NMDA and opioidergic pathways. The extract also demonstrated anxiolytic-like effects possibly by the involvement of GABAergic and serotoninergic mechanisms.

ACKNOWLEDGEMENT

I give all glory, honour and praise to the name of our Heavenly Father, whose grace and sustenance has brought me this far. I am eternally grateful!

I want to express my sincere thanks to my supervisor, Prof. Eric Woode. Your patience and counsel has produced a philosopher and a scientist you can be proud of.

Friends are scarce and priceless. I feel blessed to have my friends and fellow postgraduate colleagues, especially Juliet Fafali Tamakloe, Dr. Elvis Ofori Ameyaw and Edmund Ekuadzi. This work would not have been completed without the moments we shared together; the lengthy discussions and arguments we have had. I say thank you.

I also like to thank all the lecturers of the Department of Pharmacology and the technicians for their technical support. I thank the Centre for Scientific Research into Plant Medicine, Akuapem-Mampong, for their help in conducting the maximal electroshock seizure test.

Finally I wish to appreciate my family for their support, prayers and love. Mum, you have been a pillar; Vivian, you are love in action; Gabriel, you challenged me to reach greater heights and Stella, you have been a sweet sister.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF PLATES

ABBREVIATIONS

AEDs	Antiepileptic drugs
AMPA	Alpha-3-Hydroxy-5-Methoxy-4-Isoxazole Propionic Acid
CBZ	Carbamazepine
CNS	Central nervous system
DCS	Dextro-cycloserine
DS	Dextro-serine
DSP	Desipramine
DZP	Diazepam
EPM	Elevated Plus Maze
FLX	Fluoxetine
FST	Forced Swimming Test
GABA	Gamma Amino Butyric Acid
HLTE	Hind limb tonic extension
ICR	Imprinting Control Region
IMI	Imipramine
IPSCs	Inhibitory Post-Synaptic Currents
IGluR	Ionotropic glutamate receptors
LDB	Light-Dark Box
mGluR	Metabotropic glutamate receptors
MES	Maximal Electroshock Test
MIC	Minimum inhibitory concentration
MOE	*Mallotus oppositifolius* extract
NMDA	N-methyl- D-aspartate
p.o.	*Per os*
PTX	Picrotoxin
PTZ	Pentylenetetrazole
SSRI	Selective serotonin reuptake inhibitor
TST	Tail suspension test
Z_{add}	Theoretical ED_{50}

Chapter 1

INTRODUCTION

1.1 GENERAL INTRODUCTION

Virtually all medicines centuries before the advent of modern medicine, synthetic chemistry and the pharmaceutical industry, came from plants (Agosta, 1997). These medicinal plants continue to serve as important repositories for the discovery of novel bioactive compounds which can be utilized as lead molecules for the development of new drugs (Cragg *et al.*, 1997). Aspirin, atropine, scopolamine, taxol, theophylline, tubocurarine, vincristine and vinblastine are a few examples of medicines derived from plant sources. At least 25% of drugs in the modern pharmacopoeia are derived from plants and many others, which are synthetic analogues or built on prototype compounds isolated from plants (Cox and Balick, 1994; Jones, 1996).

The use of traditional medicine and medicinal herbs is currently enjoying a renaissance in popularity in the West as well, and in many parts of the world it is the source of primary health care. People often resort to traditional and other forms of complementary and alternative medicines for chronic conditions which do not respond well to conventional or modern drug treatments. Among these are neurological disorders such as anxiety, depression, epilepsy and pain (Spinella, 2001).

Epilepsy is one of the major neurological disorders affecting approximately 2% of the population (Pitkanen and Lukasiuk, 2009). Considerable progress in the pharmacotherapy of epilepsy has been made over the last few decades, including the introduction of new antiepileptic drugs such as retigabine, felbamate, lamotrigine, etc. (Bazil and Pedley, 1998; McCabe, 2000). However, current drug therapy of epilepsy is fraught with side-effects, teratogenic effects, long term toxicity and about a third of patients do not respond to these pharmacotherapies (Raza *et al.*, 2001; Loscher, 2002). Furthermore, there is currently no drug available which prevents the development of epilepsy (Temkin, 2001; Temkin *et al.*, 2001). Epilepsy in particular is a condition where traditional healers are very critical in providing treatment in the rural settings (Stafford *et al.*, 2008). For example, a sample in Nigeria revealed that 52% of epilepsy patients use some form of traditional medicine (Danesi and Adetunji, 1994). Considering the great reliance on traditional medicinal plants for treatment of diseases and the potential for drug discovery, it is justifiable to search for potent, effective and relatively safe plant medicines as well as to scientifically validate success claims about plants already in use by traditional medicine practitioners.

The plant *Mallotus oppositifolius* (family Euphorbiaceae) is a shrub that is commonly found in drier types of forest and secondary growth throughout the West African region. Locally it is known as 'sroti' in Ewe or 'sratadua' in Akan (Burkill, 1985). Economically, *M. oppositifolius* twig is used as chewing sticks for cleaning the teeth; the stem is used as yam stakes. In Nigeria the leaves are taken by the Hausas in cold infusion to expel tapeworm, while the decoction is a vermifuge in Ivory Coast. In Ghana, the crushed leaves are applied to inflammation of the eye during an attack of small pox. Leaf-sap is an eye-instillation in Ubangi for eye-troubles and in Congo (Brazzaville) it is administered in attacks of epilepsy. The leaves are also used for treating convulsion (Burkill, 1985).

This work seeks to investigate the anticonvulsant effect of the hydroalcoholic leaf extract of the plant in classical animal models of epilepsy namely, maximal electroshock seizure (MES) test, pentylenetetrazole (PTZ), picrotoxin (PTX), strychnine, 4-aminopyridine induced seizure tests and pilocarpine induced *status epilepticus.*

A relationship between epilepsy and psychiatric disorders like depression and anxiety has been recognized since antiquity. Statement found in Hippocrates' writings: "melancholics ordinarily become epileptics, and epileptics melancholics: what determines the preference is the direction the malady takes; if it bears upon the body, epilepsy, if upon the intelligence, melancholy "corroborates this fact. Moreover antiepileptic drugs like carbamazepine have been used in managing refractory depression (Rogawski and Löscher, 2004). This is probably because there is some overlap in the neurobiology of these conditions. Thus the effect of the extract on animal models of depression, forced swimming test, tail suspension test and the open space swim test was also investigated. The elevated plus maze, light-dark box and open field were employed to assess the anxiolytic effects of the extract. Acute and subacute toxicity studies were also carried.

1.2 THE PLANT *MALLOTUS OPPOSITIFOLIUS*

1.2.1 Name

Botanical name: *Mallotus oppositifolius*
Family: Euphorbiaceae
Local name (s): Sroti (Ewe); Osratadua (Twi)

1.2.2 Description

Mallotus oppositifolius is a dioecious shrub or a small tree that grows up to 6 to 13 m tall. The young shoots are densely stellate-hairy; older twigs almost glabrous, often purplish brown. Leaves are opposite and simple; petiole is long and short in each pair; 2.5- 11cm long when long and 0.5- 2 cm long when short, slightly thickened at the both ends; stipules are long, soon

falling; blade is broadly ovate to oblong-ovate, 3-18-21 cm × 2-13 cm, unequal in size in each pair; base shallowly cordate to rounded or truncate, margins almost entire or more or less deeply toothed or lobed, 3 veined from the base, sparingly stellate-hairy to almost glabrous, sparingly gland dotted, also with simple hair beneath. Inflorescence a terminal or axillary raceme; male inflorescence up to 10- 15 cm long, female one up to 10- 18 cm long, bracts 0.5-1.5 mm long, triangular, each 1-5 flowered. Flowers are unisexual, fragrant, petals absent, male flowers with jointed pedicel 3-7 mm long, sepals 3-4, elliptical about 2mm long, strongly reflexed, pale yellow-green, disk absent, stamens numerous, filament about 2 mm long, free, greenish white, female flowers with pedicels 2-3 mm long, extending to 2-5 cm in fruit, calyx lobes 3-5-6 ovate to lanceolate about 2 mm long, united at the base, recurved, densely short-hairy and covered with yellow glands, 3-lobed, styles 3 about 1.5 mm long, free, plemose. Fruit a deeply 3-lobed capsule 5-7 mm × 7-9 mm, short-hairy and gland-dotted 3 seeded. Seeds are almost globose, 3.5-4 mm × about 3 mm, smooth, shiny, grayish and olive-brown.

Figure 1.0 Plant showing the leaves of *M. oppositifolius*

1.2.3 Ecological and geographical distribution

Mallotus oppositifolius is widely distributed and occurs from Senegal to Ethiopia, Angola, Mozambique and Madagascar. It is also widely distributed in Ghana and Nigeria.

1.2.4 Traditional uses

In West Africa most plant parts, but especially the leaves, are commonly used for medicinal purposes. A leaf or stem bark infusions are taken as anthelmintic to expel tapeworms and to treat diarrhoea. The crushed or chewed fresh leaves, sometimes mixed with butter, are put on cuts and sores as a haemostatic and antibacterial; on skin eruptions and rashes for fast healing. They are also applied to burns for analgesic effect (Adekunle and Ikumapayi, 2006). A steam bath with the leaves is taken to treat headache, epilepsy or mental illness. Leaf sap is used as nasal drops or eye drops and the heads are massaged with the pulped leaves to treat headache. The crushed leaves or leaf sap are applied to aching teeth and inflamed eyes. The ground leaves in salted water are applied to extract poisons in snakebites and the extract is also drunk for this purpose (Farombi *et al.*, 2001). Crushed leaves or a leaf infusion are applied to treat urinary infections, venereal diseases, malaria, leprosy, chickenpox and female sterility. A leaf and fruit infusion is taken to treat dysentery and diarrhoea, or the leaves are added to food. A leaf and root decoction is drunk to treat anaemia and general fatigue. A root and leaf paste is applied to treat convulsions, stomach-ache and chest pains. An infusion of the roots together with the seeds of *Aframomum melegueta* K. Schum is taken as an enema to treat lumbago. In eastern Africa a root decoction is taken as an aphrodisiac. The root decoction and leaf sap are taken to treat pneumonia, vomiting and chest pain. *Mallotus oppositifolius* is commonly browsed by cattle. The wood is also used as firewood and to make tool handles or yam stakes; thinner stems or the bark are sometimes used as binding material and the twigs are commonly used as chew sticks. Refer to (Burkill, 1985).

1.2.5 Previous works on *M. oppositifolius*

1.2.5.1 Chemical constituents

Phytochemical screening of *M. oppositifolius* revealed the presence of secondary metabolites such as alkaloids, phenols, flavonoids, anthraquinones and cardenolides (Farombi *et al.*, 2001). Five hydrolysable tannins and cytotoxic phloroglucinol have been reported from the bark of *M. japonicus*, another mallotus species. Anthocyanins, butacyanin have also been found in the leaves. Heavy metal analysis of the leaves of *M. oppositifolius* showed that the plant did not contain heavy metals such as cadmium, zinc, lead, chromium, and nickel. The presence of

copper, iron and manganese was less than 0.95% individually (Adekunle and Ikumapayi, 2006). Rottlerin, a protein kinase C inhibitor, has also been found in its bark and leaves (Oliver, 1960).

1.2.5.2 Antimicrobial activity

M. oppositifolius leaf extract exhibited significant antimicrobial activity against *Pseudomanas aeruginosa, Baccilus subtilis* and *Staphylococcus aureus*. *M. oppositifolius* significantly enhanced antibacterial activity of amoxicillin against *Staph. aureus* and *B. subtilis* (Gbedema *et al.*, 2010).

1.2.5.3 Effect on root-knot nematode infection

The undiluted leaf extracts of *M. oppositifolius* inhibited egg hatching (100%) and caused mortality in larva even after 12 h of exposure time. The juvenile mortality increased with increase in exposure time (Okeniyi, 2010).

1.2.5.4 Antifungal Property

M. oppositifolius crude extracts (ethanolic and aqueous) had significant antifungal activity on *Aspergillus flavus, Candida albicans, Microsporum audouinii, Penicillium sp., Trichoderma mentagrophytes* and *Trichoderma sp*. The ethanolic extracts of *M. oppositifolius* used did not inhibit the growth of *T. cutaneum*. (Adekunle and Ikumapayi, 2006).

1.3 EPILEPSY, THERAPY AND DRUG SCREENING

1.3.1 Background

The word "epilepsy" comes from the Greek word ***epilepsia*** which means to be taken, seized or attacked. Epilepsy is not a single entity but an assortment of different seizure types and syndromes originating from several mechanisms that have in common the sudden, excessive, and synchronous discharge of cerebral neurons (McNamara and Puranam, 1996; Fisher *et al.*, 2005). Seizures are finite episodes of brain dysfunction resulting from abnormal discharge of cerebral neurons. This abnormal electrical activity may result in a variety of events, including loss of consciousness, abnormal movements, atypical or odd behaviour, or distorted perceptions that are of limited duration but recur if untreated. The site of origin of the abnormal neuronal firing determines the symptoms that are produced. For example, if the motor cortex is involved, the patient may experience abnormal movements or a generalized convulsion. Seizures originating in the parietal or occipital lobe may include visual, auditory, or olfactory hallucinations (Stafstrom, 2005).

Epilepsy is one of the most prevalent neurological disorders. It is estimated that over 50 million people suffer from epilepsy, 85% of whom live in developing countries (Sander and Shorvon, 1996; Stafford *et al.*, 2008). It is the second most common neurological disorder after stroke and it is estimated that approximately 2% of the population worldwide is affected by some form of epilepsy (Pitkanen and Lukasiuk, 2009). In most African communities epilepsy carries a social stigma and sufferers are often shunned or discriminated against with respect to education, employment and marriage (Baskind and Birbeck, 2005; Stafford *et al.*, 2008). This makes early treatment difficult.

Drug or vagal nerve stimulator therapy is the most widely effective mode for the treatment of patients with epilepsy, although surgery is also an option. However, for most patients, pharmacotherapy is still considered the mainstay of treatment (Simoens, 2010). Drug therapy of epilepsy with currently available antiepileptic drugs (AEDs) is often plagued with significant adverse effects such as, foetal abnormalities e.g. cleft palate, cleft lip, congenital heart disease; slowed growth rate and mental deficiency, rash, blood dyscrasias, vitamin K and folate deficiencies, loss of libido, hormonal dysfunction, and bone marrow hypoplasia. Furthermore, a significant proportion of patients (up to 40%) do not respond to these agents and this proportion is quite high in the developing countries (Regesta and Tanganelli, 1999; Kwan and Brodie, 2001). 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 increases the risk of side effects and drug interactions (Shorvon and Reynolds, 1979; Mula and Trimble, 2009). Due to lack of facilities for proper diagnosis, treatment and monitoring of serum levels of AEDs all problems with the current AED therapy of epilepsy seem more prevalent in underdeveloped countries (Tran *et al.*, 2007; Carpio and Hauser, 2009). 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 (Taylor *et al.*, 1995; Shinnar and Berg, 1996; Loscher, 2002). Only a very 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. Clearly there is a pressing need for alternative pharmacotherapies of epilepsy which are not only anticonvulsant but also antiepileptogenics and will not infringe on the patient's quality of life. Natural products and plants for that matter, used in traditional medicine can serve as an invaluable source for novel antiepileptic compounds (Meldrum, 1997; Stafford *et al.*, 2008). They may provide better alternatives to the currently prescribed medications.

1.3.2 **Pathophysiology of epilepsy**

The susceptibility for generating an epileptic seizure varies between individuals because of differences in threshold for the development of epileptic seizures from one person to the other. Any brain can elicit a seizure if provoked sufficiently. Only if seizures become recurrent and are not provoked by systemic disease, is the individual diagnosed with epilepsy. Seizure phenomenology varies from patient to patient and more than one seizure type can occur within the same patient (McNamara and Puranam, 1996; Fisher *et al.*, 2005). Irrespective of the fact that epilepsy can be caused by a variety of intracranial structural, cellular or molecular conditions and manifests itself in different ways, the epileptic seizure always reflect abnormal hypersynchronous electrical activity of neurons, caused by an imbalance between excitation and inhibition in the brain. The neuronal membrane potential is regulated by an accurate balance between excitatory postsynaptic potential (EPSP) and inhibitory postsynaptic potential (IPSP). If this balance is compromised, an epileptic seizure can be generated (Dudek and Staley, 2007). More than 100 neurotransmitters or neuromodulators have been shown to play a role in the process of neuronal excitation. L-glutamate, the major excitatory amino acid plays a major role in the spread of seizure activity by acting at more than half of the neuronal synapses in the brain. There is also an increased release of glutamate in the brain associated with seizure activity. Gamma-aminobutyric acid (GABA) is the major inhibitory neurotransmitter of the CNS. Under normal conditions the excitatory post-synaptic potentials are followed immediately by GABAergic inhibition. Neuronal hypersynchronization occurs if excitatory mechanisms dominate; either initiated by increased excitation or decreased inhibition. As the abnormal neuronal hypersynchronous activity continues, more and more neurons are activated (high frequency depolarization or repolarization), generating the epileptic seizure that can be registered in the electroencephalogram. The existence of excitatory connections between pyramidal neurons generating epileptic bursts through a positive-feedback mechanism in epileptogenic areas and the fact that neurons in some epilepsy prone regions, (e.g. the structures of the limbic system in the temporal lobe, especially the hippocampal CA3 region), possess the capability to generate ‘intrinsic bursts’, dependent on voltage dependent calcium currents or persistent sodium currents has been identified as key features of epileptogenic circuits (Dudek and Staley, 2007). Sex hormones also influence the regulation of GABAergic transmission in the CNS (Verrotti *et al.*, 2009). Animal models have shown that the infusion of oestrogens lowers the threshold for experimentally provoked seizures, and that this effect of oestrogen is intensified if a cortical lesion is already present. Progesterone has been shown to possess an inhibitory effect on spontaneous and experimentally provoked seizures. Progesterone probably works through a direct activation of the GABA complex to enhance the effect of GABA.

Additionally, progesterone possesses the capability of inhibiting glutamatergic activity. The convulsant or anticonvulsant effects of oestrogens and progesterone respectively, are demonstrated in women with catamenial epilepsy (seizure clustering around the time of menses) (Morris and Vanderkolk, 2005). There is an increase in seizure frequency, immediately before the menstrual period, which correlates with a decrease in progesterone level and an additional increase in seizure frequency immediately before the time of ovulation, correlating with a high oestrogen level with no simultaneous increase in progesterone. At the end of ovulation, simultaneously with an increase in the progesterone level, the seizure frequency decreases (Verrotti *et al.*, 2009).

1.3.3 **Classification 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 (Bienvenu *et al.*, 2002). In partial seizures the discharge begins locally and often remains localized. These may produce relatively simple symptoms such as involuntary muscle contractions, abnormal sensory experiences or autonomic discharge without loss of consciousness or they may cause more complex effects on consciousness, mood and behaviour, often termed psychomotor epilepsy. 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; Engel, 2006). 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 defecation, 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 (Engel, 2006). 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 and Tatum, 2001).

1.3.4 History of antiepileptic drugs

From 1857 to 1958, potassium bromide, phenobarbital (PB), and a variety of drugs that were derived mainly by modification of the barbiturate structure, including phenytoin (PHT), primidone (PRM), trimethadione, and ethosuximide (ESM) became the first generation antiepileptics entering the market (Krall *et al.*, 1978; Shorvon, 2009). The second generation AEDs, including carbamazepine (CBZ), valproate (VPA), and the benzodiazepines, introduced between 1960 and 1975, differed chemically from the barbiturates (Shorvon, 2009). The era of the third-generation AEDs started in the 1980s with ‘‘rational’’ developments such asprogabide, vigabatrin (VGB), and tiagabine (TGB), that were designed to selectively target a mechanism that was thought to be critical for the occurrence of epileptic seizures (Loscher and Schmidt, 1994). Other examples include retigabine (a potassium channel activator), eslicarbazepine and lacosamide. The new, third-generation AEDs have undoubtedly expanded the therapeutic options, in particular for those in need of a change in medical regimen (Kwan and Brodie, 2000; Brodie *et al.*, 2007; Marson *et al.*, 2007).

1.3.5 Mechanism of action of antiepileptic drugs

Antiepileptic effects are produced by drugs that modulate ion channels, enhance inhibition mediated by $GABA_A$ receptors and/or glycinergic systems, the regionally specific transmitter systems (including monoamines such as catecholamines, serotonin and histamine, and neuropeptides such as opioid peptides, galanin and neuropeptide Y) and the inhibitory neuromodulator adenosine (Errington *et al.*, 2005; Surges *et al.*, 2008). In addition, blockade of glutamate receptors (including those of the NMDA, AMPA, kainate and group I metabotropic (mGluR1 and mGluR5 types) can also protect against seizures in animal models. In principle, it might be possible to prevent seizures by targeting any one or a combination of these systems (Rostock *et al.*, 1996; Rogawski, 2006). Consideration will be given to three of the major mechanisms of action of antiepileptic drugs: modulation of ion channels, enhancement of GABA inhibitory neurotransmission, and attenuation of glutamate mediated excitatory transmission (Kwan *et al.*, 2001).

1.3.5.1 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. Voltage-gated sodium channel is arguably the most important ion channel responsible for depolarization of cell membranes. Modulation of the gating of brain sodium channels is believed to account, at least in part, for the ability of several AEDs to protect against generalized tonic-clonic and partial seizures (Deckers *et al.*, 2003). Phenytoin and

carbamazepine are prototype sodium channel blockers and this mechanism is shared by the newer drugs lamotrigine, felbamate, topiramate oxcarbazepine and zonisamide (Schauf, 1987; Schmutz *et al.*, 1994; Taglialatela *et al.*, 1996; Albus and Williamson, 1998; Taverna *et al.*, 1999; Ambrosio *et al.*, 2001; Remy *et al.*, 2003). By binding mainly to the inactivated state of the sodium channel these drugs 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). Their actions translate indirectly into reduced transmitter output at synapses (Leach *et al.*, 1986). AEDs might also act by blocking the persistent sodium current (current that flows due to the overlap between the voltage ranges for sodium channel activation and inactivation) (Maurice *et al.*, 2001; Taddese and Bean, 2002). It has been reported that phenytoin (Chao and Alzheimer, 1995; Segal and Douglas, 1997; Lampl *et al.*, 1998; Niespodziany *et al.*, 2004), valproate (Taverna *et al.*, 1998) and topiramate (Taverna *et al.*, 1999) inhibit the persistent sodium current at concentrations lower than those that block fast sodium current. The selective reduction of late, persistent sodium channel openings might contribute to the ability of these drugs to protect against seizures with minimal interference in normal function (Rogawski and Loscher, 2004).

Voltage-gated calcium channels are also involved in depolarization. They are often recruited in response to initial sodium-dependent action potential generation. 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 neurons, is believed to play a role in the distinctive rhythmic discharges of generalized absence seizures (Kwan *et al.*, 2001). The efficacy of ethosuximide against generalized absence seizures is believed to be mediated by blockade of the T-type calcium channel. 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).

Potassium channels also play a major role in the control of resting membrane potential, responsiveness to synaptic inputs, spike frequency adaptation and neurotransmitter release. They are therefore potential targets for antiepileptic drugs. Genetic, molecular, physiological and pharmacological evidence supports a role of some K^+ channels in the control of neuronal excitability and epileptogenesis (Wickenden, 2002). Retigabine functions through its ability to activate potassium currents (Rostock *et al.*, 1996; Rundfeldt, 1997).

1.3.5.2 Enhancement of inhibitory neurotransmission

Potentiation of inhibitory neurotransmission mediated by GABA is a key mechanism of AED action. Compared to excitatory synapses, neurons that use GABA as their neurotransmitter represent only a small fraction of neurons in key regions implicated in epileptic activity, such as the neocortex, hippocampus and amygdala (Galarreta and Hestrin, 1998). These inhibitory connections are still capable of restraining the natural tendency of excitatory neurons from undergoing the transition into synchronized epileptiform discharges. Following synaptic release, GABA acts through $GABA_A$, $GABA_B$ and $GABA_c$ receptors to bring about neuronal hyperpolarization leading to synaptic inhibition. Potassium bromide, the first effective epilepsy treatment, augments $GABA_A$ receptor-mediated inhibition by enhancing the sensitivity of $GABA_A$ receptors to GABA and increasing $GABA_A$ receptor currents (Gallagher *et al.*, 1978; Akaike *et al.*, 1989; Brunig *et al.*, 1999). Bromide salts continue to be widely used for treating epileptic dogs and cats in veterinary medicine although clinically it has been replaced by less toxic agents. Drugs that act by interacting with $GABA_A$ receptors or by modifying the activity of enzymes and transporters typically have a broad spectrum of antiepileptic activity in human seizure disorders, although, with the exception of benzodiazepine receptor agonists, they are generally ineffective in absence seizures. The actions of valproate and gabapentin (by increasing GABA synthesis and turnover) overlap with drugs that interact with GABA systems. The antiepileptic activity of benzodiazepine-like agents occurs through positive allosteric modulation of $GABA_A$ receptors containing the α2 subunit, β1 subunit (necessary for seizures protection) and effects on tonic $GABA_A$ receptor currents, which originate from GABA acting on extrasynaptic receptors (Rudolph *et al.*, 1999; Crestani *et al.*, 2000). Benzodiazepines have an important clinical role in the acute treatment of status *epilepticus* though sedation, muscle relaxation but development of tolerance and dependence limit their chronic use. Benzodiazepines, such as clonazepam have anti-absence activity by inhibiting the 3-Hz spike-and-wave activity believed to underlie the generalized absence seizures, in thalamocortical circuits) (Sohal *et al.*, 2003). Thalamic reticular neurons exert an inhibitory influence on thalamocortical relay neurons that is necessary for de-inactivation of the T-type calcium currents that underlie bursting. Benzodiazepines reduce the inhibitory output of the reticular neurons by effects on benzodiazepine-sensitive α3-containing $GABA_A$ receptors and therefore prevent absence seizure activity.

Barbiturates such as phenobarbital also act as positive allosteric modulators of $GABA_A$ receptors to shift the relative proportion of openings to favour the longest-lived open state associated with prolonged bursting (brief openings in rapid succession), thereby increasing the overall probability that the channel is open (Macdonald and Olsen, 1994). In addition,

barbiturates act on other ion channel systems, including calcium and sodium channels, and this probably contributes to their therapeutic activity and might also be a factor in their side effects (ffrench-Mullen *et al.*, 1993). Felbamate (Rho *et al.*, 1997) and topiramate (White *et al.*, 1997; Gordey *et al.*, 2000) might also act, in part, through positive modulation of $GABA_A$ receptors. The concentration of GABA in the brain is controlled by two pyridoxal-5′-phosphate-dependent enzymes, glutamate decarboxylase (GAD) and GABA transaminase (GABAT). The AED vigabatrin (γ-vinyl GABA) elevates brain GABA by acting as an irreversible suicide inhibitor of GABAT (De Biase *et al.*, 1991). The AED tiagabine is a potent and selective competitive inhibitor of GABA transporter 1(GAT1) that binds with high affinity for the transporter and prevents GABA uptake without itself being transported (Suzdak and Jansen, 1995). By slowing the reuptake of synaptically released GABA, tiagabine prolongs inhibitory postsynaptic potentials (Thompson and Gahwiler, 1992; Jackson *et al.*, 1999).

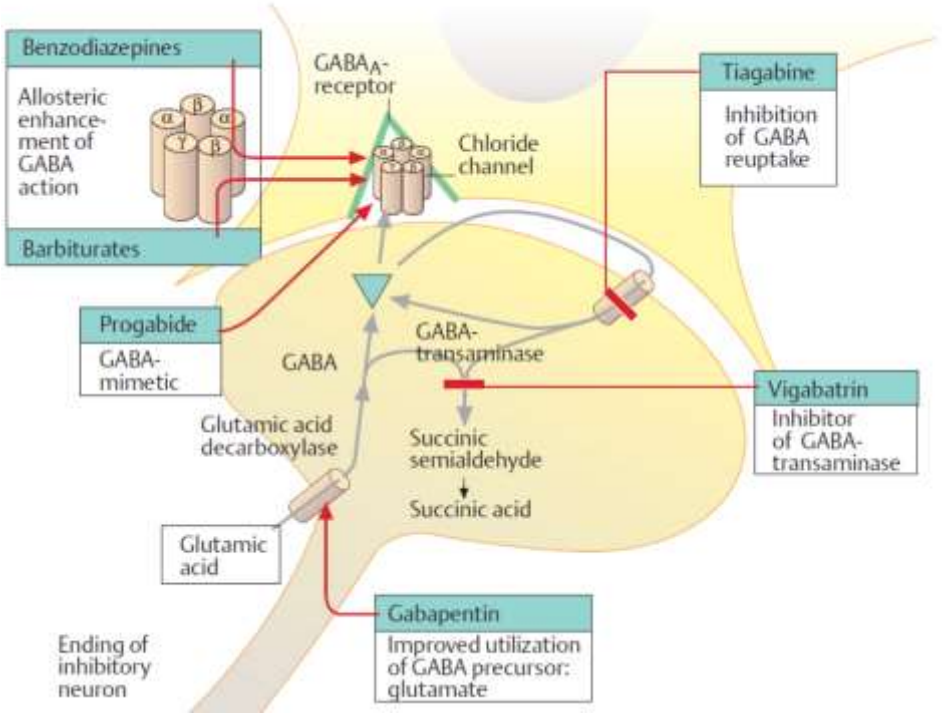

Figure 1.2 Sites of action of antiepileptics in GABAergic synapse

1.3.5.3 Attenuation of excitatory neurotransmission

Glutamate is the principal excitatory neurotransmitter in the mammalian brain. Following synaptic release, it exerts its effects on both ionotropic (AMPA, kainate and NMDA) and metabotropic receptor types. The ionotropic glutamate receptors form ligand-gated ion

channels permeable to sodium and depending on subtype and subunit 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). NMDA receptors consist of NR1 subunits combined with one or more NR2 (A–D) subunits, which form channels that are permeable to sodium and calcium ions. The activity of NMDA receptors is regulated via a strychnine-insensitive glycine-binding site and other modulatory sites, such as polyamines, Zn^{2+} and H^{+}. At resting membrane potentials, the pore of this receptor is blocked by magnesium ions, which are removed after membrane depolarization. The role of NMDA receptors in experimental epileptogenesis, neuroplasticity, seizures and excitotoxicity has been firmly established (Delorenzo *et al.*, 2005; Lason *et al.*, 2011). Antagonists of NMDA receptors, such as dizocilpine or ketamine, inhibit seizures induced by pentylenetetrazole, pilocarpine, maximal electroshock or sensory stimulation. Furthermore, they delay the development of amygdala kindling but have a weaker effect on fully developed seizures in this model. Unfortunately, both competitive and non-competitive NMDA receptor antagonists show serious undesired effects, such as psychomotor, memory and cognitive disturbances, and psychotomimetic-like effects in experimental animals and in initial clinical studies. However allosteric modulators of NMDA receptors, especially modulators that interact with the strychnine-insensitive glycine-binding site and the polyamine-binding site, are more promising as potential AEDs. The partial agonist of the glycine binding site, D-cycloserine, exerts anticonvulsant activity most likely via the desensitization of NMDA receptors. This compound also augments the seizure suppressing effects of some AEDs and, in low doses, has a positive influence on memory processes. Beneficial effects in experimental models of seizures have been observed after the concomitant administration of glycine- and polyamine-binding site antagonists. In 2008 lacosamide (LCM), an antagonist of the glycine binding site on NMDA receptors, was registered as an AED Blockade of the NMDA subtype of glutamate receptor and AMPA receptors have also been reported to contribute to the antiepileptic effects of felbamate and topiramate respectively (Deckers *et al.*, 2003).

1.3.6 **Dietary treatment of epilepsy**

A growing body of evidence demonstrates that dietary therapies for epilepsy are highly effective, with approximately 30–60% of children overall having at least a 50% reduction in seizures after 6 months of treatment (Henderson *et al.*, 2006; Neal *et al.*, 2008; Freeman, 2009; Nabbout *et al.*, 2010). Ketogenic diets may be also effective for adult *status epilepticus* and

adult epilepsy (Wusthoff *et al.*, 2010; Smith *et al.*, 2011) and as a first-line treatment of seizures associated with glucose transporter 1 deficiency (De Vivo *et al.*, 2002).

All dietary therapies used to treat epilepsy share the common characteristic of restricting carbohydrate intake to shift the predominant caloric source of the diet to fat (Huffman and Kossoff, 2006). Body tissues are thereby forced to catabolize fats as their primary source of energy, and the catabolism of fats results in ketones, hence the origin of the common descriptor for these therapies, "the ketogenic diet." The precise mechanisms by which the ketogenic diet yields its anticonvulsant effect are not known, but appear unique from the mechanisms of action for other anticonvulsants (Bough and Rho, 2007).

1.3.7 Experimental Models for Anticonvulsant screening

1.3.7.1 Acute models

The maximal electroshock (MES) test and the pentylenetetrazole (PTZ) seizure are often classified as acute animal models (Loscher, 2002). The first important neuropharmacological step in detecting the potential value of candidate anticonvulsant compounds is the classical maximal electroshock (MES) test in mice, introduced by Putnam and Merritt (Putnam and Merritt, 1937). The MES is the most widely used animal model in AED discovery, because seizure induction is simple and the predictive value for detecting clinically effective AEDs is high (Loscher, 2002). A powerful detection system is ensured when the MEST is combined with the pentylenetetrazole (PTZ) seizure test. These are the two primary bioassays employed in the in vivo screening of new anticonvulsant compounds (Krall *et al.*, 1978; Loscher *et al.*, 1988; Raza *et al.*, 2001). AEDs such as phenytoin, carbamazepine, valproic acid, that inhibit the hind limb tonic extension phase (HLTE) of the electroshock seizure in MEST are effective in the therapy of generalized tonic-clonic and partial seizures, while AEDs that inhibit seizures induced by pentylenetetrazole (PTZ) in PTZ test e.g. ethosuximide and phenobarbitone are effective in the treatment of generalized myoclonic and absence seizures (White, 1997; Raza *et al.*, 2001). The subcutaneous administration of bicuculline (BIC), picrotoxin (PTX) and strychnine (STR) are also valuable tests to induce seizures and evaluate the effectiveness and mechanisms of anticonvulsant compounds (Porter *et al.*, 1984; Raza *et al.*, 2001). Clinically efficacious drugs have been discovered by these acute models including ethosuximide, trimethadone and valproate. These show similar anticonvulsant effects in different genetic models of absence epilepsy such as Genetic Absence Epilepsy Rats from Strasbourg (GAERS) or lethargic mice (Loscher, 2002).

1.3.7.2 Chronic models

Acute models might not detect all compounds with antiepileptic activity when used alone. The MES test preselects drugs with certain mechanisms, but misses others (Meldrum, 1997). Although the MES test is often considered a mechanism-independent model (Kupferberg, 2001), it is particularly sensitive to drugs blocking sodium channels (Meldrum, 1997). This means that several clinically efficacious AEDs which act by other mechanisms (such as levetiracetam, vigabatrin and tiagabin) and were initially not screened or detected by using MES test would have been missed using this model as the only drug discovery model (Loscher and Schmidt, 1994). Also, the PTZ test might not be able to detect all antiepileptic drugs against non-convulsive seizures (Loscher, 2002). This is due to the fact that lamotrigine, which is very efficacious against non-convulsive seizures in patients, is ineffective in the PTZ test, while vigabatrin and tiagabin, which are quite effective in the PTZ test, are ineffective in patients and even aggravate non-convulsive seizures (Loscher, 2002). In this case chronic seizure models including kindling, genetic models such as GAERS or lethargic mice have been used (Loscher, 2002). Also, even after the primary screening of anticonvulsants, advanced experiments on primate models and 'Kindling in rodents' which may follow include monkey models of absence (petit mal) seizures, aluminium hydroxide induced partial or secondary generalised (grand mal) seizures in monkeys, experimental temporal lobe epilepsy in monkeys and amygdala kindled seizures in rats. Also the pliocarpine induced status epilepticus which is a model of temporal lobe epilepsy is also widely used.

1.4 DEPRESSION

1.4.1 Background

Epileptic patients often present with other psychiatric comorbidities, one of the commonest being depression. There is a general consensus that epileptic patients have a four to fivefold higher incidence of depression and up to a fivefold higher incidence of suicide than the general population (Hauser and Kurland, 1975; Standage and Fenton, 1975; Kogeorgos *et al.*, 1982).

Depression is a chronic recurring illness that affects 120 million people worldwide. Major depressive disorder (depression) is characterized by the core symptoms of depressed mood and a loss of interest and/or pleasure. Other symptoms that may be manifested include significant weight changes (loss or gain), sleep disturbances (insomnia or hypersomnia), fatigue or loss of energy, diminished ability to think or concentrate, feelings of worthlessness or guilt, recurrent thoughts of death or suicide, and psychomotor agitation or retardation (Khawam *et al.*, 2006). In industrialized societies, approximately 5% of the population experienced a major depressive

episode (MDE, defined as the occurrence of a core symptom and 4 or more other symptoms daily or almost daily for ≥2 weeks) in the past year (Andrews *et al.*, 2002). It is likely that many brain regions mediate the diverse symptoms of depression. Knowledge of the function of these brain regions under normal conditions suggests the aspects of depression to which they may contribute. Neocortex and hippocampus may mediate cognitive aspects of depression, such as memory impairments and feelings of worthlessness, hopelessness, guilt, doom, and suicidality. The striatum (particularly the ventral striatum or nucleus accumbens) and amygdala, and related brain areas, are important in emotional memory, and could mediate the anhedonia (decreased drive and reward for pleasurable activities), anxiety, and reduced motivation that predominates in many patients (Schlaepfer *et al.*, 2008). Given the prominence of so-called neurovegetative symptoms of depression, including too much or too little sleep, appetite, and energy, as well as a loss of interest in sex and other pleasurable activities, a role of the hypothalamus has also been speculated. These various brain regions operate in a series of highly interacting parallel circuits, which perhaps begins to formulate a neural circuitry involved in depression (Nestler *et al.*, 2002).

Over the past four to five decades, a number of antidepressants have been approved for use. The newer ones seem to offer better patient tolerability and safety than the older ones. Yet there are almost 40% of patients whose condition are refractory to current treatment. Currents medications still have slow onset of action (Khawam *et al.*, 2006). Though there are evidences to suggest that rapid onset antidepressants are available none have been approved yet (Lucas *et al.*, 2007; Duman and Voleti, 2012). These reasons underscore the need for alternative medicines that will cater for the needs of those with refractory depression and also provide rapid onset of action.

1.4.2 Theories of Depression

There has been a marked shift in the last decade in the understanding of the pathophysiology of major depression. In addition to the older idea that a deficit in function or amount of monoamines (the monoamine hypothesis) is central to the biology of depression, there is evidence that neurotrophic and endocrine factors also play a major role.

1.4.2.1 Monoamine Hypothesis

The monoamine hypothesis of depression suggests that depression is related to a deficiency in the amount or function of cortical and limbic serotonin (5-HT), norepinephrine (NE), and dopamine (DA) (Hirschfeld, 2000). Evidence to support the monoamine hypothesis comes from several sources. Reserpine treatment, which depletes monoamines, is associated with

depression in a subset of patients. Similarly, depressed patients who respond to serotonergic antidepressants such as fluoxetine often rapidly suffer relapse when given diets free of tryptophan, a precursor of serotonin synthesis. Patients who respond to noradrenergic antidepressants such as desipramine are less likely to relapse on a tryptophan-free diet (Hirschfeld, 2000; Yamada and Higuchi, 2002). However, depleting catecholamines in depressed patients who have previously responded to noradrenergic agents likewise tends to be associated with relapse. Administration of an inhibitor of norepinephrine synthesis is also associated with a rapid return of depressive symptoms in patients who respond to noradrenergic but not necessarily in patients who had responded to serotoninergic antidepressants (Yamada and Higuchi, 2002).

Another line of evidence supporting the monoamine hypothesis comes from genetic studies (Yamada and Higuchi, 2002). A functional polymorphism exists for the promoter region of the serotonin transporter gene, which regulates how much of the transporter protein is available. Subjects who are homozygous for the *s* (short) allele may be more vulnerable to developing major depression and suicidal behavior in response to stress. In addition, homozygotes for the s allele may also be less likely to respond to and tolerate serotoninergic antidepressants. Conversely, subjects with the *l* (long) allele tend to be more resistant to stress and may be more likely to respond to serotoninergic antidepressants (Drevets *et al.*, 1999; Yamada and Higuchi, 2002). Studies of depressed patients have sometimes shown an alteration in monoamine function. For example, some studies have found evidence of alteration in serotonin receptor numbers (5-HT_{1A} and 5-HT_{2C}) or norepinephrine (α_2) receptors in depressed and suicidal patients, but these findings have not been consistent (Drevets *et al.*, 1999). Perhaps the most convincing line of evidence supporting the monoamine hypothesis is the fact that all available antidepressants appear to have significant effects on the monoamine system. All classes of antidepressants appear to enhance the synaptic availability of 5-HT, norepinephrine, or dopamine (Elhwuegi, 2004).

The monoamine hypothesis is at best incomplete (Boerkamp and Wijnberger, 2011). Many studies have not found an alteration in function or levels of monoamines in depressed patients. In addition, some candidate antidepressant agents under study do not act directly on the monoamine system (Boerkamp and Wijnberger, 2011). These include glutamate antagonists, melatonin agonists, and glucocorticoid-specific agents. Thus, monoamine function appears to be an important but not exclusive factor in the pathophysiology of depression.

1.4.2.2 Neurotrophic Hypothesis

There is substantial evidence that nerve growth factors such as brain-derived neurotrophic factor (BDNF) are critical in the regulation of neural plasticity, resilience, and neurogenesis (Groves, 2007; Hasler, 2010). The evidence suggests that depression is associated with the loss of neurotrophic support and that effective antidepressant therapies increase neurogenesis and synaptic connectivity in cortical areas such as the hippocampus. BDNF is thought to exert its influence on neuronal survival and growth effects by activating the tyrosine kinase receptor B in both neurons and glia (Groves, 2007; Hasler, 2010). Animal and human studies indicate that depression, stress and pain are associated with a drop in BDNF levels and that this loss of neurotrophic support contributes to atrophic structural changes in the hippocampus and perhaps other areas such as the medial frontal cortex and anterior cingulate which play important roles in depression. Moreover, all known classes of antidepressants are associated with an increase in BDNF levels in animal models with chronic administration. This increase in BDNF levels is consistently associated with increased neurogenesis in the hippocampus in these animal models (Duman and Li, 2012; Voleti and Duman, 2012). Other interventions thought to be effective in the treatment of major depression, including electroconvulsive therapy, also appear to robustly stimulate BDNF levels and hippocampus neurogenesis in animal models. Human studies seem to support the animal data on the role of neurotrophic factors in stress states. Thus, the neurotrophic hypothesis continues to be intensely investigated and has yielded new insights and potential targets in the treatment of major depressive disorder (MDD) (Duman and Li, 2012; Voleti and Duman, 2012).

1.4.2.3 Neuroendocrine Factors in the Pathophysiology of Depression

Depression is known to be associated with a number of hormonal abnormalities (Cleare *et al.*, 1995; Cleare *et al.*, 1995). Among the most replicated of these findings are abnormalities in the hypothalamic- pituitary-adrenal (HPA) axis in patients with MDD (Gotlib *et al.*, 2008). Moreover, MDD is associated with elevated cortisol levels, nonsuppression of adrenocorticotropic hormone (ACTH) release in the dexamethasone suppression test, and chronically elevated levels of corticotropin-releasing hormone (Gotlib *et al.*, 2008; Pariante and Lightman, 2008). The significance of these HPA abnormalities is unclear, but they are thought to indicate a dysregulation of the stress hormone axis. More severe types of depression, such as psychotic depression, tend to be associated with HPA abnormalities more commonly than milder forms of major depression. It is well known that both exogenous glucocorticoids and endogenous elevation of cortisol are associated with mood symptoms and cognitive deficits similar to those seen in MDD (Markopoulou *et al.*, 2009). Thyroid dysregulation has also been

reported in depressed patients (Engum *et al.*, 2002). Up to 25% of depressed patients are reported to have abnormal thyroid function. These include a blunting of response of thyrotropin to thyrotropin-releasing hormone, and elevations in circulating thyroxine during depressed states. Clinical hypothyroidism often presents with depressive symptoms, which resolve with thyroid hormone supplementation (Cole *et al.*, 2002; Engum *et al.*, 2002). Thyroid hormones are also commonly used in conjunction with standard antidepressants to augment therapeutic effects of the latter (Engum *et al.*, 2002). Finally, sex steroids are also implicated in the pathophysiology of depression (Kessler, 2003; Almeida *et al.*, 2004). Estrogen deficiency states, which occur in the postpartum and postmenopausal periods, are thought to play a role in the aetiology of depression in some women. Likewise, severe testosterone deficiency in men is sometimes associated with depressive symptoms (Kessler, 2003). Hormone replacement therapy in hypogonadal men and women may be associated with an improvement in mood and depressive symptoms (Almeida *et al.*, 2004).

1.4.3 Antidepressant Drugs

1.4.3.1 Monoamine Oxidase Inhibitors

In 1952 iproniazid, a hydrazine derivative was found to inhibit monoamine oxidase (MAO). It was later used as the first antidepressant to treat depressed patients but owing to hepatotoxicity its use was discontinued (Nelson *et al.*, 1978; Timbrell, 1979). Two other hydrazine-derivative inhibitors of MAO, phenelzine (the structural analog of phenethylamine, an endogenous amine) and isocarboxazid subsequently were introduced into clinical practice. Tranylcypromine, structurally related to amphetamine, was the first MAO inhibitor unrelated to hydrazine to be discovered. The development of reversible, selective MAO inhibitors with potentially broad applications (e.g. selegiline for Parkinson's disease) was stimulated by the understanding that the early MAO inhibitors result in irreversible and nonselective blockade of both MAO-A and MAO-B, which were responsible for the metabolic breakdown of dopamine, norepinephrine, and serotonin in neuronal tissues. The nonselective MAO inhibitors in clinical use are reactive hydrazines (phenelzine and isocarboxazid) or amphetamine derivatives (tranylcypromine). Selegiline, a propargylamine, is relatively specific for MAO-B (Cesura and Pletscher, 1992). Following their oxidation to reactive intermediates by MAO, each of these "suicide" substrates interacts irreversibly to inactivate the flavin prosthetic group of the MAO enzyme (Krishnan, 2007). Cyclization of the side chain of amphetamine resulted in tranylcypromine. After formation of a reactive imine intermediate by MAO, inhibition of MAO by this cyclopropylamine derivative may involve the reaction of a sulfhydryl group in the active site of MAO. Due to the irreversible inactivation of MAO, these compounds produce long-acting

inhibition that may persist for up to 2 weeks after drug discontinuation. Short-acting, reversible inhibitors of MAO-A (RIMAs) with antidepressant activity are also available. These include a piperidylbenzofuran (Brofaromine), a morpholinobenzamide (Moclobemide) and an oxazolidinone (Toloxatone) (Delini-Stula *et al.*, 1988).

1.4.3.2 Tricyclic Antidepressants

Tricyclic antidepressants are closely related in structure to the phenothiazines and were initially synthesized in 1949 as potential antipsychotic drugs (Gallanosa *et al.*, 1981). Imipramine was found to be of no use in schizophrenia but effective in depression, so related compounds, such as clomipramine, were synthesised. In addition to their effects on amine uptake, most TCAs affect one or more types of neurotransmitter receptor, including muscarinic acetylcholine receptors, histamine receptors and 5-HT receptors. The antimuscarinic effects of TCAs do not contribute to their antidepressant effects but are responsible for their various side effects. Older tricyclic antidepressants with a tertiary-amine side chain (including amitriptyline, doxepin, and imipramine) block neuronal uptake of both serotonin and norepinephrine, whereas clomipramine is relatively selective against serotonin (Gallanosa *et al.*, 1981).

The actions of imipramine-like tricyclic antidepressants also include a range of complex, secondary adaptations to their initial and sustained actions as inhibitors of norepinephrine neuronal transport (reuptake) and variable blockade of serotonin transport (Beasley *et al.*, 1992). Tricyclic-type antidepressants with secondary-amine side chains or the N-demethylated (nor) metabolites of agents with tertiary-amine moieties (e.g., amoxapine, desipramine, maprotiline, norclomipramine, nordoxepin, and nortriptyline) are relatively selective inhibitors of norepinephrine transport. Most tertiary-amine tricyclic antidepressants also inhibit the reuptake of serotonin. It is likely that relatively selective inhibitors of norepinephrine reuptake, including atomoxetine and reboxetine, share many of the actions of older inhibitors of norepinephrine transport (Kratochvil *et al.*, 2003) such as desipramine (Delgado and Michaels, 1999). Among the tricyclic antidepressants, trimipramine is exceptional in that it lacks prominent inhibitory effects at monoamine transport, and its clinical actions remain unexplained. The tricyclic and other norepinephrine-active antidepressants do not block dopamine transport, thereby differing from CNS stimulants, including cocaine, methylphenidate, and amphetamines. Nevertheless, they may facilitate effects of dopamine indirectly by inhibiting the nonspecific transport of dopamine into noradrenergic terminals in cerebral cortex. Tricyclic antidepressants also can desensitize D2 dopamine autoreceptors, through uncertain mechanisms and with uncertain behavioral contributions. In addition to their transport-inhibiting effects, tricyclic antidepressants variably interact with adrenergic receptors.

The presence or absence of such receptor interactions appears to be critical for responses to increased availability of extracellular norepinephrine in or near synapses. Most tricyclic antidepressants have at least moderate and selective affinity for α1 adrenergic receptors, much less for α_2, and virtually none for β-receptors. The α_2 receptors include presynaptic autoreceptors that limit the neurophysiological activity of noradrenergic neurons ascending from the locus coeruleus in brainstem to supply mid- and forebrain projections. The same noradrenergic neurons provide descending projections to the spinal cord preganglionic cholinergic efferents to the peripheral autonomic ganglia. Autoreceptor mechanisms also reduce the synthesis of norepinephrine through the rate-limiting enzyme tyrosine hydroxylase, presumably through α_2 adrenergic receptor attenuation of cyclic AMP-mediated phosphorylation-activation of the enzyme. Activation of these receptors inhibits transmitter release by incompletely defined molecular and cellular actions that likely include suppression of voltage-gated Ca^{2+} currents and activation of G protein-coupled, receptor-operated K^+ currents. The α_2 receptor-mediated, presynaptic, negative-feedback mechanisms are rapidly activated after administration of tricyclic antidepressants. By limiting synaptic availability of norepinephrine, such mechanisms normally tend to maintain functional homeostasis. However, with repeated drug exposure, α_2-receptor responses eventually are diminished. This loss may result from desensitization secondary to increased exposure to the endogenous agonist ligand norepinephrine, or alternatively from prolonged occupation of the norepinephrine transporter itself via an allosteric effect, as suggested for inhibitors of serotonin transporters on serotonergic neurons (Chaput *et al.*, 1991). Over a period of days to weeks, this adaptation allows the presynaptic production and release of norepinephrine to return to, or even exceed, baseline levels (Charney *et al.*, 1987). However, long-term treatment eventually can reduce the expression of tyrosine hydroxylase as well as the norepinephrine transporter (NET) protein (Nestler *et al.*, 1990). Other adaptive changes have been observed in response to long-term treatment with tricyclic antidepressants. These include altered sensitivity of muscarinic acetylcholine receptors as well as decreases in gamma-aminobutyric acid ($GABA_B$) receptors and possibly N-methyl-D-aspartate (NMDA) glutamate receptors (Kitamura *et al.*, 1992). In addition, cyclic AMP production is increased and the activities of protein kinases altered in some cells, including those acting on cytoskeletal and other structural proteins that may alter neuronal growth and sprouting (Perez *et al.*, 1991). Transcription and neurotrophic factors also are affected, including the cyclic AMP-response-element binding protein (CREB) and brain-derived neurotrophic factor (BDNF) (Duman *et al.*, 1997). Additional changes may be indirect effects of antidepressant treatment or may reflect recovery from depressive illness. These include normalization of glucocorticoid release and glucocorticoid receptor sensitivity, as well

as shifts in the production of prostaglandins and cytokines and in lymphocyte functions (Kitamura *et al.*, 1989).

1.4.3.3 Selective Serotonin Reuptake Inhibitors (SSRIs)

SSRIs block neuronal transport of serotonin both immediately and chronically, leading to complex secondary responses. Citalopram and fluoxetine are racemates; sertraline and paroxetine are separate enantiomers. Escitalopram is the (S)-enantiomer of citalopram. Fluoxetine and its major metabolite norfluoxetine are highly active against serotonin transport and also may have antimigraine effects not found with the (R)-enantiomer of fluoxetine. The (R)-enantiomer of fluoxetine also is active against serotonin transport and is shorter acting than the (S)-enantiomer, but its clinical development was halted due to adverse electrocardiographic effects. (R)-Norfluoxetine is virtually inactive (Wong *et al.*, 1993). Structure-activity relationships are not well established for the SSRIs. However, it is known that the para-location of the CF3 substituent of fluoxetine is critical for serotonin transporter potency. Its removal and substitution at the ortho-position with a methoxy group yields nisoxetine, a highly selective norepinephrine-uptake inhibitor.

Increased synaptic availability of serotonin stimulates a large number of postsynaptic 5-HT receptor types (Azmitia *et al.*, 1995). Stimulation of 5-HT_3 receptors is suspected to contribute to common adverse effects characteristic of this class of drugs, including gastrointestinal effects (nausea and vomiting) and delayed or impaired orgasm. Stimulation of 5-HT_{2C} receptors may contribute to agitation or restlessness. An important parallel in responses of serotonin and norepinephrine neurons is that negative feedback mechanisms rapidly emerge to restore homeostasis (Azmitia *et al.*, 1995). In the serotonin system, 5-HT_1-subtype autoreceptors (types 1A and 7 at raphe cell bodies and dendrites, type 1D at terminals) suppress serotonin neurons in the raphe nuclei of the brainstem, inhibiting both tryptophan hydroxylase (probably through reduced phosphorylation-activation) and neuronal release of serotonin. Repeated treatment leads to gradual down-regulation and desensitization of autoreceptor mechanisms over several weeks (particularly of 5-HT_{1D} receptors at nerve terminals), with a return or increase of presynaptic activity, production, and release of serotonin (Blier *et al.*, 1990). Additional secondary changes include gradual down-regulation of postsynaptic 5-HT_{2A} receptors that may contribute to antidepressant effects directly, as well as by influencing the function of noradrenergic and other neurons via serotoninergic heteroceptors.

Many other postsynaptic 5-HT receptors presumably remain available to mediate increased serotonergic transmission and contribute to the mood-elevating and anxiolytic effects of this class of drugs. As in responses to norepinephrine-transport inhibitors, complex late adaptations

occur upon repeated treatment with serotonin reuptake inhibitors. These may include indirect enhancement of norepinephrine output by reduction of tonic inhibitory effects of 5-HT_{2A} heteroceptors. Similar nuclear and cellular adaptations occur as with the tricyclic antidepressants (Azmitia *et al.*, 1995; Hyman and Nestler, 1996).

1.4.3.4 Atypical Antidepressants

Atypical antidepressants represent a heterogeneous group comprising agents that interfere only weakly or not at all with monoamine reuptake (trazodone, nefazodone, bupropion, mirtazapine), preferentially block reuptake of norepinephrine (reboxetine), or act as dual inhibitors of 5-HT and norepinephrine reuptake (venlafaxine, milnacipran, duloxetine) (Horst and Preskorn, 1998). Venlafaxine appears to be as effective as tricyclic antidepressants in severe depression. Mirtazapine and mianserin are structural analogs of serotonin with potent antagonistic effects at several postsynaptic serotonin receptor types (including 5-HT_{2A}, 5-HT_{2C}, and 5-HT_3 receptors) and can produce gradual down-regulation of 5-HT_{2A} receptors (Golden *et al.*, 1998). Mirtazapine also limits the effectiveness of inhibitory α_2 adrenergic heteroceptors on serotonergic neurons as well as inhibitory α_2 autoreceptors and 5-HT_{2A} heteroceptors on noradrenergic neurons. These effects may enhance release of amines and contribute to the antidepressant effects of these drugs. Mirtazapine also is a potent histamine H1-receptor antagonist and is relatively sedating (Arko and Florjanc, 2001).

1.4.4 Animal models of depression

1.4.4.1 Forced Swimming Test

The forced swimming test, originally described by Porsolt *et al*., (1977) is the most widely used laboratory test for assessing the potential clinical antidepressant activity of drugs (Cryan *et al.*, 2002; 2005). This is largely due to its ease of use, reliability across laboratories, ability to detect a broad spectrum of antidepressants, and its capacity to meet the high through-put demands of the pharmaceutical industry (Borsini and Meli, 1988; Rupniak, 2003). The development of immobility when rodent is placed in an inescapable cylinder of water is thought to reflect the cessation of persistent escape-directed behaviour that serves various adaptive functions in response to stress (Detke *et al.*, 1997; Cryan and Mombereau, 2004).

1.4.4.2 Tail Suspension Test

The TST is based on the observation that rodents (almost always mice although gerbils and rats have been used (Chermat *et al.*, 1986; Varty *et al.*, 2003), after initial escape-oriented movements, develop an immobile posture when placed in an inescapable stressful situation. The stressful situation involves the haemodynamic stress of being hung in an uncontrollable

fashion by their tail (Thierry *et al.*, 1986). If antidepressant treatments are given prior to the test, the subjects will actively persist engaging in escape-directed behaviours for longer periods of time than after vehicle treatment. The test is usually quite short, and the amount of time they spend immobile is recorded either manually or through an automated device (Steru *et al.*, 1985). Acute antidepressant treatments decrease these immobility scores. An obvious advantage of this test is its ability to detect a broad spectrum of antidepressants irrespective of their underlying mechanism; it is inexpensive, methodologically unsophisticated and easily amenable to automation.

1.5 ANXIETY

1.5.1 Background

Anxiety is both a normal emotion and a psychiatric disorder. Anxiety is a feeling of apprehension or fear, combined with symptoms of sympathetic activity. It is a normal response to stress and only becomes a clinical problem if it becomes severe or persistent, and interferes with everyday performance. It has a lifetime prevalence of over 5% of the population (Sinclair *et al.*, 2007). A number of pharmacological theories exist which suggest that anxiety is caused by either amine or excitatory amino acid function so anxiolytics have therefore, been developed to target specific brain neurotransmitter systems. The $GABA_A$ receptor is the brain's main inhibitory receptor and so regulates the activity of many types of neurons, including dopaminergic, noradrenergic and serotonergic. There is considerable evidence that a down-regulation of $GABA_A$ function may underlie some forms of anxiety, some of which comes from imaging studies (Malizia *et al.*, 1998).

Most anticonvulsant drugs act via GABA and glutamate neurotransmission and so can serve as sources for novel anxiolytics. Benzodiapines act on $GABA_A$-BZ receptor and are effective predominantly in panic disorder (PD), general anxiety disorder (GAD) and social phobia (Malizia *et al.*, 1998). Gabapentin and pregabalin are effective in certain anxiety disorders. Pregabalin, which works via voltage-gated Ca^{2+} channels, causing decreased release of several neurotransmitters, has shown short-term efficacy GAD (Sinclair *et al.*, 2007). The anticonvulsant properties of lamotrigine are mediated via NMDA glutamate receptor antagonism. Efficacy has been shown in post-traumatic stress disorder (PTSD) (Sinclair *et al.*, 2007). Tiagabine, a $GABA_A$ reuptake inhibitor, has had mixed results in clinical trials but has shown efficacy in GAD as well as in PD.

1.5.2 **Animal models used for screening anxiolytics**

Animal tests of anxiety are used to screen for novel compounds for anxiolytic or anxiogenic activity, to investigate the neurobiology of anxiety, and to assess the impact of other occurrences such as exposure to predator odours or early rearing experiences. These models involve exposure of animals to stimuli (exteroceptive or interoceptive) that appear capable of causing anxiety in humans. Despite their apparent diversity, animal anxiety models may be grouped into two general categories involving either conditioned (e.g. Geller-Seifter conflict, potentiated startle) or unconditioned (social interaction and light/dark exploration tests) responses (Rodgers and Dalvi, 1997). An ideal model of anxiety should have predictive, face and construct validities. A model that has predictive validity should display reduced anxiety when treated with anxiolytics, while in a model with face validity, the response of an animal to a threatening stimulus should be comparable to the response known for humans, and the mechanisms underlying anxiety should be exhibited by a model with construct validity (McKinney and Bunney, 1969). Naturally, one or more models usually combine to achieve these parameters. Conditioning models require considerable training of subjects, food or water deprivation and/or the use of electric shock as an aversive stimulus. However, some of these procedures, such as conditioned defensive burying, take advantage of the natural tendency of rodents to make faster stimulus-response associations when faced with ecologically relevant (versus arbitrary) environmental challenges (Treit, 1990). The study of unconditioned responses to various forms of external threat represents a logical extension of this refinement of laboratory methods, providing a high degree of ecological validity for the research and allowing for a very much more complete behavioural characterization of the effects of experimental manipulations. Hence models involving unconditioned behavior were adopted in this study as discussed below:

1.5.2.1 The elevated plus-maze test

The elevated plus-maze (EPM) is the most popular of all currently available animal models of anxiety, and affords an excellent example of a model based on the study of unconditioned or spontaneous behaviour (File, 1992; Rodgers and Dalvi, 1997; Carobrez and Bertoglio, 2005). It was initially described by Pellow and co-workers (Pellow *et al.*, 1985) as a simple method for assessing anxiety responses by rodents. It is made of four arms (two open and two closed) that are arranged to form a plus shape. The assessment of anxiety behaviour of rodents is done by using the ratio of time spent on the open arms to the time spent on the closed arms. In the test, mice or rats are placed at the junction of the four arms of the maze, facing an open arm, and entries/duration in each arm are recorded by a video-tracking system and observer

simultaneously for 5 minutes. Other ethological parameters (i.e. rears, head dips, and stretch-attend postures) reflect anti-anxiety behaviour (Walf and Frye, 2007).

1.5.2.2 Light Dark Exploration test

The light/dark test is based on the innate aversion of rodents to brightly illuminated areas and on the spontaneous exploratory behaviour of the animals, applying mild stressors, i.e. novel environment and light (Bourin and Hascoet, 2003). The test apparatus consists of a small dark secure compartment (one-third) and a large illuminated aversive compartment (two-thirds). A natural conflict situation occurs when an animal is exposed to an unfamiliar environment or novel objects. The conflict is between the tendency to explore and the initial tendency to avoid the unfamiliar (neophobia). The exploratory activity reflects the combined result of these tendencies in novel situations. Thus, in the light/dark test, drug-induced increases in behaviours in the white part of a two-compartment box, in which a large white compartment is illuminated and a small black compartment is darkened, suggest anxiolytic activity. An increase in transitions without an increase in spontaneous locomotion is considered as anxiolytic activity. Transitions have been reported to be an index of activity exploration because of habituation over time and the time spent in each compartment to be a reflection of aversion. Classic anxiolytics (benzodiazepines) as well as the newer anxiolytic-like compounds (e.g. serotoninergic drugs or drugs acting on neuropeptides receptors) can be detected using this paradigm. It has the advantages of being quick and easy to use, without requiring the prior training of animals (Bourin and Hascoet, 2003).

1.6 JUSTIFICATION

The current pharmacotherapy for CNS disorders like epilepsy, depression and anxiety is far from optimum (Sun and Alkon, 2002; Misra *et al.*, 2003). Refractoriness and side effects are rife (Sills, 2006). In most African countries, herbal remedies are used for primary healthcare needs (Abu-Irmaileh and Afifi, 2003). These medicinal plants have proven to be important sources of bioactive molecules—one of the reasons why WHO is advocating for the incorporation of traditional medicine in the primary healthcare of developing nations (Calixto, 2000; Abu-Irmaileh and Afifi, 2003). Aside their effectiveness, they are generally considered to be safer compared to their orthodox counterparts because of the many compounds that can act antagonistically to reduce side effects and also synergistically to enhance efficacy. *Mallotus oppositifolius* is used traditionally for epilepsy, pain and treating infections in Ghana, Nigeria and Congo but there exists no scientific evidence suppoting their efficacy and safety (Burkill, 1985). The study was therefore conducted to evaluate scientifically the anticonvulsant effect

and antidepressant of the leaves of plant and to assess its possible mechanism(s) of action. This information will go a long way to help in our search for better and alternative drugs for most of these psychiatric ailments that have remained elusive.

1.7 PURPOSE AND OBJECTIVES OF STUDY

The primary aim of this study was to investigate the anticonvulsant, antidepressant and anxiolytic activity of *Mallotus oppositifolius* and other neurobehavioural effects that may be associated with antiepileptic drugs. The specific objectives of the thesis were to:

- Conduct phytochemical tests on the extract to confirm the presence of secondary metabolites
- Screen the general CNS activity of the extract
- Evaluate the anticonvulsant activity of the hydroalcoholic leaf extract in pentylenetetrazole, picrotoxin, maximal electroshock, strychnine, 4-aminopyridine - induced seizures and pilocarpine induced *status epilepticus*.
- Examine the antidepressant effect of the extract using classical acute models of depression including the tail suspension and the forced swimming tests and the chronic model, open space swim test.
- Examine the anxiolytic effect of the extract using classical models of anxiety including the elevated plus-maze, light/dark exploration tests and open field test.
- Investigate the effect of the extract on motor coordination and the rotarod test.

Chapter 2

PLANT COLLECTION, EXTRACTION AND PHYTOCHEMICAL ANALYSIS

2.1 PLANT COLLECTION AND EXTRACTION

2.1.1 Plant collection

Leaves of the plant *Mallotus oppositifolius* were collected from the campus of the Kwame Nkrumah University of Science and Technology (KNUST), Kumasi, Ghana (6°41‘6.4‘‘N, 1°33‘42.8‘‘W), in September, 2009. The leaves were authenticated at the Department of Herbal Medicine of the Faculty of Pharmacy and Pharmaceutical Sciences, KNUST, Kumasi and a voucher specimen (KNUST/FP/035/09) has been deposited at the herbarium of the Department of Herbal Medicine.

2.1.2 Extraction

The leaves were air-dried indoors for a week and pulverized with a hammer-mill. The powder was extracted by cold maceration using 70% (v/v) ethanol in water over a period of 72 h. The resulting extract was concentrated under moderate temperature (60°C) and pressure to a syrupy mass in a rotary evaporator. The syrupy mass was then dried to a dark brown solid mass on water bath and kept in a dessicator till it was ready to be used. The final yield was 9.5% (w/w). This is subsequently referred to as *Mallotus oppositifolius* extract (MOE) or extract.

2.2 PHYTOCHEMICAL TESTS

The freshly prepared hydroalcoholic extract was analyzed for phytochemical constituents as described by Trease and Evans (1989) for the detection of alkaloids, saponins, reducing sugars, sterols, tannins and terpenoids. These are described as follows:

2.2.1 Test for alkaloids

A sample of the extract (0.5 g) was boiled with 10 ml dilute hydrochloric acid for 5 min. The supernatant liquid was filtered and 3 drops of Dragendorf’s reagent was added to 1 ml of the filtrate. The mixture was shaken and the appearance of an orange-red spot precipitate indicates the presence of alkaloids.

2.2.2 Test for tannins

An amount of the extract (0.5 g) was boiled with 25 ml of water for 5 min, cooled and filtered. The volume of the filtrate was adjusted to 25 ml with water. A small quantity of water (10 ml)

and 2 drops of 1 % ferric chloride was added to 1 ml of the filtrate and observed for the appearance of a blue-black or green precipitate.

2.2.3 Test for saponins

A small amount (0.2 g) of the extract was shaken with a few mls of water in a test tube and the mixture observed for the presence of a froth which does not break readily upon standing.

2.2.4 Test for reducing sugars

A portion of the extract (0.2 g) was boiled in 5 ml of water. The mixture was cooled and filtered. An equal quantity (5 ml) of Fehling's A and B solutions were added to the filtrate, heated and observed for a red-brown precipitate.

2.2.5 Test for sterols

An amount of acetic anhydride (2ml) was added to 0.5 g of the extract with 2 ml of H_2SO_4. A colour change from violet to blue or green in the samples indicates the presence of steroids.

2.2.6 Test for terpenoids

An amount of 0.5 g of MOE was extracted with 2 ml of chloroform in a test tube followed by addition of 1ml of concentrated sulphuric acid. The reddish-brown coloration at interface shows the presence of terpenoids (Jana and Shekhawat, 2011).

2.3 RESULTS

The phytochemical screening revealed the presence of alkaloids, tannins, saponins, reducing sugars and sterols. Terpenoids were absent (Table 2.1).

Table 2.1Phytochemical constituents of the hydroalcoholic extract from the leaves of *M. oppositifolius*.

Constituent	Results
Saponins	+
Tannins	+
Alkaloids	+
Triterpenes	-
Reducing sugars	+
Sterols	+

–: Not detected, +: Present

2.4 DISCUSSION

Phytochemicals like alkaloids, flavonoids, coumarins, glycosides, gums, polysaccharides, phenols, tannins, terpenes and terpenoids are chemical compounds formed during the plants normal metabolic processes (Briskin, 2000; Palombo, 2006). Most of these secondary metabolites are potent bioactive compounds found in medicinal plant parts and have been reported to be responsible for their pharmacological effects (Briskin, 2000; Eastwood, 2001; Rogerio *et al.*, 2010). The hydroalcoholic extract of the leaves of *M. oppositifolius* contained alkaloids, saponins, sterols, tannins and glycosides, an observation consistent with works done by other investigators (Farombi *et al.*, 2001; Adekunle and Ikumapayi, 2006).

Alkaloids, the largest single class of secondary plant substances are ranked among the most efficient and therapeutically significant plant substances (Otani *et al.*, 2005; Makkar *et al.*, 2007). Pure, isolated plant alkaloids and their synthetic derivatives are used as basic medicinal agents for their CNS and analgesic e.g. morphine and codeine (*Papaver somniferum*); anti-inflammatory e.g. colchicine; anticancer e.g. vincristine and vinblastine (*Vinca roseus*); antispasmodic and bactericidal effects (Staerk *et al.*, 2002; Norn *et al.*, 2005; Yang *et al.*, 2006). Tannins are a group of polymeric/phenolic substances capable of tanning leather or precipitating gelatin from a solution; a property known as astringency (Okuda, 2005). Many physiological activities such as stimulation of phagocytic cells, host mediated antitumour activity and wide range of anti-infective action have been assigned to tannins (Okuda *et al.*, 1992; Souza *et al.*, 2007; Buzzini *et al.*, 2008; Koleckar *et al.*, 2008). Saponins are a group of naturally occurring plant glycosides, characterized by their strong foam-forming properties in aqueous solution (Vincken *et al.*, 2007; Quante *et al.*, 2010). They possess significant anti-cancer, antiinflammatory, antifungal, moluscidal, spermicidal, antidepressant effects, among others (George, 1999; Jung *et al.*, 2005; Dang *et al.*, 2009; Quante *et al.*, 2010). Sterols are cholesterol-like compounds found in plant. Plant sterols are best known for their ability to lower cholesterol level (Pollak, 1985; Katan *et al.*, 2003). They are used to boost the immune system (Bouic *et al.*, 1996; Breytenbach *et al.*, 2001). Moreover they have antidiabetic, anticancer and antimicrobial effects (Donald *et al.*, 1997; Muti *et al.*, 2003).

The pharmacological and therapeutic effects of the plant may be due to the presence of one or more of these secondary metabolites.

2.5 CONCLUSION

The hydroalcoholic extract of the leaves of *M. oppositifolius* contain alkaloids, sterols, reducing sugars, saponins and tannins.

Chapter 3
PRELIMINARY CNS SCREENING

3.1 INTRODUCTION

In the drug discovery process, it is important to assess and monitor the efficacy and safety of drugs especially in the preclinical phase (Kinter and Valentin, 2002). In 2000, the International Conference on Harmonization (ICH), determined guidelines for safety pharmacology. The Conference recommended the monitoring of the functions and effects of test drugs on the cardiovascular, respiratory and central nervous system.

The core CNS battery procedures are applied at the initial stages of the drug discovery process to help eliminate substances with a potential for CNS risk. These protocols include measuring general behavioural signs induced by test substance (Irwin test), effects on spontaneous locomotion (activity meter test), effects on neuromuscular coordination (Rotarod test), effects on the convulsive threshold (PTZ seizure test), interaction with hypnotics (Pentobarbital interaction test) and effects on the pain threshold (tail immersion test) (Porsolt *et al*., 2002).

Mallotus oppositifolius is a common shrub that is used traditionally for treating convulsion, epilepsy, treatment of parasitic, eye and kidney infections, as diuretic, pain killers, and treatment of paralysis, spasm, headache and swellings. Despite its usefulness there is no available scientific data on its CNS effects. Thus these tests were carried out on the leaves to assess the neuropharmacological effects of the leaves of the plant.

3.2 MATERIALS AND METHODS

3.2.1 Animals

Male ICR mice were obtained from the Noguchi Memorial Institute for Medical Research, Accra, Ghana and kept at the animal facility of the Department of Pharmacology, KNUST, Kumasi, Ghana. The animals were housed in groups of five in stainless steel cages (34 x 47 x 18 cm) with soft wood shavings as bedding, fed with normal commercial pellet diet (GAFCO, Tema), given water *ad libitum* and maintained under laboratory conditions. All animals used in these studies were treated according to the Guide for the Care and Use of Laboratory Animals (NRC, 1996) and were approved by the College Ethics Committee.

3.2.2 Drugs and chemicals

Diazepam (DZP), pentylenetetrazole (PTZ) caffeine (CFN), pentobarbitone (PBT), and phenobarbitone (PHE) were purchased from Sigma-Aldrich Inc., St. Louis, MO, USA. Morphine hydrochloride (MOR) was obtained from Phyto-Riker, Accra, Ghana.

3.2.3 **Irwin test**

Assessment of behaviour and physiological function was carried out similar to the primary observation procedure originally described by Irwin (1968). ICR mice (20- 25 g) were randomly divided into seven groups (n=7) and kept in the experimental environment for 7 days to acclimatize. Animals were fasted overnight, but had access to water *ad libitum*, and then treated orally with *M. oppositifolius* extract in doses of 30 - 6000 mg kg^{-1} of body weight. Mice in the control group received 10 ml kg^{-1}, *p.o*. of saline (0.9%w/v). The mice were observed at 0, 15, 30, 60, 120 and 180 min, up to 24 hours after treatment for general changes in behaviour and physiological function as well as mortality. The animals were assessed for behaviours related to neurotoxicity, central nervous system (CNS) stimulation and depression. Effects on autonomic functions were also noted, as was the lethality of the test agent. Refer to Appendix.

3.2.4 **Activity meter test**

This test is employed to measure spontaneous locomotor activity with an activity cage (Ugo Basille model 7401, Milan, Italy). ICR mice weighing (20- 25 g), n=7, were given the extract (30- 3000 mg kg^{-1}), caffeine (16 mg kg^{-1}) or diazepam (8 mg kg^{-1}) by the oral route. After 60 minutes, the animals were placed in the activity cage individually and their activities were scored every 5 minutes for 30 minutes.

3.2.5 **Rotarod test**

The effect of the extract on motor coordination was determined using the rotarod (UgoBasile, model 7600, Comerio, Varese, Italy). ICR mice were divided into 8 groups of 7 animals each after an initial screening to obtain a baseline. This screening involved placing each mouse on the rotarod prior to treatment, any mouse that fell off before the cut off time of 3 min was excluded from the experiment. The extract (30 - 3000 mg kg^{-1}, *p.o.*) were administered to groups A, B, C, D, E while the reference drugs D-tubocurarine (0.1 mg kg^{-1}, i.p.) and diazepam (8 mg kg^{-1}, *p.o.*) were administered to groups F and G respectively. Group H received distilled water (10 ml kg^{-1}, i.p.) and served as the control group. The animals were placed on the rotarod bar prior to treatment and at 0.5, 1, 1.5 and 2 h after treatment. The time in seconds for the mouse to fall off within the cut off time of 180 s was noted.

3.2.6 **Convulsive threshold test (PTZ seizure test)**

ICR mice weighing 20-25 g, were divided into 7 groups (n=7). Group 1 was given distilled water (10 ml kg^{-1}); groups 2- 6 received MOE (30- 3000 mg kg^{-1}, *p.o.*) and group 7, received diazepam (8 mg kg^{-1}, *p.o.*). Pentylenetetrazole (100 mg kg^{-1}, s.c.) was administered to the mice 30 min after distilled water or 1 h after diazepam or MOE. The mice were then placed in a

plastic observation cage and observed for 60 minutes for latency to clonic and tonic convulsions, frequency and duration of clonic and tonic convulsions for 1 h. Clonic convulsions consist of a prolonged and rapid succession of involuntary muscle contractions, while tonic convulsions are characterized by predominant abduction and extension of the limbs and rigid stretching of the body.

3.2.7 **Pentobarbitone interaction test**

The effect of MOE on pentobarbitone-induced sleeping time was studied in mice as described previously (de-Paris *et al.*, 2000). Fifty-six ICR mice were randomly divided into eight groups (n=7). The control group received distilled water (10 ml kg^{-1}). The remaining 7 groups received either MOE (30 - 3000 mg kg^{-1}, *p.o.*), diazepam (8 mg kg^{-1}, *p.o.*) or caffeine (16 mg kg^{-1}, *p.o.*). Sodium pentobarbitone (50 mg kg^{-1}, i.p.) was administered 1 hour after diazepam, caffeine or MOE. The control group received only the pentobarbitone. In a separate experiment, the effect of hepatic enzyme induction on pentobarbitone sleeping time was assessed. Briefly eight groups of mice (n=7) were pretreated with phenobarbitone (25 mg kg^{-1}, i.p.) for two days and on the third day, the first five groups were given extract (30- 3000 mg kg^{-1}, *p.o.*) and pentobarbitone (50 mg kg^{-1}, i.p.), then the rest of the groups took either, diazepam and pentobarbitone; caffeine (16 mg kg^{-1}, *p.o.*) and pentobarbitone (50 mg kg^{-1}, i.p.) or pentobarbitone alone (50 mg kg^{-1}, i.p.). In another experiment, the effect of extract (30- 3000 mg kg^{-1}) on hepatic enzyme induction or inhibition was investigated. Five groups of mice (n=7) were pretreated with extract for 2 consecutive days and on the third day, they received pentobarbitone (50 mg kg^{-1}, i.p.). Two parameters were recorded: time elapsed since the administration of pentobarbitone until the loss of the righting reflex (latency/onset of action) and the time elapsed from the loss to regaining of the righting reflex (duration of sleep).

3.2.8 **Tail immersion test**

Tail-immersion test was carried out as described (Sewell and Spencer, 1976; Luttinger, 1985) with modifications. The tail of the mouse was immersed in a water bath containing water at a temperature of 48 ± 0.5 ºC. The mouse reacts by withdrawing the tail (Bohn *et al.*, 2000). The reaction time (time taken for mice to withdraw tail) was recorded with a stop watch and a cut-off time of 10 s imposed on this measure. Mice were randomly divided into one of the following study groups (seven per group): control, morphine (10 mg kg^{-1}, i.p.) and MOE (30 - 3000 mg kg^{-1}, *p.o.*). The reaction time (T) for the study groups was taken at 0.5, 1, 2 h intervals after a latency period of 30 min (i.p.) or 1 h (*p.o.*) following the administration of the morphine

and extract. The percentage maximal possible effect (% MPE) was calculated from the reaction times by the formula:

$$\%\ \mathrm{MPE} = \frac{[\mathrm{Post} - \mathrm{drug\ latency}] - [\mathrm{Pre} - \mathrm{drug\ latency}]}{[\mathrm{Cut} - \mathrm{off\ latency}] - [\mathrm{Pre} - \mathrm{drug\ latency}]} \times 100$$

where 10 s is the cut-off latency time.

3.2.9 **Analysis of Data**

All data are presented as mean ± SEM. Data were analyzed using one-way analysis of variance (ANOVA) with drug treatment as a between-subjects factor. Whenever ANOVA was significant, further comparisons between vehicle- and drug-treated groups were performed using the Newman Keuls' *post hoc* test. GraphPad Prism for Windows Version 5 (GraphPad Software, San Diego, CA, USA) was used for all statistical analyses. $P< 0.05$ was considered significant.

3.3 RESULTS

3.3.1 **Irwin test**

Convulsions were observed in two mice in the highest dose (6000 mg kg^{-1}) after 15 min. During the 24 h period, no other toxic signs were observed except for sedation and loss of muscle coordination from the dose of 300 - 6000 mg kg^{-1}. Two mice died on the first day and after 24 hours, there was one more death in the highest dose (Table 3.1).

Table 3.1 Observations in the acute toxicity test after oral administration of *M. oppositifolius* in mice.

Dose	Mortality		Toxicity
	D/T	Latency (h)	
0	0/7	-	-
30	0/7	-	-
100	0/7	-	-
300	0/7	-	Sedation, motor incoordination
1000	0/7	-	Sedation, motor incoordination
3000	0/7	-	Sedation, motor incoordination
6000	3/7	24	Convulsion, sedation, motor incoordination, death

The hydroalcoholic extract of *M. oppositifolius*, was administered orally; each dose was administered to groups of 7 mice. D/T: dead/treated mice; -: no toxic symptoms were seen during the observation period; latency: time to death (in hours) after the dose.

3.3.2 **Activity meter test**

MOE affected spontaneous activity only at 3000 mg kg^{-1}($F_{7,47}$=55.78, P<0.0001) (Figure 3.1). Diazepam, the reference CNS depressant (8 mg kg^{-1}, *p.o.*), significantly decreased spontaneous activity in contrast to caffeine, (16 mg kg^{-1}, *p.o.*), which is the reference CNS stimulant.

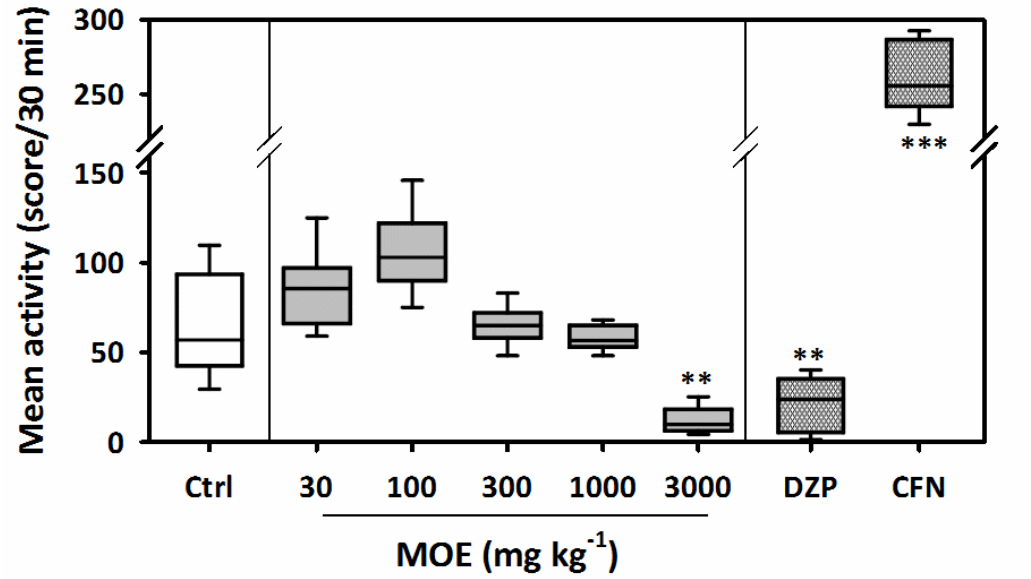

Figure 3.1 Effect of MOE (30 - 3000 mg kg^{-1}, *p.o.*), diazepam (8 mg kg^{-1}, *p.o.*) and caffeine (16 mg kg^{-1}, *p.o.*), on spontaneous activity in mice. Data was presented as mean ± S.E.M. (n=7); ***P<0.001; **P<0.01; compared to vehicle-treated group (One-way ANOVA followed by Newman Keuls' test).

3.3.3 **Rotarod**

From the time course curve, MOE caused a slight decrease in the time spent on the rotarod at lower doses but significantly decreased it at higher doses (Figure 3.2a). From the area under the curve (AUC) calculated from the time course curves, MOE caused significant motor impairment from 1000 to 3000 mg kg^{-1} ($F_{7,48}$=37.27, P<0.0001) (Figure 3.2a). Both diazepam (8 mg kg^{-1}) and D-tubocurarine (0.5 mg kg^{-1}) also caused significant motor impairment (Figure 3.2a and b).

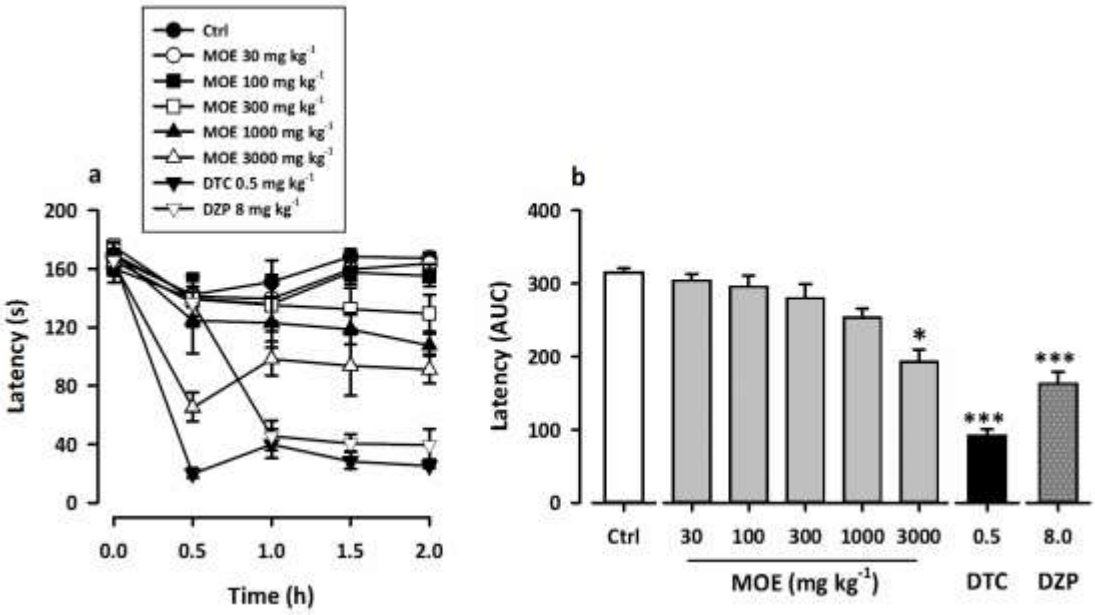

Figure 3.2 Effect of MOE (30 - 3000 mg kg^{-1}, *p.o.*), diazepam (8 mg kg^{-1}, *p.o.*) and D-tubocurarine (0.5 mg kg^{-1}, i.p.) on (a) the time course curve of the rotarod test and (b) the area under the curve (AUC), in mice. Data was presented as mean ± S.E.M. (n=7); ***P<0.001; *P<0.05; compared to vehicle-treated group (One-way ANOVA followed by Newman Keuls' test).

3.3.4 **PTZ seizure test**

MOE (30 - 3000 mg kg^{-1}), administered orally, significantly delayed both onset of clonic ($F_{8,54}$=651.2, P<0.0001) (Figure 3.3a) and tonic convulsions ($F_{8,54}$=625.1, P<0.0001) (Figure 3.3c) in mice. The frequency of both clonic ($F_{8,54}$=33.28, P<0.0001) (Figure 3.3a) and tonic convulsions ($F_{8,54}$=26.70, P<0.0001) (Figure 3.3c) was reduced. Furthermore, duration of clonic convulsions ($F_{8,54}$=26.74, P<0.0001) (Figure 3.3b) and tonic convulsions ($F_{8,54}$=52.27, P<0.0001) (Figure 3.3d) were also reduced significantly by the extract. Diazepam (0.3 - 3 mg kg^{-1}), the reference drug also demonstrated similar effects as the extract (Figure 3.3a-d).

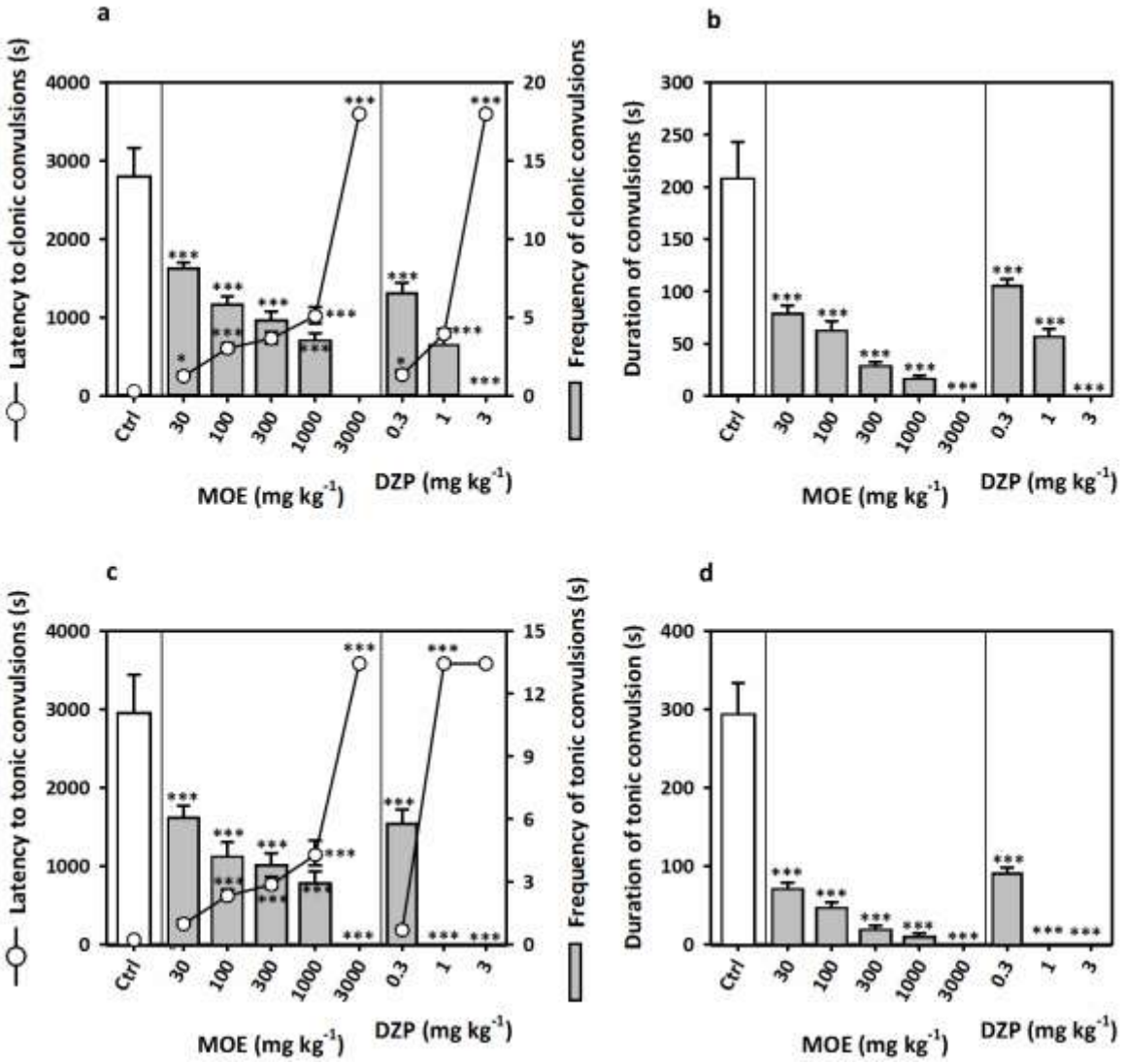

Figure 3.3 Effect of MOE (30 - 3000 mg kg^{-1}, *p.o.*) and diazepam (0.3 - 3 mg kg^{-1}, i.p.) on: (a) the latency to clonic and (c) tonic convulsions; (a and c) frequency of clonic and tonic convulsions, and (b and d) duration of clonic and tonic convulsions in the pentylenetetrazole-induced seizure test in mice. Data was presented as mean ± S.E.M. (n=7); ***P<0.001; **P<0.01; compared to vehicle-treated group (One-way ANOVA followed by Newman Keuls' test).

3.3.5 **Pentobarbital interaction test**

In the barbiturate-induced sleeping time test, the extract demonstrated a significant CNS depressant effect by causing a dose dependent increase in the duration of sleep ($F_{7,48}$=22.02, P<0.0001) (Figure 3.4a) at all doses tested except at 30 mg kg^{-1}. It however did not affect the onset of sleep compared to the control group (Figure 3.4a). Diazepam (8 mg kg^{-1}, *p.o.*), a reference CNS depressant caused a similar effect as the extract while caffeine (16 mg kg^{-1},

p.o.), the CNS stimulant, delayed onset of sleep and significantly reduced the sleeping time (Figure 3.4). MOE and diazepam had no effect on latency to sleep in phenobarbitone pretreated mice (Figure 3.5a). On the contrary caffeine reduced the latency to sleep in phenobarbitone pretreated mice (Figure 3.5a). MOE ($F_{7,48}$=22.02, P<0.0001) and diazepam significantly reduced the duration of sleep induced in mice pretreated with phenobarbitone for 2 days (Figure 3.5b). Decrease in sleep latency was reversed by MOE at doses of 1000 and 3000 mg kg^{-1} when mice were pretreated with various doses of extract for 2 days (Figure 3.6a). This treatment reversed the increase in sleep duration in untreated mice ($F_{7,48}$=22.02, P<0.0001) (Figure 3.6b).

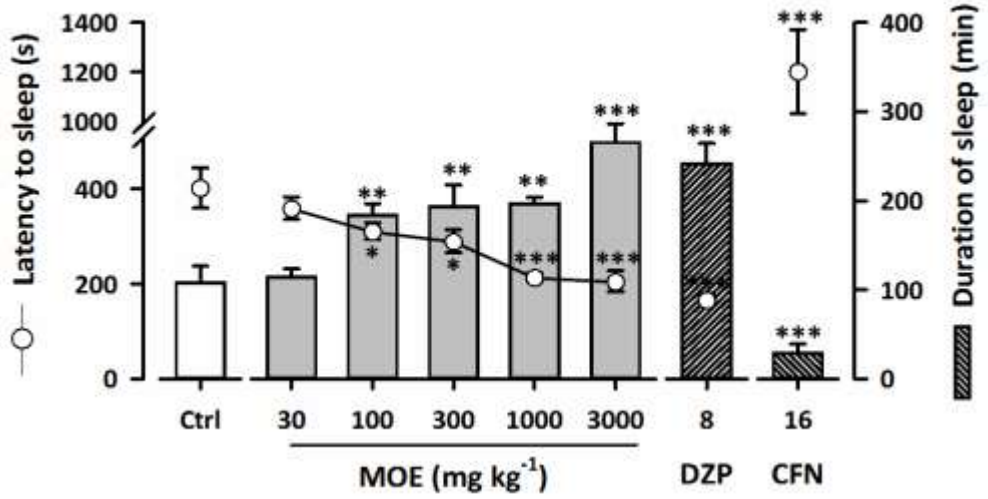

Figure 3.4 Effect of MOE (30 - 100 mg kg^{-1}, *p.o.*) and diazepam (8 mg kg^{-1}, *p.o.*) and caffeine (16 mg kg^{-1}, *p.o.*) on the latency to sleep and duration of sleep, in the pentobarbitone-induced sleeping test. Data was presented as mean ± S.E.M. (n=7); ***P<0.001; **P<0.01; compared to vehicle-treated group (One-way ANOVA followed by Newman Keuls' test).

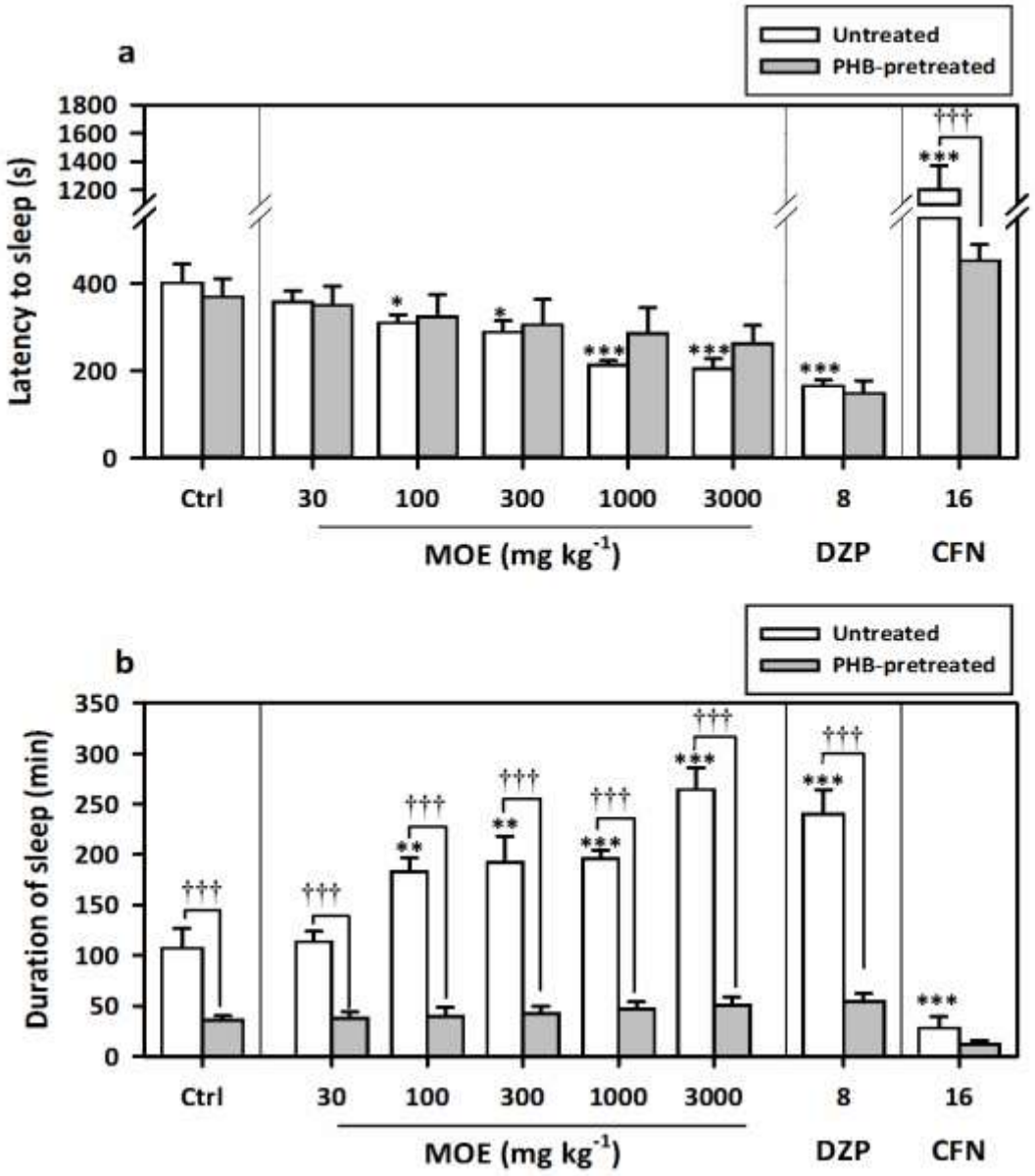

Figure 3.5 Effect of MOE (30 - 100 mg kg^{-1}, *p.o.*), diazepam (8 mg kg^{-1}, *p.o.*) and caffeine (16 mg kg^{-1}, *p.o.*) on (a) the latency to sleep in the pentobarbitone induced sleeping test. Data was presented as mean ± S.E.M. (n=7); ***P<0.001; **P<0.01; compared to vehicle-treated group (One-way ANOVA followed by Newman Keuls' test). (b) duration of sleep, after pre-treating with phenobarbitone in the pentobarbitone-induced sleeping test. Data was presented as mean ± S.E.M. (n=7); †††P<0.001; comparison between dose and treatment (Two-way ANOVA followed by Bonferroni's test).

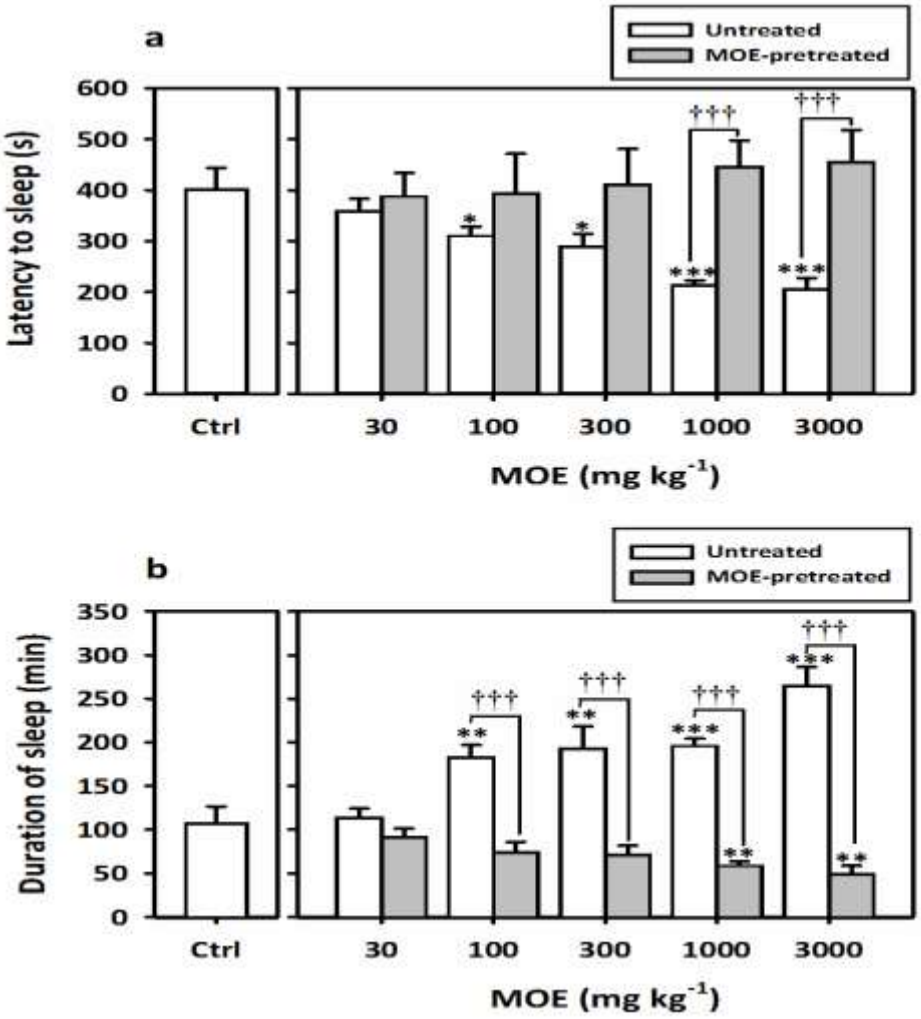

Figure 3.6 Effect of phenobarbitone on (a) the latency to sleep. Data was presented as mean ± S.E.M. (n=7); ***P<0.001; **P<0.01; compared to vehicle-treated group (One-way ANOVA followed by Newman Keuls' test). (b) duration of sleep, after pre-treatment with extract in the pentobarbitone-induced sleeping test. Data was presented as mean ± S.E.M. (n=7); †††P<0.001; comparison between dose and treatment (Two-way ANOVA followed by Bonferroni's test).

3.3.6 **Tail immersion test**

MOE exhibited significant analgesic activity by increasing the % maximal possible effect (MPE) in the tail immersion test. From the time course curve, the analgesic activity peaked at 1 h after drug treatment and reduced by the second hour although analgesic activity was still maintained. One-way ANOVA followed by Newman Keuls' test of the AUC indicated that MOE exhibited analgesic effect ($F_{6,42}$=9.069, P<0.0001) (Figure 3.7b). Similar result was obtained for morphine (10 mg kg^{-1}), the reference analgesic drug used.

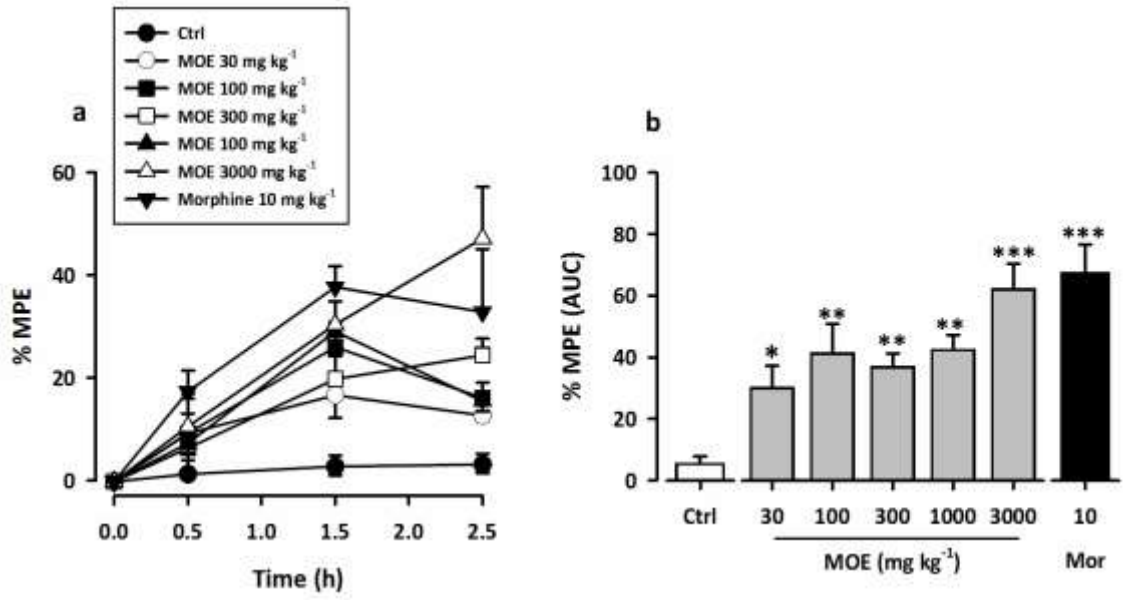

Figure 3.7 Effect of MOE (30 - 100 mg kg^{-1}, *p.o.*) and morphine (10 mg kg^{-1}, i.p.) on (a) the time course curve of the tail immersion test and (b) the area under the curve (AUC) in mice. Data are presented as mean ± S.E.M. (n=7). **P*<0.05; ***P*<0.01; ****P*<0.001 compared to vehicle-treated group (One-way ANOVA followed by Newman-Keuls' test).

3.4 DISCUSSION

Central effects of the hydroalcoholic extract of the leaves of *Mallotus oppositifolius* was investigated in the core CNS battery test. The extract caused sedation and reduced spontaneous locomotor activity. It showed analgesic, anticonvulsant properties and induces hepatic microsomal enzymes. Furthermore, hepatic microsomal enzymes metabolized the extract.

In the Irwin test, MOE exhibited sedation and motor impairment at higher doses without impairing respiration. This is indicative of possible CNS depressant activity. The Irwin test, one of the core battery tests, is used to estimate the minimum lethal dose of a test substance, the dose range for CNS responses, and the primary effects on behavior and physiological functions (Irwin, 1968; Porsolt *et al.*, 2002; Roux *et al.*, 2005; Lynch *et al.*, 2011). The results of this test are used to predict potential therapeutic activity and to select doses for subsequent tests of efficacy. Data from the Irwin test are also used to assess the risks associated with the use of this agent (Irwin, 1968; Iezhitsa *et al.*, 2002). It is possible that MOE may have potential use in CNS conditions where there is CNS excitation such as in convulsions, epilepsy or anxiety. In fact the plant is used traditionally for managing these conditions. To corroborate this, the extract showed anticonvulsant activity in the PTZ convulsive threshold test. At all doses, the extract delayed the onset of clonic and tonic convulsions; and also reduced the frequency and duration of the clonic and tonic convulsions. Any substance that delays the onset of and the

duration of clonic or tonic clonic convulsion induced by pentylenetetrazole is described as an anticonvulsant (Vellucci and Webster, 1984; De Sarro *et al.*, 1999; Sayyah *et al.*, 2004). Caution should however be exercised since at the highest dose of 6000 mg kg-1 of body weight, there were visible signs of convulsion and death in the Irwin test. Out of seven mice used, three died after 24 hours at the highest dose, suggesting that the lethal dose-50 (LD_{50}) is approximately 6000 mg kg^{-1}.

Drugs that act on the CNS may enhance, inhibit or not affect locomotor activity in mice) (Kimmel *et al.*, 2000; Wiley and Martin, 2003; El-Mas and Abdel-Rahman, 2004; Kawaura *et al.*, 2010). MOE reduced spontaneous activity in mice in the activity meter test. Locomotion can be decreased as a result of sedation, drug-induced motor impairment by test substance (Brooks *et al.*, 1999; Betarbet *et al.*, 2002; Henry *et al.*, 2002; Porsolt *et al.*, 2002). In order to ascertain whether reduction in spontaneous activity was by drug-induced motor impairment or sedation, the rotarod test was performed. From the results, it was clear that there was significant motor impairment at the dose that reduced spontaneous activity in the activity meter test. Since sedation was also observed at the dose of 3000 mg kg^{-1} in the Irwin test, it is possible that sedation may also contribute to the reduction in activity. Diazepam, a CNS depressant, reduced spontaneous activity and impaired motor coordination at the dose used while caffeine, a CNS stimulant increased the locomotor activity (El Yacoubi *et al.*, 2000; Solinas *et al.*, 2002; Wiley *et al.*, 2002; Dunne *et al.*, 2007). Many CNS depressant compounds can cause reduction in spontaneous locomotor activity in laboratory animals. Nearly all the neuroleptic agents used in psychiatry diminish spontaneous locomotor activity in all species including man (Baldessarini *et al.*, 1990; Simon *et al.*, 2000; Kinkead and Nemeroff, 2002).

Sleep-enhancing effect of substances can readily be detected in the barbiturate-induced sleeping time test by substances, which do not induce sleep even at high doses when administered alone (Montalto de Mecca *et al.*, 2000; Porsolt *et al.*, 2002; Nayak *et al.*, 2004). There is also a high correlation between the effects observed in this procedure and those observed in more complex tests and in man (Andre *et al.*, 1984; Renton, 1985; Zhao *et al.*, 2006). In the present study though MOE enhanced the duration of sleep in mice profoundly, this was not apparent in the Irwin test further supporting the fact that sleep enhancing effect of substances can be readily detected in the pentobarbitone sleep test. The prolongation of pentobarbitone induced sleep further supports the central depressant activity of the extract.

Pretreatment of mice with phenobarbitone for two days reduced the increase in sleep duration induced by the extract or diazepam. This suggests that the extract is metabolized by the hepatic enzymes (Conney, 1967; Kushikata *et al.*, 2003). Some drugs which are metabolized by the

liver can either induce or inhibit hepatic enzyme metabolism (Conney, 1967; Brockmoller and Roots, 1994; Faught, 2001; Back *et al.*, 2003; Park *et al.*, 2005). Hence, the possible effect of the extract on hepatic enzymes was assessed by pre-treating mice with the extract for two days before administering pentobarbitone. It is evident that the extract was a hepatic enzyme inducer since it reduced the sleep duration when compared to the group that received only pentobarbitone or extract and pentobarbitone. Drugs that are either metabolized by liver enzymes or induce hepatic enzymes are potential targets for drug-drug interactions. This pharmacokinetic effect of substances may result in toxicity or reduced therapeutic effect as a result of decrease in the effective concentration of drug in the plasma. On the other hand, beneficial synergistic effects can be observed through these interactions (George *et al.*, 1995; Park *et al.*, 2005).

Analgesic activity was observed from 100 - 6000 mg kg^{-1} in the Irwin test. This observation in the Irwin test was further confirmed in the tail immersion test, an acute thermal pain model (Le Bars *et al.*, 2001; Simmons *et al.*, 2002; Jones *et al.*, 2005). This supports the traditional use of the plant in managing headache and other forms of pain. Morphine, the reference analgesic, also gave similar results. Thermal nociception models such as hot plate and the tail immersion tests are used to evaluate central analgesic activity. The tail immersion model is sensitive to drugs that act spinally or supraspinally (Ramabadran *et al.*, 1989; Pal *et al.*, 1999; Muhammad *et al.*, 2012). It is possible that the analgesic effect of MOE may be due to spinal or supraspinal pathways. That MOE has sedative properties may confound the test because most pain models are based on behavioural measures which involve motor coordination (Jarvis *et al.*, 2002; Kehl *et al.*, 2003; Nassar *et al.*, 2005; McGowan *et al.*, 2009; Zhao *et al.*, 2011). However in this study, doses required for analgesia were much lower than those that caused motor impairment.

3.5 CONCLUSION

From the core CNS battery tests, MOE exhibited significant CNS depressant, analgesic and anticonvulsant effect. The LD_{50} is estimated to be approximately 6000 mg kg^{-1}. The extract reduced spontaneous activity and impaired motor coordination at high doses. MOE is a hepatic enzyme inducer and metabolized by the hepatic enzymes.

Chapter 4

ANTICONVULSANT EFFECT AND ISOBOLOGRAPHIC ANALYSIS OF DRUG INTERACTION

4.1 INTRODUCTION

Epilepsy is a heterogeneous neurological disorder that is characterized by sudden, transitory and unprovoked convulsive and non-convulsive seizures with or without loss of consciousness (McNamara and Puranam, 1996; Fisher *et al.*, 2005). Worldwide, there are over 50 million people afflicted by this disorder, approximately 50% of which can be effectively treated with the currently available anti-epileptic drugs. For another 20%, their seizures are inadequately controlled or controlled at the expense of substantial adverse effects (White, 2003; Jacoby *et al.*, 2005; Reid *et al.*, 2012). Over the last decade at least ten new antiepileptic drugs have been introduced into the market for the treatment of epilepsy with improved standard of care for a large number of patients in the form of reduced adverse events, a lower propensity for drug–drug interactions, and improved efficacy (Hachad *et al.*, 2002; Perucca, 2002; Schmidt, 2002; Bialer, 2006). Unfortunately, there still remain approximately 20–30% of the patients with refractory epilepsy (Brodie, 2001; Sander, 2003). The quest to satisfy the needs of this category of patients underscores the need to search for newer anti-epileptics.

Since prehistoric times, humans have attempted to alleviate ailments or injuries with the aid of plant parts or herbal preparations. Ancient civilizations have recorded various prescriptions of this kind. In the herbal formularies of medieval times numerous plants were promoted as remedies (Cragg *et al.*, 1997; Goldman, 2001). It is estimated that about 80% of the population in developing states use herbal remedies for primary health care. In these nations conditions like epilepsy, pain, etc. are usually treated using herbs (Mahady, 2001; Spinella, 2001; Kamatenesi-Mugisha and Oryem-Origa, 2005). One of such plants that have been used in treating epilepsy is *Mallotus oppositifolius* (Burkill, 1985).

Traditionally the leaves are used for treating convulsion and epilepsy (Burkill, 1985). Preliminary screening revealed anticonvulsant effect of the extract against PTZ-induced seizures (refer to section 3.3.4). Hence the present study was conducted to further explore the anticonvulsant potential of the hydroalcoholic extract of the leaves of *M. oppositifolius* in acute chemoconvulsant (pentylenetetrazole, picrotoxin, and strychnine) and electroshock (maximal electroshock seizure test) models. The pilocarpine model of *status epilepticus*, a human temporal lobe epilepsy model representing 70% of refractory partial seizures was also used in characterizing the anticonvulsant effect of the extract. The possible role of the benzodiazepine/$GABA_A$ receptor complex and the potassium channels in the mechanism of action of the extract was investigated.

It is common for patients in developing countries to combine herbal drugs with orthodox drugs (Zhou *et al.*, 2007). With regards to therapeutic outcomes this may be beneficial or deleterious (Camerino *et al.*, 2007; Zhou *et al.*, 2007). Isobolograghic analysis is a tool used in investigating whether drug combinations produces synergistic effect, additive or antagonistic effect (2006). Therefore an isobolographic analysis of the drug interaction between the extract and diazepam against PTZ-induced seizures was performed and the possible mechanism of action investigated.

4.2 MATERIALS AND METHODS

4.2.1 Animals

Male ICR mice were used in this experiment and experimental conditions were as described in section 3.2.1.

4.2.2 Drugs and chemicals

Diazepam (DZP), pentylenetetrazole (PTZ), picrotoxin (PTX), strychnine (STR), 4-aminopyridine and pilocarpine were purchased from Sigma-Aldrich Inc., St. Louis, MO, USA. Flumazenil (FLZ) from Roche (Brazil), carbamazepine (Tegretol®, Novartis, Basel, Switzerland).

4.2.3 Pentylenetetrazole-induced seizure test

The method used was adapted from that described by (Swinyard and Kupferberg, 1985). Male ICR mice were divided into seven groups (n=5). The extract (10, 30 and 100 mg kg^{-1}, *p.o.*) was administered to three groups while diazepam (0.1, 0.3 and 1.0 mg kg^{-1}, i.p) was given to three other groups and the last group received 10 ml kg^{-1}, *p.o.* of the vehicle (distilled water) to serve as control. All other procedures were similar to earlier procedure described in 3.2.8.

4.2.4 Picrotoxin-induced seizure test

The procedure used was the same as in the case of pentylenetetrazole-induced seizure test except that mice were administered picrotoxin, 3.2 mg kg^{-1} intraperitoneally (Swinyard, 1969; Avallone *et al.*, 2000; Mackenzie *et al.*, 2002; Ngo Bum *et al.*, 2004) 30 minutes and 1 hour after treatment with diazepam and MOE, respectively. The latency to myoclonic jerks, latency to clonic convulsions and the frequency and duration of clonic convulsions were recorded from the videos for each mouse as in pentylenetetrazole-induced convulsions.

4.2.5 Maximal electroshock seizure test

The method used has been previously described by (Toman *et al.*, 1946) and modified by (Swinyard and Kupferberg, 1985). Male ICR mice were grouped into seven groups (n=5). Three groups were treated with the extract (10, 30 and 100 mg kg^{-1}, *p.o.*), three other groups treated with carbamazepine (10, 30 and 100 mg kg^{-1}, *p.o.*) and the last grouped administered distilled water (10

ml kg^{-1}, *p.o.*), to serve as control. After 1 hour of oral drug treatments, tonic convulsions of the hind limb extremities of mice were induced by passing alternating electrical current (50 Hz, 60 mA and 0.2 s) through the ear electrodes. This was the maximal current (60 mA) that induced tonic hind limb extension in all the trial mice and it was determined previously before commencement of the experiment. The duration of tonic hind limb extension seizures was determined in each dose group.

4.2.6 **Strychnine-induced seizure test**

This method has been described previously (Lehmann *et al.*, 1988). In brief, strychnine (STR) seizures were induced in male mice by the i.p. injection of 0.5 mg kg^{-1} of the STR nitrate after one hour of extract (10 - 100 mg kg^{-1}) or 30 minutes of diazepam (0.1 - 1.0 mg kg^{-1}) administration. The latency to myoclonic jerks, the frequency and duration of convulsions were recorded for extract treated groups and the diazepam group compared with the control group (vehicle).

4.2.7 **Pilocarpine-induced *status epilepticus***

In this experiment, seizures were induced by injection of pilocarpine (300 mg kg^{-1}, i.p.) to male ICR mice, 10 in each group. MOE (10 - 100 mg kg^{-1}) or diazepam (1 - 10 mg kg^{-1}) was administered one hour or thirty minutes respectively before pilocarpine injection. To reduce peripheral autonomic effects produced by pilocarpine, the animals were pretreated with n-butyl-bromide hyoscine (1 mg kg^{-1}, 30 min before pilocarpine administration). After the injection of the pilocarpine, the animals were placed separately into the transparent plexiglass testing chamber and video recordings made as described in the pentylenetetrazole-induced seizure experiments. The latency to and duration of clonic-tonic seizures were tracked using The JWatcher software version 1.0. The ED_{50} (a measure of anticonvulsant potency) and E_{max} (a measure of efficacy) were calculated from the dose response curves plotted.

4.2.8 **Possible Mechanisms**

4.2.8.1 Involvement of GABAergic mechanisms

To investigate the probable involvement of $GABA_A$ receptors in the anticonvulsant mechanism of the extract, the effects of a selective benzodiazepine receptor antagonist, flumazenil on the anticonvulsant activity of MOE was studied. Six groups of eight mice each were selected. The first four groups received MOE (100 mg kg^{-1}, *p.o.*), diazepam (0.3 mg kg^{-1}, i.p.), flumazenil (2 mg kg^{-1}) and normal saline 30 min before the administration of PTZ (85 mg kg^{-1}, i.p.). The last two groups were given flumazenil (2 mg kg^{-1}, i.p.) 5 min before the administration of MOE (100 mg kg^{-1}, *p.o.*) or diazepam (0.3 mg kg^{-1}, i.p.) and 65 min or 35 min before the injection of PTZ (85 mg kg^{-1}, i.p.)

respectively. The dose of flumazenil was selected based on the work of Rolland *et al*., (2001). The latency to, the frequency and duration of clonic convulsions were tracked for each mouse.

4.2.8.2 Involvement of potassium channels

The 4-aminopyridine induced seizure test described by Yamaguchi and Rogawski, (1992) was used. Eighty mice were randomly divided into groups of 10 mice each. The first group received normal saline (10 mg kg^{-1} body weight, i.p.); the second, third and fourth groups were given 10, 30 and 100 mg kg^{-1} body weight *p.o*. of the extract, respectively, and the last three groups received 100, 300 and 600 mg kg^{-1} body weight of sodium valproate *p.o*. respectively. One hour later, mice in all the groups received 10 mg kg^{-1} body weight s.c. of 4-aminopyridine (4-AP). Ability of the extract/drug to protect the mice from lethality within a one hour observation period and the latencies to clonic as well as tonic convulsions were considered as indices of anticonvulsant activity.

4.2.9 Isobolographic analysis of drug interaction between extract and diazepam

Drugs given in combination may produce effects that are greater than or less than the effect predicted from their individual potencies. Isobolographic analysis is a tool consisting of a graph (isobologram), constructed on a coordinate system which is composed of the individual drug doses, a straight 'line of additivity' that is employed to distinguish additive from synergistic and antagonistic interactions .

ED_{50}s of diazepam and the extract were obtained from the pentylenetetrazole-induced seizure test for isobolographic analysis. Mice were grouped into eight (n=7). Group I mice was treated with 0.22 mg kg^{-1} diazepam i.p. (ED_{50} of diazepam) and then received 85 mg kg^{-1} of pentylenetetrazole s.c 30 minutes afterwards. Group II mice were treated with 15.37 mg kg^{-1} extract *p.o*. (ED_{50} of MOE) and then received 85 mg kg^{-1} of pentylenetetrazole s.c 1 h afterwards. Group III-VI mice were treated with a combination of diazepam and extract respectively as follows; (0.22 + 15.37) mg kg^{-1}, $\frac{0.22 + 15.37}{2}$ mg kg^{-1}, $\frac{0.22 + 15.37}{4}$ mg kg^{-1}, $\frac{0.22 + 15.37}{8}$ mg kg^{-1} followed by administration of 85 mg kg^{-1} of pentylenetetrazole. Group VII-IX mice were treated 1 mg kg^{-1} flumazenil and after 15 min a combination of diazepam and extract (0.22 + 15.37) mg kg^{-1}, $\frac{0.22 + 15.37}{2}$ mg kg^{-1}, $\frac{0.22 + 15.37}{4}$ mg kg^{-1}, followed by administration 85 mg kg^{-1} of pentylenetetrazole. Isobologram (a Cartesian plot of pairs of doses that, in combination, yield a specified level of effect) was then built by connecting the theoretical ED_{50} of diazepam plotted on the abscissa with that of extract plotted on the ordinate to obtain the additivity line. For each drug mixture, the ED_{50} (experimental) and its associated 95% confidence intervals were determined by linear regressional analysis of the log

dose–response curve (and compared by a 't'-test to a theoretical additive ED_{50}) obtained from the calculation;

$$Z_{add} = f\ (ED_{50}) \text{ of diazepam} + (1\text{-}f)\ ED_{50} \text{ of extract}$$

Where f is the fraction of the each component in the mixture and the variance (Var) of Z_{add} was calculated as:

$$\text{Var } Z_{add} = f^2\ (\text{Var } ED_{50} \text{ of diazepam}) + (1\text{-}f)^2\ \text{Var } ED_{50} \text{ of extract.}$$

From these variances S.E.M's are calculated and resolved according to the ratio of the individual drugs in the combination. A supra-additive or synergistic effect is defined as the effect of a drug combination that is higher and statistically different (ED_{50} significantly lower) than the theoretically calculated equieffect of a drug combination in the same proportion. If the ED_{50}s are not statistically different, the effect of the combination is additive and additivity means that each constituent contributes with its own potency to the total effect. The degree of interaction was calculated using fractional analysis by dividing the experimental ED_{50} (Z_{mix}) by the theoretical ED_{50} (Z_{add}). A value close to 1 is considered as additive interaction. Values lower than 1 are an indication of the magnitude of supra-additive or synergistic interactions ($Z_{mix}/Z_{add}<1$), and values higher than 1 correspond to sub-additive or antagonistic interactions (Miranda *et al.*, 2007).

4.2.10 **Rotarod test**

The effect of the extract on motor coordination was determined using the Ugo Basile Rotarod bar (model 7600, Comerio, Varese, Italy) ICR mice were divided into 7 groups of 5 animals each after an initial screening to obtain a baseline. This screening involved placing each mouse on the Rotarod prior to treatment, any mouse that fell off before the cut off time of 2 min was excluded from the experiment. The extract (10, 30 and 100 mg kg^{-1}, *p.o.*) were administered to groups A, B, C whilst the reference diazepam (0.1, 0.3, 1 mg kg^{-1}, i.p.) were administered to groups D, E and F respectively. Group G received distilled water (10 ml kg^{-1}, i.p.) and served as the control group. The animals were placed on the Rota rod bar prior to treatment and at 1, 2, 3 and 4 h after treatment. The time in seconds for the mouse to fall off within the cut off time of 120 s was noted.

4.2.11 **Statistical analysis**

All data are presented as mean ± SEM. Data were analyzed using one-way analysis of variance (ANOVA) with drug treatment as a between-subjects factor. Whenever ANOVA was significant, further comparisons between vehicle- and drug-treated groups were performed using the Newman Keuls' test. In analyzing the possible role of GABAergic mechanisms in the anticonvulsant effect of the extract, Two-way ANOVA with the Bonferroni's *post hoc* test (treatment × dose) was performed In all the tests GraphPad Prism for Windows Version 5 (GraphPad Software, San Diego, CA, USA) was used for all statistical analyses. $P < 0.05$ was considered significant. Doses

for 50% of the maximal effect (ED_{50}) and E_{max} for each drug were determined by using an iterative computer least squares method, with the following nonlinear regression (three-parameter logistic) equation:

$$Y = \frac{a + (b - a)}{\left(1 + 10^{(LogED_{50} - X)}\right)}$$

Where, X is the logarithm of dose and Y is the response. Y starts at a (the bottom) and goes to b (the top) with a sigmoid shape. The fitted midpoints (ED_{50}s) of the curves were compared statistically using F test (Miller, 2003; Motulsky and Christopoulos, 2003).

Survival curves were plotted for the 4-aminopyridine test by plotting percentage survival against time. Analysis of survival curves was done with the log-rank test.

Isobolographic calculations were performed with the program Pharm Tools Pro (version 1.27, the McCary Group Inc.). Results are presented as mean ± S.E.M. or as ED_{50} values with 95% confidence limits (95% CL). The statistical analysis of the isobolograms was performed according to Tallarida (2006) and the statistical difference between experimental and theoretical values was assessed by the Student's t test for independent means, and the *P* values < 0.05 were considered significant.

4.3 RESULTS

4.3.1 PTZ-induced seizures

The extract (10 - 100 mg kg^{-1}) exhibited a significant anticonvulsant effect in this model. MOE caused a dose dependent delay in the onset of myoclonic jerks ($F_{3,16}$=22.63, P<0.0001) (Figure 4.1a) and clonic convulsions in mice ($F_{3,16}$=31.78, P<0.0001) (Figure 4.1a). It also significantly decreased the frequency ($F_{3,16}$=10.89, P<0.0001) (Figure 4.1b) and duration of clonic convulsions ($F_{3,16}$=16.54, P<0.0001) (Figure 4.1b), in the same animal model. Diazepam (0.1 - 1.0 mg kg^{-1}), used as the reference anticonvulsant, showed similar results as the extract (Figure 4.1c, d). Comparing the ED_{50} and E_{max} values, diazepam was more potent and more efficacious than the extract in reducing the duration of seizures (Table 4.3). MOE was more efficacious than diazepam though less potent in reducing the frequency of seizures (Table 4.2) and delaying onset of convulsions (Table 4.1).

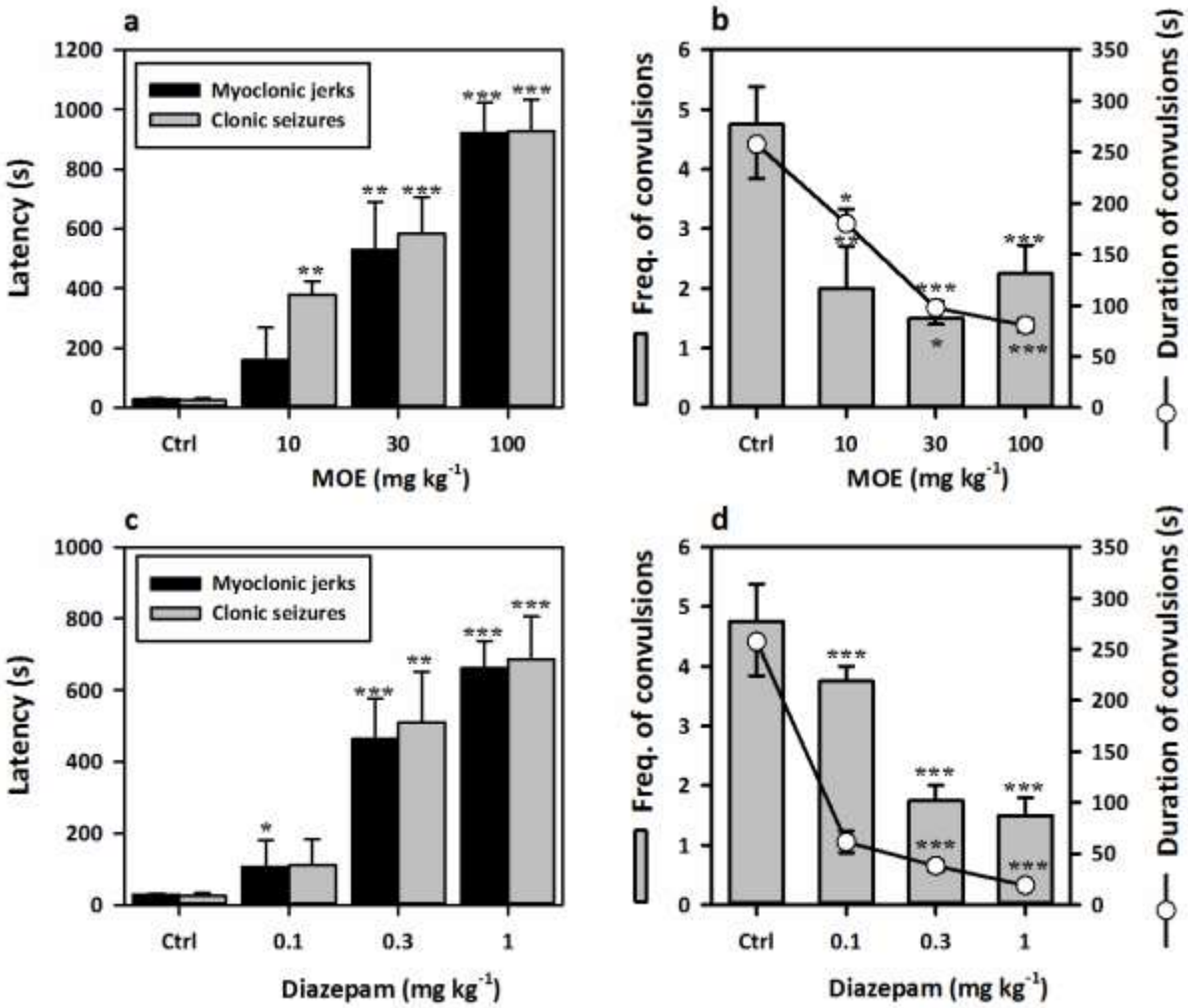

Figure 4.1 Effect of MOE (10 - 100 mg kg^{-1}, *p.o.*) and diazepam (0.1 - 1.0 mg kg^{-1}, i.p.) on (a, c) the latencies to myoclonic jerks and clonic convulsions respectively; (b and d) frequency and duration of clonic convulsions respectively, in the pentylenetetrazole-induced seizure test in mice. Data was presented as mean ± S.E.M. (n=5); ***P<0.001; **P<0.01; *P<0.05; compared to control group (One-way ANOVA followed by Newman Keuls' test).

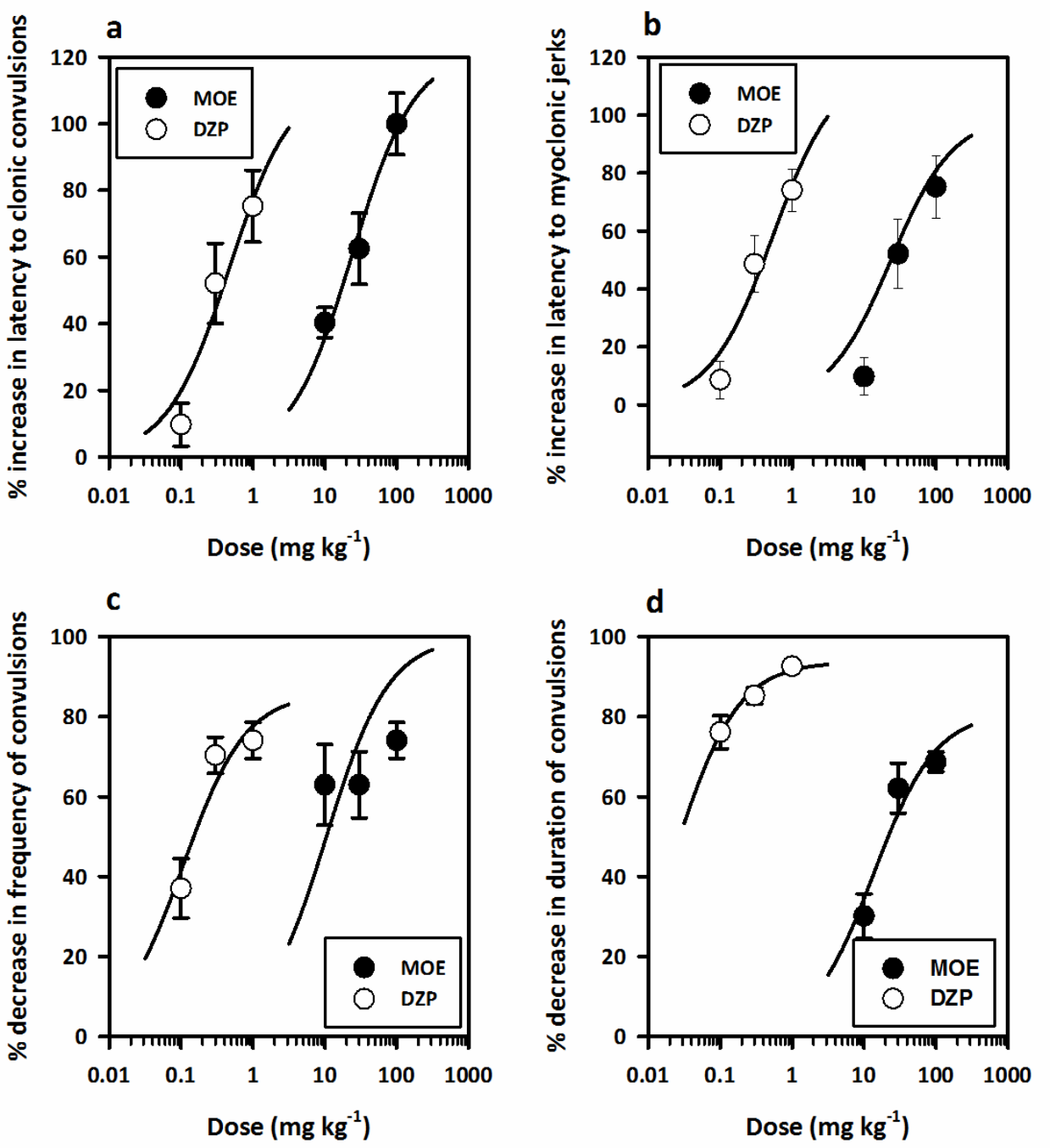

Figure 4.2 Dose–response curves for the anticonvulsant activity induced by the administration of MOE (10 - 100 mg kg^{-1}) and diazepam (0.1 - 1 mg kg^{-1}) in the pentylenetetrazol- induced seizure test in mice. (a) % increase in latency to clonic convulsions; (b) % increase in latency to myoclonic convulsions; (c) % decrease in frequency convulsions; (d) % decrease in frequency convulsions. Each point is the mean ± S.E.M. of 5 animals.

4.3.2 **Picrotoxin-induced seizures**

Extract treated groups exhibited a significant anticonvulsant effect in this model. MOE (10 - 100 mg kg^{-1}) caused a profound dose dependent delay in the onset of myoclonic jerks ($F_{3,16}$=157.0, P<0.0001) (Figure 4.3a) and clonic convulsions in mice ($F_{3,16}$=97.82, P<0.0001) (Figure 4.3a). The extract also decreased the frequency ($F_{3,16}$=8.667, P=0.0012) (Figure 4.3b) and duration of convulsions ($F_{3,16}$=22.63, P<0.0001) (Figure 4.3b) significantly. Diazepam (0.1 - 1.0 mg kg^{-1}), the reference anticonvulsant showed similar results as the extract. Diazepam was more efficacious and potent than MOE (Figure 4.4) in reducing the frequency and duration of clonic convulsions but less efficacious in delaying the onset of seizures (Table 4.2,Table 4.3 and Table 4.4).

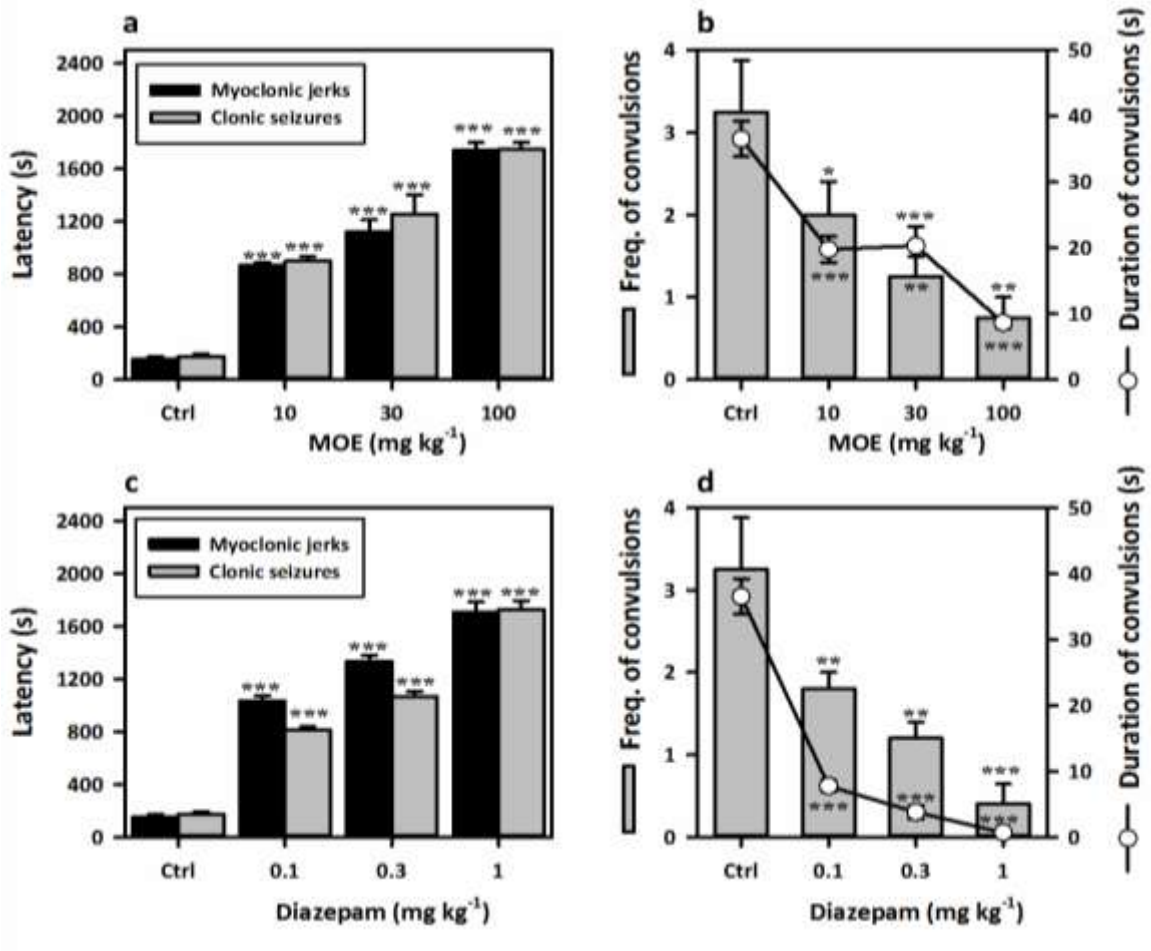

Figure 4.3 Effect of MOE (10 - 100 mg kg^{-1}, *p.o.*) and diazepam (0.1 - 1 mg kg^{-1}, i.p.) on (a, c) the latencies to myoclonic jerks and clonic convulsions; (b and d) frequency and duration of convulsions, in the picrotoxin-induced seizure test in mice. Data was presented as mean ± S.E.M. (n = 5); ***P<0.001; ** P<0.01; compared to control group (One-way ANOVA followed by Newman Keuls' test).

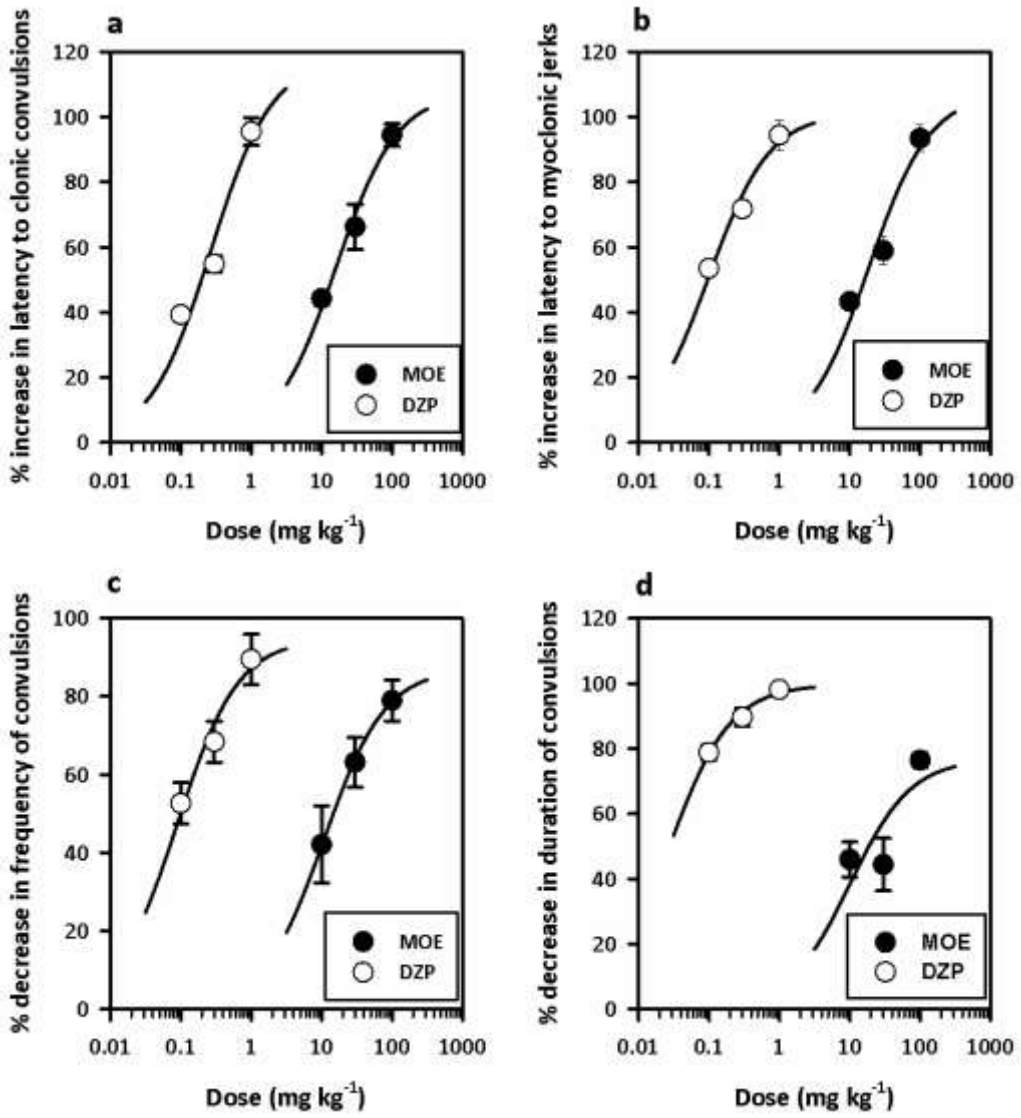

Figure 4.4 Dose–response curves for the anticonvulsant activity induced by the administration of MOE (10 - 100 mg kg^{-1}) and diazepam (0.1 - 1 mg kg^{-1}) in the picrotoxin-induced seizure test in mice. Each point is the mean ± S.E.M. of 5 animals.

4.3.3 **Maximal electroshock seizure (MES) test**

Though MOE (10 - 100 mg kg^{-1}, *p.o.*) could not prevent tonic limb extensions (TLEs) it caused a significant reduction in the duration of tonic limb extensions at all dose levels ($F_{3,19}$=132.9, P<0.0001) (Figure 4.5). Carbamazepine [CBZ], (10 - 100 mg kg^{-1}), the reference anticonvulsant significantly reduced the duration of TLEs. At 100 mg kg^{-1}, CBZ totally abolished the TLEs in mice. From the table of value of ED_{50}s and E_{max}, oral administration of carbamazepine was more efficacious and potent than the extract in the reducing the duration of convulsions (Table 4.3, Figure 4.6).

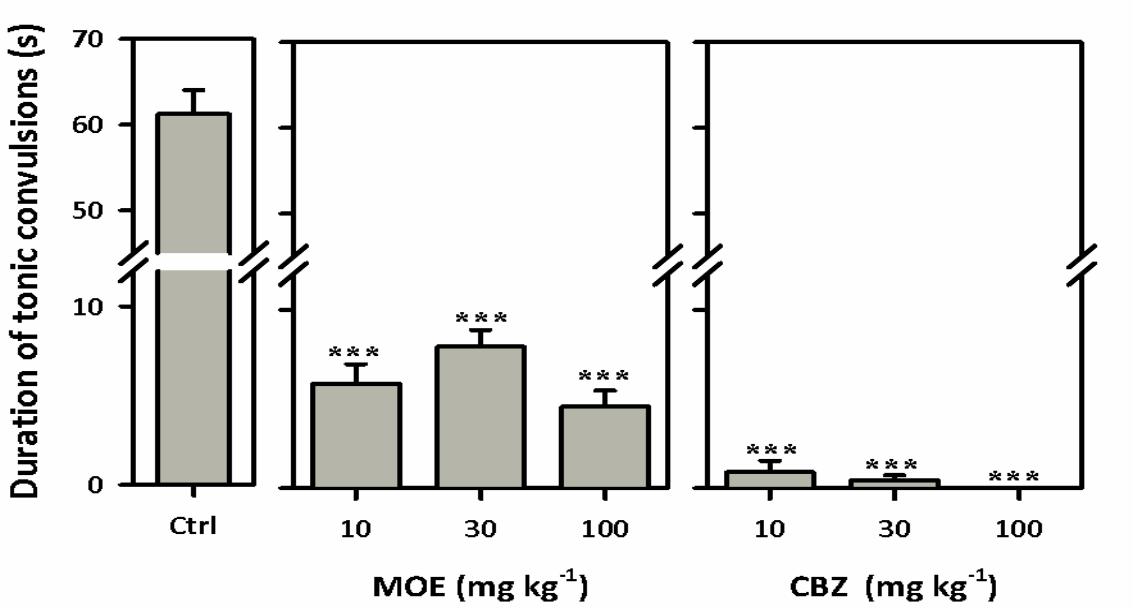

Figure 4.5 Effect of MOE (10 - 100 mg kg^{-1}, *p.o.*) and carbamazepine (10 - 100 mg kg^{-1}, *p.o.*) on the duration of tonic limb extensions maximal electroshock seizure test in mice. Data was presented as mean ± S.E.M. (n = 5); ***P<0.001; compared to control group (One-way ANOVA followed by Newman Keuls' test).

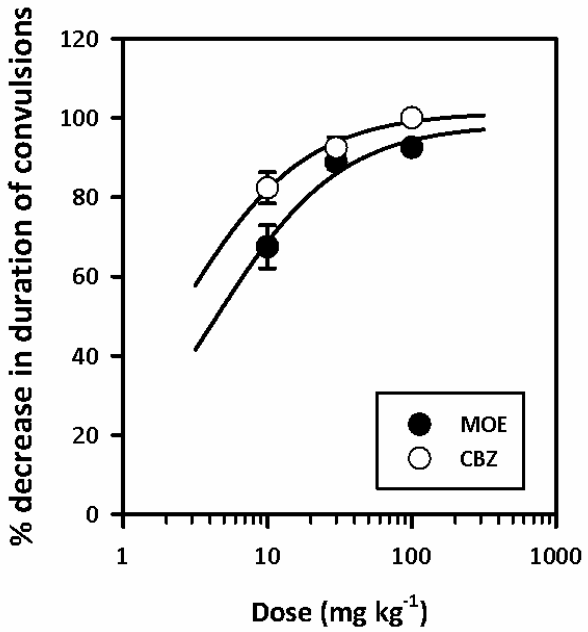

Figure 4.6 Dose–response curves for the anticonvulsant activity induced by the administration of MOE (10 - 100 mg kg^{-1}) and carbamazepine (10 - 100 mg kg^{-1}) in the maximal electroshock seizure test in mice. Each point is the mean ± S.E.M. of 5 animals.

4.3.4 **Strychnine-induced convulsions**

Extract (10 - 100 mg kg^{-1}) showed significant anticonvulsant property. It dose dependently decreased the frequency ($F_{3,25}$=11.91, P<0.0001) (Figure 4.7a) and duration of convulsions

($F_{3,36}$=12.07, P<0.0001) (Figure 4.7c). MOE also significantly delayed the onset of myoclonic jerks ($F_{3,25}$=11.85, P<0.0001) (Figure 4.7a). Diazepam (0.1 - 1 mg kg^{-1}) showed similar results as the extract but was more potent and efficacious than the extract in reducing the duration of convulsions (Table 4.3, Figure 4.8c).

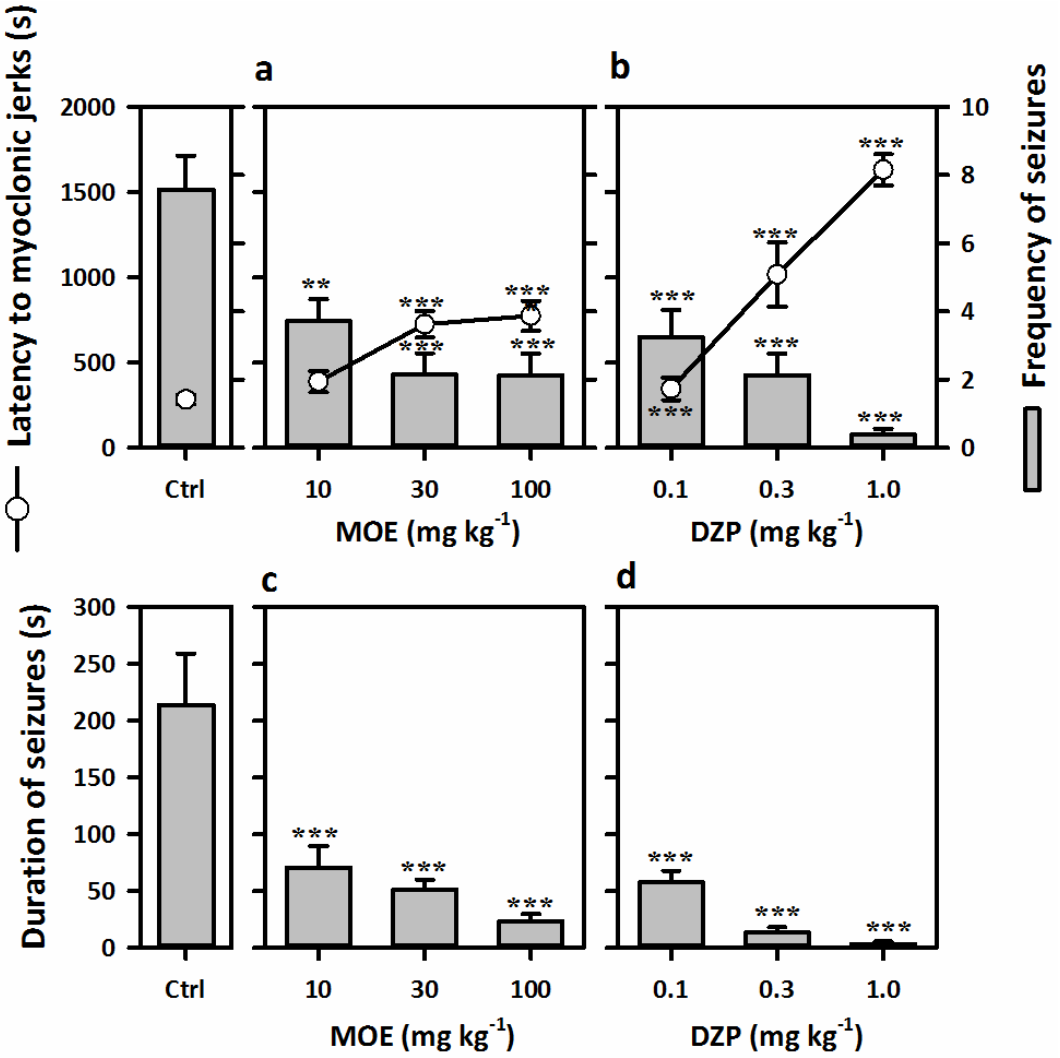

Figure 4.7 Effect of MOE (10 - 100 mg kg^{-1}, *p.o.*) and diazepam (0.1 - 1 mg kg^{-1}, i.p.) on (a, b) the latency to clonic convulsions and frequency of convulsions; (c, d) duration of convulsions, in strychnine-induced seizure test in mice. Data was presented as mean ± S.E.M. (n = 8); ***P<0.001; **P<0.01; compared to control group (One-way ANOVA followed by Newman Keuls' test).

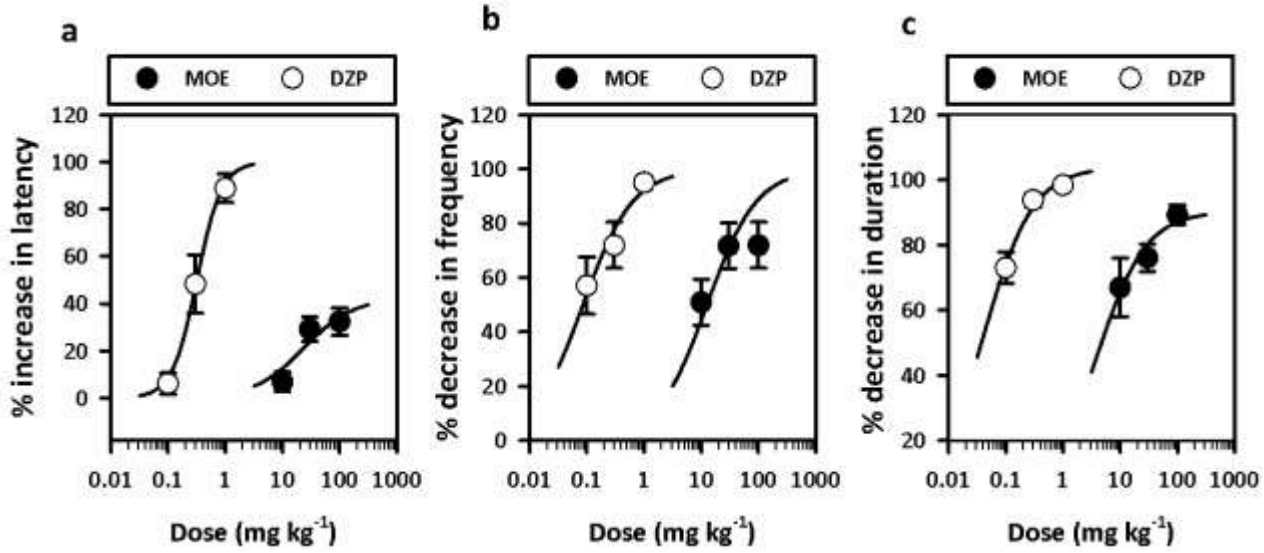

Figure 4.8 Dose–response curves for the anticonvulsant activity induced by the administration of MOE (10 - 100 mg kg^{-1}) and diazepam (0.1 - 1.0 mg kg^{-1}) in the strychnine-induced seizure test in mice. (a) % increase in latency to convulsions; (b) % decrease in frequency of convulsions; (c) % decrease in duration of convulsions. Each point is the mean ± S.E.M. of 8 animals.

4.3.5 **Pilocarpine-induced *status epilepticus***

Oral administration of MOE was effective against pilocarpine induced *status epilepticus*. One-way ANOVA followed by Newman Keuls' test revealed that the extract significantly delayed the onset of clonic convulsions ($F_{3,36}$=25.72, P<0.0001) (Figure 4.9) and the duration of clonic convulsions ($F_{3,36}$=250.5, P<0.0001) (Figure 4.9). ED_{50} and E_{max} values calculated from the dose response curves (Figure 4.10) demonstrated that MOE was less potent than diazepam in reducing the duration of convulsions and delaying the onset of convulsions but their efficacies were comparable (Table 4.3;Table 4.4 and Figure 4.10).

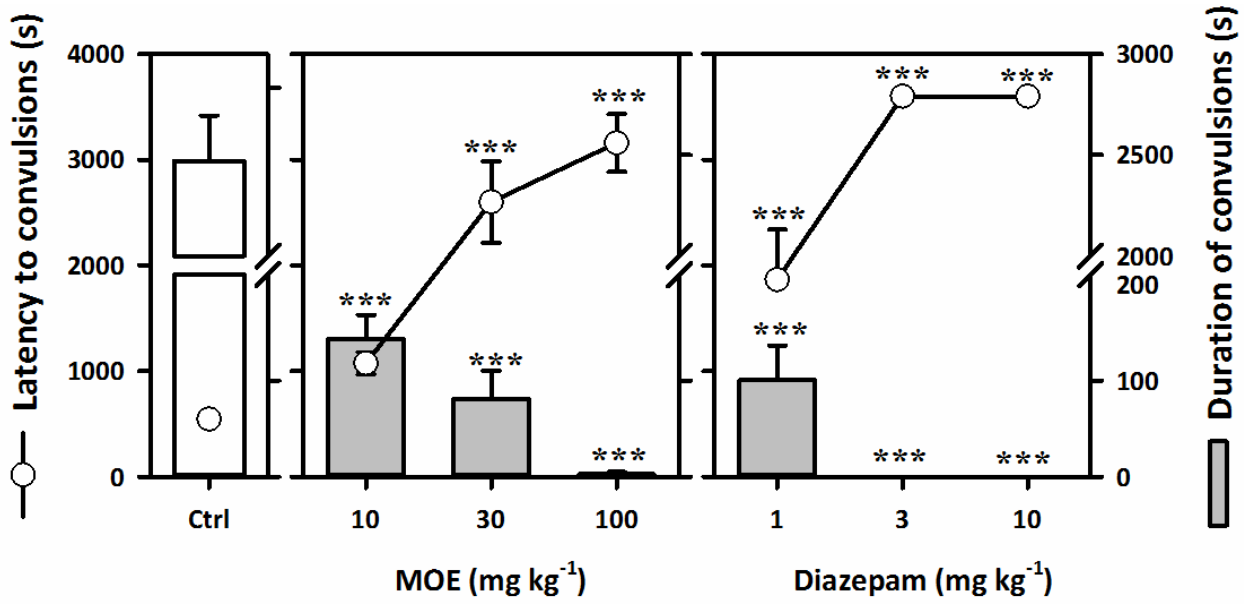

Figure 4.9 Effect of MOE (10 - 100 mg kg^{-1}, *p.o.*) and diazepam (1 - 10 mg kg^{-1}, i.p.) on the latency to and duration of clonic convulsions in the pilocarpine-induced *status epilepticus* in mice. Data was presented as mean ± S.E.M. (n = 10); ***P< 0.001; ** P< 0.01; compared to control group (One-way ANOVA followed by Newman Keuls' test).

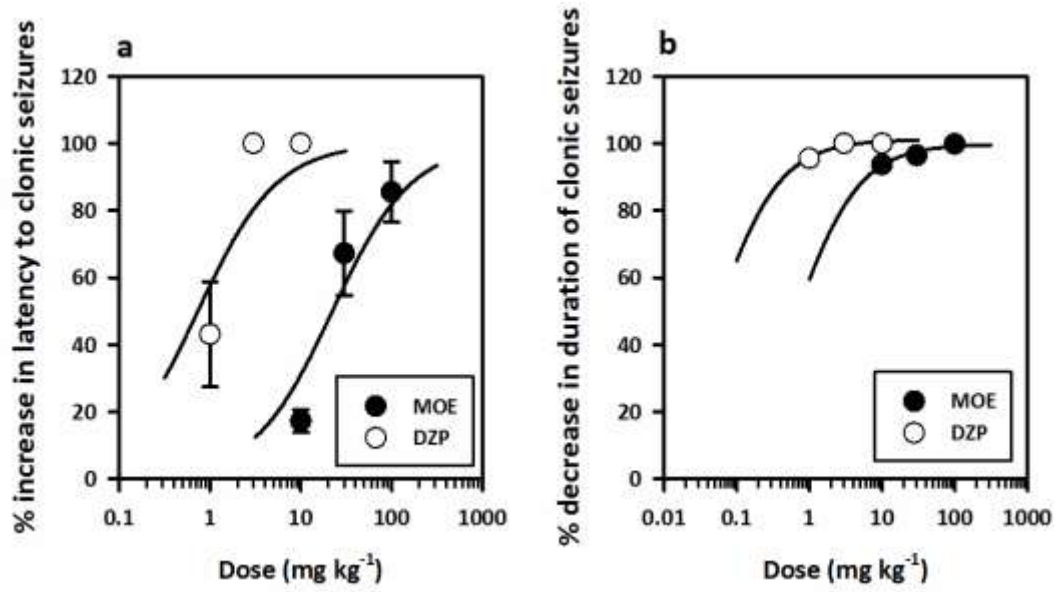

Figure 4.10 Dose–response curves for the anticonvulsant activity induced by the administration of MOE (10 - 100 mg kg^{-1}) and diazepam, (1 - 10 mg kg^{-1}) in the pilocarpine-induced *status epilepticus* in mice. (a) % Increase in latency to clonic convulsions; (b) % Decrease in duration of clonic convulsions. Each point is the mean ± S.E.M. of 10 animals.

4.3.6 **Involvement of GABAergic mechanisms**

MOE alone delayed onset of convulsions and decreased frequency and duration of convulsions similar to diazepam alone (Figure 4.11a, b and c). Flumazenil alone (2 mg kg^{-1}) did not alter the onset, frequency or duration of convulsions. Pretreatment with flumazenil, inhibited the

anticonvulsant effect of extract and diazepam. It reversed the delay in convulsion onset by 90.99%, the decrease in frequency by 78.41% and the decrease in duration of convulsions by 87.66% (Figure 4.11a, b and c).

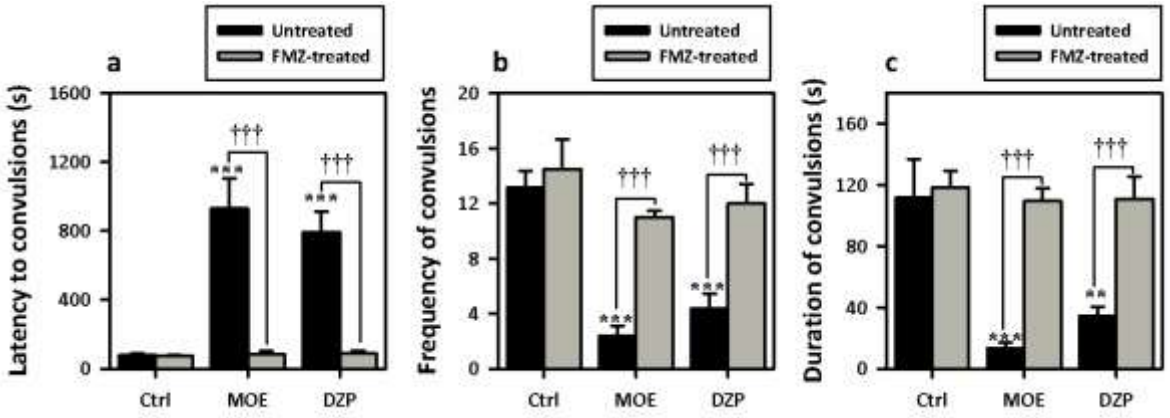

Figure 4.11 Effect of MOE (10 - 100 mg kg^{-1}, *p.o.*) and diazepam (01 - 1.0 mg kg^{-1}, i.p.) on the effect of flumazenil pretreatment on the (a) latency to (b) frequency and (c) duration of clonic convulsions in the pentyleneterazole induced seizure test in mice. Data was presented as mean ± S.E.M. (n = 8); ***P<0.001; **P<0.01; compared to control group (One-way ANOVA followed by Newman Keuls' test). †††P<0.001, comparison between treated and untreated group (Two-way ANOVA followed by Bonferroni's test).

4.3.7 Involvement of potassium channels: The 4-aminopyridine induced seizure test

Oral dose of MOE (10-100 mg kg^{-1}), showed profound anticonvulsant effect. One-way ANOVA showed that MOE dose dependently delayed the onset of clonic ($F_{3,36}$=3.764, P=0.0190) (Figure 4.12) and tonic convulsions ($F_{3,36}$=3.857, P=0.0172) (Figure 4.12). Valproate, the reference anticonvulsant (100, 300, 600 mg kg^{-1}) showed similar effects as the extract. Valproate was more potent than the extract in delaying the onset of convulsions (Table 4.2, Figure 4.14). From the survival curves, percentage survival decreased with time in the extract-treated group. The percentage survival however increased dose dependently (Figure 4.13).

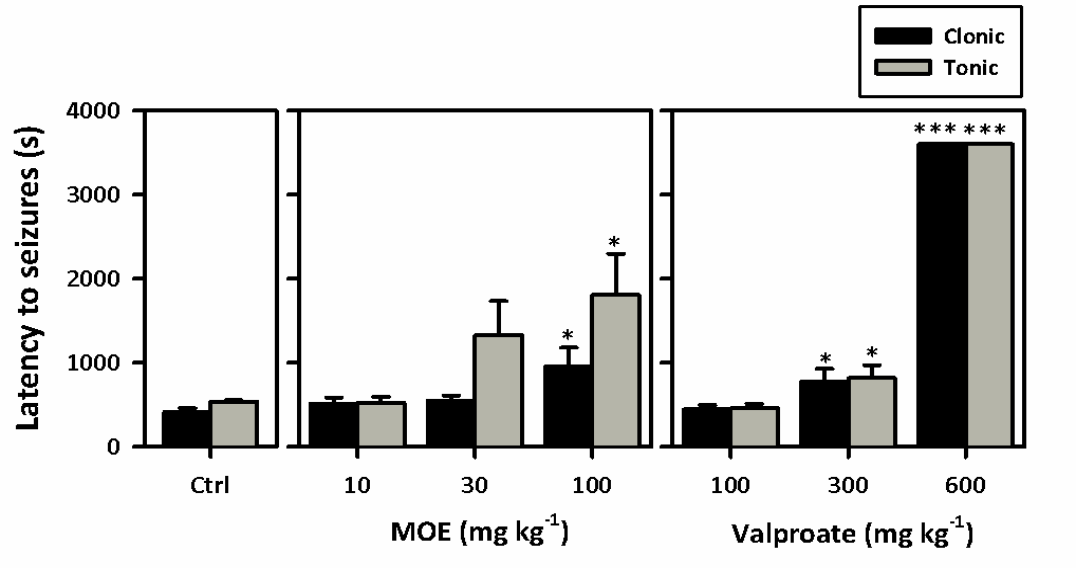

Figure 4.12 Effect of MOE (10 - 100 mg kg^{-1}, *p.o.*) and sodium valproate (100 - 600 mg kg^{-1}, *p.o.*) on the latency to clonic and tonic convulsions in the 4-aminopyridine induced seizure test in mice. Data was presented as mean ± S.E.M. (n = 10); ****P*<0.001; **P*<0.05; compared to vehicle-treated group (One-way ANOVA followed by Newman Keuls' test).

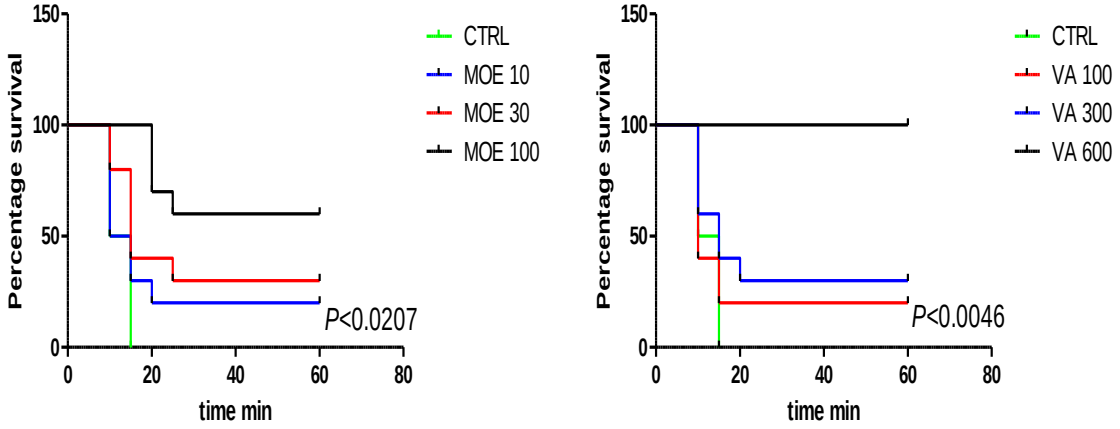

Figure 4.13 A Kaplan-Meier estimates of the percentage survival of mice for extract (MOE 10 – 100 mg kg^{-1}) and sodium valproate (VA 100 – 600 mg kg^{-1}). Each point is the mean ± S.E.M. of 10 animals.

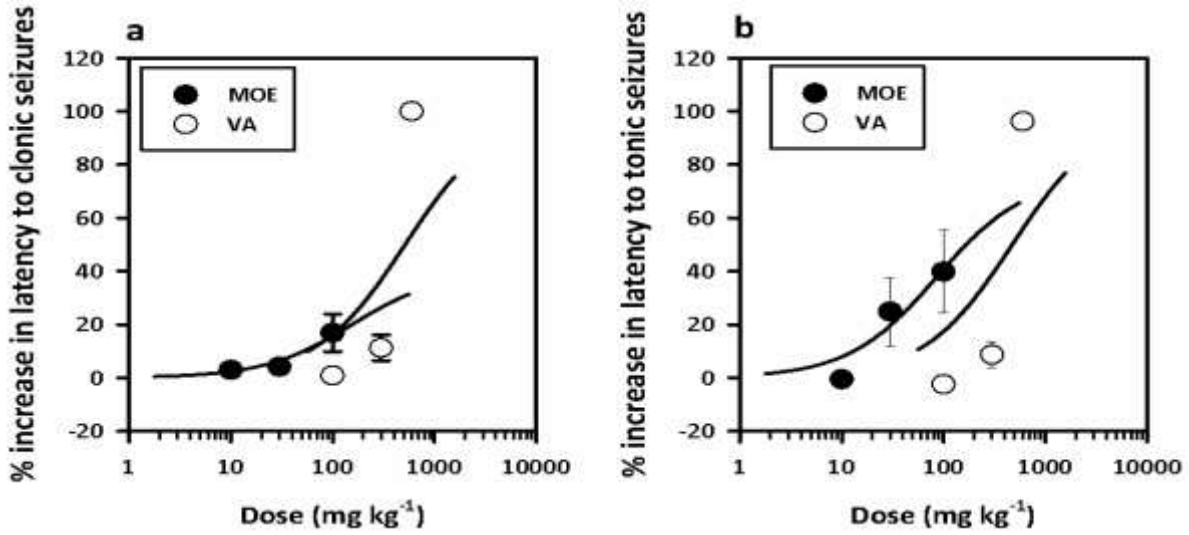

Figure 4.14 Dose–response curves for the anticonvulsant activity induced by the administration of MOE (10 - 100 mg kg^{-1}) and sodium valproate (100 - 600 mg kg^{-1}) in the 4-aminopyridine induced seizure test in mice. (a) % increase in latency to clonic convulsions; (b) % increase in latency to tonic convulsions. Each point is the mean ± S.E.M. of 10 animals.

4.3.8 **Isobolographic analysis of extract and diazepam**

Fractions of the extract and diazepam combination dose dependently decreased the frequency and duration of convulsions in the PTZ induced seizure test (Figure 4.15). Flumazenil failed to reverse the decline in the frequency and duration of convulsions by the fraction of extract and diazepam combination (Figure 4.15). The experimental ED_{50} (Z_{mix}) obtained by non-linear regression analysis was 5.49±2.92 indicating potentiation of anticonvulsant effect (Figure 4.16a). The interaction index (a measure of the degree of potentiation) displayed as the Z_{mix} lying below the line of additivity of the isobologram was 0.70 indicating synergism (Figure 4.16b).

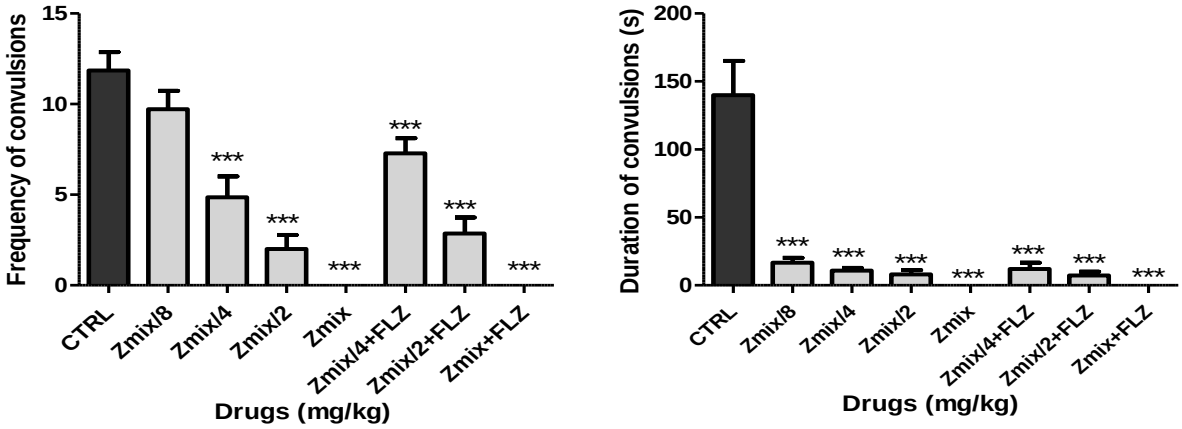

Figure 4.15 Effect of fractions of extract and diazepam combinations and their antagonism with flumazenil on frequency and duration of convulsions in the PTZ test. Each point is the mean ± S.E.M. of 7 animals. ****P*<0.001; (One-way ANOVA followed by Newman Keuls' test).

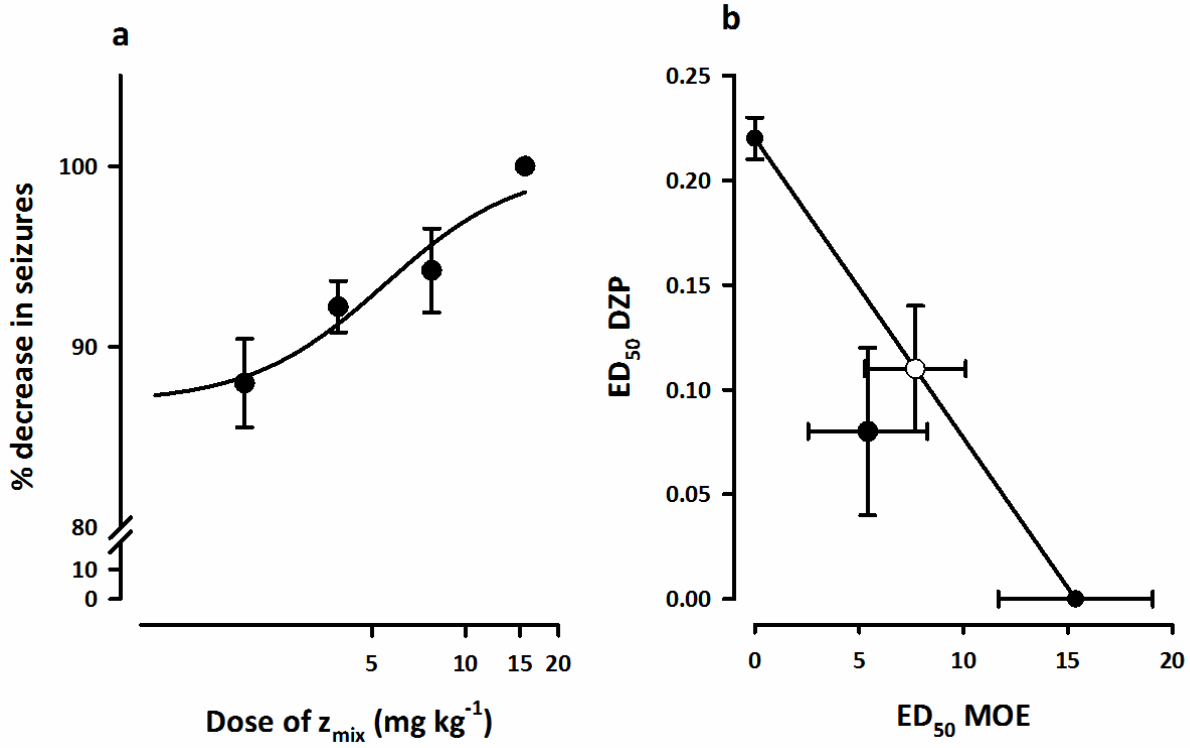

Figure 4.16 (a) Dose response curve for fractions of extract and diazepam combinations in the PTZ seizure test. (b) Isobologram for the combination of extract and diazepam in the PTZ seizure test. Filled circle represent the theoretical ED50 ± S.E.M and open circle for the experimental ED_{50} ± S.E.M.

Table 4.1 ED_{50} and E_{max} Values of the Latency to Convulsions

Drug (mg kg^{-1})	STR		PTZ		PTX		4-AP	
	ED_{50}	E_{max}	ED_{50}	E_{max}	ED_{50}	E_{max}	ED_{50}	E_{max}
MOE	23.7±26.8	42.4	24.2±12.5	122	18.7±4.8	107.5	86.1±59.6	75.7
DZP	0.3±0.1	100	0.5±0.1	113.6	0.1±0.0	101.2		
VA	-	-	-	-	-	-	47.51±154.6	100

Values are expressed as mean ± S.E.M. (n=5). The values were obtained from experiments described earlier. ED_{50}s ± S.E.M. were obtained by least-square nonlinear regression as described in material and methods.

Table 4.2 ED_{50} and E_{max} Values of the frequency of Convulsions

Drug (mg kg^{-1})	STR		PTZ		PTX	
	ED_{50}	E_{max}	ED_{50}	E_{max}	ED_{50}	E_{max}
MOE	12.6±3.9	100	15.4±3.7	100	10.9±6.1	87.2
DZP	0.1±0.1	99.8	0.2±0.0	85.9	0.1±0.0	94.8

Values are expressed as mean ± S.E.M. (n=5). The values were obtained from experiments described earlier. ED_{50}s ± S.E.M. were obtained by least-square nonlinear regression as described in material and methods.

Table 4.3 ED_{50} and E_{max} Values of the Duration of Convulsions.

Drug (mg kg^{-1})	MES		STR		PTZ		PTX	
	ED_{50}	E_{max}	ED_{50}	E_{max}	ED_{50}	E_{max}	ED_{50}	E_{max}
MOE	4.3±1.2	98.4	3.8±2.2	90.3	13.6±5.8	81.3	10.1±6.7	76.9
DZP	-	-	2.4±0.7	103.9	0.3±0.1	93.8	0.3±0.1	99.7
CBZ	2.4±0.7	101.4	-	-	-	-	-	-

Values are expressed as mean ± S.E.M. (n=5). The values were obtained from experiments described earlier. ED_{50}s ± S.E.M. were obtained by least-square nonlinear regression as described in material and methods.

4.3.9 **Rotarod**

MOE did not impair motor coordination at all dose levels. Diazepam, however, significantly impaired motor coordination at 1 mg kg^{-1} (Table 4.4).

Table 4.4 showing the latency for mice to fall off the rotarod

Drug (mg kg^{-1})	Latency to fall (s)				
	0 h	1 h	2 h	3 h	4 h
Ctrl	108.6±11.4	120.0±0.0	98.9±14.1	120.0±0.0	109.8±10.2
MOE					
10	101.6±9.9	76.1±13.6	67.5±15.5	71.0±21.9	102.2±11.4
30	119.6±33.6	111.6±8.4	97.0±15.0	97.0±17.4	50.0±11.7*
100	109.1±9.8	87.2±21.5	105.1±14.9	76.6±18.5	85.9±14.0
DZP					
0.1	112.0±5.8	96.1±10.4	82.6±4.9	97.0±9.4	100.6±11.8
0.3	110.5±5.8	81.5±10.2	75.6±6.1	60.3±12.3***	82.5±17.5
1.0	109.0±8.7	65.7±12.3**	50.1±11.6**	35.1±13.5***	65.8±12.7*

Values are expressed as mean ± S.E.M. (n=5). ***P< 0.001; ** P< 0.01

4.4 DISCUSSION

From the studies carried out, it is evident that oral administration of *Mallotus oppositifolius* leaf extract (MOE) has anticonvulsant effect in both acute generalized seizure models—pentylenetetrazole-, picrotoxin- and maximal electroshock-, strychnine-, 4-aminopyridine -induced seizure tests and partial seizure model—the pilocarpine-induced *status epilepticus*.

The maximal electroshock test is the most widely used animal model in antiepileptic drug discovery because seizure induction is simple and the predictive value for detecting clinically effective antiepileptic is high (White, 1997; Holmes, 2007; Castel-Branco *et al.*, 2009). Though the extract did not totally abolish tonic hind limb extension at all the doses used in the maximal electroshock seizure test it significantly reduced the duration of the tonic hind limb extension. Tonic hind limb extension is the universal feature of maximal electroshock in mice, rats, rabbits, cats, monkeys and humans (Raza *et al.*, 2001; Giardina *et al.*, 2005). Abolishing tonic hind limb extension in electroshock test predicts the ability of testing material to prevent the spread of seizure discharge from the epileptic focus and its effectiveness in this model correlates well with suppressing generalized tonic-clonic seizures and indicates the ability of the testing material to inhibit or prevent seizure discharge within brainstem substrate (Krall *et al.*, 1978; Porter *et al.*, 1984; Raza *et al.*, 2001). All the currently available drugs that are clinically effective in generalized tonic-clonic seizures (phenytoin, carbamazepine, phenobarbitone, valproate, lamotrigine, oxycarbamazepine, etc.) are effective in this model (Macdonald and Kelly, 1995; Castel-Branco *et al.*, 2009). Thus the extract may not be effective in completely attenuating generalised tonic-clonic seizures but may reduce the duration of these seizures.

An agent that prevents or delays the onset of clonic and tonic-clonic convulsion induced by PTZ, a GABA receptor antagonist, in animals is an anticonvulsant (Vellucci and Webster, 1984;

Amabeoku and Chikuni, 1993; Sayyah *et al.*, 2004). In the present study, the extract showed anticonvulsant effect against PTZ-induced seizures by delaying the onset of myoclonic jerks and clonic convulsions in mice. It also caused profound decrease in the frequency and duration of the clonic convulsions. This result confirmed an earlier report of the effectiveness of MOE against PTZ seizures in the preliminary CNS screening tests (Kukuia *et al*., 2012). PTZ-induced seizure model identifies compounds that can raise seizure threshold in the brain (Raza *et al.*, 2001; White, 2003; Mandhane *et al.*, 2007). Antiepileptics effective in the therapy of generalized seizures of *petit mal* type (absence or myoclonic) i.e. phenobarbitone, valproate, ethosuximide and benzodiazepines suppress PTZ-induced seizures (Loscher *et al.*, 1991; Kaminski *et al.*, 2001; White, 2003; Akula *et al.*, 2009). Thus the extract may be effective against generalized absence/myoclonic seizures.

MOE also showed anticonvulsant activity against seizures induced by picrotoxin, a $GABA_A$ receptor antagonist. PTZ and picrotoxin induce convulsions in rodents by blocking the chloride-ion channels linked to $GABA_A$ receptors, thus preventing the entry of chloride ions into the brain and consequently inhibitory transmission in the brain (Loscher and Schmidt, 1988; Mehta and Ticku, 2001). GABAergic neurotransmission plays an important role in stress, anxiety (Zwanzger and Rupprecht, 2005), pain (Rode *et al.*, 2005) and epilepsy (Perucca, 2005). Benzodiazepines and many barbiturates potentiate the inhibitory action of $GABA_A$ receptors, reducing neuronal excitability and increasing the threshold for convulsions (Loscher and Schmidt, 2006). Since MOE was effective against both pentylenetetrazole and picrotoxin-induced seizures, a possible interaction with GABAergic mechanisms was investigated by pretreating mice with flumazenil, a benzodiazepine receptor antagonist (File and Pellow, 1986; Przegalinski *et al.*, 2000), in the PTZ-induced seizure test. The reversal of the anticonvulsant effect of MOE by flumazenil in this test, confirms possible interaction with the GABA/benzodiazepine receptor complex or pathway.

Together with the $GABA_A$ receptors, the glycine receptor is responsible for mediating fast inhibitory neurotransmission in the mature central nervous system making it a potential target for antiepileptic drugs (Lopez-Corcuera *et al.*, 2001; Bowery and Smart, 2006; Webb and Lynch, 2007). Strychnine causes convulsions by antagonizing the activity of strychnine sensitive glycine receptors and increasing postsynaptic excitability and ongoing activity in the brainstem and spinal cord (Curtis *et al.*, 1967; Werman *et al.*, 1967; Wang *et al.*, 2001; Wood *et al.*, 2002). Since MOE delayed the onset of convulsions, reduced the frequency and duration of convulsions induced by strychnine, an interaction of the extract with glycine receptors/pathways is plausible. It is possible the extract may contain compounds that activate glycinergic inhibitory neurotransmission.

The importance of potassium ion channels as an inhibitory mechanism in epilepsy is well-known (Lerche *et al.*, 2001; George, 2004; Parthasarathi *et al.*, 2006). Moreover, it has been established

that blocking these potassium channels and preventing them from opening in response to membrane depolarization or deleting these channels will lower seizure threshold and can produce seizures (Zhang *et al.*, 2010; N'Gouemo, 2011). 4-aminopyridine (4-AP) is a known potassium channel blocker that produces seizures by increasing the release of glutamate and calcium while preventing GABAergic neurotransmission (Yamaguchi and Rogawski, 1992; Lohle *et al.*, 2008; Leung *et al.*, 2011). The effectiveness of the extract against 4-AP induced seizures is an indication that the anticonvulsant activity of the extract may depend on activation of potassium channels or conductance. It is also possible that the extract may be indirectly enhancing potassium conductance through its activation of GABAergic neurotransmission since activation of GABA neurotransmission can result in enhancement of potassium conductance. Retigabine, a newly approved drug for the treatment of epilepsy with broad-spectrum anticonvulsant profile in animal seizure and epilepsy models, functions through its ability to activate potassium currents (Rostock *et al.*, 1996; Rundfeldt, 1997). So far it is the only potassium channel opener that has been approved as an anticonvulsant. That MOE possibly interacts with potassium channels is an important finding since MOE may be another source of anticonvulsant compounds that will enhance potassium conductance. Rottlerin (mallotoxin), which has been found in the leaves of *M. oppositifolius* (Oliver, 1960; Adekunle and Ikumapayi, 2006), is a potent activator of the large conductance voltage and Ca^{2+} activated K^{+} channels which are implicated in epilepsy (Zakharov *et al.*, 2005; Soltoff, 2007; Wu *et al.*, 2007). It is possible that the anticonvulsant effect observed in this model may be due to the presence of rottlerin in the leaves.

Systemic administration of pilocarpine, a nonselective muscarinic agonist, is an animal model of intractable epilepsy (Turski *et al.*, 1989; Matsui *et al.*, 2000; Eglen *et al.*, 2001; Mirza *et al.*, 2003; Wirtshafter, 2006). Histological studies have shown important similarities between this model and temporal lobe epilepsy in humans; thus drugs effective in this model are potential candidates for managing temporal lobe epilepsy (Isokawa and Mello, 1991; Liu *et al.*, 1994; Wall *et al.*, 2000; Szyndler *et al.*, 2005; Perez-Mendes *et al.*, 2011). MOE exhibited potent anticonvulsant effect against *status epilepticus* induced by pilocarpine. It is therefore possible that the extract may have potential value in the management of temporal lobe epilepsy and/or other partial seizures. Muscarinic receptor stimulation is presumed to be responsible for the onset of pilocarpine-induced seizures, whereas the effect of glutamate on NMDA receptors sustains seizure activity and causes neuronal damage (Jope *et al.*, 1986; Clifford *et al.*, 1987; Turski *et al.*, 1989; Smolders *et al.*, 1997). By delaying the onset of clonic seizures in this model, it is possible that the extract may have some antimuscarinic properties and by reducing the duration of seizures, it is likely it has antagonistic effect on glutamatergic neurotransmission. It is possible that activation of potassium conductance (which can lead to inhibition in the release of glutamate) by the extract may support the antiglutamatergic theory proposed for the extract.

Triterpenic steroids, saponins and alkaloids have been reported to possess anticonvulsant activity in some experimental seizure models such as PTZ (Chauhan *et al.*, 1988; Kasture *et al.*, 2002). It is possible that one or more of the secondary metabolites in the plant—alkaloids, saponins and sterols may be responsible for the observed anticonvulsant effect of the extract (Farombi *et al.*, 2001; Adekunle and Ikumapayi, 2006, Kukuia *et. al*., 2012).

Isobolographic analysis of the drug interaction between diazepam and MOE in the PTZ induced seizures showed a significant potentiating effect suggesting that a combination of the two drugs have beneficial anticonvulsant effect. Though flumazenil pretreatment inhibited the anticonvulsant effect of the extract and diazepam individually, interestingly it failed to reverse their anticonvulsant effect when administered together. This suggests that the mechanism of action of the combination of extract and diazepam may not be due to activation of the benzodiazepine/GABA receptor complex. Other mechanisms may be involved which is yet to be elucidated.

4.5 CONCLUSION

These findings confirm the anticonvulsant effect of the extract. MOE may be acting possibly by activating GABAergic and gylcinergic systems. It may also be activating potassium channels. Antimuscarinic and antiglutamatergic effect especially antagonism of NMDA receptors are also possible mechanisms of action of MOE. The anticonvulsant effect exhibited makes MOE a likely therapeutic potential for both generalized and partial seizures.

Chapter 5
ANTIDEPRESSANT EFFECT

5.1 INTRODUCTION

Depression is an extremely common pathological complex with psychological, neuroendocrine and pathological symptoms (Newcomer *et al.*, 1990; Holmes, 2003). It is a leading cause of disability worldwide and has a very significant impact on morbidity, mortality and health care cost (Alonso *et al.*, 2004; Ustun *et al.*, 2004; Gilmour and Patten, 2007).

Perturbations in monoaminergic neurotransmission especially serotonin and noradrenaline neurotransmission are considered major culprits for the observed symptoms of depression, explaining why currently used antidepressants more or less selectively interact with the monoaminergic systems (Pacher *et al.*, 2001; Slattery *et al.*, 2004; Berton and Nestler, 2006; Yacoubi *et al.*, 2011). Unfortunately the efficacies of these medications are unsatisfactory and multiple side effects are common (Gumnick and Nemeroff, 2000; Nomura, 2003; Poleszak *et al.*, 2011). Furthermore, these drugs have slow onset of action, often requiring at least 2-4 weeks of administration before producing clinical improvement in the symptoms (Tran *et al.*, 2003; Adell *et al.*, 2005; Gourion, 2008; Machado-Vieira *et al.*, 2009). Additionally, about 30- 40% of patients have conditions refractory to current medications (Fava and Kendler, 2000; Belmaker and Agam, 2008). These reasons accentuate the need for novel therapeutic agents with less side effects and faster onset of action and wider patient spectrum (Cryan *et al.*, 2002; Nestler *et al.*, 2002). Also both preclinical and clinical studies support the role of NMDA receptor antagonists as possible therapeutic agents for depression (Trullas and Skolnick, 1990; Poleszak *et al.*, 2004; Siwek *et al.*, 2009). For instance ketamine and memantine have demonstrated rapid and profound antidepressant effects clinically (Berman *et al.*, 2000; Zarate *et al.*, 2012). Numerous behavioural studies have further demonstrated that antagonists and partial agonists at the glycine co-agonist site of the NMDA receptor have antidepressant potentials with less severe side effects (Trullas and Skolnick, 1990; Vamvakides, 1998). While the search of newer antidepressants continue, renewed interest in medicinal plants e.g. *Mallotus oppositifolius* for the treatment of many CNS disorders has been on the ascendancy (Briskin, 2000; Akhondzadeh and Abbasi, 2006; Darwish and Aburjai, 2010). Despite the use of *Mallotus oppositifolius* in treating psychiatric disorders, there is no scientific data on its antidepressant effect. Thus the present study investigated the effect of the hydroalcoholic leaf extract of the plant in acute antidepressant models- the forced swim (FST) and tail suspension tests (TST).

Investigations were carried out to ascertain whether MOE would exhibit antidepressant effect with chronic administration in the open space swim test. Though predictive of antidepressant activity, the FST and TST are unlike human depression (Cryan *et al.*, 2005; Stone *et al.*, 2008). The open

space swim test, a chronic model was chosen because it has closer resemblance to human depression while maintaining its predictive and construct validity (Sun and Alkon, 2003; Stone and Lin, 2011). Major depressive disorder (depression) itself and some antidepressants induce significant weight changes (loss or gain) and alter cognitive funtion among others (Fava, 2000; Dixon *et al.*, 2003; Nebes *et al.*, 2003; Anderson *et al.*, 2006; Biringer *et al.*, 2007). Hence the effect of the extract on cognitive function using the Morris water maze, and weight changes in the depressed rodents was examined.

The effects of the extract on the monoaminergic system (serotonin and noradrenaline) as well as the glycine/NMDA receptor complex were investigated in order to elucidate the possible mechanism(s) of action of the extract.

5.2 MATERIALS AND METHODS

5.2.1 Animals

Male ICR mice were used in this experiment and experimental conditions were as described in section 3.2.1.

5.2.2 Chemicals

Fluoxetine hydrochloride (Prozac®), was purchased from Eli Lilly and Co., Basingstoke, England. Imipramime hydrochloride (Tofranil®) and desipramine hydrochloride, D-serine, D-cycloserine α-methyldopa, reserpine, para-chlorophenylalanine were purchased from Sigma-Aldrich Inc., St. Louis, MO, USA.

5.2.3 Acute antidepressant model

5.2.3.1 Forced Swimming test

The FST was based on that described by Porsolt *et al*., (1977). Mice were divided into ten groups of five animals each and received the vehicle (water), extract (10, 30 or 100 mg kg^{-1}, *p.o.*), or the reference drugs fluoxetine (3, 10 or 30 mg kg^{-1}, *p.o.*) and imipramine (3, 10 or 30 mg kg^{-1}, *p.o.*). Thirty minutes after i.p. and 1 h after oral administration of the test compounds, mice were gently dropped individually into transparent cylindrical polyethylene tanks (25 cm high, 10 cm internal diameter) containing water (25 to 28°C) up to a level of 20 cm and allowed to swim for 5 min. Mice placed in the tank engage in escape directed activities until they assume an immobile state which represents a state of helplessness (depression). Each session was recorded by a video camera suspended approximately 100 cm above the cylinders. An observer scored the duration of immobility (when it floated upright in the water and made only small movements to keep its head above water), during the 5 min test, from the videotapes with the aid of the public domain software

JWatcher Version 1.0 (University of California, Los Angeles, USA and Macquarie University, Sydney, Australia. Available at http://www.jwatcher.ucla.edu/).

5.2.3.2 Tail suspension test

The TST was carried out as previously described Steru *et al*., (1985). Mice were allowed to acclimatize to the room for 3.5-4 h before the test. Groups of ten mice were treated with MOE (10, 30 or 100 mg kg^{-1}, *p.o.*), fluoxetine (3, 10 or 30 mg kg^{-1}, *p.o.*) and imipramine (3, 10 or 30 mg kg^{-1}, *p.o.*) or vehicle. One hour after oral administration of the test compounds, mice were individually suspended by the tail from a horizontal bar (distance from floor is 30 cm) using adhesive tape (distance from tip of tail is 1 cm). Mice hanged by the tail engage in escape directed activities until they become immobile which represent a state of helplessness (depression). Duration of immobility, defined as the absence of all movement except for those required for respiration, was recorded by an observer for 5 min from video recordings of the test as described above for forced swimming test. Mice that climbed up on their tails during the test session were gently pulled down and testing continued. Mice that continued to climb their tails were excluded from the study.

5.2.4 Chronic depression

5.2.4.1 Open space swim test

The method described by Stone and Lin (2011) with some slight modifications was used. Swimming was carried out in rat tub cages (28×26×41 cm, w×h×l) filled with 13 cm high tepid tap water (32–34 °C). Mice were swum individually for 15 min/day on 4 consecutive days in order to induce a depressive state characterized by the decrease in mobility and distance travelled by the mice. Drug treatment started from day 5, through days 7, 10, 14, 18. All swim sessions were videotaped from above. No special procedures were used to dry or warm the animals as they rapidly dried themselves with no observable episodes of shivering. The distance swum was rated as the number of quadrants of the tub entered and duration of immobility from the total time the animal was observed to float, which is defined as drifting with the tail fully extended and no motion observed in the tail or limbs.

5.2.4.2 Tail suspension test

The tail suspension test was performed 24 h after the last treatment in the open space swim test to ascertain if the behavioural effect will be sustained. The procedure used was similar to that described in section 5.2.3.2.

5.2.4.3 Spatial learning and memory task- Morris water maze

Effects of the induced depressive behavior on spatial learning and memory were evaluated with the Morris water maze task (Sun and Alkon, 2004), in an 85 cm-diameter pool (24±1 °C). The maze was divided into four quadrants. Naïve control mice were allowed to swim for 1 min in the pool without a hidden platform, the same day when the other groups were on their third day of depressive behavior induction. Twenty-four hours later, all mice were swum in two sessions a day for 4 days to find a hidden platform (5 cm diameter). The platform was centred in one of the quadrants and submerged about 2 cm below the water surface. At the start of all trials, mice were placed in the water facing the maze wall, using different starting positions, and allowed to swim until they found the platform where they remained for 20 s. A mouse that failed to find the platform within 1 min was guided there, with the maximum latency of 60 s scored. The swimming activity was observed for latency to find the platform.

5.2.4.4 Weight variation

Weights of mice were taken with a sensitive electronic balance when chronic depression was being induced and every 3 days during the treatment period. The change in weight was calculated to ascertain drug-induced changes in the weight of the mice.

5.2.5 Possible Mechanisms

Based on current knowledge of the mode of action of antidepressants and the pathophysiology of depression, the contribution of the adrenergic, serotoninergic and glutamatergic systems to the antidepressant effect of the extract were investigated.

5.2.5.1 Involvement of noradrenergic systems

Mice were pretreated with reserpine and/or α-methyldopa (α-MD) in order to investigate the possible role of noradrenergic system in the actions of MOE. The doses of α-MD and reserpine were chosen based on work done by others (van Giersbergen *et al.*, 1990; O'Leary *et al.*, 2007, Woode *et. al*., 2010). To deplete newly synthesized pools of noradrenaline (NA) and dopamine (DA), mice were treated with a single dose of α-MD (400 mg kg^{-1}, i.p.) 3.5 hours before behavioural testing. To deplete vesicular pools of NA and DA, mice were treated with a single dose of reserpine (1 mg kg^{-1}, s.c.) 24 h before behavioral testing. In an effort to deplete both the vesicular and cytoplasmic pools of NA and DA, mice were pretreated with a combination of reserpine (1 mg kg^{-1}, s.c., 24 h before behavioral testing) and α-MD (200 mg kg^{-1}, i.p., 3.5 h before behavioural testing), respectively.

5.2.5.2 Involvement of serotoninergic systems

Mice were grouped into twenty (20) groups, n=5. *p*CPA (200 mg kg^{-1}, i.p.) was administered once daily for 3 consecutive days to 10 groups of animals. On the fourth day, group 1 received saline, groups 2- 4 received MOE (10, 30 and 100 mg kg^{-1}); groups 5- 7 received fluoxetine (3, 10 and 30 mg kg^{-1}, *p.o.*), groups 8- 10 received imipramine (3, 10 and 30 mg kg^{-1}, *p.o.*). The remaining 10 groups received which did not undergo pretreatment, received extract (10, 30 and 100 mg kg^{-1}, *p.o.*), fluoxetine (3, 10 and 30 mg kg^{-1}, *p.o.*), imipramine (3, 10 and 30 mg kg^{-1}, *p.o.*) or saline on the day of experiment. The tail suspension and modified forced swim tests were used. After the tail suspension sessions, mice were taken through the modified forced swimming test. The modified forced swimming test followed the same procedure described above except that the depth of water was changed to 20 cm. For tail suspension, immobility period was scored whilst for the modified swimming test mean immobility counts, mean swimming and mean climbing counts were scored. Swimming is defined as the active horizontal movement of the mice in the swim tank and this behaviour is sensitive to serotonin-based drugs; climbing is the vertical movement of the paw of the mice along the walls of the tank and it is sensitive to adrenergic drugs.

In a separate experiment, the effects of the extract (10 - 100 mg kg^{-1}, *p.o.*) and fluoxetine (3 -30 mg kg^{-1}, *p.o.*) on 5-hydroxytryptamine (5-HTP) (200 mg kg^{-1}) induced head twitch response were studied. Seven groups of mice (n= 6) were used. The first 3 groups were given the extract, the next three fluoxetine and the last group received vehicle and after 1 h or 30 min for the drug treated groups or vehicle treated respectively, 5-HTP was administered and the number of head twitches every 5 min were counted for 30 min and also the total number of head twitches for the 30 min period.

5.2.5.3 Involvement of glycine/NMDA neurotransmission

Mice were divided into 2 groups, A and B. Groups A and B were further subdivided into 6 groups each (n=8). Shortly five groups of mice from group A received D-cycloserine (2.5 mg kg^{-1}, i.p.) and 30 min after the first three groups received an oral dose of the extract (10- 100 mg kg^{-1}) with the last two groups receiving either fluoxetine (10 mg kg^{-1}) or desipramine (10 mg kg^{-1}, i.p.). The sixth group received only D-cycloserine. Five groups of mice from group B received D-serine (600 mg kg^{-1}) and 30 min after the first three groups received an oral dose of the extract (10 - 100 mg kg^{-1}) with the last two groups receiving either fluoxetine (10 mg kg^{-1}, *p.o.*) or desipramine (10 mg kg^{-1}, i.p.). The sixth group from group B received only D-serine. The forced swim and tail suspension tests were used as described above to investigate the antidepressant mechanism. The rotarod test was used to assess whether there was any locomotor impairment after extract, D-serine or D-cycloserine administration. Pedalling behaviour was defined as the continuous movement of

the paw of the mice without moving the body while curling was defined as the raising of the head of the mouse towards its hind paws.

5.2.6 Statistics

GraphPad Prism for Windows version 4.03 (GraphPad Software, San Diego, CA, USA) was used for all data and statistical analyses. $P<0.05$ was considered statistically significant. In all the tests, a sample size of ten animals (n=10) were used. The time-course curves were subjected to two-way (treatment × time) repeated measures analysis of variance (ANOVA) with Bonferroni's post hoc test. Total immobility time, distance travelled, and time taken to find the hidden platform and change in weight for each treatment was calculated in arbitrary unit as the area under the curve (AUC). Differences in AUCs were analysed by ANOVA followed by Student-Newman-Keuls' post hoc test. Doses for 50% of the maximal effect (ED_{50}) for each drug were determined by using an iterative computer least squares method, with the following nonlinear regression (three-parameter logistic) equation:

$$Y = \frac{a + (b - a)}{\left(1 + 10^{(LogED_{50} - X)}\right)}$$

Where, X is the logarithm of dose and Y is the response. Y starts at a (the bottom) and goes to b (the top) with a sigmoid shape.

5.3 RESULTS

5.3.1 Forced swimming and Tail suspension tests

MOE (10 - 100 mg kg^{-1}, *p.o.*), administered 60 min before the test period, significantly decreased the frequency of immobility ($F_{3,19}=21.47$, $P<0.001$) (Figure 5.1a) and immobility periods of mice ($F_{3,19}=143.4$, $P<0.001$) (Figure 5.1d) in a dose dependent manner in the FST. In the TST both frequency ($F_{6,54}=0.486$, $P=0.8159$) (Figure 5.3a, b, c), and duration ($F_{6,52}=25.57$, $P<0.001$) (Figure 5.3d, e, f) of immobility decreased, indicating significant antidepressant activity. In both TST and FST, the order of antidepressant efficacy calculated from the dose response curves (Figure 5.2 and Figure 5.4), with regards to duration of immobility was imipramine>fluoxetine >MOE. From the ED_{50} values calculated, MOE was more potent in reducing duration of immobility than fluoxetine and imipramine in the TST but was the least potent in the FST (Table 5.1)

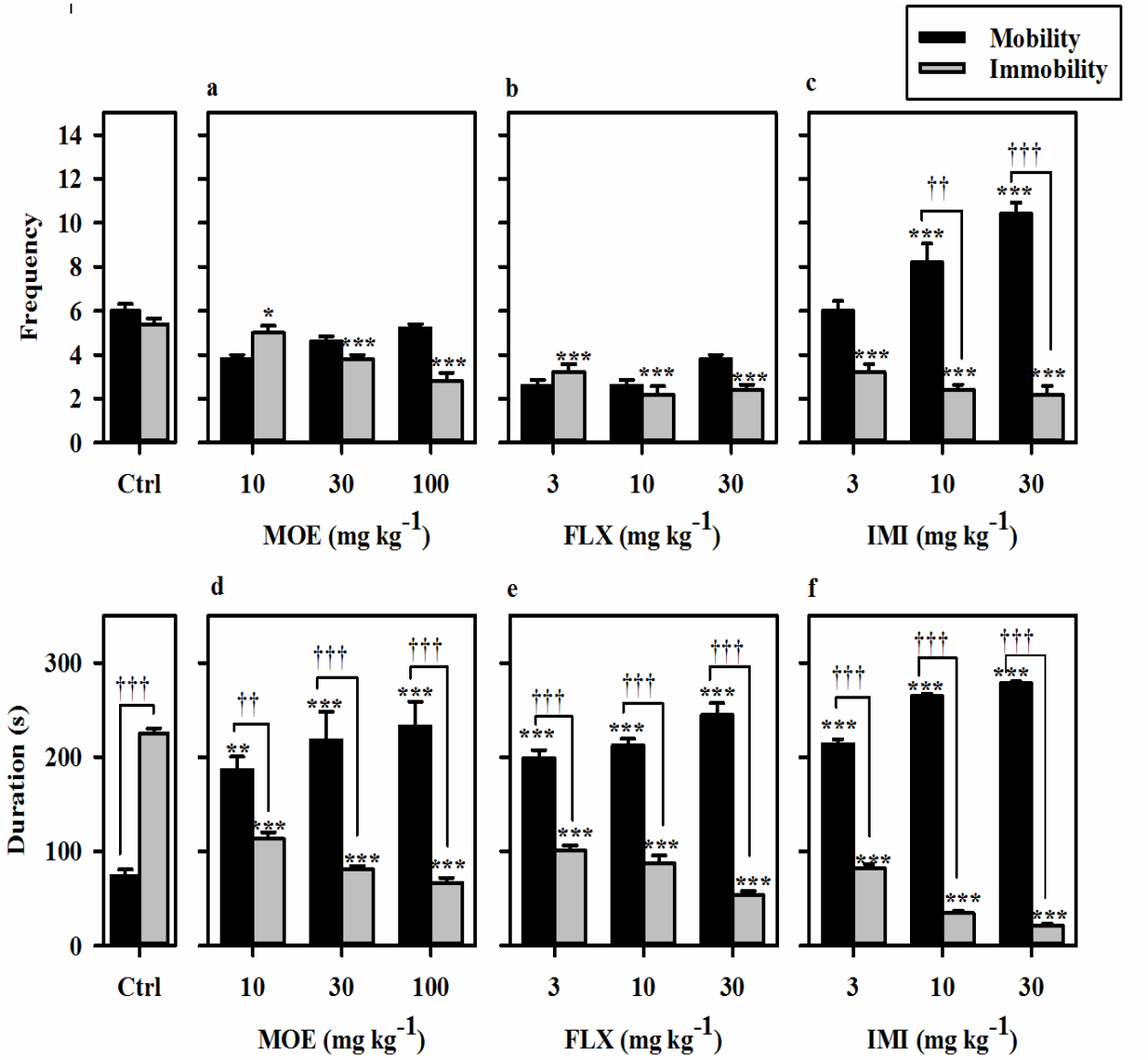

Figure 5.1 Effects of extract, MOE (10 - 100 mg kg^{-1}), fluoxetine, FLX (3 - 30 mg kg^{-1}) and imipramine, IMI (3 - 30 mg kg^{-1}) treatment on (a, b, c) the frequency of mobility and immobility and (d, e, f) duration of mobility and immobility in the FST. Data are presented as group Means ± SEM. Significantly different from control: ***P<0.001; **P<0.01; (One-way ANOVA followed by Newman Keuls' test). †††P<0.001, comparison between effect and dose (Two-way ANOVA followed by Bonferroni's test).

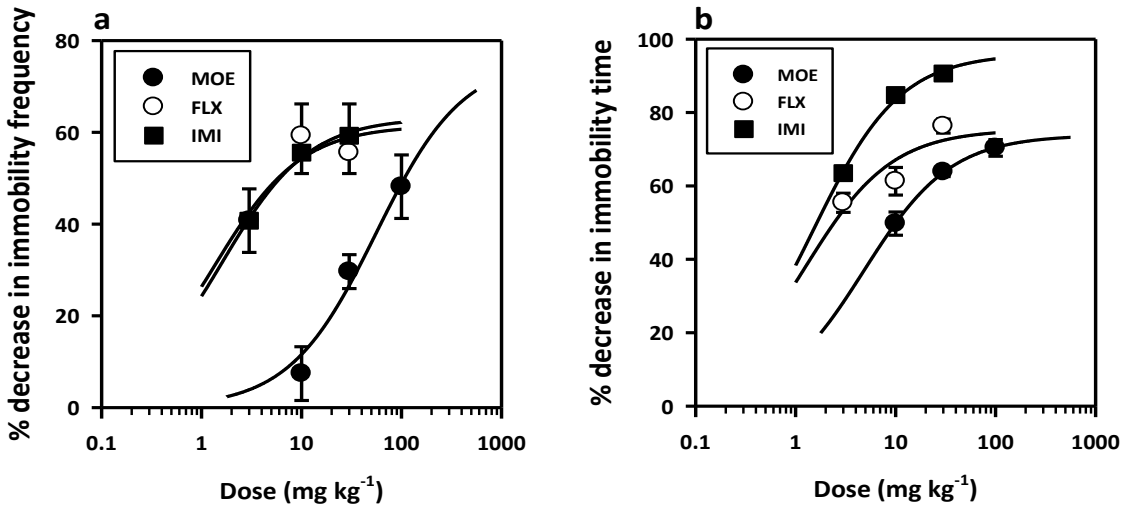

Figure 5.2 Dose–response curves of the extract, MOE (10 - 100 mg kg^{-1}), fluoxetine, FLX (3 - 30 mg kg^{-1}) and imipramine, IMI (3 - 30 mg kg^{-1}) showing (a) % decrease in frequency and (b) % decrease in immobility in the forced swimming test in mice. Each point is the mean ± S.E.M. of 5 animals.

Table 5.1ED_{50} and E_{max} values of drugs used in the forced swim (FST) and tail suspension tests (TST)

Test		Frequency		Duration (s)	
		ED_{50}	E_{max}	ED_{50}	E_{max}
FST					
	MOE	55.7±13.6	76.0	4.8±1.1	73.9
	FLX	1.3±1.3	61.5	1.2±0.5	75.4
	IMI	1.6±1.3	63.2	1.5±0.1	96.1
TST					
	MOE	82.6±13.3	40.0	1.2±1.0	59.1
	FLX	7.2±6.9	39.8	3.1±1.7	82.1
	IMI	32.3±9.2	100	1.7±0.9	85.8

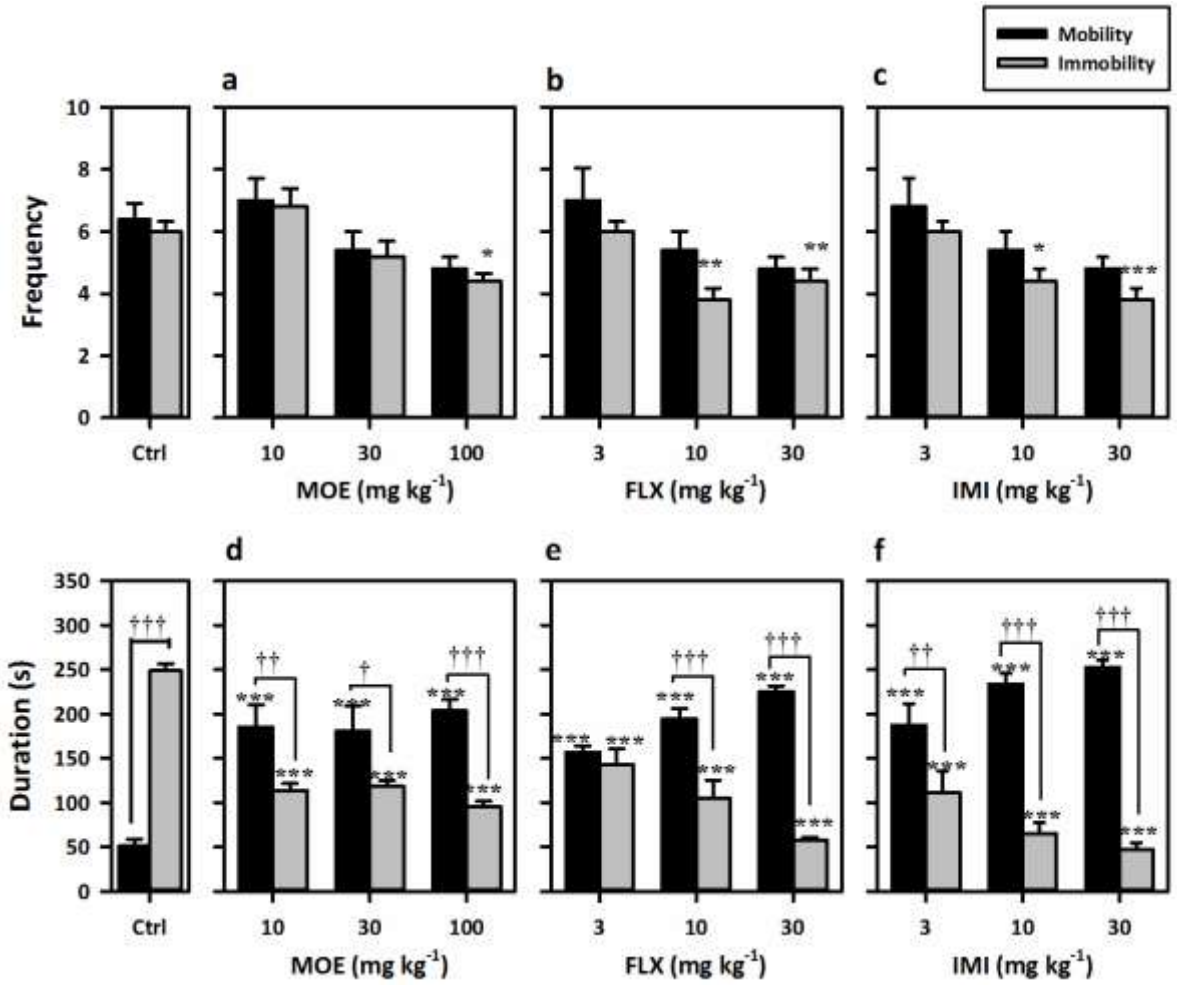

Figure 5.3 Effect of the extract, MOE (10, 30 and 100 mg kg^{-1}), fluoxetine, FLX (3, 10 and 30 mg kg^{-1}) and imipramine, IMI (3, 10 and 30 mg kg^{-1}) treatment on the (a, b, c) frequency of mobility and immobility; and (d, e, f) duration of mobility and immobility in the TST. Data are presented as group Means ± SEM. ***P<0.001; **P<0.01; compared to vehicle-treated group (One-way ANOVA followed by Newman Keuls' test). †††P<0.001, comparison effect and dose (Two-way ANOVA followed by Bonferroni's test).

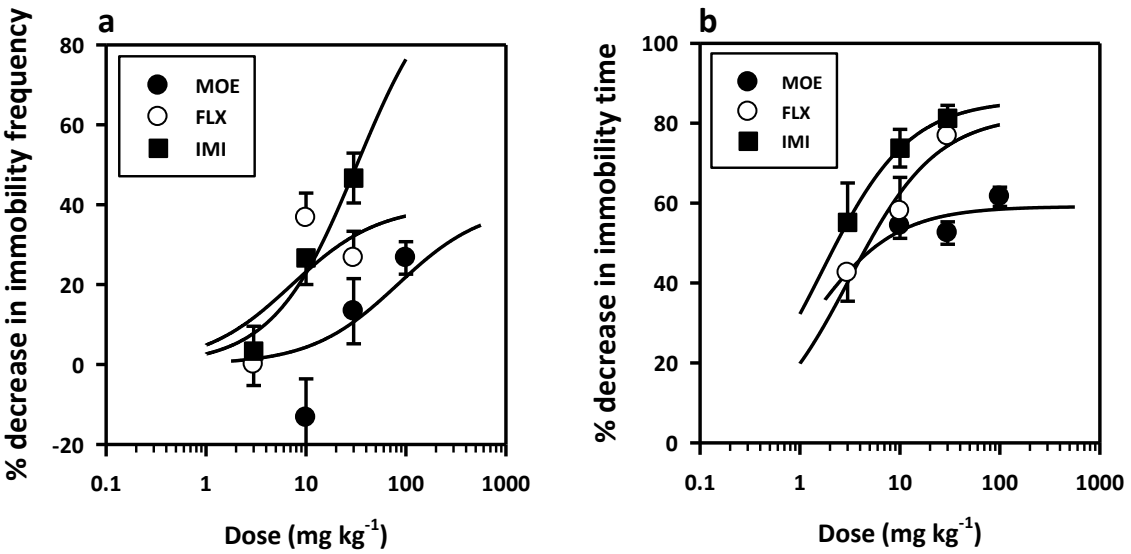

Figure 5.4 Dose–response curves of the extract, MOE (10 - 100 mg kg^{-1}), fluoxetine, FLX (3 - 30 mg kg^{-1}) and imipramine, IMI (3 - 30 mg kg^{-1}) showing (a) % decrease in frequency and (b) % decrease in immobility in the tail suspension test in mice. Each point is the mean ± S.E.M. of 5 animals.

5.3.2 **The open space swim test**

Extract showed significant antidepressant effect in mice exposed to the open space swim test. From the time course curve, decrease in immobility by extract was observed after first day of treatment and this was sustained till the fourteenth day of treatment ($F_{3,216}$=34.48, P<0.0001) (Figure 5.5a). In contrast MOE increased distance travelled by mice ($F_{3,216}$=4.05, P<0.0001) (Figure 5.6a). The graph showing the area under the curve for the extract treated group also showed a dose-dependent decrease in immobility period ($F_{3,36}$=369.8, P<0.0001) (Figure 5.5b). One way ANOVA showed that distance travelled was significantly increased in extract-treated groups at all dose levels ($F_{3,36}$=28.42, P<0.0001) (Figure 5.6b). Both imipramine (Figure 5.5c, d) and fluoxetine (Figure 5.5e, f), the standard antidepressant used, showed a decrease in immobility time after day 7 of treatment but similar effect as extract with regards to distance travelled (Figure 5.6c - f). Though fluoxetine and imipramine were more potent than the extract, the extract was more efficacious in reducing immobility time which is the more reliable index for mice. With regards to distance travelled MOE was less efficacious and potent than fluoxetine and imipramine. See (Figure 5.7 and Table 5.2).

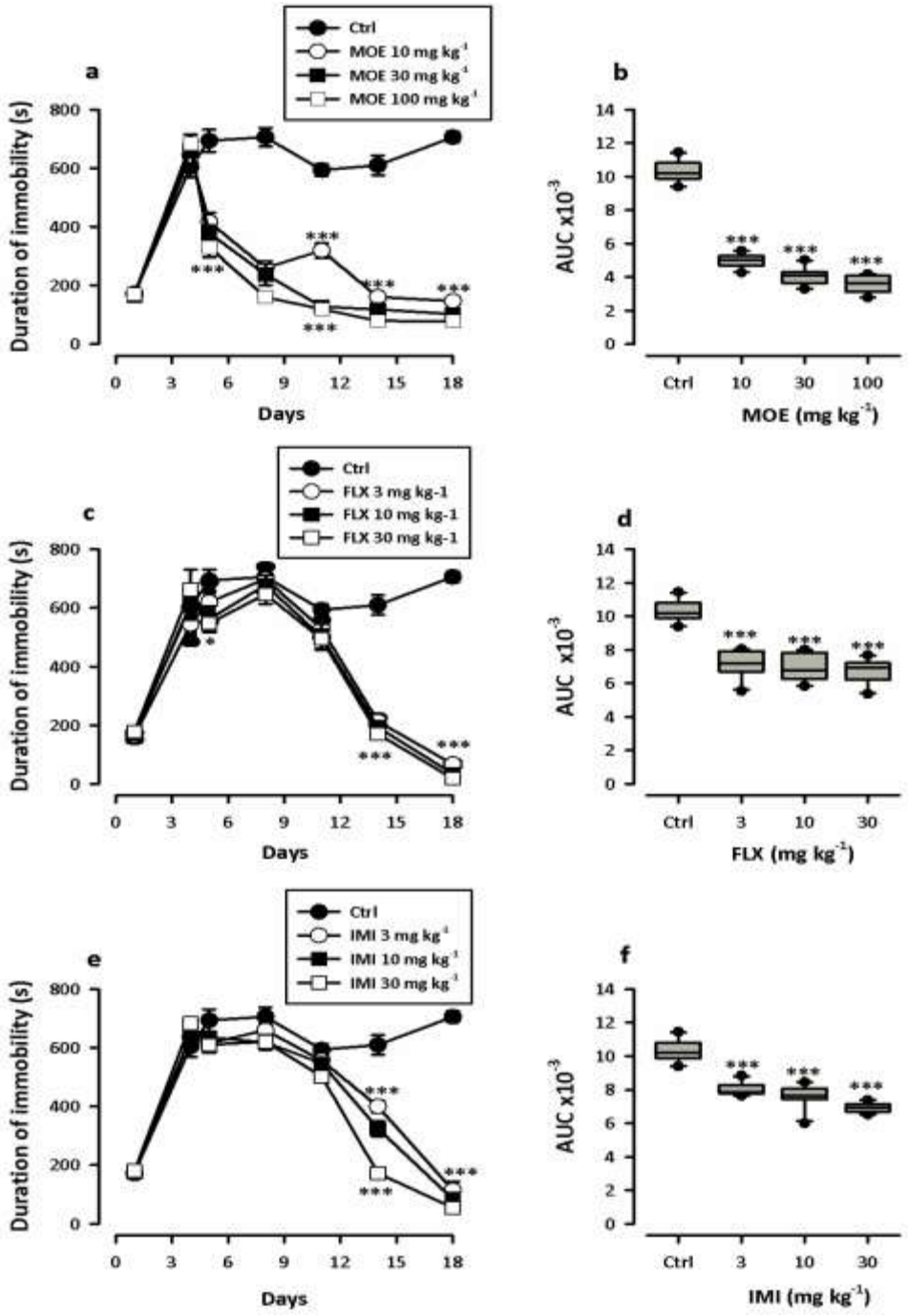

Figure 5.5 Effects of extract, MOE (10 - 100 mg kg^{-1}), fluoxetine, FLX (3 - 30 mg kg^{-1}) and imipramine, IMI (3 - 30 mg kg^{-1}) treatment on the duration of immobility in the open space swim test. Data are presented as both (a, c, e) a time course curve and the (b, d, f) Mean ± SEM of their areas under the curves (AUCs). Significantly different from control: *P<0.05, ***P<0.001 by Newman Keuls' test.

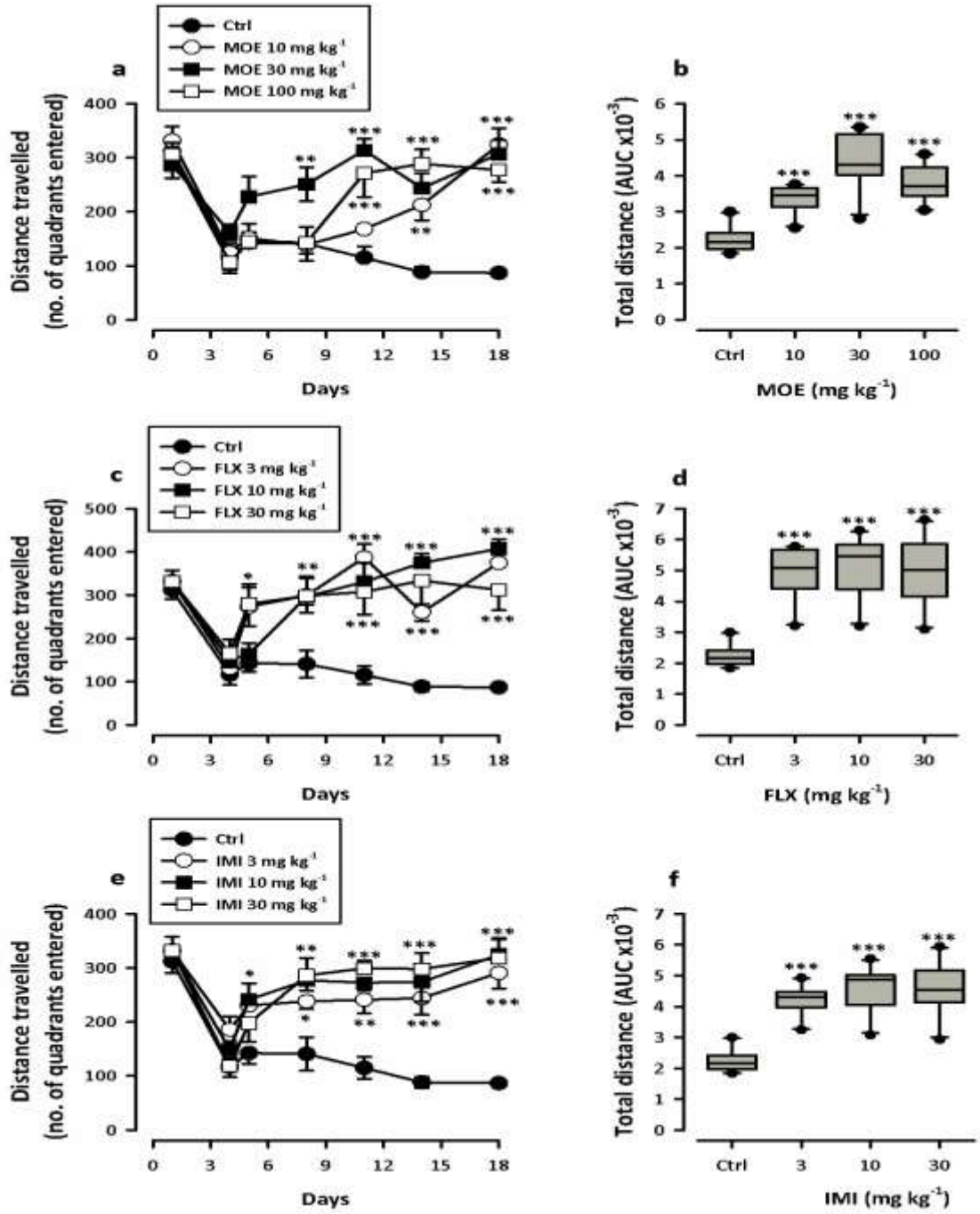

Figure 5.6 Effects of extract, MOE (10 - 100 mg kg^{-1}), fluoxetine, FLX (3 - 30 mg kg^{-1}) and imipramine, IMI (3 - 30 mg kg^{-1}) treatment on the distance travelled in the open space swim test. Data are presented as both (a, c, e) a time course curve and the (b, d, f) Mean ± SEM of their areas under the curves (AUCs). The lower and upper margins of the boxes represent the 25th and 75th percentiles, while the extended arms represent the 10th and 90th percentiles respectively. The horizontal line within the box is the median. Significantly different from control: **P*<0.05, ***P*<0.001, ****P*<0.001 by Newman Keuls' test.

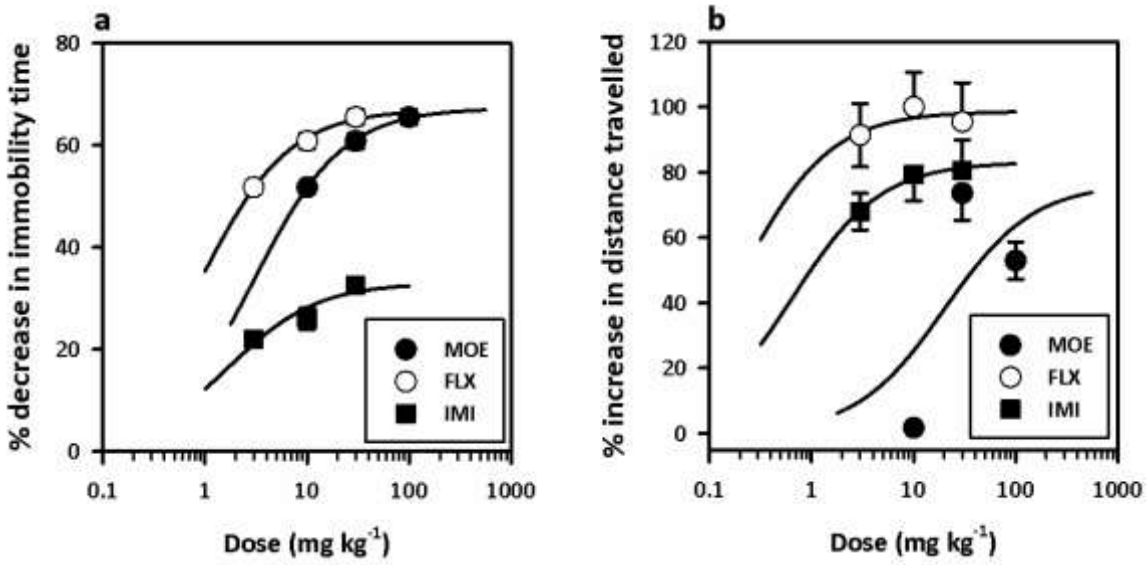

Figure 5.7 Dose–response curves showing the effect of extract, MOE (10 - 100 mg kg^{-1}), fluoxetine, FLX (3 - 30 mg kg^{-1}) and imipramine, IMI (3 - 30 mg kg^{-1}) on (a) % decrease in immobility time and (b) % increase in distance travelled in the open space swim test in mice. Each point is the mean ± S.E.M. of 10 animals.

Table 5.2 ED_{50} and E_{max} values of drugs used in the open space swim test.

Parameters	MOE		FLX		IMI	
	ED_{50}	E_{max}	ED_{50}	E_{max}	ED_{50}	E_{max}
Immobility time	3.0±0.6	67.3	0.9±0.2	67.0	1.7±0.6	32.9
Distance travelled	20.2±6.0	76.5	0.2±2.7	98.7	0.7±0.2	83.1

5.3.3 **Spatial memory and learning in the Morris water maze task.**

Extract improved spatial memory and learning task in mice exposed to open space swim test. From the time course curve, the extract significantly reduced latency for the mice to find the hidden platform from the third day ($F_{4,180}$=23.41, P<0.0001) (Figure 5.8a). Two Way ANOVA revealed that treatment had effect on the behavioural response ($F_{4,45}$=3.37, P<0.0169). The graph showing the area under the curve (AUC) revealed that the extract dose dependently reduced latency to find hidden platform ($F_{4,45}$=3.139, P<0.0233) (Figure 5.8b) . Spatial memory and learning was improved in naïve mice that were not exposed to the open space swim test. Similar results as the extract were obtained for fluoxetine. In contrast imipramine did not show any memory

improvement. See (Figure 5.8c - f). Fluoxetine was the most efficacious in improving memory, followed by the extract then imipramine (Figure 5.9).

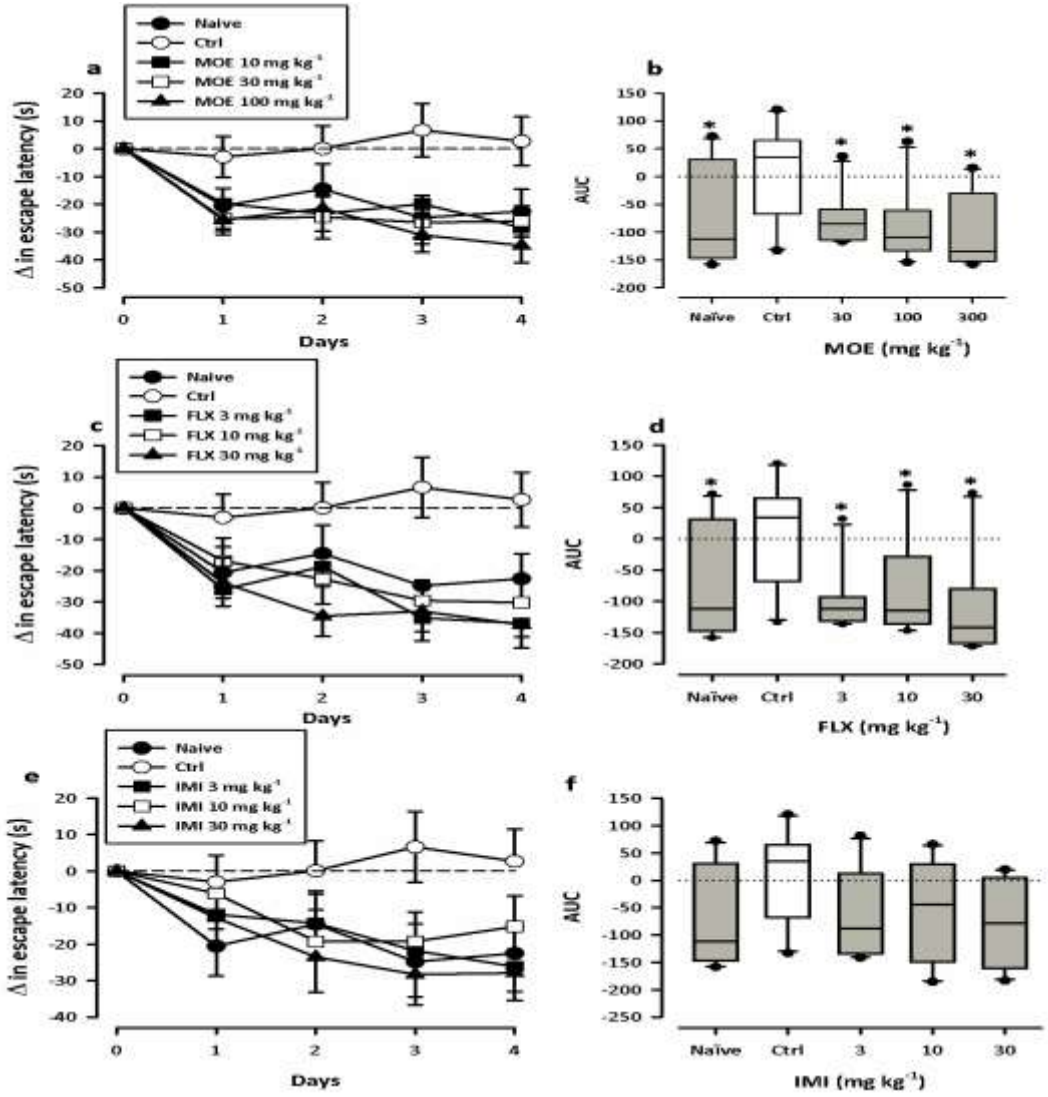

Figure 5.8 Effects of MOE (10 - 100 mg kg^{-1}), fluoxetine, FLX (3 - 30 mg kg^{-1}) and imipramine, IMI (3 - 30 mg kg^{-1}) treatment on the spatial learning and memory in the Morris water maze test. Data are presented as both (a, c, e) a time course curve and (b, d, f) the Mean ± SEM of their areas under the curve (AUCs). The lower and upper margins of the boxes represent the 25th and 75th percentiles, while the extended arms represent the 10th and 90th percentiles respectively. The horizontal line within the box is the median. Significantly different from control: **P*<0.05, ***P*<0.01, ****P*<0.001 by Newman Keuls' test.

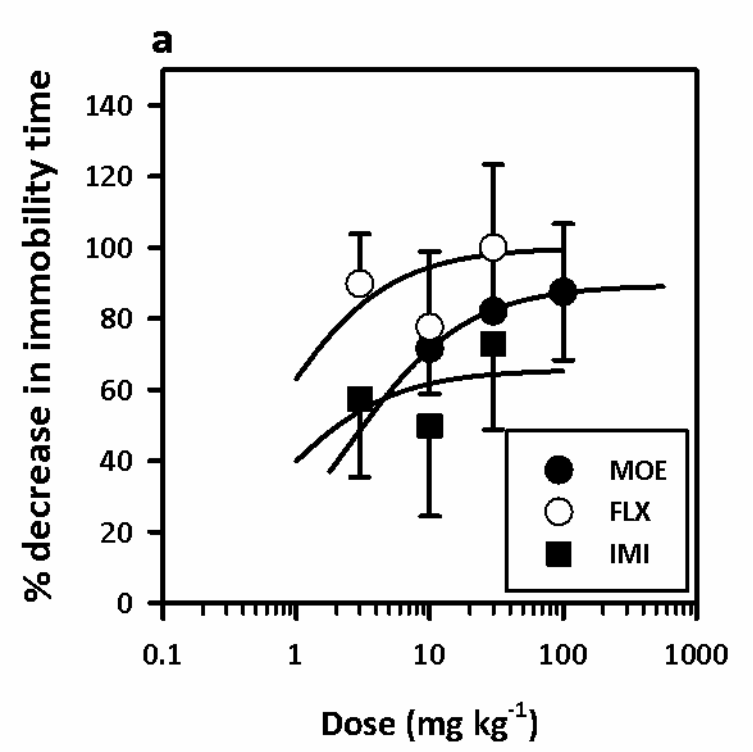

Figure 5.9 Dose–response curves showing the effect of MOE (10 - 100 mg kg^{-1}), fluoxetine (3 - 30 mg kg^{-1}) and imipramine (3 - 30 mg kg^{-1}) on (a) % decrease in the escape latency in the Morris water maze test in mice. Each point is the mean ± S.E.M. of 10 animals.

Table 5.3 ED_{50} and E_{max} values of drugs used in the Morris water maze test.

Drug	Escape Latency	
	ED_{50}	E_{max}
MOE	2.5±1.1	89.4
FLX	0.6±0.3	100
IMI	0.7±0.4	65.7

5.3.4 **Weight variation**

Extract did not have any significant effect on the weight of mice during induction of chronic depression and after the 2 weeks period of treatment (Figure 5.10a, b). Imipramine significantly reduced the weight of the mice at all dose levels whilst the lowest dose of fluoxetine significantly reduced the weight of the mice after the 2 week period (Figure 5.10c - f).

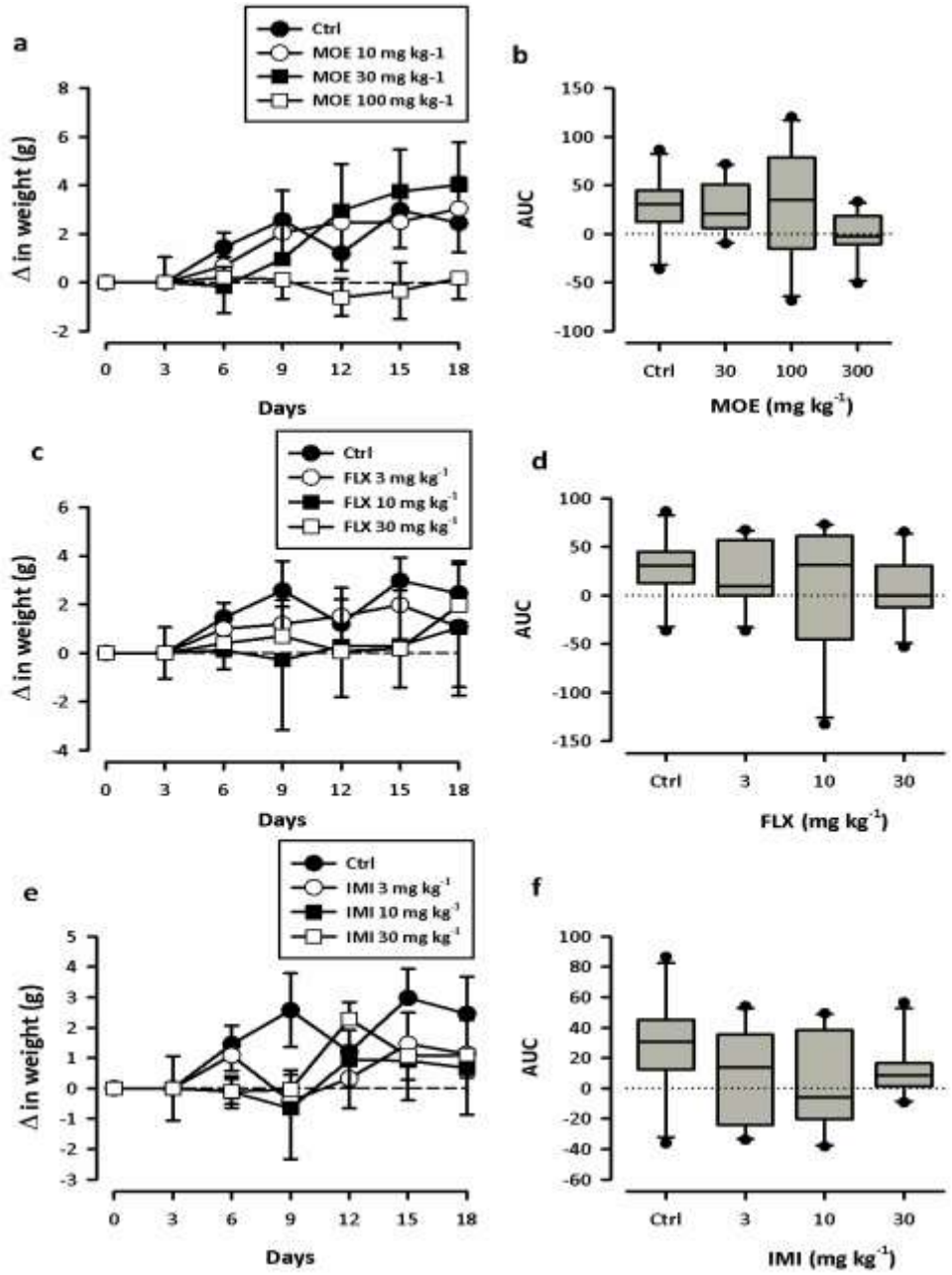

Figure 5.10 Effect of extract, MOE (10 - 100 mg kg^{-1}), fluoxetine, FLX (3 - 30 mg kg^{-1}) and imipramine, IMI (3 - 30 mg kg^{-1}) treatment on weight changes. Data are presented as both (a, c, e) a time course curve and (b, d, f) the Mean ± SEM of their AUCs (n= 10). The lower and upper margins of the boxes represent the 25th and 75th percentiles, while the extended arms represent the 10th and 90th percentiles respectively. The horizontal line within the box is the median.

5.3.5 **Tail suspension test (TST).**

The extract maintained a significant antidepressant effect 24 hours after treatment day fourteen in the TST. It demonstrated a dose dependent decrease in both the absolute immobility period and %

immobility period at all dose levels (Figure 5.11a, d). Similar results were obtained for fluoxetine and imipramine (Figure 5.11b, c, e, and f).

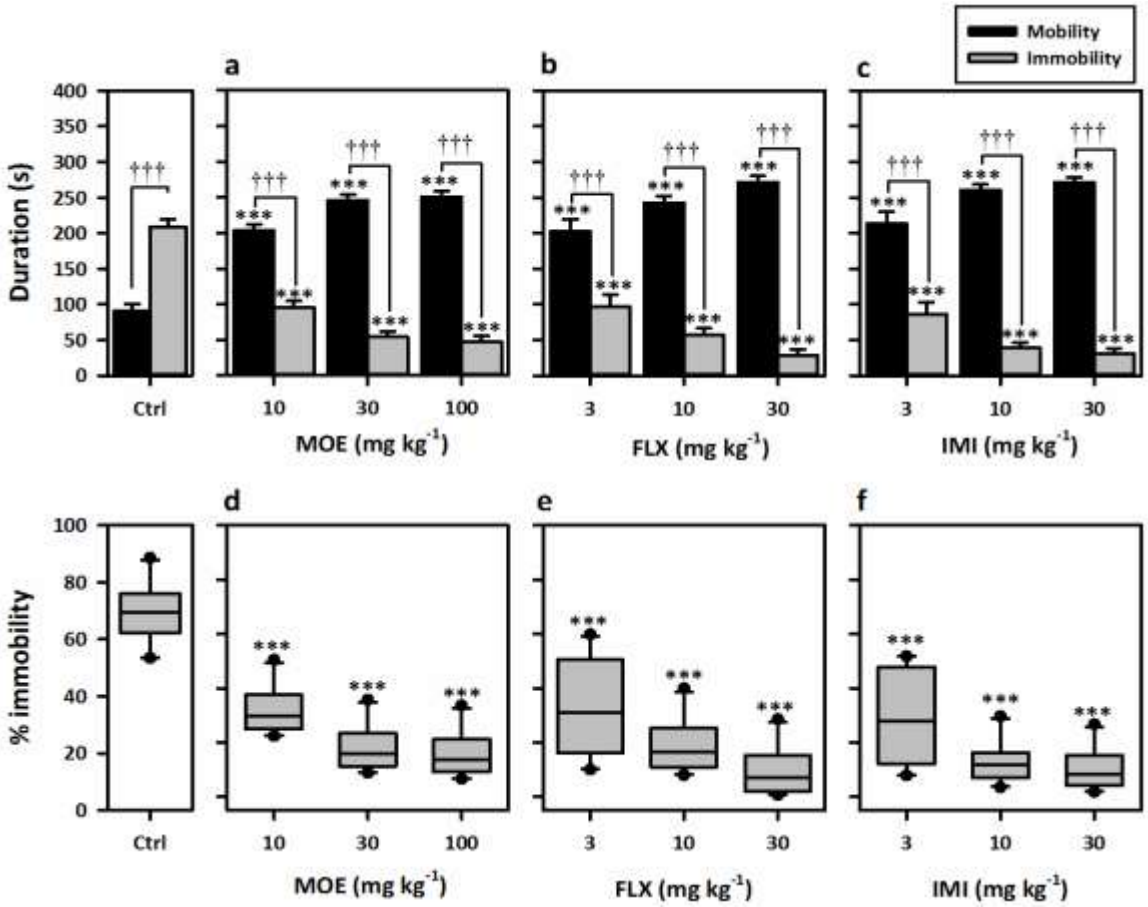

Figure 5.11 Effect of (a) the extract, MOE (10 - 100 mg kg^{-1}), (b) fluoxetine, FLX (3 - 30 mg kg^{-1}) and (c) imipramine, IMI (3 - 30 mg kg^{-1}) treatment on duration of mobility and immobility. (d - f) % Immobility of the extract, MOE, fluoxetine, FLX and imipramine, IMI treatment respectively. ***P<0.001; **P<0.01; compared to vehicle-treated group; (One-way ANOVA followed by Newman Keuls' test). †††P<0.001 comparison between immobility and mobility (Two-way ANOVA followed by Bonferroni's test). Data are presented as group Means ± SEM of 10 animals.

5.3.6 **Involvement of noradrenergic mechanisms**

Pretreatment with either reserpine (1 mg kg^{-1}, s.c.) alone, α-methyldopa (400 mg kg^{-1}, *p.o.*) alone or a concomitant administration of reserpine (1 mg kg^{-1}, s.c.) and α-methyldopa (200 mg kg^{-1}, *p.o.*) did not reverse the decrease in immobility caused by the extract, MOE (10 -100 mg kg^{-1}, *p.o.*) in the forced swim test (FST) (Figure 5.12a, d, g). Results obtained for fluoxetine (FLX) treated groups (3 - 30 mg kg^{-1}, *p.o.*) were similar to that of the extract treated groups (Figure 5.12b, e, h). In contrast, the antidepressant effect of imipramine (IMI) was reversed by either reserpine alone, α-methyldopa alone or a concomitant administration of reserpine and α-methyldopa (Figure 5.12c, f, i).

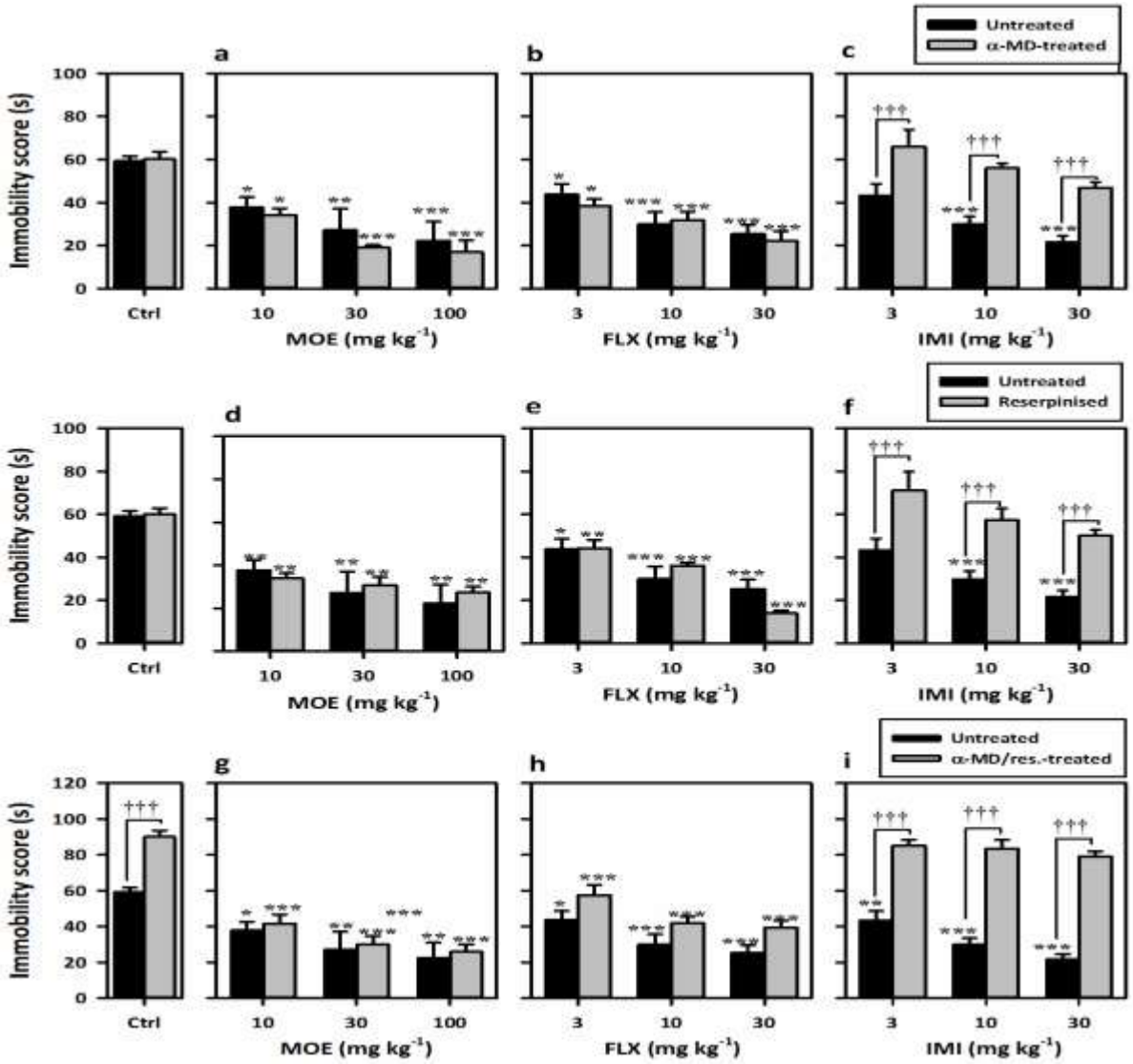

Figure 5.12 Effects of (a - c) reserpine alone; (d - f) α-methyldopa, αMD alone or (g - i) both reserpine and α-methyldopa, on duration of immobility of MOE (10 - 100 mg kg^{-1}, *p.o.*), fluoxetine (3 - 30 mg kg^{-1}, *p.o.*) and imipramine (3 - 30 mg kg^{-1}, *p.o.*), treatment in the FST. Data are presented as Mean ± SEM of 5 animals. Significantly different from control: ***P*<0.01, ****P*<0.001 by One Way ANOVA followed by Newman Keuls' test. †*P*<0.05, ††*P*<0.01, †††*P*<0.001; Significant difference between treatment and dose (Two Way ANOVA with Bonferonni *post hoc* test).

5.3.7 **Involvement of serotoninergic mechanism**

Pretreatment of mice with *p*CPA (200 mg kg^{-1}) abolished the antidepressant effect of MOE (10 - 100 mg kg^{-1}, *p.o.*) and fluoxetine, FLX (3 - 30 mg kg^{-1}, *p.o.*) but not imipramine, IMI (3-30 mg kg^{-1}, *p.o.*) in the FST. The mean counts for immobility ($F_{7,32}$=14.63; P<0.0001) (Figure 5.13a), swimming ($F_{7,32}$=44.74; P<0.0001) (Figure 5.13d) and climbing ($F_{7,32}$=1.121; P=0.3742) (Figure 5.13g) in the extract treated group after *p*CPA treatment did not show any difference when

compared with the control. Similar results as above were observed for FLX-treated groups but not imipramine (Figure 5.13b - c, e - f, h - i). In an attempt to investigate the possible involvement of 5-HT_{2A} receptor activation in the antidepressant action of the extract, mice were given 5-hydroxytryptophan after extract pretreatment to induced head twitch responses. It was observed from the time course curve that the extract as well as fluoxetine increased the head twitch responses significantly for the period of 30 minutes (Figure 5.14a, c). Response peaked after 15 minutes. One-way ANOVA followed by Newman Keuls' test of the areas under the curve (AUCs) showed a dose dependent increase in the head twitch response for both extract and fluoxetine (Figure 5.14b, d). From the dose response curve, fluoxetine (ED_{50}=6.472±0.27) was more potent in activating 5-HT_{2A} receptors than MOE (ED_{50}=41.30±4.51) though their efficacies were comparable (Figure 5.15).

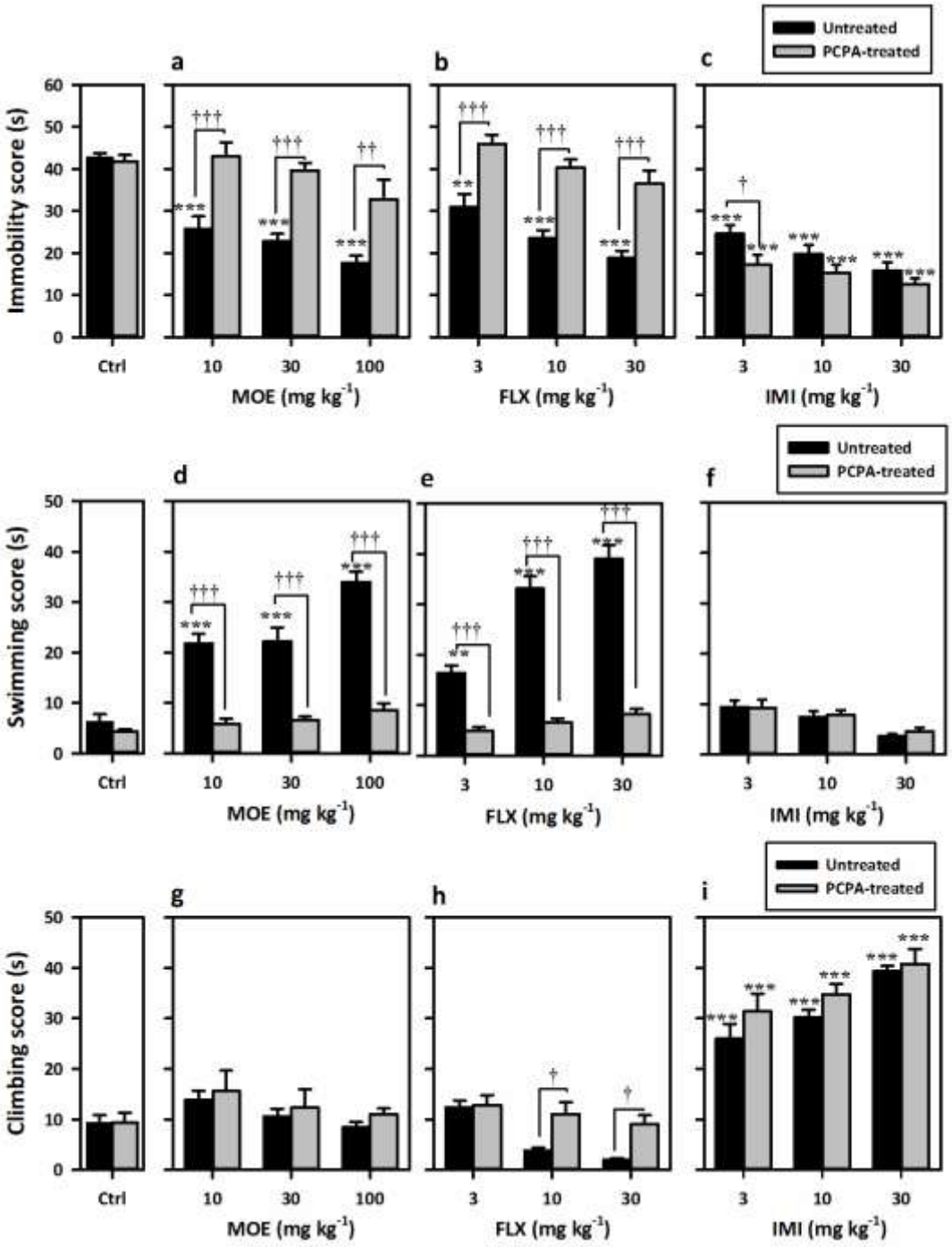

Figure 5.13 Effects of *p*CPA (200 mg kg^{-1}) pretreatment on the (a - c) mean immobility counts; (d - f) swimming counts and (g - i) climbing counts of oral doses of extract, MOE (10 - 100 mg kg^{-1}), fluoxetine, FLX (3 - 30 mg kg^{-1}) and IMI (3 - 30 mg kg^{-1}) treated groups in the FST. Data are presented as mean ± SEM. Significantly different from control: ***P*<0.01, ****P*<0.001 by Newman Keuls' test. †††*P*<0.001: (Two-way ANOVA followed by Bonferroni's post; comparison between drug treatment and dos

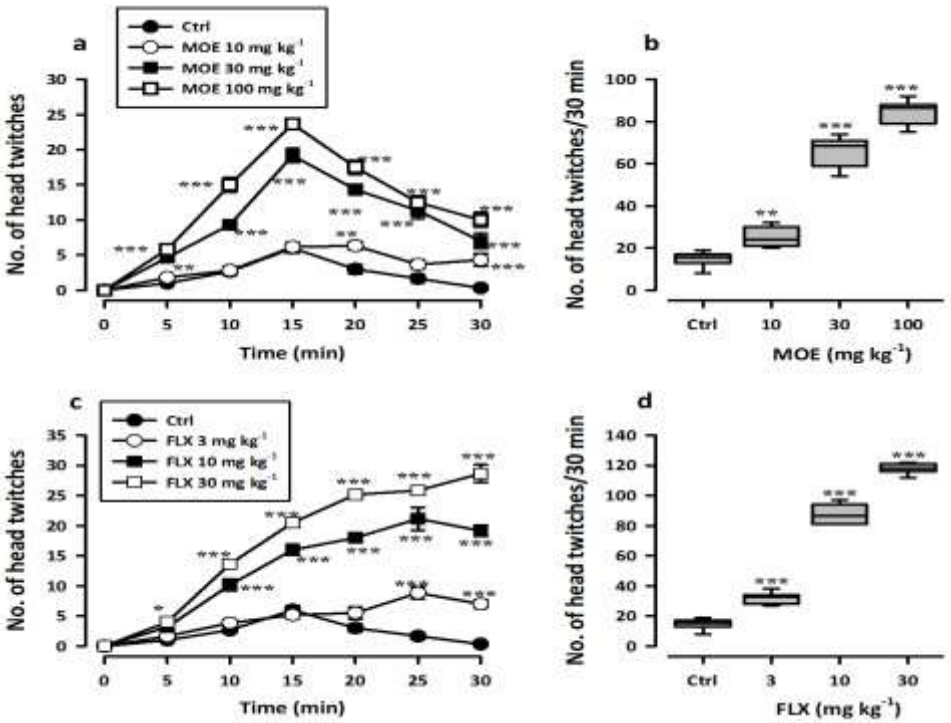

Figure 5.14 Effect of MOE (10 - 100 mg kg^{-1}, *p.o.*) and fluoxetine (3 - 30 mg kg^{-1}, *p.o.*) on the time course curve of head twitch response test (a and c) and their corresponding AUCs (b and d) in the same test represented as box plots. Data was presented as mean ± S.E.M. (n=6); ***P<0.001; **P<0.01; compared to vehicle-treated group (One-way ANOVA followed by Newman Keuls' test). †††P<0.001: (Two-way ANOVA followed by Bonferroni's test; comparison between drug treatment and dose). The lower and upper margins of the boxes represent the 25th and 75th percentiles, while the extended arms represent the 10th and 90th percentiles respectively.

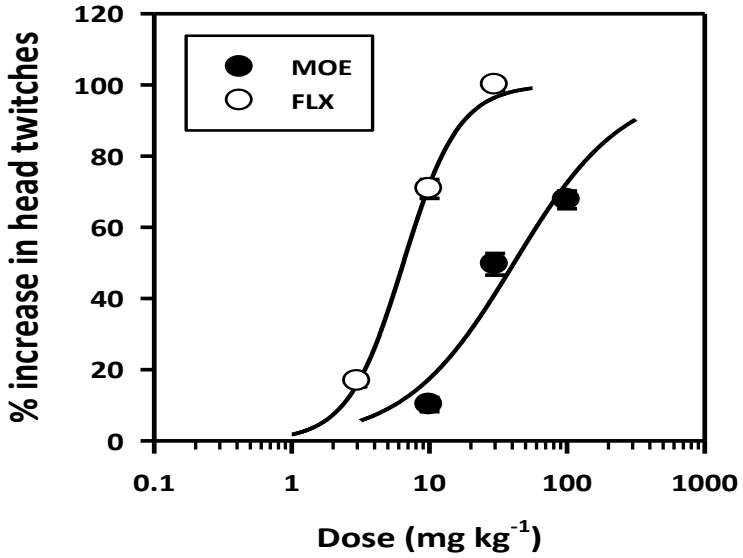

Figure 5.15 Dose–response curves of % increase in head twitches by oral dose of the extract, MOE (10 - 100 mg kg^{-1}) and fluoxetine, FLX (3 - 30 mg kg^{-1}) in response to 5-hydroxytryptophan (5-HTP) administration in mice. Each point is the mean ± S.E.M. of 6 animals.

5.3.8 **Involvement of glycine/NMDA receptor complex**

In the tail suspension test, MOE (100 mg kg^{-1},p.o.), fluoxetine, FLX (10 mg kg^{-1}, *p.o.*) and desipramine, DSP (10 mg kg^{-1}, i.p.), exhibited significant antidepressant effect by decreasing mean immobility score which was reversed by D-serine, DS, (600 mg kg^{-1}, i.p.) pretreatment (Figure 5.16a). Pretreatment with D-cycloserine, DCS, (2.5 mg kg^{-1}, i.p.) potentiated the effect of MOE and FLX (but not DSP) by further decreasing mean immobility score (Figure 5.16b). MOE alone did not affect curling score and this was not changed by DS pretreatment (Figure 5.16e). FLX and DSP alone, caused slight increase in the curling score which was reversed by DS. Pretreatment with DCS significantly increased curling score of MOE but caused only a modest increase in both FLX and DSP treated groups (Figure 5.16f). MOE, DS, DCS, FLX and DSP alone increased swinging score. DS pretreatment partially inhibited swinging behaviour by MOE but totally in FLX and DSP treated groups (Figure 5.16c). DCS pretreatment also inhibited swinging behaviour by MOE and DSP but not FLX (Figure 5.16d). In the forced swim test, MOE, FLX and DSP, decreased immobility score and this was reversed by DS pretreatment (Figure 5.17a). Pretreatment with DCS potentiated the effect of MOE and FLX (but not DSP) by further decreasing immobility score (Figure 5.17b). MOE and FLX unlike DSP, increased swimming behaviour which was inhibited by DS but increased by DCS pretreatment (Figure 5.17c and d). Climbing scores were decreased by both MOE and FLX and this was not affected by both DS and DCS pretreatment (Figure 5.17e and f). DSP on the contrary, increased climbing score which was unaffected by DCS pretreatment but decreased by DS.

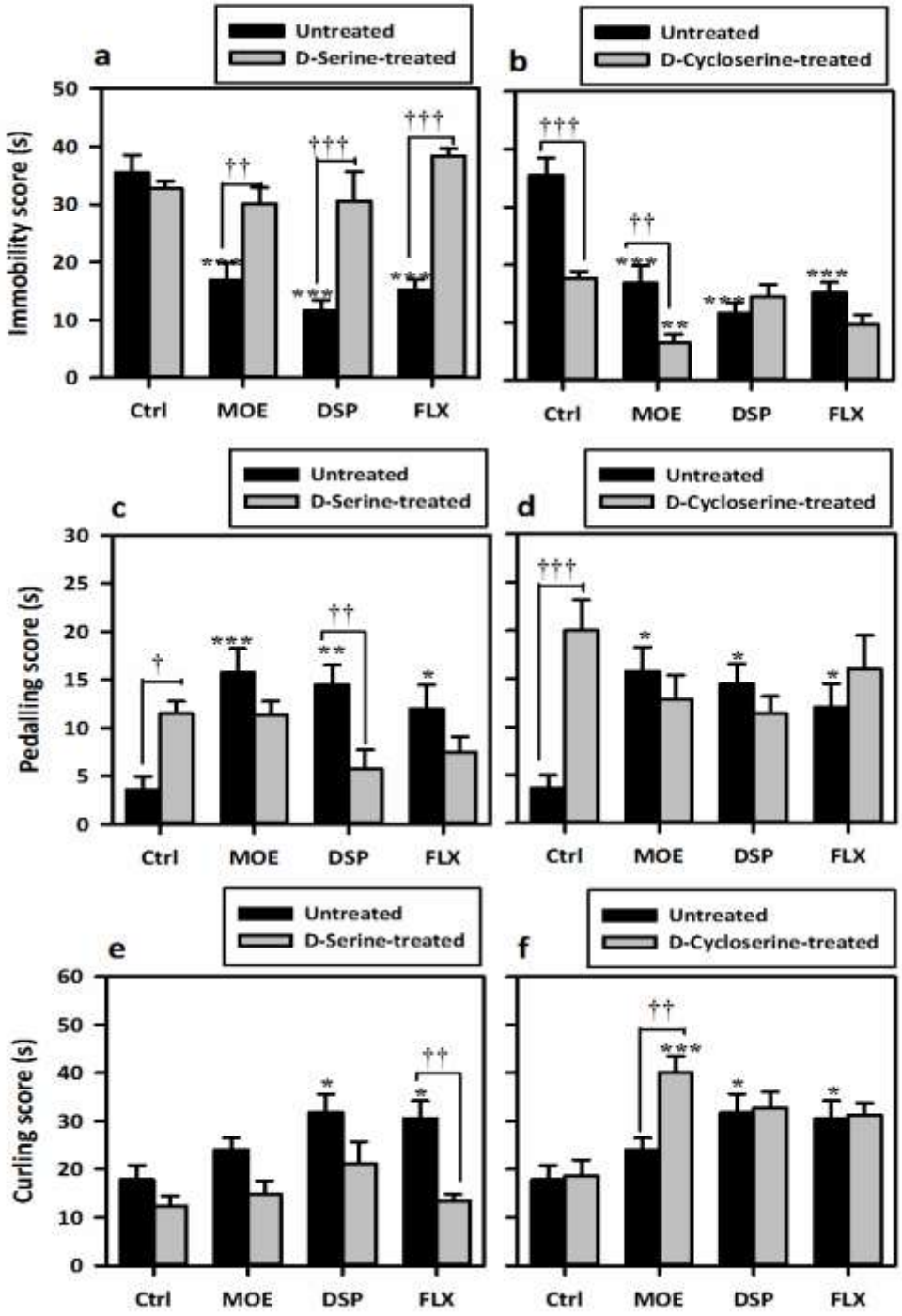

Figure 5.16 Effects of D-serine (DS) or D-cycloserine (DCS) pretreatment on (a, b) mean immobility count, (c, d) pedalling count and (e, f) curling count of extract, MOE (100 mg kg^{-1}), fluoxetine, FLX (10 mg kg^{-1}) and desipramine, DSP (10 mg kg^{-1}) treatment in the tail suspension test (TST). Data are presented as Means ± SEM. Significantly different from control: ***P*<0.01, ****P*<0.001 by Newman Keul's test. Significant difference between treatments: †*P*<0.05, ††*P*<0.01, †††*P*<0.001.

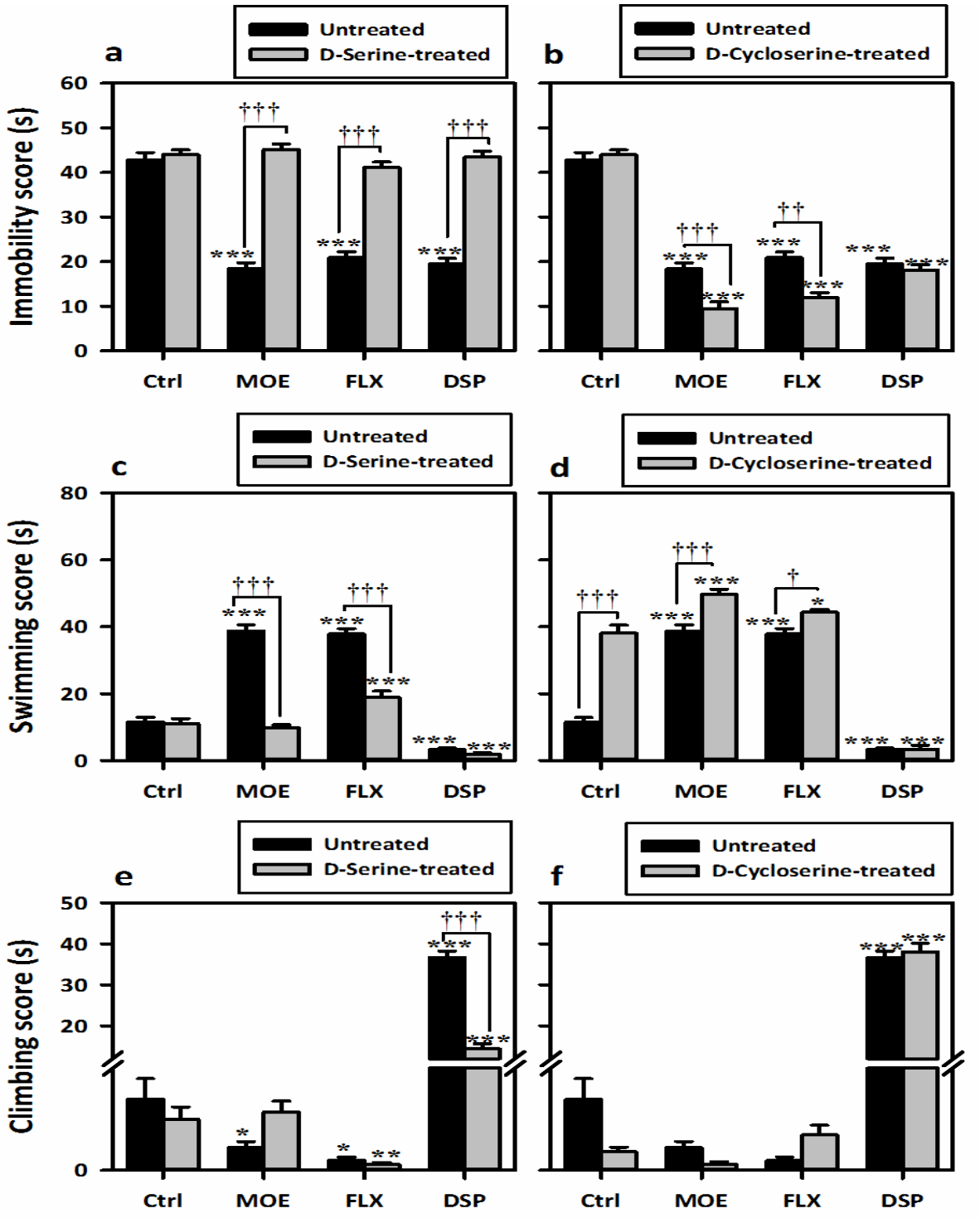

Figure 5.17 Effects of D-serine (DS) or D-cycloserine (DCS) pretreatment on (a, b) mean immobility count, (c, d) swimming count and (e, f) climbing count of extract, MOE (100 mg kg^{-1}), fluoxetine, FLX (10 mg kg^{-1}) and desipramine, DSP (10 mg kg^{-1}) treatment in the forced swimming test (FST). Data are presented as Means ± SEM. Significantly different from control: ***P*<0.01, ****P*<0.001 by Newman Keul's test. Significant difference between treatments: †*P*<0.05, ††*P*<0.01, †††*P*<0.001.

5.4 DISCUSSION

Depressed patients often have suicidal tendencies and the delay in improvement of symptomatology as observed with most antidepressants may increase mortality if medications with faster onset and sustained effect are not sought (Lucas, 2008). The goal of the present study was to ascertain first of all whether the hydroalcoholic leaf extract of *Mallotus oppositifolius* has antidepressant action and to further assess the contributions of monoamine neurotransmitters, to the behavioural effects of MOE. The possible interaction of MOE with the glycine coagonist site of NMDA receptors as a means of investigating possible effect on glutamatergic neurotransmission was also evaluated.

The results of the study demonstrated that MOE has significant antidepressant effect in both the forced swimming and the tail suspension tests. In both animal models, percentage immobility and frequency of immobility were decreased by extract treatment. Reduction in immobility has been used as the primary index for antidepressant effect of test substances in these models—almost all antidepressant in clinical use induce a decrease in immobility in rodents whilst other drugs fail to give the same response (Cryan *et al.*, 2005; Petit-Demouliere *et al.*, 2005). MOE exhibited a rapid and sustained antidepressant effect in the chronically depressed mice. The extract reduced immobility significantly and increased distanced travelled, the primary indices of antidepressant effect, in the open space swim test (Sun and Alkon, 2003; Stone and Lin, 2011).

Preclinical and clinical studies suggest that depletion of a monoamine implicated in depression pathophysiology may abolish the antidepressant effect of a substance if the substance depends on that particular monoamine for its antidepressant effect (Delgado *et al.*, 1991; 1999; O'Leary *et al.*, 2007). Hence when 5-HT was depleted by pretreating mice for 3 days with the tryptophan hydroxylase inhibitor para-chlorophenylalanine (*p*CPA), the effects of drugs that act by enhancing 5-HT neurotransmission were abolished (Eckeli *et al.*, 2000; Gavioli *et al.*, 2004) while those that act on noradrenergic pathways were not affected (Lucki *et al.*, 1994; Page *et al.*, 1999; Page and Lucki, 2002; Gavioli *et al.*, 2004). The inhibition of the antidepressant effect of MOE by *p*CPA in both TST and FST suggests that its antidepressant effect is dependent on enhancement of serotoninergic neurotransmission. The lack of antidepressant effect of fluoxetine in *p*CPA treated mice is consistent with the hypothesis that fluoxetine elicits its acute behavioural effects by increasing extracellular 5-HT after blockade of the serotonin transporter (Bymaster *et al.*, 2002). The extract and fluoxetine increased swimming score which was reversed with *p*CPA pretreatment, further supporting their action on the serotoninergic system (Page *et*

al., 1999; Chau *et al.*, 2011). Both MOE and fluoxetine did not affect mean climbing score, suggesting that their behavioural effect may not depend on noradrenergic pathways. Further evidence suggesting that the extract enhances 5-HT neurotransmission was derived from its ability to increase head twitch responses induced by 5-HTP. The head twitch response (HTR) in rodents induced by 5-hydroxytryptophan (5-HTP), a precursor of 5-HT (Corne *et al.*, 1963), is considered as a specific behavioural model for the activation of serotoninergic neuron specifically 5-HT_{2A} receptors (Schreiber and Pick, 1995). Thus it can be inferred that MOE may be acting via activating 5-HT_{2A} receptors. Fluoxetine also increased the frequency of HTRs. This result is consistent with a number of studies where fluoxetine elicited similar responses (Essman *et al.*, 1994; Xu *et al.*, 2006).

The present experiments also examined the role of noradrenaline and dopamine in the acute behavioural effects of the extract in the modified FST by using drugs that interfere with their neurotransmitter synthesis or release. Depletion with α-methyldopa, an L-aromatic amino acid decarboxylase inhibitor that inhibits the biosynthesis of catecholamines and 5-HT (DeMuth and Ackerman, 1983) failed to attenuate the behavioural effects of MOE and fluoxetine while that of imipramine was abolished. This suggests that MOE may not affect the biosynthesis of noradrenaline or dopamine. Moreover when vesicular pools were depleted by reserpine, the decrease in immobility elicited by MOE or fluoxetine was not affected. Here again the effect of imipramine was attenuated. Reserpine is an irreversible inhibitor of the vesicular monoamine transporter 2 (VMAT-2) which is located primarily within the CNS and is responsible for transporting monoamines from the cytoplasm into secretory vesicles (Metzger *et al.*, 2002; Ji *et al.*, 2007). Treatment with reserpine therefore leads to depletion of vesicular monoamine stores- both serotonin and noradrenaline (Fukui *et al.*, 2007) suggesting both serotonin and noradrenaline might be important in the antidepressant effects of imipramine. The inability of reserpine pretreatment to reverse the antidepressant effects of the extract and fluoxetine however, seem to suggest that reserpine does not affect vesicular storage of 5-HT to the same extent as that of noradrenaline. In fact this assertion is consistent with the results obtained by O'Leary *et al*., (2007) and Woode *et al*., (2010)—the former demonstrating that reserpine at the dose used produced a 93 and 95% depletion of cortical noradrenaline and dopamine content respectively, and a 78% depletion of 5-HT. To inhibit both synthesis and deplete vesicular pools of noradrenaline and dopamine, mice were pretreated with both reserpine and α-methyldopa. Results were similar to effects observed when mice were treated with reserpine alone. The work published by O'Leary *et al*., (2007) indicated that when reserpine was combined with α-methyl para-tyrosine AMPT (NE and DA biosynthetic inhibitor), a depletion of cortical DA (95%), NE (97%), and 5-HT (78%) was observed. The combination had only a modest

effect on NE and DA but failed to affect 5-HT. Though α-methyldopa was used instead of the AMPT used by O'Leary and colleagues, the results from the combined effect of reserpine and α-methyldopa did not differ significantly from when reserpine was used alone. A more recent by Woode *et al*., (2010) used α-methyldopa and similar results was observed. These results demonstrate that the antidepressant effect of MOE may not be dependent on noradrenergic neurotransmission.

Both clinical and preclinical studies support the antidepressant activity of antagonists on functional glycine/NMDA receptor complex. These compounds are thought to have lower side effect profiles compared to the competitive and non-competitive NMDA antagonists (Poleszak *et al.*, 2011). The effect of MOE on glutamatergic neurotransmission was assessed by pretreating mice with D-serine (DS), a full agonist on glycine/NMDA receptors or D-cycloserine (DCS), a partial agonist on these receptors. In both the TST and FST, DS reversed the decline in immobility by MOE and fluoxetine but DCS pretreatment potentiated the decline, demonstrating that MOE may be acting as an antagonist on the glycine/NMDA receptor complex. In contrast, the decrease in immobility of desipramine was reversed by DS but DCS had no effect on it. This suggests that the antidepressant effect of both serotonin and noradrenaline-based compounds depend on the inhibition of the glycine/NMDA receptor complex but the enhancement of antidepressant activity depends on the serotoninergic pathway and not the noradrenaline pathway (Poleszak *et al.*, 2011). This explains why the antidepressant effect of the extract, fluoxetine and desipramine was abolished by D-serine but only the effect of the extract and fluoxetine (which act via serotoninergic pathway) were potentiated by D-cycloserine. Moreover MOE increased curling score in the TST slightly (suggestive of opioidergic activity) though not significant. Pretreatment with DCS significantly increased curling score of MOE but did not affect both FLX and DSP treated groups. DS pretreatment partially inhibited pedalling behaviour by MOE but totally in FLX and DSP treated groups. DCS pretreatment also inhibited pedalling behaviour by MOE and DSP but not FLX. According to Berrocoso *et al*., (2012), opioids decrease immobility score while increasing curling behaviour in mice. This suggests that the extract on its own may have little effect on opioidergic activity but may interact with opioid receptors when combined with DCS. In the FST, MOE and FLX unlike DSP increased swimming behaviour which is sensitive to selective serotonin reuptake inhibitors (SSRIs) and 5HT agonists (Cryan and Lucki, 2000; Cryan *et al.*, 2005). This behaviour exhibited by MOE and fluoxetine treated mice was inhibited by DS but increased by DCS pretreatment further supporting the theory that antidepressant effect of serotonin based drugs depend on the inhibition of the glycine/NMDA receptor complex and the enhancement of antidepressant activity depends on the serotoninergic pathway

(Poleszak *et al.*, 2011). Climbing scores were decreased by both MOE and FLX and this was not affected by both DS and DCS pretreatment. This confirms their lack of noradrenergic activity. DSP, a selective noradrenergic reuptake inhibitor, on the contrary increased climbing score which was unaffected by DCS pretreatment but decreased by DS. It is worth mentioning that the extract, fluoxetine, desipramine alone or their combination with either D -serine or D -cycloserine did not impair motor coordination. Thus the behavioural effect observed can be attributed to drug treatment alone.

The enhancement of serotoninergic mechanisms and inhibition of glycine/NMDA systems observed in the acute models may be responsible for the antidepressant-like effect of MOE in the open space swim test. This assumption is consistent with findings that suggest that chronic treatment with antidepressants acting via serotoninergic pathways enhance serotoninergic neurotransmission (Owens, 1996; Ceglia *et al.*, 2004; Faure *et al.*, 2006). Another explanation that can be attributed to the effectiveness of MOE in the chronic model is its possible effect on neurotrophic factors. Successful antidepressant treatments following chronic administration are associated with an enhancement of hippocampal neurogenesis, increase in neurotrophin levels, and a resultant increase in hippocampal function which can be measured as neuronal response or behaviour (Duman and Monteggia, 2006; Monteggia *et al.*, 2007; Sahay and Hen, 2007). This has been substantiated by the ability of chronic SSRIs administration of fluoxetine or citalopram, along with other antidepressants and electroconvulsive shock to increase hippocampal cell proliferation and increase the expression of BDNF (Nibuya *et al.*, 1995; Malberg *et al.*, 2000; Balu and Lucki, 2009). It is possible that the sustained antidepressant effect of the extract may be partly due to effect on neurotrophic factors.

According to Stone and Lin, depressed mice in the open space swim test model respond only to chronic treatment ($\geq$ 2 weeks) and not to acute or subacute treatment (Stone and Lin, 2011). While this delay in the onset of action was observed in imipramine and fluoxetine treated mice, it was not so in the extract treated groups. Antidepressant effect in this model with regards to immobility period which is deemed the more consistent index in mice (Stone *et al.*, 2008; Stone and Lin, 2011) began on the first day of treatment. The complex array of neuropharmacological changes, such as desensitization of 5-HT autoreceptors ($5HT_{1A}$ and $5HT_{1B/D}$) and transporters, downregulation of neurotransmitter receptors, changes in signal transduction, mobilization of neurotrophins, and increases in hippocampal neurogenesis that emerge after chronic treatment with SSRIs may be involved in their clinical efficacy and the time taken for these processes to occur may contribute to the time lag in their clinical onset of action (Caldecott-Hazard and Schneider, 1992;

Pineyro and Blier, 1999; Frazer and Benmansour, 2002; Duman and Monteggia, 2006; Tardito *et al.*, 2006; Sahay *et al.*, 2007). For a faster onset of action drugs may act as antagonist at presynaptic 5-HT_{1A} receptors or bypass the inertia of the autoinhibitory mechanisms of the serotonin receptors (Zanardi *et al.*, 1997; Artigas *et al.*, 2001; Adell *et al.*, 2005). This quick effect has been the basis for adding buspirone , a partial agonist at 5-HT_{1A} receptors or pindolol, as adjuncts to SSRIs in depression (Dimitriou and Dimitriou, 1998; Keks *et al.*, 2007). The inhibitory effect of the 5-HT_{1A} autoreceptors is one of the reasons for the delay in onset of action of antidepressants affecting 5-HT systems (Blier and de Montigny, 1994; Moret, 2005). Moreover drugs that activate the $5HT_4$ receptors demonstrated faster onset of action in vitro and in animal models that were known to respond to chronic treatment only (Lucas *et al.*, 2007; 2010). Additionally, 1-aminocyclopropanecarboxylic acid, a partial agonist on the glycine/NMDA site produced a faster onset of action than biogenic amine-based agents in the chronic mild stress model (Cherkofsky, 1995; Papp and Moryl, 1996; Poleszak *et al.*, 2011). It is therefore not surprising that the extract, which enhances serotoninergic neurotransmission and antagonize glycine/NMDA system, should demonstrate a rapid onset of behavioural effect in the open space swim test. It is interesting to note that the antidepressant effect of the extract was sustained throughout the treatment period. In fact a day after the fourteenth day of treatment, the behavioural effect of the extract was still maintained in the tail suspension test, one of the widely used and validated models of antidepressant.

Depression in humans has dramatic impact on cognition, including memory and learning (Troster *et al.*, 1995; Kuzis *et al.*, 1997; Dolan, 2002; Ravnkilde *et al.*, 2002; Uekermann *et al.*, 2003). Sun and Alkon (2004) have demonstrated that the induced depressive behaviour in rodents has a lasting impact (at least for days) on learning and memory and that the impairment is sensitive to antidepressant treatment. Findings in this study support this fact. The time taken to locate the hidden platform was higher (indicative of cognitive impairment) in untreated depressed mice but reduced with treatment. In extract treated mice, naïve unswum mice or fluoxetine treated mice latency to find the hidden platform decreased on daily basis but not in the depressed untreated mice or imipramine treated mice. The role of the serotoninergic and the glycine/NMDA systems in memory has been well documented (Buhot *et al.*, 2000; Meneses, 2002; Williams *et al.*, 2002). Converging evidence indicates that activation of $5HT_{2A/2C}$ and $5HT_4$ receptor agonists prevent memory impairment and facilitate learning while their antagonists have opposite effect (Buhot *et al.*, 2000; Harvey, 2003). It is therefore not surprising that MOE should improve memory since it potentiated the 5-hydroxytryptophan-induced head twitch response, a model sensitive to $5HT_{2A}$ receptor activation (Sun *et al.*, 2003; Fantegrossi *et al.*, 2010). Though some studies

suggest that NMDA antagonists impair memory (Hadj Tahar *et al.*, 2004; Rowland *et al.*, 2005), there have also been reports about their beneficial effects on memory and learning (Si *et al.*, 2004; Parsons *et al.*, 2007; Dashniani *et al.*, 2010; Lockrow *et al.*, 2011). For instance memantine, an NMDA antagonist have been shown to be value in conditions like Alzheimer's, dementia and Parkinson's disease where memory is impaired (Rogawski, 2000; Reisberg *et al.*, 2003; Ota and Godwin, 2006; Lockrow *et al.*, 2011). It is possible that the inhibitory effect of the extract against glycine/NMDA receptor complex or pathway also contributed to the memory improvement in the Morris water maze test.

Significant weight changes (increase or decrease) are found among many depressed patients (Polivy and Herman, 1976; Anderson *et al.*, 2006). This could be attributed to the condition and/or antidepressant-induced adverse effects (Weissenburger *et al.*, 1986; Fava, 2000; Dixon *et al.*, 2003; Roberts *et al.*, 2003; Zimmermann *et al.*, 2003). Serotonin is a neurotransmitter that has significant influence on appetite and therefore weight changes (Wurtman, 1993; Halford and Blundell, 2000; Finlayson *et al.*, 2007). Considering this fact and that MOE interact with serotoninergic pathways, effect of extract on weight variation was investigated. Results indicated that the extract did not affect the weight of the mice significantly although slight weight reductions were observed at all dose levels.

5.5 CONCLUSION

This study provides support not only to the multifaceted aspects of depression as a syndrome but also to the fact that antidepressants can treat some of these comorbid states. It is also evident that the extract has a rapid and sustained antidepressant effect in the open space swim test without affecting the weight of the mice—the plant could be a possible source of bioactive compounds with faster onset of antidepressant action. Moreover the extract improved the spatial learning and memory in mice. MOE demonstrated its behavioural effects by interacting with serotoninergic pathway (e.g. activating 5-HT_{2A} receptors) as well as the glycine/NMDA receptor complex.

Chapter 6
ANXIOLYTIC EFFECT

6.1 INTRODUCTION

Anxiety is a normal and adaptive state of autonomic arousal and behavioural defence in response to threat but can become pathological when it increases in scope, magnitude, or duration (Nesse, 1999). Anxiety disorders are the most common mental illness in the world (Rabbani *et al.*, 2008). Moreover, complaints of anxiety are common among healthy individuals and have been associated with numerous negative health consequences (Muller *et al.*, 2005; Balon, 2006; Scott *et al.*, 2007), absenteeism (Hoffman *et al.*, 2008), and decreased work productivity (Sanderson *et al.*, 2007).

Drug therapy for anxiety though effective is fraught with dependence, tolerance and side effect issues (Baldwin *et al.*, 2005; Nutt, 2005). It is evident that alternative therapies are needed and plants can be invaluable sources for compounds with anxiolytic effects.

The plant *Mallotus oppositifolius*, is commonly used in Ghana for epilepsy. Sleep enhancing effect of the extract and enhancement of GABAergic inhibitory neurotransmission was observed as some of the pharmacological effects of the extract. Based on these findings, possible anxiolytic effects of the extract were investigated.

6.2 MATERIALS AND METHODS

6.2.1 Animals

Male ICR mice were used in this experiment and experimental conditions were as described in section 3.2.1.

6.2.2 Chemicals

Diazepam was obtained from Phyto-Riker, Accra, Ghana.

6.2.3 Elevated Plus-Maze test

The method used was as described for rats (Pellow *et al.*, 1985) with some modifications. Mice were grouped into seven groups (n=5); group 1 was the control, groups 2 to 4 received the extract (10 - 100 mg kg^{-1}, *p.o.*), and groups 5 to 7 received diazepam (0.1 - 1 mg kg^{-1}, i.p.). Behavioural parameters were scored from the videotapes as follows: (1) number of closed and open arm entries (absolute value and percentage of the total number); (2) time spent in exploring the open and closed arms of the maze (absolute time and percentage of the total time of testing); (3) number of head-dips- protruding the head over

the edge of either an open (unprotected) or closed (protected) arm and down toward the floor; (4) number of stretch-attend postures- the mouse stretches forward and retracts to original position from a closed (protected) or an open (unprotected) arm. An arm entry was counted only when all four limbs of the mouse were within a given arm. Behavioural parameters for all the tests were scored from videotapes with the aid of the public domain software JWatcher Version 1.0 (University of California, Los Angeles, USA and Macquarie University, Sydney, Australia. Available at http://www.jwatcher.ucla.edu/)

6.2.4 **Light/Dark Box**

This apparatus is based on the initial model described by (Belzung *et al.*, 1987; Belzung and Le Pape, 1994). Mice were grouped into seven groups (n=5); group 1 was the control, groups 2 to 4 received the extract (10 - 100 mg kg^{-1}, *p.o.*), and groups 5 to 7 received diazepam (0.1 - 1 mg kg^{-1}, i.p.). At the beginning of the experiment, mice were placed individually in the centre of the illuminated box, facing away the opening from the dark compartment. Behaviours of the animals were recorded for 5 minutes with a digital camera placed 1 m above the box. Videotapes were scored as mentioned above for the following parameters: (frequency of compartment entries, total time spent by mice in each compartment and the number of transitions).

6.2.5 **Open-field test**

The test was based on that described previously by Kasture *et al*., (2002). The animals were divided into seven groups of five animals each and received the extracts (10 - 100 mg kg^{-1}, *p.o.*), the standard drug diazepam (0.1 - 1 mg kg^{-1}, i.p.) and the vehicle. Thirty minutes after i.p. and 1 h after oral administration of the test compound, mice were placed individually in the centre of the open field and allowed to explore freely for 5 min. Each session was recorded by a video camera suspended approximately 100 cm above the arena. All animals were regularly handled before individual tests in order to minimize handling-related stress. Videotapes of the arena and the following variables were recorded: number of entries as well as the duration of stay in individual zones. Thereafter, behaviour in the open field was analyzed for 5 min with the aid of the public domain software JWatcher Version 1.0 (University of California, Los Angeles, USA and Macquarie University, Sydney, Australia. Available at http://www.jwatcher.ucla.edu/). Mean values ± SEM were calculated for each and compared to vehicle-treated animals.

6.3 RESULTS

6.3.1 Effect of MOE and DZP on mice in the elevated plus-maze

In the EPM, extract showed similar anxiolytic effect as diazepam. Administration of extract significantly increased the open arm activity by increasing open arm entries ($F_{3,16}$=13.52, P=0.0001) (Figure 6.1a) and percentage open arm entries ($F_{3,16}$=19.29, P<0.0001) (Figure 6.1c). It also increased percentage time spent in the open arm ($F_{3,\ 16}$=21.99, P<0.0001) (Figure 6.2c). The frequency and the duration of rearing were also significantly decreased by the extract. MOE decreased the number of protected stretch attend postures and protected head dips. See (Table 6.1 and Table 6.2).

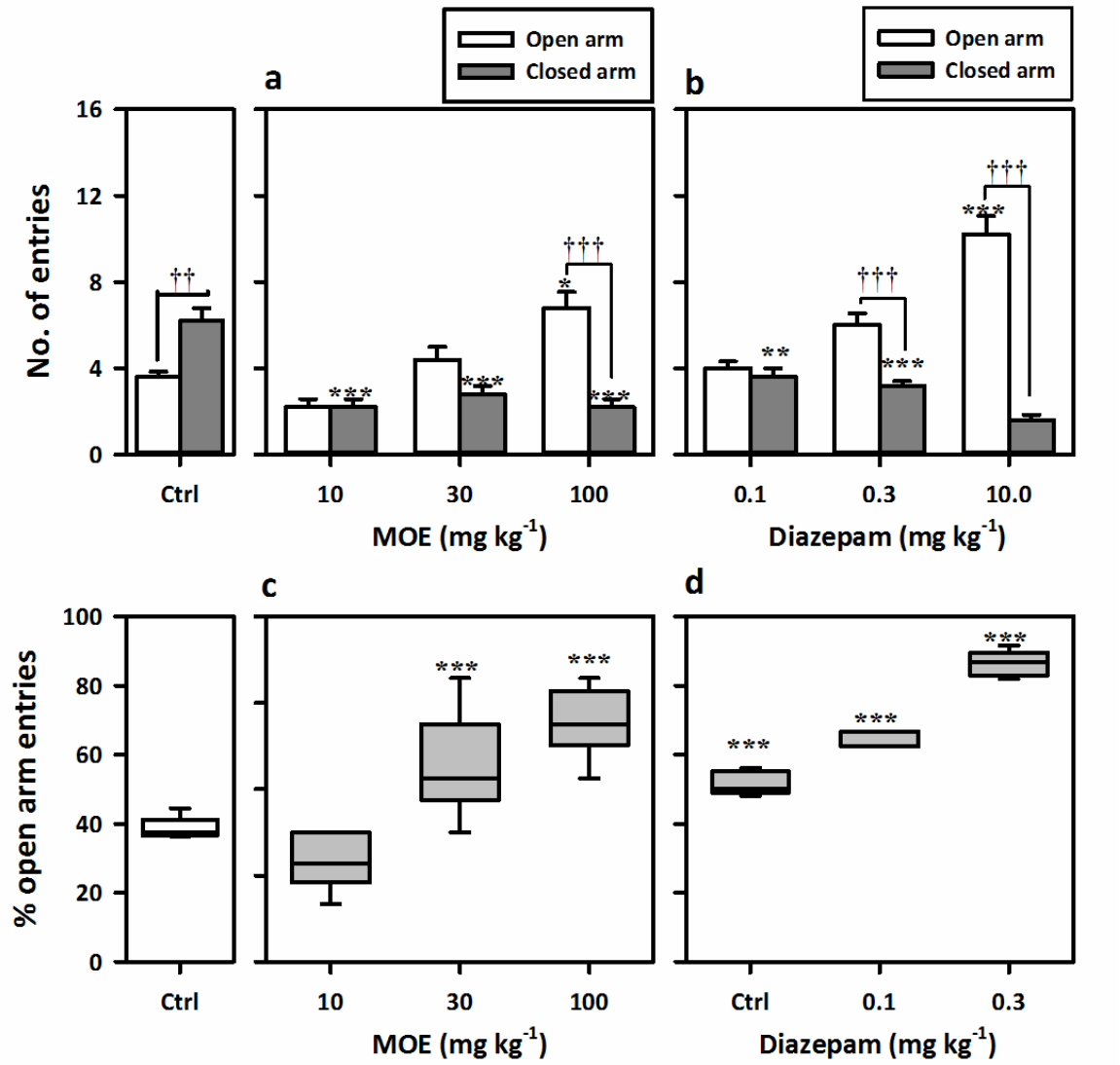

Figure 6.1 Effect of (a) the extract, MOE (10 - 100 mg kg-1) and (b) diazepam, DZP (0.1 – 1.0 mg kg-1) treatment on the open and closed arm entries; (c - d) Effect of the extract (MOE) and diazepam, treatment on % open arm entries respectively. The lower and upper margins of the boxes represent the 25th and 75th percentiles, while the extended arms represent the 10th and 90th percentiles respectively. ***P<0.001; **P<0.01; compared to vehicle-treated group; (One-way ANOVA followed by Newman Keuls' test). †††P<0.001 comparison between open and closed arm entries (Two-way ANOVA followed by Bonferroni's test). Data are presented as group Means ± SEM of 5 animals.

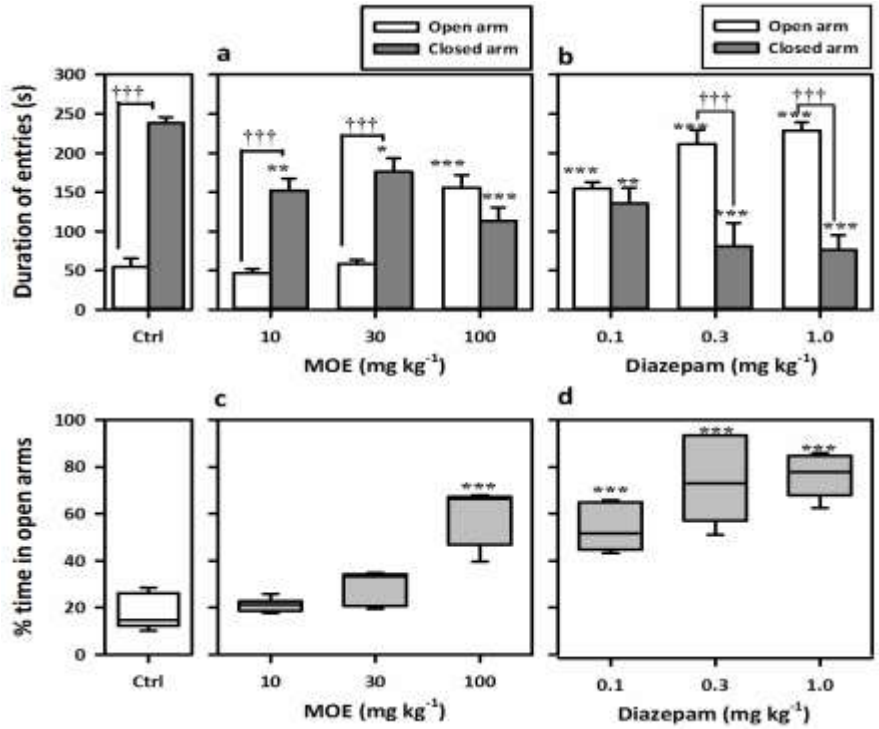

Figure 6.2 Effect of (a) the extract, MOE (10 - 100 mg kg^{-1}) and (b) diazepam, DZP (0.1 – 1.0 mg kg^{-1}) treatment on the time spent in the open and closed arm; (c - d) Effect of the extract, MOE, and diazepam, DZP treatment on % time spent in the open respectively. The lower and upper margins of the boxes represent the 25th and 75th percentiles, while the extended arms represent the 10th and 90th percentiles respectively. ***P<0.001; **P<0.01; compared to vehicle-treated group; (One-way ANOVA followed by Newman Keuls' test). †††P<0.001 comparison between open and closed arm duration (Two-way ANOVA followed by Bonferroni's test). Data are presented as group Means ± SEM of 5 animals.

Table 6.1 Effect of Drug treatment on the frequency of some ethological parameters

	Frequency				
Treatment	Rearing	PHD	UPHD	PSAP	UPSAP
Ctrl	14.8±1.2	1.8±0.2	8.2±0.6	1.4±0.3	2.2±0.2
MOE					
10	9.0±0.6**	2.2±0.2	4.8±0.2***	1.2±0.2	2.2±0.2
30	5.6±0.7***	1.2±0.2***	2.2±0.2***	0.0±0.0***	0.6±0.3***
100	5.2±0.4***	0.6±0.3***	1.4±0.3***	0.0±0.0***	0.2±0.2***
DZP					
0.1	6.8±0.5***	0.1±0.1***	4.2±0.5***	0.4±0.3***	0.6±0.3***
0.3	3.8±0.5***	0.0±0.0***	4.2±0.2***	0.0±0.0***	0.4±0.3***
1.0	3.3±0.6***	0.0±0.0***	2.4±0.3***	0.0±0.0***	0.2±0.2***

Values are mean±S.E.M. (n=5). ***P<0.001, **P<0.01 considered statistically significant from control. PHD: protected head dip; UPHD: unprotected head dip; PSAP: protected stretch attend posture; UPSAP: unprotected stretch attend posture.

Table 6.2 Effect of Drug treatment on the duration of some ethological parameters

Treatment		Duration				
		Rearing	PHD	UPHD	PSAP	UPSAP
Ctrl		14.3±1.7	0.9±0.1	4.6±2.3	1.1±0.2	1.6±0.6
MOE						
	10	9.9±1.3*	3.8±0.9**	3.9±1.8	1.6±0.4***	1.4±0.7
	30	9.4±0.7*	0.7±0.1	1.9±0.9	0.2±0.2***	0.9±0.4
	100	7.2±0.2**	0.9±0.2	0.9±0.4	0.0±0.0***	0.2±0.1
DZP						
	0.1	6.4±0.5***	0.0±0.0***	8.9±5.8	0.0±0.0***	0.4±0.3
	0.3	4.5±0.3***	0.0±0.0***	5.9±1.3	0.0±0.0***	0.4±0.3
	1.0	3.2±0.6***	0.1±0.0***	4.5±0.9	0.2±0.2***	0.0±0.0***

Values are mean±S.E.M. (n=5). ***P<0.001, **P<0.01 considered statistically significant from control. PHD: protected head dip; UPHD: unprotected head dip; PSAP: protected stretch attend posture; UPSAP: unprotected stretch attend posture.

6.3.2 **Effect of MOE and DZP on mice in the light-dark test.**

Extract treated mice exhibited anxiolytic effect in the light-dark box. MOE significantly increased the time spent by mice in the lit region ($F_{3,16}$=24.58, P<0.0001) while decreasing the time spent in the dark region (Figure 6.3a, c). There was also a significant increase in latency to leave the lit area into the dark area ($F_{3,16}$=24.32, P<0.0001) (Figure 6.3a). There was however a decrease in the number of transitions by extract treatment 100 mg kg^{-1} ($F_{3,16}$=6.82, P<0.036) (Figure 6.3a). Diazepam (0.1, 0.3 and 1.0 mg kg^{-1}), the reference anxiolytic, showed similar effects as the extract (Figure 6.3b, d).

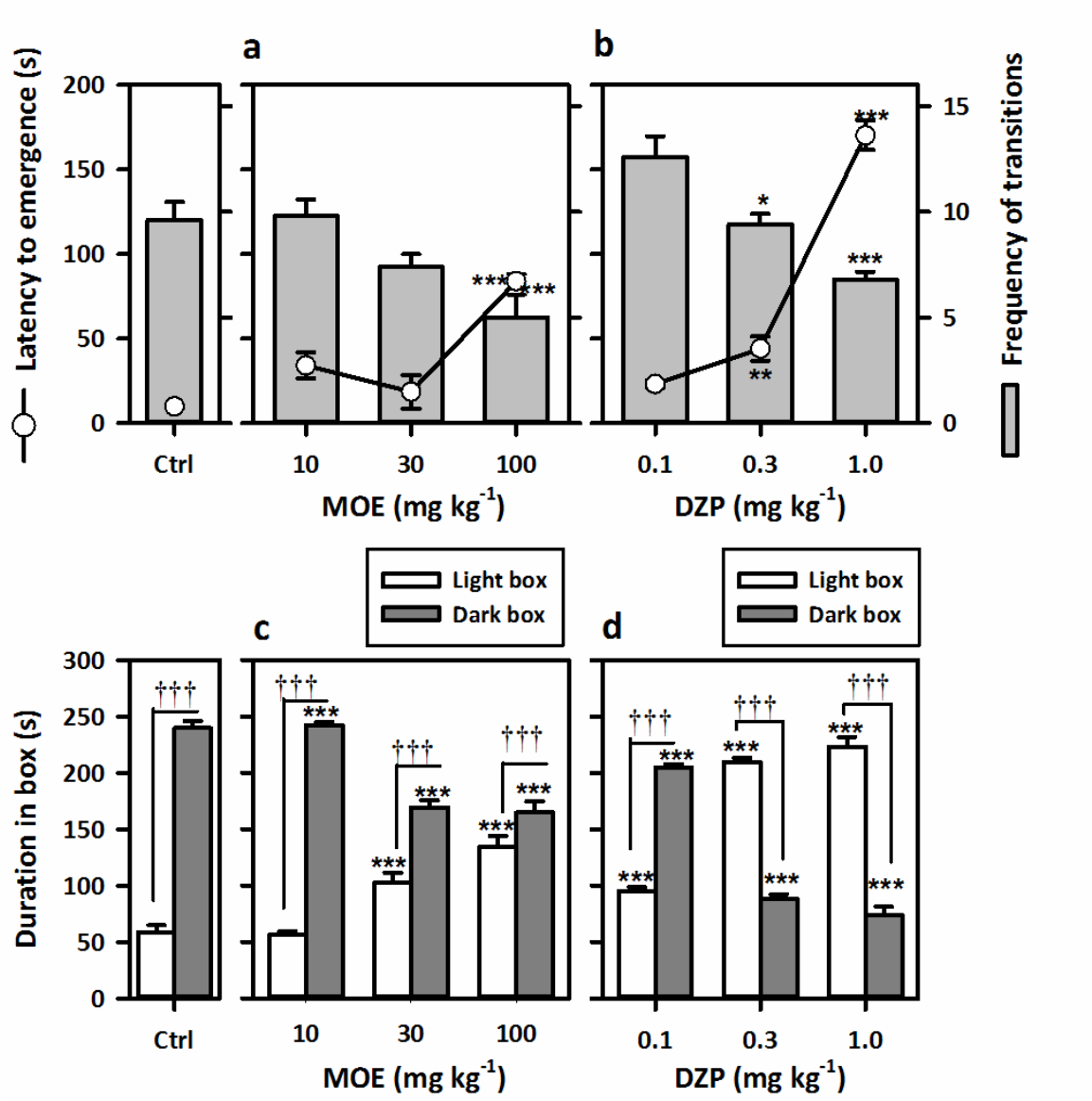

Figure 6.3 Effect of (a) the extract, MOE (10 - 100 mg kg^{-1}) and (b) diazepam, DZP (0.1 – 1.0 mg kg^{-1}) treatment on the latency to enter dark compartment and the frequency of transitions; (c - d) Effect of the extract, MOE, and diazepam, DZP treatment on time spent in the lit and dark compartments respectively. ***P<0.001; **P<0.01; compared to vehicle-treated group; (One-way ANOVA followed by Newman Keuls' test). †††P<0.001 comparison between open and closed arm duration (Two-way ANOVA followed by Bonferroni's test). Data are presented as group Means ± SEM of 5 animals.

6.3.3 **Effect of MOE and DZP on mice in the open field test**

In the open field test, all drug treated mice showed significant differences in both the number of entries into the various fields as well as the time spent in the various zone. *M. oppositifolius* treated mice exhibited anxiolytic activity similar to diazepam by significantly increasing the absolute number and percentage number of centre entries ($F_{6,28}$=49.17,

P<0.0001) (Figure 6.4) and the percentage time spent in the centre of the open field ($F_{3,15}$=36.12, *P*<0.001) (Figure 6.5).

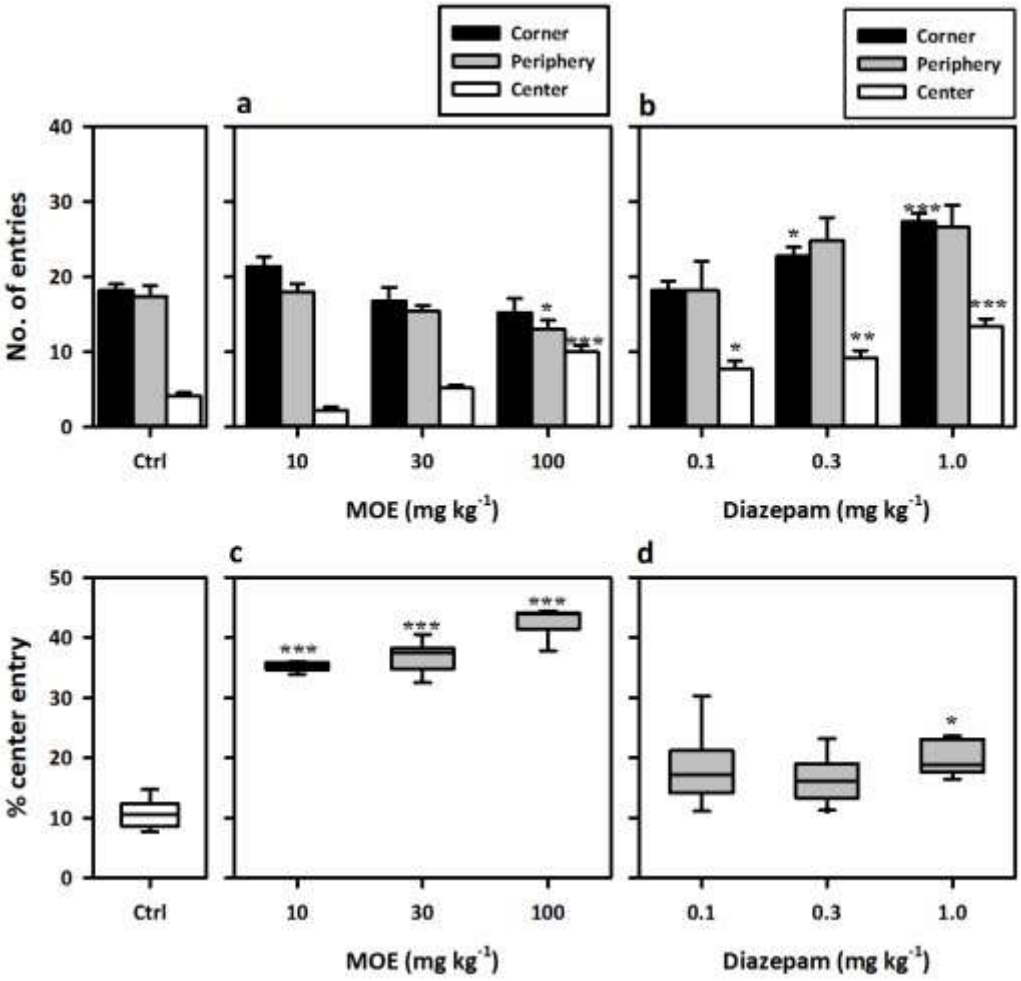

Figure 6.4 Effect of (a) the extract, MOE (10 - 100 mg kg^{-1}) and (b) diazepam, DZP (0.1 – 1.0 mg kg^{-1}) treatment on the frequency of entries into the corner, peripheral or central compartments; (c - d) Effect of the extract, MOE, and diazepam, DZP treatment on % centre entries respectively. ****P*<0.001; ***P*<0.01; compared to vehicle-treated group; (One-way ANOVA followed by Newman Keuls' test). Data are presented as group Means ± SEM of 5 animals. The lower and upper margins of the boxes represent the 25th and 75th percentiles, while the extended arms represent the 10th and 90th percentiles respectively.

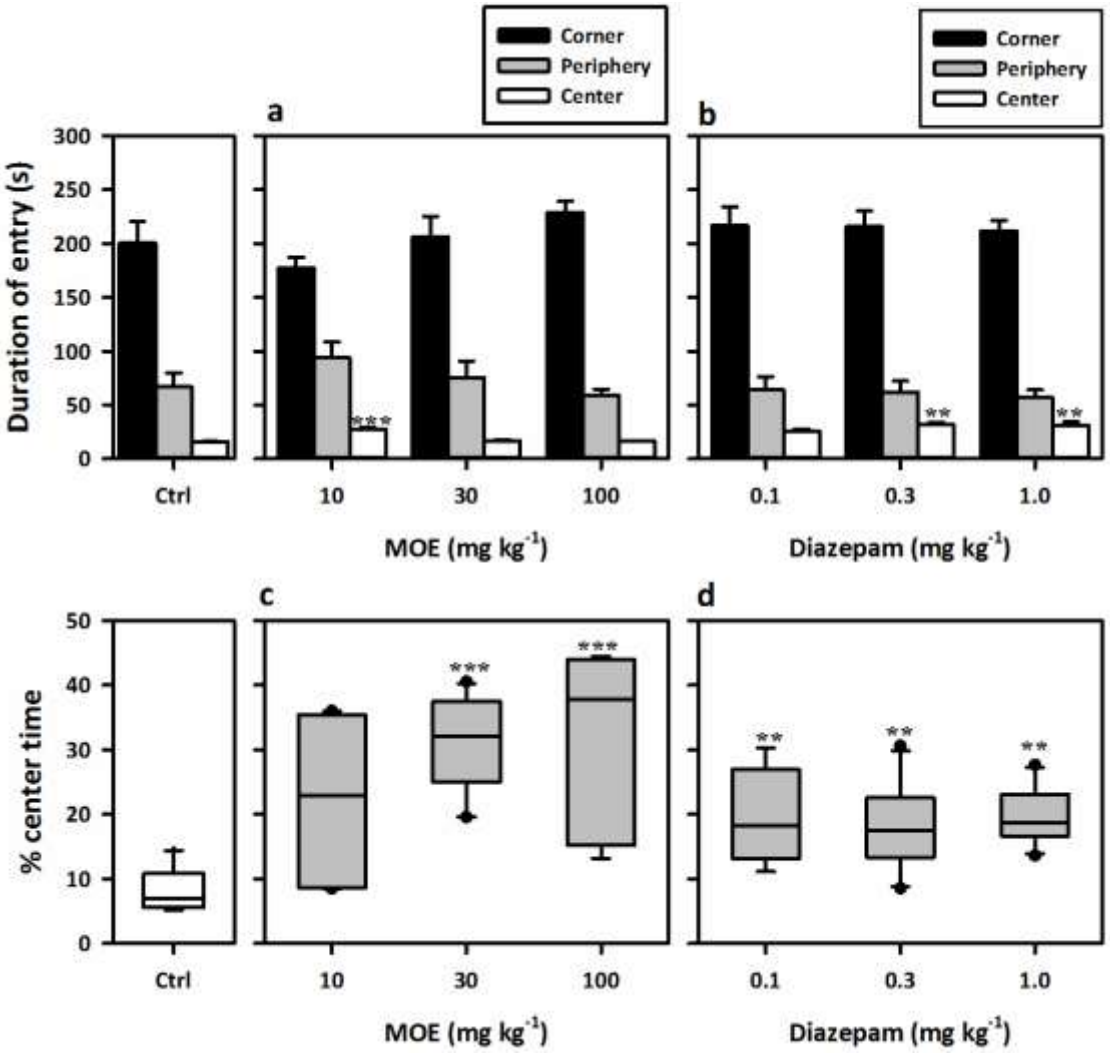

Figure 6.5 Effect of (a) the extract, MOE (10 - 100 mg kg-1) and (b) diazepam, DZP (0.1 – 1.0 mg kg-1) treatment on the time spent in the corner, peripheral or central compartments; (c - d) Effect of the extract, MOE, and diazepam, DZP treatment on % time spent in the centre respectively. The lower and upper margins of the boxes represent the 25th and 75th percentiles, while the extended arms represent the 10th and 90th percentiles respectively. ***P<0.001; **P<0.01; compared to vehicle-treated group; (One-way ANOVA followed by Newman Keuls' test). Data are presented as group Means ± SEM of 5 animals.

6.4 DISCUSSION

The present study revealed that the hydroalcoholic extract of *M. oppositifolius* has anxiolytic effects. In all the models used the extract showed an attenuation of anxiety-like behaviours.

MOE caused statistically significant increase in percentage open arm entries and time spent there in the elevated plus-maze (EPM), one of the most widely used animal models for screening putative anxiolytics (Cole and Rodgers, 1994; Dawson and Tricklebank,

1995; Wei *et al.*, 2007). This is indicative of possible anxiolytic effect. Ethological measures of risk assessment, such as stretched-attend postures head-dipping and rearing, which have been validated and shown by factor analysis to be a more predictive determinant of anxiety were also used in addition to the spatio-temporal indices (frequency and duration of arm entries) of anxiety (Rodgers and Johnson, 1995; Rodgers and Dalvi, 1997). Extract treated mice demonstrated less protected stretch attend postures, protected head dips and rearing suggestive of anxiolysis. Rodents display enhanced risk assessment behaviours even in the closed arm of the maze, suggesting that this defensive pattern is more sensitive to anxiety modulating drugs than avoidance-related measures (Rodgers and Cole, 1994; Griebel *et al.*, 1997; Rodgers and Dalvi, 1997; Setem *et al.*, 1999). Thus the anxiolytic effects of the extract were demonstrated not only by the increased open arm entries and percentage of open arm time, but also by the decreased protected stretch-attend postures, protected head dips and rearing.

The light/dark box test exploits rodents' natural aversion to bright areas compared to dark areas and their innate exploratory behaviour (File *et al.*, 2004). Thus, drug-induced increase in behaviours in the lit region and increase in transitions without an increase in spontaneous locomotion is considered to reflect anxiolytic activity (Treit, 1990; Njung'e and Handley, 1991; File, 1992; De Vry *et al.*, 1993). In the present study, the extract showed significant anxiolytic activity by delaying the time for the mice to leave the lit compartment and increasing the amount of time spent in the lit compartment. This affirms that the extract possibly contains compounds that have anxiolytic activity. Interestingly there was a decrease in transition at the dose of 30 mg kg^{-1} though previous studies indicated that this dose does not cause sedation or motor impairment which could have accounted for the decrease in transitions (Crawley, 1981). It is worth mentioning that the measurement found to be most consistent and useful for assessing anxiolytic-like action is the time spent in the lit area and not frequency of transitions, because this parameter provides the most consistent dose–effect results with drugs (Hascoet and Bourin, 1998; Lepicard *et al.*, 2000). The decrease in transition therefore should not detract from the effects observed in both the EPM and the light/dark exploration test.

A third model, the open field test, was utilized to further clarify the possible effects of the extract. The open-field model examines anxiety-related behavior characterized by the normal aversion of the animal to an open, brightly lit area (Choleris *et al.*, 2001; Mechan *et al.*, 2002). Thus, animals removed from their acclimatized cage and placed in a novel environment express anxiety and fear, by showing decreases in ambulation and exploration time in the centre of the open field with thigmotaxis (Bhattacharya and Mitra, 1991). MOE

increased the percentage time spent in the central portion of the arena and reduction in peripheral movements just like diazepam. These results confirm the anxiolytic-like effect of the extract.

6.5 CONCLUSION

The present study indicates that the hydroalcoholic extract of *M. oppositifolius* has anxiolytic effect.

Chapter 7
ACUTE AND SUBACUTE TOXICITY STUDIES

7.1 INTRODUCTION

During the last decade, use of traditional medicine has expanded globally and has gained popularity not only developing countries, but also in countries where conventional medicine is predominant (Cupp, 1999; Marinac *et al.*, 2007). Herbal medicines are widely thought to confer health benefits and safer than their orthodox counterparts (Sheehan, 1998; Cupp, 1999). With the tremendous expansion in the use of traditional medicine worldwide, safety assessments of herbal medicines have become imperative because this notion that herbal preparations are natural and therefore must safe may be misleading in the absence of scientific facts (Stedman, 2002; Seeff, 2007). For instance, neurotoxicity, cardiac toxicity, pulmonary toxicity, hepatotoxicity, and nephrotoxicity have been traced to the use of some herbal medicines (Gertner *et al.*, 1995; Stedman, 2002; Su *et al.*, 2011).

Though *Mallotus oppositifolius* is used in managing many conditions traditionally, there is no scientific data on its toxic effect on critical organs like the brain, kidneys, heart, spleen, stomach and liver. The study was therefore conducted to investigate the toxic effects of *M. oppositifolius* in general and on these organs.

7.2 METHOD

7.2.1 Acute toxicity

Male Sprague-Dawley rats (200 - 230 g) were randomly divided into seven groups (n=5) and kept in the experimental environment for an acclimation period of 1 week. The animals were fasted overnight, but with access to water *ad libitum*, and then treated orally with *M. oppositifolius* extract in doses of 30 - 3000 mg kg^{-1} of body weight. The control group received 10 ml kg^{-1} *p.o.* of water. The rats were observed up to 24 hours for general changes in behaviour and physiological function as well as mortality. The assessment of behaviour and physiological function was carried out similar to the primary observation procedure (Irwin test) originally described by Irwin (1968). In accordance with the Irwin test, the rats were observed at 0, 15, 30, 60, 120 and 180 min, and 24 h after treatment for behaviours specifically related to neurotoxicity, such as convulsions and tremor, for behaviours related to CNS stimulation, such as excitation, Straub tail, jumping, hypersensitivity to external stimuli, stereotypies, and aggressive behaviour, and for behaviours related to CNS depression, such as sedation, rolling gait, loss of balance, loss of traction, motor incoordination, hyposensitivity to external stimuli, decreased muscle tone,

akinesia, catalepsy, and hypothermia. Effects on autonomic functions, such as respiration, body temperature, salivation, urination and defecation, were also noted.

7.2.2 Subacute toxicity

Male Sprague-Dawley rats (180 – 200 g); n=5, were treated orally with *M. oppositifolius* extract in doses of 30 - 3000 mg $kg^{-1}$of body weight daily, for 14 consecutive days. Group A, the control, received 10 ml kg^{-1}*p.o.* of water daily. Group B, C and D were treated with daily doses of extract i.e. 30 - 3000 mg kg^{-1} *p.o*. respectively. The extract was prepared such that not more than 2 ml was given orally. The animals were monitored closely for signs of toxicity. Appearance and behaviour pattern were assessed daily and any abnormalities in food and water intake were noted.

7.2.2.1 Preparation of serum and isolation of organs

The rats were sacrificed on the fifteenth day by cervical dislocation, the jugular vein was cut and blood flowed freely. About 1.5 ml of blood was collected into vacuum tubes containing 2.5 µg of ethylenediaminetetraacetic acid (EDTA) as an anticoagulant for haematological assay and 3.5 ml of the blood was collected into sample tubes without anticoagulant. The blood without the anticoagulant was allowed to clot before centrifugation (4000 rpm at 4 °C for 10 min) to obtain serum, which was collected and stored at -20ºC until assayed for biochemical parameters the next day. After collecting blood, the rats were quickly dissected and the organs (spleen, liver, kidney, brain, heart and stomach) removed, freed of fat and connective tissue, blotted with clean tissue paper and then weighed on a balance.

7.2.2.2 Effect of extract on haematological parameters

Haematological parameters including haemoglobin (HGB), red blood cells (RBC), white blood cells (WBC), haematocrit (HCT), platelets (PLT), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), and mean corpuscular haemoglobin concentration (MCHC) were determined by an automatic analyzer (BC-3000 Plus Auto Haematology Analyzer, Shenzhen Mindray Bio-Medical Electronics Co. Ltd, China).

7.2.2.3 Effect of extract on serum biochemical parameters

Biochemical analyses were performed on serum collected for the determination of the following parameters: aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), total bilirubin (T-BIL), direct bilirubin (D-BIL), indirect bilirubin (I-BIL), total protein, albumin, urea and creatinine. All analyses were carried out

using an automated clinical chemistry analyser (Flexor Junior®, Vital Scientific, AC Dieren, The Netherlands).

7.2.2.4 Effect of extract on body and organ weights

Body weights of the rats were taken on days 0 and 15. The relative organ weight (ROW) of each organ was calculated as follows:

$$ROW = \frac{\text{Absolute organ weight (g)}}{\text{Rat body weight on sacrifice day (g)}} \times 100$$

7.2.2.5 Histological examination

Portions of the tissue from liver, kidney, spleen, brain, heart and stomach were used for histopathological examination. Tissues were fixed in 10 % neutral buffered formalin (pH 7.2) and dehydrated through a series of ethanol solutions, embedded in paraffin and routinely processed for histological analysis. Sections of 2 μm thickness were cut and stained with haematoxylin-eosin for examination. The stained tissues were observed through an Olympus microscope (BX-51) and photographed by INFINITY 4 USB Scientific Camera (Lumenera Corporation, Otawa, Canada).

7.2.3 **Statistics**

Data were presented as mean± SEM. The presence of significant differences among means of groups was determined by one-way ANOVA using GraphPad Prism for Windows version 5 (GraphPad Software, San Diego, CA, USA). Significant difference between pairs of groups was calculated using the Newman-Keuls' Multiple Comparison Test.

7.3 RESULTS

7.3.1 Acute toxicity

There was no mortality after 24 hour period—LD_{50} is above 3000 mg kg^{-1}. Rats did not show any signs of neurotoxicity such as convulsions and tremor. Behaviours related to central nervous system (CNS) stimulation, such as excitation, Straub tail, jumping, hypersensitivity to external stimuli, stereotypies, and aggressions were absent. In contrast there were signs of CNS depression such as sedation and motor incoordination at 1000 and 3000 mg kg^{-1}. There were no visible signs on autonomic functions such as respiration, pupil diameter, body temperature, salivation and defecation.

Table 7.1 Observations in the acute toxicity test after oral administration of *M. oppositifolius* in rats.

Dose	Mortality		Toxicity
	D/T	Latency (h)	
0	0/5	-	-
30	0/5	-	-
100	0/5	-	-
300	0/5	-	-
1000	0/5	-	Sedation, motor incoordination
3000	0/5	-	Sedation, motor incoordination

The hydroalcoholic extract of *M. oppositifolius*, was administered orally; each dose was administered to groups of 5 rats. D/T: dead/treated rats; -: no toxic symptoms were seen during the observation period; latency: time to death (in hours) after the dose.

7.3.2 Subacute toxicity

There was no death after 14 days. Behavioural signs related to toxicity were absent except for sedation at the dose of 1000 - 3000 mg kg^{-1} which lasted for the first week but receded afterwards.

7.3.2.1 Effect of extract on body weight and organ weight

At lower doses (30 – 100 mg kg^{-1}) there was a decline in the percentage change in weight during the 14 days of extract treatment. This declined was reversed from 300 to 3000 mg kg^{-1}. The extract significantly increased the weight of the rats at 3000 mg kg^{-1}, when compared to control (Figure 7.1). There was no statistical difference in the relative organ weight of control and extract-treated groups.

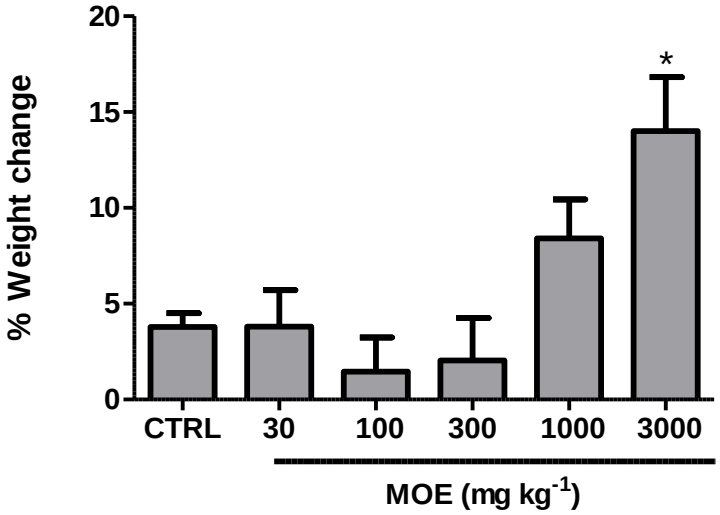

Figure 7.1 Effect of oral administration of *Mallotus oppositifolius* extract (MOE), on the % change in body weights of rats in the sub-acute toxicity test. Data are expressed as mean±S.E.M. (n=5). Treated groups were compared to controls with a one-way ANOVA followed by Newman Keuls' test.

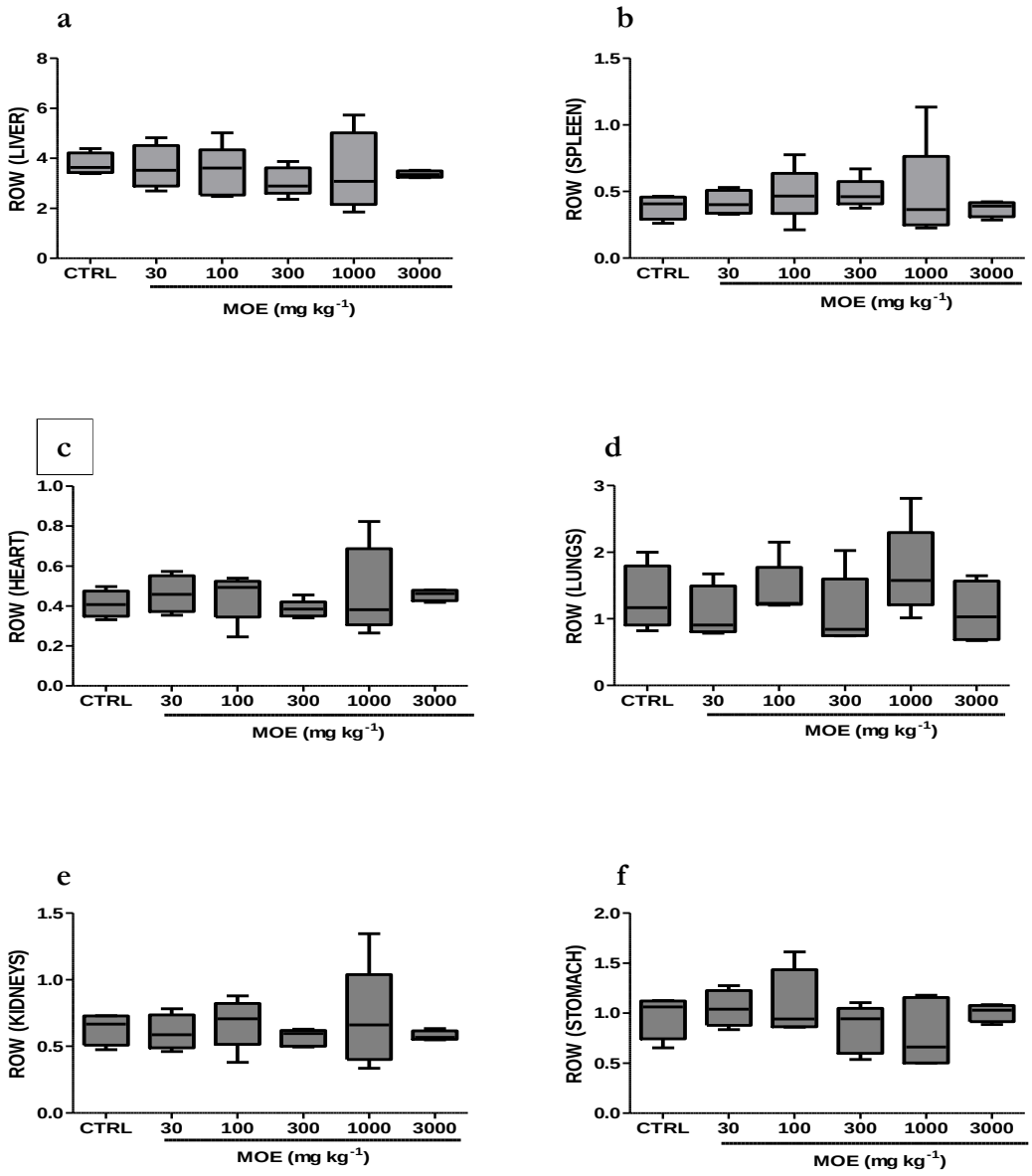

Figure 7.2 Effect of oral administration of *Mallotus oppositifolius* extract (MOE), on the relative organ weights (ROW) of rats in the sub-acute toxicity test. Data are expressed as mean±S.E.M. (n=5). Treated groups were compared to controls with a one-way ANOVA followed by Newman Keuls' test. The lower and upper margins of the boxes represent the 25th and 75th percentiles, while the extended arms represent the 10th and 90th percentiles respectively.

7.3.2.2 Effect of extract on haematological parameters

Haematological parameters did not reveal any extract induced toxicity on (Table 7.2).

7.3.2.3 Effect of extract on serum biochemical parameters

Clinical biochemical parameters indicated that the extract did not affect the synthetic function of the liver or the integrity of the hepatocytes. The levels of albumin and globulin, indicative of the liver synthetic function did not differ significantly from the control although there were slight increases. Alanine transaminase (ALT), aspartate transferase (AST) and alkaline phosphatase (ALP) levels were normal compared to control. Bilirubin levels were normal except at 300 and 3000 mg kg^{-1} where there were significant increases (P=0.0093). Urea and creatinine levels increased at 30 mg kg^{-1} but only urea increased at 100 mg kg^{-1}. The values decreased however at higher doses (300 – 3000 mg kg^{-1}) although not statistically significant. The liver profile showed general decrease in the total cholesterol, very low density lipoprotein (VLDL), and triglycerides but higher levels of high density lipoproteins (HDL). The differences were however not statistically significant compared to control. The coronary risk also decreased with extract treatment although not significantly. See Table 7.3.

Table 7.2Haematological values of control and rats treated with *M. oppositifolius* for 14 days.

Parameters	*Mallotus oppositifolius* extract (mg kg^{-1})						*F*	*P*Value
	0	30	100	300	1000	3000		
WBC (10^9/L)	12.03±1.01	10.15±2.06	12.64±0.93	8.440±0.60	13.90±2.43	13.98±1.09	$F_{5,21}$=2.144	0.0998
LYM (%)	59.40±2.09	63.73±4.14	67.04±5.71	79.70±5.32	60.04±4.89	71.30±5.58	$F_{5,21}$=2.521	0.0615
RBC (10^{12}/L)	7.19±0.21	7.32±0.53	8.23±0.28	7.36±0.53	7.61±0.66	7.39±0.17	$F_{5,21}$=0.701	0.6289
HGB (g/dL)	12.73±0.47	13.40±0.57	14.24±0.27	13.08±0.48	13.92±1.16	13.83±0.45	$F_{5,21}$=0.725	0.6126
HCT (%)	41.68±1.43	42.48±3.02	44.96±1.29	45.78±1.89	43.42±4.11	42.83±1.08	$F_{5,21}$=0.393	0.8482
MCV (fL)	57.98±1.16	58.73±0.49	56.84±0.65	62.84±3.00	56.92±0.99	57.95±0.49	$F_{5,21}$=2.235	0.0887
MCH (pg)	17.72±0.38	18.65±0.53	18.02±0.36	18.08±1.25	18.38±0.58	18.70±0.36	$F_{5,21}$=363.4	0.9246
MCHC (g/dL)	30.53±0.16	31.75±1.12	31.70±0.35	28.68±1.15	32.28±0.79	32.30±0.87	$F_{5,21}$=2.170	0.0351
RDW_CV (%)	17.50±1.36	14.00±1.18	14.00±0.08	16.36±1.72	13.70±1.30	12.83±0.23	$F_{5,21}$=02.167	0.0968
RDW_SD (fL)	35.58±1.72	32.23±1.84	30.64±0.42	38.34±4.61	30.38±1.21	29.88±0.44	$F_{5,21}$=2.135	0.1009
MPV (fL)	7.43±0.12	7.50±0.40	7.16±0.11	7.28±0.11	7.42±0.13	7.33±0.13	$F_{5,21}$=0.465	0.7977
PDW (fL)	9.03±0.18	9.55±0.69	8.80±0.23	8.84±0.22	9.02±0.19	9.05±0.13	$F_{5,21}$=0.714	0.6196
Platelets (10^9/L)	841.0±132.7	734.5±107.3	698.0±65.09	781.4±165.2	788.2±102.5	642.0±131.5	$F_{5,21}$=0.319	0.8954

Values are mean±S.E.M. (n=5). *P<0.05 considered statistically significant from control.

Table 7.3 Clinical biochemistry values of control and rats treated with *M. oppositifolius* for 14 days.

Parameters	*Mallotus oppositifolius* extract (mg kg^{-1})						*F*	*P*Value
	0	30	100	300	1000	3000		
Albumin (g/L)	30.68±0.72	33.75±1.14	32.94±2.27	34.44±0.95	31.12±1.20	31.68±0.72	1.153	0.3646
Globulin (g/L)	36.16±2.59	28.77±2.23	31.69±2.24	28.65±0.72	33.09±1.32	29.68±1.16	2.293	0.0823
Total Prot (g/L)	66.83±3.04	62.53±2.89	64.62±1.98	63.10±1.42	64.22±1.33	61.35±0.88	0.696	0.6323
A/G Ratio	0.85±0.06	1.17±0.05	1.04±0.12	1.20±0.04	0.95±0.07	1.076±0.06	2.815	0.0425
D-Bil (µmol/L)	0.48±0.02	0.37±0.05	0.36±0.16	0.26±0.08	0.38±0.13	0.68±0.24	1.183	0.3508
I-Bil (µmol/L)	0.76±0.08	1.11±0.05	1.48±0.19	1.89±0.20**	1.20±0.27	1.29±0.17	4.117	0.0092
T-Bil (µmol/L)	1.20±0.07	1.48±0.04	1.86±9.10	2.14±0.24*	1.56±0.18	1.98±0.09*	4.106	0.0093
ALT (U/L)	128.60±17.11	105.63±8.95	119.28±14.16	100.36±10.54	83.3±9.12	95.98±3.37	2.169	0.0966
AST (U/L)	183.95±16.96	162.68±16.45	174.64±1.46	164.30±17.09	129.68±13.16	150.45±4.24	1.573	0.2110
ALP (U/L)	3.25±0.74	11.50±6.50	3.20±0.41	9.80±2.78	5.6±2.69	5.00±0.35	1.744	0.1685
Urea (mmol/L)	3.78±0.40	8.36±1.99*	7.91±2.38*	6.12±0.38	6.668±0.42	4.32±0.76	3.538	0.0178
Creat. (µmol/L)	66.83±3.40	69.93±4.56*	66.56±1.30	63.80±4.72	57.42±3.58	51.98±1.49	2.932	0.0368
Chol (mmol/L)	1.84±0.15	2.24±0.10	3.57±0.30	2.03±0.15	1.736±0.05	2.05±0.11	1.345	0.2846
TG (mmol/L)	3.13±0.40	3.69±0.90	2.73±0.20	3.21±0.42	2.724±0.23	2.73±0.10	0.633	0.6766
HDL (mmol/L)	0.67±0.04	0.84±0.03	0.84±0.04	0.75±0.05	0.658±0.06	0.75±0.03	2.436	0.0685
VLDL (mmol/L)	1.43±0.18	1.68±0.41	1.24±0.09	1.46±0.19	1.236±0.10	1.24±0.042	0.644	0.6688
Coronary Risk	3.84±0.33	3.89±0.09	3.77±0.12	3.71±0.23	3.75±0.25	3.79±0.10	0.079	0.9947

Values are mean±S.E.M. (n=5). ***P*<0.01; **P*<0.05 compared to vehicle-treated group (one-way ANOVA with Newman Keuls' *post hoc* test).

7.3.2.4 Histopathological changes

Histopathological evaluation of the organs isolated from rats sacrificed at the end of the sub-acute toxicity study revealed no significant extract-related changes compared to the control animals except for some changes in the kidney.

Comparison of liver morphological structure in extract-treated rats to control (Plate 7.1) showed no remarkable abnormalities. There was no tissue necrosis or cellular degeneration. The morphology of the capsule and hepatic lobules were normal. Furthermore there was no fatty infiltration (steatosis) in the hepatocytes neither was there infiltration of inflammatory cell. Extract treatment did not affect the histomorphology of the spleen (Plate 7.2). Splenic congestion or haemorrhage, which are considered important features of toxicity in the spleen were absent. Cardiac myocytes did not suffer any injury after 14 days of extract treatment (Plate 7.3). All stomach samples showed normal zymogenic cells, parietal cells and normal grooves in the mucosa with neither atrophy nor inflammatory cell infiltration (Plate 7.4). At 30 mg kg^{-1} there were some changes in the histomorphology of the kidney but this was not apparent in doses higher than 30 mg kg^{-1} (Plate 7.5). The histology of the hippocampus showed normal cell bodies and CA1-3 regions as well as dentate gyrus (Plate 7.6).

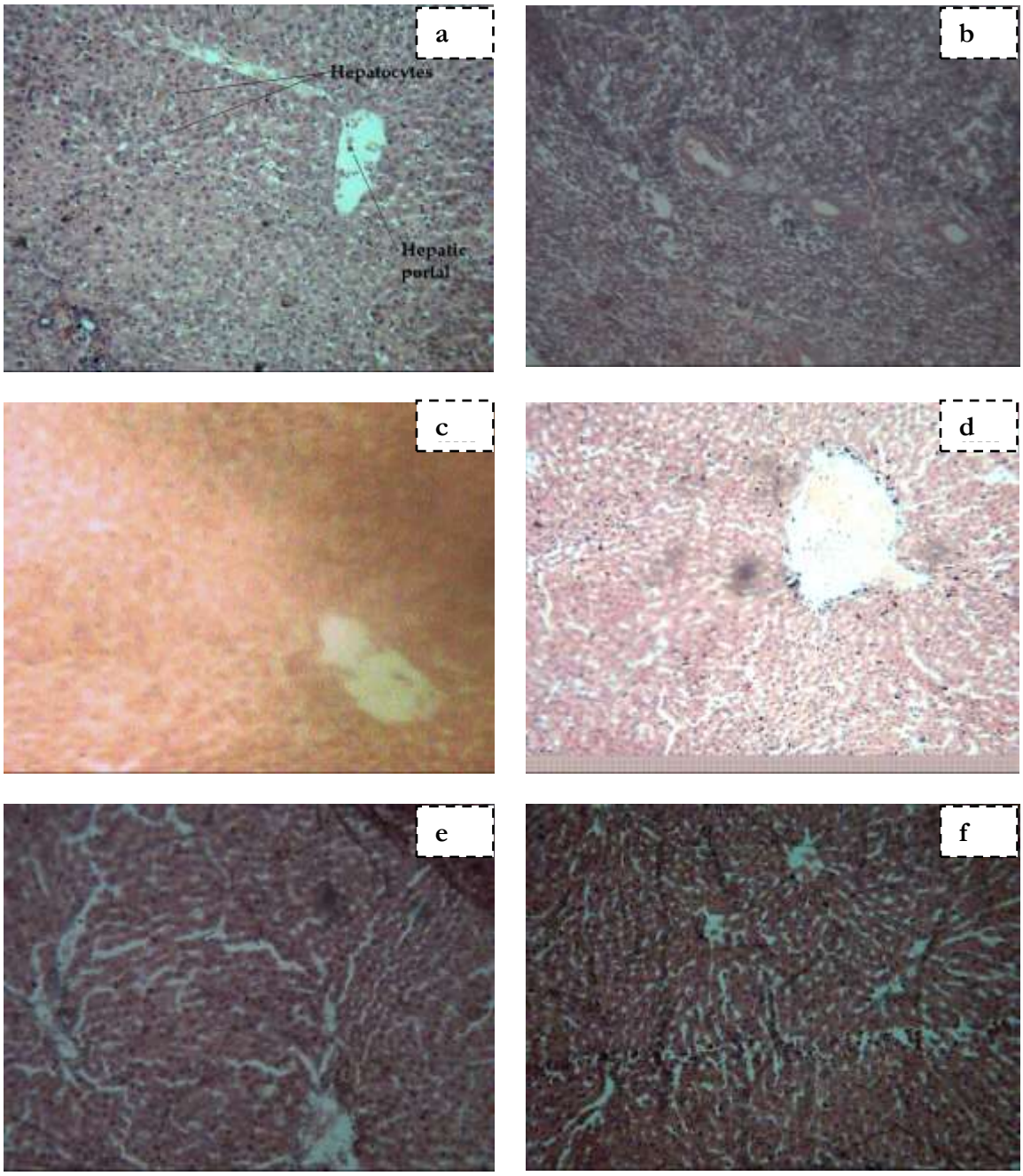

Plate 7.1 Photomicrograph of the sections of (a) the liver in control rats, and (b-f) rats treated orally with 30 - 3000 mg kg-1 of the extract for 14 days in the sub-acute toxicity study (H & E, ×400).

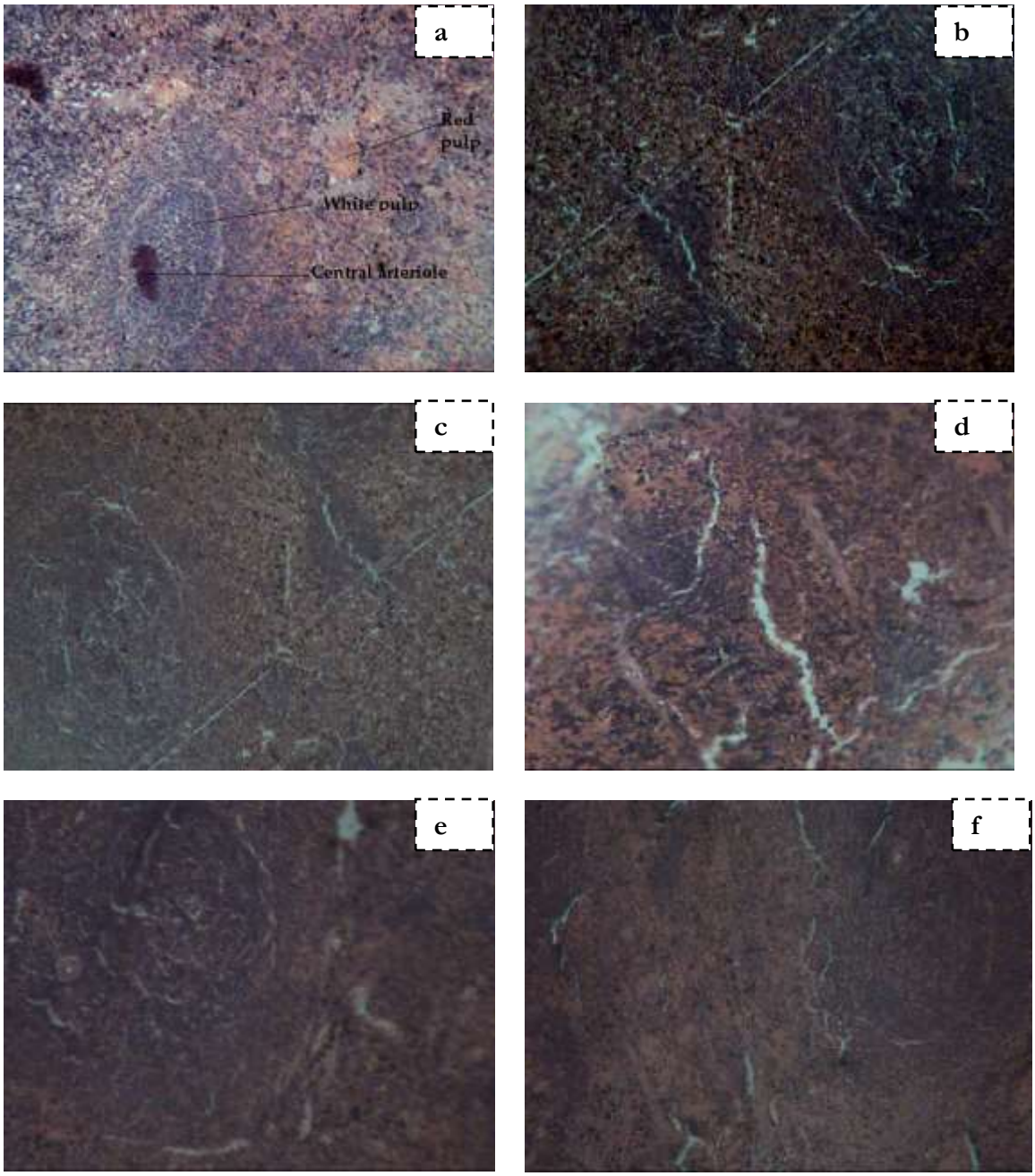

Plate 7.2 Photomicrograph of the sections of (a) the spleen in control rats, and (b-f) rats treated orally with 30 - 3000 mg kg-1 of the extract for 14 days in the sub-acute toxicity study (H & E, ×400)

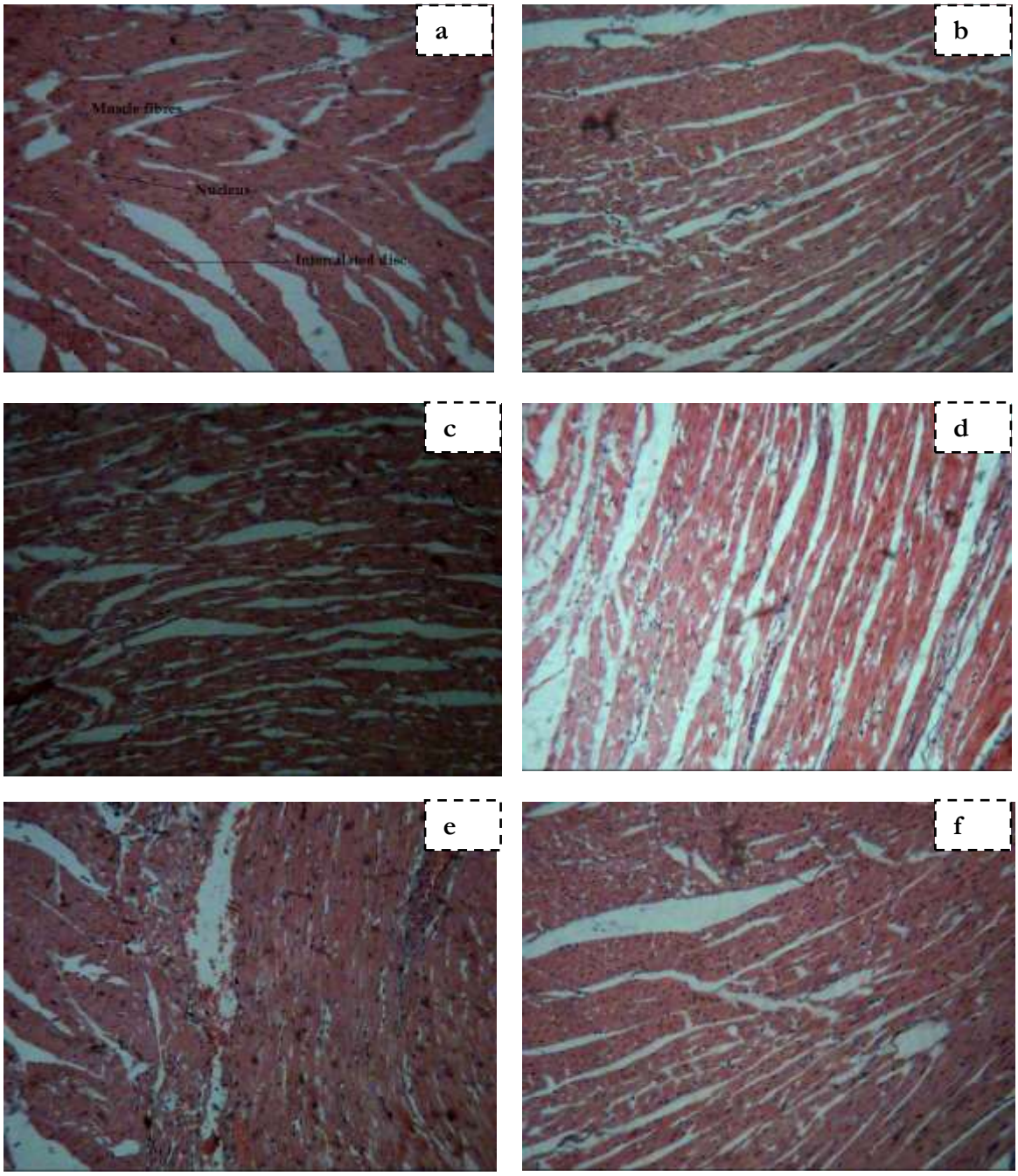

Plate 7.3 Photomicrograph of the sections of (a) the heart in control rats, and (b-f) rats treated orally with 30 - 3000 mg kg-1 of the extract for 14 days in the sub-acute toxicity study (H & E, ×400)

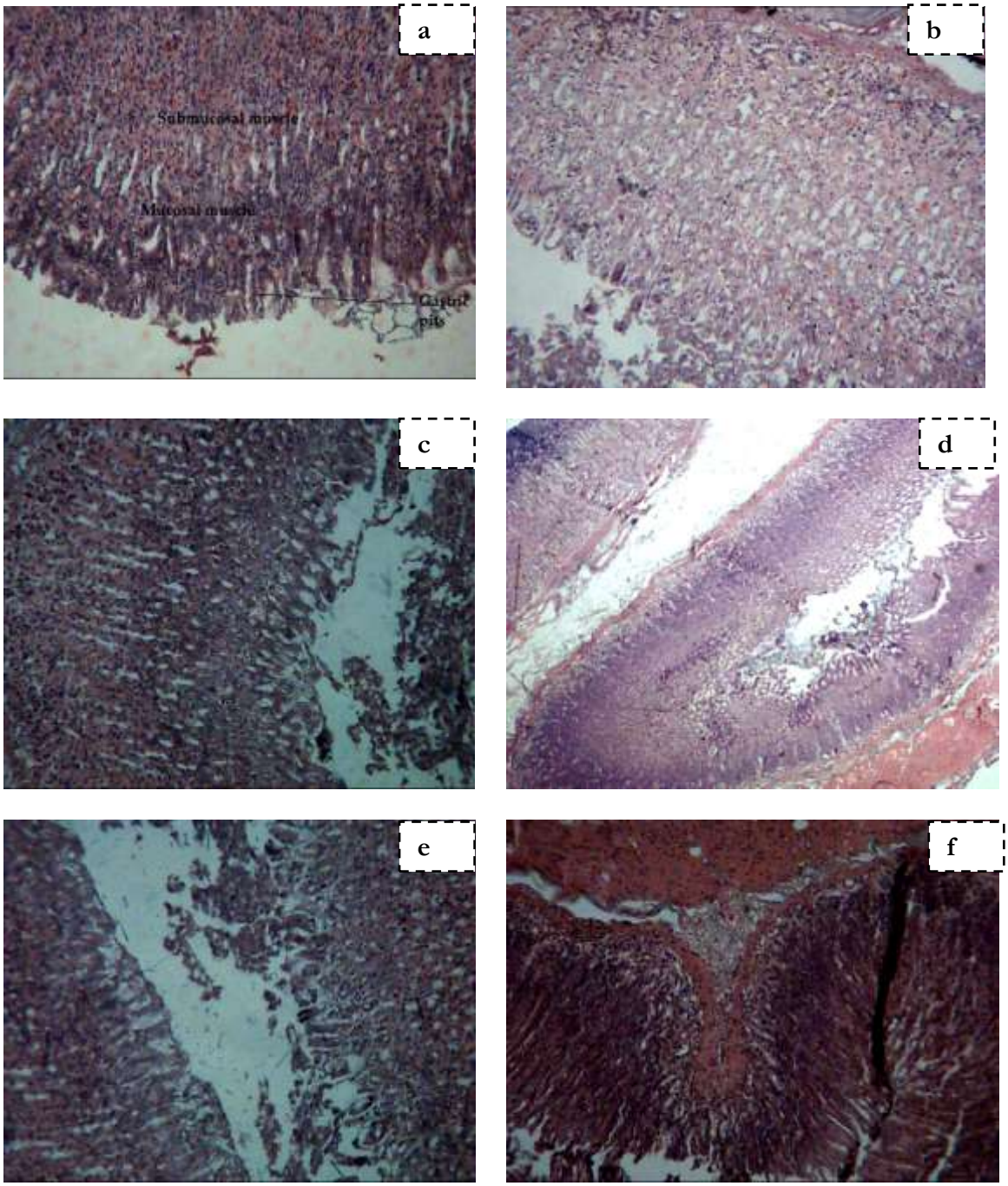

Plate 7.4 Photomicrograph of the sections of (a) the stomach in control rats, and (b-f) rats treated orally with 30 - 3000 mg kg-1 of the extract for 14 days in the sub-acute toxicity study (H & E, ×400)

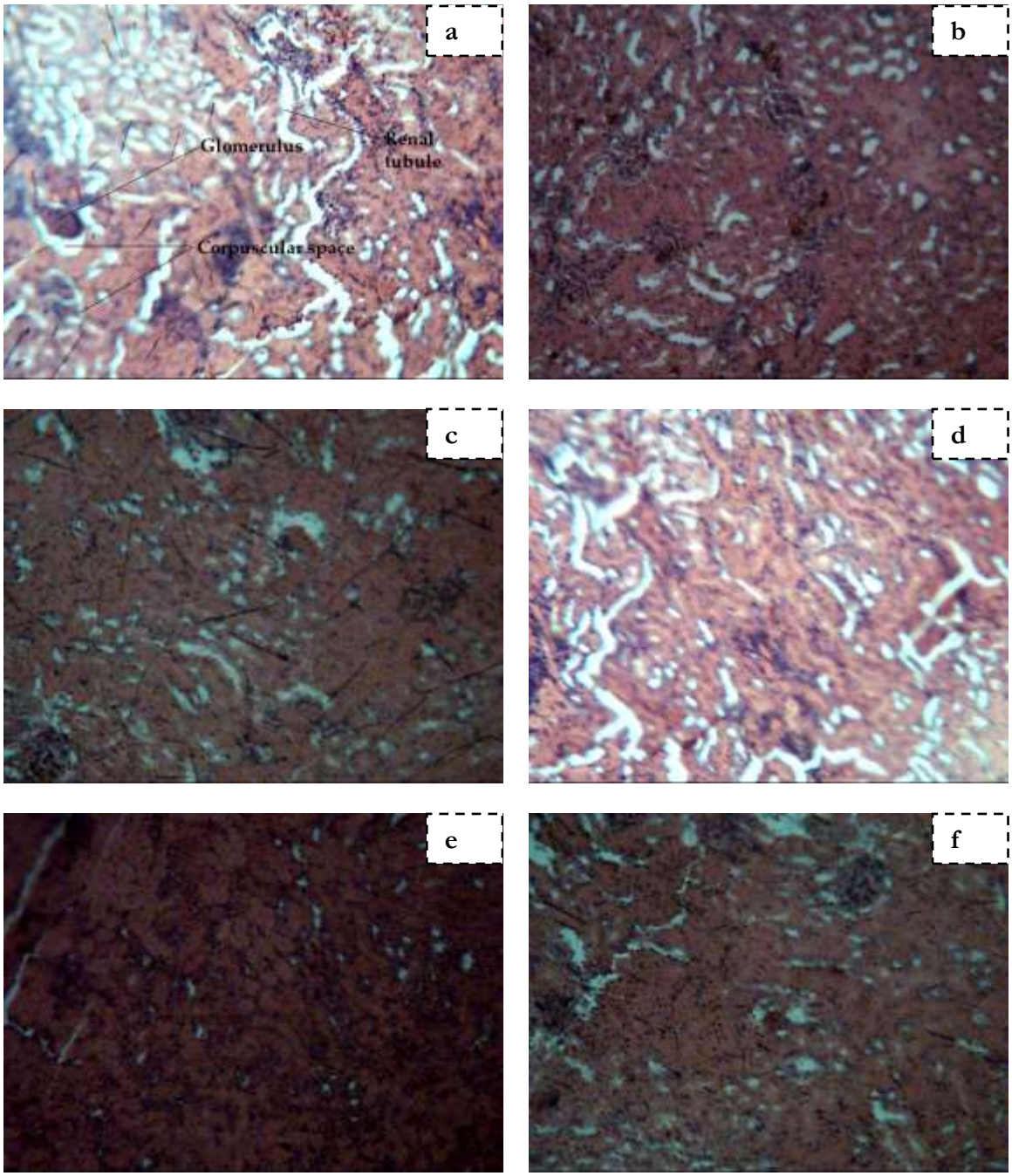

Plate 7.5 Photomicrograph of the sections of (a) the kidney in control rats, and (b-f) rats treated orally with 30 - 3000 mg kg^{-1} of the extract for 14 days in the sub-acute toxicity study (H & E, ×400)

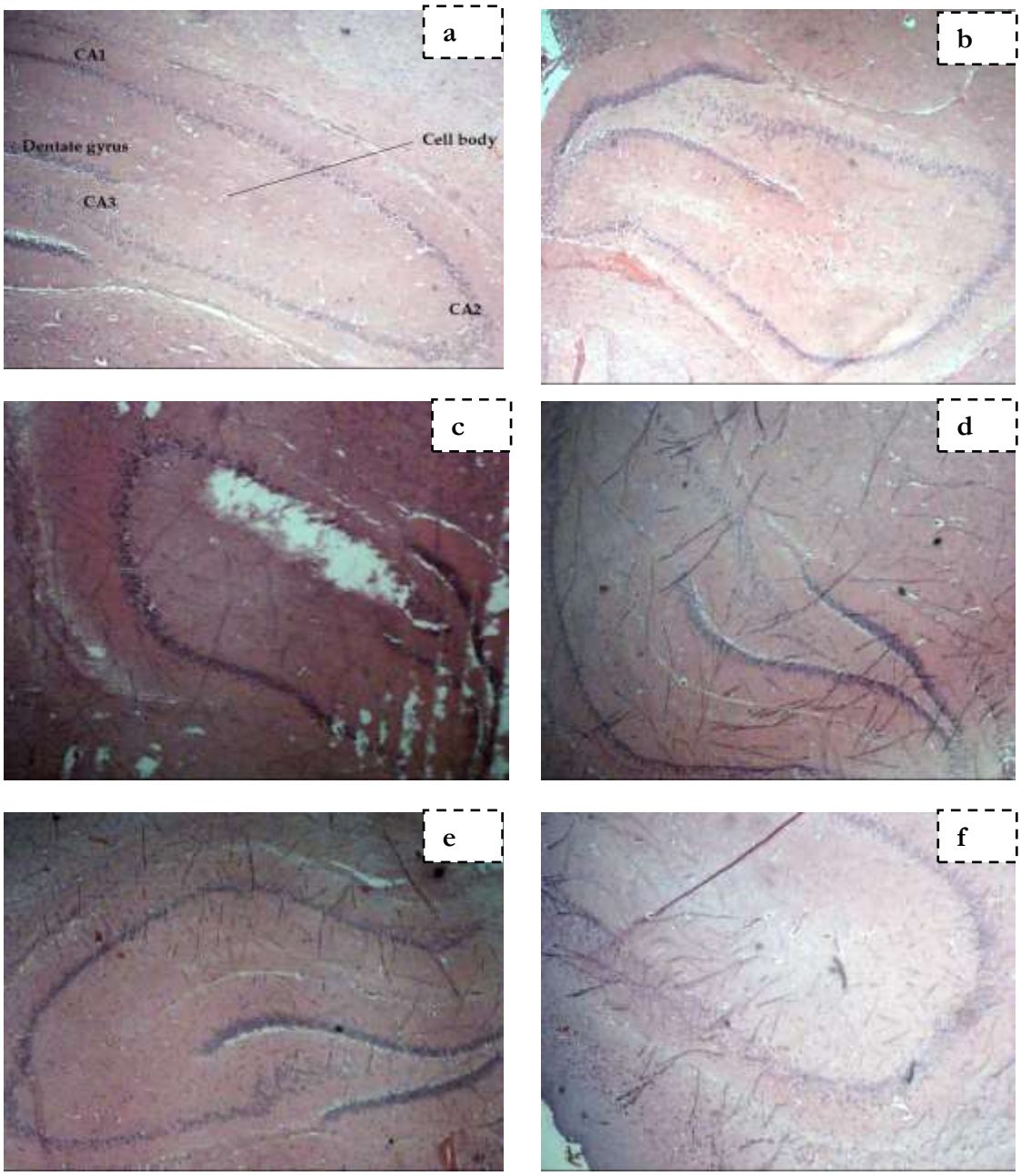

Plate 7.6 Photomicrograph of the sections of (a) the brain showing regions of the hippocampus in control rats, and (b-f) rats treated orally with 30 - 3000 mg kg-1 of the extract for 14 days in the sub-acute toxicity study (H & E, ×400)

7.4 DISCUSSION

Just as the therapeutic potential of plants can be rapid or delayed, the toxic effect can also be rapid or delayed (Anderson *et al.*, 1996; Haller *et al.*, 2002). It is therefore necessary to subject medicinal plants to safety assessment procedures to ensure their safety before they are recommended for human use.

Acute toxicity studies revealed that the extract of *M. oppositifolius* leaves was not lethal to the rats after 24 hours, indicating that the LD_{50} is above 3000 mg kg^{-1}. This suggests that the extract is relatively safe in rodents. At all doses, the extract did not show any toxic signs except sedation and motor incoordination at doses ranging between 1000 and 3000 mg kg^{-1} in the rats suggesting CNS depressant activity.

Even at low doses, cummulative toxic effects can occur with repeated dosing (Chitturi and Farrell, 2000; Niggemann and Gruber, 2003). Therefore subacute toxicity studies were carried out for 14 days to ascertain whether the extract will be toxic when administered repeatedly. All the rats survived after the fourteen days of treatment. Rats did not show any toxic signs during this period except for sedations and motor incordination at high doses (1000 and 3000 mg kg^{-1}) but these waned after some days. Water and food consumption was normal. In addition to these parameters, changes in body weight and relative organ weights were evaluated. Changes in body weight and relative organ weights provide a simple and sensitive index of toxicity after exposure to toxic substances (Teo *et al.*, 2002; Obici *et al.*, 2008). The extract treated rats did not show any significant difference in these parameters when compared to the control.

The blood is a very important tissue as far as drug action and toxicity is concerned— it is vital in the transportation and bioavailability of drug molecules. The white blood cells also fight against infection while the platelets regulate blood clotting (Heiser *et al.*, 2000; Misra *et al.*, 2003). Owing to these functions among many others, any haematological toxicity in the course of treatment may be deleterious. Thus an evaluation of the effect of the extract on some haematological parameters (Table 8.1) was conducted. MOE did not have any toxic effects on the haematological parameters measured. Histological sections of the cardiomyocytes did not show any toxic effects indicating any lack of cardiotoxic effect.

Integrity of the synthetic function of the liver was assessed by measuring total protein, albumin, globulin and albumin to globulin ratio (Rothschild *et al.*, 1988; Johnston, 1999). The extract had no toxic effect on these parameters. Levels of ALT, AST and ALP, which are used as markers of liver function, were measured (Johnston, 1999). Liver damaged is suspected when ALT and AST levels increase significantly though ALT is considered a

more sensitive marker because it is invariably found in hepatocytes (Beckingham and Ryder, 2001). Increases in ALP levels reveal possible biliary duct obstruction (Whitfield *et al.*, 1972). The levels of these markers in the extract treated rats did not differ from the control. Another test for liver function is the estimation of serum bilirubin levels (Beckingham, 2001; Beckingham and Ryder, 2001). Results from these estimates indicate the liver's ability to take up, process, and secrete bilirubin into the bile (Beckingham, 2001; Beckingham and Ryder, 2001;). There were no increases in the direct, indirect and total bilirubin levels after extract treatment except an increase in indirect and total bilirubin at 300 mg kg^{-1}. An increase in indirect and total bilirubin without commensurate increase in direct bilirubin may be an indication of prehepatic or hepatic disorder such as excessive haemolysis, cirrhosis or viral hepatitis (Ryder and Beckingham, 2001). However haematological indices did not indicate any haematological toxicity at this dose and the lack of hepatotoxicity of the extract was further confirmed by microscopic examination of the liver histology. The examinations did not reveal any signs of steatosis or necrosis. Increased bilirubin levels at 300 mg kg^{-1} may therefore be artefactual.

Kidneys play very important function in the pharmacokinetic effects of drugs especially metabolism and excretion of metabolic wastes (Lin *et al.*, 2003; Fagerholm, 2007). Aside these, the kidneys regulate blood pressure, water and electrolyte balance (Lotspeich, 1958; Finsterer *et al.*, 1988). The effect of MOE on kidney function was therefore investigated since any toxic effect on it can be detrimental or fatal. Results indicated that the extract at (30 and 100 mg kg^{-1}) increased the urea levels while only the 30 mg kg^{-1} significantly increased the serum creatinine levels compared to the control. This was however within the normal range (60 – 120 µmol/L). At higher doses however the increases of these parameters were reversed. Histological specimens did not reveal any extract induced injuries except a slight change in morphology at 30 mg kg^{-1}. This may suggest that the extract may not have any destructive effect on the kidney.

Plasma lipids such as cholesterol and triglycerides are essential for formation of cell membrane, synthesis of hormones and free fatty acids but can pose health challenges when their levels increase abnormally (Dietschy, 1998; Feroz *et al.*, 2011). Serum biochemical analysis revealed a general pattern of reduction in the levels of total cholesterol, triglycerides and very low density lipoproteins (VLDL) but increase in high density lipoproteins (HDL) though not significant statistically. The low density lipoproteins are considered ‘bad cholesterols’ because they carry a higher risk for cardiovascular incidence while the high density ones are the ‘good cholesterols’. Human studies have demonstrated that the risk for atherosclerotic disease, which can affect critical organs such as the heart,

brain and kidneys, is inversely related to blood levels of HDL (Miller and Miller, 1975; Toth, 2005). It is therefore not surprising that the coronary risk decreased since the very low density lipoproteins depreciated while the high density lipoproteins increased. This may also explain why no fatty infiltration was found in the liver or the heart histology.

The brain especially the hippocampus plays a very critical role with regards to disease conditions like epilepsy, depression and anxiety (Sahay and Hen, 2007). In these conditions research has shown morphological derangements in the hippocampus formation while some effective therapies may lead to reversal or worsening in these derangements. Thus the histology investigated possible extract induced changes in the hippocampal formation (CA1-3 and dentate gyrus). From the results it was apparent that the extract did not have any toxic effect on the brain. Visually the intensity of the cells in the hippocampus seems to be greater compared to the control. This could be possibly due to neurogenic activity in this region and may explain the effectiveness of the extract in memory improvement, depression as well as epilepsy (Duman and Monteggia, 2006; Sahay and Hen, 2007).

7.5 CONCLUSION

Oral administration of extracts from the leaves of *M. oppositifolius* is relatively safe in rats. Caution should however be exercised when extrapolating this results to man.

Chapter 8
GENERAL DISCUSSION

8.1 SUMMARY

Epilepsy, major depression and anxiety remain major health menace in the world because the burden of these non-communicable diseases is on the ascendancy (Lambert and Robertson, 1999; Jackson and Turkington, 2005). There is an overwhelming percentage of the population with conditions that are refractory to the current medications; the onset of action of current medication is slow; adverse effects and toxicity are rife (Lambert and Robertson, 1999; Jackson and Turkington, 2005). These reasons accentuate the need for alternative medications that are effective against the refractory conditions, safe, have rapid onset of action and can improve patient quality of life.

The therapeutic potential of plants in managing psychiatric and affective disorders on the other hand has been gaining credibility not only in developing nations but also the developed ones (Firenzuoli and Gori, 2007). Hence researching into the ability of medicinal plants to treat epilepsy, depression and anxiety is justified. *Mallotus oppositifolius*, a common shrub used for epilepsy and other psychiatric disorders in Ghana was effective as an anticonvulsant, antidepressant and anxiolytic in their respective models.

Central to every epileptic seizure is the imbalance between the equilibrium existing between excitation and inhibition—both enhancement of excitatory and impairment of inhibitory mechanisms can disturb this equilibrium leading to epileptic discharges (Dudek and Staley, 2007; Talathi *et al.*, 2009). Two basic mechanisms underlie the electrophysiological excitability of and the communication between neurons—axonal conduction, which is mediated by action potentials and signal transduction from cell to cell by synaptic transmission and ion channels provide the basis for the occurrence of these processes (Talathi *et al.*, 2009). Hence alterations in the function of ion channels may give rise to seizures and most of the currents antiepileptics alter ion channel processes to some extent (Mody, 1998; Jung *et al.*, 2007). Potassium channel activation or increase in potassium conductance may be a plausible mechanism of action of MOE because it was effective against 4-aminopyridine-induced seizures. When 4-aminopyridine is administered to rodents there is blockade of potassium channels which leads to increase in excitation through the release of glutamate and calcium and this forms the bedrock for the convulsant and neurodegenerative effect of 4-aminopyridine (Pena and Tapia, 2000; Sitges *et al.*, 2011). Owing to the fact that potassium channels play a major role in controlling all aspects of neuronal excitability and neurotransmitter release, any substance that activates these

channels may possess broad spectrum antiepileptic effect or be effective in painful conditions. For instance retigabine, a potassium channel activator, prevents epileptiform activity induced by 4-aminopyridine, bicuculline, low magensium (Mg^{2+}) and low calcium (Ca^{2+}) in hippocampal slices and seizures induced by PTZ, maximal electroshock, kainate, penicillin, picrotoxin and NMDA in rodents (Rostock *et al.*, 1996; Armand *et al.*, 1999; Dost and Rundfeldt, 2000). Retigabine is also effective against audiogenic seizures in DBA/2J mice, against seizures in epilepsy-prone rats and against seizures in an amygdala-kindling model (Dailey *et al.*, 1995; Rostock *et al.*, 1996; Tober *et al.*, 1996). Possibly the enhancement of potassium conductance by the extract may explain its broad spectrum anticonvulsant effect against 4-aminopyridine-, pentylenetetrazole-, picrotoxin-, strychnine and pilocarpine -induced seizures. It may also explain its analgesic effect since heperpolarization as a result of activating potassium channels may lead to analgesic effect. MOE, by activating GABAergic mechanisms in the PTZ seizure test will increase chloride conductance resulting in hyperpolarization and subsequent attenuation of seizures (Harris and Allan, 1985; Treiman, 2001). The extract may also increase chloride conductance by enhancing glycinergic activity since it was effective against seizures induced by strychnine, a glycine receptor antagonist.

That the extract was effective against 4-aminopyridine induced seizures might explain its possible inhibition of glutamatergic neurotransmission in the pilocarpine induced *status epilepticus* since 4-aminopyridine induces the release of glutamate (Sitges *et al.*, 2011). Pilocarpine induced *status epilepticus* in rodents represent a model of human temporal lobe epilepsy (Sanabria *et al.*, 2002; Curia *et al.*, 2008). Temporal lobe epilepsy is a partial seizure representing 70% of all drug-refractory epilepsies (Pitkanen, 2002). With 30% of all epilepsy cases being refractory to drug treatment, the efficacy of the extract against *status epilepticus* should be received with great glee and this information should help in the quest for isolating compounds that will treat drug-refractory epilepsies.

Clinical efficacy of an antiepileptic drug is defined as the ability of the drug to decrease the frequency of seizures by $\geq$ 50% (Cramer *et al.*, 1999; Glauser *et al.*, 2006). Since the extract demonstrated greater than 50% reduction in seizure frequency, it may be inferred that the extract will be promising as an anticonvulsant. It can also be deduced that the extract may be effective against temporal lobe epilepsy since it demonstrated activity against pilocarpine-induced *status epilepticus* and against generalized seizures because of its effectiveness in the MES, PTZ and picrotoxin-induced seizures. These effects observed attaches credibility to the traditional use of the plant in epilepsy.

Isobolographic analysis of low doses of fractions of extract and diazepam combination showed synergistic effect. However the mode of action of this combination may not be dependent on GABAergic mechanisms since flumazenil pretreatment could not inhibit the anticonvulsant effect of the combined extract and diazepam effect.

Aside its anticonvulsant effect, the extract demonstrated significant antidepressant effect in both acute and chronic models of depression. It is interesting to note that the extract exhibited a more rapid onset of action than fluoxetine and imipramine in the open space swim test, the chronic model used—effect started on the first day of drug treatment. Thus MOE may be a good source of antidepressant compounds with a more rapid onset of action than the conventional ones. Not only did the extract show a rapid antidepressant effect but it also improved spatial learning and memory in depressed mice that received treatment. Scientific evidence suggests that the incidence of suicides and hence mortality is positively correlated with the delay in symptom improvement among the depressed (Lucas *et al.*, 2007). It is possible that MOE may be a potential source of potent compounds with rapid onset of action that can help improve the quality of life of depressed patients and thus a reduction in the rate of suicides. One of the major goals of depression therapy is to manage depression-induced memory and cognitive deficits (Parikh and Lam, 2001; Reesal and Lam, 2001). Some antidepressants however can also induce memory impairment—this may confound assessment of disease improvement (Moore and O'Keeffe, 1999; Gallassi *et al.*, 2006). That the extract improves memory while demonstrating a rapid onset of action is a finding that must be exploited, especially in those at risk of cognitive dysfunction like the elderly.

Central serotoninergic mechanisms with weak opioidergic activity, devoid of central adrenergic mechanisms are responsible for the antidepressant effect observed in the acute depression models used. MOE's dependence on serotoninergic enhancement was demonstrated through the extract's ability to increase swimming score and subsequent inhibition of this behavioural effect by para-chlorophenylalanine, a 5-HTP hydroxylase inhibitor (Park *et al.*, 1994; Carrillo-Vico *et al.*, 2005). The potentiation of 5-hydroxytryptophan induced head twitch further supported the serotoninergic enhancement by the extract. By slightly increasing curling score in the tail suspension test and this effect being potentiated by D-cycloserine, a possible opioidergic activity by the extract is likely (Berrocoso *et al.*, 2012). The opiodergic activity demonstrated in the tail suspension test may also explain the antinociceptive effect of the extract in the tail immersion test, a model sensitive to morphine-like compounds or opioids (Vonvoigtlander *et al.*, 1983; Romberg *et*

al., 2003). The antinociceptive effect of the extract supports the traditional use of the leaves in the management of pain.

The antidepressant effect of MOE in both the tail suspension and modified forced swim test could be due to its antagonistic effect on the glycine/NMDA receptor complex or pathway. Antagonists of the glycine/NMDA receptor complex or pathway have demonstrated fewer side effects in comparison to their counterparts which are competitive or non-competitive antagonists (Poleszak *et al.*, 2011). Also some of these compounds have demonstrated rapid antidepressant effects in chronic depressions models and are used for conditions where memory is impaired (Poleszak *et al.*, 2011; Howard *et al.*, 2012). It can therefore be inferred that the rapid onset of action and the memory improvement may be attributable to the antagonistic effect of the extract on this pathway. This antagonistic effect may also be responsible for the anticonvulsant effect of the extract in the pilocarpine-induced *status epilepticus* and partly PTZ seizures (Eloqayli *et al.*, 2003; Costa *et al.*, 2004).

Anxiolytic effect was demonstrated by the extract in the elevated plus, light dark box and open field tests. Anxiety is one of the commonest psychiatric disorders in epileptic patients (Beyenburg and Schmidt, 2005; Caplan *et al.*, 2005). Finding a compound with combined anxiolytic and antiepileptic effect would be very important. Though examples like the benzodiazepines exist, tolerance and dependence limit their long term use (Bateson, 2002; Ashton, 2005). It will be important to further explore the anxiolytic effects of the extract. Most compounds that enhance GABA neurotransmission or cause sedation seem to demonstrate anxiolytic effect (Bateson, 2002; Donaldson *et al.*, 2007). It is therefore not surprising that the extract showed anxiolysis in rodents since it enhanced GABA neurotransmission and caused sedation.

Potential for drug interaction by the extract with other drugs must be borne in mind since it was metabolized by hepatic enzymes and demonstrated significant induction of liver enzymes. Drugs that are metabolized by by the liver may have reduced therapeutic effect or reduced toxic effect—hence there may be positive or negative effects to it (Lynch and Price, 2007; Bibi, 2008).

The extract was relatively safe in mice and rats. In mice the LD_{50} was approximately 6000 mg kg^{-1} while it was above 3000 mg kg^{-1} in rats. It also did not induce serious tissue or organ related damages in the rats.

The possible mechanism(s) by which the extract may be acting is shown below (Figure 8.1).

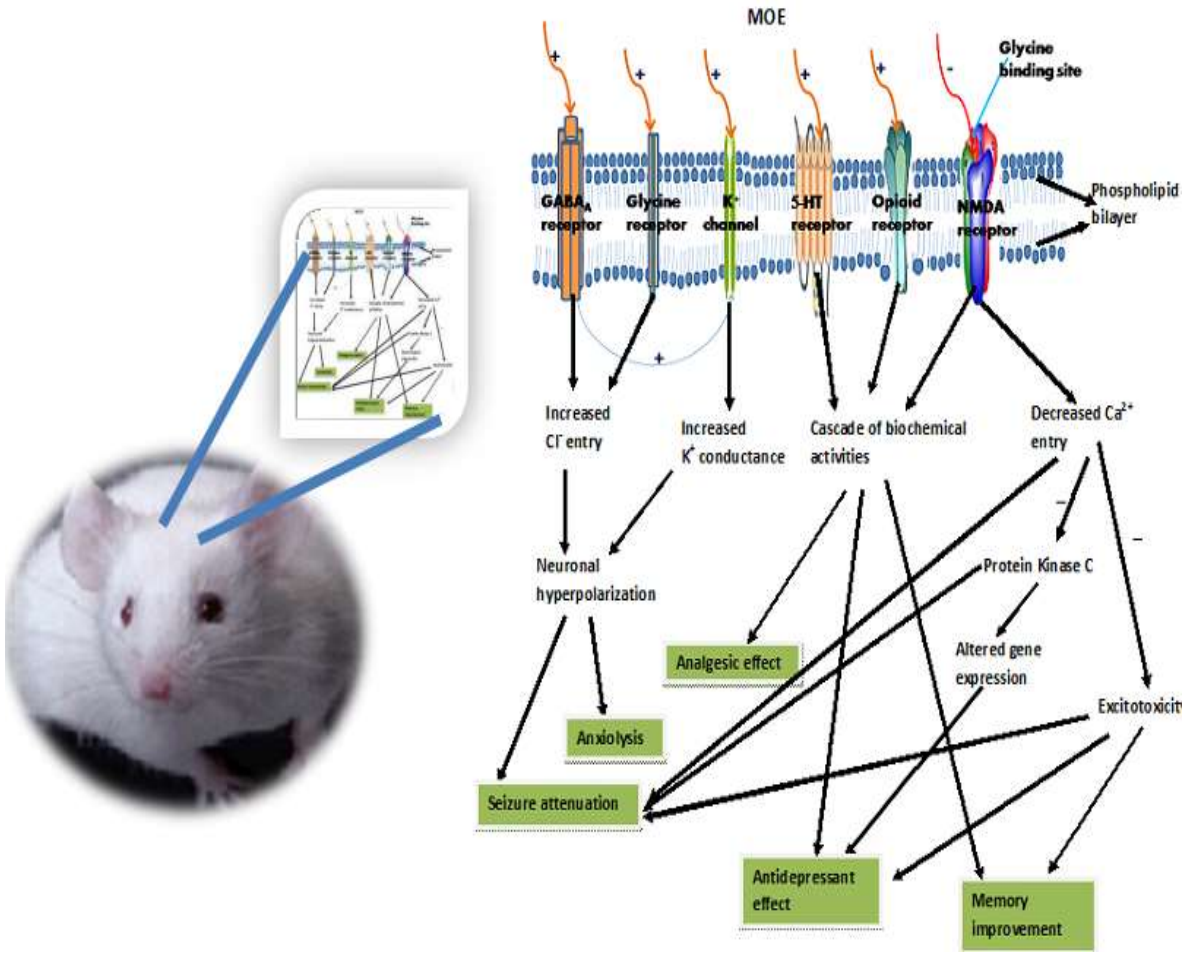

Figure 8.1 A simplified representation of the proposed mechanisms of action of the hydroalcoholic extract of the leaves of *Mallotus oppositifolius* (MOE). MOE activates $GABA_A$ and glycinergic neurotransmission causing an increase in chloride (Cl^{-1}) conductance and consequently neuronal hyperpolarization. The hyperpolarization results in seizure attenuation and anxiolysis. MOE activates K^+ channels leading to increased potassium conductance, hyperpolarization and decreased neuronal excitation (decreased excitotoxicity). This also contributes to attenuation of seizures. GABA activation can result in increased potassium conductance leading to the anticonvulsant effect observed. MOE also antagonises NMDA receptors or pathway leading to a decrease in Ca^{2+} entry. Decreased Ca^{2+} entry inhibits calcium dependent excitotoxicity which can also contribute to the anticonvulsant, antidepressant effects as well as improvement in memory. The decreased Ca^{2+} entry can also lead to altered gene expressions resulting in antidepressant effect. The interaction of MOE with the 5-HT-opioid-glycine/NMDA pathways leads to a cascade of signal transduction mechanisms which are responsible for the observed antidepressant effect, analgesic and memory improvement.

8.2 CONCLUSION

It is evident from the research conducted that the hydroalcoholic leaf extract of *Mallotus oppositifolius* has anticonvulsant, anxiolytic, and rapid sustained antidepressant properties. The extract also improved spatial learning and memory and showed analgesic effect. The extract is an inducer of liver metabolising enzymes and serves as a substrate to these enzymes.

- Enhancement of GABAergic and glycinergic neurotransmission as well as increase in potassium conductance and inhibition of glutamatergic activity was responsible for the anticonvulsant effect observed.
- Interaction with central serotoninergic-opioid-glycine/NMDA pathways accounted for the rapid antidepressant effect observed. These mechanisms may also explain the analgesic properties of the extract in the tail immersion test. Enhacement of $5HT_{2A}$ and inhibitory interaction with the strychnine insensitive glycine site of the NMDA receptor may be responsible for the memory improvement properties of the extract.
- Interplay between enhancement of GABAergic and serotoninergic neurotransmission may support the role of the extract in anxiety states.

8.3 RECOMMENDATIONS

- The extract should be fractionated to isolate the compounds responsible for the observed pharmacological effects especially the compound(s) responsible for the rapid and sustained antidepressant effect since there is so far no drug approved with such effect. Also animal models such as genetic absence epilepsy rats from Strasbourg (GAERS) or lethargic mice should be used to further confirm the possible anti-absence properties of the extract observed in the PTZ seizure test.
- Chronic toxicity should be carried out to extablish the safety of the extract.
- This work should be repeated in non-human primates in order to assess and/or confirm the scientific information gathered or discover those that were not apparent in this study.
- Further isobolographic analysis of the extract and diazepam should be carried out to elucidate the exact mechanism by which their combination demonstrated synergism.
- Other pain models should be used to fully characterize the antinociceptive properties of the extract and its mode of action`

REFERENCES

ABU-IRMAILEH, B. E. and AFIFI, F. U. (2003). Herbal medicine in Jordan with special emphasis on commonly used herbs. *J Ethnopharmacol* **89**(2-3): 193-197.

ADEKUNLE, A. A. and IKUMAPAYI, A. M. (2006). Antifungal property and phytochemical screening of the crude extracts of Funtumia elastica and Mallotus oppositifolius. *West Indian Med J* **55**(4): 219-223.

ADELL, A., CASTRO, E., CELADA, P., BORTOLOZZI, A., PAZOS, A. and ARTIGAS, F. (2005). Strategies for producing faster acting antidepressants. *Drug Discov Today* **10**(8): 578-585.

AGOSTA, W. (1997). Medicines and Drugs from Plants. *Journal of Chemical Education* **74**(857).

AKAIKE, N., INOMATA, N. and YAKUSHIJI, T. (1989). Differential effects of extra- and intracellular anions on GABA-activated currents in bullfrog sensory neurons. *J Neurophysiol* **62**(6): 1388-1399.

AKHONDZADEH, S. and ABBASI, S. H. (2006). Herbal medicine in the treatment of Alzheimer's disease. *Am J Alzheimers Dis Other Demen* **21**(2): 113-118.

AKULA, K. K., DHIR, A. and KULKARNI, S. K. (2009). Effect of various antiepileptic drugs in a pentylenetetrazol-induced seizure model in mice. *Methods Find Exp Clin Pharmacol* **31**(7): 423-432.

ALBUS, H. and WILLIAMSON, R. (1998). Electrophysiologic analysis of the actions of valproate on pyramidal neurons in the rat hippocampal slice. *Epilepsia* **39**(2): 124-139.

ALMEIDA, O. P., WATERREUS, A., SPRY, N., FLICKER, L. and MARTINS, R. N. (2004). One year follow-up study of the association between chemical castration, sex hormones, beta-amyloid, memory and depression in men. *Psychoneuroendocrinology* **29**(8): 1071-1081.

ALONSO, J., ANGERMEYER, M. C., BERNERT, S., BRUFFAERTS, R., BRUGHA, T. S., BRYSON, H., DE GIROLAMO, G., GRAAF, R., DEMYTTENAERE, K., GASQUET, I., HARO, J. M., KATZ, S. J., KESSLER, R. C., KOVESS, V., LEPINE, J. P., ORMEL, J., POLIDORI, G., RUSSO, L. J., VILAGUT, G., ALMANSA, J., ARBABZADEH-BOUCHEZ, S., AUTONELL, J., BERNAL, M., BUIST-BOUWMAN, M. A., CODONY, M., DOMINGO-SALVANY, A., FERRER, M., JOO, S. S., MARTINEZ-ALONSO, M., MATSCHINGER, H., MAZZI, F., MORGAN, Z., MOROSINI, P., PALACIN, C., ROMERA, B., TAUB, N. and VOLLEBERGH, W. A. (2004). Prevalence of mental disorders in Europe: results from the European Study of the Epidemiology of Mental Disorders (ESEMeD) project. *Acta Psychiatr Scand Suppl*(420): 21-27.

AMABEOKU, G. J. and CHIKUNI, O. (1993). Cimetidine-induced seizures in mice. Antagonism by some GABAergic agents. *Biochem Pharmacol* **46**(12): 2171-2175.

AMBROSIO, A. F., SILVA, A. P., MALVA, J. O., SOARES-DA-SILVA, P., CARVALHO, A. P. and CARVALHO, C. M. (2001). Inhibition of glutamate release by BIA 2-093 and BIA 2-024, two novel derivatives of carbamazepine, due to blockade of sodium but not calcium channels. *Biochem Pharmacol* **61**(10): 1271-1275.

ANDERSON, I. B., MULLEN, W. H., MEEKER, J. E., KHOJASTEH BAKHT, S. C., OISHI, S., NELSON, S. D. and BLANC, P. D. (1996). Pennyroyal toxicity: measurement of toxic metabolite levels in two cases and review of the literature. *Ann Intern Med* **124**(8): 726-734.

ANDERSON, S. E., COHEN, P., NAUMOVA, E. N. and MUST, A. (2006). Association of depression and anxiety disorders with weight change in a prospective community-based study of children followed up into adulthood. *Arch Pediatr Adolesc Med* **160**(3): 285-291.

ANDERSON, S. E., COHEN, P., NAUMOVA, E. N. and MUST, A. (2006). Relationship of childhood behavior disorders to weight gain from childhood into adulthood. *Ambul Pediatr* **6**(5): 297-301.

ANDRE, P., DESCOTES, J., SIMONET, R. and EVREUX, J. C. (1984). Reappraisal of the barbiturate sleeping time in mice as predictive tool for the detection of liver enzymes inhibiting drugs. *Methods Find Exp Clin Pharmacol* **6**(11): 695-699.

ANDREWS, G., SZABO, M. and BURNS, J. (2002). Preventing major depression in young people. *Br J Psychiatry* **181**: 460-462.

ARKO, M. and FLORJANC, T. I. (2001). Effects of mirtazapine on the levels of exogenous histamine in the plasma of the cat. *Pflugers Arch* **442**(6 Suppl 1): R207-208.

ARMAND, V., RUNDFELDT, C. and HEINEMANN, U. (1999). Effects of retigabine (D-23129) on different patterns of epileptiform activity induced by 4-aminopyridine in rat entorhinal cortex hippocampal slices. *Naunyn Schmiedebergs Arch Pharmacol* **359**(1): 33-39.

ARTIGAS, F., CELADA, P., LARUELLE, M. and ADELL, A. (2001). How does pindolol improve antidepressant action? *Trends Pharmacol Sci* **22**(5): 224-228.

ASHTON, H. (2005). The diagnosis and management of benzodiazepine dependence. *Curr Opin Psychiatry* **18**(3): 249-255.

AVALLONE, R., ZANOLI, P., PUIA, G., KLEINSCHNITZ, M., SCHREIER, P. and BARALDI, M. (2000). Pharmacological profile of apigenin, a flavonoid isolated from Matricaria chamomilla. *Biochem Pharmacol* **59**(11): 1387-1394.

AZMITIA, E. C., RUBINSTEIN, V. J., STRAFACI, J. A., RIOS, J. C. and WHITAKER-AZMITIA, P. M. (1995). 5-HT1A agonist and dexamethasone reversal of para-chloroamphetamine induced loss of MAP-2 and synaptophysin immunoreactivity in adult rat brain. *Brain Res* **677**(2): 181-192.

BACK, D., GIBBONS, S. and KHOO, S. (2003). Pharmacokinetic drug interactions with nevirapine. *J Acquir Immune Defic Syndr* **34 Suppl 1**: S8-14.

BALDESSARINI, R. J., MARSH, E. R., KULA, N. S., ZONG, R. S., GAO, Y. G. and NEUMEYER, J. L. (1990). Effects of isomers of hydroxyaporphines on dopamine metabolism in rat brain regions. *Biochem Pharmacol* **40**(3): 417-423.

BALDWIN, D. S., ANDERSON, I. M., NUTT, D. J., BANDELOW, B., BOND, A., DAVIDSON, J. R., DEN BOER, J. A., FINEBERG, N. A., KNAPP, M., SCOTT, J. and WITTCHEN, H. U. (2005). Evidence-based guidelines for the pharmacological treatment of anxiety disorders: recommendations from the British Association for Psychopharmacology. *J Psychopharmacol* **19**(6): 567-596.

BALON, R. (2006). Mood, anxiety, and physical illness: body and mind, or mind and body? *Depress Anxiety* **23**(6): 377-387.

BALU, D. T. and LUCKI, I. (2009). Adult hippocampal neurogenesis: regulation, functional implications, and contribution to disease pathology. *Neurosci Biobehav Rev* **33**(3): 232-252.

BASKIND, R. and BIRBECK, G. L. (2005). Epilepsy-associated stigma in sub-Saharan Africa: the social landscape of a disease. *Epilepsy Behav* **7**(1): 68-73.

BATESON, A. N. (2002). Basic pharmacologic mechanisms involved in benzodiazepine tolerance and withdrawal. *Curr Pharm Des* **8**(1): 5-21.

BAZIL, C. W. and PEDLEY, T. A. (1998). Advances in the medical treatment of epilepsy. *Annu Rev Med* **49**: 135-162.

BEASLEY, C. M., MASICA, D. N. and POTVIN, J. H. (1992). Fluoxetine: a review of receptor and functional effects and their clinical implications. *Psychopharmacology (Berl)* **107**(1): 1-10.

BECKINGHAM, I. J. (2001). ABC of diseases of liver, pancreas, and biliary system. Gallstone disease. *BMJ* **322**(7278): 91-94.

BECKINGHAM, I. J. and RYDER, S. D. (2001). ABC of diseases of liver, pancreas, and biliary system. Investigation of liver and biliary disease. *BMJ* **322**(7277): 33-36.

BELMAKER, R. H. and AGAM, G. (2008). Major depressive disorder. *N Engl J Med* **358**(1): 55-68.

BELZUNG, C. and LE PAPE, G. (1994). Comparison of different behavioral test situations used in psychopharmacology for measurement of anxiety. *Physiol Behav* **56**(3): 623-628.

BELZUNG, C., MISSLIN, R., VOGEL, E., DODD, R. H. and CHAPOUTHIER, G. (1987). Anxiogenic effects of methyl-beta-carboline-3-carboxylate in a light/dark choice situation. *Pharmacol Biochem Behav* **28**(1): 29-33.

BENBADIS, S. R. and TATUM, W. O. T. (2001). Advances in the treatment of epilepsy. *Am Fam Physician* **64**(1): 91-98.

BERMAN, R. M., CAPPIELLO, A., ANAND, A., OREN, D. A., HENINGER, G. R., CHARNEY, D. S. and KRYSTAL, J. H. (2000). Antidepressant effects of ketamine in depressed patients. *Biol Psychiatry* **47**(4): 351-354.

BERROCOSO, E., IKEDA, K., SORA, I., UHL, G. R., SANCHEZ-BLAZQUEZ, P. and MICO, J. A. (2012). Active behaviours produced by antidepressants and opioids in the mouse tail suspension test. *Int J Neuropsychopharmacol*: 1-12.

BERTON, O. and NESTLER, E. J. (2006). New approaches to antidepressant drug discovery: beyond monoamines. *Nat Rev Neurosci* **7**(2): 137-151.

BETARBET, R., SHERER, T. B. and GREENAMYRE, J. T. (2002). Animal models of Parkinson's disease. *Bioessays* **24**(4): 308-318.

BEYENBURG, S. and SCHMIDT, D. (2005). [Patients with epilepsy and anxiety disorders. Diagnosis and treatment]. *Nervenarzt* **76**(9): 1077-1078, 1081-1072, 1084-1076 passim.

BHATTACHARYA, S. K. and MITRA, S. K. (1991). Anxiolytic activity of Panax ginseng roots: an experimental study. *J Ethnopharmacol* **34**(1): 87-92.

BIALER, M. (2006). New antiepileptic drugs that are second generation to existing antiepileptic drugs. *Expert Opin Investig Drugs* **15**(6): 637-647.

BIBI, Z. (2008). Role of cytochrome P450 in drug interactions. *Nutr Metab (Lond)* **5**: 27.

BIENVENU, E., AMABEOKU, G. J., EAGLES, P. K., SCOTT, G. and SPRINGFIELD, E. P. (2002). Anticonvulsant activity of aqueous extract of Leonotis leonurus. *Phytomedicine* **9**(3): 217-223.

BIRINGER, E., MYKLETUN, A., SUNDET, K., KROKEN, R., STORDAL, K. I. and LUND, A. (2007). A longitudinal analysis of neurocognitive function in unipolar depression. *J Clin Exp Neuropsychol* **29**(8): 879-891.

BLIER, P. and DE MONTIGNY, C. (1994). Current advances and trends in the treatment of depression. *Trends Pharmacol Sci* **15**(7): 220-226.

BLIER, P., DE MONTIGNY, C. and CHAPUT, Y. (1990). A role for the serotonin system in the mechanism of action of antidepressant treatments: preclinical evidence. *J Clin Psychiatry* **51 Suppl**: 14-20; discussion 21.

BOERKAMP, K. and WIJNBERGER, A. (2011). [Looking beyond just making the animals better]. *Tijdschr Diergeneesk* **136**(7): 484-485.

BOHN, L. M., XU, F., GAINETDINOV, R. R. and CARON, M. G. (2000). Potentiated opioid analgesia in norepinephrine transporter knock-out mice. *J Neurosci* **20**(24): 9040-9045.

BORSINI, F. and MELI, A. (1988). Is the forced swimming test a suitable model for revealing antidepressant activity? *Psychopharmacology (Berl)* **94**(2): 147-160.

BOUGH, K. J. and RHO, J. M. (2007). Anticonvulsant mechanisms of the ketogenic diet. *Epilepsia* **48**(1): 43-58.

BOUIC, P. J., ETSEBETH, S., LIEBENBERG, R. W., ALBRECHT, C. F., PEGEL, K. and VAN JAARSVELD, P. P. (1996). beta-Sitosterol and beta-sitosterol glucoside stimulate human peripheral blood lymphocyte proliferation: implications for their use as an immunomodulatory vitamin combination. *Int J Immunopharmacol* **18**(12): 693-700.

BOURIN, M. and HASCOET, M. (2003). The mouse light/dark box test. *Eur J Pharmacol* **463**(1-3): 55-65.

BOWERY, N. G. and SMART, T. G. (2006). GABA and glycine as neurotransmitters: a brief history. *Br J Pharmacol* **147 Suppl 1**: S109-119.

BRANDT, C., HEILE, A., POTSCHKA, H., STOEHR, T. and LOSCHER, W. (2006). Effects of the novel antiepileptic drug lacosamide on the development of amygdala kindling in rats. *Epilepsia* **47**(11): 1803-1809.

BREYTENBACH, U., CLARK, A., LAMPRECHT, J. and BOUIC, P. (2001). Flow cytometric analysis of the Th1-Th2 balance in healthy individuals and patients infected with the human immunodeficiency virus (HIV) receiving a plant sterol/sterolin mixture. *Cell Biol Int* **25**(1): 43-49.

BRISKIN, D. P. (2000). Medicinal plants and phytomedicines. Linking plant biochemistry and physiology to human health. *Plant Physiol* **124**(2): 507-514.

BRISKIN, D. P. (2000). Medicinal Plants and Phytomedicines. Linking Plant Biochemistry and Physiology to Human Health. *Plant Physiol* **124**(2): 507-514.

BROCKMOLLER, J. and ROOTS, I. (1994). Assessment of liver metabolic function. Clinical implications. *Clin Pharmacokinet* **27**(3): 216-248.

BRODIE, M. J. (2001). Do we need any more new antiepileptic drugs? *Epilepsy Res* **45**(1-3): 3-6.

BRODIE, M. J., PERUCCA, E., RYVLIN, P., BEN-MENACHEM, E. and MEENCKE, H. J. (2007). Comparison of levetiracetam and controlled-release carbamazepine in newly diagnosed epilepsy. *Neurology* **68**(6): 402-408.

BROOKS, A. I., CHADWICK, C. A., GELBARD, H. A., CORY-SLECHTA, D. A. and FEDEROFF, H. J. (1999). Paraquat elicited neurobehavioral syndrome caused by dopaminergic neuron loss. *Brain Res* **823**(1-2): 1-10.

BRUNIG, I., SOMMER, M., HATT, H. and BORMANN, J. (1999). Dopamine receptor subtypes modulate olfactory bulb gamma-aminobutyric acid type A receptors. *Proc Natl Acad Sci U S A* **96**(5): 2456-2460.

BUHOT, M. C., MARTIN, S. and SEGU, L. (2000). Role of serotonin in memory impairment. *Ann Med* **32**(3): 210-221.

BURKILL, H. (1985). The Flora of West Tropical Africa. . **2nd Edn., Kew, Royal Botanic Gardens, London,** .

BUZZINI, P., ARAPITSAS, P., GORETTI, M., BRANDA, E., TURCHETTI, B., PINELLI, P., IERI, F. and ROMANI, A. (2008). Antimicrobial and antiviral activity of hydrolysable tannins. *Mini Rev Med Chem* **8**(12): 1179-1187.

BYMASTER, F. P., ZHANG, W., CARTER, P. A., SHAW, J., CHERNET, E., PHEBUS, L., WONG, D. T. and PERRY, K. W. (2002). Fluoxetine, but not other selective serotonin uptake inhibitors, increases norepinephrine and dopamine extracellular levels in prefrontal cortex. *Psychopharmacology (Berl)* **160**(4): 353-361.

CALDECOTT-HAZARD, S. and SCHNEIDER, L. S. (1992). Clinical and biochemical aspects of depressive disorders: III. Treatment and controversies. *Synapse* **10**(2): 141-168.

CALIXTO, J. B. (2000). Efficacy, safety, quality control, marketing and regulatory guidelines for herbal medicines (phytotherapeutic agents). *Braz J Med Biol Res* **33**(2): 179-189.

CAMERINO, D. C., TRICARICO, D. and DESAPHY, J. F. (2007). Ion channel pharmacology. *Neurotherapeutics* **4**(2): 184-198.

CAPLAN, R., SIDDARTH, P., GURBANI, S., HANSON, R., SANKAR, R. and SHIELDS, W. D. (2005). Depression and anxiety disorders in pediatric epilepsy. *Epilepsia* **46**(5): 720-730.

CAROBREZ, A. P. and BERTOGLIO, L. J. (2005). Ethological and temporal analyses of anxiety-like behavior: the elevated plus-maze model 20 years on. *Neurosci Biobehav Rev* **29**(8): 1193-1205.

CARPIO, A. and HAUSER, W. A. (2009). Epilepsy in the developing world. *Curr Neurol Neurosci Rep* **9**(4): 319-326.

CARRILLO-VICO, A., LARDONE, P. J., FERNANDEZ-SANTOS, J. M., MARTIN-LACAVE, I., CALVO, J. R., KARASEK, M. and GUERRERO, J. M. (2005). Human lymphocyte-synthesized melatonin is involved in the regulation of the interleukin-2/interleukin-2 receptor system. *J Clin Endocrinol Metab* **90**(2): 992-1000.

CASTEL-BRANCO, M. M., ALVES, G. L., FIGUEIREDO, I. V., FALCAO, A. C. and CARAMONA, M. M. (2009). The maximal electroshock seizure (MES) model in the preclinical assessment of potential new antiepileptic drugs. *Methods Find Exp Clin Pharmacol* **31**(2): 101-106.

CEGLIA, I., ACCONCIA, S., FRACASSO, C., COLOVIC, M., CACCIA, S. and INVERNIZZI, R. W. (2004). Effects of chronic treatment with escitalopram or citalopram on extracellular 5-HT in the prefrontal cortex of rats: role of 5-HT1A receptors. *Br J Pharmacol* **142**(3): 469-478.

CESURA, A. M. and PLETSCHER, A. (1992). The new generation of monoamine oxidase inhibitors. *Prog Drug Res* **38**: 171-297.

CHAO, T. I. and ALZHEIMER, C. (1995). Effects of phenytoin on the persistent Na+ current of mammalian CNS neurones. *Neuroreport* **6**(13): 1778-1780.

CHAPUT, Y., DE MONTIGNY, C. and BLIER, P. (1991). Presynaptic and postsynaptic modifications of the serotonin system by long-term administration of antidepressant treatments. An in vivo electrophysiologic study in the rat. *Neuropsychopharmacology* **5**(4): 219-229.

CHARNEY, D. S., WOODS, S. W., GOODMAN, W. K. and HENINGER, G. R. (1987). Serotonin function in anxiety. II. Effects of the serotonin agonist MCPP in panic disorder patients and healthy subjects. *Psychopharmacology (Berl)* **92**(1): 14-24.

CHAU, D. T., RADA, P. V., KIM, K., KOSLOFF, R. A. and HOEBEL, B. G. (2011). Fluoxetine alleviates behavioral depression while decreasing acetylcholine release in the nucleus accumbens shell. *Neuropsychopharmacology* **36**(8): 1729-1737.

CHAUHAN, A. K., DOBHAL, M. P. and JOSHI, B. C. (1988). A review of medicinal plants showing anticonvulsant activity. *J Ethnopharmacol* **22**(1): 11-23.

CHERKOFSKY, S. C. (1995). 1-Aminocyclopropanecarboxylic acid: mouse to man interspecies pharmacokinetic comparisons and allometric relationships. *J Pharm Sci* **84**(10): 1231-1235.

CHERMAT, R., THIERRY, B., MICO, J. A., STERU, L. and SIMON, P. (1986). Adaptation of the tail suspension test to the rat. *J Pharmacol* **17**(3): 348-350.

CHITTURI, S. and FARRELL, G. C. (2000). Herbal hepatotoxicity: an expanding but poorly defined problem. *J Gastroenterol Hepatol* **15**(10): 1093-1099.

CHOLERIS, E., THOMAS, A. W., KAVALIERS, M. and PRATO, F. S. (2001). A detailed ethological analysis of the mouse open field test: effects of diazepam, chlordiazepoxide and an extremely low frequency pulsed magnetic field. *Neurosci Biobehav Rev* **25**(3): 235-260.

CLEARE, A. J., BEARN, J., ALLAIN, T., MCGREGOR, A., WESSELY, S., MURRAY, R. M. and O'KEANE, V. (1995). Contrasting neuroendocrine responses in depression and chronic fatigue syndrome. *J Affect Disord* **34**(4): 283-289.

CLEARE, A. J., MCGREGOR, A. and O'KEANE, V. (1995). Neuroendocrine evidence for an association between hypothyroidism, reduced central 5-HT activity and depression. *Clin Endocrinol (Oxf)* **43**(6): 713-719.

CLIFFORD, D. B., OLNEY, J. W., MANIOTIS, A., COLLINS, R. C. and ZORUMSKI, C. F. (1987). The functional anatomy and pathology of lithium-pilocarpine and high-dose pilocarpine seizures. *Neuroscience* **23**(3): 953-968.

COLE, D. P., THASE, M. E., MALLINGER, A. G., SOARES, J. C., LUTHER, J. F., KUPFER, D. J. and FRANK, E. (2002). Slower treatment response in bipolar depression predicted by lower pretreatment thyroid function. *Am J Psychiatry* **159**(1): 116-121.\

COLE, J. C. and RODGERS, R. J. (1994). Ethological evaluation of the effects of acute and chronic buspirone treatment in the murine elevated plus-maze test: comparison with haloperidol. *Psychopharmacology (Berl)* **114**(2): 288-296.

CONNEY, A. H. (1967). Pharmacological implications of microsomal enzyme induction. *Pharmacol Rev* **19**(3): 317-366.

CORNE, S. J., PICKERING, R. W. and WARNER, B. T. (1963). A method for assessing the effects of drugs on the central actions of 5-hydroxytryptamine. *Br J Pharmacol Chemother* **20**: 106-120.

COSTA, M. S., ROCHA, J. B., PEROSA, S. R., CAVALHEIRO, E. A. and NAFFAH-MAZZACORATTI MDA, G. (2004). Pilocarpine-induced status epilepticus increases glutamate release in rat hippocampal synaptosomes. *Neurosci Lett* **356**(1): 41-44.

COX, P. A. and BALICK, M. J. (1994). The ethnobotanical approach to drug discovery. *Sci Am* **270**(6): 82-87.

CRAGG, G. M., NEWMAN, D. J. and SNADER, K. M. (1997). Natural products in drug discovery and development. *J Nat Prod* **60**(1): 52-60.

CRAMER, J. A., FISHER, R., BEN-MENACHEM, E., FRENCH, J. and MATTSON, R. H. (1999). New antiepileptic drugs: comparison of key clinical trials. *Epilepsia* **40**(5): 590-600.

CRAWLEY, J. N. (1981). Neuropharmacologic specificity of a simple animal model for the behavioral actions of benzodiazepines. *Pharmacol Biochem Behav* **15**(5): 695-699.

CRESTANI, F., MARTIN, J. R., MOHLER, H. and RUDOLPH, U. (2000). Mechanism of action of the hypnotic zolpidem in vivo. *Br J Pharmacol* **131**(7): 1251-1254.

CRETIN, B. and HIRSCH, E. (2010). Adjunctive antiepileptic drugs in adult epilepsy: how the first add-on could be the last. *Expert Opin Pharmacother* **11**(7): 1053-1067.

CRYAN, J. F. and LUCKI, I. (2000). Antidepressant-like behavioral effects mediated by 5-Hydroxytryptamine(2C) receptors. *J Pharmacol Exp Ther* **295**(3): 1120-1126.

CRYAN, J. F., MARKOU, A. and LUCKI, I. (2002). Assessing antidepressant activity in rodents: recent developments and future needs. *Trends Pharmacol Sci* **23**(5): 238-245.

CRYAN, J. F. and MOMBEREAU, C. (2004). In search of a depressed mouse: utility of models for studying depression-related behavior in genetically modified mice. *Mol Psychiatry* **9**(4): 326-357.

CRYAN, J. F., MOMBEREAU, C. and VASSOUT, A. (2005). The tail suspension test as a model for assessing antidepressant activity: review of pharmacological and genetic studies in mice. *Neurosci Biobehav Rev* **29**(4-5): 571-625.

CRYAN, J. F., PAGE, M. E. and LUCKI, I. (2005). Differential behavioral effects of the antidepressants reboxetine, fluoxetine, and moclobemide in a modified forced swim test following chronic treatment. *Psychopharmacology (Berl)* **182**(3): 335-344.

CRYAN, J. F., VALENTINO, R. J. and LUCKI, I. (2005). Assessing substrates underlying the behavioral effects of antidepressants using the modified rat forced swimming test. *Neurosci Biobehav Rev* **29**(4-5): 547-569.

CUPP, M. J. (1999). Herbal remedies: adverse effects and drug interactions. *Am Fam Physician* **59**(5): 1239-1245.

CURIA, G., LONGO, D., BIAGINI, G., JONES, R. S. and AVOLI, M. (2008). The pilocarpine model of temporal lobe epilepsy. *J Neurosci Methods* **172**(2): 143-157.

CURTIS, D. R., HOSLI, L. and JOHNSTON, G. A. (1967). Inhibition of spinal neurons by glycine. *Nature* **215**(5109): 1502-1503.

DAILEY, J. W., CHEONG, J. H., KO, K. H., ADAMS-CURTIS, L. E. and JOBE, P. C. (1995). Anticonvulsant properties of D-20443 in genetically epilepsy-prone rats: prediction of clinical response. *Neurosci Lett* **195**(2): 77-80.

DANESI, M. A. and ADETUNJI, J. B. (1994). Use of alternative medicine by patients with epilepsy: a survey of 265 epileptic patients in a developing country. *Epilepsia* **35**(2): 344-351.

DANG, H., CHEN, Y., LIU, X., WANG, Q., WANG, L., JIA, W. and WANG, Y. (2009). Antidepressant effects of ginseng total saponins in the forced swimming test and chronic mild stress models of depression. *Prog Neuropsychopharmacol Biol Psychiatry* **33**(8): 1417-1424.

DARWISH, R. M. and ABURJAI, T. A. (2010). Effect of ethnomedicinal plants used in folklore medicine in Jordan as antibiotic resistant inhibitors on Escherichia coli. *BMC Complement Altern Med* **10**: 9.

DASHNIANI, M., BURJANADZE, M., BESELIA, G., CHKHIKVISHVILI, N. and NANEISHVILI, T. (2010). Effects of the uncompetitive nmda receptor antagonist memantine on recognition memory in rats. *Georgian Med News*(183): 27-33.

DAWSON, G. R. and TRICKLEBANK, M. D. (1995). Use of the elevated plus maze in the search for novel anxiolytic agents. *Trends Pharmacol Sci* **16**(2): 33-36.

DE-PARIS, F., NEVES, G., SALGUEIRO, J. B., QUEVEDO, J., IZQUIERDO, I. and RATES, S. M. (2000). Psychopharmacological screening of Pfaffia glomerata Spreng. (Amarathanceae) in rodents. *J Ethnopharmacol* **73**(1-2): 261-269.

DE BIASE, D., BARRA, D., BOSSA, F., PUCCI, P. and JOHN, R. A. (1991). Chemistry of the inactivation of 4-aminobutyrate aminotransferase by the antiepileptic drug vigabatrin. *J Biol Chem* **266**(30): 20056-20061.

DE SARRO, A., CECCHETTI, V., FRAVOLINI, V., NACCARI, F., TABARRINI, O. and DE SARRO, G. (1999). Effects of novel 6-desfluoroquinolones and classic quinolones on pentylenetetrazole-induced seizures in mice. *Antimicrob Agents Chemother* **43**(7): 1729-1736.

DE VIVO, D. C., LEARY, L. and WANG, D. (2002). Glucose transporter 1 deficiency syndrome and other glycolytic defects. *J Child Neurol* **17 Suppl 3**: 3S15-23; discussion 13S24-15.

DE VRY, J., BENZ, U., SCHREIBER, R. and TRABER, J. (1993). Shock-induced ultrasonic vocalization in young adult rats: a model for testing putative anti-anxiety drugs. *Eur J Pharmacol* **249**(3): 331-339.

DECKERS, C. L., GENTON, P., SILLS, G. J. and SCHMIDT, D. (2003). Current limitations of antiepileptic drug therapy: a conference review. *Epilepsy Res* **53**(1-2): 1-17.

DELGADO, P. L. and MICHAELS, T. (1999). Reboxetine: a review of efficacy and tolerability. *Drugs Today (Barc)* **35**(9): 725-737.

DELGADO, P. L., MILLER, H. L., SALOMON, R. M., LICINIO, J., KRYSTAL, J. H., MORENO, F. A., HENINGER, G. R. and CHARNEY, D. S. (1999). Tryptophan-depletion challenge in depressed patients treated with desipramine or fluoxetine: implications for the role of serotonin in the mechanism of antidepressant action. *Biol Psychiatry* **46**(2): 212-220.

DELGADO, P. L., PRICE, L. H., MILLER, H. L., SALOMON, R. M., LICINIO, J., KRYSTAL, J. H., HENINGER, G. R. and CHARNEY, D. S. (1991). Rapid serotonin depletion as a provocative challenge test for patients with major depression: relevance to antidepressant action and the neurobiology of depression. *Psychopharmacol Bull* **27**(3): 321-330.

DELINI-STULA, A., RADEKE, E. and WALDMEIER, P. C. (1988). Basic and clinical aspects of the activity of the new monoamine oxidase inhibitors. *Psychopharmacol Ser* **5**: 147-158.

DELORENZO, R. J., SUN, D. A. and DESHPANDE, L. S. (2005). Cellular mechanisms underlying acquired epilepsy: the calcium hypothesis of the induction and maintainance of epilepsy. *Pharmacol Ther* **105**(3): 229-266.

DEMUTH, G. W. and ACKERMAN, S. H. (1983). alpha-Methyldopa and depression: a clinical study and review of the literature. *Am J Psychiatry* **140**(5): 534-538.

DETKE, M. J., JOHNSON, J. and LUCKI, I. (1997). Acute and chronic antidepressant drug treatment in the rat forced swimming test model of depression. *Exp Clin Psychopharmacol* **5**(2): 107-112.

DIETSCHY, J. M. (1998). Dietary fatty acids and the regulation of plasma low density lipoprotein cholesterol concentrations. *J Nutr* **128**(2 Suppl): 444S-448S.

DIMITRIOU, E. C. and DIMITRIOU, C. E. (1998). Buspirone augmentation of antidepressant therapy. *J Clin Psychopharmacol* **18**(6): 465-469.

DIXON, J. B., DIXON, M. E. and O'BRIEN, P. E. (2003). Depression in association with severe obesity: changes with weight loss. *Arch Intern Med* **163**(17): 2058-2065.

DOLAN, R. J. (2002). Emotion, cognition, and behavior. *Science* **298**(5596): 1191-1194.

DONALD, P. R., LAMPRECHT, J. H., FREESTONE, M., ALBRECHT, C. F., BOUIC, P. J., KOTZE, D. and VAN JAARSVELD, P. P. (1997). A randomised placebo-controlled trial of the efficacy of beta-sitosterol and its glucoside as adjuvants in the treatment of pulmonary tuberculosis. *Int J Tuberc Lung Dis* **1**(6): 518-522.

DONALDSON, M., GIZZARELLI, G. and CHANPONG, B. (2007). Oral sedation: a primer on anxiolysis for the adult patient. *Anesth Prog* **54**(3): 118-128; quiz 129.

DOST, R. and RUNDFELDT, C. (2000). The anticonvulsant retigabine potently suppresses epileptiform discharges in the low Ca ++ and low Mg++ model in the hippocampal slice preparation. *Epilepsy Res* **38**(1): 53-66.

DREVETS, W. C., FRANK, E., PRICE, J. C., KUPFER, D. J., HOLT, D., GREER, P. J., HUANG, Y., GAUTIER, C. and MATHIS, C. (1999). PET imaging of serotonin 1A receptor binding in depression. *Biol Psychiatry* **46**(10): 1375-1387.

DUDEK, F. E. and STALEY, K. J. (2007). How does the balance of excitation and inhibition shift during epileptogenesis? *Epilepsy Curr* **7**(3): 86-88.

DUMAN, R. S., HENINGER, G. R. and NESTLER, E. J. (1997). A molecular and cellular theory of depression. *Arch Gen Psychiatry* **54**(7): 597-606.

DUMAN, R. S. and LI, N. (2012). A neurotrophic hypothesis of depression: role of synaptogenesis in the actions of NMDA receptor antagonists. *Philos Trans R Soc Lond B Biol Sci* **367**(1601): 2475-2484.

DUMAN, R. S. and MONTEGGIA, L. M. (2006). A neurotrophic model for stress-related mood disorders. *Biol Psychiatry* **59**(12): 1116-1127.

DUMAN, R. S. and VOLETI, B. (2012). Signaling pathways underlying the pathophysiology and treatment of depression: novel mechanisms for rapid-acting agents. *Trends Neurosci* **35**(1): 47-56.

DUNCAN, J. S. (2002). The promise of new antiepileptic drugs. *Br J Clin Pharmacol* **53**(2): 123-131.

DUNNE, F., O'HALLORAN, A. and KELLY, J. P. (2007). Development of a home cage locomotor tracking system capable of detecting the stimulant and sedative properties of drugs in rats. *Prog Neuropsychopharmacol Biol Psychiatry* **31**(7): 1456-1463.

EASTWOOD, M. A. (2001). A molecular biological basis for the nutritional and pharmacological benefits of dietary plants. *QJM* **94**(1): 45-48.

ECKELI, A. L., DACH, F. and RODRIGUES, A. L. (2000). Acute treatments with GMP produce antidepressant-like effects in mice. *Neuroreport* **11**(9): 1839-1843.

EGLEN, R. M., CHOPPIN, A. and WATSON, N. (2001). Therapeutic opportunities from muscarinic receptor research. *Trends Pharmacol Sci* **22**(8): 409-414.

EL-MAS, M. M. and ABDEL-RAHMAN, A. A. (2004). Effects of long-term ovariectomy and estrogen replacement on clonidine-evoked reductions in blood pressure and hemodynamic variability. *J Cardiovasc Pharmacol* **43**(5): 607-615.

EL YACOUBI, M., LEDENT, C., MENARD, J. F., PARMENTIER, M., COSTENTIN, J. and VAUGEOIS, J. M. (2000). The stimulant effects of caffeine on locomotor behaviour in mice are mediated through its blockade of adenosine A(2A) receptors. *Br J Pharmacol* **129**(7): 1465-1473.

ELHWUEGI, A. S. (2004). Central monoamines and their role in major depression. *Prog Neuropsychopharmacol Biol Psychiatry* **28**(3): 435-451.

ELOQAYLI, H., DAHL, C. B., GOTESTAM, K. G., UNSGARD, G., HADIDI, H. and SONNEWALD, U. (2003). Pentylenetetrazole decreases metabolic glutamate turnover in rat brain. *J Neurochem* **85**(5): 1200-1207.

ENGEL, J., JR. (2006). ILAE classification of epilepsy syndromes. *Epilepsy Res* **70 Suppl 1**: S5-10.

ENGUM, A., BJORO, T., MYKLETUN, A. and DAHL, A. A. (2002). An association between depression, anxiety and thyroid function--a clinical fact or an artefact? *Acta Psychiatr Scand* **106**(1): 27-34.

ERRINGTON, A. C., STOHR, T. and LEES, G. (2005). Voltage gated ion channels: targets for anticonvulsant drugs. *Curr Top Med Chem* **5**(1): 15-30.

ESSMAN, W. D., SINGH, A. and LUCKI, I. (1994). Serotonergic properties of cocaine: effects on a 5-HT2 receptor-mediated behavior and on extracellular concentrations of serotonin and dopamine. *Pharmacol Biochem Behav* **49**(1): 107-113.

FAGERHOLM, U. (2007). Prediction of human pharmacokinetics - renal metabolic and excretion clearance. *J Pharm Pharmacol* **59**(11): 1463-1471.

FANTEGROSSI, W. E., SIMONEAU, J., COHEN, M. S., ZIMMERMAN, S. M., HENSON, C. M., RICE, K. C. and WOODS, J. H. (2010). Interaction of 5-HT2A and 5-HT2C receptors in R(-)-2,5-dimethoxy-4-iodoamphetamine-elicited head twitch behavior in mice. *J Pharmacol Exp Ther* **335**(3): 728-734.

FAROMBI, E. O., OGUNDIPE, O. and MOODY, J. O. (2001). Antioxidant and anti-inflammatory activities of Mallotus oppositifolium in model systems. *Afr J Med Med Sci* **30**(3): 213-215.

FAUGHT, E. (2001). Pharmacokinetic considerations in prescribing antiepileptic drugs. *Epilepsia* **42 Suppl 4**: 19-23.

FAURE, C., MNIE-FILALI, O. and HADDJERI, N. (2006). Long-term adaptive changes induced by serotonergic antidepressant drugs. *Expert Rev Neurother* **6**(2): 235-245.

FAVA, M. (2000). Weight gain and antidepressants. *J Clin Psychiatry* **61 Suppl 11**: 37-41.

FAVA, M. and KENDLER, K. S. (2000). Major depressive disorder. *Neuron* **28**(2): 335-341.

FEROZ, Z., KHAN, R. A. and AFROZ, S. (2011). Cummulative toxicities on lipid profile and glucose following administration of anti-epileptic, anti-hypertensive, anti-diabetic and anti-arrhythmic drugs. *Pak J Pharm Sci* **24**(1): 47-51.

FFRENCH-MULLEN, J. M., BARKER, J. L. and ROGAWSKI, M. A. (1993). Calcium current block by (-)-pentobarbital, phenobarbital, and CHEB but not (+)-pentobarbital in acutely isolated hippocampal CA1 neurons: comparison with effects on GABA-activated Cl- current. *J Neurosci* **13**(8): 3211-3221.

FILE, S. E. (1992). Usefulness of animal models with newer anxiolytics. *Clin Neuropharmacol* **15 Suppl 1 Pt A**: 525A-526A.

FILE, S. E., LIPPA, A. S., BEER, B. and LIPPA, M. T. (2004). Animal tests of anxiety. *Curr Protoc Neurosci* **Chapter 8**: Unit 8 3.

FILE, S. E. and PELLOW, S. (1986). Intrinsic actions of the benzodiazepine receptor antagonist Ro 15-1788. *Psychopharmacology (Berl)* **88**(1): 1-11.

FINLAYSON, G., KING, N. and BLUNDELL, J. E. (2007). Liking vs. wanting food: importance for human appetite control and weight regulation. *Neurosci Biobehav Rev* **31**(7): 987-1002.

FINSTERER, U., SCHIED, U., BUTZ, A., JENSEN, U., BEYER, A., KELLERMANN, W., UNERTL, K., FOTTNER, I. and PETER, K. (1988). [Water-electrolyte balance and kidney function for 3 weeks following severe trauma]. *Anasth Intensivther Notfallmed* **23**(1): 22-31.

FIRENZUOLI, F. and GORI, L. (2007). Herbal medicine today: clinical and research issues. *Evid Based Complement Alternat Med* **4**(Suppl 1): 37-40.

FISHER, R. S., VAN EMDE BOAS, W., BLUME, W., ELGER, C., GENTON, P., LEE, P. and ENGEL, J., JR. (2005). Epileptic seizures and epilepsy: definitions proposed by the International League Against Epilepsy (ILAE) and the International Bureau for Epilepsy (IBE). *Epilepsia* **46**(4): 470-472.

FRAZER, A. and BENMANSOUR, S. (2002). Delayed pharmacological effects of antidepressants. *Mol Psychiatry* **7 Suppl 1**: S23-28.

FREEMAN, J. M. (2009). Seizures, EEG events, and the ketogenic diet. *Epilepsia* **50**(2): 329-330.

FRENCH, J. A. (2007). Refractory epilepsy: clinical overview. *Epilepsia* **48 Suppl 1**: 3-7.

FUKUI, M., RODRIGUIZ, R. M., ZHOU, J., JIANG, S. X., PHILLIPS, L. E., CARON, M. G. and WETSEL, W. C. (2007). Vmat2 heterozygous mutant mice display a depressive-like phenotype. *J Neurosci* **27**(39): 10520-10529.

GALARRETA, M. and HESTRIN, S. (1998). Frequency-dependent synaptic depression and the balance of excitation and inhibition in the neocortex. *Nat Neurosci* **1**(7): 587-594.

GALLAGHER, J. P., HIGASHI, H. and NISHI, S. (1978). Characterization and ionic basis of GABA-induced depolarizations recorded in vitro from cat primary afferent neurones. *J Physiol* **275**: 263-282.

GALLANOSA, A. G., SPYKER, D. A., SHIPE, J. R. and MORRIS, D. L. (1981). Human xylazine overdose: a comparative review with clonidine, phenothiazines, and tricyclic antidepressants. *Clin Toxicol* **18**(6): 663-678.

GALLASSI, R., DI SARRO, R., MORREALE, A. and AMORE, M. (2006). Memory impairment in patients with late-onset major depression: the effect of antidepressant therapy. *J Affect Disord* **91**(2-3): 243-250..

GAVIOLI, E. C., VAUGHAN, C. W., MARZOLA, G., GUERRINI, R., MITCHELL, V. A., ZUCCHINI, S., DE LIMA, T. C., RAE, G. A., SALVADORI, S., REGOLI, D. and CALO, G. (2004). Antidepressant-like effects of the nociceptin/orphanin FQ receptor antagonist UFP-101: new evidence from rats and mice. *Naunyn Schmiedebergs Arch Pharmacol* **369**(6): 547-553.

GBEDEMA, S. Y., ADU, F., BAYOR, M. T., ANNAN, K. and BOATENG, J. S. (2010). Enhancement of Antibacterial Activity of ammoxicillin by Some Ghanaian Medical Plant Extracts. *Int J Pharma. Sci. Res.* **1**: 145-152.

GEORGE, A. L., JR. (2004). Inherited Channelopathies Associated with Epilepsy. *Epilepsy Curr* **4**(2): 65-70.

GEORGE, J., MURRAY, M., BYTH, K. and FARRELL, G. C. (1995). Differential alterations of cytochrome P450 proteins in livers from patients with severe chronic liver disease. *Hepatology* **21**(1): 120-128.

GEORGE, W. (1999). Recent Advances in Saponins Used in Foods, Agriculture, and Medicine. Biologically Active Natural Products, CRC Press.

GERTNER, E., MARSHALL, P. S., FILANDRINOS, D., POTEK, A. S. and SMITH, T. M. (1995). Complications resulting from the use of Chinese herbal medications containing undeclared prescription drugs. *Arthritis Rheum* **38**(5): 614-617.

GIARDINA, W. J., DART, M. J., HARRIS, R. R., BITNER, R. S., RADEK, R. J., FOX, G. B., CHEMBURKAR, S. R., MARSH, K. C., WARING, J. F., HUI, J. Y., CHEN, J., CURZON, P., GRAYSON, G. K., KOMATER, V. A., KU, Y., LOCKWOOD, M., MINER, H. M., NIKKEL, A. L., PAN, J. B., PU, Y. M., WANG, L., BENNANI, Y., DURMULLER, N., JOLLY, R., ROUX, S., SULLIVAN, J. P. and DECKER, M. W. (2005). Preclinical profiling and safety studies of ABT-769: a compound with potential for broad-spectrum antiepileptic activity. *Epilepsia* **46**(9): 1349-1361.

GILMOUR, H. and PATTEN, S. B. (2007). Depression and work impairment. *Health Rep* **18**(1): 9-22.

GLAUSER, T., BEN-MENACHEM, E., BOURGEOIS, B., CNAAN, A., CHADWICK, D., GUERREIRO, C., KALVIAINEN, R., MATTSON, R., PERUCCA, E. and TOMSON, T. (2006). ILAE treatment guidelines: evidence-based analysis of antiepileptic drug efficacy and effectiveness as initial monotherapy for epileptic seizures and syndromes. *Epilepsia* **47**(7): 1094-1120.

GOLDEN, J. P., BALOH, R. H., KOTZBAUER, P. T., LAMPE, P. A., OSBORNE, P. A., MILBRANDT, J. and JOHNSON, E. M., JR. (1998). Expression of neurturin, GDNF, and their receptors in the adult mouse CNS. *J Comp Neurol* **398**(1): 139-150.

GOLDMAN, P. (2001). Herbal medicines today and the roots of modern pharmacology. *Ann Intern Med* **135**(8 Pt 1): 594-600.

GORDEY, M., DELOREY, T. M. and OLSEN, R. W. (2000). Differential sensitivity of recombinant GABA(A) receptors expressed in Xenopus oocytes to modulation by topiramate. *Epilepsia* **41 Suppl 1**: S25-29.

GOTLIB, I. H., JOORMANN, J., MINOR, K. L. and HALLMAYER, J. (2008). HPA axis reactivity: a mechanism underlying the associations among 5-HTTLPR, stress, and depression. *Biol Psychiatry* **63**(9): 847-851.

GOURION, D. (2008). [Antidepressants and their onset of action: a major clinical, methodological and pronostical issue]. *Encephale* **34**(1): 73-81.

GRIEBEL, G., PERRAULT, G. and SANGER, D. J. (1997). CCK receptor antagonists in animal models of anxiety: comparison between exploration tests, conflict procedures and a model based on defensive behaviours. *Behav Pharmacol* **8**(6-7): 549-560.

GROVES, J. O. (2007). Is it time to reassess the BDNF hypothesis of depression? *Mol Psychiatry* **12**(12): 1079-1088.

GUMNICK, J. F. and NEMEROFF, C. B. (2000). Problems with currently available antidepressants. *J Clin Psychiatry* **61 Suppl 10**: 5-15.

HACHAD, H., RAGUENEAU-MAJLESSI, I. and LEVY, R. H. (2002). New antiepileptic drugs: review on drug interactions. *Ther Drug Monit* **24**(1): 91-103.

HADJ TAHAR, A., BLANCHET, P. J. and DOYON, J. (2004). Motor-learning impairment by amantadine in healthy volunteers. *Neuropsychopharmacology* **29**(1): 187-194.

HALFORD, J. C. and BLUNDELL, J. E. (2000). Separate systems for serotonin and leptin in appetite control. *Ann Med* **32**(3): 222-232.

HALLER, C. A., DYER, J. E., KO, R. and OLSON, K. R. (2002). Making a diagnosis of herbal-related toxic hepatitis. *West J Med* **176**(1): 39-44.

HARRIS, R. A. and ALLAN, A. M. (1985). Functional coupling of gamma-aminobutyric acid receptors to chloride channels in brain membranes. *Science* **228**(4703): 1108-1110.

HARVEY, J. A. (2003). Role of the serotonin 5-HT(2A) receptor in learning. *Learn Mem* **10**(5): 355-362.

HASCOET, M. and BOURIN, M. (1998). A new approach to the light/dark test procedure in mice. *Pharmacol Biochem Behav* **60**(3): 645-653.

HASLER, G. (2010). Pathophysiology of depression: do we have any solid evidence of interest to clinicians? *World Psychiatry* **9**(3): 155-161.

HAUSER, W. A. and KURLAND, L. T. (1975). The epidemiology of epilepsy in Rochester, Minnesota, 1935 through 1967. *Epilepsia* **16**(1): 1-66.

HEISER, P., DICKHAUS, B., SCHREIBER, W., CLEMENT, H. W., HASSE, C., HENNIG, J., REMSCHMIDT, H., KRIEG, J. C., WESEMANN, W. and OPPER, C. (2000). White blood cells and cortisol after sleep deprivation and recovery sleep in humans. *Eur Arch Psychiatry Clin Neurosci* **250**(1): 16-23.

HENDERSON, C. B., FILLOUX, F. M., ALDER, S. C., LYON, J. L. and CAPLIN, D. A. (2006). Efficacy of the ketogenic diet as a treatment option for epilepsy: meta-analysis. *J Child Neurol* **21**(3): 193-198.

HENRY, S. A., LEHMANN-MASTEN, V., GASPARINI, F., GEYER, M. A. and MARKOU, A. (2002). The mGluR5 antagonist MPEP, but not the mGluR2/3 agonist LY314582, augments PCP effects on prepulse inhibition and locomotor activity. *Neuropharmacology* **43**(8): 1199-1209.

HIRSCHFELD, R. M. (2000). History and evolution of the monoamine hypothesis of depression. *J Clin Psychiatry* **61 Suppl 6**: 4-6.

HOFFMAN, D. L., DUKES, E. M. and WITTCHEN, H. U. (2008). Human and economic burden of generalized anxiety disorder. *Depress Anxiety* **25**(1): 72-90.

HOLMES, G. L. (2007). Animal model studies application to human patients. *Neurology* **69**(24 Suppl 3): S28-32.

HOLMES, P. V. (2003). Rodent models of depression: reexamining validity without anthropomorphic inference. *Crit Rev Neurobiol* **15**(2): 143-174.

HORST, W. D. and PRESKORN, S. H. (1998). Mechanisms of action and clinical characteristics of three atypical antidepressants: venlafaxine, nefazodone, bupropion. *J Affect Disord* **51**(3): 237-254.

HOWARD, R., MCSHANE, R., LINDESAY, J., RITCHIE, C., BALDWIN, A., BARBER, R., BURNS, A., DENING, T., FINDLAY, D., HOLMES, C., HUGHES, A., JACOBY, R., JONES, R., MCKEITH, I., MACHAROUTHU, A., O'BRIEN, J., PASSMORE, P., SHEEHAN, B., JUSZCZAK, E., KATONA, C., HILLS, R., KNAPP, M., BALLARD, C., BROWN, R., BANERJEE, S., ONIONS, C., GRIFFIN, M., ADAMS, J., GRAY, R., JOHNSON, T., BENTHAM, P. and PHILLIPS, P. (2012). Donepezil and memantine for moderate-to-severe Alzheimer's disease. *N Engl J Med* **366**(10): 893-903.

HUFFMAN, J. and KOSSOFF, E. H. (2006). State of the ketogenic diet(s) in epilepsy. *Curr Neurol Neurosci Rep* **6**(4): 332-340.

HYMAN, S. E. and NESTLER, E. J. (1996). Initiation and adaptation: a paradigm for understanding psychotropic drug action. *Am J Psychiatry* **153**(2): 151-162.

IEZHITSA, I. N., SPASOV, A. A., BUGAEVA, L. I. and MOROZOV, I. S. (2002). Toxic effect of single treatment with bromantane on neurological status of experimental animals. *Bull Exp Biol Med* **133**(4): 380-383.

IRWIN, S. (1968). System analysis: a strategy for drug experimentation and evaluation. *Perspect Biol Med* **11**(4): 654-674.

ISOKAWA, M. and MELLO, L. E. (1991). NMDA receptor-mediated excitability in dendritically deformed dentate granule cells in pilocarpine-treated rats. *Neurosci Lett* **129**(1): 69-73.

JACKSON, M. F., ESPLIN, B. and CAPEK, R. (1999). Inhibitory nature of tiagabine-augmented GABAA receptor-mediated depolarizing responses in hippocampal pyramidal cells. *J Neurophysiol* **81**(3): 1192-1198.

JACKSON, M. J. and TURKINGTON, D. (2005). Depression and anxiety in epilepsy. *J Neurol Neurosurg Psychiatry* **76 Suppl 1**: i45-47.

JACOBY, A., SNAPE, D. and BAKER, G. A. (2005). Epilepsy and social identity: the stigma of a chronic neurological disorder. *Lancet Neurol* **4**(3): 171-178.

JANA, S. and SHEKHAWAT, G. S. (2011). Critical review on medicinally potent plant species: Gloriosa superba. *Fitoterapia* **82**(3): 293-301.

JARVIS, M. F., BURGARD, E. C., MCGARAUGHTY, S., HONORE, P., LYNCH, K., BRENNAN, T. J., SUBIETA, A., VAN BIESEN, T., CARTMELL, J., BIANCHI, B., NIFORATOS, W., KAGE, K., YU, H., MIKUSA, J., WISMER, C. T., ZHU, C. Z., CHU, K., LEE, C. H., STEWART, A. O., POLAKOWSKI, J., COX, B. F., KOWALUK, E., WILLIAMS, M., SULLIVAN, J. and FALTYNEK, C. (2002). A-317491, a novel potent and selective non-nucleotide antagonist of P2X3 and P2X2/3 receptors, reduces chronic inflammatory and neuropathic pain in the rat. *Proc Natl Acad Sci U S A* **99**(26): 17179-17184.

JI, J., MCDERMOTT, J. L. and DLUZEN, D. E. (2007). Sex differences in K+-evoked striatal dopamine output from superfused striatal tissue fragments of reserpine-treated CD-1 mice. *J Neuroendocrinol* **19**(9): 725-731.

JOHNSTON, D. E. (1999). Special considerations in interpreting liver function tests. *Am Fam Physician* **59**(8): 2223-2230.

JONES, C. K., PETERS, S. C. and SHANNON, H. E. (2005). Efficacy of duloxetine, a potent and balanced serotonergic and noradrenergic reuptake inhibitor, in inflammatory and acute pain models in rodents. *J Pharmacol Exp Ther* **312**(2): 726-732.

JONES, F. A. (1996). Herbs--useful plants. Their role in history and today. *Eur J Gastroenterol Hepatol* **8**(12): 1227-1231.

JOPE, R. S., MORRISETT, R. A. and SNEAD, O. C. (1986). Characterization of lithium potentiation of pilocarpine-induced status epilepticus in rats. *Exp Neurol* **91**(3): 471-480.

JUNG, H. J., KIM, S. G., NAM, J. H., PARK, K. K., CHUNG, W. Y., KIM, W. B., LEE, K. T., WON, J. H., CHOI, J. W. and PARK, H. J. (2005). Isolation of saponins with the inhibitory effect on nitric oxide, prostaglandin E2 and tumor necrosis factor-alpha production from Pleurospermum kamtschaticum. *Biol Pharm Bull* **28**(9): 1668-1671.

JUNG, S., JONES, T. D., LUGO, J. N., JR., SHEERIN, A. H., MILLER, J. W., D'AMBROSIO, R., ANDERSON, A. E. and POOLOS, N. P. (2007). Progressive dendritic HCN channelopathy during epileptogenesis in the rat pilocarpine model of epilepsy. *J Neurosci* **27**(47): 13012-13021.

KAMATENESI-MUGISHA, M. and ORYEM-ORIGA, H. (2005). Traditional herbal remedies used in the management of sexual impotence and erectile dysfunction in western Uganda. *Afr Health Sci* **5**(1): 40-49.

KAMINSKI, R. M., MAZUREK, M., TURSKI, W. A., KLEINROK, Z. and CZUCZWAR, S. J. (2001). Amlodipine enhances the activity of antiepileptic drugs against pentylenetetrazole-induced seizures. *Pharmacol Biochem Behav* **68**(4): 661-668.

KASTURE, V. S., DESHMUKH, V. K. and CHOPDE, C. T. (2002). Anxiolytic and anticonvulsive activity of Sesbania grandiflora leaves in experimental animals. *Phytother Res* **16**(5): 455-460.

KATAN, M. B., GRUNDY, S. M., JONES, P., LAW, M., MIETTINEN, T. and PAOLETTI, R. (2003). Efficacy and safety of plant stanols and sterols in the management of blood cholesterol levels. *Mayo Clin Proc* **78**(8): 965-978.

KAWAURA, K., MIKI, R., SHIMA, E., HONDA, S., SOEDA, F., SHIRASAKI, T. and TAKAHAMA, K. (2010). Antidepressant-like effect of centrally acting non-narcotic antitussive caramiphen in a forced swimming test. *Neurosci Lett* **481**(3): 188-190.

KEHL, L. J., HAMAMOTO, D. T., WACNIK, P. W., CROFT, D. L., NORSTED, B. D., WILCOX, G. L. and SIMONE, D. A. (2003). A cannabinoid agonist differentially attenuates deep tissue hyperalgesia in animal models of cancer and inflammatory muscle pain. *Pain* **103**(1-2): 175-186.

KEKS, N. A., BURROWS, G. D., COPOLOV, D. L., NEWTON, R., PAOLETTI, N., SCHWEITZER, I. and TILLER, J. (2007). Beyond the evidence: is there a place for antidepressant combinations in the pharmacotherapy of depression? *Med J Aust* **186**(3): 142-144.

KESSLER, R. C. (2003). Epidemiology of women and depression. *J Affect Disord* **74**(1): 5-13.

KHAWAM, E. A., LAURENCIC, G. and MALONE, D. A., JR. (2006). Side effects of antidepressants: an overview. *Cleve Clin J Med* **73**(4): 351-353, 356-361.

KIMMEL, H. L., GONG, W., VECHIA, S. D., HUNTER, R. G. and KUHAR, M. J. (2000). Intra-ventral tegmental area injection of rat cocaine and amphetamine-regulated transcript peptide 55-102 induces locomotor activity and promotes conditioned place preference. *J Pharmacol Exp Ther* **294**(2): 784-792.

KINKEAD, B. and NEMEROFF, C. B. (2002). Neurotensin: an endogenous antipsychotic? *Curr Opin Pharmacol* **2**(1): 99-103.

KINTER, L. B. AND VALENTIN, J. P. (2002). Safety pharmacology and risk assessment. *Fundam. Clin. Pharmacol.,* **16**: 175-182

KITAMURA, Y., ZHAO, X. H., OHNUKI, T. and NOMURA, Y. (1989). Ligand-binding characteristics of [3H]QNB, [3H]prazosin, [3H]rauwolscine, [3H]TCP and [3H]nitrendipine to cerebral cortical and hippocampal membranes of senescence accelerated mouse. *Neurosci Lett* **106**(3): 334-338.

KITAMURA, Y., ZHAO, X. H., OHNUKI, T., TAKEI, M. and NOMURA, Y. (1992). Age-related changes in transmitter glutamate and NMDA receptor/channels in the brain of senescence-accelerated mouse. *Neurosci Lett* **137**(2): 169-172.

KOGEORGOS, J., FONAGY, P. and SCOTT, D. F. (1982). Psychiatric symptom patterns of chronic epileptics attending a neurological clinic: a controlled investigation. *Br J Psychiatry* **140**: 236-243.

KOLECKAR, V., KUBIKOVA, K., REHAKOVA, Z., KUCA, K., JUN, D., JAHODAR, L. and OPLETAL, L. (2008). Condensed and hydrolysable tannins as antioxidants influencing the health. *Mini Rev Med Chem* **8**(5): 436-447.

KRALL, R. L., PENRY, J. K., WHITE, B. G., KUPFERBERG, H. J. and SWINYARD, E. A. (1978). Antiepileptic drug development: II. Anticonvulsant drug screening. *Epilepsia* **19**(4): 409-428.

KRATOCHVIL, C. J., VAUGHAN, B. S., HARRINGTON, M. J. and BURKE, W. J. (2003). Atomoxetine: a selective noradrenaline reuptake inhibitor for the treatment of attention-deficit/hyperactivity disorder. *Expert Opin Pharmacother* **4**(7): 1165-1174.

KRIGE, J. E. and BECKINGHAM, I. J. (2001). ABC of diseases of liver, pancreas, and biliary system. *BMJ* **322**(7285): 537-540.

KRISHNAN, K. R. (2007). Revisiting monoamine oxidase inhibitors. *J Clin Psychiatry* **68 Suppl 8**: 35-41.

KUKUIA, K. K. E., AMEYAW, E. O., MANTE, P. K., ADONGO, D. W. and WOODE, E. (2012). Screening of Central Effects of the Leaves of *Mallotus oppositifolius* (Geiseler) Mull. Arg. in Mice. *Pharmacologia*, **3**: 683-692

KUMAR, V. (2006). Potential medicinal plants for CNS disorders: an overview. *Phytother Res* **20**(12): 1023-1035.

KUPFERBERG, H. (2001). Animal models used in the screening of antiepileptic drugs. *Epilepsia* **42 Suppl 4**: 7-12.

KUSHIKATA, T., HIROTA, K., YOSHIDA, H., KUDO, M., LAMBERT, D. G., SMART, D., JERMAN, J. C. and MATSUKI, A. (2003). Orexinergic neurons and barbiturate anesthesia. *Neuroscience* **121**(4): 855-863.

KUZIS, G., SABE, L., TIBERTI, C., LEIGUARDA, R. and STARKSTEIN, S. E. (1997). Cognitive functions in major depression and Parkinson disease. *Arch Neurol* **54**(8): 982-986.

KWAN, P. and BRODIE, M. J. (2000). Early identification of refractory epilepsy. *N Engl J Med* **342**(5): 314-319.

KWAN, P. and BRODIE, M. J. (2000). Epilepsy after the first drug fails: substitution or add-on? *Seizure* **9**(7): 464-468.

KWAN, P. and BRODIE, M. J. (2001). Effectiveness of first antiepileptic drug. *Epilepsia* **42**(10): 1255-1260.

KWAN, P., SILLS, G. J. and BRODIE, M. J. (2001). The mechanisms of action of commonly used antiepileptic drugs. *Pharmacol Ther* **90**(1): 21-34.

LAMBERT, M. V. and ROBERTSON, M. M. (1999). Depression in epilepsy: etiology, phenomenology, and treatment. *Epilepsia* **40 Suppl 10**: S21-47.

LAMPL, I., SCHWINDT, P. and CRILL, W. (1998). Reduction of cortical pyramidal neuron excitability by the action of phenytoin on persistent Na+ current. *J Pharmacol Exp Ther* **284**(1): 228-237.

LASON, W., DUDRA-JASTRZEBSKA, M., REJDAK, K. and CZUCZWAR, S. J. (2011). Basic mechanisms of antiepileptic drugs and their pharmacokinetic/pharmacodynamic interactions: an update. *Pharmacol Rep* **63**(2): 271-292.

LE BARS, D., GOZARIU, M. and CADDEN, S. W. (2001). Animal models of nociception. *Pharmacol Rev* **53**(4): 597-652.

LEACH, M. J., MARDEN, C. M. and MILLER, A. A. (1986). Pharmacological studies on lamotrigine, a novel potential antiepileptic drug: II. Neurochemical studies on the mechanism of action. *Epilepsia* **27**(5): 490-497.

LEHMANN, J., HUTCHISON, A. J., MCPHERSON, S. E., MONDADORI, C., SCHMUTZ, M., SINTON, C. M., TSAI, C., MURPHY, D. E., STEEL, D. J., WILLIAMS, M. and ET AL. (1988). CGS 19755, a selective and competitive N-methyl-D-aspartate-type excitatory amino acid receptor antagonist. *J Pharmacol Exp Ther* **246**(1): 65-75.

LEPICARD, E. M., JOUBERT, C., HAGNEAU, I., PEREZ-DIAZ, F. and CHAPOUTHIER, G. (2000). Differences in anxiety-related behavior and response to diazepam in BALB/cByJ and C57BL/6J strains of mice. *Pharmacol Biochem Behav* **67**(4): 739-748.

LERCHE, H., JURKAT-ROTT, K. and LEHMANN-HORN, F. (2001). Ion channels and epilepsy. *Am J Med Genet* **106**(2): 146-159.

LEUNG, G., SUN, W., BROOKES, S., SMITH, D. and SHI, R. (2011). Potassium channel blocker, 4-aminopyridine-3-methanol, restores axonal conduction in spinal cord of an animal model of multiple sclerosis. *Exp Neurol* **227**(1): 232-235.

LIN, J., SAHAKIAN, D. C., DE MORAIS, S. M., XU, J. J., POLZER, R. J. and WINTER, S. M. (2003). The role of absorption, distribution, metabolism, excretion and toxicity in drug discovery. *Curr Top Med Chem* **3**(10): 1125-1154.

LIU, Z., NAGAO, T., DESJARDINS, G. C., GLOOR, P. and AVOLI, M. (1994). Quantitative evaluation of neuronal loss in the dorsal hippocampus in rats with long-term pilocarpine seizures. *Epilepsy Res* **17**(3): 237-247.

LOCKROW, J., BOGER, H., BIMONTE-NELSON, H. and GRANHOLM, A. C. (2011). Effects of long-term memantine on memory and neuropathology in Ts65Dn mice, a model for Down syndrome. *Behav Brain Res* **221**(2): 610-622.

LOHLE, M., SCHREMPF, W., WOLZ, M., REICHMANN, H. and STORCH, A. (2008). Potassium channel blocker 4-aminopyridine is effective in interictal cerebellar symptoms in episodic ataxia type 2--a video case report. *Mov Disord* **23**(9): 1314-1316.

LOPEZ-CORCUERA, B., GEERLINGS, A. and ARAGON, C. (2001). Glycine neurotransmitter transporters: an update. *Mol Membr Biol* **18**(1): 13-20.

LOSCHER, W. (2002). Animal models of epilepsy for the development of antiepileptogenic and disease-modifying drugs. A comparison of the pharmacology of kindling and post-status epilepticus models of temporal lobe epilepsy. *Epilepsy Res* **50**(1-2): 105-123.

LOSCHER, W. (2002). Current status and future directions in the pharmacotherapy of epilepsy. *Trends Pharmacol Sci* **23**(3): 113-118.

LOSCHER, W., FASSBENDER, C. P. and NOLTING, B. (1991). The role of technical, biological and pharmacological factors in the laboratory evaluation of anticonvulsant drugs. II. Maximal electroshock seizure models. *Epilepsy Res* **8**(2): 79-94.

LOSCHER, W., FISHER, J. E., NAU, H. and HONACK, D. (1988). Marked increase in anticonvulsant activity but decrease in wet-dog shake behaviour during short-term treatment of amygdala-kindled rats with valproic acid. *Eur J Pharmacol* **150**(3): 221-232.

LOSCHER, W., HONACK, D. and RUNDFELDT, C. (1998). Antiepileptogenic effects of the novel anticonvulsant levetiracetam (ucb L059) in the kindling model of temporal lobe epilepsy. *J Pharmacol Exp Ther* **284**(2): 474-479.

LOSCHER, W. and SCHMIDT, D. (1988). Which animal models should be used in the search for new antiepileptic drugs? A proposal based on experimental and clinical considerations. *Epilepsy Res* **2**(3): 145-181.

LOSCHER, W. and SCHMIDT, D. (1994). Strategies in antiepileptic drug development: is rational drug design superior to random screening and structural variation? *Epilepsy Res* **17**(2): 95-134.

LOSCHER, W. and SCHMIDT, D. (2006). New Horizons in the development of antiepileptic drugs: Innovative strategies. *Epilepsy Res* **69**(3): 183-272.

LOTSPEICH, W. D. (1958). Kidney, water and electrolyte metabolism. *Annu Rev Physiol* **20**: 339-376.

LUCAS, G. (2008). Fast-acting antidepressants: are we nearly there? *Expert Rev Neurother* **8**(1): 1-3.

LUCAS, G., DU, J., ROMEAS, T., MNIE-FILALI, O., HADDJERI, N., PINEYRO, G. and DEBONNEL, G. (2010). Selective serotonin reuptake inhibitors potentiate the rapid antidepressant-like effects of serotonin4 receptor agonists in the rat. *PLoS One* **5**(2): e9253.

LUCAS, G., RYMAR, V. V., DU, J., MNIE-FILALI, O., BISGAARD, C., MANTA, S., LAMBAS-SENAS, L., WIBORG, O., HADDJERI, N., PINEYRO, G., SADIKOT, A. F. and DEBONNEL, G. (2007). Serotonin(4) (5-HT(4)) receptor agonists are putative antidepressants with a rapid onset of action. *Neuron* **55**(5): 712-725.

LUCKI, I., SINGH, A. and KREISS, D. S. (1994). Antidepressant-like behavioral effects of serotonin receptor agonists. *Neurosci Biobehav Rev* **18**(1): 85-95.

LUTTINGER, D. (1985). Determination of antinociceptive efficacy of drugs in mice using different water temperatures in a tail-immersion test. *J Pharmacol Methods* **13**(4): 351-357.

LYNCH, J. J., 3RD, CASTAGNE, V., MOSER, P. C. and MITTELSTADT, S. W. (2011). Comparison of methods for the assessment of locomotor activity in rodent safety pharmacology studies. *J Pharmacol Toxicol Methods* **64**(1): 74-80.

LYNCH, T. and PRICE, A. (2007). The effect of cytochrome P450 metabolism on drug response, interactions, and adverse effects. *Am Fam Physician* **76**(3): 391-396.

MACDONALD, R. L. and KELLY, K. M. (1995). Antiepileptic drug mechanisms of action. *Epilepsia* **36 Suppl 2**: S2-12.

MACDONALD, R. L. and OLSEN, R. W. (1994). GABAA receptor channels. *Annu Rev Neurosci* **17**: 569-602.

MACHADO-VIEIRA, R., SALVADORE, G., DIAZGRANADOS, N. and ZARATE, C. A., JR. (2009). Ketamine and the next generation of antidepressants with a rapid onset of action. *Pharmacol Ther* **123**(2): 143-150.

MACKENZIE, L., MEDVEDEV, A., HISCOCK, J. J., POPE, K. J. and WILLOUGHBY, J. O. (2002). Picrotoxin-induced generalised convulsive seizure in rat: changes in regional distribution and frequency of the power of electroencephalogram rhythms. *Clin Neurophysiol* **113**(4): 586-596.

MAHADY, G. B. (2001). Global harmonization of herbal health claims. *J Nutr* **131**(3s): 1120S-1123S.

MAKKAR, H. P., SIDDHURAJU, P. and BECKER, K. (2007). Alkaloids. **393:** 107-111.

MALBERG, J. E., EISCH, A. J., NESTLER, E. J. and DUMAN, R. S. (2000). Chronic antidepressant treatment increases neurogenesis in adult rat hippocampus. *J Neurosci* **20**(24): 9104-9110.

MALIZIA, A. L., CUNNINGHAM, V. J., BELL, C. J., LIDDLE, P. F., JONES, T. and NUTT, D. J. (1998). Decreased brain GABA(A)-benzodiazepine receptor binding in panic disorder: preliminary results from a quantitative PET study. *Arch Gen Psychiatry* **55**(8): 715-720.

MANDHANE, S. N., AAVULA, K. and RAJAMANNAR, T. (2007). Timed pentylenetetrazol infusion test: a comparative analysis with s.c.PTZ and MES models of anticonvulsant screening in mice. *Seizure* **16**(7): 636-644.

MARINAC, J. S., BUCHINGER, C. L., GODFREY, L. A., WOOTEN, J. M., SUN, C. and WILLSIE, S. K. (2007). Herbal products and dietary supplements: a survey of use, attitudes, and knowledge among older adults. *J Am Osteopath Assoc* **107**(1): 13-20; quiz 21-13.

MARKOPOULOU, K., PAPADOPOULOS, A., JURUENA, M. F., POON, L., PARIANTE, C. M. and CLEARE, A. J. (2009). The ratio of cortisol/DHEA in treatment resistant depression. *Psychoneuroendocrinology* **34**(1): 19-26.

MARSON, A. G., APPLETON, R., BAKER, G. A., CHADWICK, D. W., DOUGHTY, J., EATON, B., GAMBLE, C., JACOBY, A., SHACKLEY, P., SMITH, D. F., TUDUR-SMITH, C., VANOLI, A. and WILLIAMSON, P. R. (2007). A randomised controlled trial examining the longer-term outcomes of standard versus new antiepileptic drugs. The SANAD trial. *Health Technol Assess* **11**(37): iii-iv, ix-x, 1-134.

MATSUI, M., MOTOMURA, D., KARASAWA, H., FUJIKAWA, T., JIANG, J., KOMIYA, Y., TAKAHASHI, S. and TAKETO, M. M. (2000). Multiple functional defects in peripheral autonomic organs in mice lacking muscarinic acetylcholine receptor gene for the M3 subtype. *Proc Natl Acad Sci U S A* **97**(17): 9579-9584.

MAURICE, N., TKATCH, T., MEISLER, M., SPRUNGER, L. K. and SURMEIER, D. J. (2001). D1/D5 dopamine receptor activation differentially modulates rapidly inactivating and persistent sodium currents in prefrontal cortex pyramidal neurons. *J Neurosci* **21**(7): 2268-2277.

MCCABE, P. H. (2000). New anti-epileptic drugs for the 21st century. *Expert Opin Pharmacother* **1**(4): 633-674.

MCGOWAN, E., HOYT, S. B., LI, X., LYONS, K. A. and ABBADIE, C. (2009). A peripherally acting Na(v)1.7 sodium channel blocker reverses hyperalgesia and allodynia on rat models of inflammatory and neuropathic pain. *Anesth Analg* **109**(3): 951-958.

MCKINNEY, W. T., JR. and BUNNEY, W. E., JR. (1969). Animal model of depression. I. Review of evidence: implications for research. *Arch Gen Psychiatry* **21**(2): 240-248.

MCNAMARA, J. O. and PURANAM, R. S. (1996). Epilepsy. Protease inhibitor implicated. *Nature* **381**(6577): 26-27.

MECHAN, A. O., MORAN, P. M., ELLIOTT, M., YOUNG, A. J., JOSEPH, M. H. and GREEN, R. (2002). A comparison between Dark Agouti and Sprague-Dawley rats in their behaviour on the elevated plus-maze, open-field apparatus and activity meters, and their response to diazepam. *Psychopharmacology (Berl)* **159**(2): 188-195.

MEHTA, A. K. and TICKU, M. K. (2001). Characterization of the picrotoxin site of GABAA receptors. *Curr Protoc Pharmacol* **Chapter 1**: Unit 1 18.

MELDRUM, B. S. (1997). Identification and preclinical testing of novel antiepileptic compounds. *Epilepsia* **38 Suppl 9**: S7-15.

MENESES, A. (2002). Tianeptine: 5-HT uptake sites and 5-HT(1-7) receptors modulate memory formation in an autoshaping Pavlovian/instrumental task. *Neurosci Biobehav Rev* **26**(3): 309-319.

METZGER, R. R., BROWN, J. M., SANDOVAL, V., RAU, K. S., ELWAN, M. A., MILLER, G. W., HANSON, G. R. and FLECKENSTEIN, A. E. (2002). Inhibitory effect of reserpine on dopamine transporter function. *Eur J Pharmacol* **456**(1-3): 39-43.

MILLER, G. J. and MILLER, N. E. (1975). Plasma-high-density-lipoprotein concentration and development of ischaemic heart-disease. *Lancet* **1**(7897): 16-19.

MILLER, J. R. (2003). GraphPad Version 4.0. Step-by-Step Examples. San Diego, CA, GraphPad Software Inc.

MIRANDA, H. F., PUIG, M. M., DURSTELER, C., PRIETO, J. C. and PINARDI, G. (2007). Dexketoprofen-induced antinociception in animal models of acute pain: synergy with morphine and paracetamol. *Neuropharmacology* **52**(2): 291-296.

MIRZA, N. R., PETERS, D. and SPARKS, R. G. (2003). Xanomeline and the antipsychotic potential of muscarinic receptor subtype selective agonists. *CNS Drug Rev* **9**(2): 159-186.

MISRA, A., GANESH, S., SHAHIWALA, A. and SHAH, S. P. (2003). Drug delivery to the central nervous system: a review. *J Pharm Pharm Sci* **6**(2): 252-273.

MODY, I. (1998). Ion channels in epilepsy. *Int Rev Neurobiol* **42**: 199-226.

MONTALTO DE MECCA, M., BERNACCHI, A. S. and CASTRO, J. A. (2000). Prevention of benznidazole-induced prolonging effect on the pentobarbital sleeping time of rats using different thiol-containing compounds. *Res Commun Mol Pathol Pharmacol* **108**(1-2): 39-48.

MONTEGGIA, L. M., LUIKART, B., BARROT, M., THEOBOLD, D., MALKOVSKA, I., NEF, S., PARADA, L. F. and NESTLER, E. J. (2007). Brain-derived neurotrophic factor conditional knockouts show gender differences in depression-related behaviors. *Biol Psychiatry* **61**(2): 187-197.

MOORE, A. R. and O'KEEFFE, S. T. (1999). Drug-induced cognitive impairment in the elderly. *Drugs Aging* **15**(1): 15-28.

MORET, C. (2005). Combination/augmentation strategies for improving the treatment of depression. *Neuropsychiatr Dis Treat* **1**(4): 301-309.

MORRIS, G. L., 3RD and VANDERKOLK, C. (2005). Human sexuality, sex hormones, and epilepsy. *Epilepsy Behav* **7 Suppl 2**: S22-28.

MOTULSKY, H. J. and CHRISTOPOULOS, A. (2003). Fitting model to biological data using linear and nonlinear regression. A practical guide to curve fitting. San Diego, CA, GraphPad Software Inc.

MUHAMMAD, N., SAEED, M. and KHAN, H. (2012). Antipyretic, analgesic and anti-inflammatory activity of Viola betonicifolia whole plant. *BMC Complement Altern Med* **12**(1): 59.

MULA, M. and TRIMBLE, M. R. (2009). Antiepileptic drug-induced cognitive adverse effects: potential mechanisms and contributing factors. *CNS Drugs* **23**(2): 121-137.

MULLER, J. E., KOEN, L. and STEIN, D. J. (2005). Anxiety and medical disorders. *Curr Psychiatry Rep* **7**(4): 245-251.

MUTI, P., AWAD, A. B., SCHUNEMANN, H., FINK, C. S., HOVEY, K., FREUDENHEIM, J. L., WU, Y. W., BELLATI, C., PALA, V. and BERRINO, F. (2003). A plant food-based diet modifies the serum beta-sitosterol concentration in hyperandrogenic postmenopausal women. *J Nutr* **133**(12): 4252-4255.

N'GOUEMO, P. (2011). Targeting BK (big potassium) channels in epilepsy. *Expert Opin Ther Targets* **15**(11): 1283-1295.

NABBOUT, R., MAZZUCA, M., HUBERT, P., PEUDENNIER, S., ALLAIRE, C., FLURIN, V., ABERASTURY, M., SILVA, W. and DULAC, O. (2010). Efficacy of ketogenic

diet in severe refractory status epilepticus initiating fever induced refractory epileptic encephalopathy in school age children (FIRES). *Epilepsia* **51**(10): 2033-2037.

NASSAR, M. A., LEVATO, A., STIRLING, L. C. and WOOD, J. N. (2005). Neuropathic pain develops normally in mice lacking both Na(v)1.7 and Na(v)1.8. *Mol Pain* **1**: 24.

NAYAK, S. S., GHOSH, A. K., DEBNATH, B., VISHNOI, S. P. and JHA, T. (2004). Synergistic effect of methanol extract of Abies webbiana leaves on sleeping time induced by standard sedatives in mice and anti-inflammatory activity of extracts in rats. *J Ethnopharmacol* **93**(2-3): 397-402.

NEAL, E. G., CHAFFE, H., SCHWARTZ, R. H., LAWSON, M. S., EDWARDS, N., FITZSIMMONS, G., WHITNEY, A. and CROSS, J. H. (2008). The ketogenic diet for the treatment of childhood epilepsy: a randomised controlled trial. *Lancet Neurol* **7**(6): 500-506.

NEBES, R. D., POLLOCK, B. G., HOUCK, P. R., BUTTERS, M. A., MULSANT, B. H., ZMUDA, M. D. and REYNOLDS, C. F., 3RD (2003). Persistence of cognitive impairment in geriatric patients following antidepressant treatment: a randomized, double-blind clinical trial with nortriptyline and paroxetine. *J Psychiatr Res* **37**(2): 99-108.

NELSON, S. D., MITCHELL, J. R., SNODGRASS, W. R. and TIMBRELL, J. A. (1978). Hepatotoxicity and metabolism of iproniazid and isopropylhydrazine. *J Pharmacol Exp Ther* **206**(3): 574-585.

NESSE, R. M. (1999). Proximate and evolutionary studies of anxiety, stress and depression: synergy at the interface. *Neurosci Biobehav Rev* **23**(7): 895-903.

NESTLER, E. J., BARROT, M., DILEONE, R. J., EISCH, A. J., GOLD, S. J. and MONTEGGIA, L. M. (2002). Neurobiology of depression. *Neuron* **34**(1): 13-25.

NESTLER, E. J., MCMAHON, A., SABBAN, E. L., TALLMAN, J. F. and DUMAN, R. S. (1990). Chronic antidepressant administration decreases the expression of tyrosine hydroxylase in the rat locus coeruleus. *Proc Natl Acad Sci U S A* **87**(19): 7522-7526.

NEWCOMER, J. W., FAUSTMAN, W. O., YEH, W. and CSERNANSKY, J. G. (1990). Distinguishing depression and negative symptoms in unmedicated patients with schizophrenia. *Psychiatry Res* **31**(3): 243-250.

NGO BUM, E., DAWACK, D. L., SCHMUTZ, M., RAKOTONIRINA, A., RAKOTONIRINA, S. V., PORTET, C., JEKER, A., OLPE, H. R. and HERRLING, P. (2004). Anticonvulsant activity of Mimosa pudica decoction. *Fitoterapia* **75**(3-4): 309-314.

NIBUYA, M., MORINOBU, S. and DUMAN, R. S. (1995). Regulation of BDNF and trkB mRNA in rat brain by chronic electroconvulsive seizure and antidepressant drug treatments. *J Neurosci* **15**(11): 7539-7547.

NIESPODZIANY, I., KLITGAARD, H. and MARGINEANU, D. G. (2004). Is the persistent sodium current a specific target of anti-absence drugs? *Neuroreport* **15**(6): 1049-1052.

NIGGEMANN, B. and GRUBER, C. (2003). Side-effects of complementary and alternative medicine. *Allergy* **58**(8): 707-716.

NJUNG'E, K. and HANDLEY, S. L. (1991). Evaluation of marble-burying behavior as a model of anxiety. *Pharmacol Biochem Behav* **38**(1): 63-67.

NOMURA, S. (2003). [Problems of current antidepressant drugs]. *Nihon Shinkei Seishin Yakurigaku Zasshi* **23**(2): 61-65.

NORN, S., KRUSE, P. R. and KRUSE, E. (2005). [History of opium poppy and morphine]. *Dan Medicinhist Arbog* **33**: 171-184.

NRC (1996). Guide for the Care and Use of Laboratory Animals. *Institute of Laboratory Animal Research Commission on Life Sciences*, The National Academies Press.

NUTT, D. J. (2005). Overview of diagnosis and drug treatments of anxiety disorders. *CNS Spectr* **10**(1): 49-56.

O'LEARY, O. F., BECHTHOLT, A. J., CROWLEY, J. J., HILL, T. E., PAGE, M. E. and LUCKI, I. (2007). Depletion of serotonin and catecholamines block the acute behavioral response to different classes of antidepressant drugs in the mouse tail suspension test. *Psychopharmacology (Berl)* **192**(3): 357-371.

OBICI, S., OTOBONE, F. J., DA SILVA SELA, V. R., ISHIDA, K., DA SILVA, J. C., NAKAMURA, C. V., GARCIA CORTEZ, D. A. and AUDI, E. A. (2008). Preliminary toxicity study of dichloromethane extract of Kielmeyera coriacea stems in mice and rats. *J Ethnopharmacol* **115**(1): 131-139.

OKENIYI, M. (2010). Effect of biological extracts on root-knot nematode (*Melloidogyne incoignita*) infection and growth of cacao seedlings. *J. Appl. Biosci.* **36**: 2346-2352.

OKUDA, T. (2005). Systematics and health effects of chemically distinct tannins in medicinal plants. *Phytochemistry* **66**(17): 2012-2031.

OKUDA, T., YOSHIDA, T. and HATANO, T. (1992). Pharmacologically active tannins isolated from medicinal plants. *Basic Life Sci* **59**: 539-569.

OLIVER, B. (1960). *Medicinal Plants in Nigeria.* **Nigerian College of Arts, Science and Technology, Lagos;** (70).

OTA, K. S. and GODWIN, T. (2006). Memantine: the next trend in academic performance enhancement? *J Am Osteopath Assoc* **106**(6): 358-359.

OTANI, M., SHITAN, N., SAKAI, K., MARTINOIA, E., SATO, F. and YAZAKI, K. (2005). Characterization of vacuolar transport of the endogenous alkaloid berberine in Coptis japonica. *Plant Physiol* **138**(4): 1939-1946.

OWENS, M. J. (1996). Molecular and cellular mechanisms of antidepressant drugs. *Depress Anxiety* **4**(4): 153-159.

PACHER, P., KOHEGYI, E., KECSKEMETI, V. and FURST, S. (2001). Current trends in the development of new antidepressants. *Curr Med Chem* **8**(2): 89-100.

PAGE, M. E., DETKE, M. J., DALVI, A., KIRBY, L. G. and LUCKI, I. (1999). Serotonergic mediation of the effects of fluoxetine, but not desipramine, in the rat forced swimming test. *Psychopharmacology (Berl)* **147**(2): 162-167.

PAGE, M. E. and LUCKI, I. (2002). Effects of acute and chronic reboxetine treatment on stress-induced monoamine efflux in the rat frontal cortex. *Neuropsychopharmacology* **27**(2): 237-247.

PAL, S., SEN, T. and CHAUDHURI, A. K. (1999). Neuropsychopharmacological profile of the methanolic fraction of Bryophyllum pinnatum leaf extract. *J Pharm Pharmacol* **51**(3): 313-318.

PALOMBO, E. A. (2006). Phytochemicals from traditional medicinal plants used in the treatment of diarrhoea: modes of action and effects on intestinal function. *Phytother Res* **20**(9): 717-724.

PAPP, M. and MORYL, E. (1996). Antidepressant-like effects of 1-aminocyclopropanecarboxylic acid and D-cycloserine in an animal model of depression. *Eur J Pharmacol* **316**(2-3): 145-151.

PARIANTE, C. M. and LIGHTMAN, S. L. (2008). The HPA axis in major depression: classical theories and new developments. *Trends Neurosci* **31**(9): 464-468.

PARIKH, S. V. and LAM, R. W. (2001). Clinical guidelines for the treatment of depressive disorders, I. Definitions, prevalence, and health burden. *Can J Psychiatry* **46 Suppl 1**: 13S-20S.

PARK, B. K., KITTERINGHAM, N. R., MAGGS, J. L., PIRMOHAMED, M. and WILLIAMS, D. P. (2005). The role of metabolic activation in drug-induced hepatotoxicity. *Annu Rev Pharmacol Toxicol* **45**: 177-202.

PARK, D. H., STONE, D. M., BAKER, H., KIM, K. S. and JOH, T. H. (1994). Early induction of rat brain tryptophan hydroxylase (TPH) mRNA following parachlorophenylalanine (PCPA) treatment. *Brain Res Mol Brain Res* **22**(1-4): 20-28.

PARSONS, C. G., STOFFLER, A. and DANYSZ, W. (2007). Memantine: a NMDA receptor antagonist that improves memory by restoration of homeostasis in the glutamatergic system--too little activation is bad, too much is even worse. *Neuropharmacology* **53**(6): 699-723.

PARTHASARATHI, U. D., HARROWER, T., TEMPEST, M., HODGES, J. R., WALSH, C., MCKENNA, P. J. and FLETCHER, P. C. (2006). Psychiatric presentation of voltage-gated potassium channel antibody-associated encephalopathy. Case report. *Br J Psychiatry* **189**: 182-183.

PELLOW, S., CHOPIN, P., FILE, S. E. and BRILEY, M. (1985). Validation of open:closed arm entries in an elevated plus-maze as a measure of anxiety in the rat. *J Neurosci Methods* **14**(3): 149-167.

PENA, F. and TAPIA, R. (2000). Seizures and neurodegeneration induced by 4-aminopyridine in rat hippocampus in vivo: role of glutamate- and GABA-mediated neurotransmission and of ion channels. *Neuroscience* **101**(3): 547-561.

PEREZ-MENDES, P., BLANCO, M. M., CALCAGNOTTO, M. E., CININI, S. M., BACHIEGA, J., PAPOTI, D., COVOLAN, L., TANNUS, A. and MELLO, L. E. (2011). Modeling epileptogenesis and temporal lobe epilepsy in a non-human primate. *Epilepsy Res* **96**(1-2): 45-57.

PEREZ, J., TINELLI, D., BIANCHI, E., BRUNELLO, N. and RACAGNI, G. (1991). cAMP binding proteins in the rat cerebral cortex after administration of selective 5-HT and NE reuptake blockers with antidepressant activity. *Neuropsychopharmacology* **4**(1): 57-64.

PERUCCA, E. (2002). Marketed new antiepileptic drugs: are they better than old-generation agents? *Ther Drug Monit* **24**(1): 74-80.

PERUCCA, E. (2005). [Pharmacotherapy of epilepsy in women]. *Zh Nevrol Psikhiatr Im S S Korsakova* **105**(11): 60-62.

PERUCCA, E., ALEXANDRE, V., JR. and TOMSON, T. (2007). Old versus new antiepileptic drugs: the SANAD study. *Lancet* **370**(9584): 313; author reply 315-316.

PERUCCA, E., FRENCH, J. and BIALER, M. (2007). Development of new antiepileptic drugs: challenges, incentives, and recent advances. *Lancet Neurol* **6**(9): 793-804.

PETIT-DEMOULIERE, B., CHENU, F. and BOURIN, M. (2005). Forced swimming test in mice: a review of antidepressant activity. *Psychopharmacology (Berl)* **177**(3): 245-255.

PINEYRO, G. and BLIER, P. (1999). Autoregulation of serotonin neurons: role in antidepressant drug action. *Pharmacol Rev* **51**(3): 533-591.

PITKANEN, A. (2002). Efficacy of current antiepileptics to prevent neurodegeneration in epilepsy models. *Epilepsy Research* **50**: 141–160.

PITKANEN, A. and LUKASIUK, K. (2009). Molecular and cellular basis of epileptogenesis in symptomatic epilepsy. *Epilepsy Behav* **14 Suppl 1**: 16-25.

POLESZAK, E., SZEWCZYK, B., KEDZIERSKA, E., WLAZ, P., PILC, A. and NOWAK, G. (2004). Antidepressant- and anxiolytic-like activity of magnesium in mice. *Pharmacol Biochem Behav* **78**(1): 7-12.

POLESZAK, E., WLAZ, P., SZEWCZYK, B., WLAZ, A., KASPEREK, R., WROBEL, A. and NOWAK, G. (2011). A complex interaction between glycine/NMDA receptors and serotonergic/noradrenergic antidepressants in the forced swim test in mice. *J Neural Transm* **118**(11): 1535-1546.

POLIVY, J. and HERMAN, C. P. (1976). Clinical depression and weight change: a complex relation. *J Abnorm Psychol* **85**(3): 338-340.

POLLAK, O. J. (1985). Effect of plant sterols on serum lipids and atherosclerosis. *Pharmacol Ther* **31**(3): 177-208.

PORSOLT, R. D., LE PICHON, M. and JALFRE, M. (1977). Depression: a new animal model sensitive to antidepressant treatments. *Nature* **266**(5604): 730-732.

PORSOLT, R. D., LEMAIRE, M., DURMULLER, N. and ROUX, S. (2002). New perspectives in CNS safety pharmacology. *Fundam Clin Pharmacol* **16**(3): 197-207.

PORTER, R. J., CEREGHINO, J. J., GLADDING, G. D., HESSIE, B. J., KUPFERBERG, H. J., SCOVILLE, B. and WHITE, B. G. (1984). Antiepileptic Drug Development Program. *Cleve Clin Q* **51**(2): 293-305.

PRZEGALINSKI, E., TATARCZYNSKA, E. and CHOJNACKA-WOJCIK, E. (2000). The influence of the benzodiazepine receptor antagonist flumazenil on the anxiolytic-like effects of CGP 37849 and ACPC in rats. *Neuropharmacology* **39**(10): 1858-1864.

PUTNAM, T. J. and MERRITT, H. H. (1937). Experimental Determination of the Anticonvulsant Properties of Some Phenyl Derivatives. *Science* **85**(2213): 525-526.

QUANTE, A., ZEUGMANN, S., LUBORZEWSKI, A., SCHOMMER, N., LANGOSCH, J., BORN, C., ANGHELESCU, I. and WOLF, J. (2010). Aripiprazole as adjunct to a mood

stabilizer and citalopram in bipolar depression: a randomized placebo-controlled pilot study. *Hum Psychopharmacol* **25**(2): 126-132.

RABBANI, M., SAJJADI, S. E. and MOHAMMADI, A. (2008). Evaluation of the anxiolytic effect of Nepeta persica Boiss. in mice. *Evid Based Complement Alternat Med* **5**(2): 181-186.

RAMABADRAN, K., BANSINATH, M., TURNDORF, H. and PUIG, M. M. (1989). Tail immersion test for the evaluation of a nociceptive reaction in mice. Methodological considerations. *J Pharmacol Methods* **21**(1): 21-31.

RAVNKILDE, B., VIDEBECH, P., CLEMMENSEN, K., EGANDER, A., RASMUSSEN, N. A. and ROSENBERG, R. (2002). Cognitive deficits in major depression. *Scand J Psychol* **43**(3): 239-251.

RAZA, M., SHAHEEN, F., CHOUDHARY, M. I., SOMBATI, S., RAFIQ, A., SURIA, A., RAHMAN, A. and DELORENZO, R. J. (2001). Anticonvulsant activities of ethanolic extract and aqueous fraction isolated from Delphinium denudatum. *J Ethnopharmacol* **78**(1): 73-78.

REESAL, R. T. and LAM, R. W. (2001). Clinical guidelines for the treatment of depressive disorders. II. Principles of management. *Can J Psychiatry* **46 Suppl 1**: 21S-28S.

REGESTA, G. and TANGANELLI, P. (1999). Clinical aspects and biological bases of drug-resistant epilepsies. *Epilepsy Res* **34**(2-3): 109-122.

REID, A. Y., METCALFE, A., PATTEN, S. B., WIEBE, S., MACRODIMITRIS, S. and JETTE, N. (2012). Epilepsy is associated with unmet health care needs compared to the general population despite higher health resource utilization--a Canadian population-based study. *Epilepsia* **53**(2): 291-300.

REISBERG, B., DOODY, R., STOFFLER, A., SCHMITT, F., FERRIS, S. and MOBIUS, H. J. (2003). Memantine in moderate-to-severe Alzheimer's disease. *N Engl J Med* **348**(14): 1333-1341.

REMY, S., URBAN, B. W., ELGER, C. E. and BECK, H. (2003). Anticonvulsant pharmacology of voltage-gated Na+ channels in hippocampal neurons of control and chronically epileptic rats. *Eur J Neurosci* **17**(12): 2648-2658.

RENTON, K. W. (1985). Inhibition of hepatic microsomal drug metabolism by the calcium channel blockers diltiazem and verapamil. *Biochem Pharmacol* **34**(14): 2549-2553.

RHO, J. M., DONEVAN, S. D. and ROGAWSKI, M. A. (1997). Barbiturate-like actions of the propanediol dicarbamates felbamate and meprobamate. *J Pharmacol Exp Ther* **280**(3): 1383-1391.

ROBERTS, R. E., DELEGER, S., STRAWBRIDGE, W. J. and KAPLAN, G. A. (2003). Prospective association between obesity and depression: evidence from the Alameda County Study. *Int J Obes Relat Metab Disord* **27**(4): 514-521.

RODE, F., JENSEN, D. G., BLACKBURN-MUNRO, G. and BJERRUM, O. J. (2005). Centrally-mediated antinociceptive actions of GABA(A) receptor agonists in the rat spared nerve injury model of neuropathic pain. *Eur J Pharmacol* **516**(2): 131-138.

RODGERS, R. J. and COLE, J. C. (1994). Anxiolytic-like effect of (S)-WAY 100135, a 5-HT1A receptor antagonist, in the murine elevated plus-maze test. *Eur J Pharmacol* **261**(3): 321-325.

RODGERS, R. J. and DALVI, A. (1997). Anxiety, defence and the elevated plus-maze. *Neurosci Biobehav Rev* **21**(6): 801-810.

RODGERS, R. J. and JOHNSON, N. J. (1995). Factor analysis of spatiotemporal and ethological measures in the murine elevated plus-maze test of anxiety. *Pharmacol Biochem Behav* **52**(2): 297-303.

ROGAWSKI, M. A. (2000). Low affinity channel blocking (uncompetitive) NMDA receptor antagonists as therapeutic agents--toward an understanding of their favorable tolerability. *Amino Acids* **19**(1): 133-149.

ROGAWSKI, M. A. (2006). Molecular targets versus models for new antiepileptic drug discovery. *Epilepsy Res* **68**(1): 22-28.

ROGAWSKI, M. A. and LOSCHER, W. (2004). The neurobiology of antiepileptic drugs. *Nat Rev Neurosci* **5**(7): 553-564.

ROGERIO, A. P., SA-NUNES, A. and FACCIOLI, L. H. (2010). The activity of medicinal plants and secondary metabolites on eosinophilic inflammation. *Pharmacol Res* **62**(4): 298-307.

ROLLAND, A., FLEURENTIN, J., LANHERS, M. C., MISSLIN, R. and MORTIER, F. (2001). Neurophysiological effects of an extract of Eschscholzia californica Cham. (Papaveraceae). *Phytother Res* **15**(5): 377-381.

ROMBERG, R., SARTON, E., TEPPEMA, L., MATTHES, H. W., KIEFFER, B. L. and DAHAN, A. (2003). Comparison of morphine-6-glucuronide and morphine on respiratory depressant and antinociceptive responses in wild type and mu-opioid receptor deficient mice. *Br J Anaesth* **91**(6): 862-870.

ROSTOCK, A., TOBER, C., RUNDFELDT, C., BARTSCH, R., ENGEL, J., POLYMEROPOULOS, E. E., KUTSCHER, B., LOSCHER, W., HONACK, D., WHITE, H. S. and WOLF, H. H. (1996). D-23129: a new anticonvulsant with a broad spectrum activity in animal models of epileptic seizures. *Epilepsy Res* **23**(3): 211-223.

ROTHSCHILD, M. A., ORATZ, M. and SCHREIBER, S. S. (1988). Serum albumin. *Hepatology* **8**(2): 385-401.

ROUX, S., SABLE, E. and PORSOLT, R. D. (2005). Primary observation (Irwin) test in rodents for assessing acute toxicity of a test agent and its effects on behavior and physiological function. *Curr Protoc Pharmacol* **Chapter 10**: Unit 10 10.

ROWLAND, L. M., ASTUR, R. S., JUNG, R. E., BUSTILLO, J. R., LAURIELLO, J. and YEO, R. A. (2005). Selective cognitive impairments associated with NMDA receptor blockade in humans. *Neuropsychopharmacology* **30**(3): 633-639.

RUDOLPH, U., CRESTANI, F., BENKE, D., BRUNIG, I., BENSON, J. A., FRITSCHY, J. M., MARTIN, J. R., BLUETHMANN, H. and MOHLER, H. (1999). Benzodiazepine actions mediated by specific gamma-aminobutyric acid(A) receptor subtypes. *Nature* **401**(6755): 796-800.

RUNDFELDT, C. (1997). The new anticonvulsant retigabine (D-23129) acts as an opener of K+ channels in neuronal cells. *Eur J Pharmacol* **336**(2-3): 243-249.

RUPNIAK, N. M. (2003). Animal models of depression: challenges from a drug development perspective. *Behav Pharmacol* **14**(5-6): 385-390.

RYDER, S. D. and BECKINGHAM, I. J. (2001). ABC of diseases of liver, pancreas, and biliary system. Other causes of parenchymal liver disease. *BMJ* **322**(7281): 290-292.

SAHAY, A., DREW, M. R. and HEN, R. (2007). Dentate gyrus neurogenesis and depression. *Prog Brain Res* **163**: 697-722.

SAHAY, A. and HEN, R. (2007). Adult hippocampal neurogenesis in depression. *Nat Neurosci* **10**(9): 1110-1115.

SAMREN, E. B., VAN DUIJN, C. M., KOCH, S., HIILESMAA, V. K., KLEPEL, H., BARDY, A. H., MANNAGETTA, G. B., DEICHL, A. W., GAILY, E., GRANSTROM, M. L., MEINARDI, H., GROBBEE, D. E., HOFMAN, A., JANZ, D. and LINDHOUT, D. (1997). Maternal use of antiepileptic drugs and the risk of major congenital malformations: a joint European prospective study of human teratogenesis associated with maternal epilepsy. *Epilepsia* **38**(9): 981-990.

SANABRIA, E. R., SILVA, A. V., SPREAFICO, R. and CAVALHEIRO, E. A. (2002). Damage, reorganization, and abnormal neocortical hyperexcitability in the pilocarpine model of temporal lobe epilepsy. *Epilepsia* **43 Suppl 5**: 96-106.

SANDER, J. W. (2003). The epidemiology of epilepsy revisited. *Curr Opin Neurol* **16**(2): 165-170.

SANDER, J. W. and SHORVON, S. D. (1996). Epidemiology of the epilepsies. *J Neurol Neurosurg Psychiatry* **61**(5): 433-443.

SANDERSON, K., TILSE, E., NICHOLSON, J., OLDENBURG, B. and GRAVES, N. (2007). Which presenteeism measures are more sensitive to depression and anxiety? *J Affect Disord* **101**(1-3): 65-74.

SAYYAH, M., NADJAFNIA, L. and KAMALINEJAD, M. (2004). Anticonvulsant activity and chemical composition of Artemisia dracunculus L. essential oil. *J Ethnopharmacol* **94**(2-3): 283-287.

SCHAUF, C. L. (1987). Anticonvulsants modify inactivation but not activation processes of sodium channels in Myxicola axons. *Can J Physiol Pharmacol* **65**(6): 1220-1225.

SCHLAEPFER, T. E., COHEN, M. X., FRICK, C., KOSEL, M., BRODESSER, D., AXMACHER, N., JOE, A. Y., KREFT, M., LENARTZ, D. and STURM, V. (2008). Deep brain stimulation to reward circuitry alleviates anhedonia in refractory major depression. *Neuropsychopharmacology* **33**(2): 368-377.

SCHMIDT, D. (2002). The clinical impact of new antiepileptic drugs after a decade of use in epilepsy. *Epilepsy Res* **50**(1-2): 21-32.

SCHMUTZ, M., BRUGGER, F., GENTSCH, C., MCLEAN, M. J. and OLPE, H. R. (1994). Oxcarbazepine: preclinical anticonvulsant profile and putative mechanisms of action. *Epilepsia* **35 Suppl 5**: S47-50.

SCHREIBER, S. and PICK, C. G. (1995). Fluoxetine for blepharospasm: interaction of serotonin and dopamine. *J Nerv Ment Dis* **183**(11): 719-721.

SCOTT, K. M., BRUFFAERTS, R., TSANG, A., ORMEL, J., ALONSO, J., ANGERMEYER, M. C., BENJET, C., BROMET, E., DE GIROLAMO, G., DE GRAAF, R., GASQUET, I., GUREJE, O., HARO, J. M., HE, Y., KESSLER, R. C., LEVINSON, D.,

MNEIMNEH, Z. N., OAKLEY BROWNE, M. A., POSADA-VILLA, J., STEIN, D. J., TAKESHIMA, T. and VON KORFF, M. (2007). Depression-anxiety relationships with chronic physical conditions: results from the World Mental Health Surveys. *J Affect Disord* **103**(1-3): 113-120.

SEEFF, L. B. (2007). Herbal hepatotoxicity. *Clin Liver Dis* **11**(3): 577-596, vii.

SEGAL, M. M. and DOUGLAS, A. F. (1997). Late sodium channel openings underlying epileptiform activity are preferentially diminished by the anticonvulsant phenytoin. *J Neurophysiol* **77**(6): 3021-3034.

SETEM, J., PINHEIRO, A. P., MOTTA, V. A., MORATO, S. and CRUZ, A. P. (1999). Ethopharmacological analysis of 5-HT ligands on the rat elevated plus-maze. *Pharmacol Biochem Behav* **62**(3): 515-521.

SEWELL, R. D. and SPENCER, P. S. (1976). Antinociceptive activitiy of narcotic agonist and partial agonist analgesics and other agents in the tail-immersion test in mice and rats. *Neuropharmacology* **15**(11): 683-688.

SHEEHAN, D. M. (1998). Herbal medicines, phytoestrogens and toxicity: risk:benefit considerations. *Proc Soc Exp Biol Med* **217**(3): 379-385.

SHINNAR, S. and BERG, A. T. (1996). Does antiepileptic drug therapy prevent the development of "chronic" epilepsy? *Epilepsia* **37**(8): 701-708.

SHORVON, S. D. (2009). Drug treatment of epilepsy in the century of the ILAE: the first 50 years, 1909-1958. *Epilepsia* **50 Suppl 3**: 69-92.

SHORVON, S. D. and REYNOLDS, E. H. (1979). Reduction in polypharmacy for epilepsy. *Br Med J* **2**(6197): 1023-1025.

SI, A., HELLIWELL, P. and MALESZKA, R. (2004). Effects of NMDA receptor antagonists on olfactory learning and memory in the honeybee (Apis mellifera). *Pharmacol Biochem Behav* **77**(2): 191-197.

SILLS, G. J. (2006). The multidrug transporter hypothesis of refractory epilepsy: corroboration and contradiction in equal measure. *Epilepsy Curr* **6**(2): 51-54.

SILVER, J. M., SHIN, C. and MCNAMARA, J. O. (1991). Antiepileptogenic effects of conventional anticonvulsants in the kindling model of epilespy. *Ann Neurol* **29**(4): 356-363.

SIMMONS, R. M., WEBSTER, A. A., KALRA, A. B. and IYENGAR, S. (2002). Group II mGluR receptor agonists are effective in persistent and neuropathic pain models in rats. *Pharmacol Biochem Behav* **73**(2): 419-427.

SIMOENS, S. (2010). Pharmacoeconomics of anti-epileptic drugs as adjunctive therapy for refractory epilepsy. *Expert Rev Pharmacoecon Outcomes Res* **10**(3): 309-315.

SIMON, V. M., PARRA, A., MINARRO, J., ARENAS, M. C., VINADER-CAEROLS, C. and AGUILAR, M. A. (2000). Predicting how equipotent doses of chlorpromazine, haloperidol, sulpiride, raclopride and clozapine reduce locomotor activity in mice. *Eur Neuropsychopharmacol* **10**(3): 159-164.

SINCLAIR, A. M., MILLER, B. and LEE, L. K. (2007). Chronic orchialgia: consider gabapentin or nortriptyline before considering surgery. *Int J Urol* **14**(7): 622-625.

SITGES, M., SANCHEZ-TAFOLLA, B. M., CHIU, L. M., ALDANA, B. I. and GUARNEROS, A. (2011). Vinpocetine inhibits glutamate release induced by the convulsive agent 4-aminopyridine more potently than several antiepileptic drugs. *Epilepsy Res* **96**(3): 257-266.

SIWEK, M., DUDEK, D., PAUL, I. A., SOWA-KUCMA, M., ZIEBA, A., POPIK, P., PILC, A. and NOWAK, G. (2009). Zinc supplementation augments efficacy of imipramine in treatment resistant patients: a double blind, placebo-controlled study. *J Affect Disord* **118**(1-3): 187-195.

SLATTERY, D. A., HUDSON, A. L. and NUTT, D. J. (2004). Invited review: the evolution of antidepressant mechanisms. *Fundam Clin Pharmacol* **18**(1): 1-21.

SMITH, M., POLITZER, N., MACGARVIE, D., MCANDREWS, M. P. and DEL CAMPO, M. (2011). Efficacy and tolerability of the modified Atkins diet in adults with pharmacoresistant epilepsy: a prospective observational study. *Epilepsia* **52**(4): 775-780.

SMOLDERS, I., KHAN, G. M., MANIL, J., EBINGER, G. and MICHOTTE, Y. (1997). NMDA receptor-mediated pilocarpine-induced seizures: characterization in freely moving rats by microdialysis. *Br J Pharmacol* **121**(6): 1171-1179.

SOHAL, V. S., KEIST, R., RUDOLPH, U. and HUGUENARD, J. R. (2003). Dynamic GABA(A) receptor subtype-specific modulation of the synchrony and duration of thalamic oscillations. *J Neurosci* **23**(9): 3649-3657.

SOLINAS, M., FERRE, S., YOU, Z. B., KARCZ-KUBICHA, M., POPOLI, P. and GOLDBERG, S. R. (2002). Caffeine induces dopamine and glutamate release in the shell of the nucleus accumbens. *J Neurosci* **22**(15): 6321-6324.

SOLTOFF, S. P. (2007). Rottlerin: an inappropriate and ineffective inhibitor of PKCdelta. *Trends Pharmacol Sci* **28**(9): 453-458.

SOUZA, S. M., AQUINO, L. C., MILACH, A. C., JR., BANDEIRA, M. A., NOBRE, M. E. and VIANA, G. S. (2007). Antiinflammatory and antiulcer properties of tannins from Myracrodruon urundeuva Allemao (Anacardiaceae) in rodents. *Phytother Res* **21**(3): 220-225.

SPINELLA, M. (2001). Herbal Medicines and Epilepsy: The Potential for Benefit and Adverse Effects. *Epilepsy Behav* **2**(6): 524-532.

STAERK, D., LYKKEBERG, A. K., CHRISTENSEN, J., BUDNIK, B. A., ABE, F. and JAROSZEWSKI, J. W. (2002). In vitro cytotoxic activity of phenanthroindolizidine alkaloids from Cynanchum vincetoxicum and Tylophora tanakae against drug-sensitive and multidrug-resistant cancer cells. *J Nat Prod* **65**(9): 1299-1302.

STAFFORD, G. I., PEDERSEN, M. E., VAN STADEN, J. and JAGER, A. K. (2008). Review on plants with CNS-effects used in traditional South African medicine against mental diseases. *J Ethnopharmacol* **119**(3): 513-537.

STAFSTROM, C. E. (2005). The role of the subiculum in epilepsy and epileptogenesis. *Epilepsy Curr* **5**(4): 121-129.

STANDAGE, K. F. and FENTON, G. W. (1975). Psychiatric symptom profiles of patients with epilepsy: a controlled investigation. *Psychol Med* **5**(2): 152-160.

STEDMAN, C. (2002). Herbal hepatotoxicity. *Semin Liver Dis* **22**(2): 195-206.

STERU, L., CHERMAT, R., THIERRY, B. and SIMON, P. (1985). The tail suspension test: a new method for screening antidepressants in mice. *Psychopharmacology (Berl)* **85**(3): 367-370.

STONE, E. A. and LIN, Y. (2011). Open-space forced swim model of depression for mice. *Curr Protoc Neurosci* **Chapter 9**: Unit9 36.

STONE, E. A., LIN, Y. and QUARTERMAIN, D. (2008). Evaluation of the repeated open-space swim model of depression in the mouse. *Pharmacol Biochem Behav* **91**(1): 190-195.

SU, T., ZHANG, L., LI, X., ZUO, L., ZHANG, P. and WANG, H. (2011). Regular use of nephrotoxic medications is an independent risk factor for chronic kidney disease--results from a Chinese population study. *Nephrol Dial Transplant* **26**(6): 1916-1923.

SUN, H. L., ZHENG, J. W., WANG, K., LIU, R. K. and LIANG, J. H. (2003). Tramadol reduces the 5-HTP-induced head-twitch response in mice via the activation of mu and kappa opioid receptors. *Life Sci* **72**(11): 1221-1230.

SUN, M. K. and ALKON, D. L. (2002). Depressed or demented: common CNS drug targets?! *Curr Drug Targets CNS Neurol Disord* **1**(6): 575-592.

SUN, M. K. and ALKON, D. L. (2003). Open space swimming test to index antidepressant activity. *J Neurosci Methods* **126**(1): 35-40.

SUN, M. K. and ALKON, D. L. (2004). Induced depressive behavior impairs learning and memory in rats. *Neuroscience* **129**(1): 129-139.

SURGES, R., VOLYNSKI, K. E. and WALKER, M. C. (2008). Is levetiracetam different from other antiepileptic drugs? Levetiracetam and its cellular mechanism of action in epilepsy revisited. *Ther Adv Neurol Disord* **1**(1): 13-24.

SUZDAK, P. D. and JANSEN, J. A. (1995). A review of the preclinical pharmacology of tiagabine: a potent and selective anticonvulsant GABA uptake inhibitor. *Epilepsia* **36**(6): 612-626.

SWINYARD, E. A. (1969). Laboratory evaluation of antiepileptic drugs. Review of laboratory methods. *Epilepsia* **10**(2): 107-119.

SWINYARD, E. A. and KUPFERBERG, H. J. (1985). Antiepileptic drugs: detection, quantification, and evaluation. *Fed Proc* **44**(10): 2629-2633.

SZYNDLER, J., WIERZBA-BOBROWICZ, T., SKORZEWSKA, A., MACIEJAK, P., WALKOWIAK, J., LECHOWICZ, W., TURZYNSKA, D., BIDZINSKI, A. and PLAZNIK, A. (2005). Behavioral, biochemical and histological studies in a model of pilocarpine-induced spontaneous recurrent seizures. *Pharmacol Biochem Behav* **81**(1): 15-23.

TADDESE, A. and BEAN, B. P. (2002). Subthreshold sodium current from rapidly inactivating sodium channels drives spontaneous firing of tuberomammillary neurons. *Neuron* **33**(4): 587-600.

TAGLIALATELA, M., ONGINI, E., BROWN, A. M., DI RENZO, G. and ANNUNZIATO, L. (1996). Felbamate inhibits cloned voltage-dependent Na+ channels from human and rat brain. *Eur J Pharmacol* **316**(2-3): 373-377.

TALATHI, S. S., HWANG, D. U., DITTO, W. L., MARECI, T., SEPULVEDA, H., SPANO, M. and CARNEY, P. R. (2009). Circadian control of neural excitability in an animal model of temporal lobe epilepsy. *Neurosci Lett* **455**(2): 145-149.

TALLARIDA, R. J. (2006). An overview of drug combination analysis with isobolograms. *J Pharmacol Exp Ther* **319**(1): 1-7.

TARDITO, D., PEREZ, J., TIRABOSCHI, E., MUSAZZI, L., RACAGNI, G. and POPOLI, M. (2006). Signaling pathways regulating gene expression, neuroplasticity, and neurotrophic mechanisms in the action of antidepressants: a critical overview. *Pharmacol Rev* **58**(1): 115-134.

TAVERNA, S., MANTEGAZZA, M., FRANCESCHETTI, S. and AVANZINI, G. (1998). Valproate selectively reduces the persistent fraction of Na+ current in neocortical neurons. *Epilepsy Res* **32**(1-2): 304-308.

TAVERNA, S., SANCINI, G., MANTEGAZZA, M., FRANCESCHETTI, S. and AVANZINI, G. (1999). Inhibition of transient and persistent Na+ current fractions by the new anticonvulsant topiramate. *J Pharmacol Exp Ther* **288**(3): 960-968.

TAYLOR, J., CHADWICK, D. W. and JOHNSON, T. (1995). Accident experience and notification rates in people with recent seizures, epilepsy or undiagnosed episodes of loss of consciousness. *QJM* **88**(10): 733-740.

TEMKIN, N. R. (2001). Antiepileptogenesis and seizure prevention trials with antiepileptic drugs: meta-analysis of controlled trials. *Epilepsia* **42**(4): 515-524.

TEMKIN, N. R., JARELL, A. D. and ANDERSON, G. D. (2001). Antiepileptogenic agents: how close are we? *Drugs* **61**(8): 1045-1055.

TEO, S., STIRLING, D., THOMAS, S., HOBERMAN, A., KIORPES, A. and KHETANI, V. (2002). A 90-day oral gavage toxicity study of D-methylphenidate and D,L-methylphenidate in Sprague-Dawley rats. *Toxicology* **179**(3): 183-196.

THIERRY, B., STERU, L., SIMON, P. and PORSOLT, R. D. (1986). The tail suspension test: ethical considerations. *Psychopharmacology (Berl)* **90**(2): 284-285.

THOMPSON, S. M. and GAHWILER, B. H. (1992). Effects of the GABA uptake inhibitor tiagabine on inhibitory synaptic potentials in rat hippocampal slice cultures. *J Neurophysiol* **67**(6): 1698-1701.

TIMBRELL, J. A. (1979). The role of metabolism in the hepatotoxicity of isoniazid and iproniazid. *Drug Metab Rev* **10**(1): 125-147.

TOBER, C., ROSTOCK, A., RUNDFELDT, C. and BARTSCH, R. (1996). D-23129: a potent anticonvulsant in the amygdala kindling model of complex partial seizures. *Eur J Pharmacol* **303**(3): 163-169.

TOMAN, J. E., SWINYARD, E. A. and GOODMAN, L. S. (1946). Properties of maximal seizures, and their alteration by anticonvulant drugs and other agents. *J Neurophysiol* **9**: 231-239.

TOTH, P. P. (2005). Cardiology patient page. The "good cholesterol": high-density lipoprotein. *Circulation* **111**(5): e89-91.

TRAN, D. S., NGOUNGOU, E. B., QUET, F. and PREUX, P. M. (2007). [Management of epilepsy in developing countries]. *Med Trop (Mars)* **67**(6): 635-643.

TRAN, P. V., BYMASTER, F. P., MCNAMARA, R. K. and POTTER, W. Z. (2003). Dual monoamine modulation for improved treatment of major depressive disorder. *J Clin Psychopharmacol* **23**(1): 78-86.

TREASE, G. E. and EVANS, W. C. (1989). Textbook of Pharmacognosy.

TREIMAN, D. M. (2001). GABAergic mechanisms in epilepsy. *Epilepsia* **42 Suppl 3**: 8-12.

TREIT, D. (1990). A comparison of anxiolytic and nonanxiolytic agents in the shock-probe/burying test for anxiolytics. *Pharmacol Biochem Behav* **36**(1): 203-205.

TROSTER, A. I., PAOLO, A. M., LYONS, K. E., GLATT, S. L., HUBBLE, J. P. and KOLLER, W. C. (1995). The influence of depression on cognition in Parkinson's disease: a pattern of impairment distinguishable from Alzheimer's disease. *Neurology* **45**(4): 672-676.

TRULLAS, R. and SKOLNICK, P. (1990). Functional antagonists at the NMDA receptor complex exhibit antidepressant actions. *Eur J Pharmacol* **185**(1): 1-10.

TURSKI, L., IKONOMIDOU, C., TURSKI, W. A., BORTOLOTTO, Z. A. and CAVALHEIRO, E. A. (1989). Review: cholinergic mechanisms and epileptogenesis. The seizures induced by pilocarpine: a novel experimental model of intractable epilepsy. *Synapse* **3**(2): 154-171.

UEKERMANN, J., DAUM, I., SCHLEBUSCH, P., WIEBEL, B. and TRENCKMANN, U. (2003). Depression and cognitive functioning in alcoholism. *Addiction* **98**(11): 1521-1529.

USTUN, T. B., AYUSO-MATEOS, J. L., CHATTERJI, S., MATHERS, C. and MURRAY, C. J. (2004). Global burden of depressive disorders in the year 2000. *Br J Psychiatry* **184**: 386-392.

VAMVAKIDES, A. (1998). [D-cycloserine is active in the adult mouse and inactive in the aged mouse, in the forced swim test]. *Ann Pharm Fr* **56**(5): 209-212.

VAN GIERSBERGEN, P. L., VAN DUINKERKEN, E., SWEEP, C. G., WIEGANT, V. M., VAN REE, J. M. and DE JONG, W. (1990). Alpha-methyldopa induces a naltrexone-insensitive antinociception and hypomotility in rats. *Br J Pharmacol* **99**(3): 467-472.

VARTY, G. B., COHEN-WILLIAMS, M. E. and HUNTER, J. C. (2003). The antidepressant-like effects of neurokinin NK1 receptor antagonists in a gerbil tail suspension test. *Behav Pharmacol* **14**(1): 87-95.

VELLUCCI, S. V. and WEBSTER, R. A. (1984). Antagonism of caffeine-induced seizures in mice by Ro15-1788. *Eur J Pharmacol* **97**(3-4): 289-293.

VERROTTI, A., D'EGIDIO, C., COPPOLA, G., PARISI, P. and CHIARELLI, F. (2009). Epilepsy, sex hormones and antiepileptic drugs in female patients. *Expert Rev Neurother* **9**(12): 1803-1814.

VINCKEN, J. P., HENG, L., DE GROOT, A. and GRUPPEN, H. (2007). Saponins, classification and occurrence in the plant kingdom. *Phytochemistry* **68**(3): 275-297.

VOLETI, B. and DUMAN, R. S. (2012). The roles of neurotrophic factor and Wnt signaling in depression. *Clin Pharmacol Ther* **91**(2): 333-338.

VONVOIGTLANDER, P. F., LAHTI, R. A. and LUDENS, J. H. (1983). U-50,488: a selective and structurally novel non-Mu (kappa) opioid agonist. *J Pharmacol Exp Ther* **224**(1): 7-12.

WALF, A. A. and FRYE, C. A. (2007). The use of the elevated plus maze as an assay of anxiety-related behavior in rodents. *Nat Protoc* **2**(2): 322-328.

WALL, C. J., KENDALL, E. J. and OBENAUS, A. (2000). Rapid alterations in diffusion-weighted images with anatomic correlates in a rodent model of status epilepticus. *AJNR Am J Neuroradiol* **21**(10): 1841-1852.

WANG, L. F., ZHAO, M. L. and LIU, Y. (2001). [Experimental pharmacodynamic study on the anti-convulsion effect of shenpu decoction]. *Zhongguo Zhong Xi Yi Jie He Za Zhi* **21**(11): 837-839.

WEBB, T. I. and LYNCH, J. W. (2007). Molecular pharmacology of the glycine receptor chloride channel. *Curr Pharm Des* **13**(23): 2350-2367.

WEI, X. Y., YANG, J. Y., WANG, J. H. and WU, C. F. (2007). Anxiolytic effect of saponins from Panax quinquefolium in mice. *J Ethnopharmacol* **111**(3): 613-618.

WEISSENBURGER, J., RUSH, A. J., GILES, D. E. and STUNKARD, A. J. (1986). Weight change in depression. *Psychiatry Res* **17**(4): 275-283.

WERMAN, R., DAVIDOFF, R. A. and APRISON, M. H. (1967). Inhibition of motoneurones by iontophoresis of glycine. *Nature* **214**(5089): 681-683.

WHITE, H. S. (1997). Clinical significance of animal seizure models and mechanism of action studies of potential antiepileptic drugs. *Epilepsia* **38 Suppl 1**: S9-17.

WHITE, H. S. (2003). Preclinical development of antiepileptic drugs: past, present, and future directions. *Epilepsia* **44 Suppl 7**: 2-8.

WHITE, H. S., BROWN, S. D., WOODHEAD, J. H., SKEEN, G. A. and WOLF, H. H. (1997). Topiramate enhances GABA-mediated chloride flux and GABA-evoked chloride currents in murine brain neurons and increases seizure threshold. *Epilepsy Res* **28**(3): 167-179.

WHITFIELD, J. B., POUNDER, R. E., NEALE, G. and MOSS, D. W. (1972). Serum -glytamyl transpeptidase activity in liver disease. *Gut* **13**(9): 702-708.

WICKENDEN, A. D. (2002). Potassium channels as anti-epileptic drug targets. *Neuropharmacology* **43**(7): 1055-1060.

WILEY, J. L., FAGALDE, R. E., BUHLER, K. G., LAVECCHIA, K. L. and BALSTER, R. L. (2002). Evaluation of 1,1,1-trichloroethane and flurothyl locomotor effects following diazepam treatment in mice. *Pharmacol Biochem Behav* **71**(1-2): 163-169.

WILEY, J. L. and MARTIN, B. R. (2003). Cannabinoid pharmacological properties common to other centrally acting drugs. *Eur J Pharmacol* **471**(3): 185-193.

WILLIAMS, G. V., RAO, S. G. and GOLDMAN-RAKIC, P. S. (2002). The physiological role of 5-HT2A receptors in working memory. *J Neurosci* **22**(7): 2843-2854.

WIRTSHAFTER, D. (2006). The selective m1 muscarinic antagonist MT-7 blocks pilocarpine-induced striatal Fos expression. *Brain Res* **1085**(1): 127-131.

WONG, D. T., BYMASTER, F. P., REID, L. R., MAYLE, D. A., KRUSHINSKI, J. H. and ROBERTSON, D. W. (1993). Norfluoxetine enantiomers as inhibitors of serotonin uptake in rat brain. *Neuropsychopharmacology* **8**(4): 337-344.

WOOD, D., WEBSTER, E., MARTINEZ, D., DARGAN, P. and JONES, A. (2002). Case report: Survival after deliberate strychnine self-poisoning, with toxicokinetic data. *Crit Care* **6**(5): 456-459.

WOODE, E., BOAKYE-GYASI, E., AMIDU, N., ANSAH, C., DUWIEJUA, M (2010). Anxiolytic and Antidepressant Effects of a Leaf Extract of *Palisota hirsuta* K. Schum. (Commelinaceae) in Mice. *International Journal of Pharmacology* **6**: 1-17.

WU, S. N., WANG, Y. J. and LIN, M. W. (2007). Potent stimulation of large-conductance Ca2+-activated K+ channels by rottlerin, an inhibitor of protein kinase C-delta, in pituitary tumor (GH3) cells and in cortical neuronal (HCN-1A) cells. *J Cell Physiol* **210**(3): 655-666.

WURTMAN, J. J. (1993). Depression and weight gain: the serotonin connection. *J Affect Disord* **29**(2-3): 183-192.

WUSTHOFF, C. J., KRANICK, S. M., MORLEY, J. F. and CHRISTINA BERGQVIST, A. G. (2010). The ketogenic diet in treatment of two adults with prolonged nonconvulsive status epilepticus. *Epilepsia* **51**(6): 1083-1085.

XU, L. F., CHU, W. J., QING, X. Y., LI, S., WANG, X. S., QING, G. W., FEI, J. and GUO, L. H. (2006). Protopine inhibits serotonin transporter and noradrenaline transporter and has the antidepressant-like effect in mice models. *Neuropharmacology* **50**(8): 934-940.

YACOUBI, M. E., POPA, D., MARTIN, B., ZIMMER, L., HAMON, M., ADRIEN, J. and VAUGEOIS, J. M. (2011). Genetic association between helpless trait and depression-related phenotypes: evidence from crossbreeding studies with H/Rouen and NH/Rouen mice. *Int J Neuropsychopharmacol*: 1-12.

YAMADA, M. and HIGUCHI, T. (2002). Functional genomics and depression research. Beyond the monoamine hypothesis. *Eur Neuropsychopharmacol* **12**(3): 235-244.

YAMAGUCHI, S. and ROGAWSKI, M. A. (1992). Effects of anticonvulsant drugs on 4-aminopyridine-induced seizures in mice. *Epilepsy Res* **11**(1): 9-16.

YANG, C. W., CHEN, W. L., WU, P. L., TSENG, H. Y. and LEE, S. J. (2006). Anti-inflammatory mechanisms of phenanthroindolizidine alkaloids. *Mol Pharmacol* **69**(3): 749-758.

ZAKHAROV, S. I., MORROW, J. P., LIU, G., YANG, L. and MARX, S. O. (2005). Activation of the BK (SLO1) potassium channel by mallotoxin. *J Biol Chem* **280**(35): 30882-30887.

ZANARDI, R., ARTIGAS, F., FRANCHINI, L., SFORZINI, L., GASPERINI, M., SMERALDI, E. and PEREZ, J. (1997). How long should pindolol be associated with paroxetine to improve the antidepressant response? *J Clin Psychopharmacol* **17**(6): 446-450.

ZARATE, C. A., JR., BRUTSCHE, N. E., IBRAHIM, L., FRANCO-CHAVES, J., DIAZGRANADOS, N., CRAVCHIK, A., SELTER, J., MARQUARDT, C. A., LIBERTY, V. and LUCKENBAUGH, D. A. (2012). Replication of Ketamine's Antidepressant Efficacy in Bipolar Depression: A Randomized Controlled Add-On Trial. *Biol Psychiatry*.

ZHANG, X., BERTASO, F., YOO, J. W., BAUMGARTEL, K., CLANCY, S. M., LEE, V., CIENFUEGOS, C., WILMOT, C., AVIS, J., HUNYH, T., DAGUIA, C., SCHMEDT, C., NOEBELS, J. and JEGLA, T. (2010). Deletion of the potassium channel Kv12.2 causes hippocampal hyperexcitability and epilepsy. *Nat Neurosci* **13**(9): 1056-1058.

ZHAO, C., LEITGES, M. and GEREAU, R. W. T. (2011). Isozyme-specific effects of protein kinase C in pain modulation. *Anesthesiology* **115**(6): 1261-1270.

ZHAO, X., CUI, X. Y., CHU, Q. P., CHEN, B. Q., WANG, X. M., LIN, Z. B., LI, X. J., KU, B. S. and ZHANG, Y. H. (2006). Potentiating effects of L-type Ca(2+) channel blockers on pentobarbital-induced hypnosis are influenced by serotonergic system. *J Neural Transm* **113**(10): 1395-1402.

ZHOU, S. F., ZHOU, Z. W., LI, C. G., CHEN, X., YU, X., XUE, C. C. and HERINGTON, A. (2007). Identification of drugs that interact with herbs in drug development. *Drug Discov Today* **12**(15-16): 664-673.

ZIMMERMANN, U., KRAUS, T., HIMMERICH, H., SCHULD, A. and POLLMACHER, T. (2003). Epidemiology, implications and mechanisms underlying drug-induced weight gain in psychiatric patients. *J Psychiatr Res* **37**(3): 193-220.

ZWANZGER, P. and RUPPRECHT, R. (2005). Selective GABAergic treatment for panic? Investigations in experimental panic induction and panic disorder. *J Psychiatry Neurosci* **30**(3): 167-175.

APPENDIX

DETAILED OBSERVATIONS IN THE IRWIN'S TEST

Dose (mg/kg)	0							30							100							300						
Time(min)	0-15	15	30	60	120	180	24h	0-15	15	30	60	120	180	24h	0-15	15	30	60	120	180	24h	0-15	15	30	60	120	180	24h
Lethality	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Convulsions	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Tremor	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Straub	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Sedation	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	+	++	++	++	++	++	0
Excitation	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Abnormal gait(rolling)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Abnormal gait(tipto	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Jumps	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Motor incoordinatio	#	0	0	0	0	0	0	#	0	0	0	0	0	0	#	0	0	0	0	0	0	#	+	+	+	++	+	0
Loss of balance	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Fore-paw treading	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Writhes	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Piloerection	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Stereotypies(sniffing	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Stereotypies(chewin	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

Stereotypies(head movements)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Head twitches	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Scratching	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Respiration	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Aggressiveness	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Fear	#	0	0	0	0	0	0	#	0	0	0	0	0	0	#	0	0	0	0	0	0	#	0	0	0	0	0	0
Reactivity to touch	#	0	0	0	0	0	0	#	0	0	0	0	0	0	#	0	0	0	0	0	0	#	0	0	0	0	0	0

Muscle tone	#	0	0	0	0	0	0	#	0	0	0	0	0	0	#	0	0	0	0	0	0	#	0	0	0	0	0	0
Loss of writhing reflex	#	0	0	0	0	0	0	#	0	0	0	0	0	0	#	0	0	0	0	0	0	#	0	0	0	0	0	0
Ptosis	#	0	0	0	0	0	0	#	0	0	0	0	0	0	#	0	0	0	0	0	0	#	0	0	0	0	0	0
Exophthalmos	#	0	0	0	0	0	0	#	0	0	0	0	0	0	#	0	0	0	0	0	0	#	0	0	0	0	0	0
Loss of grasping	#	0	0	0	0	0	0	#	0	0	0	0	0	0	#	0	0	0	0	0	0	#	0	0	0	0	0	0
Akinesia	#	0	0	0	0	0	0	#	0	0	0	0	0	0	#	0	0	0	0	0	0	#	0	0	0	0	0	0
Catalepsy	#	0	0	0	0	0	0	#	0	0	0	0	0	0	#	0	0	0	0	0	0	#	0	0	0	0	0	0
Loss of traction	#	0	0	0	0	0	0	#	0	0	0	0	0	0	#	0	0	0	0	0	0	#	0	0	0	0	0	0
Loss of corneal refle	#	0	0	0	0	0	0	#	0	0	0	0	0	0	#	0	0	0	0	0	0	#	0	0	0	0	0	0
Analgesia	#	0	0	0	0	0	0	#	+	++	++	++	++	0	#	+	++	++	++	++	0	#	++	++	++	++	++	0
Defaecation	#	0	0	0	0	0	0	#	0	0	0	0	0	0	#	0	0	0	0	0	0	#	0	0	0	0	0	0
Salivation	#	0	0	0	0	0	0	#	0	0	0	0	0	0	#	0	0	0	0	0	0	#	0	0	0	0	0	0
Lacrimation	#	0	0	0	0	0	0	#	0	0	0	0	0	0	#	0	0	0	0	0	0	#	0	0	0	0	0	0
Urination	#	0	0	0	0	0	0	#	0	0	0	0	0	0	#	0	0	0	0	0	0	#	0	0	0	0	0	0
Change in Rectal temperature	#	0	0	0	0	0	0	#	0	0	0	0	0	0	#	0	0	0	0	0	0	#	0	0	0	0	0	0

Dose (mg/kg)	**1000**							**3000**						
Time(min)	**0-15**	**15**	**30**	**60**	**120**	**180**	**24h**	**0-15**	**15**	**30**	**60**	**120**	**180**	**24h**
Lethality	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Convulsions	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Tremor	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Straub	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Sedation	+	++	++	++	++	++	0	+	++	++	++	++	++	0
Excitation	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Abnormal gait(rolling)	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Abnormal gait(tipto	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Jumps	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Motor incoordinatio	#	++	++	++	++	++	0	#	++	++	++	++	++	0
Loss of balance	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Fore-paw treading	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Writhes	0	0	0	0	0	0	0	0	0	0	0	0	0	0

Piloerection	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Stereotypies(sniffing	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Stereotypies(chewin	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Stereotypies(head movements)	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Head twitches	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Scratching	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Respiration	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Aggressiveness	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Fear	#	0	0	0	0	0	0	#	0	0	0	0	0	0
Reactivity to touch	#	0	0	0	0	0	0	#	0	0	0	0	0	0
Muscle tone	#	0	0	0	0	0	0	#	0	0	0	0	0	0
Loss of writhing reflex	#	0	0	0	0	0	0	#	0	0	0	0	0	0
Ptosis	#	0	0	0	0	0	0	#	0	0	0	0	0	0
Exophthalmos	#	0	0	0	0	0	0	#	0	0	0	0	0	0
Loss of grasping	#	0	0	0	0	0	0	#	0	0	0	0	0	0
Akinesia	#	0	0	0	0	0	0	#	0	0	0	0	0	0
Catalepsy	#	0	0	0	0	0	0	#	0	0	0	0	0	0
Loss of traction	#	0	0	0	0	0	0	#	0	0	0	0	0	0
Loss of corneal refle	#	0	0	0	0	0	0	#	0	0	0	0	0	0
Analgesia	#	++	+++	+++	++	++	0	#	++	++	++	+++	+++	0
Defaecation	#	0	0	0	0	0	0	#	0	0	0	0	0	0
Salivation	#	0	0	0	0	0	0	#	0	0	0	0	0	0
Lacrimation	#	0	0	0	0	0	0	#	0	0	0	0	0	0
Urination	#	0	0	0	0	0	0	#	0	0	0	0	0	0
Change in Rectal temperature	#	0	0	0	0	0	0	#	0	0	0	0	0	0

MOE was administered at 30, 100, 300, 1000 and 3000 mg kg^{-1}, *p.o.*; 5 rats per group were used. Data is presented as the number of animals showing symptoms during the test, with an indication of intensity for sedation, analgesia, fear, reactivity to touch, defaecation, urination, salivation and lacrimation (+ = slight increase, ++ = moderate increase,+++=marked increase). Observations were performed at 15, 30, 60, 120, 180 min and 24 hr after administration. The symptoms that did not necessitate handling were also observed up to 15 min immediately following administration. # Parameters not measured.