EVALUATION OF NOOTROPIC ACTIVITY OF METHANOL STEM EXTRACT OF *PARQUETINA NIGRESCENS* AFZEL IN MICE

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DECLARATION

I declare that the work in this Dissertation entitled 'EVALUATION OF NOOTROPIC ACTIVITY OF METHANOL STEM EXTRACT OF *PARQUETINA NIGRESCENS* AFZEL IN MICE' has been carried out by me in the Department of Pharmacology and Therapeutics. The information derived from the literature has been duly acknowledged in the text and a list of references provided. No part of this dissertation was previously presented for another degree at this or any other Institution.

Bukhari MAHMUD ..... ……

Name of Student Signature Date

CERTIFICATION

This dissertation entitled 'EVALUATION OF NOOTROPIC ACTIVITY OF METHANOL STEM EXTRACT OF *PARQUETINA NIGRESCENS* AFZEL IN MICE' by Bukhari MAHMUD meets the regulations governing the award of Master degree in Pharmacology of the Ahmadu Bello University and is approved for its contribution to knowledge and literary presentation.

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ABSTRACT

Parquetina nigrescens is a plant with numerous ethnomedicinal uses in African traditional medicine practice. In the South Western part of Nigeria, the stems and roots are roasted, powdered then mixed with pap taken as memory enhancer and for its antiaging effect. This study therefore, aimed to investigate the nootropic activity of the methanol stem extract of P. nigrescens. Phytochemical screening was carried out on the methanol extract and the median lethal dose (LD_{50}) determined using the Organisation for Economic Cooperation and Development in Europe (OECD) 425 limit test. The effect of the extract on cognition was evaluated at doses of 250, 500 and 1000 mg/kg using the elevated plus maze, Barnes maze and novel object recognition test models while the antiamnesic potential was investigated against scopolamine and diazepam induced amnesia. The effect of the extract on exploratory behaviour was also studied using the open field test and hole-board test. The behavioural cognitive enhancing effect of the extract on sub-chronically scopolamine induced cognitive deficit in mice was evaluated in an elevated plus maze and novel object recognition test thereafter, the brain tissue was assayed for malondialdehyde, superoxide dismutase, reduced glutathione and acetylcholinesterase. The results of the phytochemical screening of the extract revealed the presence of carbohydrates, tannins, saponins, phenolics, unsaturated sterols but absence of alkaloids, cardiac glycosides and triterpenes. The LD_{50} was estimated to be ≥ 5000 mg/kg. Piracetam a standard nootropic agent and the extract decreased transfer latencies at all doses on day 1 and day 2 in the elevated plus maze. In the Barnes maze, the escape latency and escape errors were decreased significantly ($p < 0.05$, $p < 0.01$) respectively by the extract at 1000 mg/kg and 250 and 1000 mg/kg respectively. The time spent in target quadrant was significantly ($p < 0.01$) increased at doses of 250 and 500 mg/kg. Piracetam decreased escape latency, escape errors and increased time spent in target quadrant. Discrimination index in the novel object recognition test was significantly ($p < 0.01$) increased by piracetam and the extract at all doses tested. Piracetam and the extract at all doses significantly ($p < 0.05$) decreased the transfer latencies increased by diazepam (0.7 mg/kg) on day 1 and non-significantly on day 2 but failed to reverse the scopolamine (1 mg/kg) induced increase in transfer latencies except for piracetam. The extract did not increase number of square cross and rearing, while number of central square cross was significantly ($p < 0.01$) increased at all doses tested in the open field test. Piracetam increased the number of square, central square cross and rearing. There was significant ($p < 0.01$) increase in the number of head dip in the hole-board test by the extract which was not observed with piracetam. In the sub-chronic study, piracetam and the extract decreased the transfer latencies on day 1 and 2 in an elevated plus maze and increase discrimination index in the novel object recognition test. The level of malondialdehyde was significantly ($p < 0.01$) reduced. Piracetam and the extract at all doses tested significantly ($p < 0.01$) increased superoxide dismutase level. The level of reduced glutathione was non-significantly increased by the extract and piracetam. The activity of the enzyme acetylcholinesterase was not decreased by the extract and piracetam. The methanol stem extract of P. nigrescens possesses nootropic-like activity and antiamnesic effect which may possibly be mediated via inhibition of the GABAergic pathway and this study provides some scientific justification for the ethnomedicinal use of the plant as a memory enhancer.

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ABBREVIATIONS

Ach	Acetylcholine
AChE	Acetylcholinesterase
AD	Alzheimer's Disease
ADHD	Attention Deficit Hyperactivity Disorder
AMPA	Alpha-amino-3-Hydroxy-5-Methyl-4-Isoxazole-propionic acid
BDZ	Benzodiazepine
BM	Barnes maze
CAMK	Calcium calmodulin related kinase
CREB	cAMP response element-binding proteins
CAMP	Cyclic adenosine monophosphate
CAT	Catalase
DI	Discrimination index
DTNB	5,5- Dithio-bis 2- Nitrobenzoic acid
DZP	Diazepam
GSH	Reduce glutathione
EPM	Elevated plus maze
GABA	Gamma aminobutyric acid
HBT	Hole board test
HD	Hutington's Disease
IU	International Unit
LD_{50}	Median lethal dose
MDA	Malondialdehyde
MSEPN	Methanol Stem Extract of *Parquetina nigrescens*
NARICT	National Research Institute for Chemical Technology

NCSC	Number of Central Square Cross
NHD	Number of head dip
NMDA	N-methyl D-aspartate
NORT	Novel object recognition test
NR	Number of Rearing
NSC	Number of Square Cross
OECD	Organisation of Economic Cooperation and Development
OFT	Open field test
PE	Primary error
PL	Primary latency
PD	Parkinson's Disease
ROS	Reactive oxygen species
SCP	Scopolamine
SPP	Species
SOD	Superoxide dismutase
TE	Total error
TL	Transfer Latency
TL	Transfer latency
TSTQ	Time spent in target quadrant
W.H.O	World Health Organisation

CHAPTER ONE

1.0 INTRODUCTION

Nootropic is sometimes called cognitive, memory, intelligence enhancer or motivational and stress management agent capable of preserving the neurones and directly affecting the level of neurotransmitters (Babija *et al.,* 2016). It is regarded to be any substance, drug or supplement with the ability of improving cognitive function, particularly executive functions, memory, creativity or motivation in healthy individuals (Lanni *et al.,* 2008; Frati *et al.,* 2015). The term nootropic is coined from the Greek words nous (mind) and trepein (to turn) (Giurgea, 1972; Gazzaniga and Michael, 2006). Nootropic is a class of psychotropic agents with selective facilitatory effect on integrative functions of the central nervous system especially on the intellectual performance, learning and memory (Chintawar *et al.,* 2002).

The cognitive enhancing effect of nootropics has led many individuals to consider them as reliable supplements in the renovation of memory in order to improve their intellectual abilities. These drugs are purportedly used primarily to treat cognitive or motor function difficulties attributable to disorders such as Alzheimer's disease (AD), Parkinson's disease (PD), Huntington's disease (HD), Attention Deficit Hyperactivity Disorder (ADHD). Among students, they are used to increase productivity despite lack of safety data about their long term usage (Babija *et al.,* 2016).

Nootropics are used to reduce neurotoxins and alleviate cognitive deficits in the damaged brain. They work by increasing the supply of neurochemicals and oxygen to the brain or stimulate nerve growth. These drugs are able to protect the brain from disruptive conditions and enhance the efficacy of neuronal function. Dietary

supplements, such as vitamins, Omega-3, iron, amino acids and other nutrients also have nootropic potential by frequently replenishing neurotransmitters and glucose in the brain (Malik *et al.,* 2007; Joshi, 2013).

'Smart drugs' have also been seen as having nootropic potentials most especially from the mass media, since they are associated with increased intelligence, motivation and mental energy. However, unlike smart drugs which can often cause side effects with long-term use, nootropics are regarded to be safe, neuroprotective and pose a very low risk of side effects when they are used appropriately. Their action is not a matter of short term central stimulation such as psychostimulants but rather of a long term metabolic facilitation, manifesting itself especially when the nerve cell metabolism is disturbed by hypoxia, trauma, intoxication and so forth (Bruno and Nicolaus, 1982; Doru, 2011).

A number of medicinal plants have been used in traditional medicine practice as brain tonic or nootropic. One of such plants with long history of ethnomedicinal usage in India traditional medicine is *Bacopa monniera* which is used in the treatment of poor cognition and lack of concentration (Russo and Borrelli, 2005).

The traditional system of medicine is replete with medicinal plants claimed to promote learning, memory and intelligence. Several medicinal plants have been used for decades in different cultures to improve memory and ameliorate aging examples of which include: *Lycium barbarum* L (China), *Corydalis spp* (Denmark), *Melissa officinalis* (Iran) among many others (Yu *et al.*, 2005; Adsersen *et al.*, 2006). Plants like *Glychyrryza glabra, Bacopa monniera, Azadiracta indica, Vitis vimifera, Albyzzia*

lebbeck, Ocimum sanctum, Phyllanthus amarus, Tinospora cordifolia and so forth have been investigated for their effect on cognition (Joshi and Parle, 2006a; Ladde *et al.*, 2011). In Nigeria, one of such medicinal plants which is claimed to have memory enhancing and antiaging effect and used among the Yorubas of the South Western Nigeria is *Parquetina nigrescens* (Elufioye *et al.*, 2012).

1.1 Statement of the Research Problem

Cognitive impairment is on the increase due to aging of the population most often accompanied with many neurodegenerative diseases like dementia, Alzheimer's disease, Parkinson's disease and so forth. These neurodegenerative diseases impact negatively on the cognitive functions of individuals thus making it a relevant issue from the scientific and public health perspectives (Ferreira *et al.*, 2006; Devi *et al.*, 2011).

According to the World Health Organization (WHO), 5% of men and 6% of women 60 years and above suffer from dementia of Alzheimer's disease (AD) worldwide (Alzheimer's Facts and Figures, 2016). In Africa, dementia and the likes are usually considered as normal part of ageing (Rhiannon *et al.*, 2012). It is one of the costliest chronic disease conditions with considerable financial, social and emotional burdens associated in caring for patients with the disease condition (Akhondzadeh and Noorozian, 2002).

There are few drugs known to improve cognitive functions. The most commonly used ones are not only stimulants like amphetamine, methylphenidate, caffeine and so forth but also have addictive potential of becoming a major public health problem (Fond *et*

al, 2015). Most of the currently used drugs (donepezil, rivastigmine, galantamine e.t.c) in restoring normal cognitive function in patients with cognitive impairment only provide symptomatic relief and do not halt the progression of the disease. The side effects associated with their use have also made them of limited benefit (Deepika *et al.*, 2010).

1.2 Justifications

Memory is important for human survival and personal identity. Diseases affecting cognitive functions will result to inability to recognise and respond appropriately to threats in the natural and social environment; perform basic motor skills and ultimately affect reasoning and decision making (Glannon, 2006).

Cognitive enhancers are in demand, whether in healthy populations or for those with cognitive deficit and current pharmacological enhancers offer only slightly modest benefits (Leslie *et al.,* 2015). Enhancing memory with nootropics will benefit individuals and promotes effective processing of information in decision making thus creating a more informed population (Glannon, 2006).

Several drugs such as lecithin, 4-Aminopyridine, piracetam all of which stimulate the release of acetylcholine have been investigated and have shown unimpressive improvement in the treatment of cognitive disorders. The use of acetylcholinesterase inhibitors such as donepezil, rivastigmine and galantamine has been shown to improve cognition only in patients with mild to moderately severe cognitive deficit. Side effects such as agitation, confusion, anorexia, weight loss and so forth have made them of limited benefit (Shetty and Woodhouse, 1999).

The traditional system of medicine has used herbs, nutraceuticals for controlling age related neurodegenerative disorders (Joshi and Parle, 2006a). More than 70% of people in developing countries of Africa use medicinal plants for their health care needs because, the modern health care delivery is beyond the reach of people most especially those living in rural areas, making them to greatly rely on readily available medicinal plants in their neighbourhoods (Birhan *et al.,* 2011).

Parquetina nigrescens is a common medicinal plant used as memory enhancer among the Yoruba people of South Western Nigeria (Elufioye *et al.,* 2012). However there is no data in the literature on its nootropic activity.

1.3 Aim and Objectives

1.3.1 Aim

To evaluate the nootropic activity of the methanol stem extract of *P. nigrescens*.

1.3.2 Objectives

i. To determine the phytochemical constituents of the methanol stem extract of *P. nigrescens*

ii. To estimate the oral median lethal dose (LD_{50}) of the methanol stem extract of *P. nigrescens*

iii. To investigate the learning and memory enhancing effect of methanol stem extract of *P. nigrescens*

iv. To assess the effect of methanol stem extract of *P. nigrescens* on exploratory behaviour

v. To investigate the acute anti-amnesic effect of the methanol stem extract of *P. nigrescens*

vi. To investigate the effect of the methanol stem extract of *P*. *nigrescens* on some biochemical markers of cognition

1.4 Research Hypothesis

Methanol stem extract of *Parquetina nigrescens* possesses nootropic activity and improved biochemical cognitive markers.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Overview of Nootropics and Cognition

The brain is responsible for controlling cognitive processes like learning, memory, thought, reasoning, consciousness, emotion and attention. Hence, preserving the healthy brain is vital for successful coordination of body activities (Lee *et al*., 2011). Nootropics are neuroprotective and are capable of improving one or more aspect of cognitive processes leading to enhancement of cognitive function (Leslie *et al.*, 2015). Nootropic is any substance be it drugs, supplements, nutraceuticals and functional foods that enhance mental function and capable of preserving the neurones from chemical and physical assault (Joshi, 2013). It is a drug having the essential characteristics of piracetam such as direct activation of the integrative activities of the brain leading to a positive action of the mind and restoring disturbed brain activity to normal (Giurgea, 1972).

The concept of nootropic and cognitive enhancers are loosely regarded as equivalent. A nootropic agent enhances learning and memory, help the brain function under disruptive conditions such as hypoxia and electroconvulsive shock, protect the brain from chemical assaults such as anti-cholinergic drugs and barbiturates, increase the efficacy of neuronal firing control mechanisms in cortical and sub-cortical regions of the brain, lack a generalized sedative, stimulatory effect, possess few to no side effects and be virtually non-toxic (Giurgea, 1980). These characteristics clearly showed the holistic concept of nootropic agent capable of activating the integrative activities of the brain. Hence, not all cognitive enhancers are nootropics but all nootropics are cognitive enhancers (Duro, 2011).

Nootropic drugs seem to be at odd with the usual principle of neuropharmacology in their mode of action. This usual principle of neuropharmacology assert that, every molecule exerting a therapeutic or behavioural effect on individual have a modulating effect on various neurotransmitters by which brain neurones communicate synaptically. Piracetam a prototype nootropic drug influences cholinergic, serotonergic, noradrenergic and glutamatergic systems. These effects of piracetam do not result from agonism or antagonism with no noticeable affinity for these neurotransmitters receptors in contrast to psychotropic drugs (Duro, 2011). Despite this odd, a common mechanism of action of nootropic is enhancing increased communication between neurons, balancing neurotransmitters level or promoting brain cell health. They may help improve energy metabolism in the neurons, support neuroplasticity, or stimulate the growth of new neurons while slowing down the rate of damage (Mali *et al.,* 2012; Adam, 2014). Other nootropics show effects of vasodilatation which means they increase the blood flow to the brain, improve electrical activity of the brain (Joshi, 2013).

The brain is the basis for thinking, wanting, perceiving, learning, memory, curiosity, and behaviour (Hideyuki *et al.,* 2000). Cognition is the term used to define these processes. It is the mental process of acquiring knowledge and understanding through thought, experience and the senses. It encompasses processes such as knowledge, attention, understanding, memory, judgment and evaluation, reasoning and 'computation', problem solving, decision making and so forth. Cognitive processes use existing knowledge and generate new knowledge (Shivani *et al.,* 2016).

Cognitive enhancement is the improvement of the information processing systems of the mind and the extension of its main capacities, which can be undertaken simply by learning, especially during early development. Interventions to improve cognitive function therefore affect attention, learning, understanding and memory and use these to guide behavior and coordinate motor output. Limiting beliefs, removing psychological barriers and increasing confidence all improve cognitive function (Shivani *et al.,* 2016). Cognitive enhancement includes both medical and psychological interventions. The conventional method for enhancing cognitive function is education and training which improve concentration and critical thinking. Medical interventions to enhance cognition involve the use of nootropic which affect physiological processes of the brain.

Learning and memory are among the most intensively studied cognitive processes in the field of neuroscience (Devi *et al.*, 2011). Memory is a fundamental mental process, without it human are capable of nothing but simple reflexes and stereo type behaviours (Hideyuki *et al.*, 2000). It is the ability to record, retain sensory stimuli or information for short or long periods of time and retrieve the same when needed (Devi e*t al.,* 2011). While, learning is a process of acquiring new information about the event occurring in the given surroundings (Markowitsch, 1988).

Memory can be classified based on different criteria viz: function (e.g Working and Reference Memory) content (e.g Declarative/Explicit and Procedural/Implicit Memory), duration (e.g Immediate or Short-Term and Long-Term or Remote Memory) and motivation (Appetitive/Reward and Aversive Memory) (Jorge, 2006). The declarative memory '(knowing what)' is concerned with events and facts, and is

available to the conscious mind; while the procedural memory '(knowing how)' is the memory needed to use previously learned skills and not available to consciousness (Hideyuki *et al.*, 2000).

2.2 Neuro-circuitry of Cognition

Learning and memory are complex phenomenon requiring the coordinated interaction of multiple brain structures such as the hippocampus, striatum, and amygdala. These structures are important for different types of memory (Squire, 1992; White and McDonald, 2002). Animal models have been used in understanding the differences in information processing by a normal and damaged brain (Sumit *et al.*, 2012). Thus, studies on the brain of animals have revealed multiple memory systems that interact competitively, cooperatively or in parallel depending on the cognitive demands and psychological nature of the task (White and McDonald, 2002; Gold, 2002).

Several neural systems in the brain function simultaneously and with some degree of independence to process and store information about events that occur during the life of an individual. Although the systems have access to much of the same information, each deals with it in a different way. The multiple parallel memory systems (MPMS) theory hypothesized three neural systems which are the hippocampus, the dorsal striatum, and the amygdala. Each system receives information, processes it in its own style, stores some of it under certain circumstances, and influences behaviour. Each of these systems is capable of performing these functions independently of the other systems. The processing style of each system is assumed to be the result of its neural architecture. Neuroscientist opined that memory must require alterations to occur in the brain (White and McDonald, 2002)

2.3 Cellular Mechanism of Learning and Memory

The most popular candidate site for memory storage is the synapse, where nerve cells communicate (Kandel *et al.,* 1991). A change in the transmission efficacy at the synapse (synaptic plasticity) has been considered to be the cause of memory. A particular pattern of synaptic stimulation is believed to induce synaptic plasticity (Hideyuki *et al.,* 2000).

Many neurotransmitters and intracellular signaling cascades have been identified to be involved in synaptic plasticity and memory (Clea and Zafar, 2014). The release of glutamate and acetylcholine from presynaptic afferents activates a range of glutamate and cholinergic postsynaptic receptors respectively. Activation of cholinergic receptors (nicotinic acetylcholine receptors and the muscarinic acetylcholine receptors) and the glutamate receptors (metabotropic glutamate receptors, NMDA receptors and AMPA (Alpha-amino- 3-Hydroxy-5-Methyl-4-Isoxazole-propionic acid) receptors) result to an increase intracellular calcium ion concentration which then lead to the activation of calcium-calmodulin related kinases (CAMKs) resulting to phosphorylation of AMPA receptors (leading to endocytosis and synaptic plasticity) and transcription factors CREB (cAMP response element-binding proteins) leading to memory formation (Clea and Bashir, 2014).

2.4 Cholinergic System in Cognition

The role of acetylcholine (ACh) in cognition was sparked by the loss of cholinergic neurones in Alzheimer's disease resulting to memory loss (Perry *et al.,* 1981). The blockade of cholinergic system in the brain leads to learning and memory impairment (Fibiger, 1991; Blokland, 1996). Conversely, cholinesterase inhibitors often effectively

reverse lesion and pharmacologically induced cognitive deficits. The behavioural results in humans complemented by animal data shows that basal forebrain ACh modulates the responsiveness of cortical neurons in cognition (Sato *et al.,* 1987; Kurosawa *et al.,* 1989). The modulation of the cortical neurone responsiveness by ACh is mediated through muscarinic receptors (Metherate *et al.,* 1992; Farkas *et al.,* 1996). Anticholinergic drugs, such as scopolamine and atropine, produce learning and memory deficits in a variety of cognitive animal models and recognition memory in humans (Ennaceur and Meliani, 1992).

However, neuronal alterations associated with cognitive deficits are not restricted to the cholinergic systems. Dysfunctions of dopamine, gamma-aminobutyric acid (GABA), noradrenalin, serotonin and histamine neurons have been identified in Alzheimer's disease (Hardy *et al.,* 1985; Schneider *et al.,* 1997). Region-selective decreases in dopaminergic, noradrenergic, or serotonergic contents are associated with the level of age-related learning and memory impairments (Stemmelin *et al.,* 2000; Birthelmer *et al.,* 2003). This phenomenon warranted the increasing interest in understanding the complex physiology of brain systems affecting cognitive processes (Patrizio *et al.,* 2004).

2.5 Gabaergic System in Cognition

Gamma-aminobutyric acid (GABA) is the primary mediator of inhibitory neurotransmission in the central nervous system. It is involved in the regulation of certain key processes in brain (Sieghart, 1995). The central GABAergic system plays key role in cognitive processes, including memory formation and consolidation (Izquierdo and Medina, 1991; Davis, 1994). Certain brain areas rich in GABA

receptors (e.g., amygdala, septum, hippocampus, entorhinal cortex) played important role in the memory processes (Rawling, 1987; Davis, 1994). Direct physical effects on these structures or local drug application of drugs mediating their effect through GABA were reported to affect both memory formation and consolidation (Izquierdo and Medina, 1991), supporting the concept of GABAergic involvement in memory regulation.

The involvement of GABAergic system in memory so far is based upon the following principles viz: the central GABAergic system is involved in memory regulation; modulation of GABAergic system at all stages from the synthesis of GABA to chloride current (including GABA binding site, various sites for binding endogenous and exogenous regulators and drugs affecting the channel) may result in significant alteration of memory and inhibition of the GABAergic system has memory-facilitating effects, while stimulation produces memory impairment (Brioni, 1993).

Studies aimed to test the specific role of central GABAergic system in memory used traditional GABA-mimetic and GABA-blocking drugs in relatively simple memory tests. Muscimol a GABA-$_A$ agonist administered to entorhinal cortex was reported to induce amnesia or block memory of habituation and inhibitory avoidance in animals. Sodium valproate an agent facilitating GABAergic function, slowed acquisition (Rayevsky and Kharlamov, 1983; Rosat *et al.,* 1992).

Indirect GABA modulators such as benzodiazepines (BDZ) agonists and barbiturate have been studied for their amnesic effect. Ethanol enhances the effects of GABA thus, producing both anxiolytic-like effects and deleterious effects on memory and

learning (Nevo and Hamon, 1995; Kalueff and Nutt, 1996). Pentobarbitone a barbiturate inhibits memory formation, slowed down acquisition and decreased accuracy in numerous animal studies (Tomaz *et al.,* 1994).

Neurosteroids have been known to have a modulating effect on GABA-$_A$ receptors (Lambert *et al.,* 1995). Steroids that are positive modulators of GABA receptors (for example, tetra-hydroprogesterone) were found to be memory-impairing agents and GABA-inhibiting steroids (such as pregnenolone-sulfate) are able to activate memory (Flood *et al.,* 1992; Mayo *et al.,* 1993).

GABA antagonists like bicuculline were found to be strong memory-activating agents. Systemic administration of convulsants such as picrotoxin, pentylenetetrazole or other GABA inhibitors has long been known to enhance behaviour and memory in a variety of tests (Breen and McGaugh, 1961; Cruz-Morales *et al.,* 1993).). Administration of BDZ antagonist flumazenil or inverse agonist like methyl- and butyl-beta-carboline 3-carboxylate resulted in significant improvement of memory and learning in various tests (Kalueff and Nutt, 1996).

2.6 Cognitive Disorders

Cognitive disorders are a category of mental health disorders that primarily affect learning, memory, perception, and problem solving. It include amnesia, dementia, and delirium, While anxiety disorders, mood disorders, and psychotic disorders can also have effect on cognitive functions. Age, stress and some emotional breakdown are some conditions that could lead to loss of memory and memory deficit disorder resulting to changes in cognition and behaviour. Various drugs such as diazepam,

alcohol, barbiturates and so forth have detrimental effect on learning and memory (Nishikant *et al.,* 2014). Cognitive disorders like Alzheimer's disease, amnesia, depression and schizophrenia are associated with impairments in learning and memory (Redy, 1997).

2.6.1 Amnesia

It is a deficit in memory which could result from brain damage, disease, or psychological trauma (Gazzaniga *et al.,* 2009). The use of various sedatives and hypnotic drugs also induce amnesia. Essentially, amnesia is loss of memory with two main types: retrograde amnesia and anterograde amnesia. Retrograde amnesia is the inability to retrieve information that was acquired before a particular date, usually the date of an accident or operation. In some cases the memory loss can extend back decades, while in others the person may lose only a few months of memory, while anterograde amnesia is the inability to transfer new information from the short-term store into the long-term store. People with this type of amnesia hardly remember things for long periods of time. These two types are not mutually exclusive. Both can occur within a patient at one time (Gazzaniga *et al.,* 2009).

2.6.2 Dementia

Dementia is a clinical syndrome characterised by progressive impairment in multiple cognitive and behavioural domains primarily memory, language and speech, visuospatial ability, executive functioning, and mood/personality severe enough to undermine daily functioning (Ross and Bowen, 2002). It is a chronic illness that arises from interplay of genetic, environmental and behavioural factors with severe adverse

effect on the quality of life. Dementia is one of the major causes of disability among elderly people (Rhiannon, 2012).

No medications have so far been shown to prevent or cure dementia *(Rafii and Aisen, 2009).* Medications may be used to treat the behavioural and cognitive symptoms but have no effect on the underlying disease process (*Solomon and Budson, 2011).*

2.6.3 Alzheimer's disease

It is a neurodegenerative disorder that is gradual in onset and relentless in progression characterised with loss of memory, ability to learn, make judgement and communicate with the social environment and carry out daily activities (Annette *et al.,* 2008). It is the most common type of dementia accounting for approximate two-third of all cases of dementia and affect about thirty five million people worldwide. In the course of the disease short term memory is first affected due to neuronal dysfunction and degeneration in the hippocampus and amygdala, neurones also degenerate and die in other cortical regions of the brain as the disease progresses (Stuchbury and Munich, 2005; Annette *et al.,* 2008).

Alzheimer's disease is characterised by two neuropathological hallmarks, the deposition of beta-amyloid containing senile plaque and neurofibrillary tangles. Inflammation is another hallmark of AD resulting from super oxide production (oxidative burst) which is an important source of oxidative stress in patients suffering from Alzheimer's disease (Retz *et al.,* 1998).

Acetylcholinesterase inhibitors (AChEI), such as donepezil, may be useful for Alzheimer type of dementia (*Bond et al., 2012)* and dementia in Parkinson's, or vascular dementia (*Solomon and. Budson, 2011).* N-methyl-D-aspartate (NMDA) receptor blockers such as memantine have proven to be of benefit but the evidence is less conclusive than for AChEI (*Bond et al., 2012).* Other drugs investigated for the disease prevention include anti-inflammatory agents, antioxidants (including vitamin E) and oestrogens (in women) (Bonner and Peskind, 2002).

2.7 Brief Pharmacology of Drugs Used

2.7.1 Piracetam

Cognitive properties of piracetam were disclosed in 1967 (Giurgea *et al.,* 1967). A number of structurally related molecules were found to be endowed with a similar pharmacological profile, with excellent tolerability (Coper and Herrmann, 1988). Piracetam-like nootropics reverse amnesia induced by scopolamine and other amnesic drugs, electroconvulsive shock and hypoxia with an unknown mechanism. In general, they show no affinity for the most important central receptors, but are able to modulate the action of most central neurotransmitters, in particular acetylcholine (Pepeu and. Spignoli, 1989; Oyaizu and Narahashi, 1999) and glutamate (Pittaluga *et al.,* 1999).

Side effects from piracetam are considered very rare. The entire racetam family of nootropics has been shown to be extremely safe with low toxicity rates. The few reported adverse effects include headache, anxiety, and insomnia. Headache is the most widely reported side effect Chaounald *et al*., 1983).

2.7.2 Scopolamine

Scopolamine is an alkaloidal drug that exerts its effects by acting as a competitive antagonist at muscarinic acetylcholine receptors. It induces cognitive deficit through competitive antagonism at low therapeutic doses. The basis for this is probably the permeation across the blood brain barrier. Scopolamine is used clinically (though less frequently than in past years) as an adjunct to surgical or obstetric procedures to induce sedation and post-procedural amnesia. It is relatively nonselective pharmacologically with respect to receptor subtypes, and the drug does not discriminate very much with respect to brain region and muscarinic receptors in induction of amnesia (Jerry, 2009).

Scopolamine is employed as the gold standard for inducing memory impairments in healthy human and animals to mimic muscarinic decline characteristic of ageing and dementia (Drachman and Leavitt, 1974; Klinkenberg and Blokland, 2010) with varying underlying mechanisms. It triggers reactive oxygen species (ROS), inducing free radical injury, increase brain malondialdehyde (MDA) levels and deterioration in antioxidant status (El-Sherbiny *et al.,* 2003; Hancianu *et al.,* 2013). Scopolamine induces neuro-inflammation by promoting high level of oxidative stress and pro inflammatory cytokines in the hippocampus (Jang *et al.,* 2013; Ahmad *et al.,* 2014). Administration of scopolamine led to marked histopathological alterations in the cerebral cortex, including neuronal degeneration (Haroutunian *et al.,* 1997; Bihaqi *et al.,* 2012).

2.7.3 Diazepam

Diazepam is a long acting benzodiazepine that exerts anxiolytic, sedative, muscle relaxant, anticonvulsant and amnesic effects (Fox *et al.,* 2011). Most of these effects are thought to result from a facilitation of the action of gamma amino butyric acid (GABA), an inhibitory neurotransmitter in the central nervous system. These effects of diazepam are dose dependent. Anxiolytic effects are seen at low doses while amnesic, muscle relaxant, sedative effects are seen at high doses (Crestani *et al.,* 2001).

The amnesic property of benzodiazepines seems not to affect the sensory and short term memory but the long term memory. The memory impairment induced by the benzodiazepine is dependent on the time course of the drug (Charles *et al*., 2013). Diazepam inhibits acetylcholine release in mouse hippocampal synaptosomes. This has been found by measuring sodium-dependent high-affinity choline uptake in mouse brain cells *in vitro*, after pre-treatment of the mice with diazepam *in vivo* (*Miller and Richter, 1985).* Diazepam increases the inhibitory processes in the cerebral cortex (*Zakusov et al., 1997).*

2.8 Medicinal Plant and Memory Enhancement

Plant constitute principal sources of medicinal agent in traditional and orthodox medicine practice, where they are used in the treatment of many ailments or serving as "lead" for structural modification and synthesis of novel compounds with improved pharmacological profile (Manuel *et al.,* 2009). During the years 2005–2007, 13 natural product related drugs were approved (Harvey, 2008). It is estimated that about 75% of useful bioactive plant derive pharmaceuticals used globally are discovered by systemic investigation of leads from traditional medicines (Tomoko *et al.,* 2002).

Disease remedy from plants sources for mankind is dated to the existence of man. The use of medicinal plant to enhance learning and memory is well known in traditional medicine practice. Medicinal plants have been traditionally used in the treatment of several ailments (mental and physical) and the ethnomedicinal importance of these plants varies among different ethnic and cultural groups (Mussema, 2006; Ayinde *et al.*, 2015). Plants such as *Musa sapientum, Piper nigrum, Baccopa monniera, Senecio albysinicus, Ficus religiosa, Dioscorea bulbifera, Ocimum sanctum* are used as memory enhancer (Mukherjee *et al*., 2007, Devi *et al*., 2011). *Bacopa floribunda, Cleome gynandra, Dalbergia lacteal, Aframomum melegueta, Digitaria debilis, Jatropha curcas, Spondias mombin, Bambusa vulgaris, Baphia nitida, Entandrophragma utilis, Parquetina nigrescens* and so forth are memory enhancing plants use among the Yoruba folk of western part of Nigeria (Elufioye *et al.*, 2012).

2.8.1 The plant (*Parquetina nigrescens),* description and distribution

It is a plant common in Nigeria and planted around houses for its value in traditional medicine practice. Some scientific findings have confirmed the efficacy of this plant in the treatment of many diseases (Agbor and Odetola, 2001).

Parquetina nigrescens Afzel, with the synonyms *Parquetina gabonica* Bail. is an herbaceous, perennial twine belonging to the Kingdom: Plantae, Phylum: Magnoliophyta, Class: Magnoliopsida, Order: Gentianales, Family: Asclepiadaceae/Periplocaceae, Genus: *Parquetina* Baill. It is usually found in the forest and often planted around houses by traditional herbal practitioners in the South Western part of Nigeria probably for its numerous medicinal applications (Ayinde *et al.*, 2015). *Parquetina nigrescens* is found in equatorial West Africa (Mabberly, 1987).

The plant commonly grows on ant-hills across the African regions, from Senegal to Nigeria, and over the Congo basin down to south tropical Africa (Burkill, 1985).

The plant *P. nigrescen* may be climbing, twinning or erect soft woody occasionally with wiry stems. It is herbaceous with tuberous rootstock, leaves opposite, entire, linear to very broad, pinnately nerve. The leaves are glabrous up to 15 cm long and 8 cm broad, dark shining green above and glaucous beneath (Hutchinson and Dalziel, 1963). The plant *Parquetina nigrescens* is commonly called African parquetina and has the following local names 'Ogbo' in Yoruba, 'Kwankwanin' 'tsa-tsumbe' in Hausa and 'Otonta' in Igbo (Gbadamosi, 2015).

2.8.1.2 Ethnomedicinal uses

The plant has been in traditional medicine practice for centuries with its leaves, roots and latex all in use (Gill, 1992). It is a medicinal plant that possesses important therapeutic properties and its various parts are used among different cultures in the management of different disease conditions. It is used to treat various types of gastro-intestinal disorders in the south western part of Nigeria. Different parts of the plant, leaves, stem, latex and root are used for the treatment of rickets, diarrhoea, skin lesions, menstrual disorders and gonorrhoea (Sofowora, 1993; Adeyemi, 1994) and sickle cell disease (Gbadamosi, 2015).



Plate I: *Parquetina nigrescens* in its Natural Habitat (www.gbif.org/species/3580801)

The plant is used for the treatment of wounds in Africa (Irvine, 1961; Mabberly, 1987). In Ghana, the leave poultice and roots are used for boils, carbuncles, and snake bites, new and old wounds among the people of Bosomtwi-Atwima-Kwanwoma area

(Christian *et al.*, 2009). The decoction of the leaves is used in the treatment of piles and diabetes (Borokini *et al.,* 2013).

In Oyo State, Nigeria, the leaves have been reputed for treatment of helminthiasis (intestinal worm), while the roots are used for the management of rheumatism (Adeyemi, 1994). Other uses include the decoction of the stem been given as cardiac tonic (Iwu, 1993; Sofowora 1993). The decoction of the leaves is used to arrest the progression of tumour proliferation in patients (Soladoye *et al.,* 2010). The leaf decoction is drunk in the evening as aphrodisiac in east Africa (Kokwaro, 1993), as herbal preparations for insanity in Nigeria and for dropsy in India (Iwu, 1993). The plant is also employed for other purpose in the Congo basin, where it is used as an arrow poison (Odetola *et al.,* 2006).

Parquetina nigrescens is one of the constituents of a commercial herbal preparation (Jubi formular) in Nigeria used in the treatment of anaemia in humans (Oyewole *et al.,* 2011). The stalk and roots are roasted or dried in clay pots, powdered then mixed with pap and taken for its memory enhancing and antiaging effect (Elufioye *et al.,* 2012).

2.8.1.3 Pharmacological investigations on Parquetina nigrescens

Many of the folkloric claims of the plant have been validated and the plant has been shown to have anti-inflammatory and analgesic (Bamidele *et al.,* 2009), anti-ulcer, antianaemic, antidiabetic (Owoyele *et al.,* 2011; Saba *et al.,* 2010), antimicrobial (Odetola *et al.,* 2006; Makanjuola *et al.,* 2010), antimalaria (Mikhail *et al,* 2014) and anticancer effect (Ayinde *et al.,* 2015).

The aqueous leaves extract have been shown to possess haematopoietic activities, increasing erythrocytes indices in anaemic rats on dose basis (Agbor and Odetola, 2005). The aqueous extract of the leaves has antidiabetic property and antianaemic effect with improved leucopoenia and thrombocytopenia associated with diabetes. The extract also reduced the erythrocyte osmotic fragility, body and organ weights (Saba *et al.*, 2010).

The aqueous extract of the leave has oxytocic effect similar to the effects of oxytocin (Datté *et al*., 1996). Aqueous extracts of leaves of *P. nigrescens* displayed a more moderate effect on the worms' viability hence, its use in the treatment of worm infestation (Dieudonné Ndjonka *et al.*, 2013). Roots, leaves and stems of *P. nigrescens* extract have antisickling effect (**Gbadamosi**, 2015). The methanol extracts of *P. nigrecens* leaves and the stem bark of *Brachystegia eurycoma* increasingly inhibited the growth of lung cancer cells with increase in concentration (Ayinde *et al.*, 2015).

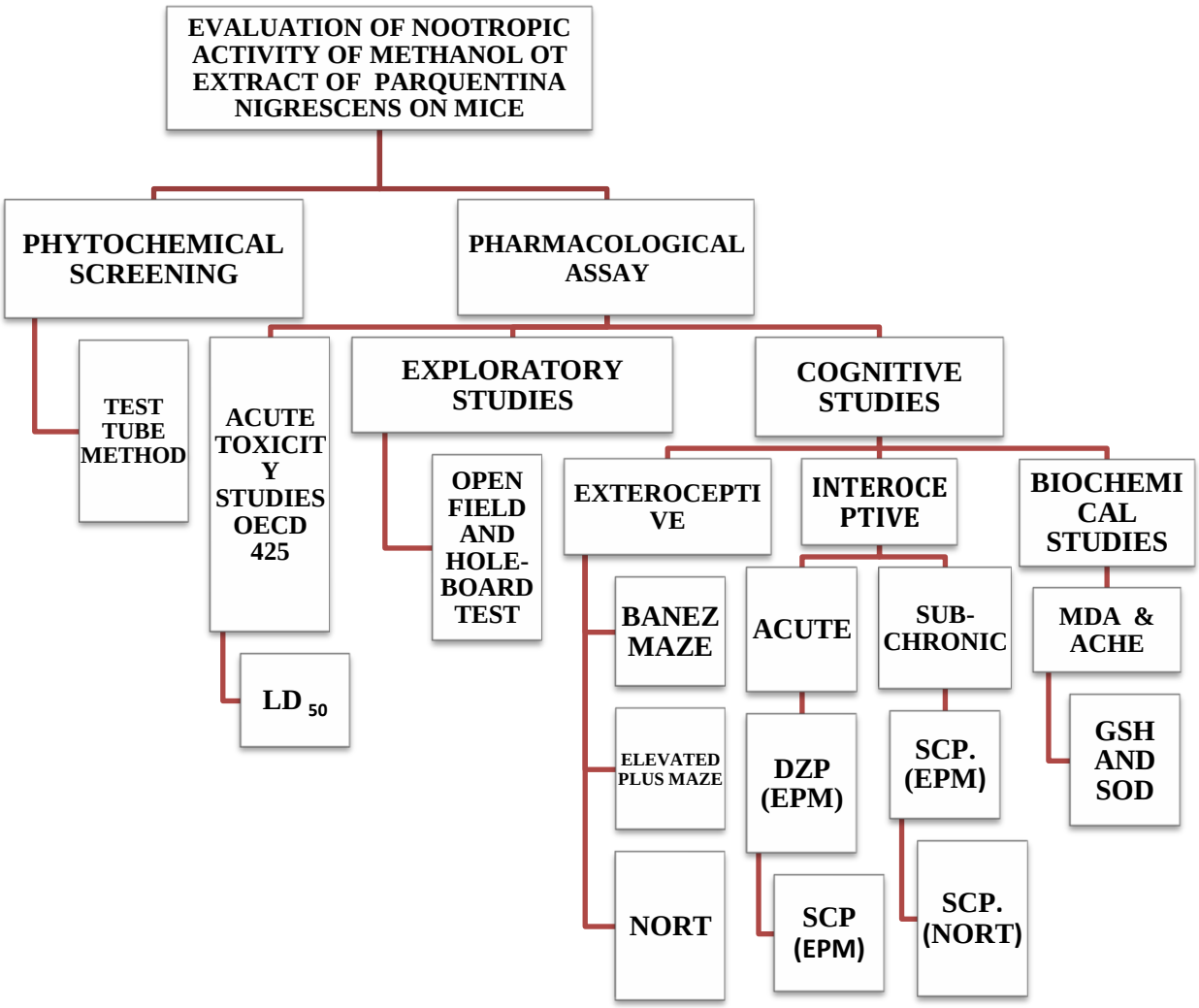
The methanol leaves extract of *P. nigrescens* slowed lipid peroxidation, increased the activities of Super Oxide Dismutase (SOD), Catalase (CAT) and protein levels in ethanol-induced ulcer rats (Owoyele ***et al.,* 2011)**. The aqueous leaves extract significantly reduced gastric acid secretion, and increased gastric mucus secretion which may be responsible for the antiulcer property (Odetola *et al.,* 2006).

Odetola *et al.,* (2006) reported that aqueous leaves extract of *P. nigrescens* showed activity against a wide range of bacterial in the following order of activity*: Staphylococcus aureus > Salmonella typhi > Proteus mirabis > Pseudomonas aeruginosa > Bacillus subtilis > Proteus vulgaris*, whereas the ethanol extract was effective only against *Pseudomonas aeruginosa and Salmonella typhi.* This

antimicrobial activity against the common pathogenic microbes, *S. aureus, S. typhi and P. aeruginosa* may account for its acclaimed potency against diarrhoea. The aqueous leave extract of *P. nigrescens* displayed a very good activity against the *Plasmodium berghei in vivo* (Mikhail *et al.*, 2014) and also produced significant analgesic, anti-inflammatory and antipyretic effect (Owoyele *et al.,* 2009).

Olatunbosun *et al.,* (2014) showed that the plant in combined extract of *P. nigrescens, Camellia sinensis and Telfaria occidentalis* has positive synergistic proliferative effects on haemopoietic multipotent stem cells in irradiated guinea pigs bone marrow. Jubi Formula® an herbal preparation comprising *P. nigrescens, Sorghum bicolor and Harungana madagascariensis* is used to treat anaemia in humans (Patrick *et al.,* 2003). The ethanol-chloroform leaves extract tested positive to flavonoids, alkaloids, tannins, saponins and reducing sugars while, chloroform extract tested positive to fat and oil and steroids. Acute toxicity study on ethanol-chloroform leaves extracts revealed an oral $LD_{50} \geq 5000$ mg/kg body weight in mice (Omoboyowa *et al.,* 2016). Methanol leaves extract of *P. nigrescens* exhibited dose and time dependent toxicity to animals. The kidney and liver tissues from rats administered with the extracts showed diseased conditions including inflammation of cells, necrotic tissues and cellular infiltration (Louis *et al.,* 2014).

2.9 Study Design



CHAPTER THREE

3.0 MATERIALS AND METHOD

3.1 Materials

3.1.1 Drugs and chemicals

Piracetam (Nootropil^{R}, UCB, Turkey), scopolamine hydrobromide (Sigma Aldrich, Germany), diazepam (Valium^{R}, Roche), gum acacia and methanol.

3.1.2 Equipment and apparatus

Elevated Plus Maze (EPM), Barnes Maze (BM), Novel Object Recognition Test (NORT) apparatus, Open Field Test (OFT) apparatus, Hole Board Test (HBT) apparatus, digital camcorder (JVC Everio 32G HDD), 80 watt bulb, stop watch, Orogastric tube, Syringes (1ml, 5ml and 10ml), Pestle and Mortar, Beaker, Vials and Animal cages.

3.1.3 Animals

Swiss Albino Mice of both sexes (18-25 g) were obtained from the Animal House Facility of the Department of Pharmacology and Therapeutics, Faculty of Pharmaceutical Sciences, Ahmadu Bello University (ABU) Zaria. The mice were maintained on standard laboratory animal feed and allowed access to water *ad libitum*. Animals were allowed to acclimatise for five days in the laboratory room and randomly selected into various groups before experimentation. Institutional animal ethics committee's approval is being obtained. Experiment was carried out between 9:00 and 18:00 hours of the day.

3.2 Method

3.2.1 Preparation of extract

3.2.1.1 Plant collection

The whole plant was collected in a bush within Samaru, Zaria, Kaduna State, Nigeria in the month of June, 2016. The freshly harvested plant material was identified by Mallam Umar Gallah at the National Institute for Chemical Research Technology (NARICT) Zaria, Kaduna State. A voucher specimen was prepared with voucher number 01624 and deposited in the herbarium unit.

Plant extraction

The stems were removed, washed and air-dried under the shade for three weeks after which it was grounded to coarse powder using mortar and pestle. The powdered sample (1000 g) was extracted with 10 litres of 70% methanol by cold maceration with occasional shaking for one week. The macerated mixture was filtered and the filtrate concentrated using a rotary evaporator. The concentrate was further dried over a water bath at a temperature of 40 ^{0}C to obtain a dried solid mass subsequently referred to as methanol stem extract from which the doses to be administered were prepared just before the experiment. The resulting residue of the methanol extract was stored in a desiccator until required for use.

3.2.2 Phytochemical analysis

Phytochemical analysis was carried out on the methanol extract using the standard protocol to determine the presence or absence of some secondary plant metabolites. The tests are as follows:

3.2.2.1 Test for carbohydrates (Molisch test)

3 drops of Molisch reagent was added to a small portion of the aqueous extract in a test tube. 3 drops of concentrated sulphuric acid was added down the side of the test tube to form a lower layer. A reddish coloured ring at the interphase indicates the presence of carbohydrate (Evans, 1996).

3.2.2.2 Test for alkaloid

Mayer: 3 drops of Mayer's reagent were added to a portion of the extract. Appearance of white or cream colour indicates the presence of alkaloid (Evans, 1996).

Dragendorff: 3 drops of Dragendorff's reagent were added to a portion of the aqueous extract. Appearance of orange or reddish brown precipitate indicates the presence of alkaloid (Evans, 1996).

3.2.2.3 Test for tannins (Ferric chloride test)

1ml of extract was diluted with 2ml of distil water. 3 drops of ferric solution was added. The occurrence of black-blue or black-green precipitate indicates the presence tannins (Evans 1996).

3.2.2.4 Test for phenolics (Ferric chloride test)

2 drops of ferric chloride solution were added to a portion of the extract, a green precipitate indicates the presence of phenolics nucleus (Evans, 1996).

3.2.2.5 Test for saponins

2ml of the extract was diluted with 4ml of distil water and shaken for 2 minutes. The occurrence of persisting foam for about 15 minutes indicates the presence of saponins (Silva *et al.*, 1997).

3.2.2.6 Test for cardiac glycosides (Keller-kiliani test)

A portion of the extract was dissolved in 1ml glacial acetic acid containing traces of ferric chloride solution. This was then transferred into a dry test tube and 1ml of concentrated sulphuric acid was added down the side of the test tube to form a lower layer at the bottom. A pale green colour in the upper acetic acid layer indicated the presence of cardiac glycosides (Evans, 1996).

3.2.2.7 Test for unsaturated sterols (Salkowski test)

2 drops of concentrated sulphuric acid was added at the side of the test tube containing 2ml of aqueous extract, immediate color change to cherry red indicated presence of unsaturated sterols (Evans, 1996).

3.2.2.8 Test for triterpenes (Lieberman Buchard test)

Equal volume of acetic anhydride was added to an aqueous portion of the extract and mixed gently. 1ml of concentrated sulphuric acid was added down the side of the test tube to form a lower layer. A colour change of blue to blue-green colour in the upper layer and reddish, pink or purple color indicated the presence of triterpenes (Evans, 1996).

3.2.3 Drug preparation and administration

3.2.3.1 Preparation

The dried methanol extract was mixed thoroughly with gum acacia in ratio of 4 to 1 using mortar and pestle. The mixture was then suspended in distilled water and serial dilution was done for the test doses (1000, 500 and 250 mg/kg). Gum acacia was suspended in distilled water to obtain 1% w/v suspension. Piracetam and scopolamine were dissolved in distilled water to obtain a concentration from which doses of 400 and 1 mg/kg respectively. Diazepam injection was diluted appropriately with distilled water.

3.2.3.2 Route of administration and volume of drug solutions administered

The methanol extract, 1% gum acacia and piracetam were administered orally. Scopolamine and diazepam were administered through the intraperitoneal route. Doses were administered in mg/kg body weight of mice in volume of 1 ml / 100 g body weight.

3.2.4 Acute toxicity study

The OECD 425 in Europe guideline limit test was adopted for the establishment of the acute toxicity profile of the methanol stem extract of *P. nigrescens.*

Briefly, five mice were dosed sequentially with 5000 mg/kg of the plant extract after they were fasted for four hours. Food was further withheld for 2 hours and they were then observed twice during the first 30 minutes after dosing, periodically during the first 24 hours for signs of toxicity such as changes in skin and fur, eyes and mucous membranes, tremors, convulsions, salivation, diarrhoea, lethargy, sleep, coma and

death during the first 4 hours then daily for two weeks. The LD_{50} was subsequently estimated.

3.2.5 Dose selection

Doses of scopolamine, piracetam and diazepam that produced the desired effects (Effective doses ED_{50}) were obtained from the outcome of the pilot study. Twenty percent of the LD_{50} from acute toxicity study of the extract was selected as the test dose from which lower doses were derived.

3.2.6 Behavioural studies

3.2.6.1 Exteroceptive model

Elevated plus maze: The method previously described by Parle and Dhingra (2003) was adopted in this study. The elevated plus maze for mice consisted of two open arms (16 cm × 5 cm) and two covered arms (16 cm × 5 cm × 15 cm) extended from a central platform (5 cm × 5 cm) and the maze elevated to a height of 45 cm from the floor.

Mice were randomly selected into five groups of six animals each. Group 1, 2, 3, 4 and 5 received 1 ml / 100 g of 1% gum acacia, 400 mg/kg piracetam, 250, 500 and 1000 mg/kg extract per body weight per oral respectively. One, two and twenty four hours after oral administration each mouse was placed at the end of an open arm, facing away from the central platform and the transfer latency (TL) was taken for training (one hour), learning (2 hours) and memory (24 hours).

Transfer latency (TL) defined as the time taken by the animal to move from the end of the open arm into one of the covered arms with all its four limbs. 60 seconds

maximum TL was assigned for each animal and while in any of the closed arm a maximum of 20 second was allowed for the animal to explore the maze and thereafter returned to its home cage. Animals that did not enter into either one of the covered arm within 60 seconds were pushed gently into one of the two covered arms and TL assigned 60 seconds. The maze was cleaned after each trial with 70% ethanol to remove olfactory cues.

Barnes maze: The method previously described by Barnes (1979) and slightly modified was adopted in this study. The maze consisted of a circular platform (95 cm in diameter) with 40 equally spaced holes (5 cm in diameter and 5 cm between holes) along the perimeter in which recessed goal box (10 cm x 20 cm) is located underneath one of the holes and the maze elevated to 105 cm above the floor. A circular start box (15 cm in height and 20 cm in diameter) usually put at the centre of the maze containing the animal for 10 seconds before each trial was used. The walls of the laboratory room were designed to allow spatial navigation.

The procedure consisted of three phases viz: habituation, acquisition and probe trials.
In maze habituation, each mouse underwent three habituation trials with an inter-trial interval of fifteen minutes. Firstly, each mouse was placed directly into the recessed goal box and allowed to remain there undisturbed for 2 minutes, thereafter returned to their home cages. In the second trial mouse was then placed on the maze surface adjacent to the goal hole, gently guided into the goal box and allowed to remain there undisturbed for 1 minute. Finally, each mouse was placed at the maze centre containing a start box for 10 seconds after which the start box was lifted away, the

mouse was gently guided into the goal box and allowed to remain undisturbed for a final 1 minute.

In the acquisition trial, the testing began 24 hours after maze habituation day. Mice were placed in the start box located at the centre of the maze. After a 10 seconds delay, the start box was lifted away and the fan switched on. Mice were allowed to explore for 2 min in order to locate and enter the recessed box. Upon entering the goal box, the fan was switched off and the mice were allowed to stay undisturbed for 20 seconds before returning to their home cage. This testing process was repeated for 3 additional trials to conclude Testing Day 1 with an inter-trial interval of 15 minutes.

On Testing Day 2 mice were randomly selected into treatment groups of six animals per group for spatial learning assessment. Group 1, 2, 3, 4 and 5 received 1 ml / 100 g of 1% gum acacia, 400 mg/kg piracetam, 250, 500 and 1000 mg/kg extract per oral respectively. 1 hour after drug administration each animal underwent four training as in Testing Day 1 with a maximum latency of 120 seconds. Primary latency and total latency, primary error and total error were taken for each animal as indices of learning and working memory respectively. Primary latency defined as the time taking to locate the target hole but no entering into the recessed box. Primary error is the number of head dip before first locating the escape hole. Total latency is time taken to locate and enter the goal box. A total error is the total number of head dip before entering the goal box. Animals that failed to find the goal location within the 2 minutes trial were gently guided into it, and allowed to remain for 20 seconds then assigned a total latency and maximum error (number of head dip before entering the recessed box). The maze was cleaned after each trial with 70% ethanol to remove olfactory cue.

Probe trial, 24 hours after day 2 trials the goal box was removed and the maze platform was divided into four quadrants viz: target quadrant, opposite quadrant, positive and negative quadrant each containing ten holes.

Each mouse was placed in a start box for 10 seconds after which it was lifted away and the mouse was allowed to explore for 90 seconds. The behaviour of the mice was recorded with the aid of a camera placed above the centre of the maze. The time spent in the target quadrant was taking as an index of spatial memory. The maze was cleaned after each trial with 70% ethanol to remove olfactory cue.

Novel object recognition test (NORT): The method described by Ennaceur (2010); Gaskin *et al.,* (2010) was adopted to assess recognition memory. The apparatus consisted of a plexiglas box of 40 cm x 40 cm x 40 cm in dimension. The NORT consist of three phases: the habituation, familiarization and testing phase.

Habituation phase, each mouse was allowed to explore the open arena without object for 2 minutes on the first day and returned to the home cage.

Familiarisation phase, two identical objects were introduced into the arena of the apparatus 20 cm apart from each other and 5 cm away from the walls of the apparatus. Each mouse was allowed to explore the identical objects for 10 minutes 24 hours after habituation and then returned to the home cage.

Testing phase, 24 hours after familiarization, one of the objects was replaced with another (a novel object) of different size and colour. Mice were randomly selected into five groups of six animals each. Group 1, 2, 3, 4 and 5 received 1 ml / 100 g of 1% gum acacia, 400 mg/kg piracetam, 250, 500 and 1000 mg/kg extract per oral

respectively. 1 hour after drug administration mice were introduced into the arena and allowed to explore for 5 minutes. The behaviour of mice was monitored with the aid of a video camera placed above the apparatus. The time for novel and familiar object exploration was taken and the discrimination index of memory was calculated as difference between novel and familiar object exploration. Object exploration is defined as the time taken for the animal's orientation towards the object with the snout, sniffing and touching the object. While climbing or sitting on the object was not considered as exploration (Aggleton *et al.*, 2010).

3.6.2.2 Acute interoceptive model studies

Scopolamine induced amnesia: Mice were randomly selected into six groups of six animals each. Group 1, 2, 3, 4, 5 and 6 received 1 ml / 100 g of 1% gum acacia; 1 ml / 100 g 1% gum acacia, 400 mg/kg piracetam, 250, 500 and 1000 mg/kg extract per oral respectively. Amnesia was induced with scopolamine hydrobromide (dose: 1 mg/kg intraperitoneally) in animals of group 2 to 6, 30 minutes after oral drug administration to the respective treatment groups. Transfer latency (TL) was recorded 30 minutes (training), 1 hour (learning) and 24 hours (memory) after scopolamine administration in an elevated plus maze as previously described.

Diazepam induced amnesia: Mice were randomly selected into six groups of six animals each. Group 1, 2, 3, 4, 5 and 6 received 1 ml / 100 g 1% gum acacia, 1 ml /100 g 1% gum acacia, 400 mg/kg piracetam, 250, 500 and 1000 mg/kg extract per oral respectively. Amnesia was induced with diazepam (dose: 0.7 mg/kg intraperitoneally) in animals of group 2, 3, 4, 5 and 6, 30 minutes after oral drug administration to the respective treatment group. Transfer latency (TL) was recorded 30 minutes (training),

1 hour (learning) and 24 hours (memory) post diazepam administration in an elevated plus maze as previously described.

3.6.2.3 Study of exploratory behaviours

Open field test: The method described by Kalueff *et al.,* (2006) was adopted for the assessment of spontaneous locomotor (horizontal) and exploratory (vertical) activity. It consisted of plywood (72 x 72 x 36 x 36 cm), one of the wall is a clean transparent plexiglas for visibility. The base was divided into 16 squares (18 x 18 cm) with blue marker and covered with transparent plexiglas (Brown *et al.,* 1999).

Mice were randomly selected into five groups of six animals each. Groups 1, 2, 3, 4 and 5 received 1 ml / 100 g 1% gum acacia, 400 mg/kg piracetam, 250, 500 and 1000 mg/kg extract per oral respectively. One hour post drug administration, each mouse was placed individually at the corner of the arena and its behaviour monitored for 5 minutes with a video camera hung 1.5 meter above the apparatus. The number of squares, number of central square crossed and rearing were recorded for each animal. The apparatus was cleaned in-between observations with 70% methanol and allowed to dry to remove any olfactory cue.

Hole-board test: The method described by File (1973) was adopted for the assessment of exploratory behaviour. The hole-board was made of a wooden panel (60 x 30 cm) provided with 16 equally spaced holes (1 cm diameter x 2 cm depth) positioned 105 cm above the table. One hour post drug administration and immediately after the open field test each mouse was placed at the side of the board and allowed to explore for 5 minutes. The number of head dips was determined. Head dip was defined as the

poking of the hole to the level of the eye (File and Wardill, 1975). The apparatus was cleaned after each test with 70% methanol to remove olfactory cue.

3.2.7 Sub-chronic interoceptive studies

Animals were grouped into 6 groups of 6 animals each. Group 1, 2, 3, 4, 5 and 6 received 1 ml / 100 g of 1% gum acacia; 1 ml / 100 g of 1% gum acacia, 400 mg/kg piracetam, 250, 500 and 1000 mg/kg of extract per oral. Amnesia was induced with scopolamine (dose: 1 mg/kg) intraperitoneally to group 2, 3, 4, 5 and 6, for 7 consecutive days 30 minutes after oral administration to the respective treatment groups according to the method described by Ramadeep *et al.,* (2015). The behavioural cognitive studies were carried out using novel object recognition task for assessment of recognition memory from day 5 to day 7 (for habituation, familiarisation and testing respectively) and elevated plus maze on day 7 (learning) and 8 (memory).

One hour after drug administration on the day 7, each mouse was placed in the novel object exploration apparatus as previously described. At the end of the task transfer latency was taken (training) immediately, then 1 hour later (learning) and 24 hours (day 8) later (memory) on the Elevated Plus Maze (EPM). 30 minutes after the EPM, mice were sacrificed by cervical decapitation, whole brain was carefully removed from the skull, weighed and homogenised in 5 ml 0.1 M phosphate buffer pH 7.4 and assayed for acetylcholinesterase, reduced glutathione, superoxide dismutase (using the rat SOD Kit), malondialdehyde (using the rat MDA ELISA Kit).

3.2.7.1 Acetylcholinesterase (AChE) assay method

An aliquot portion (0.4 ml) of the homogenate was added to a cuvette containing 2.6 ml phosphate buffer (0.1 M, pH 8) and 100 micro litre of DTNB (5,5-dithio-bis-(2-

nitrobenzoic acid). The contents of the cuvette were mixed thoroughly by bubbling air and absorbance was measured at 412 nm in an LKB spectrophotometer. When absorbance reached a stable value, it was recorded as the basal reading. 20 microlitre of substrate i.e., acetylthiocholine was added and change in absorbance recorded for a period of 10 minutes at intervals of 2 minutes. Change in the absorbance per minute was thus determined as according to the method described by Ellman *et al.,* (1961):

The enzyme activity is calculated using the following formula;

R = 5.74 x 10-4 x A/CO

Where, R = Rate in moles of substrate hydrolyzed / minute / gm tissue, A = Change in absorbance / min and CO = Original concentration of the tissue (mg / ml).

3.2.7.2 Reduced glutathione assay method

Reduced glutathione estimation in brain homogenate was measured according to the method described by Ellman *et al.,* (1959). This method was based on the development of a yellow colour when 5, 5′-dithio-bis-2-nitrobenzoic acid (DTNB) was added to the compound containing the sulfhydryl groups. To the 0.5 ml of brain homogenate was mixed with 1.5 ml of 0.2 M Tris buffer (pH-8.2) and 0.1 ml of 0.01 M DTNB and this mixture was brought to 10 ml with 7.9 ml of absolute methanol. The above reaction mixture was centrifuged at approximately 300 g at room temperature for 15 minutes. The absorbance of supernatant was read in a spectrophotometer against reagent blank (without sample) at 412 nm.

3.3. Statistical Analysis

All the results were expressed as mean ± standard error of mean (SEM). Data was analyzed using One-Way ANOVA followed by Dunnett's post hoc test for multiple comparisons and *p*-values ≤ 0.05 was considered as statistically significant.

CHAPTER FOUR

4.0 RESULTS

4.1 Phytochemical Analysis

4.1.1 Phytochemical constituents of methanol stem extract of *Parquetina nigrescens*

The result of the preliminary phytochemical screening of *Parquetina nigrescens* revealed the presence of carbohydrate, unsaturated sterol, saponins, phenolics and tannins. Cardiac glycosides, alkaloids and triterpenes were found to be absent (Table 4.1).

4.2 Acute Toxicity Study

Animals treated with methanol stem extract of *Parquetina nigrescens* showed no sign(s) of toxicity. No mortality recorded after 14 days observation period. The oral median lethal dose was estimated to be $\geq$ 5000 mg/kg.

4.3 Exteroceptive Studies

4.3.1 Effect of methanol stem extract of *Parquetina nigrescens* on transfer latency of mice in an elevated plus maze

The methanol stem extract produced a dose-dependent decrease in transfer latency on day 2 at all doses which was only significant at doses of 500 mg/kg and 1000 mg/kg ($p < 0.05$ and $p < 0.01$ respectively) and a decrease in transfer latency on day 1 at dose of 250 mg/kg and only significant ($p < 0.05$) at 1000 mg/kg. There was no decrease in transfer latency on day 1 at the dose of 500 mg/kg. Piracetam significantly decreased the transfer latency on day 1 and 2 ($p < 0.01$ and $p < 0.05$ respectively) (Figure 4.1).

4.3.2. Effect of methanol stem extract of *Parquetina nigrescens* on cognition-related behaviour of mice in a Barnes maze

The methanol stem extract of *P. nigrescens* at dose 1000 mg/kg significantly ($p < 0.05$) decreased primary latency. There was no decrease in primary latency at lower doses. Piracetam significantly ($p < 0.05$) decreased primary latency. Piracetam and the extract at dose 1000 mg/kg non-significantly decreased total latency. The extract at dose 500 mg/kg increased primary latency. There was no change in total latency at dose 250 mg/kg (Table 4.2).

The methanol stem extract significantly decreased primary and total errors at doses of 250 ($p < 0.05$) and 1000 mg/kg ($p < 0.05$ and $p < 0.01$ respectively). There was non-significant increase in primary and total errors at dose of 500 mg/kg. Piracetam non-significantly decrease total and primary errors (Table 4.2).

The methanol stem extract of *P. nigrescens* significantly improved the time spent in target quadrant at doses of 250 and 500 mg/kg ($p < 0.01$ and $p < 0.01$ respectively). There was non-significant increase in time spent in target quadrant at dose 1000 mg/kg. Piracetam non-significantly increase time spent in target quadrant (Table 4.2).

4.3.3 Effect of methanol stem extract of *Parquetina nigrescens* on object discrimination index in novel object recognition test

The methanol stem extract of *P. nigrescens* significantly ($p < 0.01$) increased object discrimination at doses of 500 mg/kg and 1000 mg/kg. There was a non-significant increase object discrimination index at dose 250 mg/kg. Piracetam significantly ($p < 0.05$) increased object discrimination index (Figure 4.2).

4.4 Exploratory Study

4.4.1 Effect of methanol stem extract of *Parquetina nigrescens* on exploratory behaviour of mice in open field test

The methanol stem extract of *P. nigrescens* did not increase the number of square cross at all doses tested. Piracetam non-significantly increase the number of square cross (Table 4.3).

The methanol stem extract of *P. nigrescens* significantly ($p < 0.01$) increased the number of central square crossing at all doses tested. Piracetam significantly ($p < 0.01$) increased the number of centre square crossed (Table 4.3).

The methanol stem extract of *P. nigrescens* did not significantly alter the number of rearing at all tested doses. Piracetam significantly ($p < 0.05$) increased the number of rearings (Table 4.3).

4.4.2: Effect of methanol stem extract of *Parquetina nigrescens* on number of head dip of mice in hole-board test

The methanol stem extract of *P. nigrescens* significantly ($p < 0.01$) increased the number of head dip at all doses tested. Piracetam did not significantly alter the number of head dip (Figure 4.3).

4.5 Anti-amnesic Studies

4.5.1 Effect of methanol Stem extract of *Parquetina nigrescens* on transfer latency of diazepam induced amnesic mice in an elevated plus maze

Diazepam non-significantly increased the transfer latency on day 1 and 2 compared to 1% gum acacia group. The extract significantly ($p < 0.05$) decreased the transfer latency on day 1 at all doses tested and non-significantly on day 2. Piracetam significantly ($p < 0.05$) decreased the transfer latency of day 1 and 2 (Figure 4.4)

4.5.2: Effect of methanol stem extract of *Parquetina nigrescens* on transfer latency of scopolamine induced amnesic mice in an elevated plus maze

Scopolamine non-significantly increase transfer latency on day 1 and day 2 compared to 1% gum acacia group. The methanol stem extract of *P. nigrescens* significantly ($p < 0.01$) increased the tranfer latency on day 1 and 2 at dose of 250 mg/kg. The extract non-significantly decreased the transfer latency on day 1 at dose 1000 mg/kg. There was no appreciable alteration in tranfser latency on day 1 and 2 at dose 500 mg/kg and on day 2 at dose 1000 mg/kg. Piracetam significantly ($p < 0.05$) decreased the tranfer latency on day 1 and non-significantly on day 2 (Figure 4.5).

4.6 Sub-chronic Studies

4.6.1 Effect of methanol stem extract of *Parquetina nigrescens* on transfer latency following sub-chronic scopolamine induced amnesic mice in an elevated plus maze

Scopolamine significantly ($p < 0.05$) increased the transfer latency on day 7 and non-significantly on day 8 compared to 1% gum acacia group. The extract at all doses tested significantly ($p < 0.05$) decreased the transfer latency on day 7 and non-

significantly on day 8 except at dose 500 mg/kg which was significant ($p < 0.05$). Piracetam significantly ($p < 0.01$) decreased the transfer latency on day 7 and 8 (Figure 4.6.).

4.6.2 Effect of methanol stem extract of *Parquetina nigrescens* on object discrimination index following sub-chronic scopolamine induced amnesic mice in novel object recognition test

Scopolamine significantly ($p < 0.01$) decreased the discrimination index compared to 1% gum acacia group. The methanol stem extract of *P. nigrescens* significantly ($p < 0.05$) increased discrimination index at dose of 250 mg/kg, non-significantly at dose of 1000 mg/kg and further decreased the discrimination index at dose 500 mg/kg. Piracetam significantly ($p < 0.01$) increased the discrimination index (Figure: 4.7).

4.6.3: Effect of methanol stem extract of *Parquetina nigrescens* on brain malondialdehyde following sub-chronic scopolamine induced lipid peroxidation

Scopolamine significantly ($p < 0.05$) increased brain MDA compared to 1% gum acacia group. The extract at all doses tested significantly ($p < 0.05$) decreased the brain MDA level compared with the scopolamine group. However the extract did not reduced brain MDA level to the level of control group. Similarly, piracetam was able to decrease the level of brain MDA significantly ($p < 0.01$) (Figure 4.8).

4.6.4: Effect of methanol stem extract of *Parquetina nigrescens* on brain superoxide dismutase following sub-chronic scopolamine induced amnesic mice

Scopolamine non-significantly decreased the level of brain SOD compared to 1% gum acacia group. The methanol stem extract significantly *(*$p < 0.05$) increased brain SOD

level at all doses tested. The level of brain SOD in the treated group was greater than that of the negative control. Piracetam non-significantly increased the level of brain SOD (Figure 4.9).

4.6.5: Effect of methanol stem extract of *Parquetina nigrescens* on brain reduced glutathione following sub-chronic scopolamine induced amnesic mice

Scopolamine non-significantly decreased brain reduced glutathione level compared to 1% gum acacia group. The methanol extract at all doses tested and piracetam non-significantly increased the brain reduced glutathione level (Figure 4.10).

4.6.6: Effect of methanol stem extract on *Parquetina nigrescens* on brain Acetylcholinesterase level following Sub-chronic scopolamine induced amnesic mice

Scopolamine significantly ($p < 0.05$) increased the brain level of acetylcholinesterase compared to 1% gum acacia group. The methanol stem extract of *P. nigrescen* at all doses tested and piracetam did not significantly alter the brain acetylcholinesterase enzyme concentration (Figure 4.11).

Table 4.1: Phytochemical constituents present in the methanol stem extract of *Parquetina nigrescens*

Secondary plant metabolites	Inference
Carbohydrate	Present
Unsaturated sterols	Present
Saponin glycosides	Present
Cardiac glycosides	Absent
Phenolics	Present
Alkaloids	Absent
Tannins	Presents
Triterpenes	Absent

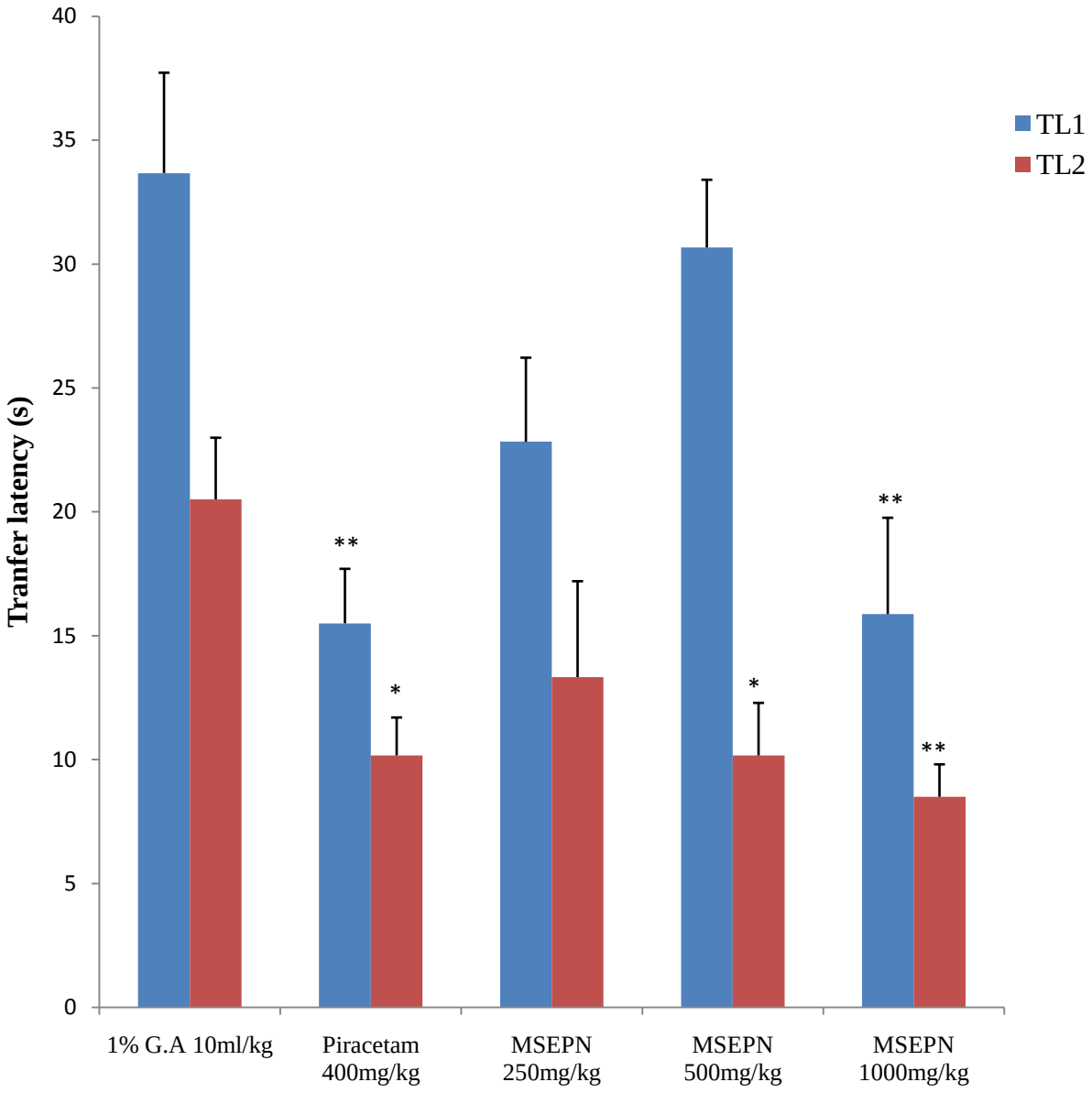


Figure 4.1: Effect of methanol stem extract of *Parquetina nigrescens* on learning and memory of mice in an elevated plus maze

Data presented as mean ± standard error of mean (n=6); * denotes $p < 0.05$; ** denotes $p < 0.01$ as compared to 1% G.A group; TL1: learning; TL2: memory; G.A: gum acacia; S: seconds; MSEPN: methanol stem extract of *P. nigrescens*

Table 4.2: Effect of methanol stem extract of *Parquetina nigrescens* on cognition-related behaviour of mice in the Barnes maze

Treatment	PL (S)	TL (S)	PE	TE	TSTQ (S)
1% G.A 10 ml/kg	57.75±6.56	66.42±6.79	10.58±1.06	10.58±0.77	27.17±4.72
Piracetam 400mg/kg	37.54*±2.92	54.96±4.85	6.83±0.94	7.25±0.98	43.17±6.78
MSEPN 250mg/kg	61.21±8.51	69.01±9.01	5.00*±0.82	6.12*±0.99	53.17**±5.94
MSEPN 500mg/kg	58.54±9.65	91.17±11.33	12.67±2.01	14.29±2.13	62.00**±3.98
MSEPN 1000mg/kg	36.08*±2.92	54.04±3.99	4.54**±0.45	5.71*±0.36	34.17±3.58

Data presented as mean ± standard error of mean (n = 6); * denotes $p < 0.05$; ** denotes $p < 0.01$ as compared to 1% G.A group; PL = primary latency; TL = total latency; TE: total error; PE: primary error; TSTQ: time spent in target quadrant; S: seconds; MSEPN: methanol stem extract of *P. nigrescens*

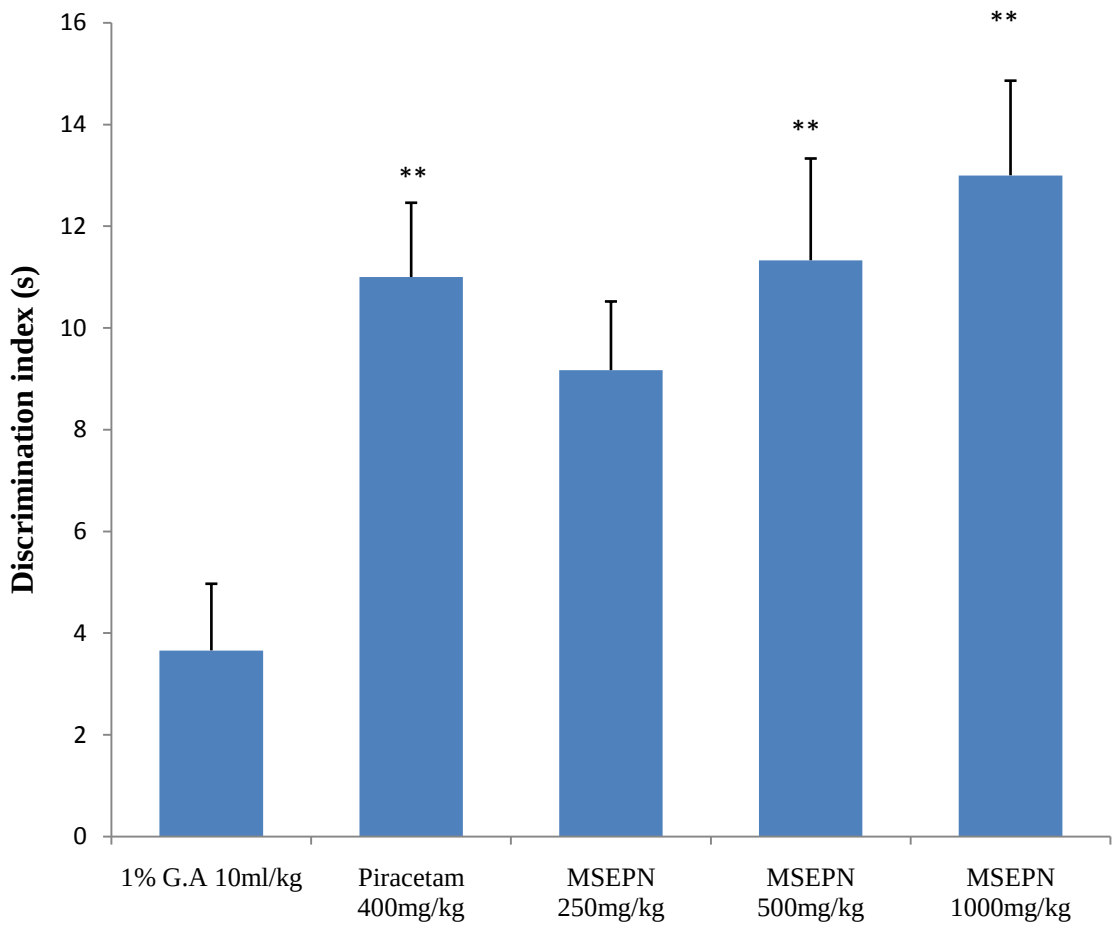


Figure 4.2: Effect of methanol stem extract of *P. nigrescens* on object discrimination of mice in novel object recognition test

Data presented as mean ± standard error of mean; (n=6); * denotes $p < 0.05$; ** $p < 0.01$ as compared to 1% G.A; S: seconds; DI: discrimination index; G.A: gum acacia; MSEPN: methanol stem extract of *P. nigrescen*s

Table 4.3: Effect of methanol stem extract of *Parquetina nigrescens* on exploratory behaviour of mice in open field test

Treatment	NSC	NCSC	NR
1% G.A 10ml/kg	87.50±4.97	0.5±0.22	32.17±2.30
Piracetam 400mg/kg	110.17±6.46	4.33**±0.49	41.50*±2.14
MSEPN 250mg/kg	86.83±8.74	2.83*±0.31	30.00±2.89
MSEPN 500mg/kg	90.00±3.83	3.00**±0.45	31.00±2.56
MSEPN 1000mg/kg	92.17±8.00	4.33**±0.84	35.17±2.20

Data presented as mean ± standard error of mean (n=6); *denote $p < 0.05$; **denote $p < 0.01$ as compared to 1% G.A; NSC: number of square cross; NCSC: number of central square cross; NR: number of rearing; G.A: gum acacia; MSEPN: methanol stem extract of *P. nigrescens*

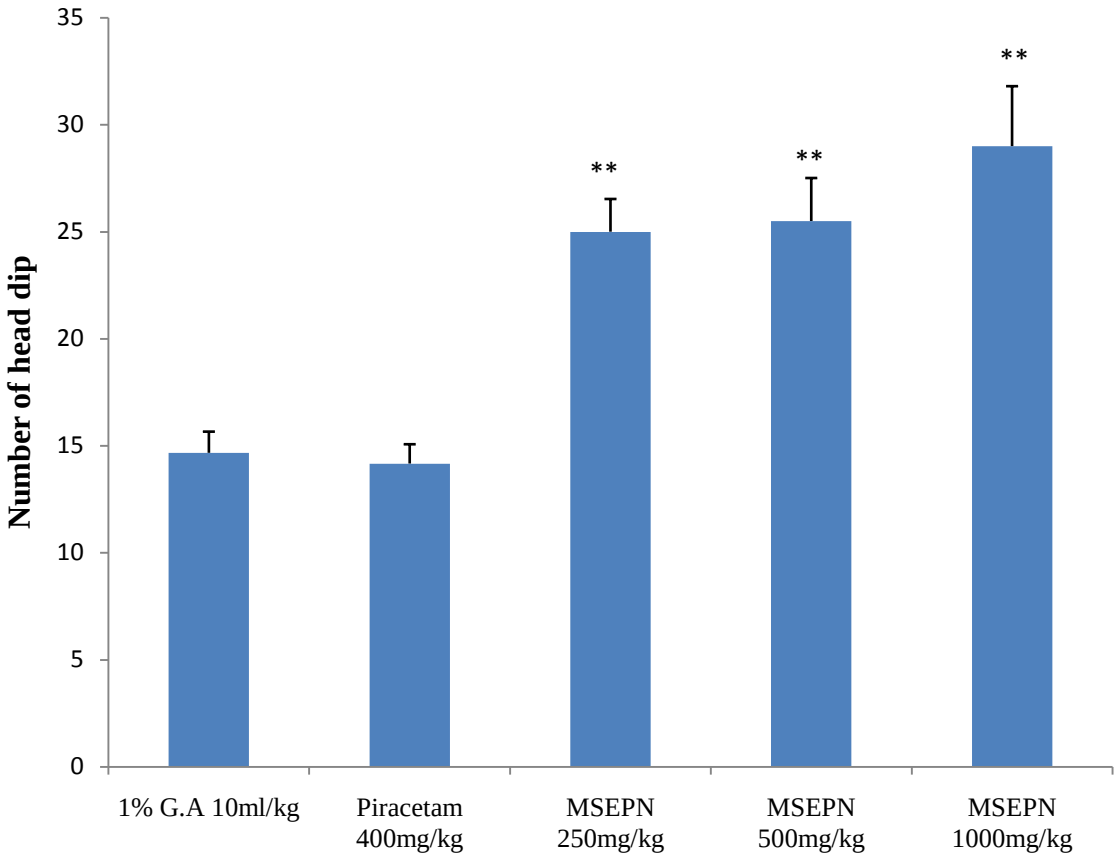


Figure 4.3: Effect of methanol stem extract of *Parquetina nigrescens* on number of head dip of mice in the hole-board test

Data presented as mean ± standard error of mean; (n=6); * denotes $p < 0.05$; ** $p < 0.01$ as compared to 1% G.A; G.A: gum acacia; MSEPN: methanol stem extract of *P. nigrescens*

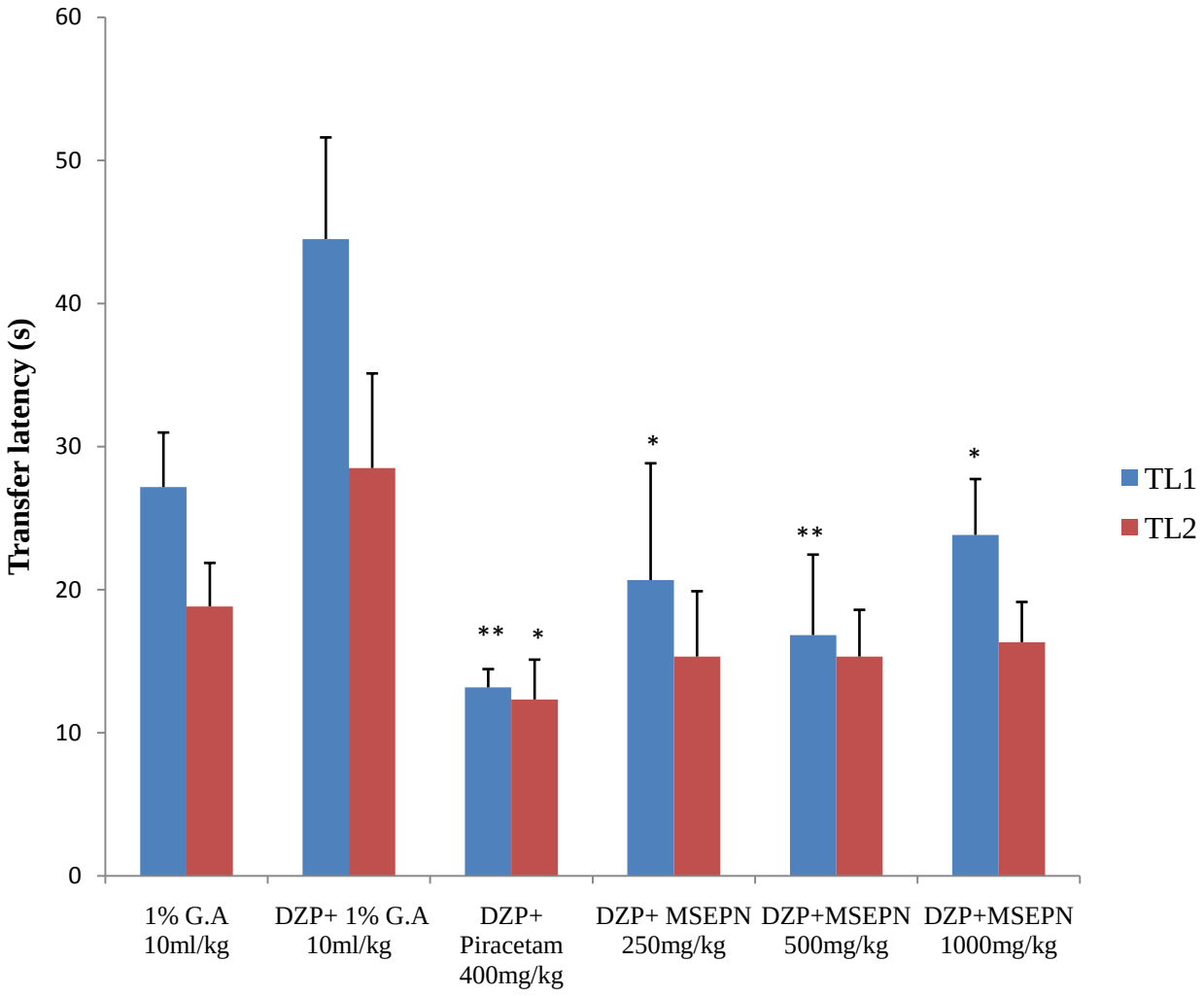


Figure 4.4: Effect of methanol stem extract of *Parquetina nigrescens* on transfer latency of diazepam induced amnesic mice in an elevated plus maze

Data presented as mean ± standard error of mean; (n=6); ** denotes $p \leq 0.01$; * denotes $p < 0.05$ as compared to DZP+1% G.A group; TL1: learning; TL2: memory; DZP: diazepam; S: seconds; G.A: gum acacia; MSEPN: methanol stem extract of *P. nigrescens*

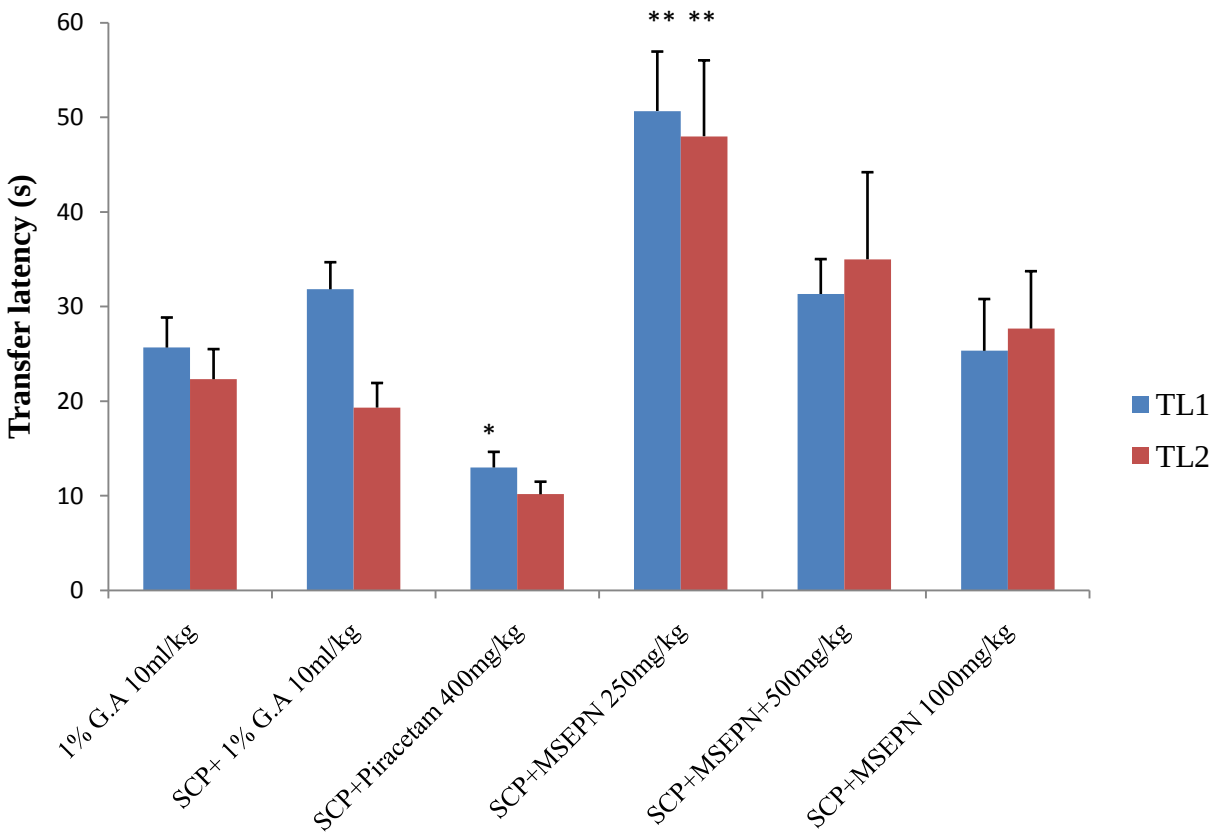


Figure 4.5: Effect of methanol stem extract of *Parquetina nigrescens* on transfer latency of scopolamine induced amnesic mice in an elevated plus maze

Data presented as mean ± standard error of mean; (n=6); ** denotes $p < 0.01$; * denotes $p < 0.05$ as compared to SCP+1% G.A group; TL1: learning; TL2: memory; SCP: scopolamine; G.A: gum acacia; S: seconds; MSEPN: methanol stem extract of *P. nigrescens*

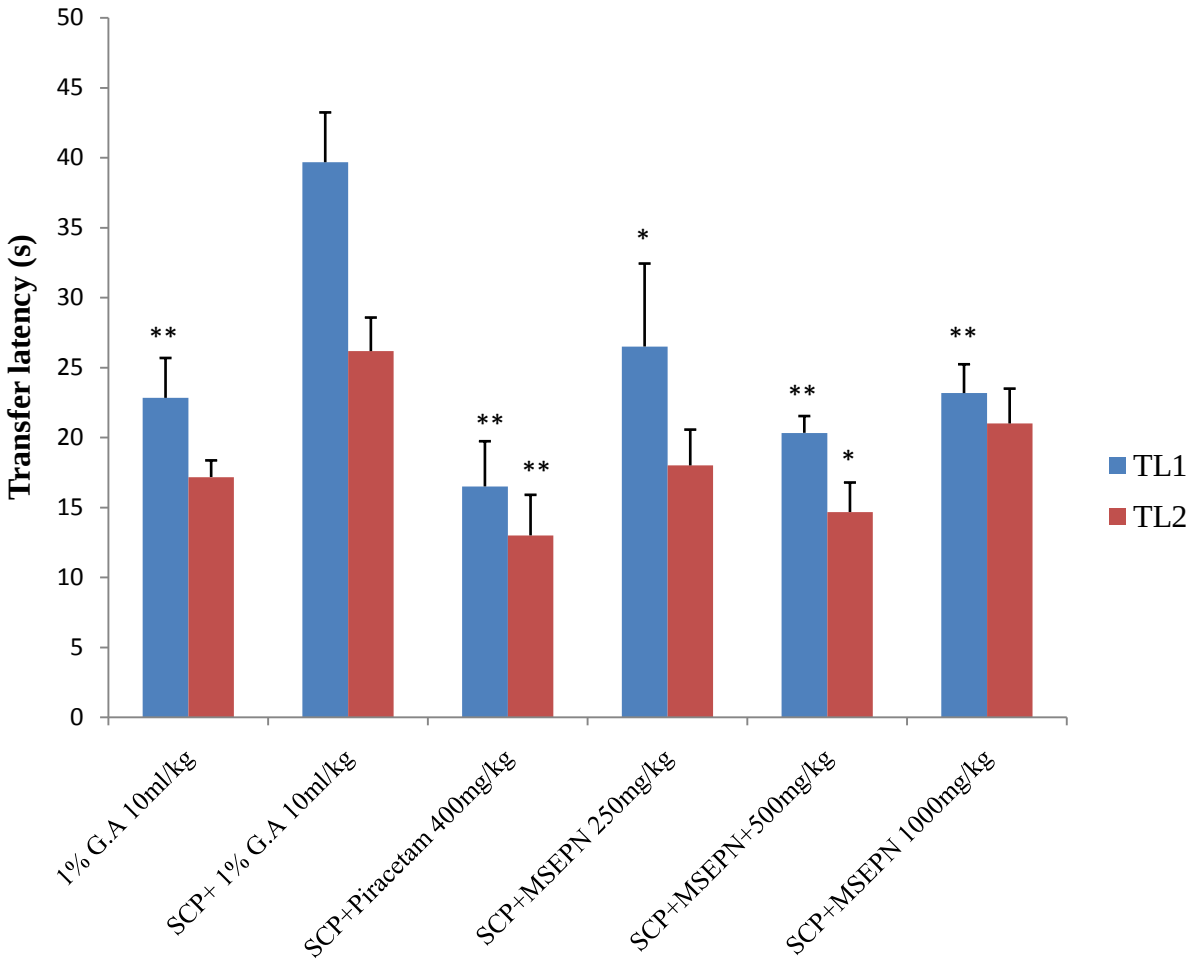


Fig. 4.6: Effect of methanol stem extract of *Parquetina nigrescens* on transfer latency following sub-chronic scopolamine induced amnesic mice in an elevated plus maze

Data presented as mean ± standard error of mean; (n=6); ** denotes $p < 0.01$; * denotes $p < 0.05$ as compared to SCP+1% G.A group; TL1: transfer latency on day 7; TL2: transfer latency on day 8; SCP: scopolamine; S: seconds; G.A: gum acacia; MSEPN: methanol stem extract of *P. nigrescens*

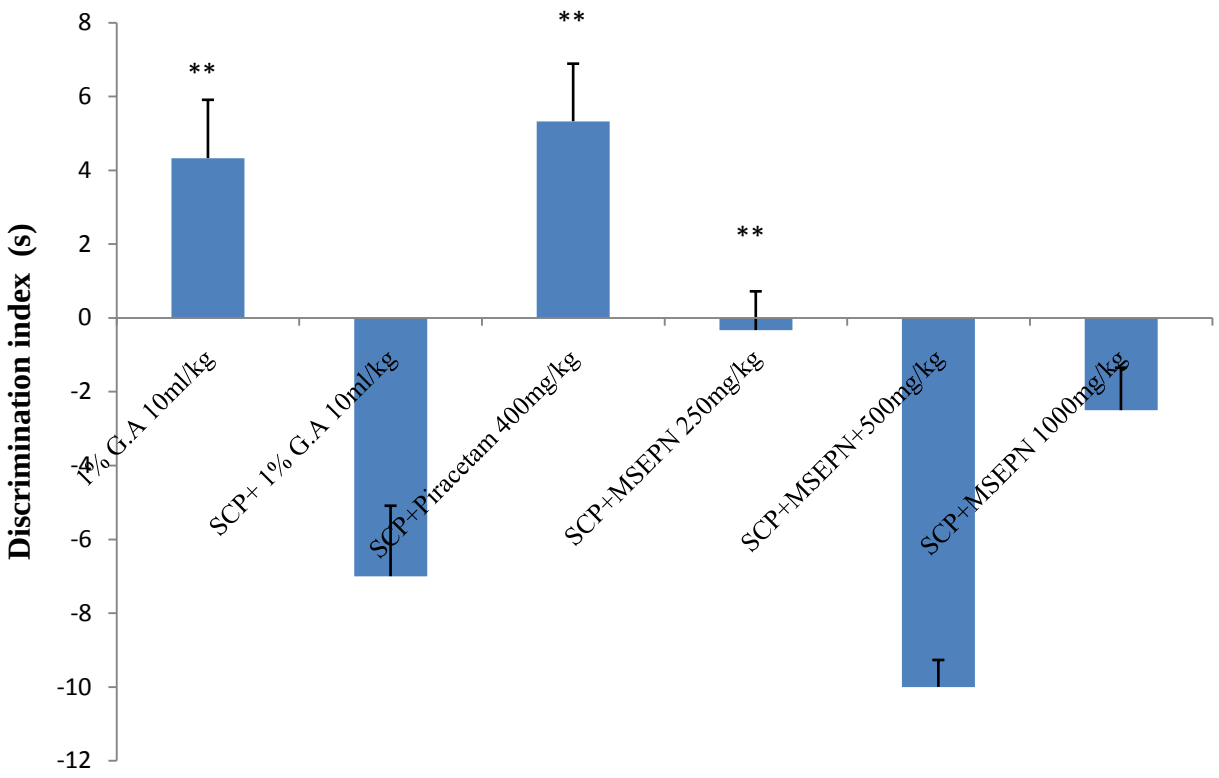


Figure 4.7: Effect of methanol stem extract of *Parquetina nigrescens* on discrimination index following sub-chronic scopolamine induced amnesic mice in novel object recognition test

Data presented as mean ± standard error of mean; (n=6); ** denotes $p < 0.01$; * denotes $p < 0.05$ as compared to SCP+1% G.A group; DI: discrimination index; SCP: scopolamine; G.A: gum acacia; S: seconds; MSEPN: methanol stem extract of *P. nigrescens*

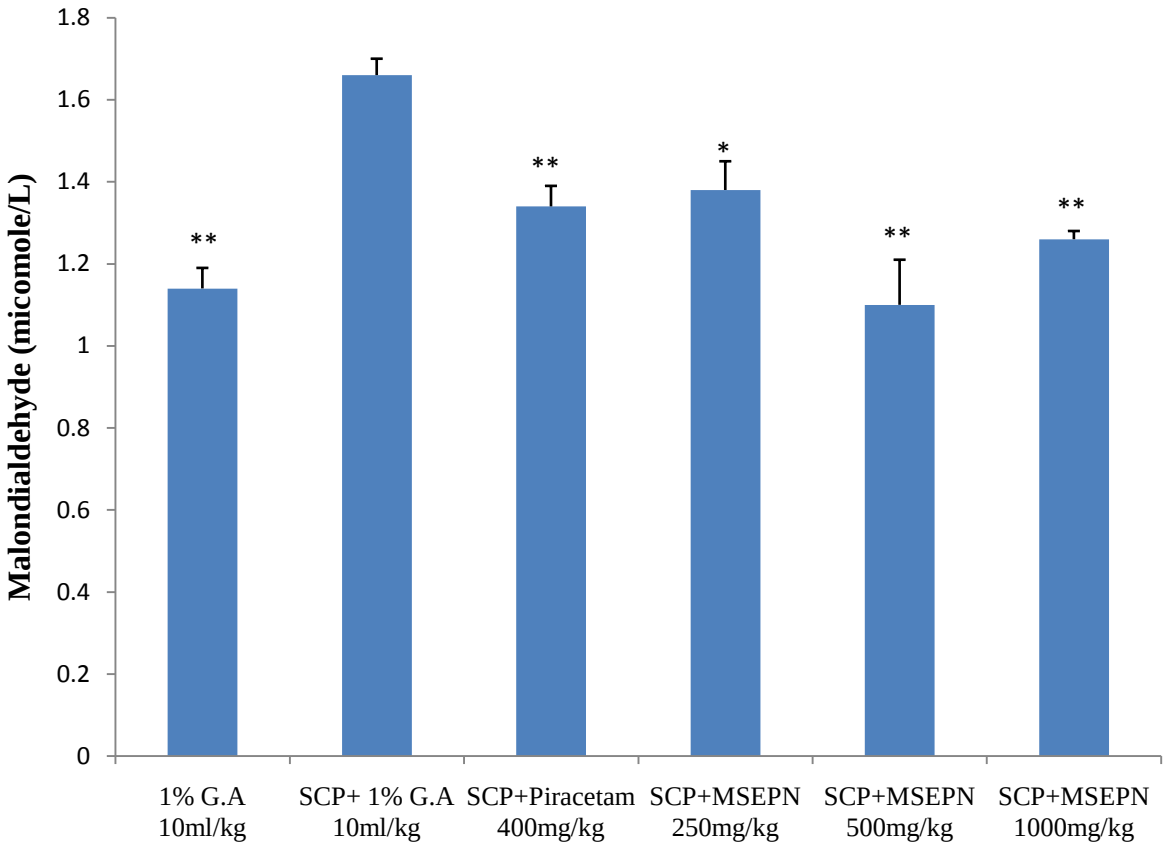


Figure 4.8: Effect of methanol stem extract of *Parquetina nigrescens* on brain malondialdehyde level following sub-chronic scopolamine induced amnesic mice

Data presented as mean ± standard error of mean; (n=6); ** denotes $p < 0.01$; * denotes $p < 0.05$ as compared to SCP+1% G.A group; SCP: scopolamine; G.A: gum acacia; MSEPN: methanol stem extract of *P. nigrescens*

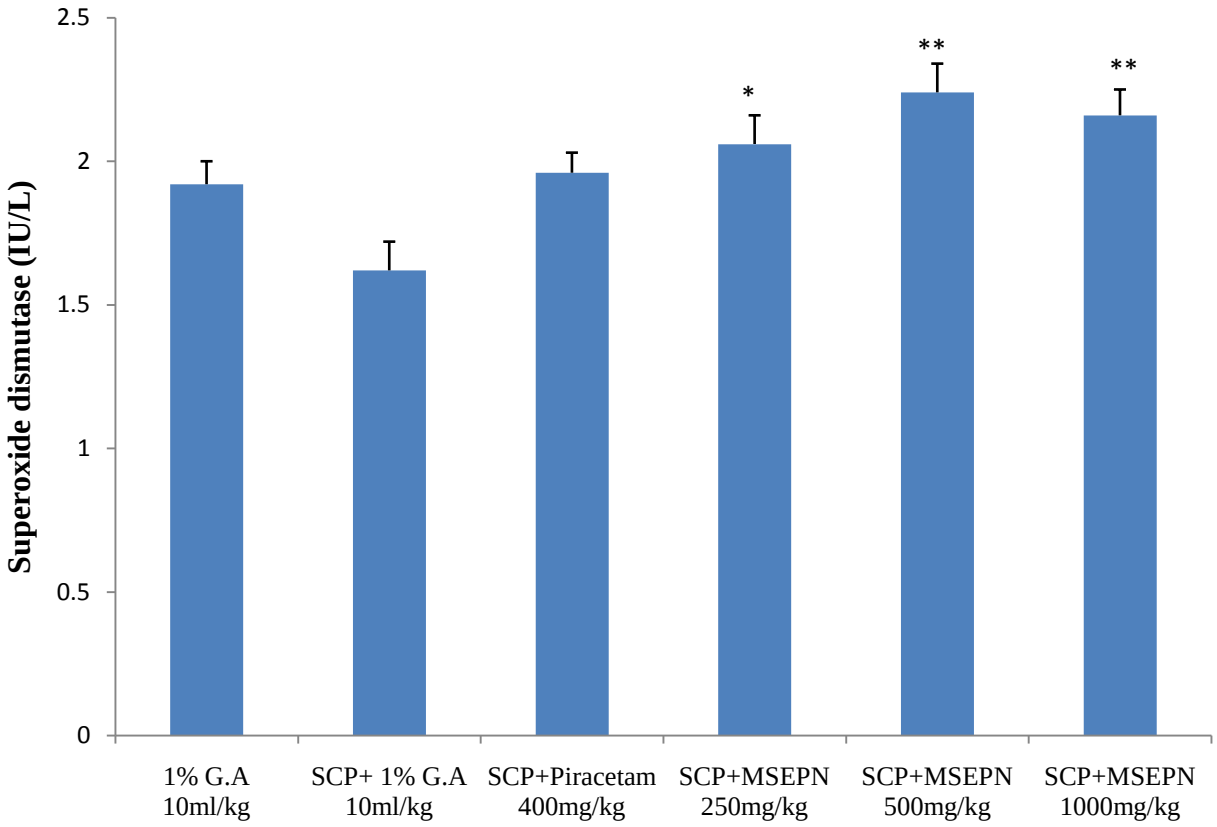


Figure 4.9: Effect of methanol stem extract of *Parquetina nigrescens* on brain super oxide dismutase level following Sub-chronic scopolamine induced amnesic mice

Data presented as mean ± standard error of mean; (n=6); ** denotes $p < 0.01$; * denotes $p < 0.05$ as compared to SCP+1% G.A group; SCP: scopolamine; G.A: gum acacia; MSEPN: methanol stem extract of *P. nigrescens*

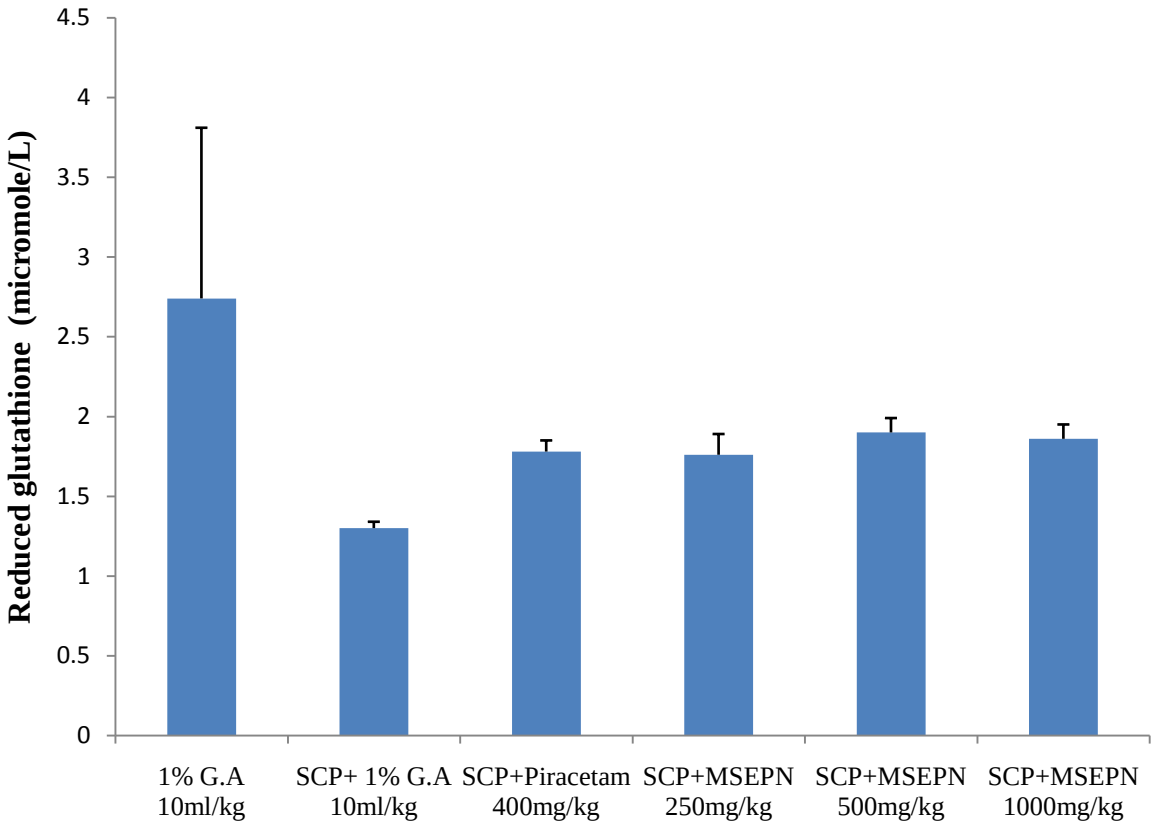


Figure 4.10: Effect of methanol stem extract of *Parquetina nigrescens* on brain reduced glutathione level following sub-chronic scopolamine induced amnesic mice

Data presented as mean ± standard error of maen; (n=6); compared to SCP+1% G.A group; SCP: scopolamine; G.A: gum acacia; MSEPN: methanol stem extract of *P. nigrescens*

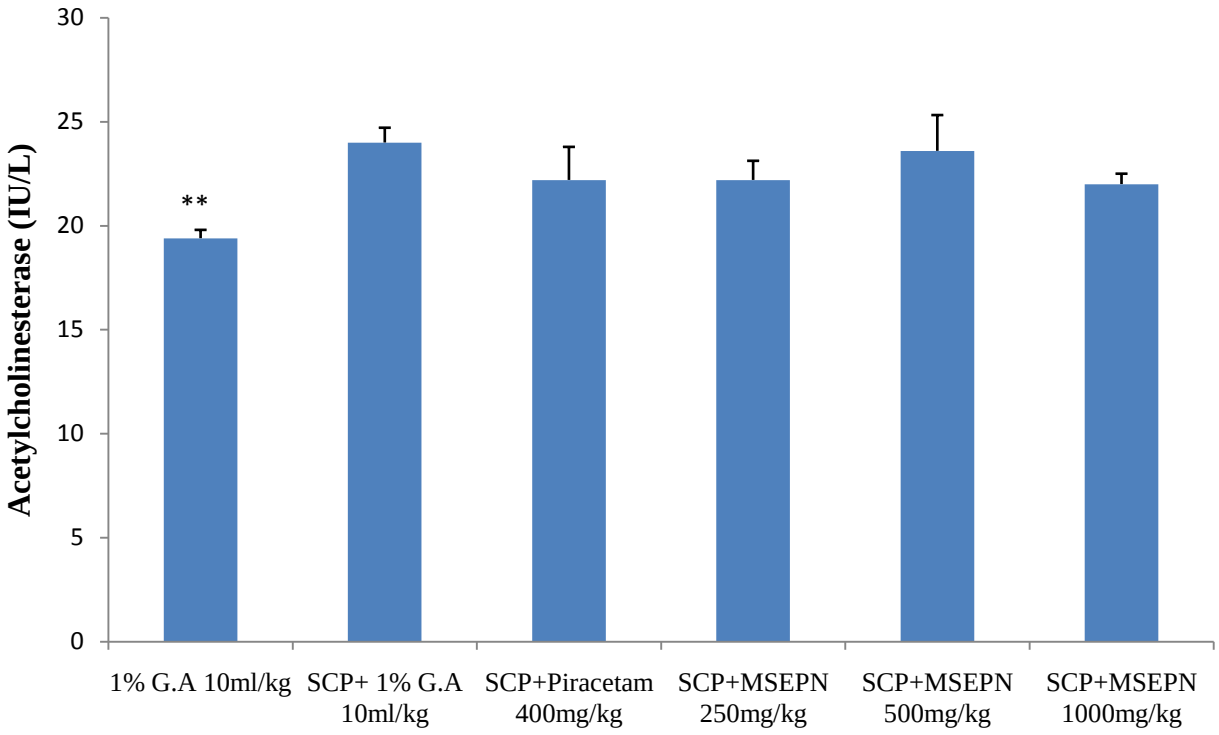


Figure 4.11: Effect of methanol stem extract of *Parquetina nigrescens* on brain acetylcholinesterase activity level following Sub-chronic scopolamine induced amnesic mice

Data presented as mean ± standard error of mean; (n=6); ** denotes $p < 0.01$; as compared to SCP+1% G.A group; SCP: scopolamine; G.A: gum acacia; MSEPN: methanol stem extract of *P. nigrescens*

CHAPTER FIVE

5.0 DISCUSSION

Parquetina nigrescens has been reported to be used in traditional medicine practice for the enhancement of memory among the people of South-Western part of Nigeria (Elufioye *et al.,* 2012). This study investigated the nootropic potential of the methanol stem extract of *P. nigrescen.*

The phytochemical screening of the methanol stem extract of *P. nigrescen* revealed the presence of carbohydrate, unsaturated sterol, tannins, saponins and phenolics which may be responsible for the observed pharmacological effects. Phenolics and tannins have been thought to have nutritional and antioxidant properties (George *et al.,* 2014). Tannins and saponins have beneficial effect on vascular health (Takagi *et al*., 1980; Rajeshwari *et al.,* 2014). Saponins are compounds known to have nootropic activities (Chintawar *et al.,* 2002). The nutritional benefits of these phytochemicals may be responsible for the enhanced cognitive function of the methanol stem extract of *P. nigrescens* (Akhisa and Kokke, 1991; Rajeshwari *et al.,* 2014).

The methanol stem extract of *P. nigrescens* is relatively non-toxic because the oral median lethal dose is $\geq$ 5000 mg/kg (Matsumura, 1985).

Elevated plus maze, Barnes maze and novel object recognition test are widely employed in assessing the effect of agents capable of improving learning and memory (Barnes, 1979; Dhingra *et al*., 2004 and Ennanceur, 2010). Elevated plus maze is a paradigm used to asses anxiety related behavioural studies and has been widely employed in pre-screening of drugs capable of enhancing cognitive functions using

transfer latency as a parameter for assessing learning and memory. It is used as a model to detect anxiolytic effects of benzodiazepine-related compounds (Silveira *et al.*, 1993; Gonzalez and File, 1997). Cognitive disruption in anxiety is thought to occur when anxiety take reins of sensory, perceptual and attentional processes in certain areas of the brain and threatening information preferentially processed over other important information (Bar-Haim *et al*., 2007). In the EPM, the extract and piracetam decreased transfer latencies on day 1 and dose dependently on day 2 compared to the control. Hence, the ability of the extract to decrease transfer latencies suggests that it improves learning and memory (Dhingra, 2004).

The Barnes maze is a less stressful test for the assessment of spatial learning and memory in the search of an escape hole using spatial cues by rodents. It assesses both long and short term memory (working and reference memory) (Barnes, 1979; Brickman and Stern, 2009). The task relies on hippocampal-dependent spatial reference memory and on the inherent tendencies of the subjects to escape from an aversive environment (Akirav *et al*., 2001). In the Barnes maze the latency to locate the target hole (escape latency) is the most widely used measure for the assessment of learning with the primary latency being more sensitive than the total latency for detecting differences in learning (Timothy and Richard, 2012). The extract improved spatial learning only at the highest tested dose, working memory and reference memory as indicated by decrease in escape latency, escape errors and time spent in target quadrant respectively. There was impairment of spatial learning and working memory at 500 mg/kg as indicated by increase in escape latency and escape errors. The observed improvement in spatial learning and memory in the Barnes maze

suggests that one or more of the phytochemicals: saponins, tannins, phenolics, unsaturated sterols and carbohydrate maybe exerting their effect on the hippocampus.

The novel object recognition test is used to assess recognition memory in rodents based on their inherent exploratory behaviour to distinguish a novel (non-familiar) object from a familiar object in the absence of externally applied rules or reinforcement. The perirhinal, entorhinal and inferior cortices play an important role in object recognition memory (Hammond *et al.,* 2004; Aggleton *et al.,* 2010) and damages affecting these areas impair performance in recognition memory tasks (Albasser *et al*., 2009). Drugs capable of improving recognition memory may affect these brain regions. In the NORT, the methanol stem extract of *P. nigrescens* at all doses tested and piracetam increase discrimination index. The increase in discrimination index by the extract suggests that it improves recognition memory (Baxter, 2010). It is therefore possible that the improvement in recognition memory of methanol stem extract of *P. nigrescens* exerts its effect on these key brain regions.

Anxiolytic and locomotive effect of a substance can affect cognitive parameters (Lowry, *et al*., 2005). The open field test is used for assessing exploratory behaviours such as locomotor activity and anxiolytic effect (Tatem *et al.,* 2014). In general, animals with impaired motor function will display decrease activity and vice versa (Nagaraju *et al.,* 2010) and this will be reflected as impaired or increased cognitive parameters in EPM, BM and NORT. The amygdala and hippocampus are the brain regions implicated in motor function in the open field test. In the open field test, the extract at all the doses tested is devoid of central nervous system stimulating or depressive effect as indicated with no significant changes in the vertical activity (rearing) and horizontal activity (number of line crossing) compared to the control. The extract showed anxiolytic effect at all the tested doses as indicated by a significant

increase in the number of centre square crossing. Piracetam showed stimulating but no sedating and anxiolytic effect as indicated by increased line crossing and rearing.

The hole-board test is used for assessing exploratory behaviour in rodents (File and Wardill, 1975) with head dip as a measure of anxiolytic effect. In the hole-board test, the extract at all doses displayed an anxiolytic effect indicated by significant increase in the number of head dip. This further corroborated the anxiolytic effect found in the open field assay. Piracetam did not show anxiolytic effect as indicated by almost the same mean value in the number of head dip with the control. This lack of observed anxiolytic effect of piracetam in the hole-board test was observed in the open field test that the increase in number of centre square crossing of piracetam may be as a result of its stimulating effect.

Nootropics are known to protect the brain from chemical assaults such as scopolamine and benzodiazepine (Giurgea, 1980). In the interoceptive studies, diazepam a benzodiazepine is known to induce cognitive deficit through the potentiation of GABAergic system by acting as a positive allosteric modulator of GABA-$_A$ receptor type. Drugs capable of blocking the action of GABA improve cognitive function in contrary to drugs potentiating the function of GABA (Kalueff and Nutt, 1996). The methanol stem extract of *P. nigrescens* was able to decrease the transfer latencies increased by diazepam. The ability of the extract to reverse diazepam induced cognitive deficit may imply its cognitive enhancement is mediated through opposition or inhibition of the GABAergic system. GABA antagonists are known to be strong memory activating agents. Administration of flumazenil a benzodiazepine antagonist or inverse agonist like methyl- and butyl-beta-carboline 3-carboxylate resulted in

significant improvement of memory and learning in various tests (Kalueff and Nutt, 1996).

Scopolamine is an anticholinergic and a nonselective competitive muscarinic antagonist capable of inducing cognitive deficit through the blockade of cholinergic function (Sherman *et al.,* 2003). The methanol stem extract of *P. nigrescens* could not decrease the transfer latencies on day 1 and 2 which was increased by scopolamine. Hence, scopolamine induced cognitive deficit could not be reversed by the extract. Interestingly, the extract further impaired cognitive function as seen with increase in transfer latency when compared with the control and the scopolamine treated groups. Piracetam significantly reversed learning and memory deficit induced by scopolamine as indicated by decrease in transfer latency on both days. The inability of the extract to reverse scopolamine induced cognitive deficit suggest that the cognitive enhancing effect may not be mediated directly through the cholinergic system.

To further investigate the non involvement of the cholinergic system opined in the acute study result, amnesia was induced sub-chronically with scopolamine to provide pharmacological model of cognitive impairment for screening drugs capable of improving or restoring normal cognitive function (Kwon *et al.,* 2010). Scopolamine is employed as the gold standard for inducing memory impairments in healthy human and animals to mimic muscarinic decline characteristic of ageing and dementia (Drachman and Leavitt, 1974; Klinkenberg and Blokland, 2010). The behavioural cognitive enhancing activity of methanol stem extract of *P. nigrescens* in sub-chronic scopolamine induced cognitive deficit in mice was carried out using the elevated plus

maze and the novel object recognition test and biochemical markers of cognitive decline were also investigated.

In the elevated plus maze, scopolamine was able to induce cognitive (learning and memory) deficit non-significantly when compared to the control by increasing the transfer latency on day 1 and 2. The methanol stem extract of *P. nigrescens* reversed the sub-chronic scopolamine induced cognitive deficit in contrary to the acute study. Piracetam also reversed the scopolamine induced cognitive impairment. The ability of the extract to reverse sub-chronic scopolamine induced cognitive deficit maybe as a result of neuroprotective effect of the extract against scopolamine induced neurodegeneration.

In the novel object recognition test, scopolamine significantly impaired recognition memory as indicated with increased negative discriminative index when compared to the control group. This impairment of recognition memory was completely reversed by piracetam as indicated by a positive value of discrimination index, while the extract reversed the recognition memory deficit only at doses of 250 and 500 mg/kg. At dose 500 mg/kg of the extract there was further impairment of recognition memory. The improvement in recognition memory in NORT may also result from neuroprotective effect of the extract.

Several mechanisms have been proposed by which scopolamine impairs cognitive function. Aside antagonising the action of acetylcholine, scopolamine increases acetylcholinesterase activity, decreases cerebral blood flow, triggers reacting oxygen species, decrease brain antioxidants, increases lipid peroxidation and induces

neuroinflammation (Tota *et al*., 2012; Ahmad *et al.,* 2014; Hancianu *et al.*, 2013). Hence, the methanol stem extract of *P*. *nigrescens* was studied for its effect on the level of malondialdehyde, superoxide dismutase, reduced glutathione and acetylcholinesterase indicative of cognitive decline.

Malondialdehyde is an end product of lipid peroxidation and a measure of free radical generation. Lipid peroxidation is an important indicator of brain neurodegeneration which affects the integrity of the neuronal membrane and function (Ramandeep *et al.,* 2015). Piracetam and all doses of the extract were able to protect the brain of the mice from scopolamine induced lipid peroxidation as indicated by the reduction in malondialdehyde level which was significantly increased by scopolamine. The ability of the extract to protect the brain against lipid peroxidation may be due to the known free radical scavenging properties of the tannins and phenolics. Scopolamine triggers reactive oxygen generation which builds up and consequently breaks down lipid membrane (Lobo *et al*., 2010, Ahmed *et al*., 2014).

Scopolamine decreases the level of endogenous antioxidants (Ramandeep *et al*., 2015). Superoxide dismutase is an important enzyme with the ability of detoxifying super oxide anions that otherwise damage the membrane of neurones and other macromolecules (Balu *et al.,* 2005). Piracetam and the extract at all doses were able to increase the level of super oxide dismutase (SOD) reduced by scopolamine.

Lipid peroxidation may be enhanced by reduction in the level of reduced glutathione (GSH) in the brain (Younes and Siegers, 1981). The enhancement of lipid peroxidation maybe as a result of increase in the level of hydrogen peroxide generated during

oxidative stress which is highly toxic to the neurones. GSH is an endogenous antioxidant against oxidative stress most especially hydrogen peroxide (Schuessel *et al.*, 2004). The extract and piracetam were able to increase the level of reduced glutathione decreased by scopolamine. The ability of the extract to increase the level of these endogenous antioxidants may results from the antioxidant properties of phenolics and tannins.

AChE catalyses the breakdown of acetylcholine, inhibitors of the enzyme increase the level of acetylcholine consequently improving cognition. Prolong administration of scopolamine increases the activity of AChE and consequently depleting the level of acetylcholine, a neurotransmitter mostly implicated cognition (Perry *et al.*, 1981). The extract at all doses and piracetam could not decrease the activity of acetylcholinesterase. This implies that the improvement of learning and memory by the methanol stem extract of *P. nigrescens* and piracetam is not mediated through the inhibition of the enzyme AChE activity. The reversal of sub-chronic scopolamine induced cognitive deficit in the behavioural studies may be due to free radical scavenging, antioxidant, nutritional properties of the of phenolics, saponins, carbohydrates and tannins present in the methanol stem extract of *P. nigrescens* and probably not through cholinergic system.

CHAPTER SIX

6.0 SUMMARY, CONCLUSION, RECOMMENDATION

6.1 Summary

Parquetina nigrescens is a plant used in Africa and in Nigeria most especially among the folks of the Western part of the country for its medicinal importance. It is used in the management of diabetes, cancer, sickle cell anaemia, rheumatism and in the treatment of pile, wound, boil and as aphrodisiac. Previous pharmacological studies have demonstrated its activity against cancer, diabetes, anaemia, ulcer and so forth.

The powdered sample of methanol stem extract of *P. nigrescens* afforded a yield of 7.97%. The methanol stem extract of *P. nigrescens* has an oral median lethal dose ≥ 5000 mg/kg. The preliminary phytochemical screening of the extract revealed the presence of tannins, saponins, phenolics, unsaturated sterols and carbohydrates and does not contain triterpenes, alkaloids and cardiac glycosides. In the cognitive study, the methanol stem extract improved learning and memory at all doses in the EPM and recognition memory in the NORT. In the BM, the extract improved spatial learning at dose 1000 mg/kg, working memory at dose 250 and 1000 mg/kg and reference memory at all doses. The methanol stem extract lacks stimulating and sedating effect in the OFT and may possess anxiolytic effect in the OFT and HBT. Acute diazepam induced amnesia was reversed significantly by the extract while that of scopolamine induced amnesia could not be reversed. In the sub-chronic study, the methanol stem extract of *P. nigrescens* reversed the scopolamine induced cognitive deficit in the EPM and in the NORT at doses of 250 and 1000 mg/kg. The extract was able to protect the brain against lipid peroxidation, increased the level of endogenous antioxidants, but did not produce a change in the level of AChE following scopolamine induced amnesia.

6.2 Conclusion

The methanol stem extract of *P. nigrescens* possesses learning and memory enhancing-like activity. The learning and memory enhancement of the plant may not be mediated directly through the cholinergic pathway. The extract also may possess anxiolytic effect. The results of this study therefore, provide the pharmacological rationale for the ethnomedicinal use of the plant as memory enhancer.

6.3 Recommendations

Based on the findings from this study and the limitations, the following are suggested:

i. Separation, isolation and purification of the extract should be carried out and individually studied for cognitive enhancing ability.

ii. Further mechanistic studies of the extract should be carried out to ascertain the inhibition of GABA system and non-involvement of cholinergic system speculated.

iii. Finally, chronic toxicity studies of the methanol stem extract should be carried out to ascertain its safety.

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APPENDIX

B. **MAHMUD**, M.G. Magaji, L.O Ayanwuyi, M.Y, Sani, A. Shehu, (2017). Nootropic Activity of Methanol Stems Extract of *Parquetina nigrescens* Afzel Asclepiadacea in Mice. Paper presented at Nigeria Association of pharmacist in Academia 15th Annual National Conference 21st to 25th August, 2017, Faculty of Pharmacy University of Lagos.

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