MODULATORY EFFECT OF AQUEOUS EXTRACT OF *PHOENIX DACTYLIFERA* LINNFRUIT ON COGNITION IN SWISS ALBINO MICE

BY

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DECLARATION

I declare that the work in this thesis titled "Modulatory Effect of Aqueous Extract of *Phoenix dactyliferaLinn*. Fruit on Cognition in Swiss Albino Mice" has been performed by me in the Department of Human Physiology under the supervision of Dr. Rabiu AbduSSALAM Magaji, and Dr. Mohammed Garba Magaji. The information derived from the literature has been duly acknowledged in the text and a list of references provided. No part of this thesis was previously presented for another degree or diploma at any university.

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Signature **Date**

CERTIFICATION

This thesis titled "Modulatory Effect of Aqueous Extract of *Phoenix dactylifera Linn* Fruit on Cognition in Swiss Albino Mice" by Yusuf Yusha'u meets the regulations governing the award of the degree of Master of science of Ahmadu Bello University Zaria and is approved for its contribution to knowledge and literary presentation.

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DEDICATION

This research work is dedicated to Almighty Allah for His uncountable blessings on me.

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Abstract

Phoenix dactylifera fruits possess essential properties such as analgesic, antioxidant, and nephroprotective activity but there is paucity of information on researches centered on the benefits of *Phoenix dactylifera* in learning and memory. This study was designed to evaluate the effects of *Phoenix dactylifera* fruit extract on spatial learning and memory using neurobehavioral paradigms of Morris water, Barnes, and elevated plus mazes as well as evaluation of acetylcholinesterase enzyme activity of the brain tissues of the mice studied. Seventy five mice of both sexes were used for the study and divided into five groups of 5 mice each. Group 1 (distilled water 10 ml/kg) served as control, group 5 (Piracetam 100 mg/kg) served as positive control. Groups 2-4 were treated with*Phoenix dactylifera* extract 1000, 500 and 250 mg/kg respectively. Treatment with aqueous extract of *Phoenix dactylifera* and Piracetam was done 1 hour prior to the experiment daily for three days (in Morris water and Barnes mazes) and two days (in elevated plus maze). Results obtained from this study revealed that *Phoenix dactylifera* fruit (1000 mg/kg) impaired learning of mice in Morris water maze ($p<0.05$), but did not impair memory in Morris water maze, Barnes maze and elevated plus maze. No statistically significant difference was seen between control group and *Phoenix dactylifera* treated groups in acetylcholinesterase activity in Morris water, Barnes and elevated plus mazes, but statistically significant difference exist between control group and Piracetam treated group in acetylcholinesterase activity ($p>0.05$). No strong correlation was observed between probe parameters of neurobehavioral paradigms (frequency of platform crossings, retention and correct head dips in Morris water, Barnes and elevated plus mazes respectively) and acetylcholinesterase activity. Acute treatment with aqueous extract of *Phoenix dactylifera* fruit impaired learning in

Morris water maze and has no effect on memory in Morris water, Barnes and elevated plus mazes

Key words: *Phoenix dactylifera*, Piracetam, Cognition, Acetylcholinesterase enzyme, Learning and Memory.

CHAPTER ONE

1.0 Introduction

One of the major functions of the brain is the flexible adaptation to our ever-changing environment. The brain possesses executive circuits which do not only monitor and maintain current behavioral goals but also incorporate new goals and rules. This updating can come in the form of a quick integration of previously acquired knowledge when a well-known stimulus informs an animal of a change in reward contingencies. Hence, such updating requires new learning.Higher cognitive abilities evolved largely in mammals (Victoria *et al*., 2014).

Cognitive neuroscientists consider memory as the retention, reactivation, and reconstruction of the experience-independent internal representation (Schwabe and Wolf, 2010). The major challenge of neuroscientists today is identifying therapies or mechanisms that can treat or reverse the effects of memory complaints and other neurodegenerative disorders. Date palm (known as *Phoenix dactylifera*) has been used intreatment of various nervous disorders and memory complaint (Vyawahare *et al*., 2009), such as Parkinson's disease via acting as dopamine agonist (Ali *et al*., 2014), Alzheimer's and Vascular dementiavia its protective role in cerebral hypoperfusion (Rohini *et al*., 2014).

Brain areas involved in the neuroanatomy of memory include the hippocampus, the amygdala, the striatum, or the mammillary bodies which are thought to be involved in specific types of memory. For example, the hippocampus is believed to be involved in spatial learning and declarative learning, while the amygdala is thought to be involved in emotional memory (Labark and Cabeza, 2006). Prefrontal cortex and basal ganlia play vital role in storing working memory (Fiona and Torkel, 2008). The mechanism via which basal ganglia store working memory might

be via dopamine modulation in ventral tegmented area and substantia nigra (Karin and Daphna, 2011).

Today, a major point of concern for research in the pharmaceutical sciences isfinding unique and efficient compounds of natural origin. Plants have been seen to possess the potential to be excellent lead structures and to serve as a basis of promising therapeutic agents for treatmentof diseases (Jabeena *et al*., 2014).Also, restoration and development of traditional Medicine should be a main concern of health plan, which is in line with WHO's call for returning to use of traditional treatments to those countries with a history of traditional medicine (Sahar *et al*., 2013). Date fruit from date palmhas been used as food for over 6000 years. Although precise origin is obscure historically, but there is evidence its' dating to 4000 BC. Resources confirm that it is indigenous to Northeast Africa. It grows a wide belt from the Atlantic Ocean through the Sahara, the Arabian Peninsula, into Iran and Pakistan (Sahar *et al.*, 2013).

1.1 Statement of Research Problems

Understanding the variations in encoding and retrieving information among individuals of the same sex and age are parts of the challenges of neuroscientists today. These variations in memory may be associated with diet or ability of an individual to utilize his neurons.Today, cognitive enhancement is one of the most controversial topics in neuroscience. Academic doping is the off-label use of nootropics for the purpose of enhancing cognitive ability or academic success (Lucke *et al.*, 2014). The use of drugs for academic doping are influenced positively or negatively by several factors. Among them are personal characteristics, drug characteristics, and characteristics of the social context. Numerous researches had shown that nootropics such as piracetam and cholinergics (e.g choline, meclofenoxate, galantamine) enhance cognition

(McDaniel *et al.,* 2002; Gualtieri *et al.,* 2002). These drugs have many side effects, not easily affordable and some are used as substances of abuse by students in colleges(Kimberly and Wen, 2014). Hence, alternatives are really needed.*Phoenix dactylifera*is a fruit acceptable and easily affordable by most people because of its sweet taste and cheapness. This research work will evaluate the effects of *Phoenix dactylifera* on cognition in mice.

1.2 Justification

Many researches have been conducted on the effects of *Phoenix dactylifera* in organ systems including the nervous system by Asadi-Shekari *et al.,* (2008), Panahi *et al.,* (2008), and Masoumeh *et al.,* (2014) among others.*Phoenix dactylifera* was found to cause significant increase in motor co-ordination and analgesic activities (Vyawahare *et al*., 2009).To what extent *Phoenix dactylifera* increases cognition is not well scientifically established. This study was specificallycarried out on cognition to investigate the traditionally claimed that *Phoenix dactylifera* has been used in treatment of various nervous disorders and memory complaints.The significant of this study centered on finding alternative source of cognitive enhancement from dietary sources, as well as reducing drug abuse by the students in colleges for academic doping.

1.3 Research Hypothesis

Aqueous extract of *Phoenix dactylifera* fruits has no effect on cognition in mice

1.4 Aim and Objectives

1.4.1 Aim

The aim of this research is to determine the effect of aquoeus extract of *Phoenix dactylifera* on cognition in mice.

1.4.2 Objectives

Specific objectives of the study are:

(i) To assess the effects of *Phoenix dactylifera* on visio-spatial memory using Morris water, Barnes and elevated plus mazes.

(ii) To determine the effect of *Phoenix dactylifera*on acetylcholinesterase enzyme activity in brain of mice using acetylcholinesterse assay kit.

CHAPTER TWO

2.0 Literature Review

2.1 Learning and Memory

Learning is the act of acquiring new, or modifying and reinforcing existing knowledge, behaviors, skills, values, or preferences and may involve synthesizing different types of information (Daniel *et al.,* 2011).Memory is the process in which information is learned, stored, and retrieved (Jarrad and Gina, 2013). Encoding allows information that is from the outside world to reach senses in the forms of chemical and physical stimuli (Tali *et al.,* 2014). Memories for facts and events are not acquired in their definite form. Rather, some post-learning processes are known to take place that gradually stabilize new memories (Florian and Anders, 2014).

2.2 Short-Term Memory

Short-term memory holds a small amount of information (typically around 7 items or even less) in mind in an active, readily-available state for a short period of time (typically from 10 to 15 seconds, or sometimes up to a minute). It involves the ability to remember and process information at the same time (Davelaar *et al*., 2005).Working memory is a form of short-term memory. It refers to the short-term storage and manipulation of sensory information that lasts for seconds. It has been considered to be a core cognitive process that underpins a range of behaviors, from perception to problem solving and action control.The quality of working memory representations determines performance which improves with brain development from childhood to early adulthood and is closely related to measures of intelligence. It has recently been proposed that working memory might better be conceptualized as a limited resource that is distributed flexibly among all items to be maintained in memory(Wei*et al*., 2014).

2.3 Long-Term Memory

Long-term memory stores information over a long period of time. It actually decays very little over time, and can store a seemingly unlimited amount of information almost indefinitely.Short-term memories can become long-term memory through the process of consolidation, involving rehearsal and meaningful association. A group of studies suggests that erasing information from long-term memory might not typically occur (Storm *et al*., 2008). Long-term memory is further subdivided into two: Declarative and Implicit memories.

2.3.1 Declarative memory

Declarative memory is memory ofeventsand facts, and refers to those memories that can be consciously recalled. It is sometimes called explicit memory, since it consists of information that is explicitly stored and retrieved, although, it is more properly to be considered as a subset of explicit memory.Declarative memory is a memory that an individual can mention in words, while explicit memory is the deliberate recall of information that individual recognizes as a memory (Kalat, 2009).

Declarative memory can be further sub-divided into episodic memory and semantic memory.Episodic memory refers to storage and retrieval of specific personal experiences in time while, semantic memory stores general or factual information suchas information remembered for a test (Wood *et al*., 2011). Episodic memory refers to an ensemble of memory processes.This is because, the content of the memory and the procedures for encoding and retrieval vary in complexity and ecological validity, while the retention time varies in delay (Anne *et al*., 2014).

2.3.2 Implicit memory

Implicit memory is sometimes referred to as unconscious memory or automatic memory. Implicit memory refers to the use of past experiences to remember things without conscious thought. The performance of implicit memory is enabled by previous experiences, irrespective of the period learned (Kim, 2014).Implicit memory can be procedural or priming. Procedural memory is a type of implicit memory that involves carrying out commonly learned tasks and skills without consciously thinking about them (Posit Science, 2014).Priming is an implicit memory effect in which exposure to one stimulus influences a response to another stimulus (Meyer and Schvanevelt, 1971).

2.4 Spatial Cognition

Spatial cognition is concerned with the acquisition, organization, utilization, and revision of knowledge about spatial environments. Spatial memory can be working, short-term or long-term memory.A person's spatial memory is required in order to navigate around a familiar location, just as a rodent’s spatial memory is needed to learn the location of food or platform at the end of a maze (Denis and Loomis, 2007).

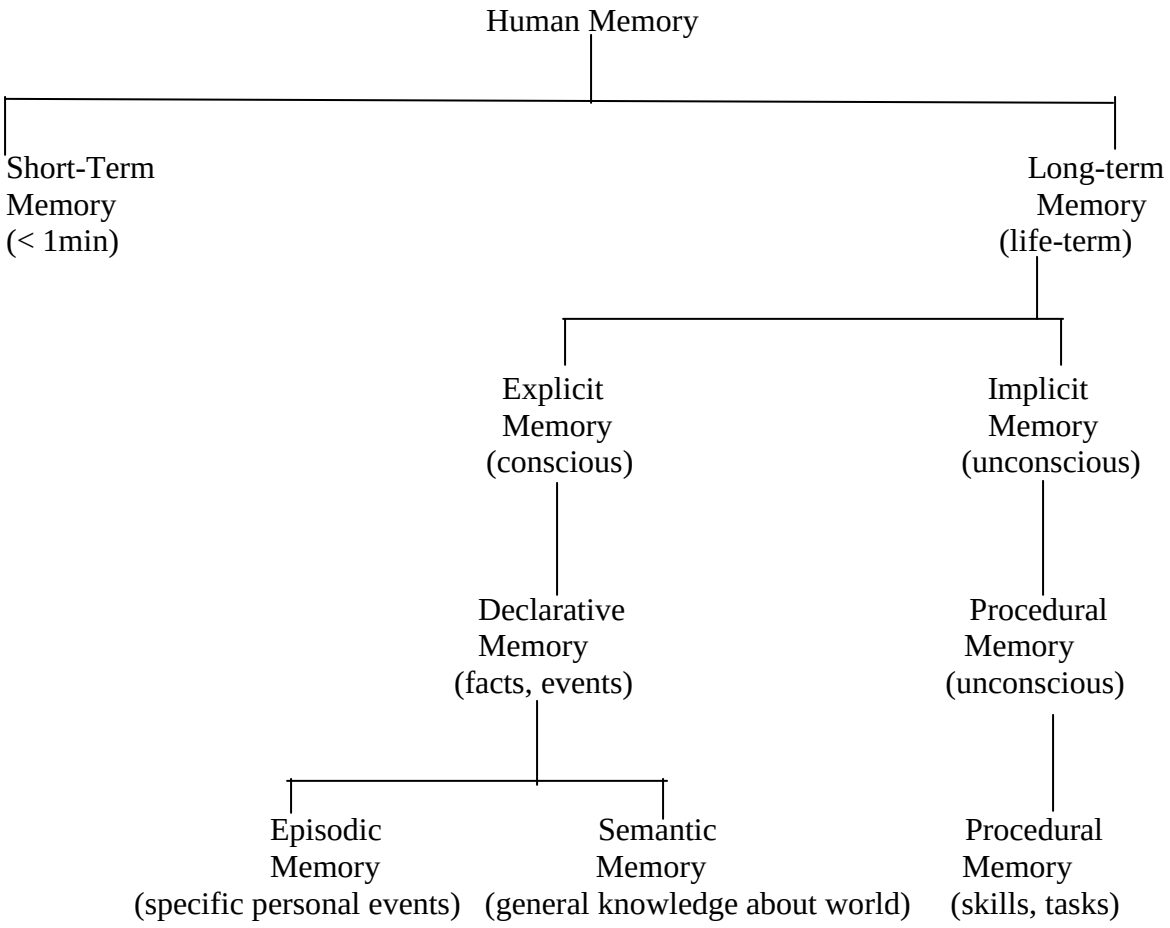

Figure 2.1: Types of Human Memory (Posit Science, 2014).

2.5 Memory Enhancers

Nootropics(also referred to as memory enhancers, neuro enhancers or cognitive enhancers) are drugs, supplements, nutraceuticals, and functional foods that improve one or more aspects of mental function, such as working memory. Examples are Piracetam, choline, galanthamine etc(Lanni *et al*., 2008).Stimulantsof memory enhance cognition in the general population, but only when used at low therapeutic doses. Relatively high doses of stimulants will result in cognitive deficits (Wood *et al*., 2014). These include: Amphetamine, Methylphenidateand Nicotine (enhancing working memory), Xanthines such as Caffeine and Eugeroics (increasing alertness).

The racetams are structurally similar compounds, such as pramiracetam, oxiracetam, coluracetam, aniracetam. piracetam and aniracetam. Piracetam and aniracetam modulate cholinergic systems by acting as positive allosteric modulators of AMPA (α-Amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid) receptors (Gualtieri *et al*., 2002).

2.6 Academic Doping

Today, cognitive enhancement is one of the most controversial topics in neuroscience.Academic doping is the off-label use of nootropics for the purpose of enhancing cognitive ability or academic success.It is often referred to asneuroenhancement or cognitive enhancement (Lucke *et al*., 2014). The use of drugs for academic doping are influencedpositively or negatively by several factors. Among them are personal characteristics, drug characteristics, and characteristics of the social context. Nootropics are normally used to treat neurocognitive disorders such as dementia (e.g Alzheimers), attention deficit disorder and schizophrenia. Neuroscientists believed that nootropics might also improve cognitive function in healthy peoplebased on their stimulant properties (Sattler *et al.,* 2014).

Presently, the main classes of drugs used as potential cognitive enhancers include psychostimulants (such as methylphenidate (MPH), amphetamine), wakefulness-promoting agents (modafinil) and glutamate activators (ampakine). Pharmacologically substances that enhance neuronal excitation(the components of the memory/learning circuits—dopamine, glutamate and/or norepinephrine) stand to improve brain function in healthy individuals beyond their baseline functioning. In particular, non-medical use of prescription stimulants such as MPH and illicit use of psychostimulants for cognitive enhancement have seen a recent rise among teens and young adults in schools and college campuses (Kimberly and Wen, 2014).

2.7 Synaptic Plasticity

Synaptic plasticity is the ability of synapses to strengthen or weaken over time, in response to alterations in their activity. Increase or decrease in the number of neurotransmitter receptors located on a synapse can lead to plastic change (Gerrow, 2010).Most mechanism-based efforts directed at improving memory have focused on synaptic plasticity and in particular the long term potentiation (LTP) effect (Gary *et al*., 2014).Synaptic plasticity in both excitatory and inhibitory synapses has been found to be dependent upon postsynaptic calcium release (Gerrow, 2010).Some types of synaptic plasticity depend on the activation of Ca^{2+}/calmodulin-dependent kinase II (CaMKII) (Enrica *et al.*, 2014).

2.7.1 Biochemical mechanism of synaptic plasticity

Synaptic plasticity involves the NMDA (N-methyl-D-aspartate receptor) and AMPA glutamate receptorsvia two molecular mechanisms. The first mechanism involves opening of NMDA channels (which relates to the level of cellular depolarization) leading to a rise in post-synaptic Ca^{2+} (Calcium ion) concentration which has been linked to long-term potentiation, LTP (as well as to protein kinase activation). Strong depolarization of the post-synaptic cell completely

displaces the magnesium ions that block NMDA ion channels and allows calcium ions to enter a cell – probably causing LTP, while weaker depolarization only partially displaces the Mg^{2+} ions, resulting in less Ca^{2+} entering the post-synaptic neuron and lower intracellular Ca^{2+} concentrations (which activate protein phosphatases and induce long-term depression, LTD) (Bear *et al*., 2007).

These activated protein kinases serve to phosphorylate post-synaptic excitatory receptors (e.g. AMPA receptors), improving cation conduction, and thereby potentiating the synapse. Also, these signals recruit additional receptors into the post-synaptic membrane, stimulating the production of a modified receptor type, thereby facilitating an influx of calcium. This in turn increases post-synaptic excitation by a given pre-synaptic stimulus. This process can be reversed via the activity of protein phosphatases, which act to dephosphorylate these cation channels (Bear *et al*., 2007).

The second mechanism depends on a second messenger cascade regulating gene transcription and changes in the levels of key proteins at synapses such as CaMKII and PKAII(Protein Kinase A type II).Within the dendritic spine, the second messenger pathway is activated leading to increased levels of CaMKII and PKAII.These protein kinases have been linked to growth indendritic spine volume and LTP processes such as the addition of AMPA receptors to the plasma membrane and phosphorylation of ion channels for enhanced permeability (Haining *et al*., 2009).

Localization of activated proteins occurs in the presence of their given stimulus which creates local effects in the dendritic spine. Calcium influx from NMDA receptors is necessary for the activation of CaMKII. This activation is localized to spines with focal stimulation and is

inactivated before spreading to adjacent spines or the shaft, indicating an important mechanism of LTP in that particular changes in protein activation can be compartmentalized to enhance the responsivity of single dendritic spines. Individual dendritic spines are capable of forming unique responses to presynaptic cells.This second mechanism can be triggered by protein phosphorylation but takes longer and lasts longer, providing the mechanism for long-lasting memory storage (Seok *et al.*, 2009).

2.7.2 Synaptic strengthening and weakening

Fundamental studies of plasticity mechanisms have shown that every brief change cycle invokes all inputs whose coordinated actions moment by moment in time are correlated with a positive behavioral outcome known as a synapse-strengthening moment followed byweakening all inputs occurring within a brief, following epoch of time referred to as a synapse-weakening moment (Cooper and Bear, 2012).

2.7.3 Long term potentiation

Long-term potentiation (LTP) is a long-lasting enhancement in signal transmission between two neurons that results from stimulating them synchronously. It is one of several phenomena underlying synaptic plasticity, the ability of chemical synapses to change their strength. As memories are thought to be encoded by modification of synaptic strength, LTP is widely considered one of the major cellular mechanisms that underlie learning and memory (Cooke and Bliss, 2007). In LTP the AMPA receptors become sufficiently excited which leads to an influx of Na^+ which leads to a dramatic depolarization of the postsynaptic cell (Excitatory Post Synaptic Potential, EPSP). This EPSP releases the Magnesium ion blocking the NMDA receptor, and allows a Calcium-glutamate molecule to enter the cell. As intracellular $[Ca^{2+}]$ increases, protein kinases such as CaMKII and protein kinase C (PKC) are activated. The activation of these two

proteins allows for the 2 major mechanisms of LTP to proceed. These two mechanisms are the phosphorylation of existing AMPA receptors (AMPAr's) thereby increasing their sensitivity, and the increase of postsynaptic AMPAr's due to kinase activity(Seok *et al.*, 2009).

2.7.4 Long term depression

Long-term depression (LTD) is one of the two widely accepted models for the cellular and molecular mechanism that underlies synaptic changes during learning and memory.LTD is an activity-dependent reduction in the efficacy of neuronal synapses lasting hours or longer following a long patterned stimulus. LTD occurs in many areas of the CNS with varying mechanisms depending upon brain region and developmental progress. LTD in the hippocampus and cerebellum have been the best characterized, but there are other brain areas in which mechanisms of LTD are understood (Archour *et al*., 2010). LTD has also been found to occur in different types of neurons that release various neurotransmitters, however, the most common neurotransmitter involved in LTD is the NMDA receptors, AMPA receptors, kainate receptors (KARs) and metabotropic glutamate receptors. It can result from strong synaptic stimulation (as occurs in the cerebellar Purkinje cells) or from persistent weak synaptic stimulation (as in the hippocampus) (Massey and Bashir, 2007).

2.8 Date Palm Fruit (*Phoenix dactylifera*)

Date palms are monocotyledon, dioecious and can grow up to an altitude of 1500 m in well-drained soilsand windpollinated member of the Arecaceae family(Manjeshwar *et al.*, 2011). For the last 7000 years,*Phoenix dactylifera* has played an important role in the day-to-day life of the people.Production, utilization and industrialization of dates worldwide today, are continuously increasing since date fruits have earned great importance in human nutrition owing to their rich content of essential nutrients (Chandrasekaran and Ali, 2013).Dates fruits are used as staple food

in the Middle East for thousands of years. Khodry, Khalas, Ruthana, Bejjou, Sukkary, Sefri, Segae, Ajwa, Hilali and Munifiare various types of *phoenix dactylifera*found worldwide. Each type of dates has shown medicinal value in various type of disease prevention. Dates and their constituents show a role in diseases prevention through anti-oxidant, anti-inflammatory, anti-bacterial activity(Arshad *et al*., 2014).

There is increase in the production of date fruits as recorded for some of the major date producing countries which include: Egypt (1,352,950 metric tons), Saudi Arabia (1,078, 300 metric tons), Iran (1,023,130 metric tons), UAE (775,000 metric tons) and Algeria (710,000 metric tons).As a high value confectionery, and as a fresh fruit, it remains an important subsistence crop marketed all over the world. It is produced largely in the hot arid regions of the world particularly in Gulf Cooperation Council (GCC) countries (Chandrasekaran and Ali, 2013).In Nigeria, annual Dates fruits production is over 21,000metric tons.The Date fruit was introduced into Nigeria via trans-Sahara trade route from NorthAfrica and Muslim pilgrims on pilgrimage to theHoly cities of Mecca and Medinain the early 17th century.Date palm ispresently grown in northern Nigerian stateslike Katsina, Kano, Sokoto, KebbiJigawa, Yobe, Borno, Gombe, Bauchi andAdamawa States (AbdulQadir *et al*., 2011).Date palm (Phoenix dactylifera) is an ovalcylindrical fruit with one stone grooved down one side. Its color varies from pale yellow to light red depending on its ripeness. The tree's trunk is made up of strong cellulose fibers (Barreveld, 1993). The development of date fruits is divided into three stages: "Khalal" (full-size, crunchy), "Rutab" (ripe, soft), and "Tamr" (fully repined, sun-dried), and dates are generally harvested at "Tamr" stage, that is after the development of Total Soluble Solids (TSS) of 60–70 brix that are edible at this stage (Chandrasekaran and Ali, 2013).

Date is easily produced and stored and is rich in nutrition. Date fruit contains a high percentage of carbohydrates (44-88%), fat (0.2-0.5%), 15 salts, protein (2.3-5.6%), vitamin, high percentage of fiber (6.4-11.5%), pigments and tannin. The weight of date pit is 5.6-11.5% of the date fruit (Al-Shahib, 1993). However, different tests indicate that constitutes and their proportions depend on the cultivated species (Amira et al., 2011). Interestingly, when compared to other cultivars of fruits such as apples, oranges, bananas and grapes (containing 0.3, 0.7, 1.0 and 1.0% proteins, respectively) dates contain higher concentrations of proteins (Al-Humaid *et al.*, 2010).

In traditional medicinal practices, dates are considered as "tonic" and "aphrodisiac."(Rohini et al., 2014). Arabs considered date palm as “tree of life” and claimed it cures memory disturbances, fever and paralysis (Rohini et al., 2011). In a study on rats in which pain threshold was measured with warm container, it was demonstrated that dates had a distinct improving effect on memory, but not on weight and pain threshold (Zafari *et al.*, 2009). Also, Nasser et al. (2011) found that Phoenix dactylifera extract-diet intake in Streptozotocin-induced diabetic rats provided protection against deterioration of the peripheral nerve.

According to studies, by Asadi-Shekaari *et al.* (2008) and Panahi *et al.* (2008), Phoenix dactylifera extract significantly inhibited neuronal damage induced by cerebral ischemia. Also they resulted that the effectiveness of Phoenix dactylifera in focal cerebral ischemia most probably is due to its antioxidant property.

The development of the fruit is classified into four stages: “Khimiri” “Khalal” “Rutab” and “Tamer”. The tamer stage is the final stage of maturation when the date has dried to a fairly firm consistency with a darker color (Alshahib and Marshall, 2003).

In the Khimiri stage, the date development is longest and lasts for a total of nine to fourteen weeks. There is a visible transition from a small berry to the characteristic oblong shape. The fruit is green in color, quite hard, with a dry weight composition of 80% moisture and 50% sugar (glucose and fructose). It is usually bitter and unsuitable for eating.In the Khalal stage, the color changes from green to greenish yellow, yellow, pink, scarlet or red. This stage extends for six weeks and the fruit is physiologically mature, hard and ripe. The fruit attains maximum weight and size at the end of this stage. A rapid increase in the sugar concentration results following decrease in the water content (around 50–85% moisture content). The Rutab stage lasts between two to four weeks. The apex starts ripening and the texture of the fruit becomes soft. The astringency from the previous stage is gradually lost and the fruit starts acquiring brown or black color. Due to the consistent loss of moisture content,the weight further decreases. There is a gain in total sugars and solids with concomitant increase in the rate of conversion of sucrose to simpler sugars. Tamar stage (full ripe stage or final stage in the ripening) is the last stage of ripening and the date appears dehydrated. The semi-dry and dry dates will have nearly 50% eachof sucrose and reducing sugars. On a bunch, the fruits ripen over a month and not simultaneously (Manjeshwar *et al.,* 2011).

2.8.1 Phytohemical composition of date palm fruits

Based on the phytochemical studies, date fruits contain anthocyanins, phenolics, sterols, carotenoids, procyanidins and flavonoids. These natural compounds are known to function as free radical scavenger, antioxidant, antimutagenic, anti-inflammatory, hepatoprotective and nephroprotective agents (Baliga *et al*., 2011).

The dates are rich in sugar (71.2–81.4% dry weight), while ash represented 1.68–3.94%; they contained low concentrations of protein and lipid (1.72–4.73% and 0.12–0.72%, respectively).

Date fruit contains significant amounts of minerals.They also contain vitamins like riboflavin, thiamine, biotin, folic and ascorbic acid that are essential for the body). The pulps are rich in iron, calcium, cobalt, copper, fluorine, magnesium, manganese, potassium, phosphorus, sodium, copper, sulfur, boron, selenium and zinc(Al Farsi and Lee, 2008). The potassium concentration is the highest(289.6–512 mg/100 g dry matter), followed in descen-ding order by calcium (123–187 mg/100 g), magnesium (56–150 mg/100 g), phosphorus (12–27 mg/100 g)and sodium (4.9–8.9 mg/100 g). Also, Date fruits contain high concentrations of aspartic acid, proline, alanine, glycine, valine and leucine; low concentrations of threonine, serine, isoleucine, tyrosine, arginine, phenylalanine and lysine and very low concentrations of methionine and histidine. This shows that dates are nutritious and can play a major role in human nutrition and health (Eman, 2014).

Date fruits contain phenolic acids that constitute one of the main classes of secondary metabolites. It contains a hydroxylated benzene ring with one or more carboxyl groups attached directly or indirectly to it.Comparative studies with fresh and dried dates have shown that a significant increase in phenolic content ensues on drying, possibly due to the degradation of tannins and maturation of degradative enzymes at higher temperatures (Alfarsi *et al.*, 2005).Flavonoids present in plants possess diverse health benefits, which includes antioxidant and radical scavenging activities, reduction of certain chronic diseases, prevention of some cardiovascular disorders and certain kinds of cancerous processes (Manjeshwar *et al.*, 2011).

2.8.1.1. *Enzymes in date palm fruits*

Enzymes that play an important role in the conversion processes that take place during formation and maturation of the date fruit include: Invertase: responsible for the inversion of sucrose into glucose. Polygalacturonase and pectinesterase both convert insoluble pectic substances into more

soluble pectins, contributing to softness of the fruit. Cellulase: breaks down cellulose into shorter chain substances with increasing solubility and eventually leading to glucose, thus decreasing fibre content.Polyphenol oxidase is responsible for biochemical changes of polyphenols to which the tannins belong; it is plays a vital role in non-oxidative browning reactions of the date fruit (FRDR, 2014).

2.9 Date Palm Fruits and Cognition

Date fruit isone of the dietary supplements which have great importance from nutritional and economic points of view and it is composed of a fleshy pericarp and seed.*Phoenix dactylifera* provides a physical benefit that is important to the brain, also known as neuroprotective or cerebroprotective effect. In general, this neuroprotective effect protects the brain from the destructive activity of the reactive oxygen species (ROS) that could come from the cell metabolism or from other exogenous sources. Hence,date fruit extracts have been recently identified as promising neuroprotective agents in several models of neurodegeneration.This neuroprotective effect is basically an extension from the previously known antioxidant effect of palm date as it operates based on similar concept. Date palm is an excellent antioxidant agent and this is due to the high concentrations of phenolic compounds, flavonoids and anthocyanins as well as the presence of selenoproteins(Asadi *et al.*, 2008;Wan and Mohd, 2013). The neuroprotection provided by date fruits might be as a result of strong antioxidants present inthem. Recent findings suggest that antioxidant agents might exert neuroprotective effects and may be promising in therapy of neurological deficits and thismight be from melatonin present it (Nasser *et al.*, 2011). Methalonic extract of*Phoenix dactylifera* fruit and pits have effectiveness on inhibition of the selective serotonin reuptake, prevent the hyperactivity of the immune system, oxidative stress profile, lipid profile, and improved the sucrose consumption, which play a role in

improving the etiology of depression (Ragaa *et al*., 2014).The dates are believed to boost immunity, relief against pains and ailments including fever, memory disturbances, nervous disorders and abdominal disorders (Vyawahare *et al.,* 2009).In a study on rats in which pain threshold was measured with warm container, it was demonstrated that dates had a distinct improving effect on memory, but not on weight and pain threshold (Zafari *et al.*, 2009).

2.10 Date Palm Fruits and Other Components of Nervous System

Hashempour, (1991) recommended that Date fruit could be used in treatment of infantile paralysis, because of its vital role instrengthening the nervous system in children. Also, Date palm fruits play role in etiology of depression. The fruit and pit were found toinhibit selective serotonin reuptake thereby, preventing immune system hyperactivity, oxidative stress profile, with lipid effects, and improve the sucrose consumption, which play a role in improving the etiology of depression (Ragaa *et al.,* 2014). The methanolic extract of *Phoenix dactylifera*improved motor co-ordination and locomotor activity in mice(Vyawahare *et al.,* 2009). Aqueous and methanolic extracts of date fruits possesses analgesic activity. They suppressed swelling in foot and adjuvant arthritis (Vyawahare *et al.,* 2009). Date fruit containsZea-xanthin which is an important dietary carotenoid that is selectively absorbed into the retinal macula lutea. In elderly populations Zea-xanthinoffers protection against age-related macular degeneration by providing antioxidant and protective light-filtering functions (USN, 2014).

2.11 Date Palm Fruits and Other Body Systems

2.11.1 Nephro-protection

Phoenix dactylifera extracts play a vital role in nephron-protection. In gentamicin-induced nephrotoxicity rat model, *Phoenix dactylifera*significantly reduced the increase in plasma

creatinine and urea concentrations ameliorated the proximal tubular damage (Arshad *et al.*, 2014).

2.11.2Haemopoietic activity:

Dose dependent increase in absolute values of red blood cells, hemoglobin, hematocrit, reticulocytes and platelet counts was observed on administration of crude methanolic and aqueous extracts of date fruit on peripheral blood parameters in Wistar rats(Onuh *et al.,* 2012).

2.11.3 Reproductive system

Date fruit enhances cervical dilation and ripening in last weeks of gestation in pregnant women.It seems that date fruit influences oxytocin receptors, stimulates the uterine muscles to respond more comfortably to oxytocins, and better prepares the uterus and cervix for delivery (Masoumeh *et al*., 2014). Onmale fertility, date palm pollen (DPP) improves the sperm count, motility, and morphology, and DNA quality with increase in weights of the testis and epididymis. DPP seems to ameliorate male infertility by improving the quality of sperm parameters(Bahmanpour *et al*., 2006), and act as a dopamine agonist to cure male infertility. It can be used as an aphrodisiac that leads to further increases in dopamine release (Ali *et al*., 2014).

2.11.4 Antimicrobial activity

Phoenix dactylifera and its constituents play a significant role in the prevention or treatment of bacterial diseases. An important study showed that the effect of methanol and acetone extracts of leaves and pits *Phoenix dactylifera* inhibited the growth of F. oxysporum, Fusarium sp., F. solani, A. alternata, Alternaria sp, Gram positive and Gram negative bacteria (Bokhari *et al.,*

2012).Another recent study in the support of *P. dactilifera* effect as antimicrobial on *KlebsiellaPneumonia* and *Escherichia coli* and also showed a role in reducing the side effects due to the use of drugs as methylprednisolone (Aamir *et al*., 2013).Another study also showed that *Phoenix dactylifera* extract has antibacterial effect against E. fecalis, indicating that this extract can be used for treating enteric diseases (Arshad *et al.,* 2014). Also, Boulenouar *et al*. (2011) found that*Phoenix dactylifera* presented some similarities concerning phytochemical screening results related to their resistance or sensibility to Foa. It was observed that the majority of bioactive compounds against Foa seem to be polyphenols present in the fruit. Thus, the natural defence mechanism in vivo against Foa is probably related to the action of polyphenols.

2.11.5 Hepatoprotection

Findings of Emna *et al.*,(2010)indicate that in vivo date palm fruit may be useful for the prevention of oxidative stress induced hepatotoxicity.Pretreatmentwithdatepalmfruitextractrestoredtheliver function afterdamageinduced by dimethoate,asrevealedbyinhibitionofhepaticlipidperoxidation,ameliorationof Superoxide dismutase (SOD),and Catalase (CAT) activities.

2.11.6 Gastrointestinal stimulatory activity:

Date pulp extract significantly and dose dependently increased GIT activity while the palm sap slightly increased it (Souli *et al*., 2014). Aqueous fruit extract of *Phoenix datylifera*showed antidiarrheal activity, it significantly decrease frequency of defecation and decreased gastrointestinal motility. It showed greater anti-enteropooling effect than standard drug loperamide (Abel *et al*., 2013).

2.11.7 Date fruit extract (DFE) and diabetes mellitus

DFE treatment shows efficacy for preventing diabetic deterioration and for improving pathological parameters of diabetic neuropathy in rats. In STZ-induced model of diabetic neuropathy, impairment of the explorative activity of the rats in an open field behavioral test and of the conduction velocity of the sciatic nerve wascounteracted with chronic treatment for 6 weeks with DFE(Nasser *et al*., 2011).

CHAPTER THREE

3.0 Materials and Methods

3.1 Animals

Seventy five (75) mice of both sexes, age 6-8weeks were purchased from the Animal House, Department of Human Physiology, Faculty of Medicine,Ahmadu Bello University(A.B.U) Zaria-Nigeria. The mice were housed in standard cages containing sawdust bedding. They were fed with pellets made from grower's mash, maize offal as binder and *water ad libitum*. The weight of the mice ranged between 18 to 26g. Twenty five mice were used in each of the Morris water, Barnes and elevated plus mazes paradigms. The same mice used in the paradigms were used for the neurochemical assay.

The animals were grouped into five groups. Each group consisting of five mice (n=5)

Group I: Animals served as the negative control group (Distilled water 10ml/kg)

Group II: Animals received Piracetam 100mg/kg orally

Group III: Animals received PDE 250mg/kg orally

Group IV: Animals received PDE 500mg/kg orally

Group V: Animals received PDE 1000mg/kg orally

3.2 *Phoenix dactylifera*

Fresh fruit of *Phoenix dactylifera* (Bejjou type) was purchased from Tudun Wada Market, Zaria. It was identified in by Mr. Musa Muhammad of the Herbarium Section of theDepartment of Biological Sciences A.B.U Zaria-Nigeria by comparing with existing Voucher Specimen Number (3493).The flesh of the date fruit was removed from the pit, then grinded using pestle and mortal. 600g was taken and dissolved in 2 litres of distilled water, then was allowed to settle for 24 hours. The filtrate was put in glass container and kept in water bath (temperature 63.1^0C) 24 hours for drying (Rohini *et al*., 2014).

3.3Drug Preparation

Piracetam 800mg was purchased fromLaboratories Sothema, Casablanca, Morocco. Manufacturing date: 24/12/2005, expirydate 11/2016. Batch number: LOT 12 24 05, UTAV 11 16, PPM 171 40.

3.4 Neurobehavioral Assay

Animals that received aqueous extract of *Phoenix dactylifera*and Piracetam were treated 1 hour prior to the commencement of the experiment daily in all the neurobehavioral paradigms

3.4.1 Morris water maze

The Morris water maze, also known as Morris water navigation task is a behavioral procedure widely used to study spatial learning and memory (Hooge and Deyn, 2001). It is an open circular

pool that is filled with water. It is a ‘maze’ in the sense that the animal must search in order to locate an escape platform that is submerged below the water surface and placed in a fixed location. Two principal axes of the maze were designated each line bisecting the maze perpendicular to one another to create an imaginary ‘+’. The end of each line demarcates four cardinal points: North (N), South (S), East (E) and West (W) making four quadrants. The diameter of the pool is 133cm

The circular pool was filled with water to a depth of about 14cm at room temperature (37^{o}C). Escape plat form was a sealed cylindrical container with a height of 13cm and diameter of 5cm. It was submerged 0.5cm below the water surface in the middle of the target quadrant.

Each animal was placed in a desired start position in the maze, facing the tank wall. A timer was started the moment the animal is released. The timer was stopped when the animal reached the platform. A trial limit 2 min per trial was adopted. Animals not finding the platform within this time limit were placed on the platform or guided to it (Cain *et al.,* 1996). Each animal was left on the platform for 20s. The animal was placed in the maze at new start position until it had 4 trials in a day with inter-trial interval of 15 minutes. On subsequent day the same trials were repeated. Then on the third day the plat form was removed and each animal undergo only one trial for 90seconds known as probe trial. The time spent in the quadrant where plat form was removed was taken as an indexof cognition. The water was changed after trials of each day of the

experiment.

Plate I:Morris water maze (Snapped by the candidate 09/12/2014)

3.4.2 Elevated plus maze for memory

An elevated plus-maze test was conducted as described by (Komada *et al.*,2008).The elevated plus-maze consisted of two open arms (25 × 5 cm) and two enclosed arms of the same size with 15-cm high wooden walls. The arms and central square are made of wood elevated 55 cm above the floor. Arms of the same type are located opposite from each other.

Each mouse was placed at the distal part of an open arm maze (5 × 5cm), facing away from the closedarms. On the training day (first day), each animal was placed at the end of one open arm, facing away from the central platform. The latency of the mouse to move from the open to the enclosed arms was recorded within 90s. Following entry into the arm, the animals were allowed to explore the apparatus for 20s. Twenty-four hours later, the second trial (retention test) was performed and the animals were observed for 90s. After each trial the maze was wiped with a cloth dipped in 70% ethanol and allowed to dry to remove any olfactory cue.

Plate II: Elevated Plus Maze (Snapped by the candidate 09/12/2014).

3.4.3 Barnes maze

Barnes maze test was first developed by Carol Barnes in 1979 (Barnes, 1979). The test was originally developed for use with rats to overcome the stress induced by swimming in the Morris water maze (MWM), and later was adapted for mice. During the task, mice will be placed in the middle of a circular table containing holes around the edges and receive negative reinforcement, in the form of bright lights, motivating them to escape to a dark cage hidden underneath one of the holes (escape box or plat form) (Aida *et al.,* 2013).

Barnes maze test consists of a habituation phase (training phase) and probe trial. Each mouse was directed to the escape box and allowed to stay for 2minutes, then placed in the centre and allowed to locate the escape box itself within period of 3 minutes. Latency to locate the escape box (primary latency), time taken to enter the escape box (total latency), number of head dips into incorrect holes before locating the escape hole (primary error),and number of head dips into incorrect holes for the whole experiment (total error) were taken. Mouse unable to locate the escape box within 3 minutes were directed towards the box and allowed to stay in for 20seconds. The same procedure was repeated in the second day. A probe phase was performed following a 48-hours delay, in which the mouse was tested for remembering what had been previously learned. In probe trial each mouse was given 90 seconds. Latency to locate hole where plat form was placed beneath, number of head dips into incorrect holes and correct hole were taken.After each trial the maze was wiped with a cloth dipped in 70% ethanol and allowed to dry to remove any olfactory cue.

Plate III: Barnes maze (Snapped by the candidate 09/12/2014)

3.5 Biochemical Analysis

3.5.1 Brain tissue preparation

Mice brain tissues were collected and prepared according to the method described by Zatta *et al.*(2002) and Habila *et al.*(2012). Treated and control animals were sacrificed by decapitation under anesthesia and brain tissue was immediately removed andplaced on an inverted Petri dish on ice.The fore brain was dissected, weighed and homogenized in 10ml of a medium containing a solution of 0.1 Msodium phosphate 10 W/V, pH 7.5.The total homogenate was centrifuged at 1000 rev/min in a refrigerated centrifuge for 7 minute. Aliquots of resulting brain homogenates were stored at -20^0 C until utilization.The supernatants were used for AChE activity determination.

3.5.2 Acetylcholinesterase assay kit

Acetylcholinesterase assay kit (Calorimetric) ab138871 was purchased from Abcam PLC United Kingdom. The acetylcholinesterase enzyme (AchE) assay was conducted at Centre of Excellence in Veterinary Epidemiology, Department of Veterinary Public Health and Preventive Medicine, A.B.U Zaria, Nigeria.

Ab138871 provides a convenient method for the detection of AchE activity. It uses DTNB (5, $5^!$ -dithiobis-(2-nitrobenzoic acid) to quantify the thiocholine produced from the hydrolysis of acetylthiocholine by AchE in brain tissues. The absorption intensity of DTNB adduct is used to measure the amount of thiocholine formed, which is proportional to the AchE activity (abcam, 2014).

3.5.3Acetylcholinesterase assay

0.6ml of assay buffer was added into the vial of DTNB to make 20×DTNB stock solution, 0.6ml of distilled water was added into the vial of acetylthiocholine to form acetylthiocholine stock solution (reaction mixture). 100µl of distilled water with 0.1% BSA (Bovine Serum Albumin) into the acetylcholinesterase standard solutions.Serial dilutions were conducted to form 1000, 300, 100, 30, 10, 3, 1 and 0 mU/ml of the acetylcholinesterase standard. These dilution solutions and acetylcholinesterase-containing test samples were put into white bottom 96-well microplate.50µl of acetylthiocholine reaction mixture was added to each well of the acetylcholinesterase standard, blank control, and test samples to make the total acetylcholinesterase assay volume of 100µl/well. The reaction was protected from light and was incubated for 30minutes at room temperature. The samples were then put in microplate reader at wavelength of 450nm and the absorbance increase was recorded.

Graph of absorbance was plotted against acetylcholinesterase enzyme concentration in mU/ml using the standard curve described in the protocol. Values of acetylcholinesterase enzyme concentration above 1000mU/ml were considered as zero. Decrease in acetylcholinesterase enzyme concentration is an indicative of increased cholinergic transmission and improved memory and vice-versa (Meerah and Mamatha, 2014).

3.6 Statistical Analysis

Results were expressed as Mean ± SEM, analyzed using factorial repeated measures and one-way ANOVA and Tukey's post-hoc test for multiple comparisons. Results for retention of elevated plus maze were presented as Mean ± SEM and analyzed by Kruskal-Walli's test. Acetylcholinesterase activity was correlated with probes of neurobehavioral tests using Pearson correlation test. Values with $P< 0.05$ were considered significant**.**

CHAPTER FOUR

4.0 Results

4.1 Assessment of Visio-Spatial Long Term Memory using Morris Water Maze

4.1.1 Latency to locate escape platform

In latency to locate escape platform,there was statistical significant difference between negative control group (distilled water 10ml/kg (54.80 ± 6.7)) and PDE 1000mg/kg(91.40 ± 5.3). Likewise, statistical significant difference was observed between Piracetam 100mg/kg, a positive control(56.80 ± 7.0) and PDE 1000mg/kg(91.40 ± 5.3)in day one and two,$F(4,20)=5.644$, $p=0.003$.In day 2, significant difference was seen between Piracetam (34.00 ± 4.8) and PDE 1000mg/kg & PDE 500mg/kg (60.00 ± 6.5 and 59.80 ± 4.0 respectively) with $p<0.05$ (Figure4.1).

4.1.2 Frequency of platform crossings

No significant difference was seen between negative control (1.8 ± 0.4) and PDE treated groups 1000, 500 and 250 mg/kg with means (2.0 ± 0.3, 0.8 ± 0.5 and 1.0 ± 1.7 respectively), $F(4,20)=0.827$, $p=0.523$. Likewise no significant difference was seen between positive control (Piracetam 100mg/kg) with mean (1.2 ± 1.6) and PDE treated groups (Table 4.1).

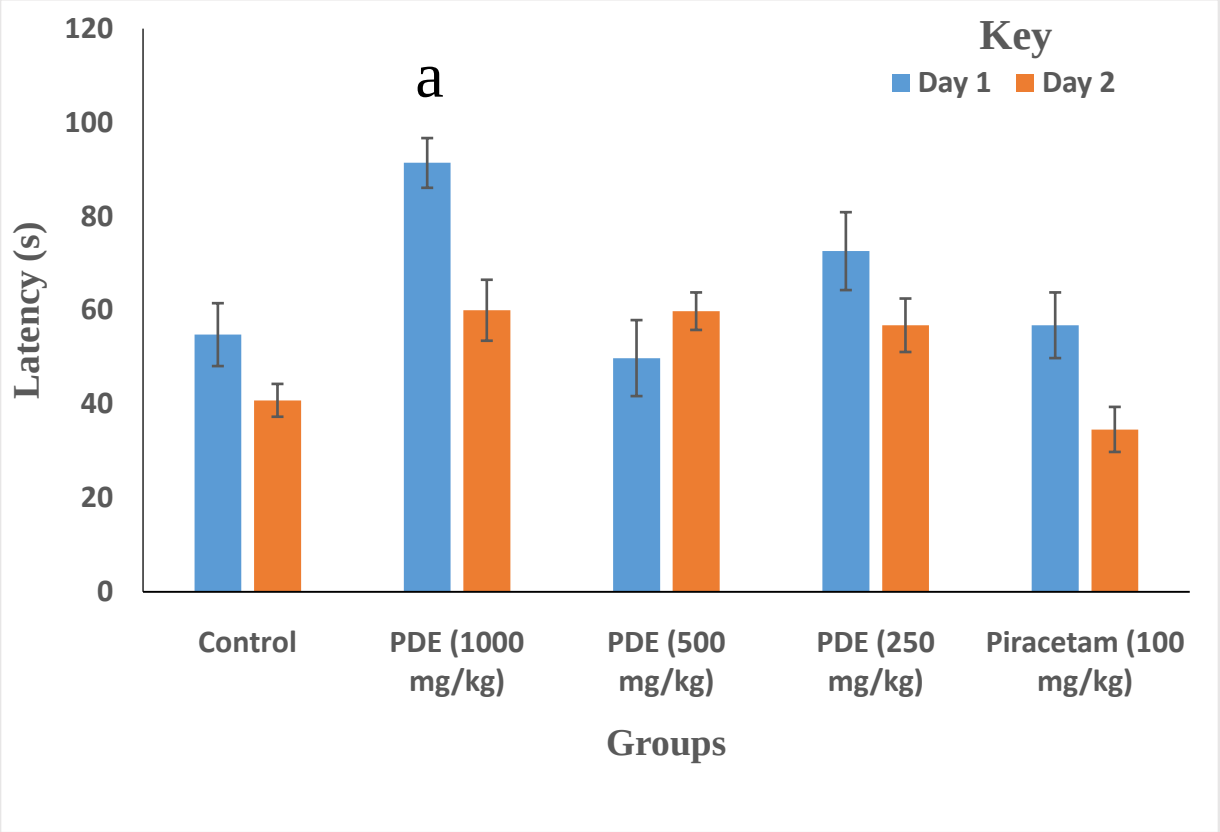

Figure 4.1: Effect of *Phoenix dactylifera* extract on learning in mice during a two-day training in Morris water maze

a: Mean difference is statistically significant when compared with control $p<0.05$ (n=5) SPSS version 20.

Table 4.1: Effect of *Phoenix dactylifera* extract (PDE) on memory in mice during one-day probe trial in Morris Water Maze (n=5)

Groups	Frequency of Platform Crossings
Control	1.8 ± 0.4
PDE (1000mg/kg)	2.0 ± 0.3
PDE (500mg/kg)	0.8 ± 0.5
PDE (250mg/kg)	1.0 ± 1.7
Piracetam (100mg/kg)	1.2 ± 1.6

The mean difference is not statistically significant when compared with control, $p > 0.05$ (n=5), SPSS version 20. PDE=*Phoenix dactylifera* extract.

4.1.3 Time spent in quadrants

There was statistically significant difference between time spent by negative control, PDE treated groups and Piracetam treated group in quadrants 2 and 3 when compared with quadrant 4 (platform's quadrant), $F(4,20)=75.826$, $p=0.000$, no significant difference was observed between time spent by all the groups in quadrant 1 when compared with quadrant 4(Table 4.2).

4.2 Assessment of Visio-Spatial Long Term Memory using Elevated Plus Maze for Memory in Mice

4.2.1 Acquisition and retention sessions

No significant difference was observed between control (81.8 ± 8.2) and PDE treated groups: 1000, 500 and 250 mg/kg (81.4 ± 8.6, 43.0 ± 16.0 and 58.8 ± 19.1, respectively), $F (4, 20)=1.552$, $p=0.226$, (Figure 4.2). There was no statistically significant difference was observed between control (23.0 ± 11.1) and PDE treated groups: 1000, 500 and 250 mg/kg (48.6 ± 17.39, 24.6 ± 16.4 and 30.8 ± 15.3, respectively), $F (4, 20) = 0.913$, $p=0.476$, (Figure 4.2).

4.3 Assessment of Visio-Spatial Long Term Memory using Barnes Maze

4.3.1 Time spent to locate escape box (Primary Latency)

No statistically significant difference was seen when primary latency of PDE treated groups: 1000, 500 and 250 mg/kg (147.8 ± 32.2, 151.8 ± 23.7 and 153.0 ± 27.0, respectively) when compared with control (136.8 ± 26.5) in day 1. Also, no significant difference was seen between PDE treated groups (178.4 ± 1.6, 178.4 ± 1.6 and 150.0 ± 30.0 respectively) when compared with control (180.0 ± 0.0) in day 2, $F (4, 20) =0.132$, $p=0.72$, (Table 4.3).

Table 4.2: Effect of *Phoenix dactylifera* extract (PDE) on memory in mice during a one-day probe trial in four-quadrants of Morris Water Maze.

Groups	Latency (s)			
	Quadrant 1	Quadrant 2	Quadrant 3	Quadrant 4
Control	15.4 ± 3.4	6.2 ± 1.0[a]	13.6 ± 1.2[a]	21.0 ± 2.9
PDE (1000mg/kg)	14.6 ± 0.7	5.4 ± 0.4[a]	10.4 ± 1.1[a]	21.2 ± 3.0
PDE (500mg/kg)	20.6 ± 1.5	10.2 ± 0.7[a]	8.6 ± 1.7[a]	19.2 ± 2.2
PDE (250mg/kg)	16.0 ± 0.6	9.2 ± 1.5[a]	14.0 ± 2.9[a]	20.8 ± 1.1
Piracetam	16.0 ± 1.5	7.8 ± 0.6[a]	11.4 ± 2.5[a]	19.4 ± 0.4

a: Themean difference is statistically significant when compared with quadrant 4 $p < 0.05$ (n=5), SPSS version 20. PDE=*Phoenix dactylifera* extract.

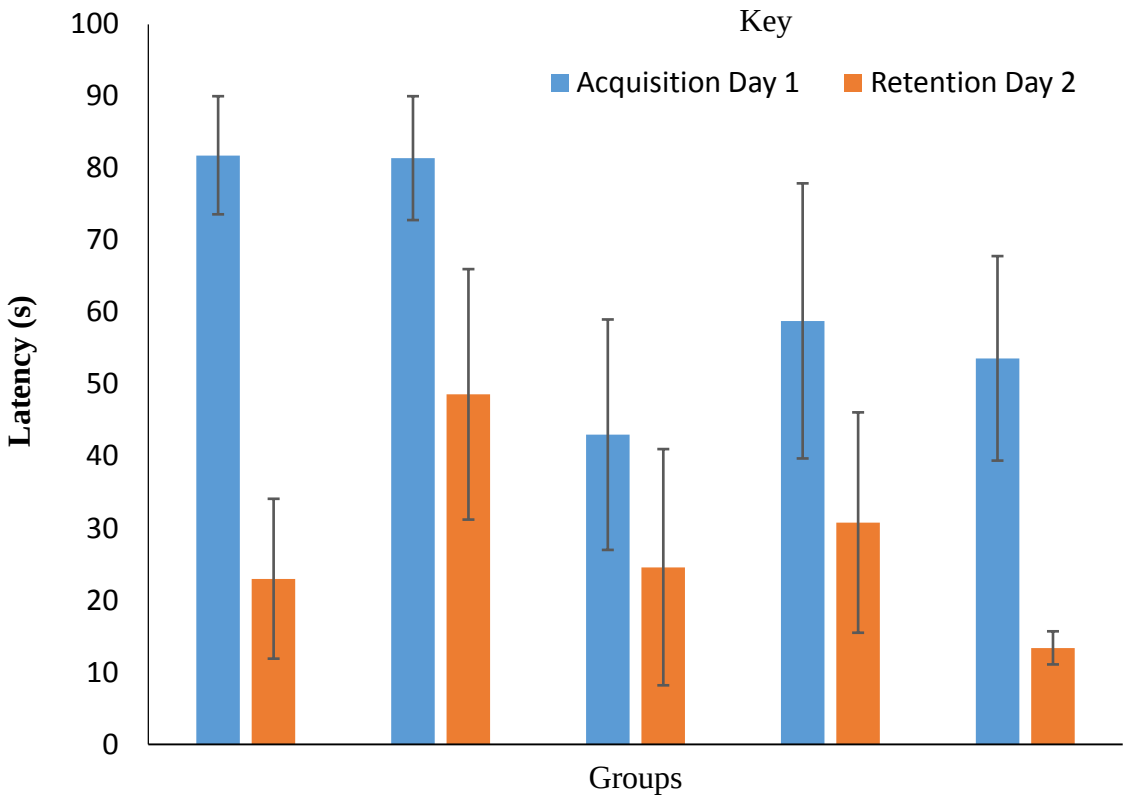

Figure 4.2: Effect of *Phoenix dactylifera* extract on learning and memory during elevated plus maze for memory.

The mean difference is not statistically significant when compared with control, $p > 0.05$ (n=5), SPSS version 20.

Table 4.3: Effect of *Phoenix dactylifera* extract (PDE) on learning in mice during a two-day acquisition session in Barnes maze- primary latency

Groups (Dose)	Primary latency (s)	
	Day 1	Day2
Control	136.8 ± 26.5	180.0 ± 0.0
PDE (1000mg/kg)	147.8 ± 32.2	178.4 ± 1.6
PDE (500mg/kg)	151.8 ± 23.7	170.4 ± 9.6
PDE (250mg/kg)	153.0 ± 27.0	150.0 ± 30.0
Piracetam (100mg/kg)	180.0 ± 0.0	118.6 ± 37.7

The mean difference is not statistically significant when compared with control 4 $p > 0.05$ (n=5), SPSS version 20. PDE=*Phoenix dactylifera* extract.

4.3.2 Total time spent to enter escape box (Total Latency)

There was no statistically significant difference between PDE treated groups: 1000, 500 and 250mg/kg (149.0 ± 3.0, 153.8 ± 23.1 and 180.0 ± 0.0, respectively) when compared with control (136.0 ± 26) in day 1. Also, no significant difference was seen between PDE treated groups (170.8 ± 9.2, 180 ± 0.0 and 180 ± 0.0, respectively) when compared with control (178.4 ± 1.6) in day 2, $F(4, 20)=1.772$, $p=0.198$, (Table 4.4).

4.3.3 Primary error

No statistically difference was seen in primary error of PDE treated groups: 1000, 500 and 250 mg/kg when compared with control in day 1 and 2 with $p>0.05$. (11.4 ± 2.8, 14.6 ± 3.0 and 7.4 ± 2.1 for PDE 1000, 500 and 250mg/kg and control 11.4 ± 1.9 in day 1, 18.6 ± 8.7, 11.4 ± 2.6 and 8.0 ± 5.0 for PDE 1000, 500 and 250mg/kg respectively, 10.4 ± 3.7 for control in day 2), (Table 4.5).

4.3.4 Total error

There was no statistically difference between the total error of PDE treated groups: 1000, 500 and 250 mg/kg when compared with negative and control in day 1 and 2 with $p>0.05$. The total error in day 1 was 9.8 ± 3.1, 15.4 ± 2.8 and 7.4 ± 2.1 for 1000, 500 and 250 mg/kg of PDE treated groups and control 11.4 ± 1.9. The total error in day 2 was 21.2 ± 5.6, 11.4 ± 2.6 and 15.2 ± 7.6 for PDE 1000, 500 and 250 mg/kg respectively, and 10.4 ± 3.7 for control, (Table 4.6).

Table 4.4: Effect of *Phoenix dactylifera* extract (PDE) on learning in mice during a two-day acquisition session in Barnes maze-total latency

Groups	Total latency (s)	
	Day 1	Day 2
Control	136.0 ± 26.5	178.4 ± 1.6
PDE (1000mg/kg)	149.0 ± 3.0	170.8 ± 9.2
PDE (500mg/kg)	153.8 ± 23.1	180.0 ± 0.0
PDE (250mg/kg)	180.0 ± 0.0	180.0 ± 0.0
Piracetam (100mg/kg)	153.4 ± 26.0	151.6 ± 28.4

The mean difference is not statistically significant when compared with control, $p > 0.05$ (n=5), SPSS version 20. PDE=*Phoenix dactylifera* extract.

Table 4.5: Effect of *Phoenix dactylifera* extract (PDE) on learning in mice during two-day acquisition session in Barnes maze- primary error

Groups (Dose)	Frequency of Primary Error	
	Day 1	Day 2
Control	11.4 ± 1.9	10.4 ± 3.7
PDE (1000mg/kg)	11.4 ± 2.8	18.6 ± 8.7
PDE (500mg/kg)	14.6 ± 3.0	11.4 ± 2.6
PDE (250mg/kg)	7.4 ± 2.1	8.0 ± 5.0
Piracetam (100mg/kg)	8.8 ± 2.2	6.2 ± 2.8

The mean difference is not statistically significant when compared with control, $p > 0.05$ (n=5), SPSS version 20. PDE=*Phoenix dactylifera* extract.

Table 4.6: Effect of *Phoenix dactylifera* extract (PDE) on learning in mice during a two-day acquisition session Barnes maze- total error

Groups (Dose)Number of Total Error				
		Day 1		Day 2
Control		11.4 ± 1.9		10.4 ± 3.7
PDE (1000mg/kg)	9.8 ± 3.1	21.2 ± 5.6		
PDE (500mg/kg)	15.4 ± 2.8	11.4 ± 2.6		
PDE (250mg/kg)		7.4 ± 2.1	15.2 ± 7.6	
Piracetam (100mg/kg)		8.0 ± 2.1	9.8 ± 3.4	

The mean difference is not statistically significant when compared with control, $p > 0.05$ (n=5), SPSS version 20. PDE=*Phoenix dactylifera* extract.

4.3.5 Latency to locate escape platform

No statistically significant difference was observed between the time taken to locate escape platform of PDE treated groups: 1000, 500 and 250mg/kg when compared with control with $p>0.05$. The latency was: 71.6 ± 26.2,67.4 ± 23.4 and 90.0 ± 0.0for 1000, 500 and 250mg/kg for PDE treated groups respectively and 83.0 ± 15.7 for control (Table 4.7).

4.3.6 Frequency of head dips in correct hole

There was no statistically significant difference was between the frequency of head dips in correct hole of PDE treated groups: 1000, 500 and 250 mg/kg when compared with control with $p>0.05$. The frequency of head dips in correct hole was: 1.4 ± 2.2, 1.6 ± 2.5 and 0.0 ± 0.0 respectively for 1000, 500 and 250 mg/kg of PDE treated groups and 2.0 ± 4.5 for control (Table 4.8).

4.3.6 Frequency of head dips in incorrect holes

No statistically significant difference was seen between the frequency of head dips in incorrect holes of PDE treated groups: 1000, 500 and 250 mg/kg when compared with control $p>0.05$. The mean frequency of head dips in incorrect hole was: 2.0 ± 0.7, 1.2 ± 1.1 and 1.4 ± 0.9 respectively for 1000, 500 and 250 mg/kg of PDE treated groups, 1.2 ± 0.8 for control (Table 4.9).

Table 4.7: Effect of *Phoenix dactylifera* extract (PDE) on memory in mice during a one-day probe trial in Barnes maze- latency to locate platform

Groups (Dose)	Latency to locate escape platform (s)
Control	83.0 ± 15.7
PDE (1000 mg/kg)	71.6 ± 26.2
PDE (500 mg/kg)	67.4 ± 23.4
PDE (250 mg/kg)	90.0 ± 0.0
Piracetam (100 mg/kg)	89.2 ± 1.8

The mean difference is not statistically significant when compared with control, $p > 0.05$ (n=5), SPSS version 20. PDE=*Phoenix dactylifera* extract.

Table 4.8: Effect of *Phoenix dactylifera* extract (PDE) on memory in mice during a one-day probe trial in Barnes maze-head dip in correct hole

Groups (Dose)	Frequency of head dips in correct hole
Control	2.0 ± 4.5
PDE (1000 mg/kg)	1.4 ± 2.2
PDE (500 mg/kg)	1.6 ± 2.5
PDE (250 mg/kg)	0.0 ± 0.0
Piracetam (100 mg/kg)	0.4 ± 0.9

The mean difference is not statistically significant when compared with control, $p > 0.05$ (n=5), SPSS version 20. PDE=*Phoenix dactylifera* extract.

Table 4.9: Effect of *Phoenix dactylifera* extract (PDE) on memory in mice during a one-day probe trial in Barnes maze-head dip in incorrect holes

Groups(Dose)	Frequency of head dips in incorrect holes
Control	1.2 ± 0.8
PDE (1000mg/kg)	2.0 ± 0.7
PDE (500mg/kg)	1.2 ± 1.1
PDE (250mg/kg)	1.4 ± 0.9
Piracetam (100mg/kg)	1.0 ± 0.7

The mean difference is not statistically significant when compared with control, $p > 0.05$ (n=5), SPSS version 20. PDE=*Phoenix dactylifera* extract

4.4 Acetylcholinesterase Enzyme Activity

4.4.1 Acetylcholinesterase enzyme activity of mice used in Morris water maze

There was a statistically significant difference in acetylcholinesterase enzyme concentration (mU/ml) between thecontrol group (distilled water 10ml/kg) and piracetam 100mg/kg ($p<0.05$). The acetylcholinesterase enzyme concentration was 12.8 ± 4.9 for control and 453.0 ± 172.4 for piracetam, $F(4,20)=6.463$, $p=0.002$. No statistically significant difference was seen between PDE treated groups when compared with control, $p>0.05$ (Table 4.10).

4.4.2 Acetylcholinesterase enzyme activity of mice used in elevated plus maze for memory

No statistically significant difference was observed in acetylcholinesterase enzyme concentration (mU/ml) between PDE treated groups and control. The acetylcholinesterase enzyme concentration was 228.0 ± 142.5, 320.0 ± 460.4 and 6.0 ± 13.4 for PDE 1000, 500 and 250 mg/kg respectively, and 200.0 ± 200.0 for control, $F (4, 20) =0.982$, $p=0.440$, values of acetylcholinesterase enzyme concentration for Piracetam were above standard (1000 mU/ml) and considered 0.00 (Table 4.11).

4.4.3 Acetylcholinesterase enzyme activity of mice used in Barnes maze

There was statistically significant difference in acetylcholinesterase enzyme concentration (mU/ml) between control group and Piracetam 100 mg/kg, AchE concentrations were 72.0 ± 4.5 and 280.4 ± 78.8 for control and Piracetam respectively, $F(4,20)=7.938$, $p=0.001$. No statistically significant difference was observed between control and PDE treated groups, $P>0.05$ (Table 4.12).

Table 4.10: Effect of *Phoenix dactylifera* extract (PDE) on acetylcholinesterase enzyme activity in mice used in Morris water maze

Groups	Acetylcholinesterase concentration (mU/ml)
Control (distilled H_2 0 10ml/kg)	12.8 ± 4.9
PDE (1000mg/kg)	8.7 ± 2.1
PDE (500mg/kg)	5.1 ± 1.2
PDE (250mg/kg)	34.0 ± 4.7
Piracetam (100mg/kg)	453.0 ± 172.4[a]

a: the mean difference is statistically significant at $p<0.05$ when compared to control, (n=5) SPSS version 20.PDE=*Phoenix dactylifera* extract

Table 4.11: Effect of *Phoenix dactylifera* extract (PDE) on acetylcholinesterase enzyme activity in mice used in elevated plus maze for memory

Groups	Acetylcholinesterase concentration (mU/ml)
Control	200.0 ± 200.0
PDE (1000mg/kg)	228.0 ± 142.5
PDE (500mg/kg)	320.0 ± 460.4
PDE (250mg/kg)	6.0 ± 13.4
Piracetam (100mg/kg)	0.0 ± 0.0

The mean difference is not statistically significant when compared with control, $p > 0.05$ (n=5), SPSS version 20. PDE=*Phoenix dactylifera* extract.

Table 4.12: Effect of *Phoenix dactylifera* extract (PDE) on acetylcholinesterase enzyme activity in mice subjected to Barnes maze paradigm of cognition

Groups	Acetylcholinesterase concentration (mU/ml)
Control	72.0 ± 4.5
PDE (1000mg/kg)	0.6 ± 0.6
PDE (500mg/kg)	45.5 ± 43.6
PDE (250mg/kg)	86.8 ± 40.9
Piracetam (100mg/kg)	280.4 ± 78.8[a]

a: the mean difference is statistically significant at $p<0.05$ when compared to control, (n=5) SPSS version 20. PDE=*Phoenix dactylifera* extract

4.5 Pearson Correlation between Acetylcholinesterase Activity and Paradigms of Cognition.

4.5.1 Acetylcholinesterase activity and frequency of platform crossings in Morris water maze

Positive correlation was seen in frequency of platform crossings of control group (r=0.865, p=0.056) and PDE 250mg/kg (r=0.566, p=0.32), while negative correlation was seen in PDE 500mg/kg (r=-0.579, p=0.32), PDE 1000mg/kg (r=-0.529, p=0.359) and Piracetam (r=-0.47, p=0.941) when compared with their acetylcholinesterase concentrations (Table 4.13)

4.5.2 Acetylcholinesterase activity and retention in elevated plus maze

There was positive correlation between retention of PDE500mg/kg and acetylcholinesterase activity (r=0.795, p=0.108). Negative correlation was observed between retention andacetylcholinesterase concentrationsof control, PDE 250 and 1000mg/kg (r=-0.023, p=0.971; r=0.274, p=0.656; r=-0.497, p=0.394, respectively) (Table 4.13).

4.5.3 Acetylcholinesterase activity and correct head dips of Barnes maze

Positive correlation was observed between correct head dips and acetylcholinesterase concentration of PDE 1000mg/kg (r=0.153, p=0.806), while negative correlation was seen between head dips and acetylcholinesterase concentrations of control, PDE 500mg/kg and Piracetam (r=-0.399, p=0.506; r=-0.371, p=0.538 and r=-0.562, p=0.324 respectively) (Table 4.13)

Table 4.13: Pearson correlation between probes and acetylcholinesterase activity

Correlation coefficient (r)			
Groups (Dose)	Morris water maze	Barnes maze	Elevated plus maze
Control	0.865	-0.399	-0.023
PDE (1000 mg/kg)	-0.529	0.153	0.274
PDE (500 mg/kg)	-0.579	-0.371	0.795
PDE (250 mg/kg)	0.566	0.000	0.274
Piracetam (100 mg/kg)	-0.470	0.324	0.000

The mean difference is not statistically significant when compared with control, $p > 0.05$ (n=5), SPSS version 20.

CHAPTER FIVE

5.0 Discussion

There have been many studies on pharmacologic effects of *Phoenix dactylifera*. In those studies, beneficial effects of this plant, including antioxidant property, effect on lowering blood sugar in diabetic patients, effects onGIT movements, effects on male infertility have been pointed out. The present study was carried out to evaluate cognitive function of *Phoenix dactylifera* in Swiss albino mice. The result of this research work has shown that PDE 1000mg/kg impaired learning inMorris water maze (MWM)and training enhanced learning of mice in MWM. This finding is in conformation with that of Hosseini *et al*., (2011) who showed that rodents, during a four days training period, progressively learn to find the hidden platform in the MWM test. This was observed by significant reduction of escape latency in the four day trained compared with one day trained animals (Hosseini *et al*., 2011).

No statistical significant difference was seen between the groups in frequency of platform crossings (an index of memory) during probe trial. This may be associated with the stationary nature of the platform during acquisition session.Perrot *et al.*(1996) showed that non- stationary hidden platform training led to faster acquisition and improved retention of the subsequent spatial task when compared with stationary hidden platform in MWM test. This finding is contrary to that of Christine *et al*. (2011) who showed that performance was better during the probe trial in animals that were unaware that the platform could be located elsewhere in MWM. This could be due to visio-spatial memory shown by the animals after training. There was statistical significant difference between time spent in quadrant 2 and 3 when compared to quadrant 4 (platform's quadrant) during probe. This conforms to finding of Christine *et al.*(2011) above showing that animals aware of location of platform persist searching in one location.

During acquisition and retention in elevated plus maze for memory, no significant difference was seen between the groups. This might be due to the natural exploratory behavior of the mice which make them to quickly escape to enclosed arm. This is in agreement with finding of Itoh *et al*.,(1990);Blatt and Takahashi, (1998)who identified that, mice in the plus-maze escaped from the open arm to the enclosed arm because of fear and anxiety, they apparently dislike open and high spaces. This showed that *Phoenix dactylifera*did not impair cognition.

In Barnes maze (BM) paradigm, no statistical significant mean difference was seen between the treatment groups and control groups in all the parameters assessed during training and probe session. However, latency to locate escape box increases in all the groups except Piracetam treated group, likewise, total error except negative control and PDE 250mg/kg groups which had decrease in total error. This might be attributed to the lesser stressful and aversive nature of the apparatus which make the animals to display high explorative behaviors and commit more errors.This conforms to the finding of Harrison*et al*.,(2009) who showed that Barnes maze is less stressful when compared with Water maze after measuring corticosterone level.Also, Lewejohann *et al.*,(2004); Sharma *et al*. (2010) showed that rodents tend to commit more errors as training increases due to less aversive nature of Barnes maze and this does not show impairment of memory. Also, *Phoenix dactylifera* did not impair memory in mice.

Piracetam treated group was found to have significantly increase acetylcholinesterase enzyme activity compared to other groups in mice subjected to Morris water maze and Barnes maze.This increase in achE activity was positively correlated with platform crossings in MWM and not correlated with performance in probe of BM. This finding suggested that Piracetam might not act via cholinergic system and this is in disagreement with the postulation of Wannicka *et al.,* (2005) who showed that Piracetam act via muscarinic receptors of acetylcholine implicated in memory.

PDE 500mg/kg and 1000mg/kg groups were found to have lower acetylcholinesterase activity compared with control in mice used in MWM and BM. This reduction in achE activity was associated with their performances in MWM and BM probe trials.The result of this research has shown clearly that PDE at doses of 500 and 1000mg/kg decreased acetylcholinesterase activity in brain homogenate (although not statistically significant). Thus, acetylcholine accumulates in the brain and enhanced memory. This agrees with recently finding of Meera and Mamatha(2014), who demonstrated that ethanolic extract of *Phoenix dactylifera* decreased acetylcholine esterase activity in brain homogenate after assessment of learning and memory enhancing activity of *Phoenix dactylifera* in rats. Also, the results are in conformation with the findings of Zafari *et al*., (2009); Vyawahare *et al*.,(2009); Wan and Moh'd (2013) who showed that Date fruits improve cognition.

CHAPTER SIX

6.0 Summary, Conclusion, Recommendation and Contribution to Knowledge

6.1 Summary

Learning is the acquisition of information and memory is the retrieval of this information. Many drugs were found to enhance cognition. Nootropics such as Piracetam, galanthamine and nicotine were found to enhance cognition and students in colleges used some of these drugs for academic doping. The mechanism through which memory is enhanced might be via improvement of cholinergic transmission.

Many plants extracts were hypothesized to possess cognitive enhancement ability. This study was design to accept or reject the hypothesis made by traditional medical practitioners that *Phoenix dactylifera* was used to enhanced memory. Many studies accept this hypothesis but not well scientifically established. This study evaluated the effect of *Phoenix dactylifera* fruit on cognition using Morris water maze, Barnes maze and elevated plus maze neurobehavioral tests. Also, acetylcholinesterase activity was assessed in the brains of the mice using achE assay kit. Results obtained had shown that *Phoenix dactylifera* fruit did not impair memory in mice in Morris water, elevated plus and Barnes maze paradigms.

6.2 Conclusion

Phoenix dactylifera fruit impaired learning in mice in Morris water maze at dose of 1000 mg/kg and did not impair memory in Morris water maze, Barnes maze and elevated plus maze for memory.

6.3 Recommendations

(i) Chronic studies of cognitive effect of date fruit using the same neurobehavioral paradigms

using different fractions of PDE.

(ii) Effect of PDE on inhibitory neurotransmitters of the brain like GABA and glycine

(iii) Assessment of other excitatory neurotransmitters like glutamate on cognitive enhancement effect of date fruit.

6.4 Contribution to Knowledge

(i) PDE at dose of 1000mg/kg impairs learning in mice subjected to Morris water maze.

(ii) This study was first to assess cognitive effect of extract of Phoenix dactylifera fruit using Barnes maze.

(iii) Acute treatment with PDE did not affect acetylcholinesterase activity in the brainof mice.

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Appendix 1: Effect *Phoenix dactylifera* extract (PDE) on learning in mice during a two-day acquisition session in Morris Water Maze.

Groups	Latency (s) in day 1	latency (s) in day 2
Control	54.80 ± 6.7	40.80 ± 3.5
PDE (1000mg/kg)	91.40 ± 5.3 [a]	60.00 ± 6.5
PDE (500mg/kg)	49.80 ± 8.1	59.80 ± 4.0
PDE (250mg/kg)	72.60 ± 8.3	56.80 ± 5.7
Piracetam (100mg/kg)	56.80 ± 7.0	34.00 ± 4.8

a: the mean difference is statistically significant at $p<0.05$ when compared to control, (n=5) SPSS version 20.

Appendix II: Effect of *Phoenix dactylifera* extract (PDE) on learning in mice during a one-day acquisition session in elevated plus maze for memory.

Groups	Acquisition (s)
Control	81.8 ± 8.2
PDE (1000mg/kg)	81.4 ± 8.6
PDE (500mg/kg)	43.0 ± 16.0
PDE (250mg/kg)	58.8 ± 19.1
Piracetam (100mg/kg)	53.6 ± 14.2

The mean difference is not statistically significant when compared with control, $p > 0.05$ (n=5), SPSS version 20.

Appendix III: Effect of *Phoenix dactylifera* extract (PDE) on memory in mice during a one-day retention session in elevated plus maze for memory.

Groups	Retention (s)
Control	23.0 ± 11.1
PDE (1000mg/kg)	48.6 ± 17.39
PDE (500mg/kg)	24.6 ± 16.4
PDE (250mg/kg)	30.8 ± 15.3
Piracetam (100mg/kg)	13.4 ± 2.3

The mean difference is not statistically significant when compared with control, $p > 0.05$ ($n=5$), SPSS version 20.