EVALUATION OF THE EFFECT OF KHAT (*Catha edulis)* Forsk ON SPATIAL LEARNING AND MEMORY IN CBA MICE USING THE MORRIS WATER MAZE TASK.

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DECLARATION

This thesis is my original work and has not been submitted for a degree in any other University.

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DEDICATION

This work is dedicated to God the Almighty for seeing me through my education and to my beloved wife Jane Wambui and Son Alvin Kimani for their unwavering patience and support during my education.

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List of abbreviations

ACTH	Adrenocorticotropic hormone
CA1	Cornu ammon 1
CA2	Cornu ammon 2
CA3	Cornu ammon 3
cAMP	Cyclic adenosine monophosphate
CNS	Central nervous system
CRE	cAMP-Response Elements
DHEA	Dehydroepiandrosterone
DNA	Deoxyribonucleic acid
DNMTS	Delayed non matching to sample
GABA	Gamma amino butyric acid
HIV	Human immuno deficiency virus
IGF-1	Insulin like growth factor-1
IPSPS	Inhibitory postsynaptic potentials
LTP	Long term potentiation
LSD	Lysergic acid diethylamide
MANOVA	Multivariate analyses of variance
mRNA	Messenger ribonucleic acid
MTL	Medial temporal lobe
MWM	Morris water maze
NMDA	N-Methyl-D Aspartate
SHR	Spontaneous hypertensive rat
WHO	World health organization

SUMMARY

Khat *(Catha edulis* Forsk) is a psychoactive plant commonly used in East Africa, horn of Africa and the Arabian peninsula. Its effects are mainly modulated through the dopaminergic system similar to amphetamines. Psychoactive substances/ drugs of abuse effect their changes centrally by acting on the brain, altering the neurotransmitter system and structurally affecting the limbic Papez system. These substances can affect learning and memory by interfering with the medial temporal lobe structures and thus affecting spatial learning and memory. This study evaluated the effect of khat on CBA mice using a Morris water maze task which is a test of spatial learning and memory. Twenty CBA mice were divided into 5 groups and administered, intraperitoneally, 0.5 mls normal saline, 40, 120, and 360 mg/kg body weight of khat extract, respectively. The doses were given once daily for 17 days during which the animals were submitted to acquisition, reversal learning and reference tests in the Morris water maze. The study also investigated the effect on learning and memory of escalating, repeated high (run) doses of khat extract given to CBA mice. Twenty nine CBA mice were divided into groups of 5 - 7 animals. The animals were first subjected to an escalating dose regime and then followed by repeated high (run) doses, or an escalating dose regime followed by single daily dose, or an escalating dose regime followed by saline or saline followed by escalating dose regime of khat extract. The mice were tested in a Morris water maze for spatial learning and memory. The escape latency, swim path length, swim speed, quadrant time and quadrant swim distances were measured by the use of stop watch and video recording tracings.

The data were analyzed using SPSS statistical package, in which multivariate analysis of variance (MANOVA) was carried out on the independent variables (days, doses,

and groups) against the dependent variables (escape latency, swim distance, swim speed). Bonferroni post hoc tests were carried out on dependent variables, and $P < 0.05$ was considered significant.

High doses of khat extract (360 mg/ kg body weight) significantly increased ($P < 0.05$) the line crossings while low doses (10, 30, 40 and 120 mg/kg) and very high doses (540 mg/kg) body weight of khat extract had no effect on line crossings in Swiss and CBA mice. High doses (120, 270, 360 mg/kg) body weight of khat extract significantly ($P < 0.05$) inhibited the centre square and rearing frequencies in the two species of mice, whereas, low doses (10, 30, 40 mg/ kg) body weight of extract significantly increased ($P < 0.05$) the two measures. Repeated khat treated CBA mice had significantly ($P < 0.05$) higher line crossings than Swiss mice treated with the same dose. Similarly, the centre square and rearing frequencies were significantly higher ($p < 0.05$) in CBA mice than the Swiss mice and the control. The study demonstrates that repeated khat causes behavioural sensitization in mice and affects locomotor behaviors similarly regardless of the dose regime.

Mice treated with higher (360 mg/kg body weight) khat extract had their learning and memory significantly ($P < 0.05$) impaired. The extract, further significantly ($P < 0.05$) impaired learning and memory of CBA mice at higher (360 mg/ kg body weight) during reversal sessions. The study shows that higher dose (360 mg/kg body weight), behavioural switching in CBA mice was interfered with as evidenced by the mice spending more time and swimming longer distances in the former platform quadrant as opposed to the current target platform quadrant.

Mice treated with escalating then repeated runs (Binge) regimen of khat extract significantly ($P < 0.05$) improved their learning but their retention were also significantly ($p < 0.05$) impaired. Khat extract administered as escalating followed by single daily dose, significantly ($P < 0.05$) improved their learning as well as memory, whereas, mice injected with saline then repeated high doses of khat extract had their learning and memory adversely affected. In addition, CBA mice treated with escalating dose followed by saline had their learning and memory impaired. The study shows that khat extract at a high dose adversely affected learning and memory though, the mechanism of this is not clear, but could involve the dopaminergic neurotransmitter system.

CHAPTER ONE

1.0 INTRODUCTION

1.1 Problem statement

Khat is used as a recreational (social drug) by the inhabitants of areas where it is grown, in East Africa and Arabian Peninsula (Patel, 2000). Fresh leaves and shoots of khat plant contain a naturally occurring alkaloid, cathinone which produces euphoric and psycho stimulant effects on the users (Luqman and Danowiski, 1976; Baasher *et al.*, 1980; Brenneisen *et al.*, 1990; Kalix, 1992).

Advances in transport, especially air transport has made khat reasonably accessible in many parts of the world (Paker, 1985; Weir, 1988; Cassanelli, 1986; Kalix, 1987, and Almotareb *et al.*, 2002). Furthermore, the flooding of refugees from the horn of Africa in the eighties to USA, Canada and other European countries has also contributed further to the spread of khat chewing practices.

It is difficult to say precisely how much khat is chewed but the practice is widespread and records of khat usage are scarce, however, according to rough estimates, five million people use khat on a daily basis (Balint, 1991) and many of them have become compulsive users, developing psychic dependence on the drug (Eddy *et al.*, 1965). In Ethiopia alone, a survey in one of the rural regions showed high dependence on the drug (Eddy *et al.*, 1965) and found the prevalence of khat usage to be about 50% of the total population (Alem *et al.*, 1999).

Globally, pure cathinone and methcathinone (cathinone analogues) have appeared as substances of abuse in more economically developed countries (Sparago *et al.*, 1996; Young and Glennon, 1998) but not used in regions where khat chewing is common.

Addictive drugs act as primary reinforcers, inducing a compulsive pattern of use by the user and khat is no exception. It causes moderate but often a persistent psychic, physical dependence or

tolerance similar to marked tolerance observed with amphetamine abuse (Kennedy et al., 1980, Kennedy, 1987). Tolerance, also develop to the sympathomimetic effects of khat (Nencini *et al.*, 1984).

The problem of khat abuse is a major concern socially, psychologically, economically and medically (Dhaifalah, 2004). The drop out rates from school has increased because the would be students leave school to go and work for and /or sell khat for short term economic gain, a situation that can lead to devastating socio economic long term effects. Similarly, individuals divert their income into khat chewing, neglecting their family needs (Kalix, 1987) and low productivity due to work place absenteeism and the after effects of its use (Halbach, 1972; 1979; Alem, 1983b; Giannin *et al.*, 1986; Kalix, 1987). Nationally, diversion of resources towards the production and /or importation and marketing of the khat has negative effects on the economy (Baasher, 1980). The cultivation of khat results in decreased production of other essential crops, thus promoting malnutrition and disease (Murad, 1983).

Although the scientific literature on cellular and psychomotor effects of amphetamine and other psychostimulants are large, comparatively, little has been published on the pharmacology of khat in controlled experiments in animals (Connor *et al.*, 2002). However, the phenylpropanolamines in khat (cathinone, cathine, norpseudoephedrine and norephedrine) have pharmacological properties similar to d-amphetamine (Zelger *et al.*, 1980) as they stimulate the release and block the re-uptake of dopamine in the CNS (Zelger and Carlini, 1981). Similarly, a lot has been written on the effects of khat being similar to amphetamine on psychomotor behavior in humans and animals; however, there are no studies that have been conducted to determine the effects of khat on learning and memory in humans and animals. The effects of khat on human cognition remains under explored, however, ethical considerations limit the nature of experimentation using human subjects and thus the use of CBA mice as the animal model in this study.

Furthermore, despite khat being classified as drug of abuse by United Nations Office on drug and crime (WHO, 1985) some countries, Kenya included, still consider it as a minor psychostimulant just like caffeine and nicotine, and indeed, it's a legal drug (Dhaifalah, 2004), a view that could blur the appreciation of the magnitude of its effects.

1.2 Study questions

1.2.2. How does Catha edulis (khat) affect the CBA mice performance of spatial learning and memory task in Morris water maze (MWM)?

1.2.3 Are there dose dependent effects of khat on CBA mice spatial learning and memory performance in MWM?

1.3 Justification

Amphetamines and other psychostimulnats, khat included, inhibit re uptake and stimulate the release of dopamine in the CNS, thereby increasing the temporal and spatial presence of dopamine at post-synaptic receptors (Klause *et al*., 2000; Safer and Krager, 1988). Brain structures such as striatal cortex, prefrontal cortices and limbic areas, have been demonstrated to undergo adaptive changes during repeated amphetamine administration and these structures have been implicated with various forms of learning and memory (Goldman-Rakie, 1987; Baddeley, 1992; Dias *et al.*, 1996; Nester and Aghajanian, 1997; Robinson and Kolb, 1997; Berke and Hyman, 2000). Post trial amphetamine treatment has been shown to enhance memory consolidation in the water maze task (Brown *et al.*, 2000), however, on the other hand, chronic administration of psychostimulant cocaine, a drug that resembles amphetamine pharmacologically, has been reported to impair water maze performance in rats (Quirk *et al*., 2001). In another study, acute treatment with amphetamine has been reported to increase memory consolidation and increase the impact of reinforcement in some learning paradigms

(McGaugh, 1989 and Killcross *et al.*, 1994). It is for these reasons therefore, the study was conducted to elucidate whether khat extract resembles amphetamines on its effect on learning and memory among other similarities.

Drugs of abuse are proving to be a major public health concern throughout the world. Indeed, they are a major cause of mortality and morbidity in the communities directly and / or indirectly and more so through crime, disease and accidents. Cathinone, a major ingredient of khat, is a psychoactive compound that has been shown to modulate its effects in humans and animals and has abuse potential.

Today, use and abuse of illicit substances is pervasive and associated with frequent co morbidities such as HIV infection and other sexually transmitted diseases, teenage pregnancy, rape, assault and murder (Allen, 2001; Odejide, 2006). The effects of these drugs contribute immensely to social economic burden of the family, the community, and more importantly, their effects and risks to the abuser. Adolescents and youths are heavily affected because at this stage in their development, they are adventurous, experimenting on almost everything. Their death and / or dependence on these psychostimulants could have major repercussions both to the family and the community.

Khat is chewed as a social drug, with hardly any restrictions in Kenya, and in most of the East African and Arabian Peninsula countries, hence the need to carry out experimental research to elucidate its effects particularly towards learning and memory. This study examined the effects of khat on learning and memory in CBA mice and may provide information that may lead to similar evaluation in humans.

1.4 Objectives and hypothesis of the study

1.4.1 Broad objective

To determine the effect of Catha edulis on CBA mice spatial learning and memory task performance in Morris Water Maze.

1.4.1.1 Specific objectives

1. To determine the effect of *Catha edulis* extract on CBA mice locomotor activity
2. To determine the effects of single daily dose of *Catha edulis* extract on acquisition training of MWM task in CBA mice
3. To determine the effects of single daily dose of *Catha edulis* extract on reversal learning
4. To determine the effects of single daily dose of khat extract on memory retention of MWM task in the CBA mice
5. To determine the effects of khat extract on post training locomotion in CBA mice
6. To determine the effects of escalating binge model dose of khat extract on acquisition and retention of MWM task by CBA mice.

1.4.2 Hypothesis

Khat has no adverse effect on learning and memory in CBA mice

CHAPTER TWO

LITERATURE REVIEW

2.1 Background

Learning is the process by which new information is acquired by the nervous system and memory is the mechanism of storage and /or retrieval of that information (Purves, 2001) The process depends on complex forms of synaptic plasticity that involves largely post synaptic changes. Most prominent among the candidate cellular mechanisms of learning in vertebrates is NMDA receptor dependent long term potentiation (LTP) (Bliss & Lømo, 1973; Bliss & Collingridge, 1993). Similarly, post synaptic mechanisms, including NMDA receptor dependent plasticity have been shown to play crucial roles in learning in invertebrates, for example, Aplysia (Robert & Glanzman, 2003). LTP is the activity dependent increase in synaptic efficiency (Bliss & Lømo, 1973) and it is now accepted that one prominent type of LTP involves the elevation of post synaptic Ca^{2+} following NMDA receptor activation (Lynch *et al.,* 1983, Collingridge *et al.*, 1983, 1988; Malenka *et al.,* 1992). The changes in synaptic strength may be critical for learning either as a mechanism for direct storage of memories, or as a process that transforms information making it suitable for long term storage (Hebb, 1949). The NMDA receptor is a voltage sensitive glutamate gated channel and it regulates calcium current essential for the induction of LTP (Collingridge & Singer, 1990).

In most mammalian brain regions, neurogenesis only occurs during development. However, within the hippocampal formation, the dentate gyrus continues to produce granule neurons throughout adulthood (Altmann, 1962; Gross, 2000). Indeed, increasing number of reports suggest that adult hippocampal neurogenesis is involved in hippocampal mediated learning since the hippocampus is implicated in various forms of memory. Furthermore, it has been shown that

conditions that increase memory performance such as enriched environment, running and physical exercise also enhance neurogenesis (Kempermann et al., 1997, 1998; Van praag *et al.*, 1999). On the other hand, situations that reduce neurogenesis such as prenatal stress or antimitotic treatment have been associated with cognitive impairments (Van praag *et al.*, 1999).

There is evidence that, drugs of abuse are capable of altering the structure and functions of structures concerned with learning and memory. For example, ethyl alcohol when ingested in large bolus in "binge drinking" can have a devastating effect on neurogenesis in the hippocampus (Aberg, 2001). But more importantly, other drugs, for example, cortisol inhibits hippocampal neurogenesis whereas dehydroepiandrosterone (DHEA) has stimulatory effect. The former can actually kill hippocampal neurons and it does so increasingly with aging and stress, but, this effect can be reversed with melatonin and insulin like growth factor (IGF-1) (Aberg, 2001).

Attention deficit hyperactivity disorder (ADHD) is a common childhood psychiatric disorder affecting between 1.3% and 5% of primary school children (Swanson *et al.*, 1998; Taylor, 1998). It is characterized by hyperactivity, inattention, and impulsivity (Himelstein *et al.*, 2000; Taylor, 1998) and problems with cognitive impulsiveness, that may be defined as planning deficits, forgetfulness, poor use of time and impetuous behavior (Sagvolden, 2000). Spontaneous hypertensive rats (SHR) have often been used as an animal model of ADHD, since they display hyperactivity, impulsiveness, impaired ability to withhold responses and poorly sustained attention in comparison with normotensive Wistar- Kyoto control rats (Russell, 2002; Sagvolden, 2000; Sergeant, 1998). In addition, reduced performance by SHR has been observed in different paradigms used to investigate learning and memory processes; Morris water maze being one of them (De Bruin *et al.*, 2003).

To date, the "dopaminergic hypothesis" based on a dysregulation in dopaminergic neurotransmission, has been the most accepted hypothesis regarding the behavioural alterations both in attention deficit hyperactive disorder patients (ADHD) and spontaneous hypertensive rat. There is considerable evidence suggesting that ADHD patients may have disturbances in dopamine uptake, storage and / or metabolism (Castellanos & Tannock, 2002). The most effective and frequently prescribed drugs for ADHD, methylphenidate and d-amphetamine, are psychostimulants that inhibit re uptake and stimulate release of dopamine in CNS, thereby increasing the temporal and spatial presence of dopamine at post synaptic receptors (Krause *et al*., 2000, Safer and Krager, 1988). Reduced performance by spontaneous hypertensive rat has been observed in different paradigms used to investigate learning and memory processes, for example Morris water maze (De Bruin *et al.,* 2000). For this reasons, it is evident that psychostimulants and drugs of abuse are capable of altering the structure and functioning of structures concerned with learning and memory.

2.2 Types of learning and memory

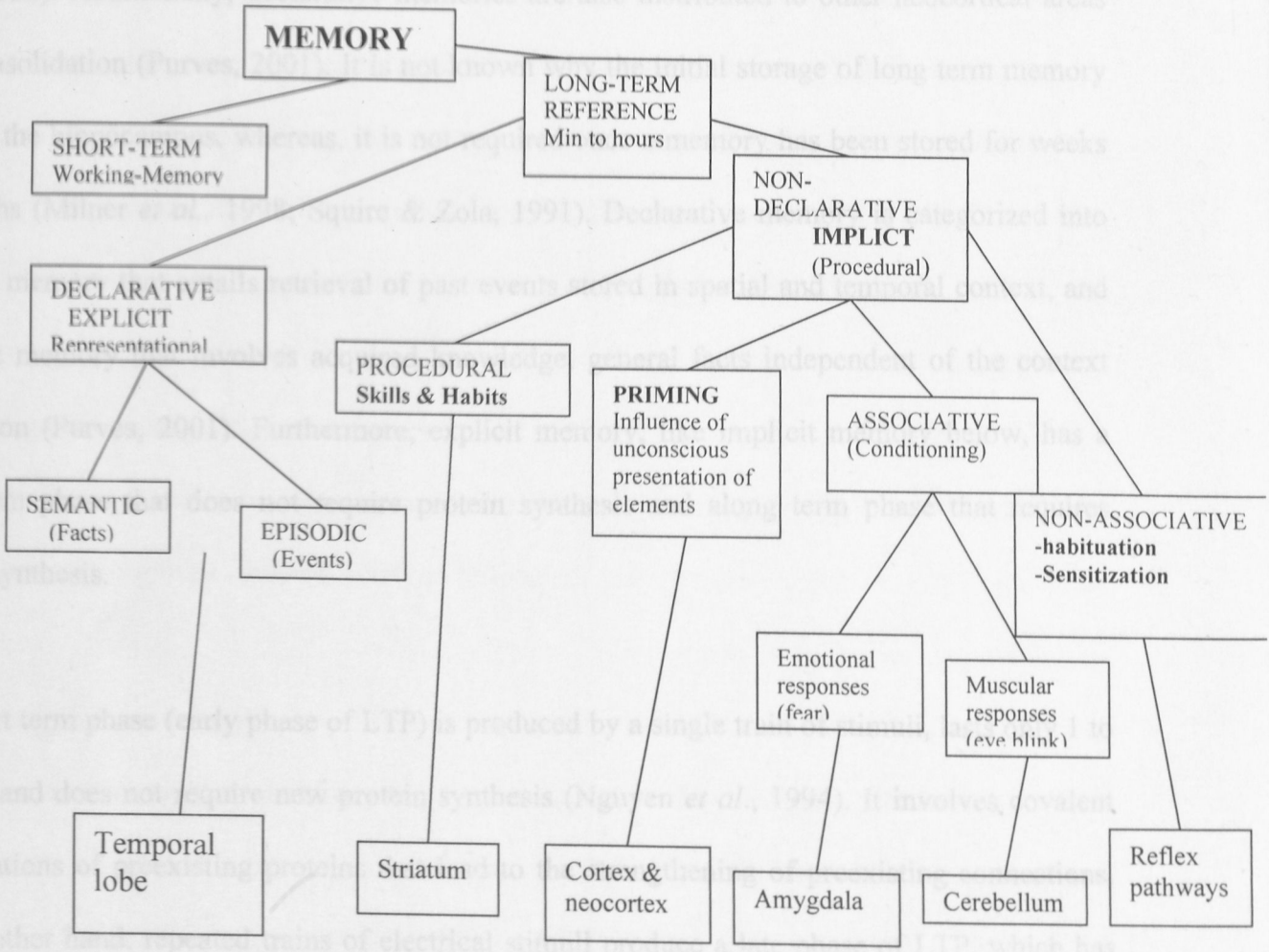


Figure 1 Taxonomy of memory according to Tülving (1983), and Squire and Knowlton (1994)

Human memory can be qualitatively divided into two different systems of information storage; these are generally referred to as declarative (explicit) and procedural (implicit) memory.

2.2.1 Declarative Memory

These are the memories we hold near and dear, they are also called explicit memories (Kandel, 2001). They require conscious recall and are concerned with memories for people, places, objects and events. They involve a specialized anatomical system in the medial temporal lobe,

and a structure deep to it, the hippocampus (Milner *et al.*, 1998; Bacskal *et al.,* 1993; Castellucci *et al.,* 1988). Additionally, declarative memories are also distributed to other neocortical areas after consolidation (Purves, 2001). It is not known why the initial storage of long term memory requires the hippocampus, whereas, it is not required once a memory has been stored for weeks or months (Milner *et al.*, 1998; Squire & Zola, 1991). Declarative memory is categorized into episodic memory that entails retrieval of past events stored in spatial and temporal context, and semantic memory that involves acquired knowledge, general facts independent of the context acquisition (Purves, 2001). Furthermore, explicit memory, like implicit memory below, has a short term phase that does not require protein synthesis and along term phase that requires protein synthesis.

The short term phase (early phase of LTP) is produced by a single train of stimuli, lasts only 1 to 3 hours and does not require new protein synthesis (Nguyen *et al.*, 1994). It involves covalent modifications of preexisting proteins that lead to the strengthening of preexisting connections. On the other hand, repeated trains of electrical stimuli produce a late phase of LTP, which has properties quite different from early phase of LTP. The late phase of LTP persists for at least a day and requires both translation and transcription. This phase of LTP like long term storage of implicit memory requires protein kinase A, mitogen – activated protein kinase and cAMP response element binding (CREB) and appears to lead to the growth of new synaptic connectors (Frey *et al*, 1993; Nguyen, 1994).

2.2.2 Procedural Memory

This is the memory of perceptual and motor skills. Procedural memory is expressed through performance without conscious recall of past episodes (Kandel, 2001). It is further divided into associative memory, for example, classical and operant conditioning; and non associative

memory such as sensory priming, habituation, sensitization skills and habits. The amygdala, cerebellum and the striatum are some of the structures that modulate procedural memory such as classical conditioning, (fear conditioning), operant conditioning and motor skills among others. Other forms of procedural learning, for example, sensory priming and habits are modulated by neocortex, and striatum. In addition, reflex loops in the brain stem and the spinal cord also modulate some form of learning and memory such as habituation and sensitization.

2.3 Temporal classification of memory

The human memory can further be temporally categorized depending on the time over which it is effective into three groups (Purves, 2001) as follows;

2.3.1 Immediate memory

This is the routine ability to hold ongoing experiences for a few seconds. The capacity of this register is very large and it involves all the modalities (visual, tactile, and verbal among others) and provides the ongoing sense of present (Purves, 2001).

2.3.2 Short term memory

This is the ability to hold information in mind for seconds to minutes once the present moment has passed. A conventional way of testing the integrity of (declarative) short term memory is to present a string of randomly ordered digits which the patients is asked to repeat. Ordinarily, the normal digit span is only 7 – 9 numbers (Purves, 2001). There is a special sort of (procedural) short term memory called working memory which refers to the ability to hold information long enough to carry out sequential actions e.g. in searching for a lost object. This memory allows the search to proceed efficiently, avoiding places already searched. Working memory is advantageous because it can readily be examined in experimental animals (Purves, 2001).

2.3.3 Long term memory

This is a type of memory in which information is retained in a more permanent form for days, weeks or even life time. There is evidence for a continual transfer of information from the short to long term memory storage as in the phenomenon of priming (Purves, 2001).

2.4 CENTRAL NERVOUS SYSTEM LEARNING MECHANISMS

Although the neurobiologic basis for learning and memory is far from understood at least five different areas of the CNS have been proposed to underlie the different aspects of memory (Brown *et al.*, 2000). Damage to one of these structures disrupts some memory categories but leaves others intact (Squire, 1987). The five neural area mostly widely studied include the hippocampus, amygdala, dorsal striatum, rhinal cortex and cerebellum. None of these neural systems is a unitary entity.

2.4.1 The hippocampus

Mice have a medial temporal lobe system, including a hippocampus that resembles that of humans and they use it much as humans do to store memory of places and objects (Kandel, 2001). It is distinguished by three distinctive regions composed of granule cells, the CA3 and CA1 regions, which are composed of pyramidal cells with different properties. Vargha Khaden and coworkers (1997), proposed an anatomical model in which they suggested that the hippocampus is necessary for remembering past experiences, while the remaining medial temporal lobe (MTL) regions are necessary for learning of factual information. Medial temporal lobe structures including the hippocampus are essential for normal memory in humans (Milner, 1970; Rempel Clower *et al.*, 1996) and animals (Cohen and Eichenbaum, 1999; Shapiro & Olton, 1996; Squire & Zola, 1996).

Cells in the hippocampus, therefore contribute to memory, however, the precise way in which they contribute to memory remains unclear (Shapiro and Eichenbaum, 1999). Furthermore, it is well established that the hippocampus contains a cellular representation of extra personal space, a kind of cognitive map of space, and lesions of the hippocampus are known to interfere with spatial tasks. More over, Lømo and Bliss (1972) discovered that the perforant path, a major pathway within the hippocampus, exhibits activity dependent plasticity, a change now called long term potentiation (Kandel, 2001), which appears to be the basis for learning and memory. In the CA1 region, LTP is induced post synaptically by activation of an NMDA receptor by glutamate. Blocking the NMDA receptor pharmacologically not only interferes with LTP but also blocks memory storage (Bliss and Lømo, 1973).

One striking sensory and / or behavioral correlate of hippocampal neuronal activity is the place field (O'Keefe and Dostrovsky, 1971). Hippocampal pyramidal cells discharge at rates that vary with the specific location of a rat as it moves through a given environment. Regions of the environment where a single cells fires at a high rate defines the place field of that cell and neurons with this pattern of activity have been called place cells (O'Keefe and Dostrovsky, 1971).

According to Eichenbaum (1999), place cells are principal hippocampal neurons that fire when an animal is in a particular location in the environment. They fire regardless of what direction the animal is facing and regardless of what the animal is doing. The locus and pattern of firing is determined by constellation of all attended spatial cues and is independent of any particular stimulus. In addition, place cells are viewed not as a collection of separate place representation but as part of a cognitive map in the hippocampus. Indeed, the primary significance of place cells as suggested by O'Keefe and Nadel (1978) is that they are elements of a Cartesian representation

of the environment, that is, the hippocampus contains three dimensional framework in which the activation of each place cell (the place field) represents the animals presence at a particular set of coordinates within the spatial reference frame.

The observation of place cells together with demonstrations that hippocampal lesions impair learning and performance in spatial discrimination tasks, led to the spatial map theory of hippocampal function. This theory holds that the hippocampus contributes to memory by encoding locations with a spatial framework (O'Keefe and Nadel, 1978). Hippocampal neurons are responsive to complex spatial cues, a characteristic which is consistent with a role for the hippocampus in spatial learning (O'Keefe & Nadel, 1978; Kubie & Ranck, 1983; Mc Naughton *et al,* 1983). While much behavioural and electrophysiological data are consistent with the spatial map theory, other results indicate that hippocampus cells formation is independent of location. Indeed, the hippocampus is needed for learning, remembering and other non spatial types of information (Winocur, 1990; Bunsey and Eichenbaum, 1995, 1996).

Learning and memory are supported by many brain systems and different aspects of experience and behaviour are encoded in parallel by many different circuits in the brain (McDonald and White, 1993). Through a variety of plasticity mechanisms, the structure and function of these circuits change as a consequence of information processing (Shapiro and Eichenbaum, 1999).
In mammalian hippocampus, the phenomenon of long term potentiation (LTP), a stimulation induced form of synaptic plasticity, has been hypothesized to reflect a potential neural mechanism for memory storage (Douglas and Goddard, 1975; McNaghton, 1983; Teyler and Discena, 1984; Eccles, 1986, McNaughton and Morris, 1987; Matthies, 1989). At the cellular level, hippocampal long term potentiation (LTP) has been implicated as a mechanism underlying learning and memory (Swanson *et a.l.,* 1982; Teyler and Disena, 1984; Morris, 1990).

Alternatively, the information processing that precedes memory encoding may require LTP (Dejonge and Racine, 1985; Squire, 1987). Bilateral damage to the human medial temporal lobe results in profound and persistent anterograde amnesia (Scoville and Milner, 1957; Milner, 1972).

Comprehensive neuropsychological and neuropathological studies of amnesic patients have provided substantial evidence that the hippocampal formation plays an essential role in normal memory function (Zola-Morgan *et al.,* 1986; Victor and Agamonils, 1990). Further, experimental support for this idea has also come from ablation studies in monkeys. These studies have identified several components of a medial temporal lobe memory system (Mishkin, 1978; Squire and Zola-Morgan, 1991). The important structures appear to be the hippocampal formation itself (comprised of the dentate gyrus, hippocampus proper and subinsular complex and entorhinal cortex) and the adjacent peripheral and parahippocampal cortices.

A major finding emerging from recent studies of the primates' medial temporal lobe memory system is that structures besides those of the hippocampal formation play a significant role in normal memory function (Zola-Morgan and Squire, 1993; Mishkin and Murray, 1994). A number of theories of hippocampal function suggest that it has a role in using the relations between ambient cues to guide movements (Hirsh, 1974; O'Keefe and Nadel, 1978; Sutherland and Rudy, 1989; Jarrard, 1993).

In a learning task, rats placed in a swimming pool in which a small escape platform was hidden just beneath the water surface, learnt to swim directly to the platform from one location on the pool edge (Morris, 1981). Rats with hippocampal dysfunction, produced by hippocampal removal (Morris *et al.*, 1982; Sutherland *et al.*, 1982; Whishaw, 1987), damage to the efferents

and afferents in the fimbria-fornix (Cassel and Kelche, 1989; Sutherland and Rodriguez, 1989) or cholinergic blockade (Sutherland *et al.*, 1982; Whishaw, 1985 a, b; Whishaw and Jomil, 1987) are severely impaired in this task. Since the same studies show that the rats remain excellent swimmers and can quickly learn to escape to a visible platform, the results suggest that the hippocampus is selectively involved in using the relational properties of ambient cues to guide movements (Whishaw *et al.*, 1995).

As first demonstrated by Krechwky (1938), problem solving involves at least two processes, discovering the tasks solution and then learning the task, similarly, in swimming pool tasks, animals must first learn to swim, discover that there is an escape, and find that effective guidance requires distal cues (Sutherland and Dyck, 1984; Whishaw and Petrie, 1988). In fact, during the first swimming trials, the rats engage in a variety of behaviours such as scrabbling at the edge, making sorties into the centre of the pool, swimming in circles, etc, therefore, suggesting that they are searching for an appropriate solution. Once a solution is found, however, new place responses, even in tests in novel locations can be acquired with in one trial (Whishaw, 1985c, 1989).

Hippocampal functioning has typically been examined with tests of spatial learning such as the Morris water maze (Upchurch and Wehner, 1988) and radial arm maze (McDonald and White, 1993). Learning in water maze, however, may depend on the amygdala as well as the hippocampus in as much as swimming in an opaque pool may arouse anxiety (Mc Naughton, 1991).

2.4.2 The Amygdala

The amygdala has been considered for years to be involved in emotions. This almond shaped part of the limbic forebrain (at the base of the temporal lobe) is also involved in learning and memory both directly and indirectly via its close association with the hippocampus. The electrical stimulation of the amygdala can increase memory retention (McGaugh & Gold, 1976). Furthermore, it has been demonstrated that memory can be modulated by post-training injections of epinephrine and nor epinephrine directly into the amygdala. The increased retention induced intra-amygdala injections of norepinephrine can be blocked by concurrent administration of beta-adrenergic antagonists such as propranolol (Gallagher *et al.*, 1981). The two effects of norepinephrine and epinephrine are dose and time dependent.

The Amygdala is composed of at least four independent units (Swanson and Petrovich, 1998). Artificial stimulation of the amygdala causes animals to demonstrate signs of strong fear or rage. Monkeys whose amygdala has been lesioned can learn to recognize objects, but have difficulty associating objects with reward or punishment. The role of amygdala in learning and memory is generally studied using tests of conditioned fear responses. In the context of conditioning, animals shocked in distinct environment display conditioned fear behaviours (e .g. freezing) when they remember the association between context and shock (Fanselow, 1990, 1994.)

2.4.3 The Dorsal Striatum (Caudate/Putamen)

This memory system is activated in operant conditioning task in which a stimulus associated with a specific motor response is reinforced. The caudate nucleus and the putamen are part of the basal ganglia and receive inputs primarily from the cerebral cortex and substantia nigra; the caudate nucleus from the association areas and the putamen from somatosensory and somatomotor gyri. Operant conditioning has been demonstrated on a single nucleus of the

somatomotor cortex (Fetz and Baker, 1973). This memory system is activated in operant conditioning tasks in which the stimulus associated with a specific motor response is reinforced. The cued win stay radial maze task has been effective in dissociating differences in the caudate/putamen from those in the amygdala and hippocampus. In this task, a light is used as a cue, and during each trial, the animal learns to enter the arm of the maze associated with the light/tone (Packard *et al.*, 1989).

2.4.4 The Rhinal Cortex

The rhinal cortex appears to be involved in object recognition (Zhu *et al*, 1995). In rats, recognition memory in the delayed non matching to sample (DNMTS) object recognition task is impaired by lesions of the rhinal cortex but not by lesions of the amygdala or hippocampus (Mumby and Pinel, 1994; Mumby *et al.,* 1992). This impairment difference suggests that the DNMTS object recognition task can be used to dissociate the role of the rhinal cortex, hippocampus and amygdala in learning. When testing object recognition, it is necessary to ensure that there is an absence of all spatial and odour cues and that the objects have no biologic significance so that the spatial and emotional learning and memory systems are not activated.

2.4.5 The Cerebellum

The cerebellum seems to be important in learning certain types of motor skill, especially those involving balance and coordination, like riding a bicycle. The interpositus nucleus of the cerebellum and the red nucleus of the mid brain are the critical intermediaries in a circuit linking cerebellum to the cerebral cortex in this classical conditioning experiment. New synapses are apparently formed in the red nucleus in association with this learning (Fetz. and Baker, 1973).

The cerebellum coordinated motor learning tasks such as the conditioned eye-blink (Thompson, 1986) and vestibuloocular reflex (Ito, 1984; Chen *et al.,* 1996). The conditioned eye-blink response have been studied in mutant mice with Purkinje cell degeneration. Rats raised in an environment where they could exercise and be active showed 23% more spines on the dendrites of Purkinje cells of the cerebellum than rats kept in cages allowing only enough space for access to food and water. Similarly, rats raised in enriched environment allowing for exploration showed increased branching of dendrites in the primary visual area of the cerebral cortex (Fetz and Baker, 1973).

2.5 Effects of hormones and neurotransmitters on learning and memory

2.5.1. Epinephrine and Norepinephrine

The generality of the effects of adrenergic compounds on memory has been supported by research on olfactory memory in honey bees. Post learning injections of norepinephrine into bees' brains affect memory: retention is enhanced by moderate doses and impaired by high doses of norepinephrine (Menzel, 1983; Menzel & Michelsen, 1986).

In one study involving humans, individuals read either an emotionally charged story or a similar story that was judged by other people as more emotionally neutral (Cahill *et al.,* 1994). Some of

the individuals were treated with propranolol which significantly impaired memory of the emotionally arousing story, but did not affect memory of the neutral story (Cahill *et al.,* 1994). These results supported the hypothesis that highly charged emotional memories require activation of beta adrenergic receptors (Cahill *et al.*, 1994). The effects of epinephrine administered either peripherally or centrally could hypothetically be mediated via increased release rates of adrenocorticotropic hormone (ACTH). However, the memory enhancing effect of post training epinephrine administration is not blocked by dexamethasone, an artificial steroid that blocks the release of ACTH. Therefore, it appears that ACTH does not mediate the memory-enhancing effect of epinephrine (McGaugh et al., 1987) although ACTH has its own potent effects on memory.

Epinephrine elevates blood glucose concentration which increases the amount of glucose that enters the neurons. Epinephrine enhances memory and its memory enhancing effects are both dose and time dependent. Responses to different concentrations of epinephrine follows an inverted U- shaped curve, that is, low and high blood levels of epinephrine impairs memory whereas moderate epinephrine enhances memory (Parsons & Gold, 1992). In tihis regard studies have reported that, the optimal dose in rats is about 0.1 mg/kg which yields a blood level of epinephrine of about 1500 picograms/ml (Gold and Van Buskirk, 1975). In addition, injections of epinephrine (1 mg/kg) are most effective in enhancing memory if given 1 minute after training and these beneficial effects diminish 60 minutes after training (Gold, 1987). Because epinephrine is released in response to stressful events a reasonable possibility is that it potentiates the effects of the noxious stimuli used to train animals in active avoidance tasks. Animals perform better in avoidance situations after receiving a moderate than after a mild foot shock. If epinephrine potentiates the effects of shock then it should be released when shock occurs, and epinephrine treatment paired with mild foot shocks should produce learning compared to that observed in

animals experiencing moderate foot shocks. Both of these effects have been demonstrated (Gold; 1987), For example, the optimal levels of epinephrine for memory enhancement in rats is 1500 picograms /ml of blood serum and this level in rats showed optimal performance in avoidance tasks (Gold, 1987). If a mild foot shock produces blood epinephrine levels of 1000 pg/ml and the shock is paired with an injection that raises epinephrine levels another 500 pg/ml, then the exogenous and endogenous epinephrine sum and the animal exhibit optimal learning (Gold, 1987).The best time to administer epinephrine is immediately after training, treating before training or when a substantial period of time has elapsed since training is not effective in enhancing memory (Gold, 1987). These temporal constraints are consistent with the hypothesis that epinephrine influences memory by potentiating the effects of noxious events.

Learning and memory involve encoding, storing and retrieving information. Epinephrine as well as other hormones could facilitate any or all of these processes. The precise mechanisms of memory have yet to be elucidated. Epinephrine is a molecule that is too large to cross the blood brain barrier, although it is produced by very few neurons in the brain as a neurotransmitter (Weil-Malharbe *et al.,* 1959). How then can epinephrine affect learning and memory processes if it cannot get to the neurons in the brain? Epinephrine must affect some process outside of the brain that subsequently influences the brain.

One of the hypotheses is that epinephrine affects memory via its effects on blood glucose levels. Furthermore, one of the many physiological consequences of epinephrine secretion in a stressed animal is hyperglycaemia. In general, glucose enhances memory in a dose dependent manner, with its dose response curve resembling an inverted U- shape (Gold, 1986; Parson's & Gold, 1992). The effects of glucose are time dependent. Injection of glucose delayed by 1 hour after training having no effect on retention and performance. Furthermore, glucose injected directly

into the brain also enhances memory (Gold, 1987). Elevated blood glucose levels permit more glucose to enter neurons, which in turn stimulates an increase in the release of acetylcholine from neurons in the brain. The neurobiological mechanism(s) by which glucose improves memory appear to involve acetylcholine release. In a study of rats in a simple T-maze, micro dialysis revealed that acetylcholine levels were elevated in glucose treated rats during memory testing (Ragozzino *et al.*, 1996).

Recent studies have confirmed that glucose enhances both memory storage and retrieval in healthy elderly humans (Manning *et al.*, 1998 b). In addition, glucose also improves learning and memory in people with Alzheimer disease as well as in adults with Down's syndrome (Korol & Gold, 1998; Manning *et al.*, 1998 a). However, rats rendered diabetic display deficits in spatial learning in Morris water maze (Biessels *et al.*, 1996).

The other hypothesis on how epinephrine modulates memory is that it activates peripheral receptors that communicate with the central nervous system (McGaugh, 1989). In order to discover which neural receptors are involved in the memory effects of epinephrine, specific receptor agonists and antagonists are used. The effects of epinephrine on memory can be blocked by both alpha and beta adrenergic antagonists (Sternberg *et al.*, 1985, 1986). In one study, rats were injected with an alpha or a beta adrenergic antagonist phenoxybenzamine or propranolol, respectively, 30 minutes before they received either an injection of epinephrine or foot shock treatment which caused endogenous levels of epinephrine to rise. The glucose concentrations did not rise very much after epinephrine or foot shock treatment. In rats pre-treated with the beta blockers propranolol, however, glucose concentrations increased after epinephrine treatment or foot shock in rats pre treated with the alpha blocker phenoxybenzamine (Manning *et al.*, 1992). The memory enhancing effects of Clenbuterol, an adrenergic agonist

that crosses the blood brain barrier can be blocked only by centrally acting beta adrenergic antagonists (Introini-Collison & Baratti, 1986). These data suggests that epinephrine acts in peripheral adrenergic receptors to initiate its effects on memory (McGaugh, 1989). The two working hypothesis explaining how epinephrine affects memory are not compatible. Epinephrine elevates blood glucose concentration which increases the amount of glucose that enters the neurones. The neurones thus release higher concentration of acetylcholine into the synapses. Epinephrine also appears to act directly on neurons to enhance their function. These direct central effects of epinephrine may reflect endocrine activity or epinephrine could affect Amemory directly (perhaps after entering the brain through the cerebro-spinal fluid thus circumventing the blood brain barrier) by acting as a central neurotransmitter (McGaugh, 1989).

2.5.2 Adrenocorticotropic hormone (ACTH)

ACTH injections in rats lacking both pituitary and adrenal glands restore learning and these effects on memory are independent of its endocrine effects (De Wied, 1974). Not only ACTH but also small pieces of the ACTH molecule devoid of any effect on the adrenal glands, affect learning (De Wied, 1974; 1977). ACTH can also protect against amnesia, failure to remember. Three experimental techniques can induce amnesia; exposing animal to carbon dioxide immediately after training, administering a strong electric shock after training or treatment with protein synthesis inhibitors (Quartermain, 1976). ACTH attenuates the amnesic effects produced by these experiments manipulations.

2.5.3 Vasopressin and Oxytocin

The Brattleboro rats have a congenital lack vasopressin and also have difficulty in learning shuttle box avoidance skills (De Wied, 1980; 1984). They forget these tasks very quickly and the deficit in their performance appears to be due to memory problems rather than learning

dysfunction. Homozygous Brattleboro rats avoid the compartment in which they have been shocked when their retention is tested immediately after the learning trial, however, if a delay occurs between training and testing, they rapidly re enter the compartment in which they had been shocked. Treatment with vasopressin immediately after the learning trial raises the subsequent avoidance behaviour of homozygous Brattleboro to the level of the heterozygous cousin which has normal vasopressin levels.

Vasopressin blocks forgetting or prolongs memory; indeed its injection can prolong memory in normal animal for noxious experience by days or weeks (De Wied, 1980). In addition, their effects of on memory are dose and time dependent (De Wied, 1984). Furthermore, the memory enhancing effects of post training treatment with vasopressin in normal rats require the presence of an intact adrenal gland or prior treatment with epinephrine (McGaugh, 1989). Both working memory and reference memory (long-term) assessed in a radial arm maze are improved by vasopressin and vasopressin antagonists (Dietrich & Allen, 1997 a, b).

Oxytocin on the other hand appears to have a variety of effects on memory function but the majority of studies have implicated oxytocin as an amnesic peptide when given intraventricularly it enhances forgetting (Bohus *et al.*, 1982). Other studies indicate that systemic injections of oxytocin can enhance memory (De Wied, 1984) but usually only in constrained situations or under specific conditions (Boccia *et al.*, 1998).Oxytocin promotes smooth muscle contraction and is therefore critical to milk let down and the uterine contractions of birth. One possible adaptive function of the amnesic properties of oxytocin is the dulling of memory of the pain associated with giving birth thereby increasing the probability that females will repeat the process (Carter *et al.*, 1992). In another study, the results have suggested that oxytocin may have intrinsic reinforcing properties (Liberzon *et al.*, 1997) and rats injected with oxytocin developed

slight place preference suggesting that oxytocin has intrinsic reinforcing properties (Liberzon *et al.,* 1997).

2.5.4 Endogeneous Opiates

Opiates attenuate pain or the emotional response to pain. Opiods have amnestic properties in avoidance learning paradigms, but seem to enhance the reward properties of trial and error learning situations. True amnestics act directly on memory storage, retrieval, or both processes. Opiods probably ameliorate the noxiousness of an aversive stimulus so that it is not perceived as aversive, thus appearing to act as amnestics. Opiate receptors may play a part in reinforcement and reward, for example, opiates such as morphine and heroin have reinforcing effects that cause some people to become addicted to the drugs. The reinforcing effects of opiods were found to be caused by activation of opiate receptors in the brain (McGaugh, 1983). This finding suggests that the release of endogenous opiates may play a role in the reinforcement of behaviour.

Neurones than contain opiate receptors are found in several regions of the brain in which electrical stimulation has reinforcing effects including the hypothalamus, nucleus accumbens and periaqueductal grey matter. Rats will press a lever repeatedly to cause opiates to be injected into their brains, thus activating these receptors. Furthermore, morphine and other opiates increase the rate of responding (bar pressing) for electrical brain stimulation whereas naloxone an opiate receptor blocker decreases the response rate (Schaefer, 1988). In addition, rats show a place preference in response to systemic injections of morphine that is, they prefer the part of a cage where they received morphine injections on previous days (Amalric *et al.*, 1987) and this effect is blocked by injection of naloxone an opiate receptor blocker. When opiate receptor agonists, e.g. morphine, beta endorphin and enkephalin are given to adult rats immediately after training in low doses, their memory was impaired in a dose and time dependent manner (McGaugh, 1989).

On the other hand, opiate antagonists ameliorate the memory impairing effects of opiates and their agonists. Studies have demonstrated that retention is enhanced by post training administration of opiate antagonists including naloxone (McGaugh, 1989). The memory enhancing effects of these opiate antagonists are also dose and time dependent and have been found in studies using several types of training tasks, including passive avoidance, active avoidance, habituation and appetitive spatial learning (McGaugh, 1989). Furthermore, opiate antagonists also block the amnestic effects of electroconvulsive shock therapy (Collier *et al.*, 1987) but have had mixed results in human patients with memory disorders (McGaugh, 1989).

The memory modulating effects both central and peripheral injections of met-enkephalin (and peripheral naloxone) are attenuated in adrenal-denervated (or demedullated) animals (Conte *et al.*, 1986). Taken together, these results suggest that peripheral injections of opiates affect memory via epinephrine. The injections of met-encephalin elevate blood glucose probably by stimulating epinephrine secretion, however, it is prudent to recall that the effects of epinephrine and glucose follow an inverted U-shaped curve, levels too high or low reduce performance on learning and memory tasks and therefore, only moderate levels of epinephrine and glucose enhance learning and memory.

2.5.5 Effects of Glucocorticoids on learning and memory

Acute stress appears to promote lasting memories. However, chronic stress has the opposite effect. Long term stress or chronic treatment with corticosterone impairs memory (Luine, 1994; Luine *et al.*, 1994; de Quervain *et al.*, 1998). Therefore, corticosterone (cortisol) appears to function as an amnestic in most of the studies reported to date (McLay *et al.*, 1998).

A study that correlated corticosterone consumption and maze performance indicated that rats consuming the most corticosterone made the most errors in a radial arm-maze (Luine *et al.,* 1993). Therefore, corticosterone treatment impairs spatial learning in rats in a variety of testing situations (McLay *et al.,* 1998) and, in addition, treatment with a progestin and glucocorticoid receptor antagonist (RU 486) infused directly into the dorsal hippocampus improved the performance of male rats in the MWM (Oitzl *et al.,* 1998). In another study, evidence indicated stress and corticosterone impairs memory retrieval (De Quervain *et al.,* 1998). Like estradiol, corticosterone causes restructuring of the hippocampus and its circuit. The hippocampus related brain structures are rich in glucocorticoid receptors. Treatment of rats with corticosterone or inducing chronic stress decreases number of pyramidal cells, dendritic length and number of dendritic branch points in the CA1 and CA3 regions of the hippocampus (Watanabe *et al.*, 1992; Woolley *et al.,* 1990; Sapolsky et al., 1985). Similarly, very low corticosterone concentration also cause degeneration in a brain region closely associated with hippocampus, the dentate gyrus (Sloviter *et al.,* 1989; Conrad & Roy, 1992). After adrenalectomy, hippocampal cell numbers decrease by approximately 50% (Gould *et al.,* 1991; Conrad & Roy, 1992; Luine, 1994). Spatial memory is impaired by adrenalectomy (Gould *et al.*, 1991). Furthermore, adult neurogenesis is chronically suppressed by repeated exposure to stress contributing to diminished spatial navigational learning (Gould *et al.,* 1998).

2.6 Effects of Sex hormones on learning and memory

The animal models of spatial learning indicate that males perform better on spatial learning tasks than females in most cases, and that hormones are likely to mediate the sex difference in performance (Gaulin & FitzGerald, 1989; Williams *et al.,* 1990). Early hormone exposure may influence visuospatial learning among humans as well. These models also show that the effects

of sex hormone on learning performance may be indirect and may be influenced by extrinsic and intrinsic factors such as stress. Indeed, an interaction between sex and the stress of novelty or electric shock has been demonstrated in recent studies (Williams *et al.,* 1990). Males generally perform better than females in the Morris water maze (Galea *et al.*, 1996). In one study, stress impaired learning of a simple associative task (i.e. eye blink) in females but not in males. In fact, stress facilitated the male's performance (Wood & Shors, 1998). From the foregone the choice of male CBA mice was to avoid the effect of estrous which arises from sex hormones with their attendant effect on learning and memory. This was an important consideration because female in comparison to male animals, undergoes estrous, which could be a confounding factor causing interpretational problem and thus affecting the validity of the results.

2.6.1 Effects of Androgens on learning and memory

Many studies conducted to investigate the role of testicular androgens in learning and memories have shown that the gonadal androgens do not affect learning and memory and this is true of humans and non human animals (Alexander *et al.*, 1998). For example, neither testosterone replacement therapy in hypogonadal men nor testosterone treatment of normal men revealed changes in learning and memory performance (Alexander *et al.,* 1998).

Sex difference in spatial learning performance has been reported for meadow Voles (*Microtus pennsylvanicus*) and deer mice (*Peromyscus maniculatus)* (Galea *et al.*, 1995; 1996). During the breeding season, males of both species out perform females, but sex difference disappears when the animals are not in breeding condition (Galea *et al.,* 1996). Testosterone seem to have some positive reinforcing properties, for example, place preference develops in rats that have testosterone injected into the nucleus accumbens (Packard *et al.*, 1997).

2.6.2 Effects of Oestrogen on learning and memory

Estradiol appears to enhance spatial memory in a reliable and subtle manner (Luine, 1994; Daniel *et al.,* 1997). However, estrogenic effects on learning and memory are complex and depend on many factors, including the timing of hormone administration and the gonadal state of e individual being assessed. Further more, estrogens appears to enhance consolidation and slightly enhance acquisition of spatial reference memory tasks (Daniel *et al.*, 1997; Luine *et al.,* 1998; Fader *et al.,* 1998). On the other hand, several studies in rats and humans have demonstrated that spatial memory is impaired during pre ovulatory portion of the ovarian cycle, when estadiol concentrations are normally high (Frye, 1995; Galea *et al.*, 1995; Korol *et al.,* 1994; Warren & Juraska, 1997).

Infusion of water soluble form of estradiol directly into the hippocampus enhances the memory of ovariectomized rats for Morris water maze but only if given immediately after training. (Packard *et al.*, 1996; Packard & Teather, 1997). Estradol receptors are located within the hippocampus especially in the CA1, CA3 and dentate gyrus regions (Loy *et al.,* 1988; Maggi *et al.,* 1989; Gould *et al.*, 1990). Oestrogen treatment and naturally high oestrogen concentrations around the time of ovulation are associated with an increase in the density of dendritic spines in the CA1 region (Gould *et al.,* 1990, Woolley *et al.*, 1990a; McEwen *et al.,* 1995). Hippocampal LTP is facilitated by oestrogen treatment in awake rats (Cordoba-Montoya & Carrer, 1997), and oestrogen also affects basal forebrain cholinergic neurones that might be important in passive avoidance, attentional tasks, as well as spatial learning (Gibbs, 1997). Oestrogen administration has been shown to enhance memory and reduce neuronal loss associated with Alzheimer disease in post menopausal women (Simpkins *et al.,* 1997) and to reduce the damage caused by blood reperfusion after a cerebral ischaemia (stroke) (Hurn *et al.*, 1995).

2.7 Paradigms used to investigate learning and memory

2.7.1 Habituation

This is the most generalized and simple type of learning. It is usually considered to be a loss of response after repeated exposure to a stimulus (Nelson, 2000). Habituation occurs in the central nervous system and is regarded as learning not to respond. The neuronal mechanism of habituation has been studied in simple invertebrates systems and involves a reduction in the amount of neurotransmitter released at the synapse. Habituation is different from fatigue or sensory adaptation. Fatigue is the loss of efficiency in the performance of a motor act after numerous rapid repetitions whereas sensory adaptation occurs at the level of sensory receptor; that is the sensory receptors repeatedly exposed to a stimulus stop sending nerve impulses to the central nervous system (Nelson, 2000). The habituation test has been used as a non associative test of learning (Platel and Porsolt, 1982).

2.7.2 Conditioned fear response (aversive learning)

Active avoidance describes a situation in which an animal must do something to avoid noxious situation (Nelson, 2000). This is a test of conditioned fear response which generally studies the role of amygdala in learning and memory. In the content of conditioning, animals shocked in a distinct environment display conditioned fear behaviours when they remember the association between the context and the shock (Fanselow, 1990, 1994). In place avoidance conditioning, animals avoid the place associated with shock (Siegfried and Frischknecht, 1989). In active avoidance conditioning, mice learn to avoid the location/place associated with fear. In passive avoidance the animal learns to suppress some behaviour that would otherwise be exhibited (Nelson, 2000), for example, rats prefer dark compartments to illuminated ones. When the rat goes into the dark compartment, which it prefers, it receives a foot shock and must travel back

into the illuminated part of the shuttle to escape the shock. In other words the rat inhibits its inclination to enter the dark compartment of the box and therefore passively avoids the unpleasant stimulus (Delprato and Rusiniak, 1991).

2.7.3 Radial arm maze

It is a common apparatus used in assessment of spatial memory. The maze is open at the top and may have 8, 12 or even 36 arms. The greater the number of arms, the more difficult the maze is. The radial arm maze has been used to examine the functioning of hippocampus on spatial learning (McDonald and White, 1993). Males use different strategies than females to solve radial arm mazes, and these strategies can be manipulated by early hormone treatment (Williams *et al.,* 1990). In one study, eight of the twelve runways of a radial arm maze were always provided with a food treat (baited); four arms were always devoid of the treats (unbaited). The solution to the maze involved making only one trip down each of the eight baited arms and avoiding the unbaited arms. This task, therefore involves long-term, or reference, memory recall which of the twelve arms were always baited, as well as short term or working memory to recall of which the eight baited arms had already been visited on that particular trial (Olton and Samuelson, 1976; Williams *et al.,* 1990).

The strategies used by rats to solve the radial arm maze were probed by manipulating potential cues in the interior of the maze (landmarks) and orientation cues outside the maze (geometry). In one study, female rats used both landmarks and geometry to solve the maze whereas males and masculinized females used only geometry to learn the task. In other words, males and masculinized females learned fewer total cues and could master the task faster than females or feminized males (Williams *et al.,* 1990). This effect is thought to be mediated by hormonal effects on the hippocampus.

2.7.4 Win stay radial maze

The cued win stay radial maze task has been effective in dissociating differences in the candate/putamen from those in amygdala and hippocampus. In this task, a light is used as the cue, and during each trail the animal learns to enter the arm of the maze associated with the light/tone (Packard *et al.*, 1989).

2.7.5 Novel Object recognition task

Object recognition memory is another model of declarative memory in which the medial temporal lobe has been implicated in primates and humans (Squire and Zola, 1996). Some studies of primates and rodents have shown the importance of the parahippocampal regions of the temporal lobe (the perirhinal, entorhinal and inferior temporal) in visual object recognition memory (Gilbert and Kesner, 2000). Excitotoxic lesions of the peripheral cortex in rats disrupt object recognition memory (Aggleton *et al.*, 1997; Lin & Bilkey, 2001) and in some studies of neuronal activation and response in rats and monkeys suggest it is cortical and not hippocampal neurons that are involved in object recognition tasks (Brown & Aggleton, 2001). However, some human and primate studies have shown that hippocampal lesion result in impaired object recognition memory (Zola *et al.*, 2000). While others have shown limited effects of hippocampal lesions on object recognition memory in primates (Zola & Squire, 2001).

The spontaneous object recognition task was developed by Ennaceur and Dalacour (1988) and takes advantage of rodents' natural tendency to explore novel objects so that there is no need for food deprivation to motivate rodents to perform (Hammond *et al.*, 2004). There are two different ways of conducting the novel object test, each of which focuses on a different type of memory depending on the time lapse between training to the novel objects and testing (Brown & Aggleton, 2001). The first method involves a short duration between introduction of the novel

objects and testing. The second type of novel object recognition task is a test of long term memory which involves a 24 hour period between trial 1 and trial 2.

2.7.6 Delayed non matching to sample (DNMTS) object recognition task

Perirhinal cortex makes an essential contribution to object recognition memory, as measured by delayed matching (or non matching) to sample tasks. In these tasks, subjects must choose a currently presented object that matches (or fails to match) an object presented previously (Murray and Richmond, 2001).The rhinal cortex also appears to be involved in object recognition (Zhu *et al.,* 1995). In rats recognition memory in the delayed non matching to sample (DNMTS) object recognition task is impaired by lesions of the rhinal cortex but not by lesions of the amygdala or hippocampus (Mumby and Pinel, 1994; Mumby *et al.,* 1992). This impairment difference suggests that the DNMTS object recognition task can be used to dissociate the role of the rhinal cortex, hippocampus and amygdala in learning.

2.7.7 The Morris Water Maze

The Morris Water Maze (Upchurch and Wechner, 1988) is paradigm that is used to assess spatial learning, a hippocampal function. It is a circular pool filled with water. Mice are trained to use extra-maze visual cues to locate an escape platform hidden just below the surface of the opaque water (Morris, 1984). The hidden platform version of the Morris water maze is test of spatial memory which is sensitive to hippocampal damage or dysfunction. While the visible platform version of the Morris water maze is a non-hippocampal task which is disrupted by dorsal striatum lesions (Mc Donald and White, 1994).

Considerable use has been made of the Morris water maze for studying spatial learning in transgenic and mutant mice (Owen *et al.,* 2000), the effects of brain injury on spatial learning (Fox *et al.,* 1998) and the effects of drugs on spatial learning (Von Lubitz *et al.,* 1993; Fordyce *et*

al., 1995). The MWM apparatus is very useful for evaluation of spatial learning and memory. It allows simultaneous evaluation of learning, spatial memory and working memory as well as motor activity by submitting the same animal to distinct and consecutive phases of training (Pettenuzzo, 2003). Distinct protocols can be run in the water maze e.g. the platform can be hidden or visible, the cues can be intra or extra-maze, the number of daily trials and number of training days can vary (Morris, 1982). Using a hidden platform, better evaluates the spatial learning and memory since animals must build a spatial map to locate the submerged platform in the pool; that is distinct from what occurs when the platform is visible.

2.8 Effects of addictive drugs on the brain.

Psychoactive drugs have their effects on the brain. The drugs act primarily on the brain at the neuronal and structural levels. At the neuronal level the drugs interfere with neurotransmitter turnover. At the structural level they alter the morphology of key brain structures. Further, the addictive drugs alter the functioning of the brain by modifying the production, release and breakdown of neurotransmitter and nueropeptides. The type of effect a given drug will exert depends on the transmitter (catecholamine, serotonin, and acetylcholine) or neuropepetides (endorphin) with which the drug predominately interacts, the nature of this interaction, and the locus of the interaction in the brain and the function that this area of the brain fulfils.

The turnover of all neurotransmitters and neuropepetides is linked through a network of feedback control mechanisms which normally results in a state of brain equilibrium or brain homeostasis. Drugs can disrupt this homeostasis and thus affect the normal functioning of specialized parts of the brain (Nahas, 1981). The brain is made up of numerous structures which have specialized functions and these are specifically altered by psychoactive drug.

2.8.1 Effects of amphetamines on learning and memory

The most effective and frequently prescribed drugs for treatment of attention deficit hyperactive disorder, is methylphenidate (Ritalin) and amphetamines which are psychostimulants that inhibit re uptake and stimulate the release of dopamine in the CNS, thereby increasing the temporal and spatial presence of dopamine at post-synaptic receptors (Klause *et al.,* 2000; Safer and Krager, 1988). One of the effects of amphetamine withdrawal is demonstrated in behavioural sensitization studies, in which withdrawn subjects exhibit an enhanced behavioural response to a single challenge of the drug compared with drug-naïve subjects and this effect can last for at least a year (Paulson *et al.,* 1991). The striatal cortex, prefrontal cortices and limbic areas are some of brain structures demonstrated to undergo adaptive change during repeated amphetamine administration. These structures have been implicated with various forms of learning and memory (Goldman-Rakie, 1987; Baddeley, 1992; Dias *et al.*, 1996; Nester and Aghajanian, 1997, Robinson and Kolb, 1997 and Berke and Hyman, 2000). Post trial amphetamine treatment has been shown to enhance memory consolidation in the water maze task (Brown *et al.,* 2000). On the other hand, chronic administration of psychostimulant cocaine, a drug that resembles amphetamine pharmacologically, has been reported to impair water maze performance in rats (Quirk *et al.,* 2001).

In another study, acute amphetamine treatment has been reported to increase memory consolidation and increase the impact of reinforcement in some learning paradigms (McGaugh, 1989 and Killcross *et al.,* 1994). This finding is in line with research that reported a lack of an effect during amphetamine withdrawal on spatial working memory (Stefans and Moghaddani, 2002). Similarly, rats treated with amphetamine performed well on reversal learning and had superior performance over the control on the final trial of the first reversal day (Russing *et al.,*

2003). In addition, acute administration of low doses of amphetamine enhances reversal learning (Weiner *et al.,* 1986; Weiner and Feldon, 1986) possibly due to an effect of the drug acting on the nucleus accumbens in enhancing behavioural switching. This hypothesis attributes a role of switching to the nucleus accumbens under the modulation of the ascending dopaminergic input from the ventral tegmental area and the glutamatergic limbic inputs originating from the hippocampal formation, entorhinal cortex, amygdala and prefrontal cortices. It is suggested that acute amphetamine disrupts this balance between the two sets of inputs resulting in enhanced behavioural switching (Weiner, 1990; Weiner and Feldon, 1997).

In one study, methamphetamine administration during early post natal development in rats resulted in impaired learning in a T-maze if administration occurs from postnatal day 1 to 10, and impaired learning in the MWM if administration occurred from postnatal days 11 to 20 (Vorhees *et al.,* 1994). In a report published by McFadyen and colleagues (2001) methyphenidate administered chronically to male CD-1 mice at a prepubertal (late juvenile) developmental stage had little or no enduring effect on locomotor, exploration, emotionality or learning in a simple visible platform water maze task and in a recent study, methylphenidate has been found to enhance working memory in humans (Mehta *et al.,* 2000).

2.9 Classification of drugs of abuse

According to American Psychiatrist Association (APA, 2000), substance abuse is defined as maladaptive use of a substance leading to impairment or distress as manifested by one of the following: curtailing of a major role obligation at work, school or at home in favour of substance abuse, the use of a substance when it is hazardous, having substance related legal problems, and continued use despite adverse social and interpersonal effects. It follows that these substances

trigger brain mechanisms which are then predisposed to their continued self administration. They produce a primary pleasurable reward (Paton, 1980), mediated, by their action upon brain reward mechanism (Freud, 1961; Olds, 1977). The effects of addictive drugs on the brain neurotransmitter and specialized functions of the limbic structure produce neuropsychological anomalies, which result in behaviour alterations. These are numerous and differ according to the drug. Addictive drugs are classified on the basis of their effects on brain and behaviour (Nahas; 1981). The effects are primarily mediated through alteration of brain neurotransmitters in the areas of the limbic systems that have been associated with the pleasure reward mechanism of the brain; these mechanisms control motivation and behaviour.

2.9.1 Major Psycho stimulants

This class of drug cause increased arousal, awareness and insomnia. Major psycho stimulants are the most profound reinforcers in rhesus monkeys, which will press a lever more than 4,000 times in order to get single injection of cocaine. When given free access to cocaine or amphetamines, the animals will immediately self-administer high daily doses. Man's propensity to take drugs of abuse is shared with other mammals (Johanson & Balster, 1978; Johanson, 1978).

2.9.2 Minor Psycho stimulants

This class of drug of abuse cause moderate arousal, awareness and insomnia. It includes drugs like nicotine and caffeine. These drugs given in moderate amounts do not produce any measurable symptoms of neuropsychological toxicity (Nahas, 1981).

2.9.3 Opiates and their Derivatives

These drugs produce pleasurable effect on brain reward mechanism and they are associated with ability to dissipate unpleasant feelings, decrease anxiety and detachment from the world. They are also powerful with respect to their analgesic property i.e. by relieving pain. They decrease arousal and awareness and thus cause sleepiness. There are withdrawal effects after drug discontinuation. With opiates, monkeys self administer the drug by gradually raising the daily dose over a period of weeks until they reach a steady state. For the opiates, the withdrawal symptoms seem to result mainly from an imbalance or alteration of autonomic nervous system controlled by hypothalamus (Pradhan & Dutta, 1977). Drugs in this group include opium, heroin, morphine and synthetic opiate agonists among others.

2.9.3 Depressants

This category of drug includes barbiturates, alcohol, benzodiazepines and marijuana (THC). Intoxicating concentration of ethanol enhance the function of gamma amino butyric acid type A ($GABA_A$) receptors, the major inhibitory neurotransmitter in the brain. (Allan and Hams, 1986; Ticku *et al.*, 1986). They decrease arousal and awareness causing sleepiness, especially barbiturates. In addition, alcohol causes motor in coordination and tremor to the user, but alcohol in small amounts does not produce measurable symptoms of neuropsychological toxicity. However, if alcohol is suddenly deprived to the addicted user, he/she comes down with withdrawal syndrome (Rosthein, 1973).

2.9.4 Hallucinogens

This class of drug includes mescaline, psilocybine, lysergide (LSD), and phencyclidine among others. They distort the sensory perception and therefore, the user experiences hallucinations. The manifestation could be mild or severe reflecting profound distortion of the brain function,

including alterations in the receiving sensory areas of the cerebral cortex. Hallucinogens are not self administered by non human primates as opposed to other drugs, such as, the psycho stimulants and opiates.

2.9.5 Antidepressants or antipsychotic

These are medical drugs used in the treatment of severe mental illness like, schizophrenia or endogenous depression. Drugs grouped in this class include amitryptylline, imipramine, chlorpromazine, butyrophenone, perphenazine among others. These drugs tried on monkeys are never self administered and the animals learn to avoid manoeuvres that results in administration of the drugs. (Griffith *et al*., 1978; Johanson and Uhlenhuth, 1978).

2.9.6 Non-Narcotic analgesics

This class of drugs includes non steroidal anti-inflammatory drugs such as acetylsalicylic acid, phenybutazone and sodium salicylate to mention a few. They are generally used medically to relieve pain.

2.9.7 Solvents

These are broad range of volatile substances which are inhaled. They include glues, solvents, gases (e.g. nitrous oxide) and aerosol propellants among others. Many of these are organic, petroleum based products. They typically produce central nervous system (CNS) depression, although they can cause excitability. Chronic use can lead to cerebral or cerebellar dysfunction, and death can occur as a result of asphyxiation or arrhythmias.

2.9.1.1 KHAT

2.9.1.1.1 Introduction (Background)

Khat, *Catha edulis* forsk (family celestraceae), is a flowering evergreen shrub or small tree that grows wild or is cultivated in certain regions of East Africa and Southern Arabia (Patel, 2000). It has been known for centuries in East Africa and the Middle East, indeed it is indigenous to Ethiopia and Somalia (Carlini, 2003; Connor *et al.,* 2002)

The chewing of khat leaves is common in certain countries of East Africa and the Arabian Peninsula. It has been used for recreational purposes (Kennedy, 1987) for its valued psycho stimulant effects (Baasher, 1980). Khat must be chewed while fresh and that is the reason why it is usually wrapped in banana leaves immediately after picking, to preserve its potency (Elmi, 1983). Fresh khat leaves are crimson – brown and glossy, but become yellow green and leathery as they age. They also emit a strong smell. The most favoured part of the leaves is young shoots near the top of the plant. However, leaves and stems at the middle and lower sections are also used.

2.9.1.1.2 Geographical distribution

Several million people are estimated to be frequent users of khat (Kalix and Braenden, 1985) and its consumption is increasing (Kennedy, 1987). The prevalence varies widely between the various khat using countries (Mancioli and Parrinello, 1967; Omolo and Dhadphale, 1987). In Somalia, Elmi, (1983) estimated that about 18% in the southern and 55% of the population in the North were consumers. Kennedy, (1987) estimated that approximately 50 – 60% of women chew khat more than once a week compared with 80 – 85% of men. In countries such as Yemen and Somalia many houses have a room specifically used for chewing khat (Basher, 1980). In Yemen,

khat is regarded as beneficial, though it was considered desirable to prevent the young generation developing the habit (Mckee, 1987).

In Kenya, khat is grown in Meru district and proposed active ingredient are named reflecting the sample source as merucathinone, pseudomerucathine and merucathine (Brenneisen and Geisshusler, 1987), however, the concentration of these compounds in the plant is quite low (Kalix, 1991). Khat has several local names, miraa in Kenya, qat, African salad, Africa tea, gomba among others. Locally, there are different variety depending on quality with giza being the best quality and most expensive and kangeta and kata, the latter being less expensive and not fresh. The market value of the leaves correlates with cathinone content (Kalix and Braeden, 1988) and season.

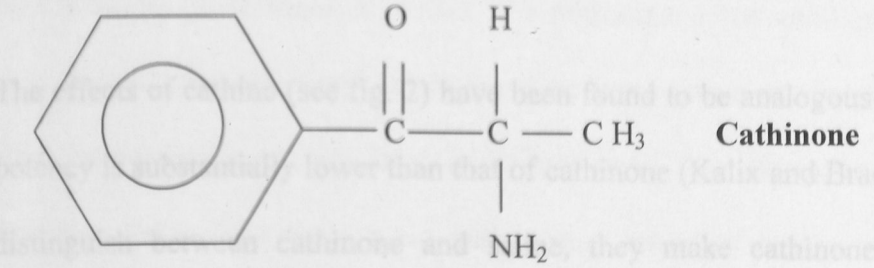
Substance abuse involving khat cuts across all ages. Youths and young adults between the ages of 16 – 25 years constitute the majority in khat consumption. These findings are reported to correspond to observation in Kenya (Adugna *et al.,* 1994) and other neighbouring countries, Uganda, Tanzania and Ethiopia (Selassie and Gebre, 1996; Adugna *et al*., 1994).

The habitual use of khat is often compulsive, a fact that is illustrated by the tendency of many chewers to secure their daily supply of the leaves at the expense of vital needs. Indeed, the habit may give rise to moderate but often persistent psychic dependence on this drug (Eddy *et al*., 1965). There are however, only minor withdrawal symptoms after prolonged khat use: these include lethargy, mild depression, slight trembling and recurrent bad dreams (Halbach, 1972; Kennedy *et al.,* 1980).

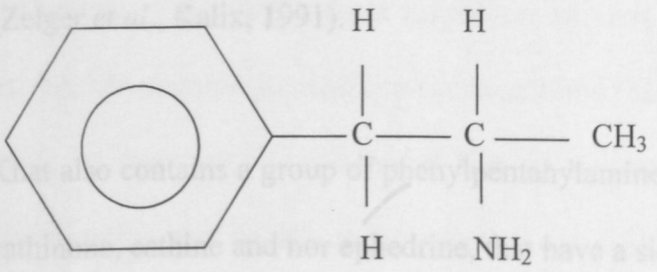
2.9.1.1.3 Active ingredients of khat

The first attempts to isolate the active compounds of khat were made in the 1800's (Fluckiger & Gerock, 1987). Wolfes, (1930) identified cathine in the khat leaves and up to about 1960s; cathine was generally believed to be the main active compound of khat (Alles *et al.,* 1961). However, Von Brucke (1941) pointed out that the concentration of cathine in the leaves is insufficient to account for the symptoms produced by that portion of the drug, and therefore, the presence of another alkaloid in khat had to be taken into consideration.

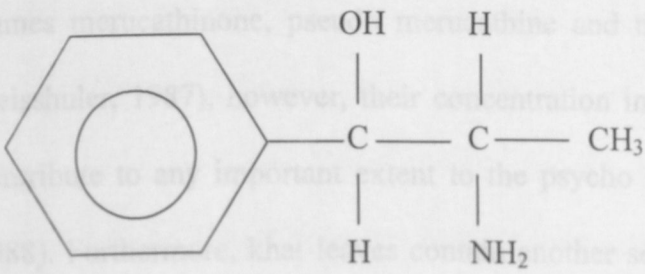
A careful re-investigation by the United Nation Narcotics Laboratory culminated with the isolation in 1975 of the cathinone (UN documents 1975). Cathinone is mainly present in young leaves which explain why the khat users prefer fresh leaves (Guantai and Maitai, 1982; Schorno *et al*., 1982). The findings led to the conclusion that cathinone is a biosynthetic precursor which accumulates in young leaves, but in adult leaves and during the wilting process, undergoes enzymatic reduction to cathine and norephedrine (Kalix, 1991). Indeed, cathinone is rather labile compound which explains why it was discovered so late inspite of its relatively simple structure (Kalix, 1991).



Cathinone



Amphetamine



Cathine (norpseudoephedrine)

Fig. 2: The chemical structure of Cathinone; Amphetamine; (+) norpseudoephedrine

Cathinone is structurally similar to D-amphetamine (see fig. 2) and also to cathine. Indeed, fresh khat leaves contains both ingredients, and those left unrefrigerated beyond 48 hours, contain

mainly cathine which explains the users preference for fresh leaves. Because of the similarity of their structure and their physiological effects, cathinone has been named a natural amphetamine. The only chemical difference between the two is that, oxygen double bond substitutes the two hydrogen molecules in the beta carbon of the amphetamine side chain.

The effects of cathine (see fig. 2) have been found to be analogous to those of cathinone, but its potency is substantially lower than that of cathinone (Kalix and Braeden, 1985). In rats trained to distinguish between cathinone and saline, they make cathinone appropriate response upon substitution of cathinone by cathine; however cathine is in such experiments eight times less potent than cathinone (Glennon *et al.,* 1984). The more and intense action of cathinone compared to cathine, is explained by its higher lipid solubility, facilitating quicker access into the CNS (Zelger *et al*., Kalix, 1991).

Khat also contains a group of phenylpentanylamines that are analogs of the phenylpropylamines, cathinone, cathine and nor ephedrine, that have a side chain containing an additional element that is unsaturated. These substances were discovered in the khat from the Meru district in Kenya, the names merucathinone, pseudo merucathine and merucathine were proposed (Brenneisen and Geisshuler, 1987), however, their concentration in khat plant is quite low. They appear not to contribute to any important extent to the psycho stimulant effect of khat leaves (Kalix *et al.,* 1988). Furthermore, khat leaves contain another series of alkaloid called the cathedulins which have a molecular weight ranging from 600 to 1200 (Baxter *et al*., 1979), so far they have not been pharmacologically assessed (Kalix, 1991). Other compounds have been isolated from khat such as tannins, amino acids and significant amounts of vitamin C, magnesium and beta carotene (Kalix, 1984; Kalix and Braeden, 1985; Kennedy, 1987).

2.9.1.1.4 THE PHARMACOLOGY OF KHAT

2.9.1.1.4.1 Absorption

Khat is rapidly absorbed after oral administration (WHO, 1985). Indeed buccal mucosal plays a major role (80%) in the absorption of all the three alkaloids (cathinone, cathine and norephedrine) (Toennes *et al.*, 2003). The stomach and / or small intestine receive the swallowed juice and are probably responsible for the second phase of absorption (Toennes *et al.*, 2003). Khat is metabolized in the liver with only small fraction appearing in the urine (Kalix and Braeden, 1985). The more rapid and intense action of cathinone compared with cathine, is explained by its higher lipid solubility facilitating access into the CNS (Zelger *et al.*, 1980.).

2.9.1.1.4.2 Mechanism of action

Cathinone, which is the main ingredient of khat, is structurally similar to amphetamine and indeed, has pharmacological profile resembling that of amphetamine (Kalix, 1992; Wilder *et al.*, 1994). It has been demonstrated that cathinone operates through the same mechanism as amphetamine, that is, it acts by releasing catecholamines from presynaptic storage sites (Kalix, 1992). Furthermore, they inhibit re uptake and stimulate release of dopamine in the CNS, thereby increasing the temporal and spatial presence of dopamine at the presynaptic receptors (Krause *et al.*, 2000; Safer and Krager, 1988).

2.9.1.1.5 EFFECTS OF KHAT ON BODY SYSTEMS

Historically, khat has been used for medicinal purposes (Kennedy *et al.*, 1983) as well as an aphrodisiac (Marhetts, 1967; Krikorian, 1984) and also for recreational purposes (Kennedy, 1987).

2.9.1.1.5.1 Psychoactive effects of khat

Khat is most valued for its stimulant effects (Baasher, 1980). The stimulatory effects of khat are perceived by the user as an increase in alertness, energy, relief from fatigue, feeling of elation and improved ability to communicate (Baasher, 1980). Additionally, they have a feeling of enhanced imaginative ability, capacity to associate ideas and an increase in self confidence (Kalix, 1991). Objectively, khat use induces a state of mild euphoria and excitement often accompanied by loquacity (Laurent, 1962; Margetts, 1967). High doses may induce hyperactivity and frank manic behaviour and in exceptional cases it may result in toxic psychosis (Kalix, 1991) and paranoid (Critchlow & Serfert, 1987). Other effects of khat on the CNS are insomnia, hyperthermia and increased respiration. It is also an effective anorectic, which largely explains the malnutrition observed in the habitual chewers (Lebras and Fretillere, 1965; Halbach, 1972). The peripheral effects of khat are the sympathomimetic type. El-Guindy (1971) demonstrated increases in temperature and pulse rate as well as mydriasis in 30 people chewing khat.

2.9.1.1.5.2 Effects on circulatory system

The cardiovascular effects of cathinone have been examined in anaesthetized dogs and confirmed to be analogous to those of amphetamine (Kohli & Goldberg, 1982).

Cathinone administration to anaesthetized cats or rats have been found to cause substantial increase in blood pressure and that it had a positive inotropic and chronotopic effects in isolated guinea pig heart (Kalix ,1991). It has pressor effects on arteries, constriction of the vas deferens (Kalix and Braeden, 1985; Kroll, 1979) as well as producing excitation and increased activity (WHO, 1980). The peripheral effects of khat being the sympathomimetic types, may lead to arrhythmias and an increase in blood pressure depending on the amount and quality of the material absorbed (Halbach, 1972; Nencini *et al.*, 1984). Furthermore, the cardiovascular response to effort is exaggerated after khat consumption (Galkin and Mironycher, 1964; Lebras

& Fretillere, 1965). Khat has also been suspected to cause myocardiat infarction (Alkadi *et al.*, 2002)

2.9.1.1.5.3 Effects on respiratory system

Khat causes an increase in respiration possibly by acting on the respiratory center. Additionally, it increases metabolic rate and oxygen consumption by the user (Yanagita 1979, WHO, 1980). Indeed, recent findings have shown that khat stimulates the respiratory center and bronchodilation, explaining the feeling of comfort for asthmatic users (Dhaifalah and Santavy, 2004). There is increased prevalence of respiratory problems in men resulting from associated heavy smoking during khat session (Kennedy *et al.*, 1983) as co morbidity.

2.9.1.1.5.4 Effects on gastrointestinal system

Gastro intestinal tracts effects are common, such as anorexia and constipation (Giannin *et al.*, 1986). Constipation and malnutrition is probably related to high tannin and norpseudoephedrine content of the leaves (Kalix, 1991; Halbach, 1972; Dhaifalah, 2004). Anorexia leads to malnutrition and increased susceptibility to infectious diseases, especially tuberculosis (Kalix, 1987). Khat chewing has been associated with development of periodontal brownish staining of teeth (Kalix, 1987; Dhaifalah, 2004). In addition, Khat chewing has been suspected to cause oral squamous cell carcinoma (Nasr and Khatri, 2000) and hemorrhoidal disease (Al-Hadrani, 2000).

2.9.1.1.5.5 Effects on urinary system

Khat has been reported to cause relaxation of the bladder wall and the closure of internal sphincter (Dhaifalah, 2004). Khat chewing has been associated with inhibition of mictiturition and decrease in maximum urine flow rate (Nasher *et al.*, 1995).

2.9.1.1.5.6 Effects on endocrine system

Endocrinologically associated effects of khat are variable (Giannin *et al.,* 1986). Hyperglycemia associated with khat may occur in diabetes (Luqman & Danowiski, 1976), reduced birth weight of babies (Kalix, 1987) and inhibition of lactation (Luqman and Danowiski, 1976) have been reported in khat chewing mothers, possibly resulting from increased dopamine production (Larret, 1961). Khat has also been associated with an increase in adrenocorticotrophic hormone and growth hormone, as with amphetamines (Nencini *et al.,* 1983). More recent studies have reported an increase in testosterone levels but reduction of plasma prolactin and cortisol in olive baboon (*Papio anubis*. Cercopithecidae) after oral administration of khat (Mwenda *et al*, 2005). Invitro studies of crude khat on isolated mouse interstitial cells showed that low concentrations of khat extracts (0.06, 0.6 and 6 mg/ml) enhanced while high (30 mg/ml and 60 mg/ml) suppressed testosterone production (Nyongesa *et al,* 2007)

2.9.1.1.5.7 Effects on reproductive system

Historically khat has been used as an aphrodisiac (Margetts, 1967; Krikorian, 1984). However, studies have reported divergent results in this area, for example, according to Halbach, (1979) khat increases libido, causes impotence and spermatorrhoea among others. Other findings report the opposite effects, an impairment of sexuality (WHO Advisory group, 1980). Islam *et al.* (1990) reported loss of libido and decreased semen output in people who chew khat. Furthermore, deleterious effects of khat on semen parameters including, sperm morphology have been observed in khat users, especially those who have consumed it for a long time (El-Sshoura *et al.,* 1995). Khat has been reported to cause reproductive abnormalities in animals. Male mice treated with khat extract showed reduced rate of fertility, whereas in females, khat treatment induced post- implantation loss during the first week of pregnancy (Tariq *et al.,* 1986). In addition, khat has been shown to decrease sperm volume, sperm count, motility and fertilization

of eggs in roosters (Hammouda, 1978) and increase in the frequency of abnormal sperm in mice (Qureshi *et al.*, 1998). More recent studies have reported that, crude khat extract affected the viability of cell in isolated mouse interstitial cells in a dose related manner (Nyongesa *et al,* 2007). Khat has also been shown to cause chromosomal abnormalities, decrease mitosis and decrease synthesis of DNA, RNA and total protein in studies conducted with rats (De Hondt *et al.*, 1984).

2.9.1.1.6 BEHAVIOURAL EFFECTS OF KHAT

Cathinone the main ingredient of khat has been shown to maintain drug seeking behaviour in rats habituated to amphetamine and monkeys trained to lever press for cocaine injection (Yanagita, 1979). Similarly, rats injected with cathinone, display the same stereotypical behaviour and hyperlocomotion observed with amphetamines (Zelger *et al.*, 1980). Locomotion stimulation induced by cathinone in mice is characterized by a dose effect relationship resulting in an inverted U- shape curve, a feature that is typical and similar to hyper motility of the amphetamine type. The locomotor effects could be antagonized by the same substances as that for amphetamine, for example, haloperidol and pimozide (Kalix, 1982). Recent studies have shown that khat like amphetamine and cocaine can induce seizures and at low doses (3.7 g/kg b.wt) it reduces the pentylenetetrazol (PTZ) induced seizure threshold (Patel et al, 2004).

2.9.1.1.7 SOCIO-ECONOMIC EFFECTS OF KHAT

Individuals who are users of khat commonly divert their income into khat chewing therefore neglecting their families' needs (Kalix, 1987) and indeed, the average family income can be halved to support the habit (Baasher and Sadoun, 1983). In addition, khat has been implicated as causal factor for family instability (Elmi, 1983), divorce. (Baasher and Sadoun, 1983),

prostitution and criminal behaviour (Elmi, 1983). The cultivation of khat, results in the decreased production of other more essential crops like cereals, promoting malnutrition and disease (Murad, 1983). Khat chewing leads to low productivity due to absenteeism and the after effects of the use (Halbach, 1987, 1979; Elmi, 1983 b; Giannin *et al.*, 1986; Kalix, 1987). However, khat is a major source of revenue (Kalix, 1991).

The concomitant use of alcohol to counteract the stimulant and insomniac effects of khat (Kennedy, 1987, Omolo & Dadphale, 1987) raises the risk of complication from the two drugs.

CHAPTER 3

3.0 MATERIALS AND METHODS

3.1 The Study Area.

This study was conducted at the Department of Medical Physiology, Chiromo Campus, of the University of Nairobi. The experiments were carried out in a laboratory measuring (4.64 x 3.78 x 3.2 m). The light and dark cycle was maintained by turning on the lights at 0800 hrs and turning off at 1700 hrs during the work. All the procedures on mice were carried out during the light cycle. The cages with animals and their accessories were kept in the animal house. To avoid interference with the results, only the principle investigator, the supervisors and the assistant were allowed into the laboratory where the testing was done.

3.2 Experimental animals

Male Swiss mice (n=18), weighing 20-35 gms, 5-6 weeks old bred in the department were used in the open field test (experiment I) to examine the effects of khat extract on locomotor activity and to determine the dose to be used in subsequent experiments.

Male CBA mice (n=20), weighing 20 – 35gms, 5 – 6 weeks old from Department of Biochemistry were used in experiment II. A further 29 male CBA mice weighing 20 – 35gms, 5 – 6 weeks old were used during experiment III. The animals were caged in groups of 5 – 7 in 30 x 15 x 12 cm metallic wire meshed cages, which were put on a raised surface, 0.75m from the floor, in the animal house. The beddings (wood shavings) were changed every three days. They were fed with standard rodent pellets (Unga Feeds, Nairobi) and fresh tap water ad libitum in the home cages. The mice used in the experiments were handled for five minutes every day for the first two weeks before the experiments to habituate them to the handling, investigator and the experimental environment. Furthermore, they were allowed to acclimatize to the testing area for

(15-30 minutes) before injection with khat or normal saline. All the experiment started at 0800 hours every day. The study was conducted in accordance with the internationally accepted principles for laboratory animal use and care (NIH publication # 85 – 23 revised 1985) and the FELASA C guidelines.

3.3 Experimental design

This was a randomized experimental study carried out using simple random sampling technique aimed at generating data on the effects of khat (Catha edulis) on learning and memory in the CBA mice on Morris Water Maze task.

3.3.1 Experiment 1 (Open field test)

The aim of this experiment was to determine the appropriate dose of khat extract to be used in the subsequent Morris water maze task experiments. This was necessary because in the spatial learning and memory, escape latency, swim distance and the velocity are the key variables measured and they are affected by the locomotor activity of the animal. Taken together it was important to establish which dose affected locomotor activity significantly to avoid the problem of confounding the MWM results. A poor learning and memory results could be due to inhibition of locomotion rather than an actual effect on learning and memory. The dose of khat extract tested was established by a 3n progression formula starting from 10 mg/ kg body weight. The following doses were tested, 10, 30, 90, 270, 360, and 540 mg/ kg body weight. The 360 mg/ kg body weight dose was chosen to capture effects that would have been elicited by the 270 to 540 mg/ kg body weight khat extract but probably missed because of the wide interdose gap.

3.3.2 Experiment 2 (Morris water maze task following single daily dose khat extract injection)

In the second set of experiment, mice were divided into four groups: control group injected with 0.5 mls normal saline, and three treatment group injected i.p in 3n progression, of khat extract daily: 40, 120, 360mg/kg body weight, respectively. The volume of khat extract was made up to a volume of 0.5 mls per injection with normal saline for all the animals. Mice were injected with khat extract 10 minutes before the Morris water maze tasks were commenced.

3.3.3 Experiment 3 (Morris water maze task following escalating then multiple high khat extract dose)

In this experiment, mice were divided into five groups: control group were injected with 0.5 mls normal saline i.p, and four treatment groups were injected with escalating dose followed by repeated high doses of khat extract (binge model) or 0.5 mls normal saline. The volume of khat extract was made up to a volume of 0.5 mls per injection for all the animals using normal saline.

3.4 Khat Material Preparation and Extraction

Fresh bundles of khat (Catha edulis), shoots and small branches were purchased from Westlands (Nairobi) local market for each experiment. During experiment 1, two bundles of khat leaves were purchased. They were crushed on a glass plate, weighed (90.74 gms) and then crushed with pestle and mortar. The chopped leaves were put into a flask and 800 mls of methanol was added to cover the leaves completely. Methanol was used as extraction solvent as it is able to remove polar molecules. During experiment 2, three bundles of khat leaves were purchased; they were chopped, crushed and weighed (140.96 gms). The leaves were covered with 1500mls of methanol. During the third experiment, two bundles of khat extract were purchased, after chopping they weighed (84.68 gms). A volume of 610 mls of methanol was added to cover the

leaves completely. The mixtures were stirred gently and then left to stand overnight. They were then coarse filtered using a piece of gauze roll, to separate the big particles, followed by fine filtering using Watman's filter paper No.1 to remove the fine particles. The methanol was removed with a Rotavac evaporator at a temperature of 65°C, at a speed of 100 rpm and 240 Pascal pressure vacuum. The distillation took about 2.5 hours and it was considered complete, when no drops of methanol were coming out. The complete removal of methanol yielded 32.47 gms in a volume of 31.42 mls for experiment I, 61.30 gms in a volume of 60.30 mls for experiment II and, 43.42 gms in a volume of 42.0 mls for experiment III. The extract was weighed and the volume determined to calculate the dosage at mg/kg body weight. The extract was put into small bottles, stored at 0 - 8°C and covered with aluminium foil to avoid degeneration. Every bottle was used specifically for one day until the experiment was over.

3.5 APPARATUS

3.5.1 The Morris Water Maze

The Morris water maze modified for mice was used (Taylor *et al.,* 1996). It consisted of a circular polypropylene pool (112 cm in diameter X 25cm in depth). The pool was filled to a depth of 12.5 cm with tap water and the temperature was maintained at 25°c using a coil water heater. The water was made opaque with the addition of non toxic white paint (Xpress colour & Screen LTD) to camouflage the escape platform. A stable circular platform measuring 11.5cm in diameter and painted white acted as the escape platform. It had a rough surface which allowed the animals to climb onto it easily. The platform was submerged 1cm below the surface of the water and was, therefore, hidden from the animals view. The pool was divided into four quadrants that is; Northwest, Northeast, Southeast and Southwest. Boundaries of the quadrants were marked on the edges of the pool with a masking tape and labeled North, South, East and West. The pool was located in a room measuring 4.64 M x3.78 M. On each of the walls of the

room, posters with drawing were mounted to provide visual cues which could be used by the mice to develop spatial map to navigate to the platform.

The mice were put into the maze using a plastic mug by the investigator at different starting position, randomly selected. The performance of the mice in the water maze was recorded using a video camera (Type-Image video camera CAM) mounted 2 meters above the water maze, which was connected to video camera recorder (Toshiba) and 14 inch television (JVC Supermulti). The escape latency was measured using a stop watch (model 3000 ADANAC) and expressed in seconds. The stopwatch was started immediately the mouse was placed into the water at the start position, and stopped when it stepped on to the escape platform. The swim path length (swim distance), the distance covered by the mouse from start position until it located the platform was determined by replaying the video tape and tracing the mouse swim path using marker pen on a tracing paper mounted on the TV using masking tape. The length of the swim path was determined using a map reader and the distance obtained by multiplying by 5.65, the ratio of true maze diameter and that of the maze on the TV. It was expressed in centimeters. The pool was emptied after five days and refilled again for subsequent experiments.

3.5.2 The Open Field Test

The open field test (Walsh & Cummins, 1979) provides simultaneous measures of locomotion, exploration, and anxiety. The number of line crosses and the frequency of rearing are usually used as measures of locomotor activity, but are also measures of exploration and anxiety. A high frequency of these behaviors indicates increased locomotion and exploration or a lower level of anxiety or both (Brown *et al.*, 1999).

The apparatus was constructed of plywood painted white and measured 72 x 72cm x 36cm height. One of the walls was clear Plexiglas through which the mice could be viewed. Black lines

were drawn on the floor with a marker dividing the floor into a grid. The lines divided the floor into sixteen 18 x 18cm squares.

The central square was used because some mouse strains have high locomotor activity and cross the lines of the test chamber many times during a test session. Additionally, the central square has sufficient space surrounding it to give meaning to the central location as being distinct from the other locations (Carey, Mc Fadyen & Brown, 2000). The open field was located in a 4.64 x 3.78 M test room with sufficient lighting.

Mice were placed into the center of the open field and then allowed to habituate and to explore the apparatus for 15 minutes prior to being injected with khat extract or saline. After 15 minutes, mice were returned to their home cages and the open field was cleaned with 70% ethyl alcohol and permitted to dry between tests. Measures of the line crossing, center square entries, center square duration and rearing (Brown *et al.,* 1999) were scored at an interval of 5 minutes. The mice were then injected with khat at different doses and then returned to the open field and scored for the above measures at an interval of 5 minutes for 40 to 50 minutes.

Center square duration was measured manually by use of a stop watch, while the line crossing was scored by the investigator and an assistant. Furthermore, to maintain validity, the mice activities were video taped with a video camera (Image video camera CAM) mounted 2 m above the field and then connected to the VCR and TV.

3.6 PROCEDURE

3.6.1 EXPERIMENT I

3.6.1.1 Open Field Test

The purpose of this experiment was to determine the appropriate dose of khat that would not inhibit mice locomotor activity. A total of 18 white mice were used. They weighed 20 - 35gms and they were 5 – 6 weeks old. The mice were divided into six groups, of three mice each. They were handled and allowed to the experimental environment (habituated) for one week before being exposed to the test for one full week. On the test day each mouse was placed in the center square of the open field and the following variables were scored by the investigator: line crossing, rearing, and frequency to the center square at an interval of 5 minutes for 15 minutes. After 15 minutes the mouse was injected with khat extract intraperitoneally according to the group dosage: 10, 30, 90, 270, 360 and 540 mg/kg body weight with an injection volume of 0.5 mls.

3.6.2 EXPERIMENT II- MORRIS WATER MAZE TEST 1

3.6.2.1 Learning / Acquisition

The purpose of this experiment was to determine the effects of pretraining administration of khat on spatial learning and memory of CBA mice in MWM task.

A total of 20 mice, 20 – 30gm, aged 5 – 6 weeks were used. They were divided into groups of four mice (groups I, II, III & IV). The groups were injected 0.5 saline, 40mg, 120mg and 360mg/kg body weight khat extract, respectively, 15 minutes before the test.They were subjected to daily sessions of 4 trials per day for 4 days to find a submerged platform that was located in the center of South East (SE) quadrant of the Morris Water maze tank. The platform remained there throughout the training period (4 days). During each trial, the mouse was placed randomly in one of the start (launch) locations (N, S, W, and E). The order of start locations was varied

randomly so that each block of four trials (sessions) in any given sequence was not repeated on consecutive days. Each mouse was allowed 60 seconds to search for the platform. The latency to find the platform (escape latency) and swim path was recorded using a video camera. Once the mouse located the platform, it was permitted to remain on it for 30 seconds. If the mouse did not find the platform within the 60 seconds it was guided to it and allowed to remain on it for 30 seconds. After each trial, the mouse was removed, dried in a paper towel and put back in its home cages. For each mouse the inter trial interval was 5 minutes. The trials were carried out between 0800hrs to 1130 hrs.

3.6.2.2 Probe trial (memory) 1

On day five the platform was removed and each mouse was subjected to a probe trial (60 seconds). The mouse was placed in the water maze from the North (N) position because during acquisition phase the platform was in the South East (SE) position. The time spent in the quadrant of the former platform position and the proportion of swim distance in the platform quadrant was determined from the video recording.

3.6.2.3 Reversal learning phase

The purpose of this experiment was to determine the effects of administration of khat extract on reversal learning in CBA mice on MWM task. After seven days of daily khat extract injection following experiment I, a reversal training phase was carried out in which the mice were trained for 4 days (4 trials per day) to find a hidden platform which was located diagonally opposite the original location North West (NW) quadrant. The escape latency and swim path were recorded as described in experiment I. On the fifth day, all the mice were subjected to a probe trial. Each mouse was placed in the South (S) position in the water maze and allowed 60 seconds of

swimming and the time and distance spent in the target and the opposite platform quadrant and the swim distance were determined from the video recording.

3.6.3. EXPERIEMNT III-MORRIS WATER MAZE TEST 2

The purpose of this experiment was to determine the effect of escalating dose binge model of khat extract administration on learning and memory in CBA mice on MWM task. Twenty nine (29) mice weighing 20 – 35gm aged 5 – 6 weeks were grouped in 5 groups. They were injected with khat extract as follows: 10, 60, 110, 160, 210, 260, 310 and 360 mg/kg in a n escalating regimen to mimic an escalating dose model.

The animals were exposed to gradually escalating doses of the stimulant to a maximum of three doses per day for 3 days, and two doses on day 4, to mimic the common usage pattern of high dose stimulant abusers (Gawin, 1991; Angrist, 1994b; Gawin and Khalsa, 1996) (table 1). In such a dose regimen, tolerance develops to the sympathomimetic effects of the stimulants, and the users are able to survive higher doses (Fischman and Schuster, 1974, 1977; Schuster and Fischman, 1975; Schmidt *et al.,* 1985b; Angrist 1994b) and thus increase both the dose and frequency of administration, presumably to achieve and maintain high levels of the euphoria produced by these drugs (Angrist, 1987, 1994b; Gawin and Khalsa, 1996). Thus the escalating doses frequently leads to a high dose binge pattern of administration and prevailing evidence suggest that psychosis is most frequently associated with this pattern of stimulant abuse (Davies and Schlemmer, 1980; Angrist, 1994 b; Gawin and Khalsa, 1996).

To simulate this condition, mice were exposed to gradually escalating doses of khat extract before multiple daily administrations of relatively high doses of the drug. The regime was selected according to the previous study in which Segal and Kuczenski (1997), exposed rats to

gradually escalating doses of d-amphetamine before multiple daily administration of relatively high dose.

3.6.3.1 Escalating dose phase

In the open field test, the effects of different khat extract doses on locomotor activity were tested to establish the maximum doses that did not produce locomotor dysfunction. The dose up to 360 mg/kg body weight was found to be suitable. Therefore, the dose pattern that was followed in this experiment was guided by the open field test results and the schedule was adopted from Segal and Kaczenski (1997) as follows:

Table 1 – Escalating dose injection schedule

Day	Time		
	8 am	2 pm	8 pm
1	10 mg/kg bwt	60 mg/kg bwt	110 mg/kg bwt
2	110 mg/kg bwt	160 mg/kg bwt	210 mg/kg bwt
3	210 mg/kg bwt	260 mg/kg bwt	310 mg/kg bwt
4	310 mg/kg bwt	360 mg/kg bwt	

For the escalating cycles, mice received their injection each day for four days beginning with a 10mg/kg body weight dose of a khat extract and ending with 360mg/kg body weight on the fourth day of the cycle. During the run phase, animals received four injections of 360mg/kg body weight every two hours beginning at 8 am and ending at 2 pm. The mice were exposed to this

regime for another four days and then tested on the MWM. The animals were monitored continuously throughout the course of the treatment.

Group 1 mice **N = 7.**

The mice in this group weighed 20-25mgs; they were all male aged between 5-6weeks old. From day one to day four they were injected with the escalating dose of khat extract. After 15minutes they were submitted to Morris Water Maze whose platform was submerged in the SE quadrant for all the doses. Therefore, there were three sessions every day of four trials each, on day 5 they were run on a probe trial in which the submerged platform was removed and the mouse was placed at the N start point. The swim distance and the time spent in each of the four quadrants were interpreted as a memory strategy with the quadrant where mice swam and spent most as a memory for the quadrant location. From day 6 through to day 9 the mice were subjected to run regimen in which they were injected with 360mg/kg body weight of khat extract every two hours for four runs, during which they were subjected to Morris Water Maze test and therefore there were four sessions of four trials per day. On day 10 the mice were subjected to a probe trial in which the platform had been removed, distance and time spent in the four quadrants were determined.

Group 2 mice **N = 5.**

The animals received escalating dose of khat extract as above and then were subjected to MWM test as above from day 1 to day 4. On day 5, the probe trial was carried out the same way as that of group one. However, from day 6 through to day 9, mice were injected with a single daily dose of 360mg/kg body weight of khat extract then subjected to 4 sessions of MWM tests. On day 10, they were subjected to a probe trial similar to group 1 mice.

Group 3 mice **N = 6**

The mice were injected with escalating dose of khat extract as for the two former groups and then subjected to MWM tests as above from day 1-4. On day 5, they were injected with normal saline 0.5 mls intraperitoneally and then a probe trial was carried out. From day 6 through to day 9, the mice were injected with 0.5 mls normal saline (4 doses) and then exposed to 4 sessions of trials as for the former groups. Probe trial was also carried out as for the other group.

Group 4 **N = 6**

The animals were injected with 0.5 mls normal saline as from day 1 – 4 then subjected to MWM tests as for the other groups. On day 5 probe trial was carried out as for the former groups. From day 6 through to day 9 the mice were injected with high dose (360mg/kg) of khat extract for four runs. They were then subjected to 4 sessions of MWM tests of 4 trials each. On day 10 probe trial was carried out similar to the other groups.

Group 5 (control) **N = 5**

The mice were injected with 0.5 mls normal saline for all the acquisition days and subjected to MWM tests the same way as the other treatment groups. On the fifth day the probe trial was carried out similar to the treatment groups. The control animals were also injected with 4 doses of 0.5mls normal saline and submitted to 4 sessions of MWM trainings from the sixth to ninth day. The probe trial was further conducted on the tenth day similar to the other groups.

3.6.4 Data recording

Escape latency was determined manually by use of stop watch and was expressed in seconds. Swim distance was determined by mounting a tracing paper on the TV screen with a masking tape and by tracing the movements of each mouse with a marker. From the tracings swim

distance was determined using a map measure and then expressed in centimeters. The actual swim distance was calculated as real maze diameter divided by maze diameter multiplied by tracing distance, that is multiplied by of 5.65. The swim speed was calculated as a distance (cm) divided by the time (seconds). The escape latency and the swim distance were recorded in the MWM data sheet (appendix 2).

3.6.5 Data analysis

The data collected was entered and organized for analysis. All values of escape latency, swim distance and velocity were expressed as mean ± standard error of the mean (SEM).

Escape latency and swim distance data for difference in the mean among groups and time period was analyzed by MANOVA followed by Bonferroni multiple comparison post hoc tests. Statistical significance was set at $P < 0.05$

CHAPTER FOUR

4.0 RESULTS

4.1 Effects of khat on locomotor activity (open field test)

The results of the effects of khat extract on locomotor activity of Swiss mice are presented in figures 4a, 4b, and 4c. The locomotor activity of all the mice were evaluated before and after injecting them with 10, 30, 90, 270, 360 and 540 mg/kg body weight doses of khat extract, respectively. The two phases (pre and post khat extract injection) were analysed together and the line crossings, centre square frequency and rearing frequency scored as the measure of locomotor activity. There was reduction of activity between doses and over the 60 minutes duration.

A multivariate analysis of variance test yielded a highly significant effect of doses (F (5, 13) = 27.4; $p < 0.001$) and time (F (11, 49) = 9.18; $p < 0.001$) on locomotor activity. The interaction of doses and time, however, was not significant (F (33, 62) = 0.79; $p = 0.79$). The changes across doses were highly significant (F (5, 13) =16.93; $p < 0.001$) but, the changes across time were not significant (F (11, 49) = 1.39; $p = 0.189$). A post hoc test with Bonferroni procedure revealed that the mean line crossing of mice treated with 360 mg/kg body weight khat extract (42.18 ± 24.83) was significantly higher than mice treated with other doses ($p < 0.001$), (fig.4a). On the other hand, the changes of line crossing across time were evident, but, were not significant ($p > 0.05$).

The results indicated that there was dose dependent manner inhibition of line crossing at all other doses except 360 mg/kg body weight. There was a slight reduction in line crossing over the first 15 minutes (pre injection) as the animal acclimatized to the test equipment. This was followed with depressed line crossing due to the effects of the drug which was reduced after 30 minutes

post khat extract injection. The reason for this is probably because the levels of khat decreased in the body due to metabolism and thus the declining line crossing.

The centre square frequency of the Swiss mice is shown in Figure 4b. The result show significant decrease of centre square frequency across dose and time (F (5, 13) =2.34; $p < 0.05$) and (F (11, 49) =2.75; $p < 0.05$), respectively. However, the interactions of dose and time was not significant (F (33, 62) = 0.75; $p = 0.83$). A post hoc test revealed that the mean centre square frequency of mice treated with 30, 90, 360 and 540 mg/kg body weight of khat decreased significantly ($p < 0.05$) over time compared to pre injection phase. On the other hand, khat extract at doses 10 and 270 mg/kg body weight had no significant ($p > 0.05$) effect in reducing centre square frequency in Swiss mice. Furthermore, centre square frequency decreased significantly ($p < 0.05$) across time with significant decrease ($p < 0.05$) occurring between 15 to 35 minutes in these post injection phase. It appears that khat extract abolished centre square frequency completely between 15 minutes and 35 minutes, which returned after 45 minutes.

The results of rearing frequency of the Swiss mice are shown in figure 4c. They show an increase in rearing frequency in the first 15 minutes which was absent in the subsequent 15 to 35 minutes. This phase was followed by gradual regain of locomotor activity.

A multivariate analysis showed that the change on rearing frequency across doses (F (5, 13) = 2.36; $p < 0.05$) and time (F (11, 49) = 8.63; $p < 0.05$) was significantly different. However, the interaction of dose and time was not significant (F (33, 62) = 0.75; $p = 0.83$) with rearing. Furthermore, post hoc test revealed that the rearing frequency of mice treated with 30, 90, 270, 360 and 540 mg/kg body weight was significantly ($p < 0.05$) reduced compared to pre injection phase. Similarly, mice treated with 10 mg/kg body weight khat extract had reduced rearing frequency but the difference was not significance.

Further test revealed that rearing frequency increased significantly ($p < 0.05$) during the first 10 minutes of pre injection phase; this could be attributed to acclimatization and less anxiety. In addition, though the rearing decreased between 15 – 45 minutes during khat extract injection phase, the decrease was not significance ($p > 0.05$). After, this there was an increase in rearing frequency.

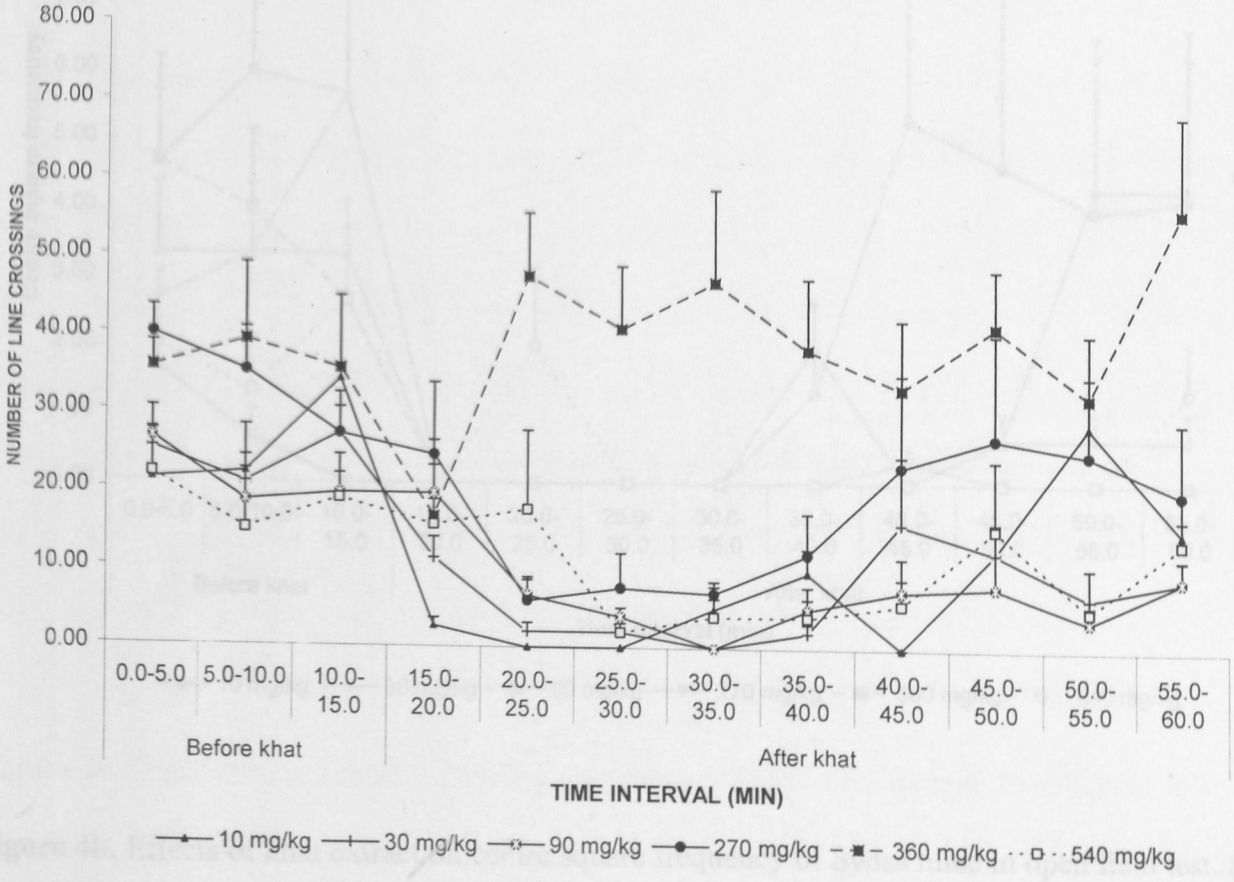


Figure 4a. Effect of khat extract on line crossing of Swiss mice in open field test. The mice were injected with (10, 30, 90, 270, 360 and 540 mg/kg b.wt of khat extract) then tested in an open field. (n = 3, mice in each group). Data are presented as mean ± s.e.m of line crossing between doses and over 60 minutes duration. Mice injected with 360 mg/kg b.wt khat extract had higher ($p < 0.05$) line crossing than mice treated with other doses and control group.

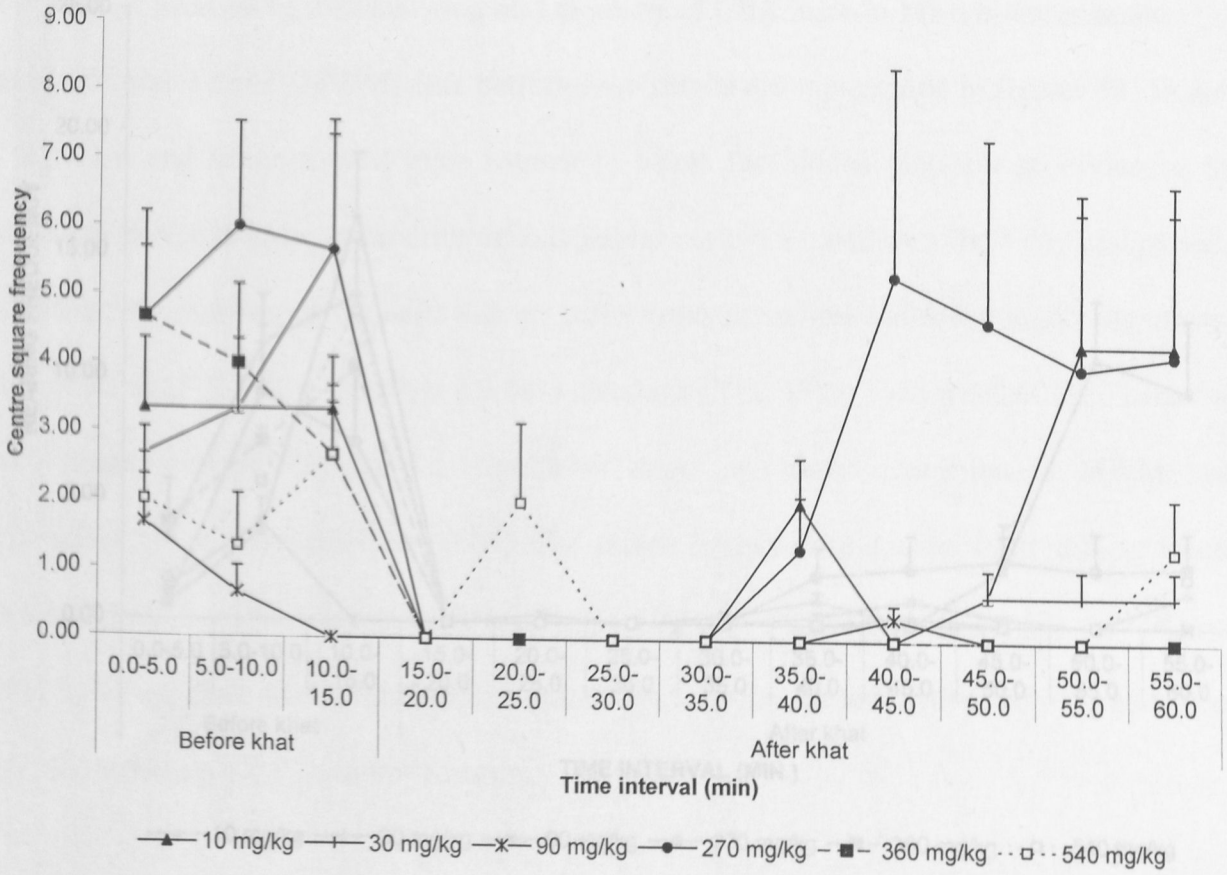


Figure 4b. Effects of khat extract on centre square frequency of Swiss mice in open field test. (n = 3 mice in each group). Data are presented as mean ± s.e.m of centre square frequency between doses (10, 30, 90, 270, 360, & 540 mg/ kg b.wt khat extract) and over 60 minutes. Mice injected with 10 and 270 mg/kg b.wt. had higher ($p < 0.05$) centre square frequency. In addition, the centre square frequency decreased significantly ($p < 0.05$) across time with significant ($p < 0.05$) decrease occurring between 15 to 35 minutes after injection.

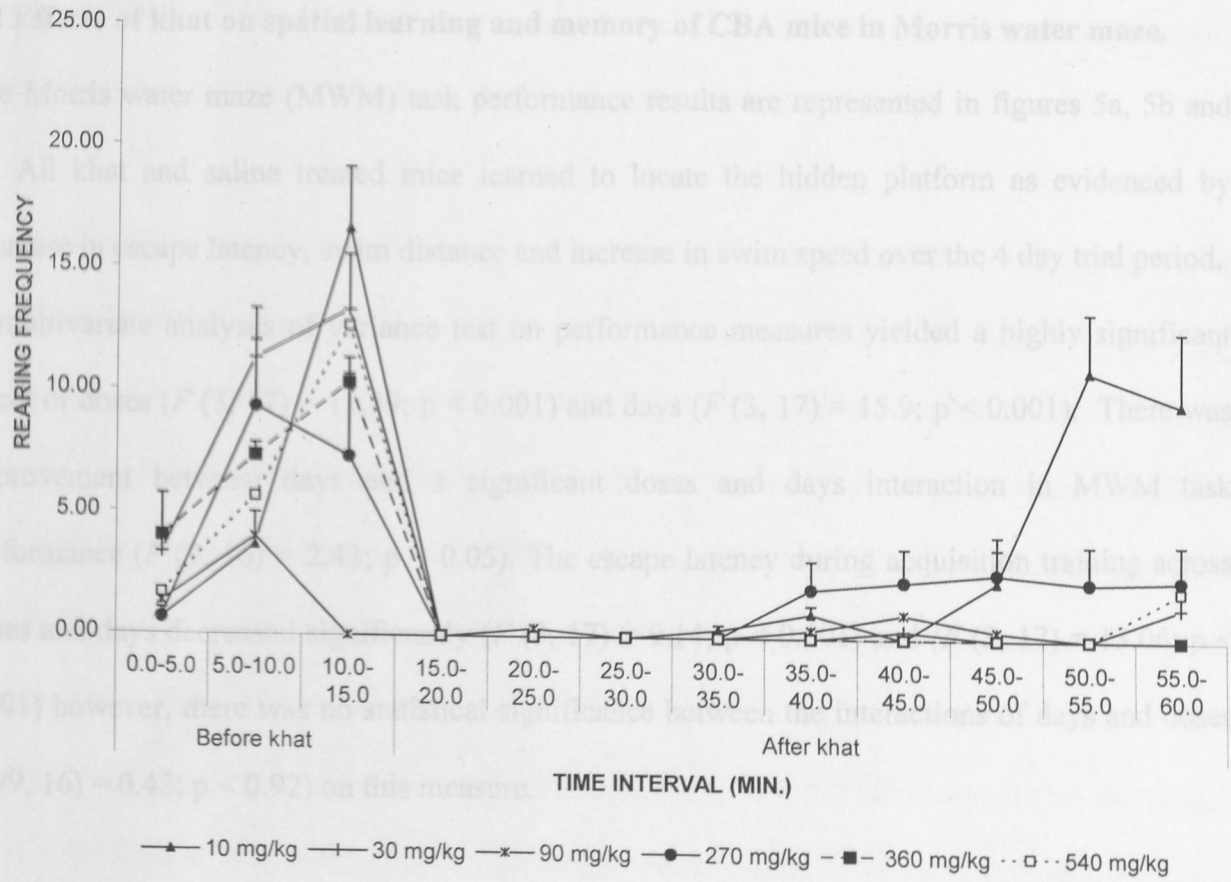


Figure 4c. Effect of khat extract on rearing frequency of Swiss mice in open field test. (n = 3 mice in each group). Data are presented as mean ± s.e.m of rearing frequency between doses (10, 30, 90, 270, 360, & 540 mg/ kg b.wt) khat extract over the 60 minutes period. Multivariate analyses of variance showed that rearing frequency decreased with time and across the dose. Mice treated with 30, 90, 270, 360, &540 mg /kg b.wt khat extract had significantly ($p < 0.05$) reduced rearing frequency compared to pre injection phase.

4.2 Effects of khat on spatial learning and memory of CBA mice in Morris water maze.

The Morris water maze (MWM) task performance results are represented in figures 5a, 5b and 5c. All khat and saline treated mice learned to locate the hidden platform as evidenced by decrease in escape latency, swim distance and increase in swim speed over the 4 day trial period. A multivariate analyses of variance test on performance measures yielded a highly significant effect of doses (F (3, 17) = 11.39; $p < 0.001$) and days (F (3, 17) = 15.9; $p < 0.001$). There was improvement between days and a significant doses and days interaction in MWM task performance (F (9, 16) = 2.43; $p < 0.05$). The escape latency during acquisition training across doses and days decreased significantly (F (3, 17) = 9.14; $p < 0.001$) and (F (3, 17) = 13.06; $p < 0.001$) however, there was no statistical significance between the interactions of days and doses (F (9, 16) = 0.42; $p < 0.92$) on this measure.

A post hoc test with Bonferroni multiple comparison procedure revealed that the mean escape latency was significantly ($p < 0.05$) higher in mice treated with 120 and 360 mg/kg body weight of khat extract compared to the controls and 40 mg/kg body weight khat treated mice . The escape latency improvement for all the mice was more pronounced on day 3 (18.26 ± 3.22) but not significantly different though, it was markedly improved compared to day 2 (28.29 ± 3.28). The improvement on day 3 was facilitated by the marked improvement of mice treated with 40 mg/kg body weight of khat extract (8.88 ± 2.67) which in the previous day (29.14 ± 3.9) had shown very small improvement and thus the general smaller improvement on that day by the mice. The control group and mice treated with 40 mg/kg body weight of khat appeared not to have improved on escape latency on 4th day as compared to other groups. From the results, it appears that mice treated with normal saline and 40 mg/kg body weight respectively had attained their lowest escape latency by day 3 (9.84 ± 0.73) and (8.8 ± 1.06) compared to day 4 (10.10 ±

0.52) and (9.68 ± 0.56). However, mice treated with 120 mg and 360 mg/kg body weight of khat extract required more days to achieve their minimum escape latency.

The swim distance measure of Morris water maze task during acquisition training decreased significantly across doses and days (F (3, 17) = 6.43; $p < 0.05$) and (F (3, 17) = 14.24; $p < 0.001$). However, the interaction of dose and days was not significant for this measure (F (9, 17) = 1.08; $P < 0.389$). Post hoc multiple comparison test showed that the mean swim distance of 120 mg/kg body weight khat extract treated mice (542.88 ± 54.61) was significantly ($p < 0.05$) higher than mice treated with 40 and 360 mg./kg body weight of khat extract and control group, respectively. The swim distance improved with days in a dose dependent manner. Swim distance between days improvement was more marked ($p = 0.54$) on 3rd day for all the groups. This improvement was more evident due to the improvement by mice treated with 40 mg/kg body weight of khat extract. This is in contrast to swim distance recorded on 2nd and 4th days which had very minimal improvement because of the mice treated with 360 mg/kg body weight of khat extract had shown marginal improvement in swim distance measure over the first 3 days but showed remarked improvement on day 4. A similar pattern to the escape latency by mice treated with 40 mg/kg boy weight of khat extract was observed with swim distance measurement in which the mice improved marginally on the last day. Their swim distance was higher however, it was not significant. The results show that it takes a few more days for the measures of swim distance to converge and achieve the lowest value for the mice treated with 40 and 360 mg/ kg body weight khat treated mice and the control as opposed to the extra days that would be required for the converge at the of escape latency values

The swim speed (velocity) increased significantly (F (3, 17) = 6.53; $p < 0.05$) across doses. The swim speed across days increased as training progressed however, the increase was not

significant (F (3, 17) =1.48; $p < 0.229$). In addition, no significant difference (F (9, 17) = 0.740; $p < 0.671$) was observed on swim speed with interaction of dose and days.

A post hoc test showed, that mean swim speed of mice treated with 360 mg/kg body weight khat extract (13.03 ± 0.640) was significantly ($p < 0.05$) lower than in mice treated with 40, 120 mg/kg body weight khat and the control group. Whereas, the swim speed increased as training progressed, it was not sustained across all doses (groups). The 360 mg/kg body weight khat extract treated mice swim speed increased across days while for the other groups, it increased then decreased. For example, the swim speed of mice treated with 40 mg/kg body weight increased on day 2, decreased on day 3 and then improved on day 4. Similarly, the swim speed of mice treated with 0.5 mls normal saline and 120 mg/kg body weight khat increased marginally on day 2 and 3 and then decreased on the 4th day.

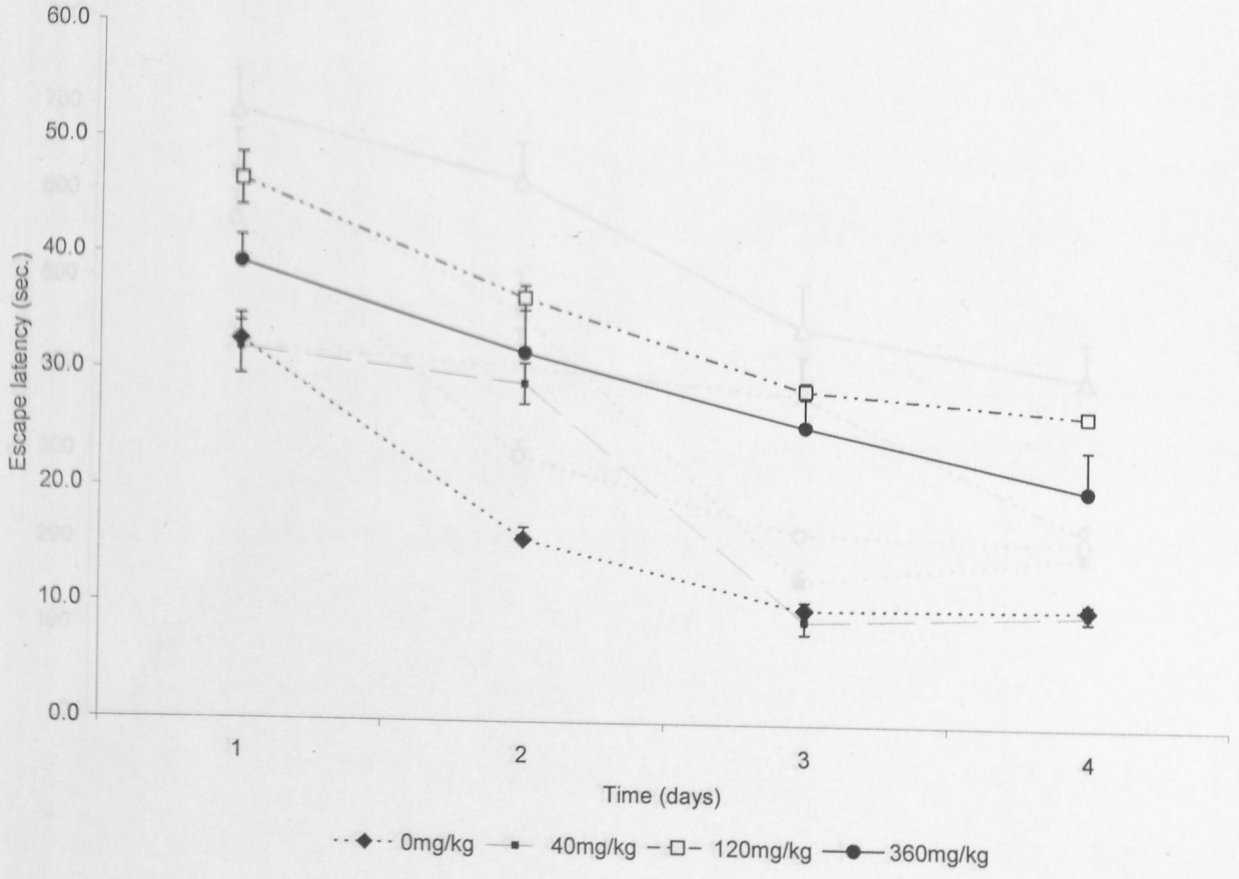


Figure 5a. Effects of khat extract on escape latency of CBA mice over 4 day trial period in a MWM task. Mice were injected with (0.5 mls normal saline, 40, 120, & 360 mg/ kg b.wt) of khat extract then subjected to the MWM test. (n = 5 mice in each group). Data are presented as mean ± s.e.m.of escape latency. Mice injected with 120 and 360mg /kg b.wt khat extract had higher ($p < 0.05$) escape latency than other doses and the control group. Furthermore, the mean escape latency decreased significantly ($p < 0.001$) over days for all the animals

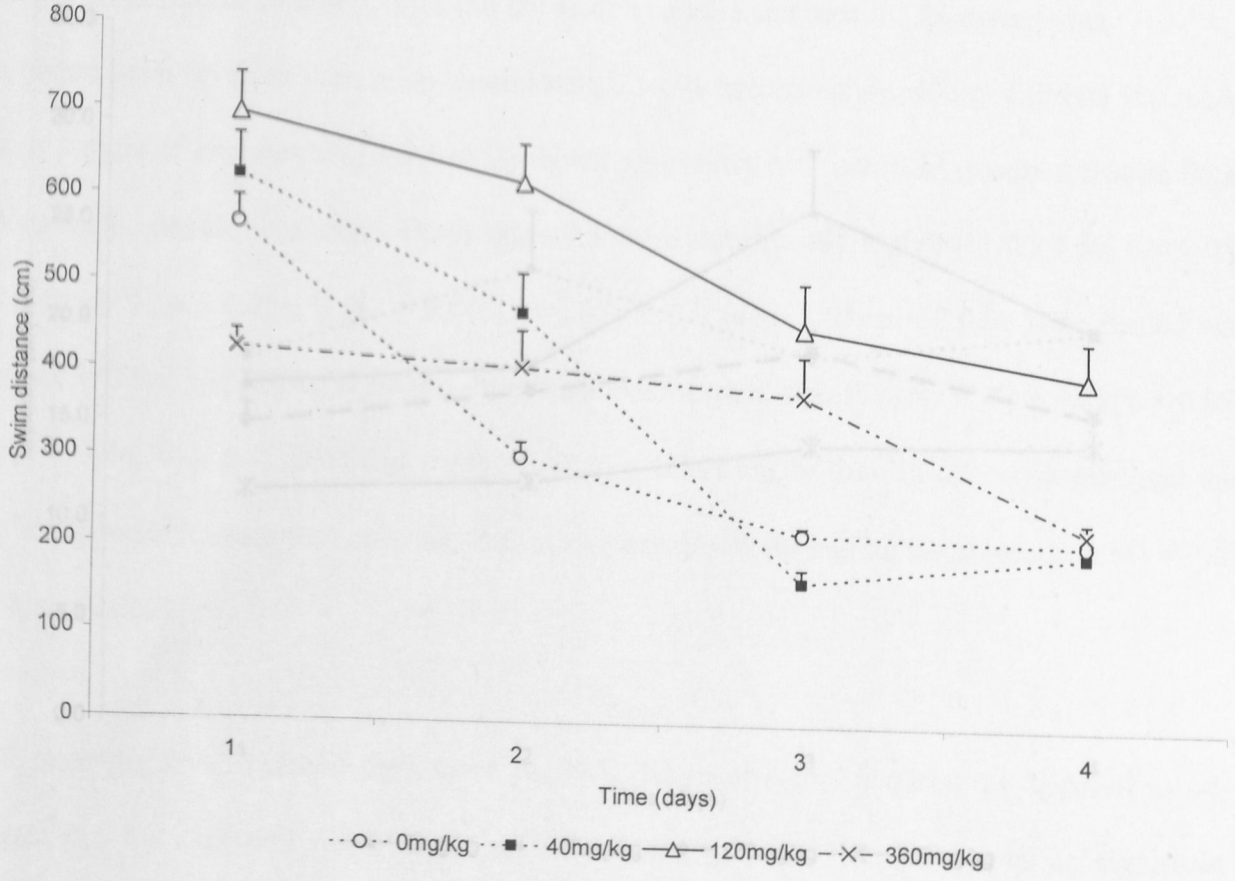


Figure 5b. Effects of khat extract on swim distance of CBA mice over 4 day trial in a MWM task.. Mice were injected with (0.5 mls normal saline, 40, 120, & 360mg) kg/b.wt of khat extract. (n = 5 mice in each group). Data are presented as mean ± s.e.m of swim distance to find the platform. Mice injected with 120mg/kg b.w.t khat extract had longer swim distance ($p < 0.05$) compared to the other doses and control. Furthermore, the swim distance decreased significantly ($p < 0.001$) over days for all the animals

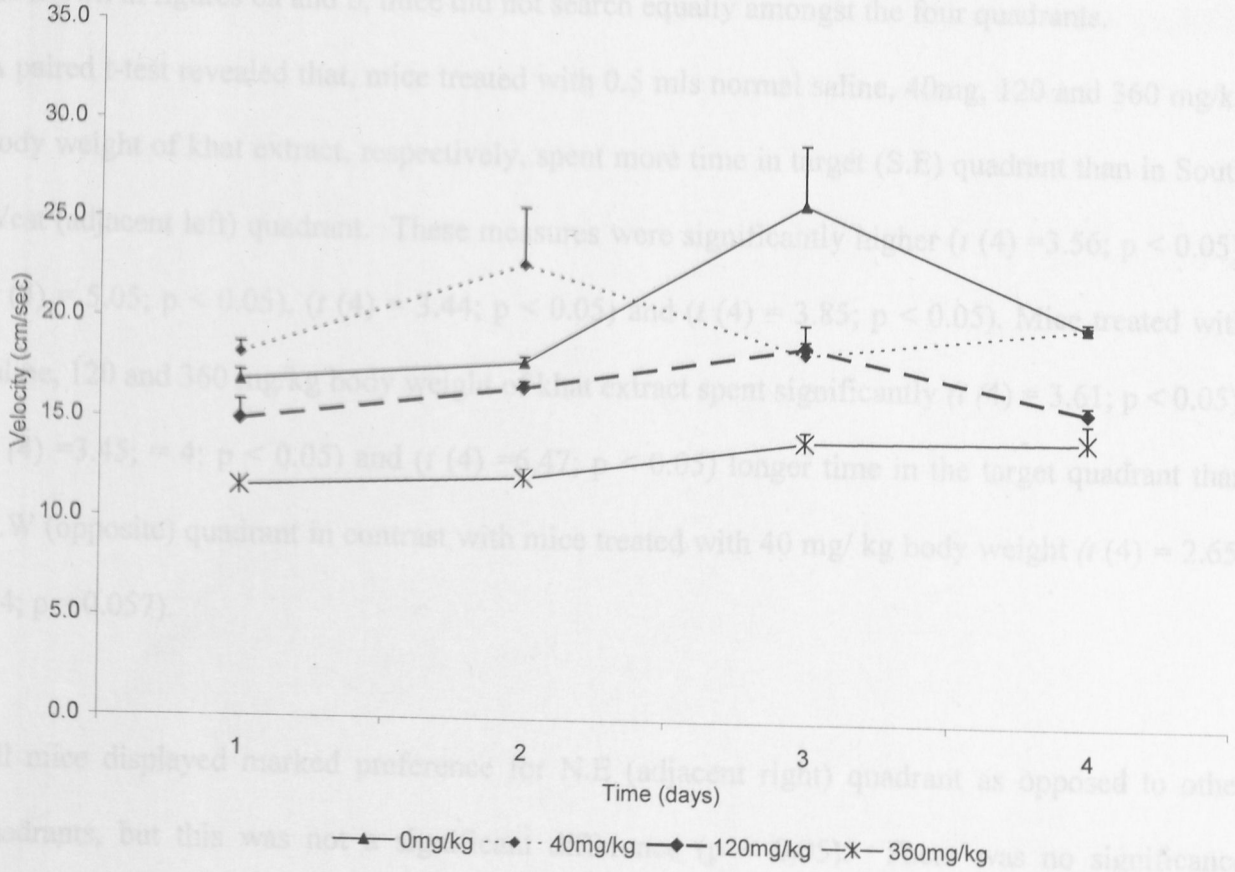


Figure 5c. Swim speed of CBA mice during 4 day trial in a MWM task. Mice were injected with 0.5 mls normal saline, 40, 120, & 360 mg/kg b.wt khat extract. (n = 5 mice in each group). Data are presented as mean ± s.e.m of swim speed. Mice injected with 360 mg / kg b.wt khat extract had significantly ($p < 0.05$) slower swim speed than the other doses and control group

4.2.1 Effects of khat on spatial memory retention of CBA mice in MWM (probe trial 1)

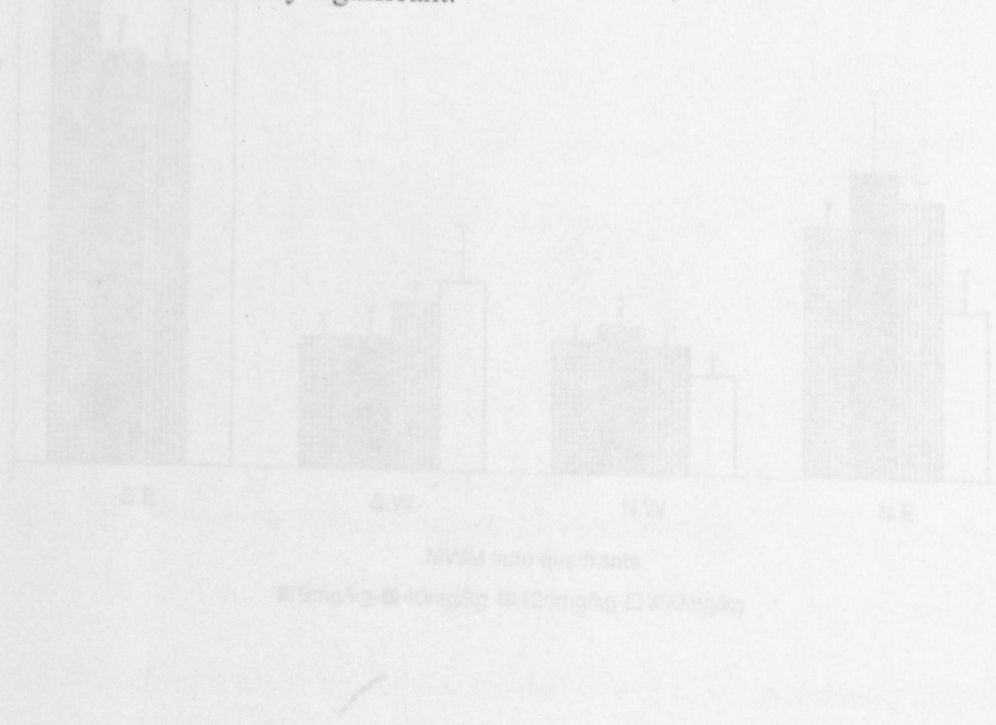
As shown in figures 6a and b, mice did not search equally amongst the four quadrants.

A paired t-test revealed that, mice treated with 0.5 mls normal saline, 40mg, 120 and 360 mg/kg body weight of khat extract, respectively, spent more time in target (S.E) quadrant than in South West (adjacent left) quadrant. These measures were significantly higher (t (4) =3.56; $p < 0.05$), (t (4) = 5.05; $p < 0.05$), (t (4) = 3.44; $p < 0.05$) and (t (4) = 3.85; $p < 0.05$). Mice treated with saline, 120 and 360 mg/kg body weight of khat extract spent significantly (t (4) = 3.61; $p < 0.05$), (t (4) =3.45; = 4; $p < 0.05$) and (t (4) =6.47; $p < 0.05$) longer time in the target quadrant than N.W (opposite) quadrant in contrast with mice treated with 40 mg/ kg body weight (t (4) = 2.65; = 4; $p = 0.057$).

All mice displayed marked preference for N.E (adjacent right) quadrant as opposed to other quadrants, but this was not a significant difference ($p < 0.05$). There was no significance difference between time spent in target quadrant and N.E (adjacent right) quadrant ($p > 0.05$) among mice treated with 40 and 120 mg/kg body weight khat extract and the control. On the contrary, mice treated with 360 mg/ kg body weigt khat extract did not show this pattern (t (4) = 6.15; $p < 0.05$

All mice groups except the ones teated with 120 mg/ kg body weight, had longer swim distance in the target (S.E.) quadrant and had significantly ($p < 0.05$) longer swim distance in target quadrant than in S.W.(adjacent left) and N.W.(adjacent right) quadrants, respectively. However, mice treated with 360 mg/kg body weight khat extract had longer swim distance in target quadrant than S.W. and N.W. and N.E quadrant (t (4) =2.8; $p < 0.05$), (t (4) = 7.8; $p < 0.05$) and (t (4) = 16.08; $p < 0.001$ respectively.

All mice treated with 120 and 360 mg/kg body weight of khat extract had significantly ($p < 0.05$) longer swim distance in the target quadrant compared with N.E (adjacent right) quadrant, however, mice treated with control and 40 mg/kg body weight khat extract showed preference for N.E quadrant ($t(4) = 1.62$; $p = 0.180$) as well as the target quadrant though the distance in the N.E quadrant was statistically significant.



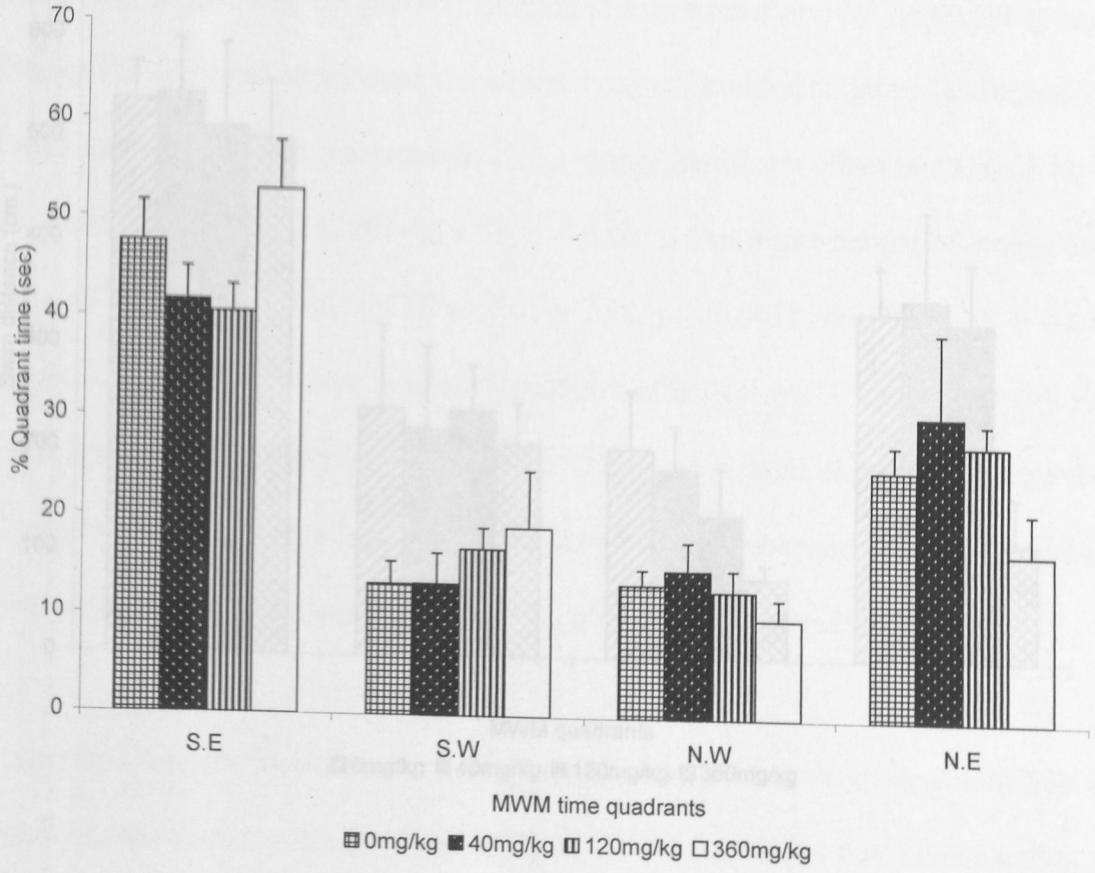


Figure 6 a. Effects of khat extract on quadrant time of CBA mice during probe trial to assess spatial memory acquisition in MWM task. Mice were injected with 0.5 mls normal saline, 40, 120 & 360mg /kg b.wt khat extract respectively. (n = 5 mice in each group). Data are presented as mean ± s.e.m of % quadrant time (sec.) Mice injected with 0.5 mls saline, 40, 120 and 360 mg/ kg b.wt khat extract spent more time ($p < 0.05$) in the target (S.E) quadrant than in other quadrants. Mice treated with 120 and 360 mg/ kg b.wt and saline spent significant ($p < 0.05$) time in target quadrant than in N.W (opposite) quadrant as opposed to mice treated with 40 mg/kg b.wt. Mice treated with 360 mg/ kg b.w.t khat extract did not show preference for N.E quadrant as compared to other doses and control.

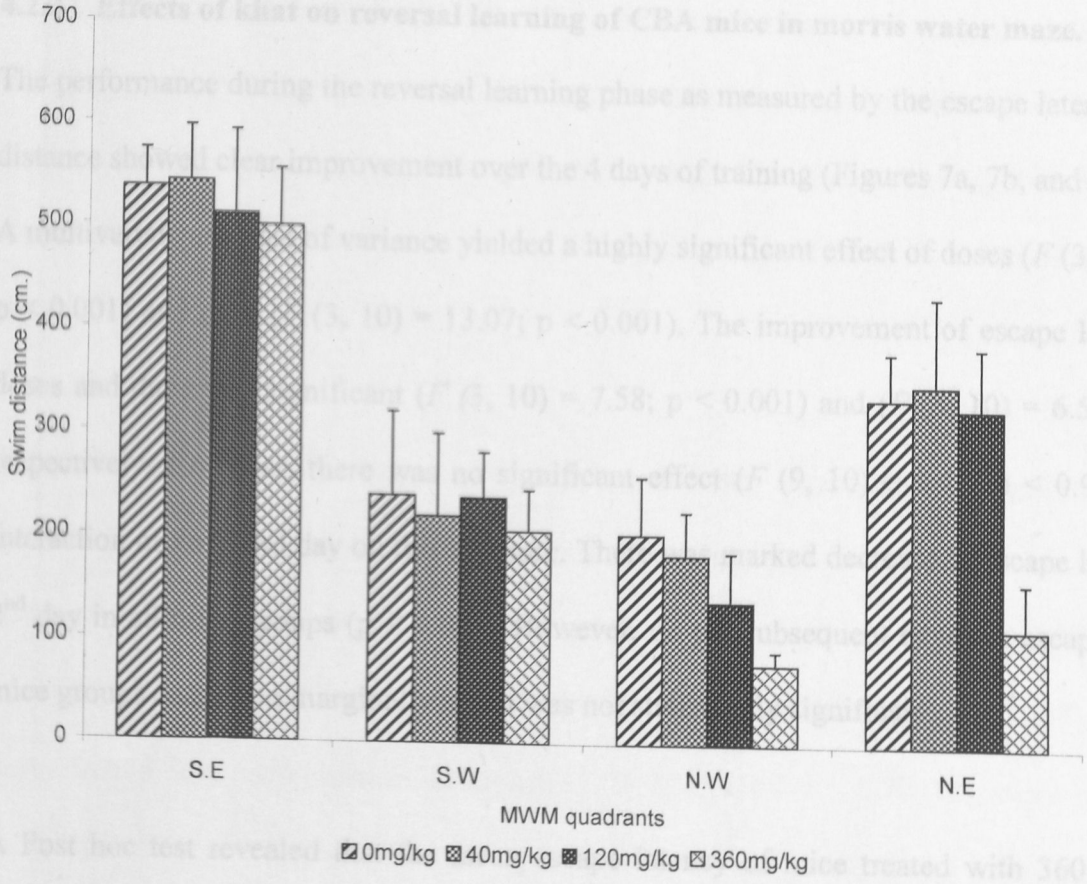


Figure 6 b The effect of khat extract on swim distance of CBA mice during spatial memory assessment in MWM task acquisition. (n = 5 mice in each group). Data are represented as mean ± s.e.m of quadrant swim distance (cms). Mice injected with 0.5 mls saline, 40mg, and 120 mg/kg b.wt khat extract swam longer ($p < 0.05$) in the target (S.E) quadrant than mice treated with 360 mg/kg b.wt khat extract. All mice treated with 360mg /kg b.wt khat extract had longer ($p < 0.05$) swim distance in target quadrant than in N.E (adjacent right) quadrant as opposed to controls an other treatment groups.

4.2.2 Effects of khat on reversal learning of CBA mice in morris water maze.

The performance during the reversal learning phase as measured by the escape latency and swim distance showed clear improvement over the 4 days of training (Figures 7a, 7b, and 7c)

A multivariate analysis of variance yielded a highly significant effect of doses (F (3, 10) = 10.14; $p < 0.001$) and days (F (3, 10) = 13.07; $p < 0.001$). The improvement of escape latency across doses and days was significant (F (3, 10) = 7.58; $p < 0.001$) and (F (3, 10) = 6.54; $p < 0.05$), respectively. However, there was no significant effect (F (9, 10) = 0.42; $p < 0.921$) with the interaction of dose and day on this measure. There was marked decrease in escape latency on the 2nd day in all mice groups ($p < 0.05$). However, on the subsequent days the escape latency for mice groups decreased marginally and it was not statistically significant.

A Post hoc test revealed that the mean escape latency of mice treated with 360 mg/kg body weight of khat extract was significantly higher (38.69 ± 5.58, $p < 0.05$) compared to mice treated with other doses and control. This group of mice improved their escape latency on the second day but there after the latency was poor. It appears that mice treated with 40 mg and 120 mg/kg body weight of khat extract and the control needed only one more day to reduce their escape latencies to the lowest level, however, it could take more trials for 360 mg / per kg body weight khat treated mice to achieve its lowest level if the pattern was to be repeated. This is an indication of interference of khat to behavioural switching in mice.

The swim distance across doses decreased over days (Fig 7b). MANOVA yielded significant effect of doses (F (3, 10) = 3.84; $p < 0.05$) and days (F (3, 10) = 11.77; $p < 0.001$) but no significant difference (F (9, 10) = 0.42; $p = 0.919$) with interaction of dose and days on swim distance. There was marked improvement in the swim distance on the 2nd day of reversal learning in all mice groups ($p < 0.05$) but, on the subsequent days, the improvement was

marginal for animals treated with lower khat extract doses and the control while mice treated with 360 mg/kg weight khat extract did not show any improvement there after. A post hoc test revealed that mice treated with 360 mg/kg body weight khat extract had significantly ($p < 0.05$) longer swim distance than the control but not significantly different from the other khat treated mice.

The swim speed of mice treated with 120 mg and 360 mg/kg body weight of khat extract decreased while mice treated with 40 mg/kg body weight and the control increased consistently with days (Fig. 7c). The changes on swim speed across doses were significant (F (3, 10) = 7.16; $p < 0.001$) but no statistically significant difference (F (3, 10) = 0.63; $p < 0.597$) yielded over days. In addition, no significant difference (F (9, 10) = 0.69; $p < 0.709$) on swim speed with the interaction of dose and days was observed. A post hoc test revealed that mice treated with 360 mg/kg body weight had significantly ($p < 0.05$) reduced swim speed than the other groups and the control. Taken together (escape latency, swim distance and swim speed), the results indicated that mice treated with higher dose (360 mg/ kg) of khat extract poorly acquired MWM task compared to lower dose and the control

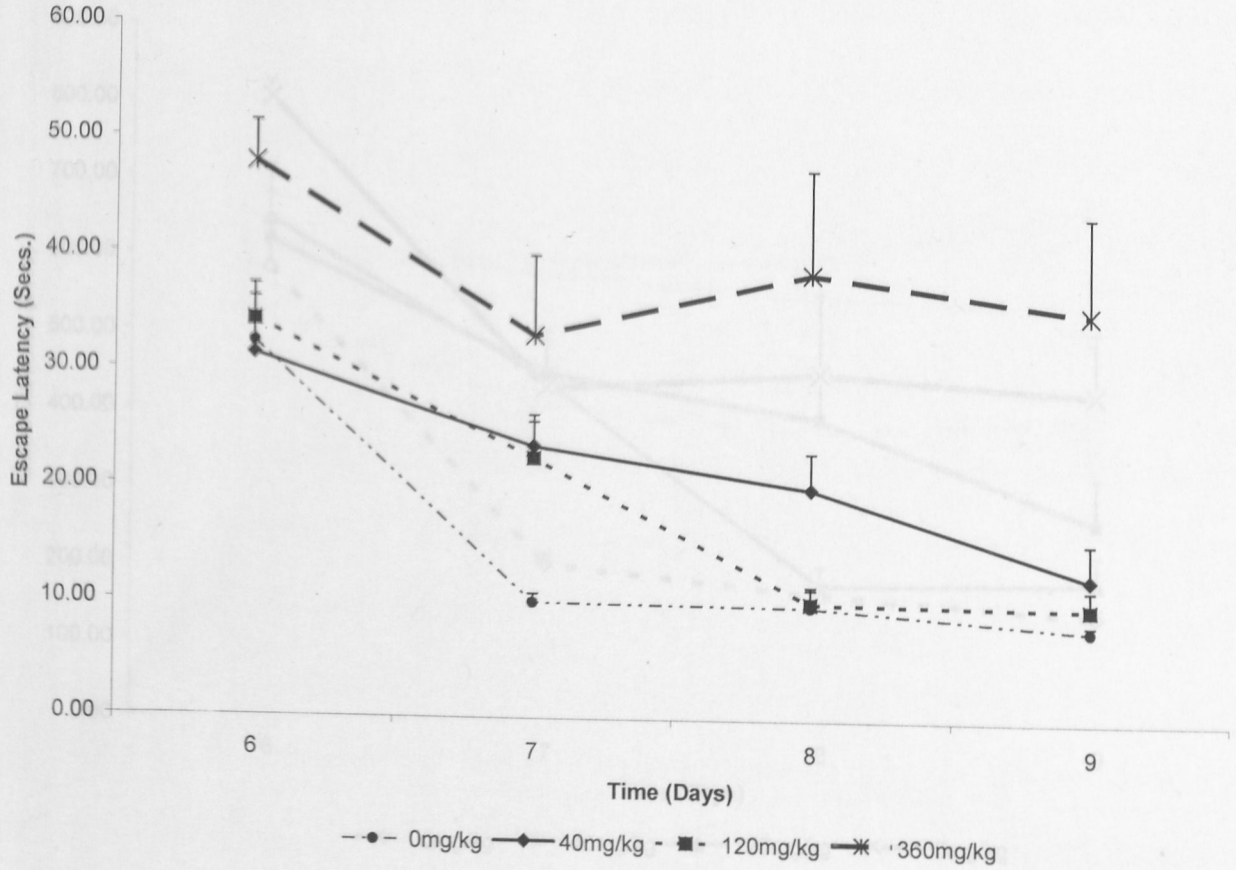


Figure 7 a. Effects of khat extract on escape latency of CBA mice during reversal learning trial of MWM task. (n = 2 – 5 mice in each group). Data are presented as mean ± s.e.m of escape latency (secs). Escape latency were significantly higher ($p < 0.05$) in mice treated with 360 mg/kg b.wt of khat extract compared with the other doses and the control group. The escape latency, improved significantly over days and marked improvement was shown on 2nd day ($p < 0.05$)

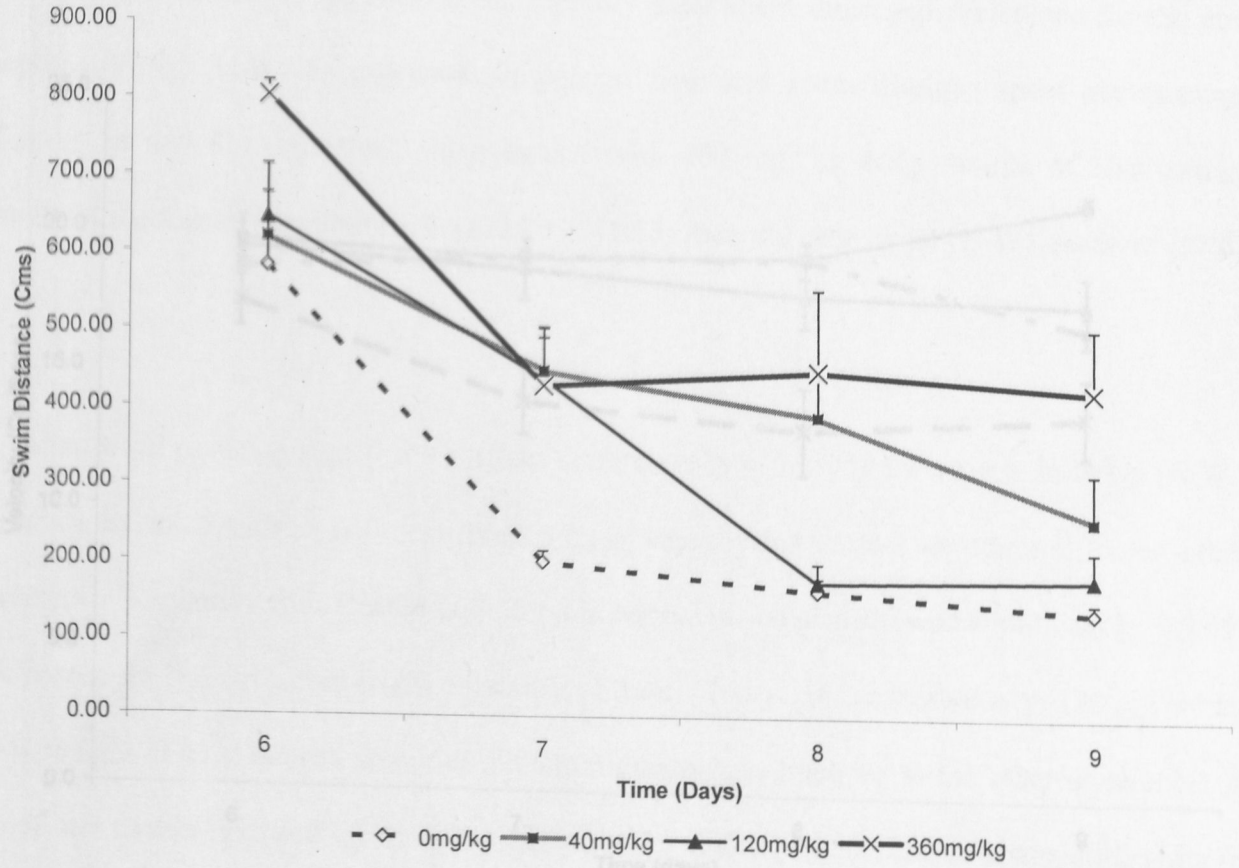


Figure 7 b. Effects of khat extract on swim distance of CBA mice during reversal spatial learning and memory of MWM task. (n = 2 – 5 mice in each group). Data are presented as mean ± s.e.m of swim distance (cms.). Mice treated with 360 mg/kg body weight khat extract, had longer swim distance ($p < 0.05$) than the other doses and the control.

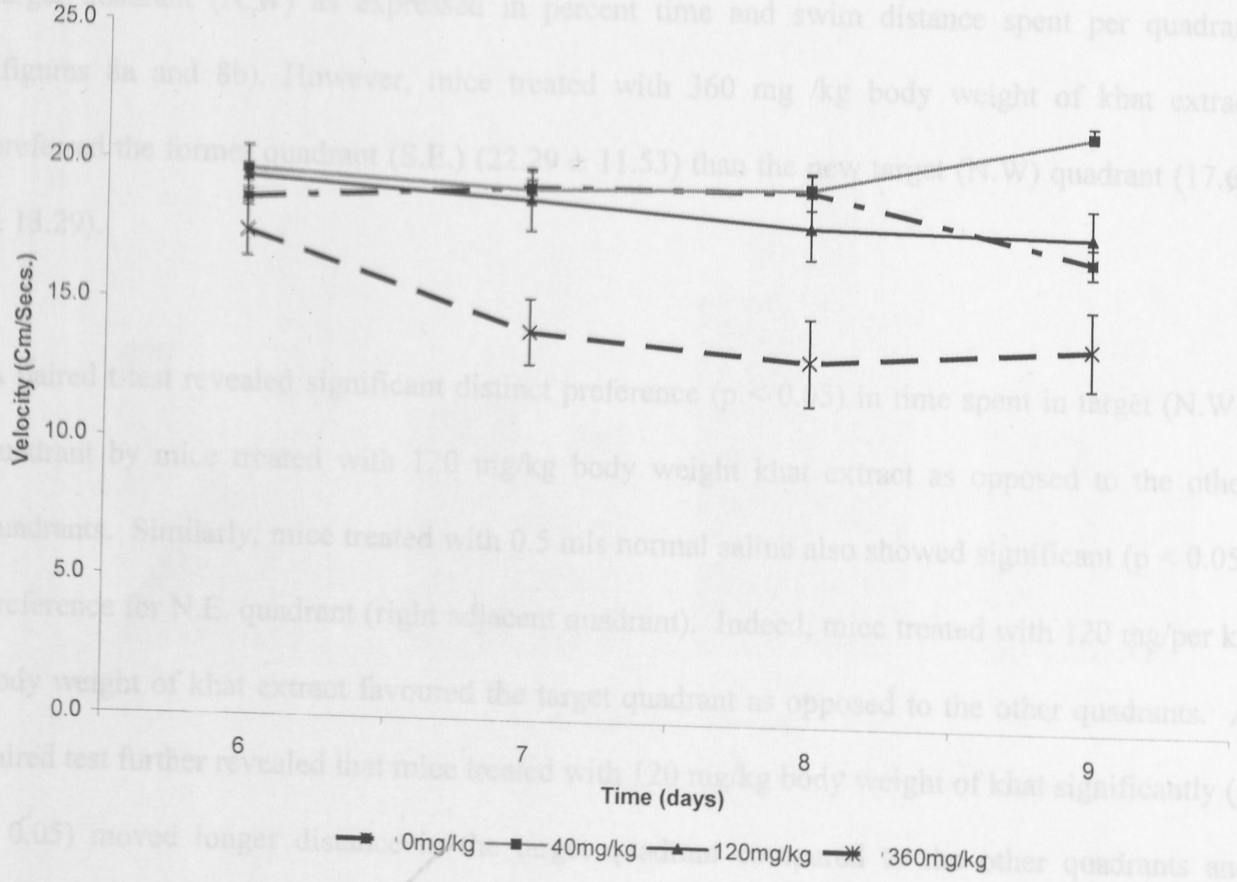


Figure 7 c. Effects of administration of (0.5 mls normal saline, 40, 120 or 360 mg/kg b.wt) khat extract on swim speed of CBA mice during reversal spatial learning and memory of MWM task. (n = 2 - 5 mice in each group). Data are presented as mean ± s.e.m. Mice injected with 360 mg khat had slower ($p < 0.05$) swim speed than the other doses and control.

4.2.3 Effects of khat on spatial memory retention during reversal learning

The khat and saline groups on reversal memory assessment displayed preference for the new target quadrant (N.W) as expressed in percent time and swim distance spent per quadrant (figures 8a and 8b). However, mice treated with 360 mg /kg body weight of khat extract preferred the former quadrant (S.E.) (22.29 ± 11.53) than the new target (N.W) quadrant (17.62 ± 13.29).

A paired t-test revealed significant distinct preference ($p < 0.05$) in time spent in target (N.W.) quadrant by mice treated with 120 mg/kg body weight khat extract as opposed to the other quadrants. Similarly, mice treated with 0.5 mls normal saline also showed significant ($p < 0.05$) preference for N.E. quadrant (right adjacent quadrant). Indeed, mice treated with 120 mg/per kg body weight of khat extract favoured the target quadrant as opposed to the other quadrants. A paired test further revealed that mice treated with 120 mg/kg body weight of khat significantly ($p < 0.05$) moved longer distance in the target quadrant compared to the other quadrants and comparred with other groups of mice and the control. All mice injected with 360 mg/kg of khat extract, swam (236.05 ± 156.95) in the target quadrant and (328 ± 165.0) in the former target quadrant (S.E quadrant). However, the difference was not significant statistically ($p > 0.05$).

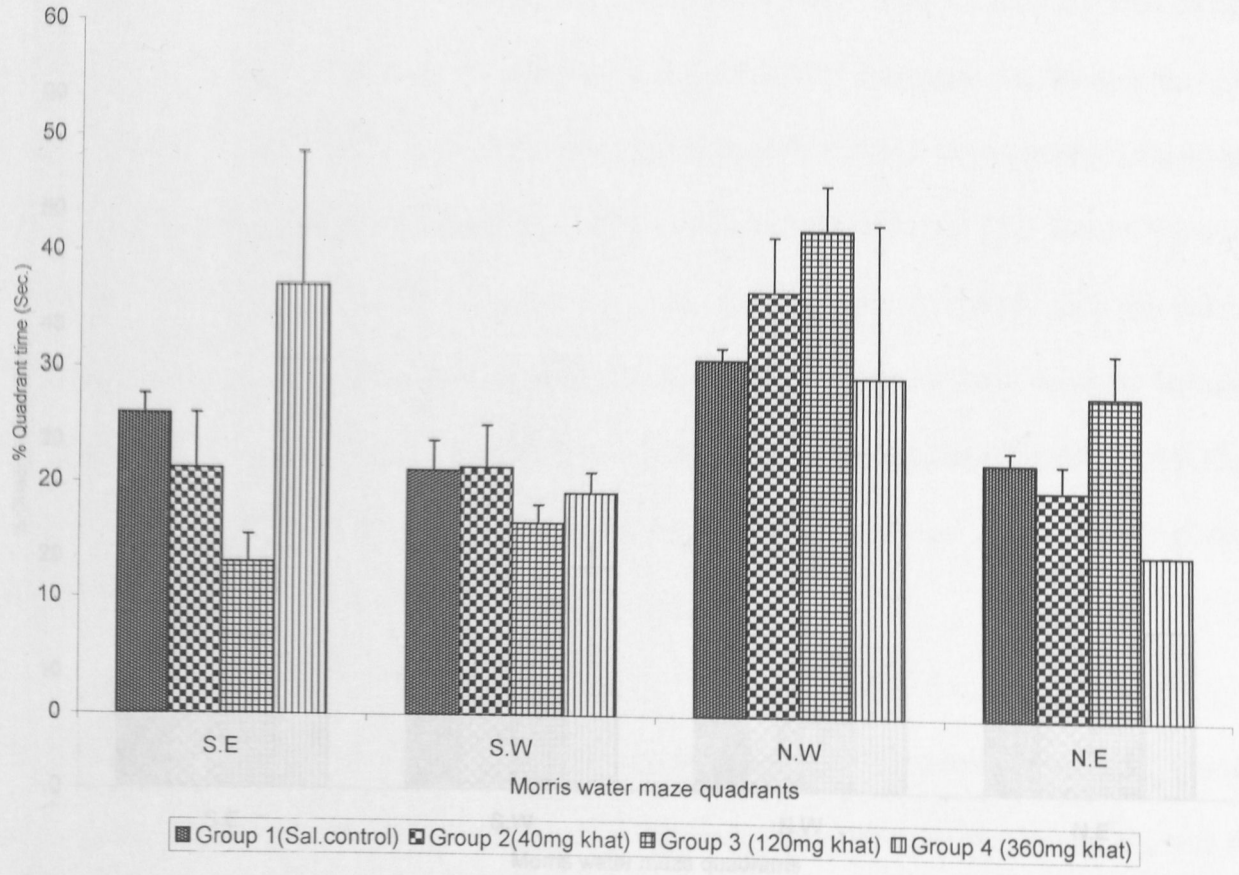


Figure 8 a. Effects of khat extract on quadrant time of CBA mice during reversal spatial memory assessment of MWM task acquisition. Mice were injected with (0.5 mls normal saline, 40, 120 or 360 mg/kg b.wt khat extract). (n = 2 - 5 mice in each group). Data are represented as mean ± s.e.m of % quadrant time (secs.). Mice injected with saline, 40 and 120 mg khat extract spent more time in the target (N.W) quadrant (21.97 ± 3.23) than (12.11 ± 2.97) in the former target (S.E) quadrant. Mice injected with 360 mg of khat spent more time (17.62 ± 13.29) in the target quadrant and (22.29 ±11.53) in the former target (S.E) quadrant

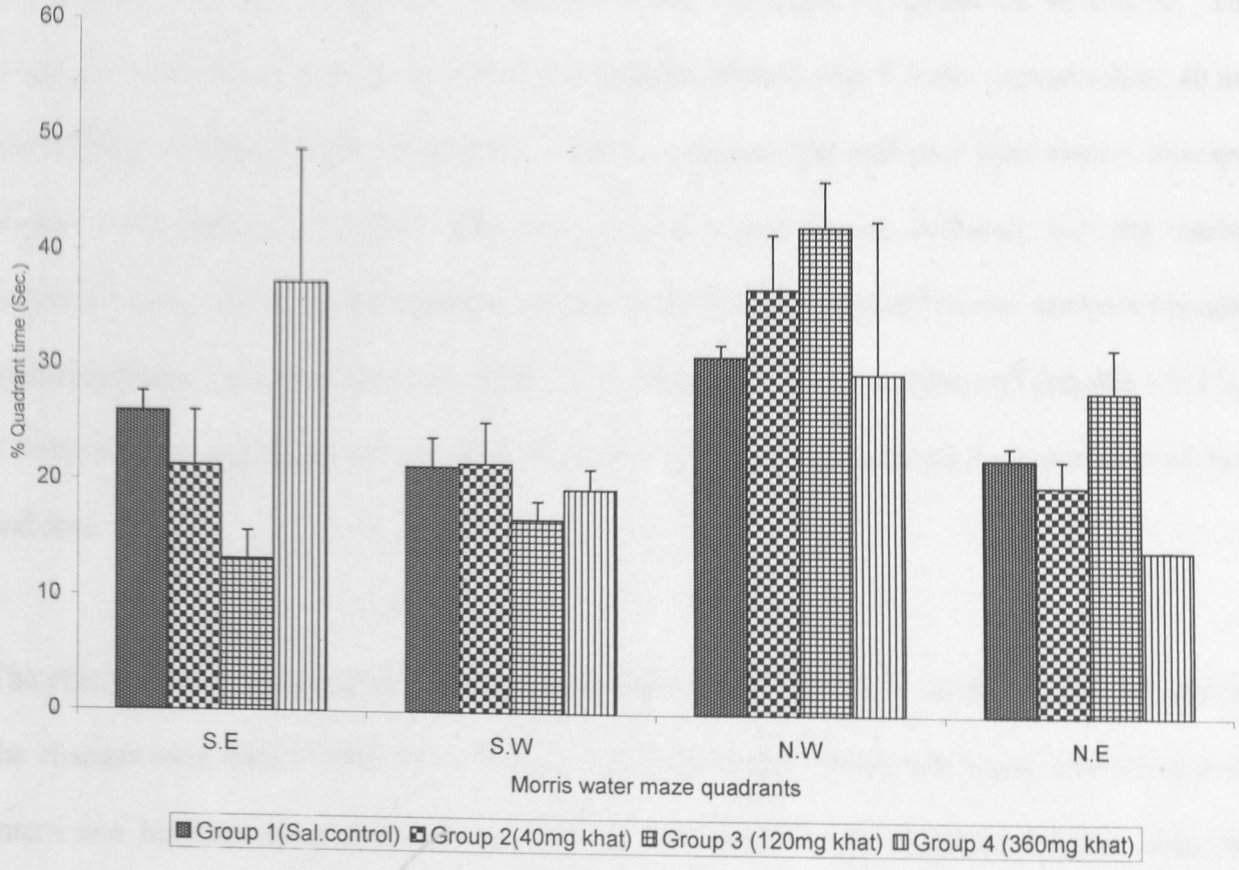


Figure 8 b Effects of khat extract on quadrant swim distance of CBA mice during reversal spatial memory assessment in MWM task acquisition. Mice were injected with (0.5 mls normal saline, 40, 120 or 360 mg/kg b.wt khat extract). (n = 2 - 5 mice in each group). Data are represented as mean ± s.e.m of quadrant swim distance (Cm.). All mice injected with saline, 40 and 120 mg khat had longer swim distance in target (N.W) quadrant (491.31 ± 81.27) than in the former target (S.E) quadrant (263.67 ± 63.22). All mice injected with 360mg/ kg b.wt of khat extract had shorter swim distance (236.05 ± 156.95) in the target quadrant than in the former quadrant (328.90 ±165.0)

4.3 Locomotor behaviour of CBA mice after Morris water maze and khat extract treatment

The results of the effects of khat extract on locomotor activity after 17 days of khat extract injection and 10 days of exposure to MWM task are illustrated in figures 9a, 9b and 9c. The behaviour was evaluated before and after injecting the animals with 0.5 mls normal saline, 40 mg and 120 mg of khat extract respectively. The two phases (pre and post khat extract injection phases) were analysed together. The line crossing, centre square frequency and the rearing frequency were scored as the measure of locomotor activity. A multivariate analysis revealed high significant change across doses (F (2, 12) = 18.82; $p < 0.001$) and time (F (10, 45) = 3.17; $p < 0.05$) with no significant (F (14, 47) = 0.94; $p = 0.52$) change between the interaction of dose and time.

The change on line crossing across doses was significant (F (2, 12) = 10.64; $p < 0.05$) however, the changes over time (F (10, 45) = 0.66; $p = 0.76$) was not statistically significant nor was the interaction between dose and time ($p < 0.05$). A post hoc test revealed that the mean line crossing of 40 mg/kg body weight of khat extract treated mice (17.08 ± 20.44) was significantly ($p < 0.001$) lower than 120 mg/ kg b.wt khat extract treated mice (34.04 ± 34.67, $p < 0.05$) and control group (41.03 ± 24.67, $p < 0.001$). (Fig. 9 a). These effects on line crossing appeared to be dose dependent. The mean line crossing decreased with time as the animal became habituated during pre injection phase and also during the 1st 20 minutes post khat injection time, however, the decrease was not statistically significant.

The centre square frequency results for the CBA mice are illustrated in figure (9 b). The results show decreased activity across doses. The centre square frequency changed significantly (F (2, 12) = 5.74; $p < 0.05$) across doses but there was no significant change across time (F (10, 45) = 0.52; $p = 0.88$) on this measure. The interaction between dose and time was not significant (F

(14, 47) = 0.73; p = 0.74) on centre square frequency. A post hoc test revealed that the mean centre square frequency for mice treated with 120 mg/kg body weight khat extract (0.88 ± 0.73) was significant ($p < 0.001$) than control group (figure 9 b). Mice treated with 120 mg/kg body weight of khat extract had reduced centre square frequency compared with mice treated with 40 mg/kg body weight of khat extract (0.88 ± 0.73) and (2.8 ± 2.14) respectively.

The rearing frequency changed significantly across doses (F (2, 12) = 23.89; $p < 0.001$) and (F (10, 45) = 2.64; $p < 0.05$) across time. There was no significant difference (F (14, 47) = 0.75; p = 0.76) with the interaction of dose and time on rearing frequency. A post hoc test showed that mice treated with 40 mg and 120 mg/kg body weight of khat extract had significantly ($p < 0.001$) lower rearing frequency than the control (0.58 ± 0.66) and (0.22 ± 0.36) vs. (5.6 ± 3.13). In all mice, however, there were changes in rearing frequency across time but the changes were not significance ($p > 0.005$).

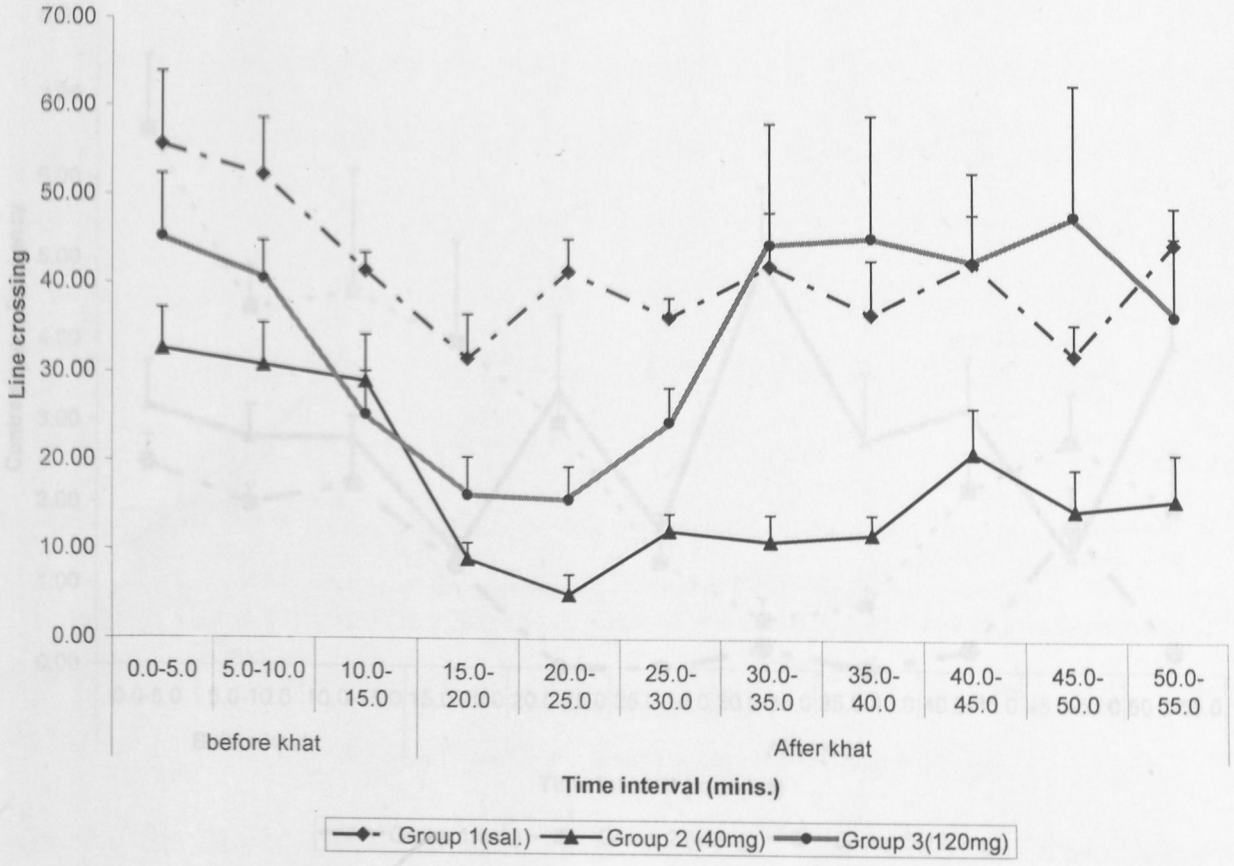


Figure 9 a. The effects of khat extract on locomotor activity after 17 days of i.p administration including 10 days of Morris water maze test. Mice were injected with (0.5 mls saline, 40 0r 120 mg/ kg b.wt khat extract), (n = 4 - 5 mice in each group). Data are presented as mean ± s.e.m of line crossing. Mice treated with 40 mg/kg b.wt had significantly fewer ($p < 0.001$) line crossing than control and mice treated with 120 mg/kg b.wt khat extract.

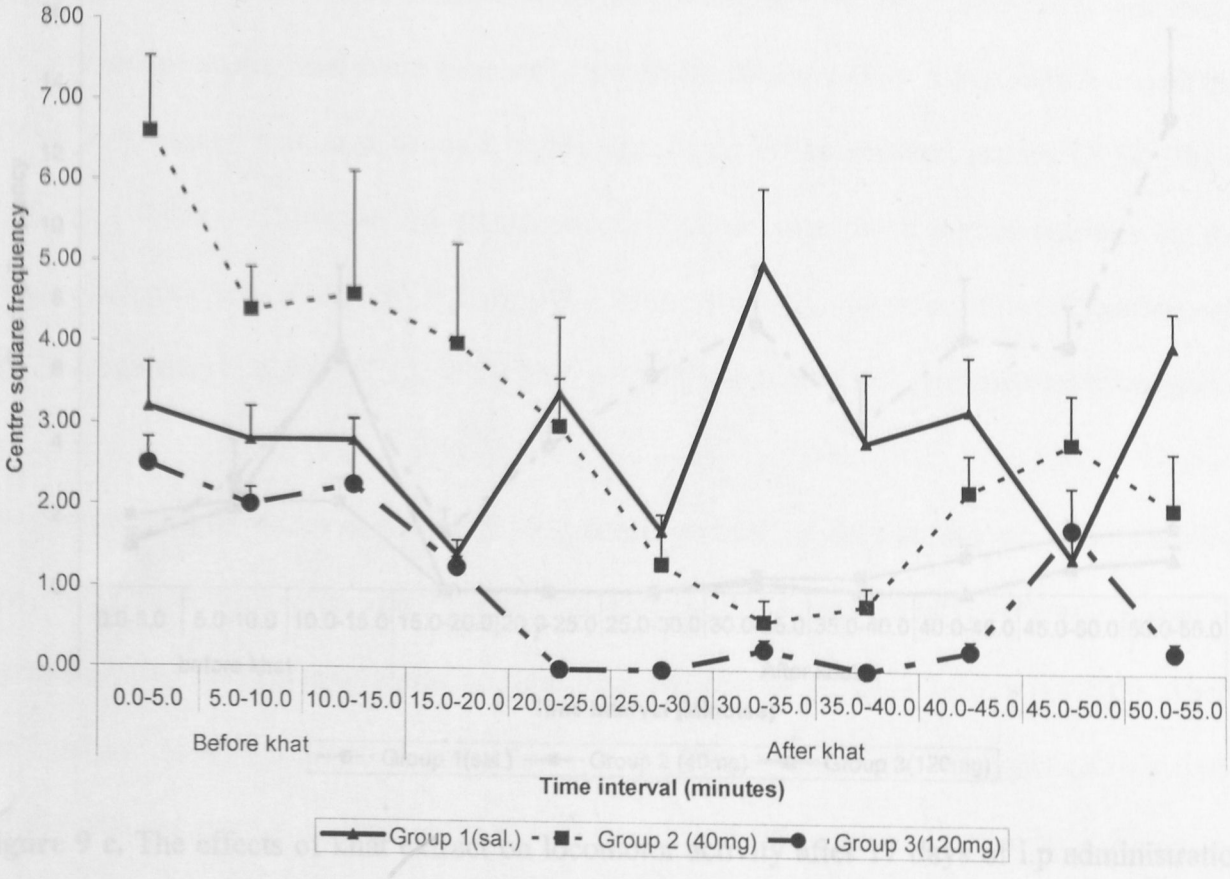


Figure 9 b. The effects of khat extract on locomotor activity after 17 days of i.p administration including 10 days of the Morris water maze test. Mice were injected with (0.5 mls saline, 40 or 120 mg/ kg b.wt khat extract); (n = 4 - 5 mice in each group). Data are presented as mean ± s.e.m of centre square frequency. Mice treated with 120 mg khat extract had fewer ($p < 0.001$) centre square frequencies than control and mice treated with 40 mg khat.

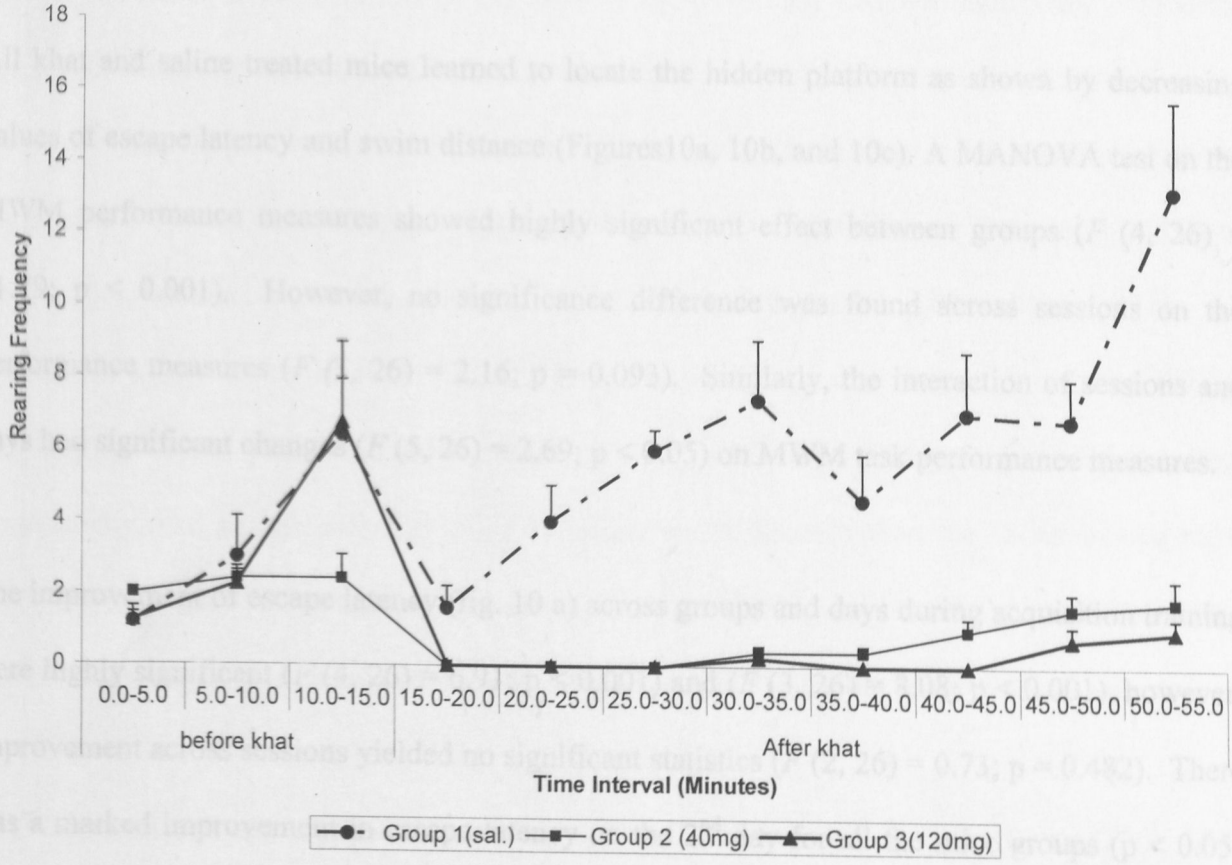


Figure 9 c. The effects of khat extract on locomotor activity after 17 days of i.p administration including 10 days of Morris water maze test. Mice were injected with (0.5 mls saline, 40 or 120 mg/ kg b.wt khat extract); (n = 4 - 5 mice in each group). Data are presented as mean ± s.e.m rearing frequency. Mice treated with 40 or 120 mg khat had a fewer ($p < 0.001$) rearing frequency than the control.

4.4.1 Effects of escalating khat dose regimen on acquisition learning and memory of CBA mice.

All khat and saline treated mice learned to locate the hidden platform as shown by decreasing values of escape latency and swim distance (Figures10a, 10b, and 10c). A MANOVA test on the MWM performance measures showed highly significant effect between groups (F (4, 26) = 11.79; $p < 0.001$). However, no significance difference was found across sessions on the performance measures (F (3, 26) = 2.16; $p = 0.093$). Similarly, the interaction of sessions and days had significant changes (F (5, 26) = 2.69; $p < 0.05$) on MWM task performance measures.

The improvement of escape latency (fig. 10 a) across groups and days during acquisition training were highly significant (F (4, 26) = 6.91; $p < 0.001$) and (F (3, 26) = 8.08; $p < 0.001$), however, improvement across sessions yielded no significant statistics (F (2, 26) = 0.73; $p = 0.482$). There was a marked improvement in escape latency on the 2nd day for all the mice groups ($p < 0.05$) and the changes were sustained up to the 4th day, but it was marginal. Similarly, there was significant improvement (F (5, 7) = 2.295; $p < 0.05$) in escape latency with the interaction of days and session. Despite the improvement of escape latency across doses and sessions, the difference was not significant (F (8, 26) = 0.94; $p = 0.482$) and (F (2, 26) =0.73; $p = 0.482$).

A post hoc test with Bonferroni revealed that (Fig. 10 a) the mean escape latency decreased significantly ($p < 0.05$) with days. The tests further revealed that, the escape latency improved with escalating doses, but between doses improvement was not significant ($p > 0.05$) and mice treated with 10 mg, 60 mg/kg body weight of khat extract had higher ($p < 0.05$) escape latency compared to saline treated mice group. On the other hand, mice treated with 310 mg/kg body weight of khat extract had lower escape latency compared with saline treated mice ($p < 0.05$).

The swim distance improved across days for all the mice groups (F (3, 26) = 8.49; $p < 0.01$) but the improvement across sessions (F (2, 26) = 2.12; $p < 0.135$) was not significant. However, there was a significance effect (F (5, 26) =2.35; $p < 0.05$) of days, sessions interaction on the swim distance. A post hoc test with Bonferroni test revealed that, the swim distance improved significantly ($p < 0.001$). The days with marked changes on swim distance were day 1 and day 2 ($p < 0.001$), but there was further improvement on day 4, though not significant ($p = 0.971$). There was improvement of swim distance across doses but the interdose improvement was not significant. On the other hand, mice treated with 10, and 60 mg/kg body weight of khat extract, respectively, had significantly ($p < 0.001$) longer swim distance than the saline treated mice. Mice treated with 310 mg/kg body weight of khat had significantly shorter swim distance than the control group and mice treated with doses between 10-110 mg/kg body weight khat.

The improvement of swim speed across days for all the mice was not significant (F (3, 26) = 2.84; $p < 0.052$) nor were the changes across sessions (F (2, 26) = 0.070; $p = 0.93$). Similarly, the interaction of sessions, days and doses was not significant for swim speed ($p = 0.17$).

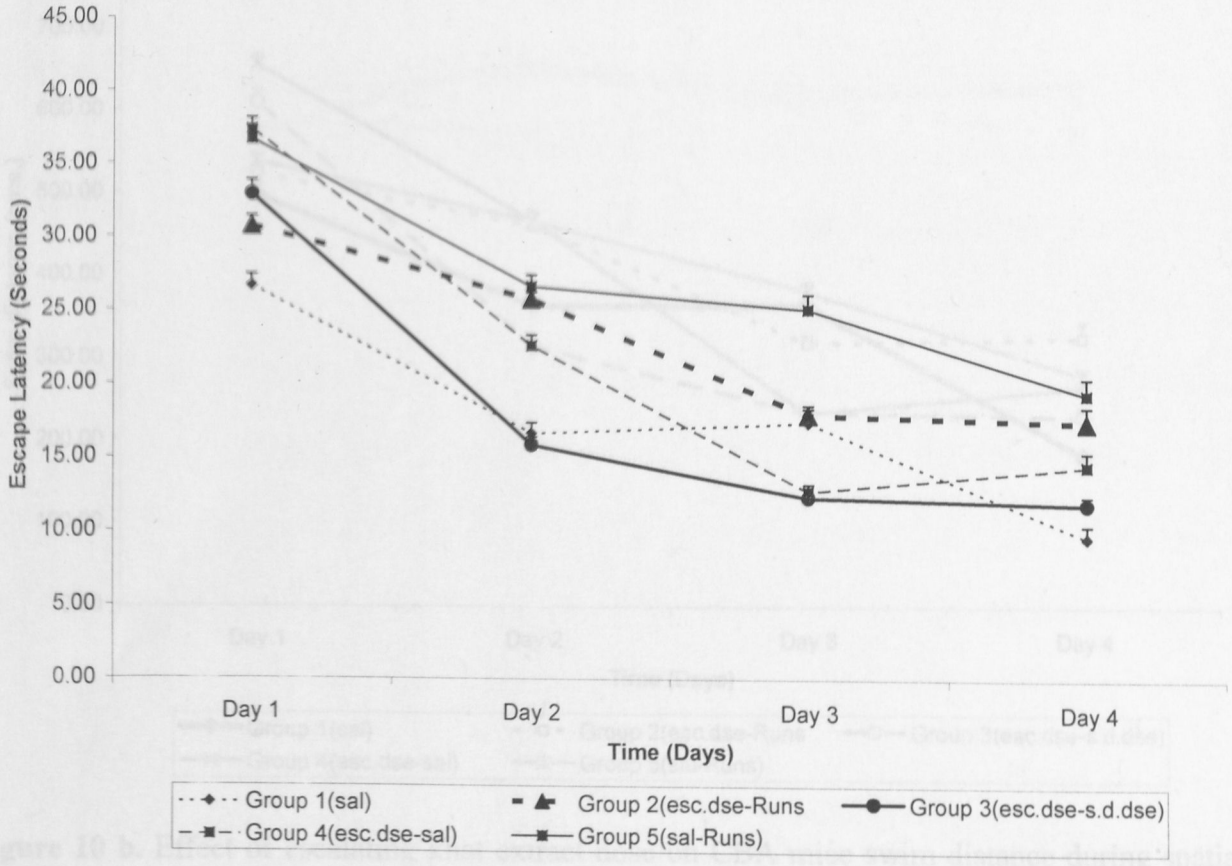


Figure 10 a. Effect of escalating khat extract dose on CBA mice escape latency during spatial learning and memory in MWM task. Mice were injected with 0.5 mls normal saline and escalating khat doses. (n = 5 -7 in each group). Data are presented as a mean ± s.e.m of escape latency. There was improvement ($p < 0.001$) in escape latency over days across all doses and control. Most reduction of escape latency was observed on the 2nd day ($p < 0.05$)

Meaning of Dose Regimes

Esc. dse-Runs: The group was injected with escalating then multiple high khat extract dose regime

Esc.dse-s.d.dse: The group was injected with escalating then single daily high khat extract dose regime.

Esc.dse-sal: The group was injected with escalating then multiple saline khat extract dose regime.

Sal-Runs: The group was injected with normal 0.5mls normal saline then multiple high khat extract dose regime.

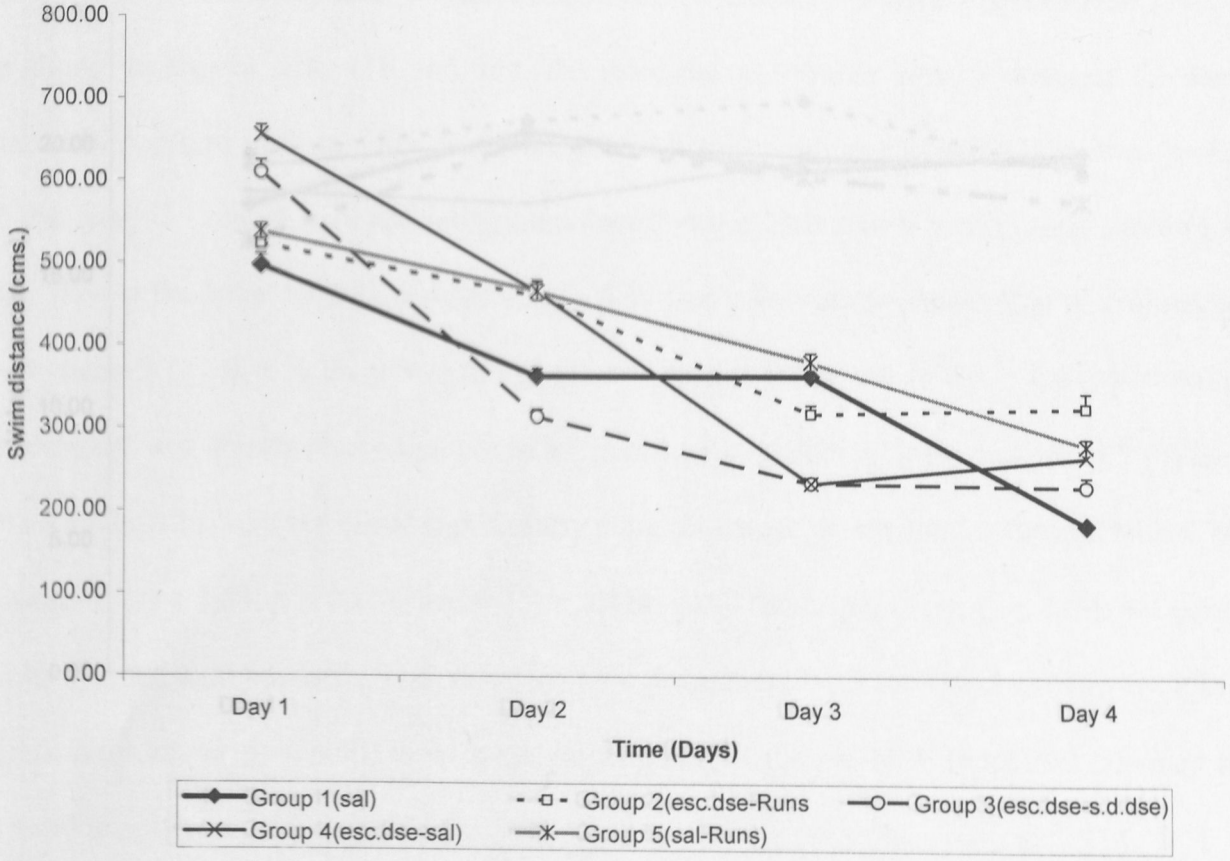


Figure 10 b. Effect of escalating khat extract dose on CBA mice swim distance during spatial learning and memory assessment in MWM task. Mice were injected with 0.5 mls normal saline and escalating khat doses. (n = 5 - 7 in each group). Data are presented as a mean ± s.e.m of swim distance. There was decrease ($p < 0.001$) in swim distance over days across all doses and control group. Marked decrease in swim distance was observed on the 2^{nd} day ($p < 0.001$)

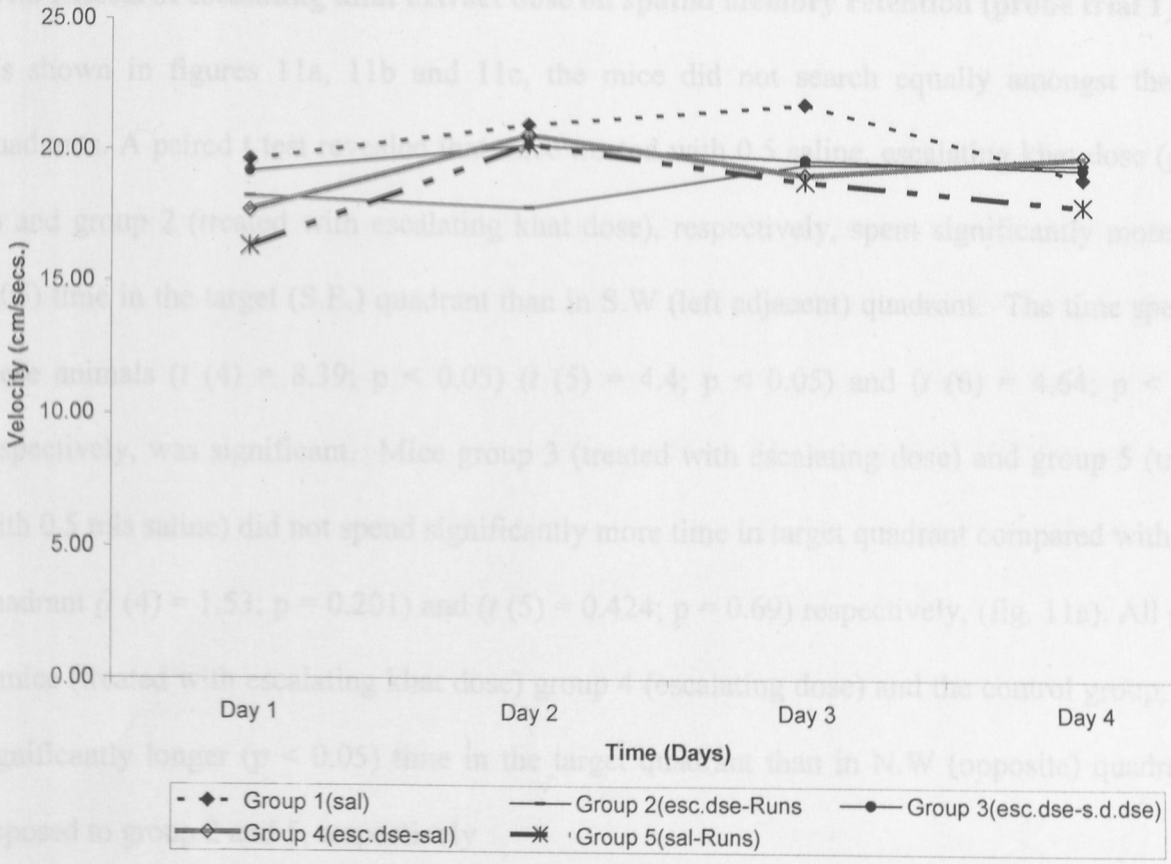


Figure 10 c. Effect of escalating khat extract dose on CBA mice swim speed during spatial learning and memory assessment in MWM task. Mice were injected with 0.5 mls normal saline and escalating khat doses. (n = 5 - 7 in each group). Data are presented as a mean ± s.e.m of swim speed. There was no improvement ($p < 0.052$) of swim speed over days across all doses and control group.

4.4.2 Effects of escalating khat extract dose on spatial memory retention (probe trial 1)

As shown in figures 11a, 11b and 11c, the mice did not search equally amongst the four quadrants. A paired t test revealed that mice treated with 0.5 saline, escalating khat dose (group 4) and group 2 (treated with escalating khat dose), respectively, spent significantly more ($p < 0.05$) time in the target (S.E.) quadrant than in S.W (left adjacent) quadrant. The time spent by these animals (t (4) = 8.39; $p < 0.05$) (t (5) = 4.4; $p < 0.05$) and (t (6) = 4.64; $p < 0.05$) respectively, was significant. Mice group 3 (treated with escalating dose) and group 5 (treated with 0.5 mls saline) did not spend significantly more time in target quadrant compared with S.W. quadrant (t (4) = 1.53; $p = 0.201$) and (t (5) = 0.424; $p = 0.69$) respectively, (fig. 11a). All group 3 mice (treated with escalating khat dose) group 4 (escalating dose) and the control group, spent significantly longer ($p < 0.05$) time in the target quadrant than in N.W (opposite) quadrant as opposed to group 2 and 5, respectively

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All mice groups spent most of their time in N.E (right adjacent) quadrant as opposed to other quadrants but this was not significant ($p > 0.05$). Indeed, some mice groups, for example, group 4 (treated with normal saline) spent almost half the time in the two quadrants (16.95 ± 3.49 vs 14.9 ± 3.93 seconds). Mice treated with escalating dose (groups 3 and 4) spent more time in the N.E. quadrant as opposed to target quadrant (28.04 ± 4.64 vs 21.76 ± 3.72) and (26.9 ± 5.03 vs. 24.27 ± 4.62), respectively. Similarly, mice treated with escalating dose (group 2) spent more time in the N.E. quadrant as compared to target quadrant but the difference was not significant (21.63 ± 3.00 vs 18.91 ± 3.41). No significance preference was noted with all mice concerning the other two quadrants. A further t -test on swim distances (Fig 11 b) revealed that mice treated with escalating khat dose (group 2 and 3) and the control had significantly ($p > 0.05$) longer distance in the target quadrant than the S.W. quadrants. Mice treated with escalating khat dose

(group 3) and the control had longer swim distance ($p < 0.05$) in the target quadrant than N.W. quadrant.

All mice treated with escalating khat dose saline and control had preference for N.E. quadrant as shown by longer swim distance in N.E. quadrant and, further confirmed, by lack of statistical significance. The mice groups treated with escalating doses (group 2, 3 and 4) had longer swim distances in N.E. quadrant than the S.E. quadrant (291.4 ± 37.20 vs 260.73 ± 52.91) and (428.08 ± 81.41 vs 402.3 ± 38.62) and (428.78 ± 72.4 vs 388.27 ± 111.69), respectively.

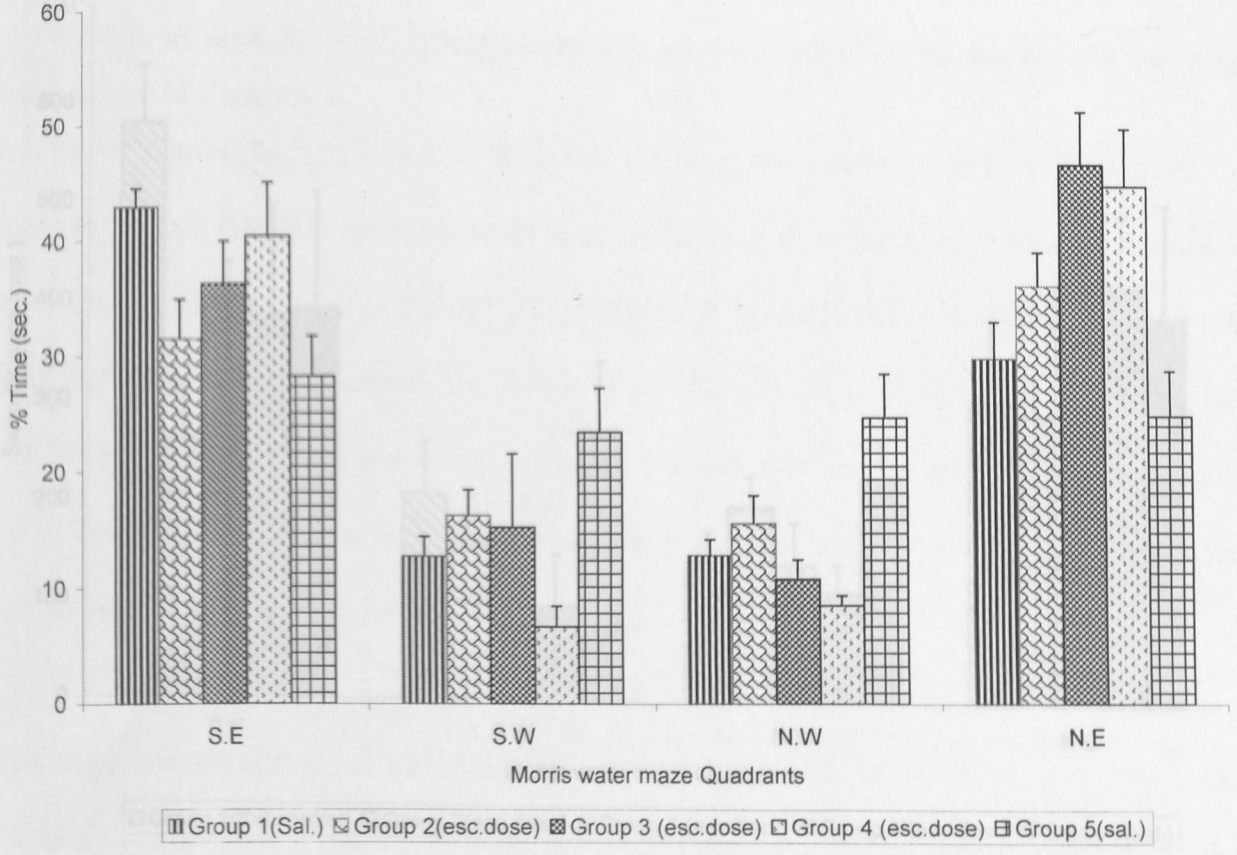


Figure 11 a. Effects of escalating khat extract dose on quadrant time of CBA mice during spatial memory assessment of MWM task. Mice were injected with escalating doses of khat extract or saline (n = 5 - 7 in each group). Data are presented as a mean ± s.e.m of % quadrant time (Secs.). Mice treated with escalating doses (group 2 & 4) and control (group 1) had longer ($p < 0.05$) time in target quadrant (S.E) than in N.E quadrant compared with mice treated with escalating dose (group 3) and saline (group 5). Mice treated with escalating khat (group 3 & 4) and control (group 1) allocated longer ($p < 0.05$) time in the target (S.E) quadrant than N.W quadrant compared to group 2 & 5 respectively.

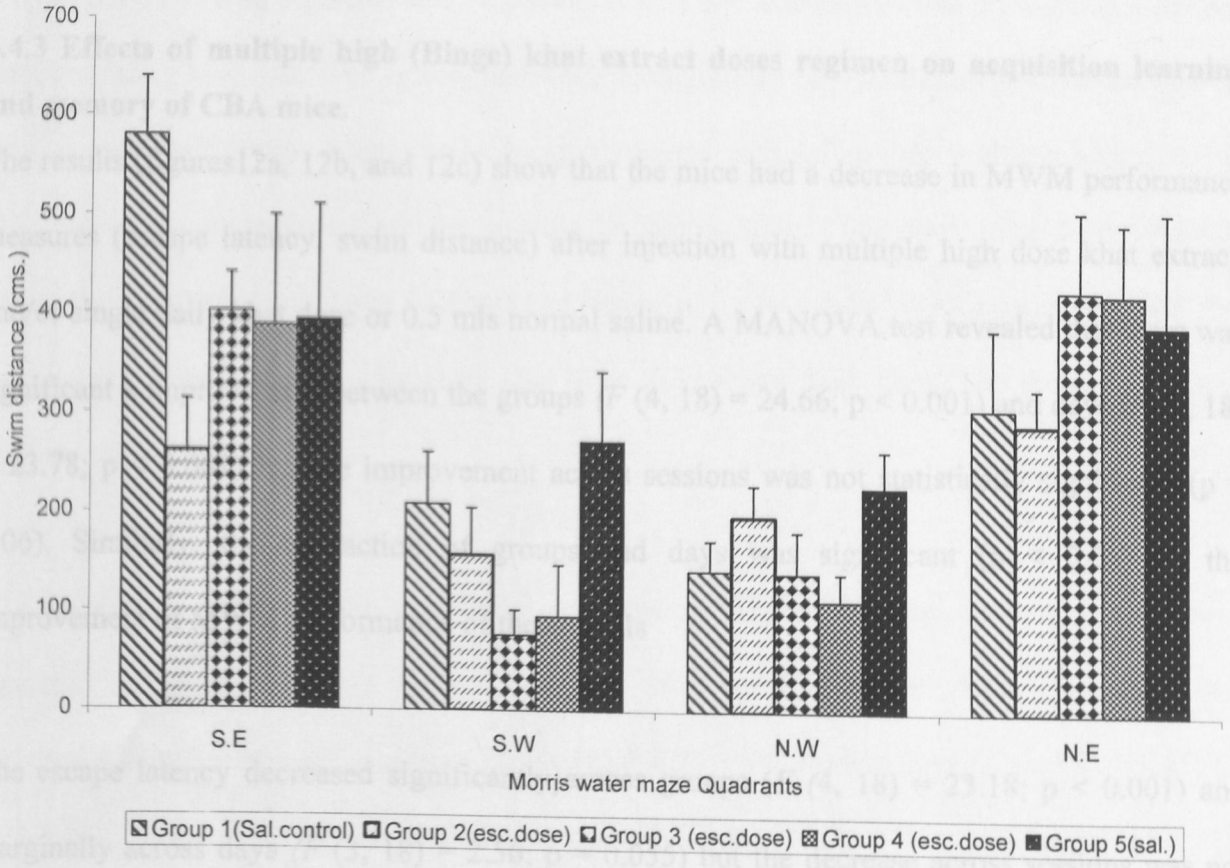


Figure 11 b. Effects of escalating khat extract dose on quadrant time of CBA mice during spatial memory assessment of MWM task acquisition. (n = 5 - 7 mice in each group). Data are presented as a mean ± s.e.m of quadrant swim distance (cm). Mice treated with escalating dose (group 2, & 3) and control had longer ($p < 0.05$) swim distances in target (S.E) quadrant than in S.W quadrant compared with other doses. Mice treated with escalating dose (group 3) and control had significant longer ($p < 0.05$) swim distance in target quadrant than in N.W quadrant compared to other groups. All mice whether treated with escalating dose or saline had no significant difference ($p > 0.05$) in swim distance observed in target quadrant compared to N.E quadrant.

4.4.3 Effects of multiple high (Binge) khat extract doses regimen on acquisition learning and memory of CBA mice.

The results (figures12a, 12b, and 12c) show that the mice had a decrease in MWM performance measures (escape latency, swim distance) after injection with multiple high dose khat extract, and/or single daily khat dose or 0.5 mls normal saline. A MANOVA test revealed that there was significant a improvement between the groups (F (4, 18) = 24.66; $p < 0.001$) and days (F (3, 18) = 23.78; $p < 0.001$) but the improvement across sessions was not statistically significant ($p = 0.06$). Similarly, the interaction of groups and days was significant ($p < 0.05$) on the improvement of MWM performance of the animals

The escape latency decreased significantly across groups (F (4, 18) = 23.18; $p < 0.001$) and marginally across days (F (3, 18) = 2.56; $p = 0.055$) but the decrease across sessions was not significant (F (3, 18) = 0.85; $p = 0.47$). The interaction of groups, days, and sessions was not significant (F (24, 18) = 0.42; $p = 0.99$) with regard to escape latency. A post hoc test revealed that mice group 4 (administered with escalating khat dose then saline) had significantly higher escape latency ($p < 0.001$) than the other groups. Similarly, mice groups 5 (treated with saline then multiple high khat dose) had significantly high ($p < 0.001$) escape latency than control and other groups; however, the escape latency was lower than that of group 4. The escape latency decreased across days, however, the changes was not significant and there was no specific day where a marked change was observed.

The swim distance decreased across groups (F (4, 18) =16.78; $p < 0.001$) and days (F (3, 18) =16.57; $p <0.001$), however, improvement across sessions was not significant (F (3, 18) = 0.39; $p < 0.76$). Furthermore, the interaction of group, day and session was not significant (F (24, 33)

= 0.66; p = 0.89) with regard to swim distance. A post hoc test showed that mice group 4 (treated with escalating khat dose then saline) had significantly longer ($p < 0.001$) swim distance than the control and other groups except mice group 5 (treated with saline then multiple high doses of khat) which when compared was not statistically significance . The mice group 5 (treated with saline then multiple high doses of khat) had significantly ($p < 0.05$) higher swim distance than mice group 3 (treated with escalating khat dose then single daily dose) and the control group (treated with 0.5 mls saline).

The decrease in swim distance was significant across days ($p < 0.05$), and, there was a marked decrease on the 2nd day (day 7) ($p < 0.001$) as compared to the other days. The swim speed decreased significantly across groups (F (4, 18) = 4.3; $p < 0.05$) and days (F (3, 18) = 5.61; $p < 0.05$) but the decrease across session was not significant (F (3, 18) = 1.78; p = 0.15). The interactions between group, day and session was not significant (F (24, 33) = 1.1; p = 0.34) with regard to the swim speed.

A post hoc test revealed that the swim speed of mice group 2 (treated with escalating khat dose then multiple high dose khat) and mice group 4 (treated with escalating khat dose then saline had significant ($p < 0.05$) reduced swim speed than the control group 1, however, compared to the other groups their swim speeds were lower, but the difference was not significant.

Furthermore, the decrease in swim speed across days was significant with noticeable decrease on second day (day 7) ($p < 0.05$). The decrease lasted to the last day, however, on these days the changes were not significant.

= 0.66; p = 0.89) with regard to swim distance. A post hoc test showed that mice group 4 (treated with escalating khat dose then saline) had significantly longer ($p < 0.001$) swim distance than the control and other groups except mice group 5 (treated with saline then multiple high doses of khat) which when compared was not statistically significance . The mice group 5 (treated with saline then multiple high doses of khat) had significantly ($p < 0.05$) higher swim distance than mice group 3 (treated with escalating khat dose then single daily dose) and the control group (treated with 0.5 mls saline).

The decrease in swim distance was significant across days ($p < 0.05$), and, there was a marked decrease on the 2nd day (day 7) ($p < 0.001$) as compared to the other days. The swim speed decreased significantly across groups (F (4, 18) = 4.3; $p < 0.05$) and days (F (3, 18) = 5.61; $p < 0.05$) but the decrease across session was not significant (F (3, 18) = 1.78; p = 0.15). The interactions between group, day and session was not significant (F (24, 33) = 1.1; p = 0.34) with regard to the swim speed.

A post hoc test revealed that the swim speed of mice group 2 (treated with escalating khat dose then multiple high dose khat) and mice group 4 (treated with escalating khat dose then saline had significant ($p < 0.05$) reduced swim speed than the control group 1, however, compared to the other groups their swim speeds were lower, but the difference was not significant.

Furthermore, the decrease in swim speed across days was significant with noticeable decrease on second day (day 7) ($p < 0.05$). The decrease lasted to the last day, however, on these days the changes were not significant.

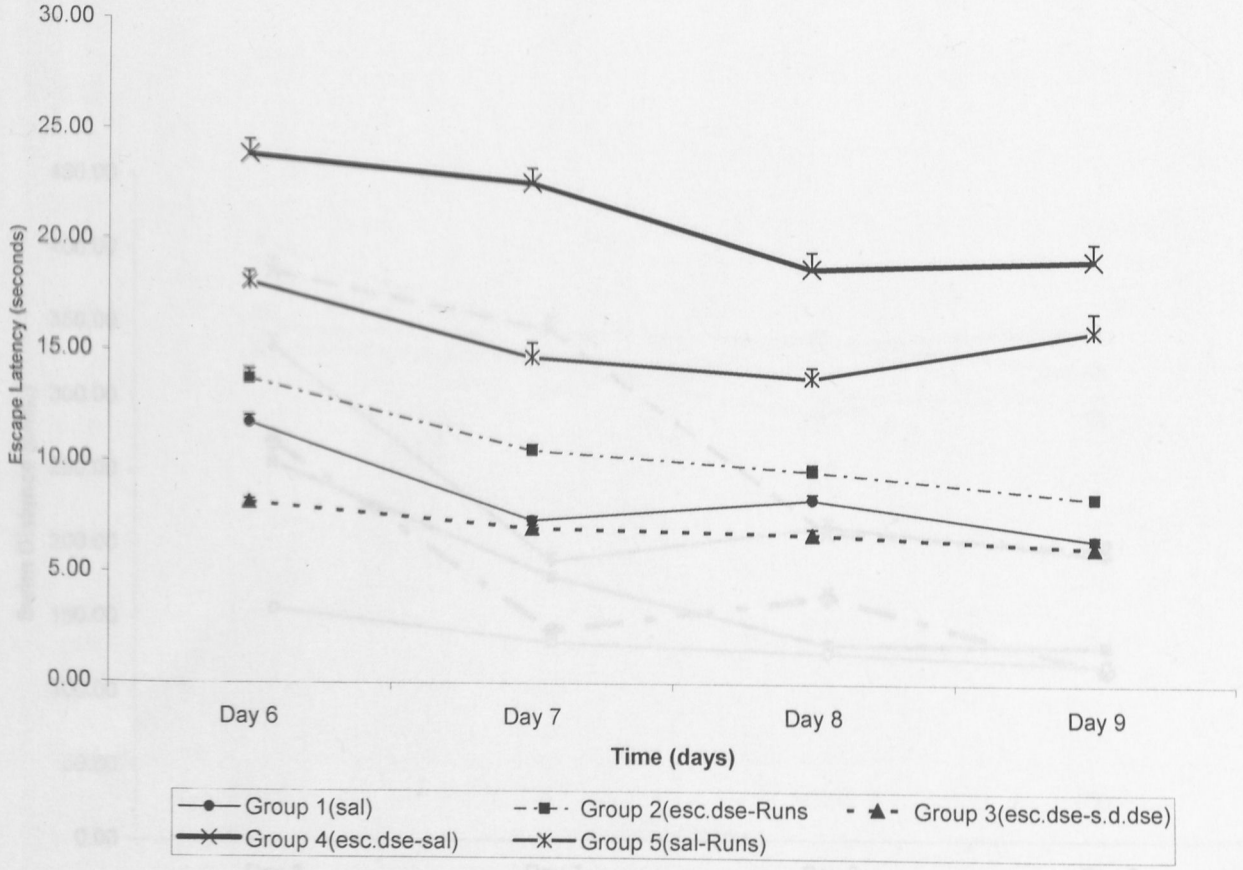


Figure 12 a. Effects of repeated high khat extract dose on escape latency of CBA mice during spatial learning and memory in MWM task. Mice were injected with (0.5 mls normal saline, and / or repeated high (binge) dose khat extract). (n =3 - 7 mice in each group). Data are presented as mean ± s.e.m of escape latency. Escape latency significantly improved ($p < 0.05$) over days. There was significant improvement ($p < 0.001$) on the 2nd day. Mice group 4 (injected with escalating khat dose then normal saline) had higher ($p < 0.001$) escape latency than the other groups. Mice group 5 (injected with normal saline then multiple high khat doses) had higher ($p < 0.05$) escape latency than the other groups.

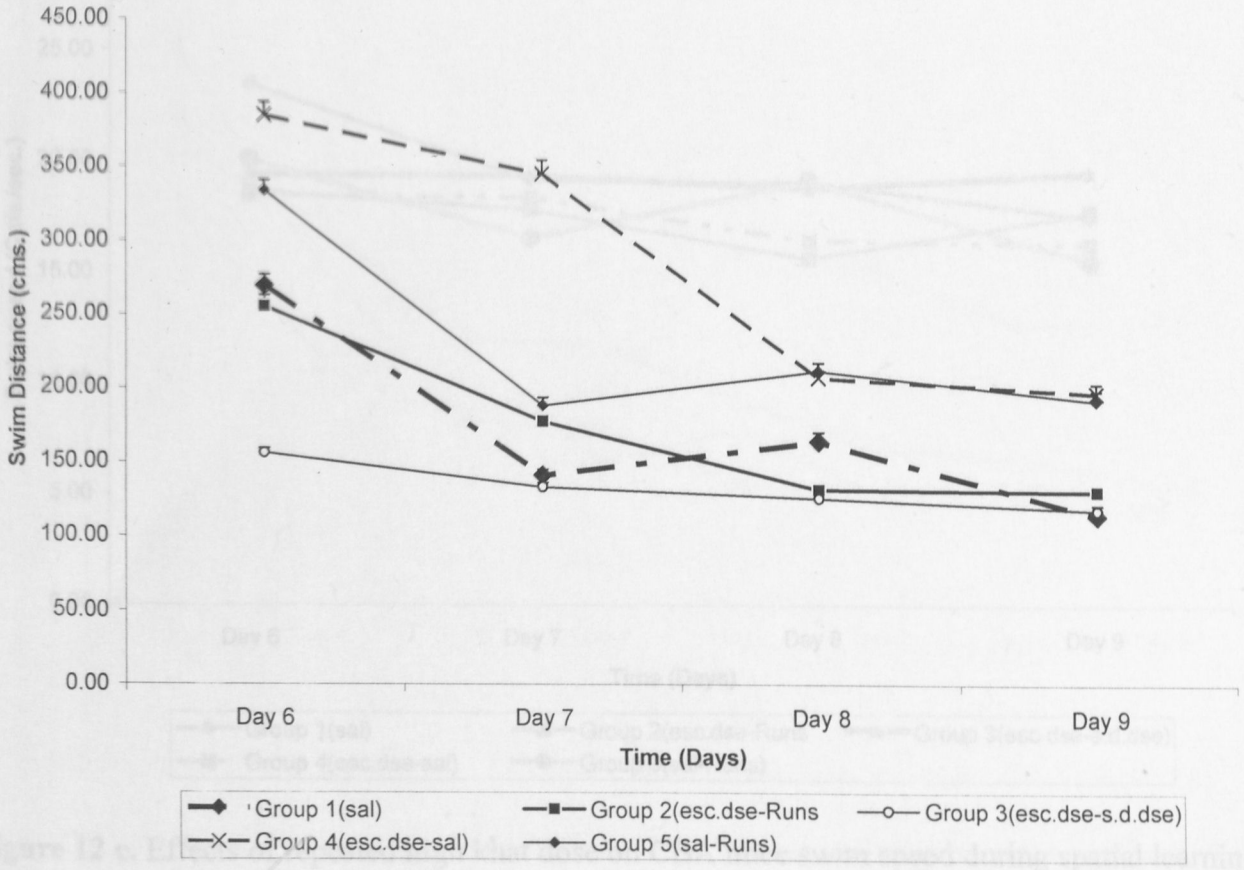


Figure 12 b. Effects of repeated high khat extract dose on CBA mice swim distance during spatial learning and memory in MWM task. Mice were injected with (0.5 mls normal saline, and / or multiple high (binge) dose (360) mg/kg b.wt khat extract). (n = 3 - 7 in each group). Data are presented as mean ± s.e.m of swim distance. Swim distance, significantly improved ($p < 0.05$) over days. All mice, had significant decrease ($p < 0.001$) in swim distance on the 2nd day. Mice group 4 showed longer ($p < 0.001$) swim distance than control and other groups. Mice group 5 swam longer ($p < 0.05$) than other groups and the control.

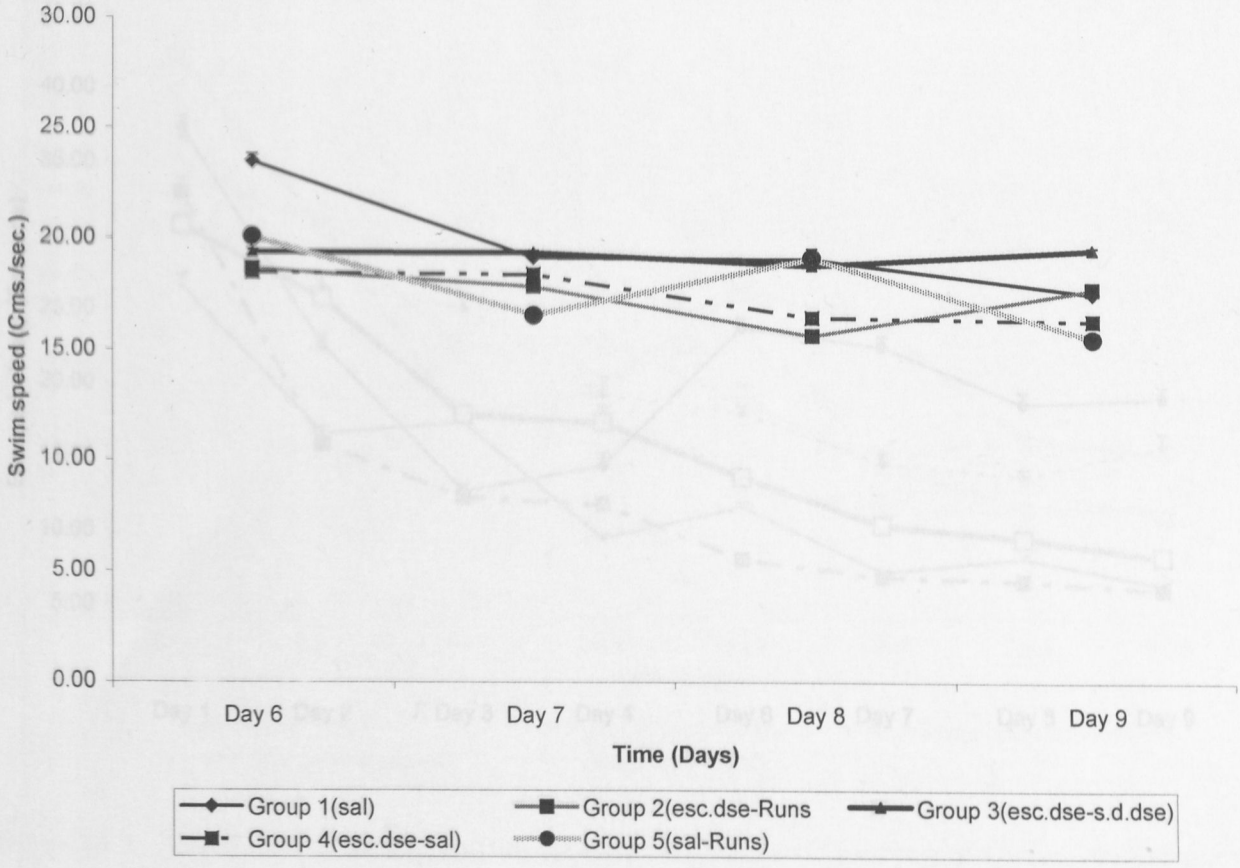


Figure 12 c. Effects of repeated high khat dose on CBA mice swim speed during spatial learning and memory in MWM task. Mice injected with (0.5 mls normal saline, and / or multiple high (360mg/kg b.wt) (binge) dose khat extract. (n =3 - 7 mice in each group). Data are presented as mean ± s.e.m of swim speed. Swim speed significantly improved ($p < 0.05$) over days and it was strongly significant ($p < 0.001$) on the 2nd day. Mice group 2 (injected with escalating khat dose then multiple high khat doses) and mice group 4 (injected with escalating khat dose then normal saline) had slower ($p < 0.05$) swim speed than the other groups.

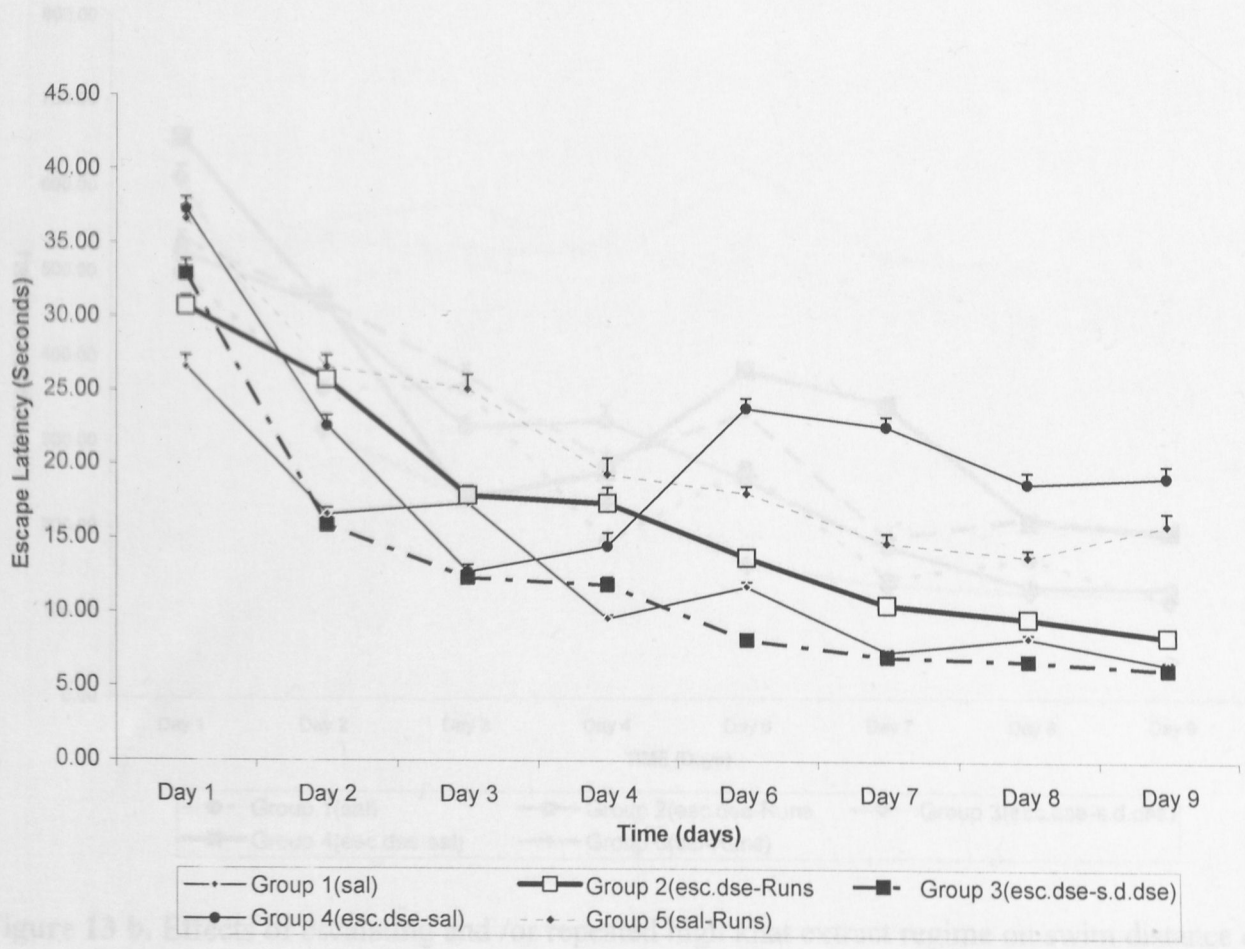


Figure 13 a. Effects of escalating and /or repeated high khat extract regime on escape latency of CBA mice during spatial learning and memory in MWM task. (n = 5 - 7 in each group). Data are presented as a mean ± s.e.m of escape latency. Escape latency of mice treated with escalating dose then 0.5 mls saline (group 4) and mice treated with saline then repeated high khat dose (group 5) was significantly longer ($p < 0.001$) than the other groups and control.

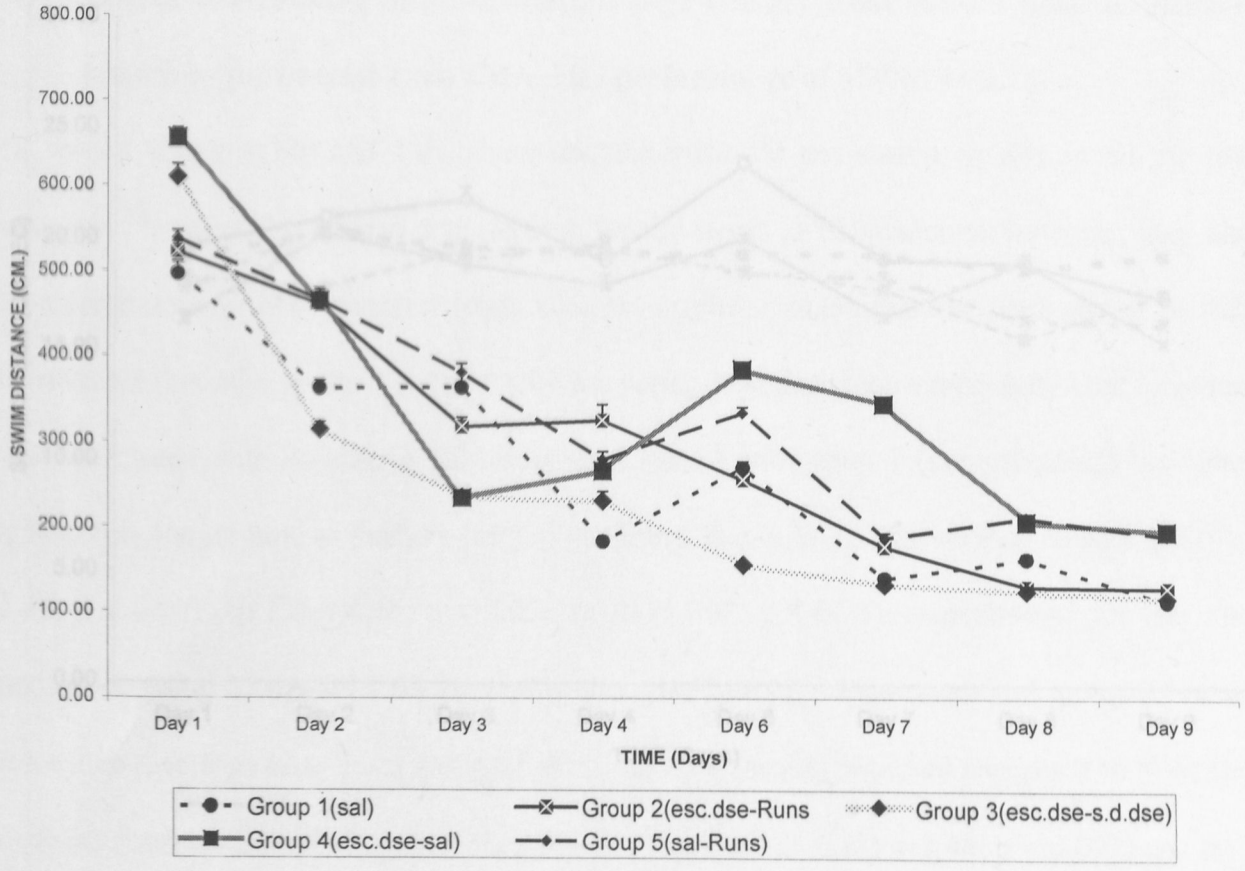


Figure 13 b. Effects of escalating and /or repeated high khat extract regime on swim distance of CBA mice during spatial learning and memory in MWM task. (n = 5 - 7 in each group). Data are presented as a mean ± s.e.m of swim distance. Swim distance of mice treated with escalating dose then 0.5 mls saline (group 4) and mice treated with saline then repeated high khat dose (group 5) was longer ($p < 0.001$) than the other groups and control

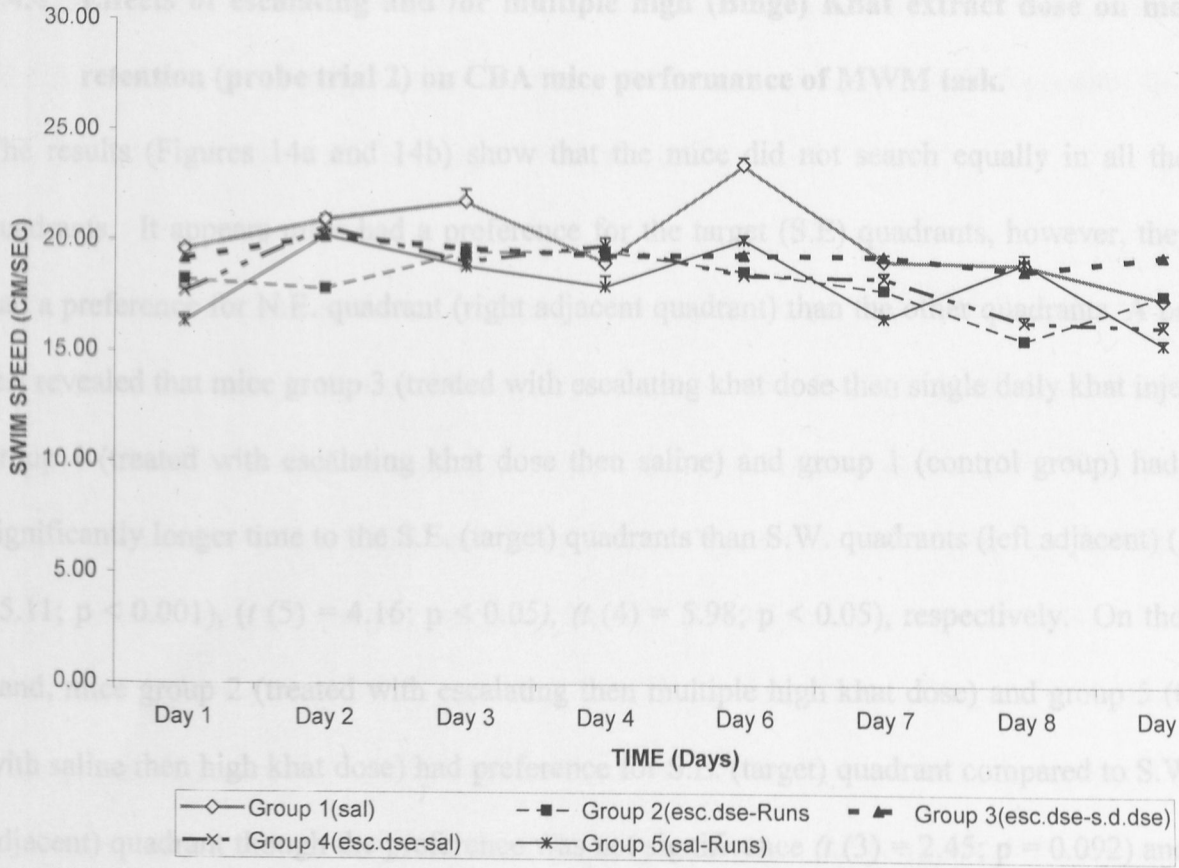


Figure 13 c. Effects of escalating and /or repeated high khat extract regime on swim speed of CBA mice during spatial learning and memory in MWM task.. (n = 5 - 7 in each group). Data are presented as a mean ± s.e.m of swim speed. Swim speed of mice treated with escalating dose then repeated high khat dose (group 2) and mice treated with escalating then single high daily khat dose (group 3) and mice treated with 0.5 mls saline then repeated high khat dose showed reduced ($p < 0.05$) swim speed than the other groups and the control.

4.4.4 Effects of escalating and /or multiple high (Binge) Khat extract dose on memory retention (probe trial 2) on CBA mice performance of MWM task.

The results (Figures 14a and 14b) show that the mice did not search equally in all the four quadrants. It appears mice had a preference for the target (S.E) quadrants, however, they also had a preference for N.E. quadrant (right adjacent quadrant) than the other quadrants. A paired t test revealed that mice group 3 (treated with escalating khat dose then single daily khat injection) group 4 (treated with escalating khat dose then saline) and group 1 (control group) had spent significantly longer time to the S.E. (target) quadrants than S.W. quadrants (left adjacent) (t (4) = 15.11; $p < 0.001$), (t (5) = 4.16; $p < 0.05$), (t (4) = 5.98; $p < 0.05$), respectively. On the other hand, mice group 2 (treated with escalating then multiple high khat dose) and group 5 (treated with saline then high khat dose) had preference for S.E. (target) quadrant compared to S.W. (left adjacent) quadrant though the preference was not significance (t (3) = 2.45; $p = 0.092$) and (t (1) = 0.43; $p = 0.74$), respectively.

Further t test revealed that mice group 3 (treated with escalating then single daily khat dose), group 4 (treated with escalating then saline) and the control group 1 had significantly longer time in the S.E. (target) quadrant than in N.W. (opposite) quadrant at (t (4) = 10.89; $p < 0.0001$) and (t (5) = 5.88; $p < 0.05$) and (t (4) = 7.03; $p < 0.05$), respectively. Similarly, mice group 5 (treated with saline then multiple high khat dose) spent significantly (t (1) = 14.38; $p < 0.05$) higher time in the S.E. (target) quadrant than in the N.W. (opposite) quadrants. All mice groups (2,3,4,5) treated at same point with khat extract had a bias for N.E. (right adjacent) quadrant as opposed to the control group which had preference for target quadrant compared to the other quadrants (23.78 ± 7.7 vs. 24.13 ± 6.53), (28.70 ± 2.11 vs. 22.18 ± 3.46), (26.40 ± 2.68 vs. 17.17 ± 3.92) and (24.75 ± 0.35 vs. 1 5.60 ± 15.60) compared to the control group at (33.88 ± 3.59 vs. 13.74 ± 3.8) at (t (4) = 2.89; $p < 0.05$). On the other hand, mice group 5 (treated with saline then

multiple high khat doses) showed bias for SW (left adjacent) quadrant (24.75 ± 0.35 vs. 17.65 ± 16.45) similar to N.E. (right adjacent) quadrant but biased against NW (opposite) quadrant (24.75 ± 0.35 vs. 1.75 ± 1.25) at (t (1) = 14.38; $p < 0.05$).

A further t test revealed that mice searched differently in the quadrant based on the measure of swim distances with preference for target quadrant and bias for N E (right adjacent quadrant) (Fig. 12 b). Mice group 3 (treated with escalating then daily khat doses), group 4 (treated with escalating khat doses then saline) and group 1 (control group) had allocated significantly longer swim distance in the S E (target) quadrants then SW (left adjacent) quadrant at (t (4) = 13.23; $p < 0.001$) and (t (5) = 4.00; $p < 0.05$) and (t (4) = 6.33; $p < 0.05$), respectively. On the other hand, mice group 2 (treated with escalating then multiple high khat dose) and mice group 5 (treated with saline then multiple high khat dose) had preference for S.E (target) quadrant compared to S.W (left adjacent) quadrant, however, the preference was not significant (t (3) = 2.1; $p = 0.982$) and (t (1) = 0.822; $p = 0.56$), respectively.

Mice group 3 (treated with escalating then single daily dose), group 4 (treated with escalating then saline) and the control group 1 had significantly longer distance in the SE (target) quadrant than in NW (opposite) quadrants at (t (4) = 19.59; $p < 0.001$) and (t (5) = 4.47; $p < 0.05$) and (t (4) = 9.48; $p < 0.05$), respectively. Similarly, mice group 2 (treated with escalating then multiple high khat doses) and mice group 5 (treated with saline then multiple high khat doses) had longer swim distances in the S.E (target) quadrant than the N.W (opposite) quadrant, however, the values were not significant (272.63 ± 93.88 vs. 100.33 ± 36.28) and (200.6 ± 115 vs. 10.0 ± 7.00) at (t (3) = 1.34; $p = 0.274$) and (t (1) = 1.56; $p = 0.364$), respectively.

Mice group 3 and control group had marginally longer distance in the S.E (target) quadrant compared to the N.E (right adjacent) quadrant at (t (4) = 2.71; p = 0.54) and (t (4) = 3.57; p < 0.05), respectively, however, compared to other quadrants they showed biased for this quadrant. Mice group 5 (treated with saline then run doses) showed bias for the N.E (right adjacent) quadrant with regard to swim distance than the other groups at (t (1) = 16.95; p < 0.0001). Mice group 2 (treated with escalating then multiple high khat doses) showed a bias for the N.E (right adjacent) quadrant by having longer swim distance than the target quadrant at (282.47 ± 42.34 vs. 272.63 ± 93.88), respectively.

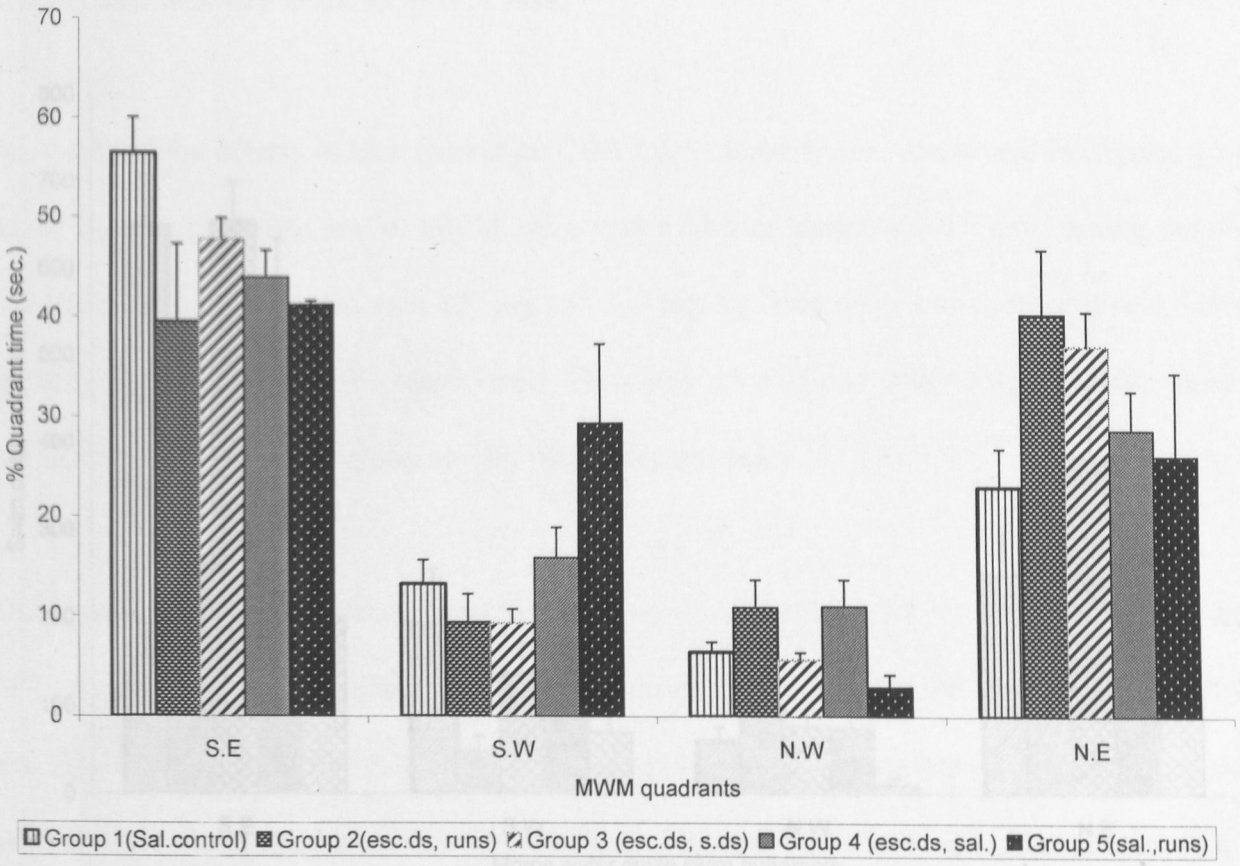


Figure 14 a. Effects of escalating and /or repeated high khat extract dose regime on CBA mice quadrant time during spatial memory assessment of MWM task**.** Mice were injected with (escalating –run, escalating-single daily dose, escalating-saline run, saline-runs or saline-saline regimen). (n = 4-7 mice in each group). Data are presented as mean ± s.e.m of % quadrant time. Mice treated with escalating then single daily dose (group 3), escalating dose then saline (group 4) spent longer ($p < 0.05$) time in the target quadrant than in S.W quadrant compared with other group. All mice except escalating –run treated mice spent longer ($p < 0.05$) time in target quadrant than in N.W quadrant. All mice treated with the different regime of khat dose had no significant ($p > 0.05$) differences in time spent in target quadrant as compared with N.E quadrant as opposed to the controls.

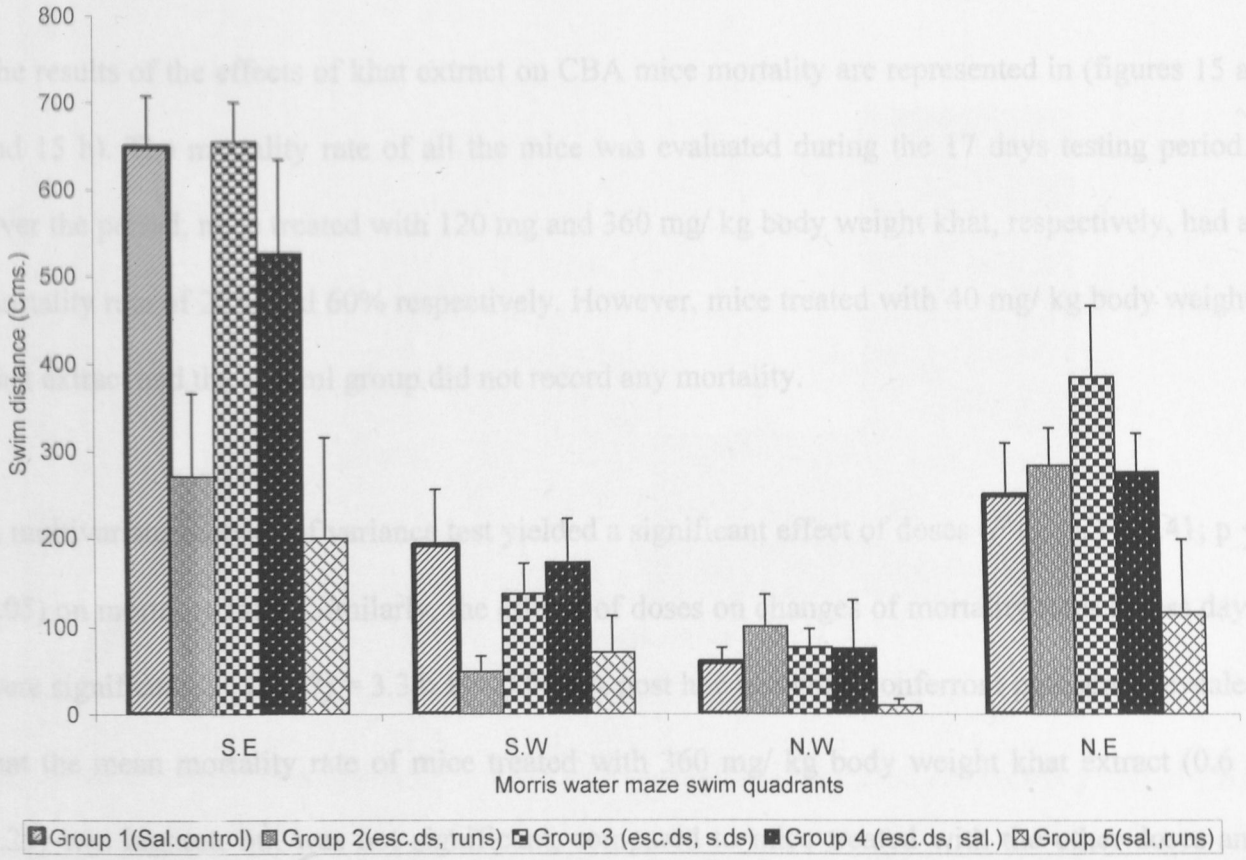


Figure 14 b. Effects of escalating and /or repeated high khat extract dose regime on CBA mice quadrant swim distance during spatial memory assessment in MWM task. Mice were injected with (escalating –run, escalating-single daily dose, escalating-saline run, saline-runs or saline-saline regimen). (n = 4 - 7 mice in each group). Data are presented as mean ± s.e.m of (a) quadrant swim distance. Mice treated with escalating then single daily dose (group 3), escalating then saline (group 4), and control had longer swim distance ($p < 0.05$) in the target quadrant than in S.W quadrant. Mice treated with saline then run doses (group 5), saline then saline (group 1), had shorter swim distance in N.E quadrant than in target quadrant compared to the groups.

4.5 Effects of single daily khat extract dose administration on CBA mice mortality during learning and memory trials of MWM task.

The results of the effects of khat extract on CBA mice mortality are represented in (figures 15 a and 15 b). The mortality rate of all the mice was evaluated during the 17 days testing period. Over the period, mice treated with 120 mg and 360 mg/ kg body weight khat, respectively, had a mortality rate of 20% and 60% respectively. However, mice treated with 40 mg/ kg body weight khat extract and the control group did not record any mortality.

A multivariate analysis of variance test yielded a significant effect of doses (F (3, 17) = 3.41; $p < 0.05$) on mortality rates. Similarly, the effects of doses on changes of mortality rates across days were significant (F, (3, 17) = 3.34; $P < 0.05$). A post hoc test with Bonferroni procedure revealed that the mean mortality rate of mice treated with 360 mg/ kg body weight khat extract (0.6 ± 0.25) was highest, but was not significant, compared to mice treated with the other doses and control. In addition, the mean survival days of mice treated with 360 mg/ kg body weight khat extract (15.2 ± 0.92) was lowest, than mice treated with 40mg, 120 mg/kg body weight khat extract and control respectively at (17 ± 0) , (16.2 ± 0.8) and (17.0 ± 0). The survival rates by days were 100% for mice treated with 40mg/ kg body weight khat extract and the control group. In all the animals that died, there was no post mortem done to determine the cause of death.

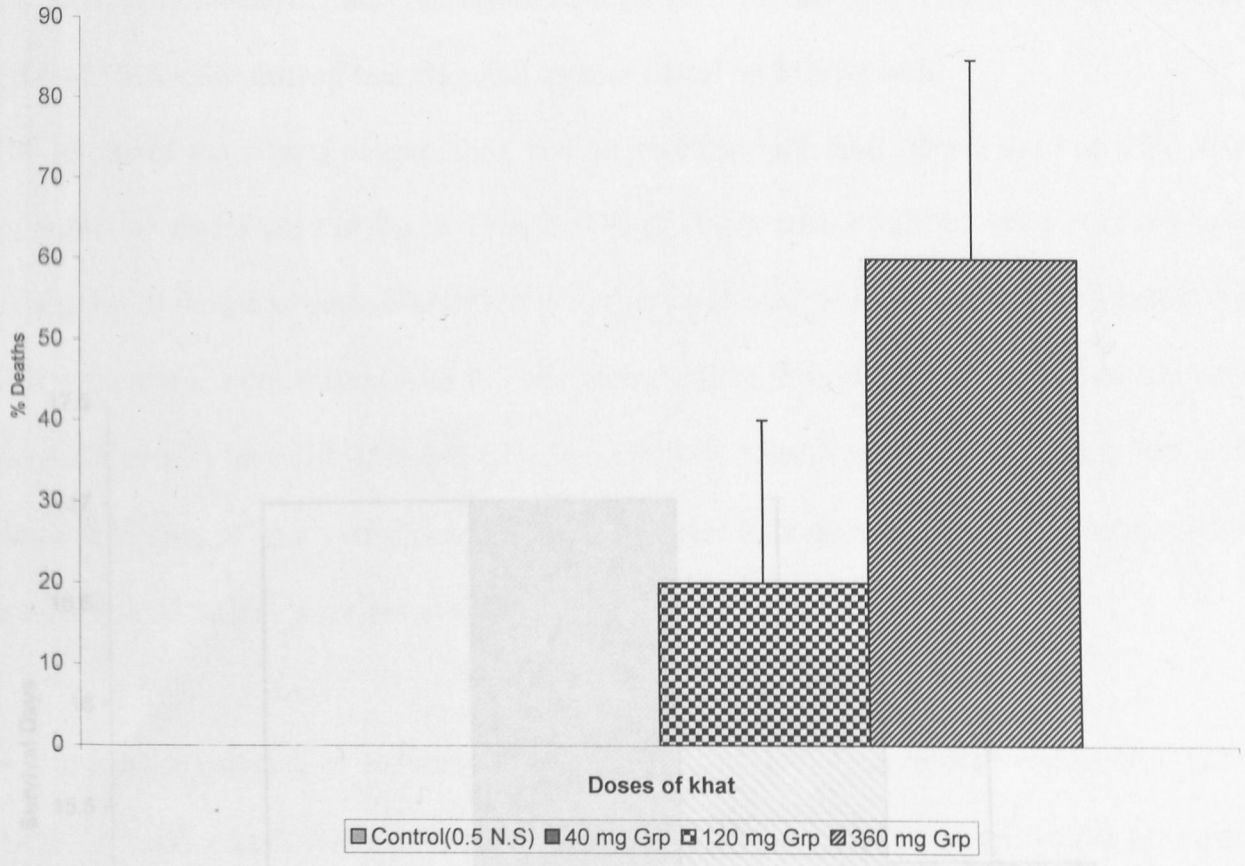


Figure 15 a. Effects of single daily dose of khat extract administration on mortality rate of CBA mice during learning and memory trial in MWM task. (n = 5 mice in each group). Data are presented as mean ± s.e.m. of deaths. Mice treated with 120 and 360 mg/kg b.wt khat extract showed mortality rate of 20 and 60%, respectively, compared to the other doses and control which did not record any mortality.

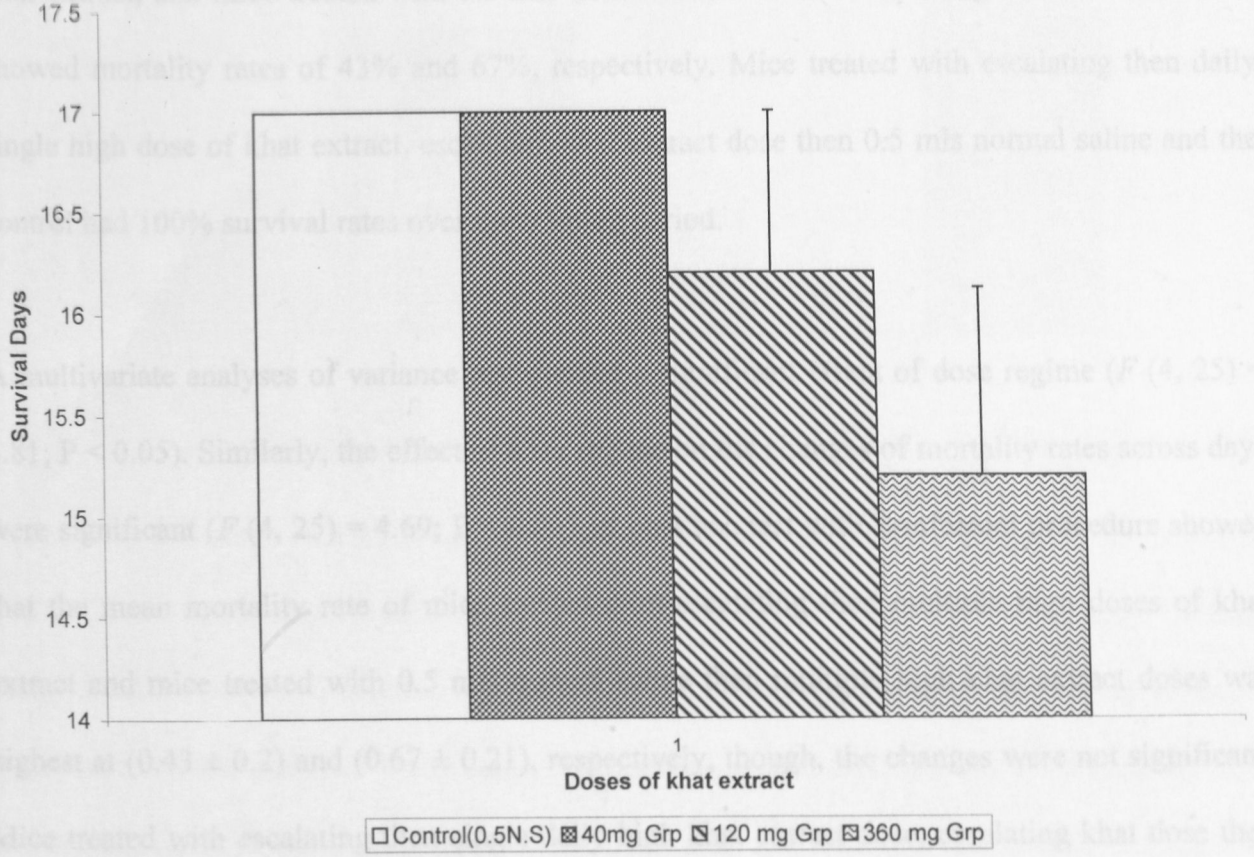


Figure 15 b. Effects of single daily dose of khat extract administration on survival rate of CBA mice during learning and memory trial in MWM task. (n = 5 mice in each group). Data are presented as mean ± s.e.m. of survival days. Mice treated with 360 mg/kg b.wt khat extract showed lowest survival rate by days (15.2 ± 0.92) as compared to the other days.

4.6. Effects of escalating and /or repeated high khat extract dose regime on the mortality rates of CBA mice during learning and memory trial on MWM task.

The results of the effects of escalating and /or repeated high khat extract dose on CBA mice mortality are represented in figure 15 a, b. The mortality rates of all the mice were evaluated during the 10 days trial period. Over the period, mice treated with escalating then repeated high khat extract, and mice treated with 0.5 mls normal saline then multiple high khat extract doses showed mortality rates of 43% and 67%, respectively. Mice treated with escalating then daily single high dose of khat extract, escalating khat extract dose then 0.5 mls normal saline and the control had 100% survival rates over the training period.

A multivariate analyses of variance test yielded a significant effect of dose regime (F (4, 25) = 4.81; $P < 0.05$). Similarly, the effect of dose regime on the changes of mortality rates across days were significant (F (4, 25) = 4.69; $P < 0.05$). A post hoc test with Bonferroni procedure showed that the mean mortality rate of mice treated with escalating then multiple high doses of khat extract and mice treated with 0.5 mls normal saline then multiple high khat extract doses was highest at (0.43 ± 0.2) and (0.67 ± 0.21), respectively, though, the changes were not significant. Mice treated with escalating then single daily high khat extract dose, escalating khat dose then 0.5 mls normal saline, and the control, did not show any mortalities. The mean survival days of mice treated with escalating then multiple high khat extract doses, normal saline then multiple high khat extract dose regime, had lowest survival days at (9.29 ± 0.42), and (9.50 ± 0.22) compared to the other dose regime and the control group at (10.0 ± 0.0) each.. Indeed, all the other dose regime treated mice achieved a 100% survival rate over the testing period.

Figure 16 a. Effects of escalating and /or repeated high khat extract dose regime on the mortality rate of CBA mice during learning and memory trial in MWM task. (n = 3 - 7 mice in each group). Data are presented as mean ± s.e.m. of deaths. Mice treated with escalating then repeated high khat extract showed mortality rate of 43%. Mice treated with 0.5 mls normal saline then repeated high khat extract regime showed 67% mortality rate compared to the other regime and control which showed no mortality rates.

Figure 16 b. Effects of escalating and /or repeated high khat extract dose regime on the survival rate of CBA mice during learning and memory trial in MWM task. (n = 3 - 7 mice in each group). Data are presented as mean ± s.e.m. of survival days. Mice treated with escalating then repeated high khat extract and. mice treated with 0.5 mls normal saline then repeated high khat extract showed the lowest survival rate by days (9.29 ± 0.42) and (9.50 ± 0.22), respectively, compared to the other regime and control which showed no mortality rates at all.

CHAPTER FIVE

5.0 DISCUSSION

The results of this study indicate that acute intraperitoneal administration of khat enhanced locomotor activity in a dose and time dependent manner with the highest line crossing at 360 mg/kg body weight of khat (moderate khat dose). The khat used in this study show an inverted U-shaped dose response curve, consistent with an inverted U-shaped response observed with amphetamines (Connor *et al.,* 2002). The low dose khat extract inhibited locomotor activity and moderate dose increased locomotor activity in mice, whereas, very high doses were locomotor inhibitory.

The findings, though in agreement with effects of amphetamine, did show some differences especially on centre square and rearing frequency, both of which are measures of locomotor activity. Whereas, the line crossing showed an inverted U – shaped response, the rearing frequency and centre square frequency were abolished in a dose dependent manner. Thus, it seems probably that the effects of whole khat extract with its many constituents, would differ from amphetamine with regard to the production of motor and probably other behaviours. The dose range adopted for this experiment were wide (3n progression), with the highest dose being 54 times the lowest, this was to cover full dose response range, however, some inter dose responses could have been missed hence the introduction of intermediate dose like 360mg.

The effects of khat extract exhibited were consistent and time dependent, indeed, the maximum effects were observed up to 35 minutes, which is consistent with the fact that the maximum effect of cathinone is within 5 - 30 minutes upon intraperitoneal administration in rats (Schechter, 1989). Psycho stimulants have shorter half life, and are more quickly metabolized in

rodents quicker than in humans (Melega *et al.,* 1995, Srinivas *et al.,* 1992; Wargin *et al.*, 1983). Furthermore, peak plasma concentrations occur at about 10 minutes following subcutaneous injection of cathinone in rats, compared to 15 to 30 minutes following oral administration (Cho *et al.,* 1999; Wargin *et al.,* 1983).

The locomotor activities are modulated by neurotransmitter dopamine and serotonin. Cathinone the active compound of khat is associated directly or indirectly with dopamine or serotonin release, by its action on dopamine or serotonin transporters function (Banjaw *et al.*, 2003). In addition, cathinone is regarded as a dopamine releaser and acts through D_1 type dopamine receptors in mediating its reinforcing effects (Kalix, 1990). It is also documented that cathinone and its close analog amphetamine, increase the efflux of [^{3} H] dopamine from slices of rat striatum (Zelger and Carlini, 1983). It have also been demonstrated that the motor activities induced by S-(-) cathinone in experimental animals are associated with dopamine release (Glennon and Schowalter, 1981; Valterio and Kalix, 1982; Calcagnetti and Schechter, 1992). On the other hand, head twitches in mice and head shakes in rats are quantifiable motor behaviours closely linked to brain serotonin mechanisms. It has also been documented that S-(-) cathinone could also release serotonin (Nielsen, 1985; Fleckenstein *et al.,* 199). The locomotor behaviours like head twitches were not evaluated in this study.

Since acute and chronic treatment of animals with psychostimulnats can affect locomotor behaviour, this factor had to be ruled out before evaluating the results of Morris water maze task. This was important to avoid bias in the interpretation of behaviours alterations presented by drug treated CBA mice. In this study reduced swim speed due to khat would have been misinterpreted as poor learning and vice versa. The results obtained in this study were varied, that neither of the khat doses inhibited all the locomotor parameter nor enhanced all of them, a finding that helped

to set the doses (40 mg, 120 mg and 360 mg) as appropriate for the subsequent Morris water maze experiments.

The present study represented an attempt to evaluate the effects of khat extract administration on acquisition spatial learning and memory of CBA mice on the Morris Water maze task followed by reversal learning. The schedule of single daily pre training administration of khat was effective in impairing learning and memory in a dose related way.

The study demonstrated selective impaired learning but improved memory at dose 120 mg/kg body weight as compared to both impaired learning, memory and reduced swim speed at dose 360 mg/kg body weight dose. However, at low dose (40 mg/kg body weight) there was increased enhancement of spatial learning and memory than at 120 and 360 mg/kg b.wt but similar to the control

The finding of decreased swim speed in Morris water maze at dose 360 mg contradicted the open field test in the previous study in which mice injected with 360 mg had increased line crossing. Nevertheless, the results confirm that the improved spatial learning and memory in MWM is not due to locomotor alterations. The results that lower doses of khat extract substantially improved spatial learning and memory are consistent with De Bruin et. al (2003) findings in which, they concluded that caffeine can reverse attention deficit in spontaneous hypertensive rats and facilitate their spatial learning. The findings are also consistent with, the findings that acute amphetamine administration increases memory consolidation and increases the impact of reinforcement in some learning paradigms (Mc Gaugh, 1989; Killcross *et al.*, 1994).

The results of reversal learning experiment in which the hidden platform was moved to the opposite quadrant, demonstrated learning and memory impairment at higher khat extract

administration (dose 360mg).The escape latency, swim distance and velocity were substantially affected depicting poor reversal spatial learning and memory. Further more on the probe trial test the mice treated with higher dose khat did not discriminate between the former target quadrant and the current quadrant. This was a demonstration that higher dose khat extract causes preserverative behavior. Perserverative behaviour is a deficit in switching behaviour from one mode of responding to another, it expresses disturbance of executive function (Devain *et al.,* 1996; Kirkby, 1969) and it is associated with lesions in the striatum and basal ganglia. It is also interpreted as an inability to inhibit on going action or as a failure to initiate next response (Devain *et al.,* 1996).

The results of this study demonstrated that the locomotor behaviour of CBA mice after, sub chronic intraperitoneal administration of khat and submission to MWM was affected in dose related manner. The khat dosage range used in the experiment was narrow (40 to 120 mg) thus, it was difficult to conclude which pattern of dose response could be adopted by mice injected with 360mg/kg b.wt the results because of high mortality. Nevertheless, the results of this experiment appeared to agree with the previous study on locomotor behaviour, in which low doses of khat extract inhibited locomotor behaviour and high doses increased the behaviour with and higher doses inhibited the locomotor activity. The other results were also consistent in that the centre square frequency and rearing frequency were enhanced at lower khat extract doses and abolished as the dose was increased, while the line crosses increased with high doses and vice versa.

The effects of khat on post Morris Water maze CBA mice locomotor behavior though dose dependent, did not show any time relationship. This was contrary to the observations made on acute administration of khat on locomotor. This could probably be accounted for by habituation, a decrease in response following the same amount of stimulation. Nevertheless, the results were

important because they confirmed that the effects of khat extract on CBA mice in MWM was not due to changes of the locomotor behaviour but neural behavioural alterations. The range of drug response was narrowly tested because at dose 360 mg/ kg b.wt the mice mortalities were high. However, behavioural alterations can also be demonstrated in animals in the absence of any specific challenge, including reduced motivation as well as specific cognitive impairments (Barr and Philips, 1999, Lin *et al.,* 1999; Lin *et al.,* 2002).There is evidence to suggest that changes observed are linked to altered dopaminergic transmission in the prefrontal cortex and /or striatum (Robinson and Becker, 1986; Pezze *et al*., 2002). Such neurochemical change represents a form of neuro-adaptive changes developed during repeated psychostimulants exposure.

The brain structures demonstrated to undergo adaptive changes developed during repeated amphetamine administration, the striatal complex, prefrontal cortices and limbic arrears, have all been implicated in various forms of learning and memory (Gold man- Rakic, 1987; Baddeley 1992; Dias *et al.,* 1996; Nestler and Aghayanian, 1997; Robinson and Kolb, 1997; Berke and Hyman, 2000).

The results of effects of escalating run dose regime on acquisition learning and memory demonstrated selective impairment on learning and memory. Escalating run dose treated mice demonstrated reduced escape latency and swim distance an indication of improved learning but had impaired memory. In addition, the animals treated with escalating khat dose showed decreased velocity, an indicator that their locomotor activity could be impaired. However, mice treated with escalating dose followed by saline demonstrated impaired learning. On the other hand, mice subjected to escalating dose followed by single daily injections of khat had improved learning and memory all through the MWM test. Moreover, mice injected with escalating khat dose followed by saline demonstrated impaired learning but improved memory on second probe

trial. The same case applied to mice subjected to normal saline followed by escalating dose, that though they had impaired learning, their memory improved depicting selective impairment effects of khat on learning and sparing memory

The results demonstrated selective impairment of learning in mice treated with escalating dose followed by saline. This is probably due to withdrawal effects of khat impairing learning but sparing memory. The findings are consistent with a study that reported that acute amphetamine increased memory consolidation and enhanced the impact of reinforcement in some learning paradigms (Mc Gaugh, 1989; Killcross *et al.*, 1994). However, the results also contradicted findings by Russig who reported that escalating dose amphetamine withdrawal have little effect upon the acquisition of the MWM task by Wister rats (Russig *et al.*, 2003

The variance in the finding can probably be accounted for, by the difference in the test procedures. The results further demonstrated impairment of learning and memory in mice treated with saline followed with high dose khat regimen. This was probably because of high levels of brain concentrations of khat compounds causing neurotoxicity. This argument can be supported by study of Segal and Kuczenski (1997), in which mice were injected with high doses amphetamine in repeated runs in absence of previous dose treatment, and this resulted in debilitation or death of most animals (Segal and Kuczenski, 1997). Indeed, in this study majority of animals died (60%) by day 9 in the group treated with saline followed by repeated run doses. However, mice treated with escalating khat doses followed by repeated runs had improved learning and memory (probe test 1) at day 5. In addition, they had improved learning in the acquisition phase that followed, however, their memory following repeated runs was impaired (probe test 2) at day 10. The mixed findings appear to be because of tolerance and sensitization

of locomotor component of the response, are consistent with findings by Segal and Kuczenski (1997).

The results of impaired memory demonstrated during probe trial could also be attributed to neurotoxicity of the repeated high doses of khat extract. All mice treated with escalating dose followed by single daily doses of khat demonstrated improvement of both learning and memory. Again, these findings can be attributed to admixture of tolerance, particularly in the acquisition phase and sensitization of the locomotor component of the response (Segal and Kuczenski, 1997).

In summary, the study demonstrates that single high dose khat extract causes increased locomotor behavior in Swiss mice. Similarly, repeated high dose khat extract cause increased locomotor and behavioral sensitization. However, the two khat extract administration regime affected measures of locomotor behavior differentially. The study further showed that khat extract effects on learning and memory are diverge depending on the dose; khat extract at lower dose improves learning and memory whereas at high doses it is selective in either impairing learning or memory or both. The extract administered at higher single daily doses impairs learning and memory and causes perseverative behaviors. Escalating then repeated high dose (run) khat extract regime improves learning but impairs reference memory, whereas, escalating then single daily high dose khat extract regime improved both learning and reference memory. The study showed that khat extract has differential effects on measures of both locomotor behavior and learning and memory, possibly because of its many ingredients, and differences in administration regime and mice species used.

The study calls for further experiments, to establish the effect of khat on spatial learning and memory using rats, other paradigms of learning and memory using mice and rats. Studies should be conducted to investigate the mechanism of effect of khat on learning and memory.

Appendix 1

Morris Water Maze Launch Schedule form

DAY 1

Mouse Group	Location 1	Location 2	Location 3	Location 4
1	N	S	E	W
1	E	W	S	N
1	W	E	N	S
1	S	N	W	E
1	N	W	S	E
2	W	S	E	N
2	S	N	W	E
2	S	E	N	W
2	E	N	W	S
2	N	E	W	S
3	S	N	E	W
3	E	W	S	N
3	W	S	N	E
3	N	S	E	W
3	E	W	S	N
4	W	E	N	S
4	S	N	W	E
4	N	W	S	E
4	W	S	E	N
4	S	E	N	W

Appendix 2

Moris Water Maze Acquisition data form

Mouse No. ..... **Date Started** .....

Experimenter .....

Acquisition with invisible platform

Day	Trial	Launch	Latency

Mouse No. ..... **Date Started** .....

Experiment .....

Acquisition with invisible platform

Day	Trial	Launch	Latency

Appendix 3

Morris Water Maze probe trial data form

Mouse No. …….. **Date Started** ……

Experiment ……..

Probe Trial with no platform

Day	Trial	Launch	Latency

Mouse No. …….. **Date Started** ……

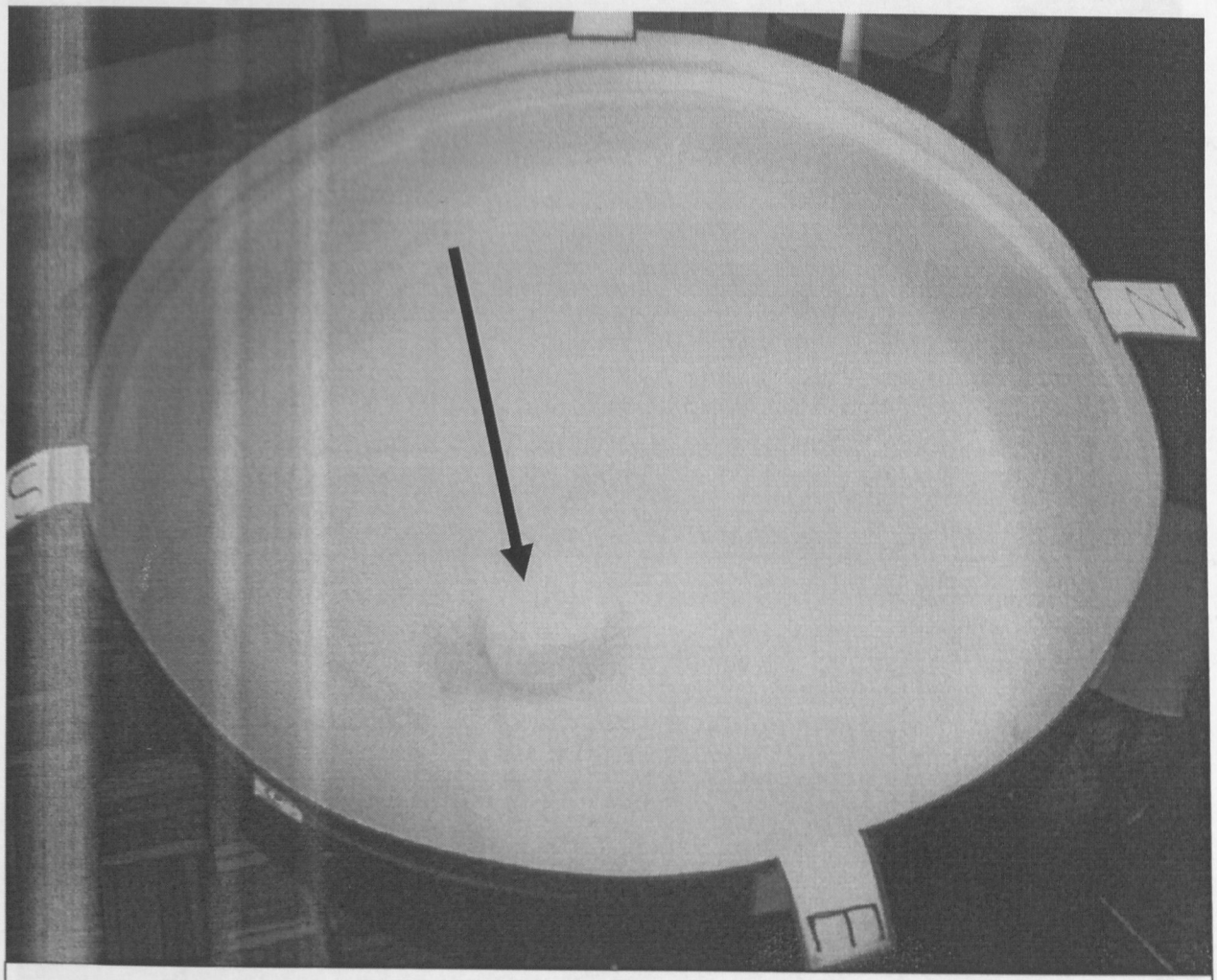
Experiment ……..

Probe Trial with no platform

Day	Trial	Launch	Latency

Appendix 4

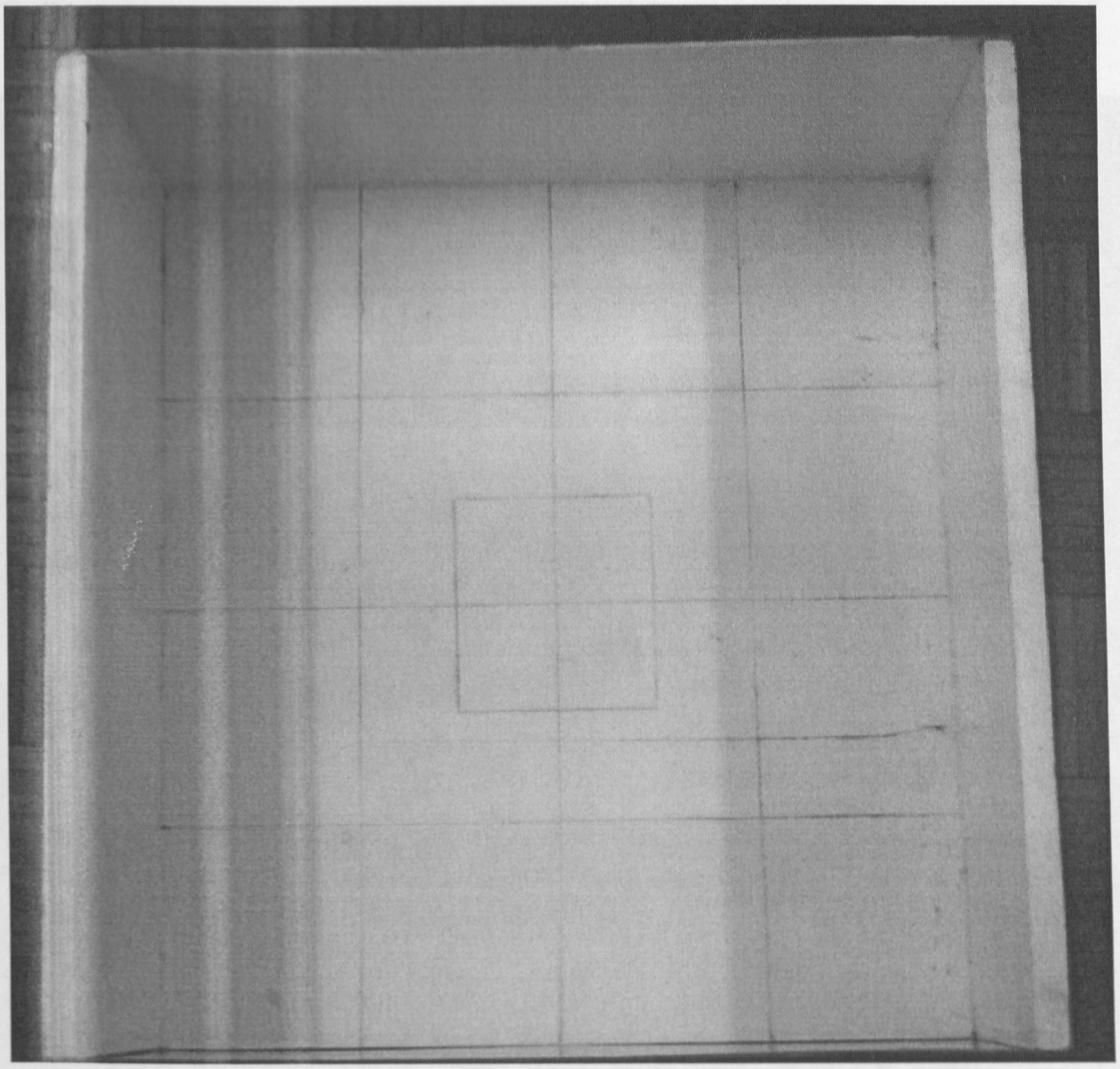
Morris Water Maze Tank



Arrow indicate escape platform

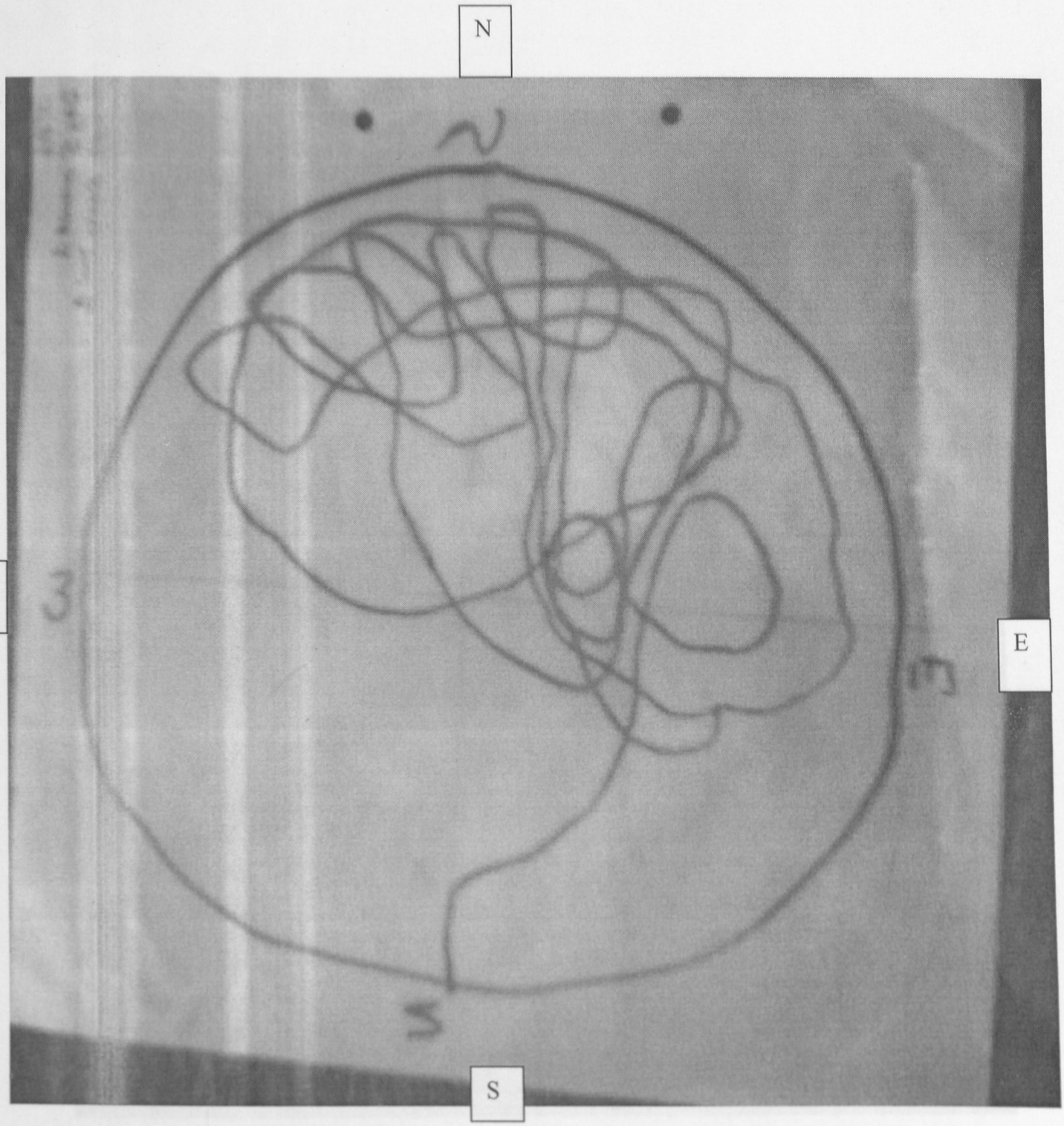
Appendix 5

Open field box



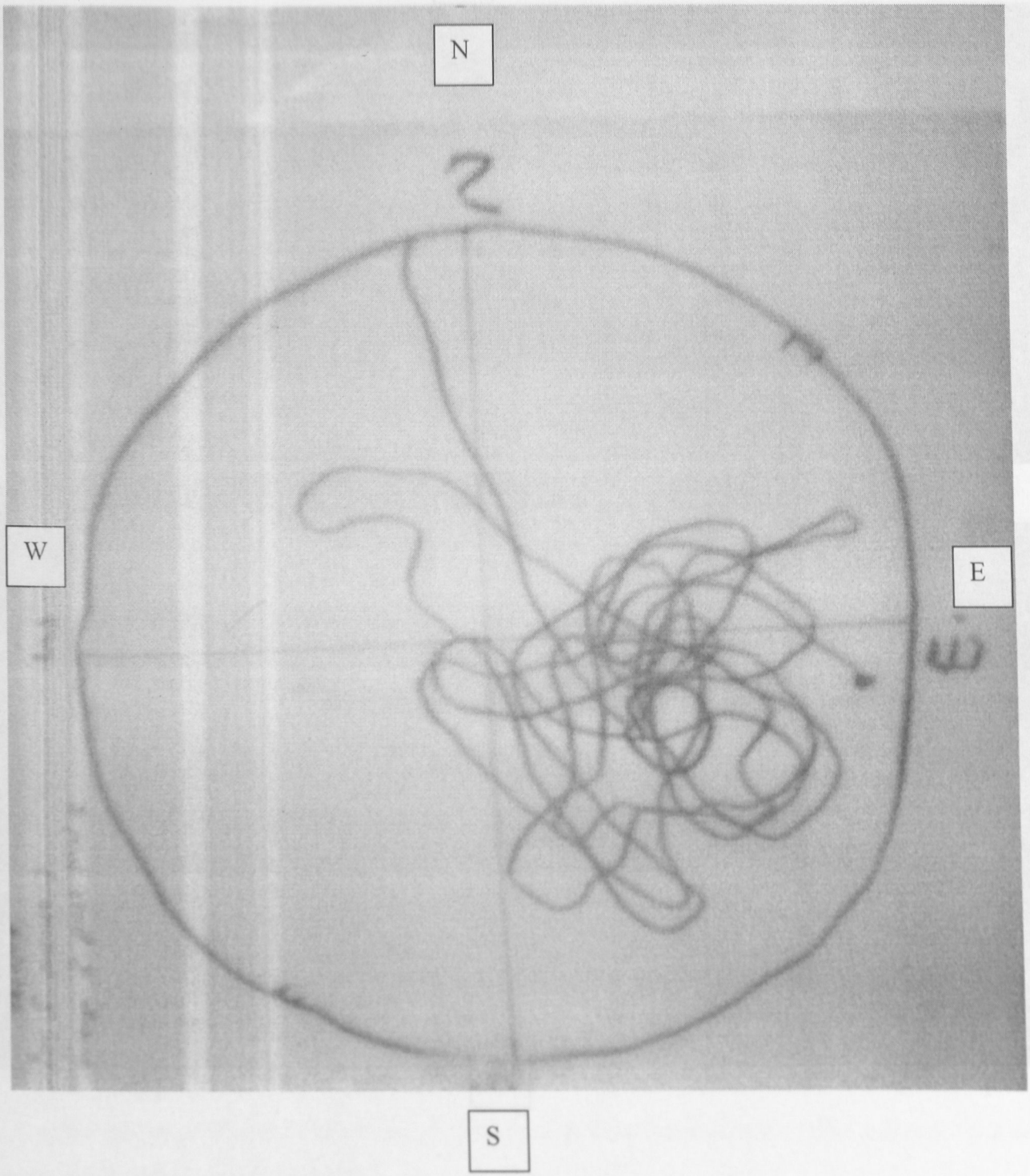
Appendix 6

Mouse Swim Path tracing



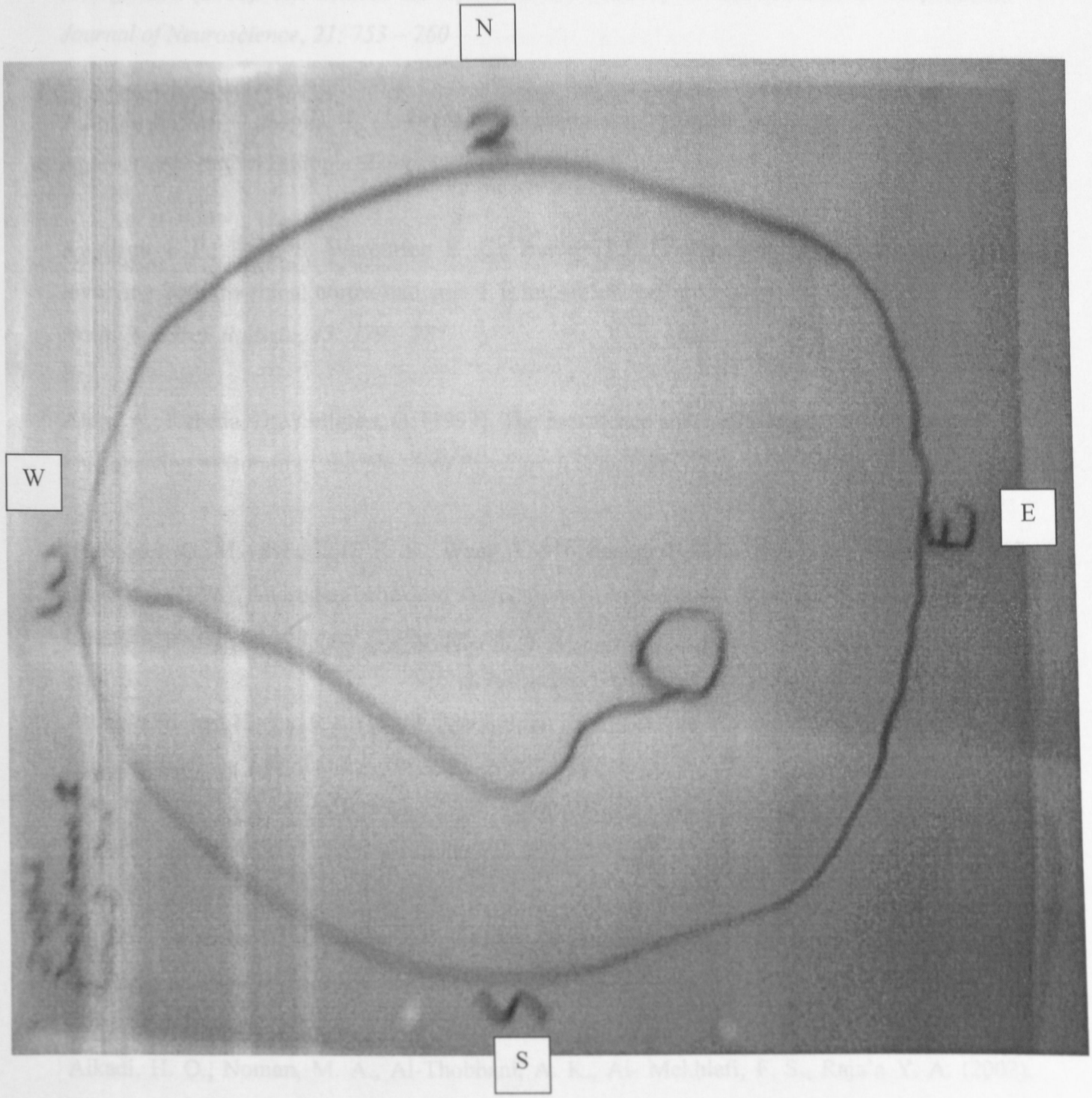
Appendix 7

Mouse Swim path tracing during probe trial



Appendix 8

Mouse Swim path tracing during acquisition training



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