Interaction of *Catha edulis* (Vahl) Forssk. ex Endl. (Khat) and the Endocannabinoid system on spatial learning and memory in mice

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A Thesis Submitted to the Department of Pharmacology and Clinical Pharmacy, School of Pharmacy, College of Health Sciences, Addis Ababa University, in partial fulfillment for the requirements of a Master of Science Degree in Pharmacology

Addis AbabaUniversity
Addis Ababa, Ethiopia
June, 2023

Addis Ababa University

School of Graduate Studies

This is to certify that the dissertation prepared by Hilina Melese Argaw, entitled: "Interaction of *Catha edulis* (Vahl) Forssk. ex Endl. (Khat) and the Endocannabinoid system on spatial learning and memory in mice" and submitted in partial fulfillment of the requirements for the Degree of Master of Science in Pharmacology complies with the regulations of the University and meets the accepted standards with respect to originality and quality.

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Abstract

Interaction of *Catha edulis* (Vahl) Forssk. ex Endl. (Khat) and the Endocannabinoid system on spatial learning and memory in mice

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Numerous studies have looked into the neurocognitive impact of cannabinoid ligands. The effect of *Catha edulis* (Vahl) Forssk. ex Endl**.** (Khat) on learning and memory is also researched by few scholars, although the findings were inconsistent. Moreover, the interaction of cannabis with other drugs of abuse is well studied. Indeed, recently, it was reported that the neurobehavioral effects of khat, such as motor tasks, working memory and anxiety-like behaviors are modulated by the endocannabinoid system. However, it is not known whether such modulation is apparent in spatial learning and memory. To this effect, mice 6–8 weeks old (6 per group) and a single dose of either crude khat extract orally (300mg/kg) alone or the cannabinoids intraperitonially (WIN-55,212-2 (1 mg/kg), JWH133 (5 mg/kg), cannabidiol (5 mg/kg), AM251 (1 mg/kg) and AM630 (1 mg/kg)) alone and in combination with crude khat were administerd according to their respective groups. Controls were administered with 0.5 ml of 2% Tween 80 in water. Spatial learning and memory were assessed using a battery of tests, including the Radial arm maze, Multiple T maze and the Morris water maze. Parameters including latency, correct/incorrect decision, reference memory error, and working memory error were determined. The data were analyzed using the Statistical Package for the Social Sciences and considered statistically significant when the P value was $\leq$ 0.05. The study revealed that acute khat exposure does not have a substantial effect on spatial learning and memory. Except for JWH 133, lone administration of Cannabidiaol and WIN-55,212-2 resulted in significant enhancement and impairment of cognition, respectively. Whlist, co-administration of khat with cannabinoid agonists attenuates the effect produced by the agonist regardless of the direction of change (enhanced or reduced cognition). Co-administration with antagonists, however, has a pro-cognitive effect. Especially the cannabinoid 1 receptor inveres agonist/antagonist AM251 augmented khat's effect on spatial learning and memory more precisely.

Key words; Khat, Endocannabinoid system, Cannabinoid receptor antagonists, Cannabinoid receptor agonists, Cannabinoid receptors, Radial arm maze, Multiple T maze, Morris water maze, Spatial learning and memory.

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Acknowledgments

First and foremost, I must express my praise to the Almighty God, the creator of everything, for giving me strength and endurance throughout all the up and downs.

Next, without the assistance of the following benevolent people, this work would not have been feasible.

I owe a special debt of gratitude to Professor Ephrem Engidawork, who served as my thesis advisor and who put in a lot of effort to offer me advice, comments, and suggestions throughout the entire process. His vast knowledge and wealth of experience have significantly advanced this research.

My special appreciation goes to Professor Emmanuel Onaivi and Dr. Berhanu Geresu for their logistic support and purposeful advice, which have been invaluable.

I also applaud the sage guidance, unwavering support, and compassion of my husband, Naod Zerihun.

I would also like to thank Debre Berhan University for sponsoring my study and Addis Ababa University for funding this laboratory work. Last but not least, the Department of Pharmacology and Clinical Pharmacy for granting permission to use the necessary laboratory facilities.,and Mrs. Fantu Assefa and Mr.Mola Wale, for their cooperation and technical support during the laboratory work, should be acknowledged.

Abbreviations and acronyms

2AG	2-Arachydonyl Glycerol
AC	Adenylate cyclase
ACh	Acetylcholine
CBD	Cannabidiol
CBN	Cannabinol
cAMP	Cyclic Adenosine mono phosphate
CREB	cAMP response element-binding protein
CBR	Cannabinoid Receptors
DNMS	Dependent Delayed Nonmacho Sample
DSE	Depolarization Induced Suppression of Excitation
DSi	Depolarization Induced Suppression of Inhibition
EC	Endocannabinoid
ERK1/2	Extracellular signal-regulated kinase 1/2,
FAAH	Fatty Acid Amide Hydrolase
GPCR	G-Protein Coupled Receptors
JNK	c-Jun N-terminal kinase
ITI	Inter-trial interval
LTD	Long Term Depression
LTP	Long Term Potentiation
MAGL	Mono Acyl Glyceryl Lipase
MAPK	Mitogen Activated Protein Kinase
MTM	Multiple-T-maze

MWM	Morris Water Maze
N-APE	N-Arachidonoyl-Phosphatidylethanolamine
NMDA	N-methyl-d-aspartate
PLD	Phospholipase D
PKA	Protein kinase A
PI3K	Phosphatidyl Inositol-3-Kinase
RAM	Radial Arm Maze
Δ9-THC	Delta-9-Tetrahydrocannabinol

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1. Introduction

1.1 Background

Majority of the scientists working in the field of neuroscience concur that learning and memory take place in the brain. Modern learning theories in both psychology and neuroscience are built on the ideas of Aristotle, which have been improved upon over the last two thousand years. According to Aristotle, experience is the source of learning and he believed that memory relies on the creation of associations, which are governed by three criteria; contiguity, frequency, and similarity (Gluck et al., 2016). Although learning and memory are two different aspects of a single system for storing information about our experiences, learning emphasizes the acquisition of information while memory emphasizes its retention. In another way, learning is defined as the process of acquiring memory, and memory is referred to as a behavioral change brought on by an experience. Learning and memory are closely related; someone cannot learn from an experience without first recording it (learning), and cannot remember the experience without keeping a record of it (memory) (Okano et al., 2000; Liberman, 2012).

Memory can be classified based on its content or the types of memory as declarative and procedural while concerning time as short-term memory (STM) and long-term memory (LTM) (Meneses et al., 2011; Seifu and Engidawork, 2019).

Declarative memories are those that can be accessed consciously and whose contents can be verbally described; as a result, they are also known as explicit memories. Even though they are not conscious, procedural memories can still have an impact on one's behavior. These memories are known as implicit memories because they operate on an entirely unconscious level (Liberman, 2012).

Declarative and procedural memory differ primarily in terms of the type of information to be retained, the method of encoding the information, and the rate at which the information is forgotten (Goshen, 2001). The regions of the brain where these two memory categories are processed differ significantly from one another. Declarative memories rely on the hippocampus, whereas non-declarative memories rely on regions like the striatum and amygdalad (Okano et al., 2000, Seifu and Engidawork, 2019).

On the other hand, STM and LTM, basically differ in terms of their duration and/or capacity of storage. Only short-term memory exhibits temporal decay and chunk capacity restrictions (Cowan, 2009). Spatial memory is another sort of memory that cannot be strictly categorized under one of the aforementioned classification subsystems. In fact, it combines elements of declarative, procedural, and both LTM and STM (Seifu and Engidawork, 2019). Spatial memory is the ability to store and retrieve information in the brain that is required for both route planning to a desired location and recollection of where an object is or where an event took place. The hippocampus and surrounding medial temporal lobes are parts of the brain that are necessary for the creation of spatial representations of the environment (Burgess and Bisby, 2021).

Around the late 18th century, the Swedish botanist gave the first botanical description of the plant khat and he named it *Catha edulis*, in which the label ‘‘edulis’’ denotes that the plant is edible. *Catha edulis* (Vahl) Forssk. ex Endl (Khat) belongs to the family of *Celastraceae*, which includes 60–70 genera and 850–900 species. It is an evergreen flowering, glabrous shrub, or tree 2–25 m high with reddish stems (Figure 1) (Getasetegn, 2016). The plant grows in altitudes between 1500 and 2500 m and it can be harvested four times per year if it is well irrigated and cropped (Engidawork, 2017).

Its local name varies in different countries, for instance, it is called qat in Yemen, chat in Ethiopia and miraa in Kenya. There are numerous local brands in Ethiopia, but Aweday and Beleche are thought to be the most potent ones (Abdelwahab et al., 2015; Seifu and Engidawork, 2019). The habit of chewing khat is ingrained in many societies. The majority of khat chewers use fresh leaves, hold them in their cheeks to release their chemicals, and then ingest the juice, which is thought to have stimulant and euphoric properties (Patel, 2000).

According to estimates, there are five to ten million regular khat users worldwide (Patel, 2000), with the majority of them living in East Africa and the Arabian Peninsula (Abdelwahab et al., 2015). Particularly in Ethiopia, the overall lifetime prevalence of khat chewing is estimated to be 27.31% (Ayano et al., 2019). A recent cross-sectional study conducted in Hosahna, Ethiopia revealed that the overall prevalence of khat chewing is about 58%, of which 68.4% reported doing so every day and 31.5% reported doing so infrequently. Based on this study khat consumption is strongly correlated with being a Muslim, having a male gender, earning a lot of money, and engaging in khat consumption with friends and family (Rather et al., 2021). Khat’s impact on

health should not be overlooked either (Eticha et al., 2016). Despite this, khat may offer some health advantages for disorders like diabetes mellitus and other conditions (Engidawork, 2017).

Figure 1: The khat leaves

Numerous different compounds are present in the plant extract in significant amounts, including alkaloids, terpenoids, flavonoids, sterols, glycosides, tannins, amino acids, vitamins, and minerals (Abdelwahab et al., 2015). Phenylalkylamines and cathedulins are thought to be the main constituents of alkaloids found in khat. Since they weren't found in more than 43 *Celastraceae* species that were studied, the main phenylalkylamines, cathinone and cathine appear to be unique to khat (Engidawork, 2017). The principal psychoactive component of the plant, cathinone, is found in fresh leaves, and it makes the plant structurally and pharmacologically similar to amphetamine (Mohammed et al., 2014; Abdelwahab et al., 2015). As a result, cathinone has frequently been referred to as the "natural amphetamine". However, its potency is only one-third that of amphetamine (Engidawork, 2017).

Khat has a variety of effects on various bodily systems that can be either harmful or beneficial khat's effects on the peripheral and central nervous systems resemble amphetamine in many ways. An increase in respiration, body temperature, blood pressure, heart rate, and mydriasis are just a few of the sympathomimetic peripheral effects. The effects on the central nervous system, on the other hand, include euphoria, alertness, and a sense of well-being. Additionally, it may cause drowsiness, numbness, and poor concentration. At higher doses, it may also result in hyperactivity

and unstoppable talking (Engidawork, 2017). According to Patel (2000), khat intoxication may be accompanied by psychiatric manifestations like manic-like behavior, schizophrenic form psychosis, or paranoid symptoms, which are also common with amphetamine intoxication.

On the other hand, cannabinoids are a class of substances that stimulate the body's cannabinoid receptors. These chemicals may be endogenous, synthetic, or derived from plants. The plant *Cannabis sativa* (cannabis) (Figure 2) has been widely exploited both for recreational and medicinal purposes in diverse cultures for centuries (Ulugol, 2014). Analgesic, antispasmodic, antiemetic, antimalarial and anti-rheumatic effects are some of the medicinal applications of the plant (Shevyrin and Morzherin, 2015).

There are over 70 phytocannabinoids identified in the cannabis plant, but delta 9 tetrahydrocannabinol (Δ^9-THC), cannabidiol (CBD), and cannabinol (CBN) are the most prevalent and well-researched (Zou and Kumar, 2018), Especially, Δ^9-THC is majorly responsible for the psychoactive effect seen in the plant (Koob et al., 2014). But CBD, which is the precursor of Δ^9-THC, and CBN, which is the breakdown product of THC, lack the psychoactive effect (Ulugol, 2014).

Figure 2: Leaves of *Cannabis sativa.*

The establishment of synthetic cannabimimetic drugs has made it easier to characterize the pharmacology of an endogenous system that reacts to cannabis. However, a cannabinoid G-protein coupled receptor (GPCR) that cannabinoid compounds act at in the brain was accidentally

discovered, and this discovery marked the beginning of a rise in cannabinoid research (Scotter et al., 2010). The endocannabinoid (EC) system in humans is composed of the two known G-protein coupled (Zou and Kumar, 2018) cannabinoid receptors (CBR) (CB1R and CB2R), the natural EC agonists i.e., arachidonoyl ethanolamine (anandamide) and 2-arachidonoyl glycerol (2 AG) and the enzymes that synthesize and degrade ECs (Lupu, et al., 2019).

ECs are considered to be the derivative of arachidonic acid (Rodríguez et al., 2005). Though anandamides and 2-AG are extensively researched and pertinent ECs, there are other ECs, including virodhamin and noladin that have little significance, as a psychotropic agent, due to their very low concentration in the brain. (Koob et al., 2014).

ECs are indeed unlikely to be stored in synaptic vesicles due to their enhanced lipophilic nature. Consequently, they are synthesized as needed in the postsynaptic components of neurons. This occurs in response to depolarization by receptor-stimulated synthesis from membrane lipid precursors, and they are released from cells immediately after their production. ECs are thought to function more like neuromodulators than neurotransmitters as a result of this biochemistry. (Stelt et al., 2003).

There are at least two types of CBR in mammalian tissues i.e., CB1R and CB2R. Several other receptors, including other GPCRs, ion channels, and nuclear receptors, have been reported to interact with cannabinoids even though only CB1R and CB2R are generally recognized as CBRs. (Stelt et al., 2003).

The central nervous system (CNS) contains large amounts of CB1R, mostly at GABAergic projections. The binding of ligands with that of CB1R, which are largely found in the frontal cortex, hippocampus, basal ganglia, cerebellum, and striatum produces significant psychotropic effects and also may mediate inhibition of neurotransmitter release because of their presynaptic localization. (Lupu et al., 2019, Stelt et al., 2003).

Conversely, CB2R is primarily present in lymphoid tissues and immune cells (such as splenic macrophages and T and B-type lymphocytes), as well as kidneys, bones, peripheral nerves, and some vessels. It was previously thought that CB2R were only found on the periphery, but recent research has revealed that CB2R have also been found within the CNS on neurons of the striatum, brain stem, and hippocampus, as well as on microglial cells, with their expression primarily related

to inflammatory conditions (Lupu et al., 2019). Moreover, CB2Rs are expressed in the dopamine neurons of the mouse brain and have been connected to drug addiction, synaptic plasticity, eating disorders, psychosis, depression, and autism spectrum disorders (Liu et al., 2017).

The signaling mechanism of cannabinoid receptors is quite a complex process. In N18TG2 cells, R-(+)-methanandamide and ECs activated the CB1R, which facilitated the inhibition of adenylate cyclase (AC) activity (Howelet and Mukhopadhyay, 2000). According to Mu et al., (1999), stimulation of CB1Rs activates A-type and inwardly rectifying potassium channels, possibly via AC/Gi/o proteins, which inhibits cAMP-dependent protein kinase A (PKA). It has been demonstrated that cannabinoids control potassium current (inward/outward) by phosphorylating the potassium channels through the action of PKA (Stelt et al., 2003). N-type, P/Q-type, and D-type potassium channels are all inhibited by CB1R activation (Howelet and Mukhopadhyay, 2000). In COS-7 cells cotransfected with CB1Rs and Gα subunits, it has also been shown that CB1Rs activate phospholipase C through G proteins (Ho et al., 1999). In cultured cerebellar granule neurons, activation of CB1Rs increases N-methyl-d-aspartate (NMDA)-mediated calcium release from inositol 1,4,5-triphosphate-gated intracellular stores (Netzeband et al., 1999). In N18TG2 and NG108-15 cells, activation of CB1Rs by cannabinoid agonists results in a quick, momentary rise in intracellular free Ca2+ (Sugiura et al., 1997, 1996, 1999).

Furthermore, it has been demonstrated that cannabinoids control axonal growth, synaptogenesis, and neurogenesis since it boosts the activity of mitogen-activated protein kinases (MAPK) (Basavarajappa, 2017). In nonneuronal U373MG astrocytoma cells and host cells expressing recombinant CB1Rs, extracellular signal-regulated kinase 1/2 (ERK1/2) (p42/p44) activation was seen, and it was mediated by CB1R and the Gi/o protein (Bouaboula et al., 1995). Likewise, In C6 glioma and primary astrocyte cultures, activation of the Gi/o protein via CB1R by Δ9-THC and HU-210 activated p42/p44 MAPK (Sanchez et al., 1998; Guzman & Sanchez, 1999). One possible mechanism for MAPK activation could involve a pathway in which CB1R-mediated Gi/o activation leads to the recruitment of Phosphatidyl Inositol-3-Kinase (PI3K). CB1R-mediated release of βγ subunits leads to the activation of PI3K, resulting in tyrosine phosphorylation and activation of Raf-1 and the phosphorylation of MAPK (Basavarajappa, 2007). Studies using human vascular endothelial cells that express endogenous CB1Rs and CHO cells that express recombinant CB1Rs demonstrate that CB1R stimulation activates p38 MAPK. In CHO cells

expressing recombinant CB1Rs, activation of the CB1R by Δ9-THC resulted in activation of the c-Jun N-terminal kinases (Rueda et al., 2000).

CB1R-mediated signaling also involves synaptic plasticity, learning and memory. Acute CB1R activation by synthetic cannabinoids in adult animals impaired Calcium/calmodulin-dependent protein kinase type IV and cAMP response element-binding protein (CREB) activation, leading to long term potentiation (LTP) and learning and memory deficits (Basavarajappa & Subbanna, 2014). CREB activation, LTP, and a number of behavioral phenotypes, including those related to learning and memory, were all enhanced in studies using CB1R KO mice (Basavarajappa et al., 2014; Basavarajappa & Subbanna, 2014).

On the other hand, activation of CB2R produces similar signaling effects to that of CB1R activation except it does not involve either the blockade of the voltage-gated calcium channel or activation of potassium channels to mediate the inhibitory effect (Stelt et al., 2003).

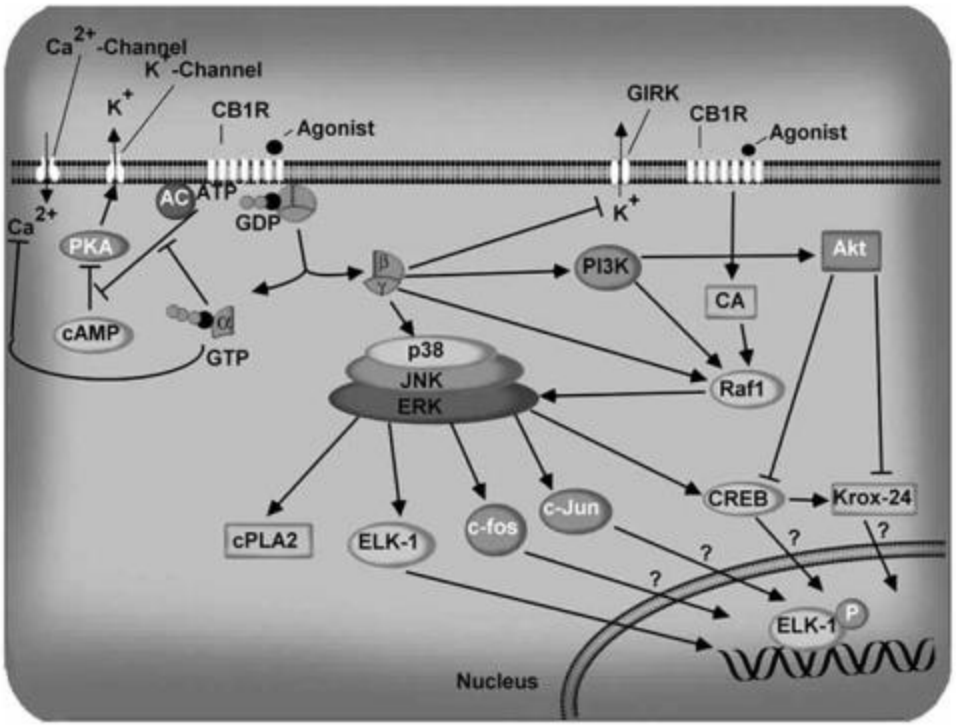

Figure 3: Schematic representation of CB1R signaling.

CB1 receptors are G-protein-coupled proteins with a 7-transmembrane domain found in cell membranes. N-, P/Q-, and L-type Ca2+ channels are among those that CB1 receptors inhibit. It is believed that the βγ subunits of the G-protein act on GIRK and PI3K while the α subunits of the G-protein act on Ca2+ channels and adenylyl cyclase (AC). K+ channel phosphorylation is reduced as a result of decreased activation of the cAMP-dependent protein kinase A (PKA) brought on by the inhibition of AC and the subsequent drop in cAMP. A (→) symbol denotes stimulatory effects, while a(⊥)symbol denote inhibitory effects. Adapted from Basavarajappa, 2007.

1.1 Statement of problem

People who chew khat believe it improves cognitive abilities such as memory and learning (Seifu and Engidawork, 2019). However, studies on laboratory animals revealed controversial results that contradicted the widely held beliefs of society. Furthermore, people use khat in conjunction with other drugs of abuse such as cannabis. The interaction of cannabis with other drugs of abuse is well studied. Indeed, recently, it was reported that the neurobehavioral effects of khat, such as motor tasks, working memory and anxiety-like behaviors are modulated by the endocannabinoid system. However, it is not known whether such modulation is apparent in spatial learning and memory. Most users of concurrent khat and cannabis, do so for their calming and euphoric effects. Nevertheless, users experience side effects from using the two at the same time. Technically, the specific effects and reactions brought on by the regular use of khat and cannabis depend on whether one consumes more cannabis in relation to khat or vice versa. In general, combining khat and cannabinoids can increase the risk of developing or worsening psychosis, as well as sedation, lethargy, respiratory depression, nausea, and other side effects brought on by the synergistic effects of the two substances (Geresu, 2015; Mihretu et al., 2017).

1.2 Hypothesis

Concurrent use of khat and cannabinoid agonists causes significant impairment in learning and memory than using either agent alone.

1.3 Research questions

1. What is the effect of khat exposure on spatial learning and memory in mice?
2. What is the effect of cannabinoid ligands on spatial learning and memory in mice?
3. What is the effect of concurrent exposure to khat and cannabinoid ligands on spatial learning and memory in mice?

1.4 Objectives of the thesis

1.4.1 General Objective

- ✓ To investigate the interaction between khat and the cannabinoid system in modulating spatial learning and memory in mice.

1.4.2 Specific Objectives

- ✓ To assess the interaction of khat and cannabinoid receptor ligands on learning and memory using MTM
- ✓ To assess the interaction of khat and cannabinoid receptor ligands on learning and memory using MWM.
- ✓ To assess the interaction of khat and cannabinoid receptor ligands on learning and memory using RAM

1.5 Significance of the study

All the studies conducted todate have attempted to determine how lone administration of khat extracts and cannabis affect memory and learning. But, the concurrent use of the two and their interaction in affecting spatial learning and memory is not investigated. Thus, there is a need to know the interaction that exists between khat and cannabinoids with the aim of whether khats' effect on learning and memory is modulated by the EC system. Therefore, the current study offers the first concrete evidence of the interaction that exists between khat and cannabinoids as well as whether khats' effect on spatial learning and memory is modulated by the EC system or not. As a result, the current study serves as a baseline for stimulating further research in this area.

1.6 Scope of the study

This thesis is limited to the effect of acute administration of khat and cannabinoid ligands impact on learning and memory. So the followings are its scope:

- ✓ To assess spatial learning, short term and long term memory using MWM.
- ✓ To assess spatial learning, short term and long term memory using MTM.
- ✓ To assess spatial short term and long term memory using RAM

The effect of sub-acute/sub-chronic administration of khat and cannabinoid ligands impact on spatial learning and memory will be done in future studies.

1.7 Limitations

Due to budget constraints, subacute studies were not done, which is the main limitation of the current study.

1.8 Structure of the thesis

This thesis is comprised of six basic chapters. The first chapter briefly introduces the main idea of the finding, as well as some background information on the research problem and the research's core components. A thorough analysis of earlier works that are connected to the thesis is provided in the second chapter. The third chapter delves into the Materials, methods and experimental design used during the experiment. The fourth chapter is all about the outcome obtained in the acute phase study using the a battery of tests. The fifth chapter presents the discussion part and the final chapter includes the conclusion and recommendations.

2. Literature review

The interaction between drugs of abuse and learning is highly complex because these drugs are associated with the formation of strong but maladaptive memories that encourage drug use and addiction. Abuse-related drugs are usually connected to interruptions in learning. Acute or first-time drug use may encourage the development of maladaptive memories connecting drug effects with environmental triggers. These memories may have a major behavioral regulating effect, encouraging drug-seeking behavior and relapse. Over time, drug addiction hijacks the learning and memory pathways in the brain, causing long-lasting changes in the circuits underlying normal learning processes (Kutulu and Gould, 2017).

Preclinical and clinical studies have done a satisfactory job of examining how drugs of abuse including amphetamine, nicotine, cocaine, alcohol, opiates, and cannabis affect learning and memory. However, there have only been a few laboratory research done on the cognitive effects of khat, and those tend to have conflicting findings. Furthermore, khat is frequently combined with other drugs of abuse, (Mihretu et al., 2017). Although, the cognitive effect of concurrent use has not been researched. In this literature review the cognitive effect of khat, cannabis and their interaction will be discussed.

2.1 Khat and learning and memory

People chew khat to exploit its positive effect on memory and thinking (Engidawork, 2017). There are few studies conducted on determining the effect of khat on learning and memory, but these studies report varying findings to each other as well as to the widely recognized belief of people (Seifu and Engidawork, 2019). The effect of khat on learning and memory could be due to its effect on the serotonergic and dopaminergic networks. Neurotransmitters such as serotonin play a crucial role in learning and memory. Studies involving human volunteers reported memory impairment following the administration of acute doses of serotonin agonists. Conversely, dopamine plays an antagonistic role to that of serotonin i.e., it has a facilitatory role in cognitive functions like learning and memory (Kimani and Nyongesa, 2008). Geresu and Engidawork (2010) also demonstrated that khat reversed haloperidol-induced but not morphine-induced catalepsy, indicating khat exerts its effect through acting on the monoaminergic system.

Kimani and Nyongesa (2008) reported that different doses of khat had selective effects on learning and memory in mice via dopamine and 5-HT neurotransmission by using the Morris water maze model (MWM). According to their study, low doses of khat do not affect learning but impair memory. The reason behind this is maybe a low dose of khat extract resulted in the secretion of insignificant amounts of 5-HT which, therefore, impacted no effect on learning, whereas a high dose impairs learning but improves memory. Improved memory at a high dose can be explained in the sense that khat extract increased dopamine neurotransmission with less secretion of 5-HT.

Mohamed et al. (2014) investigated the effect of acute, subacute and subchronic administration of khat on spatial learning and memory as well as the respective morphological changes in the hippocampus. According to their study, learning and long-term memory was not affected by all of the three phases, nonetheless, short-term memory was impaired only by sub-chronic administration. The authors argued that these effects were observed due to the effect of khat on monoaminergic transporters, thereby differentially regulating the two neurotransmitters, dopamine, and serotonin, known to play an opposite role. Although the study reported a significant reduction in the weight of the brain and prosencephalon, the morphometric analysis did not reveal changes in the geometric properties of the dentate gyrus granular cells, suggesting the neural process underlying learning and memory was not affected. The authors suggested that the possible reason for the reduction in the weight of the brain could be associated with the vasoconstrictive and anorexic effects of khat.

Moreover, Seifu and Engidawork (2019) also reported that neither the crude extract nor the fractions (alkaloidal and non-alkaloidal) of khat had a detectable effect on learning and memory in both single as well as repeated dose studies. Although there appeared to be a seemingly conflicting report in the literature, the bulk of the evidence seems to favor the marginal effect of khat on learning and memory.

2.2 Cannabinoids and Memory

The brain's EC system has a significant impact on regions that are crucial for memory and learning. Numerous studies have suggested that the EC system may hinder learning and memory functions by influencing the neural networks in the striatum, frontal cortex, and hippocampus. Furthermore, they might be able to affect LTP due to their capacity to inhibit the release of neurotransmitters like glutamate. Thus, it is entirely reasonable to suggest that CB1R-mediated inhibition of

glutamate release may be a key mechanism affecting learning and memory functions (Varvel et al., 2005).

Besides that, the EC system has a significant impact on synaptic plasticity, which is thought to be the foundation for learning and memory in which organisms can alter a subsequent response to stimuli. Synaptic plasticity refers to the ability of the synapse to change or remodel itself in structure or function in response to specific patterns of activation. (Varvel et al., 2005).

Depending on the involvement of GABA and glutamate neurotransmitters, the EC system may induce short-term synaptic plasticities such as depolarization-induced suppression of inhibition (DSI) and depolarization-induced suppression of excitation (DSE), , respectively. Furthermore, the EC system affects long-term synaptic plasticity, including LTP and long-term depression (LTD). Activation of the CB1R inhibits both LTP and LTD (Rodríguez et al., 2005). The hippocampus' glutamatergic synapses are thought to play a role in the formation of memory through LTP and LTD. According to Akirav (2011), cannabinoids appear to function by lowering glutamate release below the threshold required to activate the NMDA receptors needed for LTP and LTD.

Slanani et al. (2005) investigated the influence of ECs on LTP in the CA1 region of the hippocampus. CB1R blockade favors LTP in the hippocampus in which LTP was elicited by moderate stimulations (20 or 50 pulses).

To precisely examine how various cannabinoids affect memory tasks, several studies have been carried out in both humans and rodents. For instance, Varvel et al. (2001) used the MWM to investigate the influence of synthetic Δ9-THC on mice's cognitive abilities. According to their study, administration of Δ9-THC causes impairment of both spatial working and reference memory of mice. Extremely high doses of the Δ9-THC were able to produce significant disruption of the reference memory, in contrast only a lower dose of the drug causes a deficit in the working memory of mice. This notion is supported by a study conducted by Lichtman and Martin (1996), where the cannabinoid antagonist SR141716A (1–10 mg/kg) prevents spatial memory deficits, which was caused by Δ9-THC administration (2–6 mg/kg i.p.), in a dose-dependent manner.

Using the radial arm maze, Rubino et al. (2009) hypothesized that the impact of cannabinoids, specifically on hippocampal morphology and neuroplasticity, may serve as a potential mechanism

for the impairment of spatial memory. According to the study, pretreated rats had significantly lower levels of NMDA receptors, pre- and postsynaptic protein expression, and the astroglial marker glial fibrillar acid protein. Concerning this fact, there are some contradictory findings. According to Suliman et al. (2017), Δ9-THC 's ability to raise the concentration of markers involved in all stages of neurogenesis, particularly in the hippocampus, may help rats perform better on cognitive tasks.

Comparable results were also obtained from studies done on humans. The cognitive impact Δ9-THC on human participants was researched by Morisson et al. (2009). When the drug was administered acutely, it was found to significantly impair working and episodic memory, as well as enhance anxiety-like behavior. Rimonabant, a CB1R antagonist, reverses the effects caused by CBR agonists such as Δ9-THC, WIN-55,212-2, CP 55,940, and anandamide, demonstrating the involvement of CB1Rs-related mechanisms (Geresu et al., 2019).

Ample literature supports the claim that study participants who have been exposed to cannabinoids on a long-term basis won't experience any deficits after receiving the drug acutely. The possible justification for this could be chronic exposure to cannabinoids may result in tolerance to the adverse consequences of cannabinoids on memory tasks (Ramaekers et al., 2011). But compared to the mature human and animal brains, the still-developing brain appears to be more affected morphologically and functionally by long-term and early cannabis use (Schoeler and Bhattacharyya, 2013).

Moreover, the age of the user must be taken into account because cannabinoids have different effects on adolescents and adults. For instance, chronic Δ9-THC injection in young rats results in a long-lasting response in adulthood and increased sensitivity to Δ9-THC induced learning impairments (Winsauer et al., 2010).

According to a recent study using the Barnes maze task, only adolescent rats are significantly affected by single-dose administration of Δ9-THC in terms of spatial memory acquisition, and its behavioral effects on adults are negligible. (Gibula-Tarlowska et al., 2020). Similarly, chronic administration of CP 55,940 to rats produced permanent memory impairment in adolescents but not in adult rats (O'Shea et al., 2004).

Rimonabant, a CB1R antagonist, reverses the effects caused by CBR agonists such as Δ9-THC, WIN-55,212-2, CP 55,940, and anandamide, demonstrating the involvement of CB1Rs-related mechanisms (Geresu et al., 2019).

The influence of cannabinoids on the cholinergic system may be another potential mechanism for the learning and memory deficits they cause. The EC system affects the release of acetylcholine (ACh) from the presynaptic cholinergic terminal as well as it may inhibit the uptake of choline into the hippocampus, thereby inhibiting ACh synthesis (Varvel and Lichtman, 2005).

Goonawardena et al. (2010) determined the interaction between cannabinoid and cholinergic systems. To study short-term memory, they subjected rats receiving WIN-2 or cholinergic medications to a hippocampal-dependent delayed nonmacho sample (DNMS) task and captured hippocampal single-unit activity in vivo. Performance of the DNMS and hippocampal principal cell firing and bursting were markedly reduced. This supports the idea that cannabinoid-modulated cholinergic activity could be one of the mechanisms underlying memory deficit since the acetylcholinesterase inhibitor, rivastigmine, reversed these short-term memory deficits and normalized hippocampal discharge rates. In general, the possible mechanism by which cannabinoids and ECs affect learning and memory may be through modifying the release of other neurotransmitters like glutamate and acetylcholine or directly affecting the CB1Rs in the hippocampus (Castellano et al., 2003).

2.3 Khat- cannabinoid interaction

There are several shreds of evidence that point to the EC system's role in modulating the rewarding behavior of many other non-cannabinoid psychoactive drugs. The EC system shows interaction with the drug of abuse like opioids, nicotine, alcohol and cocaine (Solinas et al., 2007). But only a few studies have been conducted to show the possible interaction that exists between the EC system and khat. The first behavioral evidence was presented by Geresu et al. (2016), where co-administration of khat and WIN55,212-2 resulted in increased parameters measured in the locomotion, anxiety-like behavior, and working memory tests, which was reversed by the cannabinoid antagonists. Further work by the same group (Geressu et al., 2019) demonstrated that while attenuation of MPTP-induced motor deficit was enhanced, locomotor activity was decreased by co-administration of khat and JWH-133 in wild-type mice. Cell type-specific deletion of CB2R on dopaminergic neurons, however, increased the hyper locomotor behavior of khat extract,

reinforcing the notion CB2R activation modulates khat-induced motor activity. On the other hand, JWH-133 did not affect the khat-induced increase in tyrosine hydroxylase immune reactivity and dopamine transporter mRNA in wild-type mice, suggesting selective interaction of the two pathways in the brain neurocircuitry system.

Although the interaction between the cannabinoids and khat on motor tasks, working memory and anxiety-like behaviors are well studied (Geressu et al., 2016, 2019), their interaction on spatial learning and memory is not yet investigated. Therefore, the current study aims to investigate the effect of the interaction of the cannabinoid system and khat on spatial learning and memory using a battery of tests.

3. Materials and Methods

3.1 Drugs, chemicals and reagents

The phytocannabinoid and negative allosteric modulator of CB1Rcannabidiol (CBD); the non-selective cannabinoid receptor agonist, WIN-55,212-2; the CB2R agonist, JWH133; the CB1R inverse agonist/antagonist, AM251; and the CB2R inverse agonist/antagonist, AM630 were purchased from Sigma-Aldrich Chem. Co. (St. Louis, Mo, USA). Chloroform, diethyl ether, Tween 80, and distilled water were procured from their respective local vendors found in Ethiopia. All of the drugs and chemicals were of analytical grade.

3.2 Experimental Animals

Healthy Swiss Albino mice of either sex 6-8 weeks of age and 25-30 g weight were used. The animals were bred at the animal house of the School of Pharmacy, College of Health Sciences, Addis Ababa University. Plastic cages with standard wood chip bedding were used to maintain the animals. All the requirements including the average room temperature, a light/ dark cycle of 12/12 h and food and water were maintained. For animals assigned to MTM and RAM, access to standard laboratory food pellets and tap water was restricted for 12 h before commencement of the experiment, while MWM animals were given free access to both. Ethical clearance was obtained from an Ethical review committee of the College of Health Sciences, School of Pharmacy, AAU (ERB/SOP/398/14/2022). Internationally accepted guidelines were used for handling animals and performing all experimental procedures (NIH, 2011).

3.3 Collection of the plant material

Bundles of fresh khat leaves were purchased from a local market in Aweday, a town located 515 km to the east of Addis Ababa, which is known to be one of the natural habitats for the potent variety of khat in Ethiopia. Tips of the fresh leaves and twigs were trimmed and wrapped in a plastic bag at Aweday. The plastic bags were then placed in an ice box and transported to the laboratory of the Department of Pharmacology and Clinical Pharmacy, School of Pharmacy, Addis Ababa University. The leaves were then preserved in a deep freezer (-20 ^{o}C) until extraction. The plant was identified by a taxonomist and a voucher specimen (collection number: ME001) was deposited in the herbarium of the College of Natural and Computational Sciences, Addis Ababa University for future reference.

3.4 Extraction of the plant material

The extraction of khat was done as previously reported (Admassie and Engidawork, 2011). Briefly, the leaves were thinly minced with a knife in a dim environment, weighed using an electronic digital scale, and then added to an Erlenmeyer flask containing organic solvents, namely, a mixture of diethyl ether and chloroform (3:1). The solvents were applied in a quantity that would cover the crushed plant material in the flask. The flask's contents were continually agitated using a rotary shaker at 120 rpm (New Brunswick Scientific Co, USA) for 24 h while it is covered with aluminum foil. The mixture was filtered using Whatman No. 1 filter paper (90 mm diameter, Whatman Ltd., England) and the solvents were allowed to evaporate by keeping the filtrate in a hood for 24 h. The yield was calculated and found to be 1% and the concentrated extract was stored in an airtight container and preserved at -20°C in a deep freezer until use.

3.5 Grouping and dosing of animals

Animals were randomly assigned to ten groups, each with six mice per group. Group I (NCON) served as negative control and received 2% Tween 80. Group II (KT) was administered with khat (300 mg/kg). The non-selective cannabinoid receptor agonist WIN-55,212-2 (1 mg/kg) was administered to Group III (WIN). Group IV (KT+WIN) was administered with WIN-55,212-2 (1 mg/kg) and khat (300 mg/kg). The CB2R agonist JWH133 (5 mg/kg) was administered to Group V (JW). Group VI (KT+JW) received JWH133 (5 mg/kg) and khat (300 mg/kg). Group VII (CBD) received CBD 5 mg/kg. Group VIII (KT+ CBD) received khat (300 mg/kg) and CBD (5mg/kg) Group IX (KT+AM251) received khat (300 mg/kg) and the CB1R antagonist AM251 (1 mg/kg). Group X (KHAT+AM630) was administered khat (300 mg/kg) and the CB2R antagonist, AM630 (1 mg/kg). Dose selection for khat and the synthetic cannabinoids was based on previous studies (Geresu and Engidawork, 2010, 2016; Hayase, 2013; Onaivi et al., 1990), while the dose for the phytocannabinoid, CBD was selected by performing a pilot study.

Khat was weighed, combined with Tween 80 (2 % v/v), vortexed constantly, and then given orally by oral gavage as a fine suspension. Weighed doses of WIN-55,212-2, JWH133, CBD, AM251 and AM630 were intraperitoneally delivered after being diluted with Tween 80 (2% v/v) to the necessary concentration. The maximum volume administered was 10 ml/kg. Control animals received the same volume of the vehicle for the same amount of time as khat or the medications. In combination treatments, the cannabinoid ligands were administered 40 min after khat

administration. To prevent light decomposition, khat sample containers, including syringes, were wrapped in aluminum foil. The experiment consisted of an acute phase, during which the animals were given a single dose of khat and other medications that work on the cannabinoid system.

3.6 Morris water maze test

The Morris water maze (MWM) is a negative reinforcement test in which rodents perceive swimming in water as an aversive stimulus while viewing escape as positive reinforcement. In this experiment, a black circular polypropylene pool (150 cm in diameter and 50 cm in depth) was used. It was loaded with tap water and the water temperature was kept at 25 °C ± 1 °C by using an electric heater during the experiments. An escape platform (10 cm in diameter and 30 cm in height) made up of circular stainless steel with its surface covered in black plastic was used. It was hidden from the animals by being submerged 1cm below the surface of the water during the days of the experiment.

In this experiment, the procedures described elsewhere (Seifu and Engidawork, 2019) were followed. Four quadrants (north-west (NW), north-east (NE), south-east (SE), and south-west (SW)) were used to allow the animals to swim from different directions to the target quadrant in which the escape platform was located. The escape platform was located in the NW direction throughout the experiment. With masking tape labeled North (N), South (S), East (E), and West (W), imaginary quadrant boundaries were marked on the pool's edges. Throughout the training and testing periods, several mazes and distal extra maze cues, including colorful posters, were mounted on the wall of the pool and the room. The cues may be able to assist the animals in creating a spatial map that will allow them to find the platform. All testing was done at roughly the same time each day to minimize performance variability caused by the time of the day. The experiment began at 4:00 pm every day. A video camera mounted above the water maze was used to record the animals' performance in the MWM.

Habituation

The animals were habituated first to reduce the stress associated with the water in the pool. Habituation was done immediately after the animals received treatment. During this phase, the animals were dipped and allowed to swim for about 60 sec. The animals were guided to the hidden platform if they could not find it on their own. Staying on the platform for at least 3 sec was defined

as finding it. This procedure was repeated until the mice remained on top of the platform for 30 sec and done in the absence of any cues in the environment. This stage is not regarded as a session of spatial acquisition. Additionally, mice were moved to the testing room an hour before each test day.

Acquisition

An acquisition test was performed for four consecutive days, with four trials done on each day. A 5 min of inter-trial interval (ITI) was considered. Cues that improve the animal's memory were placed on the wall of the tank as well as the room's wall. In each trial, the animals were introduced into the pool from different quadrant points. The mice were then allowed to remain on the platform for approximately 30 sec, with the platform remaining in the same location throughout the phase. The animal is left on the platform to help it to remember its location in relation to the surrounding cues and to help it orient itself in space. The time the mouse took to move from the start position to the escape platform was determined by using a stopwatch. If the animal did not find the platform within the time limit, it was placed on the platform for 30 sec. To prevent overexertion and hypothermia, the amount of time spent in the pool was restricted during each trial. Additionally, towels and a rodent heater (21025 Comerio VA, Italy at 38 °C,) were used to dry the animals before being returned to their home cage. Furthermore, to prevent the growth of pathogenic bacteria, the pool's water was regularly changed.

Probe trial

A probe trial was conducted in the absence of the hidden platform. The animals were brought in from the S quadrant and allowed to swim in the pool for roughly 60 sec. The length of time spent in the target area was then measured using a video recorder. Time spent in the target quadrant was taken as a measure of memory formation. STM probe trial was carried out on the 5^{th} day, 24 h after the final day of acquisition., while LTM probe trial was performed on the 12^{th} day, 7 days after the STM probe trial.

3.7 Radial Arm maze

The radial arm maze (RAM), which was employed in this experiment to evaluate spatial memory, consists of eight horizontal arms (57x11 cm) that are arranged radially around a central platform. Many extra and intra-maze cues surrounded the maze. A door (20 cm high) is located at the

entrance of each arm. The doors, central platform, and all of the arms were constructed of stainless steel and had removable glass covers. A video recorder was installed in the vicinity, enabling later analysis of the animals' performance.

A positive reinforcement (food bait), as opposed to the MWM, was used to test spatial learning and memory. The win shift protocol and the four-arm baited version were employed. Four of the arms were not baited; entry into one of the four un-baited arms was regarded as a reference (long-term) memory error. Reentry into a baited arm was considered to be a working (short-term) memory error because the food had already been consumed and the arm's status had changed from baited to not baited. Once an animal learned the protocol, its performance remained stable and the effects of treatments on memory were tested. The procedures described elsewhere (Zou et al., 1998; Acebes et al., 2011, Suarez et al., 2021) were used with minor modifications.

Habituation

This phase took place over four days and involved a single 5-min trial. During this, the animals were placed on the maze's central platform and allowed to explore the entire maze. When an animal's four paws fully encircle an arm, it is said to have the animal entered that arm. A food pellet was placed at the end of each arm and the maze was cleaned with dilute alcohol at the end of each experimental trial. No cues were used during the habituation phase. The experiment was terminated either when the animal consumed the food pellets in every arm or after 5 min elapsed.

Acquisition

To encourage food searching, mice were deprived of food for 12 h before the test. The acquisition phase was done for 5 consecutive days, i.e., a single trial per day. The food pellet was placed at the end of only the four arms of the maze. The remaining four arms were never baited and the same position was utilized throughout the phase. In this phase, intramaze and extramaze cues were used and the "win-shift" acquisition protocol was applied. After 5 min elapsed or when all the food pellets had been eaten, the mice were removed from the maze and returned to their home cage. To eliminate any potential olfactory cues, the entire maze was cleaned with diluted alcohol solution right after each trial. Each day after the test was finished, the animals were fed according to their body weight (120 g/kg), which was the recommended amount to maintain body weight while keeping them hungry for the test the next day. The working memory errors were used to measure

the acquisition status of the animals. While STM and LTM were measured by analyzing the working and reference memory error, respectively.

3.8 Multiple T maze test

A wooden multiple-T maze (MTM) (150x130 cm) and the path (15cm high and 8 cm wide) containing seven choice points were used to assess spatial learning and memory. Mice were trained to choose the goal box in which the food reward is kept. The procedures described elsewhere were followed (Seifu and Engidawork, 2019).

Habituation

The purpose of the habituation phase was to familiarize animals with the maze environment. During the habituation phase, animals were placed in the start box and then allowed to explore the maze for 2 min.

Acquisition

After being habituated, an acquisition test was done by fasting the animals for at least 12 h for enhancing foraging behavior. To evaluate the mice's learning potential during the acquisition phase, a total of 16 trials were administered to them in daily sessions of 4 trials over the course of 4 days. Each trial consisted of a 5-min session with an ITI of 20 min. All the trials were conducted after 4:00 pm across the 4 training days and animals arrived at the experiment room 1 h before the experiment began. The experiment was deemed to be over when the mice reached the goal box before the allotted five minutes had passed and were given consent to consume the food pellet there as a reward, or when the allotted five minutes had passed. The animal was guided if it didn't get to the goal box within the allotted five minutes. To eliminate any potential olfactory cues, the entire maze was cleaned with diluted alcohol solution right after each trial. Each day after the test was finished, the animals were fed according to their body weight (120 g/kg), which was the recommended amount to maintain body weight while keeping them hungry for the test the next day. The time taken for the mice to travel from the start box towards the goal box plus the number of wrong decisions were recorded by using a video recorder and stopwatch for later analysis.

Probe trials

On the fifth day, 24 h after the final day of acquisition, a probe trial was conducted to evaluate spatial STM. A 5 min single probe trial was given to each mouse. The mice were left to starve for 12 h before the test to stimulate their food search. To eliminate potential cues for the following mouse, the entire maze was cleaned with diluted alcohol solution right after each trial. A stopwatch and video recording were both used to measure the latency or the amount of time the mouse took to move from the start box to the goal box, as well as the number of incorrect decisions. On the 12^{th} day, 7 days after the STM probe trial, a spatial LTM probe trial was conducted. For LTM also, the same STM procedure was used.

3.9 Statistical analysis

The data were analyzed using the Statistical Package for the Social Sciences (SPSS), version 26.0 software. Data from the study are presented as mean ± standard error of the mean. The paired sample t-test, one-way ANOVA, and two-way repeated measure ANOVA, pairwise comparison test were used to analyze the data from the maze study. In all models, acquisition training (learning) was analyzed using one-way ANOVA and two-way repeated measures ANOVA and/or pairwise comparison test. For the memory test, paired sample t-test was used to analyze quadrant preference in MWM, and one-way ANOVA was used to analyze differences in a mean between groups for latency in MWM and MTM. For analysis of Working memory error (WME) and Reference memory errors (RME) One-way ANOVA, Two-way repeated measure ANOVA and pairwise comparison test were employed. The level of significance was set at 0.05.

4. Results

4.1 Performance in MWM

4.1.1 *Effect on learning*

Table 1 shows the mean latency to find the hidden platform in the acquisition phase of MWM task. All groups had mastered the task as their mean time to locate the hidden platform decreased across the 4-days of training. According to a one-way ANOVA analysis, there was a significant difference among treatment groups in learning the task i.e. finding the hidden platform, on each training day. A Two-way repeated measure ANOVA test on the performance measures revealed a highly significant effect of treatment groups ((F (9, 50)= 4.04, p=0.01) and days (F(2.5, 126.2)=36.15 $p<0.001$). Despite the days' worth of improvement in the mean latencies, the within-subjects effect showed no statistically significant interaction between treatment groups and days.

Post hoc Tukey's multiple comparison tests revealed that there was no statistically significant difference between the negative control group and khat treated group across the four days of training in finding the hidden platform. Treatment with cannabinoid ligands, however, produced a different pattern. Whilst lone as well as a combination treatment of JWH133 did not produce any detectable difference, lone CBD (p=0.011) but not the combination of (KT-CBD) produced a significantly higher performance compared to controls. Next, the performance of mice treated with a combination of khat and cannabinoid ligands was compared with khat treated ones. Accordingly, no significant difference was seen across the four training days between mice treated with khat alone and those treated with a combination of khat and CBR agonists. Interestingly, a Two-way repeated measure ANOVA showed significantly higher performance in mice treated with concurrent khat and CBR invese agonists/antagonists AM251 (p=0.004) and AM630 (p.=0.014) when compared to negative control groups across the four training days but not with khat-treated groups.

Table 1: Mean latency to find the hidden platform in the acquisition phase in the Morris water maze task

	Mean latency (sec)				
Groups	**D1**	**D2**	**D3**	**D4**	**D1-D4**
NCON	47.94 ± 6	40.46 ± 7	36.25 ± 8	33.33 ± 8	39.49 ± 6
KT	36.00 ± 6	26.54 ± 7	29.38 ± 9	27.46 ± 7	29.84 ± 6
WIN	43.44 ± 7	36.79 ± 6	40.79 ± 5	20.13 ± 5	43.40 ± 6
KT-WIN	39.38 ± 8	22.92 ± 7	23.92 ± 7	15.71 ± 3	25.48 ± 6
JW	39.50 ± 2	17.67 ± 2	17.50 ± 4	10.17 ± 2	21.20 ± 6
KT-JW	37.17 ± 8	25.67 ± 4	22.00 ±5	17.00 ± 2	25.46 ± 6
CBD	28.83 ± 3	14.33 ± 2^{a1*}	12.50 ± 3	14.17 ± 3	17.46 ± 6$^{a1\#}$
KT-CBD	49.92 ±4	33.54 ± 4	27.83 ± 5	15.96 ± 2	31.81 ± 6
KT-AM251	23.67 ± 2	16.67 ± 2	8.33 ±2^{a1*}	12.50 ± 4	15.29 ± 6$^{a2\#}$
KT-AM630	28.00 ± 5	14.33 ± 2^{a1*}	14.17 ± 1	15.33 ± 4	17.95 ± 6$^{a1\#}$

One-way ANOVA and two-way repeated measures ANOVA were used in the statistical analysis, and all results are mean ± SEM (n=6); NCON: vehicle (2% Tween 80); KT: khat 300mg/kg; WIN: win 55,212-2 1mg/kg (non-selective cannabinoid receptor agonist); JW: JWH133 5mg/kg (selective cannabinoid 2 receptor agonist), CBD: Cannabidiol 5mg/kg (phytocannabinoid, negative allosteric modulator of CB1R); AM251; Cannabinoid 1 receptor inverse agonist/ antagonist (1mg/kg); and AM630 Cannabinoid 2 receptor inverse agonist/antagonist (1mg/kg). [1]: $p<0.05$; 2: $p<0.005$; a, compared to vehicle (2% Tween 80); *: one-way ANOVA; [#]: Two-way repeated measure ANOVA.

4.1.2 *Effect on memory*

Tables 2 (STM) and 3 (LTM) show the probe (retention) trial result of mice in MWM for acute dose administration of khat and cannabinoid ligands.

Regarding STM, which was performed on the 5th day of the experiment, Paired sample t-test revealed a highly significant ($t=5.07$, $p<0.001$) difference in time spent at the target quadrant versus the adjacent quadrant. Mice, from all treatment groups, spent more time in the target quadrant (NW) than in the adjacent quadrant (NE).

One-way ANOVA analysis revealed mice treated with a vehicle did not spend a significantly different time at the target quadrant compared to mice treated with khat. Likewise, no statistically significant difference was seen between groups that were treated with the vehicle and cannabinoid ligands. Thus, the effect of khat was neither significantly inhibited nor significantly enhanced when combined with the cannabinoid ligands.

Similarly, in the LTM study, a paired sample t-test revealed a highly significant ($t=4.02$, $p<0.001$) time spent at the target quadrant than the adjacent quadrant in all groups. Between-group comparison, however, did not show apparent differences among the different groups.

Table 2: Performance of mice in the short-term memory test using the Morris water maze experiment

	Probe Trial: Mean Latency(sec)	
	STM test (D5)	
Groups	**Time at TQ**	**Time at AQ**
NCON	20.83 ± 5	16.5333 ± 2
KT	15.50 ± 4	11.3333 ± 2
WN	11.50 ± 1	12.5833 ±1
KT-WN	16.83 ± 1	10.8333 ± 1
JW	31.83 ± 4	11.0000 ± 3
KT-JW	21.83 ± 2	9.5833 ± .7
CBD	17.00 ± 3	7.0000 ± 1
KT-CBD	14.33 ± 2	8.7500 ± 1
KT-AM251	30.83 ± 4	8.0000 ± 1
KT-AM630	16.17 ± 1	14.8333 ± 2

Paired sample T-test and One-way ANOVA were used in the statistical analysis, and all results are mean ± SEM (n=6); NCON: vehicle (2% Tween 80); KT: khat 300mg/kg; WIN: win 55,212-2 1mg/kg (non-selective cannabinoid receptor agonist); JW: JWH133 5mg/kg (selective cannabinoid 2 receptor agonist), CBD: Cannabidiol 5mg/kg (phytocannabinoid, negative allosteric modulator of CB1R); AM251; Cannabinoid 1 receptor inverse agonist/ antagonist (1mg/kg); and AM630 Cannabinoid 2 receptor inverse agonist/antagonist (1mg/kg).

Table 3: Performance of mice in the long term memory test using the Morris water maze experiment

	Probe Trial MWM: Mean Latency(sec) LTM test (D12)	
Groups	**Time at TQ**	**Time at AQ**
NCON	16.33 ± 3	12.3667 ± 1
KT	13.00 ± 3	11.0833 ± 2
WN	16.00 ± 1	13.1667 ± 2
KT-WN	12.67 ± 2	16.7500 ± 1
JW	16.50 ± 2	9.6667 ± 1
KT-JW	10.33 ± 2	9.7500 ± 1
CBD	19.17 ± 1	6.9167 ± 1
KT-CBD	13.67 ± 3	14.5000 ± 3
KT-AM251	23.17 ± 1	6.6667 ± 1
KT-AM630	25.33 ± 4	9.8333 ± 2

Paired sample T-test and One-way ANOVA were used in the statistical analysis, and all results are mean ± SEM (n=6); NCON: vehicle (2% Tween 80); KT: khat 300mg/kg; WIN: win 55,212-2 1mg/kg (non-selective cannabinoid receptor agonist); JW: JWH133 5mg/kg (selective cannabinoid 2 receptor agonist), CBD: Cannabidiol 5mg/kg (phytocannabinoid, negative allosteric modulator of CB1R); AM251; Cannabinoid 1 receptor inverse agonist/ antagonist (1mg/kg); and AM630 Cannabinoid 2 receptor inverse agonist/antagonist (1mg/kg).

4.2 Performance in MTM

4.2.1 *Effect on learning*

Tables 4 (mean latency) and 5 (mean wrong decisions) show the acquisition performance on MTM results for acute dose administration of khat and cannabinoid ligands. One-way ANOVA analysis revealed that most of the groups had learned the task as their mean time to find the goal box and errors on the way to the goal box had dropped across the 4-days of training but without significant intergroup variation. The Post hoc Tukey's multiple comparison tests revealed that neither khat nor cannabinoid agonists produced a statistically significant difference compared to vehicle-treated groups in both measures. Moreover, compared to khat, concomitant administration of khat and cannabinoid agonists did not produce significant learning across the four training days. A further analysis i.e. a pairwise comparison using estimated marginal mean of a two-way repeated measure ANOVA was conducted and based on this further analysis, co-administration of khat with

AM251 significantly enhanced (58%, p=0.007) learning and reduced the number of mean wrong decisions committed compared to khat alone and which is consistent with what was seen in MWM. Furthermore, mice treated with WIN committed a significantly (p=0.006) large number of mean wrong decisions across the 4 training days compared to the vehicle-treated mice.

Table 4: Mean latency to find the goal box in the acquisition phase of the Multiple T maze experiment

	Mean latency (sec)				
Groups	**D1**	**D2**	**D3**	**D4**	**D1-D4**
NCON	84.58 ± 43	108.83 ± 42	111.83 ± 47	87.67 ± 25	98.23±30
KT	123.42 ± 21	60.25 ± 20	65.33 ± 19	97.00 ± 33	86.50±30
WIN	190.17 ± 40	109.17 ± 42	81.75 ± 44	98.50 ± 45	119.89±30
KT-WIN	77.75 ± 20	61.58 ± 20	58.67 ± 20	45.08 ± 12	60.77±30
JW	80.17 ± 28	93.92 ± 46	90.67 ± 47	77.17 ± 45	85.48±30
KT-JW	102.25 ± 34	51.33 ± 8	133.58 ± 22	105.58 ± 29	98.19±30
CBD	63.25 ± 22	28.83 ± 5	49.92 ± 23	35.33 ± 12	44.33±30
KT-CBD	114.75 ± 41	115.75 ± 36	75.25 ± 27	185.75 ± 42	122.87±30
KT-AM251	88.00 ± 25	30.75 ± 4	22.08 ± 5	44.17 ± 23	46.25±30
KT-AM630	65.25 ± 21	97.83 ± 29	31.83 ± 4	14.92 ± 2	52.46±30

One-way ANOVA, two-way repeated measures ANOVA and pair wise comparison using estimated marginal means were used in the statistical analysis, and all results are mean ± SEM (n=6); NCON: vehicle (2% Tween 80); KT: khat 300mg/kg; WIN: win 55,212-2 1mg/kg (non-selective cannabinoid receptor agonist); JW: JWH133 5mg/kg (selective cannabinoid 2 receptor agonist), CBD: Cannabidiol 5mg/kg (phytocannabinoid, negative allosteric modulator of CB1R); AM251; Cannabinoid 1 receptor inverse agonist/ antagonist (1mg/kg); and AM630 Cannabinoid 2 receptor inverse agonist/antagonist (1mg/kg).

Table 5: Mean number of wrong decisions to find the goal box in the acquisition phase of the Multiple T maze experiment

Mean wrong decisions					
Groups	**D1**	**D2**	**D3**	**D4**	**D1-D4**
NCON	2.33 ± .7	3.58 ± .8	3.17 ± 1	1.67 ± .5	2.69 ± .7
KT	4.75 ± .9	3.17 ± .5	3.08 ± .7	2.67 ± 1	3.42 ± .7
WN	6.33 ± 1	5.25 ± .7	4.25 ± 1	3.00 ± 1	4.71 ± .7[al#]
KT-WN	4.67 ± 1	3.17 ± .6	2.58 ± .4	2.00 ± .3	3.10 ± .7
JW	3.25 ± .8	2.83 ± .6	2.42 ± .6	1.50 ± .5	2.50 ± .7
KT-JW	3.50 ± .7	2.92 ± .8	4.42 ± .7	3.00 ± .5	3.46 ± .7
CBD	3.08 ± .6	1.50 ± .4	1.92 ± .5	.92 ± .6	1.85 ± .7
KT-CBD	5.00 ± 1	3.92 ± 1	2.08 ± .6	3.75 ± .9	3.69 ± .7
KT-AM251	1.83 ± .3	2.25 ± .7	.67 ± .3	1.00 ± .5	1.44 ± .7[bl#]
KT-AM630	3.50 ± .9	3.25 ± .7	2.08 ± .7	.17 ± .1	2.25 ± .7

One-way ANOVA, two-way repeated measures ANOVA and pair wise comparison using estimated marginal means of two-way reapeted measure ANOVA were used in the statistical analysis, and all results are mean ± SEM (n=6); NCON: vehicle (2% Tween 80); KT: khat 300mg/kg; WIN: win 55,212-2 1mg/kg (non-selective cannabinoid receptor agonist); JW: JWH133 5mg/kg (selective cannabinoid 2 receptor agonist), CBD: Cannabidiol 5mg/kg (phytocannabinoid, negative allosteric modulator of CB1R); AM251; Cannabinoid 1 receptor inverse agonist/ antagonist (1mg/kg); and AM630 Cannabinoid 2 receptor inverse agonist/antagonist (1mg/kg).[1]: $p < 0.01$, a:compared to vehicle (2% Tween 80), b; compared to Khat, [#]: pairwise comparison

4.2.2 *Effect on memory*

On day 5 STM was assessed and based on a one-way ANOVA analysis, groups that were treated either with khat or cannabinoid ligands did not show significant intergroup variation in the latency (Table 6) to find the goal box and in the number of mean wrong decisions (Table 7) committed in the way to goal box compared to vehicle-treated mice. Khat treatment tended to reduce latency by about 50% compared to controls, although the difference failed to reach statistical significance. The effect of khat was further enhanced (54%) when combined with AM251. Longer latency was observed with mice treated with WIN,55-212-2. In making incorrect decisions, all groups

invariable made the same number of decisions, with mice treated with WIN,55-212-2 either alone or in combination with khat showing a relatively higher count.

Table 6: Performance of mice in the short term memory test (mean latency to find the goal box) using Multiple-T-maze experiment

Probe Trial MTM: Mean Latency(sec)	
STM (D5)	
GROUPS	**MEAN ±SEM**
NCON	71.83 ± 45.7
KT	36.00 ± 11.9
WN	94.67 ± 41.7
KT-WN	45.83 ± 15.7
JW	16.83 ± 3.9
KT-JW	22.67 ± 3.1
CBD	73.67 ± 47.4
KT-CBD	44.67 ± 15.2
KT-AM251	16.67 ± 2.2
KT-AM630	32.83 ± 5.3

One-way ANOVA was used in the statistical analysis, and all results are mean ± SEM (n=6); NCON: vehicle (2% Tween 80); KT: khat 300mg/kg; WIN: win 55,212-2 1mg/kg (non-selective cannabinoid receptor agonist); JW: JWH133 5mg/kg (selective cannabinoid 2 receptor agonist), CBD: Cannabidiol 5mg/kg (phytocannabinoid, negative allosteric modulator of CB1R); AM251; Cannabinoid 1 receptor inverse agonist/ antagonist (1mg/kg); and AM630 Cannabinoid 2 receptor inverse agonist/antagonist (1mg/kg).

Table 7: Performance of mice in the short term memory test (mean number of wrong decisions) using Multiple-T-maze experiment

Probe Trial MTM: Mean wrong decisions (sec) STM(D5)	
Groups	**MEAN ±SEM**
NCON	4.33 ± 1.2
KT	6.17 ± .7
WN	8.00 ± 1
KT-WN	7.67 ± .8
JW	3.50 ± 1
KT-JW	6.00 ± .5
CBD	4.83 ± 1
KT-CBD	5.67 ± 1
KT-AM251	3.00 ± 1
KT-AM630	4.50 ± 1

One-way ANOVA was used in the statistical analysis, and all results are mean ± SEM (n=6); NCON: vehicle (2% Tween 80); KT: khat 300mg/kg; WIN: win 55,212-2 1mg/kg (non-selective cannabinoid receptor agonist); JW: JWH133 5mg/kg (selective cannabinoid 2 receptor agonist), CBD: Cannabidiol 5mg/kg (phytocannabinoid, negative allosteric modulator of CB1R); AM251; Cannabinoid 1 receptor inverse agonist/ antagonist (1mg/kg); and AM630 Cannabinoid 2 receptor inverse agonist/antagonist (1mg/kg).

Regarding LTM, ANOVA showed that no significant difference exists among groups in the mean latency to find the goal box (Table 8). LTM was also assessed by analyzing the mean wrong decisions done on the way to the goal box. Similarly, no significant intergroup variation was observed by one-way ANOVA between mice treated with vehicle and khat and/or cannabinoid agonists. Moreover, a combination of khat and cannabinoid ligands did not produce a significant effect in LTM compared to groups that took khat alone (Table 9).

Table 8: Performance of mice in the long term memory test (mean latency to find the goal box) using Multiple-T-maze experiment

Probe Trial MTM: Mean latency (sec) LTM(D12)	
Groups	**MEAN ±SEM**
NCON	75.00 ± 45
KT	76.17 ± 45
WN	83.00 ± 45
KT-WN	92.83 ± 42
JW	27.83 ± 3
KT-JW	113.17 ± 59
CBD	63.83 ± 47
KT-CBD	186.17 ± 51
KT-AM251	18.83 ± 2
KT-AM630	16.33 ± 2

One-way ANOVA were used in the statistical analysis, and all results are mean ± SEM (n=6); NCON: vehicle (2% Tween 80); KT: khat 300mg/kg; WIN: win 55,212-2 1mg/kg (non-selective cannabinoid receptor agonist); JW: JWH133 5mg/kg (selective cannabinoid 2 receptor agonist), CBD: Cannabidiol 5mg/kg (phytocannabinoid, negative allosteric modulator of CB1R); AM251; Cannabinoid 1 receptor inverse agonist/ antagonist (1mg/kg); and AM630 Cannabinoid 2 receptor inverse agonist/antagonist (1mg/kg).

TABLE 9: Performance of mice in the long term memory test (mean number of wrong decisions) using Multiple-T-maze experiment

Probe Trial MTM: Mean wrong decisions	
LTM(D12)	
Groups	**MEAN ± SEM**
NCON	8.00 ± 2
KT	7.50 ± .9
WN	12.33 ± 1
KT-WN	7.00 ± 1
JW	4.83 ± 1
KT-JW	5.00 ± 1
CBD	2.17 ± .4
KT-CBD	5.67 ± 2
KT-AM251	3.83 ± .8
KT-AM630	3.00 ± .7

One-way ANOVA was used in the statistical analysis, and all results are mean ± SEM (n=6); NCON: vehicle (2% Tween 80); KT: khat 300mg/kg; WIN: win 55,212-2 1mg/kg (non-selective cannabinoid receptor agonist); JW: JWH133 5mg/kg (selective cannabinoid 2 receptor agonist), CBD: Cannabidiol 5mg/kg (phytocannabinoid, negative allosteric modulator of CB1R); AM251; Cannabinoid 1 receptor inverse agonist/ antagonist (1mg/kg); and AM630 Cannabinoid 2 receptor inverse agonist/antagonist (1mg/kg).

4.3 Performance in RAM

4.3.1 *Working memory error*

WME is considered when the mice re-enter into the baited arms, which serves as a measure of STM status of mice. Based on one-way ANOVA post hoc Tukeys analysis no statistically significant difference was observed among groups. Despite no significant intergroup variation being seen, groups that were treated with khat showed a 7.6% reduction in the mean number of WME compared to negative control groups. However, a further analysis i.e. a two-way repeated measure ANOVA pairwise comparison test using estimated marginal means showed that there was a significantly small number of mean WME committed by mice treated with khat and the antagonist AM251 across the 5 days of training compared to groups that took vehicle (p=0.023) and khat (p=0.046) (Table 10).

Table 10: Performance of mice in the short term memory test (Working memory errors) using Radial arm maze experiment.

	Working Memory Errors					
Groups	**D1**	**D2**	**D3**	**D4**	**D5**	**D1-D5**
NCON	5.28 ± 1	5.56 ± .7	6.67 ± 1	9.17 ± 2	5.00 ± 1	6.33 ± 2
KT	5.83 ± 1	4.44 ± 1	6.39 ± 2	5.56 ± 1	7.01 ± 2	5.84 ± 2
WN	7.22 ± 2	5.28 ± 1	8.61 ± 3	4.72 ± 1	5.28 ± 2	6.22 ± 2
KT-WN	5.00 ± 2	4.83 ± 1	3.33 ± 1	2.50 ± .6	5.00 ± 1	4.13 ± 2
JW	7.22 ± 1	2.22 ± 1	2.50 ± .7	1.67 ± .7	3.06 ± 1	3.33 ± 2
KT-JW	4.67 ± .9	4.50 ± 1	4.17 ± 1	4.00 ± 1	2.50 ± .9	3.96 ± 2
CBD	3.61 ± 1	5.00 ± 2	6.11 ± 2	6.39 ± 2	5.00 ± 1	5.22 ± 2
KT-CBD	8.83 ± 2	4.67 ± .8	5.33 ± 1	3.83 ± 1	5.28 ± 2	5.59 ± 2
KT-AM251	3.33 ± .5	3.61 ± .6	2.44 ± .2	2.00 ± .4	.83 ± .3	2.44 ± 2$^{a1\#b1\#}$
KT-AM630	5.28 ± 1	5.56 ± 1	6.39 ± 1	6.18 ± 2	5.28 ± .7	5.74 ± 2

One-way ANOVA, two-way repeated measures ANOVA and pair wise comparison using estimated marginal means of two-way repeated measure ANOVA were used in the statistical analysis, and all results are mean ± SEM (n=6); NCON: vehicle (2% Tween 80); KT: khat 300mg/kg; WIN: win 55,212-2 1mg/kg (non-selective cannabinoid receptor agonist); JW: JWH133 5mg/kg (selective cannabinoid 2 receptor agonist), CBD: Cannabidiol 5mg/kg (phytocannabinoid, negative allosteric modulator of CB1R); AM251; Cannabinoid 1 receptor inverse agonist/ antagonist (1mg/kg); and AM630 Cannabinoid 2 receptor inverse agonist/antagonist (1mg/kg).).[1]: $p < 0.05$, a: compared to vehicle (2% Tween 80), b; compared to Khat, [#]: pairwise comparison

4.3.2 *Reference memory error*

RME is used to assess LTM and is considered when the mice enter into the never baited arms. Based on one-way ANOVA post hoc Tukeys analysis no statistical significance was observed among groups (Table 11). Further analysis by a two-way repeated measure ANOVA pairwise comparison test using estimated marginal means showed that there was a significantly minimal number of mean wrong decisions made by mice treated with khat and the antagonists AM251($p=0.23$) and AM630 ($p=0.32$) across the 5 days of training compared to groups that took the vehicle. Likewise, mice treated with CBD also committed a significantly ($p=0.049$) small number of RME compared to vehicle-treated groups based on the pairwise comparison.

Table 11: Performance of mice in the long term memory test (Reference memory errors) using Radial arm maze task.

	Reference memory errors					
Groups	**D1**	**D2**	**D3**	**D4**	**D5**	**D1-D5**
NCON	10.54 ± 1	6.78 ± 1	11.39 ± 1	14.44 ±1	11.39 ±1	10.91 ± 1
KT	10.00 ± 1	8.06 ± 1	7.22 ± 1	7.50 ±1	9.58 ±1	8.47 ± 1
WN	13.33 ± 3	6.94 ± 1	10.83 ± 2	8.89 ±1	8.33 ±1	9.67 ± 1
KT-WN	7.17 ± 1	8.17 ± 1	10.17 ± 1	9.33 ±.8	8.89 ±1	8.74 ± 1
JW	9.44 ± 1	6.67 ± 2	9.17 ± 1	7.22 ±2	5.28 ±1	7.56 ± 1
KT-JW	10.83 ± 1	8.61 ± 1	7.78 ± 1	8.06 ±1	9.17 ±1	8.89 ± 1
CBD	8.33 ± 1	10.33 ± .8	7.83 ± 1	5.83 ±1	6.39 ±2	7.74 ± 1[a1#]
KT-CBD	6.94 ± 1	6.39 ± 1	9.44 ± 2	9.17 ±2	8.89 ±2	8.16 ± 1
KT-AM251	8.50 ± 2	6.17 ± 1	7.00 ± 1	7.00 ±1	7.50 ±1	7.2 ± 1[a1#]
KT-AM630	8.61 ± 1	8.33 ± 1	5.56 ± 1	8.33 ±1	6.39 ±2	7.4 ± 1[a1#]

One-way ANOVA, two-way repeated measures ANOVA and pair wise comparison using estimated marginal means of two-way repeated measure ANOVA were used in the statistical analysis, and all results are mean ± SEM (n=6); NCON: vehicle (2% Tween 80); KT: khat 300mg/kg; WIN: win 55,212-2 1mg/kg (non-selective cannabinoid receptor agonist); JW: JWH133 5mg/kg (selective cannabinoid 2 receptor agonist), CBD: Cannabidiol 5mg/kg (phytocannabinoid, negative allosteric modulator of CB1R); AM251; Cannabinoid 1 receptor inverse agonist/ antagonist (1mg/kg); and AM630 Cannabinoid 2 receptor inverse agonist/antagonist (1mg/kg).[1]: $p < 0.05$, a: compared to vehicle (2% Tween 80), [#]: pairwise comparison

5. Discussion

The impact of khat on spatial learning and memory has previously been thoroughly evaluated by a few scholars. The endogenous systems involved and the molecular basis for the effects of khat in combination with other substances have rarely been the subject of study. Therefore, the goal of the current investigation was to determine the interaction of khat extract with the EC system in modulating spatial learning and memory in mice after giving khat and cannabinoid ligands concurrently.

In the present study, three batteries of tests were utilized, from which learning was assessed by using MWM and MTM, while memory was investigated by using all three models i.e. MWM, MTM and RAM. In the MWM and MTM task, all groups had shown a reduction in the measured values across the four training days and this demonstrates that learning had taken place. In both of the two learning models khat extract did not show a significant effect on spatial learning. It did also have no effect on spatial memory in the RAM.

The finding that khat did not have a discernible effect on learning and memory is consistent with previous studies (Mohammed et al., 2014; Assefa et al., 2018; Seifu and Engidawork, 2019, Geresu et al., 2016). More briefly, in a thorough study conducted in Ethiopia (Mohammed et al., 2014), learning and memory were assessed using MWM, MTM, and other behavioral paradigms after mice were exposed to acute, subacute, and subchronic doses of khat, revealed that acute and subacute exposure of crude khat extract had no impact on learning and memory. The paradigms used and the environment of this investigation was essentially identical to the current study so regarding the effect of khat extract on spatial learning, the present study is consistent with this earlier finding.

In addition to this, a recent study conducted in the same setting using the same battery of tests (Seifu and Engidawork, 2019) showed that crude, alkaloid, and nonalkaloid fractions of khat had no impact on learning and memory. This is further corroborated by a study (Assefa et al., 2018) using the MTM test in mice, which found that acute khat exposure did not increase spatial learning and memory in comparison to control groups. Additionally, it did not demonstrate a discernible decline in incorrect decision-making in the MTM test.

Furthermore, a study that was done using another model i.e. Y-maze, revealed that crude khat extract did not affect working memory compared to controls (Geresu et al., 2016).

Unlike to the presnt finding, Kimani and Nyongesa (2008) revealed inconsistent findings i.e, compared to low and moderate dosages, the high dose of khat extract considerably ($P \leq 0.05$) enhanced accuracy for spatial memory in the MWM task. This study has demonstrated that khat extract has a selective effect on spatial learning and memory, with low doses having no effect on learning but impeding memory, and high doses having no effect on learning but enhancing memory.

In light of these findings and those of earlier research, it is reasonable to conclude that acute exposure to khat extracts has no impact on learning and memory. The reason behind khats' insignificant effect may be due to its effect on serotonergic and dopaminergic neurotransmission, since these neurotransmissions in the hippocampus may have opposing effects on learning and memory (Kimani and Nyongesa 2008).

Treatment of animals with the phytocannabinoid, CBD, a negative allosteric modulator of CB1 receptors, significantly reduced the mean latency to find the hidden platform in the MWM task as well as the reference memory errors in RAM compared to the negative control group, while its combination with khat attenuated this effect. Studies conducted in transgenic Alzheimer's disease models demonstrated that prophylactic and therapeutic chronic CBD administration improves spatial acquisition of previously rewarded places (Chesworth et al., 2022, Amini and 40 Abdolmaleki, 2022). In addition, a study conducted on adolescent mice, to investigate the developmental effect of CBD, also resulted in an increased rate of spatial learning but not spatial memory using the Barens maze (Kaplan et al., 2021).

Although these studies show CBD's procognitive effects, the underlying mechanisms are not known. Recent reports raise the possibility of some of these mechanisms. In APPxPS1 mice, long-term CBD administration can boost the immune system and raise hippocampal autophagy (Hao and Feng, 2021). In addition to increasing microglial migration and decreasing nitrite production, an enhanced immune response by CBD may also improve cognition in APPxPS1 mice by facilitating amyloid-beta phagocytosis and lowering hippocampal amyloid-beta plaque load (Watt et al., 2020; Hao and Feng, 2021). As CBD pretreatment inhibits amyloid beta-mediated LTP deficits in mouse hippocampal slices, it is also possible that CBD ameliorates hippocampal

synaptic plasticity deficits to enhance spatial learning (Hughes and Herron, 2019). But, the current study uses acute dose and normal bred animals, so future research studies would examine the potential mechanism(s) of CBD after acute administration on spatial learning and memory.

Treatment with WIN55,212-2 increased number of incorrect decisions in the MTM compared to controls, which once again the effect was attenuated with co-administration with khat. Chronic exposure to WIN55,212-2 was shown to reduce performance in MWM, which was dependent on extent of cell proliferation in the dorsal hippocampus (Abboussi et al., 2014). Although cell proliferation was not assessed in the present study, acute exposure to WIN also produced a deficit in MTM performance. Taken together, both findings suggest that both acute and chronic exposure to WIN might have a negative impact on performance in spatial learning tasks.

By contrast, the selective CB2R agonist, JWH133, did not produce any substantial effect, suggesting that cannabinoids modulate learning and memory via CB1 than CB2 receptors. Indeed, i.p administration of WIN55,212-2 (1mg/kg and 3mg/kg) brings about memory deficit via a CB1R mediated mechanism in the MWM paradigm (Robinnson et al., 2010) is consistent with this notion.

Interestingly, learning (MWM, MTM), STM (RAM) and LTM (RAM) were significantly enhanced when khat was coadministered with the CB1R inverse agonist/antagonist, AM251. Moreover, inhibiting the CB2R by AM630 revealed similar results, particularly in spatial learning (MWM) and LTM (RAM).

These findings collectively indicate that khat appears to enhance learning and memory when combined with the antagonists and the same effect does not seem to be observed when it is combined with agonists. From these observations it is plausible to assume that the interaction could be mediated in a CB1-depndent and -independent manner. Although the current study is the first to the best of our knowledge to examine the interaction between khat and cannabinoid ligands in modulating spatial learning and memory, similar results were reported from earlier studies (namely that inhibition of CB1R by an antagonist significantly enhances both acquisition and memory consolidation) (Bialuk and Winnicka, 2011; Wise et al., 2009, Torres et al., 2021).

In general, the hypothesis initially proposed is rejected since the concurrent administration of khat and the CBR agonists did not produce significant impairment rather, it lacks any cognitive effect compared to either khat or control group in all the models used. Likewise, khat does not have a

substantial effect i.e. it neither impaired nor improved spatial learning and memory. In contrast to khat, the synthetic nonselective CBR agonist WIN 55,212-2 caused a deficit in learning and memory while CBD, a negative allosteric modulator of CB1R, greatly improved spatial learning and LTM. When given alongside with khat, the cognitive effects of both CBD and WIN 55,212-2 were reversed and rendered ineffective. Contrary to this, when administered alone or in combination with khat, the selective CB2R agonist JWH133 had no effect on cognition. Furthermore, the inhibition of brain CBRs by inverse agonists/antagonists dramatically improved khat's effects on spatial learning and memory. Particularly, the brain CB1R inhibitory impact of AM251 results in a much enhanced effect on spatial learning and memory. This indicates that the EC system has a negative modulatory effect on the cognitive effects of khat. The reversal of the effect of CBD and WIN 55,212-2 but not JWH133 when coadministered with khat mainly manifests that the cognitive effects of khat mainly modulated at the point of CB1R. This was further reinforced by the significantly improved substantial spatial learning and memory that were observed from concurrent khat and AM251 treatment. Overall, based on the current study, it is possible to conclude that there is interaction between khat and the cannabinoid system and this interaction mainly revolves around the point of CB1R.

6. Conclusion and recommendations

6.1 Conclusion

The research presented here showed how, depending on the paradigm used, the EC system plays a role in modulating khat's effects on spatial learning and memory. We generated further evidence that acute khat exposure does not have a substantial effect in spatial learning and memory. Co-administration of khat with cannabinoid agonists attenuate the effect produced by the agonist regardless of the direction of change (enhanced or reduced cognition). Co-administration with antagonists, however, will have a pro-cognitive effect. Yet, more research is needed to determine the precise mechanism through which the EC system interacts with and modifies the effects of khat on spatial learning and memory.

6.2 Recommendations

- Subacute administration of Khat and cannabinoid ligands' impact on modulation of khats' effect on acquisition and memory performance needs to be investigated.
- Interaction at the molecular level should be investigated.

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