Cognitive-Enhancing, Anti-Lipid Peroxidation, Qualitative Phytochemistry, And Toxic Effects of The Aqueous Aerial Part Extract of *Launaea cornuta* (Hochst. Ex Oliv. And Hiern.)

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J56/37921/2020

A Research Thesis Submitted in Partial Fulfilment of the Requirements for the Award of a Master of Science Degree in Pharmacology and Toxicology

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September 2023.

DECLARATION

I declare that this thesis is my original work and has not been previously presented in this University or any other Institution for the award of a degree, or any other award.

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Course Name: MASTER OF SCIENCE IN PHARMACOLOGY AND TOXICOLOGY

Title of the work: 'COGNITIVE-ENHANCING, ANTI-LIPID PEROXIDATION, QUALITATIVE PHYTOCHEMISTRY, AND TOXIC EFFECTS OF THE AQUEOUS AERIAL PART EXTRACT OF *LAUNAEA CORNUTA* (HOCHST. EX OLIV. AND HIERN.)'

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DEDICATION

I would to dedicate this work to my Father and Mother who have been a strong pillar in my life. They have been an endless support to me and have ensured that I have had a quality life and education all through. They have also been a great source of encouragement all along the way.

ACKNOWLEDGEMENTS

I wish to acknowledge God for the strength and grace throughout my journey. I also would like to express my gratitude to my Parents, Henry and Joyce Maina, for the great emotional and financial support granted in the whole course. I sincerely acknowledge the support of my Supervisors Prof. James Mbaria (University of Nairobi) and Dr. Irene Kamanja (Egerton University) for their continuous guidance throughout this project.

My sincere gratitude goes to Dr. Gervason Moriasi, Mount Kenya University for the participation in laboratory work and for assistance in sourcing of reagents. I would also like to acknowledge Nelson Mandela, Mount Kenya University for availing himself for assistance in the project.

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ABBREVIATIONS AND ACRONYMS

Abbreviation/Acronym	Meaning
$\bar{x}$	Mean
°C	Degrees Celsius/Centigrade
Ach	Acetylcholine
AchE	Acetylcholinesterase
AD	Alzheimer's Disease
ADHD	Attention Deficit Hyperactivity Disorder
AMPA	α-amino-3-hydroxyl-5-methyl-4-isoxazole-propionate
ANOVA	Analysis of Variance
AOT	Acute Oral Toxicity
Aβ	Amyloid Beta
EEG	Electroencephalogram
FDA	Food and Drug Administration
HIV	Human Immunodeficiency Virus
MDA	Malondialdehyde
Mg/Kg bw	Milligrams per Kilogram body weight
MWM	Morris Water Maze
NMDA	N-Methyl-D-Aspartate
OECD	Organization for Economic Development and Cooperation
p. o	*Per os*
PD	Parkinson's Disease
ROS	Reactive Oxygen Species
SEM	Standard Error of the Mean
TBARS	Thiobarbituric Acid Reactive Substances
UDP	Up-and-Down
WHO	World Health Organization

ABSTRACT

Currently, there is no cure for dementia and its associated complications like cognitive impairment, and the available medicines only offer symptomatic relief without altering the course of the disease. Conventionally utilised armamentaria for managing cognitive deficits suffer various drawbacks, such as low- efficacy, adverse effects, unaffordability, and inaccessibility which limit their clinical applications, hence the need for alternative therapies, especially from natural sources. Despite the longstanding ethnomedicinal usage of various plants to treat cognitive deficits, their pharmacological efficacy and safety have not been validated empirically. Therefore, this study was designed to investigate the acute oral toxicity, cognitive-enhancing, and anti-lipid peroxidation effects, and qualitative phytochemistry, of the aqueous aerial part extract of *Launaea cornuta* (Hochst. Ex Oliv. And Hiern.) based on its ethnomedicinal background. The acute oral toxicity effects of the aqueous aerial part extract of *L. cornuta* were investigated in Swiss albino mice using the up-and-down procedure described by the Organisation for Economic Cooperation and Development guideline number 425. The Morris Water Maze (MWM) technique was adopted to determine the cognitive-enhancing effects of the extract in Ketamine-induced cognitive-impaired mice. After that, the concentration of malondialdehyde (MDA) in the whole brain of experimental mice involved in the Morris Water Maze (MWM) experiment was determined using the Thiobarbituric Acid Reactive Substances (TBARS) method described previously. Qualitative phytochemical screening of the study extract was performed according to standard procedures. In this study, the aqueous aerial part extract of *L. cornuta* did not cause any clinical signs of acute oral toxicity in mice at all the tested doses and the median lethal concentration (LD_{50}) was deemed to be >2000 mg/Kg bw, depicting its safety. The results further showed that the studied plant extract significantly ($P<0.05$) averted the Ketamine-induced cognitive impairment in mice dose-dependent, as witnessed by the reduced escape latencies and navigation distances and longer latencies in the target quadrant during the probe trial during the Morris Water Maze experiment. The extract significantly ($P<0.05$) reduced the malondialdehyde concentrations in mice in a dose-dependent fashion, inferring its anti-lipid peroxidation, hence antioxidative stress efficacy, partly explaining its ameliorative effects of ketamine-induced cognitive deficits in experimental mice. The results also revealed that the aqueous aerial part extract of *L. cornuta* contains various phytochemicals, such as flavonoids and phenols, associated with cognitive enhancement and antioxidant efficacy, among other pharmacologic effects. Therefore, this study concluded that the aqueous aerial part extract of *L. conuta* does not cause any observable clinical signs of acute oral toxicity in Swiss albino mice, possesses cognitive-enhancing and anti-lipid peroxidation effects, and contains various phytochemicals responsible for the reposted bioactivities, among other pharmacologic effects. Further empirical studies are recommended to determine and characterise the extract's specific cognitive-enhancing amalgams, specific action mechanisms, and complete toxicity profiles.

CHAPTER ONE

INTRODUCTION

1.1 Background information

Dementia represents multiple debilitating neurodegenerative syndromes, characterised by progressive deterioration of cognitive function in the brain (World Health Organisation, 2019). Patients suffering from dementia present memory and learning deficits, altered behaviour, impaired thinking, reasoning and judgement, and impaired physical and professional functioning, which affect their quality of life (Pink *et al.*, 2018). Although advancing age is a risk factor for dementia, with those over 60 years being the most affected, dementia is not an inevitable consequence of ageing, and can occur in persons of all ages, social classes, and gender due to an interplay of various predisposing factors. Such factors include genetics, environmental triggers like particulate matter pollution, traumatic injuries, developmental abnormalities, commodities such as diabetes mellitus, among others (Kuo *et al.*, 2020; Mendez, 2017; Nichols *et al.*, 2022; Ru *et al.*, 2021).

Epidemiological studies show that Alzheimer's Disease (AD) is the most common type of dementia and the fifth-leading cause of death among the elderly (≥65 years) (Alzheimer's Association, 2022). Currently, over 50 million persons are living with dementia-associated cognitive abnormalities worldwide, whereby over two-thirds of the affected reside in low- and medium-income countries, especially those located in Sub-Saharan Africa and Southeast Asia (LMICs) (Alzheimer's Association, 2022). The burden of dementia and its associated sequelae is unquantifiable, considering the inadequacy of healthcare infrastructure and resources, and the high poverty levels, especially in Sub-Saharan Africa and Southeast Asia, where a disproportionately high burden of disease is reported. Worryingly, it is projected that over 152

million persons will have dementia, especially AD, by 2050 if appropriate mitigation measures are not implemented (Kivipelto *et al.*, 2018).

Currently, dementia has no cure, and the conventional armamentaria only offer symptomatic relief without altering the disease course (BMJ Publishing group Ltd., 2018; Duong *et al.*, 2017; Mcshane *et al.*, 2019; Pink *et al.*, 2018; Szeto and Lewis, 2016). Cholinergic agonists-the main class of drugs for cognitive impairment, especially cholinesterase inhibitors, prevent the degradation of acetylcholine at the synaptic cleft to maintain nerve firing, hence enhancing cognition. However, these therapies exhibit low efficacy, have a short half-life, requiring higher and more frequent dosing, which, unfortunately, is associated with adverse effects, limiting their clinical significance (BMJ Publishing group Ltd., 2018; Duong *et al.*, 2017; Mcshane *et al.*, 2019; Pink *et al.*, 2018; Szeto and Lewis, 2016). Besides, the conventional medications for dementias such as AD and associated cognitive deficits are relatively inaccessible and unaffordable to the most vulnerable patients, especially those living in Sub-Saharan African countries, which account for over 80 % of the global disease burden (Mattap *et al.*, 2022; Nichols *et al.*, 2022). Moreover, recent research reports indicate that there is a significantly higher financial and emotional burden borne by dementia patients, their caregivers, health facilities, and countries, either directly or indirectly, owing to its devastating nature. Notably, the reported costs are underestimated considering the inadequacy of reliable data, especially from the Low- and Medium-Income Countries (LMICs) (Alzheimer's Association, 2022; Mattap *et al.*, 2022). Considering the challenges of conventional dementia therapy, alternative stratagems for preventing, slowing its progression, or averting its devastating effects are currently being sought (Shah *et al.*, 2016).

Plant-based products and extracts are a viable alternative source of efficacious and safe lead compounds for drug development owing to their longstanding ethnomedicinal applications in healthcare. Besides, plant-based products are arguably accessible, affordable, efficacious, and safer (when validated) than synthetic drugs, hence their prominence worldwide (Baradaran *et al.*, 2012; Shakya, 2016; Singhal *et al.*, 2012). Ample research has demonstrated that medicinal plants synthesise numerous bioactive amalgams, some of which have led to the discovery of potent medicines in modern medicine (Bagetta, 2012; Fürst and Zündorf, 2014; Jamshidi-Kia *et al.*, 2018). However, there is insufficient empirical data to validate their healing claims and safety. Additionally, various safety concerns regarding medicinal plants and their preparations have been raised, hence prevaricating their integration into conventional practice (George, 2011). For instance, the lack of proper legislative framework, unclear preparation procedures, storage, labelling, marketing, and dosage regimens for each disease, and empirical data on herb-herb and herb-conventional drug interactions, are the main hindrances to the recognition of traditional medicine practice, its advancement, and integration into conventional healthcare in many countries (Abdullahi, 2011; Gakuya *et al.*, 2020; Zhang *et al.*, 2012).

Launaea cornuta Hochst (Ex Oliv. and Hiern.) is a small, erect herb of the Asteraceae (Compositae)commonly known as the 'bitter lettuce' and grows up to 1.5 Metres above the ground (Kokwaro, 2009). The plant is indigenous to Kenya and many African countries. It is locally (Kenya) known as *'mchunga'* in Swahili, *'Muthunga'* (Meru, Kikuyu, and Embu), *'Mnyinya'* in Taita and *'Achak'* in Luo (Maundu and Tengnas, 2005). It is used to treat microbial infections, such as gonorrhoea, typhoid, inflammatory conditions like swollen testicles, earache, stomach pains, chronic joint pains, diabetes, hypertension, and memory decline, among other applications (Karau *et al.*, 2014; Wambugu *et al.*, 2011).

Previous research shows that the aqueous root extract of *L. cornuta* has considerable antioxidant and anti-inflammatory efficacy and contains various bioactivate phytocompounds (Akimat *et al.*, 2021), with potential cognitive-enhancing efficacy (Moriasi *et al.*, 2020a). Even though *L. cornuta* has been used extensively to treat various diseases, including dementia and associated complications in traditional medicine, its pharmacological efficacy and potential have not been validated empirically. Therefore, this study investigated the cognitive-enhancing, anti-lipid peroxidation, toxic effects, and qualitative phytochemistry of the aqueous aerial part extract of *L. cornuta* in an endeavour to validate its healing claims and as a potential source of safe and efficacious therapies for dementia and associated complications.

1.2 Statement of the problem and justification of the study

Cognitive impairment is among the leading causes of disability and debility in more than 35.6 million persons globally, especially those aged over 65 years (World Health Organization (WHO), 2019). Dementia-associated cognitive impairment is not only debilitating to the affected subjects by affecting their health, socioeconomic, and professional life, but also traumatises their dependants, caregivers, immediate family, and the wider society in terms of management, financial burden, and the associated psychological and emotional burden (Baird *et al.*, 2017; Hugo and Ganguli, 2014; Santos *et al.*, 2018). Sadly, despite the attempts to develop curative therapies for dementia-associated cognitive impairment, there has not been any notable success and the clinical trials have been disappointing.

The current drugs approved by the United States (US) Food and Drug Administration (FDA) for the management of cognitive impairment in dementia patients are only palliative rather than curative, hence do not alter the course of disease (Amir *et al.*, 2008; Casey *et al.*, 2010; Szeto and Lewis, 2016). Besides, the commonly prescribed drugs against cognitive impairment, such as Donepezil, Memantine, and Rivastigmine, cause severe diarrhoea, nausea, dizziness,

headache, among other adverse effects in patients, which limit their clinical significance. Additionally, other drugs, like Galantamine, which are used presently have proved to be ineffective in ameliorating cognitive impairment in most of the affected patients (Alzheimer Association, 2019; Casey *et al.*, 2010; Szeto and Lewis, 2016). Moreover, these conventionally prescribed medicines cause addiction, thereby leading to overdose, and further exacerbating their side effects with deleterious sequelae in patients (Kalapatapu *et al.,* 2017). Despite the attempts to elucidate and develop curative therapies based on pathological findings, no therapeutic cure is yet available and clinical trials of some synthetic medications have been disappointing (Casey *et al.*, 2010). Consequently, the need for alternative and complementary stratagems is warranted. Worryingly, the number of persons suffering from cognitive impairment is feared to increase to over 152 million by 2050, if no remedial measures are taken to avert the upward trend (WHO, 2019).

Medicinal plants offer a viable alternative source of efficacious and safe lead compounds, owing to their composition of various bioactive phytochemicals, and marked ethnomedical utilisation with appreciable level of efficacy across human history. Indeed, the WHO report posits that over 80 % of the world population, especially in the low- and medium-income countries, like Kenya utilize folklore medicine to treat various diseases, possibly due to the presumed efficacy, easy accessibility, affordability, and safety (WHO, 2013). However, despite the vast ethnomedical claims about various plants in treating cognitive deficits and associated ailments, only a few have been validated empirically (Palhares *et al.*, 2015). Various plants, including *L. cornuta*, have been utilised in traditional medicine to prevent dementia, and even reverse its devastating effects, like cognitive impairment (Chang *et al.*, 2016; Tang *et al.*, 2013; Tewari *et al.*, 2018; Tian *et al.*, 2010; Karau *et al.*, 2014); however, there is scanty empirical data to validate these healing claims (Chang *et al.,* 2016).

Also, various concerns regarding the efficacy and safety of medicinal plants have been raised, which have hindered empirical research, and the integration of herbalism into conventional healthcare (George, 2011; Nasri and Shirzad, 2013). For instance, there are no clear dosage regimens of herbal preparations for specific diseases, and crucial information regarding preparation procedures, labelling, contraindications, and interaction effects elusive, besides, there is a lack of clear legislative framework in many countries guiding traditional medicine practice, which affect its credibility. Thus, evaluation of toxicity profile of promising plants such as *L. cornuta* may help to elucidate their medicinal potential and safety and help to authenticate their folkloric usage.

Therefore, this study was carefully designed to empirically investigate the cognitive-enhancing and anti-lipid peroxidation efficacy of the aqueous aerial part extract of *L. cornuta*, its phytochemical composition and acute oral toxicity to offer valuable data, which will form a basis for the discovery of efficacious, safe, and affordable, curative therapies for cognitive impairment and other associated neurologic syndromes.

1.3 Study objectives

1.3.1 General objective

The main objective of this study was to investigate the cognitive-enhancing, anti-lipid peroxidation, qualitative phytochemistry, and acute oral toxicity effects of the aqueous aerial part extract of *L. cornuta*.

1.3.2 Specific objectives

This study focused on the following specific objectives.

i. To determine the acute oral toxicity effects of the aqueous aerial part extract of *L. cornuta* in Swiss albino mice.

ii. To determine the *in vivo* cognitive-enhancing effects of the aqueous aerial part extract of *L. cornuta* in ketamine-induced cognitive-impaired Swiss albino mice.

iii. To determine the effects of the aqueous aerial part extract of *L. cornuta* on malondialdehyde (MDA) profiles in ketamine-induced cognitive impaired Swiss albino mice *ex vivo*.

iv. To determine the qualitative phytochemical composition of the aqueous aerial part extract of *L. cornuta.*

1.4 Research questions

The following research questions guided the present study.

i. Does the aqueous aerial part extract of *L. cornuta* cause acute oral toxicity effects in Swiss albino mice?

ii. Does the aqueous aerial part extract of *L. cornuta* have *in vivo* cognitive-enhancing activities in ketamine-induced cognitive-impaired Swiss albino mice?

iii. What are the effects of aqueous aerial part extract of *L. cornuta* on *ex vivo* MDA profiles in the brain of ketamine-induced cognitive-impaired experimental mice?

iv. What is the qualitative phytochemical composition of the aqueous aerial part extract of *L. cornuta*?

CHAPTER TWO

LITERTURE REVIEW

2.1 Dementia and its consequences

Dementia is a mental disorder where an individual loses the ability to think, remember and reason to the point where it interferes with individual's day-to-day activities (Moriasi *et al.*, 2020b). The cognitive functioning is impaired causing emotional instability that may cause the victim to fail to control their emotions and may change their personality. Dementia has got different levels which include the mildest level to the most severe phase that is characterized by complete loss of cognitive functioning to a level where one is assisted in everything even on simple basic daily activities. Dementia prevalence increases with age advancement for instance it is estimated that one third of aged people (65 years and above) suffer from this mental disorder. However, it is not obvious that aging must trigger dementia as most old people do not have dementia.

The cause of dementia to larger extent is unknown, however, it is attributed to changes or abnormalities in the brain. Previous research points out the cause to rare genetic mutations occurring in some individuals. Dementia manifests in different forms varying from one individual to another and depending on the form or the type of dementia. The symptoms include, taking longer to process and accomplish normal daily activities, being paranoid and experiencing hallucinations, losing directions in familiar environment, losing stability while walking, making poor uninformed judgements, confusion, and memory loss (Moriasi *et al.*, 2020a).

Types of dementia include Alzheimer's disease, Lewy body dementia, frontotemporal dementia, vascular dementia, Creutzfeldt-Jakob disease, Parkinson's diseases, Normal pressure hydrocephalus, and mixed dementia (two or more types of dementia manifesting in one

individual). Alzheimer's disease is the commonest of all and its cause is build-up of some proteins such as tau tangles and amyloid plaques. Lewy body dementia is as result of build-up proteins like alpha synuclein also known as Lewy bodies. Vascular dementia is believed to be caused by conditions which interfere with supply of blood and oxygen to brain cells or damages that occur in the blood vessels. Creutzfeldt-Jakob disease is fatal and believed to be caused by build-up of prion proteins because of consuming dairy products from cows contracting mad cow disease. Parkinson's disease like Lewy body dementia is due to increased amounts of synuclein protein or Lewy bodies in the body particularly in the nerve cells. Normal pressure hydrocephalus result from fluid filling up some parts of brain and thus making it gorged (Alzheimer's Association, 2010; 2011; 2012; 2013; 2015).

2.2 Conventional management of dementia-associated cognitive deficits

Dementia is managed conventionally through various interventions such as application of therapy procedures, genetic engineering, learning/educational methods, memory aids like mnemonics, and the use of technology like smartphones. The major mechanisms that have been proved to be effective include mechanical, pharmacological, and genetic methods. The ultimate goal is to restore cognitive functioning or improve its impaired functioning. The use of modern technology in conjunction with mechanical cognitive enhancement involves enhancing and supporting brain function (s). This includes the use of neuroprosthetics such as cochlear implants, deep brain stimulation, external brain stimulation such as electroencephalogram (EEG) electrode array, and chips implanted in the brain for hippocampal memory-mediation (Moriasi *et al.*, 2020a).

The pharmacological method of cognitive improvement involves using medications and other compounds to boost brain function (s). Patients' ability to concentrate, focus, and stay awake while taking brain stimulants like Ritalin and Provigil, which are frequently recommended to

treat a variety of mental problems, has been shown to improve in cases of attention deficit hyperactivity disorder (ADHD). In otherwise healthy people, ampakines—compounds linked to the stability of -amino-3-hydroxyl-5-methyl-4-isoxazole-propionate (AMPA) glutamate receptors—are now being investigated to improve learning and long-term memory retention. (Lynch and Gall, 2006). It is recommended to use acetylcholinesterase inhibitors, such as donepezil (Aricept) and tacrine (Cognex), for symptoms related to memory, thinking, and other dementia associated symptoms like language problems. The goal of this strategy is to stop acetylcholine from degrading and preserve it as a neurotransmitter to promote cholinergic transmission. Despite the significant success of this strategy, delayed adverse outcomes brought on by these medications recur in 50% of the population after some time (6 months to 2 years) (Mcshane *et al.*, 2019).

2.3 Complementary and alternative management of dementia-associated cognitive deficits

Traditional medicine has since been used to manage cases of dementia and other diseases in the world, long before introduction of artificial medicine. Even though they come from all around the plant kingdom, some families and genera of plant species are better than others at curing diseases (Maroyi, 2013). Herbs are thought to be more beneficial than synthetic pharmaceuticals since they are readily biodegradable, non-narcotic in nature, have fewer negative impacts on the environment, and are generally more affordable (Umapathy *et al.*, 2010). Additionally, plants are available, whereas synthetic and conventional medications are more expensive (Saleem *et al.*, 2011).

Herbs' therapeutic effects have been attributed to a variety of phytochemical substances, which act singly or in combination to slow down aging, stop cognitive impairment, prevent memory loss, and otherwise benefit those who suffer from dementia (Akram and Nawaz, 2017). Some

of the plants used to manage dementia include *Valeriana officinalis, Mysristica fragrans, Ferula asafoetida, Evolvulus alsinoides, Pelargonium graveolens, Punica grantum, Zizzyphus vulgaris, Rheum officinale, Bacopa monnieri, Cinchona officinalis and Juglans regia*. Some of these plants for instance *Cinchona officinalis* and *Juglans regia* have been confirmed to have action against cholinesterase activity (Xu *et al.*, 2009; Jazayeri *et al.* 2014). However, most of these plants have not been validated empirically.

Alkaloids, flavonoids, tannins, and phenolic chemicals are just a few examples of the secondary metabolites that medicinal plants can produce that exact medicinal effect (Chandhur *et al.*, 2011). These chemical substances are synthesized from simple components like glucose and water (Ramakrishna and Ravishankar, 2011). These substances may serve as a starting point for the creation of safe and effective medicines.

Some plants have toxic effects for instance according to Ifeoma and Oluwakanyinsol (2013), certain alkaloids have been found to disrupt neurotransmitter systems. Nonetheless, little is known on the hazardous side effects of the bulk of these medicinal plants. As a result, it's important to evaluate herbal products' levels of safety and support their application. Toxic effects of herbs are revealed using toxicity assays, allowing users of herbal medicines to prevent their negative consequences.

2.4 *Launaea cornuta*

2.4.1 Plant description

Launaea cornuta Hochst (Ex Oliv. and Hiern.) is a small erect herb belonging to Asteraceae (Compositae) family, which grows up to 1.5 meters above the ground. The plant, which is native to many African nations, is frequently referred to as "bitter lettuce." Locally, it is referred to as "*Mchunga*" in Swahili, "*Muthunga*" (Meru, Kikuyu, and Embu), "*Mnyinya*" in Taita, and

"*Achak*" in Luo (Maundu and Tengnäs, 2005). A photograph of *L. cornuta* is shown in Plate 2.1.

Plate 2.1: Photograph of *L. cornuta* captured *in situ*
(Source: Mercy Maina; Captured on 15th December 2021 at Irangi forest in Embu County, Kenya)

2.4.2 Ethnomedicinal uses

Among other uses, *L. cornuta* is used in Kenyan traditional medicine to treat bacterial infections like gonorrhoea and typhoid as well as inflammatory diseases like swollen testicles, earaches, stomach pains, chronic joint problems, diabetes, hypertension, and memory loss (Wambugu *et al.*, 2011; Karau *et al.*, 2014). The concoctions of roots and leaves are boiled and taken under instruction of traditional herbal practitioner. Previous research shows that *L. cornuta* is endowed with pharmacologically active phytochemicals, including flavonoids, phenols, ascorbic acid, among others, which possess antioxidant and cognitive enhancing properties (Karau *et al.*, 2014; Machocho *et al.*, 2014; Misonge *et al.*, 2015). It is based on this ethnomedical background that this plant was selected for the present study.

2.4.3 Phytochemistry and biological activities

According to Akimat *et al.* (2022), *L. cornuta*'s aqueous root extract underwent qualitative phytochemical analysis, which identified the presence of tannins, cardiac glycosides, anthraquinones, alkaloids, terpenoids, flavonoids, steroids, and phenols. However, it was discovered that saponins and coumarins were either non-existent or barely detectable. In addition, Akimat *et al.* (2022) argued that due to its capacity to prevent oedema (brought on by carrageenan), albumin denaturation, proteinase activity, and ability to maintain the integrity of red blood cells, the aqueous root extract has anti-inflammatory properties. It was also proved to have antioxidant activities through Ferric-Reducing Antioxidant Power (FRAP) and 1,1-Diphenyl-2-Picryl Hydrazyl (DPPH) FRAP and DPPH assays.

Based on the available literature, it is notable that despite the diverse ethnomedicinal applications of *L. cornuta* and the reported bioefficacies, its cognitive-enhancing efficacy has not been investigated and validated empirically. Moreover, its safety and toxicity profile has not been sufficiently evaluated to guide further research, which may lead to the development of efficacious and safe armamentaria for various diseases, especially cognitive impairment.

CHAPTER THREE

MATERIALS AND AMETHODS

3.1 Collection of plant material and processing

The aerial parts of *L. cornuta* were collected, with the help of a local herbalist, from Irangi forest in Embu County, Kenya, where the plant grew naturally (GPS: 0.3472° S, 37.4853° E; Figure 3.1). The plant was selected for this study based on its ethnomedicinal use to treat oxidative stress-associated disorders and dementia. Voucher specimens of the plant were prepared and identified taxonomically at the East Africa Herbarium at the National Museums of Kenya (REF: NMK/BOT/CTX/1/3). Duplicate voucher specimens were deposited for future reference. The collected aerial parts of the study plant were transported to Mount Kenya University, at the Department of Pharmacology and Pharmacognosy, chopped and then dried under shade at room temperature ($25^{o}C \pm 1$) for 14 days. Occasional grabbling was done to facilitate uniform drying. The dried plant material was ground using an electric plant mill into a course powder, packaged in khaki envelopes, and stored at room temperature awaiting extraction (Moriasi *et al.,* 2021; Akimat *et al.,* 2021).

Figure 3.1: Map of Kenya showing Irangi forest in Embu County, from where the plant sample was obtained (*Adapted from Google maps*).

3.2 Extraction procedure

The extraction procedure described by Harborne (1998) and modified by Moriasi *et al.* (2021c) was adopted in this study. In brief, 100 g of the powdered plant material was mixed with 500 ml of distilled water and heated at 60 ^{o}C for five minutes. The concoction was cooled to room temperature and then filtered *in vacuo* through Whatman No. 1 filter papers using a Buchner funnel. The filtrate was transferred into freeze-drying flasks at volumes of 200 ml and

lyophilised (freeze-dried) for 48 hours in a freeze-dryer. The dry lyophilised extract was transferred into a clean, dry pre-weighed universal bottle, weighed, and then stored in a refrigerator (2-8 ^{o}C) awaiting analysis. The percentage yield of the extract was calculated according to the formulae (Eqn. 1) described by Truong *et al.*, (2019) and found to be 14.7 %.

$$\% \text{ Yield} = \frac{\text{weight of the extract}}{\text{weight of the macerated sample}} \text{x } 100 \ldots\ldots\ldots\ldots [Eqn. 1]$$

3.3 Experimental animals

A total of 50 Swiss-Albino mice (male and female) between 4 and 5 weeks old, and weighing 22-28 grams were obtained from the Kenya Agricultural and Livestock Research Organisation Veterinary Science Research Institute (KALRO-VSRI) animal breeding section. The animals were kept in standard conditions (12-hour-day and 12-hour-night cycle) in cages measuring 30 cm × 20 cm × 13 cm, in which softwood shavings were spread as bedding material. The animals were fed on standard rodent pellets purchased from Unga Feeds Limited and clean water *ad-libitum.* The animals were acclimatised for one week before experimentation, handled humanely during the study, and disposed of according to the guidelines described by the National Research Council (National Research Council, 2011).

3.4 Acute oral toxicity study

The acute oral toxicity effects of the aqueous aerial part extract of *L. cornuta* were investigated according to the Up-and-Down-Procedure (UDP) stipulated by the OECD (OECD, 2008). Briefly, four randomised groups of experimental mice (five mice per group) were fasted for four hours and then weighed before dosing. The normal control group (Group I) received normal saline (10 ml/Kg bw), while mice in the experimental groups (Group II-IV) were orally administered with the study extract at dose levels of 175, 550, and 2000 mg/Kg bw, in a stepwise manner, according to the OECD guideline. After that, wellness parameters, such as

the appearance of skin fur, salivation, mucous membrane, lethargy, eyes, convulsions, diarrhea, coma, tremors, sleep, mortality, and body weight, were observed and monitored keenly after 30 minutes, 4 hours, 24 hours, 48 hours, 7 days, and 14 days, and recorded. The median lethal dose (LD_{50}) was estimated, and the extract's safety was appraised based on the standard guidelines (OECD, 2008).

3.5 Determination of *in vivo* cognitive-enhancing effects of the test extract

3.5.1 Morris Water Maze Task

The Morris Water Maze technique (Morris, 2008; Vorhees and Williams, 2006) as modified by Moriasi *et al.* (2020b) was adopted to determine the cognitive-enhancing effects of the aqueous aerial part extract of *L. cornuta* in a ketamine-induced dementia mouse model. Briefly, clean water in which 750 g of fat-free powdered milk was mixed was poured into a maze measuring 110 cm in diameter by 45 cm in height up to a height of 30 cm from the bottom. The temperature of the maze was maintained at 26±1 °C throughout the experimental period. The maze was emptied and cleaned daily before being refilled. The maze was virtually sub-divided into four equal quadrants, which were labelled as North (N), South (S), East (E), and West (W). A white cylindrical platform measuring 6 cm in diameter by 29 cm high was placed in the NW quadrant and submerged 1 cm below the water surface.

A digital video camera was affixed 1.5 metres directly above the maze and used to record each mouse as it performed the task. Each mouse was subjected to two 60 s training sessions with visible and invisible platforms for two days before the experimentation day. In subsequent days, experimental mice were accorded two trial sessions, with an intertrial break of 20 min, each day for four days consecutively. When the animal located the escape platform, it was allowed to rest on it for 10 s before being removed and placed in a holding cage. If the animal failed to locate the platform within 60 s, it was gently guided to the platform using a wooden

rod and left and allowed to rest and explore the maze for 20 s before being removed from the maze. The starting point and the escape platform location remained constant for the experimental period.

3.5.2 Preparation of the administration drugs

Donepezil, Ketamine, and Normal saline were purchased from a local pharmacy and prepared in normal saline according to the OECD guidelines described by Erhierhie *et al.* (2014). The same guidelines were adopted in preparing the studied plant extract doses, which were selected based on a pilot study, before dosing.

3.5.3 Experimental design

A completely controlled randomised experimental study design was adopted from which an experimental design was drawn. In brief, thirty experimental mice were randomly allotted six treatment groups, each consisting of five mice. Group I (Normal control) mice were administered 200 µl of normal saline (10 ml/Kg BW; *p.o*).*;* Group II (Negative control) mice received 200 µl of normal saline (10 ml/Kg BW; *p.o*). and ketamine (1 mg/Kg bw; *i.p*) after 45 minutes.*;* Group III (Positive control) mice were administered Donepezil (1 mg/Kg bw; *p.o*) and Ketamine (1 mg/Kg bw; *i.p*) after 45 minutes.; Groups IV, V and VI mice were administered with 50, 100 and 200 mg/Kg bw; *p.o.*, respectively, of the aqueous aerial part extract of *L. cornuta* and Ketamine (1 mg/Kg bw; *i.p*) after 45 minutes.

All mice were subjected to the MWM task 30 minutes after administration of Ketamine (used to induce cognitive impairment) and allowed to navigate and search for the submerged escape platform for a maximum of 60 seconds. The respective video clips, recorded during each task trial performed by the mice, were uploaded into the Any-Maze software version 7.3 from where quantitative data for escape latency and navigation distance (during the acquisition trials) and

latency in the target (NW) quadrant (during probe trial) were derived, as indicators of cognitive status, and analysed statistically.

3.6 *Ex vivo* determination of the effects of the study extract on MDA profiles

After the Morris water maze experiment, the mice were sacrificed, and the whole brain was quickly dissected at 4 °C, eviscerated with cold normal saline, and stored at -80 °C until use. The brain samples were defrosted and homogenised in 10 ml of cold phosphate buffer (0.1 M, pH 7.4) and aliquoted to determine malondialdehyde (MDA) levels. The Thiobarbituric Acid Reactive Substances (TBARS) technique described by (Buege and Aust, (1979) and Ohkawa *et al.* (1979) was used to determine the MDA concentration in the brain samples. In brief, the reaction mixtures comprised 0.8 % Thiobarbituric acid (1.5 ml), 20 % acid acetic acid glacial (1.5 ml; pH 3.5), 8.1 % sodium dodecyl sulphate (0.2 ml), and 0.1 ml of homogenized brain tissue. The mixtures were heated at 100 ^{o}C for 1 hour and then cooled to room temperature before n-butanol/pyridine (15:1) mixture (5 ml) and distilled water (1 ml) were added. The mixtures were shaken vigorously using a vortex mixer and centrifuged at 2,500 rpm for exactly 20 minutes, after which the supernatant of each portion was aspirated carefully, and its absorbance was measured at λ_{532} nm. A molar extinction coefficient of 1.56×10^{5} M− 1 cm− 1 was used to compute the MDA concentration in samples and expressed as µmol/g tissue (Ohkawa *et al.*, 1979).

3.7 Qualitative phytochemical screening

Qualitative tests for the presence of tannins, saponins, alkaloids, glycosides, anthraquinones, terpenoids, steroids, flavonoids, coumarins, and phenols in the aqueous aerial part extract of *L. cornuta* were performed using standard phytochemical screening procedures described by Harborne (1998), Trease and Evans (2009), Bello *et al.* (2013), Jared *et al.* (2018), and Moriasi *et al.* (2020b) as follows.

3.7.1 Test for Saponins

The aqueous aerial part extract of *L. cornuta* weighing about 0.5 g was boiled in a test tube with 5 ml of distilled water before being allowed to cool. The foam that appears after shaking and lasts for more than two minutes suggests the existence of saponin (Trease and Evans, 2009; Moriasi *et al.*, 2020b).

3.7.2 Test for Carbohydrates

The aqueous aerial part extract of *L. cornuta* was dissolved in 5 ml of distilled water filtered and examined for the presence of carbohydrates using the Benedict's test as follows. About 5 ml of the extract filtrate was mixed with 5 ml of the Benedict's reagent and heated gently heated in a water bath set at 95 ^{o}C for three minutes. Precipitation that is orange or red suggests the presence of reducing sugars (Jared *et al.*, 2018).

3.7.3 Test for Amino Acids

About 100 mg of the extract was mixed with 10 mg of distilled water and filtered using Whatman paper No. 1. Then, 3 drops of ninhydrin solution were added to 2 ml of aqueous filtrate and the mixture heated at 90 ^{o}C in a water bath for three minutes. The presence of amino acids is shown by the appearance purple tint (Jared *et al.*, 2018).

3.7.4 Flavonoids

About 100 mg of the extract was mixed with 10 ml of ethanol (70 %) and warmed gently. Then, 1 ml was aspirated and mixed with five drops of strong hydrochloric acid and observed. The presence of flavonoids is shown by an immediate development of a red colour. After that, two additional techniques were used to validate the presence of flavonoids. About 10 ml solution of the test extract was hydrolysed with 10 % sulfuric acid and divided into two portions. The first portion was diluted with ammonia solution and observed. The presence of flavonoids is indicated by development of a greenish-yellow colour. To the remaining portion, 1 ml of

diluted sodium carbonate solution was added and observed. A pale-yellow colouration indicated the presence of flavonoids (Haborne, 1998; Moriasi *et al.*, 2020b).

3.7.5 Test for Cardiac Glycosides

About 200 mg of the extract was mixed with 5 ml of chloroform evaporated to dryness. Then, 0.5 ml of concentrated sulphuric acid and 0.4 ml of glacial acetic acid (with few drops of $FeCl_3$) were added and mixed. The presence of cardiac glycosides is revealed by the blue acetic layer (Jared *et al.*, 2018)

3.7.6 Test for Tannins

In this test 500 mg of the aqueous extract was heated with 5 ml of distilled water before being filtered. After that, 3 drops of 0.1% $FeCl_3$ were added to the filtrates, and the appearance of a blue precipitate suggests the presence of tannins (Trease and Evans, 2009; Moriasi *et al.*, 2020b).

3.7.7 Test for Phenols

The extract (100 mg) was weighed, mixed with 10 ml of 70 % ethanol, and heated in a water bath for 5 minutes. The extract was filtered while hot and the supernatant was cooled under flowing tap water. Then, 5 drops of 5 % ferric chloride were added to 2 ml of the filtrate, mixed, and observed. A green precipitate in the sample indicates the presence of phenols (Trease and Evans, 2009).

3.7.8 Test for Coumarins

The test extract (200 mg) was warmed with 2 ml of absolute ethanol for five minutes, and the mouth of the test tube was covered with a Whatman filter paper that had been soaked in 10% NH_4OH solution. The paper was removed and examined under ultraviolet light (365 nm). A yellow fluorescence indicates the presence of coumarins in the sample (Kumar *et al.*, 2013)

3.7.9 Test for Alkaloids

The extract (100 mg) was mixed with 5 ml of 1% HCL, warmed, and filtered. About three drops of the Meyer's reagent was added to 2 ml of the filtrate and observed. The presence of alkaloids is shown by the formation of cream-colored precipitate. Similarly, about 3 drops of Dragendorff's reagent was added to 2 ml of the filtered extract in another test tube, mixed, and observed. A reddish-brown precipitate suggests that alkaloids are present (Onyancha *et al.*, 2018)

3.7.10 Test for Steroids

Three drops of the Liebermann-Burchard reagent were added to a 1 ml of extract, mixed, and observed for the presence of steroids. In this test, production of a reddish-purple tint indicates the presence of steroids in the test sample (Jared *et al.*, 2018).

3.7.11 Test for Anthraquinones

In this test, 200 mg of the extract was mixed 5 ml of benzene and filtered using a Whatman paper. Then, 5 ml of 10 % ammonium hydroxide was added into the filtrate and observed for the appearance of a violet colour in the ammoniacal layer, which denoted the presence of anthraquinones (Moriasi *et al.*, 2020b).

3.8 Ethical Considerations

This study was ethically approved and conducted in accordance to the guidelines of the Biosafety Animal Use and Care Committee of the Faculty of Veterinary Medicine of the University of Nairobi (BAUEC/2022/336). Also, a research permit was granted by the National Commission for Science Technology and Innovation (NACOSTI/P/22/17850).

3.9 Data management, statistical analysis, and presentation

Quantitative data from the Morris water maze experiment and TBARS assay (MDA profile) was tabulated on an Excel spreadsheet (Microsoft 365) and then exported to GraphPad Prism statistical software version 9.4 for analysis. The data was analysed descriptively, and the results were presented as $\bar{x} \pm SEM$. One-Way analysis of variance (ANOVA) and Tukey's *post hoc* test were performed to determine differences in means among the study groups and for pairwise comparison and separation of mean at $\alpha_{0.05}$, and the results were presented in bar graphs. Qualitative data from the acute oral toxicity study was described and interpreted according to the OECD guideline 425 (OECD, 2008). Qualitative phytochemical screening results were tabulated and described.

CHAPTER FOUR

RESULTS

4.1 Acute oral toxicity effects of the aqueous aerial part extract of *L. cornuta* in Swiss albino mice

In this study, the aqueous aerial part extract of *L. cornuta*, at all the three tested dose levels (175 mg/Kg bw, 550 mg/Kg bw, and 2000 mg/Kg bw), did not cause any observable clinical signs of acute oral toxicity in the experimental mice. Additionally, all the dosed animals remained normal, without any adverse behavioural changes, throughout the 14-day experimental period. No mortality in the experimental mice was recorded in this study. Therefore, based on the OECD guidelines, LD_{50} of the studied plant extract was envisaged to be >2000 mg/Kg bw. Table 4.1 presents the findings of the acute oral toxicity study of the studied plant extract.

Table 4.1: Acute oral toxicity of the aqueous aerial part extract of *L. cornuta* in Swiss albino mice

Wellness parameter	Observation at various time frames											
	30 min- 2 Hrs.		4 Hrs.		24 Hrs.		48 Hrs.		7 days		14 days	
	EM	CM	EM	CM	EM	CM	EM	CM	EM	CM	EM	CM
Skin and Fur appearance	N	N	N	N	N	N	N	N	N	N	N	N
Feeding	N	N	N	N	N	N	N	N	N	N	N	N
Body weight gain	N	N	N	N	N	N	N	N	N	N	N	N
Faecal matter consistency	N	N	N	N	N	N	N	N	N	N	N	N
Urination and urine appearance	N	N	N	N	N	N	N	N	N	N	N	N
Mucous membrane appearance	N	N	N	N	N	N	N	N	N	N	N	N
Itching	-	-	-	-	-	-	-	-	-	-	-	-
Salivation	N	N	N	N	N	N	N	N	N	N	N	N
Sleep	N	N	N	N	N	N	N	N	N	N	N	N
Convulsions and tremors	-	-	-	-	-	-	-	-	-	-	-	-
Breathing	N	N	N	N	N	N	N	N	N	N	N	N
Coma	-	-	-	-	-	-	-	-	-	-	-	-
Somatomotor activity	N	N	N	N	N	N	N	N	N	N	N	N
Aggression	-	-	-	-	-	-	-	-	-	-	-	-
Grooming	N	N	N	N	N	N	N	N	N	N	N	N
Eyes	N	N	N	N	N	N	N	N	N	N	N	N
Teeth	N	N	N	N	N	N	N	N	N	N	N	N
Mortality/Death	-	-	-	-	-	-	-	-	-	-	-	-

EM: Experimental mice treated with either 175 mg/Kg bw, or 550 mg/Kg bw, or 2000 mg/Kg bw of the aqueous aerial part extract of *L. cornuta*; CM: Control mice treated with 10 ml/Kg BW of Normal saline only; n=3 mice per group at each experiment step.

4.2 Cognitive-enhancing efficacy of the aqueous aerial part of *L. cornuta* in ketamine-induced cognitive-impaired mice

In this study, the efficacy of the studied plant extract in ameliorating the ketamine-induced cognitive impairment in experimental mice. The time taken by each experimental mouse to locate the escape platform, or complete the task (escape latency), and the distance covered by each experimental mouse from the starting point to the platform location or completion of the Morris water maze task (Navigation distance) were determined as indicators of learning capacity. Besides, the time spent in the target (NW) quadrant by each experimental mouse during the probe trial was determined as a measure of memory retention and retrieval in the Morris water maze experiment.

4.2.1 Effect of the aqueous aerial part extract of *L. cornuta* on Escape Latency of ketamine-induced cognitive-impaired mice

In the first day (Day 1), no significant difference between the escape latency of mice treated with 50 mg/Kg bw of the aqueous aerial part extract of *L. cornuta* and those in the negative control group was observed ($P>0.05$; Figure 4.1). Similarly, the differences among the escape latencies of mice treated with 100 mg/Kg bw and 200 mg/Kg bw of the studied plant extract and those in the normal and positive control groups were not significant in the first day (Day 1) ($P>0.05$; Figure 4.1). However, the escape latencies recorded in the negative control mice and those administered with 50 mg/Kg bw of the studied plant extract were significantly higher than those recorded in all the other experimental mice in the first day (Day 1) ($P<0.01$; Figure 4.1).As shown in Figure 4.1, no significant differences in escape latency were observed between mice, which received 100 mg/Kg bw of the aqueous aerial part extract of *L. cornuta* and those in the normal control group in the second day (Day 2) ($P>0.05$). Likewise, in the second day (Day 2), the differences between the escape latency observed between mice treated with 200 mg/Kg bw of the studied plant extract and those in the normal and positive control

groups were insignificant ($P>0.05$; Figure 4.1). The negative control group mice took a significantly higher escape latency than all the other experimental mice to complete the Morris water maze task in the first experimental day (Day 1) ($P<0.05$; Figure 4.1). Notably, the results showed a significant dose-dependent decrease in the escape latency of mice administered with the aqueous aerial part extract of *L. cornuta* in the second experimental day (Day 2) ($P<0.05$; Figure 4.1).

In the third and fourth days (Day 3 and Day 4), respectively, the escape latencies taken by mice treated with the studied plant extract at doses of 100 mg/Kg bw and 200 mg/Kg bw, and those in the normal and positive control groups were comparable ($P>0.05$; Figure 4.1). At the same period, the escape latencies of the negative control mice were significantly higher than those of all the other experimental mice in this study ($P<0.05$; Figure 4.1). Also, significantly higher escape latencies were recorded in mice administered with the studied plant extract at a dose of 50 mg/Kg bwb compared with those obtained in mice treated with 100 mg/Kg bw and 200 mg/Kg bw of the plant extract and those in the normal and positive control groups in the third and fourth days, respectively ($P<0.05$; Figure 4.1).

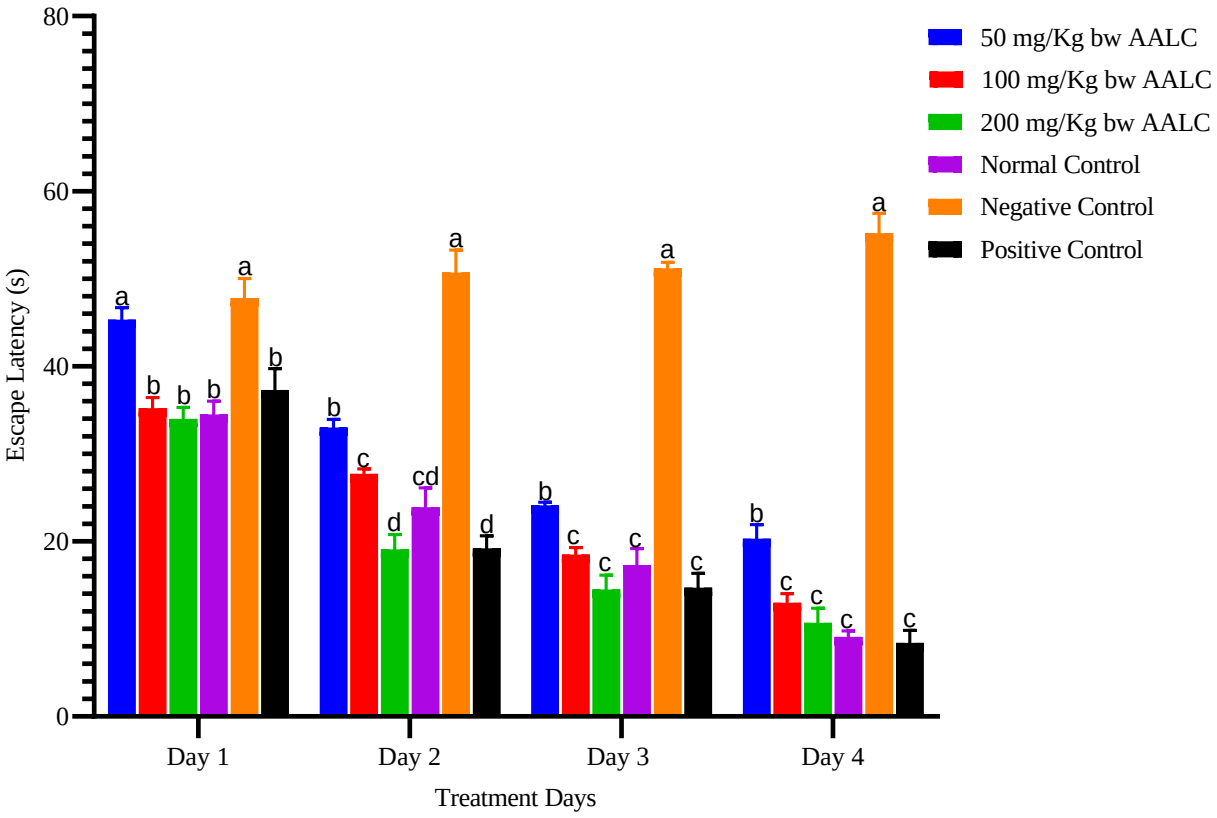

Figure 4.1: Escape latencies of ketamine-induced cognitive impaired mice treated with the aqueous aerial part extract of *L. cornuta*

Values are plotted as $\bar{x} \pm SEM$; Bars with similar alphabets within the same day are not significantly different (P>0.05), while those having different alphabets within the same day are significantly different (P<0.05) (One-Way ANOVA with Fisher's LSD *post hoc* test. AALC: Aqueous aerial part extract of *L. cornuta*).

Besides, the escape latencies of mice in each experimental group were compared across the four days. The results showed significant decreases in escape latencies of the normal control group mice from the first day (Day 1) through to the fourth day (Day 4) (P<0.05; Figure 4.2). Further, no significant differences (P>0.05) in escape latencies of the negative control group mice were observed throughout the four-day study period as shown in Figure 4.3. Moreover, significant daily decreases in escape latency were observed in the positive control group mice through to the fourth day of experimentation (P<0.05; Figure 4.4).

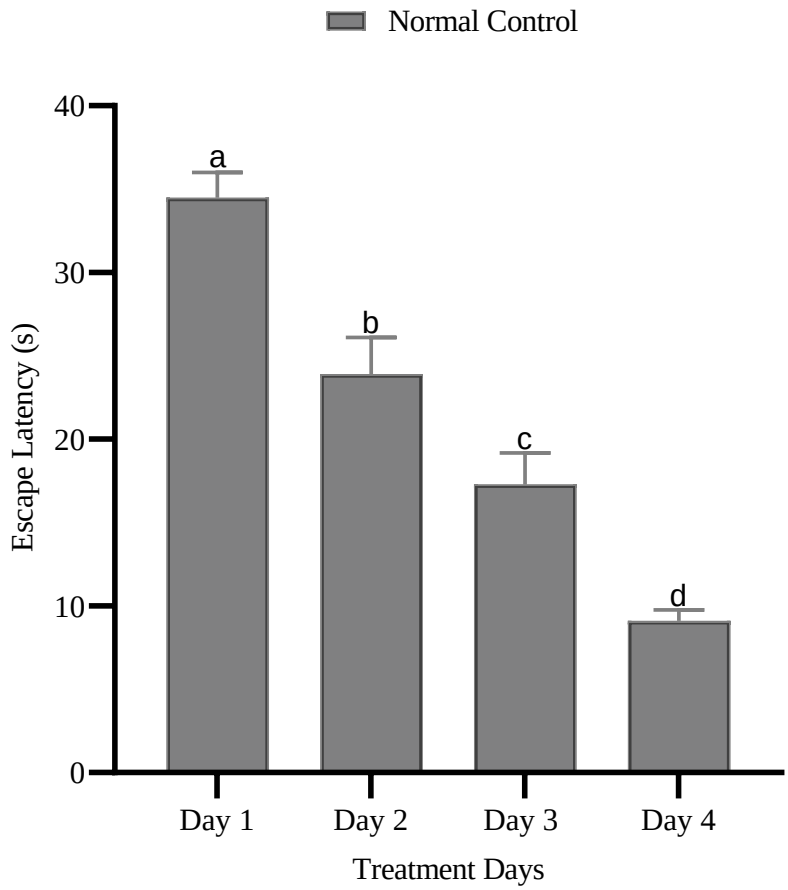

Figure 4.2: Comparison of the escape latencies of the normal control group mice across the four-day experimentation period

Values are plotted as $\bar{x} \pm SEM$; Bars with different alphabets across the treatment day are significantly different (P<0.05; One-Way ANOVA with Fisher's LSD *post hoc* test).

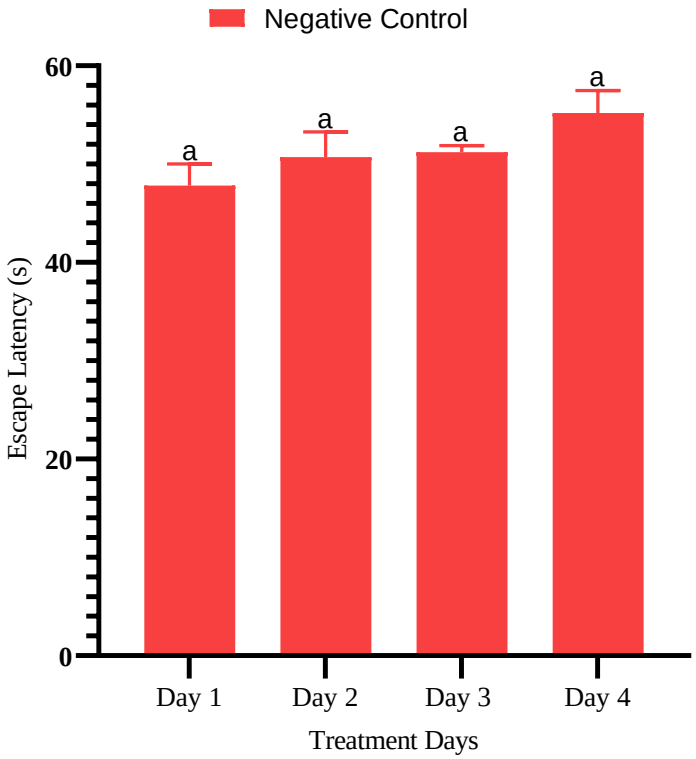

Figure 4.3: Comparison of the escape latencies of the negative control group mice across the four-day experimentation period

Values are plotted as $\bar{x} \pm SEM$; Bars with similar alphabets across the treatment day are not significantly different (P>0.05; One-Way ANOVA with Fisher's LSD *post hoc* test).

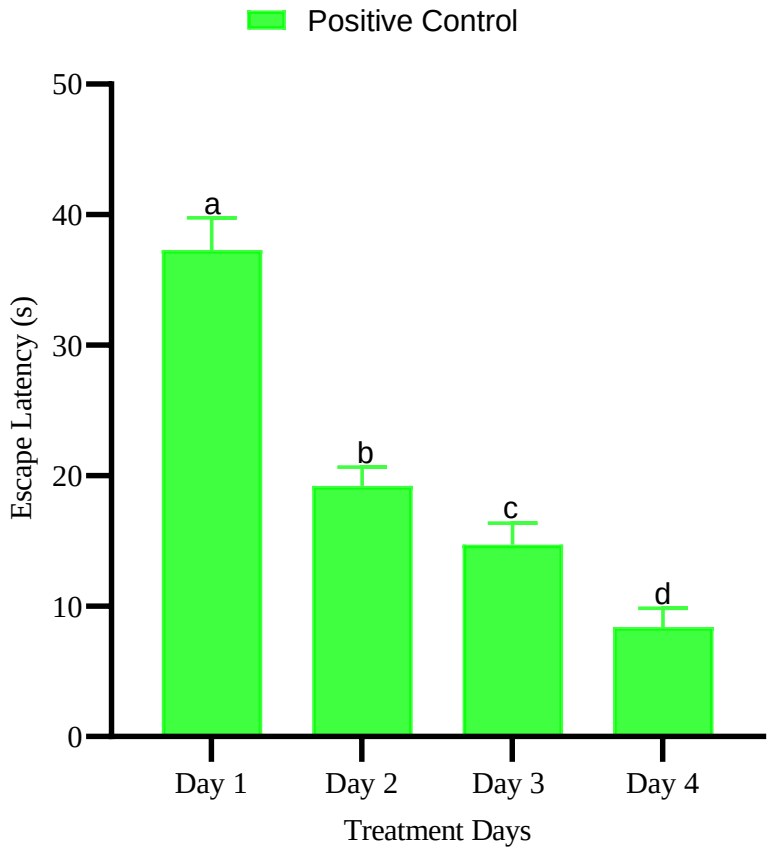

Figure 4.4: Comparison of the escape latencies of the positive control group mice across the four-day experimentation period

Values are plotted as $\bar{x} \pm SEM$; Bars with different alphabets across the treatment day are significantly different (P<0.05; One-Way ANOVA with Fisher's LSD *post hoc* test).

The escape latencies of ketamine-induced cognitive-impaired mice, which were treated with the aqueous aerial part extract of *L. cornuta,* at each dose level, were compared across the four-day study period. The results revealed significant (P<0.05) decreases in escape latency of cognitive-impaired mice treated with 50 mg/Kg bw of the studied plant extract from the first day (Day 1), through to the third (Day 3) and fourth (Day 4) days, respectively (Figure 4.5). However, no significant difference between the escape latency in the third day (Day 3) and fourth day (Day 4) was observed in mice which received 50 mg/Kg bw of the study extract (P>0.05; Figure 4.5).

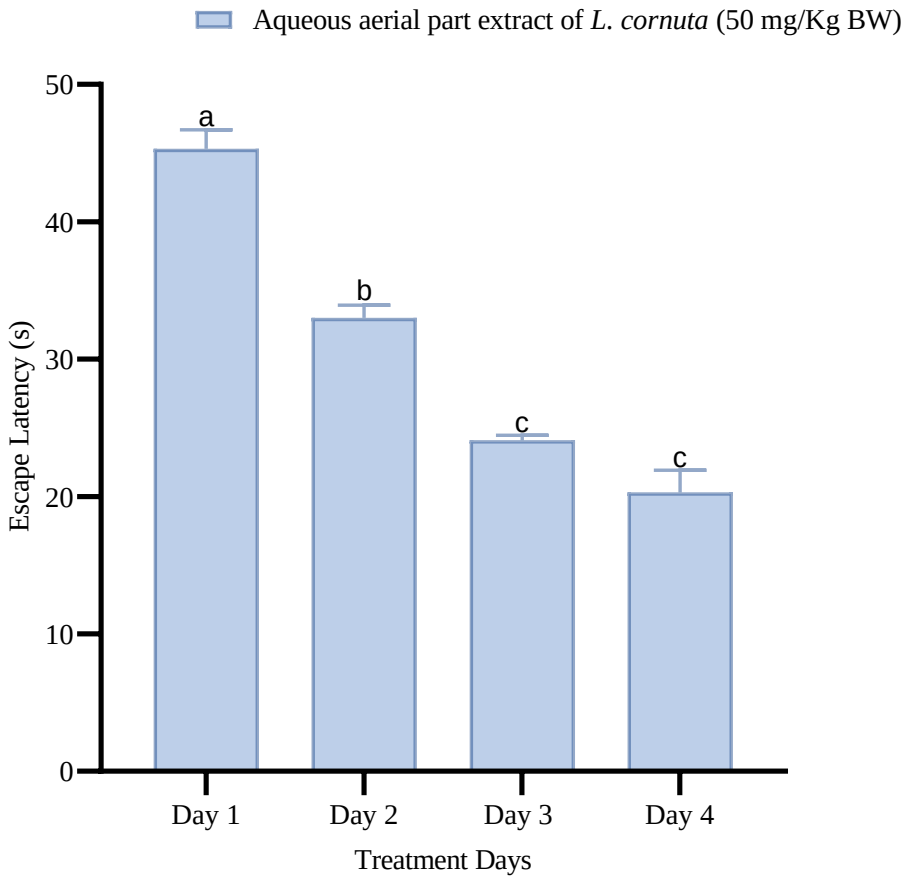

Figure 4.5: Comparison of the escape latencies of the ketamine-induced cognitive-impaired mice treated with 50 mg/Kg bw of the aqueous aerial part extract of *L. cornuta* across the four-day experimentation period

Values are plotted as $\bar{x} \pm SEM$; Bars with different alphabets across the treatment day are significantly different (P<0.05) while those with similar alphabets are not significantly different (P>0.05) (One-Way ANOVA with Fisher's LSD *post hoc* test).

In this study, significant decreases in escape latency, from the first day (Day 1) to the fourth day (Day 4) were observed in cognitive-impaired mice, which were treated with the aqueous aerial part extract of *L. cornuta* at a dose of 100 mg/Kg bw (P<0.05; Figure 4.6).

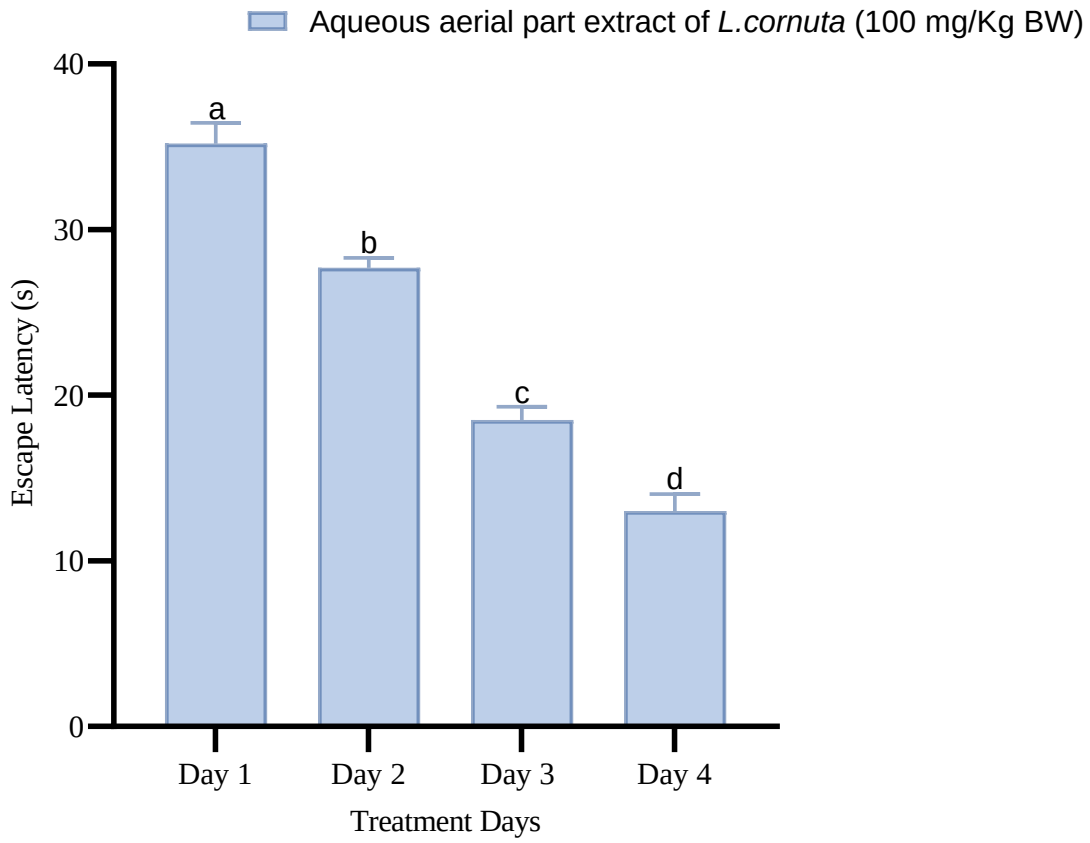

Figure 4.6: Escape latencies of the ketamine-induced cognitive-impaired mice treated with 100 mg/Kg bw of the aqueous aerial part extract of *L. cornuta* during the four-day experimentation period

Values are plotted as $\bar{x} \pm SEM$; Bars with different alphabets across the treatment day are significantly different (P<0.05), while those with similar alphabets are not significantly different (P>0.05) (One-Way ANOVA with Fisher's LSD *post hoc* test).

As shown in Figure 4.7, the escape latency of mice treated with the aqueous aerial part extract of *L. cornuta* at a dose of 200 mg/Kg bw decreased significantly from the first to the fourth day (P<0.05). However, no significant differences in escape latency were observed in mice administered with 200 mg/Kg bw of the studied plant extract between the second and third day and between the third and fourth day respectively (P>0.05; Figure 4.7).

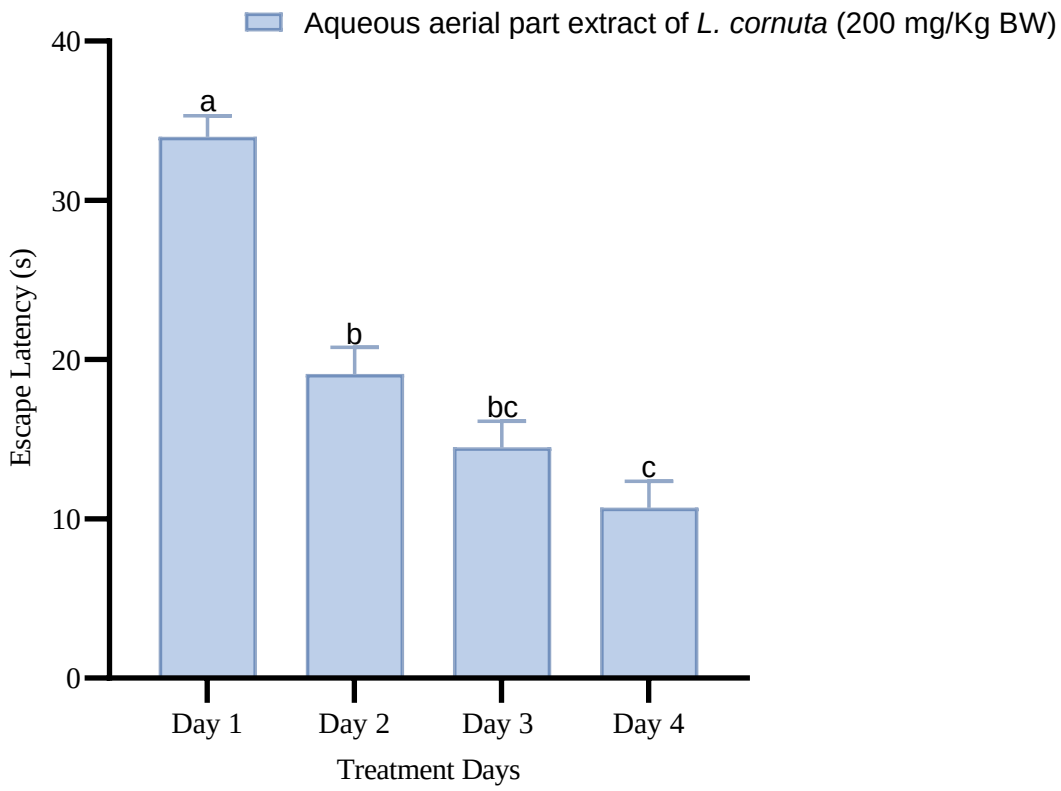

Figure 4.7: Escape latencies of the ketamine-induced cognitive-impaired mice treated with 200 mg/Kg bw of the aqueous aerial part extract of *L. cornuta* during the four-day experimentation period

Values are plotted as $\bar{x} \pm SEM$; Bars with different alphabets across the treatment day are significantly different (P<0.05), while those with similar alphabets are not significantly different (P>0.05) (One-Way ANOVA with Fisher's LSD *post hoc* test).

4.2.2 Effect of the aqueous aerial part extract of *L. cornuta* on the Navigation distance of ketamine-induced cognitive-impaired mice

The present study measured the navigation distances covered by all experimental mice participating in the Morris water maze task were measured at each day throughout the experimental period. The results showed no significant differences in navigation distances covered by mice treated with 50 mg/Kg bw and 100 mg/Kg bw of the studied plant extract and

those in the normal control group in the first day (Day 1) ($P>0.05$; Figure 4.8). Similarly, in the first day, no significant difference in the navigation distance covered by the experimental mice, which received 100 mg/Kg bw and 200 mg/Kg bw of the aqueous aerial part extract of *L. cornuta* and those in the positive control group ($P>0.05$; Figure 4.8). However, the results revealed that the negative control group mice covered significantly longer navigation distances than all the other mice participating in the Morris water maze task in the first day ($P<0.05$; Figure 4.8).

In the second day, the navigation distances covered by mice administered with the studied plant extract at doses of 100 mg/Kg bw and 200 mg/Kg bw, and those in the normal and positive control groups were not significantly different ($P>0.05$); however, these distances were significantly ($P<0.05$) shorter than those covered by mice treated with 50 mg/Kg bw of the studied plant extract and those in the negative control group (Figure 4.8). Notably, the negative control group mice covered significantly longer navigation distances than those covered by all the other groups of mice as shown in Figure 4.8.

The results revealed a significant dose-dependent reduction in navigation distances covered by cognitive-impaired mice treated with the aqueous aerial part extract of *L. cornuta* in the third day ($P<0.05$; Figure 4.8). It was also noted that the navigation distances covered by mice treated with 100 mg/Kg bw and 200 mg/Kg bw were significantly shorter than those covered by the positive control and negative control group mice in the third day ($P<0.05$; Figure 4.8). Overall, the negative control group mice covered significantly longer navigation distance than all the other mice in the third day ($P<0.05$; Figure 4.8).

A significant dose-dependent reduction in navigation distance covered by cognitive-impaired experimental mice administered with the studied plant extract was observed in the fourth day

of the study (P<0.05; Figure 4.8). No significant difference between the navigation distance covered by mice which received 200 mg/Kg bw of the study extract and those in the positive control group(P>0.05) was observed in the fourth day as shown in Figure 4.8. However, the negative control group mice covered a significantly longer navigation distance, while the normal control group mice covered a significantly shorter navigation distance than all the other mice in the same day (Day 4) (P<0.05; Figure 4.8).

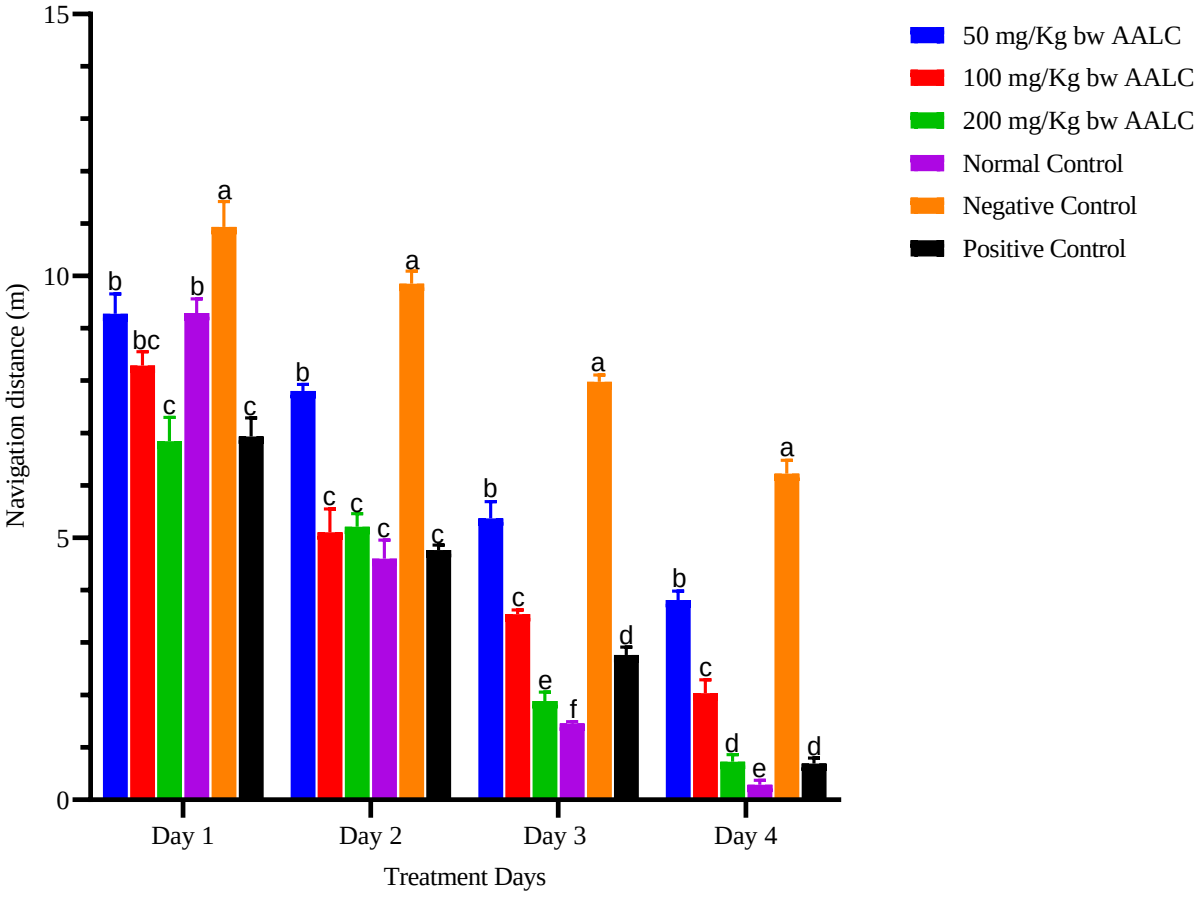

Figure 4.8: Navigation distances covered by ketamine-induced cognitive impaired mice treated with the aqueous aerial part extract of *L. cornuta*

Values are plotted as $\bar{x} \pm SEM$; Bars with similar alphabets within the same day are not significantly different (P>0.05), while those having different alphabets within the same day are significantly different (P<0.05) (One-Way ANOVA with Fisher's LSD *post hoc* test). AALC: Aqueous aerial part extract of *L. cornuta.*

A comparison of the navigation distances covered by each experimental group of mice was performed across the four-day study period. The results significantly progressive reduction in the navigation distance covered by the normal control group mice from the first to the fourth day ($P<0.05$; Figure 4.9). As shown in Figure 4.10, the measured navigation distance covered by the negative control mice was not significantly different between the first and second day of experimentation ($P>0.05$). However, the negative control mice's navigation increased significantly in the third and fourth day, respectively ($P<0.05$; Figure 4.10). The results further showed significant daily reductions in navigation distance covered by the positive control group mice across the study period ($P<0.05$; Figure 4.11).

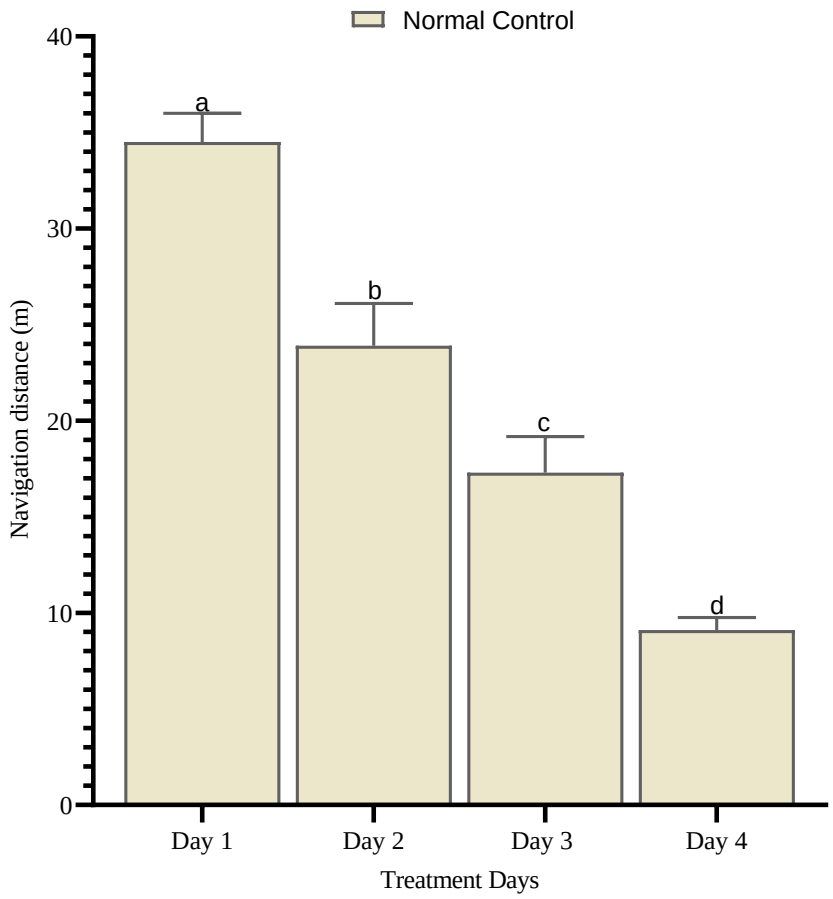

Figure 4.9: Comparison of navigation distances covered by the normal control group mice across the four-day experimentation period

Values are plotted as $\bar{x} \pm SEM$; Bars with different alphabets across the treatment day are significantly different (P<0.05; One-Way ANOVA with Fisher's LSD *post hoc* test).

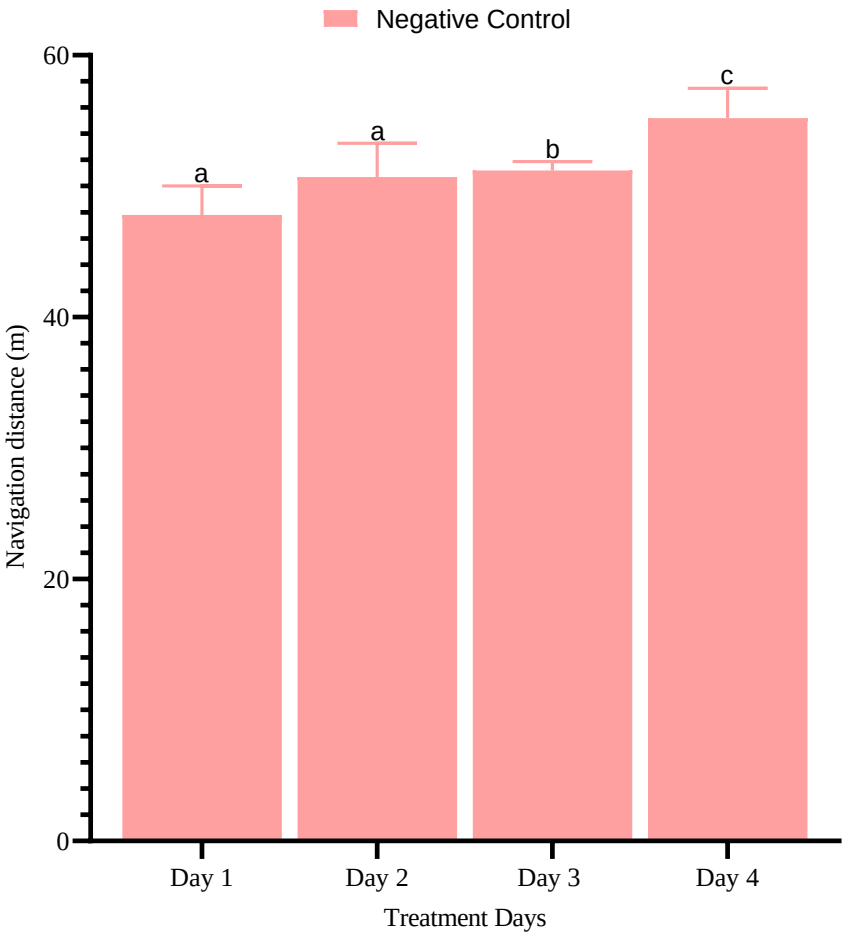

Figure 4.10: Comparison of navigation distances covered by the negative control group mice across the four-day experimentation period

Values are plotted as $\bar{x} \pm SEM$; Bars with different alphabets across the treatment day are significantly different (P<0.05) while those with similar alphabets are not significantly different (P>0.05) (One-Way ANOVA with Fisher's LSD *post hoc* test).

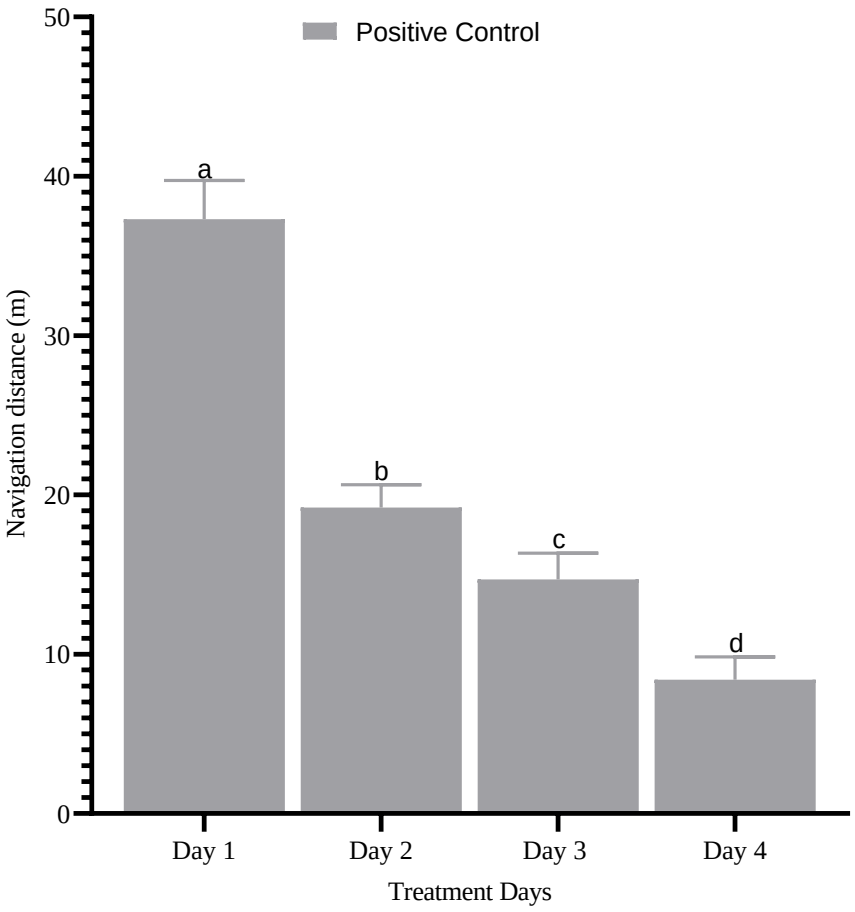

Figure 4.11: Comparison of navigation distances covered by the positive control group mice across the four-day experimentation period

Values are plotted as $\bar{x} \pm SEM$; Bars with different alphabets across the treatment day are significantly different (P<0.05; One-Way ANOVA with Fisher's LSD *post hoc* test).

The navigation distances covered by cognitive impaired mice, which were treated with the aqueous aerial part extract of *L. cornuta* at each studied dose level were compared across the experimentation days. The results showed a significant reduction in navigation distances covered by mice administered with 50 mg/Kg bw of the studied plant extract throughout the study period (P<0.05; Figure 4.12). Similarly, significant daily reductions (P<0.05) in navigation distances covered by mice which received 100 mg/Kg bw (Figure 4.13) and 200 mg/Kg bw (Figure 4.14) were observed across the study period.

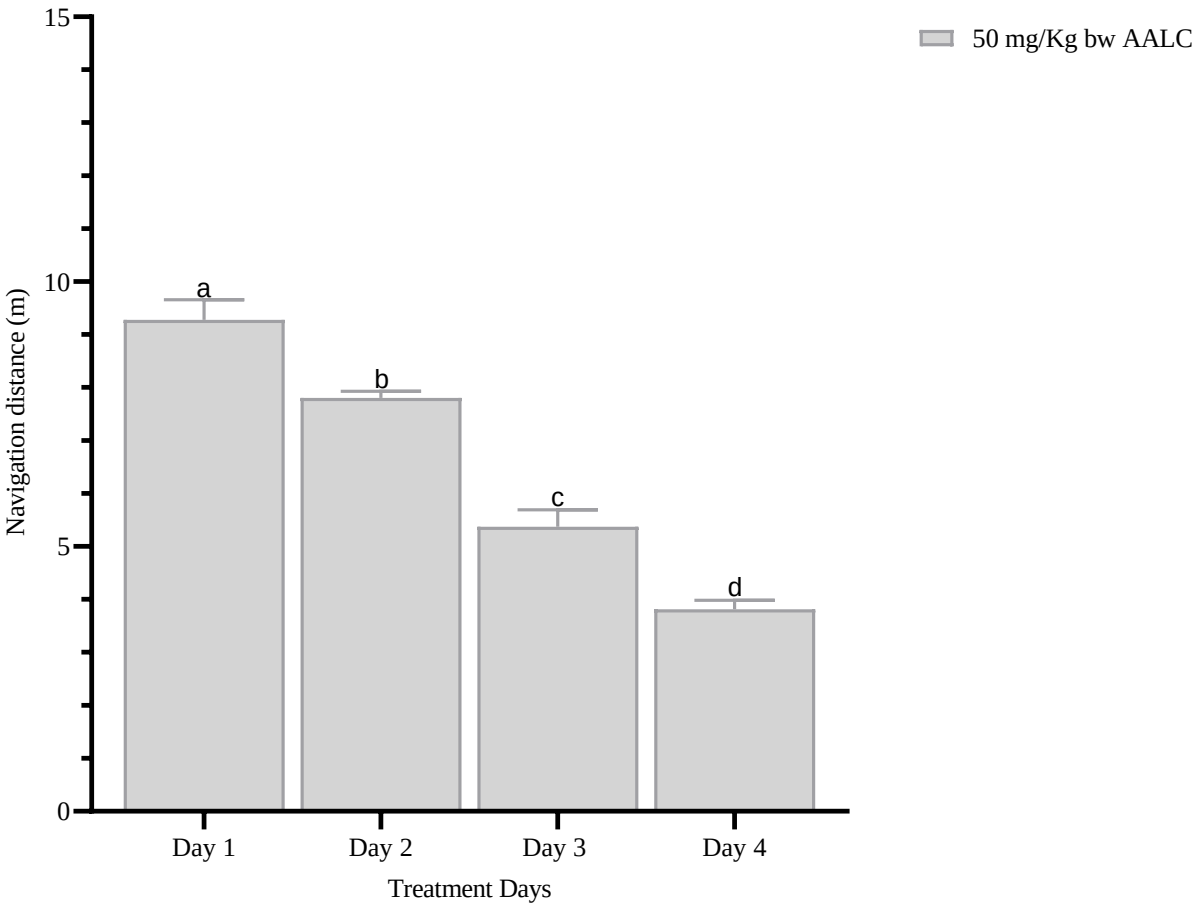

Figure 4.12: Comparison of navigation distances covered by mice administered with 50 mg/Kg bw of the aqueous aerial part extract of *L. cornuta* across the four-day experimentation period

Values are plotted as $\bar{x} \pm SEM$; Bars with different alphabets across the treatment day are significantly different (P<0.05; One-Way ANOVA with Fisher's LSD *post hoc* test). AALC: Aqueous aerial part extract of *L. cornuta.*

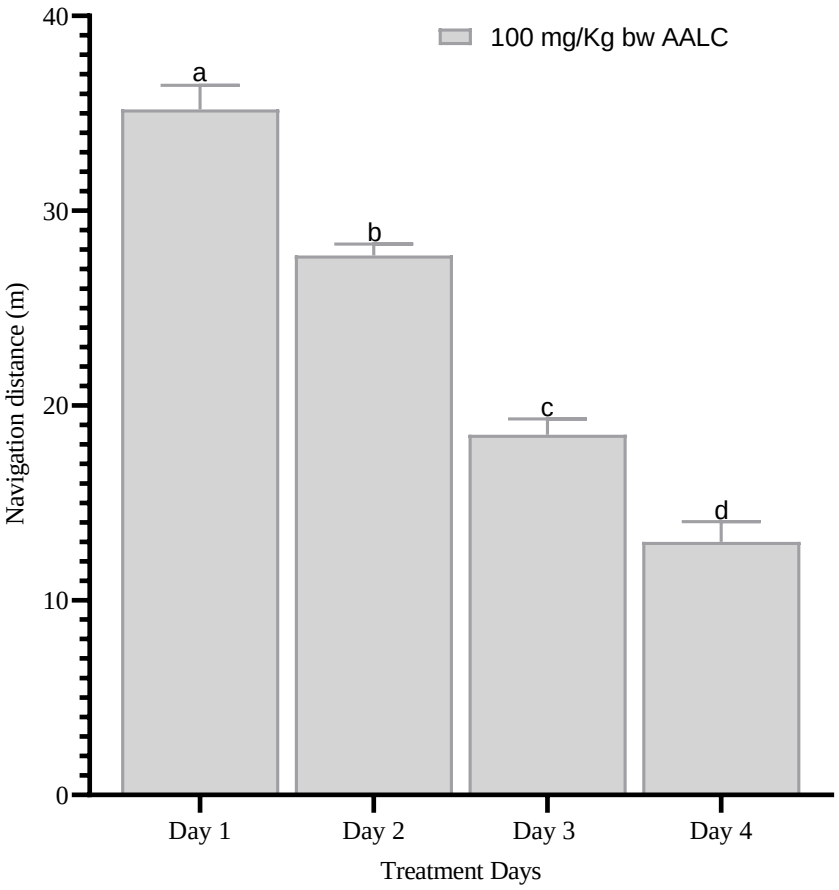

Figure 4.13: Comparison of navigation distances covered by mice administered with 100mg/Kg bw of the aqueous aerial part extract of *L. cornuta* across the four-day experimentation period

Values are plotted as $\bar{x} \pm SEM$; Bars with different alphabets across the treatment day are significantly different (P<0.05; One-Way ANOVA with Fisher's LSD *post hoc* test). AALC: Aqueous aerial part extract of *L. cornuta.*

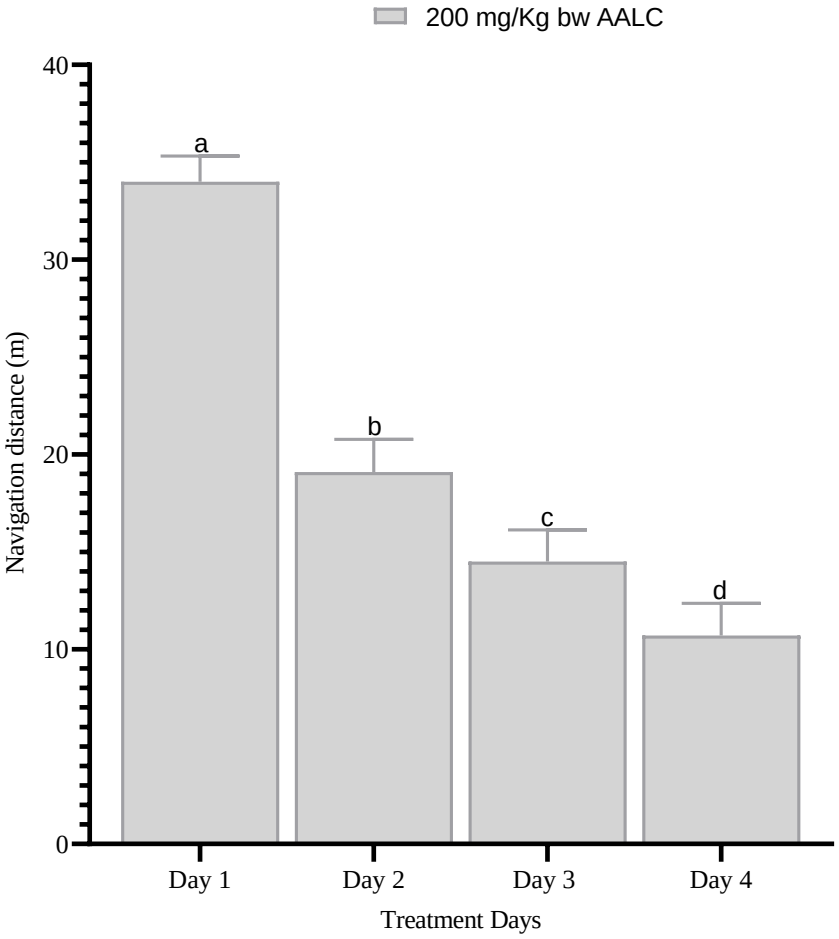

Figure 4.14: Comparison of navigation distances covered by mice administered with 200 mg/Kg bw of the aqueous aerial part extract of *L. cornuta* across the four-day experimentation period

Values are plotted as $\bar{x} \pm SEM$; Bars with different alphabets across the treatment day are significantly different (P<0.05; One-Way ANOVA with Fisher's LSD *post hoc* test). AALC: Aqueous aerial part extract of *L. cornuta.*

4.2.3 Effect of the aqueous aerial part extract of *L. cornuta* on latency in the target (NW) quadrant of the ketamine-induced cognitive-impaired mice

On the final experimentation day, the time spent in the target quadrant (NW), where the escape platform was previously located, by the experimental mice was determined to appraise their memory retention and retrieval capacity. In this study, cognitive-impaired mice, which were treated with the aqueous aerial part extract of *L. cornuta* recorded a significant dose-dependent

increase in latency in the NW quadrant (P<0.05; Figure 4.15). Besides, the latency of mice treated with 50 mg/Kg bw of the studied plant extract and those in the normal and positive control groups were not significantly different (P>0.05; Figure 4.15). Notably, the negative control group mice spent significantly shorter latency in the NW quadrant than the latencies spent in the same quadrant by all the other mice (P<0.05; Figure 4.15).

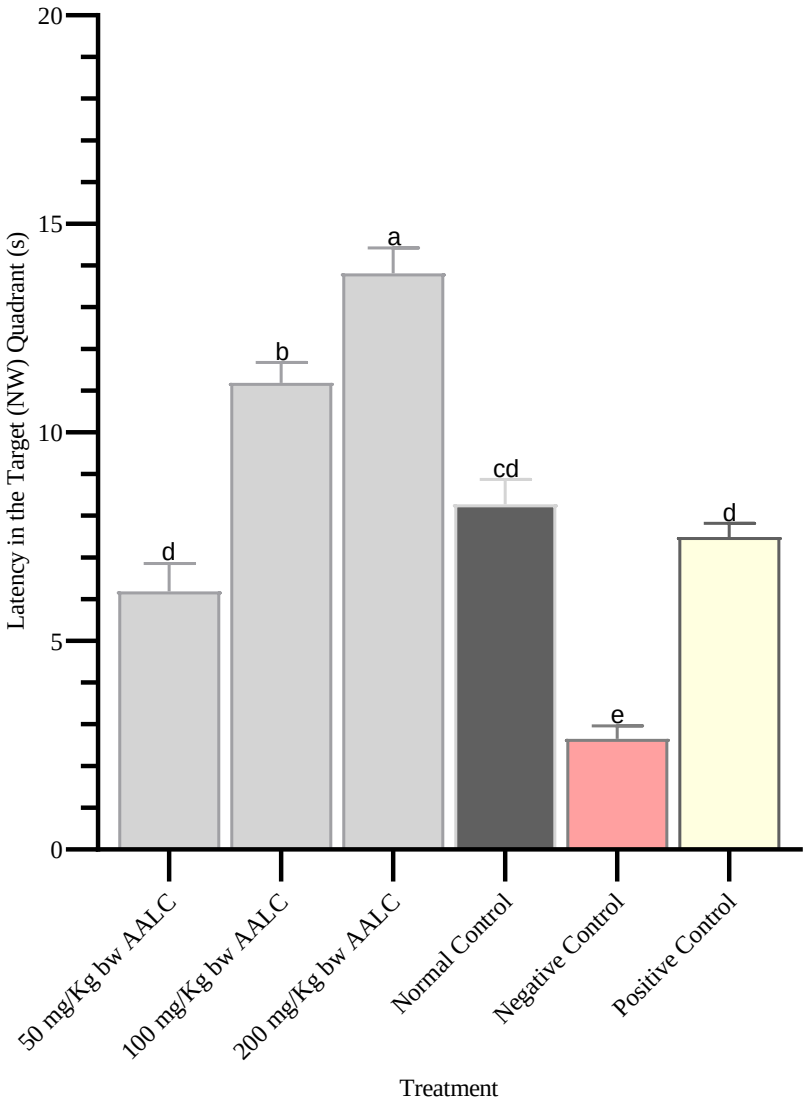

Figure 4.15: Latency of the ketamine-induced cognitive impaired mice treated with the aqueous aerial part extract of *L. cornuta* in the target (NW) Quadrant during the probe trial

Values are plotted as $\bar{x} \pm SEM$; Bars with similar alphabets are not significantly different (P>0.05), while those having different alphabets are significantly different (P<0.05) (One-Way ANOVA with Fisher's LSD *post hoc* test). AALC: Aqueous aerial part extract of *L. cornuta.*

4.3 Effects of the aqueous aerial part extract of *L. cornuta* on malondialdehyde (MDA) levels in the ketamine-induced cognitive-impaired mice

The MDA levels in the brain samples of the experimental mice, which were subjected to the four-day Morris water maze task were determined in the present study. The results showed no significant differences in MDA concentrations in brain samples from mice treated with the studied plant extract, at a dose of 200 mg/Kg bw, and those in the normal and positive control groups (P>0.05; Figure 4.16).

Notably, a significant dose-dependent decrease in MDA concentration was observed in samples derived from mice treated with the studied plant extract (P<0.05; Figure 4.16). However, the brain samples of negative control group mice had significantly higher MDA concentrations than all the other analysed samples (P<0.05) as shown in Figure 4.16.

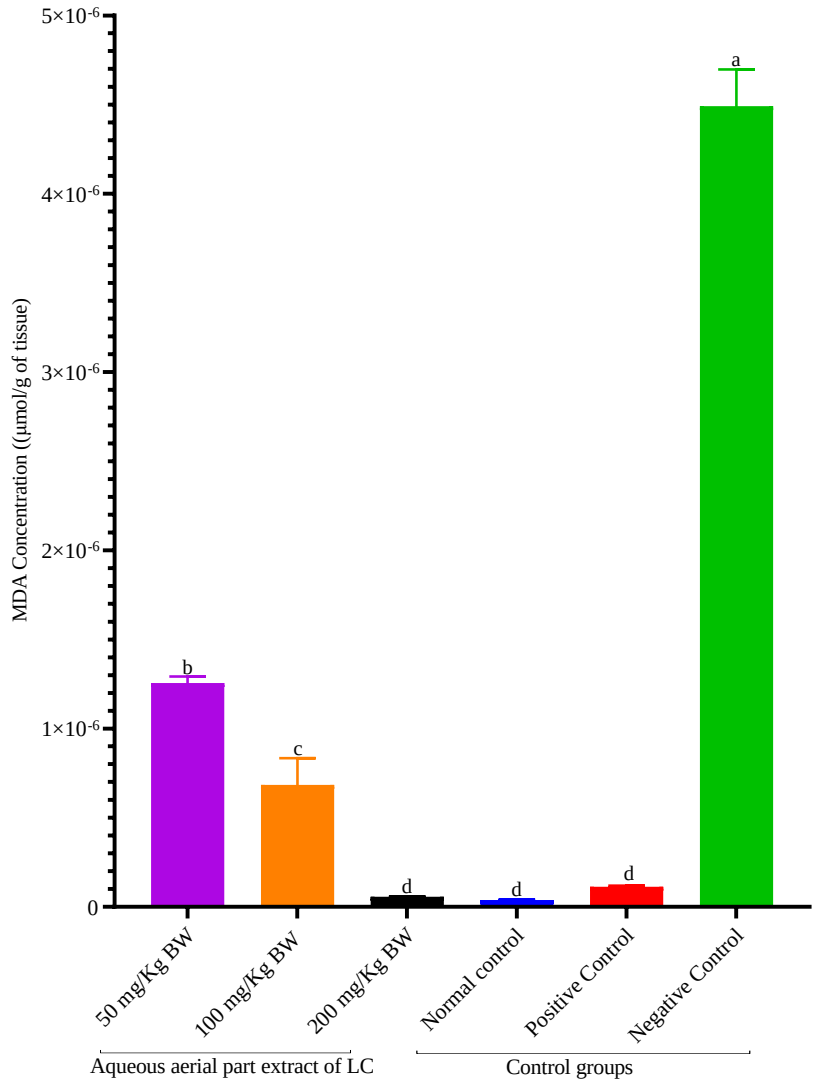

Figure 4.16: Concentration of MDA in brain samples from ketamine-induced cognitive impaired mice treated with the aqueous aerial part extract of *L. cornuta*

Values are plotted as $\bar{x} \pm SEM$; Bars with similar alphabets are not significantly different (P>0.05), while those having different alphabets are significantly different (P<0.05) (One-Way ANOVA with Fisher's LSD *post hoc* test). LC: *L. cornuta.*

4.4 Qualitative phytochemical composition of the aqueous aerial part extract of *L. cornuta*

In this study, qualitative phytochemistry revealed the presence of saponins, carbohydrates, amino acids, flavonoids, phenols, alkaloids, and steroids (Table 4.2). However, tannins and anthraquinones were not detected in the studied plant extract (Table 4.2).

Table 4.2: Qualitative phytochemical composition of the aqueous aerial part extract of *L. cornuta*

Phytochemical	Observation
Saponins	+
Carbohydrates	+
Amino Acids	+
Flavonoids	+
Cardiac Glycosides	+
Tannins	-
Phenols	+
Coumarins	+
Alkaloids	+
Steroids	+
Anthraquinones	-

+: Present; -: Absent

CHAPTER FIVE

DISCUSSION, CONCLUSIONS, AND RECOMMENDATIONS

5.1 Discussion

The acute oral toxicity effects of the studied plant extract were investigated according to the up-and-down procedure described by the OECD (OECD, 2008). In this method, a drug agent or extract is considered safe if it does not elicit toxicity-associated signs such as coma, tremors, discolouration of the mucosa, excessive salivation, diarrhoea, morbidity, among others, and death upon oral administration into experimental animals at doses ≤ 2000 mg/Kg bw (LD_{50}> 2000mg/Kg bw) (Bedi and Krishan, 2019; OECD, 2000).

In this study, no observable signs of acute oral toxicity, or mortality were observed in all animals which were treated with the studied plant extract at doses of 175 mg/Kg bw, 550 mg/Kg bw, and 2000 mg/Kg bw, throughout the 14-day experimentation period. Thus, it was considered safe and its LD_{50} value was estimated to be greater than 2000 mg/Kg bw according to the OECD guidelines (OECD, 2008). These findings corroborate those of Akimat *et al.* (2021) who observed that the root extract of *L. cornuta* were non-toxic to experimental mice. The confirmation of the safety of the aqueous aerial part extract of *L. cornuta* in this study is important considering its extensive usage as food (vegetable) and remedy for various diseases in traditional medicine (Karau *et al.*, 2014; Misonge *et al.*, 2015). Thus, this study partly demonstrates that the aqueous aerial part extract of *L. cornuta* is safe when orally administered; however, further toxicological investigations are recommended to establish its toxicity profile and safety.

The Morris water maze (MWM) technique (Vorhees and Williams, 2006) was used to determine the cognitive-enhancing effects of the aqueous aerial part extract of *L. cornuta* in ketamine-induced cognitive-impaired mice. This method was selected based on its extensive usage in evaluating spatial learning and memory in antidementia studies, with high accuracy, precision, and reproducibility. Moreover, using the MWM technique helps to determine alterations in the central cholinergic system, and cognitive-associated biochemical parameters, based on a single task (Moriasi *et al.*, 2020b).

Ketamine was used to induce cognitive deficits, akin to those observed in dementia patients. Ketamine is a non-competitive N-Methyl-D-Aspartate (NMDA) receptor antagonist, which disrupts the glutamate neurotransmitter, whose activity is essential for learning, memory, emotion, and pain perception by the brain (Zanos *et al.*, 2018). Besides, ketamine antagonistically interacts with muscarinic, monoaminergic, and nicotinic receptors, and neuronal sodium and calcium ion channels, interfering with cognition and pain recognition (Farahmandfar *et al.*, 2017; Mion and Villevieille, 2013). In the MWM experiments, the negative effects of ketamine in experimental mice are evidenced by the increased escape latency time, navigation distance, which indicate impaired learning and reduced latency in the target quadrant, indicating poor memory, which characterise cognitive deficits akin those observed in dementias (Moriasi *et al.*, 2020a; Moriasi *et al.*, 2020b). Therefore, a drug agent or plant extract capable of preventing or ameliorating the ketamine-induced effects, especially cognitive deficits, may be a potential cognitive-enhancing and antidementia therapy.

The present study's findings showed significant dose-dependent reductions in escape latencies and navigation distances of ketamine-induced cognitive-impaired mice. Besides, the significant reduction in escape latencies and navigation distances were observed in each group

of mice, except the negative control group across the four-day experimentation period. These findings indicate a successive amelioration of ketamine-induced learning and memory deficits by the aqueous aerial part extract of *L. cornuta* and the reference drug (Donepezil), underscoring their cognitive-enhancing efficacy.

The dose-dependent efficacy of the studied pant extract in averting the ketamine-induced cognitive deficits may be attributed to the varied concentration of bioactive compounds associated with cognitive enhancement, such as those reported in previous studies (Moriasi *et al.*, 2020b). The increased latency of the cognitive-impaired mice treated with the studied plant extract, and the reference drug, in the target quadrant, depicts memory-enhancing efficacy. Donepezil exerts its cognitive-enhancing efficacy by inhibiting the activity of acetylcholinesterase enzyme, which subsequently increases the acetylcholine levels (Naik *et al.*, 2009). Additionally, donepezil has been shown to restore altered redox homeostasis in AD and allied neuropathies (Atukeren *et al.*, 2017; Saxena *et al.*, 2008).

The high neurotransmitter levels at the pre- and post-synaptic clefts maintains thereby maintaining nerve firing in the central nervous system (Batool *et al.*, 2016; Gutierres *et al.*, 2014). Similarly, increasing and maintaining the activity of the glutamate neurotransmitter at the postsynaptic cleft increases nerve firing, which translates to enhanced cognitive function in patients (Ide *et al.*, 2019). Perhaps the observed efficacy of the aqueous aerial part extract of *L. cornuta* may be partly mediated through mechanisms, which increase cognitive-associated neurotransmitter concentrations and activity, as well as receptor function, thereby promoting nervous signalling and nerve health.

Considering that spatial learning and memory depend on the integrity of the hippocampus and cerebral cortex, the studied plant extract may have averted ketamine-induced damage through

various mechanisms, such as fostering mitochondrial health and function, quenching oxidative, mitigating neuroinflammation, fostering nerve health, among others. These effects are mediated by the various pharmacologically active phytocompounds, which may act individually or synergistically on multiple targets (Moriasi *et al.*, 2020b, 2021). Besides, a recent study showed that blockade of the NLRP3/Caspase 1 axis attenuates the ketamine-induced hippocampal pyro ptosis and cognitive impairment in neonatal rats (Zhang *et al.*, 2021). Perhaps, the cognitive-enhancing efficacy of the studied plant extract may be partly mediated through this mechanism.

Extensive research shows that ketamine induces oxidative stress in the hippocampus and cerebral cortex, the brain regions responsible for cognition, causing neuronal cell death, and impairing neuronal development (Félix *et al.*, 2016; Kalopita *et al.*, 2021; Liang *et al.*, 2018; Réus *et al.*, 2017). Damage to these brain sites following antimuscarinic agent administration such as scopolamine and ketamine impairs learning, memory creation, retention, and retrieval (Boon *et al.*, 2005; Genzel *et al.*, 2017; Hou *et al.*, 2004; Lee *et al.*, 2015; Rahimzadegan and Soodi, 2018). Therefore, drug agents or plant extracts, which can thwart oxidative stress in the brain, may ameliorate cognitive deficits. It is thus arguable that the aqueous aerial part extract of *L. cornuta* effectively attenuated oxidative stress in the hippocampal and neocortical regions, resulting in proper cognitive functioning of these regions.

Lipid peroxidation and its associated adducts causes oxidative damage to cellular machinery leading to a plethora of disease conditions, including neuroinflammation, brain cell death, among other complex maladies (Farmer and Mueller, 2013; Shabana *et al.*, 2020; Willey *et al.*, 2014). Malondialdehyde (MDA) is the major product of lipid peroxidation and is considered significant marker of oxidative damage in the body (Ahmad *et al.*, 2013; Moriasi *et al.*, 2020a,

2020b). As the level of lipid peroxidation-induced cell damage increases, the production and concentration of MDA also increases proportionately. Earlier studies have demonstrated that cognitive-impaired mice present high MDA concentrations, depicting the muscarinic and NMDA receptor antagonists in exacerbating oxidative brain damage (Hritcu *et al.*, 2014; Ionita *et al.*, 2017; Moriasi *et al.*, 2020b).

The brain samples obtained from experimental mice treated with the aqueous aerial part extract of *L. cornuta* had significantly lower MDA levels, which were comparable to those of samples obtained from the positive and normal control group mice. Notably, the brain samples of the negative control group mice had significantly higher MDA levels than all the others, implying that the ketamine-induced cognitive impairment may be partly linked to lipid peroxidation (Liang *et al.*, 2018). Markedly, the studied plant extract considerably normalised the MDA levels, depicting its anti-lipid peroxidation potential. This efficacy is attributable to the antioxidant-associated phytocompounds in the studied plant extract, which may be exerting their effects either solely or synergistically (Sajjad *et al.*, 2019).

Qualitative phytochemical screening of the aqueous aerial part extract of *L. cornuta* was also performed in this study. The results revealed the presence of various phytochemicals, including flavonoids, phenols, steroids, coumarins, among others. These findings are consistent with those of Akimat *et al.* (2021) who reported the presence of various phytochemicals in the aqueous root extract of *L. cornuta.* However, tannins and anthraquinones were absent in the aqueous aerial part extract of *L. cornuta* in the present study, which differ from an earlier study (Akimat *et al.*, 2021) which indicate their presence in the aqueous root extract of the same plant. Phytochemical research has shown that production of phytochemicals in plants is influenced by the climatic conditions, part of the plant, season, and the nature of biotic and

abiotic stresses affecting the plant at a given time (Altemimi *et al.*, 2017; Koche *et al.*, 2016; Xu, 2019; Zhang *et al.*, 2015), which may partly explain the differences observed in this study.

Research has demonstrated that antioxidant-associated phytochemicals, such as phenols, flavonoids, coumarins, among others possess the widest spectra of pharmacologic activity (Huang, 2018; Lam *et al.*, 2016). These phytochemicals maintain the pro- and antioxidant homeostasis in the body, which averts oxidative cell damage. It is now apparent that oxidative stress initiates and exacerbates many diseases, including diabetes, inflammation, neurodegenerative disorders, cancer, among other devastating diseases in the body (Gracia *et al.*, 2017; Liguori *et al.*, 2018; Rahman *et al.*, 2012). Thus, antioxidant therapy has been valorised as the most viable strategy to prevent the onset of reverse oxidative in the body. Additionally, the consumption of these antioxidants through fruit and vegetable diets, medicinal plants, and through supplementation helps to promote health (Flynn and Ranno, 1999; Lourenço *et al.*, 2019).

The amenability of the brain to oxidative damage is attributable to its high lipid composition, especially the polyunsaturated fatty acids (PUFAs), whose rancidity produces toxic adducts such as MDA and other advanced glycated end-products (AGEs), which drive its damage (Sevastre-Berghian *et al.*, 2017; Tsai and Huang, 2015). Research has affirmed that oxidative brain cell and neuronal damage are the main mediators of dementia pathology (Huang *et al.*, 2016; Onyou, 2013). For instance, disproportionate concentrations of free radicals have been shown to attack biological membranes, impairing their architecture and function as exemplified in neurodegenerative diseases, inflammation, diabetes mellitus, among other complex pathologies (Liguori *et al.*, 2018; Pizzino *et al.*, 2017). Besides, free radicals modify proteins producing pathological adducts, as the tau and β-amyloid evident in AD (Tan *et al.*, 2018).

It is therefore suggestive that the antioxidant phytochemicals in the studied plant extract played significant roles in ameliorating the ketamine-induced cognitive impairment by maintaining the redox homeostasis, averting lipid peroxidation, promoting neuronal cell health, and by modifying the interaction of ketamine with its receptors. Besides, the antioxidant phytocompounds have been shown to modulate neurotransmitter concentration and activity in the central cholinergic system and other brain regions, thereby enhancing cognition. Nevertheless, further empirical investigations on the specific amalgams from the aqueous aerial part extract of *L. cornuta* and their mechanisms of bioactivity may provide crucial insights into its antidementia potential.

5.2 Conclusions

Based on the study findings, the following conclusions were made.

i. The aqueous aerial part extract of *L. cornuta* does not cause acute oral toxicity effects in Swiss albino mice.

ii. The aqueous aerial part extract of *L. cornuta* has considerable *in vivo* cognitive-enhancing effects in ketamine-induced cognitive-impaired Swiss albino mice.

iii. The aqueous aerial part extract of *L. cornuta* has significant malondialdehyde (MDA) profile -lowering effects in ketamine-induced cognitive impaired Swiss albino mice.

iv. The aqueous aerial part extract of *L. cornuta* contains various phytochemicals associated with cognitive-improvement and anti-lipid peroxidation.

Therefore, all the research questions formulated in this study were answered accordingly.

5.3 Recommendations

Considering the potential of the studied plant extracts as demonstrated by the findings reported herein, the following recommendations were made.

i. Further toxicological investigations should be conducted using other experimental methods and clinical settings to exhaustively appraise extract's safety profile.

ii. Specific mechanisms through which the studied plant extract exerts its cognitive-enhancing effects should be explored and demystified.

iii. Extensive studies to decipher the anti-lipid peroxidation and associated anti-oxidative stress mechanisms of the studied plant extract should be conducted.

iv. Characterisation of the specific phytochemicals responsible for the cognitive-enhancement and anti-lipid peroxidation and their specific mechanisms of bioactivity should be done.

REFERENCES

Abdullahi, A. A. (2011). Trends and challenges of traditional medicine in Africa. *African Journal of Traditional, Complementary and Alternative Medicines*, ***8*(5 SUPPL.)**, 115–123. https://doi.org/10.4314/ajtcam.v8i5S.5.

Ahmad, A., Singhal, U., Hossain, M. M., Islam, N., and Rizvi, I. (2013). The role of the endogenous antioxidant enzymes and malondialdehyde in essential hypertension. *Journal of Clinical and Diagnostic Research*, ***7*(6),** 987–990. https://doi.org/10.7860/JCDR/2013/5829.3091.

Akimat, E. K., Omwenga, G. I., Moriasi, G. A., and Ngugi, M. P. (2021). Antioxidant, Anti-Inflammatory, Acute Oral Toxicity, and Qualitative Phytochemistry of The Aqueous Root Extract of *Launaea cornuta* (Hochst. Ex Oliv. andamp; Hiern.). *Journal of Evidence-Based Integrative Medicine*, ***26,*** 2515690X2110645. https://doi.org/10.1177/2515690X211064585.

Altemimi, A., Lakhssassi, N., Baharlouei, A., Watson, D. G., and Lightfoot, D. A. (2017). Phytochemicals: Extraction, isolation, and identification of bioactive compounds from plant extracts. *Plants*, ***6*(4), 42,** 1-23. https://doi.org/10.3390/plants6040042.

Alzheimer Association. (2019). Early Signs and Symptoms of Alzheimer's. *Alzheimer's ..... https://www.alz.org/alzheimers-dementia/10_signs. Accessed on 17th March 2023.*

Alzheimer's Association. (2022). 2022 Alzheimer's Disease Facts and Figures*: More Than Normal Aging: Understanding Mild Cognitive Impairment*. **18(4)**:700-789. https://doi.org/10.1002/alz.12638.

Qaseem A, Snow V, Cross JT Jr, Forciea MA, Hopkins R Jr, Shekelle P, Adelman A, Mehr D, Schellhase K, Campos-Outcalt D, Santaguida P, Owens DK, (2008). Current pharmacologic treatment of dementia: A clinical practice guideline from the American college of physicians and the American academy of family physicians. *Annals of Internal Medicine*, ***148*(5)**, 370–378. https://doi.org/10.7326/0003-4819-148-5-200803040-00008.

Atukeren, P., Cengiz, M., Yavuzer, H., Gelisgen, R., Altunoglu, E., Oner, S., Erdenen, F., Yuceakın, D., Derici, H., Cakatay, U., and Uzun, H. (2017). The efficacy of donepezil administration on acetylcholinesterase activity and altered redox homeostasis in Alzheimer's disease. *Biomedicine and Pharmacotherapy*, ***90,*** 786–795. https://doi.org/10.1016/j.biopha.2017.03.101.

Bagetta, Giacinto. (2012). *Herbal medicines: development and validation of plant-derived medicines for human health*. *CRC Press (1st Ed)*. **2012**, 519 https://doi.org/10.1201/b11208.

Baird, C., Lovell, J., Johnson, M., Shiell, K., and Ibrahim, J. E. (2017). The impact of cognitive impairment on self-management in chronic obstructive pulmonary disease: A systematic review. *Respiratory Medicine*, ***129,*** 130–139. https://doi.org/10.1016/j.rmed.2017.06.006.

Baradaran, A., Rabiei, Z., Rafieian, M., and Shirzad, H. (2012). A review study on medicinal plants affecting amnesia through cholinergic system. *Journal of HerbMed Pharmacology*, ***1*(1),** 3–9.

Batool, Z., Sadir, S., Liaquat, L., Tabassum, S., Madiha, S., Rafiq, S., Tariq, S., Batool, T. S., Saleem, S., Naqvi, F., Perveen, T., and Haider, S. (2016). Repeated administration of almonds increases brain acetylcholine levels and enhances memory function in healthy rats while attenuates memory deficits in animal model of amnesia. *Brain Research Bulletin*. https://doi.org/10.1016/j.brainresbull.2015.11.001.

Bedi, O., and Krishan, P. (2019). *Investigations on acute oral toxicity studies of purpurin by application of OECD guideline 423 in rodents*.

Bello, M. O., Agbendeh, Z. M., and Jacob, A. G. (2013). The comparative studies of phytochemical screening of *Piliostigma thonningii* root and leaves extract. *Pelagia Research Library Asian Journal of Plant Science and Research*, ***2013*****(3)**, 74–77. www.pelagiaresearchlibrary.com.

BMJ Publishing group Ltd. (2018). *Pharmacological management for people with dementia*. Editorial.

Boon, W. C., Diepstraten, J., van der Burg, J., Jones, M. E. E., Simpson, E. R., and van den Buuse, M. (2005). Hippocampal NMDA receptor subunit expression and watermaze learning in estrogen deficient female mice. *Molecular Brain Research*. https://doi.org/10.1016/j.molbrainres.2005.07.004.

Buege, JA., and Aust, SD. (1979). The Mechanism of NADPH-dependent Lipid Peroxidation. *Methods in Enzymology* ***254*****(13)**, 5892–5899.

Casey, D. A., Antimisiaris, D., and O'Brien, J. (2010). Drugs for Alzheimer's disease: are they effective? *P* and *T: A Peer-Reviewed Journal for Formulary Management*, ***35*****(4)**, 208–211.

Chang, D., Liu, J., Bilinski, K., Xu, L., Steiner, G. Z., Seto, S. W., and Bensoussan, A. (2016). Herbal Medicine for the Treatment of Vascular Dementia*: An Overview of Scientific Evidence*. ***2016***.

Council, N. R. (2011). *Committee for the update of the guide for the care and use of laboratory animals. Guide for the care and use of laboratory animals*. https://doi.org/10.1163/1573-3912_islam_DUM_3825.

Duong, S., Patel, T., and Chang, F. (2017). Dementia: What pharmacists need to know. *Canadian Pharmacists Journal*, ***150*****(2)**, 118–129. https://doi.org/10.1177/1715163517690745.

Erhierhie, Oghenesuvwe, Earnest., Ekene, N. E., and Daniel Lotanna, A. (2014). Guidelines on dosage calculation and stock solution preparation in experimental animals' studies. *Journal of Natural Sciences Research Www*, ***4*****(18)**, 2225–2921.

Farahmandfar, M., Akbarabadi, A., Bakhtazad, A., and Zarrindast, M. R. (2017). Recovery from ketamine-induced amnesia by blockade of GABA-A receptor in the medial prefrontal cortex of mice. *Neuroscience*, ***344***, 48–55. https://doi.org/10.1016/j.neuroscience.2016.02.056.

Farmer, E. E., and Mueller, M. J. (2013). ROS-Mediated Lipid Peroxidation and RES-Activated Signaling. *Annual Review of Plant Biology*, ***64*****(1),** 429–450. https://doi.org/10.1146/annurev-arplant-050312-120132.

Félix, L. M., Vidal, A. M., Serafim, C., Valentim, A. M., Antunes, L. M., Campos, S., Matos, M., Monteiro, S. M., and Coimbra, A. M. (2016). Ketamine-induced oxidative stress at different developmental stages of zebrafish (Danio rerio) embryos. *RSC Advances*, ***6*(66),** 61254–61266. https://doi.org/10.1039/c6ra08298j.

Flynn, B. L., and Ranno, A. E. (1999). Pharmacologic management of Alzheimer disease. Part II: Antioxidants, antihypertensives, and ergoloid derivatives. *Annals of Pharmacotherapy*, ***33*(2)**, 188–197. https://doi.org/10.1345/aph.17172.

Fürst, R., and Zündorf, I. (2014). Plant-Derived Anti-Inflammatory Compounds: Hopes and Disappointments regarding the Translation of Preclinical Knowledge into Clinical Progress. *Mediators of Inflammation*, ***2014*(March 2015)**, 1–9. https://doi.org/10.1155/2014/146832.

Gakuya, D. W., Okumu, M. O., Kiama, S. G., Mbaria, J. M., Gathumbi, P. K., Mathiu, P. M., and Nguta, J. M. (2020). Traditional medicine in Kenya: Past and current status, challenges, and the way forward. *Scientific African*, ***8*,** e00360. https://doi.org/10.1016/j.sciaf.2020.e00360.

Genzel, L., Rossato, J. I., Jacobse, J., Grieves, R. M., Spooner, P. A., Battaglia, F. P., Fernández, G., and Morris, R. G. M. (2017). The Yin and Yang of Memory Consolidation: Hippocampal and Neocortical. *PLoS Biology*. https://doi.org/10.1371/journal.pbio.2000531.

Geoffrey Karau, M., Machocho, A., Ng, P., Kamau, aru, Muriira Karau, G., Nyagah Mwaniki Njagi, E., King, A., Machocho, ori, and Nyawira Wangai, L. (2014). Efficacy and Safety Assessment of *Launaea cornuta* Extracts in the Management of Diabetes Mellitus. *American Journal of Medicine and Medical Sciences*, ***2014*(1),** 1–7. https://doi.org/10.5923/j.ajmms.20140401.01.

George, P. (2011). Concerns regarding the safety and toxicity of medicinal plants - An overview. *Journal of Applied Pharmaceutical Science*, ***1*(6)**, 40–44.

Gracia, K. C., Llanas-cornejo, D., and Husi, H. (2017). *CVD and Oxidative Stress*. https://doi.org/10.3390/jcm6020022.

Gutierres, J. M., Carvalho, F. B., Schetinger, M. R. C., Agostinho, P., Marisco, P. C., Vieira, J. M., Rosa, M. M., Bohnert, C., Rubin, M. A., Morsch, V. M., Spanevello, R., and Mazzanti, C. M. (2014). Neuroprotective effect of anthocyanins on acetylcholinesterase activity and attenuation of scopolamine-induced amnesia in rats. *International Journal of Developmental Neuroscience*. https://doi.org/10.1016/j.ijdevneu.2013.12.006.

Harborne, J. B. (1998). Phytochemical Methods A Guide To Modern Techniques Of Plant Analysis, Third Edition. In *Chapman* and *Hall*. https://doi.org/10.1017/CBO9781107415324.004.

Hou, Q., Gao, X., Zhang, X., Kong, L., Wang, X., Bian, W., Tu, Y., Jin, M., Zhao, G., Li, B., Jing, N., and Yu, L. (2004). SNAP-25 in hippocampal CA1 region is involved in memory consolidation. *European Journal of Neuroscience*, ***20*(6),** 1593–1603. https://doi.org/10.1111/j.1460-9568.2004.03600.x.

Hritcu, L., Noumedem, J. A., Cioanca, O., Hancianu, M., Kuete, V., and Mihasan, M. (2014). Methanolic extract of Piper nigrum fruits improves memory impairment by decreasing brain oxidative stress in amyloid beta(1-42) rat model of Alzheimer's disease. *Cellular and Molecular Neurobiology*, ***34*(3)**, 437–449. https://doi.org/10.1007/s10571-014-0028-y.

Huang, D. (2018). Dietary antioxidants and health promotion. *Antioxidants*, ***7*(1)**, 7–9. https://doi.org/10.3390/antiox7010009.

Huang, W. J., Zhang, X., and Chen, W. W. (2016). Role of oxidative stress in Alzheimer's disease (review). *Biomedical Reports*, ***4*(5),** 519–522. https://doi.org/10.3892/br.2016.630.

Hugo, J., and Ganguli, M. (2014). Dementia and Cognitive Impairment. Epidemiology, Diagnosis, and Treatment. *Clinics in Geriatric Medicine*, ***30*(3)**, 421–442. https://doi.org/10.1016/j.cger.2014.04.001.

Ide, S., Ikekubo, Y., Mishina, M., Hashimoto, K., and Ikeda, K. (2019). Cognitive Impairment That Is Induced by (R)-Ketamine Is Abolished in NMDA GluN2D Receptor Subunit Knockout Mice. *International Journal of Neuropsychopharmacology*, ***22*(7),** 449–452. https://doi.org/10.1093/ijnp/pyz025.

Ionita, R., Postu, P. A., Beppe, G. J., Mihasan, M., Petre, B. A., Hancianu, M., Cioanca, O., and Hritcu, L. (2017). Cognitive-enhancing and antioxidant activities of the aqueous extract from *Markhamia tomentosa* (Benth.) K. Schum. stem bark in a rat model of scopolamine. *Behavioral and Brain Functions*, ***13*(1)**, 1–13. https://doi.org/10.1186/s12993-017-0123-6.

Jamshidi-Kia, F., Lorigooini, Z., and Amini-Khoei, H. (2018). Medicinal plants: Past history and future perspective. *Journal of HerbMed Pharmacology*, ***7*(1)**, 1–7. https://doi.org/10.15171/jhp.2018.01.

Jared, M., Bibiane, A., Gervason, A., Lameck, N., and Japhet, K. (2018). The Antibacterial, Antioxidant and Phytochemical Composition of *Combretum tanaense* (J. Clark) Root Extracts. *European Journal of Medicinal Plants*, ***23*(4),** 1–8. https://doi.org/10.9734/ejmp/2018/40956.

Kalapatapu, R. K., Delucchi, K. L., Wang, S., John, D., Nelson, E. E., and Kramer, J. H. (2017). *Dementia versus Primary Progressive Aphasia*. ***35*(1),** 36–41. https://doi.org/10.1080/10550887.2015.1102026.Substance.

Kalopita, K., Armakolas, A., Philippou, A., Zarros, A., and Angelogianni, P. (2021). Ketamine-induced neurotoxicity in neurodevelopment: A synopsis of main pathways based on recent *in vivo* experimental findings. In *Journal of Anaesthesiology Clinical Pharmacology*, **37 (1),** pp. 37–42. https://doi.org/10.4103/joacp.JOACP_415_19.

Kivipelto, M., Mangialasche, F., and Ngandu, T. (2018). Lifestyle interventions to prevent cognitive impairment, dementia and Alzheimer disease. In *Nature Reviews Neurology* 14(11), pp. 653–666). Nature Publishing Group. https://doi.org/10.1038/s41582-018-0070-3.

Koche, D., Shirsat, R., and Kawale, M. (2016). An overview of major classes of phytochemicals: Their type and role in disease prevention. *Hislopia Journal*, ***9*(1)**, 2016.

Kokwaro, J. O. (2009). *Medicinal Plants of East Africa* (Third edit). University of Nairobi Press.

Kuo, C. Y., Stachiv, I., and Nikolai, T. (2020). Association of late life depression, (Non-) modifiable risk and protective factors with dementia and alzheimer's disease: Literature review on current evidences, preventive interventions and possible future trends in prevention and treatment of dementia. In *International Journal of Environmental Research and Public Health* 17(20), pp. 1–24). MDPI AG. https://doi.org/10.3390/ijerph17207475.

Lam, V., Hackett, M., and Takechi, R. (2016). Antioxidants and dementia risk: Consideration through a cerebrovascular perspective. *Nutrients*, ***8*(12)**, 1–18. https://doi.org/10.3390/nu8120828.

Lee, J. S., Kim, H. G., Lee, H. W., Han, J. M., Lee, S. K., Kim, D. W., Saravanakumar, A., and Son, C. G. (2015). Hippocampal memory enhancing activity of pine needle extract against scopolamine-induced amnesia in a mouse model. *Scientific Reports*, ***5,*** 1–10. https://doi.org/10.1038/srep09651.

Liang, J., Wu, S., Xie, W., and He, H. (2018). Ketamine ameliorates oxidative stress-induced apoptosis in experimental traumatic brain injury via the Nrf2 pathway. *Drug Design, Development and Therapy*, ***12***, 845–853. https://doi.org/10.2147/DDDT.S160046.

Liguori, I., Russo, G., Curcio, F., Bulli, G., Aran, L., Della-Morte, D., Gargiulo, G., Testa, G., Cacciatore, F., Bonaduce, D., and Abete, P. (2018). Oxidative stress, aging, and diseases. *Clinical Interventions in Aging*, ***13***, 757–772. https://doi.org/10.2147/CIA.S158513.

Lourenço, S. C., Moldão-Martins, M., and Alves, V. D. (2019). Antioxidants of natural plant origins: From sources to food industry applications. *Molecules*, ***24*(22)**, 14–16. https://doi.org/10.3390/molecules24224132.

Machocho, A., Karau, G., Njagi, E., Koech, L., and Wangai, L. (2014). *Profiling of the chemical compounds in ethyl acetate extracts of Launaea cornuta* asteraceae by GC-MS. ***1*(5)**, 1–5. https://doi.org/10.13040/IJPSR.0975-8232.1(5).296-00.

Mattap, S. M., Mohan, D., McGrattan, A. M., Allotey, P., Stephan, B. C. M., Reidpath, D. D., Siervo, M., Robinson, L., and Chaiyakunapruk, N. (2022). The economic burden of dementia in low- and middle-income countries (LMICs): a systematic review. In *BMJ Global Health*, **7(4).** BMJ Publishing Group. https://doi.org/10.1136/bmjgh-2021-007409.

Mcshane, R., Westby, M., Roberts, E., Minakaran, N., Schneider, L., Farrimond, L., Maayan, N., Ware, J., and Debarros, J. (2019). Memantine for dementia [Systematic Review]. *Cochrane Database of Systematic Reviews*, *1*. https://doi.org/10.1002/14651858.CD003154.pub6.www.cochranelibrary.com.

Mendez, M. F. (2017). What is the Relationship of Traumatic Brain Injury to Dementia? In *Journal of Alzheimer's Disease*, **57 (3),** pp. 667–681. IOS Press. https://doi.org/10.3233/JAD-161002.

Mion, G., and Villevieille, T. (2013). Ketamine Pharmacology: An Update (Pharmacodynamics and Molecular Aspects, Recent Findings). In *CNS Neuroscience and Therapeutics*, **19(6),** pp. 370–380). https://doi.org/10.1111/cns.12099.

Misonge, J. O., Kinyanjui, J. G., Kingori, W. M., and Mwalukumbi, J. M. (2015). *Phytochemical screening and cytotoxicity evaluation of Launaea Cornuta H. (Asteraceae) using brine shrimp*. ***3*(4)**, 116–120.

Moriasi, G. A., Ireri, A. M., and Ngugi, M. P. (2020). *In vivo* Cognitive-Enhancing, Ex Vivo Malondialdehyde-Lowering Activities and Phytochemical Profiles of Aqueous and Methanolic Stem Bark Extracts of *Piliostigma thonningii* (Schum.). *International Journal of Alzheimer's Disease*, ***2020***, 1–15. https://doi.org/10.1155/2020/1367075.

Moriasi, G., Ireri, A., and Ngugi, M. (2020b). Cognitive-Enhancing, Ex Vivo Antilipid Peroxidation and Qualitative Phytochemical Evaluation of the Aqueous and Methanolic Stem Bark Extracts of Lonchocarpus eriocalyx (Harms.). *Biochemistry Research International*, ***2020*,** 1–16. https://doi.org/10.1155/2020/8819045.

Moriasi, G., Ireri, A., and Ngugi, M. P. (2020c). *In vitro* antioxidant activities of the aqueous and methanolic stem bark extracts of *Piliostigma thonningii* (Schum.). *Journal of Evidence-Based Integrative Medicine*, ***25***, 2515690X20937988.

Moriasi, G., Kibiti, C., and Ngugi, M. (2021). *In vivo* Antidiabetic Efficacy, Mineral Element Composition, and Qualitative Phytochemistry of the Aqueous Leaf Extracts of Pentas zanzibarica (Klotzsch.) Vatke and *Olea europaea* subspecies *africana* (Mill.). *Journal of Advanced Biotechnology and Experimental Therapeutics*, ***4*(3),** 334. https://doi.org/10.5455/jabet.2021.d134.

Morris, R. (2008). Morris water maze. *Scholarpedia*, ***3*(8),** 6315. https://doi.org/10.4249/scholarpedia.6315.

Naik, R. S., Hartmann, J., Kiewert, C., Duysen, E. G., Lockridge, O., and Klein, J. (2009). Effects of rivastigmine and donepezil on brain acetylcholine levels in acetylcholinesterase-deficient mice. *Journal of Pharmacy and Pharmaceutical Sciences*, *12*(1), 79–85. https://doi.org/10.18433/J3MK59.

Nasri, H., and Shirzad, H. (2013). Toxicity and safety of medicinal plants. *Journal of HerbMed Pharmacology*, ***2*(2),** 21–22.

Nichols, E., Steinmetz, J. D., Vollset, S. E., Fukutaki, K., Chalek, J., Abd-Allah, F., Abdoli, A., Abualhasan, A., Abu-Gharbieh, E., Akram, T. T., al Hamad, H., Alahdab, F., Alanezi, F. M., Alipour, V., Almustanyir, S., Amu, H., Ansari, I., Arabloo, J., Ashraf, T., … Vos, T. (2022). Estimation of the global prevalence of dementia in 2019 and forecasted prevalence in 2050: an analysis for the Global Burden of Disease Study 2019. *The Lancet Public Health*, ***7*(2)**, e105–e125. https://doi.org/10.1016/S2468-2667(21)00249-8.

OECD. (2000). Guidance document on the recognition, assessment, and use of clinical signs as humane endpoints for experimental animals used in safety evaluation. *Series on Testing and Assessment*, **39**.

OECD. (2008). Test No. 425: Acute Oral Toxicity: Up-and-Down Procedure. In *Test* (Issue October). OECD. https://doi.org/10.1787/9789264071049-en.

Ohkawa, H., Ohishi, N., and Yagi, K. (1979). Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. *Analytical Biochemistry*, ***95*(2)**, 351–358. https://doi.org/10.1016/0003-2697(79)90738-3.

Onyou, H. (2013). *Role of Oxidative Stress in Parkinson' s Disease*. ***22*(1),** 11–17.

Palhares, R. M., Drummond, M. G., and Alves, S. (2015). Medicinal Plants Recommended by the World Health Organization: *DNA Barcode Identification Associated with Chemical Analyses Guarantees Their Quality*. PLoS ONE 10(5):1-29 (e0127866). https://doi.org/10.1371/journal.pone.0127866.

Patrick Maundu, and Bo Tengnas. (2005). Useful Trees and Shrubs for Kenya. In *World Agroforestry Centre: **Vol. No:35*.**

Pink, J., O'Brien, J., Robinson, L., and Longson, D. (2018). Dementia: Assessment, management and support: Summary of updated NICE guidance. *BMJ (Online)*, ***361*(June)**, 1–6. https://doi.org/10.1136/bmj.k2438.

Pizzino, G., Irrera, N., Cucinotta, M., Pallio, G., Mannino, F., Arcoraci, V., Squadrito, F., Altavilla, D., and Bitto, A. (2017). Oxidative Stress: Harms and Benefits for Human Health. *Oxidative Medicine and Cellular Longevity*, ***2017*.** https://doi.org/10.1155/2017/8416763.

Rahimzadegan, M., and Soodi, M. (2018). Comparison of memory impairment and oxidative stress following single or repeated doses administration of scopolamine in rat hippocampus. *Basic and Clinical Neuroscience*, ***9*(1)**, 5–14.

Rahman, T., Hosen, I., Islam, M. M. T., and Shekhar, H. U. (2012). Oxidative stress and human health. *Advances in Bioscience and Biotechnology*, ***03*(07),** 997–1019. https://doi.org/10.4236/abb.2012.327123.

Réus, G. Z., Simões, L. R., Colpo, G. D., Scaini, G., Oses, J. P., Generoso, J. S., Prossin, A. R., Kaddurah-Daouk, R., Quevedo, J., and Barichello, T. (2017). Ketamine potentiates oxidative stress and influences behavior and inflammation in response to lipolysaccharide (LPS) exposure in early life. *Neuroscience*, ***353*,** 17–25. https://doi.org/10.1016/j.neuroscience.2017.04.016.

Ru, M., Brauer, M., Lamarque, J. F., and Shindell, D. (2021). Exploration of the Global Burden of Dementia Attributable to PM2.5: What Do We Know Based on Current Evidence? *GeoHealth*, ***5*(5).** https://doi.org/10.1029/2020GH000356.

Sajjad N., Wani A., Hassan S., Ali R., Hamid R., Ba., G., and Ea, B. (2019). Interplay of antioxidants in Alzheimer's disease. *Review Article Journal of Translational Science J Transl Sci*, **6(February)**. https://doi.org/10.15761/JTS.1000313.

Santos, T., Lovell, J., Shiell, K., Johnson, M., and Ibrahim, J. E. (2018). The impact of cognitive impairment in dementia on self-care domains in diabetes: A systematic search and narrative review. *Diabetes/Metabolism Research and Reviews*, ***34*(6)**. https://doi.org/10.1002/dmrr.3013.

Saxena, G., Singh, S. P., Agrawal, R., and Nath, C. (2008). Effect of donepezil and tacrine on oxidative stress in intracerebral streptozotocin-induced model of dementia in mice. *European Journal of Pharmacology*, ***581*(3),** 283–289. https://doi.org/10.1016/j.ejphar.2007.12.009.

Sevastre-Berghian, A. C., Făgărăsan, V., Toma, V. A., Bâldea, I., Olteanu, D., Moldovan, R., Decea, N., Filip, G. A., and Clichici, S. v. (2017). Curcumin reverses the diazepam-induced cognitive impairment by modulation of oxidative stress and erk 1/2/nf-κ b pathway in brain. *Oxidative Medicine and Cellular Longevity*, ***2017***. https://doi.org/10.1155/2017/3037876.

Shabana, Shahid, S. U., and Sarwar, S. (2020). The abnormal lipid profile in obesity and coronary heart disease (CHD) in Pakistani subjects. *Lipids in Health and Disease*, ***19*(1)**. https://doi.org/10.1186/s12944-020-01248-0.

Shah, H., Albanese, E., Duggan, C., Rudan, I., Langa, K. M., Carrillo, M. C., Chan, K. Y., Joanette, Y., Prince, M., Rossor, M., Saxena, S., Snyder, H. M., Sperling, R., Varghese, M., Wang, H., Wortmann, M., and Dua, T. (2016). Research priorities to reduce the global burden of dementia by 2025. In *The Lancet Neurology* (**Vol. 15, Issue 12,** pp. 1285–1294). Lancet Publishing Group. https://doi.org/10.1016/S1474-4422(16)30235-6.

Shakya, A. K. (2016). Medicinal plants*: Future source of new drugs Medicinal plants : Future source of new drugs*. *January*. https://doi.org/10.13140/RG.2.1.1395.6085.

Singhal, A., Bangar, O., and Naithani, V. (2012). Medicinal plants with a potential to treat Alzheimer and associated symptoms. *International Journal of Nutrition, Pharmacology, Neurological Diseases*, ***2*(2)**, 84. https://doi.org/10.4103/2231-0738.95927.

Tan, B. L., Norhaizan, M. E., Liew, W. P. P., and Rahman, H. S. (2018). Antioxidant and oxidative stress: A mutual interplay in age-related diseases. *Frontiers in Pharmacology*, ***9*(OCT)**, 1–28. https://doi.org/10.3389/fphar.2018.01162.

Tang, C. T., Belani, L. K., Das, S., and Jaafar, M. Z. (2013). *Treatment of dementia with herbs : A short review*. ***164*(1),** 43–46. https://doi.org/10.7417/T.2013.1511.

Tewari, D., Stankiewicz, A. M., Mocan, A., and Sah, A. N. (2018). *Ethnopharmacological Approaches for Dementia Therapy and Significance of Natural Products and Herbal Drugs*. ***10*(February),** 1–24. https://doi.org/10.3389/fnagi.2018.00003.

Tian, J., Shi, J., Zhang, X., and Wang, Y. (2010). Herbal therapy:*A new pathway for the treatment of Alzheimer ' s disease*. 2–5.

Trease, G. E., Evans, W. C. (2009). Pharmacognosy 16th edition. *W.B. Sanders Company Ltd., New York.*, 42–44. 221–229, 246–249, 304–306, 331–332, 391–393.

Truong, D. H., Nguyen, D. H., Ta, N. T. A., Bui, A. V., Do, T. H., and Nguyen, H. C. (2019). Evaluation of the use of different solvents for phytochemical constituents, antioxidants, and *in vitro* anti-inflammatory activities of *Severinia buxifolia*. *Journal of Food Quality*, *2019*. https://doi.org/10.1155/2019/8178294.

Tsai, M. C., and Huang, T. L. (2015). Thiobarbituric acid reactive substances (TBARS) is a state biomarker of oxidative stress in bipolar patients in a manic phase. *Journal of Affective Disorders*, *173*, 22–26. https://doi.org/10.1016/j.jad.2014.10.045.

Vorhees, C. v., and Williams, M. T. (2006). Morris water maze: Procedures for assessing spatial and related forms of learning and memory. *Nature Protocols*. https://doi.org/10.1038/nprot.2006.116.

Wambugu, S. N., Mathiu, P. M., Gakuya, D. W., Kanui, T. I., Kabasa, J. D., and Kiama, S. G. (2011). Medicinal plants used in the management of chronic joint pains in Machakos and Makueni counties, Kenya. *Journal of Ethnopharmacology*, ***137*(2)**, 945–955. https://doi.org/10.1016/j.jep.2011.06.038.

WHO. (2013). WHO herbal sale rates. ***2013*,** 26.

Willey, J. Z., Gardener, H., Moon, Y. P., Yoshita, M., DeCarli, C., Cheung, Y. K., Sacco, R. L., Elkind, M. S. V., and Wright, C. B. (2014). Lipid profile components and subclinical cerebrovascular disease in the northern Manhattan study. *Cerebrovascular Diseases*, ***37*(6)**, 423–430. https://doi.org/10.1159/000362920,

World Health Organisation. (2019). *Risk reduction of cognitive decline and dementia: WHO guidelines*.

World Health Organization (WHO). (2019). Risk Reduction of Cognitive decline and Dementia. In *WHO*. Geneva.

Xu, C. (2019). Trends in phytochemical research. *Journal of Food Biochemistry*, ***43*(6)**. https://doi.org/10.1111/jfbc.12913.

Szeto, YYJ., and Lewis, JGS. (2016). Current Treatment Options for Alzheimer's Disease and Parkinson's Disease Dementia. *Current Neuropharmacology*, ***14*(4)**, 326–338. https://doi.org/10.2174/1570159x14666151208112754.

Zanos, P., Moaddel, R., Morris, P. J., Riggs, L. M., Highland, J. N., Georgiou, P., Pereira, E. F. R., Albuquerque, E. X., Thomas, C. J., Zarate, C. A., and Gould, T. D. (2018). Ketamine and ketamine metabolite pharmacology: Insights into therapeutic mechanisms. *Pharmacological Reviews*, ***70*(3)**, 621–660. https://doi.org/10.1124/pr.117.015198.

Zhang, J., Wider, B., Shang, H., Li, X., and Ernst, E. (2012). Quality of herbal medicines: Challenges and solutions. In *Complementary Therapies in Medicine*. https://doi.org/10.1016/j.ctim.2011.09.004.

Zhang, Y. J., Gan, R. Y., Li, S., Zhou, Y., Li, A. N., Xu, D. P., Li, H. bin, and Kitts, D. D. (2015). Antioxidant phytochemicals for the prevention and treatment of chronic diseases. In *Molecules* (Vol. 20, Issue 12, pp. 21138–21156). MDPI AG. https://doi.org/10.3390/molecules201219753.

Zhang, Z., Bai, H., Ma, X., Shen, M., Li, R., Qiu, D., Li, S., and Gao, L. (2021). Blockade of the NLRP3/caspase-1 axis attenuates ketamine-induced hippocampus pyroptosis and cognitive

impairment in neonatal rats. *Journal of Neuroinflammation*, ***18*(1)**. https://doi.org/10.1186/s12974-021-02295-9.

APPENDICES

Appendix 1: Institutional Ethical Approval

UNIVERSITY OF NAIROBI
FACULTY OF VETERINARY MEDICINE
DEPARTMENT OF VETERINARY ANATOMY AND PHYSIOLOGY

P.O. Box 30197,
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Direct Line. 4448648

REF: FVM BAUEC/2022/336

Mercy Rispa Wambui.
Dept. of PHP & Toxicology
University of Nairobi
01/01/2022

Dear Mercy,

RE: Approval of proposal by Faculty Biosafety, Animal use and Ethics committee

"Cognitive-Enhancing Anti Lipid peroxidation, Qualitative Photochemistry and Toxic effects of aqueous aerial part extract of *Launaea cornuta*".
Mercy Rispa Wambui J56/37921/2020

We refer to your MS.c proposal submitted to our committee for review and your application letter dated 16th December 2021.We have reviewed your application for ethical clearance for the study. The number of mice, animal husbandry practices and protocol used to determine cognitive-enhancing properties of the aqueous aerial part extract of L. cornuta using the Morris Water Maze (MWM) technique using a controlled randomized experimental design meets the minimum standard of the Faculty of Veterinary medicine ethical regulation guidelines.
We also note that registered Veterinary surgeons will supervise the study.
We hereby give approval for you to proceed with the project as outlined in the submitted proposal.

Yours sincerely,

Kaluwa

Dr. Catherine Kaluwa, Ph.D
Chairperson, Biosafety, Animal Use and Ethics Committee,
Faculty of Veterinary Medicine,
University of Nairobi

Appendix 2:Research Permit

REPUBLIC OF KENYA

NATIONAL COMMISSION FOR SCIENCE,TECHNOLOGY & INNOVATION

Ref No: **725249**

Date of Issue: **15/June/2022**

RESEARCH LICENSE

This is to Certify that Dr.. MERCY RISPA WAMBUI MAINA of University of Nairobi, has been licensed to conduct research in Nairobi on the topic: COGNITIVE-ENHANCING, ANTI-LIPID PEROXIDATION, QUALITATIVE PHYTOCHEMISTRY, AND TOXIC EFFECTS OF THE AQUEOUS AERIAL PART EXTRACT OF LAUNAEA CORNUTA for the period ending : 15/June/2023.

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725249

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Director General
NATIONAL COMMISSION FOR SCIENCE,TECHNOLOGY & INNOVATION

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E-mail: dg@nacosti.go.ke / registry@nacosti.go.ke
Website: www.nacosti.go.ke

Appendix 3: Plant authentication certificate

NATIONAL MUSEUMS OF KENYA

WHERE HERITAGE LIVES ON

19th May 2022

REF: NMK/BOT/CTX/1/2

Dr. Rispa Maina

University of Nairobi (UoN)

Dear Madam,

PLANT IDENTIFICATION

The plant specimens you brought to East African Herbarium (EA) for identification has been determined as follows:

Botanical Name: *Launaea cornuta* (Hochst. ex Oliv. & Hiern) C.Jeffrey

Family: Asteraceae

Thank you for consulting the East African Herbarium for plant identification and confirmation.

Yours Sincerely,

Dr. Peris Kamau

For: Head, Botany Department

Address P.O. Box 40658 00100 GPO Nairobi Telephone +254 20 4448930/3. +254 20 3742161/4. +254 20 3742131/4. Fax +254 20 3741424. Website www.museums.or.ke

Heliyon 9 (2023) e15487

Contents lists available at ScienceDirect

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Research article

Acute oral toxicity, cognitive-enhancing and anti-lipid peroxidation efficacy, and qualitative phytochemistry of the aqueous aerial part extract of *Launaea cornuta* (Hochst. ex. Oliv. &Hiern) C. Jeffrey

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ARTICLE INFO

Keywords:
Morris water maze
Thiobarbituric acid reactive substances
Malondialdehyde
Dementia
Phytochemicals
Up-and-down procedure

ABSTRACT

At present, there is no cure for dementia or its related cognitive impairments. Available treatments only provide symptomatic relief and do not alter the disease's progression and they suffer serious drawbacks limiting their clinical use, hence the need for alternative therapies. Although *Launaea cornuta* has been used traditionally to treat cognitive deficits, its pharmacological efficacy and safety have not been empirically validated, prompting this study. Acute oral toxicity of the extract was examined in Swiss albino mice using the up-and-down procedure described by the Organisation for Economic Cooperation and Development guideline number 425. The Morris water maze technique was adopted in assessing cognitive-enhancing effects of the extract in ketamine-induced cognitive-impaired mice. The malondialdehyde concentrations in the whole brain of experimental mice involved in the MWM experiment were measured to determine the extract's anti-lipid peroxidation efficacy. Qualitative phytochemical screening of the extract was performed using standard procedures. Our results showed that the test extract was safe and did not cause any clinical signs of acute oral toxicity in mice at all doses (LD50 > 2000 mg/kg BW). Moreover, the extract significantly improved cognitive function in ketamine-induced cognitive-impaired mice in a dose-dependent manner, as indicated by reduced escape latency, navigation distance, and longer latency in the target quadrant during the probe trial. The extract also significantly reduced malondialdehyde concentrations in mice in a dose-dependent manner, demonstrating its antioxidative stress efficacy. The studied extract contained various phytochemicals associated with cognitive enhancement and antioxidant efficacy, among other pharmacologic effects. Further empirical studies are needed to determine and characterise the extract's specific cognitive-enhancing compounds, specific mechanisms of action, and complete toxicity profiles.

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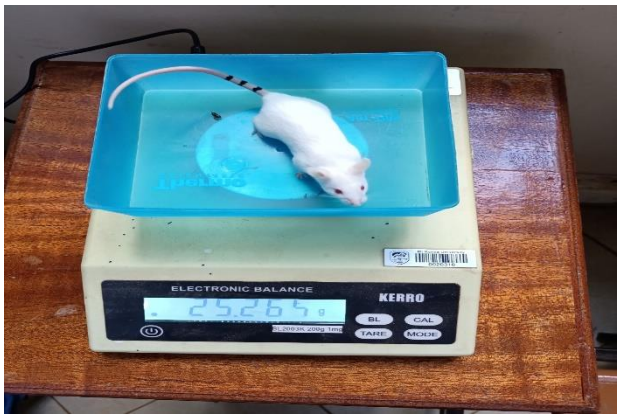
https://doi.org/10.1016/j.heliyon.2023.e15487
Received 21 November 2022; Received in revised form 2 April 2023; Accepted 12 April 2023
Available online 15 April 2023

Appendix 4: Selected Photo Gallery

Sample container labelling

Animal sacrificing



Animal weighing

TURN IT IN REPORT

Cognitive-Enhancing, Anti-Lipid Peroxidation, Qualitative Phytochemistry, And Toxic Effects of The Aqueous Aerial Part Extract of Launaea cornuta (Hochst. Ex Oliv. And Hiern.)

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