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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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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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1. Introduction

Dementia is a debilitating syndrome characterised by progressive deterioration of cognitive function in the brain [1]. The affected individuals present with memory and learning deficits, altered behaviour, impaired thinking, reasoning and judgement, and impaired physical and professional functioning [2]. Although advancing age is a risk for dementia, with those over 60 years being the most affected, dementia is not an inevitable consequence of ageing. In fact, research has shown that dementia can affect people of all ages, social classes, and gender due to an interplay of predisposing factors, such as genetics, environmental triggers like particulate matter pollution, traumatic injuries, developmental abnormalities, commodities such as diabetes mellitus, among others [3–6]. Alzheimer's disease (AD) is the commonest dementia and the fifth cause of fatalities among the elderly (≥65 years) [7]. Currently, AD affects >50 million individuals worldwide, with majority residing in low- and medium-income countries (LMICs) [7]. It is projected that over 152 million persons will have dementia, especially AD, by 2050 if appropriate mitigation measures are not implemented [8].

To date, there is no effective drug that can cure AD, and the available ones do not modify the disease course, and generally manage the associated symptoms [2,9–12]. The symptomatic relief offered by cholinesterase inhibitors is due to their ability to inhibit the activity of acetylcholinesterase, which promotes nerve firing by ensuring acetylcholine availability. Nonetheless, this strategy is confounded by many drawbacks, including low efficacy, short half-life, and frequent dosing of the drugs, often at higher doses, and devastating side effects [2,9–12]. In addition, these medicines are not easily accessible and affordable to most patients living in the LMICs, which are characterised by a high disease burden [5,13]. Moreover, recent reports indicate a significantly higher financial 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 LMICs [7,13].

Considering the challenges of conventional dementia therapy, alternative stratagems for preventing, slowing its progression, or averting its devastating effects are currently being sought [14]. Plant-based products and extracts are a feasible alternate source of valuable lead compounds for drug development [18–20] owing to their longstanding ethnomedicinal applications in healthcare, and their numerous phytochemicals. They are easily available, inexpensive, effective, and relatively safer than conventional medicines, as evidenced by their reputation in various ethnicities worldwide [15–17]. However, there is a paucity of experimental data to substantiate their therapeutic claims and safety. Moreover, the medical fraternity have a skeptical attitude towards the safety of herbal preparations, which, in turn prevaricate their integration into modern medicine [21]. Particularly, inadequate legislation, preparation methods, storage, labelling, marketing, dose regimens for each ailment, and interactions between herbs and conventional drugs severely impede the growth of traditional medicine practice and its integration into contemporary healthcare practice [22–24].

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 [25]. The plant is indigenous to Kenya and many African countries. It is referred to as "*Mchunga*" in Swahili, "*Muthunga*" (Meru, Kikuyu, and Embu), "*Mnyinya*" in Taita, and "Achak" in Luo [26]. In addition to other uses, it is used to treat gonorrhoea, typhoid, inflammatory diseases including enlarged testicles, earaches, stomach-aches, chronic joint problems, diabetes, hypertension, and memory loss [27,28]. Past studies have shown that this plant is highly effective in reducing inflammation and free radicals, and it also contains antioxidant phytocompounds [29] that may improve cognitive function [30]. Even though *L. cornuta* has been used extensively to treat dementia and associated complications, among other ailments in traditional medicine, there is a paucity of empirical information to appraise its efficacy and medicinal value. As a result, we examined the aqueous aerial part extract of *L. cornuta*'s cognitive-improving, anti-lipid peroxidation, toxic, and qualitative phytochemical properties in order to confirm its purported therapeutic benefits and as a potential source of safe and effective treatments for dementia and its complications.

2. Materials and methods

2.1. Collection of plant materials and processing

The aerial parts of *L. cornuta* were collected on 4th August 2021, with the help of a renowned herbalist, from Irangi forest in Embu County, Kenya, where the plant grew naturally. Voucher specimens of the plant were prepared, identified taxonomically, and archived at the East Africa Herbarium at the National Museums of Kenya (REF: NMK/BOT/CTX/1/3). 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 °C ± 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.

2.2. Extraction procedure

The extraction procedure described by Harborne [31] and modified by Moriasi et al. [32] 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 °C for 5 min. The concoction was cooled to room temperature and then filtered *in vacuo* through Whatman No. 1 filter papers (Lot No# 221175) using a Buchner funnel. The filtrate was transferred into freeze-drying flasks at volumes of 200 ml and lyophilised (freeze-dried) for 48 h in a Modulyo Freeze Dryer (Cat No# SuperModulyo230; Edwards-England). The dry lyophilised extract was transferred into a clean, dry pre-weighed universal

bottle, weighed using an analytical digital scale (Model No# Shimadzu ATY224) and then stored in a Samsung refrigerator (2–8 °C) awaiting analysis. The percentage yield of the extract was calculated according to the formulae (Eqn. (1)) described by Truong et al. [33].

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

2.3. Experimental animals

Swiss-Albino mice between 4 and 5 weeks old were obtained from the Kenya Agricultural and Livestock Research Organisation (KALRO) animal breeding section. The animals were kept in standard conditions (12-h-day and 12-h-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 and clean water *ad-libitum.* The animals were acclimatised for 72 h before experimentation, handled humanely during the study, and disposed of according to the guidelines described by the National Research Council [34].

2.4. Acute oral toxicity study

Acute oral toxicity was investigated according to the Up-and-Down-Procedure (UDP) stipulated by the OECD [35]. Briefly, four randomised groups of experimental mice (five mice per group) were fasted for 4 h 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 guidelines [35]. After that, wellness parameters, such as the appearance of skin fur, salivation, mucous membrane, lethargy, eyes, convulsions, diarrhoea, coma, tremors, sleep, mortality, and body weight, were observed and monitored keenly after 30 min, 4 h, 24 h, 48 h, 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 [35].

2.5. Determination of in vivo cognitive-enhancing effects of the test extract

2.5.1. Morris Water Maze task

We adopted the Morris Water Maze technique [36,37] as modified by Moriasi et al. [30] in this study. 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 warmth of the maze was kept 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 m 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 submerged 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 allowed to rest and explore the maze for 20 s before being removed by the researcher. The starting point and the escape platform location remained constant for the experimental period.

2.5.2. Preparation of the administration drugs

Donepezil ([Pr]ARICEPT® RDT, Pfizer Inc. Canada), Ketamine (Pfizer Inc. USA), and Normal saline (Infusion Kenya Ltd.) were bought from an accredited local pharmacy and reconstituted as illustrated previously [38]. Extract doses were selected upon a pilot study and prepared using a previously illustrated procedure [38].

2.5.3. 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 min*;* Group III (Positive control) mice were administered Donepezil (1 mg/kg BW; *p.o*) and Ketamine (1 mg/kg BW; *i.p*) after 45 min; 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 min. All mice were subjected to the MWM task 30 min 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 s. 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.

2.6. 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 stowed at −80 °C until use. The brain samples were defrosted and homogenized 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 [39] and Ohkawa et al. [40] was used to determine the MDA concentration in the brain samples. In brief, the reaction mixtures comprised 0.8% Thiobarbituric acid (Lot No# SG53121901; Loba Chemie) (1.5 ml), 20% acid acetic acid glacial (CAS No# 64-19-7; Sigma-Aldrich, Germany) (1.5 ml; pH 3.5), 8.1% sodium dodecyl sulphate (CAS No# 151-21-3; Sigma-Aldrich, Germany) (0.2 ml), and 0.1 ml of homogenized brain tissue. The mixtures were heated at 100 °C for 1 h and then cooled to room temperature before n-butanol (CAS No# 71-36-3; Sigma-Aldrich, Germany): pyridine (CAS No# 110-86-1; Supelco-Merck) (15:1) mixture (5 ml) and distilled water (1 ml) were added. The mixtures were shaken vigorously using a vortex mixer (Labtech) and centrifuged at 2500 rpm for exactly 20 min, after which the supernatant of each portion was aspirated carefully, and its absorbance was measured at λ_{532} nm using a Shimadzu 1601 double-beam UV–Vis Spectrophotometer (Shimadzu, Germany). 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 [40].

2.7. Qualitative phytochemical screening

The presence of flavonoids, tannins, anthraquinones, saponins, terpenoids, alkaloids, glycosides, sterols, coumarins, phenols, carbohydrates, and amino acids in the aqueous aerial part extract of *L. cornuta* was determined as per standard phytochemical screening procedures described by Harborne [31], Trease and Evans [41], and Bello et al. [42].

2.8. Ethical considerations

This study was ethically approved by 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).

2.9. 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.

Table 1
Acute oral toxicity appraisal of the aqueous aerial part extract of *L. cornuta*.

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, 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.

3. Results

3.1. Acute oral toxicity appraisal

The results showed that, at all the three tested dose levels (175 mg/kg BW, 550 mg/kg BW, and 2000 mg/kg BW) of the plant extract, did not cause any observable clinical signs of acute oral toxicity in the experimental mice. 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 guideline (No. 427), LD_{50} of the studied plant extract was envisaged to be 2000 mg/kg BW. Table 1 presents the findings of the acute oral toxicity study of the studied plant extract.

3.2. Cognitive-enhancing efficacy

We investigated the extract's efficacy in ameliorating the ketamine-induced cognitive impairment in experimental mice by measuring 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). Besides, we determined the time spent in the target (NW) quadrant by each experimental mouse during the probe trial to measure memory retention and retrieval in the Morris water maze experiment.

3.2.1. Escape latency

On 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 ($P > 0.05$; Fig. 1). Similarly, the differences between 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 on the first day (Day 1) ($P > 0.05$; Fig. 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 on the first day (Day 1) ($P < 0.01$; Fig. 1).

As shown in Fig. 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 on the second day (Day 2) ($P > 0.05$). Likewise, on 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 ($P > 0.05$; Fig. 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 on the first experimental day (Day 1) ($P < 0.05$; Fig. 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* on the second experimental day (Day 2) ($P < 0.05$; Fig. 1).

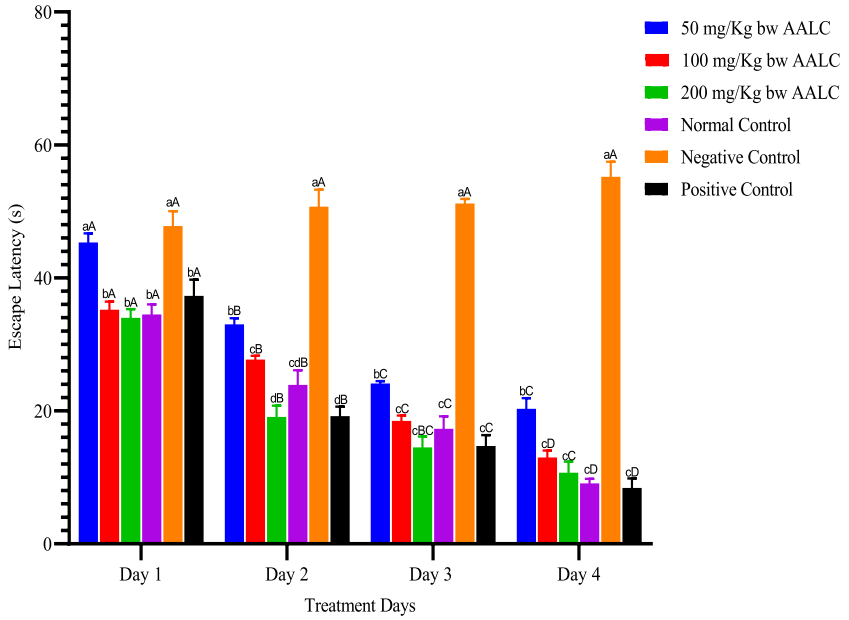

Fig. 1. Escape latencies of ketamine-induced cognitive impaired mice treated with the aqueous aerial part extract of *L. cornuta*. Values are plotted as $\bar{x} \pm SEM$; Bars with similar lowercase alphabets within the same day and those with similar uppercase alphabets across the treatment days for each experimental group are not significantly different ($P > 0.05$), while those having different lowercase alphabets within the same day and those with different uppercase alphabets across the treatment days for each experimental group are significantly different ($P < 0.05$) (One-Way ANOVA with Fisher's LSD *post hoc* test). AALC: Aqueous aerial part extract of *L. cornuta*.

On 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 ($P > 0.05$; Fig. 1). During 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 ($P < 0.05$; Fig. 1). Also, significantly higher escape latencies were recorded among extract-treated mice at doses of 50 mg/kg BW, 100 mg/kg BW, and 200 mg/kg BW, and those in the normal and positive control groups in the third and fourth days, respectively ($P < 0.05$; Fig. 1).

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) ($P < 0.05$; Fig. 1). 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 Fig. 1. Moreover, the positive control group mice observed significant decreases in escape latency until the fourth day of experimentation ($P < 0.05$; Fig. 1).

The escape latencies of ketamine-induced cognitive-impaired mice 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 (Fig. 1). However, no significant difference between the escape latency on the third day (Day 3) and fourth day (Day 4) was observed in mice which received 50 mg/kg BW of the study extract ($P > 0.05$; Fig. 1). 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 ($P < 0.05$; Fig. 1). As shown in Fig. 1, 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 the third and fourth day, respectively ($P > 0.05$; Fig. 1).

3.2.2. Navigation distance

The present study measured the navigation distances covered by all experimental mice participating in the Morris water maze task daily 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 on the first day (Day 1) ($P > 0.05$; Fig. 2). Similarly, on 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 ($P > 0.05$; Fig. 2). 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 on the first day ($P < 0.05$; Fig. 2).

On 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

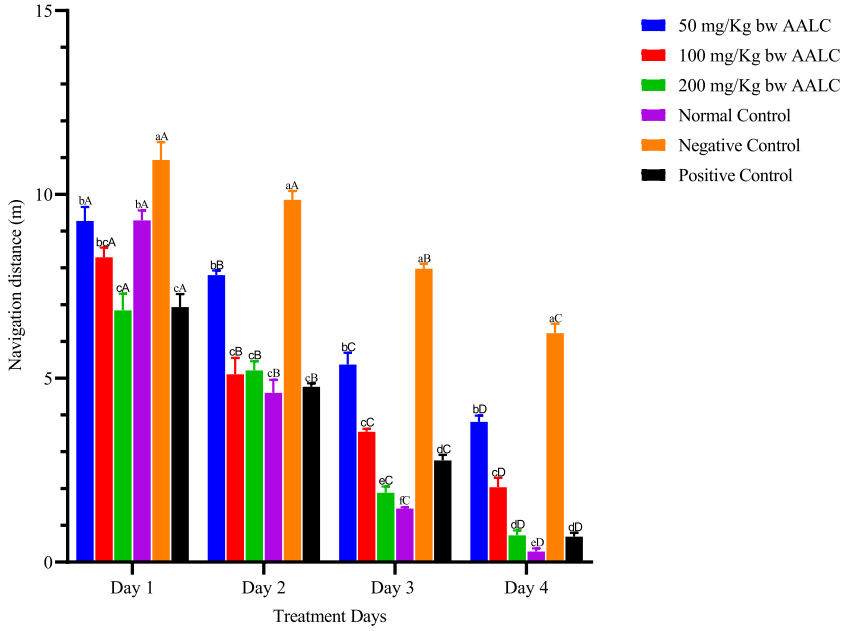

Fig. 2. Navigation distances covered by ketamine-induced cognitive impaired mice treated with the aqueous aerial part extract of *L. cornuta*. Values are plotted as $\bar{x} \pm SEM$; Bars with similar lowercase alphabets within the same day and those with similar uppercase alphabets across the treatment days for each experimental group are not significantly different ($P > 0.05$), while those having different lowercase alphabets within the same day and those with different uppercase alphabets across the treatment days for each experimental group are significantly different ($P < 0.05$) (One-Way ANOVA with Fisher's LSD *post hoc* test). AALC: Aqueous aerial part extract of *L. cornuta*.

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 (Fig. 2). Notably, the negative control group mice covered significantly longer navigation distances than those covered by all the other groups of mice in the second day, as shown in Fig. 2.

The results revealed a significant dose-dependent reduction in navigation distances covered by cognitive-impaired mice administered the plant extract on the third day ($P < 0.05$; Fig. 2). 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 on the third day ($P < 0.05$; Fig. 2). Overall, the negative control group mice covered significantly longer navigation distance than all the other mice on the third day ($P < 0.05$; Fig. 2).

A significant dose-dependent reduction in navigation distance covered by cognitive-impaired experimental mice administered with the studied plant extract was observed on the fourth day of the study ($P < 0.05$; Fig. 2). 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 on the fourth day, as shown in Fig. 2. 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 on the same day (Day 4) ($P < 0.05$; Fig. 2).

The navigation distances covered by each experimental group of mice were compared 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 ($P < 0.05$; Fig. 2). As shown in Fig. 2, 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 on the third and fourth day, respectively ($P < 0.05$; Fig. 2). The results further showed significant daily reductions in navigation distance covered by the positive control group mice across the study period ($P < 0.05$; Fig. 2).

The navigation distances covered by cognitively impaired mice 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 ($P < 0.05$; Fig. 2). Similarly, significant daily reductions ($P < 0.05$) in navigation distances covered by mice which received 100 mg/kg BW (Fig. 2) and 200 mg/kg BW (Fig. 2) were observed across the study period.

3.2.3. Latency in the target (NW) quadrant

On the final experimentation day, the experimental mice determined the time spent in the target quadrant (NW), where the escape

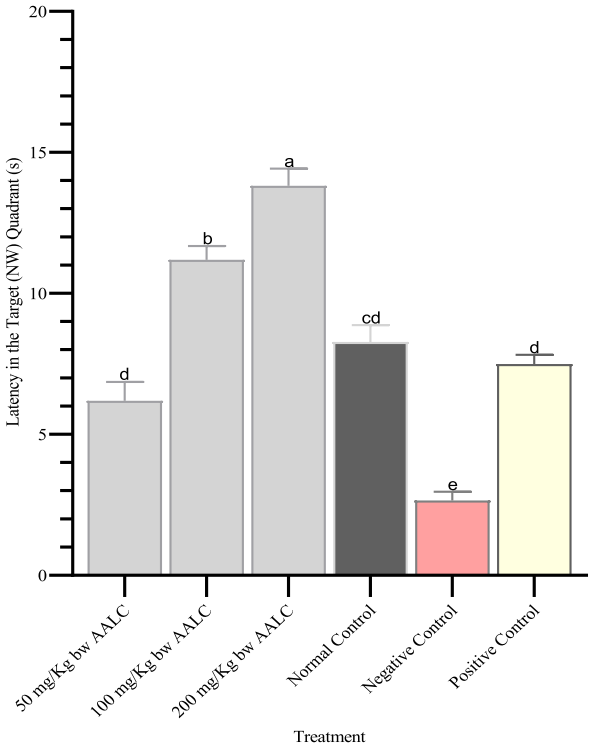

Fig. 3. Latency of the ketamine-induced cognitive impaired mice treated with the aqueous aerial part extract of L. cornuta in the target (NW) Quadrant during the probe trial. Values are plotted as $\bar{x} \pm SEM$; Bars with similar alphabets are not significantly different ($P > 0.05$), while those 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*.

platform was previously located, to appraise their memory retention and retrieval capacity. In this study, cognitive-impaired mice administered with the test extract recorded a significant dose-dependent increase in latency in the NW quadrant ($P < 0.05$; Fig. 3). 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 ($P > 0.05$; Fig. 3). 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 ($P < 0.05$; Fig. 3).

3.3. 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 200 mg/kg BW and those in the normal and positive control groups ($P > 0.05$; Fig. 4). Notably, a significant dose-dependent decrease in MDA concentration was observed in samples derived from mice treated with the studied plant extract ($P < 0.05$; Fig. 4). 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 Fig. 4.

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

This study's qualitative phytochemistry revealed the presence of saponins, carbohydrates, amino acids, flavonoids, phenols, alkaloids, and steroids (Table 2). However, tannins and anthraquinones were not detected in the studied plant extract (Table 2).

4. Discussion

The rapidly increasing population of persons with dementia and its associated complications, especially cognitive impairment in the world is worrying [8]. Moreover, the crippling nature of cognitive impairment, the absence of effective treatments, and the difficulties in managing it call for alternative strategies to either stop, slow down, or reverse its pathological sequelae [2,9–12]. Although medicinal plants and the products made from them are a potential source of safe and effective treatments for cognitive impairments, there is a lack of empirical data to support these treatments' safety and efficacy, which might serve as a direct antidementia drug

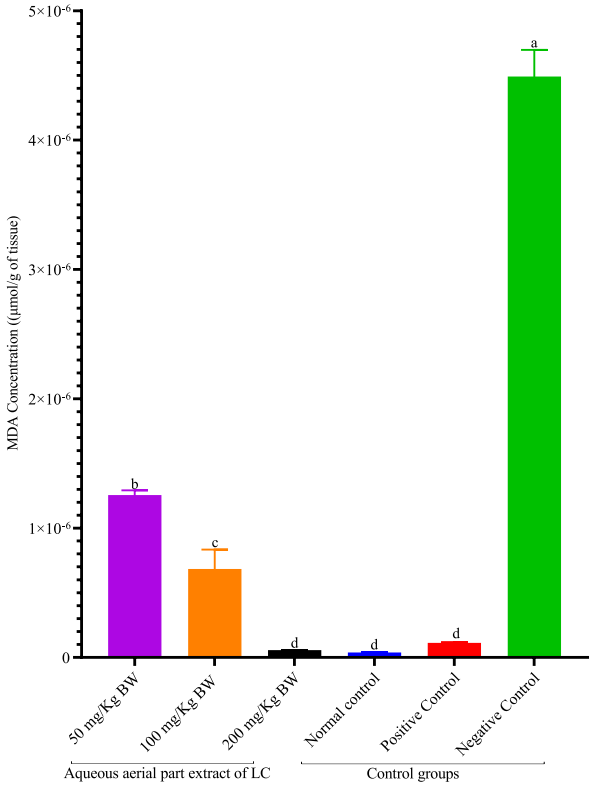

Fig. 4. Concentration of MDA in brain samples from ketamine-induced cognitive impaired mice treated with the aqueous aerial part extract of *L. cornuta*. Values are plotted as $\overline{x} \pm SEM$; Bars with similar alphabets are not significantly different ($P > 0.05$), while those having different alphabets are significantly different ($P < 0.05$) (One-Way ANOVA with Fisher's LSD post hoc test). LC: L. cornuta.

Table 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.

research and development [43,44]. In light of this, we examined the *Launaea cornuta* aqueous aerial part extract's acute oral toxicity, cognitive-enhancing, anti-lipid-peroxidation, and qualitative phytochemistry as a possible source of effective and safe treatments for cognitive impairments.

The acute oral toxicity effects of the studied plant extract were investigated according to the up-and-down procedure described by the OECD [35]. In this method, a drug agent or extract is considered safe if it does not elicit clinical signs of acute toxicity such as coma, tremors, discolouration of the mucosa, excessive salivation, diarrhoea, morbidity, among others, and death upon oral administration into experimental animals at doses $\leq$2000 mg/kg BW (LD_{50} > 2000 mg/kg BW) [45,46]. This is the most preferred method for evaluating the safety of plant extracts and chemicals in experimental animals, as it is easy to follow and yields precise and reproducible results, which are easily extrapolatable to humans [35,45,46].

In this study, no observable signs of acute oral toxicity or mortality were observed in all animals 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 [35].

These results support those reported by Akimat et al. [29] indicating the non-toxicity of the aqueous root extract of *L. cornuta* in mice. Given the widespread use of *L. cornuta* as vegetables, and a medicine for numerous ailments in traditional medicine [26,28,47], it was imperative to appraise its safety. However, beyond our findings, we suggest that more toxicological studies be conducted to determine toxicity profile and safety at greater dosages, when mixed with other extracts, and in clinical settings.

We adopted the MWM technique [37] based on its extensive usage in evaluating spatial learning and memory in antidementia studies, with high accuracy, precision, and reproducibility to appraise the cognitive-enhancing potential of *L. cornuta* aqueous aerial part extract. The MWM technique helps to determine alterations in the central cholinergic system and cognitive-associated biochemical parameters based on a single task [30].

Ketamine, a non-competitive *N*-Methyl-D-Aspartate (NMDA) receptor antagonist, causes cognitive abnormalities, akin those observed in dementia sufferers [48]. It also interferes with cognition and pain perception leading to notable neurological deficits [49,50]. In the MWM experiments, the adverse effects of Ketamine in experimental mice are evidenced by the increased escape latency time and navigation distance, as demonstrated by the negative control group mice in this study, which indicate impaired learning and reduced latency in the target quadrant, indicating poor memory, which characterises cognitive deficits akin to those observed in dementias [30,51]. Therefore, a drug agent or plant extract capable of preventing or ameliorating the ketamine-induced effects, especially cognitive deficits, maybe a potential cognitive-enhancing and antidementia therapy.

We observed significant dose-dependent reductions in escape latencies and navigation distances of ketamine-induced cognitive-impaired mice treated with the extract. Additionally, over the experiment course, mice treated with either the extract or Donepezil travelled shorter distances and took shorter escape latencies in the MWM experiment, demonstrating their amelioration of ketamine-induced learning and memory deficits. The varying concentration of bioactive chemicals linked to cognitive improvement, such as those described in earlier research [52,53], may be responsible for the dose-dependent effectiveness of the examined plant extract in thwarting the ketamine-induced cognitive impairments.

The increased latency of the cognitive-impaired mice treated with the studied plant extract and the reference drug in the target quadrant depicts their promotion of memory retention and its retrieval. Donepezil exerts its cognitive-enhancing efficacy by inhibiting the activity of the acetylcholinesterase enzyme, which subsequently increases the acetylcholine levels, thus sustaining nerve firing [54]. Additionally, Donepezil has been shown to restore altered redox homeostasis in AD and allied neuropathies [55,56]. Perhaps the observed efficacy of the examined extract may be partly mediated through various mechanisms, which increase cognitive-associated neurotransmitter concentrations and activity, as well as receptor function, fostering mitochondrial health and function, maintain the cortical and hippocampal integrity, quenching oxidative, mitigating neuroinflammation, fostering nerve health, among others [49,57–60]. These effects are mediated by the various pharmacologically active phytocompounds, which may act individually or synergistically on multiple targets [30,32].

Research demonstrates that the cerebral cortex and hippocampus are amenable to Ketamine-induced oxidative stress [61–64], which kill neuronal cells, impede neuronal growth, and ultimately impair cognition [65–69]. Therefore, drugs which can quench

oxidative stress in the brain, can also ameliorate cognitive abnormalities. It is thus arguable that the tested extract effectively attenuated oxidative stress in the hippocampal and neocortical regions, resulting in proper cognitive functioning of these regions.

Lipid peroxidation and its associated adducts cause oxidative damage to cellular machinery leading to a plethora of disease conditions, including neuroinflammation, and brain cell death, among other complex maladies [70–73]. Malondialdehyde (MDA) is the primary product of lipid peroxidation and is considered a significant marker of oxidative damage in the body [73,74]. As lipid peroxidation-induced cell damage increases, so does MDA generation and its concentration. Moreover, cognitively impaired rodents have elevated MDA levels, implying that muscarinic and NMDA receptor blockers exacerbate oxidative brain injury [75–77]. MDA levels in brain samples from experimental mice treated the test extract were considerably lower than in 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 exacerbates lipid peroxidation and its associated sequelae [63]. 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 exert their effects solely or synergistically [78].

The brain is amenable to oxidative damage due 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 [79]. Disproportionate amounts of free radicals harm cellular membranes, impairing their structure and function leading to neuropathies, inflammatory diseases, among other devastating conditions [80,81]. Furthermore, free radicals alter proteins, resulting in toxic adducts such as the tau and β-amyloid found in Alzheimer's disease [43,82]. Antioxidant phytochemicals, such as phenols, flavonoids, and coumarins, among others, maintain redox homeostasis in the body, preventing oxidative cell damage [81,83,84]. Therefore, consuming these antioxidants through fruits and vegetables and medicinal plants like *L. cornuta*, or supplementation may promote health [85,86].

Phytochemical research has shown that the climatic conditions influence the production of phytochemicals in plants, part of the plant, season, and the nature of biotic and abiotic stresses affecting the plant at a given time [87,88], which may partly explain the differences observed in this study. The results revealed the presence of various phytochemicals, including flavonoids, phenols, steroids, coumarins, among others in the examined extract. These findings are consistent with those of Akimat et al. [29] who reported the presence of various phytochemicals in the aqueous root extract of *L. cornuta.* However, tannins and anthraquinones were absent in the present study, which differ from an earlier study [29], indicating indicting the varied distribution of these phytochemicals, which may be influenced by various factors [87,88].

Therefore, the antioxidant phytochemicals in the studied plant extract, especially phenols, flavonoids, and coumarins played significant roles in ameliorating the ketamine-induced cognitive impairment by maintaining the redox homeostasis, averting lipid peroxidation, promoting neuronal cell health, and modifying the interaction of Ketamine with its receptors [17,30,51,52]. 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 [30].

Considering this study did not determine the specific concentrations of the detected phytochemicals, and their specific mode(s) of anti-lipid peroxidation and cognitive-enhancing activities, further empirical investigations on this line may provide crucial insights into the antidementia potential of the *L. cornuta* aqueous extract.

5. Conclusions and recommendations

Based on the study findings, the aqueous aerial part extract of *L. cornuta* does not cause acute oral toxicity in Swiss albino mice. Additionally, the studied extract possesses significant cognitive enhancing and anti-lipid peroxidation efficacy in a ketamine-induced cognitive-impaired mouse model, and various phytochemicals of pharmacological value. Further studies to quantify, isolate, and characterise the specific cognitive-enhancing and anti-lipid peroxidation amalgams and their specific mechanisms of bioactivity, and their toxicity profiles are recommended to appraise the antidementia of the studied plant extract.

Author contribution statement

Mercy Maina; Gervason Moriasi: Conceived and designed the experiments; Performed the experiments; Analysed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.

James Mbaria; Irene Kamanja: Conceived and designed the experiments; Analysed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.

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Data availability statement

Data included in article/supplementary material/referenced in article.

Declaration of interest's statement

The authors declare no conflict of interest.

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