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Original Research

Neuroprotective effects of *Launaea taraxacifolia* leaf extracts on aluminium chloride-induced motor dysfunction

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Abstract

Aluminium is a neurotoxic metal implicated in neurodegenerative disorders. This study investigated the neuroprotective potential of ethanol leaf extract and solvent fractions of *Launaea taraxacifolia* on motor coordination and balance in aluminium chloride-induced neurotoxicity in rats. Sixty-six adult female Wistar rats (110–180 g) were divided into 11 groups (n=6). Group 1 (control) received distilled water, while Group 2 (neurotoxic control) received aluminium chloride (100 mg/kg) orally. Groups 3–11 were co-administered aluminium chloride (100 mg/kg) with donepezil (2.5 mg/kg), ethanol extract (274, 548, and 822 mg/kg), n-hexane (548 mg/kg), dichloromethane (548 mg/kg), ethyl acetate (548 mg/kg), or aqueous fraction (548 mg/kg) for 21 days. Neurobehavioural tests, including the wire hang and beam walking tests, assessed motor function. Aluminium chloride significantly ($p<0.05$) reduced hanging time, increased foot slips, and prolonged crossing time compared to the control group. Treatment with ethanol extract and fractions significantly ($p<0.05$) improved these parameters, suggesting enhanced motor coordination and balance. The results demonstrate that *L. taraxacifolia* extracts mitigated aluminium-induced motor impairments, supporting its potential as a neuroprotective agent.

Keywords: Neuroprotection; ethanol leaf extract; *Launaea taraxacifolia*; motor coordination; aluminium chloride; neurotoxicity

1. Introduction

Aluminium chloride neurotoxicity is a well-established model of neurodegeneration. Aluminium is a trivalent cation and toxic metal that is highly ubiquitous as an environmental toxicant and in lots of medical agent, food products including pieces of bread, cakes, glace fruits, dairy products [1]. Aluminium builds up lots of tissues, including the brain, kidney, liver and ultimately results in certain medical conditions such as Alzheimer's disease and other neurological problems such as dementia [2].

Aluminium exhibits a wide variety of intracellular targets in neurons that affect physiological functions [3]. The brain regions most affected by aluminium neurotoxicity are those involved in learning and memory, which could be attributed to the unique distribution of transferring receptors and neuroanatomical linkages between brains regions involved in cognitive functions. It is established that aluminium toxicity is due to imbalances between free radicals and anti-oxidant formation, leading to oxidative stress [2]. Oxidative stress and changes in energy metabolism and mitochondrial function are the first events that make the brain sensitive to aluminium accumulation [4]. Oxidative stress is associated with a significant reduction in antioxidant activity: superoxide dismutase, catalase, glutathione peroxidase, glutathione reductase and glutathione-S- transferase with enhanced activity of nitric oxide (NO) levels in some parts of the brain [5]. A novel investigation is focused on the mechanisms of neuroprotection and many substances have been tested on animal models of diseases but potential drugs have not yet been found.

Research over the years has revealed that *Launaea taraxacifolia* possess important pharmacological activities [6]. Studies have demonstrated that *L. taraxacifolia* leaf extract has antioxidant potentials to protect against oxidative stress [7]. The phytochemical screening of *L. taraxacifolia* conducted by Anyanwu et al. [7] revealed that the plant is rich in alkaloids, tannins, flavonoids, saponins, phenols, steroids, cardiac glycosides and terpenoids, which are common chemical constituents of many traditionally prepared herbal medicines and leafy vegetables. Currently, many researchers have resorted to plant as possible source for obtaining novel bioactive agents in combating these disorders. The leaves of *L. taraxacifolia* are endowed with saponins, terpenoids, cardiac glycosides, steroids, tannins, flavonoids, anthocyanins, phenolic acids, ascorbic acid, lycopene and β-carotene [6]. Ololade et al. [8] have isolated 47 compounds from methanolic extract of the *L. taraxacifolia* leaves. The most abundant chemical components found were palmitic acid, methyl-11-octadecenoate, erythritol, glycerol, linoleic acid methyl ester, and phytol. The objective of the present study is to assess the neuroprotective effects of ethanol leaf extract and various solvent fractions of *L. taraxacifolia* on motor coordination and balance in aluminium chloride neurotoxic rats.

2. Materials and Methods

2.1 Drugs and chemicals

Donepezil (donepezil hydrochloride tablet, 5 mg), an acetyl cholinesterase inhibitor, was sourced from Smick Laboratories Ltd, Mumbai, India. Aluminium chloride ($AlCl_3$) was purchased from Sigma-Adrich, USA. The polar and non-polar solvents (ethanol, n-hexane, dichloromethane, ethyl acetate and n-butanol) were acquired from Sigma- Adrich, USA. All the drug solutions were freshly prepared before use. All the regents used in this study were of analytical grade.

2.2 Collection of identification and authentication of plant material

Launaea taraxacifolia was collected from the laboratory environment of the Department of Pharmacognosy and Natural Medicine, University of Uyo, Uyo, Nigeria. It was identified at the herbarium of the Department of Botany and Ecological Studies, Faculty of Science, University of Uyo, Uyo, Nigeria. The specimen (with Voucher No: UUPH 10 (P)) was deposited in the Herbarium, Department of Pharmacognosy and Natural Medicine, Faculty of Pharmacy, University of Uyo, Uyo, Nigeria.

2.3 Preparation of the extract of plant material

The leaves of *L. taraxacifolia* were collected, washed, cut into small pieces, and dried in open air in the laboratory. The dried leaves were pulverized into coarse powder using mortar and pestle. The powdered plant material was weighed and transferred into a transparent air-tight container prior to extraction. Thereafter, 740 g of pulverized leaves was extracted by maceration in 4 L of 70 % ethanol. The mixture was shaken time to time to ensure complete extraction of the plant’s bioactive ingredients. The mixture was separated by filtration using Whatman filter paper size 1. The filtrate was concentrated to dryness in a water bath at 45 °C. The ethanol extract of *L. taraxacifolia* was weighed and stored in a deep freezer at -4 °C.

2.4 Partitioning of ethanol extract of *L. taraxacifolia*

Ethanol extract (136 g) was partitioned successively into five solvent fractions (n-hexane, dichloromethane (DCM), ethyl acetate, n-butanol, and water) using separating funnel starting. The solution was continually stirred and decanted until clear supernatant was obtained. The fractions were concentrated using a rotary evaporator at 40 ℃. The concentrated fractions were transferred into small beakers (50 mL) and further concentrated in a water bath at 45 ℃ to ensure that all the solvents that were used during partitioning were removed. The fractions were refrigerated at 4 ℃ until needed.

2.5 Determination of qualitative phytoconstituents

Quantitative phytochemical analysis was conducted to determine the total phenolic content, total flavonoid content and total tannin content of the ethanol extract and fractions of *L. taraxacifolia* using methods as previously described [9], [10].

2.5.1 Determination of total phenolic content

The phenolic content of the plant was determined spectrophotometrically with Folin-Ciocalteu reagent using a modified method reported elsewhere [11]. An aliquot of extract and fractions (0.5 mL) was mixed with 2.5 mL of 10 % Folin-Ciocalteu reagent and 2 mL of Na_2CO_3. The resulting mixture was vortexed for 15 seconds and incubated at 40 ºC for 30 minutes for colour development. The absorbance of the sample was measured at 765 nm using a UV-visible spectrophotometer (Hewlett Packard). Total phenolic content is expressed as mg/g gallic acid equivalent from the calibration curve using the equation $y = mx + c$ where y is the absorbance and x is the gallic acid equivalent (mg/g).

2.5.2 Determination of total flavonoid content

The total flavonoids content was estimated using the procedure described by Zhishen et al. [12]. A total of 1 mL of plant extracts and fractions were diluted with 200 µL of distilled water separately followed by the addition of 150 µL of sodium nitrite (5 %) solution. This mixture was incubated for 5 minutes and then 150 µL of aluminium chloride (10 %) solution was added and allowed to stand for 6 minutes. Then 2 mL of sodium hydroxide (4 %) solution was added and made up to 5mL with distilled water. The mixture was shaken well and left to stand for 15 minutes at room temperature. The absorbance was measured at 510 nm. Appearance of pink colour showed the presence of flavonoids content. The total flavonoid content was expressed as rutin equivalent mg RE/g extract and fractions on a dry weight basis using the standard curve. All assays were carried out in triplicate.

2.5.3 Determination of total tannin content

Exactly 0.1 mL of ethanol extract and fractions of *L. taraxacifolia* was added to 1.0 mL of 2, 4-dinitrophenylhydrazine (2, 4 – DNPH). It was allowed to stand for 30 minutes, and the absorbance was read in triplicate at 515 nm, using distilled water as blank. Tannic acid was used as a reference and for the calibration curve. Result was expressed in microgram per milligram of tannic acid equivalent [13].

2.6 Experimental design

Prior to the start of the experiment, the animals were randomly divided into eleven groups of 6 rats each and treated for 21 days as shown in Table 1.

2. 7 Change in body weight measurement

Animals' body weight was measured and noted on the first day and last day of the experimentation. The body weight was calculated in comparison to the initial body weight on the first day of the experimentation.

2. 8 Wire hang test

This test was used to assess the forelimb strength (strength of the muscle). The wire hang test was carried out on day 18. For this test, a wire cage was used. The animal was placed on the top of the wire cage. Afterwards, the wire cage was turned upside down. The wire cage was held at a height approximately 0.5-8.6 m above a soft padded surface, high enough to prevent the rat from jumping down, but not high enough to cause harm in the event of fall. The hanging time, that is the latency(ies) to fall from the wire to the flat soft pad was measured. When the hanging time was over 60 seconds, the rat was released from the wire and the time was recorded as 60 seconds. The trial was conducted three times for each rat [13].

Table 1: Method of $AlCl_3$ neurotoxicity in rats

S/N	Group	Treatment	Duration of Treatment
1.	Control	10 mg/kg body weight of distilled water	Daily for 21 days
2.	$AlCl_3$ (Neurotoxic Control)	100mg/kg body weight of $AlCl_3$	Daily for 21 days
3.	$AlCl_3$ + Donepezil	100 mg/kg body weight of $AlCl_3$ and 2.5 mg/kg body weight of Donepezil	Daily for 21 days
4.	$AlCl_3$ + Ethanol extract (LD)	100mg/kg body weight of $AlCl_3$ and 274mg/kg body weight of ethanol extract (Low dose) of *L. taraxacifolia*	Daily for 21 days
5.	$AlCl_3$ + Ethanol extract (MD)	100mg/kg body weight of $AlCl_3$ and 548mg/kg body weight of ethanol extract (Middle dose) of *L. taraxacifolia*	Daily for 21 days
6.	$AlCl_3$ + Ethanol extract (HD)	100 mg/kg body weight of $AlCl_3$ and 822mg/kg body weight of ethanol extract (High dose) of *L. taraxacifolia*.	Daily for 21 days
7.	$AlCl_3$ + N-hexane fraction	100 mg/kg body weight of $AlCl_3$ and 548mg/kg body of n-hexane fraction of *L. taraxacifolia*	Daily for 21 days
8.	$AlCl_3$ + DCM fraction	100 mg/kg body weight of $AlCl_3$ and 548mg/kg body weight of DCM fraction of *L. taraxacifolia*.	Daily for 21 days
9.	$AlCl_3$ + Ethyl acetate fraction	100 mg/kg body weight of $AlCl_3$ and 548mg/kg body weight of ethyl acetate fraction of *L. taraxacifolia*.	Daily for 21 days
10.	$AlCl_3$ + N-butanol fraction	100 mg/kg body weight of $AlCl_3$ and 548mg/kg body weight of n-butanol fraction of *L. taraxacifolia*.	Daily for 21 day
11.	$AlCl_3$ + Aqueous fraction	100 mg/kg body weight of $AlCl_3$ and 548mg.kg body weight of aqueous fraction of *L. taraxacifolia*.	Daily for 21 days

Key: LD = Low dose; MD = Middle dose; HD = High dose

2. 9 Beam walking test

The beam walking test was used for the assessment of motor coordination particularly the hindlimb. Firstly, the animals were placed in one corner of the narrow beam (2.3 cm x 120 cm) and allowed to walk across the narrow beam from one end to the other end at least three times. During testing, the rats were given 3 minutes to traverse the beam. The number of foot slips and the time taken to cross the beam (crossing time) in each trial were recorded. The trial was conducted three times for each rat [14].

2.10 Statistical analysis

Data were expressed as mean ± standard error of mean (SEM). Differences between mean values were evaluated by analysis of variance (ANOVA), followed by Tukey's post-hoc test for pairwise comparisons. Weight differences were evaluated using paired student t-test. Values of $p<0.05$ were considered statistically significant. GraphPad Prism 7.0 software (Graph Pad Inc., USA) was used for the statistical analysis.

3. Results and Discussion

3.1 Results

3.1.1 Phytochemicals in ethanolic EXTRACT and fractions of L. taraxacifolia

The total phenolic content, total flavonoid content, and total tannin content of ethanol extract and fractions of *L. taraxacifolia* are presented in Tables 2, 3, and 4, respectively.

The total phenolic content for the extract, n-hexane fraction, DCM fraction, ethyl acetate fraction, n-butanol fraction and aqueous fraction were 37.63, 6.33, 10.80, 16.74, 19.75 and 31.05 mg GAE/g, respectively (Table 2). The total flavonoid content for the extract, n-hexane fraction, DCM fraction, ethyl acetate fraction, n-butanol fraction and aqueous fraction were 595.56, 77.78, 112.22, 183.33, 155.56 and 183.33 mgQE/g, respectively (In Table 3). The total tannin content for the extract, n-hexane fraction, DCM fraction, ethyl acetate fraction, n-butanol fraction and aqueous fraction were 66.51, 12.96, 13.40, 19.40, 29.40 and 101.40 mg TAE/g, respectively (Table 4).

Table 2: Total phenolic content of ethanol extract and fractions of *L. taraxacifolia*

Samples	Mean Absorbance	Total Phenolic Content (mgGAE/g)
Ethanol Extract	0.627±0.002	37.63
n-hexane fraction	0.242±0.001	6.33
DCM fraction	0.297±0.002	10.80
Ethyl acetate fraction	0.370±0.002	16.74
n-butanol fraction	0.407±0.003	19.75
Aqueous fraction	0.546±0.001	31.05

Table 3: Total flavonoid content of ethanol extract and fractions of *L. taraxacifolia*

Sample	Mean Absorbance	Total Flavonoid Content (mgQE/g)
Ethanol Extract	0.552±0.002	595.56
n-hexane fraction	0.086±0.001	77.78
DCM fraction	0.117±0.002	112.22
Ethyl acetate fraction	0.181±0.001	183.33
n-butanol fraction	0.156±0.003	155.56
Aqueous fraction	0.181±0.001	183.33

Table 4: Total tannin content of ethanol extract and fractions of *L. taraxacifolia*

Sample	Mean Absorbance	Total Tannin Content (mgTAE/g)
Ethanol Extract	2.082±0.001	66.51
n-hexane fraction	1.841±0.003	12.96
DCM fraction	1.843±0.001	13.40
Ethyl acetate fraction	1.870±0.002	19.40
n-butanol fraction	1.915±0.001	29.40
Aqueous fraction	2.239±0.002	101.40

3.1.2 Body weight experiments

From the result (Table 5), the percentage change in body weight was significantly decreased ($p < 0.05$) in the $AlCl_3$ (neurotoxic control), $AlCl_3$ + donepezil, $AlCl_3$ + ethyl acetate fraction, and $AlCl_3$ + Aqueous fraction treated groups when compared with the control group respectively. Percentage change in the body weight was significantly increased ($p<0.05$) in the $AlCl_3$ + ethanol extract (LD), $AlCl_3$ + ethanol extract (HD), $AlCl_3$ + n-hexane fraction and $AlCl_3$ + DCM fraction treated groups when compared with the control group, respectively.

3.1.1 Wire hang test outcomes

The effect of ethanol extract and fractions of *L.taraxacifolia* on hanging time during wire hang test is presented in Figure 1. From the result, the hanging time was significantly ($p<0.05$) decreased in the $AlCl_3$ (neurotoxic control), $AlCl_3$ + donepezil and $AlCl_3$ + DCM fraction when compared with the control. Hanging time was significantly ($p<0.05$) increased in the $AlCl_3$ + ethanol extract (HD) treated group when compared with the control. Hanging time was significantly($p<0.05$) increased in $AlCl_3$ + donepezil, $AlCl_3$ + ethanol extract (LD), $AlCl_3$ + ethanol extract (MD), $AlCl_3$ + ethanol extract (HD), $AlCl_3$ + ethyl acetate fraction, $AlCl_3$ + n-butanol fraction and $AlCl_3$ + aqueous fraction when treated group compared with $AlCl_3$ (neurotoxic control) treated group. Hanging time was significantly ($P<0.05$) increased in $AlCl_3$ + ethanol extract (LD) and $AlCl_3$+ ethyl acetate fraction treated groups when compared with the $AlCl_3$ + donepezil treated group. Hanging time was significantly ($p<0.05$) decreased in $AlCl_3$ + DCM fraction treated group when compared with $AlCl_3$ + donepezil treated group. Hanging time was significantly ($p<0.05$) decreased in the $AlCl_3$ + DCM fraction treated group when compared with $AlCl_3$ + ethanol extract (MD) treated group. Hanging time was significantly ($p<0.05$) decreased in $AlCl_3$ +aqueous fraction treated group when compared with $AlCl_3$ + ethanol extract (HD). Hanging time was significantly ($p<0.05$) increased in the $AlCl_3$ + n-butanol fraction and $AlCl_3$ + aqueous fraction treated groups when compared with compared with $AlCl_3$ + DCM fraction treated group.

Table 5: Effect of ethanol extract and fractions of *L. taraxacifolia* on body weight *

Group	Mean Initial Body Weight (g)	Mean Final Body Weight (g)	Mean Change in Body Weight (g)	% Mean Change in Body Weight
Control	166.8±2.55	178.5±4.03	11.7±0.10	7.0±0.05
$AlCl_3$ (Neurotoxic control)	169.5±4.82	137.2±6.02[a]	-32.3±0.17	-19.1±0.11[a]
$AlCl_3$ + Donepezil	173.2±1.42	180.8±3.82[a]	7.6±0.23	4.4±0.12[a]
$AlCl_3$ + Ethanol extract (LD)	162.7±0.61	178.5±2.81[a]	15.8±0.33	9.7±0.07[a]
$AlCl_3$ + Ethanol extract (MD)	151.5±0.45	162.7±0.56[a]	11.2±0.34	7.4±0.16
$AlCl_3$ + Ethanol extract (HD)	137.7±2.45	173.3±5.62[a]	35.6±0.71	25.9±0.20[a]
$AlCl_3$ + n-Hexane	122±1.50	154±3.72[a]	32±0.18	26.2±0.10[a]
$AlCl_3$ + DCM	132±4.59	146.2±3.65[a]	14.2±0.05	10.8±0.01[a]
$AlCl_3$ + Ethyl acetate	164.8±2.60	169.8±4.24[a]	5±0.01	3.0±0.00[a]
$AlCl_3$+ n-Butanol	155.4±5.13	175.2±2.80[a]	19.8±0.02	12.7±0.00[a]
$AlCl_3$+ Aqueous fraction	160.3±1.78	164.5±3.09[a]	4.2±.007	2.6±0.02[a]

*Values are expressed as mean ± SEM (n = 6); a = $p < 0.05$ when compared with the control

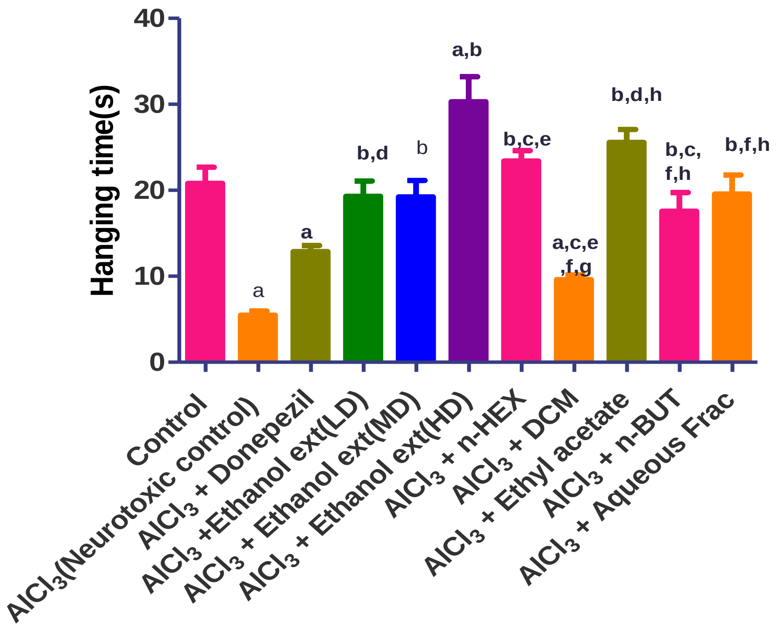

Figure 1: Effect of ethanol extract and fractions of *L. taraxacifolia* on hanging time during wire hang test. Columns represent mean ± SEM, n = 6, a = $p<0.05$ when compared with control, b = $p<0.05$ when compared with $AlCl_3$ (neurotoxic control), C=$p<0.05$ with compared with $AlCl_3$ + Ethanol extract (LD), d = $p<0.05$ when compared with $AlCl_3$ + Donepezil, e = $p<0.05$ when compared with $AlCl_3$ + Ethanol extract (MD), f = $p<0.05$ when compared with $AlCl_3$ + Ethanol extract (HD), g = $p<0.05$ when compared $AlCl_3$ + n-Hexane, h = $p<0.05$ when compared with $AlCl_3$ + DCM.

3.1.1 Beam waking test outcomes

The effect of ethanol extract and fractions of *L. taraxacifolia* on the number of foot slips while walking on the beam during the beam walking test for control and treatment groups is reported in Figure 2. From the result, the number of foot slips was significantly ($p<0.05$) increased in $AlCl_3$ (neurotoxic control) treated group when compared with the control. Number of foot slips was significantly ($p<0.05$) decreased in the $AlCl_3$ + donepezil, $AlCl_3$ + ethanol extract (LD), $AlCl_3$ + ethanol extract (MD), $AlCl_3$ + ethanol extract (HD), $AlCl_3$ + n-hexane fraction, $AlCl_3$ DCM fraction, $AlCl_3$ +ethyl acetate fraction, $AlCl_3$ + n-butanol fraction and $AlCl_3$ + Aqueous fraction treated group when compared with the $AlCl_3$ (neurotoxic control), respectively.

3.1.1 Crossing time during the beam walking test

The effect of ethanol extract and factions of *L.taraxacifolia* on crossing time during the beam walking test for control and treatment groups is reported in Figure 3. From the result, crossing time was significantly ($p<0.05$) increased in $AlCl_3$ (neurotoxic control) treated group when compared with the control. Crossing time was significantly ($p<0.05$) decreased in the $AlCl_3$ + donepezil, $AlCl_3$ + ethanol extract (LD), $AlCl_3$ + ethanol extract (MD), $AlCl_3$ + ethanol extract (HD), $AlCl_3$ + n-hexane fraction, $AlCl_3$ + DCM fraction, $AlCl_3$+ ethyl acetate fraction, $AlCl_3$ + n-butanol fraction and $AlCl_3$ + aqueous fraction treated groups when compared with the $AlCl_3$ (neurotoxic control) treated group, respectively.

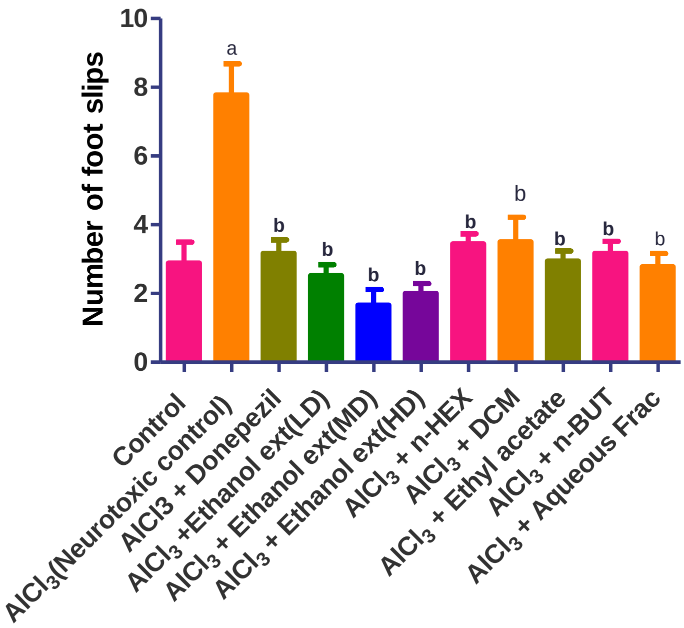

Figure 2: Effect of ethanol extract and fractions of *L.taraxacifolia* on the number of foot slips while walking on the beam during the beam walking test for control and treatment groups. Column represents mean ± SEM, n = 6, a = $p<0.05$ when compared with control, b = $p<0.05$ when compared with $AlCl_3$ (neurotoxic control).

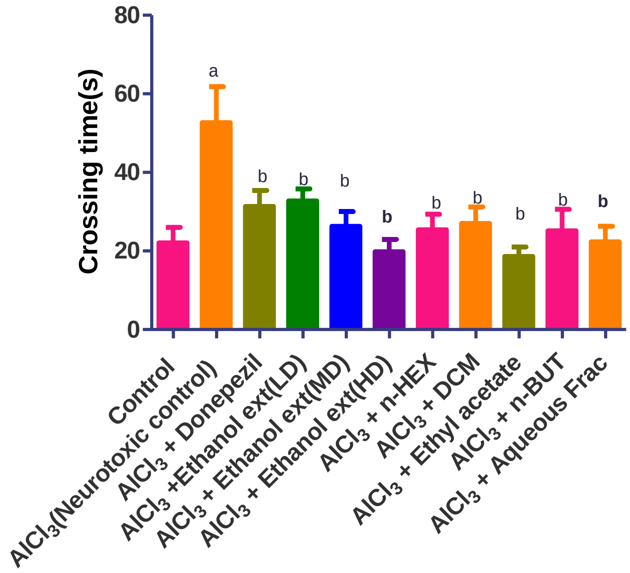

Figure 3: Effect of ethanol extract and fractions of *L.taraxacifolia* on crossing time during the beam walking test for control and treatment groups. Column represents mean ± SEM, n = 6, a = $p<0.05$ when compared with control, b = $p<0.05$ when compared with $AlCl_3$ (neurotoxic control).

3.2 Discussion

In this study, findings demonstrate that administration of aluminium chloride induced alterations in overall body weight as well as neurobehavioural deficits. Changes in the body weight are often considered an important indication of the general health status of animals and are a useful evaluator of the adverse effect of drugs and metal toxicity [15]. Result from this study revealed significant decrease in percentage change in body weight in the $AlCl_3$ (neurotoxic control), donepezil, ethyl acetate, and aqueous fraction treated groups when compared with the control group respectively. The significant decrease in percentage change in the body weight observed during aluminium neurotoxicity was possible due to depletion of nutrients, attenuation of protein synthesis and dysregulation of metabolic activities which is in agreement with a previous study reported by Oyewole and Oladele [16]. Accumulating evidence shows that metal toxicity can lead to substantial brain and body weight loss in rats if left untreated [17]. Results also revealed significant increase in percentage change in body weight in the ethanol extract (LD), ethanol extract (HD), n-hexane fraction and DCM fraction treated groups when compared with the control group respectively. The significant increase in percentage change in the body weight observed was possible because the ethanol extract (low dose and high dose), n-hexene and DCM fractions of *L.taraxacifolia* protected against body weight loss induced by aluminium. This suggests that that *L. taraxacifolia* extract and certain fractions may have a neuroprotective effect on aluminium chloride neurotoxicity in rats, which may be related to their ability to reverse the body weight loss. This agrees with a previous study conducted by Enogieru and Omoruyi [18].

The effect of ethanol extract and fractions *L. taraxacifolia* on hanging time during wire hang test was reported in this study. The hanging time was used to evaluate the strength of the muscle. Result from this study revealed that the hanging time was significantly decreased in the $AlCl_3$ (neurotoxic control), donepezil and DCM treated groups when compared with the control respectively. $AlCl_3$ at a dose of 100 mg/kg significantly decreased the hanging time (muscle strength) in the $AlCl_3$ treated group. The effect was observed in the wire hang test when the muscle weakness was about 3-fold more than in the control animals. Aluminium chloride caused neurodegeneration, leading to muscle weakness, motor impairment and balance deficits. Rats treated with aluminium chloride had a significant decrease in hanging time, indicating impaired motor function. *L. taraxacifolia* extract and fractions were used to evaluate their neuroprotective effects. Ethanol extract and fractions of *L. taraxacifolia* may improve cognimotor functions (e.g., attention, memory and learning) by reducing aluminium induced neuroinflammation and oxidative stress. Ethanol extract and fractions may improve visuospatial functions (e.g., spatial memory and navigation) by reducing aluminium chloride induced neurodegeneration. Donepezil, ethanol extract (low middle and high doses), n-hexane, ethyl acetate, n-butanol and aqueous fraction significantly increased the hanging time when compared with $AlCl_3$ treated group. In this study, donepezil, ethanol extract (low, middle and high doses), n-hexane, ethyl acetate, n-butanol and aqueous fractions of *L.taraxacifolia* offered significant neuroprotection against muscle weakness. DCM fraction of *L.taraxacifolia* offered the least neuroprotective effect against muscle weakness. Hanging time in the ethanol extract (low, middle and high doses), n-hexane, and ethyl acetate fraction treated groups was increased when compared with the donepezil treated group respectively. This suggests that the neuroprotective effect exhibited by ethanol extract (low, middle and high doses), n-hexane fraction and ethyl acetate fraction were more significant than the standard drug (donepezil). In the wire hang test, the extracts were evaluated for their ability to prevent neurodegeneration and improve motor function in rats. The results suggest that ethanol extract (high dose) had the most potent neuroprotective effect followed by ethyl acetate fraction, n-hexane fraction, ethanol extract (low and middle doses), aqueous fraction, n-butanol fraction and donepezil. DCM fraction showed the least neuroprotective activity.

The effect of ethanol extract and fractions of *L.taraxacifolia* on the number of foot slips while walking on the beam during the beam walking test was reported in this study. In the beam walking test, the extracts were evaluated for their ability to improve motor coordination and balance in rats. The time taken to complete the task and the foot slips predict motor coordination deficit with the number of foot slips having the potential to predict clinical sedation and is being used to evaluate motor coordination deficit. The fall from the beam, the number of foot slips and the time taken to cross the beam are indices of neurotoxicity [14]. Result from this study revealed a significant increase in the number of foot slips in the $AlCl_3$ (neurotoxic control) treated group when compared with the control. Aluminium chloride at a dose of 100 mg/kg in the $AlCl_3$ treated group significantly affected the rats' behaviour in the beam walking test, indicating motor coordination impairment. The increase in the number of foot slips suggest a motor coordination deficit which is similar to clinical sedation [14]. The rats treated with aluminium chloride had a significant increase in the number of foot slips and crossing time, indicating impaired motor coordination and balance. Result from this study also revealed significant decrease in the number of foot slips in the donepezil, ethanol extract (low, middle and high doses), n-hexane, DCM, ethyl acetate, n-butanol and aqueous fraction treated groups when compared with $AlCl_3$ (neurotoxic control) treated group respectively. This suggests that donepezil, ethanol extract (low middle and high doses), n-hexane, DCM, ethyl acetate, n-butanol and aqueous fraction significantly improved motor coordination and balance. Findings from this study also revealed that the neuroprotective effect exhibited by ethanol extract (low, middle and high doses) against motor deficit were more significant than the standard drug (donepezil).

The effect of ethanol extract and fractions of *L. taraxacifolia* on crossing time during the beam walking test was reported in this study. The beam walking test has been used to evaluate motor coordination and balance through the animal's ability to cross a graduated series of narrow beam until reaching a safe platform [14]. In the beam walking model, fine motor coordination is evaluated during a spontaneous motor task [14]. Result from this study revealed a significant increase in crossing time in the $AlCl_3$ (neurotoxic control) treated group when compared with the control. This suggests that rats exposed to aluminium chloride at a dose of 100 mg/kg body weight showed motor coordination and balance deficit by displaying poor performance on the beam while walking indicating ataxia and dystonic characteristics which is consistent with the findings of a previous study by Kitaura et al. [19]. Result also revealed a significant decrease in crossing time in the donepezil, ethanol extract (low, middle and high doses), n-hexane, DCM, ethyl acetate, n-butanol and aqueous fraction treated groups when compared with the $AlCl_3$ (neurotoxic control) treated group respectively. This suggests that ethanol extract (low, middle and high doses), n-hexane, DCM, ethyl acetate, n-butanol and aqueous fractions improved motor coordination and balance. The animals (rats) displayed fine motor coordination and good performance on the beam while walking. Finding from this study also revealed that the neuroprotective effect exhibited by ethanol extract (high dose), ethyl acetate, n-butanol and aqueous fractions in terms of motor coordination and balance were more significant than the standard drug (donepezil). The improved motor coordination and balance may be due to the neuroprotective effects of the extracts, which could be related to their antioxidant and anti-inflammatory properties. The various fractions of *L. taraxacifolia* exhibited neuroprotective effects, improving muscle strength, motor coordination and balance in aluminium neurotoxic rats.

4. Conclusion

Launaea taraxacifolia leaf extract and its solvent fractions (n-hexane, dichloromethane, ethyl acetate, n-butanol, and aqueous) demonstrated significant neuroprotective effects against aluminium chloride-induced neurotoxicity in rats, improving motor coordination and balance. These findings suggest that bioactive compounds present in the plant extracts may mitigate aluminium-induced neuromuscular impairments through antioxidative, anti-inflammatory, or neuroregenerative mechanisms. The improvement in motor function observed in the wire hang and beam walking tests indicates potential therapeutic applications in managing neurodegenerative conditions associated with motor deficits. Furthermore, the differential efficacy among solvent fractions highlights the importance of fractionation in isolating potent neuroactive compounds. This study underscores the therapeutic potential of *L. taraxacifolia* as a source of novel neuroprotective agents. These findings also provide a scientific basis for the traditional use of L. taraxacifolia in managing neurodegenerative disorders, promoting its development into nutraceuticals or pharmaceutical formulations for neuroprotection.

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Conflict of Interests

None declared.

Author Contributions

All authors have read and approved the final version of the manuscript.

Ethical Approval

This study was done in accordance with international guidelines for care and use of laboratory animals (National Research Council, 2011) and approval of Faculty of Basic Medical Sciences Research and Ethical Committee (FBMSREC), University of Uyo, Uyo, Nigeria with the reference number: UU-FBMSREC-2024-002.

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