

Original Research Article

Neem Leaf supplement ameliorates depressivelike behaviour ~~in~~ with neurodegeneration in Alzheimer's Disease model of Adult Wistar rats

ABSTRACT

Aim of the study: Currently, reports linked neuropathological changes in Alzheimer's Disease (AD) to be a risk factor to depression, there is a need to development natural therapeutics to avert AD neuropathogenesis. Neem leaf has strong antioxidant. This study is aimed at ascertaining depressive like disorders in AD model while evaluating mechanism through which Neem averts AD neurodegeneration in the Fronto-cerebellar cortex and potential depressive like behaviour.

Methodology: Twenty (20) adult male Wistar rats were used, and grouped into a control group (A), neurodegenerative model (B), 200mg/kg Neem leaf supplement (C) and 200mg/kg Neem leaf treated AD model (D). Each group contained five animals (n=5). Neurobehavioural changes for reward memory and depressive like behaviour was evaluated using Y-mazed (open arm reward test) and the tail suspension test (TST). The frontal and cerebellar cortices were excised, fixed and processed for H and E stain, Cresyl fast violet (CFV) stain for Nissl and astrocytes immunohistochemistry using glial fibrillary acidic protein (GFAP). Behavioural test data were analyzed using ANOVA and test for significance done using post-hoc @p<0.05.

Results: AD model of neurodegenerative demonstrates depressive behaviour characterized by a increase in time spent to reach reward arm, increase in immobility time in the TST, a decrease number of reward arm entries associated with loss of cognitive function attributed to loss of neurons, neuron necrosis and chromatolysis, astrocyteic proliferation due to oxidative tissue damage in the fronto-cerebellar cortex. However, Neem supplementation mitigates against aforementioned neuropathological presentation resulting in an improved neurocognition, neuron survival, decline in astrocytic proliferation and decline in depressive like behavior as compared to AD model.

Conclusion: Neem alleviates depressive like behaviour associated with neurocognitive impairment the activation of astrocytic-neuronal interaction which protecting neurons against oxidative stress mediated chromatolysis, inflammation while strengthening neural circuits for cognitive function that averts memory impairment and depressive like behaviour.

Keywords: Depression, Astrocytes, Chromatolysis, Neem leaf supplement, Neuroinflammation, Alzheimer's disease, Memory impairment, fronto-cerebellar cortex

Formatted: Font: Not Bold

1. INTRODUCTION

Comment [SG1]: Pls take care spacing alignments throughout the manuscript

Memory and mental disorders pathological presentation is somehow similar, due to the mediator of this disease progression which is oxidative tissue damage resulting in loss of neurons, neuroinflammation, dysfunctional neural circuits resulting in memory and mood disorders [1]. There are various reports that there is a correlation between depression and memory disorder [2]., 2020) which implies that there is an interplay between the pathogenesis of memory and mood disorders [3,4]. Various studies have linked neuropathological changes in AD causing neurocognitive impairment to be a risk factor to developing depressive disorders due to defects in neuronal structures, neurochemistry and neural circuit [5,6]. However, the pathogenic mechanism of this correlation is yet to be fully understood. Therefore, research needs to focus on the interplay between memory and mood disorders while developing and effective therapeutics to combat this menace. Alzheimer disease (AD) is the most common form of dementia affecting the aging population [7] and it is of growing concern due to lack of a precise treatment or cure for it [8] and it has been reported to be associated with mood disorders such as depression and anxiety like behaviors [9,10]. It is important to develop a drug that is effective in managing these disorders as epidemiology report on the prevalence of AD is that more than 46 million people are affected, and this number is predicted to increase to 130 million by 2050 as a result of the growth of the aging population in the world [11]. Neurotoxins are biological or chemical substances that primarily alters neuron activity causing changes in behavioral, emotional, movement abnormalities; examples of these toxins are lead, manganese, Aluminum, nitric oxide, glutamate, tetrodotoxin etc. [12-14]. Aluminum toxicity is associated with Alzheimer's disease, a study reported an increase in aluminum contents in the post mortem brains of people with dementia and AD [12]. It has been study to model AD in animal experiments [13,14] because of its ability to easily penetrate the blood brain barrier [12,15]. Animal model to represent possible neuropathological changes in human diseases can be designed to evaluate therapeutics, cognitive or emotional changes that could be translated to human, in a quest to understanding diseases mechanisms and in new drug development [16]. Behavioural testing is designed using animal model to test for anxiety -like behaviour using open field test or depression like behaviour using Tail suspension test [16]. Natural products with strong antioxidant potential are sorted to ameliorate pathogenesis and progression of neurodegenerative disorders. Neem leaves have many beneficial and medicinal properties [17] such as anti-inflammatory, anti-oxidant, antibacterial and cognition enhancing properties [18-20] linked to limonoids present in its leaves [21]. Since, there are hypothesis circulating that depression is associated with AD [22]. This study seeks to ascertain if depressive like behavior is seen in Aluminum chloride induced model of AD, and determine the role of Neem in averts the neuropathological changes seen in the Fronto-cerebellar cortex.

Formatted: Font: Not Bold

Formatted: Font: Not Bold

2. MATERIAL AND METHODS

Experimental Animals:

Twenty (20) healthy adult male Wistar rats were obtained from the National Veterinary Research Institute (NVRI) Vom in Jos-South Local Government Area of Plateau State, Nigeria. All experimental investigations in this study were done in compliance with guidelines

to humane animal care standard outline according to the "Guide for the Care and Use of Laboratory Animals" [23].

Experimental Animals Care: The 20 Adult male Wistar rats weighing between 120-150g were kept in well-ventilated cages in the Animal House of Bingham University, Department of Anatomy, Karu, Nassarawa State, Nigeria. They were kept in standard laboratory condition; maintained at 35.5-37.0°C ambient temperature and 12:12 hours light and dark cycle respectively. They were allowed to acclimatize for a period of two weeks and they were given water *ad libitum* in addition to rat pellets [Vital UAC feeds, Nasarawa State Nigeria].

Compound of Study:

Neem Leaf [Herbal Capsule : Neem Leaf herbal dietary Supplements (Nature's Way Brands LLC, Green Bay, USA) was procured from HealthPlus Pharmacy, FCT Abuja, Nigeria. The drug dose used in this research was at 200mg/kg body weight of each rat. The Neem herbal capsule is taken daily in humans at a dose of 475mg/kg body weight in which two capsules are taken daily by the average human weighing 70kg as recommended by the manufacture; Nature's Way Brand LLC, Green Bay, USA. The rats were given 1ml of the Neem tablet dissolve in normal saline

Aluminum Chloride: The salt was procured obtained from the Chemical Sciences Department of Bingham University,. Aluminum treatment dose was 200mg/kg [14]. Scientific reports have shown that aluminum can be used as an experimental model for Alzheimer's disease [14,24] as it has been detected in the post mortem brain samples of diagnose with dementia and AD [12].

Experimental Animal Grouping and Protocol

Experimental design and duration

Twenty (20) adult Male Wistar Rats with mean weight of 120g were randomly selected and distributed into four (4) groups of five (n=5) animals each. The Control /normal group given only vehicle (normal saline) is Group A, while the experimental groups are Groups B-D.

Group A: Control treated with 1 ml of normal saline, pelleted rat feed and water *ad libitum* for 10 days
Group B: Neem leaves supplement treated group (200mg/kg) and pelleted rat feed ;water *ad libitum* for 5 days

Group C: Animal model of Neuro-degeneration (oral 200mg/kg AICl3), pelleted rat feed and water *ad libitum* for 5 days

Group D:-Neurodegenerative repair model(AICl3+Neem); pretreated with 200mg/kg of AICl3 for 5 days and posttreated with 200mg/kg of Neem leaves supplement treated group orally for 5 days

Behavioral testing: Behavioural testing for the Animals was done in compliance with "Guidelines for the Use of Animals in Behavioural Projects in Schools [25] and Guidelines for the care and use of mammals in neuroscience and behavioral research [26].

One open arm reward test in Y-maze : to test for animal to locate the food reward in the open arm using Y-maze test. The Y-maze is a spontaneous alternation test that measures spatial working memory and is also used as a test to measure short term memory, and general locomotor activity The Y-maze composed of three arms spaced equally, each

having an angle of 120°, 41cm long and 15cm high. The floor of each arm is 5cm wide. Rats were placed in one of the arm compartments and allowed to move freely until its tail has completely entered another arm. The numbers of arm entries were recorded manually, with the arms being labeled A, B, and C. an alternation is defined as entry into all three arms consecutively. A reward was placed in an arm tagged "A" the number of times and duration it takes each rat to reach the reward arm was recorded. Each animal was tested for 5 minutes and the apparatus was cleaned and allowed to dry. The same procedure was used for the reward paradigm [27].

Tail Suspension Test for measuring Depression: The tail-suspension test is a behavioral test useful in the screening of potential antidepressant drugs, and assessing of other manipulations that are expected to affect depression related behaviors compounds [28,29]. The test is based on the fact that animals subjected to the short-term, inescapable stress of being suspended by their tail, will develop an immobile posture, this immobility is defined as the absence of initiated movements and includes passive swaying. Our laboratory manufactured a tail suspension boxes made of wood with the dimensions. Each rat was suspended within the three walled rectangular box by the tail with tape from the lever (vertical bar) and position such that the base of their tail is aligned with the bottom of the bar. The tail-suspension test is a valuable tool in drug discovery for high-throughput screening of prospective antidepressant..Each mouse is given 1 trial that last 6 minutes. The total duration of immobility is calculated as the time the force of the mouse's movements is below a preset threshold [28].

Experimental Animal Euthanasia: Twenty – four hours after the last dose administration, Animals were randomly selected from each group and final body weight taken, using an analytic weighing scale (P.M Hana Ltd, Hong Kong, China). The experimental animals were then euthanized via cervical dislocation and decapitation [30], according to AVMA Guidelines for the Euthanasia of Animals, [31].

Brain tissue collection and preservation for histological tissue processing:

The brains were carefully exposed from the cranial vault by dissecting the cranial bone using a bone forceps. The wet brain samples were weighed and fixed in 10% formalcalcium for 24 hours [32,33]. Then the frontal cortex and the cerebellum excised carefully using neuroanatomical markings described by Paxinos and Watson Brain Stereotaxis mapping [34].

Histological processing of the frontal and cerebellar cortices: Histological processing and staining of sections of the hippocampus and frontal cortex was done under supervision and guidance in the Histopathology Laboratory, National Hospital Garki, Abuja using an automated tissue processor (Leica TP1020; Leica Microsystems, Germany).The automated tissue processor time was set, to allowed timed sequential transfer of tissues in the tissue processing basket from one stage of histological tissue processing as described by Bancroft et al., [33] The processed tissues were embedded in paraffin wax and serial section done using a Rotatory Microtome (Leica RM 2125; Leica Microsystems, Germany) set at 5µm tissue thickness. These section on the slides are ready for histological, histochemical and immunohistochemical staining procedure described by Bancroft and Gamble, [32].

Haematoxylin and Eosin (H and E) staining method: The hematoxylin and eosin stain (H&E) is the most widely used histological stain, this study used it to display the general

histoarchitecture of the Frontal and cerebellar cortices according to Giri [35] and Memudu and Adewumi [36]

Cresyl Fast Violet (CFV) for Nissl Bodies staining procedure: Cresyl fast violet is an inorganic compound which is a basic dye and is used as a common stain in histology. The stain is commonly used to identify the neuronal structure in brain and spinal cord tissue. The Nissl substance appears dark blue due to the staining of ribosomal RNA, giving the cytoplasm a mottled appearance according to Bancroft and Gamble, [32] method.

Glial Fibrillary Acidic Protein (GFAP) staining procedure: GFAP is an intermediate filament protein that is expressed by numerous cell types of the central nervous system (CNS) including astrocytes and ependymal cells. The following primary antibodies were used Novocastra-mouse monoclonal: GFAP-antibody Leica Microsystems-Novocastra™, United Kingdom (1:100 dilutions) and the secondary antibody (Novocastra biotinylated secondary antibodies; biotinylated donkey anti-goat IgG, 1:200). The peroxidase-coupling was done using avidin-biotin complex (ABC Kit, Vector Laboratories, and Burlingame, CA). The immunoreaction product was visualized with 3,3'-diaminobenzidine (DAB, Dako) for chromogen development. The counterstain was done using Mayer's Haematoxylin and mounting media-DPX (Distrene Plasticizer Xylene). Negative controls were performed by omitting the primary antibody [32, 36].

Statistical analysis: Data set were analyzed using GraphPad Prism 7 (GraphPad software, Inc., LA Jolla, CA). Student's *t*-tests were used for all pairwise comparisons and one-way ANOVAs were used for all multiple comparisons followed by the *post hoc* Tukey test. For all analyses, differences were considered significant when *p*-values were lower than 0.05 and significant effects are indicated by asterisks (**p* < 0.05). Data were expressed as mean ± standard deviation (SD).

Photomicrography of histological, histochemical and immunohistochemical results: Sections were visualised with Leica digital microscope and digital images were captured using a light microscope digital camera (MV 500 Cameroscope™ (5.1 MP)) attached to the PC where the images were stored. The images were captured with X40 objective lenses and the phototube of the MV 500 colored digital camera used is X10 of megapixel 5.0. The images were captured and stored using the joint photographic export group (JPEG) format for analysis

3. RESULTS

Physical Observation: During the research, the control group showed normal physical characteristics, grooming and maintained a steady appetite but the aluminum treated (neurodegenerative model) animals' physical appearance is poor, reddish eyeballs, loss of appetite, weight lost, poor social behaviour/ mood and grooming seen in hair loss and distorted fur arrangement (dull fur). These physical characteristics occurred in the first 5 days of aluminum treatment but Neem

treatment progressively alter these characteristics making the animals improve in feeding habit thereby restoring appetite and body weight, reduced eye and nose bleeding also improve grooming and social behaviour.

Body weight changes: The final weight of the control increased when compared with AlCl₃ treated AD model, at $p < 0.05$ (Fig 1B). There was no significance difference between the final body weights of the control and the Neem supplement treated (C). The Neem supplement treated AlCl₃ - AD model (****D) had a significance increase in final body weight when compared with AlCl₃ treated AD model (B). The final weight in the control (A) had a significant decline when compared with the neem supplement treated and AlCl₃ treated (D) (** $p < 0.0059$). The final weight of AlCl₃ treated (B) declined as compared with the weight of Neem supplement treated and AlCl₃ treated (D) (**** $p < 0.0001$). The Neem supplement group (C) had a decline in final body weight as compared with Neem supplement treated and AlCl₃ treated (D) at $p < 0.05$ (** $p < 0.0006$). However, A vs C was not statistically significant ($p < 0.6895$). However, when comparing the initial and final weights, it was deduced that AlCl₃ treated (B) group had a decline in final weight as compared with the initial weight at $p < 0.05$.

Neem treated reversed depressive like behaviour induced by AlCl₃ in memory reward task and tail suspension test: The time spent to get to the reward arm reduced significantly in the control (A) as compared with AlCl₃ treated (B) (**A vs B: $p < 0.0018$) and AlCl₃ +Neem treated (D) group (*A vs B: $p < 0.0486$). The time spent to get the reward increased in B significantly as compared with Neem treated (C) and the AlCl₃ +Neem treated (D) groups (**B vs C: $p < 0.0027$; **B vs D: $p < 0.0062$). Neem treated (C) had a reduction in time spent to get to reward arm as compared with the AlCl₃ +Neem treated (D) (**C vs D: $p < 0.0005$) as shown in fig. 2A. The number of times experimental animals visited the reward arm increased in the control (A) as compared with AlCl₃ treated (B) (*A vs B: $p < 0.0109$); but the AlCl₃ treated (B) had a significance reduction in the number of reward arm entries when compared with Neem treated (C) and the AlCl₃ +Neem treated (D) groups (**B vs C: $p < 0.0029$; *B vs D: $p < 0.0143$) as seen in fig. 2B. however, Neem treated (C) had a significance as compared to the AlCl₃ +Neem treated (D) (**C vs D: $p < 0.0021$). In the tail suspension test for anxiety like behaviour in experimental animals used in this study there was no significance difference in the animals for the pretest tail suspension test for anxiety like behaviour (Fig 3 A). However, the test showed that the control (A) group had a reduction in time immobility time as compared with the AlCl₃ treated (B) (***A vs B $p < 0.0008$) as seen in Fig 3B. There was no significant difference between the control (A) and the Neem treated (C) at $p < 0.05$. The control (A) group had a significant decline in immobility time as compared with the AlCl₃ +Neem treated (D) group (**A vs D $p < 0.0048$). The AlCl₃ treated (B) had a significant increase in immobility time as compared with Neem

Formatted: Subscript

Comment [SG2]: Pls follow this everywhere

Formatted: Subscript

treated (C) and AlCl₃ +Neem treated (D) groups at p<0.05 (** B vs C; D p<0.0059; p< 0.0052). Neem treated (C) had a significant decline in immobility time when compared with the AlCl₃ +Neem treated (D). (*C vs D p<0.0282).

Neem reversed AlCl₃ induced neurodegeneration and chromatolysis: The histomorphology of the frontal cortex showed the presence of Nissl positive neurons within the dense non-vacuolated neuropil and non-necrotic neurons in the control (Fig 4. A1 and A2) as compared with AlCl₃ treated (B) group characterized by vacuolated scanty neuropil with pyknotic and chromatolyzed neurons. Neem treated (C) had numerous non -necrotic neurons and Nissl positive neurons. The neem in the neem treated AlCl₃ induced AD model demonstrated its neuroprotective potential by preventing aluminum induced Nissl bodies chromatolysis and necrosis in FC neurons. The histomorphology of the cerebellar cortex showed the presence of Nissl positive neurons within the dense non-vacuolated neuropil and non-necrotic neurons in the control (Fig. 5A1 and A2) as compared with AlCl₃ treated (B) group characterized by vacuolated scanty neuropil with pyknotic and chromatolyzed neurons. Neem treated (C) had numerous non -necrotic neurons and Nissl positive neurons. The neem in the neem treated AlCl₃ induced AD model demonstrated its neuroprotective potential by preventing Aluminum induced Nissl bodies chromatolysis and necrosis in FC neurons.

Neem attenuated proliferation of reactive astrocytes in AlCl₃ induced neurodegeneration: The FC and CB had numerous reactive astrocytes as compared with the control and neem treated groups (Fig 6 A1 &A2 as well as B1 and B2). Neem mediated a reduction in neuroinflammatory biomarker expression of reactive astrocytes similar to the control and neem treated groups.

4. DISCUSSION

This study was done to demonstrate the depressive -like behaviour in AD model in animal while reporting the potential role of Neem leaf supplement to acts as an anti-depressant to ameliorates the neuropathogenesis and neurobehavioural changes observed *in vivo* by evaluating histological, histochemical, immunohistochemical and neurobehavioural changes.

In this study, morphological changes in the body weight showed that the aluminum chloride treated rats had a significant decline in body weight as compared with the control and neem treated. This finding supports Memudu et al., [14], Buraimoh et al., [23] and Buraimoh and Ojo, [37] studies. The neem treated reversed the aluminum induced weight loss causing an increased in food consumption and body weight gain. But during the study, at the onset of neem administration, those group had a decline in feeding habit linked with the toxicity and bitterness of the Neem supplement that they progressive adjusted to causing an improved feeding and body weight gain.

The neuropathological changes in the frontal and cerebellar cortices demonstrating interplay between Aluminum mediated oxidative neuron tissue damage and neem supplementation. The AD model had a marked necrosis, severe loss of neuron cell integrity, chromatolysis, pericellular spaces and vacuolations within the neuropil and loss of neurite which affect synaptic connectivity in the frontal cortex [14,38] as displayed in the cyto-architecture of the fronto-cerebellar cortex in Fig 4B2 & 5B1 using H and E stain and severe chromatolysis (Fig 4A2 & 4B2) demonstrating neuronal cell death and loss of neuron histological integrity. This supports reports made by Memudu et al., [14] and Giancarlo et al., [39] a general pathological characterization of aluminum induced AD neuropathogenesis. But upon administration of neem leaf supplement, this aforementioned pathogenesis was interrupted by its polyphenolic and other flavonoid compounds such as quercetin, azadirachtin, nimbinolin, nimbin, nimbidin, nimbidol and salannin, in neem leaves having rich source of antioxidant [40,41]. Cresyl fast violet demonstrates Nissl bodies (aggregates of ribosome of rough endoplasmic reticulum) involve in protein synthesis, an important cellular process for neurons and neurotransmitters build up. A loss or decline in aggregation of rough endoplasmic reticulum causes neuronal body to swell pushing the nucleus toward the periphery of the cell. In this study, AD model results in loss of Nissl aggregation demonstrated by the marked chromatolysis seen in the perikarya of the neurons in the fronto-cerebellar cortex which was averted by neem leaf supplementation in the neem treated neurodegenerative model this implies that neem leaf reversed Nissl body chromatolysis, characterized by presence of normal Nissl positive neurons in fronto-cerebellar cortex which implies more ribosome synthesis for neuron tissue repair and neurotransmitter synthesis [14,42].

To evaluate for correlation between spatial memory for reward arm and mood behaviour using the open arm reward Y-maze test [127]. It was observed that AD model spent more time to locate the reward arm while the number of times visiting the reward arm reduced during the test as compared to AD model treated with neem leaf supplement. This indicates that aluminum induced AD model animal depicts depressive behavior based on the number of time to locate the reward arm as compared to the control and neem treated groups. This implies that AD model predisposes animals to developing depressive like behaviour. Furthermore, they were also subjected to the tail suspension test a model to test for behavioral despair or depressive -like behaviour [16]. In this paradigm, an increase time spent immobile (immobility time) indicates depressive-like behaviour and a compound with potential antidepressant drug will cause a decrease in immobility time or increase escape directed behaviour [17]. In this study, AD model during the TST had an increased in immobility time or decline in escape directed behaviour which indicates depressive like behaviour. The observed changes correlate with the neuropathological changes in fronto-cerebellar cortex of AD model characterized by loss of neuron integrity and neurite outgrowth required for synaptogenesis and neurocognition function this correlates with report by Pan et al., [1] and Sáiz-Vázquez et al., [22] that neurodegeneration in AD disruption neurons structural, loss of neurons and cognitive decline that predisposes to depressive like behavior. In this study, both open arm reward Y-maze test and TST validates that AD model can predispose to depressive like behavior in animal model. The neem leaf supplement treated AD model reversed this neuro-behavioural changes in AD model and this correlates with Raghavendra et al., [17] finding that neem leaves as an effective therapeutic for cognitive enhancement and anti-depressant. Furthermore, astrocytes activity in the fronto-cerebellar cortex was demonstrated through intermediate filament glial fibrillary acidic protein (GFAP) immunohistochemistry [36]. Astrocytes are non-neuronal cells important in neuron homeostasis but in neurodegenerative condition, astrocytes produce

proinflammatory mediators [43] which are immunohistochemically demonstrated in the expression of the intermediate filament glial fibrillary acidic protein (GFAP). Astrocytes play an important role as a biomarker for neuroinflammation during neurodegenerative and therapeutic study because they are used as diagnostic indicators or effective targets for the treatment of neurodegenerative diseases [44,45]. A number of reports on the role of astrocytes in neurodegenerative disorders such as depression and dementia such as Alzheimer's disease whereby they increase or decline in response to neurotoxins and neurotherapeutics for neuron survival [46]. In this study, AD model had a marked expression of astrocytes indicating neuroinflammatory response to the neurotoxin for neuroprotection and survival, this response was attenuated in the neem supplement treatment groups of the fronto-cerebellar cortex this implies that neem antioxidant role mediates anti-inflammatory response conducted by its active compound thereby mopping off free radicals generated by Aluminum neurotoxicity results in a moderate expression of astrocytes for neuroprotection, repair and survival from the remnant of the toxins circulating in the brain milieu. This correlates with the protection of neurons and neurite growth in neem treated AD model which correlates with an improvement in neurocognitive behaviour for spatial memory and improved mobility time in Y maze and Tail suspension test mediated by protection of neural circuits in depression and Alzheimer's disease (AD) [47,48]. In this study, aluminum toxicity induces neuroinflammatory reactions in the cerebello-frontal cortex indicated by an increased proliferation of reactive astrocytes a biomarker for neuroinflammatory activities and this supports reports made by Kamel *et al*, [49]. Neem leaf supplementation inhibits the progression of reactive astrocytes demonstrated in the observed mild expression of astrocytes this indicated its anti-inflammatory properties [17].

LIST OF FIGURES and legends

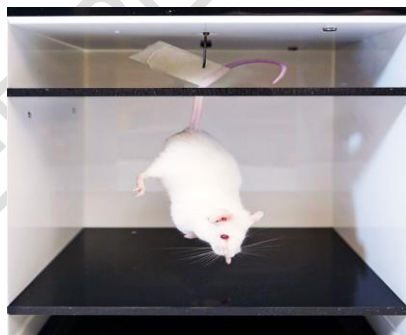


Figure 1: Illustration of the tail suspension test

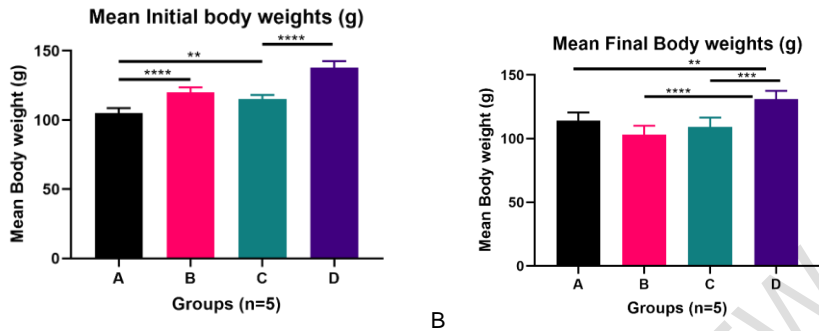


Figure 2: Graphical representation of the **mean initial and final body weights** of the experimental animals used. Data analyzed using one-way ANOVA and expressed as Mean \pm Standard Deviation (Mean \pm SD). Statistical Significance is taken at $P < 0.05$ (*) using Tukey post-hoc test. Legend: A- Control; B: AIC13 treated; C- Neem treated, and D: AIC13 +Neem treatment.

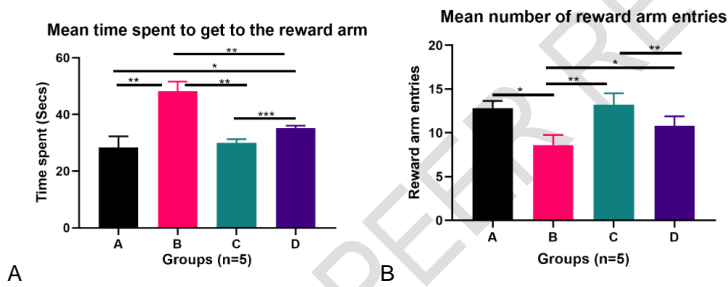


Figure 3: Graphical representation of **mean time to get reward and number of reward arms entries of the experimental animals** used in this study. Data analyzed using one-way ANOVA and expressed as Mean \pm Standard Deviation (Mean \pm SD). Statistical Significance is taken at $P < 0.05$ (*) using Tukey Post hoc test Legend: A- Control; B: AIC13 treated; C- Neem treated, and D: AIC13 +Neem treatment.

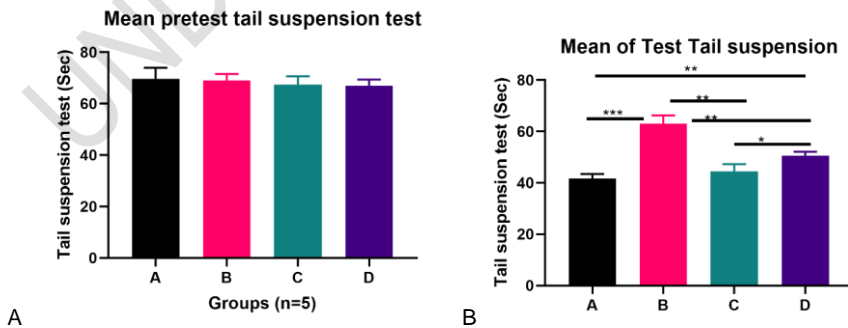


Figure 4: Graphical representation of **mean pretest and test tail suspension test for anxiety like behaviour in experimental animals** used in this study. Data analyzed using

one-way ANOVA and expressed as Mean \pm Standard Deviation (Mean \pm SD). Statistical Significance is taken at $P < 0.05$ (*) using Tukey Post hoc test. Legend: A- Control; B: AICI3 treated; C- Neem treated, and D: AICI3 +Neem treatment.

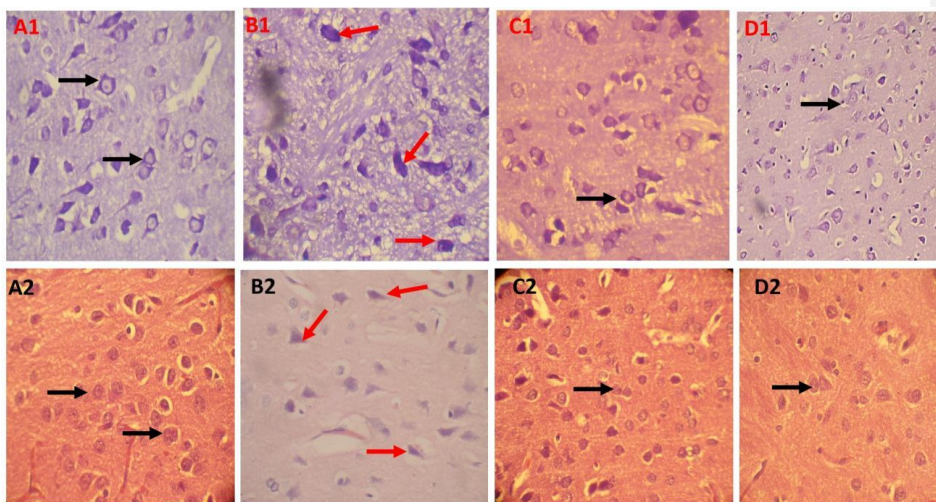


Fig 5: Photomicrograph of the frontal cortex of adult male Wistar rats stained with CFV stain (A1-D1) and H and E stain (A2-D2) stain. Legend: A- Control; B: AICI3 treated; C- Neem treated, and D: AICI3 +Neem treatment. Mag. X400. Scale bar: 50 μ m. Red arrows: Necrotic or degenerated neurons with chromatolysis; Black arrows: normal neurons with their centrally located nucleus and neurites extension.

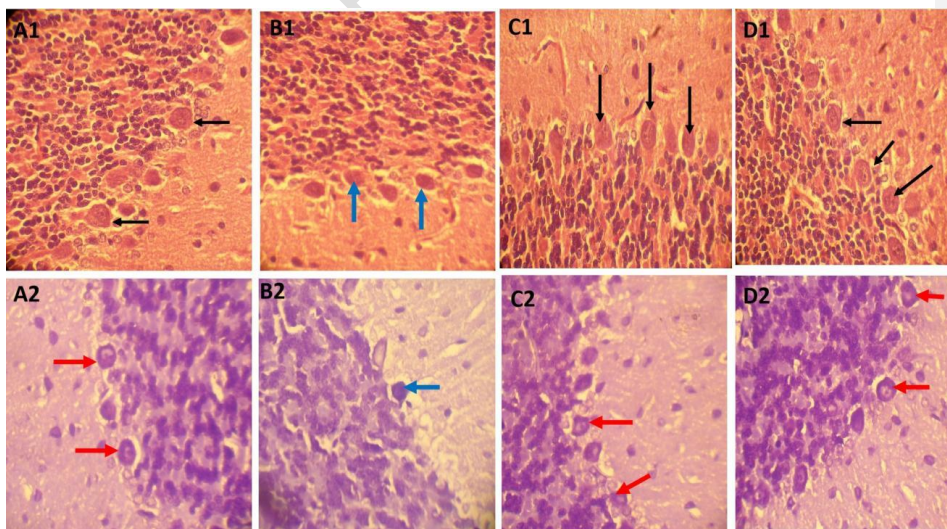


Fig 6: Photomicrograph of the Cerebellar cortex stained with H and E (A1-D1) and CFV (A2-D2) stain of adult male Wistar rats Legend: A- Control; B: AlCl₃ treated; C- Neem treated, and D: AlCl₃ +Neem treatment. Mag. X400. Scale bar: 50µm. Red arrows: Nissl positive Purkinje neurons; Black arrows: normal Purkinje neurons; Blue arrows: Necrotic or degenerated neurons characterized by chromatolysis

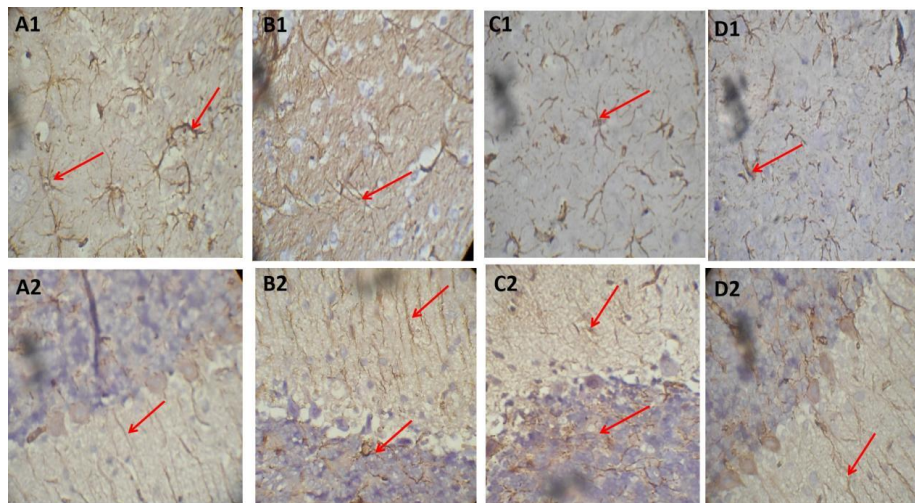


Fig. 7: Photomicrograph of the frontal cortex (A1-D1) and Cerebellar cortex (A2-D2) of adult male Wistar rats of stained with GFAP (glial fibrillary acidic protein) Legend: A- Control; B: AlCl₃ treated; C- Neem treated, and D: AlCl₃ +Neem treatment. Mag. X400. Scale bar: 50µm. The FC and CB had numerous reactive astrocytes as compared with the control and neem treated groups (Fig 6 A1 & A2 as well as B1 and B2). Neem mediated a reduction in neuroinflammatory biomarker expression of reactive astrocytes similar to the control and neem treated groups. Legend: Red arrow- Astrocytic processes

4. CONCLUSION

There is a correlation between AD and mood disorders such as depression. Neem leaf supplement potential to avert aluminum induced neurocognitive impairment and depressive like behaviour is via the activation of astrocytic-neuronal interaction which protecting neurons against chromatolysis, inflammation while strengthening neural circuits for cognitive function that averts memory impairment and depressive like behaviour

CONSENT

It is not applicable.

ETHICAL APPROVAL

Animal Ethic committee approval has been collected and preserved by the author(s).

Comment [SG3]: Pls provide Ethical committee approval registration number

REFERENCES

1. Pan PY., Bölte S, Kaur P, Jamil, S. & Jonsson, U. Neurological disorders in autism: A systematic review and meta-analysis. *Autism* 2020; 20, 1362361320951370
2. Mogle, J., Hill, N.L., Bhargava, S. et al . Memory complaints and depressive symptoms over time: a construct-level replication analysis. *BMC Geriatr.* 2020; 20, 57
3. Maramis M.M., Mahajudin M.S., Khotib J (2021). Impaired Cognitive Flexibility and Working Memory Precedes Depression: A Rat Model to Study Depression. *Neuropsychobiology* 80:225–233
4. Valverde AP, Camargo A, Rodrigues ALS. Agmatine as a novel candidate for rapid-onset antidepressant response. *World J Psychiatr* 2021; 11(11): 981-996 [PMID: 34888168 DOI: 10.5498/wjp.v11.i11.981]
5. Lee TH, Choi JH, Shin BC, Shin HE, Son HR. Correlation between Dementia and Depressive Disorder. *KJFP* 2018;8:327-330
6. Fang, Y., Ding, X., Zhang, Y. et al. Fluoxetine inhibited the activation of A1 reactive astrocyte in a mouse model of major depressive disorder through astrocytic 5-HT₂BR/ β -arrestin2 pathway. *J Neuroinflammation* 19, 23 (2022).
7. Yuan, C., Chen, H., Wang, Y., Schneider, J. A., Willett, W. C., & Morris, M. C. (2021). Dietary carotenoids related to risk of incident Alzheimer dementia (AD) and brain AD neuropathology: a community-based cohort of older adults. *Am J Clin Nutr.* 113(1), 200–208. <https://doi.org/10.1093/ajcn/ngaa303>
8. Ellouze I, Sheffler J, Nagpal R, Arjmandi B. Dietary Patterns and Alzheimer's Disease: An Updated Review Linking Nutrition to Neuroscience. *Nutrients.* 2023; 15(14):3204. <https://doi.org/10.3390/nu15143204>
9. Yu O, Jung B, Go H, et al (2020). Association between dementia and depression: a retrospective study using the Korean National Health Insurance Service-National Sample Cohort database *BMJ Open* 2020;10:e034924.
10. Lanza, C., Sejunaite, K., Steindel, C., Scholz, I., Riepe, M.W. Cognitive profiles in persons with depressive disorder and Alzheimer's disease, *Brain Communications*, Volume 2, Issue 2, 2020, fcaa206
11. Solch, R.J.; Aigbogun, J.O.; Voyiadjis, A.G.; Talkington, G.M.; Darensbourg, R.M.; O'Connell, S.; Pickett, K.M.; Perez, S.R.; Maraganore, D.M. Mediterranean diet adherence, gut microbiota, and Alzheimer's or Parkinson's disease risk: A systematic review. *J. Neurol. Sci.* 2022, 434, 120166
12. Exely; T. Vickers. (2014). *Journal of Medical case reports*. "Elevated brain Aluminium and early onset of Alzheimer's disease in an individual occupationally exposed to Aluminium: a case report.8:41.
13. Akinrinade, I.D., Memudu, A.E., Ogundele, O.M (2015). Fluoride and Aluminium disturb neuronal morphology, transport functions, cholinesterase, lysosomal, and cell cycle activity. *Pathophysiology* (22): 105 -115.
14. Memudu Adejoke Elizabeth, Pantong Samson and Osahon Roli Itohan (2020). Histomorphological evaluations on the frontal cortex extrapyramidal cell layer following administration of N-Acetyl cysteine in aluminum induced neurodegeneration rat model. *Metab Brain Dis* 35, 829–839 (2020). <https://doi.org/10.1007/s11011-020-00556-91>
15. Brenner S. Aluminum may mediate Alzheimer's disease through liver toxicity, with aberrant hepatic synthesis of ceruloplasmin and ATPase7B, the resultant excess free copper causing brain oxidation, beta-amyloid aggregation and Alzheimer disease. *Med Hypotheses.* 2013 Mar;80(3):326-327
16. Belovicova, 2017 K., Bogi, E., Csatosova, K., & Dubovicky, M. (2017). Animal tests for anxiety-like and depression-like behavior in rats. *Interdisciplinary toxicology*, 10(1), 40–43. <https://doi.org/10.1515/intox-2017-0006>

Comment [SG4]: Pls. follow uniform reference style

Formatted: Font: Not Bold, (Asian) Chinese (Simplified, PRC)

17. Raghavendra, M., Acharya, S.B., Rituparna, M., Kumar, S (2013). Role of aqueous extract of *Azadirachta indica* leaves in an experimental model of Alzheimer's disease in rats. *International Journal of Applied Basic Medical Research*. **3** (1):37-47. Doi:10.4103/2229-516x.112239
18. Fakunle P.B; Ajibade A.J; Oyewo E .B and Adeyemi O. H. (2012). A Study of Some Effects of Aqueous Extract of Neem (*Azadirachta Indica*) Leaves on the Lead Acetate Induced Neurotoxicity in the Superficial Layers of Superior Colliculus of Adult Wistar Rats (*Rattus norvegicus*).
19. Sujarwo, W/, Keim, A.P., Caneva, G., Toniolo, C., Nicoletti, M.(2016). Ethnobotanical uses of neem leaves in Bali (Indonesia) and the Indian subcontinent in relation with historical background and phytochemical properties. *Journal of ethnopharmacology*. 189:186-93. Doi: 10.1016/j.jep.2016.05.014.
20. Sinha, J.D., Nandaha, D.S.K., Jaiswal, N., Vasudeva, A., Prabha, T.s., Singh, P.U (2017). Antibacterial effect of *Azadirachta indica* (Neem) or *Curcuma longa* (turmeric) against *Enterococcus faecalis* compared with that of 5% sodium hypochlorite or 2% chlorhexidine in vitro. *Bull Tokyo Dent Coll*. **58** (2): 103-109. Doi:10.2209/tdcpublication.2015-0029
21. Datta A, Fernando L.N, Grun I.U, Kwasniewski M.T.(2017). Comparison of two absorbent based de-bittering procedures for neem a Tea-effect on polyphenols, anti-oxidant capacity, color and volatile profile. *Plant foods human nutrition*. 72(1):88-95. Doi:10.1007/s11130-016-0505-9.
22. Sáiz-Vázquez, O.; Gracia-García, P.; Ubillos-Landa, S.; Puente-Martínez, A.; Casado-Yusta, S.; Olaya, B.; Santabárbara, J. Depression as a Risk Factor for Alzheimer's Disease: A Systematic Review of Longitudinal Meta-Analyses. *J. Clin. Med.* 2021, 10, 1809
23. National Research Council (2011). Guide for the care and use of laboratory animals. (8th Edition). Washington, DC: The National Academies Press. page 123-124.
24. Buraimoh AA, Ojo SA, Hambolu JO (2011) Effects of Oral Administration of Aluminum Chloride on the Histology of the Hippocampus of Wistar Rats. *Current Research Journal of Biological Sciences*, 3(5):509-515. ISSN: 2041-0778.
25. American Psychological Association, Animal Research and Ethics in 2012 :: American Psychological Association (APA) Guidelines for Ethical Conduct in the Care and Use of Animals (2012).
26. National Research Council (2003). Guidelines for the care and use of mammals in neuroscience and behavioral research. Washington, DC: The National Academies Press
27. Onalapo, O.J., Abiodun, O.R., Akanji, O.O., Mosaku, T.J., Onalapo, A.Y. (2012). Elevated plus maze and Y-maze behavioral effects of subchronic, oral low dose monosodium glutamate in Swiss albino mice. *IOSR Journal of Pharmacy and Biological Sciences*. ISSN: 2278-3008. Vol.3, issue 4. Pp21-27
28. Cryan JF, Mombereau C, Vassout A. The tail suspension test as a model for assessing antidepressant activity: review of pharmacological and genetic studies in mice. *Neurosci Biobehav Rev* 2005, 29: 571-625
29. Can, A., Dao, D.T., Terrillon, C.E., Piantadosi, S.C., Bhat, S., Gould, T.D. (2012). The tail suspension test. *Journal of Visualized Experiments*. (59): 3769. Doi: 10.3791/3769
30. Carbone L, Elizabeth T .C; Elizabeth M .Y; Diana B .B; Krista A. L; John M .P; Jamie A. A; Youngho S; Anisha D .G; and James D .W (2012). Assessing cervical dislocation as a human Euthanasia Method in Mice. *J.Am.Assoc Lab Anim Sci*. 51 (3): 352-356
31. AVMA Guidelines for the Euthanasia of Animals, (2013):: AVMA Guidelines for the Euthanasia of Animals: 2013 Edition American Veterinary Medical Association on Euthanasia. (2007). AVMA Guidelines
32. Bancroft, J.D and Gamble, M (2008). Theory and practice of Histological techniques. 6th Edition London Churchill Livingstone: 374 – 375
33. Bancroft, John D., Christopher Layton; S Kim Suvarna (2013). Bancroft's theory and practice of histological techniques Oxford] : Churchill Livingstone Elsevier: 7th ed

34. Paxinos and Watson Brain Stereotaxis mapping (Paxinos and Watson, 2006)::; Paxinos, George, and Charles Watson. The rat brain in stereotaxic coordinates: hard cover edition. Access Online via Elsevier, 2006.
35. Giri D. (2015). *Histopathology*. "Hematoxylin and Eosin staining: principle, procedure and interpretation.
36. Memudu, A.E and Adewumi, (2021). Alpha-lipoic acid ameliorates scopolamine-induced memory deficit and neurodegeneration in the cerebello-hippocampal cortex. *Metabolic Brain Disease*: 36 (7); 1729–1745. DOI: 10.1007/s11011-021-00720-9
37. Buriamoh, A.A., Ojo, S.A. (2014). Effects of aluminum chloride exposure on the body weight of wistar rats. *Annals of Biological Sciences*. 2 (2):66-73. ISSN:2348-1927.
38. Berk M, Dean OM, Cotton SM, Jeavons S, Tanius M, Kohlmann K, Hewitt K, Moss K, Allwang C, Schapkaitz I, et al (2014). The efficacy of adjunctive Nacetylcysteine in major depressive disorder: a double-blind, randomized, placebo-controlled trial. *J Clin Psychiatry*.75(6):628–636
39. Giancarlo A, Alessandra A, Giovanna B, Giulio V, Marina C, Luisa B, Francesco S (2018) NAcetylcysteine as an antioxidant and disulphide breaking agent: the reasons why, *Free Radical Research*. 52(7):751-762, DOI: 10.1080/10715762.2018.1468564
40. Schmutterer. (2014). The Neem tree and other Melaiaceous plants. Sources of unique Natural Products for Integrated Past Management, Medicine industry & other purposes.
41. Alzohairy, M.A. (2016). Therapeutics role of *Azadirachtaindica* (Neem) and their active constituent in disease prevention and treatment. *Evidence-Based Complementary and Alternative Medicine.eCAM* 2016: 7382506. Doi:10.1155/2016/7382506
42. Blaylock, R. (2012). Aluminum induced immunoexcitotoxicity in neurodevelopmental and neurodegenerative disorders. *Current Inorganic Chemistry*.2 (1):46-53. Doi:10.2174/1877944111202010046.
43. Jana, M., Dasgupta, S., Ghorpade, A., Pahan, K. (2017). Neuroimmune Pharmacology- Astrocytes, Oligodendrites, and Schwann cells. Pp69-88.
44. KunyuLi , Jiatong Li , Jialin Zheng , Song Qin. Reactive Astrocytes in Neurodegenerative Diseases. *Aging and disease*. 2019, 10(3): 664-675
45. Paumier, A., Boisseau, S., Jacquier-Sarlin, M., Pernet-Gallay, K., Buisson, AA., Albriex, M (2022). Astrocyte–neuron interplay is critical for Alzheimer's disease pathogenesis and is rescued by TRPA1 channel blockade, *Brain*, 145 (1): 388–405
46. Dolotov OV, Inozemtseva LS, Myasoedov NF, Grivennikov IA. Stress-Induced Depression and Alzheimer's Disease: Focus on Astrocytes. *International Journal of Molecular Sciences*. 2022; 23(9):4999
47. Zhou X., Qian, X., Li, X., Fan, Y., Liping, W., Jie, T. (2019). Astrocyte, a Promising Target for Mood Disorder Interventions. *Frontiers in Molecular Neuroscience*. 12
48. Liao, Y., Xing, Q., Li, Q. et al. Astrocytes in depression and Alzheimer's disease. *Front. Med*. 15, 829–841 (2021). <https://doi.org/10.1007/s11684-021-0875-0>
49. Kamel E.S; Mostafa N. (2013). *The Egyptian Journal of Histology*. "Effects of aluminum chloride on the hippocampus of adult rats and the possible protective role of Nigella sativa: a histological and immunohistochemical study". Volume 36-Issue 2-p 505-513.