Asian Journal of Research in Medical and Pharmaceutical Sciences

Volume 13, Issue 1, Page 31-46, 2024; Article no.AJRIMPS.111677
ISSN: 2457-0745

Neem Leaf Supplement Ameliorates Depressive Like Behaviour in Alzheimer's Disease Model in Adult Male Wistar Rats

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Authors' contributions

This work was carried out in collaboration among all authors. Author AEM designed the study, supervised the research, performed the statistical analysis, and wrote the first draft of the manuscript,performs histological analysis, histo-morphometric analysis.. Author FAA performed the statistical analysis, conducted the research and manage literature search. Author GMJ performed the statistical analysis, wrote the protocol, conducted the research and manage literature search, performs histological analysis, histo-morphometric analysis. Author RPA managed the literature searches, and proofreading the manuscript drafts. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/AJRIMPS/2024/v13i1245

Open Peer Review History:
This journal follows the Advanced Open Peer Review policy. Identity of the Reviewers, Editor(s) and additional Reviewers, peer review comments, different versions of the manuscript, comments of the editors, etc are available here: https://www.sdiarticle5.com/review-history/111677

Original Research Article

Received: 12/11/2023
Accepted: 17/01/2024
Published: 24/01/2024

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ABSTRACT

Aim of the Study: Currently, reports linked neuropathological changes in Alzheimer's Disease (AD) to be a risk factor in depression. There is a need to develop natural therapeutics, with strong antioxidant property like Neem leaf, to avert AD's neuropathological and behavioural changes using animal model induced by neurotoxin aluminium chloride. 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 possible depressive like behaviour.

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

Results: Aluminum induced neurodegeneration similar to AD pathology characterized by loss of neurons, chromatolysis, proliferation of astrocytes and decline in cognitive function in addition to exhibiting depressive like behaviour seen in a decrease number of reward arm entries, increase in time spent to reach reward arm and immobility time in the TST attributed to loss of cognitive function relative to loss of neurons integrity in the fronto-cerebellar cortex. However, neem leaf supplementation mitigates against AD model neuropathological and neurobehavioural presentations resulting in an improved neurocognition, neuron survival or repair, decline in astrocytes proliferation and decline in depressive like behavior as compared to Aluminum induced AD model.

Conclusion: Neem leaf alleviates depressive like behaviour associated with neurocognitive impairment through the interaction between neuron-astrocytes to protect neurons against aluminum induced neuroinflamamation and strengthens neural circuit for cognitive function.

Keywords: Depression; astrocytes; chromatolysis; neem leaf supplement; neuroinflammation; Alzheimer's disease; memory impairment; fronto-cerebellar cortex.

1. INTRODUCTION

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] which implies that there is a interplay between the pathophysiology 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 moods disorders while developing an 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 resulting in changes in behavioral, emotional and movement abnormalities; examples of these toxins includes 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]. Aluminum 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 helps to demonstrate possible neuropathological changes in human diseases in order to study or evaluate therapeutics, cognitive or emotional changes that could be translated to human, in a quest to understanding diseases mechanisms and in new drug discovery [16]. Behavioural testing for anxiety or depressive like behaviour includes open field test, Y -maze with close arm and Tail suspension test [16]. Natural products with strong antioxidant potential are sorted to ameliorate pathophysiology and progression of neurodegenerative disorders. Neem leaves have many beneficial and medicinal properties [17] such as anti-inflammatory, anti-oxidant, antibacterial and cognitive properties [18-20] linked to phytochemicals such as limonoids present in its leaves [21]. Since, there are hypothesis circulating that depression is a 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 leaf supplementation to avert neuropathological features in the fronto-cerebellar cortex and neurobehavioral changes seen through behavioural testing in the animal.

2. MATERIALS AND METHODS

2.1 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].

2.1.2 Experimental animals care

The 20 Adult male Wistar rats weighing between 120-130g were kept in well-ventilated cages in the Animal House of Bingham University, Department of Anatomy, Karu, Nasarawa State, Nigeria. They were kept in standard laboratory condition; maintained at room 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].

2.2 Compound of Study

2.2.1 Procurement of Neem leaf supplement capsule

Neem leaf herbal dietary supplement (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 Wistar 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 adult with average weight of 70kg as recommended by the manufacture (Nature's Way Brand LLC, Green Bay, USA).

2.2.2 Aluminum chloride

The salt was procured obtained from the Chemical Sciences Department of Bingham University, Aluminum treatment dose was given at 200mg/kg [14]. There are a number of scientific reports demonstrating 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 individual diagnose with dementia and AD [12].

2.3 Experimental Animal Grouping and Protocol

2.3.1 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 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 libtum* for 10 days.

Group B: Neem leaf supplement treated group (200mg/kg) and pelleted rat feed;water *ad libtum* for 10 days

Group C: Animal model for AD given 200mg/kg of AlCl3 orally for 5 days along with pelleted rat feed and water *ad libtum.*

Group D: AD (neurodegenerative) repair model, pretreated with 200mg/kg of AlCl3 for 5 days and post-treated with oral 200mg/kg of Neem leaf supplement for 10 days.

2.3.2 Behavioral testing

Behavioural testing for the animals was done in compliance with the guidelines for the use of animals in behavioural research [25] and guidelines for the care and use of mammals in neuroscience and behavioral research [26]. Behavioural test paradigm includes Y-maze and Tail suspension test.

2.3.3 One open arm reward test in Y-maze

This was done to test for animals ability 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. 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].

2.3.4 Tail Suspension test for measuring depression

The tail-suspension test is a behavioral test useful in the screening of potential antidepressant drugs [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 (Fig. 1). 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].

2.4 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 [30], according to AVMA Guidelines for the Euthanasia of Animals, [31].

2.5 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% formol calcium 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].

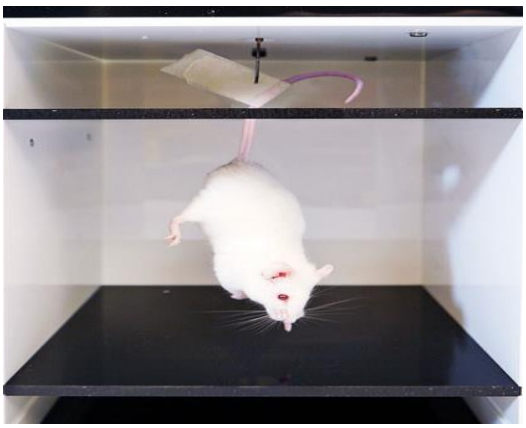


Fig. 1. Illustration of the tail suspension test apparatus designed for use in this study

2.5.1 Histological processing of the frontal and cerebellar cortices

Histological processing and staining of sections of the cerebellum and frontal cortices 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. The tissue sections on the slides are ready for histological, histochemical and immunohistochemical staining procedure dome according procedures described by Bancroft and Gamble, [32].

2.5.2 Haematoxylin and Eosin (Hand E) staining method

The Haematoxylin and Eosin stain (H&E) is the most widely used histological stain to display the general histoarchitecture of the frontal and cerebellar cortices according to Giri [35] and Memudu and Adewumi [36].

2.5.3 Cresyl Fast Violet (CFV) for nissl bodies staining procedure

Cresyl fast violet is a basic dye used as a common stain in histology identify Nissl or chromatophilic bodies in the neuronal structure in brain and spinal cord tissue. The Nissl substance appears deep purple due to the staining of ribosomal RNA, giving the cytoplasm a mottled appearance according to Bancroft and Gamble, [32] method.

2.5.4 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 counter stain was done using Mayer's Haematoxylin and mounting media-DPX (Distrene Plasticizer Xylene). Negative controls were performed by omitting the primary antibody [32,36].

2.6 Statistical Analysis

Data set were analyzed using GraphPad Prism 7 (GraphPad software, Inc., LA Jolla, CA). Student's *t*-tests were used for all paired comparisons and one-way ANOVAs were used for all multiple comparison 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).

2.7 Photomicrography of Histological, Histochemical and Immuno-histochemical Results

Tissue sections were visualized with a Leica digital microscope and digital images were captured using a light microscope digital camera (MV 500 Cameroscope ™) attached to the PC where the images were stored. The images were captured and stored using the joint photographic export group (JPEG) format for analysis.

3. RESULTS

3.1 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 was poor characterized by distorted fur arrangement (dull fur), reddish eyeballs, loss of appetite, weight lost, poor social behaviour/ mood and grooming. These physical characteristics occurred in the first 5 days of aluminum treatment but Neem leaf treatment progressively alters these characteristic features making the animals improve in feeding habit thereby restoring appetite, body weight, reduced red eye and nose bleeding in addition to an improved grooming and social behaviour.

3.2 Body Weight Changes

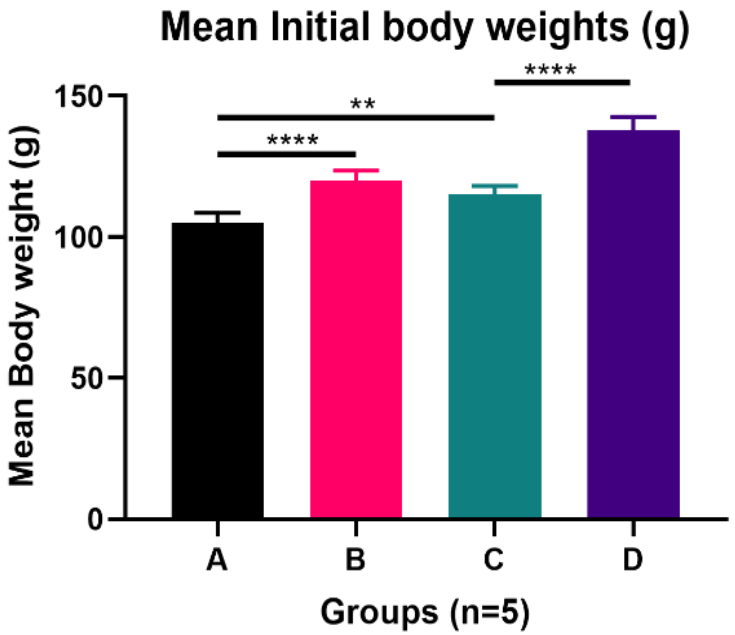
The final weight of the control group increased when compared with aluminum induced AD model at p<0.05 (Fig. 1B). There was no significance difference between the final body weights of the control and the Neem leaf supplement group (C). The neem leaf supplement treated AD model (D) had a significance increase in final body weight when compared with aluminum induced AD model (B). The final weight in the control (A) had a significant decline when compared with the neem leaf supplement treated aluminum induced AD model (D) (**p<0.0059). The final weight of aluminum induced AD model (B) declined as compared with the weight of Neem leaf supplement treated aluminum induced AD model (D) (****p<0.0001). The Neem leaf supplement group (C) had a decline in final body weight as compared with Neem leaf supplement treated and aluminum induced AD model (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 aluminum induced AD model (B) group had a decline in final weight as compared with the initial weight at p<0.05.

3.3 Neem Leaf Supplement Reversed Depressive Like Behaviour Associated with AD neuropathology

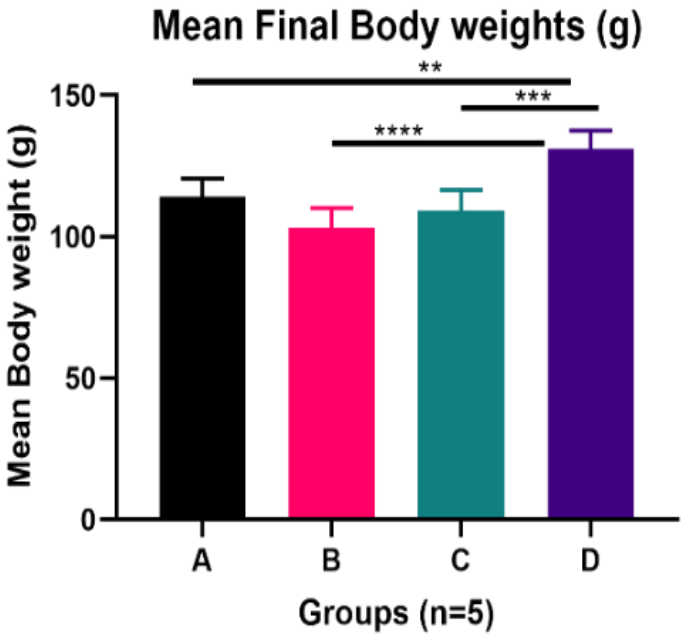
The time spent to get to the reward arm reduced significantly in the control (A) as compared with aluminum induced AD model (B) (**A vs B: p< 0.0018) and Neem leaf supplement treated aluminum induced AD model (D) group (*A vs B:p<0.0486). The time spent to get the reward increased in B significantly as compared with Neem treated (C) and the Neem leaf supplement treated aluminum induced AD model (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 group 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 aluminum induced AD model (B) (*A vs B: p<0.0109); but the aluminum induced AD model (B) had a significance reduction in the number of reward arm entries when compared with Neem leaf treated (C) and the Neem leaf treated aluminum induced AD model (D) groups (**B vs C: p< 0.0029; *B vs D: p<0.0143) as seen in Fig. 2B. However, neem leaf treated group (C) had a significance increase as compared to the Neem leaf treated aluminum induced AD model (D) (**C vs D: p<0.0021). In the tail suspension test for depressive like behaviour in experimental animals used in this study there was no significance difference in the animals for the pretest tail suspension test (Fig 3 A). However, the test session showed that the control (A) group had a reduction in the immobility time as compared with the aluminum induced AD model (B) (***A vs B p<0.0008) as seen in Fig 3B. There was no significant difference between the control (A) and the Neem leaf treated (C) at p<0.05. The control (A) group had a significant decline in immobility time as compared with the Neem leaf treated aluminum induced AD model (D) group (**A vs D p<0.0048). The aluminum induced AD model (B) had a significant increase in immobility time as compared with Neem treated (C) and Neem leaf treated aluminum induced AD model (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 group D. (*C vs D p<0.0282).

3.4 Neem Leaf Reversed Aluminum 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 aluminum induced AD model (B) group characterized by vacuolation in a neuropil with pyknotic and chromatolyzed neurons. Neem leaf treated group (C) had numerous non -necrotic neurons and Nissl positive neurons. The neem leaf treated AD model (group D) demonstrated neuroprotective potential of neem leaf by preventing aluminum induced Nissl bodies chromatolysis and necrosis in frontal lobe cortical neurons. The histomorphology of the cerebellar cortex showed the presence of Nissl positive pyramidal neurons within the dense non-vacuolated neuropil and non-necroticpyramidal neurons in the control (Fig. 5A1 and A2) as compared with the aluminum induced AD model (B) group characterized by vacuolated scanty neuropil with pyknotic and chromatolyzed pyramidal neurons. Neem treated (C) had numerous non -necrotic neurons and Nissl positive neurons. However, neem leaf treated AD model (D) demonstrated its neuroprotective potential by preventing Aluminum induced Nissl bodies chromatolysis and necrosis in fronto-cerebellar cortical neurons.



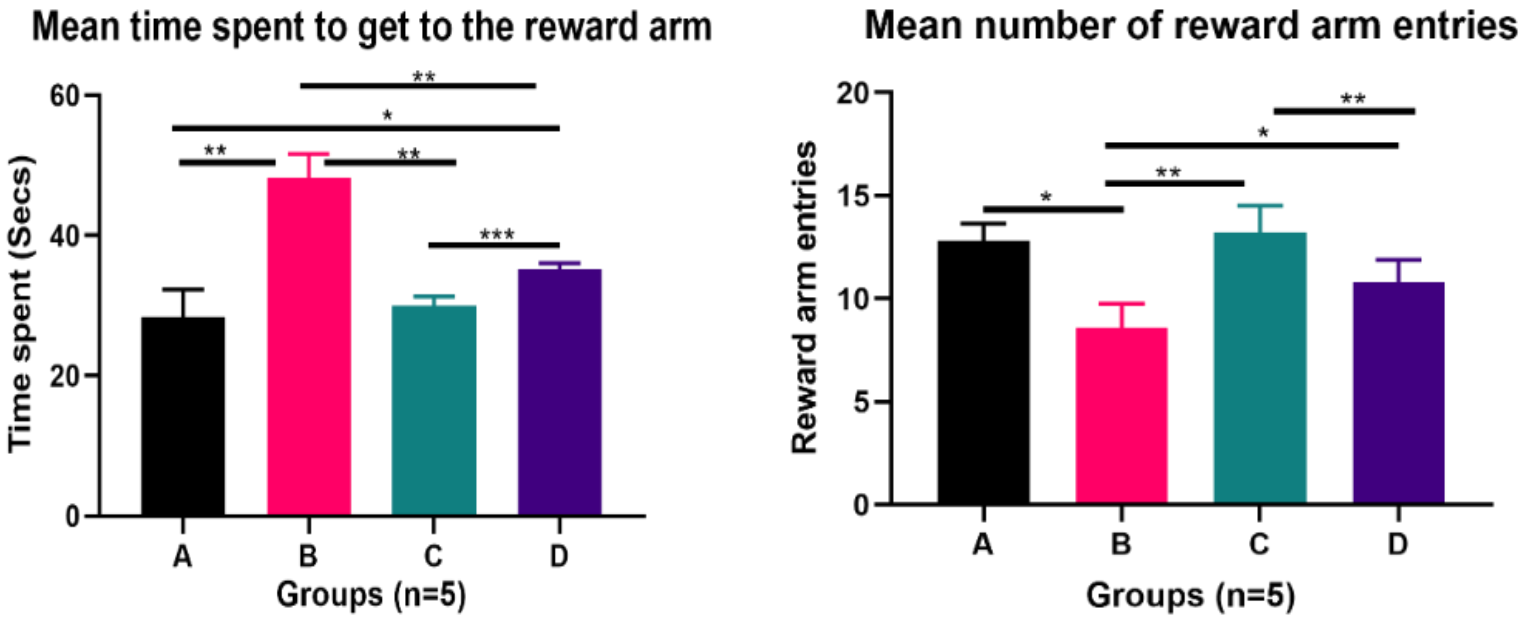
A



B

Fig. 2. Graphical representation of the mean initial and final body weights of the experimental animals used

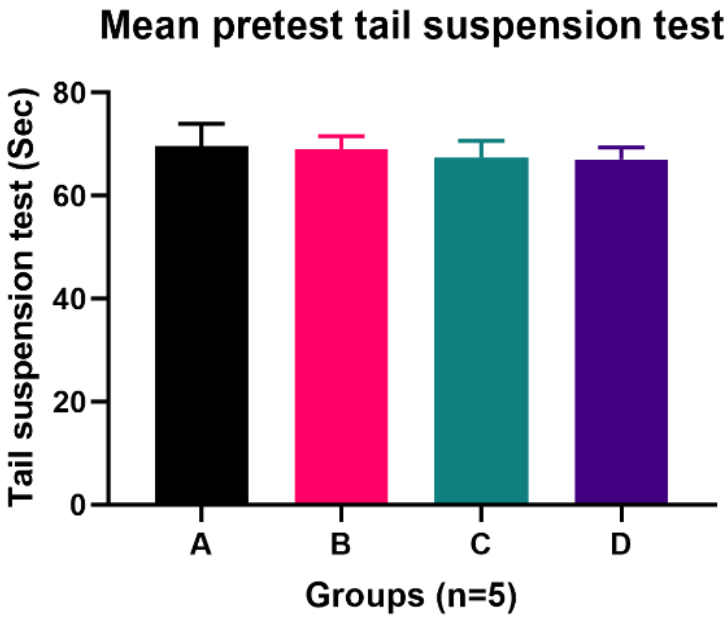
Data analyzed using one-way ANOVA and expressed as Mean ± Standard Deviation (Mean ± SD). Statistical Significance is taken at P<0.05 (*) using Tukey post-hoc test. Legend: A: Control group B: 200mg/kg oral Neem leaf supplement treated group for 10 days C: 200mg/kg of AlCl3 induced animal model for AD and D: AD (neurodegenerative) repair model treated with oral 200mg/kg oral of Neem leaf supplement for 10 days



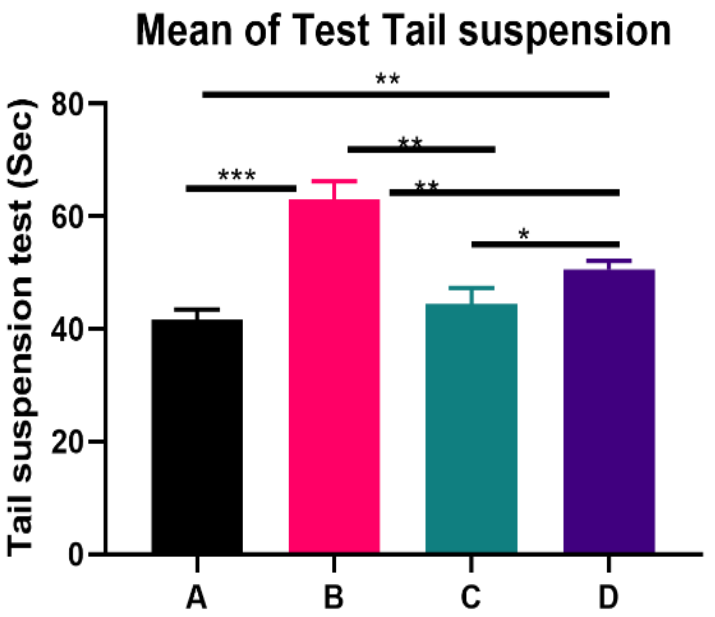
A B

Fig. 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 ± Standard Deviation (Mean ± SD). Statistical Significance is taken at P<0.05 (*) using Tukey Post hoc test. Legend: Legend: A: Control group B: 200mg/kg oral Neem leaf supplement treated group for 10 days C: 200mg/kg of AlCl3 induced animal model for AD and D: AD (neurodegenerative) repair model treated with oral 200mg/kg oral of Neem leaf supplement for 10 days



A



B

Fig. 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 ± Standard Deviation (Mean ± SD). Statistical Significance is taken at P<0.05 (*) using Tukey Post hoc test. Legend: Legend: A: Control group B: 200mg/kg oral Neem leaf supplement treated group for 10 days C: 200mg/kg of AlCl3 induced animal model for AD and D: AD (neurodegenerative) repair model treated with oral 200mg/kg oral of Neem leaf supplement for 10 days

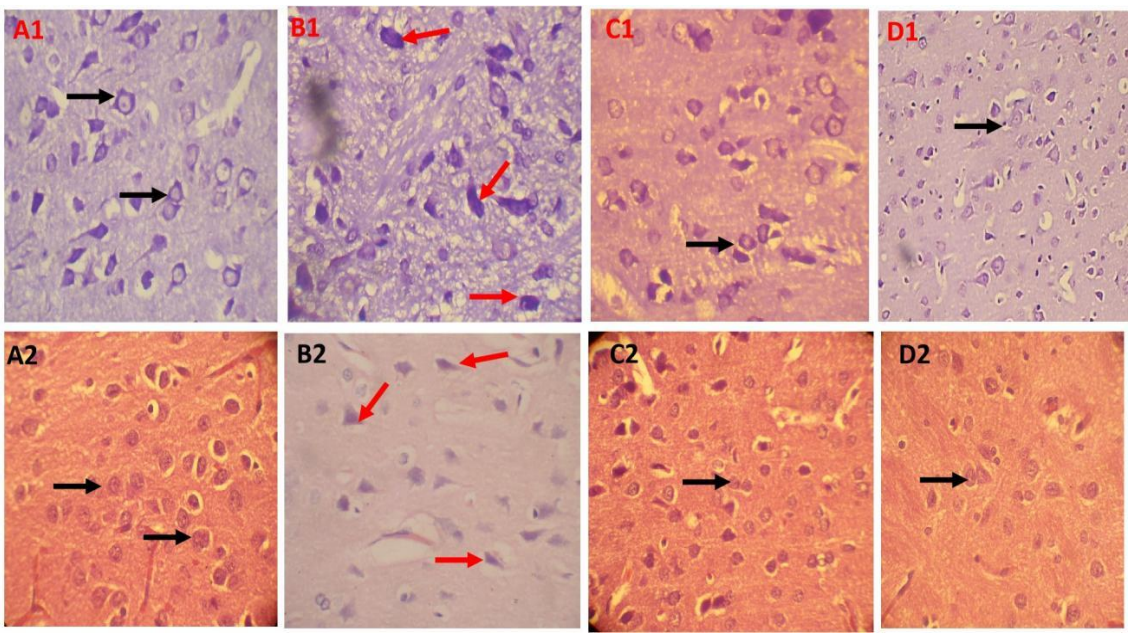


Fig. 5. Photomicrograph of the frontal cortex of adult male Wistar rats stained with CFV stain (A1-D1) and H and E stain (A2-D2) stain

Legend: Legend: A: Control group B: 200mg/kg oral Neem leaf supplement treated group for 10 days C: 200mg/kg of AlCl3 induced animal model for AD and D: AD (neurodegenerative) repair model treated with oral 200mg/kg oral of Neem leaf supplement for 10 days. Mag. X400. Scale bar: 50µm. Red arrows: Necrotic or degenerated neurons with chromatolysis; Black arrows: normal neurons with their centrally located nucleus and neurites extension

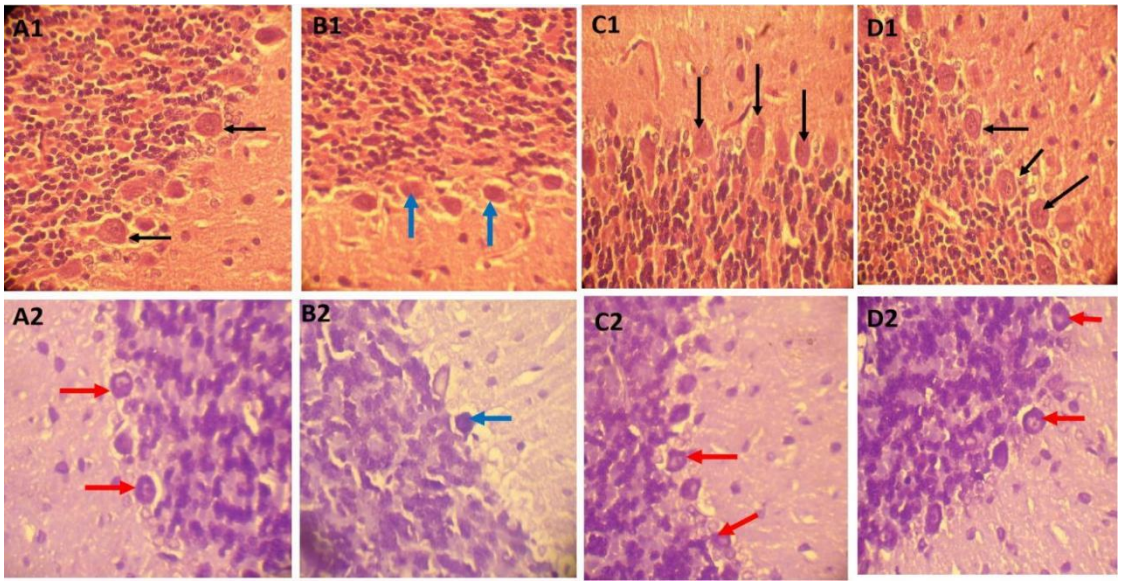


Fig. 6. Photomicrograph of the cerebellar cortex stained with H and E (A1-D1) and CFV (A2-D2) stain of adult male Wistar rats

Legend: A: Control group B: 200mg/kg oral Neem leaf supplement treated group for 10 days C: 200mg/kg of AlCl3 induced animal model for AD and D: AD (neurodegenerative) repair model treated with oral 200mg/kg oral of Neem leaf supplement for 10 days.t. 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

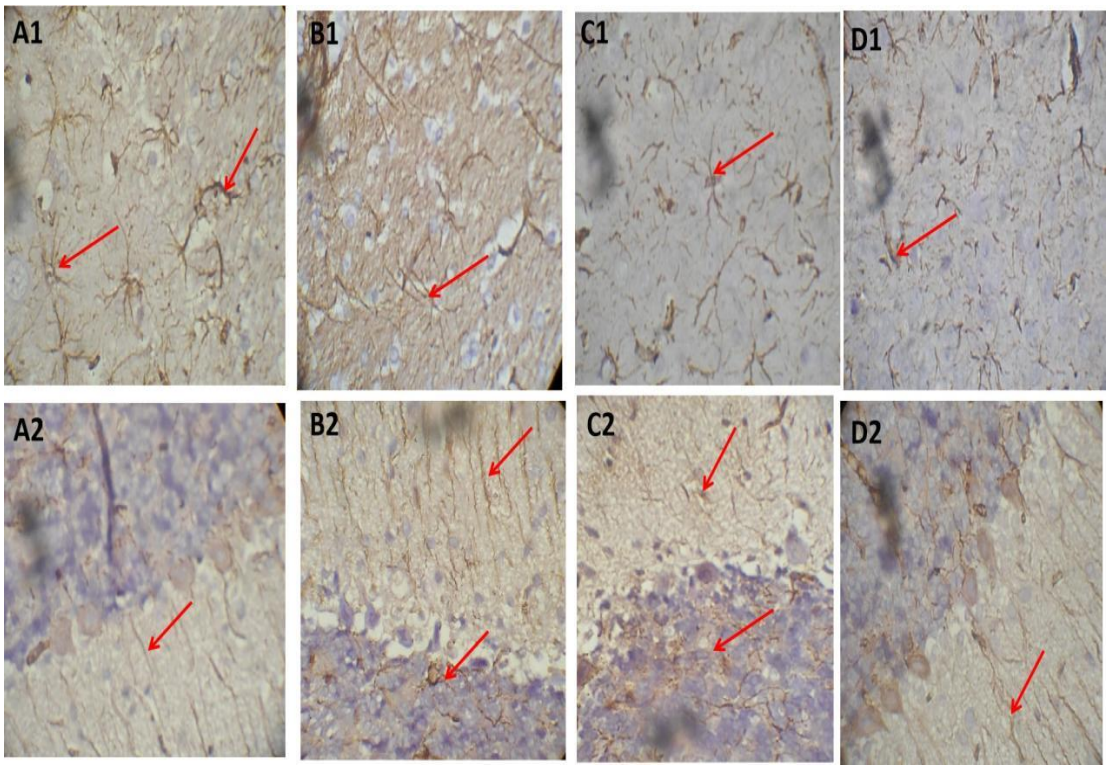


Fig. 7. Photomicrograph of the frontal cortex (A1-D1) and Cerebellar cortex (A2-D2) of adult male Wistar rats of stained with GFAP (glial fibrillary acidic protein)

Legend: A: Control group B: 200mg/kg oral Neem leaf supplement treated group for 10 days C: 200mg/kg of AlCl3 induced animal model for AD and D: AD (neurodegenerative) repair model treated with oral 200mg/kg oral of Neem leaf supplement for 10 days. 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. Leegnd: Red arrow- Astrocytic processes

3.5 Neem Leaf Supplement Attenuates Proliferation of Reactive Astrocytes

Neem leaf supplement attenuates proliferation of reactive astrocytes in aluminum induced AD an indicator for anti-inflammatory role: The FC and CB cortices in AlCl3 induced animal model for AD had numerous reactive astrocytes as compared with the control and neem leaf treated groups (Fig 6 A1 &A2 as well as B1 and B2). Neem leaf treatment mediates reduction in neuro-inflammatory response to aluminum intoxication and AD pathology demonstrated by a decline in the expression of reactive astrocytes similar to the control and neem leaf 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 act as an anti-depressant to ameliorates the neuropathophysiology and neurobehavioural deficits observed 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 lead treated groups. This finding supports Memudu et al., [14], Buraimoh et al., [23] and Buraimoh and Ojo, [37] studies. The neem leaf treated reversed 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 bitterness of the Neem leaf supplement that they progressive adjusted to taking during the period of administration causing an improved feeding and body weight gain.

The neuropathogical changes in the frontal and cerebellar cortices demonstrating interplay between aluminum mediated oxidative neuron damage and neem leaf supplementation. The AD model had a marked necrosis, severe loss of neuron cell integrity, chromatolysis, pericellular spaces and vacuolations within in 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 support reports made by Memudu et al., [14] and Giancarlo et al., [39] a general pathological characterization of aluminum induced AD neuropathology. However, upon administration of neem leaf supplement, this aforementioned pathological features were interrupted by neem leaf polyphenolic and flavonoid compounds such as quercetin, azadirachtin, nimbolinin, nimbin, nimbidin, nimbidol and salannin source of antioxidant [40,41]. Cresy 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 AD 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 the correlation between spatial memory for reward arm and mood behaviour (depressive like behaviour) using the open arm reward Y-maze test [27]. 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 using 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 hence demonstrating 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 this indicates depressive like behaviour. The observed changes correlates with the neuropathological changes in fronto-cerebellar cortex of AD model characterized by loss of neuron integrity and neurite outgrowth required for synaptogenesis, neurite circuit formation and neurocognition function this correlates with reports made by Pan et al., [1] and Sáiz-Vázquez et al.,[22] that neurodegeneration in AD disruption neurons structural and cognitive function that predisposes to depressive like behavior. In this study, both open arm reward Y-maze test and TST validates that AD model can predisposes to depressive like behavior in AD 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 therapeutics 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 plays an important role as a biomarker for neuroinflammation during neurodegenerative and therapeutic study because they are use as a diagnostic indicator 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 like Alzheimer's disease whereby they increase or decline in response to neurotoxins and neurotherapeutics for neuron survival [46]. In this study, AD model had an 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 leaf antioxidant role mediates anti-

inflammatory response conducted by its active compound thereby mobbing off free radicals generated by aluminum neurotoxicity resulting in a moderate expression of astrocytes for neuroprotection, repairs and survival from the remnant of the toxins circulating in the brain milieu. This results in protection of neurons and neurite growth in neem leaf treated AD model which correlates to an improvement in the neurocognitive behaviour for spatial memory and improved mobility time in Y amaze 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 fronto-cerebellar cortex indicated by an increased proliferation of reactive astrocytes a biomarker for neuroinflammatory activities and this supports report made by Kamel et al, [49]. Neem leaf supplementation inhibits progress proliferation of reactive astrocytes demonstrated in the observed mild expression of astrocytes this indicated its anti-inflammatory properties [17].

5. CONCLUSION

There is a correlation between AD and depressive like behaviour. Neem leaf supplement helps to avert aluminum induced neurocognitive impairment and depressive like behaviour by reducing neuroinflammation, improving neuron survival and neural circuits for cognitive function.

CONSENT

It is not applicable.

ETHICAL APPROVAL

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

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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Peer-review history:
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