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Determination of Astrocyte Reaction Using Glial Fibrillary Acidic Protein (GFAP) Following Aluminium Chloride Induced Hippocampus Damage of Adult Wistar Male Rats Treated with Ethanolic Extracts of *Carpolobia lutea* at Different Doses

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Background: Aluminium is a well-established neurotoxicant involved in the etiology of neurodegenerative diseases. *Carpolobia lutea*, commonly called cattle stick or poor man's candle belongs to the plant family polygalaceae. It is a small tree native to West and Central tropical Africa and it is one of the medicinal plants that play a major role in the health care system of developing countries such as Nigeria.

Aim of the Study: This study was aimed at investigating astrocyte reaction in aluminium chloride induced neurotoxicity in the hippocampus in adult male wistar rats treated with ethanolic extracts of *C.lutea* at different doses.

Materials and Methods: Thirty wistar rats weighing 180-200 g were used for this study. The animals were randomized into five groups of six rats each. Group A rats received only animal feed. Groups B,C,D and E were given 100 mg/kg bw of aluminium chloride intraperitoneally five times a week for three weeks to induce neurotoxicity.In order to reverse the neurotoxicity,. Group C was treated with 10 mg/kg bw of donepezil as standard drug while group D and E were treated with 200 mg/kg and 400 mg/kg of *Carpolobia lutea* respectively for 14 days. Histopathological study was done on the hippocampus of the rat brain and thereafter hematoxylin and eosin staining were carried out. Immunohistochemical studies for GFAP was done using Novocasra™ Novolink ™polymer detection system and appropriate primary monoclonal antibodies. Image J cell counter tool was used to note the number of GFAP positive cells across all groups.

Results: The result from our histopatholological study shows that lower dose (200 mg/kg/bw) of ethanolic extract of the leaf of *Carpolobia lutea* have better protection on the cytoarchitecture of the CA_1, CA_3, and dentate gyrus of the hippocampus than 400 mg/kg/bw of the plant extract. From our study, it is evident that 200 mg/kg of *Carpolobia lutea* reduced the GFAP immunoreactivity in the hippocampus of $AlCl_3$ induced neurotoxicity. When we compared the result of 200 mg/kg/bw of *C. lutea* with 10 mg/kg per body weight of donepezil (standard drug), it was observed that *C. lutea* had a slight reduced GFAP immunoreactivity than the standard drug(donepezil). The lower dose, 200 mg/kg of ethanolic extract of the leaf of *Carpolobia lutea* have a better neuroprotective benefit than the standard drug on the hippocampus

Conclusion: In conlusion, 200 mg/kg of *Carpolobia lutea* have the tendencies of protecting the neurons in the hippocampus from degenerating as a result of aluminium chloride induced neurotoxicity compared to the standard drug (10 mg/kg donepezil).

Keywords: Glial fibrillary acidic protein; hippocampal damage; Wistar male rats; ethanolic extracts; Carpolobia lutea.

1. INTRODUCTION

1.1 Background of the Study

"Medicinal plants play a major role in the health care system of developing countries such as Nigeria. Despite their high usage, most of these plants lack information on, active chemical constituent, quality, clinical studies, safety and efficacy of which *Carpolobia lutea* is one of them" [1].

"Carpolobia lutea, commonly called cattle stick or poor man's candle belongs to the plant family polygalaceae" [2]. "It is a small tree native to West and Central tropical Africa. It is common in rainforest and Guinea savannah of Sierra Leone and Cameroon. This shrub reaches up to 5 m in height and occurs as a dense overgrowth or an evergreen shrub or small tree. The common names, which the plant is known include cattle stick (English), Abekpok Ibuhu (Eket), Ikpafum, Ndiyan, Nyayanga (Ibibio), Agba or Angalagala (Igbo) and Egbo oshunshun (Yoruba)" [3].

"Herbalists in Nigerian tribes use the essence of the root as an aphrodisiac and the treatment of genitourinary infections, gingivitis, and waist pains. The tree extract could pave the way for new drugs to tackle patient symptoms but without the unwanted side effects associated with some current treatments" [4].

"Neurodegenerative diseases represent a huge health burden globally, placing pressure on health services and having a negative impact on the lives of patients and their families" [5].

"Researchers and drug companies are racing to discover new treatments for these disorders and

have begun looking to plant extracts as a potential source of novel drugs. In patients with Alzheimer's disease and other diseases such as Parkinson's disease and myasthenia gravis, the activity of the neurotransmitter acetylcholine, is increased, leading to problems with memory and attention" [6].

"Current drugs — called acetylcholinesterase inhibitors — reduce the normal breakdown of acetylcholine. Extensive research is underway to find new versions of these drugs but with additional beneficial properties" [7].

"According to a study published in Pharmacognosy Review, It is an accepted and commonly utilized herbal booster of libido. It is used to cure male infertility and to boosts libido thereby augmenting male sexual functions or it is used to induce penile erection, and enhance male virility [8]. The chewing stick prepared from the stem and root of *Carpolobia lutea* is patronized because it boosts male sexual performance" [9].

"The leaf essential oil contains a variety of terpenoids, while polyphenols and triterpenoid saponins have been isolated from the root and leaf extracts respectively. Other ethnomedicinal uses include curing of stomach ailments, rheumatism, fever, pains, insanity, dermal infection, venereal diseases; to promote childbirth; and as a taeniafuge and vermifuge" [10,11].

"It has also been reported to possess other anti-inflammatory, anti-arthritic, antimicrobial, antimalarial, and analgesic properties. This could be particularly important in Alzheimer's disease as there is more evidence emerging that Alzheimer's patients have inflammation in the brain" [12].

"Aluminium is a well-established neurotoxicant involved in the etiology of neurodegenerative diseases" [13]. "It is an abundant metal on earth with easy access to the human body through agro chemicals, water, food additives, utensils, deodorants and drugs. In the brain, aluminum predominantly accumulates in the hippocampus and frontal cortex, regions known to be particularly susceptible in Alzheimer's disease" [14,15]. It induces misfolding of cytoskeleton proteins which leads to the formation of amyloid beta plaques and tau neurofibrillary tangles [16] in the brain. "Aluminium supplementation causes neurodegeneration and apoptotic neuronal loss along with cognitive dysfunction, as it is a potent cholinotoxin. Normally, cholinergic activity is necessary for the acquisition and retrieval of learning and memory skills" [17]. "Hence patients with AD normally demonstrate impaired performance in various cognitive tasks. Various animal studies have also shown that prolonged exposure to aluminium can cause neurochemical, neurobehavioral and neuropathological changes in the brain, which impair the learning ability of the rats" [18,19].

1.2 Aim of the Study

This study was aimed at investigating astrocyte reaction in aluminium chloride induced neurotoxicity in the hippocampus in adult male wistar rats treated with ethanolic extracts of *C. lutea* at different doses.

2. MATERIALS AND METHODS

2.1 Extraction Procedures

The leaves of *Carpolobia lutea* were removed from their stalks. The leaves were oven dried for 72 hours and pulverized. Dried sample was ground into powder mechanically using manual grinder.

Thereafter, 400 g of the macerated plant powder was soaked in 1000ml of absolute alcohol and kept in a container for 48 hours. The mixture was shaken vigorously at intervals for another 2 hours, to allow complete extraction.

The resulting mixture was rapidly filtered through whatmann filter paper to obtain a homogenous filtrate.

This filtrate was concentrated in a vacuum at low temperature (37-40^{0}C). The extract was later reconstituted in distilled water at a concentration of 1g/ml before administration. The extract was refrigerated until use.

2.2 Standard Drug

A standard drug known for its therapeutic management of neurotoxicity in the hippocampus, namely donepezil was used to ascertain and compare with the potency of ethanolic extract of C*arpolobia lutea* against aluminium chloride induced neurotoxicity. A tablet of this drug was dissolved in 20ml of distilled water and mixed thoroughly.

2.3 Recruitment of the Study Population

Thirty Wistar rats weighing between 180-200 g were used for this study. The animals were obtained from the animal house of the Department of Biological Sciences of Rivers State University. They were housed in standard cages and left to acclimatize for 14 days under natural conditions in the animal house of the Department of Human Anatomy of Rivers State University before the commencement of the experiment. The animals were fed with vitakraft complete nutrition rat feed and water ad libitum.

2.4 Study Design

The animals were randomized into five groups (A, B, C, D, & E) of six rats each. Rats in group A received only animal feed and water. All rats in groups B, C, D, & E were injected with aluminium chloride ($AlCl_3$) at 100mg/kg bw intraperitoneally five times a week for three weeks [20].

The ethanolic extract of *Carpolobia lutea* and donepezil were administered orally by gavage.

Group B received 100mg/kg bw of aluminium chloride [20].
Group C received 10mg/kg bw of donepezil [21].
Group D received 200mg/kg per body weight of ethanolic extract of *Carpolobia lutea*.
Group E received 400mg/kg per body weight of ethanolic extract of *Carpolobia lutea*.

The administration of *Carpolobia lutea* and donepezil lasted for 14 days. On the 12^{th},13^{th} and 14^{th} day, the rats were subjected to neuro-behavioural tests after which they were sacrificed by cervical dislocation on the 15^{th} day.

2.5 Surgical Procedures

At the end of behavioural studies, animals were euthanized by cervical dislocation. Blood was quickly obtained by cardiac puncture and processed for antioxidant studies. Also, their brains were rapidly excised. Whole brain tissues of two animals per group were fixed in 10% neutral buffer formalin. All tissues were processed for rapid routine paraffin wax embedding for histopathological and immunohistochemical studies.

2.6 Histopathological Studies

After sacrifice, the brains of two rats from each group were fixed whole in 10% formal saline. After 48hours, hippocampus was excised from each rat brain for histological assessment using H&E.

The fixed tissues were dehydrated to remove water which is not miscible with paraffin (the embedding medium). The tissues were placed in ascending grades of alcohol (70%, 80%, 90%, and absolute). This was done to prevent water from rushing out of the tissue, which may distort and damage the cell structure. The time for each grade of alcohol was one hour and in absolute (100%) it was changed twice, one hour each time. After dehydration **t**he tissues were cleared in 2 changes of xylene for 30 mins each to remove alcohol which removed water from tissue. This was done because paraffin used for impregnation and embedding is not miscible with alcohol.

The tissues were then passed through four changes of molten paraffin wax at constant temperatures of 46-68ºC in an oven of paraffin bath. This was to prepare the tissue for the embedding medium and to confirm firmness to it. This will facilitate the process of sectioning. Metal blocks were taken and filed with paraffin wax and tissues were placed in it immediately with forceps, the face to be cut facing downward. When the paraffin cools, a thin scum of solid paraffin is formed on the bottom of the block which was now immersed in water to solidify and then removed for sectioning.

The solid paraffin blocks were then taken to the rotary microtome where excess paraffin wax was first trimmed off and the tissues cut at 5µm. Paraffin sections were taken to a water bath set at 45^0C to straighten it. The side of the glass slide to receive the section was made sticky by rubbing with egg albumin. The paraffin sections were allowed to float in the water bath to straighten out the wrinkles. Water was drained off and the slide was put in an incubator for the sections to be completely fixed on the slide and allow to dry.

2.7 Staining: Hematoxylin and Eosin Staining Technique

The procedure for H&E staining as described by (41, 42) was adopted. The sections were dewaxed in xylene, two changes of xylene for 2 minutes each and afterwards rehydrated in descending grades of alcohol, 100%, 95%, 90%, 70%, 50% ethanol for 2 minutes each.

Tissues sections were then rinsed in distilled water and stained in haematoxylin for 10-15minutes. Afterwards sections were rinsed in distilled water for 2-3 minutes and examined to confirm sufficient degree of staining. Excess stain was removed or differentiated in 1% HCL acid alcohol for a second or two as the acid breaks the mordant dye linkage.

The sections are again rinsed in distilled water for 2-3 minutes to regain the blue colour. Sections were then stained in 1% aqueous eosin for about 3-5 minutes. Surplus stain was washed off in distilled water.

Stained sections were then mounted in distrene plasticizer xylene (DPX) using clean glass cover slide and placed under the microscope for examination. Photomicrographs were taken using the photographic light microscope (National optical).

2.8 Immunohistochemical Studies

Immunohistochemical studies for GFAP was carried out using Novocastra ™ Novolink™ polymer detection system and appropriate primary monoclonal antibodies.

2.8.1 Immunohistochemistry using ImmPRESS™ HRP polymer system

The non-biotin, enzymatic, one-step detection kit, ImmPRESS™ Polymerized Reporter Enzyme Staining System (Vector® Labs, USA), provides very high sensitivity staining with very minimal background inteference in immunohistochemical applications. The ImmPRESS™ Reagent uses an innovative, exclusive approach to conjugate horseradish peroxidase (HRP) micropolymers to affinity-purified, extensively cross-adsorbed secondary antibodies.

2.8.2 Reagents supplied (#MP-7401; Vector® Labs, USA)

- ImmPRESS™ (Peroxidase) Polymer Anti-Rabbit IgG Reagent (made in horse, ready-to-use).
- 2.5% Normal Animal (Horse) Serum for blocking (ready- to-use).

2.8.3 ImmPRESS™Immunohistochemical detection

The ImmPRESS™ Reagent is ready-to-use. It requires no mixing or tittering of the ImmPRESS™ reagent to obtain optimal immunohistochemical staining. The staining procedure is performed at room temperature. For optimal performance, the ImmPRESS™ Reagent is equilibrated to room temperature before use. Phosphate buffered saline (PBS) was used as wash buffer.

2.9 Staining Protocol

The protocol was performed as previously described (Erukainure et al., 2019); [22].

- Paraffin embedded sections where deparaffinized with xylene, and rehydrated through descending grades of ethanol (100%, 95%, 70 % ethanol) and taken to water.
- Heat-mediated antigen retrieval was performed using a citrate-based antigen unmasking solution, pH 6.0 (Vector®, Burlingame, CA, USA; #H3300) in a steamer for 30 mins.
- Wash sections in PBS for 2 mins
- Endogenous peroxidase blocking in 0.3 % hydrogen peroxide solution in PBS for 10 mins.
- Wash sections in PBS for 2 mins
- Sections were incubated in animal free blocker for 20 mins for protein blocking.
- Sections were then incubated at room temperature for 2 hours in primary rat antibodies: GFAP (ThermoFisher, USA; #16825-1-AP), Sections were incubated in ImmPRESS™ HRP Anti-Rabbit IgG (Peroxidase) Polymer Reagent, made in horse for 30 mins
- Wash in PBS for 5 mins x 2
- Colour is developed with DAB Peroxidase (HRP) Substrate Kit (Vector® Labs, USA)
- Sections were rinsed well in tap water
- Sections were counter-stained in hematoxylin
- Sections dehydrated through ascending grades of ethanol (70%, 95%, 100%), cleared in Xylene and mounted with Permount (Fischer Scientific, USA).

Sections without primary antibodies were similarly processed to control for immunohistochemistry procedures. No specific immunoreactivity was detected in control sections.

2.10 Photomicrography and Image Analysis

Stained sections were viewed under a Leica DM750 Digital Light microscope and digital

photomicrographs of slides were taken by an attached Leica ICC50 camera. Image Analysis and Processing for Java (Image J), a public domain software sponsored by the National Institute of Health (USA), was used to analyze and quantify photomicrographs. Image J cell counter tool was used to identify and quantify number of intact and degenerating neurons in H&E stained sections. Also the Image J cell counter tool was used to note the number of GFAP positive cells.

2.11 Statistical Analysis

All other data obtained were analysed using one way ANOVA followed by student newman-keuls (SNK) for post test. All behavioural studies were analysed using two-way repeated measures (2-RM) ANOVA, with time as the repeated measures variable.

3. RESULTS

3.1 Haematoxylin and Oesin (H & E) Staining Results

The histological studies were carried out using the Haematoxylin and Oesin (H & E) method. A part of the brain was studied, the hippocampus (CA1, CA3 and DG). The histology of the hippocampus, dentate gyrus and their components are presented in Figs. 1, 2 and 3.

The control group, show typical histological features of the hippocampal CA1 and CA3. There are three distinct layers: stratum radiatum, pyramidalis and oriens. The pyramidal layers are composed of large pyramidal neurons with large round nucleus and prominent nucleoli with glial cells interspaced within the neurons as can be seen in Figs. 1 and 2.

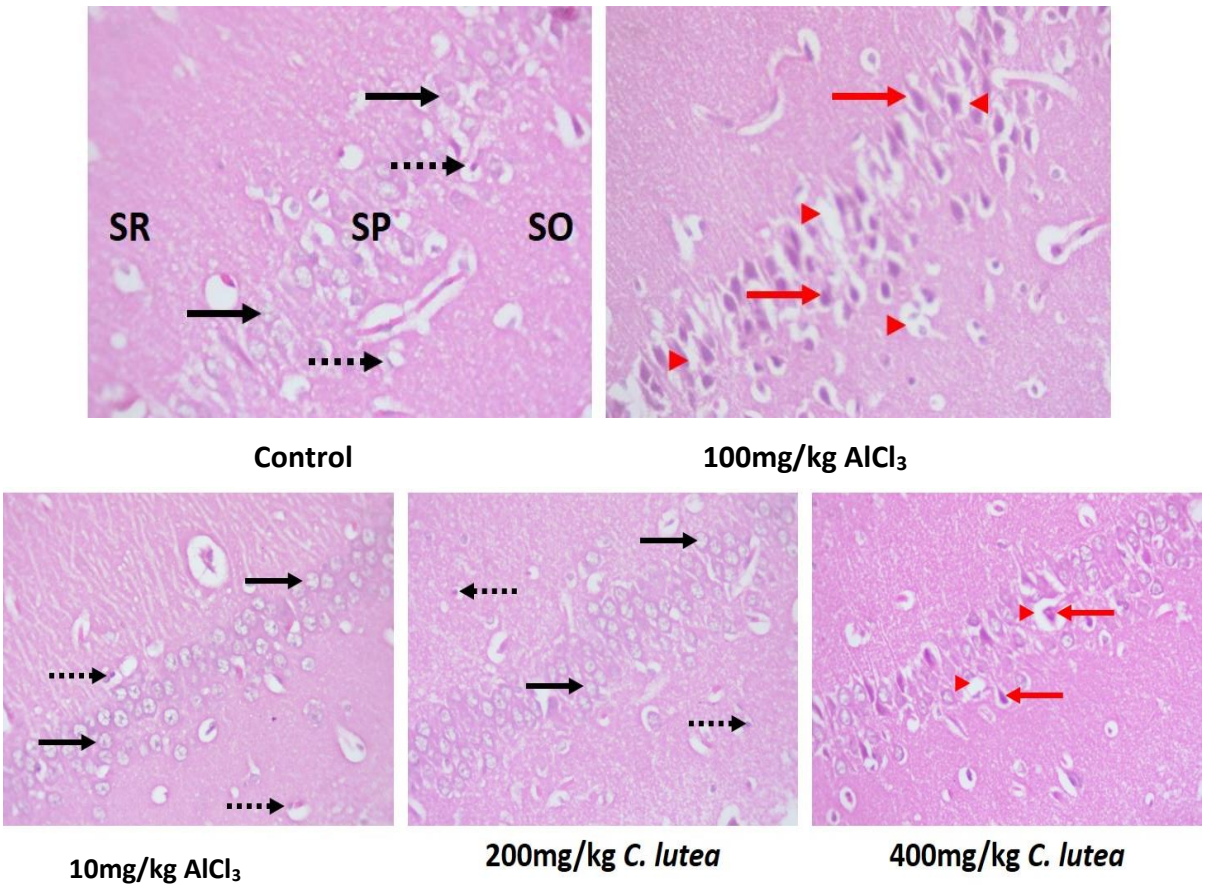

Fig. 1. Histological changes in the hippocampal CA1 of experimental groups

H&E 400x magnification. SR – stratum radiatum; SP – stratum pyramidalis; SO – stratum oriens; black arrows – intact pyramidal neurons; broken/dotted arrows – glial cells; red arrows – pyknotic neurons; red arrow heads – perinuclear spaces

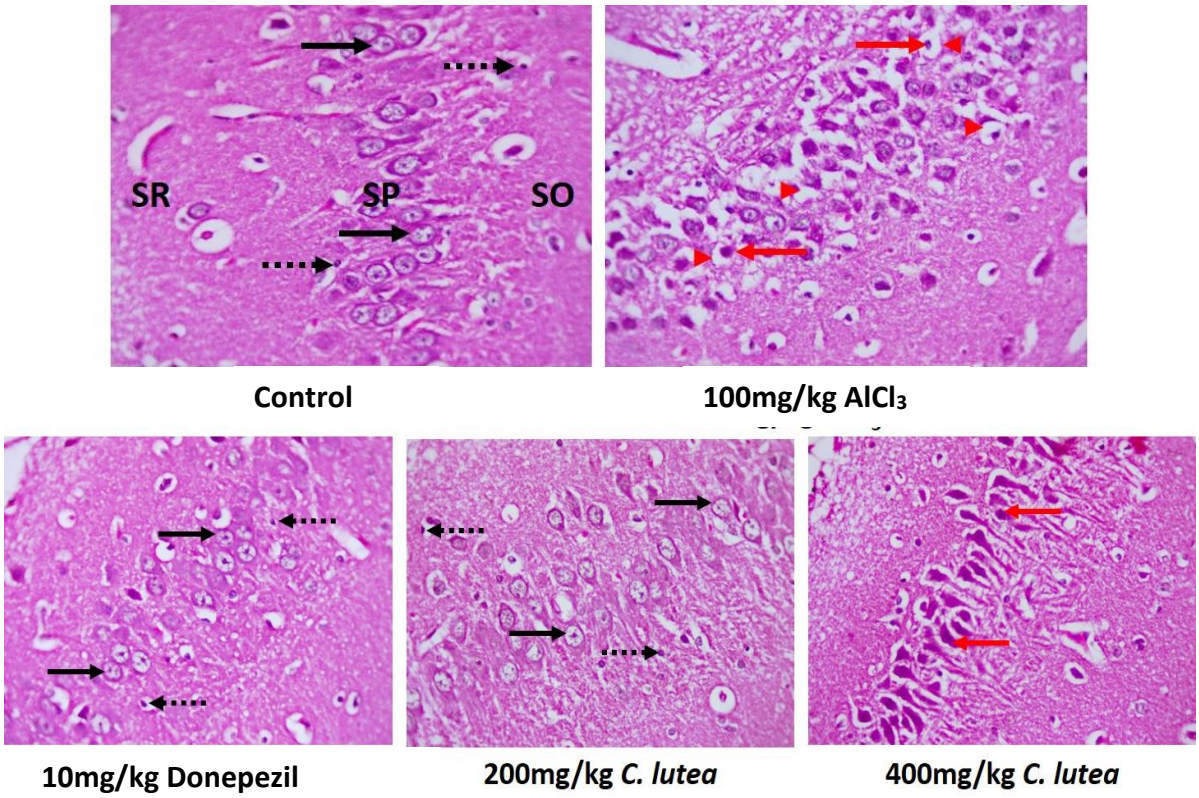

Fig. 2. Histological changes in the hippocampal CA3 of experimental groups

H&E 400x magnification. SR – stratum radiatum; SP – stratum pyramidalis; SO – stratum oriens; black arrows – intact pyramidal neurons; dotted/broken arrows – glial cells; red arrows – pyknotic neurons; red arrow heads – perinuclear spaces

The negative control group and the treated groups were observed for features that characterize degenerating process in neurons, as can be seen in light microscopy study of H & E stained sections [23,24]. These features include;

1. Prominent eosinophilic cytoplasm with or without shrunken nuclei
2. Pyknotic nuclei and
3. Neuron swelling and/ or vacuolation within the cytoplasm

The 100 mg /kg of $AlCl_3$ group showed degenerative features characterized by pyknotic cells with deeply stained nuclei. There were obvious perinuclear spaces and vacuoles formed from pyknotic neurons and lost neurons respectively as can be seen in Figs. 1 and 2. The aforementioned degenerative features were also present, albeit mildly in the 400 mg/kg of *Capolobia lutea* group when compared to control as seen in Figs. 1 and 2. Conversely, the 10 mg/kg of donepezil and 200 mg/kg of *C. lutea* showed no tissue alteration when compared to control.

Dentate Gyrus as seen in Fig. 3, the control group exhibits typical histological charactersistics; polymorphic, granular and molecular layers. The granular layer is made up of many neurons with small spherical nuclei and glial cells scattered among them.

The 100 mg/kg of $AlCl_3$ group exhibit degenerative characteristic such as pyknotic cells with darkly coloured nuclei. Perinuclear gaps and vacuoles are clearly visible, generated by pyknotic neurons and lost neurons, respectively. The aforementioned degenerative characteristics are present, however weakly in the 400 mg/kg C. lutea group just as it is in CA1 and CA3.

When compared to the control group, the 10 mg/kg of donepezil and 200 mg/kg C. lutea groups exhibit no tissue modification as can be seen in Fig. 3.

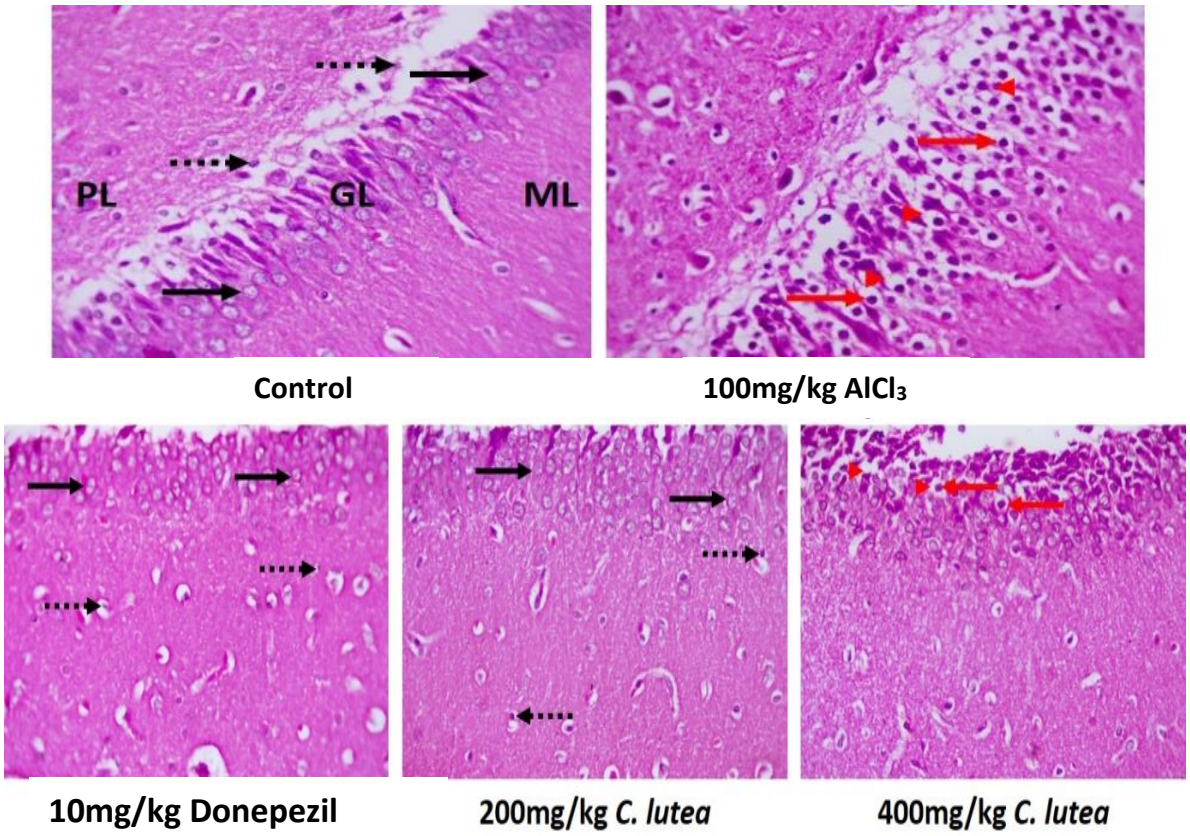

Fig. 3. Histological changes in the hippocampal DG of experimental groups

H&E x400 magnification. PL – polymorphic layer; GL -granular layer; ML – molecular layer; black arrows – intact granular neurons; dotted/broken arrows – glial cells; red arrows – pyknotic neurons; red arrow heads – perinuclear spaces

We can therefore infer that the lower dose of ethanolic extract of the leaf of *Carpolobia lutea* gave better protection on the cytoarchitecture of the hippocampus.

3.2 Results of Immunohistochemistry Studies

Using image J cell counter tool, Immunohistochemical analyses with one way ANOVA was used for this study.

3.2.1 GFAP – CA1

Immunohistochemical analysis with one-way ANOVA demonstrated significant changes in the number of GFAP-positive cells [F (4, 47) = 8.249, p< 0.0001] in the hippocampal CA1 region following $AlCl_3$ exposure in rats. Post-hoc analysis using Tukey's test revealed that rats subjected to 100mg/kg $AlCl_3$(p <0.0001) and 400 mg/kg *C. lutea*, (p < 0.01) had considerably more GFAP-positive cells in hippocampal CA1 than in the control. In contrast, when compared to 100 mg/kg $AlCl_3$-exposed rats, the number of GFAP-expressing cells in the hippocampus CA1 of 10 mg/kg donepezil- (p <0.01) and 200 mg/kg *C. lutea*-treated (p <0.0001) rats was considerably lower. Further, there was an observable, but not significant reduction in the number of hippocampal CA1 GFAP-positive cells in the 200 mg/kg *C. lutea*-treated group compared to 10 mg/kg donepezil. Similar observation was made between 400 mg/kg *C. lutea*-treated group and 100 mg/kg $AlCl_3$-exposed rats.

3.2.2 GFAP – CA3

Immunohistochemical analysis with one-way ANOVA demonstrated significant changes in the number of GFAP-positive cells [F (4, 45) = 9.037, p< 0.0001] in the hippocampal CA3 region following $AlCl_3$ exposure in rats. Post-hoc analysis with Tukey's test indicated that there was significantly more number of GFAP-positive cells in the hippocampal CA3 of rats exposed to

100 mg/kg $AlCl_3$ (p< 0.001) and 400 mg/kg *C. lutea* (p< 0.01) compared to the control. Conversely, in comparison to 100 mg/kg $AlCl_3$-exposed rats, there was significantly lower number of GFAP-expressing cells in the hippocampal CA3 of 10 mg/kg donepezil- (p< 0.05) and 200 mg/kg *C. lutea*-treated (p< 0.001) rats. Furthermore, there was an observable, albeit insignificant reduction in the number of hippocampal CA3 GFAP-positive cells in the 200 mg/kg *C. lutea*-treated group compared to 10 mg/kg donepezil. Similar observation was made between 400 mg/kg *C. lutea*-treated group and 100 mg/kg $AlCl_3$-exposed rats (Fig. 6).

3.2.3 GFAP – DG

Immunohistochemical analysis with one-way ANOVA demonstrated significant changes in the number of GFAP-positive cells [F (4, 30) = 11.33, p< 0.0001] in the hippocampal DG region following $AlCl_3$ exposure in rats. Post-hocTukey test revealed that rats subjected to 100mg/kg $AlCl_3$ (p <0.0001) and 400 mg/kg *C. lutea* (p < 0.05) had considerably more GFAP-positive cells in the hippocampus DG than in controls. In contrast to 100mg/kg AlCl3-exposed rats, 10 mg/kg donezepil- (p <0.001) and 200 mg/kg *C. lutea*-treated (p <0.0001) rats had a considerably lower number of GFAP-expressing cells in the hippocampal DG. Furthermore, there was an observable, albeit insignificant reduction in the number of GFAP-positive cells in the hippocampal DG of 200 mg/kg *C. lutea*-treated group compared to 10 mg/kg donepezil. Similar observation was made between 400 mg/kg *C. lutea*-treated group and 100 mg/kg $AlCl_3$-exposed rats (Fig. 8).

4. DISCUSSION

Concerns continually exist globally on neurodegenerative diseases which sometimes could be traced to dangerous environmental contaminants like aluminium; its compounds and products [25]. Aluminum is a well established neurotoxicant involved in the etiology of neurodegenerative diseases such as dialysis associated encephalopathy, Alzheimer's disease, Parkinson's disease, amyotropic lateral sclerosis, multiple sclerosis and autism [26-28].

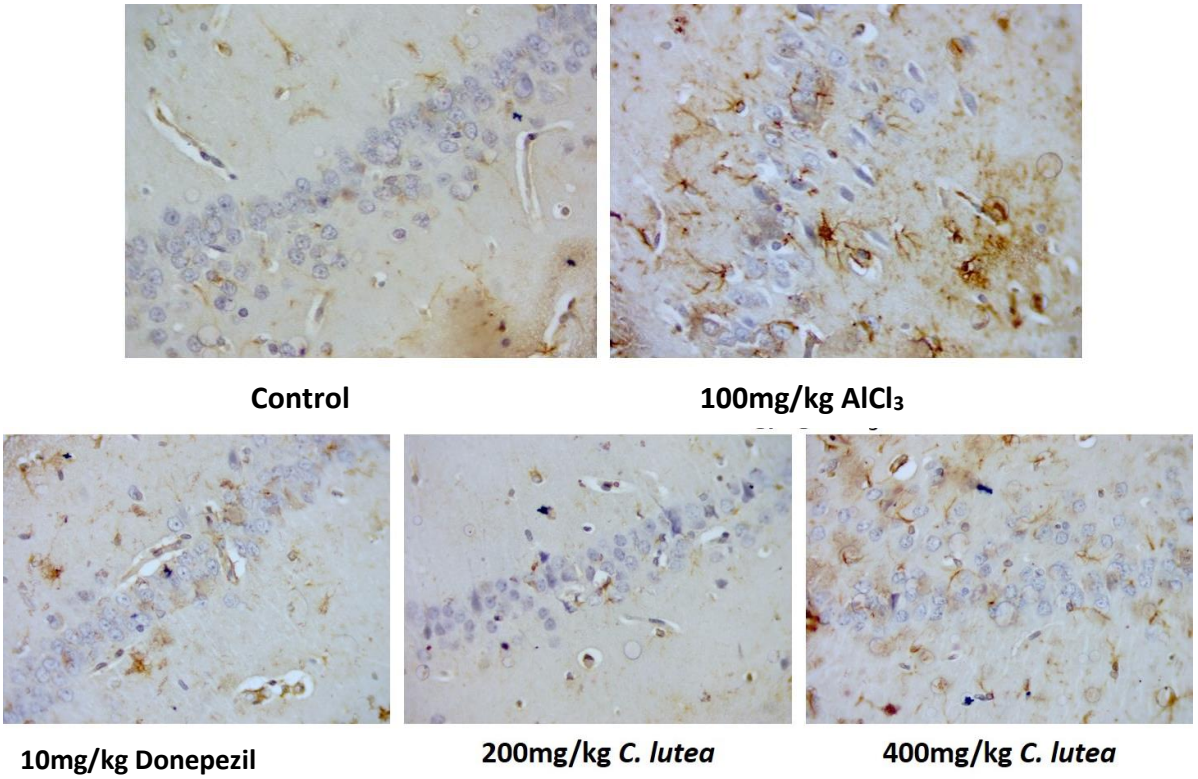

Fig. 4. Immunohistochemical demonstration of GFAP in the hippocampal CA1 of rats exposed to experimental drugs. 400x magnification. Brown-stained cells are GFAP-expressing astrocytic cells

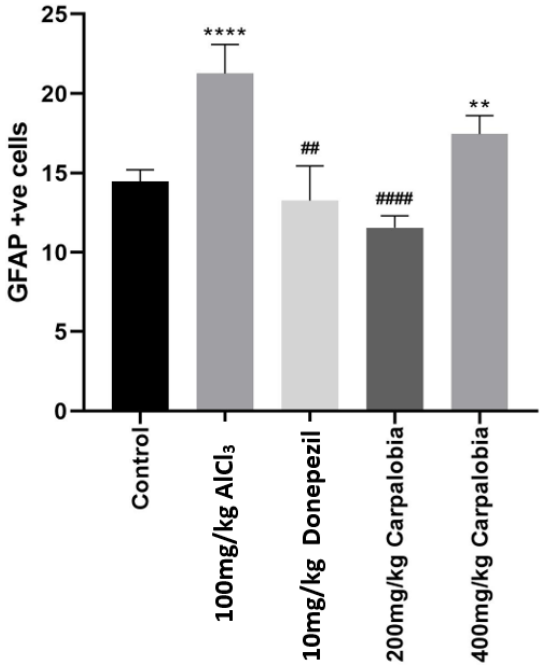

Fig. 5. Bar graphs depict the number of cells positive for GFAP in experimental rats. Each column represents mean ± S.E.M. Data were analysed using one-way ANOVA followed by Tukey's post-test. ****p < .0001, **p < .01 versus Control; ####p < .0001, ##p < .01 versus 100 mg/kg $AlCl_3$

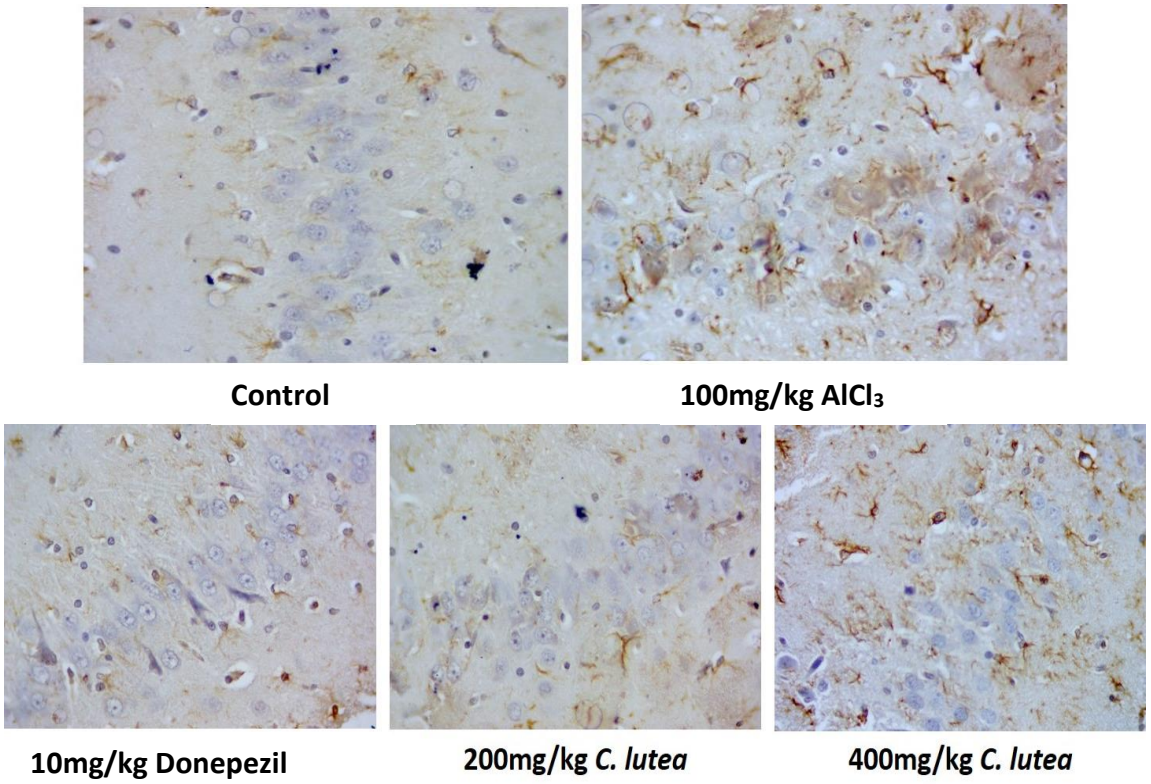

Fig. 6. Immunohistochemical demonstration of GFAP in the hippocampal CA3 of rats exposed to experimental drugs. 400x magnification. Brown-stained cells are GFAP-expressing astrocytic cells

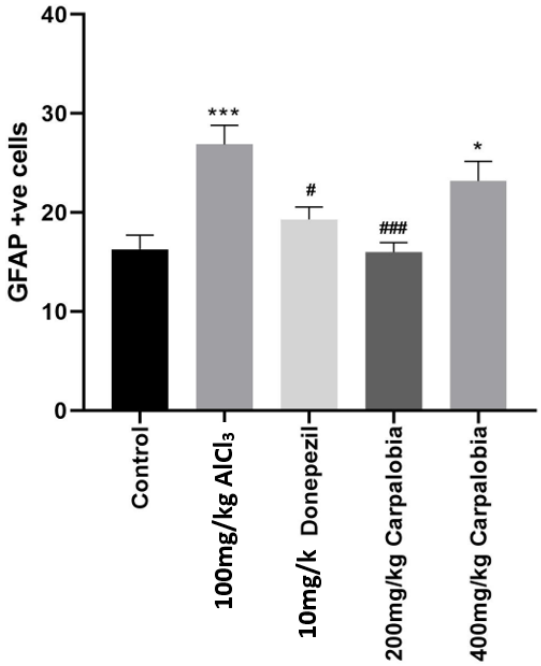

Fig. 7. Bar graphs depict the number of cells positive for GFAP in experimental rats. Each column represents mean ± S.E.M. Data were analysed using one-way ANOVA followed by Tukey's post-test. ***p < .001, *p < .05 versus Control; ###p < .001, #p < .05 versus 100mg/kg $AlCl_3$

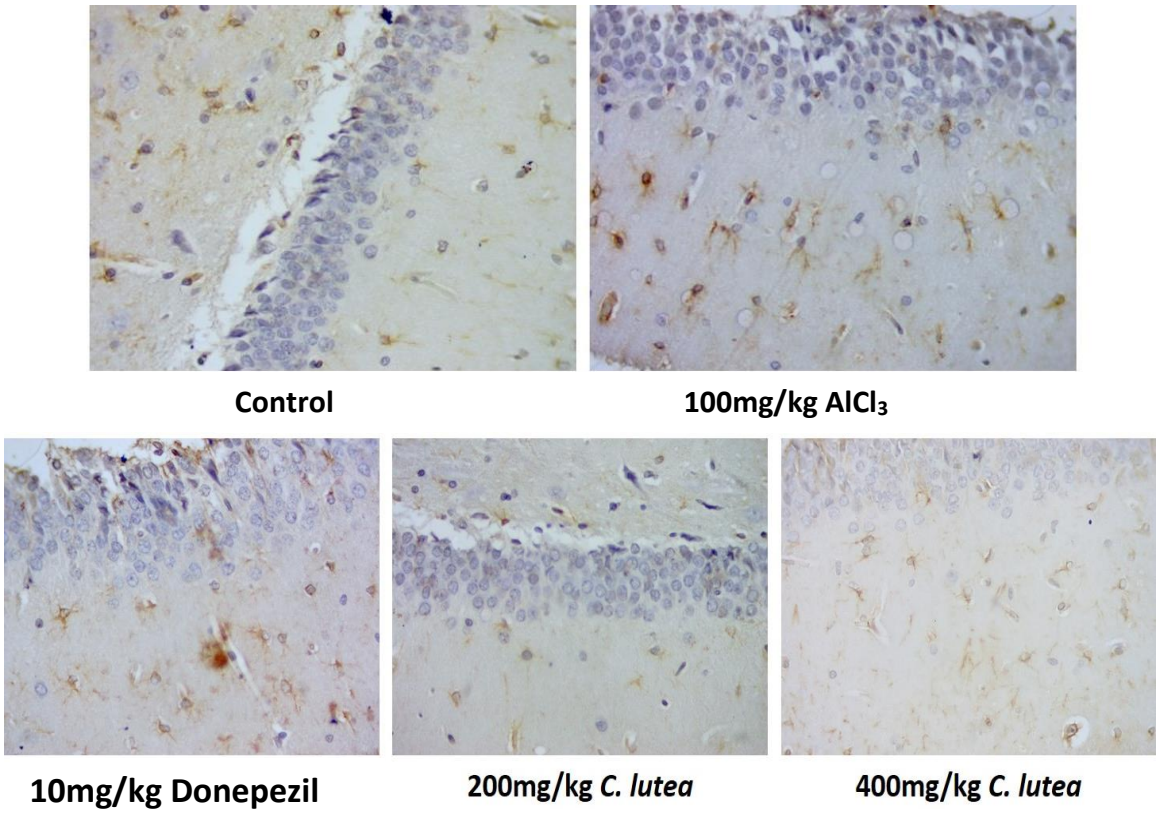

Fig. 8. Immunohistochemical demonstration of GFAP in the hippocampal DG of rats exposed to experimental drugs. 400x magnification. Brown-stained cells are GFAP-expressing astrocytic cells

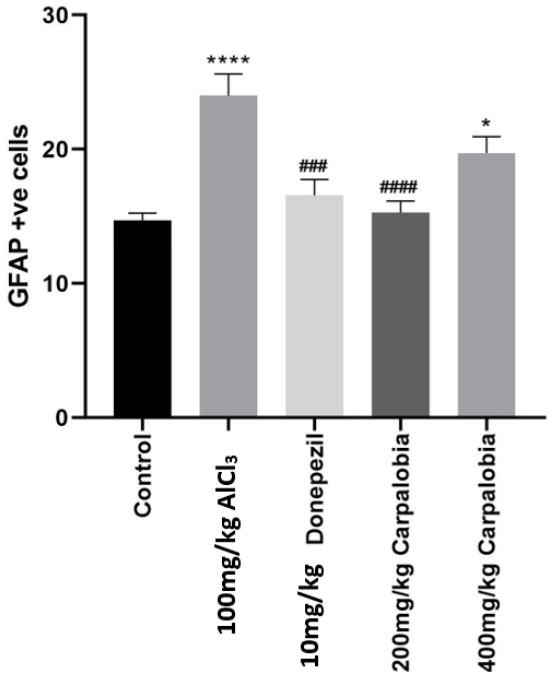

Fig. 9. Bar graphs depict the number of cells positive for GFAP in experimental rats. Each column represents mean ± S.E.M. Data were analysed using one-way ANOVA followed by Tukey's post-test. ****p < .0001, *p < .05 versus Control; ####p < .0001, ###p < .001 versus 100 mg/kg $AlCl_3$

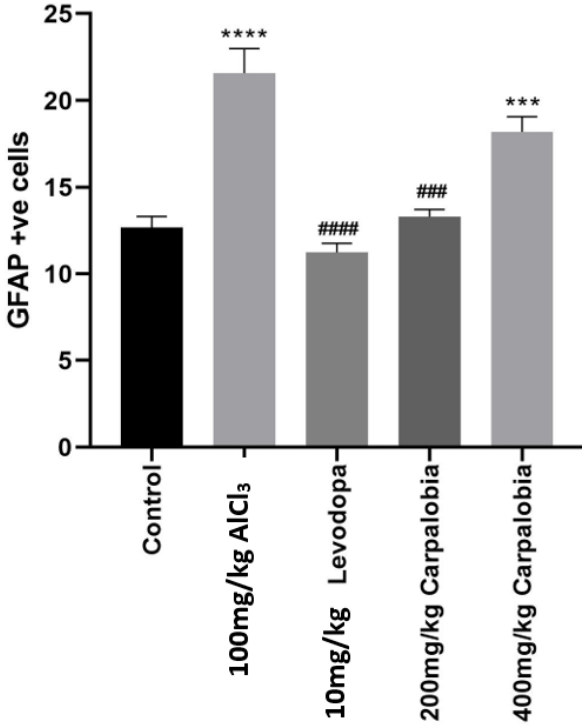

Fig. 10. Bar graphs depict the number of cells positive for GFAP in experimental rats. Each column represents mean ± S.E.M. Data were analysed using one-way ANOVA followed by Tukey's post-test. ****p < .0001, ***p < .001 versus Control; ####p < .0001, ###p < .001 versus 100 mg/kg $Alcl_3$

GFAP is a very sensitive marker for rapid astrocytic response to injury and disease [29]. In the present study, there was significant changes in the number of GFAP positive cells in the hippocampus CA_1 and CA_3 region and the dentate gyrus following $AlCl_3$ exposure in rats when compared to the control. There was an increased GFAP positive cells which is an indication of increased astrocytic reaction which occur as a result of neurotoxicity that may lead to neuroinflammation in the hippocampus. The hippocampus and dentate gyrus which are known for their role in learning and memory would invariably be impaired.

However, there was significantly reduced number of GFAP expressing cells in the hippocampal CA1, CA3 and dentate gyrus in the groups that received 10 mg/kg donepezil and 200mg/kg *C. lutea*. Furthermore, it was observed that there was an insignificant reduction in the number of hippocampal CA1, and CA3 GFAP positive cells in the 200 mg/kg *Carpolobia lutea* compared with the standard donepezil group. It may be suggested that neuroinflammation could be suppressed which may arise from neurotoxcity from aluminium chloride. The 400 mg/kg of *C. lutea* group had an increased GFAP reactive cells with a corresponding decrease in the number of hippocampal CA1 and CA3 GFAP positive cells though insignificant to the $AlCl_3$. This implies that higher dose of *C. lutea* could trigger-astrocytic reactivity similar to $AlCl_3$ group.

It is noteworthy that the major functional phenotypes associated with astrocytes reactivity include neuroinflammation and impaired glutamate [30]. Astrocytes reactivity is a hallmark of neuroinflammation that arises with Alzheimer disease [28].

Glutamate is an excitatory neurotransmitter found throughout the central nervous system especially in the hippocampus. It maintains optimal levels within the extracellular space. As such it is important in memory and learning [31].

Previous studies, revealed that exposure of co - cultured neurons and astrocytes to aluminium resulted in significant metal accumulation on both cells, whereas aluminium induced apoptosis was revealed only in astrocytes [32]. Jangra et al., [33] indicated how oral administration of $AlCl_3$ (100 mg/kg/b.w) for 42 days significantly elevated the levels of activity of cholinesterase and expression of amyloid precursor protein, amyloid beta in the hippocampus. Raj et al., [34] asserted that aluminium chloride is a neurotoxin which generates neuropathologies and cognitive depletion resembling aging and Alzheimer disease. Also d-galactose +$AlCl_3$ led to neurotoxicity assessed on the basis of neuroinflammation.glial cell activation, neuronal damage in mice hippocampus [35]. Ezzat et al., [36] indicated an upward glial fibrillary acidic protein (GFAP) immunoexpression in rat brain hippocampus. Chronic $AlCl_3$ administration raised the proinflammatory cytokines (interleukin-1 beta and tumor necrosis factor alpha level and increased acetlycholinesterase activity and reduced the BDNF content in the hippocampus [37]. Zheng et al., (2019) suggested that exposure to $AlCl_3$ can cause neuroinflammation that may result in loss of spine in the hippocampus and thereby leading to learning and memory deficits. Huo et al., [38] posited that $AlCl_3$ induced mitochondrial damage, NLRP3 inflammasome activation and apoptosis. Another study demonstrated that Aluminium accelerates neuronal loss and astrogliosis in rain brain [39,40].

From the present study, it is evident that 200 mg/kg of *C. lutea* reduced the GFAP immmuno reactivity when compared to 10 mg/kg of donepezil, the standard drug although statistically insignificant. It could be said that lower dose of *C. lutea* may have the tendencies of reversing impaired memory and learning and diseases associated with it. Our findings correlates with Noah et al., (2024). who suggested that *C. lutea* reverses the cardinal symptoms of ketamine-induced schizophrenia in a dose-dependent fashion by modulating the oxido-inflammatory and neurotransmitter-related mechanisms. Also our study is in consonance with Adeniran et al., (2022) who suggested that *C. lutea* root extract improved steroidogenic activity in male wistar rats exposed to cadmium.

5. CONCLUSION

In conclusion, 200 mg/kg of *Carpolobia lutea* have the tendencies of protecting the neurons in the hippocampus from degenerating as a result of aluminium chloride induced neurotoxicity compared to the standard drug (10 mg/kg donepezil).

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc.) and text-to-image

generators has been used during the writing or editing of this manuscript.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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