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Neuroprotective Effect of *Stachytarpheta angustifolia* Methanol Extract Against Aluminium Chloride ($AlCl_3$) Induced Alzheimer's Disease in Albino Rats

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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

Aim: This study was aim to evaluate the neuroprotective effect of *Stachytarpheta angustifolia* methanol extract on aluminium chloride induced Alzheimer's disease in Albino rats.
Study Design: This is an experimental study.
Location and Duration of the Study: The research was conducted at the Central Research Laboratory of Nasarawa State University, Keffi, Nigeria from 7th October, 2022 to 12th January, 2023.
Methodology: The whole plant was extracted using soxhlet extraction method with methanol as solvent. Thirty Albino rats of both sexes were divided into six groups with five rats in each.

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Aluminum chloride ($AlCl_3$) was used to induced Alzheimer and treated with vitamin E and various doses of the plant extract. Behavioural test was carried out using T-maze to assess learning and memory. Antioxidant parameters (SOD, CAT, GPx, GSH, MDA and NO), cyclooxygenase-2 (COX-2) and acetylcholinesterase (AChE) were determined using ELISA kits. Histopathological examination was carried out using hematoxylin and eosin.

Results: The results showed a significant (p = .05) decrease in the correct percentage number of alternations and a significant (p = .001) increase in latency time spent in the maze in the aluminum chloride ($AlCl_3$) induced and untreated group compared to the control. There were significant (p = .05) decreased in SOD, CAT activities and GSH (p = .001) level. While, there were significant increase in MDA (p = .001), NO (p = .01), COX-2 (p = .05) and AChE (p = .05) compared to the control. Treatment with the plant extract significantly (p = .001) increased the SOD, CAT, GPx and GSH compared to the aluminum chloride ($AlCl_3$) group. While, MDA, NO, COX-2 and AChE decreases significantly (p = .001) compared to the aluminum chloride ($AlCl_3$) group. Histopathological examination of hippocampus sections tallied with the biochemical parameters.

Conclusion: These results may justify the ethnomedicinal use of *Stachytarpheta angustifolia,* especially in the treatment of Alzheimer's disease.

Keywords: Antioxidant; anti-inflammation; cyclooxygenase-2; acetylcholinesterase.

1. INTRODUCTION

"Alzheimer's disease (AD) is a progressive disorder of neurological dysfunction which mainly affects movement, memory, speech, and cognitive ability" [1]. "The disease is characterized by degeneration in the cholinergic neurons found in the amygdala, hippocampus, cerebral cortex, and basal ganglia of the brain, which leads to reduced synthesis and secretion of the neurotransmitter acetylcholine (Ach) and deposition of Amyloid Beta (Aβ), causing dementia" [1]. "Impairment of short-term memory is usually the first clinical feature, whereas retrieval of distant memories is preserved relatively well into the course of the disease and when the condition progresses, additional cognitive abilities are impaired, such as the ability to calculate and use common objects and tools" [2].

"Exposure to aluminum (Al) could produce clinical and pathological features of Alzheimer's disease (AD) leading to numerous cellular signaling pathways of the brain by inducing oxidative stress and inflammation" [3]. "Oxidative stress (OS) decreased antioxidants enzymes activity under pathological conditions, resulting in relatively excess reactive oxygen species (ROS), causing cytotoxicity, which leads to tissue damage and is linked to neurodegenerative diseases and other pathological diseases" [4]. "Previous studies have strongly linked Al accumulation in the brain and progression of AD-like symptoms such as aggregation of hyper-phosphorylated tau-protein which consists of neurofibrillary tangles (NFTs) and accumulation of insoluble amyloid-β (Aβ) proteins as Aβ plaques" [5].

"In Nigeria, some plants or vegetables that are part of our diet contain useful medicinal substances" [6]. "These medicinal plants are widely used for the research of new drugs as they represent a rich source of compounds with pharmacological properties" [7]. "Many bioactive compounds extracted from herbal plants have the capacity to prevent neuronal injures and have become a vital therapeutic option for associated brain disorders" [1]. "Neuroprotective, antioxidant, and anti-inflammatory effects were demonstrated for green and black tea, *Allium cepa*, grape, *Ginkgo biloba* and saffron" [8].

The genus *Stachyterpheta* Vahl (Verbenaceae), known as "gervão" in English includes about 100 species widely distributed in tropical and subtropical America with few members in tropical Asia, Africa and Oceania [9]. "This genus is represented by three species in West Africa and in Nigeria: *S. cayannensis* (Rich.) Vahl., *S. indica* (Linn.) Vahl. and *S. angustifolia* (Mill.) Vahl." [10]. *Stachytarpheta angustifolia* is a seasonal plant that has been reported to possess various phytochemicals including; Saponins, Alkaloids, flavonoids, terpenoids, glycosides, quinones, phenol, tannins, steroids and coumarins which possessed antioxidant, anti-inflammatory and analgesic properties [7]. In North Central Nigeria, this plant is used traditionally for the treatment of asthma, epilepsy, pneumonia, antihypertensive and anti-fever. Thus, this study was conducted to evaluate the neuroprotective effect of the methanol extract on aluminium chloride ($AlCl_3$) induced Alzheimer in albino rats.

Fig. 1. Image of *Stachyterpheta angustifolia* plant

2. MATERIALS AND METHODS

2.1 Plant Sample Collection and Extraction

The whole plant (roots, stem and leaves) of *Stachytarpheta angustifolia* was harvested, washed and identified using Virtual Botanic Garden (VIRBOGA) Dataset Identifier (Identification number 788). The plant was air-dried at room temperature (25^{o}C) under laboratory conditions for two weeks. 500 g of the dried powder was filled in the porous cellulose thimble and subjected to soxhlet extraction using 99.8% methanol for 12 hours at 65^{o}C, followed by filtration through a Whatman No. 1 filter paper. The methanol extract obtained was concentrated to dryness at 45^{o}C using a rotary evaporator under reduced pressure. The concentrated extract was 109 g and was stored at 4^{o}C for further use [11].

2.2 Experimental Animals

Thirty adult Albino rats of both sexes weighing 200--250 g were assigned randomly for the study. The rats were housed in cages of 5 rats each and allowed acclimatization to laboratory status for one week before the experiment commenced [12]. Animals were maintained at room temperature and with a 12h light/12h dark cycle and allowed *ad libitum* access to water.

2.3 Experimental Protocol

A Completely Randomized Designed (CRD) was used with five replicates assigned to each group with the oral administration of aluminium chloride ($AlCl_3$) to induce Alzheimer and treatment daily for eight weeks according to Thenmozhi *et al*., [13]. Group 1: control, group 2: $AlCl_3$ and untreated, group 3: $AlCl_3$ and treated with 100 mg/kg vitamin E. group 4, 5 and 6: $AlCl_3$ and treated with 25, 50 and 75 mg/kg of the extract respectively, which was calculated using Ratio and Proportion Method [14].

2.4 Cognitive Test

T-maze alternation test was used to assess the cognitive ability of the rats according to Deacon and Rawlins [15]. The T-maze is an elevated or enclosed apparatus in the form of a T placed horizontally. This protocol details a method for using a T-maze to assess the cognitive ability of rodents. Animals are started from the base of the T and allowed to choose one of the goal arms abutting the other end of the stem. If two trials are given in quick succession, on the second trial the rodent tends to choose the arm not visited before, reflecting the memory of the first choice. This is called ‘spontaneous alternation’. This tendency can be reinforced by making the animal hungry and rewarding it with a preferred food if it alternates. During the behavioural test, the rats were pre-tested for 1 week before the commencement of the behavioural test. For each animal, six trials (T1 to T6) were carried out and the rate of visiting the same arm explored in the previous trial was perseveration (error) and choosing a different arm as a correct alternation.

2.5 Collection and Preparation of Tissue Homogenates

After the experimental period, the rats were sacrificed and their brains were removed and the

whole brain of each rat was dissected, thoroughly washed with ice-cold isotonic saline. One portion of the brain was used to prepare tissue homogenate in 0.1 M phosphate buffer (pH 8, stored 2-8 ℃) for biochemical parameters. "The homogenate was centrifuged at 3000 g for 10 min and the supernatant was used for biochemical analysis" [16].

2.6 Biochemical Analysis

Superoxide dismutase (SOD) was determined using Cayman's Superoxide Dismutase Assay Kit (No: 706002) which utilizes a tetrazolium salt for the detection of superoxide radicals generated by xanthine oxidase and hypoxanthine. Catalase activity was determined using the Cayman Catalase Assay Kit (No: 707002) which utilizes the peroxidation function of CAT for the determination of enzyme activity. Glutathione Peroxidase (GPx) was determined using the Cayman Glutathione Peroxidase Assay Kit (No: 703102) which measures GPx activity indirectly by a couple of reactions with Glutathione Reductase (GR). Reduce glutathione (GSH) was determined using the Cayman Glutathione Assay Kit (703002) which utilizes a carefully optimizing enzymatic recycling method of glutathione reductase (GR) for the quantification of GSH which measures the absorbance of TNB at 405-414 nm.

Lipid Peroxidation was determined using Cayman TBARS Assay Kit (No:100090550) which provides a sample, reproducible and standardized tool for assaying lipid peroxidation in the sample and measured colorimetrically at 530-540 nm. Nitric oxide (NO) was determined using Cayman's Nitrate/Nitrite Colorimetric Assay Kit (No: 780001) which provides an accurate and convenient method for measurement of total nitrate/nitrite concentration at 540-550 nm. Cyclooxygenase 2 (COX-2) was determined using Cayman COX colorimetric inhibitor screening assay kits (No: 701050) which measures the peroxidase component of COXs colorimetrically by the appearance of oxidized N, N, N`, N`-tetramethyl-p-phenylenediamine (TMPD) at 590nm. Acetylcholinesterase activity was determined using the Acetylcholinesterase Inhibitor Screening kit (No: MAK324) which is based on an improved Ellman method, in which thiocholine produced by the action of acetylcholinesterase forms a yellow colour with 5,5'-dithiobis (2-nitrobenzoic acid) (DTNB) and measured at 412 nm.

2.7 Histopathology Examination

Hematoxylin and eosin (H and E) stains were used for histopathological examination of the second portion of the brain using a light microscope [17]. The brain tissue was fixed in formalin buffer (10%), washed and dehydrated using serial dilutions of alcohol (methyl, ethyl and absolute ethyl). The specimens were cleared in xylene and embedded in paraffin in a hot air oven at 56 ℃. Paraffin beeswax blocks were prepared for sectioning at 4 µm using a microtome. The obtained tissue sections were collected on glass slides, deparaffinized and stained with H and E then view under the microscope.

2.8 Statistical Analysis

Statistical analysis was made by Statistical Package for Social Science (SPSS, version 24). The results were expressed as mean ± SEM. Multiple group comparisons shall be performed using one-way analysis of variance (ANOVA) followed by Dunnett's test in order to detect intergroup differences. A significant difference was determined when p =.05, p =.01 and p =.001.

3. RESULTS AND DISCUSSION

3.1 Results

3.1.1 Effect of *S. angustifolia* methanol extract on cognitive function

The results of the behavioural test are shown in Fig. 2. The percentage (%) number of correct alternations in the $AlCl_3$ group shows a significant (p = .01) decrease in the maze as compared with the control group. Vitamin E, 25, 50 and 75 mg/kg doses of the plant extract treated groups showed a significant (p = .01) increase of correct % alternation in the maze as compared with the $AlCl_3$ group. The duration of elapsed time in the maze was significantly (p = .001) increased in the $AlCl_3$ group as compared with the control group. Treatment with vitamin E, 25, 50 and 75 mg/kg doses of the plant extract improves the elapsed time as there was a significant (p = .001) decrease in the elapsed time of the maze as compared with the $AlCl_3$ group.

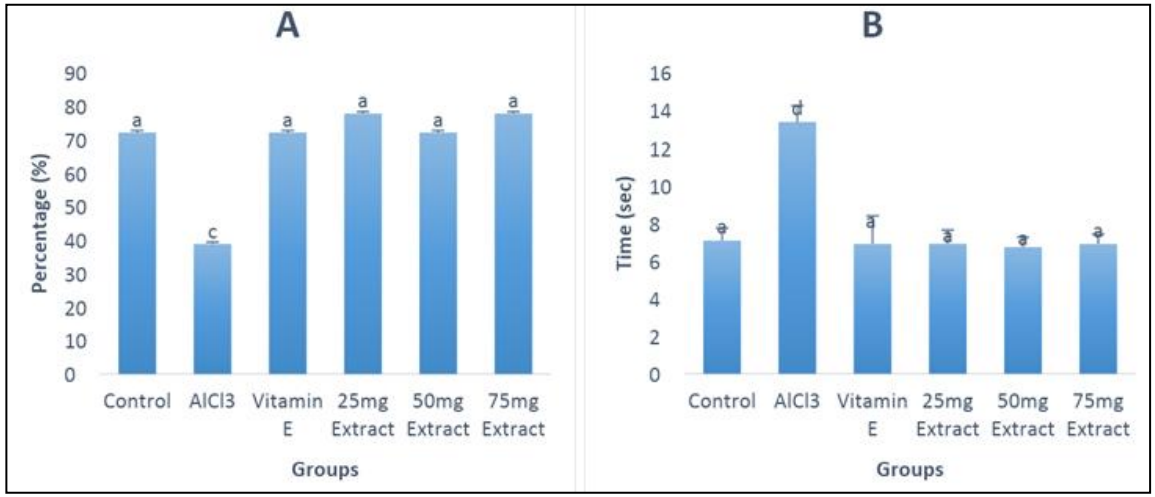

Fig. 2. Effect of *S. angustifolia* methanol extract on cognitive function

Key: A = Correct (%) Alternation, B = Latency Time Spent in the Maze. Letters assigned as superscript different from 'a' showed significant different. c = (p = .01), d = (p = .001)

Table 1. Effect of *S. angustifolia* methanol extract on brain antioxidants

Group	SOD (nmol/min/ml)	CAT (nmol/min/ml)	GPx (nmol/min/ml)	GSH (µM)	MDA (µM)	NO (µM)
Control	325.82±25.36^{a}	1652.72±111.24^{a}	307.57±15.98^{a}	23.07±1.82^{a}	1.12±0.03^{a}	529.14±33.99^{a}
$AlCl_3$	255.79±03.70^{b}	1227.15±264.15^{b}	310.94±44.46^{a}	15.67±0.98^{d}	1.91±0.11^{d}	655.56±45.46^{c}
Vit E	415.76±93.05^{a}	1178.07±27.99^{b}	393.76±92.35^{a}	20.30±1.15^{b}	1.92±0.24^{d}	670.80±00.33^{d}
25 mg	578.52±86.23^{c}	1465.11±68.52^{a}	352.83±81.72^{a}	21.35±0.88^{a}	1.19±0.14^{a}	732.97±27.49^{d}
50 mg	852.21±57.92^{d}	1549.95±308.21^{a}	591.19±58.71^{b}	23.66±0.69^{a}	0.96±0.08^{a}	591.00±31.40^{a}
75 mg	1227.37±48.62^{d}	1939.55±69.24^{a}	723.83±67.44^{d}	24.63±0.78^{a}	0.85±0.18^{a}	471.81±38.88^{a}

Results expressed in mean ± SD (n = 3). Letters assigned as superscript different from 'a' showed significant different. b = (p =.05), c = (p = .01) and d = (p =.001)

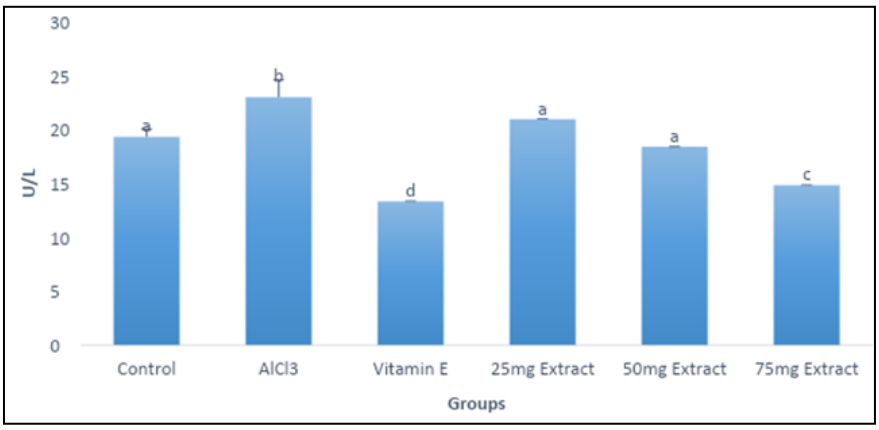

Fig. 3. Effect of *S. angustifolia* extract on cyclooxygenase-2 (COX-2)
Results expressed in mean ± SD (n = 3). Letters assigned as superscript different from 'a' showed significant different. b = (p =.05), c = (p = .01) and d = (p =.001)

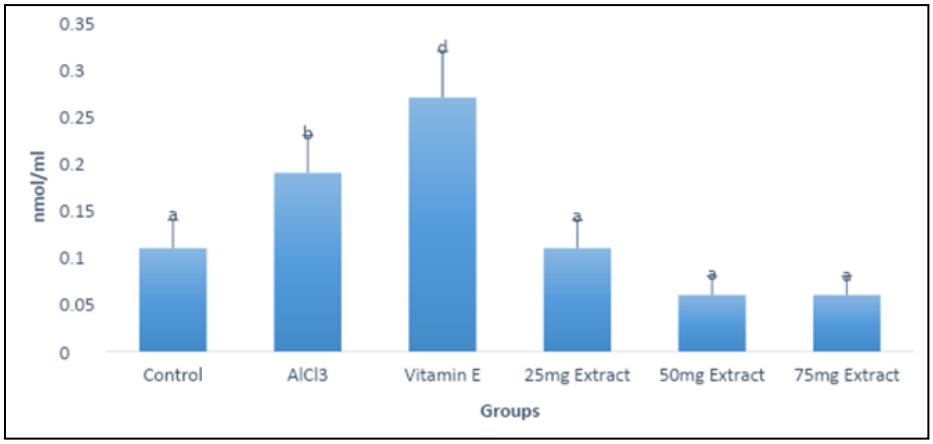

Fig. 4. Effect of *S. angustifolia* methanol extract on acetylcholinesterase (AChE)
Results expressed in mean ± SD (n = 3). Letters assigned as superscript different from 'a' showed significant different. b = (p =.05), c = (p = .01) and d = (p =.001)

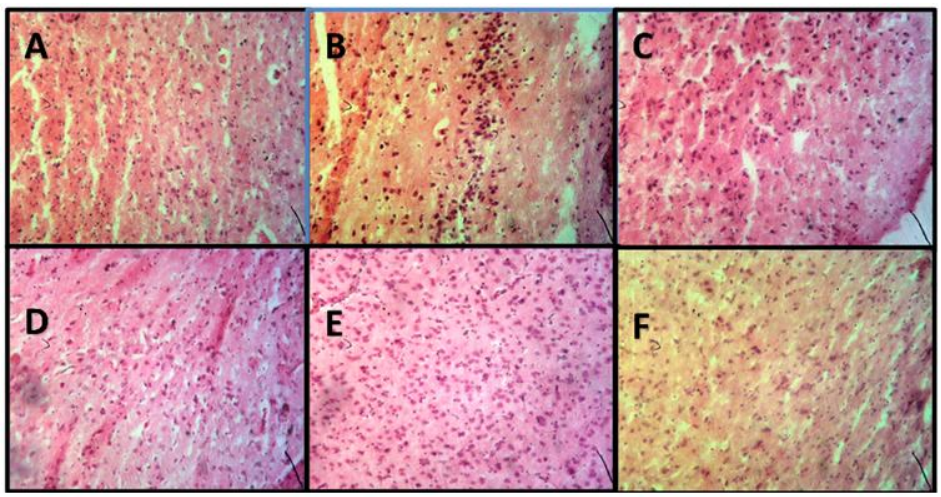

Fig. 5. Effect of *S. angustifolia* methanol extract on brain histology
Key: A = Control, B = AlCl $_3$, C = Vitamin E, D = 25 mg extract, E = 50 mg extract, F = 75 mg extract

3.1.2 Effect of *S. angustifolia* methanol extract on brain antioxidants

The assessment of *in vivo* antioxidants in Table 1 showed a significant (p = .05) reduction in SOD and CAT activity (p = .001) and the levels of GSH in the $AlCl_3$ induced group. Also, there were significant (p =.001) increases in MDA and (p = 0.01) NO levels in the $AlCl_3$ induced groups, respectively. However, treatment with the plant extract increases the SOD and Gpx enzyme activity significantly (p = .001), CAT (p = 0.05) and decreases the MDA and NO significantly (p = .05) compared to the $AlCl_3$ induced group. Treatment with vitamin E only increases the SOD activity significantly (p = .05) compared to the $AlCl_3$ induced group.

3.1.3 Effect of *S. angustifolia* extract on cyclooxygenase-2 (COX-2)

The inflammatory enzyme (COX-2) activity showed significant (p =.05) decrease in the $AlCl_3$ induced group compared to the control. Treatment with the plant extract showed significant (p = .01) reduction of the enzyme activity compared to the $AlCl_3$ induced group. However, he standard drug (vitamin E) showed highly significant (p = .001) decrease of the enzyme activity.

3.1.4 Effect of *S. angustifolia* methanol extract on acetylcholinesterase (AChE)

Significantly (p = 0.05) increased in the $AlCl_3$ induced group as compared to the control was observed. Treatment with the plant extract restored the AChE activity as there was a significant (p = 0.05) as compared to the $AlCl_3$ induced group. However, vitamin E administered group increases the AChE activity significantly (p = 0.01) as compared to the $AlCl_3$ induced group.

3.1.5 Effect of *S. angustifolia* methanol extract on brain histology

The histopathological representative photomicrographs of the brain hippocampus showed high power magnification (x100). The hippocampal section of the control group presented healthy pyramidal neurons. The hippocampus section of $AlCl_3$ group presented degenerating densely and tightly packed shrunken dark pyramidal cells with an oval or triangular nucleus. However, the hippocampal section treated with vitamin E and 25, 50 and 75 mg of plant extract showed intact cells with no sign of degeneration.

3.2 Discussion

"Aluminum (Al) is a ubiquitous metal and has been implicated in the etiology of neurodegenerative disorders and cognitive dysfunction, where it exacerbates brain oxidative damage, causes neuronal inflammation; and induces impairment in working memory, visuoperception, attention and semantic memory" [18]. "Al is especially vulnerable to oxidative stress resulting from elevated levels of free radicals and diminished levels of antioxidants following toxicity" [19]. "Different behavioral procedures were used to assess the effects of acute $AlCl_3$ intoxication on cognitive functions and significant memory lapses were observed in $AlCl_3$ administered rats" [20].

Previous report has shown that *Stachyterpheta angustifolia* methanol extract possessed Saponins and Alkaloids in copious amounts, flavonoids, terpenoids, glycosides and quinones in moderate amounts; while, phenol, tannins, steroids, and coumarins in low amounts [7].

The results of the present study significantly (p = .01) decreased the number of correct % alternations and significantly (p = .001) increases latency time to reach the arm of the T-maze compared to the control group. This affirms the previous report by Linardaki et al. [21] that learning and memory disturbance occur following Al administration in rats. However, treatment with the plant extract significantly (p = .05) increased the correct % alternations and also decreased the latency time in the T-maze significantly (p = .01) compared to the $AlCl_3$ group which was similar to the vitamin E treated group supporting similar finding in black pepper and Betalain by enhancing the memory in the $AlCl_3$ induced neurotoxicity mouse model [1].

"Excessive oxidative damage induced by Al toxicity results in neuronal injury and interrupts the brain's antioxidant defense system" [22]. "Thus, an imbalance between oxidative stress and antioxidant defenses is linked to acute cognitive impairment and may be an early sign of AD progression" [23]. The administration of $AlCl_3$ in the present study resulted in marked oxidative stress in the brain tissues as indicated by retarding the activities of SOD and CAT significantly (p = .05) and GSH (p = .001), then significant (p = .01) increase levels of MDA and NO compared to the control group. This induction of oxidative stress in brain tissues in response to sub-chronic exposure by Al affirms previous

works accomplished by many authors [1,24,19]. Treatment with methanol extract of *S. angustifolia* showed significant SOD and GPx (p .001), CAT (p = .05) and GSH (p = .01) increased and significant MDA (p = .001) and NO (p = .01) decreased compared to the $AlCl_3$ group supporting the antioxidant effect of the *S. angustifolia* methanol extract previously reported by Ashikaa *et al*., which may link to the presence of phenolic, flavonoid, alkaloid, and terpenoid compounds in the extract since they can readily donate hydrogen atoms to the radicals to neutralize them [7]. This antioxidant effect was higher than the standard drug (vitamin E) used. Therefore, this plant extract's neuroprotective activity may have seen mediated by its antioxidant properties [25].

Previous reports suggested that increased COX-2 activity in the brain has been associated with AD clinical manifestations including amyloidosis and dementia [26]. Administration of $AlCl_3$ in the present study showed a significant (p = .05) increase in COX-2 activity in the rats' brains compared to the control group. Treatment with the methanol extract of *S. angustifolia* showed a significant (p = .01) dose-dependent decrease in COX-2 activity. Although, this decrease was lower than the standard drug (vitamin E) used as it showed a highly significant (p = .001) decrease in COX-2 activity. The anti-inflammatory effects of the plant extract in inflammation-related disorders might be attributed to the presence of active phytoconstituents; flavonoids, saponins, and terpenoids [7]. Therefore, this inhibition of COX-2 may improve amyloid-β-mediated suppression of memory and synaptic plasticity in the brain since COX-2 activity has been associated with AD [27].

The results of acetylcholinesterase (AChE) in the present study showed a significant (p=.05) elevation in the activity of AChE with the administration of $AlCl_3$ for 8 weeks as compared to the control group. This was in accordance with the previous work by Shunan et al., [1], Aboelwafa et al., [19] and Doungue et al., [27]. Treatment with methanol extract of *S. angustifolia* decreases the AChE activity significantly (p = .05) compared to the $AlCl_3$ group. However, the standard drug (vitamin E) treated group significantly (p = .001) elevated the AChE activity. This result confirms previous findings that medicinal plants are reported to show AChE inhibitory activity due to the presence of phenolic and flavonoid content [28]. Thus, AChE inhibition activity of this extract suggests that the extract could be used for the management of AD since the inhibition of the cholinesterase enzyme from breaking down ACh, increases both the level and duration of the neurotransmitter action [29].

Histopathological examination of brain tissue in $AlCl_3$ group showed neuronal degeneration of the hippocampus characterized by degenerated shrunken cells with dark stains as compared to the control group. This result affirms the previous report by Laraib et al., [20] that chronic administration of $AlCl_3$ shrunk neuronal cells. These hippocampus morphological changes could be due to damage to neurons and gliosis as a result of oxidative stress, and this may affect various enzymes responsible for the synthesis and destruction of the neurotransmitters [30]. Treatment with the plant extract showed normal hippocampus morphology and was comparable vitamin E treated group. This suggests that pyramidal cell injury in the hippocampus of $AlCl_3$ induced group is more pronounced than in the treatment groups which tallied with the biochemical markers.

4. CONCLUSION

Based on the findings of this study, $AlCl_3$ promotes oxidative damage in the brain tissues which leads to neuronal cell death and alters brain neurochemistry leading to symptoms of neurodegeneration as it affects cognitive function. However, the methanol extract of *S. angustifolia* was able to prevent the deleterious effects of $AlCl_3$ as demonstrated by having antioxidant effect and decreased in COX-2 and AChE inhibitory activities. This neuroprotective effect of the extract may be due to the presence of saponin, phenol, flavonoid, alkaloid, and terpenoid with potent antioxidant, anti-inflammatory and acetylcholinesterase inhibition properties. Hence, the extract could serve as a potent source of drug for prevention and management of AD disease. Further studies will be required for investigations of the fractions of *S. angustifolia* in order to isolate potential lead for prophylaxis and therapeutic use.

CONSENT

It is not applicable.

ETHICAL APPROVAL

All experimental procedures were made according to Standard Operating Procedure for

Nasarawa State University Keffi Animal Care and Use Research Ethics Committee (NSUK-ACUREC).

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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