Neuroprotective effect of *Andrographis paniculata* (burm.f.) leaf extract in aluminum chloride-induced alzheimer's disease in mice

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Abstract

The socio-economic burden and poor quality of life associated with Alzheimer's disease (AD), coupled with the high cost of available treatment options, prompted the search for alternatives from plants. This study investigated the neuroprotective effect of *Andrographis paniculata* in Aluminum chloride- induced Alzheimer's disease in mice model. Thirty female mice were randomly allocated into six groups (n=5) following a 14-day induction of groups 2-6 with aluminum chloride ($AlCl_3$). Group 1 (AD free) received only distilled water while groups 2 to 6 were orally treated with distilled water, 1 mg/kg donepezil, 125 mg/kg, 250 mg/kg and 500 mg/kg of *Andrographis paniculata* extract respectively. T-maze and Morris water maze experiments were utilized to investigate the cognitive behaviors of the mice. *A. paniculata* treated mice showed an improved dose dependent and significant ($p \leq 0.05$) alternation between the two arms of the T maze when compared to the poor alternation observed with group 2(AD positive) but untreated group. The results also showed that treatment with *A. paniculata* significantly ($p \leq 0.05$) reduced the time taken to reach the Morris water maze escape platform dose-dependently. The AD model group, however sluggishly roamed the water and failed to reach the escape platform within the stipulated time despite the intensive prior trainings. *A. paniculata* thus, has a promising neuroprotective potential against aluminum chloride-induced Alzheimer's disease.

Keywords: Alzheiemers disease; *Andrographolide paniculata*; Morris water maze; T maze

Introduction

Alzheimer's disease, a progressive neurodegenerative disorder is the most common cause of dementia, accounting for 60-80% of dementia cases globally (Jadhav & Kulkarni, 2023; Monfared *et al*., 2022; Auti & Kulkarni, 2019; Mayer *et al*., 2018). Patients with AD have cholinergic deficits, provoked by the accumulation of beta-amyloid plaques and neurofibrillary tangles in the brain (Ko *et al*., 2018;Small & Bullock, 2011). Although age is the primary risk factor for Alzheimer's disease, genetic and environmental factors also play a significant role in disease development. Such development has been reported to include: the disruption of blood brain barrier, impaired brain metabolism (Zhong *et al*., 2019), impairment of cellular autophagy (Friedman *et al*., 2015), unsettling influence of calcium homeostasis and expanded oxidative stress (Sanabria-Castro & Alvarado-Echeverria, 2017).

High levels of certain heavy metals in our environment like aluminium has been implicated in the development and progression of Alzheimers disease (Bhattacharjee *et al*., 2014; Garcia *et al*., 2010 ;Walton, 2010). High content of aluminium in the brain has also been shown to cause similar pathology and biochemical alterations as seen during AD, such as oxidative stress, neuroinflammation, amyloid aggregation and neurofibrillary tangles(Zheng *et al*., 2016).

Management of AD involves pharmacotherapy and psychosocial support, in addition to treatment of comorbid conditions (Cotterell & Brown, 2012). Such pharmacological management includes the use of acetylcholinesterase inhibitors such as galantamine, donepezil, and rivastigmine, all of which provide only symptomatic relief (Holtzman *et al*., 2011;Korolev, 2014). The therapeutic management of AD is saddled with many side effects ranging from dizziness, gastrointestinal irritation, and headache to severe hepatic damage (Korolev, 2014). Due to the high prevalence of AD among our elderly and the continuous exposures to side effects of present treatment options, there is a pressing need for rapid development of new drugs especially herbal remedies which have

been reported with fewer side effects and are less expensive (Etti *et al*., 2016).

Andrographis paniculata (AP), commonly known as king of bitters (Nyeem *et al.*, 2017) is an annual herbaceous plant with many reported medicinal properties (Okhuarobo *et al*., 2014). The plant constitutes of diterpenoids, flavonoids and polyphenols. Its principal compounds, andrographolide, neoandrographolide and 14-deoxy-11,12-didehydroandrographolide have been reported with potent anti-inflammatory, antioxidant, and immunomodulatory properties *(Lim et al.*, 2012). However, there are limited data on the therapeutic potential of the plant's leaf extract in the management of Alzheimer's disease.

Materials and methods

Extraction

Newly grown leaves of *Andrographis paniculata* were collected from Akpabuyo located in Cross River State, Nigeria. The leaves were authenticated by a taxonomist, Professor Margaret Emmanuel Bassey as *Andrographis paniculata* (Burm.f.) Nees Acanthaceae and was deposited in the Pharmacognosy herbarium with the voucher number UUPH 1 (h). Thereafter, it was dried and pulverized into fine powder. A 0.72 kg weight of the powdered leaf sample was macerated using an extraction tank and extracted using 3.5 L of 80% methanol (Sigma Aldreich, USA). The extract, 91.4 g obtained was stored in a sealed container at 4^0C in a refrigerator and the percentage yield was calculated using:

$$\%\, Yield = \frac{We}{Ws} * 100 \text{----------} \quad (1)$$

Where: We is Weight of dried extract obtained (g) and Ws is the Initial weight of leaf sample (g)

Acute toxicity of methanol leaf extract of A. paniculata

The determination of the median lethal dose (LD50) of the leaf extract of *A. paniculata* was carried out using Lorke's method (Lorke, 1983). The tested doses were: 3000 mg/kg, 4000 mg/kg and 5000 mg/kg. The mice were monitored for any sign of toxicity and mortality within 24-hour (Twaij & Al-Dujail, 2014)

Animal experimentation

Swiss female mice weighing between 25g to 32g were obtained from the animal house of the Department of Pharmacology and Toxicology at the University of Uyo, Nigeria.

Throughout the experimental period, the mice were kept in standard cages at room temperature with a 12-hour cycle of light and darkness. They were provided with rodent pellet diet and water as needed. Experimental procedures were conducted in accordance with the Guidelines for the Care and Use of Laboratory Animals and the ARRIVE guidelines, and were approved by the Faculty of Pharmacy Institutional Animal Care and Use Committee at the University of Uyo.

Induction of Alzheimers Disease

Thirty female mice were divided into six groups, each consisting of five animals (n=5). Group I served as the normal healthy control, while the remaining groups (II-VI) were administered aluminum chloride solution (100 mg/kg) daily at an hour interval prior to treatment with different doses (125 mg, 250 mg and 500 mg) of AP for 14 consecutive days (Auti & Kulkarni, 2019).

Spontaneous alternation assessment using T-maze

This test is a common method in behavioural research used to evaluate spatial working memory in animals. It included both the acquisition and testing phase (D'Isa *et al*., 2021). In the acquisition phase, the mice were given two trials (two sample run per trial) per day for two days with 10 minutes interval. This was to enable them freely explore the T-maze and to establish a baseline behaviour. Food was placed on both arms of the maze. During the training phase, each mouse was placed at the base of the T- maze stem and the slide door of one arm was closed. In the testing phase, both arms were opened. A spontaneous alternation behaviour was observed whenever the animal chooses the arm opposite to its previous choice, indicating a successful working memory. Howbeit, if the animal chooses the same arm, then there was no spontaneous alternation. A solution of ethanol (80%) was used to clean the entire T-maze apparatus. The interval of 10 secs between each run was given and different trials per animal was performed for five days. The time spent in the different arms of the T-maze, and the number of entries in the different arms were also recorded.

Morris water maze (MWM)

To study the cognitive behaviours of the animals, the Morris water maze, a well-established behavioural task (Morris, 1984);(Auti & Kulkarni, 2019) was utilized. The test comprised of two phases, acquisition phase and testing phase with the aim of

evaluating the escape latency (the time the tested animals take to locate the hidden platform after being released into the maze) of the animals. A large circular swimming pool tank consisting of four quadrants (NW, NE, SE and SW) containing water (25˚C) was used (Auti & Kulkarni, 2019). The goal for the rodent was to locate a hidden platform submerged just beneath the water's surface. The submerged platform was placed 1 cm above the water. During the acquisition phase, a transparent platform was placed above the water surface and the animals were placed facing the wall of the tank. They were allowed to swim for 60 seconds. If the animals reached the platform within the 60 seconds, they were allowed to remain on the platform for 10 seconds. If the mice failed to reach the platform during the 60 seconds, they were guided to the platform and then allowed to remain for 10 seconds. The mice were trained for four days, with four trials per day at 5 minutes interval. As the training progressed, the mice were expected to improve their performance by locating the platform more quickly and following a more direct path. After the acquisition phase, the mice underwent a testing phase that assessed their memory retention of the platform location. Here, the submerged platform was 1cm below the level of the water. The animals were placed in the quadrant facing the wall of the tank and the water was made opaque using milk, the animals were also allowed to swim for 60 seconds. If the animals reached the platform within the 60 seconds, they were allowed to remain on the platform for 10 seconds. If the mice failed to reach the platform during that period, they were guided to the platform and then allowed to remain for 10 seconds. This phase was carried out for five days. A mouse with good spatial memory will spend more time searching in the correct quadrant, indicating that it remembered where the platform was previously located. The escape latency was then calculated by measuring the time taken to locate the hidden platform by the animals.

Statistical analysis

Data collected from this study were analysed using Graph Pad Prism Software version 5.0 for windows (Graph Pad software, San Diego California USA). Results were expressed as mean ± standard error of mean. The Significant difference between treatment groups and controls were evaluated by performing a one-way ANOVA followed by Tukey’s multiple comparison post-hoc test.

p≤0.05 were considered statistically significant.

Result

Percentage yield and acute toxicity studies of A. paniculata leaf Extract

The percentage yield of 70% methanol *A. paniculata* leaf extract was calculated to be 12.7% from an initial weight of 720 g. No sign of toxicity was observed after the oral administration of any of the oral doses (3000 mg/kg, 4000 mg/kg and 5000 mg/kg) of *Andrographis paniculata*. The median lethal dose was thus concluded to be greater than 5000 mg/kg. This finding is consistent with that of Worassuttayangkurn *et al.* (2019) and Mohammed *et al*.,(2015).

***Andrographis paniculata* Promotes Spontaneous alternation in $AlCl_3$ –induced Alzheimer's disease model**

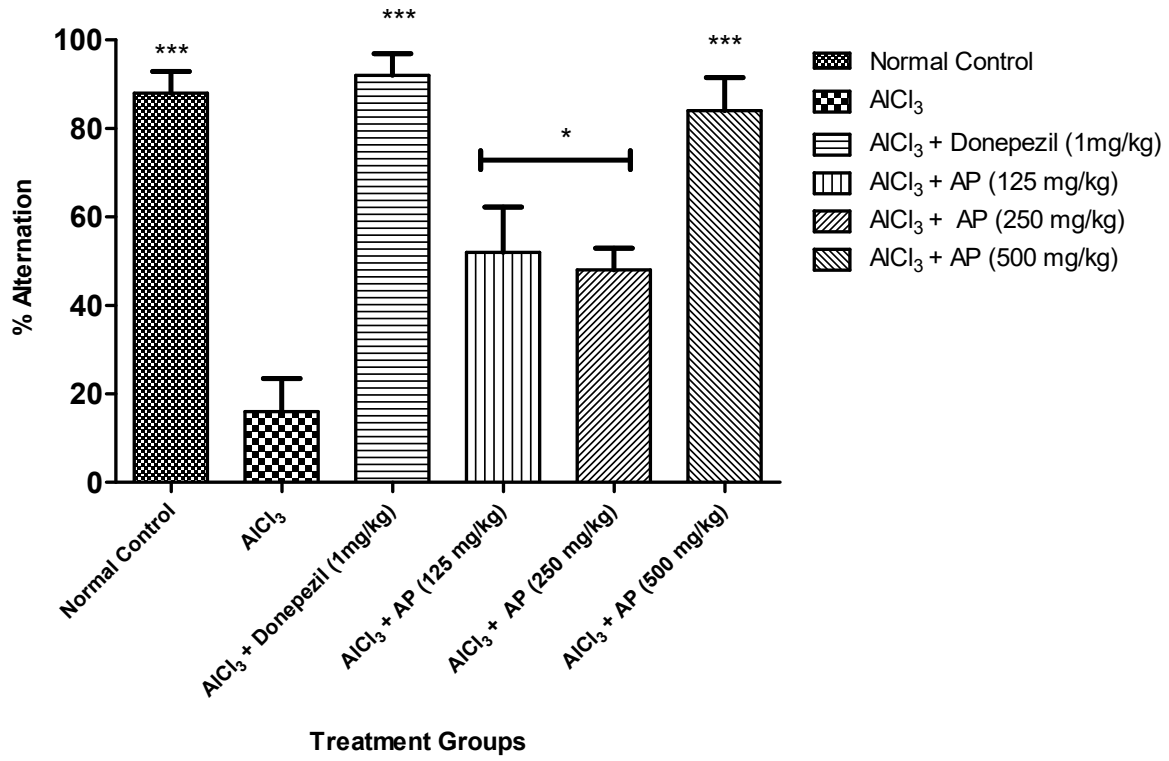

Figure 1 Percentage alternation between $AlCl_3$ - treated group and *Andrographis paniculata* extract- treated groups. Data are expressed as mean ± SEM for five rats in each

group. *p < 0.05 or ***p < 0.001 vs. $AlCl_3$ –untreated control group. SEM, Standard error of mean

There was a huge improvement in the behaviour of the AP-treated animal groups. These mice spontaneously alternated the two arms of the T maze freely. This alternation was observed to be concentration dependent as mice in the 500 mg/kg *Andrographis paniculata* showed better alternative behaviour ($p < 0.001$) than the low and medium doses ($p < 0.05$). This behaviour was similar to those in the donezepil-treated group, which was not significantly different ($p > 0.05$) from the healthy animal group. $AlCl_3$ –untreated group showed very poor alternation. The mice persistently chose the same arm of the T maze amidst the prior training sessions. However, we observed that the percentage of spontaneous alternation was better with the mice group treated with 125 mg/kg of AP than the groups treated with 250 mg/kg.

***Andrographis paniculata* Reduced the mean time taken to reach Morris water maze platform**

The primary measure in the MWM test is the escape latency, a relative measure of the cognitive abilities of the animal to learn and remember the platform location.

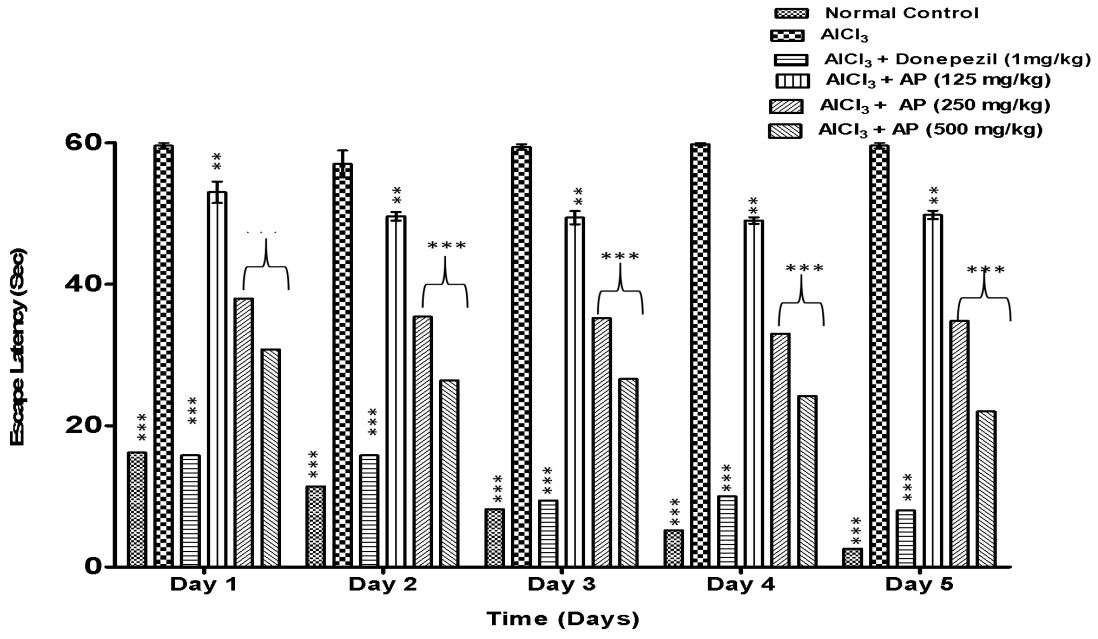

Fig 2 **Time taken to reach the rescue platform in Morris water maze**. **Data are expressed as mean ± SEM for five mice in each group. *p < 0.05 or **p < 0.01 ***p < 0.001 vs. $AlCl_3$ – untreated control group. SEM, Standard error of mean**

The escape latency in the extract-treated groups significantly decreased when compared to the $AlCl_3$ untreated AD group. All the female mice in the untreated AD group sluggishly roamed in the water until rescued. They were unable to locate the platform within the 60 seconds allotted for each trial.

Discussion

The prior exposure of the tested animals to the aluminum chloride induced significant memory deficit which manifested as altered spatial learning as seen in the MWM and memory impairment as evident with the absence of spontaneous alternation seen in the T-maze exploration. This observation was consistent with the findings of Brown and Lockwood (Brown *et al*., 2005) that aluminium chloride induced neuroinflammation is implicated in cognitive impairment. The poor percentage alternation and escape latency experienced by the untreated model group (Fig 1 and 2) could be attributed to the damage on the animal's hippocampus, the earliest and severely damaged brain area reported in AD (Khakpai *et al*., 2013). The hippocampus has been reported to be highly enriched in cholinergic, monoaminergic and glutamatergic axon terminals, and these neurotransmitters abnormality are considered to be closely related to AD (Serrano *et al*., 2014). The disparity observed between the 125 mg/kg group and the 250 mg/kg groups might be due to various factors such as the specific biological response to the different concentrations, individual metabolism variations, or other unknown variables. It could also be related to solubility issue since the components of the extract may not have infinite solubility in the vehicle which was aqueous. Further research and controlled studies are therefore recommended to determine the exact reasons for this observed difference.

Spatial memory task which was evaluated using the Morris water maze revealed a poor performance in group II. This observation reflected an impairment in learning the location of the platform despite the prior

trainings that the animals received during the acquisition phase when compared to a better performance by their normal littermates of group 1 (Fig 2). Research has shown that this mental retardation is induced by cholinergic deficits (Ko et al., 2018), (Small & Bullock, 2011). Partial memory abilities like those in the hidden platform learning test have been shown to be highly dependent on hippocampal function (Oostra & Nelson, 2006). Clark *et al*., (2005) earlier reported that a hippocampal lesion would impair spatial learning. The decrease in escape latency, a parameter used in accessing spatial memory showed an improvement in spatial learning and memory of the mice following treatment with different doses of the extract as well as with donepezil. These results revealed an improved mental performance in *Andrographis paniculata*-treated mice and was seen to be dose-dependent. We suspect that the improvement in spatial memory may be triggered by a principal phytochemical, andrographolide, a major constituent of AP (Serrano *et al*., 2014) which had been reported to be potent in suppressing neuronal apoptosis and enhancing hippocampal signalling (Wang *et al*., 2019).

Conclusion

It is therefore obvious that intervention with *Andrographis paniculata* restored the hippocampal lesion created by aluminum chloride administration. Hence, the leaf extract of *Andrographis paniculata* possesses promising neuroprotective properties in Alzheimer's mice model.

Conflict of Interest

The authors declare no conflict of interest.

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