DOI: https://dx.doi.org/10.4314/jaaps.v11i1.2

J. Afr. Ass. Physiol. Sci. 11(1): 17-28, July 2023

Journal of African Association of Physiological Sciences

Official Publication of the African Association of Physiological Sciences

https://www.ajol.info/index.php/jaaps

Research Article

Anticholinesterase activity and Antioxidant Effect of Vitamin E in Aluminium Chloride Induced Toxicity in Drosophila Melanogaster

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Keywords:

Aluminium chloride toxicity, Alzheimer's disease, Vitamin E

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Received:17 March 2023
Revised: 12 June 2023
Accepted: 18 June 2023

ABSTRACT

Background: Aluminium chloride ($AlCl_3$) toxicity has been reported to be linked with impaired locomotion, memory, learning, oxidative stress and impairment of cholinergic function which are synonymous with features seen in Alzehmiers disease (AD). Vitamin E has been put forward as a possible therapeutic intervention for AD. However, there are controversies as to whether Vitamin E is beneficial in the management of AD. Anticholinesterase activity and antioxidant potential of vitamin E was evaluated in aluminium chloride induced toxicity in *Drosophila Melanogaster*.

Methods: A 2.5mg dose of Vitamin E was considered the appropriate standard for this study after exposure of flies to varying doses of vitamin E in a 15-day survival study. Group I served as control while group II were treated with 40mM aluminium chloride ($AlCl_3$) via their diet. Group III were treated with 2.5mg of Vitamin E via their diet and Group IV were co-administered with 40 mM $AlCl_3$ and 2.5mg of Vitamin E via their diet. The flies were maintained on these treatments at room temperature for seven (7) days. Negative geotaxis was carried out to assess for locomotor performance (climbing activity). The impact of 40 mM $AlCl_3$ and/0r 2.5mg of Vitamin E on the survival rate of flies was also evaluated by carrying out a 15-day survival study At the end of the experimental period, the flies were homogenized and the supernatants were used to assay for, malonaldehyde (MDA) concentration, acetylcholinesterase (AChE), superoxide dismutase (SOD), catalase (CAT) and glutathione S-transferase (GST) activities.

Results: $AlCl_3$ significantly reduced ($P<0.05$) the survival rate, decreased the climbing activity of flies, elevated MDA concentration and AChE activities of flies. SOD, CAT and GST activities were also significantly reduced ($P<0.05$) in $AlCl_3$ treated flies. In the co treatment protocol, vitamin E was able to significantly improve ($P<0.05$) the survival rate, improved their climbing activity and ameliorated $AlCl_3$ increase in AChE activity and MDA concentration in these flies. In addition, vitamin E significantly attenuated ($P<0.05$) $AlCl_3$ induced decrease in SOD, CAT and GST activities.

Conclusion: This study has shown that vitamin E has both antioxidant and anti-cholinesterase activities and could be of therapeutic benefits against $AlCl_3$ induced toxicity and associated diseases like Alzheimer's disease.

Introduction

Alzheimer's disease (AD) is a progressive neurodegenerative disease that is symptomatically characterized by memory loss, multiple cognitive impairment, disturbance in language and executive function and by several neurobehavioral symptoms (Assal and Cummings, 2002). The etiology of AD is unclear though, however, several factors such as β- amyloid peptide, tau protein aggregation, toxicity of transition metals, oxidative stress, Inflammation and cholinergic impairment have been implicated in the pathogenesis of AD (Barnham *et al.*, 2004; Cummings, 2004 and Heppner, 2015). Aluminum (Al) is a highly pervasive environmental metal used industrially for the production of various plastic materials, cooking wares and roofing sheets. Aluminium chloride ($AlCl_3$) has been used to model neurotoxicity and replicate AD in various animal models (Bondy, 2010). Aluminium chloride ($AlCl_3$) toxicity has been reported to be associated with impaired locomotor performance, learning, memory, oxidative stress and cholinergic function all of which are typical symptoms encountered in AD patients (Ribes *et al.,* 2008).Cholinergic impairmentis characterized by a decline in acetylcholine (Ach) concentration in the synapse of the AD brain and this has been reported to be responsible for the impairment of memory and other cognitive functions reported in AD subjects (Mukherjee *et al.*, 2007). Since augmentation of acetylcholine levels in the brain has proven to be an effective therapeutic strategyin AD patients, acetylcholinesterase (AChE); an enzyme which hydrolyzes acetylcholine, has become an important therapeutic target in the management of AD (Taylor and Radić, 1994). Acetylcholinesterase inhibitors (AChEI) are currently among the best available pharmacotherapeutic agents for treatment of AD symptoms (Pueyo and Calvo, 2011). Prolonged use of these drugs, however, elicit severe side effects like diarrhoea, vomiting, dyspepsia, anorexia, muscle cramps, fatigue, insomnia, dizziness, headache and asthenia (Ohbe *et al.,* 2018) advocating for the search of alternative compounds with anticholinesterase potential but less undesirable effects.

Vitamin E is an essential micronutrient and considerably the most effective lipid soluble vitamin found in biological systems (Atef, 2011). It is composed of tocopherols and tocotrienols and plays an important role in maintaining the integrity of cell membranes, mopping up of free radicals (Singh *et al*., 2013) and cell signalling and gene regulation (Galli *et al.*, 2017). Vitamin E has been reported to possess antioxidant (Wolf, 2005) and anti-inflammatory properties (Ahsan *et al.*, 2014). It has also been proposed as a potential clinical intervention in the management of AD (Browne *et al.*, 2019) with many medical practitioners adding vitamin E supplements to their standard treatment regimen of Alzheimer's disease (Shahat *et al.*, 2015). There are, however, controversies as to whether vitamin E is beneficial in the management of AD. Some studies have argued vitamin E to be beneficial in the management of AD (Zandi et al., 2004, Devore *et al*., 2010, Basambombo *et al.*, 2017) whilst others [Lloret *et al.* (2009) and Farina *et al.* (2012)] have reported that vitamin E treatment was unable to exert positive effects in AD management. Since improving acetylcholine levels in the brain and reducing oxidative stress play a crucial role in the management of AD, the current study aimed to investigate the anticholinesterase activity and antioxidant effect of vitamin E in an aluminium chloride induced toxicity model of Alzheimer's disease in *Drosophila Melanogaster*.

2. Materials and methods

2.1 Chemicals

All chemicals and reagents utilized in this experiment were of analytical grade. Aluminum chloride and vitamin E were purchased from Pyrex chemicals in Benin-City, Edo state, Nigeria. Additional chemicals utilized such as acetylthiocholine iodide, 5,5′-dithiobis-2-nitro-benzoicacid (DTNB), 1-chloro-2,4-dinitrobenzene (CDNB), were purchased from Sigma Aldrich (St. Louis, USA).

2.2. Drosophila melanogaster stock and culture

Drosophila melanogaster (Harwich strain) were obtained from the *Drosophila* Laboratory of the University of Ibadan, Oyo State, Nigeria. The flies were reared on a cornmeal medium which contains1% w/v agar, 1% w/v brewer's yeast, 1% w/v powdered milk, 2% w/v sucrose, and 0.08% v/w nipagin at room temperature (24 °C) under a 12 h dark/light cycle condition at the *Drosophila*

laboratory of the Central Research Laboratory, University of Benin, Benin City. The same strain of *Drosophila melanogaster was* utilized throughout the course of the experiment.

2.3 Survival study

In order to determine appropriate dose of vitamin E to utilize for this study, and the effect $AlCl_3$ toxicity on survival of flies, a 15 days survival study was carried out. Flies were divided into groups with 50 flies per group. Group 1 served as our control and receive their normal corn meal diet, Group 2 were treated with 40 mM $AlCl_3$ while Group 3 and 4 were treated with 2.5mg and 5mg of vitamin E respectively (via their diet) for 15days. The flies were observed daily for mortality, and the survival rate was determined by counting the number of living flies left during the 15 days period (Abolaji *et al*., 2014). The survival rates was analyzedusing the Mantel-Cox log-rank test followed by the Bonferroni correction technique 2.5mg concentration of Vitamin E produced the least mortality in flies than 5mg which informed the choice of 2.5mg concentration of vitamin E used for the ameliorative part of this study.

2.4. Experimental layout

A total of 200 flies (both genders) were divided into 4 groups with 50 flies per vial as described below.

Group I: Control flies reared on Corn meal diet (basal diet).
Group II: Flies treated with 40mMol of $AlCl_3$via their basal diet.
Group III: Flies treated with 2.5mg of Vitamin E via their basal diet.
Group IV: Flies co-treated with 40mMol of $Alcl_3$+2.5mg of Vitamin E via their diet.

The choice of concentration for $AlCl_3$was based on previous studies on aluminum toxicity in *Drosophila melanogaster* [Wu *et al.,* 2012; Ogunsuyi, 2020,]. These flies were kept on these treatments at room temperature (24^{o}C) for seven (7) days. All experiments were carried out in five replicates.

2.5. Negative geotaxis assay

This assay is used to determine locomotor performance or climbing activity of flies. This assay was carried out as previously described by Abolaji *et al.,* 2018. Ten (10) flies from each group were immobilized under mild ice anesthesia. They were subsequently placed separately in labeled vertical glass columns (length 15 cm; diameter 1.5 cm). After the recovery from the ice exposure, the bottom of the column was gently tapped, and the flies were allowed to climb. The number of flies that climbed up to and above the 6 cm mark of the column in 6 seconds as well as those that remained below this mark after this time was recorded. Climbing activity was scored by expressing the proportion (%) of flies above the 6 cm mark. After 1-min interval, this procedure was repeated. A total of three repetitions were carried.

2.6. Preparation of samples for biochemical assays

At the end of the experimental period (7days), the living flies were carefully transferred to an empty vial before the anesthesia to prevent dead flies from being introduced and afterward these flies were anaesthetized in ice then weighed. The unconscious flies were then homogenized in 0.1 M potassium phosphate buffer of pH 7.4 (1:10).They were later centrifuged at 4,000g for 10 min at 4 °C and the supernatants obtained were used for the following biochemical assays: Malondialdehyde (MDA), superoxide dismutase (SOD), catalase (CAT), glutathione S-transferase (GST) and acetylcholinesterase (AChE) activities. The various biochemical parameters were analysed spectrophometrically using a DW-721G VIS Spectrophotometer. All reagents and chemicals used for this study were purchased from Sigma Aldrich (St. Louis, MO), USA.

2.7. Determination of biochemical indices

Superoxide dismutase (SOD) Activity
SOD activity was determined by methods described by Kostyuk and Potapovich, (1989) which involves monitoring inhibition of autooxidation of quercetin. The reaction mixture contained 10 µL of the sample, 15% quercetin, 20 mM phosphate buffer (pH 7.8), 0.08 mM EDTA and 8 Mm N,N,N,N tetramethylethylenediamine (TEMED). The

reaction was monitored for 3min at 406 nm using a DW-721G VIS Spectrophotometer. The results were expressed as the amount of protein required to inhibit quercetin auto-oxidation (µmol/min/mg protein).

Determination of catalase activity

Catalase activity was measured utilizing the methods described by of Aebi, (1984) which involves mixing 10 µL of the sample (in a 1:50 dilution) and 50 mM potassium phosphate buffer (pH 7.0) followed by 300 mM H_2O_2. The loss in absorbance of H_2O_2 was monitored for 2 min at 240 nm and was subsequently used to calculate catalase activity which was expressed as µmol of H_2O_2 consumed per minute per milligram of protein.

Determination of Glutathione S-Transferase (GST) activity

The activity of GST was evaluated using 1-chloro-2,4-dinitrobenzene (CDNB) as substrate as described by Habig and Jakoby (1974). The reaction mixture contained 270 microliter of solution A (which contains 0.25 M potassium phosphate buffer (pH 7.0) + 0.1 M GSH + 2.5 mM EDTA), 10µL of 25 mM CDNB and 20µL of the sample (1:5 dilution). This mixture was monitored using a DW-721G VIS Spectrophotometer for 5 mins (at 10s intervals) at 340 nm, and the results were expressed as µmol per minute per milligram protein.

Determination of lipid peroxidation

Lipid peroxidation was determined according to the method of Ohkawa *et al.* (1979). The mixture contained 40µL of the supernatant, 100µL of 0.67% thiobarbituric acid, 5µL of 10 mM butyl-hydroxytoluene (BHT), 300µL of 1% O-phosphoric acid and 55µL of distilled water. This was followed by a 45 mins incubation time at 90°C and the absorbance was measured at 535 nm. The results were expressed as µmol MDA formed per milligram protein.

Determination of acetylcholinesterase activity

Acetylcholinesterase activity was measured using the method previously described by Ellman *et al.* (1961). The reaction mixture contained 30µL of the sample, 1 mM DTNB, 0.1 M of potassium phosphate buffer (pH 7.4) and 0.8 mM acetylthiocholine. Thismixture was monitored for 2 min (at 30 s interval) at 412 nm. The enzyme activity was then evaluated as µmol of acetylthiocholine hydrolyzed per min per milligram protein.

2.8. Statistical analysis

Statistical analysis was carried out using the GraphPad Prism 7.0 software. Data was presented as Mean ± SEM. One-way Analysis of variance (ANOVA) was used to assess the significant differences among multiple groups under various treatments, followed by a Turkey's multiple comparison post hoc test. A *P value* ≤0.05 was taken as statistically significant.

RESULTS

3.1. Survival rate of flies exposed to $AlCl_3$ and Vitamin E

There was a significant decrease in survival rate of flies exposed to 40mMol concentration of $AlCl_3$ (68%) when compared with those of the control (92%) ($P < 0.05$). In flies co-treated with 40mM of $AlCl_3$ and 2.5mg of Vitamin E, there was a significant improvement in the survival rate of flies (77%) ($P< 0.05$) when compared with those treated with $AlCl_3$only (68%). No significant difference was observed in the percentage survival rate of flies treated 2.5mg of Vitamin E (87%) when compared with the control (92%) ($P>0.05$).

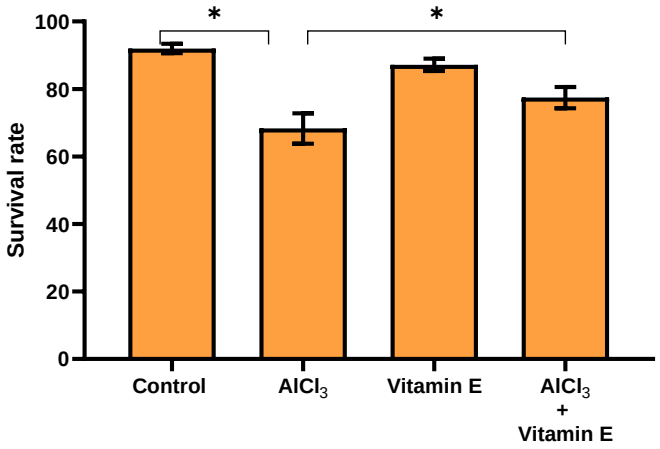

Figure 1: Percentage representation of the effect of $AlCl_3$ and Vitamin E on the survival rate of flies. Data are presented as mean SEM (n = 5, 50 flies/vial). * $P< 0.05$

3.2. Effect of $AlCl_3$ and vitamin E on the locomotor performance (climbing activity) of flies (negative geotaxis)

There was a significant reduction in climbing activity of flies treated with $AlCl_3$when compared with those of the control ($P< 0.05$). However, in flies that were co-administered with 40mMol of $AlCl_3$ and 2.5mg of Vitamin E, a significant improvement in the climbing activity was observed when compared with flies treated with $AlCl_3$ only. No significant difference was observed in flies that were treated with Vitamin E only when compared with the control ($P>0.05$).

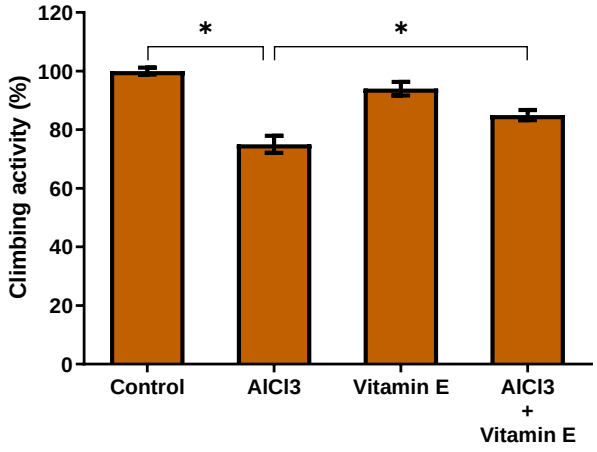

Figure 2: Effect of $AlCl_3$ and Vitamin E on climbing activity of flies. Data are presented as mean ±SEM (n = 5, 50 flies/vial).* $P< 0.05$

3.3. Malonaldehyde concentration in flies treated with $AlCl_3$ and vitamin E

MDA concentration was significantly increased in flies treated with $AlCl_3$ only ($P<0.05$). In the group that was treated with vitamin E only, there was no significant difference in MDA activity when compared with the control ($P>0.05$). In flies co-administered with 40mM of $AlCl_3$ and 2.5mg of Vitamin E, there was a significant reduction ($P<0.05$) in MDA concentration when compared with flies that receive $AlCl_3$ only.

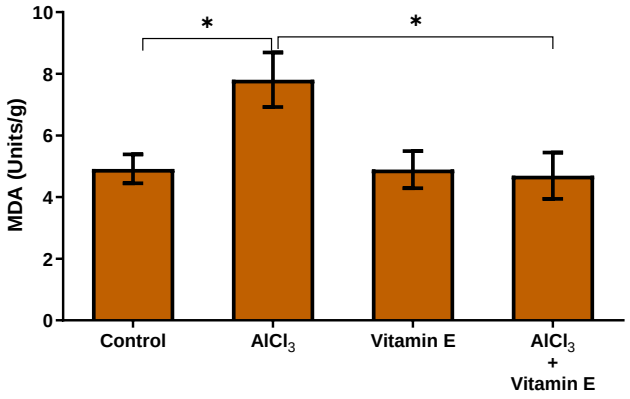

Figure 3: Effect of$AlCl_3$ and Vitamin E on malonaldehyde concentration. Data are presented as mean ± SEM (n = 5, 50 flies/vial). * $P< 0.05$

3.4. Superoxide dismutase (SOD) activity of flies treated with $AlCl_3$ and Vitamin E.

There was a significant decrease in SOD activities in flies treated with $AlCl_3$ only when compared with those of the control ($P< 0.05$) while in flies that were co-administered with40mM of $AlCl_3$ and 2.5mg of Vitamin E, a significant increase in SOD ($P< 0.05$) was observed. There was no significant difference in SOD activity in flies that received 2.5mg of Vitamin E only when compared with those of the control. No significant difference was observed in flies treated with Vitamin E only and the control ($P>0.05$).

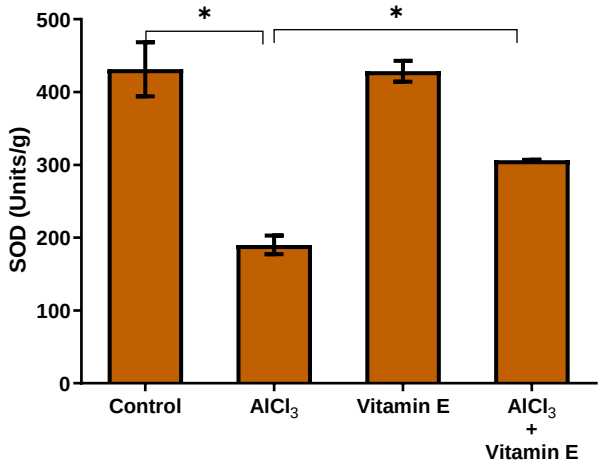

Figure 4: Effect of$AlCl_3$ and vitamin E on superoxide dismutase activity. Data are presented as mean ± SEM (n = 5, 50 flies/vial). * $P< 0.05$

3.5. Catalase (CAT) activity of flies treated with $AlCl_3$ and Vitamin E.

$AlCl_3$ significantly decrease the catalase activity of flies when compared with those of the control ($P< 0.05$). In flies co-treated with 40mM of $AlCl_3$ and 2.5mg of Vitamin E, there was a significant increase in catalase activity when compared with those that received $AlCl_3$ only ($P< 0.05$). No significant difference in catalase activity in flies that received Vitamin E only when compared with those of the control.

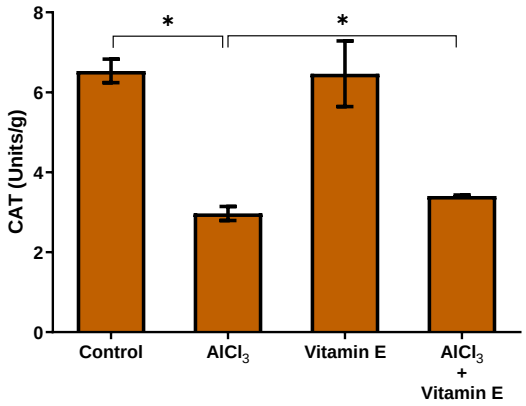

Figure 5: Effect of$AlCl_3$ and Vitamin E on catalase (CAT) activity. Data are presented as mean ± SEM (n = 5, 50 flies/vial). * $P< 0.05$.

3.6. Glutathione-S-transferase (GST) activity of flies treated with $AlCl_3$ and Vitamin E

GST activity was significantly reduced in flies treated with $AlCl_3$ only when compared with those of the control ($P< 0.05$). However, there was a significant increase in GST activity in flies co-treated with 40mM of $AlCl_3$ and 2.5mg of Vitamin E ($P>0.05$) when compared with those treated with $AlCl_3$ only. No significant difference was observed in flies treated with Vitamin E only and the control ($P>0.05$).

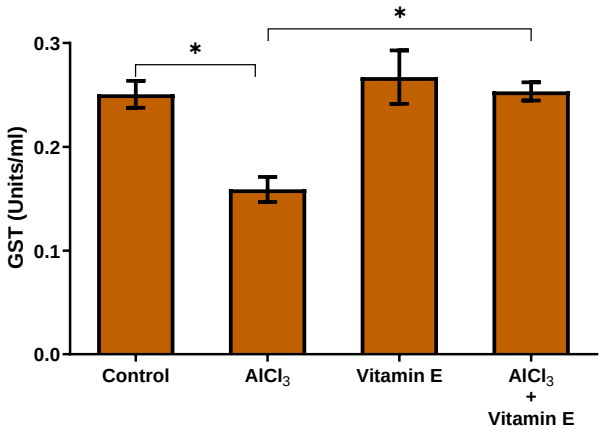

Figure 6: Effect of$AlCl_3$ and Vitamin E on Glutathione S-transferases (GSTs) activity. Data are presented as mean ± SEM (n = 5, 50 flies/vial). * $P< 0.05$

3.7.Acetylcholinesterase (AChE) activity of flies treated with $AlCl_3$ and Vitamin E

There was a significant increase in AChE activity in flies treated with 40mMol of $AlCl_3$ only when compared with those of the control ($P<0.05$). However, in flies co treated with $AlCl_3$ and 2.5mg of Vitamin E, there was a significant reduction in AChE activity ($P<0.05$) when compared to flies that were treated with $AlCl_3$ only.

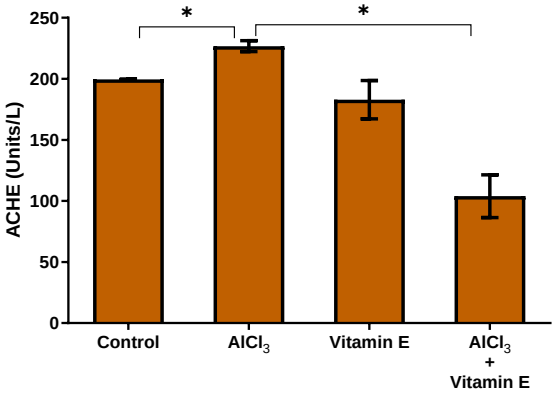

Figure 7: Effect of $AlCl_3$ and Vitamin E on acetylcholinesterase (AChE) activity. Data are presented as mean ± SEM (n = 5, 50 flies/vial). * $P< 0.05$

DISCUSSION

Aluminium toxicity has been linked with several neurodegenerative diseases including AD (Bondy, 2010). Oxidative stress and cholinergic impairment have been strongly implicated in the pathogenesis of AD. Cholinergic impairment results in the decline of acetylcholine in synapses of the brain and as such, current therapeutic methods have been focused on augmenting acetylcholine levels in the brain either through blocking its hydrolysis by using AChE inhibitors or by using cholinomimetic substances (Prerna, 2010) to improve cholinergic transmission. The unwanted side effects of AChE inhibitors have led to the search of alternative compounds with anticholinesterase and antioxidant potential. Hence, this study was carried out to investigate the anticholinesterase potential and antioxidant effect of Vitamin E in $AlCL_3$ Induced-toxicity model of Alzheimer's disease in *Drosophila Melanogaster*.

To investigate the impact of $AlCl_3$on cholinergic transmission and a possible anti-cholinesterase activity of vitamin E, the effects of $AlCl_3$ and Vitamin E on the activity of acetylcholinesterase (AChE); an enzyme which plays a pivotal role in cholinergic neurotransmission was evaluated. AChE activity was significantly increased in $AlCl_3$ treated flies. This is in agreement with previous studies by Zatta *et al*., (2002), Adedayo *et al*. (2020) and Ogunsuyi *et al*. (2020). The significant increase in AChE activity could result in a possible reduction in acetylcholine in synapses resulting in impaired cholinergic transmission. Acetylcholine plays an important role in memory, learning, locomotion and motor function (Day *et al.*, 1991) and this decrease in acetylcholine could also be responsible for the impaired locomotion also observed in this study in flies treated with $AlCl_3$. Remarkably, in this study, in flies co-treated with $AlCL_3$ and Vitamin E, the later was able to significantly reduce AChE activity in these flies thus indicating that Vitamin E hasanti-cholinesterase potential. The significance of this finding is that by Vitamin E could potentially improve acetylcholine levels and availability in synapses and consequently improve cholinergic neurotransmission. This anticholinesterase potential of Vitamin E is suggestive that Vitamin E could be beneficial in the management of Alzheimer's disease. Similar findings have been observed and reported by Thomé *et al.* (2011) where they reported that Vitamin E decreased acetylcholinesterase activities in the brain of rats exposed to diluted sidestream smoke.

Since oxidative stress also play a crucial role in the pathogenesis of Alzheimer's disease (Zhao and Zhao, 2013), oxidative stress markers and endogenous antioxidant enzymes (SOD, CAT and GST) were evaluated in flies. MDA is a marker of lipid peroxidation and indicator of oxidative stress (Ayala et al. 2014). A significant increase in malonaldehyde (MDA) concentration was observed in flies treated with $AlCl_3$, a finding which is in agreement with those from previous studies by Exley, (2004) and Adedayo *et al. (*2020). Aluminium chloride is able to stimulate increased production of reactive oxygen species (ROS) which would result in the induction of iron-mediated lipid peroxidation (Exley, 1999; 2004). Vitamin E was able to ameliorate $AlCl_3$ increased MDA concentration in flies thus justifying their ability to neutralizes peroxyl radicals and reduce lipid peroxidation. Several authors have reported similar findings on the ability of Vitamin E to mop up free radicals and reduce MDA [Morris *et al*., 2005, Thomé *et al.,* 2011 and Niki, 2014]. Morris *et al*. (2005) specifically reported that Vitamin E was able to decrease lipid peroxidation susceptibility by 60% in AD patients when compared with the controls in their study.

Superoxide dismutase (SOD) serves as the first gatekeeper of the antioxidant defense system (Oyebode *et al.,* 2020). Superoxide dismutase catalyzes the dismutation of superoxide anion to hydrogen peroxide (H_2O_2) which is in turn then converted to water (H_20) and oxygen (O_2) by the action of catalase. In this study, $AlCl_3$ significantly inhibited SOD activities in flies. A similar finding has also been previously reported by Oboh et al. (2020). The reduction in SOD is due to the pro-oxidant effect of $AlCl_3$ leading to increase production of reactive oxygen species (superoxide anions) which are then converted to H_2O_2 by SOD. In flies co-treated with $AlCl_3$ and Vitamin E, a significant increase in SOD activity was observed. This finding is indicative of the antioxidant nature of vitamin E which prevents oxidative damage by scavenging free radicals (Matrková and Remeš, 2014) hence protecting endogenous antioxidants

(SOD, CAT AND GST) from the impacts of reactive oxygen species (ROS). The antioxidant property of Vitamin E has been reportedly related to the hydroxyl group of its aromatic ring, which donates hydrogen to neutralize free radicals (Wang and Quinn, 1999; Brigelius-Flohé, 2009).

Catalase is a hem-containing enzyme that catalyzes the conversion of H_2O_2 into oxygen and water, thus reducing the risk associated with oxidative stress mediated damage (Pigeolet *et al.*, 1990; Abolaji *et al.*, 2017). In this study, there was a significant reduction in catalase activity in flies treated with $AlCl_3$. This reduction in catalase activity indicates an increase vulnerability or susceptibility of these flies to oxidative stress and damage (Fridovich and Freeman, 1986). In this study, a significant increase in catalase activity was observed in flies co-treated with Vitamin E and $AlCl_3$when compared with those treated with $AlCl_3$ only thus further justifying the free radical scavenging properties and antioxidant potential of Vitamin E. Similar findings on the ability of Vitamin E to improve CAT and SOD activities following $AlCl_3$ has been previously reported in rats by Lablack *et al.* (2020).

Glutathione-S-transferases (GST) are group of enzymes that detoxify both endogenous compounds and foreign chemicals such as pharmaceuticals and environmental pollutants (Nerbert and Vasiliou, 2004). They catalyze the conjugation of GSH with toxic products of phase I detoxification thereby converting them to less harmful forms in order to minimize oxidative damage in tissues (Abolaji *et al.*, 2015). GST also play vital roles in the regulation of processes involved in the survival of organisms to oxidative stress (Farombi *et al.*, 2018). In this study, $AlCl_3$ reduced GST activities in flies as previously reported by in other studies with flies (Oyetayo *et al.*, 2020) and rats (Katyal *et al*. 1997). This reduction in GST activity insinuates the impairment of the flies' ability to completely detoxify $AlCl_3$ and combat oxidative stress. In this study, however, Vitamin E was able to improve GST activity in flies thus further establishing its antioxidant potential and ability to protect against oxidative damage by ROS. This improvement of GST activity by vitamin E following $AlCl_3$ has also been previously reported by El-Demerdash (2004).

Impaired locomotion (negative geotaxis) is a marker of neurodegeneration (Oyetayo *et al.*, 2020) and a feature of AD. As observed in this study, flies treated with $AlCl_3$ exhibited locomotor deficit with reduced climbing activity (negative geotaxis).This finding is in agreement with those from previous studies in flies (Oboh *et al.*, 2020; Oyetayo *et al.*, 2020) and in rats (Erazi et al., 2010; Nampoothiri *et al.* 2015).The impaired locomotion seen in $AlCl_3$ treated flies can be attributed to impaired cholinergic transmission earlier reported in this study. Acetylcholine plays an important role in the regulation locomotion and motor function (Day *et al*., 1991) and alteration in AChE activity can affect acetylcholine availability andinterrupt locomotion activity (Halmenschelager and Rocha, 2018). In this study, Vitamin E was able to improve locomotor performance (climbing activity) and ameliorate locomotion deficits seen in $AlCl_3$ treated flies. This improvement in locomotion by Vitamin E can be linked to its anti-cholinesterase activity earlier reported in this study.

In this study, $AlCl_3$ significantly reduced the survival rate of flies. This finding is indicative of its toxic effect and agrees with similar findings in flies by Kijak *et al.* (2014) of the toxic effect on $AlCl_3$ on the survival rate of flies. Impaired cholinergic neurotransmission and reduction in antioxidant enzymes in $AlCl_3$ treated flies has been previously reported to be responsible for the decreased survival rate of these flies (Oboh *et al.*, 2020). However, when vitamin E was used together with $AlCl_3$, amelioration of $AlCl_3$-induced mortality can be reasonably attributed to the presence of the vitamin E. Vitamin E is known to increase endogenous antioxidant enzymes and improve cholinergic transmission in flies

CONCLUSION

The results from this study have shown that vitamin E possesses antioxidant and anticholinesterase properties and could be of therapeutic benefits against $AlCl_3$ induced toxicity and associated diseases like Alzheimer's disease. Also, further studies investigating the effect of Vitamin E on amyloid β-protein (Aβ) and tau proteins which are also pathological hallmarks or features of

Alzheimer's will further strengthen its use in the management of Alzheimer's disease.

Acknowledgements

The authors wish to acknowledge Mr. James Osakue and Mr Ayodele Abayomi for their assistance during this research.

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