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Original Research Article

CATECHIN MITIGATES OXIDATIVE STRESS, NEUROINFLAMMATION, AND CONNECTOME DEGENERATION IN A *Drosophila melanogaster* MODEL OF VINCRISTINE-INDUCED NEUROTOXICITY

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ABSTRACT

Millions of people worldwide suffer from Parkinson's disease, typified by death of dopaminergic neurons in the substantia nigra, which results in motor dysfunction and other crippling symptoms. Vincristine is an important substance in anticancer treatments, and misuse or prolonged use can lead to Parkinson's disease symptoms. Catechin, being part of the flavonoid polyphenol group, has strong anti-inflammatory and antioxidant qualities and is usually added to diets and taken as a supplement. This study aims to evaluate the protective effects of catechin against vincristine-induced neurotoxicity in a Drosophila melanogaster model, with a specific focus on reducing oxidative stress, suppressing neuroinflammation, and preventing connectome degeneration. Three-day-old flies were split into 5 separate groups of thirty flies per group for 7 days. During the 7-day period of survival tests, the flies were given varying amounts of vincristine (1ml, 2ml, and 3ml/diet) and catechin (50, 100, 200 µM). The flies were therefore exposed to 200 µM catechin and 3ml of vincristine for another 7days. Neurobehavioral assays, including crawling, climbing, phototaxis, and chemotaxis, were assessed. Biochemical analyses, utilising nitric oxide and MDA as oxidative stress markers, were also conducted. Additionally, microanatomical studies using H&E were performed. In flies exposed to vincristine-induced toxicity, a significant elevation of oxidative stress and a decrease in antioxidant level was observed. When catechin was introduced, the result was reversed. Histologically, there was neurodegeneration and expression of pyknotic cells in vincristine-induced parkinsonism but when co-treated with catechin, the cells were preserved. These findings demonstrated catechin as a promising neuroprotective and neurodegenerative agent by ameliorating vincristine-induced Parkinsonism and by regenerating the pyknotic cells in the *Drosophila melanogaster* model.

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INTRODUCTION

Parkinson's disease PD) is a common neurodegenerative disorder characterized by tremor, rigidity, and bradykinesia, with mobility limitation appearing in some patients as the disease progresses. Also, it is related to autonomic dysfunction, neurobehavioral disorders and cognitive impairment [1]. PD is caused by the degeneration of **dopaminergic neurons** in the **substantia nigra and the**

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presence of Lewy bodies, leading to reduced dopamine levels in the brain, which affects movement coordination. The chance of developing PD increases with age, affecting about 0.5-1% of persons aged 65-69 and 1-3% of those 80 and older [2].

Vincristine is a chemotherapeutic drug which is used to treat acute lymphoblastic leukaemia, Hodgkin's disease, non-Hodgkin's lymphoma, neuroblastoma, etc. [3] Vincristine, a chemotherapeutic agent, has been shown to induce neurotoxicity in rats, leading to Parkinsonism-like symptoms [4]. This is consistent with previous studies that have shown that vincristine can cause neurotoxicity in both animal models and humans [5][6]. Recent studies demonstrate that vincristine is a potential inducer of Parkinsonism, as it shows characteristics such as bradykinesia, stiffness, tremor, and postural instability [7].

Vincristine elicits its cytotoxicity via interaction with tubulin by preventing assembly into microtubules, interfering with the formation of the mitotic spindle [8]. While effective for the purpose of targeting fast-proliferating tumour cells, this practice concurrently causes neuronal cell damage that leads to neurotoxicity [9]. Vincristine exerts its neurotoxicity in an intimate relationship with the dynamics of microtubules, critical for intracellular transport and neuronal integrity. The breakdown of microtubules causes neuronal dysfunction and degeneration by affecting axonal transit of vital proteins and organelles [10]. Moreover, vincristine causes mitochondrial stress through enhanced Reactive Oxygen Species (ROS) generation, which further leads to oxidative damage and neuronal death [11].

Neuroinflammation has a great influence on vincristine-induced neurotoxicity. Vincristine was previously shown to activate astrocytes and microglia, leading to the release of pro-inflammatory cytokines such as interleukin-6 and tumour necrosis factor-alpha [12].

These inflammatory mediators accelerate neurodegeneration through synapse dysfunction and neuronal injury. Besides, such glial activation might disturb the integrity of the blood-brain barrier, allowing the permeability for inflammatory agents and toxic metabolites to the central nervous system [13]. A relation between vincristine-induced neurotoxicity and α-synuclein aggregation has also been investigated. The protein α-Synuclein, linked to Parkinson's disease, aggregates abnormally in neurons after exposure to vincristine, showing that vincristine might induce PD-like pathology [14]. Aggregation in the substantia nigra can lead to disruption of synaptic transmission and eventually neuronal death [15]. Compared to idiopathic PD, vincristine-induced Parkinsonism may have a more rapid onset and is potentially reversible after discontinuation of treatment [16].

Catechins, a polyphenolic compound, are the flavanols of the flavonoid family found in many plants. The dietary source of these compounds is mainly cocoa products, wine and Green tea. Catechins possess a powerful antioxidant property, though they can be a pro-oxidant in cells. Therefore, Catechins may offer a therapeutic effect against diseases caused by oxidative stress due to the antioxidant properties they possess [17].

Drosophila melanogaster, commonly known as "fruit fly", is an interesting model system that has more than 65-70% of human disease-causing genes, making it an ideal model organism for studying neurodegenerative diseases [18].

In this study, we assessed the protective effects of catechin against vincristine-induced neurotoxicity in a Drosophila melanogaster model, with a specific focus on reducing oxidative stress, suppressing neuroinflammation, and preventing connectome degeneration.

MATERIALS AND METHODS

Animals Used and Maintenance

Drosophila melanogaster (Harwich strain) gotten from the Drosophila Research Laboratory, University of Ibadan, were used in this study. The flies were maintained and reared in Eagles' research laboratory, LAUTECH, on cornmeal medium containing (3.08% w/v corn flour, 2% w/v brewer's yeast, 1% w/v agar, 0.08% w/v nipagin and 93.92% distilled water) at constant temperature (22-24ºC) and humidity (60–70 %) under 12 h dark/light cycle conditions [19].

Chemicals

Catechin was purchased from Sigma, USA. Vincristine injection was purchased under Doctor's prescription from Kunle Ara Pharmacy, Ibadan, Oyo state, Nigeria.

The survival (pilot) rate of flies after drug exposure

To evaluate the concentration as well as duration of treatment with drugs, flies (male and female) that were within the range of a day to three days old were split into groups of which each contains 30 flies. control group (containing 2.0% distilled water), vincristine was administered at (1ml/100ml diet, 2ml/100ml diet and 3ml/100ml diet, respectively), Catechin at (50, 100 and 200 µM) for seven days. Mortality was recorded daily. Afterwards, analysis of the surviving flies was conducted and plotted as a percentage. According to the result of the data analyzed, 200 µM of catechin and 3ml/100ml diet of vincristine was chosen as our lethal dose.

Treatment of *Drosophila melanogaster*

D. melanogaster a day to three days old, 30 flies in a vial (n = 5) were treated with Catechin and Vincristine at selected doses. Flies in group A which is the control group received diet with 2.0% distilled water, the flies in Group B were given diet mixed with vincristine at 3ml/100ml diet, those in Group C flies were given diet containing Catechin at 200 µM, Group D flies were treated with diet containing Vincristine co-treated with catechin (3ml/100ml diet and 200 µM), Group E flies were treated with vincristine for 3 days and Catechin for 4 days (3 ml/100 ml diet and 200 µM).

Climbing rate of the flies

The climbing activities of control and treated flies was conducted utilizing the negative geotaxis assay [20]. 10 flies of

control, vincristine, catechin, vincristine + catechin and vincristine (pre)+ catechin (post) were immobilized using ice anesthesia placed in a labelled vertical vial (15 x15cm) separately. Once they regained their activeness after the ice exposure (for about 20 min), the vial was gently shaken for the flies to be at the bottom of the vial, and the number of flies that climbed up to the marked part of the vial (20 cm high from the base) for 20 seconds was recorded. This procedure was done three times at one minute interval [21].

Crawling Analysis

The crawling rate of treated *Drosophila melanogaster* larva were evaluated. The larva was allowed to crawl around in a shaded petri dish for 5 minutes; the analysis was repeated twice for each group. The crawled traces were then measured using ImageJ software.

Phototaxis

This was carried out according to [22] with some modifications. A connected vial (diameter, 2.5cm; height, 9.5 cm) and test tube (20 cm) were used, with a light source (15 W) creating a gradient from ~3,000 lux to ~700 lux. 10 flies in each group were placed in the vial, sealed with cotton, and acclimatized to darkness for 30 minutes. Following acclimatization, the vial was gently shaken for the flies to be at the bottom of the vial. The setup was divided into a light-exposed section and a dark section using a black opaque covering. Upon illumination, the number of flies that migrated to the light-exposed side was recorded after 2 minutes.

Chemotaxis

The test was conducted in a dark room 15 cm away from a visible-light source parallel to the vial, and it was turned on to provide visibility during the whole trial. Ten flies were put in a screw-capped vial that was 2.5 x 9.5 cm in diameter and 20 cm long. The vial was left in the dark room for half an hour, parallel to the light source. The flies were then dispersed at random. After that, the screw cap was carefully taken off, the cover was filled with volatile repellent and benzaldehyde, and a screw cap holding 1 milliliter of 100 mM benzaldehyde dissolved in 1.5% agar was put back in. After that, for ten minutes, the flies in the third segment of the vial were recorded every 2 mins [22].

Sample preparation for biochemical assays

For biochemical assays evaluation, 50 flies (containing both male and female) were treated with Catechin, vincristine, vincristine co-treated with catechin, and controls (containing distilled water) in **the** diet for 7 days as described in the survival analysis section. On the 8th day, the flies were immobilized in ice, weighed, then homogenized in 0.1M phosphate buffer with **a** pH of 7.0, and centrifuged at 4000rpm for 10 minutes at 4 ºC in a Biofuge Sorvall Fresco centrifuge HRH-CERID LAUTECH. Subsequently, the supernatant was aliquoted into labelled micro-centrifuge tubes and was used for the determination of the activities of superoxide dismutase (SOD), acetylcholinesterase (AChE), malonaldehyde (MDA), glutathione (GSH), catalase (CAT), and nitric oxide (NO) levels. Notably, all the assays for each of the 3 replicates of the flies were done in duplicates.

Protein concentration determination

Protein concentration of the samples **was** determined using **the** Biuret method and utilizing Bovine Serum Albumin (BSA) as standards **described** by [23].

Acetylcholinesterase activity determination

The acetylcholinesterase activity was determined using the method of [24]. The test was carried out using: 1 mM DTNB, 0.1M phosphate buffer with **a** pH of 7.0 and 0.8 mM acetylthiocholine.

The reaction was examined for 2 minutes (30s interval) at a w avelength of 412 nm. The enzyme activity was estimated as µ mol of acetylthiocholine hydrolyzed/minute/mg protein.

Nitrite levels determination

Nitrite was quantified as an indicator of nitric oxide (NO) production according **to** the Griess method as described by [25]. A freshly prepared Griess reagent was achieved by mixing equal volumes of 1% sulphanilamide (in 5% phosphoric acid) and 0.1 % N-(**1-naphthyl**) ethylene diamine dihydrochloride. 50 µL of 2X diluted supernatant was further diluted with 50 µL of distilled water in a microtitre plate before incubation with Griess reagent (100 µL) in the dark for 10 minutes at room temperature. The nitrite concentration was evaluated from the sodium nitrite standard curve and expressed as µM nitrite/mg protein.

Glutathione Level Determination

Glutathione (GSH), an antioxidant maker, was quantified in tissue homogenate supernatant [26]. Briefly, 100 µL of the supernatant was diluted twenty times diluted in 0.15M Tris-KCl buffer, and deproteinized with 500 µL of trichloroacetic acid (30%). The cocktail was centrifuged in a bench-top centrifuge at 4000 rpm at room temperature for 10 minutes. Deproteinized supernatant (100 µL) was mixed with 100 µL of 0.0006M 5', 5' -Dithios-nitrobenzoic acid (DTNB) in the microplate.

Lipid peroxidation level determination

The quantification of Malondialdehyde was done as an index of lipid peroxidation using thiobarbituric reacting substances (TBARs) assay of by the method of [27]. 100 µL of the supernatant was diluted twenty times in 0.15M Tris-KCl buffer, and deproteinized with 500 µL trichloroacetic acid (30%). The mixture was centrifuged in a bench-top centrifuge at 4000 rpm at room temperature for 10 minutes. 200 µL of the supernatant was aliquoted into a micro-centrifuge tube, followed by the addition of 200 µL thiobarbituric acid (0.75%). The cocktail was heated for 1 hour at 80 °C. The tubes were cooled using ice, and 200 µL was aliquoted in a microtitre plate, and the absorbance was measured at 532 nm wavelength. The

concentration of TBARS in the tissues was expressed as ηmol MDA/ mg protein.

Catalase activity determination

Catalase activity of the flies supernatants was carried out as described by [28]. Briefly, 50 µL of 2X diluted supernatant was pipetted into a microtitre plate, then 50 µL of the cocktail containing 65 mmol/mL of H_2O_2 in sodium-potassium phosphate buffer (60 mM, pH 7.4) was added. The enzymatic reaction was incubated for 3 minutes and terminated with 100 µL of ammonium molybdate (64.8 mM) in sulfuric acid. The absorbance at wavelength 405 nm was measured. The catalase enzyme activity unit was expressed as U/ mg protein.

Superoxide Dismutase Determination

The SOD activity was carried out by the method of [29]. Superoxide dismutase activity is evaluated based on its inhibition ability of autoxidation of adrenaline in sodium carbonate buffer at pH 10.7. 50 µL of 2X diluted supernatant was pipetted into a microtitre plate containing 150 µL of carbonate buffer. The reaction started when the addition of freshly prepared 0.3mM adrenaline (30 µL) to the solution. 50 µL of distilled water was used as a blank sample. The changes in absorbance at 495 nm were recorded every 60 seconds for 1minutes 20 seconds. The activity of SOD was expressed as U/mg protein.

Immunostaining of the brain of *D. melanogaster*

For histological examination under a light microscope, treated flies were kept in 10% neutral buffered formalin. The brains were prepped for hematoxylin and eosin staining following deparaffinization. Pathologists then used a Binocular light microscope to inspect the slides and gave their interpretation while keeping the treated and control flies hidden [30].

Statistical Analysis

All the experiments were done in at least 3 replicates. The data are shown as the Mean ± SEM. A One-way Analysis of variance (ANOVA) was utilized to check for the significant differences among the groups under different treatments, followed by Tukey's post hoc and Fisher's LSD test where appropriate. For all groups, p-values < 0.05 were considered statistically significant. "a" represents significance against the control (Distilled water) group, "b" represents significance against the vincristine group, while "c" represents a significant difference in relation to Catechin using the GraphPad Prism5.0 software.

RESULTS

Vincristine reduced the survival rate of the flies, while catechin increased the survival rate after exposing them to the treated diet for seven days.

To opt for the concentrations of vincristine and catechin to be utilized in the main study, we exposed flies to varying concentrations of VCR (1ml/100ml diet, 2ml/100ml diet and 3ml/100ml diet, respectively) and CAE (50, 100 and 200 µM) to evaluate the survival rate for seven days. As shown in Figure 1, a 7-day exposure of flies to VCR caused significant reductions in survival rates when compared with the control (Figure 1A). The reduction was most pronounced in the 3ml/100ml diet group. Exposure to CAE 3 (200µM) increased the survival rate of *D. melanogaster* compared to other doses, CAE 2 (100 µM) and CAE 1 (50µM), and control, as shown in Figure 1 B.

Neurobehavioral Results

Climbing activity and crawling

As shown in Figure 2A, vincristine did not significantly impair the climbing ability of *Drosophila melanogaster* compared to the control group, with no notable differences observed across treatment groups. However, vincristine significantly reduced the crawling ability of third instar larvae compared to the control, as depicted in Figure 2 B. In contrast, catechin treatment significantly improved the crawling ability relative to the vincristine group. However, co-treatment with catechin does not improve the crawling rate compared to the control group. Rather, a significant decrease was observed when compared to the catechin group. The distances covered by the larvae are presented in Table 1.

Phototaxis

As depicted in Figure 3, Vincristine exposed group decreases in the number of flies moving towards the light on the left side compared to the control group, whereas the co-treatment with catechin group have significant increase in the number of flies moving towards the light on the left side compared to the control and vincristine groups. The movement of the flies towards the light on the right side follows a similar trend to that of the left side, although no significant differences were observed. This suggests a visual impairment of vincristine and the rescue potential of catechin.

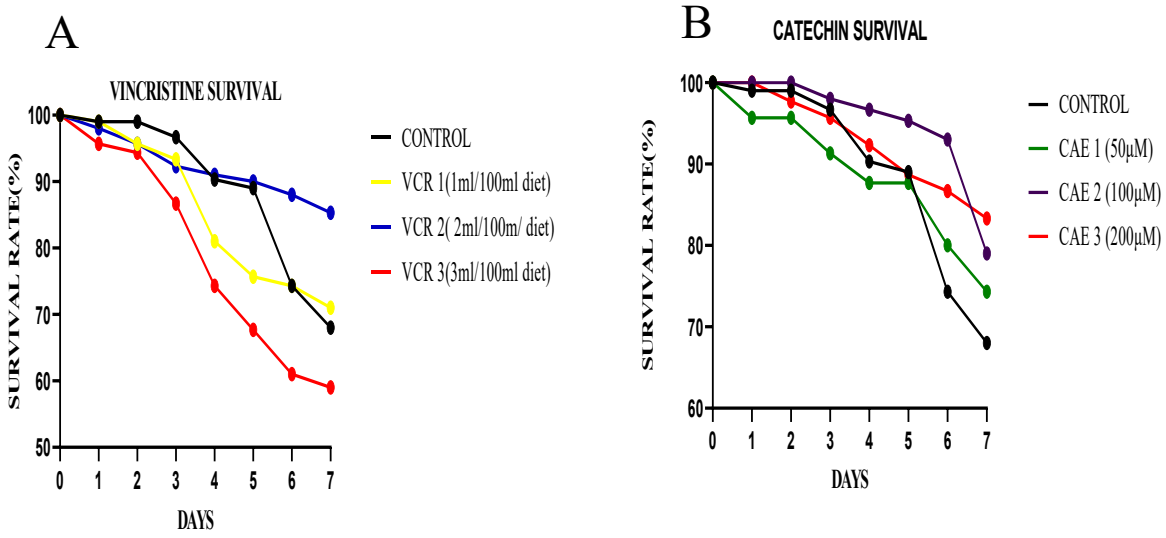

Figure 1: Survival rate of *D. melanogaster* exposed to Catechin (A) and Vincristine (B) for 7 days. CAE: Catechin, VCR: Vincristine.

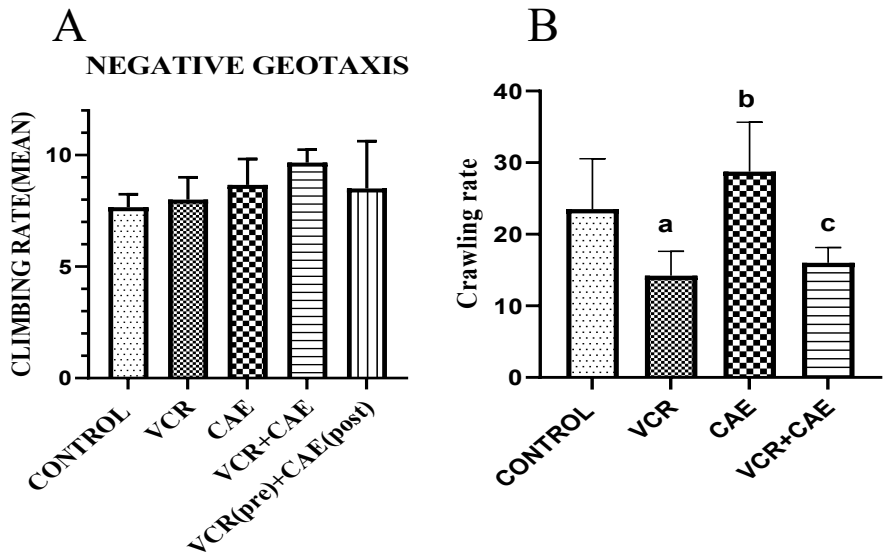

Figure 2: Quantitative data illustrating the effect of vincristine and catechin on the climbing rate (A) and crawling rate (B) of *Drosophila melanogaster* following 7 days of exposure to vincristine and co-treatment with catechin are presented.
Data for climbing rate (A) is expressed as Mean ± SEM from 30 flies per vial, with three replicates per treatment group. Data for the crawling rate (B) is expressed as Mean ± standard error of the mean (SEM) from 10 flies per vial, also with three replicates per treatment group. Statistical differences among the groups were shown as follows: "a" denotes a significant difference compared to the control group ($p < 0.05$), "b" indicates a significant difference compared to the vincristine group, and "c" represents a significant difference compared to the catechin group. VCR: Vincristine and CAE: Catechin.

Table 1: Illustration of the effect of vincristine and catechin on the crawling rate of third instar larvae after 7 days of exposure.

Groups	CTRL	VCR	CAE	VCR+CAE
Distance crawled (cm)	25	10	17	16

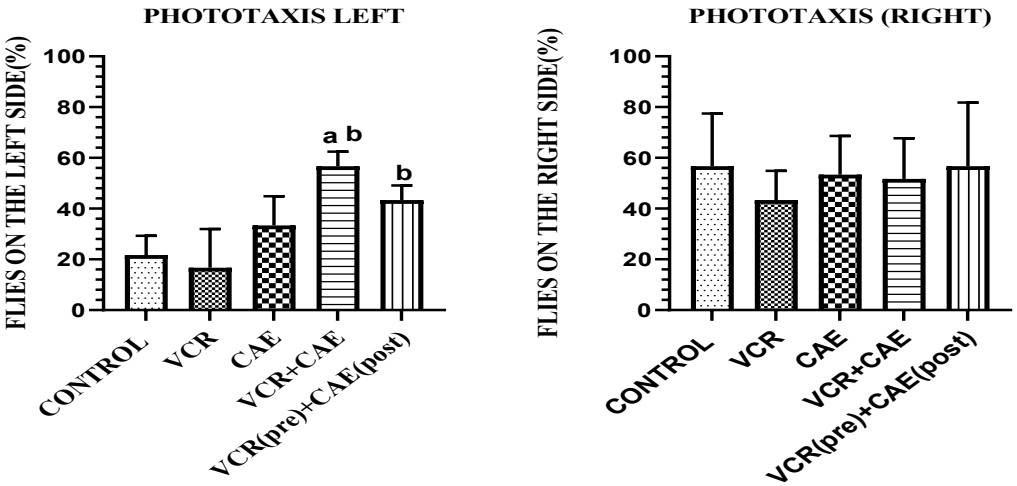

Figure 3: Quantitative data illustrating the effect of vincristine and catechin on the phototaxis response of the adult flies of *D. melanogaster* following 7 days **of** exposure to vincristine and co-treated with catechin. Data are expressed as Mean ± standard error of the mean (SEM) from 10 flies per vial**,** and the process was done on both sides. Statistical differences among the groups were shown as follows: "a" denotes a significant difference compared to the control group ($p < 0.05$), "b" indicates a significant difference compared to the vincristine group.

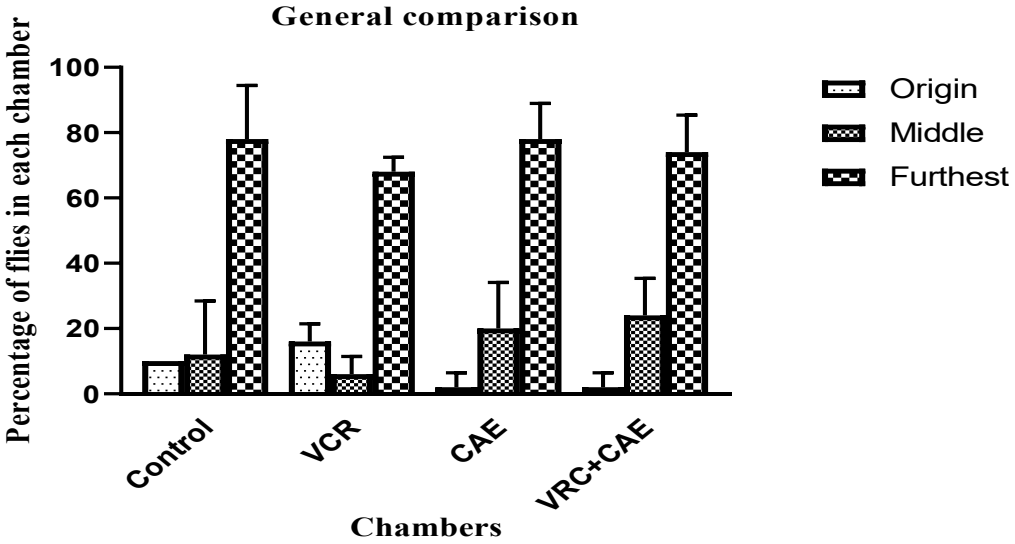

Figure 4: Quantitative data illustrating the effect of vincristine and catechin on the chemotaxis response of the adult flies of *D. melanogaster* following 7 days of exposure to vincristine and co-treated with catechin. Data are expressed as Mean ± standard error of the mean (SEM) from 10 flies per vial, and the process was carried out for 10 mins. In all the groups, differences with a p-value < 0.05 were considered statistically significant. Statistical differences among the groups were shown as follows: '*' represents significant, '**' represents moderately significant, '***' represents highly significant, and '****' represents too significant.

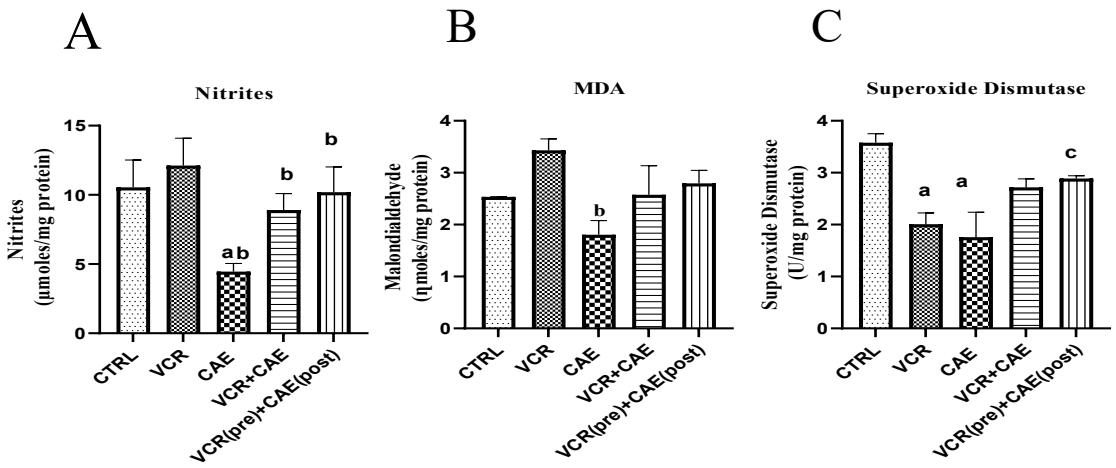

Figure 5: Effect of Vincristine and Catechin on NO level (A), Malondialdehyde MDA (B), and SOD level (C) in *D. melanogaster* exposed to Vincristine-induced toxicity for 7 days. Data are expressed as Mean ± SEM of 50 flies/vial with three replicates per treatment group. Statistical differences among the groups were shown as follows: "a" denotes a significant difference compared to the control group ($p < 0.05$), "b" indicates a significant difference compared to the vincristine group, and "c" indicates a significant difference compared to the catechin group. VCR: Vincristine; CAE: Catechin; NO: Nitric oxide; MDA: Malondialdehyde; SOD: Superoxide dismutase.

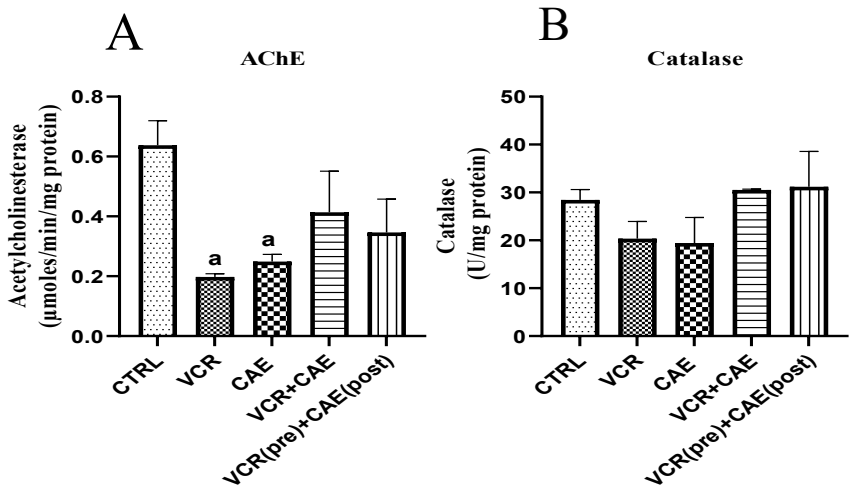

Figure 6: Effect of Catechin and Vincristine on Acetylcholinesterase (AChE) (A) and Catalase Enzyme Activity (B) in *Drosophila melanogaster* Following a 7-Day Exposure to Vincristine-Induced Toxicity. Data are presented as mean ± standard error of the mean (SEM) from 50 flies per vial, with three independent replicates per treatment group. Statistical differences among the groups were shown as follows: "a" denotes a significant difference compared to the control group ($p < 0.05$). VCR: Vincristine; CAE: Catechin.

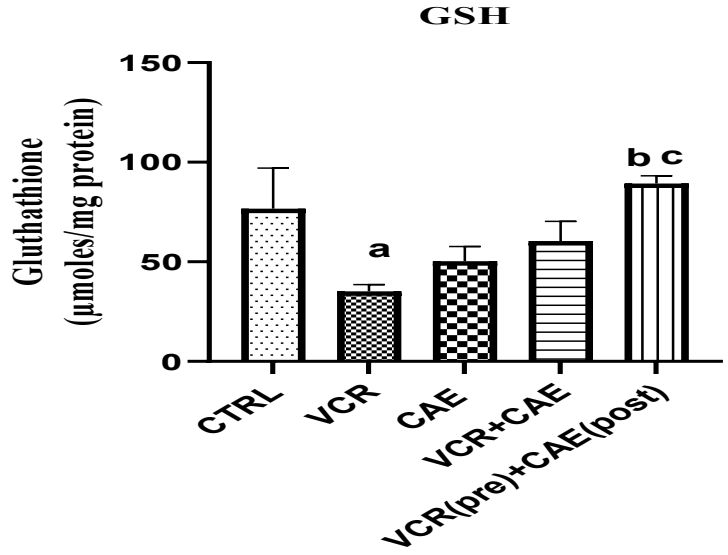

Figure 7: Effect of Catechin and Vincristine on GSH level in *D. melanogaster* exposed to vincristine-induced toxicity for 7 days. Data are presented as mean ± standard error of the mean (SEM) from 50 flies per vial, with three independent replicates per treatment group. Statistical differences among the groups were shown as follows: "a" denotes a significant difference compared to the control group ($p < 0.05$), "b" indicates a significant difference compared to the vincristine group. VCR: Vincristine; CAE: Catechin

Chemotaxis

The ability to sense the irritant agent (benzaldehyde) was observed for each group as illustrated in Figure 4, revealing the number of flies found in the division chamber (Origin, middle and furthest). The combined result**,** as observed in Figure 4 show a notable decrease in the flies found in the furthest chamber away from benzaldehyde compared to other groups, while co-treatment with catechin **maintains** a normal olfaction sensitivity as the control group. This result **suggests** that vincristine may induce olfaction impairment.

Biochemical Results

Nitric oxide (NO), malondialdehyde (MDA), and superoxide dismutase (SOD) Results

Nitric oxide (NO), malondialdehyde (MDA), and superoxide dismutase (SOD) were assessed, as presented in Figure 5. In flies exposed to vincristine-induced toxicity, there was a notable increase, though not significant, in NO (4.46µMol/mg protein) and MDA levels (2.60 nMol/mg protein) compared to the control group (11.15µMol/mg protein and 2.41nMol/mg protein, respectively). However, SOD (1.70 U/mg protein) levels significantly decrease compared to the control group (3.06 U/mg protein). On the contrary, catechin significantly reduces the oxidative stress level in Figure 5 A and B when compared to the vincristine group. Co-treatment with catechin significantly reduces the oxidative level in NO, while a notable reduction was observed in MDA. Also, the SOD level was increased but not significantly when compared to those exposed to vincristine.

Enzymatic Activity Markers

As illustrated in Figure 6, acetylcholinesterase (AChE) (Fig. 6A) and catalase (Fig. 6 B) represent anti-inflammatory and antioxidant enzymes, respectively. No tangible alteration was noticed in catalase activity across the experimental groups (Figure 6 B). However, AChE activity was significantly reduced in the vincristine-treated group (0.17µMol/min/mg protein) and catechin-treated group (0.33µMol/min/mg protein) compared to the control (0.66µMol/min/mg protein) group. Co-treatment with catechin increases the enzymatic activity but not significantly (0.26µMol/min/mg protein and 0.22µMol/min/mg protein, respectively) compared to the vincristine exposed group (0.17µMol/min/mg protein).

Antioxidant Markers

Glutathione (GSH), an established antioxidant marker, is presented in Figure 7. Flies exposed to vincristine-induced toxicity exhibited a significant decrease in GSH levels (35.21 µMol/mg protein) compared to the control group (88.66 µMol/mg protein). Concomitant co-treatment with catechin revealed no significant increase in GSH levels (26 µMol/mg protein). However, serial cotreatment (pre and post) significantly increased GSH levels (66.54 µMol/mg protein) compared to both the vincristine-only (35.21 µMol/mg protein) and catechin-only (72.35 µMol/mg protein) groups.

Histological Results

H&E photomicrograph at 400 X magnification of flies exposed to vincristine and catechin for seven days.

Immunostaning Results (α-synuclein)

α-synuclein immunostaining photomicrograph at X400 magnification of flies exposed to vincristine and catechin for seven days.

DISCUSSION

Tremor, rigidity, and bradykinesia are the hallmarks of Parkinson's disease, a prevalent neurological condition. As the disease worsens, some individuals experience a reduction in their mobility. Additionally, it is linked to cognitive impairment, neurobehavioral problems, and autonomic dysfunction [1]. Lewy bodies and the dopaminergic neurons loss in the striatal tissue, called the substantia nigra are the pathophysiology of PD, which lowers dopamine levels in the brain and impairs motor coordination. Additional symptoms include constipation, frequent periodic limb movements during sleep, mood disturbances, excessive salivation, sleep disruption, and loss of smell [31]. Although neurodegeneration affects cells located in other areas of the neural network as well as nigral dopaminergic neurons, PD is pathologically characterized when the loss of nigrostriatal dopaminergic innervation [2]. *Drosophila melanogaster* is a popular in vivo model for studying neurodegenerative illnesses like AD and is thought to be an effective model for studying ageing-related problems. The short lifespan and ease of handling are the primary causes of this [32]. In view of its effects on neuronal activity, vincristine, which is mainly used in chemotherapy, has demonstrated potential relevance to neurodegenerative illnesses [8]. According to [33], its mechanisms, such as microtubule disruption and axonal transport interference, resemble pathogenic alterations observed in tauopathies and other neurodegenerative illnesses [34], indicating similar pathways with diseases like Parkinson's and Alzheimer's. Furthermore, oxidative stress and mitochondrial dysfunction brought on by vincristine may further connect it to neurodegenerative pathways outside of oncology [35]. Its potential as a treatment for various illnesses is still mostly unknown.

Catechin, a plant-derived flavonol family secondary metabolite popularly found in nature [36] has been assumed to protect neurons from severe oxidative stress, perhaps preventing neurodegenerative diseases. Given that dysregulated iron metabolism was recently found to be a major pathogenic feature of Parkinson's disease, epigallocatechin gallate's iron-chelating properties may be essential for its protective effects against neurodegenerative diseases [37]. This implies the possibility of integrating knowledge from neurotoxic and neuroprotective substances to enhance comprehension and maybe address the mechanisms behind neurodegenerative diseases [38]. This study aims to assess catechin's neuroprotective potential in reducing vincristine-induced Parkinsonism in *Drosophila melanogaster*, with an emphasis on dopaminergic neuron maintenance, oxidative stress reduction, and motor function restoration.

This study showed that catechin prolongs the lifespan and enhances the rate of survival of the flies. The anti-ageing property of this compound was not investigated in this study; however, the increased survival rate of the flies implies that this compound possesses anti-ageing properties. Ageing is a progressive biological process that leads to physiological decline and diminished cellular function, resulting in increased vulnerability to age-related diseases such as AD and PD, and ultimately culminating in death [39]. Although the ageing process is complex, studies have shown that a decline in lifespan that is age-related is associated with various factors, such as the generation of reactive oxygen species (ROS) [40]. Vincristine greatly reduced the survival rate and caused the death of most of the *D. melanogaster* [41].

Progressive decline in learning, memory, and motor function are clinical features of Parkinsonism, and neurodegenerative diseases and disorders tend to influence locomotor activity [42]. To find out more about the therapeutic effect of catechin on vincristine-dysfunctional locomotor activities such as crawling and climbing, neurobehavioral tests were performed in this research [5]. The distance *Drosophila melanogaster* larvae covered in each time can be used to measure their locomotor activity. Thus, the capacity to move further was one of the preliminary tests on the impact of Vincristine and Lewy body expression on the region of the brain that is responsible for motor function [43]. Relative to Vincristine diet larvae, third-instar larvae maintained on a diet cultivated by Vincristine and co-treated with Catechin, both concomitantly and serially, showed greater crawling activity accompanied by inhibition of acetylcholinesterase (AChE) activity. Acetylcholine, a neurotransmitter crucial to the control of motor function and movement, is hydrolyzed by acetylcholinesterase [44]. By inhibiting acetylcholinesterase (AChE) activity, vincristine may cause neuronal damage by resulting in the build-up of acetylcholine (ACh), especially in areas that regulate movement in the brain of the flies [45]. Flies that were treated to vincristine showed AChE inhibition in alignment with this study, and it is likely to have interfered with normal cholinergic neurotransmission and potentially contributed to changes in locomotor behaviour [19]. It is also interesting to note that these flies showed an unusual increase in climbing activity in the VCR group compared to the control group, though the activity of AChE was lowered greatly in the vincristine-treated group relative to the other groups. Moreover, unlike the vincristine treated group. The groups co-treated with catechin, both concomitantly and sequentially with vincristine, showed even greater AChE activity. Nevertheless, a non-significant result was observed in the climbing activity of the flies, which might be due to the acute exposure done in this study. But the AChE result suggests that vincristine might impair locomotion, and catechin has a very good potential of reversing its effect [46].

Normal dopaminergic and cholinergic transmission in each of these regions is required for the usual neuronal functioning along the afferent visual pathway, extending from the retina to the thalamus and cerebral cortex. Altered dopaminergic and cholinergic activity in visual afferent pathways is caused by Parkinson's disease [47]. In this study, the phototactic behavior

of *D. melanogaster* was observed. Vincristine significantly disturbed the phototactic response of flies towards the light on the left side when compared to the control group. Conversely, Catechin alone treatment induced an increased response towards light when compared with the control. Notably, co-treatment of Vincristine and Catechin resulted in a highly significant recovery of phototaxis when compared to Vincristine and the control group. Further, serial (pre and post) treatment of Catechin and Vincristine exposed flies showed statistically significant restoration of phototactic behavior towards the light on the left side, suggesting a protective therapeutic effect of Catechin. Towards the light on the right side, a similar trend was observed, although no significant differences. This suggests a visual impairment of vincristine and the rescue potential of catechin. According to this study, there are alterations in visual afferent connections in early Parkinson's disease, and atrophy and/or loss of function in neurons in the visual cortex, thalamus, and retina [47].

Abundance studies suggest that olfactory disturbances in Parkinson's disease may have diagnostic utility for the differentiation of PD from other locomotion disorders. Data shown suggested that olfactory ability is differentially impaired in distinct Parkinsonian syndromes [48]. Though Parkinsonian syndromes are not distinguished from each other in this study, the chemotaxis assay was conducted to measure the olfactory-motor response of *Drosophila melanogaster* under differing treatment conditions. Most of the vincristine exposed flies remained in the origin with benzaldehyde, indicative of decreased chemotactic response or altered sensory perception. In contrast, in the control group and the co-treated with catechin group, the majority of the files migrated away from the origin zone, which showed a higher sensitivity or attractiveness to the irritant. This suggests that vincristine may induce olfaction impairment. The presence of Lewy bodies also explains vincristine group flies' decreased sensitivity to the smell of the irritant, implying olfactory loss that seemed to correspond to Parkinson's disease, as demonstrated through immunostaining of their brain tissue [48].

Biochemical examinations were employed to assess the modulatory activity of catechin extract (CAE) on the levels of activity of acetylcholinesterase (AChE), glutathione (GSH), nitric oxide (NO), catalase, malondialdehyde (MDA), and superoxide dismutase (SOD) in *Drosophila melanogaster* Parkinsonism models. Overproduction of reactive oxygen species (ROS) contributes to the heightened oxidative stress, which can further degrade the neuronal integrity and neurotransmitter communication, particularly of acetylcholine and dopamine, thus suppressing motor coordination [49]. Reduced activity of AChE was found to impair cholinergic neurotransmission, which is further associated with cognitive deficits in Parkinson's disease [50]. The findings of this study showed that AChE activity was significantly lower in the vincristine and catechin groups compared to the control. Co-treatment with catechin flies shows a significant increase in AChE levels compared with the vincristine group. These findings suggest that AChE activity can be enhanced by dietary treatment with catechin and enhance cholinergic dysfunction [51].

Antioxidant enzymes such as GSH, SOD, and catalase play crucial roles in protecting cells from oxidative damage and promoting cell survival [52][53]. In the vincristine exposed group, the GSH decreases significantly compared to the control group. Co-treatment with catechin increased the GSH levels significantly compared to vincristine, indicating the antioxidative capacity of catechin in overcoming vincristine induced oxidative stress. Similarly, SOD activity significantly reduces in the vincristine and catechin groups compared to the control. Co-treatment with CAE, however, significantly increases SOD activity compared to the vincristine exposed group, demonstrating that catechin is protective against oxidative imbalance induced by vincristine.

Catalase is a crucial antioxidant enzyme found in nearly all living organisms exposed to oxygen [54]. It plays a vital role in cellular defence by catalyzing the decomposition of hydrogen peroxide, a harmful by-product of metabolic processes, into water and oxygen [55]. This rapid reaction helps protect cells from oxidative damage and maintains redox balance [56]. Catalase activity in this study decreases in the vincristine and catechin groups compared to the control. Co-treatment with catechin notably increases catalase levels compared to the vincristine group. These results collectively indicate that catechin can re-establish the activity of critical antioxidant enzymes GSH, SOD, [57]. and catalase, suggesting a strong protective role against vincristine-induced oxidative damage in *D. melanogaster* [58].

Oxidative stress markers such as nitric oxide (NO) and malondialdehyde (MDA) have been widely employed as main indicators of cellular oxidative damage, reflecting lipid peroxidation and the activity of reactive nitrogen species in pathologic states [59]. Nitric oxide plays important roles in a wide range of physiological processes; however, it is also employed as an indirect marker of oxidative stress, particularly in biological systems. In such stress conditions, NO readily reacts with the superoxide radicals to form peroxynitrite, a potent oxidizing species capable of inducing damage to cellular macromolecules like proteins, lipids, and DNA [60]. The accumulation of NO and its subsequent reaction with superoxide anion can also generate toxic nitrite species, which can contribute to the development of various disease conditions [61]. In this study, vincristine exposed groups notably increase in NO level compared to the control group. However, co-treatment with catechin reduces the Vincristine-induced elevation of NO level. MDA level increases notably in vincristine exposed flies compared to the control group, and co-treatment with catechin reduces the MDA level compared to vincristine. These findings suggest that vincristine induces oxidative stress while catechin reduces oxidative stress [62].

Depletion of cellular integrity and the presence of dead or degenerated cells were observed in the brain tissue of vincristine exposed flies, likely due to the neurotoxic effects of

vincristine. In contrast, co-treatment with catechin appeared to mitigate this cellular damage, promoting partial restoration of tissue structure and reducing cell death. The observed cellular and tissue damage may be attributed to increased oxidative stress induced by vincristine exposure [63]. Lewy bodies were observed in the brain tissue of vincristine exposed flies, indicating protein aggregation and neurodegenerative diseases are likely caused by a combination of oxidative stress, protein misfolding, and impaired proteasomal degradation [64]. Co-treatment with catechin, however, effectively cleared such Lewy body-like inclusions, indicating a neuroprotective role. Lewy bodies are a hallmark pathological feature of Parkinson's disease [65], which highlights the Parkinsonian-like action of vincristine as well as the therapeutic potential of catechin in mitigating such neurotoxicity.

CONCLUSION

This study provides convincing evidence that vincristine induces Parkinsonian-like neurotoxicity in *Drosophila melanogaster* by the symptoms of reduced survival, impaired locomotor and neurobehavioral function, oxidative stress, acetylcholinesterase (AChE) dysregulation, Lewy body formation, and degeneration of cells. These symptoms all closely resemble signature features of Parkinson's disease, including dopaminergic neuron dysfunction and disruption of cholinergic transmission. Interestingly, co-treatment with catechin showed a significant neuroprotective effect, which was indicated by improved survival rate, better locomotor performance (crawling, climbing, phototaxis, and chemotaxis), normalization of AChE activity, and antioxidant enzyme levels (GSH, SOD, and catalase). Additionally, catechin treatment brought down cellular damage to a considerable extent and removed Lewy body-like inclusions, indicating its potential to reverse Vincristine-induced neurodegeneration. Overall, this research highlights the significance of *Drosophila melanogaster* as a possible model for neurodegeneration and highlights the potential of Catechin as a drug for neuroprotective interventions against Parkinsonian-like pathology induced by chemotherapeutic agents.

AUTHORS' CONTRIBUTION

Conceptualization: OOA; Funding acquisition: AGA, OIA, ICA, DIA, GTJ; Investigation: OOA; Supervision: OOA, YSO, Emmanuel O.A; Visualization: OOA, YSO, EOA; Writing – original draft: AGA, OIA, ICA, DIA, GTJ; Writing – review & editing: YSO, EOA.

CONFLICT OF INTEREST

The authors declare no competing interests.

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