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Original Article

Adansonia digitata Fruit Pulp-Derived Procyanidins Mitigated Ecotoxicant-Induced Neurotoxicity in *Drosophila*

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ABSTRACT

Nervous systems experience negative impacts of environmental toxicants such as heavy metals in all forms, leading to cognitive impairment with consequent death. This study examined the rescue role of *Adansonia digitata* fruit pulp (ADFP) derived procyanidins against copper oxide (Cu^{2+})-induced neurotoxicity. Three to four-day-old flies of both sexes were used to evaluate the neuroprotective effect of the procyanidin-rich fraction of ADFP. Flies were exposed to copper oxide for seven days, followed by ADFP procyanidins-rich fraction administration. Longevity, behavioral, and biochemical assays were carried out to ascertain the antioxidative and neuroprotective activities of the fraction. A decrease in survival rate in the flies fed with Cu^{2+} only was observed, compared to the control groups. The reduction in the activity of studied enzymes, dopamine, and total thiol levels was observed in the Cu^{2+}-exposed flies, with concomitant increase in nitric oxide and H_2O_2 levels. Procyanidin administration to the flies prior to Cu^{2+} exposure improved their emergence rate, survival rate, and locomotor function, by hindering the noxious effect of Cu^{2+} on the experimental flies. Procyanidins protected the flies against Cu^{2+} neurotoxicity as evidenced by behavioral assay and redox status results, suggesting that ADFP holds protection potential against the neurotoxic effect of ecotoxicological agents.

Keywords: Adansonia digitata, Drosophila melanogaster, Neurotoxicity, Oxidative stress markers, Procyanidins.

1 Introduction

Neurodegeneration has been considered a condition related to the deterioration of nerve cells, ascribing to progressive damage of neurons with consequent loss of their structures and functions with consequent death. Neuronal degeneration gives rise to a few hundred non-communicable disorders known as nervous system disorders, mostly caused by exposure to ecotoxicological or neurotoxic agents, and they were neglected due to lower incidence factors [1]. Copper (Cu^{2+}) is a vital element required in minute quantity for proper biological functions in organisms such as viruses, bacteria, and humans. The trace element influences essential biological functions including but not limited to erythrocyte maturation, respiration, and antioxidant activities in the organisms [2,3]. Copper is also an essential cofactor necessary for various biological processes such as catalytic activities of enzymes involved in protein synthesis, oxidative phosphorylation, and inflection of neurotransmission. Others include modulation of activities in some enzymes like dopamine–β-hydroxylase, cytochrome oxidase, superoxide dismutase (Cu-Zn-SOD), and ceruloplasmin [3,4]. However, as a neurotoxic agent, copper toxicity manifests upon excessive ingestion which culminates in the free radicals production via Haber-Weiss or Fenton reactions. Free radicals are implicated in oxidative stress processes that affect neuronal integrity with eventual death due to loss of structure and function over time [2,4].

Additionally, disrupted regulation of copper in the living system weighs down key biological functions including lipid peroxidation, and generates noxious alkenals known as 4-hydroxy nonenal that inhibit vital enzymes in the Krebs cycle such as alpha-keto-glutarate dehydrogenase and pyruvate dehydrogenase. A copious number of studies revealed that the surrounding environment constitutes a reasonable quantity of Cu^{2+}. Nearly 40 % of diet-sourced copper originates from cereals, white potatoes, dried beans, yeast bread, and tomatoes in addition to a higher percentage from water such as drinking, ground, surface, and seawater [3]. The aforementioned sources of Cu^{2+} have been associated with oxidative stress, neurotoxicity in flies, and neurodegeneration as a whole. It was also known that

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consumption of Cu^{2+} in large quantities affects cognitive functions and has been connected to pathogenesis and pathological progression in many neurological conditions due to consequent oxidative damage to neurons via lipid peroxidation, generation of free radicals, and mitochondrial volatility [5, 6]. In recent years, there has been a growing number of explorations of plant-sourced bioactive compounds particularly phenolics and flavonoids for their protective potential in mitigating the pathological initiation and progression of numerous human diseases where oxidative stress is implicated [7].

Procyanidins constitute a significant category of bioactive compounds recognised for their health advantages in humans. These plant metabolites show potential in the management of chronic metabolic disorders, including cancer, diabetes, and cardiovascular disease, by mitigating oxidative stress-related cell damage [8]. *Adansonia digitata* Linn (Baobab) as one of the curative plants rich in procyanidins, is known for its medicinal effects on various diseases including metabolic disorders, neuronal disorders, and microbial-related disorders, among others, in humans, lower animals, and flies such as *Drosophila melanogaster* [9]. Moreover, the protective potentials of ADFP-derived procyanidins in relation to ecotoxicant-induced neuronal damage have been poorly investigated. In this study, the neuroprotective effect of procyanidins from *A. digitata* was evaluated against Cu^{2+}-induced oxidative and neuronal damage in *D. melanogaster*. These flies have become a crucial model for biomedicals and neuroscience research with a long history of usage [9].

2 Methods and Materials

2.1 Materials

The fruit of *A. digitata* was acquired from a rural market of a specific location and was identified by a herbarium specialist. Chemicals such as acetylthiocholine iodide, 5,5-dithiobis-2-nitrobenzoate, and 1-chloro-2, 4-dinitrobenzene were obtained in Sigma Company in the United States. Copper sulfate and other pertinent reagents were obtained from Inqaba Biotech Company, Nigeria, and they were all of analytical-grade quality.

2.1.1 Adansonia digitata fruit pulp ethanolic extract preparation

To prepare the ADFP extract, the fruit hardback was broken, and the pulps were released and shed. It was then reduced to fine particles using a pestle and mortar, and the resulting sample was sieved to obtain a powder. A quantity (500 g) of the powdered sample was soaked in 1.5 L methanol for 72 hours for effective migration of phenolics to the solvent and avoidance of saturation. Whatman filter paper size No. 1 was employed for the filtration of the extract with a subsequent reduction of the solvent to dryness on a rotary evaporator and water bath, which was then kept in the refrigerator for further use.

2.2 Bioassay-guided chromatographic fractionation of ADFP extract

A 20-gram sample of dry extract was dissolved in ethanol and then transferred to a column containing silica. The column was then eluted with a gradient of ethanol and water, starting with pure ethanol and ending with pure water, using the following ratios: ethanol-water (100/0%), ethanol-water (70/30%), ethanol-water (50/50%), ethanol-water (30/70%), and water (0/100%). The process of separation was evaluated using thin-layer chromatography upon collection of fractions at regular time intervals, as described by [10]. The compounds were observed using a p-anisaldehyde reagent on UV light with wavelengths of 250 to 370 nm. The Thin Layer Chromatography resulting pattern guided the combination of fractions from the column, and they were reduced to dryness on a rotary device and water bath. The study measured the entire procyanidins contents and assessed the modulatory effects of the fractions using glutathione-S-transferase, superoxide dismutase (SOD), and acetylcholinesterase (AChE). The fractions obtained from 100 percent ethanol had the highest contents of procyanidins. The same fraction demonstrated a stronger inhibition effect on AChE and enhanced the GST and SOD activities. The resulting fraction was produced in large quantities and utilized for the bioassay section of the experiment.

2.3 Flies Culture and Stocking

A University of Ibadan Medical College professor generously donated the experimental flies *(*Harwich strain of *D. melanogaster)*, originally from the United States National Species Stock Center in Ohio. The flies were cultured in a laboratory under standard *Drosophila* culture conditions of 12 hours of dark/light cycle, room temperature, and 60 – 70% relative humidity. They were fed a cornmeal diet of 1% agar-agar, 1% sucrose, 1% brewer's yeast, 0.08% methylparaben content as a preservative, and a reasonable quantity of water.

2.4 Treatment with ADFP-derived procyanidins and Exposure to Cu^{2+}

Wild-type flies (both sexes) of one to three days old were grouped into nine vials of approximately fifty flies each. The groups were fed accordingly with a diet incorporated with the procyanidins-rich fractions of various concentrations (12.5, 25, 50, 100, 250, 500, 750, and 1000) mg/kg diet for seven days. Thereafter, the effective and working concentration(s) of the fraction was determined using survival rate and eclosion assay. Furthermore, the flies were subjected to 1 mM Cu^{2+} accordingly for seven (7) days following their treatment with the most effective concentration(s). The concentration of Cu^{2+} used in this study was established by an acute toxicity experiment in comparison with previously reported literature [3]. A 1mM dose of copper ion successfully induced neuronal toxicity and oxidative-related damage in the experimental *flies*.

2.5 Behavioral assay

2.5.1 Evaluation of Locomotor Dysfunction

The locomotive ability of experimental flies was assessed accordingly. Briefly, both control and treated flies were anesthetized and subjected to a glass column that was vertically erected with the same dimension. Sequel to their immediate recovery, the bottoms of the columns were tapped gently, and the flies walked towards the top. Those that successfully covered 6 cm upward in 6 seconds or less and the remaining that couldn't be recorded accordingly. The final scores were obtained as the average of the flies' number that crossed the line at the designated time and were presented as a percentage of the total quantity of flies. The process involved only 20 flies per group and was repeated up to three times at intervals of 60 seconds.

2.5.2 Determination of longevity and survival rate

The lifespan extension of the experimental flies was evaluated using the daily mortality record. The data generated were subjected to statistical analysis according to the reported literature by [11].

2.5.3 Assessment of rate of pupation

The rate of emergence of flies' offspring in the fraction pre-treated group co-exposed to Cu^{2+} was investigated according to the previous report [11].

2.5.4 Preparation of Sample and biomarkers investigations in the Flies

Following the ADFP-derived procyanidins' pre-treatment and Cu^{2+} exposure, 50 treated and control flies in each group were anesthetized on ice for 1-2 minutes. The flies were homogenized after being transferred to an Eppendorf tube using a buffer of 0.1M and pH 7.4, in a ratio of 1:10 insects/volume (µL) of a buffer. After the homogenate centrifugation for ten minutes at high speed under cold temperature, the supernatant was separated into a new tube immediately and stored separation of supernatant to the new containers was done immediately and stored at 20 °C for further use.

2.5.5 Assessment of protein content in D. melanogaster

The total protein concentration of the control and treated groups was assessed with an albumin content in the serum of bovine called BSA as a standard, according to the reported literature [3] with little changes.

2.5.6 Total thiol Evaluation

To determine the content of total thiol in the supernatants, 20 µL, 35 µL, and 35 µL of the sample, 1 mM DNTB, and distilled water were mixed with 510 µL 0.1 mM buffer of physiological pH (7.4) in a fresh tube. The tube was kept under 25 °C for 30 mins, and absorbance was taken at 412 nm wavelength as highlighted by [12].

2.5.7 Evaluation of acetylcholinesterase function

The estimation of acetylcholinesterase activity was done according to the previously described method. Firstly, 25 ml of each of the DTNB reagents and that of the sample were mixed with 0.5 ml of 0.1 mM buffer of physiological pH (7.4) in a fresh tube, and 10 ml of the substrate (acetylthiocholine) was then introduced and mixed thoroughly. The content was subjected to spectrophotometric measurement for 3 minutes, and the change in optical density at 400 nm. Conversion of the substrate (acetylcholine) to product per minute per milligram protein was considered as a means of measuring the activity of the enzyme acetylcholinesterase.

2.5.8 Assessment of antioxidant enzymes activities (SOD, Catalase, GST)

We meticulously observed the increase in absorbance at a wavelength of 340 nm to assess the activity of glutathione S-transferase. A 50-µL sample was transferred into a tube that already had 20 µM of CDNB and reduced glutathione in it. The alteration in absorbance signifies the establishment of a combination of reduced glutathione and CDNB. 0.1 µM phosphate buffer at pH 7.0 and 8.8 µM H_2O_2 (3%) were mixed in an Eppendorf tube to make a 1 ml reaction mixture. We conducted this experiment to assess the catalase activity. The addition of a 10 g protein aliquot to the tube initiated the reaction, which was visible at 240 nm for 3 minutes. We assessed the activity of superoxide dismutase (SOD) using the previously documented inhibitory method [13]. Finally, we added 0.15% quercetin that had been dissolved in dimethyl formamide to a tube that already had 4 grams of protein, 8 milligrams of N, N, N, N-tetramethyl ethylenediamine (TEMED), and 0.016 milligrams of sodium phosphate buffer at pH 7.4. We measured the optical density at 406 nm for three minutes and quantified it as the amount of protein required to prevent 50% quercetin auto-oxidation.

2.5.9 Assessment of dopamine concentration

Dopamine concentration was determined using ENZ-KIT188-0001 dopamine ELISA kit **(Farmingdale, New York,** USA) according to the procedure described on the enclosed template in the kit. The optical density of the sample was taken on an ELIZA reader (BioTek Synergy) at a wavelength of 450 nm, and the result was analyzed.

2.5.10 Assessment of Nitric Oxide Concentration

A 5% phosphoric acid also known as Griess reagent was combined with 1% sulphanilamide and 0.1% N-(1-naphthyl) ethylenediamine dihydrochloride for the quantification of nitric oxide level on the bases of enzymatic interconversion of nitrate to nitrite. A 500 µL of Griess reagent was combined with 500 µL of sample and kept under ambient temperature for 20 mins. Spectroscopic measurement of absorbance was performed at the wavelength of 550 nm, and the concentration of nitric oxide was deduced using a sodium nitrate standard curve.

2.5.11 Assessment of hydrogen peroxide level

The concentration of H_2O_2 was obtained accordingly. In brief, in a tube, 100 nm sorbitol, 100 mM xylenol orange, 25 mM H_2SO_4, and 250 mM ammonium ferrous sulfate were combined to form the FOX solution known as Oxidation-Xylenol Orange of Ferrous (FOX). A portion of the sample (590 µL) was aliquoted into a fresh tube, and 10 µL of supernatant was introduced, mixed thoroughly, and kept under ambient conditions for 30 mins. The absorbance of the mixture was recorded at 560 nm, followed by a calculation of the concentration of H_2O_2 which is presented in µmol/mg of protein.

2.6 Statistical analysis of data

To analyze the data statistically, a one-way analysis of variance (ANOVA) was conducted using GraphPad Prism version 9, which originated in the USA (San Diego, CA). statistical significance of the data was considered when $p<0.05$ was considered statistically significant at the 95% confidence interval. The results were presented as mean ± standard deviation (SD) and all the experiments were replicated five times (n = 5).

3 Results

3.1 ADFP fraction rich in procyanidins prolongs fruit flies' lifespan

Incorporation of *Adansonia digitata* fruit pulp (ADFP) procyanidins-rich fraction into the diet results in an increase in the rate of survival of adult *D. melanogaster* of both sexes. Figure 1below shows that the flies that received the fraction for 7 days at different concentrations had a higher survival rate compared to the untreated control and vehicle groups during the experimental period of 75 days. The groups that received a 3.0 mg/10g diet exhibited higher longevity among the treatment groups.

3.2 Effective dose for the experiment

The appropriate and effective dose of ADFP procyanidins fraction used in the present study was determined using the rate of new flies' emergence after treatment. Figure 2 below revealed the fraction's effect on the investigated flies treated with different concentrations, in which a 3.0 mg/ 10 g diet seemed to have triggered a significant increase in the emergence rate above other concentrations and control groups.

3.3 Influence of varying concentrations on selected biochemical parameters

To further ascertain the effectiveness of the varying concentrations, some antioxidant parameters and neurotoxic markers were analyzed. A remarkable rise in the activities of catalase (Figure 3a), glutathione-s-transferase (GST) (Figure 3b), and superoxide dismutase (SOD) (Figure 3c) as well as total thiol level (Figure 3d) was observed in the group fed with 3.0 mg/g diet with a concurrent decrease in the hydrogen peroxide level (Figure 3e) in the flies.

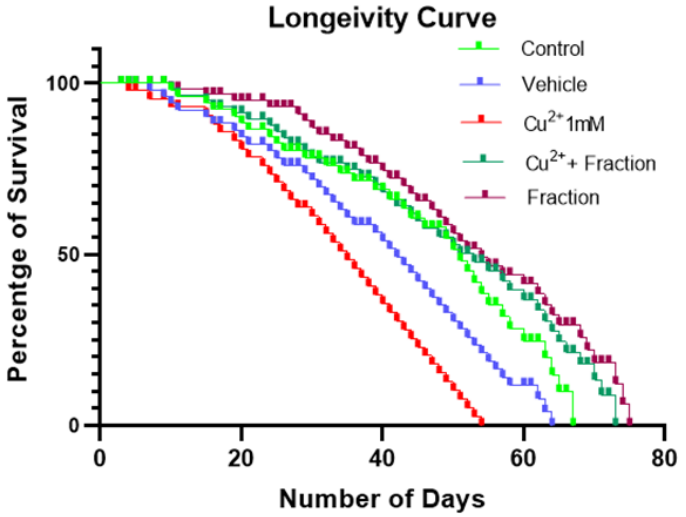

Figure 1: The survival rate of flies pretreated with a procyanidins-rich fraction of ADFP before their exposure to copper oxide. Death in each vial was recorded every 24 hours, and an average was taken for the analysis.

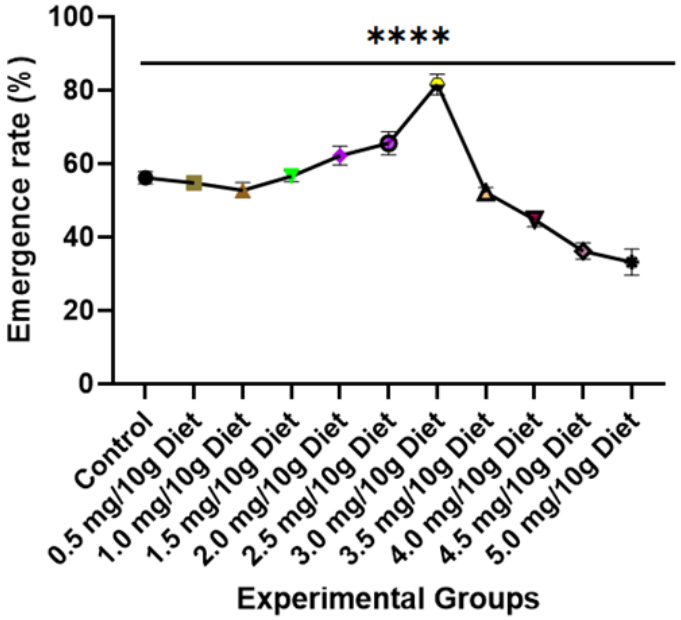

Figure 2: illustrates the determination of the effective and working concentration of the chosen fraction based on the rate of the flies' emergence (s). The most effective portion among the rest showed the highest emergence rate in percentage. Statistical significance was considered at $p<0.05$ in the experiments that were conducted in triplicate.

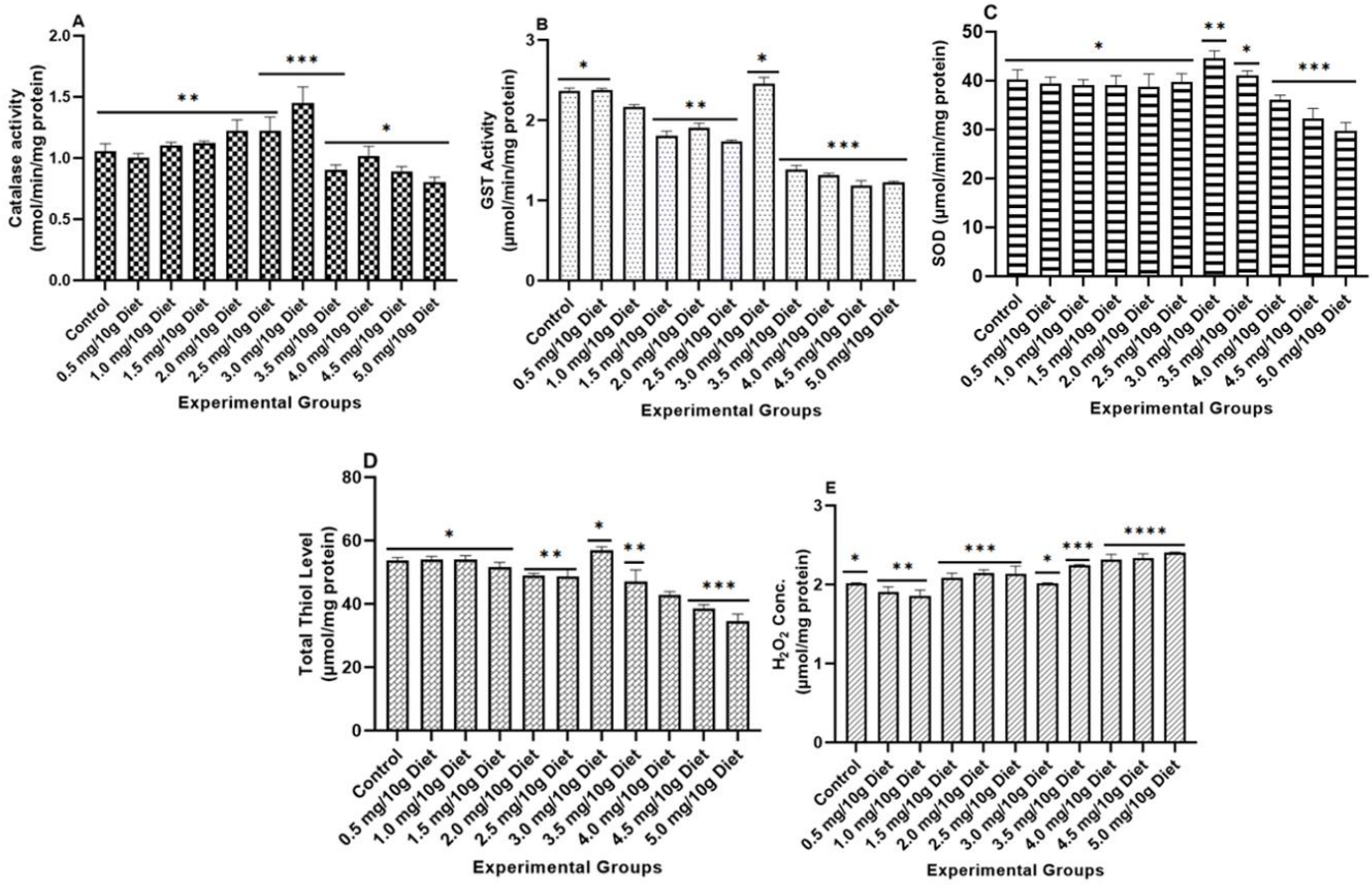

Figure 3: Effect of varying concentrations of ADFP procyanidin fraction on antioxidant parameters to establish an effective and working fraction concentration. Statistical significance was considered at $p<0.05$ in the experiments that were conducted in triplicate.

3.4 Influence of varying concentrations on cognitive-related biomarkers

The fraction, at 3.0 mg/ 10g diet and below, maintained the level of nitric oxide (Figure 4a), dopamine (Figure 4b), and the activity of acetylcholinesterase (Figure 4c) and improved the neuromuscular strength (Figure 4d) in a healthy environment. Based on the evidence obtained, a 3.0 mg/ 10g diet was regarded as the most effective dose and hence considered a working concentration throughout the study. On the other hand, as previously mentioned, the 1 mM lethal dose (LD_{50}) of Cu^{2+} for the neurotoxic agent was adapted from [3].

3.5 Neuroprotective and enhancement effect of ADFP phenolic-rich fraction in the flies

Figure 5 illustrates the curtailing effect of the fraction against Cu^{2+} harmful effects, which induced neurotoxicity in *D. melanogaster*. Sequel to the 7-day flies' pretreatment with the 3.0 mg/ 10g diet fraction and their exposure to 1 mM Cu^{2+}, we observed that the phenolic-rich fraction prevents a substantial rise ($p<0.05$) in H_2O_2 (Fig. 5a) together with nitric acid (Figure 5b) levels, resulting from the exposure of the flies to the noxious environment when compared with the 1 mM Cu^{2+} only fed (positive control) group. The activity of acetylcholinesterase (AChE) (Figure 5c) also seemed to be reduced remarkably ($p<0.05$) in the fraction pretreated before Cu^{2+} exposed group in comparison with the positive control. The fraction depicts a clear protection against Cu^{2+}-induced neurotoxicity in the experimental flies.

3.6 ADFP-derived procyanidins regulate Dopamine levels

Figure 6 depicts the protective effect of ADFP fraction rich in procyanidins on dopamine levels in the body and head of *D. melanogaster*. We recorded a remarkable ($p<0.05$) decrease in dopamine concentrations in the fly exposed to 1 mM Cu^{2+}. Upon pretreatment with the fraction before exposure to the neurotoxic agent, the production level was maintained at the normal level despite the presence of the toxic agent. The trend of dopamine production in the head was similar to that of body homogenate (Figure 6a and b). The result indicates that the plant metabolites stifled the degeneration of dopamine due to the presence of environmental toxicants. Thus, maintains the neurotransmitter level and protects the neuron cells' activities by enhancing their signaling process.

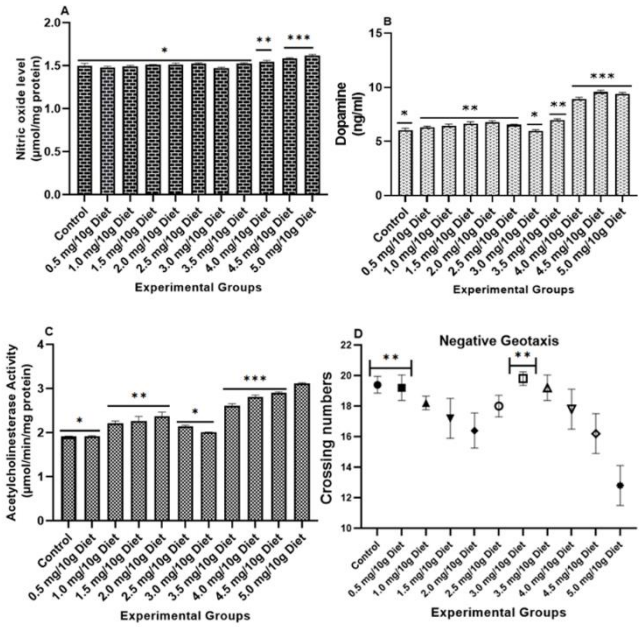

Figure 4: Effect of varying concentrations of ADFP procyanidins fraction on cognitive function and psychomotor parameters to establish an effective and working fraction concentration. Statistical significance was considered at $p<0.05$ in the experiments that were conducted in triplicate.

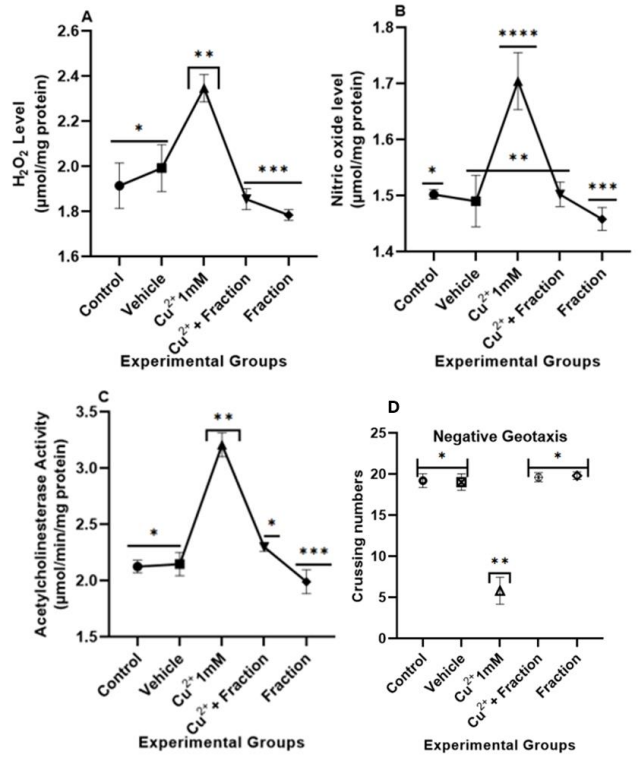

Figure 5: Protective abilities of ADFP procyanidins fraction against the pro-noxious effect of Cu^{2+} in *D. melanogaster*, where the fraction decreases the levels of (A) H_2O_2, (B) NO, and (C) AChE. Activity. The fraction also improved the (D) locomotor activity in the flies. Statistical significance was considered at $p<0.05$ in the experiments that were conducted in triplicate.

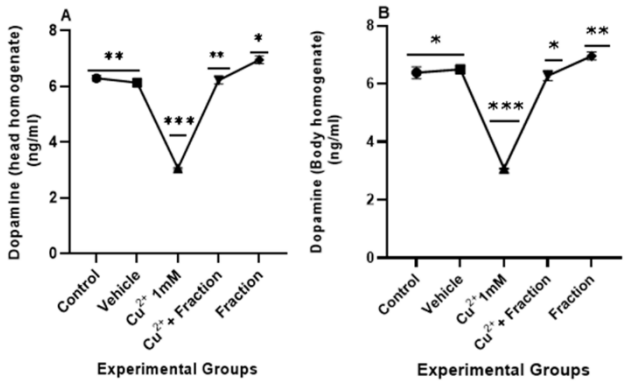

Figure 6: Dopamine concentration in the brain (A) and body (B) homogenates of *D. melanogaster* pre-treated with procyanidin-rich fraction of *A. digitata* fruit pulp. Statistical significance was considered at $p<0.05$ in the experiments that were conducted in triplicate.

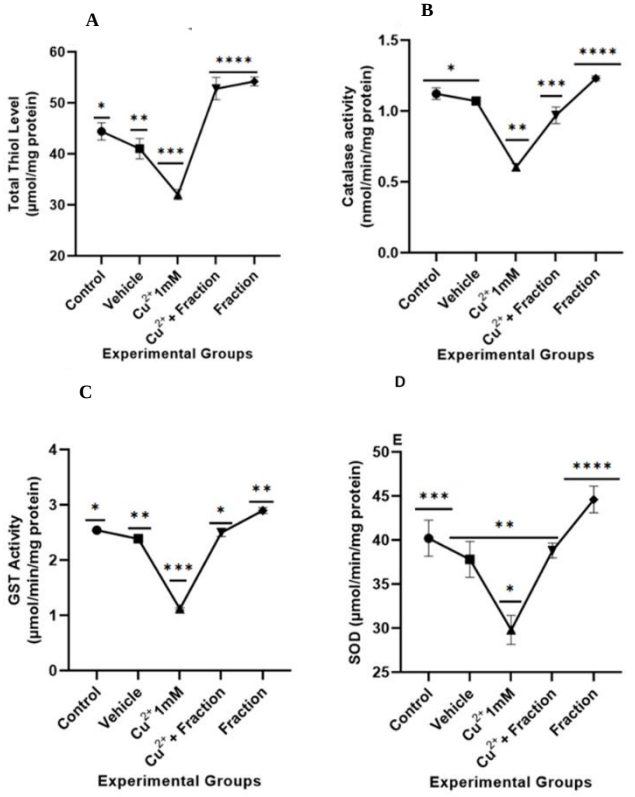

Figure 7: Protective ability of ADFP procyanidins fraction against the pro-noxious effect of Cu^{2+} in *D. melanogaster*, the fraction triggered the increase in the (A) total thiol level and the activities of (B) catalase, (C) GST, and (D) SOD, the important markers in the antioxidant system of the flies. Statistical significance was considered at $p<0.05$ in the experiments that were conducted in triplicate.

3.7 Antioxidant effect of ADFP phenolic-rich fraction in flies

Figure 7 reveals the modulatory effect of ADFP fraction rich in

procyanidins on antioxidant-related marke rom the flies under a noxious ecosystem. In comparison with the Cu^{2+}-only exposed flies, a significant ($p<0.05$) increase in the total thiol level (Figure 7a) and the activities of catalase (Figure 7b), glutathione-s-transferase (Figure 7c), and Mn superoxide dismutase (Figure 7d) were observed in the fraction-fed flies before exposure to the harmful Cu^{2+}. Indicating the antioxidant enhancement effect of the ADFP procyanidins-rich fraction.

4 Discussion

The present findings focused on the impact of *A. digitata*-derived procyanidins in protecting neuronal integrity against the noxious effect of copper oxide. Here, we observed that the procyanidins protected the experimental flies against the consequent detrimental effect of $^{2+}$-induced oxidative damage and neurotoxicity. Copper i an important microelement necessary for the growth and development of organisms and it is involved in other many biologically related functions such as erythrocyte maturation, and gaseous exchange in animals and plants among others. However, when taken in excess, the essential trace element could lead to the development of numerous chronic diseases where oxidative stress is implicated. It was also reported that excessive intake of Cu^{2+} triggers loss of memory and is involved in the pathogenesis of brain diseases [14]. The protective properties and antioxidant ability of phytochemicals are crucial for selecting medicinal plants to fight against the development of neurological and other life-threatening disorders [15, 16]. This study revealed that pretreatment of *Drosophila melanogaster* with ADFP procyanidins led to significant obstruction of Cu^{2+} noxious effect through enhancement of the activities of catalase, SOD, and the neuromuscular system in the fly. The neuroprotective potential of the plant metabolites was also evaluated using behavioral and biochemical assays that included an assessment of oxidative status through antioxidant markers and neuromuscular function indices. Studies have shown that free radical scavenging activities of intracellular antioxidant molecules synthesized via the secondary activation of ARE/Nrf-2 complex is a possible mechanism through which most phytochemicals with antioxidant properties exert their protective activities [17, 18]. Our findings also demonstrated preventive effects against depletion of total thiols level (GSH inclusive) and GST activity due to exposure of the flies to Cu^{2+}. In the present study, exposure to Cu^{2+} had a discernible impact on the level of nitric oxide (nitrate and nitrite) in *D. melanogaster*. The procyanidins abrogated the rise in the NO (nitrite and nitrate) level in the fly upon treatment prior to the Cu^{2+} exposure. Cu^{2+} can accelerate the Fenton reaction, which results in the production of hydroxyl free radicals with other reactive oxygen species as byproducts of the interaction between Cu^{2+} and H_2O_2 [19]. Here, the H_2O_2 accumulation with the concurrent decrease of catalase activity in *D. melanogaster* was observed due to the presence of Cu^{2+}. The procyanidins content appears to possess the respective radical scavenging and antioxidant effects based on its capacity to protect Cu^{2+}-induced reduction of catalase action and rise in H_2O_2 level. Additionally, it is imperative to note that exposure of flies to Cu^{2+} decreased the fly's emergence rates. However, their pre-treatment with the phenolics-rich fraction prior to exposure showed enhanced emergence rates compared to controls, as a result of the flies' suspected improved fecundity. The studied metabolites caused a decrease in the activity of acetylcholinesterase (AChE) triggered by Cu^{2+}-induced cytotoxicity. AChE inhibits cholinergic-base neurotransmission by hydrolyzing ACh to choline and acetate, thereby hindering signal transmission to the peripheral nervous system [20]. The role of the cholinergic system particularly in the etiology of neuronal disintegration-related diseases in elderly people has been sufficiently clarified, and the inhibitory effect of the contents on AChE activity in the present study is consistent with a study on similar compounds reported earlier in rodents and in flies, revealing decreased AChE activity and mRNA expression levels of the enzyme [21]. Hence, the procyanidins' inhibitory effect on AChE activity may cause the rise in acetylcholine levels within synaptic clefts, resulting in positive and effective cholinergic system function in flies. Dopamine (DA) levels in the head and the body homogenates appeared to be unexpectedly higher in the flies exposed to Cu^{2+}. DA is an organic catecholamine found within an organism that has a special role in the organization of cognition, learning, emotion, and movement. It is located in the substantia region of nigra in the brain and spinal cord [22]. DA signaling performs a variety of tactical roles in memory and learning particularly in fruit flies. Thus, altered DA levels may have a detrimental effect on cognition and memory [23]. There have been several reported pathogenetic processes that could cause DA-ergic dysfunction in disease conditions, revealing that A-oligomers have toxic and harmful effects on neuronal organization and neurons' signal transmission, killing neurons in the neocortex and hippocampi [24]. Also, it has been proven that copper and dopamine can combine to form a complex, enabling copper to be delivered to cells with the capacity to take up dopamine [25]. The ADFP methanolic fraction rich in procyanidins proved to be a potential neuroprotective agent due to its capacity to mitigate the noxious effects of eco-toxicants in flies as depicted in Figure 8. This could easily be reflected in humans due to the remarkable similarities between the two organisms.

Conclusion

Put together, the findings showed that the ADFP-derived procyanidins have protective effects *in vivo* against Cu^{2+}-induced neurotoxicity, which is attributed to its antioxidative effect supported by its modulatory role of oxidative stress, antioxidant regulation, and improvement of *D. melanogaster's* altered dopamine level. To prevent or reduce the severity of neurological diseases where oxidative stress is implicated, procyanidins of *A. digitata* may be regarded as an effective potential neuroprotective agent. The findings also supported the value of using fruit flies as a model to explore potential protective and therapeutic molecules against oxidative stress-related damages that could lead to neurodegenerative disease development, which severely reduces the quality of life and well-being of living organisms.

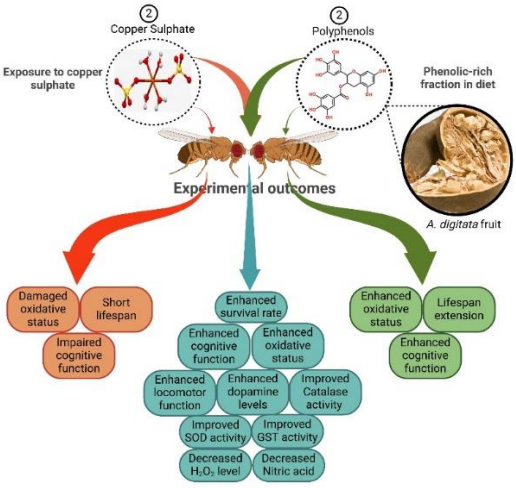

Figure 8: A proposed ADFP-derived procyanidins mechanistic action against Cu^{2+}-induced neurotoxicity in the *D. melanogaster* model.

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Author's contribution

Mohammed Sani Jaafaru: conceived the idea and designed the experiments; Ramiar Kamal Kheder, Mohammed Sani Jaafaru, and Soran Kayfi: performed the experiments and analyzed the data; Mohammed Sani Jaafaru, Louna Altrijman, and Tola Abdulsatar Faraj: drafted and proofread the paper; all the authors read and approved the submission of the final manuscript.

Conflict of interest

There is no competing interest associated with the manuscript.

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