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Research Article

Antioxidant Capacity and Effect of Coconut Water on $Alcl_3$ Amnesic-Induced Drosophila Melanogaster

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Abstract

Coconut water (CW) has gained wide attraction as a known functional food that offers additional benefits to its basic nutritional value. CW is employed in the management of oxidative stress-associated diseases for its natural antioxidant features. This study sought to investigate CW antioxidant capacity and effect upon its dietary inclusion in an $Alcl_3$-induced amnesic *Drosophila melanogaster*. CW was extracted from the coconut fruit cotyledon and supplemented in the flies' diet for five days. The CW extract antioxidant activity was examined *in vitro* through the total phenol, total flavonoid content, ferric reducing power, iron-chelating ability, ABTS* and DPPH* scavenging ability assays. Likewise, the anti-lipid peroxidation potential of CW extract was also measured *in vivo* using *D. melanogaster*. The CW extract has a total phenol content of 1.48 ± 0.43 (mg/g GAE) and a total flavonoid content of 0.53 ± 0.02 (mg/g QE) which could be attributed to its scavenging ability against ABTS* and DPPH* *in vitro* with an increase in extract concentration. Similarly, a positive trend was detected in the ferric reducing antioxidant power and iron-chelating ability tests. Furthermore, dietary inclusion of CW in groups fed with 0.1% and 1% CW lowers Malondialdehyde (MDA) concentration significantly ($p < 0.01$ and $p < 0.0001$ respectively) in $Alcl_3$ induced flies. This correlates with CW's ability to considerably ($p < 0.05$) reduced MDA *in vitro* in a concentration-dependent manner (0.01 – 0.03 mg/mL). These findings provide substantial information that

affirms CW's natural antioxidant ability, thus bolstering its therapeutics usage.

Keywords: Antioxidant; Coconut water; *Drosophila melanogaster*; Free radicals; Phenolic compound

1. Introduction

Coconut fruit, botanically called *Cocos nucifera*, is a member of the Arecaceae family, which provides numerous health benefits beyond its nutritional content [1]. Coconut water has gained attention as a functional food with its application in health and medicine, supported by increasing scientific evidence [2]. This coconut water is the plant's liquid endosperm and an outstanding natural drink with a caloric of 174/100g. Coconut water contains unique beneficial ingredients, namely sugars, vitamins, minerals, amino acids, and plant hormones which justifies its wide applications [3, 4]. The phytohormone Cytokinins' present in coconut water influences the plant cell division and confers its anti-aging effects [5]. According to some experimental research and reviews, coconut water has been revealed to contain minerals and aromatic compounds as its primary composition [3, 6] Young coconut water (about six months) contains estrogen-like compounds that prevent Alzheimer's disease in menopausal women [7], while the matured coconut water of about 12 months has displayed hypoglycemic effect and also reduced oxidative stress in rats [8].

Reactive oxygen species (ROS) are released as a byproduct during various cellular metabolism. Consequently, continuous exposure to environmental stresses results in oxidative stress and eventually death [9, 10]. Oxidative stress contributes to neurodegeneration and plays an essential role in Alzheimer's disease (AD) and Parkinson's disease (PD) pathogenesis [11]. Neurodegenerative diseases are often age-associated, usually characterized by cognitive decline, memory loss and behavioral disturbances [12]. The brain antioxidant defensive system is poor and thus more susceptible to free radical's attack [13]. The cells counteract this attack under normal conditions via homeostatic balance regulation, but the cell loses the ability in a disease condition [14]. This is characterized by accumulated free radicals and antioxidant system dysfunction, ultimately causing oxidative stress [15].

Compounds with high phenolic content easily donate hydroxyl hydrogen due to the resonance stabilization [16], scavenge free radicals, activate the antioxidant system, and chelate metals [17,18]. Natural compounds such as polyphenolic compounds derived from plants act as antioxidants that confer beneficial health functions [19]. These compounds function by reducing or neutralizing the formation of free radicals and hence protect the cell from oxidative damage [20, 21]. Phenolic compounds are important phytochemicals that exhibit several bioactive properties, including antioxidant activity [22]. Many studies have reported the quantification and identification of phenolic compounds in different kinds of fruits and vegetables, but only a few studies have reported coconut water antioxidant activity [23, 24]. However, a study conducted by Chang and Wu [25] reported the identification of phenolic compounds.

Drosophila melanogaster, a fruit fly now commonly used as a multipurpose model organism for biomedical science research due to its economic

advantage to culture in the laboratory, rapid generation time, shorter life cycle, high production rate and genetic modifications possibilities [26]. Its lifespan is between 40-120 days, depending on diet and environmental stress conditions. Some diets, such as those rich in free saccharides and cholesterol, can reduce fruit flies life span [27]. This present study was designed to examine the free radical scavenging property of coconut water and the antioxidant effect of its dietary inclusion in an oxidative-induced *D. melanogaster*.

2. Methods

2.1 Sample preparation

Figure 1a shows a sample of fresh *Cocos nucifera* fruits (thirty) purchased from a native market in Akungba-Akoko, Ondo State, Nigeria. The plant was identified and authenticated at the Department of Plant Science and Biotechnology, Adekunle Ajasin University, Akungba, Akoko, Ondo State. The shell of the coconut fruit was removed and washed properly to avoid contamination. The cotyledon was broken (Figure 1b) to extract the water. It was then broken, and the water was collected in labeled vials, kept in a deep freezer until use.

2.2 Experimental design

D. melanogaster Harwich strain (both gender, five days old) were divided into four groups containing 40 flies each. Group I was placed on a normal diet, while groups II-IV were placed on a basal diet containing: 10mM Al, 0.1% CW and 1%CW as shown thus:

Figure 1: a. Fresh Coconut fruit b. Cotyledon of *C. nucifera* (Kannaian *et al*., 2020).

Groups	**Diet**
I	Normal diet (positive control)
II	Normal diet + 10mM Al^{3+} (from $Alcl_3$)
III	Normal diet + 10mM $Alcl_3$ + 0.1% CW
IV	Normal diet + 10mM $Alcl_3$ + 1% CW

The flies were exposed to these treatments for five days and sustained at ambient temperature. All experiments were done in triplicates [19].

2.3 *Drosophila melanogaster* stock culture

Wild type *D. melanogaster* (Harwich strain) stock culture was obtained from Drosophila research laboratory, Department of Biochemistry, Adekunle Ajasin University, Akungba-Akoko, Ondo State, Nigeria. The flies were grown on a normal diet made up of cornmeal medium containing 1% w/v brewer's yeast and 0.08% v/w nipagin at constant temperature and humidity (25 ± 1oC; 60% relative humidity respectively) under 12 h dark/light cycle conditions. All the experiments were executed with equivalent *D. melanogaster* strain [28].

2.4 Diet preparation

The basal diet was based on the traditional cornmeal medium containing 1% w/v brewer's yeast, 2% w/v sucrose, 1% w/v powdered milk, 1% w/v agar and 0.08% v/w nipagin. The diet was prepared once a week. The coconut water supplemented diet was prepared by adding 0.1mg/g and 1.0 mg/g coconut water, respectively. The media were then mixed and distributed into vials [19].

2.5 Animal transfer for new emergence and treatment

The flies were transferred every five days to prevent overpopulation and contamination and also to breed new flies. The following method was employed for the transfer of the flies from old jars to new jars. A funnel was placed on the new jar, while the old jar was gently tapped on a soft padded surface (towel) so that the flies fall to the bottom of the jar. The jar mouth's cotton plug was quickly removed and then placed on the inverted funnel and slightly banged on the padded surface. Thus, the flies were transferred into a new feed [19].

2.6 Preparation of sample for biochemical assays

Using a Teflon homogenizer, the anesthetized flies were homogenized in 0.1M phosphate buffer, pH 7.4and, the resulting homogenates were centrifuged at 10,000g, 4°C for 10 minutes in a Kenxin refrigerated centrifuge Model KX3400C (KENXIN Intl. Co., Hong Kong). After that, the supernatant was removed from the pellet into an Eppendorf tube used for some bioassays.

2.7 Determination of biochemical parameters

Total phenol content was quantified as described by Singleton *et al*. [29], total flavonoid content as stated by Meda *et al*. [30], but with slight modifications. Free radical scavenging activity of coconut water was investigated through the 2, 2-azino-bis-3-ethylbenzothiazoline-6-sulfonate (ABTS) and 2, 2-diphenyl -1- picrylhydrazyl assays as described by Re *et al*. [31] and by Brand-Williams *et al*. [32], respectively. While the Ferric reducing antioxidant power was determined using the method of Oyaizu [33], Iron chelating ability was measured according to Minotti and Aust [34] method, with a slight modification by Puntel *et al*. [35].

2.8 Anti-lipid peroxidation assay

A modified [36] method was employed for the anti-lipid peroxidation assay. A reaction mixture containing 30 µL of 0.1M pH 7.4 Tris-HCl buffer, coconut water (0-100 µL) and 30 µL of 250 µM freshly prepared $FeSO_4$ was mixed with 100 µL S1 fraction briefly. The volume was top-up to 300 µL with distilled water and incubated at 37°C for 1hour. 300 µL 8.1% Sodium dodecyl sulphate (SDS) was added to the reaction mixture for colour reaction development, afterward followed by the addition of 500 µL of acetic acid/HCl (pH 3.4) and

500 µL 0.8% thiobarbituric acid (TBA) mixture. This mixture was incubated at 100℃ for 1hour. The produced thiobarbituric acid reactive species (TBARS) from the reactions were measured at 532nm using a JENWAY UV-Visible spectrophotometer. The absorbance was compared against the malondialdehyde (MDA) standard curve.

2.9 Statistical analysis

Data were pooled and expressed as mean ± standard deviation (SD). All results were statistically analyzed using the Graph pad PRISM (V.5.0) software. Levels of significance were accepted at $p < 0.05$, $p < 0.01$, and $p < 0.001$ for One-way analysis of variance (ANOVA), while the IC_{50} (concentration of extract that will cause 50% reducing activity) was determined using linear regression analysis [37].

3. Results

Table 1 presents the total phenol and total flavonoid content of coconut water as 1.48 ± 0.43 (mg/g GAE) and 0.53 ± 0.02 (mg/g QE) respectively.

Figure 1 reveals the 2, 2-azino-bis-3-ethylbenzothiazoline-6-sulfonate (ABTS) radical scavenging ability of coconut water extract (0 – 400 µg/mL) expressed in mmol. TEAC/g. The extract scavenged ABTS* significantly at $p < 0.05$ in a concentration-dependent manner. Figure 2, the 2, 2-diphenyl -1- picrylhydrazyl (DPPH) radical scavenging ability of coconut water was presented. The extract scavenged DPPH radical in a concentration-dependent manner (0-133 µg/mL).

The ferric reducing antioxidant property of coconut water was determined and expressed as ascorbic acid equivalents (Figure 3). The result showed that coconut water reduced Fe^{3+} to Fe^{2+} by an observed increase in Fe^{2+} with extract concentration. Likewise, Figure 4 presents the Fe^{2+} chelating ability of this coconut water extract. The result revealed that coconut water chelated Fe^{2+} in a dose-dependent manner.

The effect of coconut water extract against 250mM Fe_2SO_4 - induced lipid peroxidation in Drosophila melanogaster homogenate flies is presented in Figure 5. Fe_2SO_4 significantly ($p < 0.05$) increased malondialdehyde (MDA) level in the fly homogenate (125 ± 2.06). However, coconut water significantly ($p < 0.05$) decreased the MDA content in Fe^{2+}-stressed homogenate flies in a dose-dependent manner (0.01 – 0.03 mg/mL).

Figure 6 shows the effect of dietary inclusion of coconut water on brain malondialdehyde (MDA) content in $Alcl_3$ induced amnesic flies. The result reveals that the MDA content in flies fed with $Alcl_3$ was significantly ($p < 0.05$) higher than the control group. However, 0.1% and 1% CW dietary inclusion significantly lowers ($p < 0.01$ and $p < 0.001$ respectively) the MDA content against the control without CW.

Table.1: Total phenol and total flavonoid content of Coconut water.

CW	
Total phenol (mg/g GAE)	1.48 ± 0.43
Total flavonoid (mg/g QE)	0.53 ± 0.02
Values represent means of triplicate readings.	

Key: CW Coconut water.

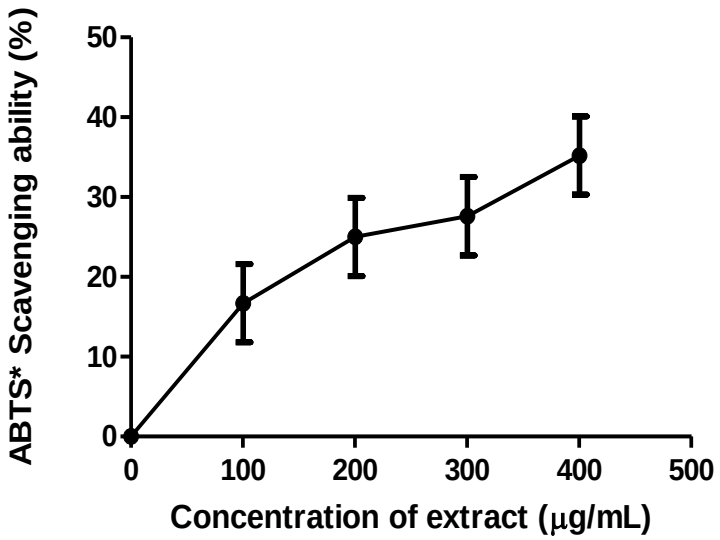

Figure.1: 2, 2-azinobis-3-ethylbenzothiazoline-6-sulfonate (ABTS*) radical scavenging ability of coconut water extract.

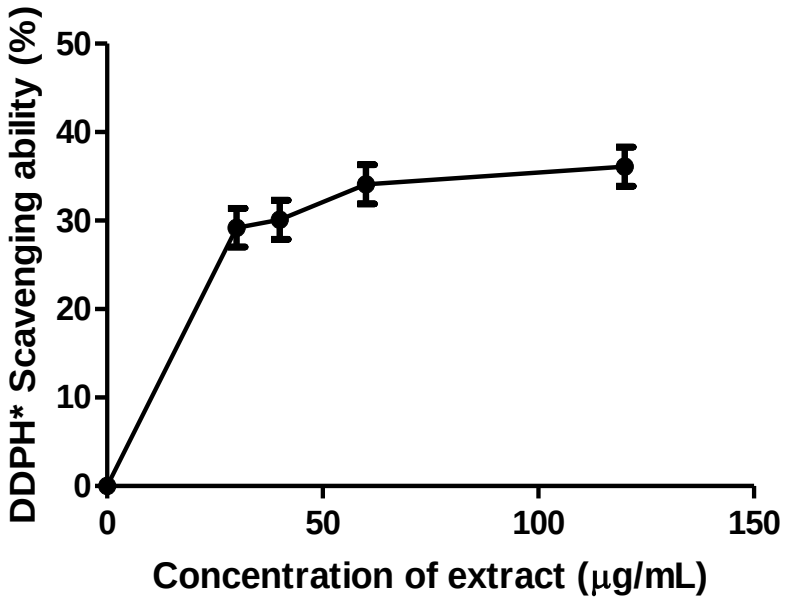

Figure.2: 2, 2-diphenyl -1- picrylhydrazyl (DPPH) radical scavenging ability of coconut water.

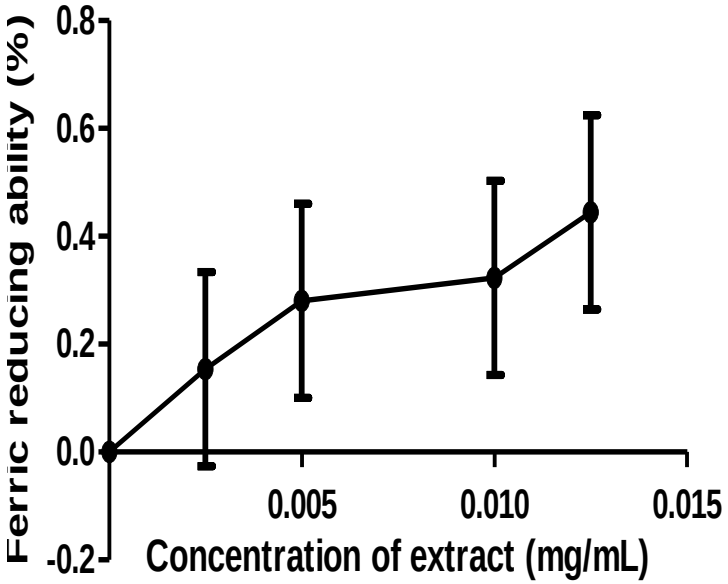

Figure.3: Ferric reducing ability of Coconut water.

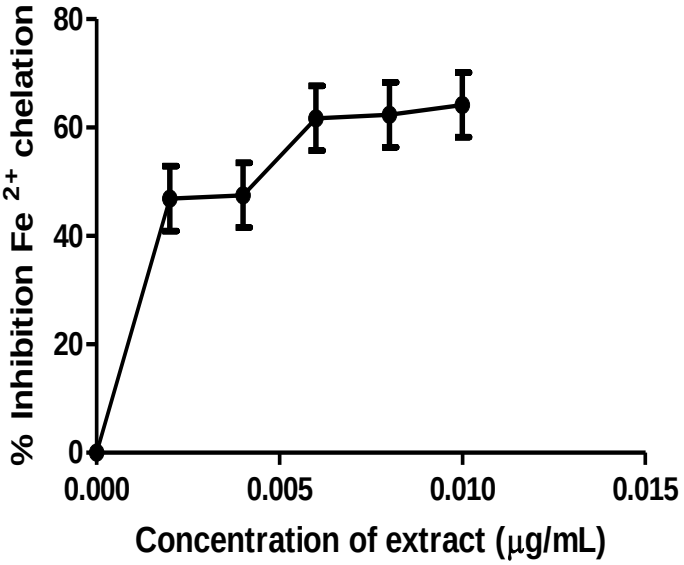

Figure.4: Fe^{2+} chelating ability of Coconut water.

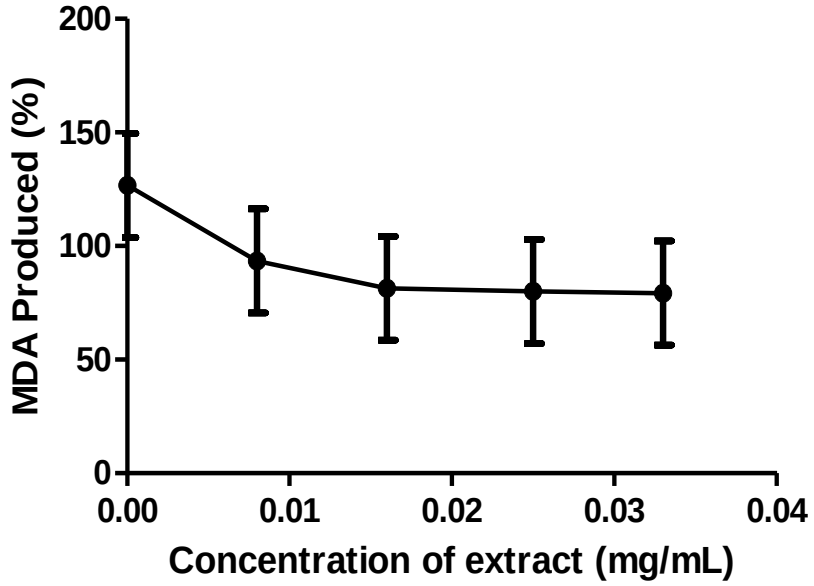

Figure.5: Inhibition of Fe- induced lipid peroxidation in *Drosophila melanongaster* by Coconut Water

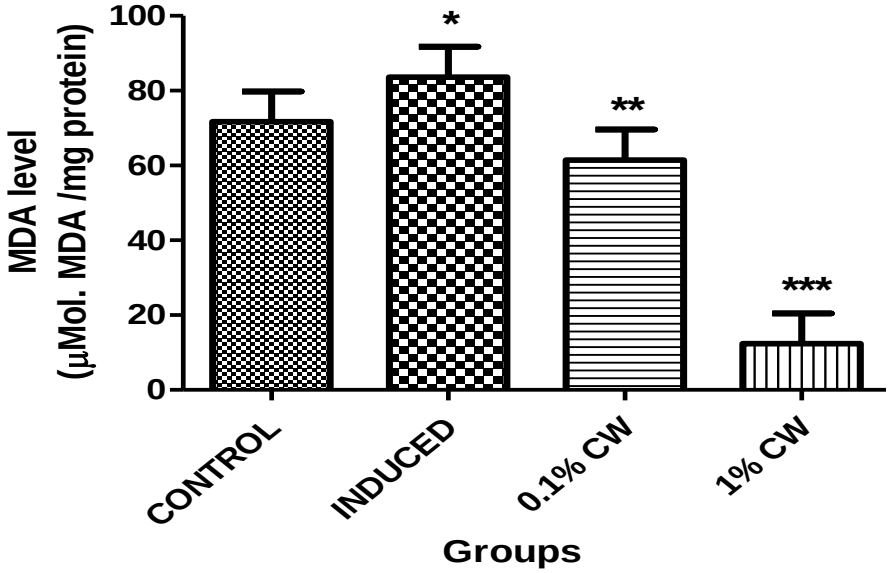

Figure.6: Effect of dietary inclusion of coconut water on brain MDA level in $Alcl_3$ induced amnesic *Drosophila melanongaster*. Values represent mean ± SD. *Values are significantly different at $p < 0.05$, **Values are significantly different at $p < 0.01$, ***Values are significantly different at $p < 0.001$.

4. Discussion

Over the years, coconut has been extensively explored for its use in different fields. Results from this study revealed high phenol and flavonoid content, consistent with a study on the phytochemical analysis of *Cocos nucifera* endosperm by Offor *et al*. [38], conferring its antioxidant ability to significantly lower cellular oxidative stress (Oboh *et al*. [28]. Flavonoids also function as signaling molecules exerting a positive

effect on cognitive function [40]. Phenolic compounds derived mostly from plants possess an antioxidant potential to manage oxidative stress-associated diseases like Alzheimer's and other neurodegenerative diseases [41, 42].

The scavenging ability and the reducing power of coconut water (CW) could be attributed to the total flavonoid and total phenol content. The increase in the scavenging ability and the reducing power of CW in a dose-dependent manner could also be due to the total phenolic concentration [18]. DPPH is a stable nitrogen-centered free radical donor that is stabilized by accepting an electron or hydrogen [43]. The ABTS and DPPH radical scavenging ability of CW results revealed that the extract can prevent radical-induced oxidative damage because phenolic compounds possess hydrogen-donating abilities to function as an antioxidant. Thus, reducing stable DPPH radical [44] and converting the coloured ABTS cation into a colourless form [45].

A similar trend was also observed when the antioxidant activity of the coconut water sample was assessed by the ferric reducing antioxidant power (FRAP) assay, a new antioxidant defence mechanism that is affected by the transfer of electrons and a hydrogen atom [45]. The antioxidants present in the extract can reduce ferric ion (Fe^{3+}) to ferrous ion (Fe^{2+}), which forms a blue complex that is absorbed at 700 nm [46]. Accordingly, several reports have established a correlation between the health benefit of polyphenolic-rich food and its antioxidant effects [47]. Additionally, current findings show that phenolic compounds exhibit health-promoting effects ranging from radicals scavenging to metal chelation responsible for lipid peroxidation [48]. Several animal models have been used to investigate the oxidative stress hypothesis of aging [49].

Specific reports have stated the implication of reactive oxygen species generation and oxidative stress in reducing *D. melanogaster's* life span [50]. The TBA assay was employed to measure the CW's scavenging ability of radicals generated in lipid peroxidation. The study revealed that both concentrations (0.1% and 1.0%) dietary inclusions of CW significantly reduce MDA content in the tissue homogenate, which agrees with Das *et al.* [51], who reported that coconut water significantly reduces free radical generation and has antioxidant activity. The increase in the scavenging activity correlates with a decrease in the MDA level as the induction progresses with the utilization of CW by the brain. Studies have shown that amnestic mild cognitive impaired patient' exhibit patterns of memory impairment and oxidative stress, similar to those observed in $Alcl_3$-induced amnesic flies' [52, 53]. Therefore, a reduction in the brain MDA (marker of lipid peroxidation) level of flies treated with CW extract indicates a marked improvement in the brain antioxidant status, which could be due to phenolic present in the extract (Table 1). This shows that coconut water extract is capable of inhibiting the formation of lipid peroxidation.

5.Conclusion

The obtained results show that coconut water extract has antioxidant property, inhibited prooxidant-induced TBARS production, scavenged free radicals, chelated Fe^{2+} *in vitro* and was also able to reduce MDA level in the brain of $Alcl_3$ induced amnesic *D. melanogaster* fed with coconut water supplemented diet. These abilities could be linked to the action of polyphenolic compounds

present in it. Therefore, this study suggests that coconut water could provide a cheap therapeutic means for neurodegenerative disease management/treatment. Further studies should be done to isolate and characterize bioactive compounds present in coconut water.

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