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ORIGINAL ARTICLE

Fatty acid composition and antioxidant effect of coconut oil in *Drosophila melanogaster*

Oluwatoyin Veronica Odubanjo[1] · **Ayo Emmanuel Oluwarotimi**[1] · **Comfort Oluwatosin Ayeni**[1] · **Henry Oluwatosin Akingbola**[1] · **Precious Taye Olabisi**[1]

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Abstract

Coconut oil (CO) has gained interest in western medicine due to its promising therapeutic approach in the treatment of Alzheimer's disease as well as combating oxidative stress-induced neurodegeneration. Using a wet extraction process, CO was extracted from fresh coconut milk. Flies were raised on CO-supplemented diet for 5 days, and the locomotor performance and survival rate were examined afterwards. The antioxidant activity of CO extract was investigated in vitro; in vivo using *Drosophila melanogaster* and the CO fatty acid (FA) composition quantified using gas-chromatography mass spectrometry (GC-MS). The survival rate and locomotor performance of the *D. melanogaster* reduced significantly at higher concentration of CO (1%)-supplemented diet. CO exhibits significant antioxidant ability by scavenging DPPH and ABTS radicals in a dose-dependent manner. Malondialdehyde (MDA) content was also significantly reduced in vitro in a dose-dependent manner. Likewise, groups II and III fed with supplemented 0.1% and 1% CO reduce MDA level significantly ($p < 0.05$) in $AlCl_3$-induced flies. GC-MS quantification revealed eight FAs with myristoleic acid (C14:0) as the most abundant, followed by stearic acid (C18:0). The major fatty acids established in this study are not retained as fat in the body but used to generate energy. These observed results prove the antioxidant ability of phytochemicals in CO. In conclusion, coconut oil is a good source of phenolic compounds and healthy FA that confers its therapeutic use.

Keywords Antioxidant · Fatty acids · *Drosophila melanogaster* · Coconut oil · Neurodegeneration

Introduction

Coconut (*Cocos nucifera*) is a perennial and flowering plant (Fig. 1) of the family Arecaceae. It is known for its comestible and medicinal properties (DebMandal and Mandal 2011).

✉ Ayo Emmanuel Oluwarotimi
emmanuel.ayobamidele@yahoo.com

Oluwatoyin Veronica Odubanjo
oluwatoyin.odubanjo@aaua.edu.ng

Comfort Oluwatosin Ayeni
comfytee001@gmail.com

Henry Oluwatosin Akingbola
henry.oluwatosin01@gmail.com

Precious Taye Olabisi
olabisitaye12809@gmail.com

[1] Department of Biochemistry, Adekunle Ajasin University, P.M.B 01, AkungbaAkoko, Ondo State, Nigeria

Cocos nucifera has a variety of uses and is categorized as a functional food because it provides many health benefits beyond its nutritional content (Anosike and Obidoa 2010). Coconut oil (CO) is one of the most important components of coconut associated with health. It is nutritious and obtained from freshly mature kernel milk (Kumalaningsih and Padaga 2012). CO is raw, chemically unprocessed, and is proposed to be safe for human consumption (Gopala et al. 2010). The CO extracted using a wet extraction process from fresh coconut milk has higher antioxidant properties and phenolic content compared with the coconut oil obtained from the drying method (Ghani et al. 2018). The wet extraction method of CO helps retain the bioactive component such as polyphenols and vitamin E compared with copra oil (Rohman et al. 2019). In addition, CO is produced through coconut milk fermentation with the addition of microbes (*Lactobacillus plantarum* and *Lactobacillus fermentum*) as starter's culture (Prasad and Satheesh 2014).

Reactive oxygen species (ROS) are toxic molecules, capable of causing higher rates of oxidative damage in tissue

Fig. 1 Coconut (*Cocos nucifera*). **a** Apical part of the tree with green coconut. **b** Fruit halves (DebMandal and Mandal 2011)

components of amino acids, proteins, lipids, and DNA (Sharma 2015). The effect of oxidative stress is worthy of notice in neurodegenerative disorders such as AD and PD (Li et al. 2015). The etiology of most neurodegenerative disorders is partially understood. Malondialdehyde (MDA), one of the lipid peroxidation products, has been shown to be elevated in patients with AD and PD (Shichiri 2014). The two main lines of defense against oxidative stress are endogenous antioxidant molecules and repair enzymes that neutralize most detrimental effects of these oxidizing species (Abolaji et al. 2015). The exogenous antioxidants are derived mainly from food and medicinal plants (Zhang et al. 2016).

Bioactive compounds, such as polyunsaturated and monounsaturated fatty acids, phenolic compounds, antioxidants vitamins (A, C, and E), and phytosterols from plant sources possess antioxidant ability exerting the health benefit functions by reducing or neutralizing free radical formation, thus protecting the cell from oxidative damage and related diseases (Siriwardhana et al. 2013; Dulloo 2011). These natural antioxidants from plant materials are mainly polyphenols, carotenoids, and vitamins (Baiano and del Nobile 2015) and they generally have a wide range of biological effects, especially in the treatment of neurodegenerative diseases (Li et al. 2016). CO is capable of preserving normal tissue and serum concentrations of lipid profile by trapping reactive species in aqueous components such as plasma and vascular walls of the lymphatic system due to its high polyphenol content (DebMandal and Mandal 2011). The consumption of a diet rich in phenolic compounds (e.g., fruits, vegetables) has been reported to provide defense against neurological diseases (Mahayothee et al. 2016) by providing the ability to control and modulate many cellular processes such as signaling, apoptosis, and redox balance (Narayanankutty et al. 2018).

Fruit fly (*Drosophila melanogaster*) is an arthropod belonging to the *Drosophilidae* family. Since its discovery more than 100 years ago, *D. melanogaster* has become a standard for biological research in particular in genetics and molecular biology (Abolaji et al. 2013). *Drosophila* is now considered an important biological model for the study of clinical toxicology (Paula et al. 2013), and it was used to explicate the structural basis of human diseases (Sudati et al. 2013). It also acts as a valuable model for evaluating the therapeutic agents against many human diseases (Adedara et al. 2015a, 2015b). Drosophila has distinct biological features comparable with that of vertebrates (Lehmann 2018), and it is known to replace the use of higher animals in biomedical research such as toxicity studies (Musselman et al. 2013).

Gas chromatography columns with various polarities are designed to analyze compounds with a corresponding polarity spectrum. Chromatographic columns with various polarities should be used to assess the composition of an essential oil extract in a systematic way (Chen et al. 2009). One of the most important considerations for various vegetable oils is the unsaturation and saturation content of the fatty acids (Dauqan et al. 2011). Edible oils are natural mixtures of plant origin that consist of glycerol-derived ester mixtures with a fatty acid chain (Kostik et al. 2012). CO is extracted using either hot or cold pressing techniques, and the process used has been stated to affect the quality and grade of the oil (Vysakh et al. 2014). In this present study, the cold-pressed extracted coconut oil was assayed for the geotactic behavior, antioxidant capacity in vitro and in vivo (dietary inclusion in an aluminum oxidative-induced neurotoxic *D. melanogaster*), and its fatty acid composition quantification using gas-chromatography mass spectrometry (GC-MS).

Materials and methods

Sample collection and preparation

Fresh thirty *C. nucifera* fruits were purchased from a local market in Akungba-Akoko, Ondo State, Nigeria. The shells were broken to remove the coconut meat and the water. The coconut meats were cut into smaller pieces, grated, and subsequently matched with an electric blender. The coconut milk was then filtered with cheesecloth, left to curd for about 24 h before being separated into two layers (oil and water). Upon removing water, the curd and oil were put in an oven for melting (70 °C), and a spoon was used to scoop the oil from the curd. A pure virgin coconut oil was obtained by filtering the extracted oil into a sterile container and then labeled. *Cocos nucifera* fruits used were selected based on ethnopharmacological survey in folklore on its neuroprotective ability.

Chemicals and reagents

Fungicide (nipagin), nutrient agar, aluminum chloride, 70% ethanol, distilled water, ortho-phosphoric acid, N-naphthyl ethylene diaminedihydro-chloride (NEDD), acetylthiocholine (substrate), potassium persulfate, Trolox, 5,5-dithio- bis (2-nitro-benzoic) acid (DTNB), sodium dihydrogen/disodium hydrogen phosphate, distilled water, 1,10,orthophenanthroline, 2,2-diphenyl-1-picrylhydrazyl (DPPH), FeSO4, KFeCN, and trichloroacetic acid (TCA) were the chemicals and reagents used. All chemicals and reagents employed were of analytical grade.

Equipment

Cooking pot, wooden stirrer, measuring cylinder, glass jars, beakers, blenders, knives, dry plastic container, pH meter, centrifuge, water bath, Spectrophotometer, Eppendorf tubes, micro-pipette, weighing balance, freezer, cotton plugs, vials, spoons, ice block trays, ice block packs, test tube racks, test tubes, gas cooker, cornmeal, brewer's yeast, analytical weighing balance, freeze dryer, and GC-MS QP 2010 were the equipment used.

Drosophila melanogaster stock culture

Wild type *D. melanogaster* (Harwich strain) stock culture was obtained from Drosophila Research Laboratory, Functional Food and Nutraceutical Unit, Department of Biochemistry, Federal University of Technology Akure, Nigeria. The flies were maintained and reared 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 ± 1 °C; 60% relative humidity respectively) under 12 h dark/light cycle conditions in the Drosophila Research Laboratory, Adekunle Ajasin University Akungba, Ondo State, Nigeria. All the experiments were carried out with the same *D. melanogaster* strain (Oboh et al. 2016).

Experimental design

Drosophila melanogaster Harwich strain (both gender, 5 days old) was 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 10 mM Al, 0.1% CO, and 1% CO as shown in Table 1.

Table 1 The four groups and their respective diets

Groups	Diet
I	Normal diet (control)
II	Normal diet + 10 mM Al^{3+} (from $AlCl_3$)
III	Normal diet + 10 mM $AlCl_3$ + 0.1% CO
IV	Normal diet + 10 mM $AlCl_3$ + 1% CO

The flies were exposed to these treatments for 5 days and sustained at ambient temperature. All experiments were done in triplicates.

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 oil-supplemented diet was prepared by adding 0.1 and 1.0 μL/L of the coconut oil to the basal diet respectively. The media were then mixed and distributed into vials.

Animal transfer for new emergence and treatment

The flies were transferred every 5 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 fell to the bottom of the jar. The cotton plug on the jar mouth 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.

Survival study

A study was carried out to assess the effect of dietary inclusion of coconut oil on the survival rate of flies after 5 days of exposure. Flies were divided into 3 groups containing 40 flies each. Each group was exposed to different dietary inclusions of coconut oil (0.1% and 1.0%). On a daily basis for the first 5 days, the mortality rate was observed and the survival rate was measured by counting the dead flies. The data were afterwards evaluated and plotted as aggregate mortality and percentage survival after the treatment period (Abolaji et al. 2014; Adedara et al. 2016).

Locomotor performance measurement (negative geotaxis)

The flies' locomotor performance was evaluated using the negative geotaxis (Le Bourg and Lints 1992). The flies in each group were immobilized briefly in ice and transferred into a clean tube of 11 cm length and 3.5 cm diameter for a period of 5 days, and each tube was labeled accordingly. After immobilization for 10 min, they were allowed to recover and taped at the tube's bottom thereafter. The total number of flies that crossed the 6-cm line within a period of 30 s was observed and recorded. The results were expressed as the percentage of flies that escaped beyond a minimum distance of 6 cm in 30 s during three independent experiments.

Preparation of tissue homogenate

The ice-immobilized and homogenized flies in 0.1 M phosphate buffer and pH 7.4 were centrifuged at 10,000×*g*, 4 °C for 10 min in a Kenxin refrigerated centrifuge model KX3400C (KENXIN Intl. Co., Hong Kong). Subsequently, the supernatant was separated from the pellet into labeled Eppendorf tubes and used for various biochemical assays.

Determination of biochemical parameters

CO free radical scavenging ability was measured using ABTS assay according to Re et al. (1999) and DPPH assay according to Brand-Williams et al. (1995). Ferric reducing antioxidant power was measured as described by Oyaizu (1986), and iron-chelating ability was assessed using the modified method of Minotti and Aust (1987) by Puntel et al. (2005).

Anti-lipid peroxidation assay

A modified Ohkawa et al. (1979) method was employed for the anti-lipid peroxidation assay. A reaction mixture containing 30 μL of 0.1 M pH 7.4 Tris-HCl buffer, oil extract (0–100 μL), and 30 μL of 250 μM freshly prepared FeSO4 (prooxidant) 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 1 h. A total of 300 μL 8.1% sodium dodecyl sulfate (SDS) was added to the reaction mixture for color reaction development, afterwards 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 °C for 1 h. The produced thiobarbituric acid reactive species (TBARS) from the reactions was measured at 532 nm using a JENWAY UV-visible spectrophotometer. The absorbance was compared against the malondialdehyde (MDA) standard curve.

GC-MS quantification of fatty acids

Analysis of fatty acid methyl esters (FAME) was performed on a GC-MS QP 2010 by Shimadzu equipped with a splitless injector. A fused silica Zebron ZB-FFAP capillary column (30 m × 0.25 mm ID, 0.25 μm film thickness) was used to separate the FAMEs using helium as the carrier gas at flow rates of 1 mL/min and a split ratio of 1:10. The injector temperature was 250 °C. The oven temperature was programmed at 100 °C for a hold of 10 min and increased to 225 °C at a rate of 7 °C/min and hold at the final temperature for 10 min. The LabSolution software was used to achieve the GC-MS operation control. MS spectra were attained at range width m/z 40–500, interface temperature 255 °C, ion source temperature 210 °C, solvent cut time 3 min, event time 0.20, and scan speed 2500. Authentic-used standards of fatty acid methyl esters (FAME) were from NIST 14 and 14s (National Institute of Standards and Technologies, Mass Spectra Libraries). Comparing their retention time and equivalent chain length with standard FAME, the sample FAME peaks were identified.

Statistical analysis

The results of triplicate readings were pooled and expressed as mean ± standard deviation (SD). One-way analysis of variance (ANOVA) was used to analyze the results, with levels of significance accepted at $p < 0.05$, $p < 0.01$, and $p < 0.001$. All statistical analyses were carried out using the Graph pad PRISM (V.5.0) software. IC50 (concentration of extract that will cause 50% reducing activity) was determined using linear regression analysis (Zar 1984).

Result and discussion

The effect of 0.1% and 1% of coconut oil dietary inclusions on 5-day survival of *D. melanogaster* is presented in Fig. 2. This shows that 4.67% and 26.67% reduction was observed in the survival rate of flies fed with supplemented CO (0.1% and 1% respectively) compared with the control. This effect was significant at $p < 0.001$ in flies fed with 1.0% CO, suggesting that coconut oil could induce some toxicological effects at higher concentrations. The decline in the survival rate of flies at higher dietary inclusion percentage (1%) could be attributed to a significant elevation of ROS produced in the flies (Oboh et al. 2016). Similarly, the locomotor performance (negative geotaxis) of flies fed with CO supplement diet (0.1% and 1.0%) is presented in Fig. 3. This reveals that the locomotor performance of flies fed with 1.0% CO significantly decreases ($p < 0.001$) in contrast to the control group, while there was no significant ($p < 0.05$) decrease in the locomotor performance of flies fed with 0.1% of CO in contrast to the control group.

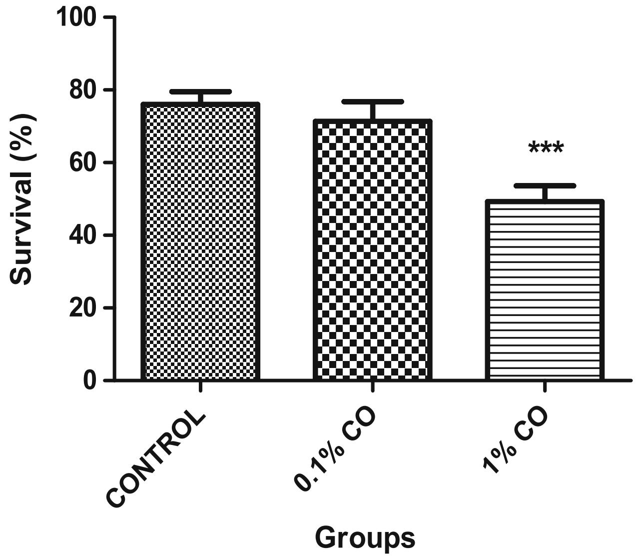

Fig. 2 Day 5 survival rate (%) of *D. melanogaster* fed diet supplemented with coconut oil (CO). Valuesrepresent mean ± SD. ***Value is significantly different at $p < 0.001$

These imply that CO could offer some medicinal properties at lower concentration and toxicological effects at higher concentrations, which may be due to high lauric acid in CO that act differently to other biologically active MCFAs (Boemeke et al. 2015; Neelakantan et al. 2020). In addition, according to a report from the Asian and Pacific Coconut Community (2016), coconut oil has been associated with elevated low-density lipoprotein (LDL) cholesterol concentration due to its high saturated fats, and its intake has been linked with high risk of cardiovascular disease (CVD). The locomotive performance disorder might be due to a high quantity of glycosides identified for being neurotoxic and cardiotoxic (David et al. 2013).

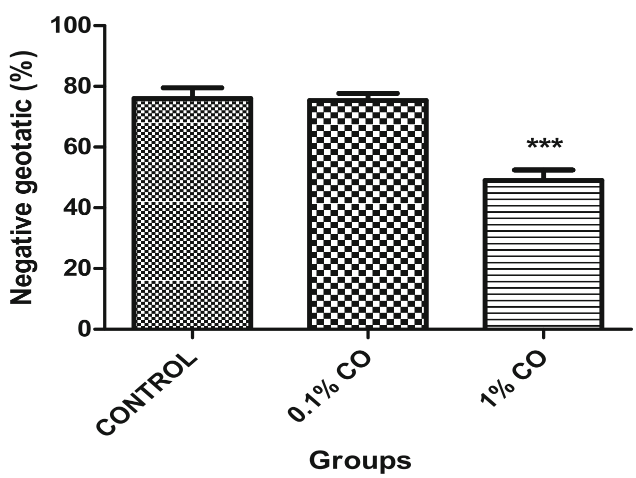

Fig. 3 Percentage locomotor performance (negative geotaxis) of *Drosophila melanogaster* fed diet supplemented with coconut oil (CO). Values represent mean ± SD. ***Value is significantly different at $p < 0.001$

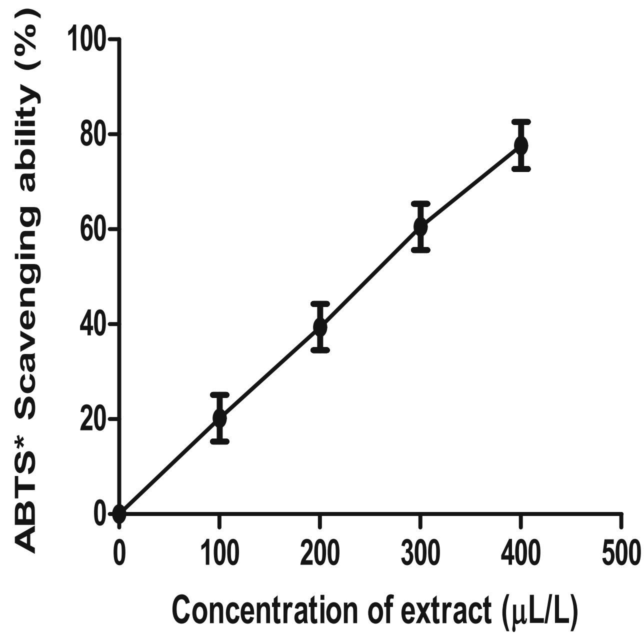

Fig. 4 ABTS radical scavenging ability of coconut oil (*Cocos nucifera*)

Different radicals produced in the living cell through molecular metabolism have the potential to impede normal activity and trigger different ailments (Phaniendra et al. 2015). Therefore, a balance must be maintained between free radicals and antioxidants. Using coconut oil as a nutritional source balances the oxidant-antioxidant ratio. Figure 4 shows the 2,2-azino-bis-3-ethylbenzothiazoline-6-sulfonate (ABTS) radical scavenging ability of coconut oil extract (0–400 µL/L) expressed as trolox equivalent antioxidant capacity (TEAC). Coconut oil was able to scavenge ABTS* in a dose-dependent manner at the concentrations tested. The result of the 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging ability of CO is presented in Fig. 5. The DPPH scavenging ability of the CO extract increases with

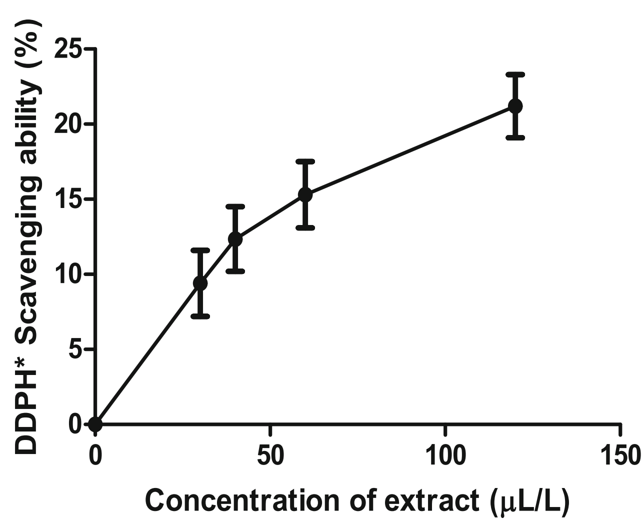

Fig. 5 The DPPH radical scavenging ability of coconut oil (*Cocos nucifera*)

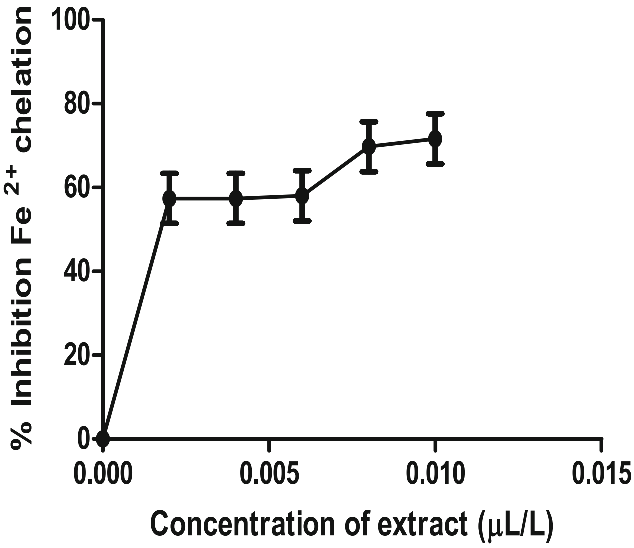

Fig. 6 Fe^{2+} chelating ability of coconut oil (*Cocos nucifera*)

concentration (0–133 μL/L). These results showed that CO possesses the ability to scavenge both ABTS and DPPH radicals in a dose-dependent manner, thus able to prevent radical-induced oxidative damage consistent with a report by Marina et al. (2014) who studied the antioxidant activities of coconut oil. Accordingly, several reports have established a correlation between the health benefit of a polyphenolic-rich food plant and their antioxidant properties (Piluzza and Bullitta 2011; Saeed et al. 2012) and the possible mechanism behind the phenolic activity could be the redox properties of their OH groups (Dai and Mumper 2010).

A similar trend was observed in the results presented in Figs. 6 and 7 (Fe^{2+} chelating ability of coconut oil extract and ferric reducing the power of the of CO extract) respectively. Evidence has proposed that transition metals, particularly iron (Fe), are a potent catalyst of free radicals. Fe accumulates in the brain with age and disease, increasing oxidative damage potential (Oboh et al. 2014). The result of the iron-chelating

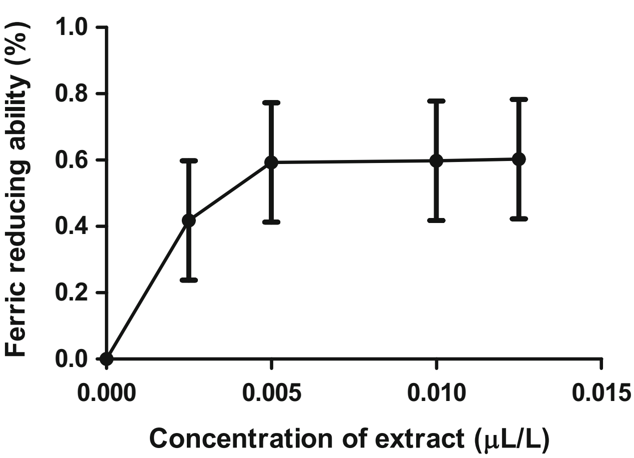

Fig. 7 Ferric reducing ability of coconut oil level in $AlCl_3$-induced amnesic *Drosophila melanogaster*

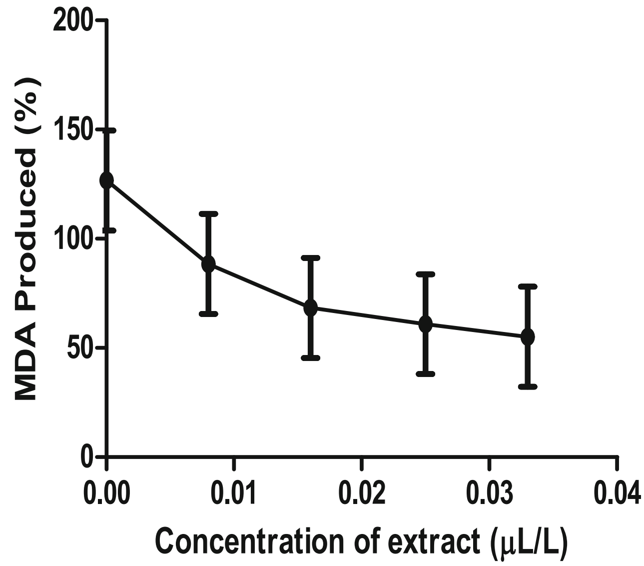

Fig. 8 Inhibition of Fe-induced lipid peroxidation in *Drosophila melanogaster* by coconut oil

ability of the CO extract follows a dose-dependent manner indicating that CO can chelate radical-induced metals in the brain cells. The highest chelation was observed at the highest dose of the extract, which could be due to its higher phenolic content, also reported in several studies (Narayanankutty et al. 2016). Likewise, the reducing antioxidant property of the CO extract, expressed as ascorbic acid equivalents, was studied by reducing power assay. This result reveals that CO was able to reduce ferric ion (Fe^{3+}) to ferrous ion (Fe^{2+}) with an increase in concentrations as a result of antioxidants present in the extract. This is in agreement with a report by Ahmad et al. (2015) who described the relationship between antiradical and antioxidant activities of virgin coconut oil using West African tall variety.

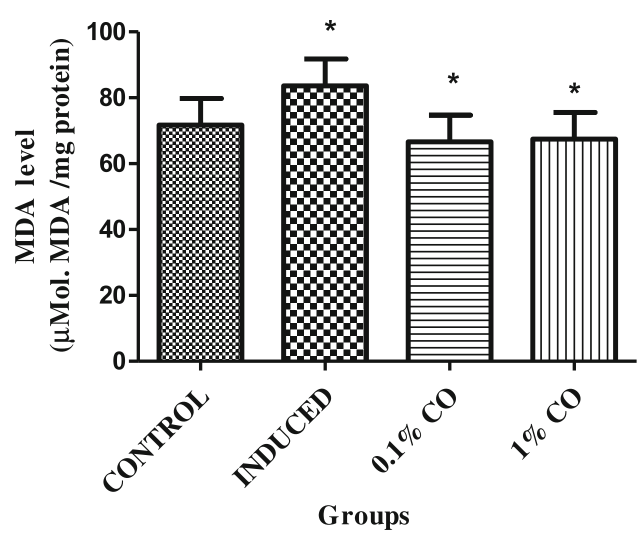

Fig. 9 Effect of coconut oil on Brain MDA level in $Alcl_3$-induced amnesic *Drosophila melanogaster*

Table 2 The fatty acid component of coconut oil

S/N	Ret time (min)	Area	Grp ID	Fatty acids present	(%) Composition
1	7.323	27.02557	1	Caproic acid methyl ester	0.62[c]
2	11.713	1096.01831	1	Caprylic acid methyl ester	11.85[d]
3	17.496	8.07479	1	Lauric acid methyl ester	0.14[b]
4	19.848	5171.34473	2	Myristoleic acid methyl ester	*61.85*[f]
5	20.994	15.82353	1	Palmitic acid methyl ester	0.14[b]
6	22.967	2107.28467	1	Stearic acid methyl ester	*25.25*[e]
7	24.044	14.03049	2	Elaidic acid methyl ester	0.06[a]
8	27.386	11.77059	3	Linoleic acid methyl ester	0.09[a]
					100.00

Keys: Grp IDs 1, 2, and 3 have group names saturated fatty acids, monounsaturated fatty acids, and polyunsaturated fatty acids respectively. Values with different alphabets are significantly ($p < 0.05$) from one other

The free form of Fe had been linked to the etiology of neurodegenerative conditions because it can cross the blood–brain barrier in conditions of extracellular overload which can initiate lipid oxidation via the Fenton reaction (Mills et al. 2010). Incubation of the flies homogenate with Fe^{2+} caused a significant ($p < 0.05$) increase in malondialdehyde (MDA) level, indicating oxidative damage induced by free radicals generated by Fe^{2+} as presented in Fig. 8. However, MDA was reduced significantly by CO extract in a concentration-dependent manner which agrees with an earlier report by Oboh et al. (2014) that coconut oil prevents Fe^{2+}-induced lipid peroxidation. Figure 9 shows the effect of coconut oil dietary inclusion in $AlCl_3$-induced amnesic flies. The brain MDA content of $AlCl_3$-induced group was significantly ($p < 0.05$) higher than the normal control group. However, the groups fed with supplemented CO (0.1% and 1%) diet have significant ($p < 0.05$) reduction in MDA content compared with the control. Furthermore, the antioxidant activity of CO extract confers the ability to exert a protective effect on the brain against free radical attack due to high polyunsaturated fatty acid (PUFA) content present in the brain (Mushtaq and Wani 2013).

Figure 10 and Table 2 shows the chromatogram from the GC-MS study and the list of fatty acid components in the coconut oil, respectively. The findings indicated that coconut oil has a total amount of eight (8) fatty acids. The coconut oil peaks are as follows: (1) caproic acid (C6:0), (2) caprylic acid (C8:0), (3) lauric acid (C12:0), (4) myristoleic acid (C14:0), (5) palmitic acid (C16:0), (6) stearic acid (C18:0), (7) elacidic (C18:1), and (8) linoleic acid (C18:2). In the extracted oil, the concentrations of myristoleic acid and stearic acid were higher than other fatty acids. As a result, myristoleic acid methyl ester

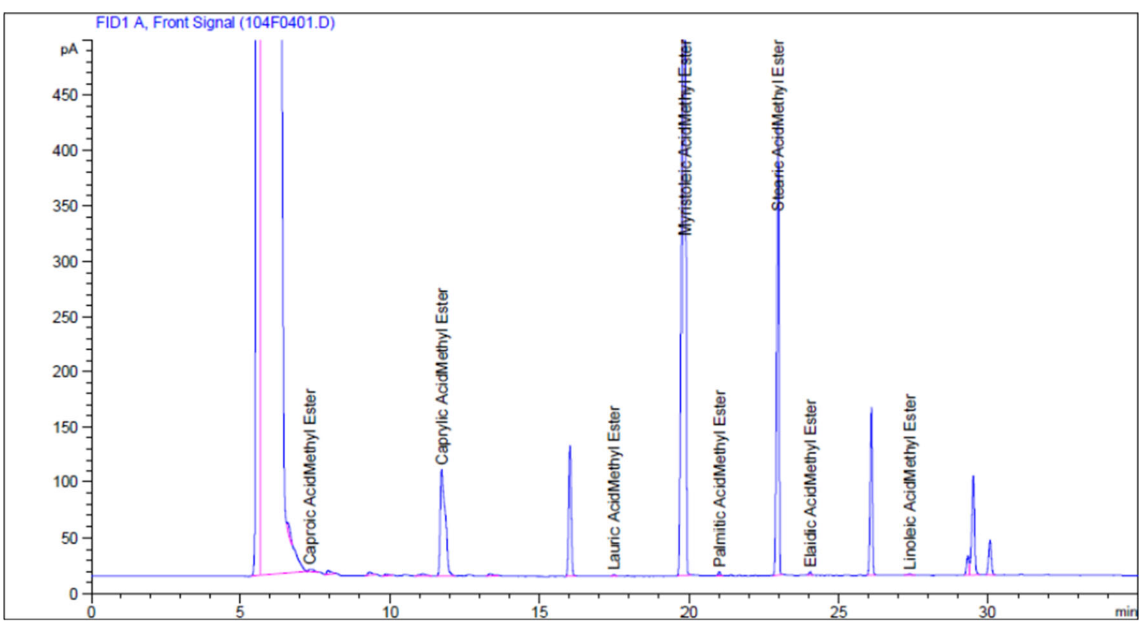

Fig. 10 Chromatogram showing the fatty acid composition of coconut oil

has the highest percentage of fatty acids with the highest peak area (pA 5171), followed by stearic acid (pA 2107), and lauric acid was the fatty acid portion with the least peak area (pA 8). The percentage of the fatty acids in this study is consistent with those present in extracted coconut oil in a study by Wallace (2018). Medium-chain fatty acids (MCFA) such as lauric, myristoleic, palmitic, capric, stearic, oleic, and caprylic acids are easily digestible in this coconut oil extract (DebMandal and Mandal 2011) and have beneficial effects on health (Gopala et al. 2010). Like other fats, these fatty acids do not pass through the bloodstream but are transported directly to the liver, where they are oxidized and converted into energy (DebMandal and Mandal 2011). They are also known to activate Lactobacilli and can enhance cognition in patients with type I diabetes (Zhou et al. 2014; Suryani 2016).

Conclusion

Coconut fruit products are commonly used in medicine for the treatment of various diseases around the world. The obtained results show that the CO extract contains high composition of safe saturated fatty acid (myristoleic acid, lauric acid, and stearic acid). Therefore, this study suggests that high consumption of coconut oil in *D. melanogaster* could induce some toxicological effects, while moderate–low consumption could be beneficiary and offer the well-reported medicinal effects attributed to coconut oil.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval This study article does not contain any studies with animals performed by any of the authors.

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