ORIGINAL RESEARCH

# Virgin Coconut (*Cocos nucifera*) Oil Attenuates Rotenone-Induced Toxicity in Fruit Flies (*Drosophila melanogaster*)

**Dosumu OO*, Akang EN, Idowu OK and Adeyemi GJ.**
Department of Anatomy, College of Medicine, Faculty of Basic Medical Sciences, University of Lagos, Lagos, Nigeria.

**Address for correspondence:**
Dr. Olufunke O. Dosumu
Department of Anatomy, Faculty of Basic Medical Sciences, College of Medicine, University of Lagos, Lagos, Nigeria.
Email: olufunkedosumu@gmail.com

**To cite this article:** Dosumu OO, Akang EN, Idowu OK and Adeyemi GJ. Virgin Coconut (*Cocos nucifera*) Oil Attenuates Rotenone-Induced Toxicity in Fruit Flies (*Drosophila melanogaster*). Journal of Basic and Social Pharmacy Research, 2021;2(1):26-37

## ABSTRACT

**Background:** Parkinson's disease (PD) is a multifactorial neurodegenerative disease with pathogenic mechanisms traceable to oxidative damage and mitochondrial dysfunction. Rotenone, a chemical compound commonly found in pesticides, has been found to inhibit mitochondrial complex-I and initiate PD-like symptoms in mammals and several invertebrates. Virgin Coconut Oil (VCNO) obtained from the coconut fruit has been found to possess anti-oxidative and anti-inflammatory properties.
**Objectives:** The present study evaluated the effect of VCNO on rotenone-induced Parkinsonism in fruit flies- *Drosophila melanogaster* (*D. melanogaster*).
**Methods:** Canton special (CS) strains of *D. melanogaster,* aged between 1 to 3 days were orally exposed for 7 days to 0, 250, 500 and 750 µM rotenone diet for toxicity assay, and 0, 2.5, 5 and 10 % *w/w* VCNO diet for longevity assay. Thereafter, 5 % VCNO diet was selected for evaluation against 500 µM rotenone. Subsequently, behavioural test (negative geotaxis), markers for redox status and enzyme activities were evaluated.
**Results:** The results showed that rotenone induced toxicity in the flies, while VCNO increased the lifespan of *D. melanogaster* in a dose-dependent manner. In addition, VCNO ameliorated rotenone-induced locomotor deficits, elevated MDA, as well as the depleted GSH levels. It also mitigated the inhibited activities of SOD, CAT and ATPase in the flies.
**Conclusions:** VCNO protected *D. melanogaster* against rotenone-induced toxicity by extending longevity, preventing locomotor deficits and reducing oxidative stress.

**Keywords:** *Drosophila melanogaster,* Fruit flies, motor skills, Parkinson's disease, Rotenone, Virgin Coconut Oil.

## INTRODUCTION

Parkinson's disease (PD), an age-related progressive neurodegenerative disease caused by several multifactors[1], is characterized by various motor and non-motor deficits majorly due to loss of dopaminergic neurons, depletion of dopamine and the presence of Lewy Bodies in surviving neurons[2,3]. Although, the etio-pathogenic mechanism of PD is not yet clearly defined, oxidative damage, inflammation and mitochondrial dysfunction that may be traced to a combination of predisposition by DNA and

environmental factors have been implicated[2-4].
Research has revealed that exposures to environmental toxicants such as pesticides contribute to the onset of PD symptoms[5,6]. Rotenone, a chemical compound commonly found in pesticides, has been found to inhibit mitochondrial complex-I and initiate PD-like symptoms in humans, rodents and invertebrates[7-9]. Rotenone being lipophilic in nature is able to cross the blood brain barrier and induce neurodegenerative syndromes[10,11]. In previous studies, treatment of *D. melanogaster* for 7 days with sub-lethal doses of rotenone resulted in loss of dopaminergic neurons, dopamine levels depletion and locomotor deficits[7,12].
*D. melanogaster* also known as the fruit fly has been used as a model organism of study in various biomedical disciplines. It has been revealed that about 75% of the genes responsible for human diseases have homologs in *D. melanogaster*[13]. Due to high rate of reproduction, short generation time, and low cost of maintenance, *D. melanogaster* has been considered worthy as a model organism to study complex pathways relevant in biomedical research, including neurodegenerative diseases[14].
Levodopa (L-dopa) among other anti-parkinsonism drugs has been used to effectively treat symptoms of PD[15]. L-dopa which is the most effective drug for PD only guarantees about 50% improvement in symptomatic relief and it is only effective for a period of 2 to 3 years[15]. Thereafter the effects of L-dopa may wear off as PD progresses[16]. Since oxidative stress and inflammation have been implicated in the pathogenesis of pesticide-induced model of PD[17-19], the search for natural products with anti-oxidative and anti-inflammatory properties is ongoing. This is because they can serve as suitable prophylactic and/or therapeutic candidates in the development and/or progression of toxicant-induced PD[20,21].
Virgin coconut oil (VCNO) has been found to be rich in carbohydrates (66%), dietary fibres, phytosterols, tocopherols, minerals[22,23] and active polyphenol compounds, which are strong inhibitors of lipid peroxidation[24]. VCNO has also been reported to have high levels of lauric acid (41%–54.5%)[25] with potent antioxidative, anti-inflammatory and anti-stress properties[26]. The present work was therefore carried out to evaluate the effect of VCNO on rotenone-induced toxicity in *D. melanogaster*.

## MATERIALS AND METHODS

### Chemicals

Rotenone (98% purity) and L-dopa (99% purity) were procured from Shaanxi Phoenix Tree, Biotech Co., Ltd., China. All other chemicals and reagents used were of analytical grade.

### Extraction of virgin coconut oil

VCNO used in this study was extracted from the coconut meat as described by Dosumu *et al.* [27] The coconut (*Cocos nucifera*) fruits were locally purchased from Idi- Araba market, Lagos, Nigeria, and samples were authenticated at the Botany Department of the University of Lagos, Nigeria. Four of the coconut fruits were cracked open, the meat was removed from the shells, chopped into very small pieces and ground in an electric blender (Master Cheff electronics, China). The ground mixture was sieved out with a fine cloth, and the filtrate (coconut milk) was left to stand undisturbed for 24 hours to facilitate the demulsification process. The upper layer which contained the oil was then gently scooped and heated at 70 ºC using an oven (Saisho, China) to remove moisture. The resultant oil was then allowed to cool down, filtered and stored at room temperature until needed for the study.

### Fly stock and culture

Ethical clearance for this study was obtained from the Animal Care and Use Research Ethics Committee of the College

of Medicine, University of Lagos, Nigeria and was assigned the approval number: CMUL/ACUREC/09/20/771. CS strain of *D. melanogaster* of Bloomington *Drosophila* Stock Center, Indiana, USA, was obtained from the Department of Pharmacology and Therapeutics, University of Lagos, Nigeria in 2019. The flies were maintained and reared on cornmeal medium containing brewer's yeast (1% w/v), agar-agar (1% w/v), and nipagin (preservative, 0.08% v/w) at constant temperature (22 ± 2 °C) under 12 h dark/light cycle in the *Drosophila* Laboratory, Department of Anatomy, University of Lagos, Nigeria.

**Determination of effective doses**

To determine the concentrations of exposure to rotenone and VCNO, 1-3 day old flies of both sexes were sorted into different groups of 40 flies each and administered rotenone (0, 250, 500 and 750 µM diet) and VCNO (0, 2.5, 5 and 10 % diet) for 7 days toxicity and longevity assays respectively. Daily mortality was recorded, climbing test was conducted for the survivors of the rotenone toxicity test and data were analyzed and plotted as percentage of dead flies in toxicity test and percentage of live flies in the longevity test. At the end of this phase of the experiment 500 µM rotenone and 5% VCNO were selected as the effective doses.

**Treatment of *Drosophila melanogaster* and sample preparation for analysis**

Both sexes of *D. melanogaster* of about1 - 3 days old were sorted into six groups (CTRL (control), VCNO, ROTE (rotenone), ETHA (ethanol), ROTE/VCNO and ROTE/LDPA (rotenone/levodopa)) of 40 flies each with five replicates per group. Flies in the control group were exposed to untreated diet, the ETHA group was exposed to diet treated with 2.0% ethanol (the vehicle for ROTE and LDPA groups), VCNO group was exposed to diet containing 5% VCNO, ROTE group was exposed to 500 µM rotenone diet, ROTE/VCNO group was exposed to 5% VCNO with 500 µM rotenone and ROTE/LDPA group was exposed to diet containing 80 mg/kg levodopa[28] with 500 µM rotenone for 7 days. Thereafter, test for locomotor functions (negative geotaxis) was conducted, after which the flies were anesthetized by freezing, weighed and homogenized in 0.1 M phosphate buffered saline with pH 7.2 at ratio of 1 mg:10 µL, and cryo-centrifuged (4°C) at 2500 rpm for 15 min[29]. Thereafter, the supernatant was transferred into labeled Eppendorf® tubes, and used for the evaluation of markers for oxidative stress and antioxidant status [malondialdehyde (MDA), superoxide dismutase (SOD), glutathione (GSH), catalase (CAT)] and ATPase activity.

**Assessment of negative geotaxis assay**

To evaluate locomotor performance, twenty flies from each group were placed in vertical tubes, covered with parafilm and allowed to acclimatize for 15 min. Thereafter, each tube was turned upside down, and the flies were gently tapped to the bottom of the test tube. The number of flies that climbed up to17.5 cm height of the tube in 8 s and the flies below the mid mark were recorded. The scores represented the mean of the number of flies at the top ($n_{top}$) expressed as a percentage of the total number of flies ($n_{tot}$). This procedure was repeated three times at 1 min interval[30,31].

**Malondialdehyde (MDA) Quantification.**

To quantify MDA levels the supernatant from the homogenate was analyzed by spectrophotometry following the method of Karatas *et al.*[32] with little modifications. The eyes of flies were previously removed to avoid the interference of eye pigments with MDA absorbance[33].

**Determination of glutathione levels**

Reduced glutathione (GSH) levels were estimated according to the method described by Sedlak and Lindsay[34]. One millilitre (1.0 ml) of supernatant was treated

with 0.5 ml of Ellman's reagent (19.8 mg of 5,5-dithiobisnitro benzoic acid (DTNB) in 100 ml of 0.1% sodium nitrate) and 3.0ml of phosphate buffer (0.2 M, pH 8.0). The absorbance was read at 412 nm. $\sum = 1.34 \times 10^4\ M^{-1}\ cm^{-1}$.

**Determination of Superoxide Dismutase (SOD) activity**

SOD activity was determined by its ability to inhibit the auto-oxidation of epinephrine determined by the increase in absorbance at 480 nm as described by Sun and Zigma[35]. The reference cuvette contained 2.95 ml buffer, 0.03 ml of substrate (epinephrine) and 0.02 ml of water. Enzyme activity was calculated by measuring the change in absorbance at 480 nm for 5 min. $\sum = 4020\ M^{-1}\ cm^{-1}$.

**Determination of catalase activity**

Catalase activity was determined according to the method of Aebi[36]. The reaction medium contained 170 µL of 50 mM phosphate buffer (pH 7.0), 20 µL of 300 mM hydrogen peroxide, and 10 µL of sample (1:50 dilution). Subsequently, the decrease in absorbance of $H_2O_2$ ($\Sigma = 39.4\ mM^{-1}\ cm^{-1}$) at a wavelength of 240 nm was recorded for 2 min (10 s interval) using a SpectraMax plate reader (Molecular Devices). Thereafter, the activity of catalase was calculated and expressed in µmol of $H_2O_2$ consumed/min/mg of protein.

**Evaluation of ATPase activity**

ATPase activity was measured as previously described by Swank *et al.*[37] and Cammarato *et al.*[38] and each sample was run in three replicates. ATPase activity was determined by defining Vmax and Km values for each sample and calculating the mean ± SD for their ratio (Vmax/Km)[39].

**Statistical analysis**

All data were reported as mean ± SEM. The statistical analyses and graphs were done using Graph Pad Prism 8 software (Graph pad software Inc., USA). Comparisons were done using a one-way ANOVA with Tukey's *post hoc* test. Significance was set at $p < 0.05$.

**RESULTS**

**Effect of rotenone-induced toxicity in *D. melanogaster***

There was a statistically significant ($p < 0.05$) increase in percentage toxicity in all treated groups compared to the control group. Severity of rotenone toxicity appeared to be dose dependent. Exposure of the flies to rotenone (250, 500 and 750 µM) for 7 days increased the number of deaths by 11.3%, 17.5% and 27.5% respectively when compared to the control (Fig. 1A). In the climbing assay conducted for the survivors thereafter, there was a statistically significant ($p < 0.05$) decrease in the numbers of flies that could climb above the height of 17.5 cm in both 500 and 750 µM, while 250 µM of rotenone treated group showed no significant difference in the numbers of flies that crossed the height of 17.5 cm during the negative geotaxis test compared to the control. The performances of the control, 250, 500 and 750 µM groups were 95.0%, 81.3%, 52.5% and 53.8% respectively (Fig. 1B). From the study, since 750 µM rotenone-treated diet caused death in more than 50% of the fly population, while the 250 µM did not significantly induce locomotor deficits, the concentration selected to induce PD in the study was therefore 500 µM.

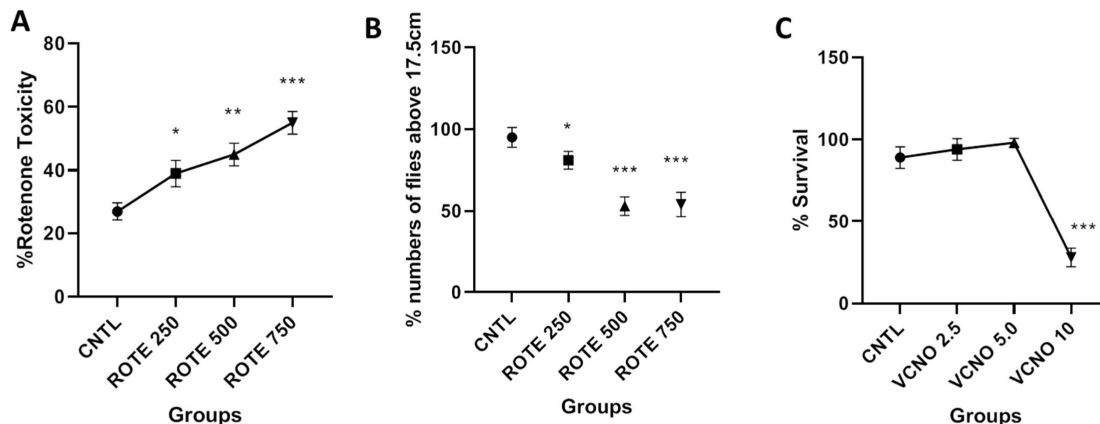


**Figure 1:** Rotenone toxicity (A), Climbing test (B), VCNO survival rate (C). Data is presented as Mean ± S.E.M. at *p< 0.05, **p< 0.01, ***p< 0.001 compared to control using One Way ANOVA followed by Tukey's Post-Hoc Test. (CNTL: Control, ROTE: Rotenone, VCNO: Virgin coconut oil).

**Effect of VCNO on survival rate of *D. melanogaster***

Exposure of flies to VCNO diet (2.5 and 5.0%) for 7 days showed numerical increase in rate of survival (prolonged longevity of flies by 1.3% and 7.5% respectively) which was however not significant when compared with the control (Fig. 1C). The high rate of death recorded in the 10% VCNO diet was due to trapping of flies by the oily diet rather than toxicity of VCNO, as a result the 5.0% VCNO diet was selected for investigation on rotenone-induced PD in the flies.

**Effect of VCNO on rotenone-induced locomotor deficit in PD models of *D. melanogaster***

There was statistically significant (p< 0.05) decrease in climbing activities of flies in the ROTE and ROTE/VCNO and ROTE/LDPA groups during the negative geotaxis test compared to the control group (Fig. 2A). There was no statistically significant difference between ETHA and VCNO groups in the climbing activities of the flies compared to the control group. However, there was a statistically significant (p< 0.05) improvement in climbing activities of flies in the ROTE/VCNO and ROTE/LDPA groups during the test compared to ROTE group. There was no statistically significant difference in climbing activity between ROTE/VCNO and ROTE/LDPA groups.

**Effect of VCNO on rotenone-induced elevation of MDA and depletion of GSH in PD models of *D. melanogaster***

There was a statistically significant (p< 0.05) increase in MDA level (Fig. 2B) accompanied by a statistically significant (p< 0.05) decrease in GSH levels (Fig. 2C) in the ROTE, ROTE/VCNO and ROTE/LDPA group compared with the control while there was no statistically significant difference between ETHA and VCNO groups. Conversely, there was a statistically significant (p< 0.05) lowering of MDA level as well as a statistically significant (p< 0.05) elevation of GSH level in ROTE/VCNO and ROTE/LDPA groups compared to ROTE group. There was no statistically significant difference in MDA and GSH levels between ROTE/VCNO and ROTE/LDPA groups.

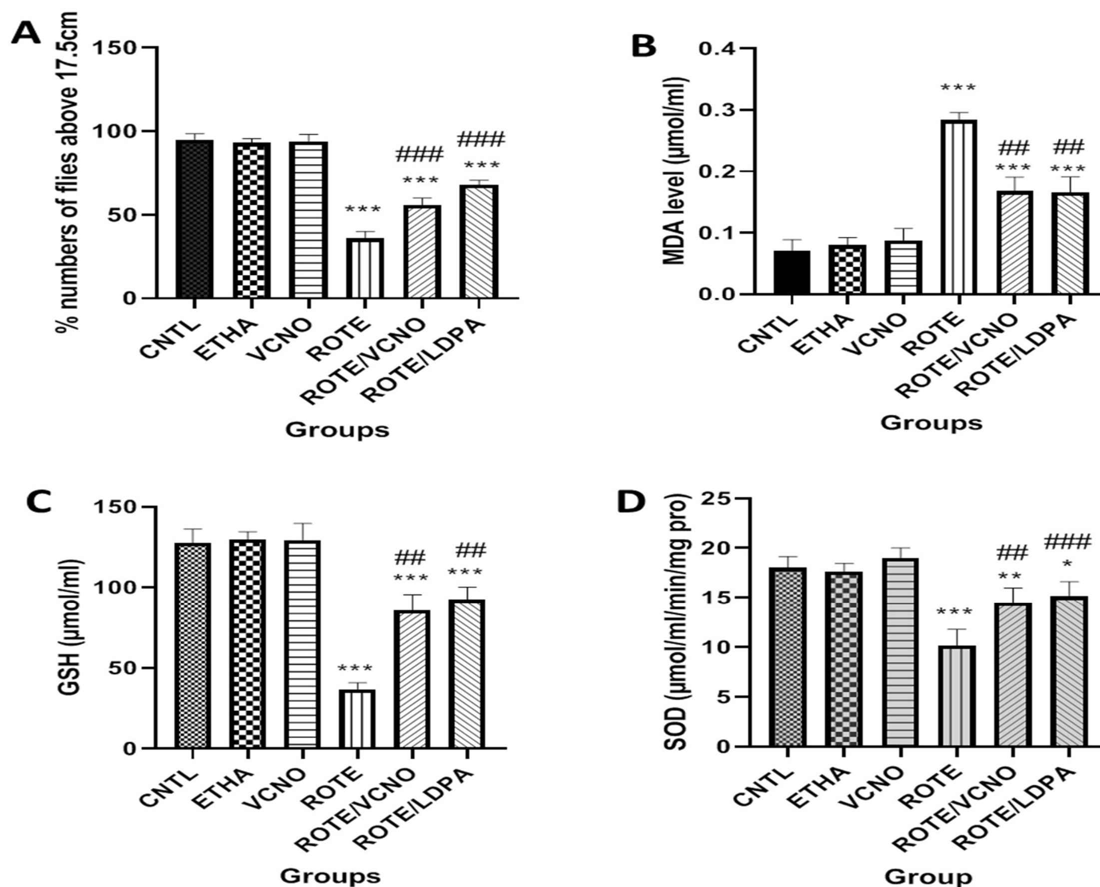


**Figure 2:** Negative geotaxis test (A), MDA levels (B), GSH levels (C), SOD activity (D). Data is presented as Mean ± S.E.M. at *p< 0.05, **p< 0.01, ***p< 0.001 compared to control; #p< 0.05, ##p< 0.01, ###p<0.001 compared to ROTE group using One Way ANOVA followed by Tukey's Post-Hoc Test. (CNTL: Control, ETHA: Ethanol, VCNO: Virgin coconut oil, ROTE: Rotenone, LDPA: L-dopa).

**Effect of VCNO on rotenone-induced inhibition of SOD, CAT and ATPase activities in *D. melanogaster***

There was a statistically significant ($p < 0.05$) decrease in SOD (Fig. 2D), CAT (Fig. 3A) and ATPase (Fig. 3B) activities of the ROTE, ROTE/VCNO and ROTE/LDPA groups when compared to the control group, while there were no statistically significant differences in SOD, CAT and ATPase activities among other treated groups compared to control. However, the groups treated with ROTE/VCNO and ROTE/LDPA showed statistically significant ($p < 0.05$) improvement in SOD, CAT and ATPase activities compared to the ROTE group. When these parameters were compared in the groups treated with ROTE/VCNO and ROTE/LDPA the difference was not statistically significant.

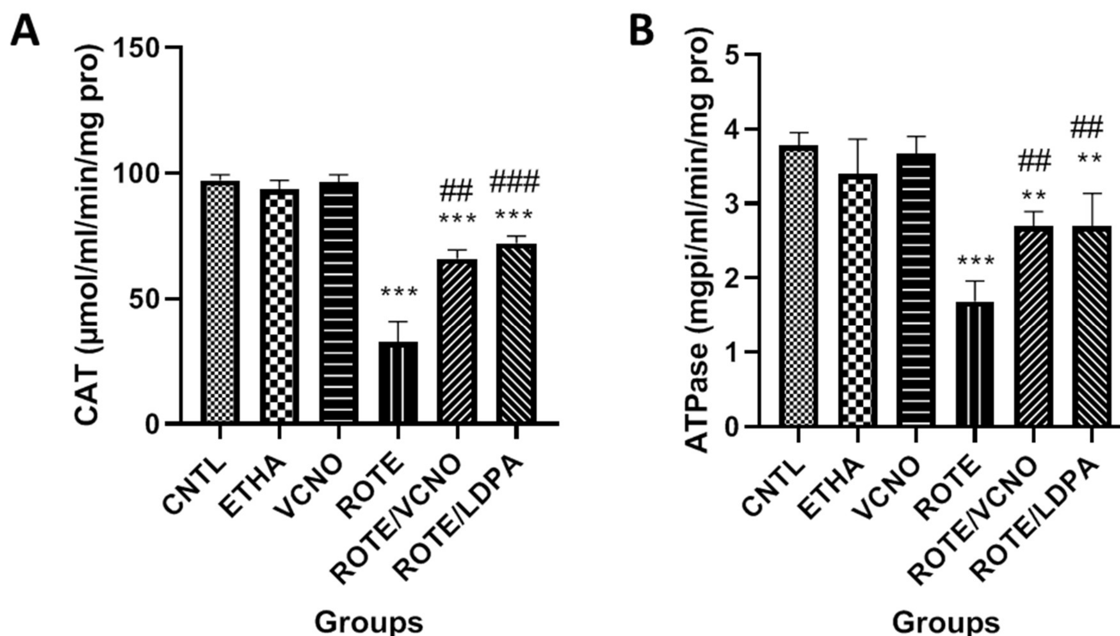


**Figure 3:** CAT activity (A), ATPase activity (B). Data is presented as Mean ± S.E.M. at *p< 0.05, **p< 0.01, ***p< 0.001 compared to control; #p< 0.05, ##p< 0.01, ###p<0.001 compared to ROTE group using One Way ANOVA followed by Tukey's Post-Hoc Test. (CNTL: Control, ETHA: Ethanol, VCNO: Virgin coconut oil, ROTE: Rotenone, LDPA: L-dopa).

## DISCUSSION

In recent times, Drosophila has gained wider acceptance as a model organism not only in the understanding of the mechanisms surrounding the pathophysiology of neurodegeneration but also in the screening of myriads of phytochemicals for their efficacy in experimentally-induced neurodegenerative diseases[40]. The present study revealed the ameliorative potentials of VCNO in rotenone-induced toxicity in fruit flies. VCNO (2.5 and 5.0% *w/w* diet) was able to prolong longevity in the treated flies by 1.3% and 7.5% respectively. This effect may be attributed to the polyphenols present in the oil, which are reportedly beneficial in the improvement of oxidative stress involved in the etiopathogenesis of various types of diseases including PD[41]. Indeed, science is beginning to establish a close relationship between polyphenols and longevity. It is now becoming clearer that these molecules have the potential to regulate cellular senescence in aging and in longevity[22,42]. In a nutshell, polyphenols have the ability to modulate some of the evolutionarily conserved hallmarks of aging such as oxidative stress, inflammation, cell senescence and autophagy[42].

Rotenone like other established neurotoxins may lead to the development of PD-like symptoms and pathology[43,12]. It is believed that rotenone exerts its toxic effects by inhibiting mitochondrial complex-I[17,44]. From our study, rotenone-exposed flies showed significant locomotor deficits (a major symptomatic feature of PD), evident in the decreased climbing ability of the flies. This result agreed with the findings of Sudati *et al.*[12] who reported that 7 days exposure to 500 µm rotenone diet induced locomotor deficits in *D. melanogaster*[12]. Zhang *et al.*[45] in their study have related energy deficits to

poor locomotor performance[45]. In addition, the vulnerability of neurons to mitochondrial damage as a result of their high metabolic activity and energy requirement may also result in locomotor deficits as observed in the flies[46]. Interestingly however, co-treatment with VCNO ameliorated the rotenone-induced locomotor deficits in the treated flies. VCNO is reportedly rich in medium chain triglycerides (such as lauric acid and myristc acid among others) which can be rapidly metabolized to ketone bodies that could serve as an alternative source of energy in situations where glucose metabolism or glucose uptake is deficient[47,48]. It is also possible that the natural antioxidants in VCNO such as polyphenols and tocopherols have the ability to act as an electron transporter for mitochondrial complex-I. These potentials of VCNO may have contributed to the positive response in the motor skills recorded in the flies.

Additional evidence suggests that pesticides such as rotenone can induce PD-like pathology such as Lewy body formation, dopaminergic neuron degeneration, decrease in striatal dopamine levels and alteration in GSH homeostasis[49]. Alterations in the expression of genes associated with the sporadic form of the disease have also been associated with rotenone[12]. Summarily, the effects mediated by rotenone can be summed up as oxidative stress linked with mitochondrial dysfunction and ROS overproduction[17]. In concert with these reported effects, the present study showed that rotenone-fed diets increased MDA levels in the flies accompanied by depleted GSH levels and inhibited SOD and CAT activities, thereby significantly hampering the ability of the flies to combat oxidative stress as observed in the study. The primary role of SOD in living organisms is to facilitate the conversion of superoxide anion to less damaging compounds which are then broken down to water by the action of CAT. While rotenone inhibited the activities of these enzymes similar to previous studies[40,50,31]. The diminutive activities of SOD and CAT- a consequence of rotenone toxicity was however mitigated in flies co-treated with VCNO and rotenone confirming the antioxidant property of the oil.

Lipid peroxidation has been associated with injuries to DNA, proteins and lipid components of tissues, which in turn have been linked with several diseases in living organisms[51]. GSH is an important antioxidant in living organisms that prevents damage to cellular components caused by reactive oxygen species and peroxides[52]. The decreased GSH levels that accompanied the significant rise in MDA levels in rotenone-fed flies, in the present study may account for the inhibition of ATPase activity the resultant impairment of ATP production needed for locomotion. These consequences can be attributed to the ripple effects of oxidative stress-induced mitochondrial dysfunction. In addition, it should be noted that mitochondria as cellular organelles do not synthesize GSH but replenishes their pool of mitochondrial GSH (mGSH) from the cytoplasm[53]. Hence it is logical to infer that if the cytosolic GSH pool is depleted, it will affect the mitochondrial pool in the long run and consequently affect ATP production since it is regulated by the mGSH[54]. These deleterious effects observed in the rotenone-fed flies were however ameliorated by co-treatment with VCNO and the effects were comparable to that elicited by levodopa, a standard drug used in the treatment of PD. These effects may be attributed to the polyphenols present in the oil. Indeed, it has been reported that flavonoid compounds, which is a class of plant polyphenols (including ferulic acid and p-coumarins which are present in coconut oil[55]), can enhance the uptake of cysteine by uncoupling their uptake from the cystine/glutamate

antiporter thereby increasing cell survival as well as GSH levels[56].

The role of dietary supplementation as an approach to mitigating deficits associated with neurodegeneration is an emerging field[48]. Hence, it may be premature to speculate the exact mechanism of action of VCNO and its constituents. However, it is possible that the protective mechanism elicited by VCNO is as a result of its free radical scavenging and antioxidant properties.

## CONCLUSION

In conclusion, we report that VCNO treatment ameliorated the deleterious effects of rotenone-induced toxicity and oxidative stress in *D. melanogaster* and could potentially be a disease-modulating agent.

## ACKNOWLEDGMENTS

The authors are grateful to Dr. Ismaila Ishola of the Department of Pharmacology and Therapeutics, University of Lagos, Nigeria, for providing the strain of flies used for the study. The authors declare that there are no conflicts of interest to disclose with regards to this research.

## REFERENCES

1. Tibar H, El Bayad K, Bouhouche A, Ait Ben Haddou EH, Benomar A, Yahyaoui M, *et al.* Non-Motor Symptoms of Parkinson's Disease and Their Impact on Quality of Life in a Cohort of Moroccan Patients. Front Neurol., 2018; 9:170.
2. Jellinger KA. Neurobiology of Non-Motor Symptoms in Parkinson Disease. J Neurol. Disord. Stroke, 2014; 2:1032.
3. Gökçal E, Gür VE, Selvitop R, Yildiz GB and Asil T. Motor and Non-Motor Symptoms in Parkinson’s Disease: Effects on Quality of Life. Noro. Psikiyatr. Ars., 2017; 54:143–148.
4. Hertz C and Mollereau B. Disturbance of endoplasmic reticulum proteostasis in neurodegenerative diseases. Nat. Rev. Neurosci., 2014; 15:233–49.
5. de Lau L and Breteler M. Epidemiology of Parkinson’s disease, Lancet Neurol., 2006; 5:525–35.
6. Davie CA. A review of Parkinson’s disease, Br Med Bull 2008;86:109–27.
7. Coulom H and Birman S. Chronic exposure to rotenone models sporadic Parkinson's disease in Drosophila melanogaster. J Neurosci., 2004; 24:10993–8.
8. Cannon J and Greenamyre J. Neurotoxic in vivo models of Parkinson's disease recent advances. Prog. Brain Res., 2010; 184:17–33.
9. Khadrawy Y, Salem A, El-Shamy K, Fadl N and Hosny N. Neuroprotective and therapeutic effect of caffeine on the rat model of Parkinson's disease induced by rotenone. J Diet Suppl., 2017; 14:553–72.
10. Swarnkar S, Singh S and Mathur R. A study to correlate rotenone induced biochemical changes and cerebral damage in brain areas with neuromuscular co-ordination in rats. Toxicol., 2010; 272:17–22.
11. Santiago RM, Barbieiro J and Lima MMS. Depressive-like behaviors alterations induced by intranigral MPTP, 6-OHDA, LPS and rotenone models of Parkinson's disease are predominantly associated with serotonin and dopamine. Prog. Neuropsychopharmacol. Biol. Psychiatry, 2010; 34:1104–14.
12. Sudati JH, Vieira FA, Pavin SS, Dias GRM, Seeger RL, Golombieski R, *et al.* Valeriana officinalis attenuates the rotenone-induced toxicity in Drosophila melanogaster. Neurotoxicol., 2013; 37:118–26.

13. Ugur B, Chen K and Bellen HJ. Drosophila tools and assays for the study of human diseases. Dis. Model Mech., 2016; 9:235–44.
14. Lee S, Bang SM, Lee JW and Cho KS. Evaluation of traditional medicines for neurodegenerative diseases using Drosophila models. Evid. Based Compl. Alternat. Med., 2014; 967462.
15. Zhuo C, Zhu X, Jiang R, Ji F, Su Z, Xue R, *et al.* Comparison for Efficacy and Tolerability among Ten Drugs for Treatment of Parkinson's Disease: A Network Meta-Analysis. Sci. Rep., 2017; 7:45865.
16. Sveinbjornsdottir S. The clinical symptoms of Parkinson's disease. J Neurochem., 2016; 139:318–24.
17. Cicchetti F, Drouin-Ouellet J and Gross RE. Environmental toxins and Parkinson's disease: what have we learned from pesticide-induced animal models? Trends Pharmacol. Sci., 2009; 30:475–83.
18. Anusha C, Sumathi T and Joseph LD. Protective role of apigenin on rotenone-induced rat model of Parkinson's disease: suppression of neuroinflammation and oxidative stress mediated apoptosis. Chem. Biol. Interact., 2017; 269:67–9.
19. Venkatesh GV, Rajasankar S, Ramkumar M, Dhanalakshmi C, Manivasagam J, Thenmozhi A, *et al.* Agaricusblazei extract abrogates rotenone-induced dopamine depletion and motor deficits by its anti-oxidative and anti-inflammatory properties in Parkinsonic mice. Nutr. Neurosci., 2017; 1–10.
20. Panchal K and Tiwari AK. Drosophila melanogaster "a potential model organism" for identification of pharmacological properties of plants/plant-derived components. Biomed. Pharmacother., 2017; 89:1331–45.
21. Nagpal I and Abraham SK. Ameliorative effects of gallic acid, quercetin and limonene on urethane-induced genotoxicity and oxidative stress in Drosophila melanogaster. Toxicol. Mech. Methods, 2017; 27:286–92.
22. Manivannan A, Bhardwaj R, Padmanabhan S, Suneja P, Hebbar K and Kanade S. Biochemical and nutritional characterization of coconut (*Cocos nucifera* L.) haustorium. Food Chem., 2018; 238:153-9.
23. Djurasevic S, Jasnic N, Prokic M, Grigorov I, Martinovic V, Djordjevic J, *et al.* The protective role of virgin coconut oil on the alloxan-induced oxidative stress in liver, kidney and heart of diabetic rats. Food Funct., 2019; 10:2114–24.
24. Dosumu OO, Akinola OB and Akang EN. Alcohol-induced testicular oxidative stress and cholesterol homeostasis in rats - The therapeutic potential of virgin coconut oil. Middle East Fertil. Soc. J, 2012; 122–128.
25. Suryani S, Sariani S, Earnestly F, Marganof M, Rahmawati R, Sevindrajuta S, *et al.* A Comparative Study of Virgin Coconut Oil, Coconut Oil and Palm Oil in Terms of Their Active Ingredients. Processes, 2020; 8:402.
26. Yeap S, Beh B, Ali N, Yusof H, Ho W, Koh S, *et al.* Antistress and antioxidant effects of virgin coconut oil in vivo. Exp. Ther. Med., 2015; 9:39–42.
27. Dosumu OO, Duru FIO, Osinubi AA, Oremosu AA and Noronha CC. Influence of virgin coconut oil (VCNO) on oxidative stress, serum testosterone and gonadotropic hormones (FSH, LH) in chronic ethanol ingestion. Agric. Biol. J N Am., 2010; 1:1126–32.

28. Jansen R, Brogan B, Whitworth A and Okello E. Effects of Five Ayurvedic Herbs on Locomotor Behaviour in a Drosophila melanogaster Parkinson's Disease Model. Phytother. Res., 2015; 28:1789–95.
29. Abolaji A, Adedara A, Adie M, Vicente-Crespo M and Farombi E. Resveratrol prolongs lifespan and improves 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine-induced oxidative damage and behavioural deficits in Drosophila melanogaster. Biochem. Biophys. Res. Commun., 2018; 503:1042–8.
30. Vang L, Medvedev A and Adler J. Simple Ways to Measure Behavioral Responses of *Drosophila* to Stimuli and Use of These Methods to Characterize a Novel Mutant. PLoS One, 2012; 7:37495.
31. Farombi E, Abolaji A, Farombi T, Oropo A, Owoje O and Awunah M. Garcinia kola seed biflavonoid fraction (Kolaviron), increases longevity and attenuates rotenone-induced toxicity in *Drosophila melanogaster*. Pestic. Biochem. Physiol., 2018; 145:39–45.
32. Karatas F, Karatepe M and Baysar A. Determination of free malondialdehyde in human serum by high-performance liquid chromatography. Anal. Biochem., 2002; 311:76–9.
33. Saraiva MA, Ávila E, da Silva GF, Macedo GE, Rodrigues NS, Vieira P, *et al.* Exposure of *Drosophila melanogaster* to Mancozeb Induces Oxidative Damage and Modulates Nrf2 and HSP70/83. Oxid. Med. Cell. Longev., 2018; 11:456928.
34. Sedlak J and Lindsay R. Estimation of total, protein-bound, and nonprotein sulfhydryl groups in tissue with Ellman's reagent. Anal. Biochem., 1968; 25:1192–205.
35. Sun M and Zigma S. An improved spectrophotometric assay of superoxide dismutase based on ephinephrine antioxidation. Anal. Biochem., 1987; 90:81–9.
36. Aebi H. Catalase in vitro. Meth. Enzymol., 1984; 105:121–6.
37. Swank D, Bartoo M, Knowles A, Iliffe C, Bernstein S, Molloy J, *et al.* Alternative exon-encoded regions of Drosophila myosin heavy chain modulate ATPase rates and actin sliding velocity. J Biol. Chem., 2001; 276:15117–24.
38. Cammarato A, Dambacher CM, Knowles AF, Kronert WA, Bodmer R, Ocorr K, *et al.* Myosin transducer mutations differentially affect motor function, myofibril structure, and the performance of skeletal and cardiac muscles. Mol. Biol. Cell, 2008; 19:553–62.
39. Rao D, Kronert W, Guo Y, Hsu K, Sarsoza F and Bernstein S. Reductions in ATPase activity, actin sliding velocity, and myofibril stability yield muscle dysfunction in Drosophila models of myosin-based Freeman–Sheldon syndrome. Mol. Biol. Cell, 2019; 30:30–41.
40. Manjunath MJ, Muralidhara. Standardized extract of *Withania somnifera* (Ashwagandha) markedly offsets rotenone-induced locomotor deficits, oxidative impairments and neurotoxicity in Drosophila melanogaster. J Food Sci. Technol., 2015; 52:1971–81.
41. Boemeke L, Marcadinti A, Busnello FM and Gottschall CBA. Effects of Coconut oil on human health. Open J Endocr. Metab Dis., 2015; 5:84–7.
42. Russo GL, Spagnuolo C, Russo M, Tedesco I, Moccia S and Cervellera C. Mechanisms of aging and potential role of selected polyphenols in extending

health span. Biochem Pharmacol., 2020; 173:113719.

43. Goldman JG, Stebbins GT, Bernard B, Stoub TR, Goetz CG and de Toledo-Morrell L. Entorhinal cortex atrophy differentiates Parkinson's disease patients with and without dementia. Mov. Disord., 2012; 27:727–34.
44. Norenberg MD and Rao KV. The mitochondrial permeability transition in neurologic disease. Neurochem. Int., 2007; 50:983–97.
45. Zhang JL, Souders CL, Denslow ND and Martyniuk CJ. Quercetin, a natural product supplement, impairs mitochondrial bioenergetics and locomotor behavior in larval Zebrafish (*Danio rerio*). Toxicol. Appl. Pharmacol., 2017; 327:30–8.
46. Wang XH, Souders CL, Zhao YH and Martyniuk CJ. Mitochondrial bioenergetics and locomotor activity are altered in Zebrafish (*Danio rerio*) after exposure to the bipyridylium herbicide diquat. Toxicol. Lett., 2018; 283:13–20.
47. Cunnane SC, Courchesne LA, St. Pierre V, Vandenberghe C, Pierotti T, Fortier M, *et al.* Can ketones compensate for deteriorating brain glucose uptake during aging? Implications for the risk and treatment of Alzheimer's disease. Ann N Y Acad. Sci., 2016; 1367:12-20
48. Nafar F, Clarke JP and Mearow KM. Coconut oil protects cortical neurons from amyloid beta toxicity by enhancing signalling of cell survival pathways. Neurochem. Int., 2017; 105:64–79.
49. Garrido M, Tereshchenko Y, Zhevtsova Z, Taschenberger G, Bahr M and Kugler S. Glutathione depletion and overproduction both initiate degeneration of nigral dopaminergic neurons. Acta Neuropathol., 2011; 121:475–85.
50. Akinyemi AJ, Oboh G, Ogunsuyi O, Abolaji AO and Udofia A. Curcumin-supplemented diets improve antioxidant enzymes and alter acetylcholinesterase genes expression level in Drosophila melanogaster model. Metab. Brain Dis., 2017; 33:369–75.
51. Fu L, Liu KK, Sun MA, Tian CP, Sun R, Betanzos CM, *et al.* Systematic and quantitative assessment of hydrogen peroxide reactivity with cysteines across human proteomes. Mol. Cell. Proteomics, 2017; 16:1815–28.
52. Dringen R. Metabolism and functions of glutathione in brain. Prog, Neurobiol, 2000; 62:649–71.
53. Vesce S, Jekabsons MB, Johnson-Cadwell LI and Nicholls DG. Acute Glutathione Depletion Restricts Mitochondrial ATP Export in Cerebellar Granule Neurons. J Biol, Chem., 2005; 280: 38720–8.
54. Marí M, de Gregorio E, de Dios C, Roca-Agujetas V, Cucarull B, Tutusaus A, *et al.* Mitochondrial Glutathione: Recent Insights and Role in Disease. Antioxidants, 2020; 9:909.
55. Marina AM, Man YB, Nazimah SA and Amin I. Antioxidant capacity and phenolic acids of virgin coconut oil. Int J Food Sci. Nutr., 2009; 60(2):114-23
56. Maher P, Lewerenz J, Lozano C and Torres JL. A novel approach to enhancing cellular glutathione levels. J Neurochem., 2008; 107:690–700.