Original Research

Protective role of resveratrol, a natural polyphenol, in sodium fluoride-induced toxicity in *Drosophila melanogaster*

Amos O Abolaji[1], Victor O Ajala[1], Janet O Adigun[1], Isaac A Adedara[1], Hellen W Kinyi[2] and Ebenezer O Farombi[1]

[1]Department of Biochemistry, Molecular Drug Metabolism and Toxicology Unit, Faculty of Basic Medical Sciences, College of Medicine, University of Ibadan, Ibadan 20028, Nigeria; [2]Department of Biochemistry, School of Biomedical Sciences, Kampala International University, Kampala 20131, Uganda

Corresponding author: Amos O Abolaji. Email: ao.abolaji@ui.edu.ng

Impact statement

D. melanogaster was used to understand the impact of NaF on lifespan and emergence rate as well as the rescue role of resveratrol. These parameters are difficult to carry out in previously used models such as rodents. This further enforces in part, the suitability of *D. melanogaster* in studying NaF-induced toxicity and the therapeutic effects of drugs.

Additionally, we found that resveratrol rescued *D. melanogaster* from oxidative stress-induced by sodium fluoride (NaF) administration. This study is of public health significance as it indicated that the consumption of fruits rich in resveratrol such as grapes may offer protective role against inadvertent exposure to NaF and related chemicals.

Abstract

Sodium fluoride (NaF) is used in water fluoridation and dental products such as mouth rinses and toothpastes. Resveratrol is a natural polyphenol with antioxidant and anti-inflammatory properties. The present study was carried out to evaluate the toxicity of NaF and the protective role of resveratrol in *Drosophila melanogaster*. For longevity assay, Harwich strain of *D. melanogaster* was treated with NaF (0, 10, 30, 50, 70 and 90 mg/kg diet) throughout the lifespan and daily mortality recorded. Then, flies were again treated with similar doses of NaF for seven days to evaluate survival rate and oxidative stress markers. Thereafter, 60 mg resveratrol/kg diet was selected to determine its ameliorative role in NaF (70 mg/kg)-induced toxicity in flies: Group A (control), Group B (60 mg resveratrol/kg diet), Group C (70 mg NaF/kg diet), and Group D (resveratrol, 60 mg/kg diet) + NaF, 70 mg/kg diet). Thereafter, Glutathione-S-transferase (GST), catalase and acetylcholinesterase (AchE) activities, as well as total thiol (T-SH), nitrites/nitrates and hydrogen peroxide (H_2O_2) levels were determined. The results showed that resveratrol prevented NaF-induced elevation of H_2O_2 and nitrites/nitrates levels, as well as catalase activity. In addition, resveratrol restored NaF-induced inhibition of GST and AChE activities and depletion of T-SH content ($P < 0.05$). Conclusively, resveratrol offered protective benefit against NaF-mediated toxicity in flies due to its antioxidant and anti-inflammatory properties.

Keywords: *Drosophila melanogaster*, sodium fluoride, resveratrol, oxidative stress, antioxidants

***Experimental Biology and Medicine* 2019; 244: 1688–1694. DOI: 10.1177/1535370219890334**

Introduction

Fluoride is a trace element that is widely distributed in nature. Its compounds are commonly used in the production of fluoridated dental products and drinking water. The main sources of human exposure to fluoride include drinking water, food, toothpastes, mouth rinses, drugs, fluoride dust, and fumes released from industries.[1,2] Indeed, fluoride is considered an important environmental pollutant that poses serious health risks to plants, animals, and humans globally.[3] Excessive exposure to fluoride is well known to cause fluorosis—a condition that results from cumulative poisoning of soft tissues such as muscles, liver, and nervous system.[4] Several mechanisms by which fluoride elicit toxicity include increased production of free radicals, lipid peroxidation, inflammation, and altered antioxidant defense systems.[3]

Resveratrol (3,5,4′-trihydroxystilbene) is a polyphenol commonly found in grapes, wine, peanuts, and soy.[5] It is known to possess antioxidative, anticarcinogenic, and anti-inflammatory properties.[6,7] In a previous study, we reported that resveratrol extended the lifespan of flies by up to 41.9%.[8] Other investigators have also shown that this

phytochemical increased the longevity of yeast[9] and obese male mice by 70 and 26%, respectively, and thus it was regarded as an antiaging agent.[10]

Drosophila melanogaster is the most significant invertebrate model organism to human considering the gene sequence similarity.[11] *D. melanogaster* is used as bioindicator for detection of contaminants as well as evaluation of biological activity of pharmacological agents.[12] Moreover, it is recommended by the European Centre for the Validation of Alternative Methods (ECVAM) for promoting the 3Rs (reduction, refinement and replacement) of laboratory animal usage in toxicity studies, and testing.[13]

Earlier studies demonstrated that resveratrol protected against fluoride-induced hepatotoxicity and neurotoxicity in mice and rats.[14–16] Hitherto, there is no study in the literature on the rescue role of resveratrol on fluoride toxicity with respect to longevity and emergence rate in rodents. Also, studies on the use of *D. melanogaster* on NaF-induced oxidative stress and the preventive effects of resveratrol are scarce in the literature. Therefore, as an alternative to conventional rodent models, the present study investigated the biochemical relevance of resveratrol protective role against fluoride toxicity in *D. melanogaster*.

Materials and methods

Chemicals

Resveratrol was procured from AK Scientific, 30023 Ahern Ave, Union City, CA 94587, U.S.A.). Sodium fluoride, reduced glutathione (GSH), 1-chloro- 2,4-dinitrobenzene (CDNB), 5′,5′-dithiobis(2-nitrobenzoic acid), and acetylthiocholine iodide were purchased from Sigma Aldrich (St. Louis, MO, USA). All chemicals procured were of analytical grade.

D. melanogaster culture

D. melanogaster (wild-type, Harwich strain) were maintained on diet consisting of cornmeal, agar-agar (1% w/v), brewer's yeast (1% w/v), and nipagin (preservative, 0.08% v/w). Flies were maintained at temperature ($23 \pm 2^{\circ}C$) under 12-h dark/light cycle in the *Drosophila* Laboratory, Department of Biochemistry, University of Ibadan, Nigeria.

Treatment of flies and preparation of samples

To select appropriate NaF doses, longevity and survival assays were carried out in one to three days old flies of both genders. The flies were divided into six groups each containing five replicates/group with 50 flies/vial and treated with NaF (0, 10, 30, 50, 70, and 90 mg/kg diet). Resveratrol dose of 60 mg/kg diets was selected based on our previous study.[8] Then, seven days duration was chosen to investigate the toxic effects of NaF (0, 10, 30, 50, 70 and 90 mg/kg diets) in *D. melanogaster*. Thereafter, flies were anesthetized in ice, weighed, and homogenized in 0.1 M phosphate buffer (pH 7.4, ratio of 1 mg:10 μL). Following centrifugation at 4000 *g* for 10 min at 4 °C, the supernatants obtained were used for the determination of total protein, hydrogen peroxide (H_2O_2), total thiol (T-SH), nitric oxide (NO, nitrate and nitrate) levels as well as catalase, glutathione-S-transferase (GST), and acetylcholinesterase (AChE) activities. The data obtained were used to select NaF (70 mg/kg) in another experiment to evaluate the rescue role of resveratrol (60 mg/kg resveratrol) on NaF-induced toxicity.

Determination of negative geotaxis and emergence rate of flies

We evaluated locomotor performance (negative geotaxis) of flies by using the method of Feany.[17]

The emergence rate of *D. melanogaster* offspring after exposure to NaF was carried out as previously described.[8,18]

Determination of oxidative stress and antioxidant parameters, nitrite level, and acetylcholinesterase activity

Protein determination was carried out as described by Lowry *et al*.[19] Total thiol content was estimated by the method of Ellman.[20] Glutathione S-transferase activity was evaluated according to the procedure of Habig and Jakoby.[21] Catalase activity was determined by the method of Aebi.[22] Acetylcholinesterase activity was evaluated with the method of Ellman *et al*.[23] Hydrogen peroxide level was determined according to the method of Wolff.[24] The amount of nitric oxide (nitrate and nitrite) in supernatants was measured following Griess reaction method.[25]

Statistical analysis

The data are presented as the Mean± SEM. One-way analysis of variance (ANOVA) was used to assess the significant differences among multiple groups under various treatments, followed by Dunett's post hoc test. In all the groups, differences were considered statistically significant among groups when $P < 0.05$, using the GraphPad Prism5.0 software.

Results

Longevity, survival rate, emergence of offspring, and acetylcholinesterase activity in D. melanogaster exposed to graded concentrations of NaF

The influence of different concentrations of NaF on the lifespan, emergence of offspring, and neurobehavioral parameters of *D. melanogaster* are presented in Figure 1. Exposure of *D. melanogaster* to NaF at 10, 30, 70, and 90 mg/kg diet resulted in a dose-dependent decrease in the lifespan by 12.5, 12.5, 67.5, and 70% respectively, in comparison with the control (Figure 1(a)). Further, treatment of flies with NaF for seven consecutive days resulted in a significant ($P < 0.05$) decreases in the survival and emergence rates of flies at 70 and 90 mg/kg diet (Figure 1(b)). In addition, NaF inhibited AChE activity at doses of 30, 50, 70, and 90 mg/kg diet in comparison with the control ($P < 0.05$; Figure 1(d)).

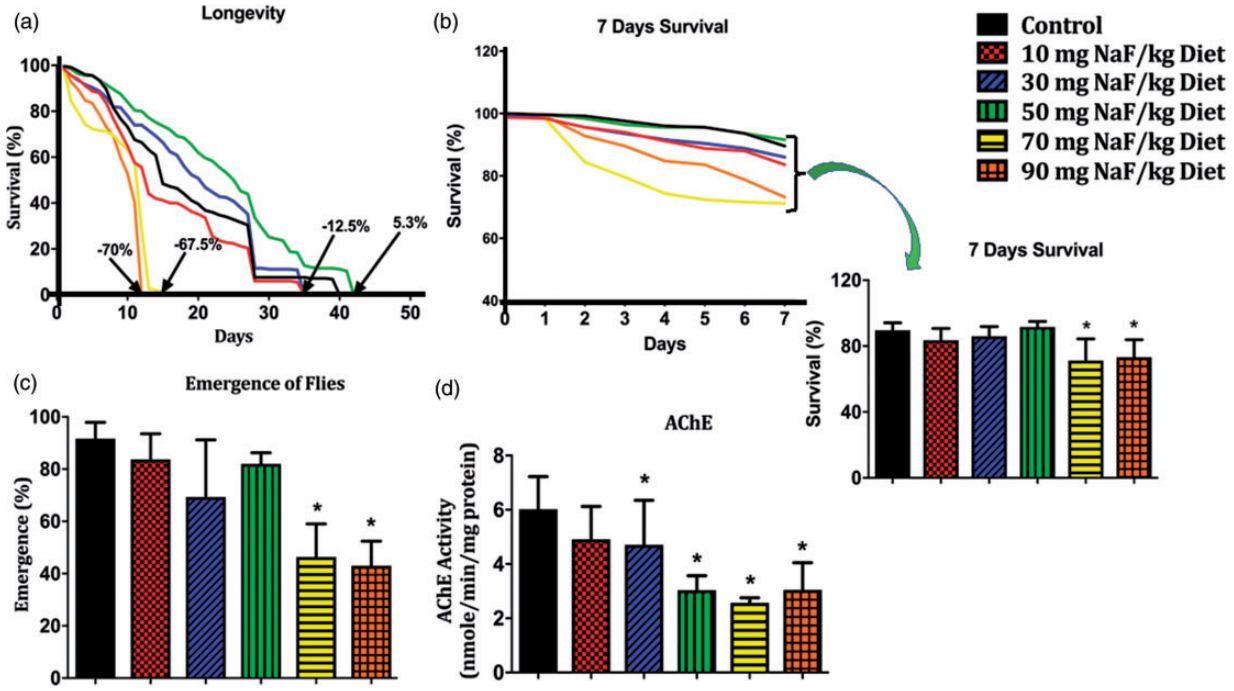

Figure 1. Effects of NaF on the survival, emergence and AChE activity of *D. melanogaster*. Effects of NaF (10, 30, 50, 70, and 90 mg/kg diet) on the longevity curve (a) of and seven days survival curve (b); offspring emergence (c) and AChE activity (d) of *D. melanogaster*. Data in (c) and (d) are presented as Mean ± SEM of 50 flies per vial (five replicates per group). *Significant difference compared with control group ($P < 0.05$). NaF: sodium fluoride; AChE: acetylcholinesterase. (A color version of this figure is available in the online journal.)

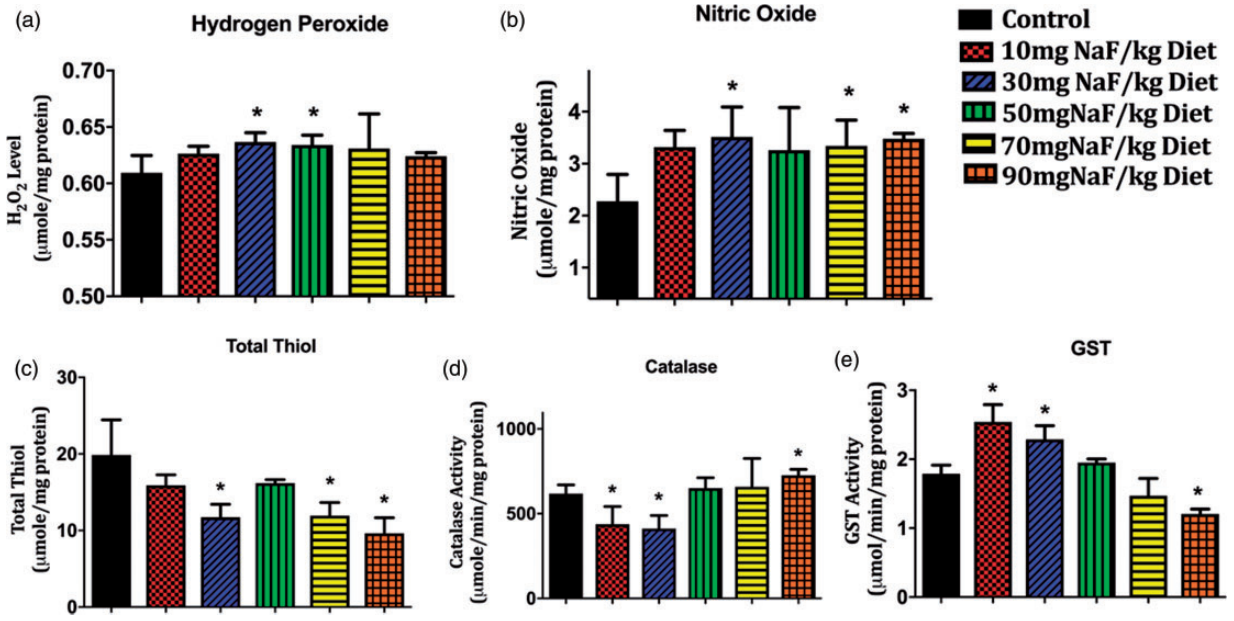

Figure 2. Antioxidant status in *D. melanogaster* exposed to graded concentrations of NaF. Levels of hydrogen peroxide (a), nitrites/nitrate (b) and total thiol (c) and activities of catalase (d) and GST (e) in *D. melanogaster* after seven days of treatment with NaF (10, 30, 50, 70, and 90 mg/kg diet). Data are presented as Mean ± SEM of 50 flies per vial (5 replicates per group). *Significant difference compared with control group ($P < 0.05$). NaF: sodium fluoride; GST: glutathione-S- transferase. (A color version of this figure is available in the online journal.)

Antioxidant status in D. melanogaster exposed to graded concentrations of NaF

The influence of NaF on antioxidant status in *D. melanogaster* exposed to NaF for seven consecutive days is presented in Figure 2. Exposure to NaF significantly ($P < 0.05$) increased H_2O_2 level (Figure 2(a)) at 30 and 50 mg/kg diets as well as NO level (Figure 2(b)) at 30, 70, and 90 mg/kg diets. Marked decrease in total thiol level (Figure 2(c)) was noted at NaF doses of 30, 70, and 90 mg/kg diet. Further, NaF (10 and 30 mg/kg diet) significantly decreased catalase activity (Figure 2(d)) but increased GST activity (Figure 2 (e)) after seven days of treatment. In contrast, exposure to

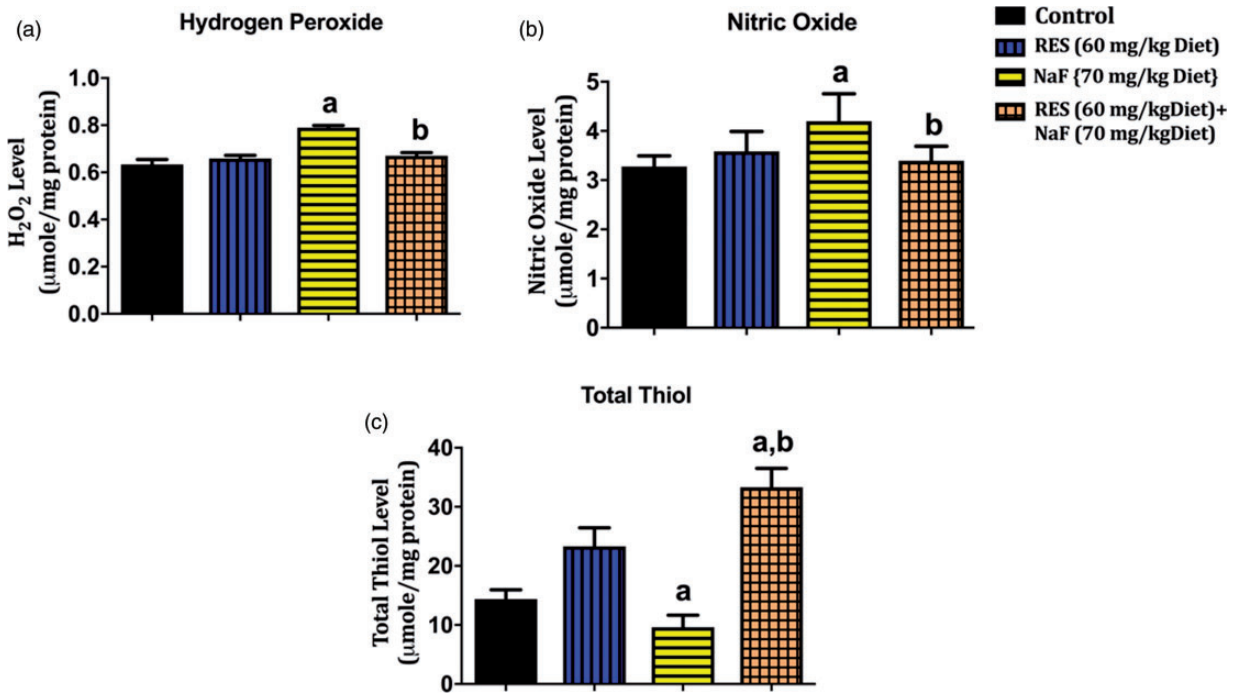

Figure 3. Effects of resveratrol and NaF on hydrogen peroxide, nitrites/nitrates, and total thiol levels in *D. melanogaster*). Resveratrol blunts NaF-induced increase in levels of hydrogen peroxide (a) and nitrites/nitrates (b) and depletion of total thiol content (c) in *D. melanogaster* after seven days of treatment with NaF (10, 30, 50, 70, and 90 mg/kg diet). Data are presented as Mean ± SEM of 50 flies per vial (five replicates per group). a: Significant difference compared with control group; b: Significant difference compared with NaF group ($P < 0.05$). NaF: sodium fluoride. (A color version of this figure is available in the online journal.)

NaF (90 mg/kg diet) increased catalase activity but decreased GST activity diet when compared with control ($P < 0.05$; Figure 2(d) and (e)).

Resveratrol blunts NaF-induced elevations of hydrogen peroxide, nitric oxide levels, and total thiol depletion in D. melanogaster after seven days of treatment

Figure 3 shows the effects of resveratrol supplementation on the levels of H_2O_2, NO, and T-SH in flies co-exposed to NaF for seven consecutive days. Resveratrol blunted NaF-induced elevations of H_2O_2 (Figure 3(a)) and NO (nitrites and nitrates) levels (Figure 3(b)), as well as depletion in T-SH content (Figure 3(c)) in the treated flies when compared with the control group ($P < 0.05$).

Resveratrol blunts NaF-induced disruption of antioxidant enzymes and acetylcholinesterase activities in D. melanogaster after treatment for seven days

Figure 4 shows the ameliorative role of resveratrol supplementation on the antioxidant status and AChE activity in flies co-exposed to NaF for seven consecutive days. The treatment-related effects of resveratrol alone on the antioxidant status and AChE activity in the flies were not statistically significant when compared with the control (Figure 4 (c)). Although, NaF exposure increased catalase activity (Figure 4(a)) and inhibited GST (Figure 4(b)) and AChE activities (Figure 4(c)) in the treated flies when compared with the control group, the dietary supplementation with resveratrol markedly abrogated NaF-mediated toxicity as evidenced by the restoration of the antioxidant status and AChE activity in *D. melanogaster* ($P < 0.05$).

Discussion

Fluorosis is a progressive degenerative disorder induced by extreme intake of fluoride either by environmental pollution or natural sources via drinking water.[26] Excessive intake of fluorides has been linked with changes in the steady-state of free radicals and altered antioxidant defense systems.[27] Here, we evaluated the toxicity of NaF and the rescue role of resveratrol using *D. melanogaster* as an alternative to mammalian model. We found that NaF (10, 30, 70, and 90 mg/Kg diet) reduced the lifespan of *D. melanogaster* by 12.5, 12.5, 67.5, and 70% respectively. In addition, NaF induced accumulation of hydrogen peroxide and total nitrate/nitrate levels, reduced emergence rate of flies, inhibited acetylcholinesterase activity, and disrupted antioxidant homeostasis in flies. Interestingly, these effects of NaF were substantially ameliorated in flies co-treated with resveratrol (60 mg/kg).

The observation that NaF (10, 30, 70, and 90 mg/kg diet) reduced the lifespan of *D. melanogaster* by 12.5, 12.5, 67.5, and 70% respectively implied that these doses heightened aging in the flies as the treatment was throughout the lifespan of the flies. Previous studies by Hamza *et al.*[28] and Miranda *et al.*[29] demonstrated chronic toxic effects of NaF in mice and rats for 30 and 60 days. In this study, using *Drosophila*, we reported the toxic effects of lifetime exposure to NaF which may suggest inherent toxicity arising from lifetime human exposure to NaF. Consequently, we chose seven days treatment regimen and duration since, beyond

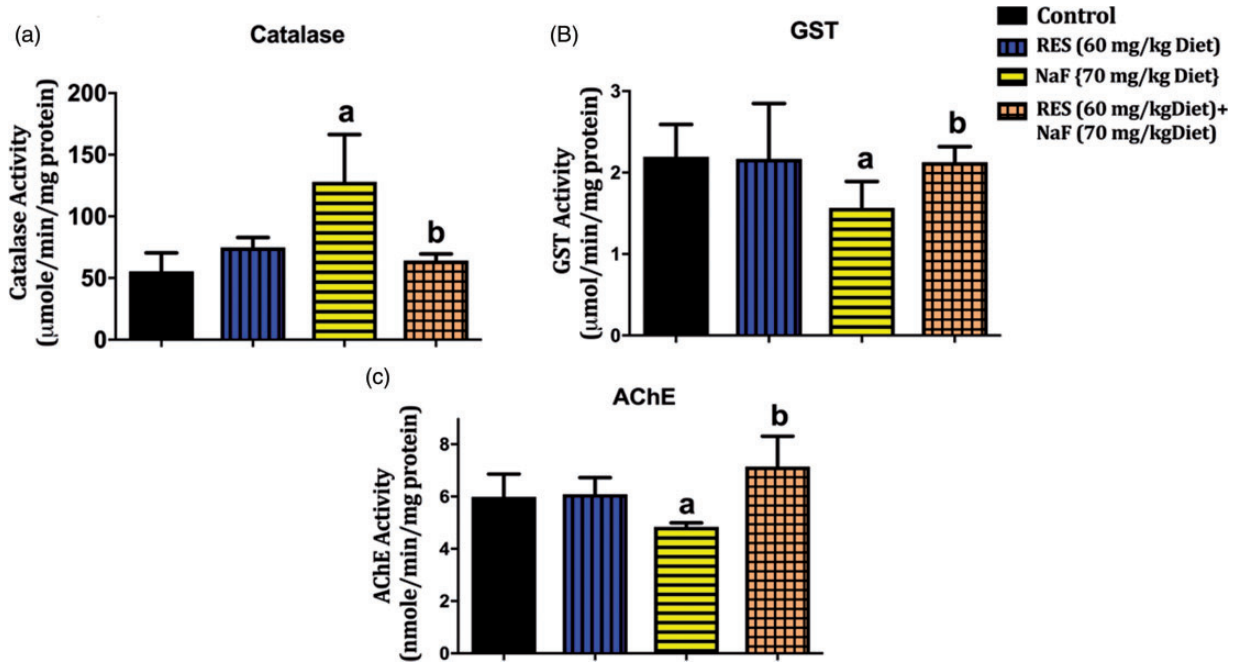

Figure 4. Effects of resveratrol and NaF on antioxidant enzymes and acetylcholinesterase activities in *D. melanogaster.* Resveratrol blunts NaF-induced increase in catalase activity (a) and inhibition of GST (b) and AChE activities in *D. melanogaster* after seven days of treatment with NaF (10, 30, 50, 70, and 90 mg/kg diet). Data are presented as Mean ± SEM of 50 flies per vial (five replicates per group). a: Significant difference compared with control group; b: Significant difference compared with NaF group ($P < 0.05$). NaF: sodium fluoride; GST: glutathione-S-transferase; AChE: acetylcholinesterase. (A color version of this figure is available in the online journal.)

this day, significant mortalities were recorded in flies exposed to 70 and 90 mg/kg doses of NaF. The seven-day survival assay carried out showed that NaF (70 and 90 mg/kg diet) significantly reduced the survival of flies compared with the control. This therefore implies that chronic, acute, and lifetime exposures to overwhelmingly high doses of NaF are potentially deleterious.

Further, the emergence of offspring of flies significantly reduced only in the flies treated with 70 and 90 mg/kg diet of NaF. This informed the choice of 70 mg/kg diet of NaF to investigate the ameliorative role of resveratrol (60 mg/kg diet) in flies after seven days of treatment. This finding is significant in *Drosophila* as NaF impacts the fecundity and slows down the rate of metamorphosis in the flies. Although, findings by Zhou *et al.*[30] and Chioka *et al.*[31] demonstrated the impact of NaF on the male and female reproductive system, focus was on the sperm production and hormones respectively.

In the nervous system of insects, acetylcholinesterase is involved in the modulation of cholinergic transmission. It terminates nerve impulses by catalyzing the hydrolysis of acetylcholine, which is an excitatory neurotransmitter at synapses.[32] We observed that NaF (30, 50, 70 and 90 mg/kg) inhibited acetylcholinesterase activity in flies after treatment for seven days. Our data agree with the report of Dutta *et al.*[33] in which AChE activity was inhibited in the larva of flies exposed to NaF. This effect would have caused accumulation of acetylcholine which may lead to overstimulation of the acetylcholine receptors. Indeed, a correlation between nervous activity and acetylcholine has been reported when acetylcholinesterase activity is inhibited.[34] However, in *D. melanogaster* co-treated with NaF (70 mg/kg diet) and resveratrol (60 mg/kg diet), the activity of acetylcholinesterase was restored.

Moreover, NaF increased H_2O_2 level and disrupt catalase activity in *D. melanogaster*. Under an increased condition of hydrogen peroxide, it can react with Fe^{2+} via Fenton reaction to generate hydroxyl radical (HO), which is a strong reactive oxidant.[35]Catalase, a heme-containing enzyme,[36] functions to degrade H_2O_2 to water and molecular oxygen, thus protecting the cell from the harmful effects of hydroxyl radical.[37]The NaF-induced disruption of catalase activity can be described as a biphasic effect in which doses of 10 and 30 mg/kg diets inhibited catalase activity, while higher doses increased its activity. However, the fact that resveratrol restored NaF-induced elevation of hydrogen peroxide level and disruption of catalase activity are further indications that it possesses both antioxidative and free radical scavenging properties as previously reported.[8]

Glutathione-S-transferases (GSTs) are phase II family of antioxidant enzymes that catalyze the conjugation of reduced glutathione with electrophiles. Apart from this, they play a vital role in the survival of organisms during a condition of oxidative damage.[38]Thus, the observed NaF-induced disruption of total GST activity might imply impaired detoxification capacity of the flies. This might be the reason for the increased mortality recorded in the seven days of the survival study. Interestingly, resveratrol (60 mg/kg diet) restored NaF-induced disruption of GST activity in *D. melanogaster*, thus further confirming its antioxidative property.

Total thiols are a group of organic compounds containing sulfhydryl group.[39]Thiols are good reductants and they

are found in albumin as well as cysteine-derived molecules such as homocysteine, glutathione, and γ-glutamylcysteine. During conditions of oxidative stress, thiols are oxidized to form disulfides between protein thiol groups as well as thiols with low molecular weight. Thus, the thiol/disulphide homeostasis is vital in order to maintain antioxidant protection, oxidative balance, and regulation of the activity of antioxidant enzymes.[40,41] The observation that NaF depleted total thiol level further implies oxidative stress and impairment of the normal physiology of the flies since a decrease in the level of thiols has been noted in various medical disorders.[42] In the co-exposure paradigm, resveratrol caused a complete restoration of total thiol level depicting its antioxidative property. This is in accordance with Abolaji *et al*.[8]

Lastly, resveratrol prevented NaF-induced accumulation of nitrite/nitrate level in *D. melanogaster*. NO synthases produce nitric oxide via the conversion of L-arginine to NO and citrulline. Although, NO plays essential role in several physiological processes, nonetheless, if it accumulates, it can react with superoxide anion to produce toxic nitrite anion, thereby causing tissue damage.[43]Thus, the apparent suppression of NaF-mediated increase in NO concentration by resveratrol further suggests its anti-inflammatory property.

In conclusion, the oxidative stress induced by NaF was due to the alteration in the free radicals-antioxidant balance in the flies. However, the co-administration of NaF with resveratrol suggests that resveratrol offers ameliorative role by reducing mortality, restoring AChE activity, and alteration of antioxidant status in *D. melanogaster*, a non-target organism. These beneficial effects are partly associated with its free radical scavenging and antioxidant properties. This study therefore further reflected the suitability of *D. melanogaster* as a model to study fluoride-induced toxicity and the ameliorative role of phytochemicals such as resveratrol.

Authors' contributions: AOA, EOF, IIA and HWK conceived the research ideas and AOA and EOF supervised the Project. VOA and JOA performed the experiments. AOA, VOA, JOA and IIA participated in data analysis. AOA, IIA and HWK wrote and edited the manuscript. AOA and IIA reviewed and edited the final version of the manuscript.

ACKNOWLEDGEMENTS

We thank Professor JBT Rocha for his valuable comments on the manuscript.

DECLARATION OF CONFLICTING INTERESTS

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

FUNDING

This work was supported by Company of Biologists/ DrosAfrica (UK) and Cambridge-Africa Alborada Research Funds U.K. via the grants provided to procure six dissecting microscopes and Thermofisher Refrigerated Centrifuge respectively used in this project.

ORCID iD

Amos O Abolaji https://orcid.org/0000-0002-4203-452X

REFERENCES

1. Ozsvath DL. Fluoride and environmental health: a review. *Rev Environ Sci Biotechnol* 2009;**8**:59–79
2. USNRC. *Health effects of ingested uoride*. Washington D.C.: National Research Council, National Academy Press, 1993
3. Zuo H, Chen L, Kong M, Qiu L, Lü P, Wu P, Yang Y, Chen K. Toxic effects of fluoride on organisms. *Life Sci* 2018;**198**:18–24
4. Chouhan S, Flora SJ. Effects of fluoride on the tissue oxidative stress and apoptosis in rats: biochemical assays supported by IR spectroscopy data. *Toxicology* 2008;**254**: 61–7
5. Pastor RF, Restani P, Di Lorenzo C, Orgiu F, Teissedre PL, Stockley C, Ruf JC, Quini CI, GarcìaTejedor N, Gargantini R, Aruani C, Prieto S, Murgo M, Videla R, Penissi A, Iermoli RH. Resveratrol, human health and winemaking perspectives. *Crit Rev Food Sci Nutr* 2019;**59**:1237–55
6. Burns J, Yokota T, Ashihara H, Lean ME, Crozier A. Plant foods and herbal sources of resveratrol. *J Agric Food Chem* 2002;**50**:3337–40
7. Yeh CB, Hsieh MJ, Lin CW, Chiou HL, Lin PY, Chen TY, Yang SF. The antimetastatic effects of resveratrol on hepatocellular carcinoma through the downregulation of a metastasis-associated protease by SP-1 modulation. *PLoS One* 2013;**8**:e56661
8. Abolaji AO, Adedara AO, Adie MA, Vicente-Crespo M, Farombi EO. 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
9. Howitz KT, Bitterman KJ, Cohen HY, Lamming DW, Lavu S, Wood JG. Small molecule activators of sirtuins extend *Saccharomyces cerevisiae* lifespan. *Nature* 2003;**425**:191–6
10. Baur JA, Pearson KJ, Price NL, Jamieson NL, Lerin C, Kalra A. Resveratrol improves health and survival of mice on a high-calorie diet. *Nature* 2006;**444**:337–42
11. Bier E. *Drosophila*, the golden bug, emerges as a tool for human genetics. *Nat Rev Genet* 2005;**6**:9–23
12. Adedara IA, Klimaczewski CV, Barbosa NB, Farombi EO, Souza DO, Rocha JB. Influence of diphenyl diselenide on chlorpyrifos-induced toxicity in *Drosophila melanogaster*. *J Trace Elem Med Biol* 2015;**32**:52–9
13. Benford DJ, Hanley AB, Bottrill K, Oehlschlager S, Balls M, Brance F, Castegnara JJ, Descotes J, Hemminiky K, Lindsay D, Schilter B. Biomarkers as predictive tools in toxicity testing: the report and recommendations of ECVAM workshop 40. *Altern Lab Anim* 2000;**28**:119–31
14. Atmaca N, Atmaca HT, Kanici A, Anteplioglu T. Protective effect of resveratrol on sodium fluoride-induced oxidative stress, hepatotoxicity and neurotoxicity in rats. *Food Chem Toxicol* 2014;**70**:191–7
15. Pal S, Sarkar C. Protective effect of resveratrol on fluoride induced alteration in protein and nucleic acid metabolism, DNA damage and biogenic amines in rat brain. *Environ Toxicol Pharmacol* 2014;**38**:684–99
16. Sharma C, Suhalka P, Bhatnagar M. Curcumin and resveratrol rescue cortical-hippocampal system from chronic fluoride-induced neurodegeneration and enhance memory retrieval. *Int J Neurosci* 2018;**13**:1–15
17. Feany MB, Bender WW. A *drosophila* model of Parkinson's disease. *Nature* 2000;**404**:394–8
18. Farombi EO, Abolaji AO, Farombi TH, Oropo AS, Owoje OA, Awunah MT. *Garcina kola* seeds biflavonoid fraction (kolaviron), increases longevity and attenuates rotenone induced toxicity in *Drosophila melanogaster*. *Pestic Biochem Physiol* 2018;**145**:39–45
19. Lowry OH, Rosebrough NJ, Farr AL, Randall RJ. Protein measurement with the folin phenol reagent. *J Biol Chem* 1951;**193**:265–75
20. Ellman GL. Tissue sulfhydryl groups. *Arch Biochem Biophys* 1959;**82**:70–7

21. Habig WH, Jakoby WB. Assays for differentiation of glutathione-S-transferases. *Meth Enzymol* 1981;**77**:398–405
22. Aebi H. Catalase in vitro. Methods Enzymol 1984;105:121-126
23. Ellman GL, Courtney KD, Andres V, Feathers-Stone RM. A new and rapid colorimetric determination of acetylcholinesterase activity. *Biochem Pharmacol* 1961;**7**:88–95
24. Wolff SP. Ferrous ion oxidation in presence of ferric ion indicator xylenol orange for measurement of hydroperoxides. *Meth Enzymol* 1994; **233**:182–9
25. Eleftherianos I, More K, Spivack S, Paulin E, Khojandi A, Shukla S. Nitric oxide levels regulate the immune response of *Drosophila melanogaster* reference laboratory strains to bacterial infections. *Infect Immun* 2014;**82**:69–81
26. Song C, Zhao J, Fu B, Li D, Mao T, Peng W, Wu H, Zhang Y. Melatonin-mediated upregulation of Sirt3 attenuates sodium fluoride-induced hepatotoxicity by activating the MT1-PI3K/AKT-PGC-1α signaling pathway. *Free Radic Biol Med* 2017;**112**:616–30
27. Ameeramja J, Panneerselvam L, Govindarajan V, Jeyachandran S, Baskaralingam V, Perumal E. Tamarind seed coat ameliorates fluoride induced cytotoxicity, oxidative stress, mitochondrial dysfunction and apoptosis in A549 cells. *J Hazard Mater* 2016;**301**:554–65
28. Hamza RZ, Nahla S, El-Shenawy, Ismail HAA. Protective effects of blackberry and quercetin on sodium fluoride-induced oxidative stress and histological changes in the hepatic, renal, testis and brain tissue of male rat. *J Basic Clin Phys Pharm* 2014;**26**:3
29. Miranda GHN, Gomes BAQ, Bittencourt LO, Aragão WAB, Nogueira LS, Dionizio AS, Buzalaf MAR, Monteiro MC, Lima RR. Chronic exposure to sodium fluoride triggers oxidative biochemistry misbalance in mice: effects on peripheral blood circulation. *Oxid Med Cell Longev* 2018;**2018**:8379123
30. Zhou Y, Zhang H, He J, Chen X, Ding Y, Wang Y, Liu X. Effects of sodium fluoride on reproductive function in female rats. *Food Chem Toxicol* 2013;**56**:297–303
31. Chioca LR, Müller JC, Boareto AC, Andreatini R, Dalsenter PR. Sodium fluoride does not alter sperm production or sperm morphology in rats. *Braz Arch Biol Technol* 2012;**55**:257–262
32. Charpentier A, Fournie D. Levels of total acetylcholinesterase in *Drosophila melanogaster* in relation to insecticide resistance. *Pestic Biochem Phys* 2001;**70**:100–7
33. Dutta M, Rajak P, Khatun S, Roy S. Toxicity assessment of sodium fluoride in *Drosophila melanogaster* after chronic sub-lethal exposure. *Chemosphere* 2017;**166**:255–66
34. Colhoun EH. The physiological significance of acetylcholine in insects and observations upon other pharmacologically active substances advances in insect. *Physiology* 1963;**1**:1–46
35. Farina M, Avila DS, Rocha JBT, Aschner M. Metals, oxidative stress and neurodegeneration: a focus on iron, manganese and mercury. *Neurochem Int* 2013;**62**:575–94
36. AebiH Catalase in vitro. *Meth Enzymol* 1984;**105**:121–6
37. Yadav SS, Kumar R, Khare P, Tripathi M. Oxidative stress biomarkers in the freshwater fish, *Heteropneustes fossilis* (bloch) exposed to sodium fluoride: antioxidant fefense and role of ascorbic acid. *Toxicol Int* 2015;**22**:71–6
38. Abolaji AO, Kamdem JP, Lugokenski TH, Farombi EO, Souza DO, da Silva Loreto EL, Rocha J. Ovotoxicants 4-vinylcyclohexene 1,2-monoepoxide and 4-vinylcyclohexene diepoxide disrupt redox status and modify different electrophile sensitive target enzymes and genes in *Drosophila melanogaster*. *Redox Biol* 2015;**5**:328–39
39. Garcia-Garcia A, Zavala-Flores L, Rodriguez-Rocha H, Franco R. Thiol-Redox signaling, dopaminergic cell death, and Parkinson's disease. *Antioxid Redox Signal* 2012;**17**:1764–84
40. Jones DP, Liang Y. Measuring the poise of thiol/disulfide couples in vivo. *Free Radic Biol Med* 2009;**47**:1329–38
41. Erel O, Neselioglu S. A novel and automated assay for thiol/disulphide homeostasis. *Clin Biochem* 2014;**47**:326–32
42. Sener S, Akbas A, Kilinc F, Baran P, Erel O, Aktas A. Thiol/disulfide homeostasis as a marker of oxidative stress in rosacea: a controlled spectrophotometric study. *Cutan Ocul Toxicol* 2019;**38**:55–8
43. Buchwalow I, Schnekenburger J, Samoilova V, Boecker W, Neumann JK. Tiemann New insight into the role of nitric oxide pathways in pancreas. *Acta Histochem Cytochem* 2018;**51**:167–72

(Received September 1, 2019, Accepted November 4, 2019)