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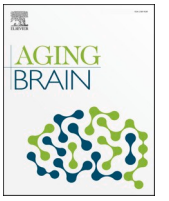

In the quest for the ideal sweetener: Aspartame exacerbates selected biomarkers in the fruit fly (*Drosophila melanogaster*) model of Alzheimer's disease more than sucrose

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A B S T R A C T

This study evaluated the effect of dietary inclusions of aspartame and sucrose on some selected behavioral and biochemical indices linked with Alzheimer's disease in a transgenic fruit fly (Drosophila melanogaster) model expressing human amyloid precursor protein and secretase. Flies were raised on a diet supplemented with sucrose and aspartame for 14 days. Thereafter, the flies were assessed for their survival rate, learning and memory, as well as locomotor performance, 14 days post-treatment. This was followed by homogenising the fly heads, and the homogenates were assayed for acetylcholinesterase and monoamine oxidase activities, as well as levels of lipid peroxidation, reactive oxygen species, and total thiol. The results showed aspartame at all levels of dietary intake and a high proportion of sucrose significantly aggravated the mortality rate, locomotor deficiency, and impaired biomarkers of oxidative stress and antioxidant status in the transgenic flies, while no significant effect was found on acetylcholinesterase activity or memory function. These findings therefore suggest that while low dietary inclusions of sucrose are tolerable under AD-like phenotypes in the flies, high inclusions of sucrose and all proportions of aspartame tested aggravated mortality rate, locomotion and oxidative stress in the flies.

Introduction

Sweeteners are compounds that interact with the taste buds to evoke a characteristic response and enhance the perception of sweetness. Sweeteners have the ability to impact sweet taste by masking the taste of materials to which they are added [46]. Sweeteners are categorised into two (2) categories: nutritive and artificial sweeteners. Nutritive sweeteners (NSs) contain carbohydrates and also provide energy (4 kcal g1 and an average of 2 kcal g1 for sugar alcohols). Nutritive sweeteners provide calories or energy to the diet at about 4 calories per gramme. Examples of nutritive sweeteners are table sugar (sucrose), honey, etc. They are further subdivided into monosaccharides or disaccharides, which provide 4 kcal/g, and sugar alcohols (polyols), which provide 2 kcal/g on average[44].

Sucrose is a white sugar that contains at least 99.7% sucrose, which is a disaccharide composed of 50% glucose and 50% fructose, and it is absolutely not necessary in our diet because it is highly refined and thus devoid of nutrients such as vitamins, minerals, and trace elements [47]. Sugars are found naturally (intrinsic) in all fruits, vegetables, and dairy foods, or they are added (extrinsic) to foods during processing or preparation for human consumption [48].

Sugars found in foods include glucose, fructose, galactose, sucrose, and maltose. Artificial or non-nutritive sweeteners are often

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used as an alternative to sugar. These sweeteners are energy- and calorie-free. It is recommended that non-nutritive artificial sweeteners (NAS) be used instead of nutritive sweeteners such as sugar to reduce overall carbohydrate and calorie intake [20].

Aspartame is an artificial sweetener that is 200 times sweeter than sucrose and is widely used as a sugar substitute in foods and beverages. Aspartame is a methyl ester of the dipeptides aspartic acid and phenylalanine. It is composed of substances normally found in the diet and the body. The acceptable daily intake (ADI) is limited to 40 mg/kg/day [4]. Aspartame has a molecular formula of C14H18N205. Aspartame has grown in popularity as a substitute for sucrose since the FDA of the United States Department of Health and Human Services declared it safe for human consumption [7]. Aspartame is an odourless, white powder that is 200 times sweeter than sucrose and dissolves easily in water. Aspartame has been linked to the worsening of diabetes, headaches, seizures, depression, arthritis, and other medical problems [12].

Neurodegenerative diseases have diverse pathophysiologies with characteristic causes of cognitive impairment, behavioural dysfunction, and memory loss, as well as speech and movement disorders[43]. Ageing, oxidative stress, DNA damage, and inflammation are some of the consistent risk factors leading to neurodegenerative disease. [26]. Alzheimer's disease is one of the neurodegenerative diseases and a common cause of dementia in adults. Its major symptoms are retrogressions in memory, learning, and other cognitive functions. AD is characterised by the buildup of misfolded proteins in the brain in the form of plaques [27]. The accumulation of beta-amyloid peptide leads to the cell death of the neurons, which causes oxidative stress and inflammation[5]. Studies have shown that consumption of sweeteners has been linked with Alzheimer's disease and stroke.

The fruit fly (Drosophila melanogaster) is an arthropod of the family Drosophilidae and the order Diptera (two-winged insects). The use of drosophila as a model organism in biology and medicine has a long history, including its famous use by Gregor Mendel in studying hereditary traits [30], trait formation [31], nervous system development, and even human disease [14]. In recent years, due to ethical concerns and calls for a reduction in the use of mammals for laboratory research, Drosophila has emerged as one of the foremost non-vertebrate models for biological research. Studies have shown that >70% of human disease-causing genes are conserved in drosophila[37,39].

Diet remains critical for a number of human diseases, including Alzheimer's disease (AD). Specifically, studies have shown that diets could produce a wide range of effects on the disease, from pathogenesis to progression and therapeutic interventions[38,36,11]. Therefore, the dietary regimen in AD is a critical factor. Sweeteners remain a key component of human diets. While caloric sweeteners such as sucrose are popular, their use is often limited due to a number of associated health complications for prolonged use, making non-caloric sweeteners a possible alternative; nevertheless, non-caloric sweeteners in general and aspartame in particular have not been completely devoid of health controversies pertaining to their safety and contributions to diseases [19,16]. In this study, using the fruit fly model of AD, we investigated the effects of aspartame and sucrose on memory index and selected biochemical parameters to provide information on the suitability of these sweeteners under AD-like phenotypes.

2. Materials and methods

2.1. Chemicals and reagents

Aspartame was sourced from SinoSweet corporation limited (Yixing city, China), and sucrose was sourced from Symrise GmbH and Company, Germany. Except stated otherwise, all other chemicals and reagents were of analytical grades and the water was glass distilled.

2.2. Fruit fly (Drosophila melanogaster) stock culture

Wild type *D. melanogaster* (Oregon strain) stock culture from the National Species Stock Centre (Bowling Green, OH, USA), was used. The transgenic fly used (Bloomington stock *P{UAS-APP$_{695}$-N-myc}, P{UAS:BACE1}* (BL#33798)) was obtained from University of Drexel, Philadelphia, USA, designed by the GAL4 UAS system to express human APP and BACE-1 in their central nervous system (otherwise referred to as Aβ fly). Bloomington stock P{GawB}elav^{C155} (BL#458) GAL4 line was used to drive the human APP and BACE-1 protein expression in the nervous system of the Aβ fly as previously described [13,28,28,34]. All fly stocks were cultured as previously described [34].

2.3. Experimental design

This study was carried out in two experiments conducted simultaneously. In the first experiment, wild-type (Harwish strain) flies, 8–10 days old, were divided into 7 groups containing 40 flies each. Group 1 was control group fed basal diet without sweetener inclusion. Groups 2–4 were flies fed dietary inclusions of 0.2, 0.5 and 1.0% sucrose, while groups 5–7 were flies fed dietary inclusions of 0.01, 0.02 and 0.05% aspartame. In the second experiment, group 1 was wild strain control flies fed basal diet, group 2 was transgenic control flies fed a basal diet; groups 3–5 were transgenic flies fed dietary inclusions of 0.2, 0.5, and 1.0% sucrose; and groups 6–8 were transgenic flies fed dietary inclusions of 0.01, 0.02, and 0.05% aspartame. Other sources of sweeteners were not included in the basal diet. The basal diet is made up of corn meal medium containing 1% w/v brewer's yeast and 0.08% v/w nipagin. The flies were exposed to these treatments for 14 days, and the vials containing flies were maintained in an incubator at room temperature before being used for different assays. The age of the flies was chosen to ensure the adult onset of dementia in the transgenic flies [33]. The choice of the percentage inclusions of the sweeteners is based on our preliminary results (data not shown) indicating a relatively stable survial rate in both fly strains.

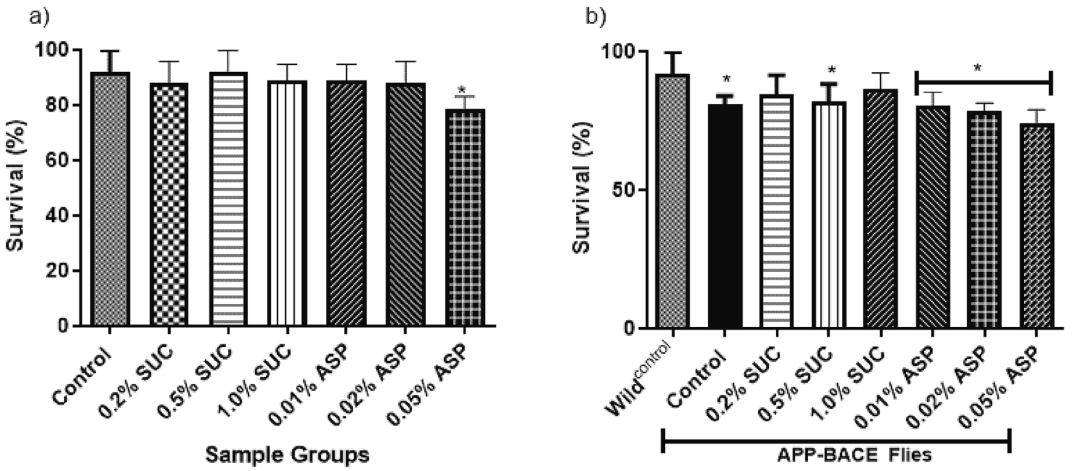

Fig. 1. Effect of Sucrose and Aspartame on Survival of a) wild type (Harwich strain) *D. melanogaster*; b). Transgenic APP-BACE flies *Values are significantly different ($p < 0.05$) compared to a) control; b) wild control Keys: 0.2% SUC- 0.2% Sucrose, 0.5% SUC- 0.5% Sucrose, 1% SUC- 1% Sucrose, 0.01% ASP- 0.01% Aspartame, 0.02% ASP- 0.02% Aspartame, 0.05% ASP- 0.05% Aspartame.

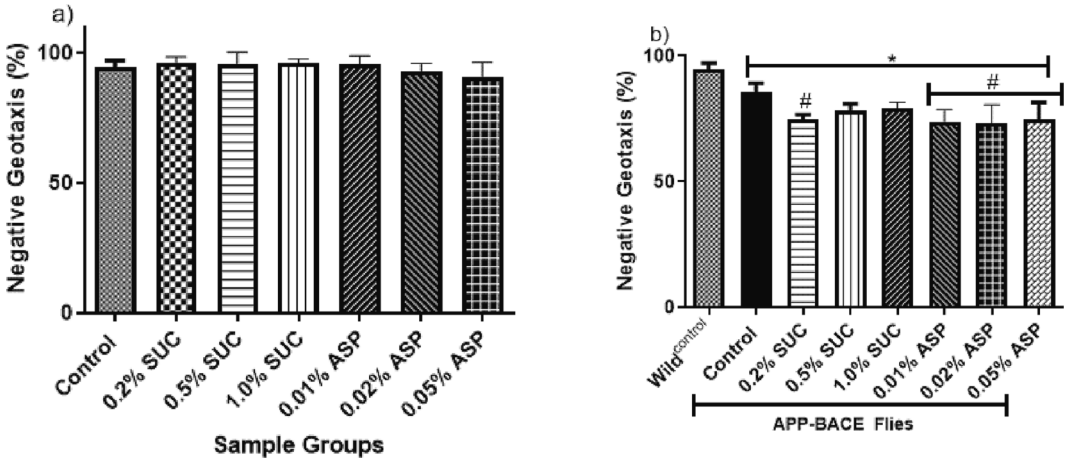

Fig. 2. Effect of Sucrose and Aspartame on Locomotor Performance of a) wild type (Harwich strain) D. melanogaster; b). Transgenic APP-BACE flies *Values are significantly different ($p < 0.05$) compared to a) control; b) wild control #Values are significantly different ($p < 0.05$) compared to b) Control Keys: As described in Fig. 1.

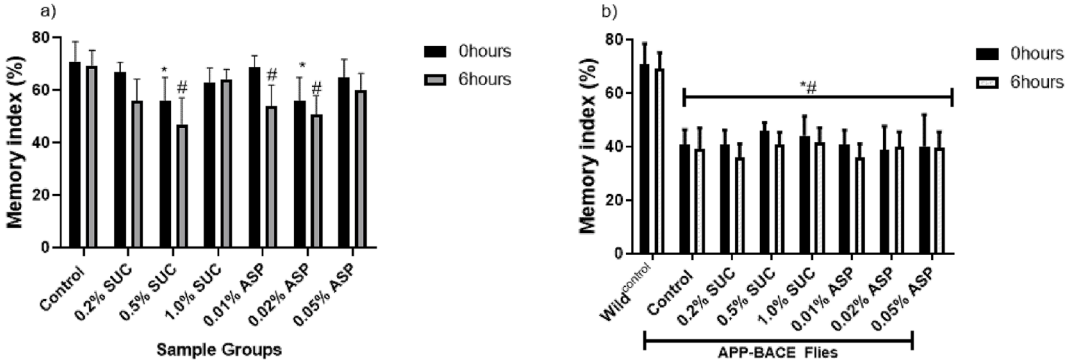

Fig. 3. Effect of Sucrose and Aspartame on Memory Index of a) wild type (Harwich strain) D. melanogaster; b). Transgenic APP-BACE flies *Values are significantly different ($p < 0.05$) compared to wild control at 0 hr # Values are significantly different ($p < 0.05$) compared to wild control at 6 hr Keys: As described in Fig. 1.

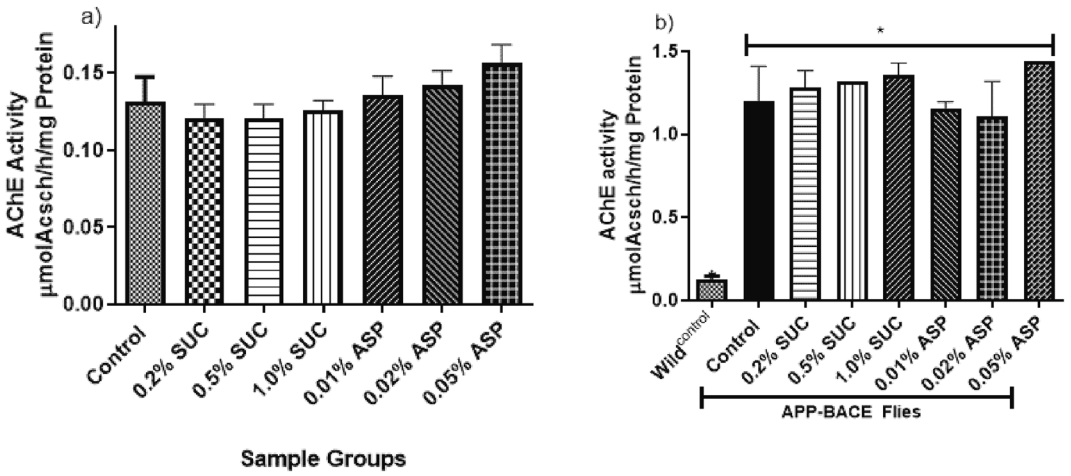

Fig. 4. Effect of Sucrose and Aspartame on Acetylcholinesterase (AChE) Activity in a) wild type (Harwich strain) D. melanogaster; b). Transgenic APP-BACE flies *Values are significantly different ($p < 0.05$) compared to a) control; b) wild control Keys: As described in Fig. 1.

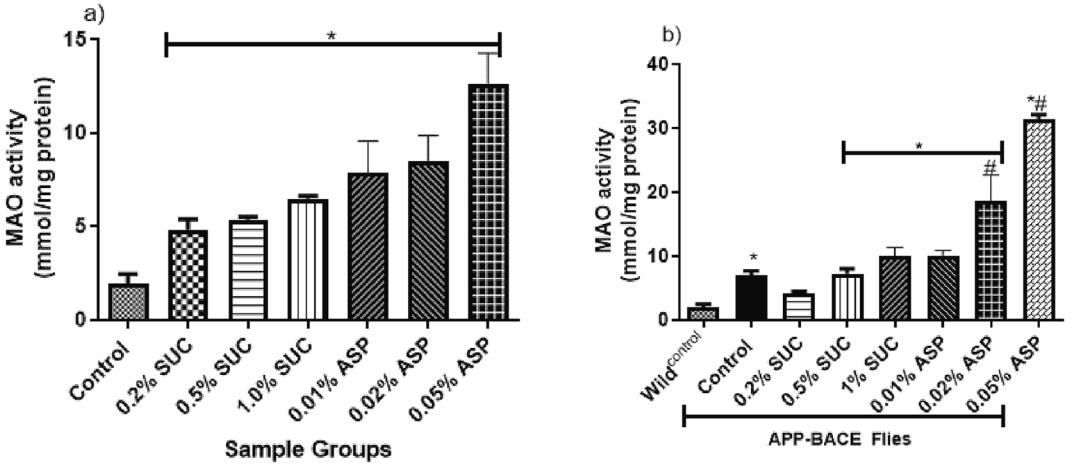

Fig. 5. Effect of Sucrose and Aspartame on Monoamine oxidase (MAO) Activity in a) wild type (Harwich strain) D. melanogaster; b). Transgenic APP-BACE flies *Values are significantly different ($p < 0.05$) compared to a) control; b) wild control #Values are significantly different ($p < 0.05$) compared to b) Control Keys: As described in Fig. 1.

2.4. Fly survival rate assessment

In this present study, the survival rate of the wild control ($W^{Control}$) flies, transgenic (Aβ) flies and Aβ flies treated with dietary inclusions of either aspartame or sucrose were also assessed throughout the duration of the experiment. Flies were separated into six groups as described above in the experimental design section. Following that, flies were counted every day to pool their survival rate at the end of the experiment, which was stated as a percentage of the total fly population for each group.

2.5. Measurement of locomotor performance by negative geotaxis test

As stated previously [1], the movement of the control and treated flies was assessed using the negative geotaxis test. Prior to being moved to the cylindrical tube for the locomotor evaluation, flies were initially immobilised in ice. Flies were tested for movement after being immobilised by counting the proportion of those that crossed the 6 cm line after 30 s in a sterilised tube.

2.6. Memory retention assay

The flies were subjected to the aversive phototaxic suppression assay [3]as previously reported by Ogunsuyi et al., [34]. In brief, this assay made use of a T Maze which paired the bitter taste of quinine with attraction for light. Quinine (180 µL of 1 µM) was carefully

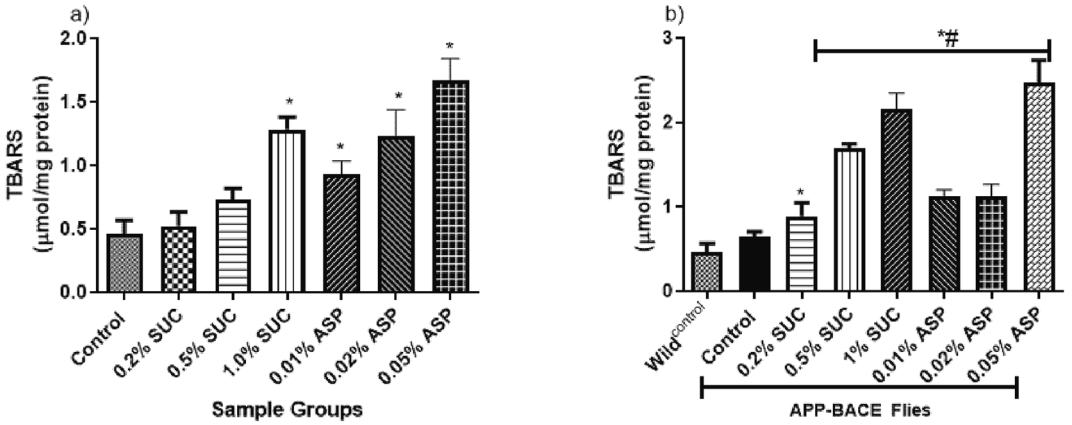

Fig. 6. Effect of Sucrose and Aspartame on Lipid peroxidation Level in a) wild type (Harwich strain) D. melanogaster; b). Transgenic APP-BACE flies *Values are significantly different ($p < 0.05$) compared to a) control; b) wild control #Values are significantly different ($p < 0.05$) compared to b) Control Keys: As described in Fig. 1.

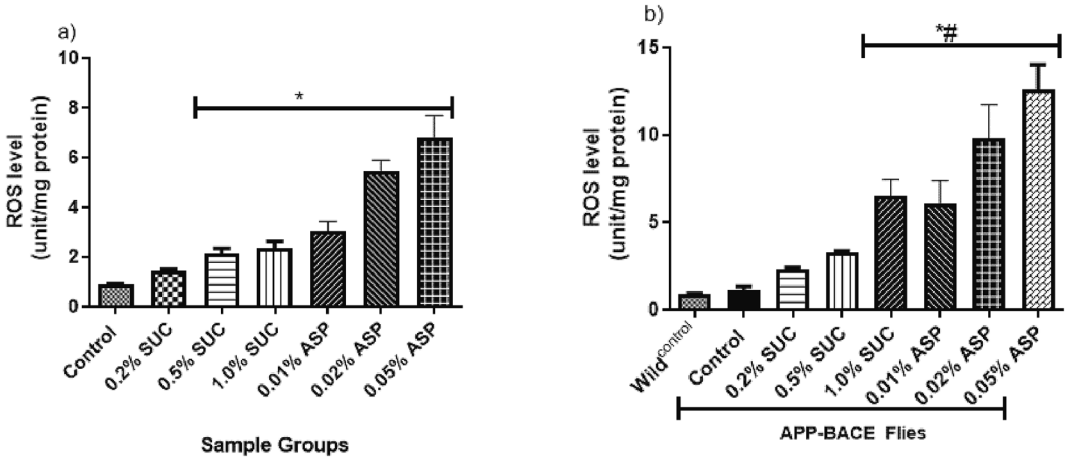

Fig. 7. Effect of Sucrose and Aspartame on Reactive Oxygen Species (ROS) Level in a) wild type (Harwich strain) D. melanogaster; b). Transgenic APP-BACE flies *Values are significantly different ($p < 0.05$) compared to a) control; b) wild control #Values are significantly different ($p < 0.05$) compared to b) Control Keys: As described in Fig. 1.

applied to filter paper and placed into the lighted chamber. The phototoxic flies were allowed free access into the lighted chamber with quinine-treated filter paper. After 45 s, the fly was tapped back to the dark chamber, and the process was repeated. Flies that failed to walk to the lighted chamber within 45 s were scored as "Pass". The experiment was repeated at time 0 and 6 hrs to serve as the memory index.

2.7. Fly head tissue homogenate preparation

Experimental flies were immobilized in ice and the head rapidly dissected. The head tissue homogenate was prepared as previously described [34]. T The total protein content of fly homogenates was determined using Bradford's 1976 Coomassie blue method, which used bovine serum albumin (BSA) as a standard.

2.8. Acetylcholinesterase (AChE) activity assay

The activity of acetylcholinesterase was determined according to the Ellman method [18]. The reaction mixture was made up of 10 mM potassium phosphate buffer (pH 7.4), 1.0 mM DTNB, 30 µL of homogenate, and 0.8 mM acetylthiocholine iodide. The AChE activity was monitored at 412 nm and expressed as µmol AcSch/h/mg protein.

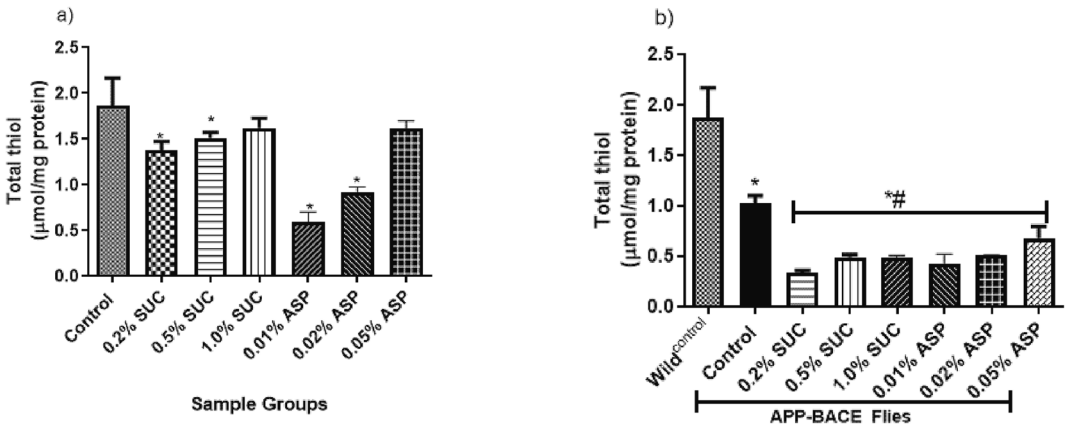

Fig. 8. Effect of Sucrose and Aspartame on Total thiol Level in a) wild type (Harwich strain) D. melanogaster; b). Transgenic APP-BACE flies *Values are significantly different ($p < 0.05$) compared to a) control; b) wild control #Values are significantly different ($p < 0.05$) compared to b) Control Keys: As described in Fig. 1.

2.9. Monoamine oxidase (MAO) activity assay

The MAO activity was measured according to a previously reported method (Turski *et al.* 1973) as previously reported [33]. In brief, the reaction mixture contained 0.025 M phosphate buffer (pH 7.0), 0.0125 M semicarbazide, 10 mM benzylamine, and 100 µL of fly tissue homogenate After 30 min incubation, acetic acid was added and incubated for 3 min in boiling water bath followed by centrifugation. The resulting supernatant (1 mL) was mixed with equal volume of 2, 4-dinitrophenylhydrazine, and 1.25 mL of benzene was added after 10 min incubation at room temperature. After separating the benzene layer, it was mixed with an equal volume of 0.1 N NaOH. Alkaline layer was decanted and incubated at 80 °C for 10 min. The orange–yellow color developed was measured at 450 nm in a UV/visible spectrophotometer. The MAO activity was expressed as mmoL of amines produced per mg protein (mmoL/mg protein).

2.10. Lipid peroxidation assay

This was carried out by the method of Ohkawa *et al.* [35]as previously reported [33]. In brief, 0.3 mL of tissue homogenate was added to 0.3 mL of 8.1% Sodium dodecyl sulfate (SDS), 0.5 mL HCL/acetic acid (pH = 3.4) and 0.5 mL of Thiobarbituric acid (TBA) and the mixture was incubated at 100 °C for 1 h. The resulting thiobarbituric acid reactive species (TBARS) was quantified at 532 nm in a spectrophotometer, calculated as malondialdehyde (MDA) equivalent and expressed as µmoL of MDA produced per mg protein (µmoL/mg protein).

2.11. Measurement of reactive oxygen species

The method described by Hayashi et al. [23], with slight modifications, was used to measure the amount of reactive oxygen species in the fly tissues interms of H_2O_2 content. The reaction consists of 130 µL of sodium acetate buffer and 15 µL of tissue homogenate, which was incubated at 37 °C for 5 minutes. Thereafter, 100 µL of n-n-diethyl-para-phenylenediamine (DEPPD) reagent (a solution consisting of 6 mg/ml of DEPPD and 4.37 µM of ferrous sulphate, dissolve in 0.1 M sodium acetate, pH 4.8) was added and the absorbance read at 505 nm in a microplate reader. ROS levels in the fly tissues were calculated using a standard H_2O_2 calibration curve and reported as Unit/mg protein, where 1 unit = 1 mM H_2O_2.

2.12. Determination of the total thiol content

Determination of the level of total thiol content in tissue homogenate was done by the method of Ellman [17]. The reaction mixture was made up of a final reaction volume of 200 µL consisting of 85 µM potassium phosphate buffer (pH 7.4), 20 µL of tissue homogenate, and 0.5 mM 5,5′-dithiobis-(2-nitrobenzoic acid) (DTNB). This was followed by 30-minute incubation at room temperature, and the absorbance was taken at 412 nm. The total thiol content was subsequently calculated and expressed as µmol/mg protein.

2.13. Data analysis

The results of replicate readings were pooled and expressed as mean ± standard deviation (SD). One-way analysis of variance (ANOVA) was used to analyze the results (except for the memory retention assay where two-way ANOVA was used), followed by Tukey post hoc test, with levels of significance accepted at $p < 0.05$. All statistical analysis was carried out using the software Graph pad PRISM (V.5.0).

3. Results

The effects of dietary inclusions of sucrose (0.2–1.0%) and aspartame (0.01–0.05%) on the survival rate of both wild type (Harwich strain) and transgenic AD model D. melanogaster are shown in Fig. 1. It was observed that in the wild-type flies, only groups administered 0.05% aspartame exhibited a significantly reduced (p 0.05) survival rate compared to the control. However, in the transgenic flies, there was a significantly reduced survival rate in the transgenic fly control, and the transgenic flies were administered 0.5% sucrose and aspartame (0.01–0.05%). Furthermore, the effects of dietary inclusions of sucrose and aspartame on the locomotor performance of *D. melanogaster* are shown in Fig. 2. It was observed that there was no significant difference in locomotion capacity in wild flies, while a reduction in locomotion capacity was observed in transgenic fly control and those fed dietary inclusions of the sweeteners, which is evident by a reduction in negative geotaxis. It is important to note that transgenic flies fed dietary inclusions of aspartame (0.01–0.05%) exhibit a further significant reduction (p 0.05) in their locomotion compared to the transgenic control flies.

Fig. 3 shows the effect of the sweeteners on the memory index of the flies. This showed that the memory index reduced significantly in wild flies (Fig. 3a) fed dietary inclusions of 0.5% sucrose and 0.02% aspartame compared to the control at both 0 hr and 6 hr analysis. However, in the transgenic flies (Fig. 3b), both the transgenic control flies and transgenic flies fed dietary inclusions of glucose (0.2–1.0%) and aspartame (0.01–0.05%) exhibited significantly reduced memory index at both 0 hr and 6 hr analysis.

The effects of dietary inclusions of sucrose and aspartame on the AChE activity of D. melanogaster are shown in Fig. 4. This study showed that in the wild flies (Fig. 4a), there was no significant difference ($p > 0.05$) in the AChE activity among the flies fed dietary inclusions of both sucrose (0.1–0.5%) and aspartame (0.01–0.05%) when compared to the control. In the transgenic flies (Fig. 4b), both the transgenic control flies and the transgenic flies fed dietary inclusions of the sweeteners exhibited significantly elevated (p 0.05) AChE activity compared to the wild control flies; nevertheless, there was no significant difference ($p > 0.05$) among the transgenic flies fed the sweeteners compared to the transgenic control flies. Fig. 5 shows the effect of the sweeteners on MAO activity in both wild and transgenic flies. In Fig. 5a, it was revealed that flies fed both sucrose (0.1 and 0.5%) and aspartame (0.01–0.05%) exhibited significantly higher MAO activity compared to the control. Further, as shown in Fig. 4b, a similar elevated MAO activity was observed in transgenic control flies and transgenic flies fed dietary inclusions of the sweeteners. It is also important to note that in transgenic flies fed dietary inclusions of 0.02% and 0.05% aspartame, the MAO activity was significantly elevated compared to the transgenic control flies.

Fig. 6a presents the effect of the sweeteners on lipid peroxidation in wild flies. This showed that aspartame at all levels of dietary inclusion (0.01–0.05%) led to a significantly elevated lipid peroxidation level when compared to the control. On the contrary, for dietary inclusion of sucrose, a significantly elevated lipid peroxidation level was only observed in flies with dietary inclusion of 0.5% sucrose. In the transgenic AD fly model, there was no significant difference between the wild control flies and the transgenic control flies in terms of their lipid peroxidation levels (Fig. 5b). Nevertheless, transgenic flies fed all levels of dietary inclusions of aspartame (0.01–0.05%) exhibited significantly higher lipid peroxidation levels when compared to the transgenic control flies. A similar significant elevation in the levels of lipid peroxidation was only observed in the transgenic flies fed dietary inclusions of 0.5% and 1.0% sucrose when compared to the transgenic control flies. The results obtained for the effects of the sweeteners on reactive oxygen species (ROS) levels in wild (Fig. 7a) and transgenic (Fig. 7b) flies are quite similar to those obtained for lipid peroxidation as earlier described. However, in the transgenic flies, significant elevations in the ROS level were observed for all levels of aspartame diet inclusions (0.01–0.05%) and dietary inclusions of 1.0% sucrose only.

The total thiol content showed that for the wild-type flies (Fig. 8a), dietary inclusions of sucrose (0.2% and 0.5%) and aspartame (0.01% and 0.02%) led to a significant reduction in the total thiol contents. Further, in the transgenic flies (Fig. 8b), there was a significant reduction in the total thiol content in the transgenic control flies compared to the wild control flies. In addition, transgenic flies fed dietary inclusions of sucrose (0.2–1.0%) and aspartame (0.01–0.05%) also exhibited significantly reduced total thiol contents compared to the transgenic control flies.Fig. 8.

4. Discussion

Alzheimer's disease commonly involves decline in cognitive function, which begins to appear as memory loss[15]. In this study, we confirmed this AD-like phenotype in the transgenic flies, resulting in their significantly reduced memory index compared to the wild-type flies. The use of the aversive phototaxis suppression assay to monitor learning and memory in flies has been well reported [3,2,33,21]. The findings of this study also agree with our earlier findings on reduced memory index in these transgenic fly models [32,34]. Furthermore, similar neurodegenerative phenotypes have been reported by Greeve et al. [22] in triple transgenic flies expressing human APP (hAPP), human β-secretase (hBACE), and Drosophila -secretase presenilin (dPsn). Also, a memory decline was reported in flies expressing human A42 [24]. Similarly, the fact that all the transgenic flies fed dietary inclusions of both aspartame and sucrose also exhibited a significantly reduced memory index compared to the wild-type flies further shows that the loss in memory conditions is preserved but not aggravated.

Considering the pivotal role the cholinergic system plays in learning and memory, and specifically AChE being a therapeutic target in AD, AChE activity was monitored in the fly heads to support the observed memory phenotype. This finding corresponds with the acetylcholinesterase activity in the fly head, in which, while all the transgenic fly groups exhibited significantly elevated AChE activity, there was no significant difference between AChE activity in the transgenic control flies and transgenic flies fed dietary inclusions of both aspartame and sucrose. This, therefore, could help explain the possible mechanism behind the observed effects on the memory index. On the contrary, in wild-type flies, flies fed dietary inclusions of glucose at 0.5% and aspartame at 0.02% exhibited a significant decline in memory index. Nevertheless, this does not correspond with the observed head AChE activity, in which no significant

difference was observed among the treatment groups. While the reason for these observations might not be fully understood at this level, it has given information to suggest the involvement of the sweeteners more in pathogens than the progression of the pathological phenotypes. Also, it suggests the involvement of other metabolic pathways in the observed findings.

Cognitive dysfunction and AD are multi-etiological diseases involving several metabolic and signalling pathways and pathological indices. To further investigate these in this study, investigations were made into the effects of the sweeteners on markers of oxidative stress and redox homeostasis. The observed significant elevation in lipid peroxidation level in the wild-type flies fed dietary inclusion of sucrose at the highest percentage inclusion of 1.0% and all ranges of aspartame inclusion (0.01–0.05%) suggest that the sweeteners elicit redox imbalance through the generation of free radical-mediated oxidative stress. This was further substantiated by a corresponding finding obtained for the elevated levels of reactive oxygen species (ROS) and reduced total thiol content. ROS are known to be a major cause of redox imbalance by eliciting oxidative stress chain reactions, including the oxidation of macromolecules, as observed in lipid peroxidation, protein carboxylation, and DNA damage [42]. These could therefore suggest that these sweeteners, at these percentage inclusions, induce ROS-mediated oxidative stress and lipid peroxidation in the fly head. Ashok and Sheeladevi [6] had earlier reported that aspartame (although at a chronic administration of 40 mg/kg b.w.) elicits oxidative stress and neurodegenerative apoptosis in rat brains. It is therefore not surprising to see similar effects in the transgenic flies. The observed significant elevation of lipid peroxidation and ROS levels in transgenic flies fed dietary inclusions of 1.0% sucrose and aspartame (0.01–0.05%) compared to the transgenic flies suggests that the sweeteners at these proportions further aggravated oxidative stress and redox imbalance in the flies. This is very critical considering the role oxidative stress plays in the progression of AD and dementia.

Previous studies have shown a direct link between free radical-mediated oxidative stress and the pathogens and progression of AD [9,10]. Consequently, antioxidant-based therapies, either through augmentation of endogenous antioxidants or dietary intervention, have been well justified for the prevention and management of AD and dementia [40]. Thiols serve as a major source of endogenous antioxidants for mitigating the deleterious effects of free radicals. The significant reduction in total thiol content, particularly in transgenic flies fed dietary inclusions of both sweeteners compared to the transgenic control group, could further support the aggravation of redox imbalance and oxidative stress in the flies.

The impairment in monoamine oxidase (MAO) activity in both wild-type and transgenic flies fed dietary inclusions of both sweeteners could further support the mechanism of dysregulation in redox homeostasis by the sweeteners in both the wild and transgenic flies. Studies have shown that elevated MAO activity contributes to the generation of ROS, which are byproducts of their enzymatic activity on biogenic amines [25]. Consequently, the significantly elevated MAO activity observed, particularly in transgenic flies fed dietary inclusions of aspartame (0.02 and 0.05%), could have contributed to the aggravated level of oxidative stress in the flies when compared to the transgenic control group.

Furthermore, MAO is critical to the monoamine neurotransmitter systems, particularly dopamine-mediated neurotransmission, and have been implicated in different neurodegenerative diseases, including those involving cognitive dysfunction like AD [41,8] and neuromuscular dysfunction like PD [45]. In this study, we observed in the transgenic flies a significant reduction in their locomotor performance. Particularly in flies fed aspartame at all proportions of inclusions, a significantly reduced locomotor performance was observed, suggesting a further aggravative effect of this sweetener in the AD fly model.

Put together, the elevated markers of oxidative stress (lipid peroxidation, ROS, and MAO), coupled with the reduction in endogenous antioxidants (total thiol) in transgenic flies fed dietary inclusions of both sucrose and aspartame, could provide mechanisms to support a state of aggravated redox imbalance and oxidative stress in the flies. Considering the role of oxidative stress in cell viability and toxicity, it comes as no surprise that this group of flies also experienced an aggravated mortality rate. Nevertheless, the reduced memory index in the transgenic flies was not significantly aggravated in the transgenic flies fed dietary inclusions of both sweeteners.

5. Conclusion

This study has shown that dietary inclusions of aspartame and sucrose aggravated the elevated markers of oxidative stress and reduced endogenous antioxidants in both wild-type and transgenic AD fruit fly (Drosophila melanogaster) models. These aggravated effects were most profound in flies fed dietary inclusions of aspartame (0.01–0.05%) and 1.0% sucrose. However, we found no evidence of aggravation in the impaired memory function but for the locomotor performance in these flies. This could therefore provide evidence to support the idea that aspartame and a high proportion of sucrose could aggravate AD-like phenotypes in the fruit fly model. Further studies are, however, encouraged to provide more justifications for these findings.

Practical application.

Sweeteners are a key component of human diets. While caloric sweeteners such as sucrose are popular, their use is often discouraged due to a number of associated health risks, particularly for prolonged use, making non-caloric sweeteners possible alternatives; nevertheless, non-caloric sweeteners in general and aspartame in particular have not been completely devoid of health controversies relating to their safety and contributions to diseases. In this study, using the fruit fly model of AD, we investigated the effects of aspartame and sucrose on survival rate, memory index and selected biochemical parameters to provide information on the suitability of these sweeteners under experimental AD-like phenotypes.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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