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RESEARCH ARTICLE

African mistletoe (*Tapinanthus bangwensis* Lor.) infestation improves the phenolic constituents, antioxidative and antidiabetic effects of almond (*Terminalia catappa* Linn.) host leaf in sucrose-rich diet-induced diabetic-like phenotypes in fruit fly (*Drosophila melanogaster* Meigen)

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Abstract

Almond is a medicinal plant commonly infested with mistletoe, and its leaves are consumed as food and/or medicine due to their health-promoting benefits. Mistletoe infestation may interfere with the chemical composition and biological activities of host plants. Therefore, we investigated the influence of mistletoe infestation on polyphenol constituents, antioxidative and hypoglycemic activities of almond leaves in sucrose-rich diet (SRD)-induced diabetic-like phenotypes in the *Drosophila melanogaster* model. Infested almond leaf (IAL) had higher polyphenol constituents; ellagic acid and isorhamnetin were the most abundant phenolic acid and flavonoid constituents in the almond leaves. However, this study revealed significant ($P < 0.05$) infestation-induced increase in some phenolic acids (*p*-hydroxybenzoic, protocatechuic, gallic, and chlorogenic acids) and flavonoids (apigenin and catechin) with known antioxidative, antidiabetic, and neuroprotective properties. Moreover, IAL had higher *in vitro* antioxidant and inhibition of α-amylase, α-glucosidase, and acetylcholinesterase (AChE) activities. The SRD-fed flies had significant ($P < 0.05$) decrease in percentage survival and locomotion and increase in weight. In addition, elevated levels of glucose, trehalose, insulin-like peptides and triglycerides coupled with increased oxidative and AChE activities were obtained. However, treatment with the leaves significantly ($P < 0.05$) lessened the negative alterations caused by SRD with IAL having higher hypoglycemic activities. Hence, we suggest that improved antidiabetic activities of IAL could be related to its higher phenolic constituents.

KEYWORDS
Almond, diabetes, *Drosophila melanogaster*, mistletoe, phenolics, sucrose

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1 | INTRODUCTION

Almond (*Terminalia catappa* Linn) is a large tree, widely spread throughout the subtropical and tropical zones of the Indian and Pacific Oceans of the world with maritime climates. It is also found in areas with coastal vegetation, especially strandline communities and beach forests including rocky shores and edges of mangrove swamps. It belongs to the Combretaceae family and has been widely used in the traditional African, Asian, and Indian medicine (Fyhrquist et al., 2002). It is often grown as an ornamental tree and for its deep shade; however, its leaves, barks, and roots are used in traditional medicine for various health-promoting purposes (Subashinee & Siriwardhana, 2002). The health benefits of the leaves had been linked to the presence of bioactive compounds, most importantly phenolic acids and flavonoids compounds (Chanda et al., 2013). The leaves are mostly used due to their anti-indigestion, hepatoprotective, antiperoxidation, antidiabetic, antidysentery, and memory-enhancing effects (Ankur & Neelesh, 2017). Almond is one of the common plant trees with mistletoe infestation. African mistletoe (*Tapinanthus bangwensis* Lor.) are hemi-parasites that bear evergreen leaves that photosynthesize. They grow on a variety of host trees of medicinal and economic importance, and depend on their host mainly for water and mineral nutrients (Milius, 2000). There are several reports suggesting that mistletoe infestation could possibly cause principal changes in the phenolic profiling and biological activities of host plants. Therefore, mistletoe infestation could alter the chemical constituents of host plants with attendant differences in their biological activities (Cuevas-Reyes et al., 2017; Edagbo et al., 2012).

Over the years, simple organisms like fruit fly (*Drosophila melanogaster* Meigen) have become widely acceptable and used as model organisms for nutritional studies by exploring the effects of diet on life span, fecundity, behavior, redox status, and other key parameters linked to human degenerative diseases (Lee et al., 2008). Studies have shown that fruit fly can be used as a model organism for studying diabetes mellitus, most importantly, type-2 diabetes, due to an increased level of circulating and stored sugar in response to increased dietary sugar (Broughton et al., 2005; Rulifson et al., 2002). Although sucrose is commonly used in preparing *Drosophila* laboratory food, reports from previous studies showed that rearing fruit fly on high sucrose diet resulted in increased expression levels of *Drosophila* insulin-like peptides and oxidative events coupled with higher glucose, trehalose, and triglyceride levels (Ecker et al., 2017; Oyeniran, Ademiluyi, & Oboh, 2020). These are similar to the peculiar characteristics observed in mammalian insulin resistance during diabetic conditions (Musselman et al., 2011, 2013). Furthermore, previous studies had established that fruit flies are used as models for studying neurodegeneration due to their similar homolog with human and, thus, flies' AChE perform similar neurochemical transmission (Lenz et al., 2013; Nichols, 2006; Wolf & Rockman, 2011). Therefore, AChE were evaluated due to complications of type-2 diabetes in the brain. Hence, this study investigated the influence of mistletoe infestation on phenolic constituents, *in vitro* antioxidative, and inhibition of α-amylase, α-glucosidase, and AChE activities of almond leaves. In addition, we evaluated the survival, development, and behavioral parameters, and antioxidant and hypoglycemic activities of infested and noninfested almond leaf in sucrose-rich diet (SRD)-induced diabetic-like phenotypes in a *Drosophila melanogaster* model.

2 | MATERIALS AND METHODS

2.1 | Almond leaf collection and preparation

The fresh leaves of mistletoe infested and noninfested almond tree were harvested at a local farm plantation in Akure. Authentication of the leaves was done by Mr Momoh at the Centre for Research and Development, Federal University of Technology, Akure, Nigeria, with voucher number 0152. The leaves were sorted, washed under running tap, and subsequently freeze dried. The freeze-dried samples were pulverized to fine powder using an electric blender. The samples were kept in an airtight container prior to subsequent analysis.

2.2 | Reagents

Sucrose, *N*-*N*-diethyl-para-phenylenediamine (DEPPD), 1-chloro-2,4-dinitrobenzene (CDNB), 5,5-Dithiobis-(2-nitrobenzoic acid) (DTNB), trichloroacetic acid (TCA), thiobarbituric acid (TBA), hydrogen peroxide, trehalase, acetylthiocholine iodide, and acetic acid were obtained from Sigma-Aldrich (Merck KGA, Darmstadt, Germany). All other chemicals and reagents used were of analytical grade, and the water used was glass distilled.

2.3 | Preparation of aqueous extracts

One gram of powdered samples from infested almond leaves (IAL) and noninfested almond leaves (NIAL) were weighed, separately dissolved in 100 mL distilled water, and extracted at room temperature for 12 h with continuous agitation on an orbital shaker. The extracts were filtered using muslin cloth and filter paper, and filtrates were centrifuged at 3,000 × *g* for 5 min. The supernatants were dried on a rotatory evaporator at 40°C for 6 h, the different solutions were later freeze-dried and the respective percentage yields were 22.63% and 19.84%. The extracts were further redissolved in distilled water at a stock concentration of 10 mg/mL and kept at −4°C for *in vitro* analysis (Ademiluyi et al., 2018).

2.3.1 | Polyphenols quantification

Total phenol content was quantified in the aqueous extracts of IAL and NIAL samples according to the method of Singleton et al. (1999). The reaction mixture consisted of appropriately diluted aqueous extracts, 10% Folin reagent, and 7.5% sodium carbonate; incubation was done at 45°C for 40 min and absorbance was taken at 765 nm. Total flavonoid content was determined using the method of Meda et al. (2005). The

mixture was made up of appropriately diluted extracts, methanol, 10% $AlCl_3$ and 1 M potassium acetate; this was incubated at 25°C for 30 min and absorbance was taken at 415 nm. HPLC-DAD quantification of phenolic compounds was carried out in two-stage extraction processes according to the method of Kelley et al. (1994) and Provan et al. (1994). In stage 1, the samples were centrifuged at 5,000 × *g*, washed with water, centrifuged again, and the resulting supernatant was heated at 90°C for 2 hours. After cooling, the extract was titrated with 4 M HCl to pH < 2.0, centrifuged, and the supernatant was saved for subsequent purification. The residue was extracted further in stage 2, the residue was extracted with 4 M NaOH and heated to 160°C. The mixture was allowed to cool, filtered, the supernatant was collected, and the residue was washed with water. The supernatants were combined and adjusted to pH < 2.0 with 4 M HCl. For purification, an aliquot (5-15 mL) of the various supernatants was passed through a conditioned Varian (Varian Associates, Harbor City, CA) Bond Elut PPL (3 mL size with 200 mg packing) solid-phase extraction tube at 5 mL/min attached to a Visiprep (Supelco, Bellefonte, PA). The tubes were then placed under vacuum (-60 kPa) until the resin was thoroughly dried after which the phenolic compounds were eluted with 1 mL of ethyl vials. The PPL tubes were conditioned by first passing 2 mL of ethyl acetate followed by 2 mL of water (pH < 2.0). The purified phenolic extracts (1 mL: 10:1 split) were analyzed for its phenolic composition by comparison with phenolic standards.

2.3.2 | *In vitro* assays

The inhibition of 2-azinobis-3-ethylbenzo-thiazoline-6-sulfonate (ABTS) radical by the aqueous extracts of IAL and NIAL was determined in a reaction mixture containing adequate diluted leaf extracts and $ABTS^{\bullet+}$ solution; incubation was done in the dark for 15 min and absorbance was taken at 734 nm (Re et al., 1999). Ferric (Fe^{3+}) reducing antioxidant property (FRAP) determination was carried out in a reaction consisting of suitably diluted extracts, phosphate buffer (0.2 M, pH 6.6) and 1% potassium ferricyanide, and incubation was done at 50°C for 20 minutes. This was followed by the addition of 10% trichloroacetic acid, centrifuged at 3,000 × *g* for 10 minutes, 0.1% $FeCl_3$ was added to the resultant supernatant, and absorbance was read at 700 nm (Oyaizu, 1986). The inhibition of 1, 1-diphenyl–2 picrylhydrazyl (DPPH) radical was evaluated in a reaction consisting of diluted aqueous extracts and DPPH*, incubation was done in the dark for 30 min, and absorbance was measured at 516 nm (Gyamfi et al., 1999). The inhibition of hydroxyl (OH) radical was carried out in a reaction mixture made of diluted aqueous leaf extracts, 20 mM deoxyribose, phosphate buffer (100 mM, pH 7.0), 0.5 mM $FeSO_4$ and water. This was incubated at 37°C for 30 minutes; 28% TCA and 0.6% TBA was subsequently added. Incubation was done at 100°C for 20 min and absorbance was read at 532 nm (Halliwell & Gutteridge, 1981).

Furthermore, the ability of the aqueous extracts of IAL and NIAL to reduce malondialdehyde (MDA) produced from Fe^{2+}-induced lipid peroxidation (LPO) was evaluated in a reaction mixture consisting of fly tissue homogenate, Tris–HCl buffer (0.1 M, pH 7.4), appropriate proportions of extracts and freshly prepared 0.25 mM $FeSO_4$. Incubation was done at 37°C for 1 h; thereafter, 8.1% sodium dodecyl sulfate (SDS), acetic acid/HCl buffer (pH 3.4), and 0.8% TBA was added. The mixtures were incubated at 100°C for 1 h, and thiobarbituric acid reactive species (TBARS) content was measured at 532 nm (Ohkawa et al., 1979). The α-amylase inhibitory activity was assessed in a reaction made of appropriately diluted aqueous leaf extracts, fly tissue homogenate, and 20 mM sodium phosphate buffer (6 mM NaCl, pH 6.9). This was incubated at 25°C for 10 min; 1% starch solution was added, incubation was done at 25°C for 10 min and, thereafter, DNSA was added. This was incubated at 100°C for 5 min, followed by the addition of distilled water and absorbance was taken at 540 nm (Adefegha et al., 2020). The inhibition of α-glucosidase was also carried out in a reaction consisting of suitable proportions of extracts, fly tissue homogenate, and 0.1 M phosphate buffer (pH 6.9); incubation was done at 25°C for 10 min; thereafter, 3 mM glutathione and 10 mM PNPG solution was added, incubated at 25°C for 5 min, and absorbance was read at 405 nm (Adefegha et al., 2020). The inhibition of AChE was assessed in a reaction made of adequate proportion of the aqueous extracts, 1.7 mM of DTNB, phosphate buffer (0.1 M, pH 7.4), fly tissue homogenate, and 1.4 mM of acetylthiocholine iodide, incubated at 25°C for 20 min, and absorbance was measured at 412 nm for 3 min at 15-s intervals (Ellman et al., 1961; Oyeniran, Ademiluyi & Oboh, 2020).

2.4 | *Drosophila melanogaster* stock and culture conditions

Fruit fly (Oregon wild fly strain) was obtained from the National Species Stock Center (Bowling Green, OH, USA). The flies were reared on corn meal medium containing brewer's yeast (1%), powdered milk (1%), agar (1%), and methyl-p-hydroxybenzoate (0.08%) at constant temperature (23°C ± 1°C) and 60% relative humidity in an incubator with 12-h dark/light cycle at the Drosophila Research Laboratory, Functional Foods, Nutraceuticals and Phytomedicine Unit, Department of Biochemistry, Federal University of Technology, Akure. The first instar larvae (L1) obtained from this strain was used due to their increased feeding and rapid growth rate in comparison with adult flies.

2.4.1 | Experimental design

Adult female fruit flies of 5 days old were separated into new glass jars containing basal diet on which they laid eggs, the flies were removed from the jars after 12 h. After 24 h, the emerged first instar larva (L1) were divided into six groups containing 50 flies (L1) each. Group I was placed on basal diet alone, groups II and III were placed on diet containing IAL and NIAL samples (the aqueous leaves freeze-dried extracts) at a final concentration of 10 mg/g diet, group IV was placed on basal

diet containing 30% w/v sucrose, while groups V and VI were placed on diet containing 30% w/v sucrose and treated with IAL and NIAL samples (1% w/v diet) and reared until 5 days of adulthood. The dietary inclusion for sucrose and almond leaves samples were based on previous toxicological tests performed in adult flies, the live flies were counted daily for 20 days and plotted as percentage live flies (data not shown). The concentration of sucrose (30%) and the leaves (1% w/v diet) that compares with the survival rate of the control flies was used for this study (data not shown). The treatment lasted for 14 days, and the newly emerged 5-day-old adult flies were collected and used for the various analyses. This consisted of more than four main studies and five vials were used per group.

2.4.2 | Eclosion and survival rate

The rate of larva eclosion and the survival rate of the emerged adult flies were monitored by counting the number of adult flies daily from the 9th to 14th days of treatment. The data obtained were analyzed and expressed as a percentage survival (Farombi et al., 2018).

2.4.3 | Negative geotaxis

The climbing activity of the newly emerged adult flies was evaluated. Twenty newly emerged 3-day-old adult flies were immobilized on ice. After the recovery period, the flies were tapped softly to the bottom of a marked glass column, and the flies that climbed up above the 6 cm mark of the column in 6 s were recorded. This process was repeated three times at 1-min interval. The data obtained were expressed as percentage locomotor performance (Abolaji et al., 2015).

2.4.4 | Weight of flies

Five newly emerged adult flies of 3-day-old hatched from the larva (L1) were separated and weighed in groups of male and female on an AMPUT high-precision microbalance. The weight of a fly was extrapolated and expressed as weight of flies in μg (Ecker et al., 2017).

2.4.5 | Collection of hemolymph and determination of glucose and trehalose levels

The hemolymph of 5-day-old adult flies was collected according to the method of Ecker et al. (2017). The flies were immobilized on ice, wings were cut off, and then placed in 200 μL Eppendorf tubes that contain small holes and were centrifuged within 2000 μL Eppendorf tubes in Kenxin refrigerated centrifuge Model KX3400C at 3,000 × *g*, 4°C for 5 min. The hemolymph obtained was diluted 50 times with glucose/trehalose buffer (5 mM Tris [pH 6.6], 137 mM NaCl, and 2.7 mM KCl) and heated at 70°C for 5 min. The level of glucose in the flies' hemolymph was determined using the Sigma Glucose Randox Assay kit and processed according to the manufacturer's procedure. Trehalose level was measured after digesting the hemolymph with porcine trehalase at 37°C for 24 hours; it was estimated by subtracting the sample-free glucose from the trehalase-treated sample. Absorbance was taken at 546 nm, and the standard was used for the calculation and expressed in mM/L.

2.4.6 | Preparation of tissue homogenates

The whole flies were immobilized on dry ice and manually homogenized in cold sodium phosphate buffer (100 mM, pH 7.4), 1:10 placed on dry ice. The homogenates obtained were centrifuged at 10,000 × *g*, −4°C for 10 min in an ice-cold centrifuge. The supernatants were later decanted, kept at −4°C and used for the following analysis.

Triglyceride level determination

The level of triacylglycerol in the flies' whole body was quantified using the Sigma Triglyceride Randox Assay kit and processed according to the manufacturer's procedure. This reaction consisted of 10 μL tissue homogenate and 1,000 μL triglyceride test kit. Absorbance was taken at 546 nm, and the standard was used for the calculation and expressed in mM/L.

Insulin-like peptide level determination

The level of insulin-like peptides in the flies was quantified using the Accu bind ELISA microwells insulin test system and processed according to the manufacturer's procedure. This test reaction consisted of 50 μL tissue homogenate and 100 μL insulin enzyme reagent; it was gently mixed by swirling, covered, and incubated at room temperature for 2 h. The content was discarded and washed three times with 350 μL wash buffer, 100 μL of working substrate buffer was added, incubated at room temperature for 15 min, and 50 μL stop solution was added. Absorbance was taken at 450 nm, and the calculation was done using standard calibration curve and expressed in nLU/mL.

Total protein estimation

The Coomassie blue method, which uses bovine serum albumin as standard, was used to determine the total protein content of the flies. The assay reaction consisted of 20 μL sodium phosphate buffer (0.1 M, pH 7.4), 20 μL flies tissue homogenate, and 200 μL Coomassie blue. This was incubated at 25°C for 30 min, and absorbance was measured at 595 nm in a SpectraMax plate reader (Molecular Devices, CA, USA). The protein concentration was calculated from standard calibration curve versus absorbance graph (Bradford, 1976).

Reactive oxygen species (ROS) level determination

ROS level was determined by estimating the amount of hydrogen peroxide (H_2O_2, an important indicator of oxidative stress) produced. The assay reaction was made up of 10 μL of flies' tissue homogenate and 183 μL of *N*-*N*-diethyl-para-phenylenediamine (DEPPD). The absorbance was taken at 505 nm using a SpectraMax plate reader (Molecular Devices, CA, USA); ROS level was calculated from the H_2O_2

standard calibration curve and expressed as μM H_2O_2 produced/mg protein (Hayashi et al., 2007).

TBARS determination

The reaction mixture contained 50 μL flies' tissue homogenate, 300 μL 8.1% SDS, 500 μL acetic acid, and 500 μL 0.8% TBA. This was boiled at 100°C for 60 min and allowed to cool. They were later centrifuged at 8,000 × *g* for 10 min, and the absorbance of the supernatants was measured at 532 nm. The TBARS level was calculated and expressed as μM/mg protein (Puntel et al., 2005).

Total thiol content determination

The assay reaction contained 150 μL sodium phosphate buffer (0.1 M, pH 7.4), 40 μL tissue homogenate, and 10 μL DTNB (10 mM). This was incubated in dark for 30 min, and absorbance was taken at 405 nm in a SpectraMax plate reader (Molecular Devices, CA, USA). The total thiol level was subsequently calculated from the GSH standard calibration curve and expressed as μM GSH produced/mg protein (Ellman, 1959).

Glutathione S-transferase activity determination

The assay mixture consisted of 250 μL sodium phosphate buffer (100 mM, pH 7.4), 10 μL of 25 mM GSH, 30 μL of flies' tissue, and 10 μL of 25 mM CDNB. The mixture was measured at 340 nm for 30 min at 30-s intervals in a SpectraMax plate reader (Molecular Devices, CA, USA), and the enzyme activities were calculated and expressed as milliunits (mU)/mg protein (Abolaji et al., 2014; Habig & Jakoby, 1981).

Superoxide dismutase (SOD) activity determination

The reaction mixture consisted of 50 μL flies' tissue homogenate, 100 μL sodium carbonate buffer (100 mM, pH 10.2), and 50 μL adrenaline. The absorbance was measured at 480 nm for 5 min at 30-s intervals in a SpectraMax plate reader (Molecular Devices, CA, USA), and the enzyme activities were calculated and expressed as mU/mg protein using the adrenaline standard calibration curve (Jewett & Rocklin, 1993).

Acetylcholinesterase (AChE) activity determination

The reaction mixture consisted of 40 μL flies tissue homogenate, 20 μL of 25 mM DTNB, 40 μL phosphate buffer (100 mM, pH 7.4), and 20 μL of 8 mM acetylthiocholine as a substrate. The absorbance was measured at 412 nm for 30 min at 30-s intervals using a SpectraMax plate reader (Molecular Devices, CA, USA). AChE activity was calculated and expressed as μM of acethiocholine hydrolyzed/min/mg protein (Ellman et al., 1961).

2.5 | Data analysis

The results were expressed as mean ± standard deviation (SD). Statistical analysis was done using one-way analysis of variance (ANOVA) followed by the Tukey post hoc test. Differences between the groups were regarded significant at $P < 0.05$, and all the analyses were done using GraphPad Prism (V.5.0).

TABLE 1 Total phenol, total flavonoid, ABTS* scavenging ability, Fe^{3+} reducing property, and IC_{50} values for DPPH* and OH* scavenging abilities, inhibition of lipid peroxidation, α-amylase, α-glucosidase, and AChE activities of the aqueous extracts of mistletoe infested and noninfested almond leaves

Parameters	Infested almond	Noninfested almond
Total phenol (mg GAE/g)	28.89 ± 0.57^b	21.53 ± 0.91^a
Total flavonoid (mg QUE/g)	23.95 ± 0.17^b	12.27 ± 0.34^a
ABTS* scavenging ability (μM TEAC/g)	63.57 ± 0.57^b	49.95 ± 0.19^a
Ferric reducing antioxidant property (mg AAE/g)	12.88 ± 0.43^b	10.93 ± 0.59^a
IC_{50} values (mg/mL)		
DPPH	1.75 ± 0.05^b	1.82 ± 0.06^a
OH	0.67 ± 0.02^b	0.71 ± 0.02^a
Lipid peroxidation	0.06 ± 0.00^a	0.05 ± 0.00^a
α-Amylase	0.53 ± 0.01^b	0.77 ± 0.02^a
α-Glucosidase	0.45 ± 0.01^a	0.48 ± 0.01^a
AChE	0.19 ± 0.01^b	0.23 ± 0.01^a

Values represent mean ± standard deviation of triplicate readings. Superscripts with different alphabets along the same row are significantly ($P < 0.05$) different.

3 | RESULTS

The total phenol and total flavonoid contents of the aqueous extracts of IAL and NIAL are presented in Table 1. The result showed that IAL had significantly ($P < 0.05$) higher total phenol and total flavonoid contents. Figure 1A and 1B and Table 2 show the result obtained for the HPLC-DAD phenolic characterization of IAL and NIAL. Several phenolic compounds were present in these leaves, and the most abundant and flavonoids constituents were p-coumaric acid, ferulic acid, syringic acid, ellagic acid, naringenin, isorhamnetin, and myricetin, while the phenolic acids (protocatechuic, p-hydroxybenzoic, gallic, and chlorogenic acids) and flavonoids (catechin and apigenin) were largely increased due to mistletoe infestation; however, IAL had significant ($P < 0.05$) higher phenolics composition.

The $ABTS^{\bullet+}$ scavenging ability of the aqueous extracts of IAL and NIAL as presented in Table 1 indicated that IAL had significantly ($P < 0.05$) higher (63.57 μM TEAC/g) $ABTS^{\bullet+}$ scavenging ability. The result obtained for FRAP as depicted in Table 1 showed that IAL extract had significantly ($P < 0.05$) higher (12.88 mg AAE/g) ferric reducing property. The DPPH* scavenging ability of the IAL and NIAL aqueous extracts as shown in Figure 2A and Table 1 revealed that the extracts reduced DPPH* in a concentration-dependent pattern (0%–72.67%) with the IAL aqueous extract (IC_{50} = 1.75 mg/mL) having significantly higher ($P < 0.05$) DPPH* scavenging ability. The inhibition of hydroxyl radical (OH*) by the aqueous extracts of IAL and NIAL was also carried out, and the result revealed that the extracts scavenged OH* in a concentration-dependent manner (0%–57.85%) with the IAL extract (IC_{50} = 0.67 mg/mL) having significant ($P < 0.05$) higher OH* inhibition

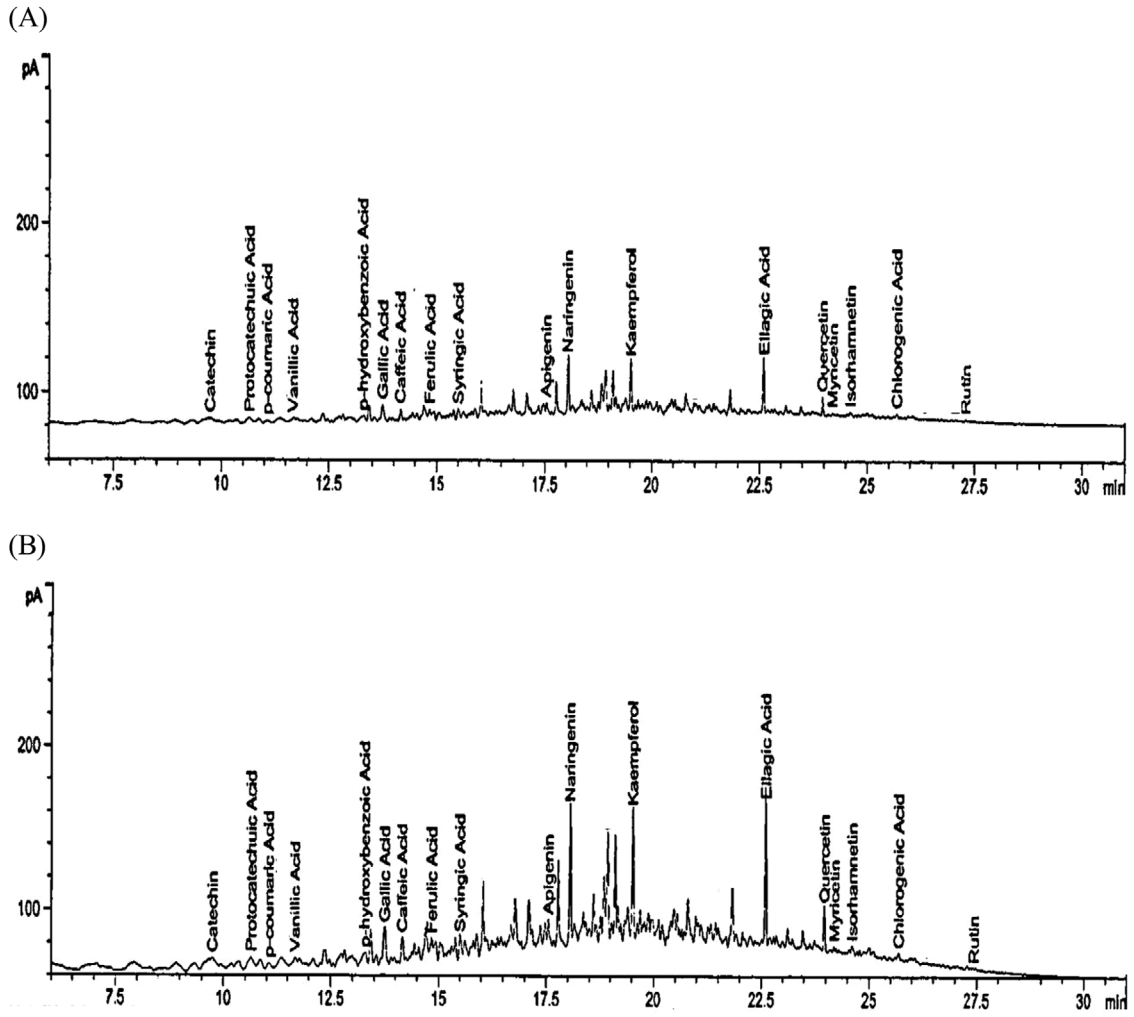


FIGURE 1 (A) Representative high-performance liquid chromatography profile of phenolic constituents of (A) infested almond leaves and (B) noninfested almond leaves.

(Figure 2B and Table 1). The result of lipid peroxidation as depicted in Figure 2C and Table 1 revealed that incubation of fruit fly homogenate in the presence of 250 μM Fe^{2+} caused significant ($P < 0.05$) increase in MDA content (137.68%); however, the IAL and NIAL extracts were able to reduce the MDA content in a concentration-dependent manner (0%–57.43%), but there was no significant ($P < 0.05$) difference in their IC_{50} values.

The inhibition of α-amylase activity by IAL and NIAL extracts as presented in Figure 2D and Table 1 showed that both extracts inhibited α-amylase activity in a concentration-dependent manner (0%–61.68%) with the IAL extract ($IC_{50} = 0.53$ mg/mL) having significant ($P < 0.05$) higher α-amylase inhibitory activity. Figure 2E and Table 1 show the α-glucosidase inhibitory activity of the leaves extracts, and the result revealed that the extracts inhibited the activity of α-glucosidase in a concentration-dependent manner (0%–72.46%); however, there was no significant ($P < 0.05$) difference in their IC_{50} values. In like manner, the extracts inhibited AChE (0-83.34%) activity in a concentration-dependent manner with IAL extract ($IC_{50} = 0.19$ mg/mL) having significant ($P < 0.05$) higher AChE inhibitory activity (Figure 2F and Table 1).

The survival of the emerged adult flies from the first instar larva (L1) as presented in Figure 3A revealed that SRD caused significant ($P < 0.05$) decrease in the flies survival rate when compared with the normal control. However, treatment of SRD-fed flies with IAL and NIAL improved their rate of survival with the IAL having significantly higher survival rate ($P < 0.05$) when compared with the sucrose control. The locomotor performance of the emerged flies as presented in Figure 3B revealed that there was significant ($P < 0.05$) reduction in the climbing activity of the sucrose-fed flies when compared with the normal control flies. However, treatment with both IAL and NIAL did not cause significant ($P < 0.05$) improvement in climbing activity in the sucrose-fed flies. The weight of the emerged flies was measured in groups of male and female (Figure 3C and 3D), and this revealed that the SRD caused significant ($P < 0.05$) elevated flies weight when compared with the normal control. No significant ($P < 0.05$) reduction was obtained in the weight of both male and female flies upon treatment with IAL and NIAL when compared with sucrose control flies.

The glucose level of emerged flies from larva fed with sucrose, infested and noninfested almond leaves as presented in Figure 4A

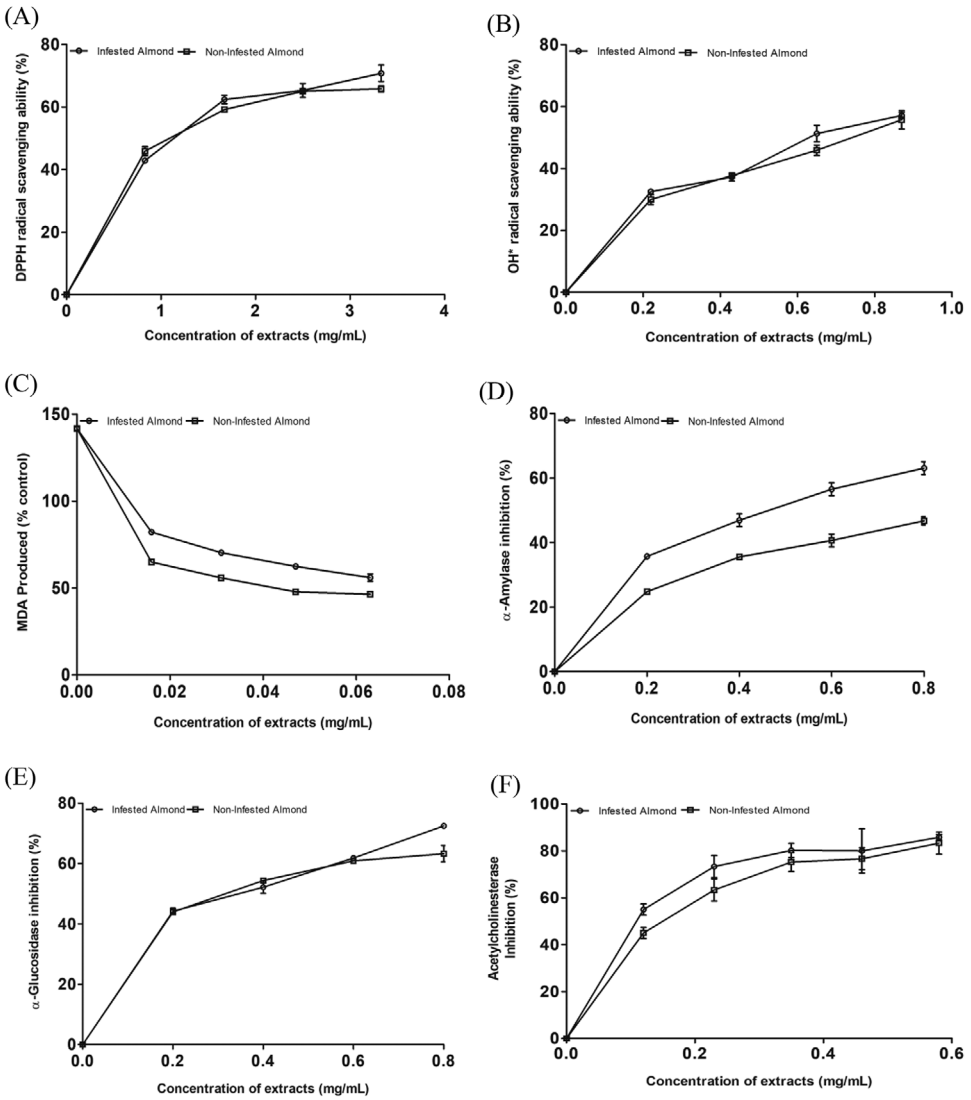


FIGURE 2 Effects of the aqueous extracts of mistletoe infested and noninfested almond leaves on (A) DPPH and (B) OH radicals scavenging activities. (C) Reduction of MDA produced from Fe^{2+} induced lipid peroxidation, (D) α-amylase, (E) α-glucosidase, and (F) acetycholinesterase inhibition.

revealed that treatment of normal flies with IAL produced significant ($P < 0.05$) reduction in the level of glucose, and all the sucrose-fed flies had significantly elevated($P < 0.05$) levels of glucose when compared with the normal control. However, treatment with IAL and NIAL caused significant ($P < 0.05$) reduction in the level of glucose when compared with the sucrose control with NIAL producing significant ($P < 0.05$) glucose-lowering effect when compared with IAL. The trehalose level of the emerged flies as shown in Figure 4B revealed that treatment of normal flies with IAL and NIAL did not produce significant ($P < 0.05$) trehalose reduction. Trehalose level was significantly ($P < 0.05$) increased in all the sucrose groups when compared with the normal control; however, treatment with NIAL produced significantly ($P < 0.05$) decrease trehalose level when compared with the sucrose control. The insulin-like peptides level of the emerged flies from larva fed with sucrose, infested and noninfested almond leaves is depicted in Figure 4C. The sucrose-fed groups had significantly ($P < 0.05$) higher insulin-like peptides level when compared with the normal control. There was no significant ($P < 0.05$) difference obtained in the treated groups of both normal and sucrose fed flies. The effects of sucrose, infested and noninfested almond leaves on triglyceride levels were depicted in Figure 4D. The treatment of normal group with IAL produced significantly ($P < 0.05$) lower triglyceride levels when compared with the normal control while the normal NIAL group had significantly ($P < 0.05$) higher triglyceride levels when compared with the IAL group. The sucrose control flies group had significantly ($P < 0.05$) higher triglyceride levels when compared with the normal control; however, treatment with IAL and NIAL did not produce significant ($P < 0.05$) triglycerides-lowering effects when compared with sucrose control.

The ROS level of the emerged flies from larva fed with sucrose, infested and noninfested almond leaves is shown in Figure 5A. This revealed that treatment of the normal group with IAL caused significant ($P < 0.05$) reduction in the flies ROS level when compared

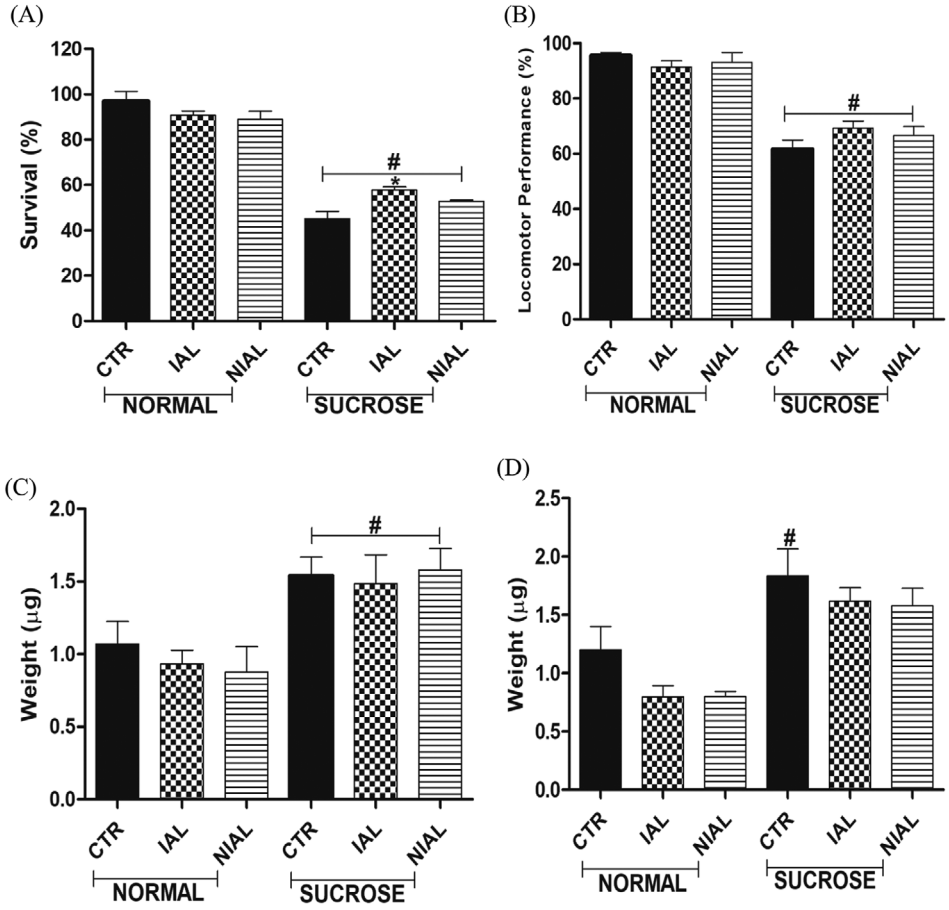


FIGURE 3 Effects of treatment on (A) survival, (B) locomotor, (C) weight of 3-day-old male and (D) female emerged flies from larva fed with sucrose, infested and noninfested almond leaves. Values represent mean ± SD ($n = 5$).
Key: CTR, control; NIAL, noninfested almond leaves; IAL, infested almond leaves; #, significant difference at $P < 0.05$ from normal control; *, significant difference at $P < 0.05$ from sucrose control.

with the normal control while the normal NIAL group had significantly ($P < 0.05$) higher ROS level when compared with the IAL group. SRD caused significantly ($P < 0.05$) elevated levels of ROS in the sucrose control group when compared with the normal control while treatment with IAL and NIAL produced significant ($P < 0.05$) reduction in the flies ROS level when compared with the sucrose control. The result obtained for the TBARS level as depicted in Figure 5B revealed that there was a significant ($P < 0.05$) reduction in the TBARS level of the IAL-treated groups in both normal and SRD-fed flies when compared with the normal and sucrose controls. The total thiol level of the emerged flies from larva fed with sucrose, infested and noninfested almond leaves as presented in Figure 5C revealed that treatment of the normal group with both IAL and NIAL produced significant ($P < 0.05$) elevated thiol levels when compared with the normal control. SRD brought about a significant ($P < 0.05$) reduction in the thiol content of the sucrose control flies. However, treatment with IAL caused significantly ($P < 0.05$) elevated thiol level when compared with the normal and sucrose controls while treatment with NIAL in both groups resulted in significant ($P < 0.05$) reduced thiol levels when compared with IAL group.

The glutathione-S-transferase (GST) activity of emerged flies from larva fed with sucrose and the almond leaves as depicted in Figure 5D revealed that treatment of flies with IAL and NIAL in the normal group caused significantly ($P < 0.05$) elevated GST activity while SRD produced significantly ($P < 0.05$) reduced GST activity when compared with normal control. However, treatment of the sucrose group with IAL and NIAL did not cause significantly ($P < 0.05$) elevated GST activity when compared with both normal and sucrose controls. The SOD activity of the emerged flies as presented in Figure 5E revealed that treatment of the normal group with IAL and NIAL caused significantly ($P < 0.05$) elevated SOD activity when compared with normal control. However, SRD did not produce significantly ($P < 0.05$) reduced SOD activity; nevertheless, treatment with IAL caused significantly ($P < 0.05$) elevated SOD activity when compared with sucrose control while treatment with NIAL caused significant ($P < 0.05$) reduction in SOD activity when compared with IAL. The AChE activity of the emerged flies from larva as shown in Figure 5F revealed that treatment of the normal groups with IAL and NIAL did not cause significant ($P < 0.05$) decrease in AChE activity. However, SRD brought about significantly ($P < 0.05$) elevated AChE activity when compared with

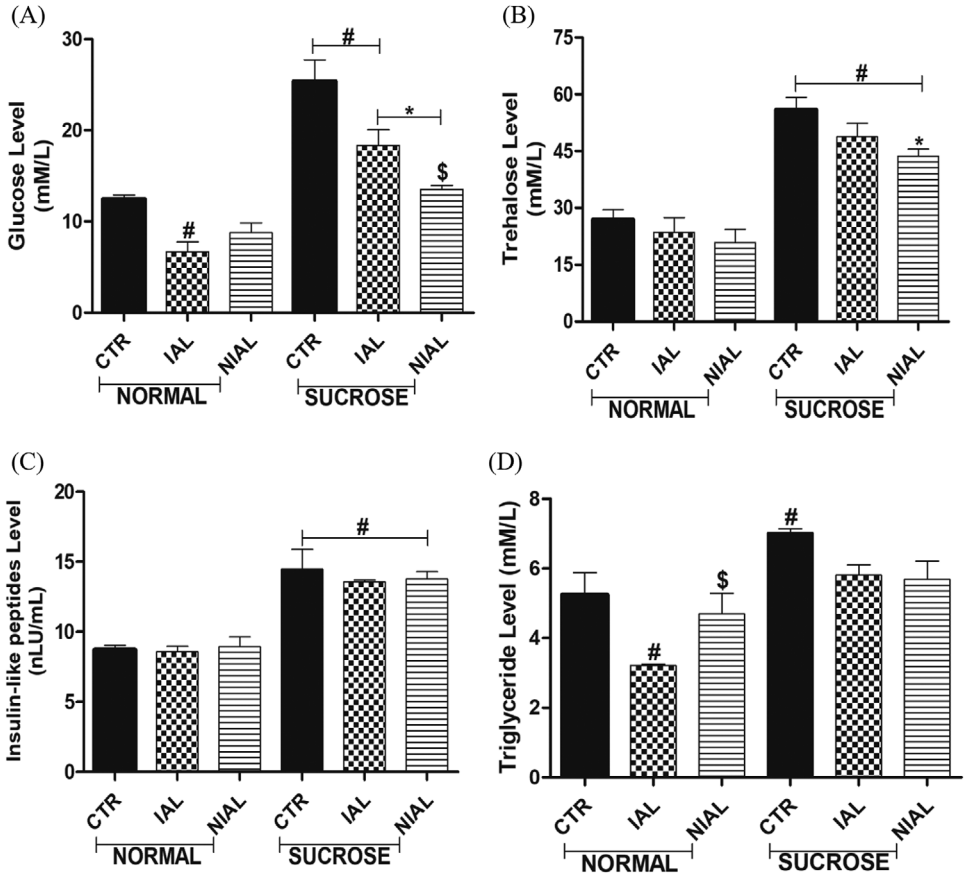


FIGURE 4 Effects of treatment on (A) glucose, (B) trehalose, (C) insulin-like peptides, and (D) triglycerides levels of emerged flies from larva fed with sucrose, infested and noninfested almond leaves. Values represent mean ± SD ($n = 5$).
Key: CTR, control; NIAL, noninfested almond leaves; IAL, infested almond leaves; #, significant difference at $P < 0.05$ from normal control; *, significant difference at $P < 0.05$ from sucrose control; $, significant difference at $P < 0.05$ from IAL in either normal or sucrose group.

the normal control while treatment with IAL and NIAL produced significantly ($P < 0.05$) reduced AChE activity when compared with sucrose control.

4 | DISCUSSION

African mistletoes grow on a large number of trees, most importantly, plants of medicinal importance such as almond tree. Almond leaves have found application in traditional medicine in the management of various metabolic diseases including diabetes (Subashinee & Siriwardhana, 2002). Previous studies had proposed that mistletoe infestation could possibly cause variation in the chemical constituents of host plants, thus altering their biological activities in comparison with their noninfested counterparts (Cuevas-Reyes et al., 2017; Edagbo et al., 2012). This is because the chemical constituents, particularly the phenolic constituents, determine to a large extent the biological activities of plants; the variation caused by mistletoe infestation would likely produce significant differences in the biological activities of infested plants (Obatomi et al., 1994; Oyeniran et al., 2020). In addition, several studies have shown that fruit fly can be used as a model organism for studying some human degenerative diseases such as type-2 diabetes and neurodegeneration (Broughton et al., 2005; Ecker et al., 2017). Hence, we investigated the influence of mistletoe infestation on phenolic constituents, *in vitro* antioxidative and α-amylase, α-glucosidase, and AChE inhibitory activities of almond leaves. In addition, we evaluated the survival, development, and behavioral parameters and antioxidant and hypoglycemic activities of infested and noninfested almond leaf in SRD-induced diabetic-like phenotypes in the *Drosophila melanogaster* model.

Phenolic acids and flavonoids are the most active constituents in green plants with greater ability to promote well-being and fight oxidative stress-related diseases (Ademiluyi et al., 2018). Mistletoe infestation caused significant variation in the total phenol, total flavonoid, and phenolic acids and flavonoids composition of almond leaves. The higher polyphenols composition obtained in IAL could be attributed to the positive influence of mistletoe infestation on phenolic constituents of almond host plants. This suggests that higher amounts of polyphenols were being produced as a defense mechanism in the host plant. Some of the several identified phenolic compounds, most importantly chlorogenic acid, p-coumaric acid, ellagic acid, ferulic acid-protocatechuic acid, syringic acid, quercetin, myricetin, kaempferol,

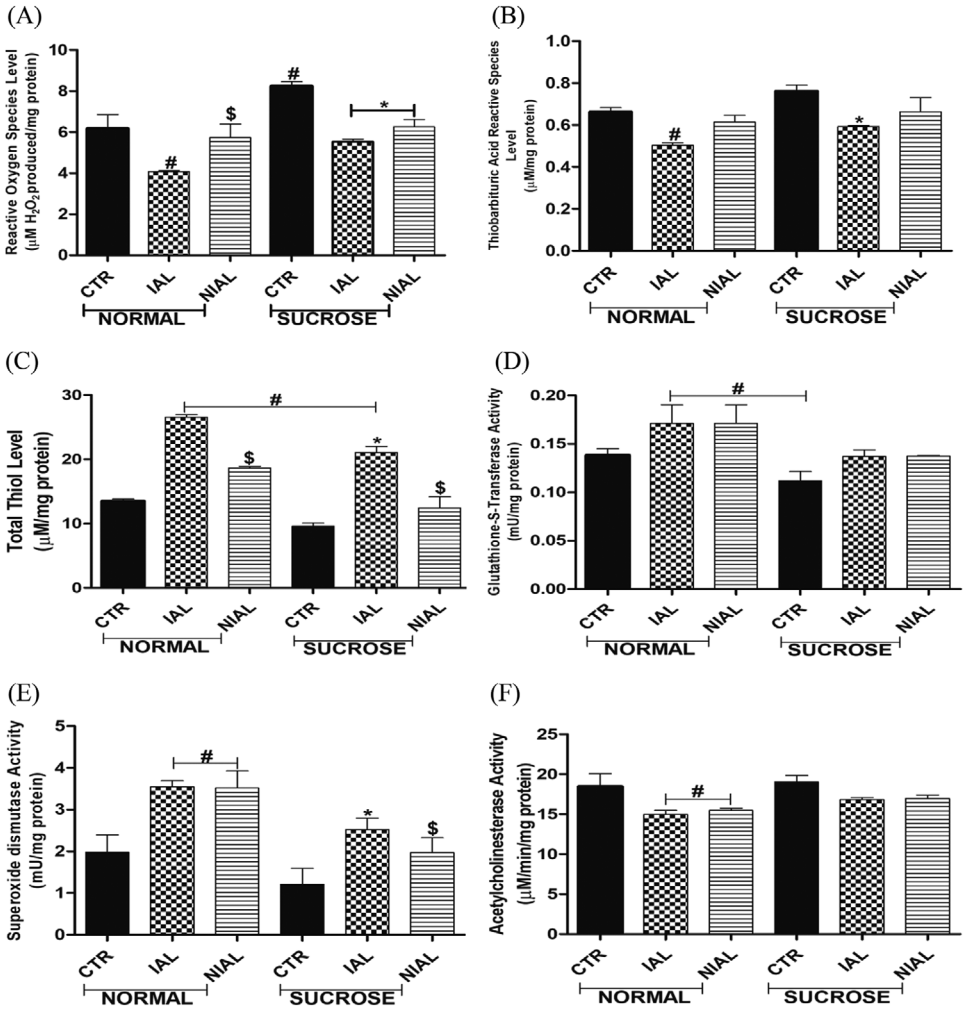


FIGURE 5 Effects of treatment on (A) reactive oxygen species, (B) thiobarbituric acid reactive species, and (C) total thiol levels. (D) Glutathione-S-transferase, (E) superoxide dismutase, and (F) AChE activities of emerged flies from larva fed with sucrose, infested and noninfested almond leaves. Values represent mean ± SD ($n = 5$).
Key: CTR, control; NIAL, noninfested almond leaves; IAL, infested almond leaves; #, significant difference at $P < 0.05$ from normal control; *, significant difference at $P < 0.05$ from sucrose control; $, significant difference at $P < 0.05$ from IAL in either normal or sucrose group.

and catechin, are acceptable compounds being employed in the mitigation of degenerative diseases due to their ability to ameliorate oxidative stress, diabetes, inflammation, depression, and other neurological deficits (Dajas et al., 2003; Szwajgier et al., 2017). However, the phenolic acids (*p*-hydroxybenzoic, protocatechuic, gallic, and chlorogenic acids) and flavonoids (apigenin and catechin) that were increased due to mistletoe infestation had known antioxidative, antidiabetic, and neuroprotective properties. Specifically, quercetin is the most abundant dietary flavonoids in green leaves with cardioprotective, anticancer, antitumor, antiulcer, antiallergy, antiviral, anti-inflammatory activity, antidiabetic, gastroprotective, antihypertensive, neuroprotective, immunomodulatory, and anti-infective activities (Lakhanpal & Rai, 2007). The catechins have an important role in maintaining homeostasis (Matsui, 2015); in addition, their cardioprotective, neuroprotective, and antidiabetic effects have been reported (Hashemipour et al., 2017; Thielecke & Boschmann, 2009). Reports have shown that gallic acid (3, 4, 5-trihydroxybenzoic acid) is employed as potent antioxidative, antimalarial, antimicrobial, antitumor, and anti-inflammatory agents (Kawada et al., 2001; Ohno et al., 2001). *P*-hydroxybenzoic acid (4-hydroxybenzoic acid) has been reported to possess antioxidative, antimicrobial, antimutagenic, estrogenic, antisickling, hypoglycemic, and anti-inflammatory properties (Manuja et al., 2013). In addition, p-coumaric acid has been reported to exert antioxidant, antimutagenesis, antigenotoxicity, anti-inflammatory, and antimicrobial activities (Akdemir et al., 2017; Ferreira et al., 2019).

The *in vitro* antioxidant activities of the aqueous extracts of IAL and NIAL is indicated by their Fe^{3+} reducing properties and inhibition of ABTS, DPPH, and OH radicals and Fe^{2+} induced lipid peroxidation. The higher antioxidant activities observed in IAL might be attributed to the greater amounts of polyphenolic compounds in these leaves. This correlates with earlier reports of Oboh et al. (2014) and Oyeniran et al. (2020b), whereby antioxidant activities of plant foods were

TABLE 2 Phenolic constituents of mistletoe infested and noninfested almond leaves

Compounds	Amount (mg/100 g)		Retention time (min)
	Infested	Noninfested	
Catechin	1.94 ± 0.01^{b}	0.01 ± 0.00^{a}	9.74
Protocatechuic acid	17.59 ± 0.27^{b}	0.27 ± 0.01^{a}	10.65
P-coumaric acid	96.43 ± 0.64^{a}	133.58 ± 0.59^{b}	11.06
Vanillic acid	16.22 ± 0.15^{a}	18.49 ± 0.60^{a}	11.68
P-hydroxybenzoic acid	16.73 ± 0.26^{b}	0.01 ± 0.00^{a}	13.29
Gallic acid	1.88 ± 0.02^{b}	0.01 ± 0.00^{a}	13.75
Caffeic acid	6.89 ± 0.05^{a}	7.05 ± 0.11^{a}	14.16
Ferulic acid	128.41 ± 0.43^{a}	124.89 ± 0.39^{a}	14.84
Syringic acid	58.45 ± 0.26^{a}	105.92 ± 0.15^{b}	15.51
Apigenin	1.74 ± 0.00^{b}	0.09 ± 0.00^{a}	17.55
Naringenin	94.67 ± 0.41^{a}	123.36 ± 0.41^{b}	18.06
Kaempferol	49.00 ± 0.82^{a}	43.98 ± 0.15^{a}	19.52
Ellagic acid	147.92 ± 0.48^{a}	338.49 ± 0.20^{b}	22.60
Quercetin	96.39 ± 0.18^{b}	89.43 ± 0.20^{a}	23.97
Myricetin	79.51 ± 0.59^{b}	62.42 ± 0.11^{a}	24.20
Isorhamnetin	131.23 ± 0.15^{b}	117.21 ± 0.49^{a}	24.62
Chlorogenic acid	47.44 ± 0.58^{b}	13.50 ± 0.18^{a}	25.70
Rutin	41.68 ± 0.32^{a}	35.44 ± 0.51^{a}	27.80

Values represent mean ± standard deviation of triplicate readings. Superscripts with different alphabets along the same row are significantly ($P < 0.05$) different by the Turkey test.

found to be directly proportional to the amount of phenolic compounds present.

Fruit flies have been employed in diabetic-like–related research (Ecker et al., 2017; Oyeniran et al., 2020). Thus, we evaluated the effects of the aqueous extracts of IAL and NIAL on key enzymes linked to diabetes in fruit flies tissue homogenate *in vitro*. α-Amylase and α-glucosidase are key carbohydrate hydrolyzing enzymes, which have been shown to play critical roles in the metabolic regulation of glucose, and the inhibition of these enzymes serves as a major therapeutic target in the control of postprandial hyperglycemia (hyperglycemia negatively affects the cholinergic enzymes; Capiotti et al., 2014, Oyeniran et al., 2020). In addition, therefore, we evaluated the inhibitory effects of IAL and NIAL extracts on AChE activity *in vitro*. The inhibition of these enzymes by the leaves extracts corresponds with the result obtained for polyphenolic compounds and antioxidant properties in these studies. This could be attributed to the fact that polyphenol compounds in plants foods contribute greatly to the inhibition of these critical enzymes (Ademiluyi et al., 2018; Oboh et al., 2014).

Furthermore, reports from previous studies have shown that dietary inclusion of high amount of sucrose in fruit fly meal could induce diabetic-like phenotypes and, thus, the various human metabolism disorders observed in type-2 diabetes could be studied using the *Drosophila melanogaster* model (Adefegha et al., 2020; Ecker et al., 2017). In like manner, the dietary consumption of plants has been related with extension of lifespan and promotion of growth, development, and general health (Zaku et al., 2015). The observed reduction in the survival rate of all the SRD-fed flies implies toxicity that could have been possibly due to the diabetic-like disease condition. The observed improved survival rate in the SRD-treated flies, particularly the IAL group, could be attributed to their rich phenolic compounds. This report is in agreement with the report of Ikhwanuddin et al. (2014), whereby the treatment of water with almond leaves was reported to improve the survival rates of black tiger shrimp (*P. monodon*). Likewise, the SRD-fed flies had impaired locomotion activities; this further suggests an ongoing disease condition in the flies. However, upon treatment with the leaves, improved locomotor performance was obtained. This observation correlates with previous reports whereby plants were shown to improve movement impairments in rats and flies (Akinyemi et al., 2017; Chatchada et al., 2013). Moreover, the SRD-fed flies had significantly increased weight in both male and female groups of flies used in this study, and this implies obesity. The results obtained here are consistent with earlier studies whereby SRD was reported to cause obesity (Musselman et al., 2011). However, treatment with IAL and NIAL caused little weight reduction in the female flies groups. This trend corresponds with earlier reports whereby plants were shown to be able to ameliorate obesity, and this could possibly be attributed to the weight-reducing properties of the plant phenolic compounds (Souravh et al., 2014). In addition, the weight of flies obtained in this study was lower than the weight reported by Ecker et al. (2017); this could possibly be due to differences in the strain of flies used.

Furthermore, to establish that SRD (30%) caused metabolic disorder during flies development, the levels of glucose, trehalose, insulin-like peptides, and triacylglycerols were measured in the flies. We observed that SRD brought about significantly elevated ($P < 0.05$) levels of glucose, trehalose, insulin-like peptides, and triacylglycerides; these parameters are important biochemical markers of diabetes, which indicates glucose metabolic imbalances. Our report corresponds with previous studies where SRD was reported to stimulate the noticeable biochemical signals of type-2 diabetes in flies (Ecker et al., 2017; Pasco & Leopold, 2012). The increased levels of these biochemical signals were ameliorated by the dietary inclusion of IAL and NIAL in the flies. This agrees with the report of Ahmed et al. (2005), where almond leaves were found to have very good antidiabetic activities. However, the observed higher ameliorative effects exhibited by IAL could be attributed to its higher phenolic compound, as previous reports have suggested that phytochemicals in many medicinal and plant foods interact with critical biomarkers involved in carbohydrate metabolisms (Ademiluyi & Oboh, 2012; Ademiluyi et al., 2018).

It is believed that hyperglycemic conditions could trigger increased production of free radicals with consequent elevated oxidative events (Ademiluyi et al., 2018; Ahmed et al., 2005). Thus, it is imperative to determine the effects of SRD and the leaves on some oxidative stress biomarkers: enzymatic and nonenzymatic antioxidant compounds. Increased production of ROS and accelerated lipid peroxidation has been reported during diabetes (Ecker et al., 2017; Oyeniran, Ademiluyi et al., 2020). Likewise, we observed that SRD caused

elevated levels of ROS and TBARS. This correlates with previous reports where increased ROS and TBARS levels were obtained in living organisms fed with sugar-rich diets (Folmer et al., 2002). The reduction obtained in the treated flies might be due to the antioxidant activities of the phenolic constituents; however, the observed higher ROS and TBARS-reducing capabilities of IAL could also be attributed to its higher polyphenolic composition. Furthermore, previous studies had indicated that the thiol compounds form part of the major component of the total body nonenzymatic antioxidants and, thus, they are largely involved in the mopping up of increased free radicals generated during disease conditions (Prakash et al., 2009). The observed decreased total thiol content obtained in the SRD-fed flies might be because there are increased oxidative events in these flies, and this further suggests an ongoing disease condition that could have possibly arisen from the SRD. However, upon treatment with IAL and NIAL, the total thiol content increased to almost the same level with the nonsucrose-fed flies. In addition, the ability of IAL to bring about increased thiol content in comparison with its NIAL counterpart might be attributed to its higher polyphenols, as polyphenols are able to increase cellular antioxidant activities (superoxide dismutase (SOD) catalyzes the degradation of superoxide radical (O_2^-) to hydrogen peroxide (H_2O_2) and oxygen (O_2; Abolaji et al., 2014). Reduction in the activities of GST and SOD was obtained in the SRD-fed flies and the treated flies had improved antioxidant enzyme activities. The trend obtained in these results correlates with the reports of Chanda et al. (2013) and Ahmed et al. (2005; Oboh et al., 2014). Enzymatic antioxidants play vital roles in producing counteractive effects against generated free radicals. GST is involved in the neutralization of intracellular peroxides and electrophilic oxidants where almond leaves were reported to be good sources of natural antioxidants. Recent studies have shown that diabetic patients are at higher risk of developing neurodegenerative diseases when compared with individuals without diabetes due to its interaction with the central nervous system (CNS), which results in the induction of brain pathological conditions (Bauduceau et al., 2010). Thus, the effects of IAL and NIAL on critical enzyme (AChE) linked to neurodegeneration were investigated, and the observed elevated level of AChE activity in the sucrose control flies supports the fact that the CNS is poorly affected in hyperglycemic conditions. The IAL and NIAL were able to reduce the AChE activity; therefore, these leaves might be able to provide protection against brain pathological condition that could arise from diabetes. These neuroprotective effects have been related to plants bioactive polyphenolic compounds, particularly flavonoids (Dajas et al., 2003).

5 | CONCLUSION

A report from this study has shown that mistletoe infestation brought about increased polyphenol composition in almond leaves, and this culminated in improved antioxidative and hypoglycemic activities in IAL. We further established the fact that feeding *Drosophila melanogaster* with SRD leads to increased expression of diabetic-like phenotypes in fruit fly. However, almond leaves with or without mistletoe infestation could be taken as functional food in order to mitigate diabetes and its associated brain complications.

ETHICAL STATEMENT

Currently, there are no ethical restrictions in the use of fruit fly as a model for research. However, the use of the fruit fly as a model organism is in compliance with the 3Rs protocol of the European Centre for the Validation of Alternative Methods on 3Rs-Refinement, replacement and reduction.

CONFLICTS OF INTEREST

The authors report no conflict of interest.

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REFERENCES

Abolaji, A. O., Kamdem, J. P., Lugokenski, T. H., Farombi, E. O., Souza, D. O., daSilva, L. E. L., & Rocha, J. B. T. (2015). 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 Biology*, *5*, 328–339.

Abolaji, O. A., Kamdem, J. P., Lugokenski, T. H., Nascimento, T. K., Waczuk, E. P., Farombi, E. O., da Silva, L. É., & Teixeira Rocha, J. B. (2014). Involvement of oxidative stress in 4-vinylcyclohexene-induced toxicity in Drosophila melanogaster. *Free Radical Biology and Medicine*, *71*, 99–108.

Adefegha, S. A., Ogunsuyi, O. B., & Oboh, G. (2020). Purple onion in combination with garlic exerts better ameliorative effects on selected biomarkers in high-sucrose diet-fed fruit fly (Drosophila melanogaster). *Comparative Clinical Pathology*, *29*, 713–720. https://doi.org/10.1007/s00580-020-03117-9

Ademiluyi, A. O., Aladeselu, O. H., Oboh, G., & Boligon, A. A. (2018). Drying alters the phenolic constituents, antioxidant properties, α-Amylase and α-Glucosidase inhibitory properties of Moringa (Moringa oleifera) leaf. *Food Science and Nutrition*, *6*, 2123–2133.

Ademiluyi, A. O., & Oboh, G. (2012). Phenolic-rich extracts from selected tropical underutilized legumes inhibit α-amylase, α-glucosidase, and angiotensin I converting enzyme *in vitro*. *Journal of Basic Clinical Physiology and Pharmacology*, *23*(1), 17–25. https://doi.org/10.1515/jbcpp-2011-0005

Ahmed, S., Swamy, V., Dhanapal, P., & Chandrashekara, V. (2005). Antidiabetic activity of Terminalia catappa Linn. leaf extracts in alloxan-induced diabetic rats. *International Journal of Pharmacy and Technology*, *4*, 36–39.

Akdemir, F. E., Albayrak, M., Çalik, M., Bayir, Y., & Gulcin, I. (2017).The protective effects of p-coumaric acid on acute liver and kidney damages induced by cisplatin. *Biomedicines*, *5*, 18.

Akinyemi, A. J., Oboh, G., Ogunsuyi, O., Abolaji, A. O., & Udofia, A. (2017). Curcumin-supplemented diets improve antioxidant enzymes and alter acetylcholinesterase genes expression level in Drosophila melanogaster model. *Metabolic Brain Disorder*, *33*, 369–375. https://doi.org/10.1007/s11011-017-0100-7

Ankur, J., & Neelesh, M. (2017). Memory enhancing activity of hydroalcoholic extract of Terminalia catappa leaves. *Journal of Drug Delivery and Therapeutics*, *7* (7), 197–199.

Bauduceau, B., Doucet, J., Bordier, L., Garcia, C., Dupuy, O., & Mayaudon, H. (2010). Hypoglycaemia and dementia in diabetic patients. *Diabetes and Metabolism*, *36*(3), S106–S111.

Bradford, M. M. (1976). A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Analytical Biochemistry*, *72*, 248–254.

Broughton, S. J., Piper, M. D., Ikeya, T., Bass, T. M., Jacobson, J., Driege, Y., Martinez, P., Hafen, E., Withers, D. J., Leevers, S. J., & Partridge, L. (2005). Longer lifespan, altered metabolism, and stress resistance in Drosophila from ablation of cells making insulin-like ligands. *Proceedings of the National Academy of Science of the United States of America*, *102*(8), 3105–3110.

Capiotti, K. M., De Moraes, D. A., Menezes, F. P., Kist, L. W., Bogo, M. R., & Da Silva, R. S. (2014). Hyperglycemia induces memory impairment linked to increased acetylcholinesterase activity in zebrafish (Danio rerio). *Behavioral Brain Research*, *274*, 319–325. https://doi.org/10.1016/j.bbr.2014.08.033

Chanda, S., Rakholia, K., Dholakia, K., & Baravalia, Y. (2013). Antimicrobial, antioxidant, and synergistic properties of two nutraceutical plants: Terminalia catappa L. and Colocasia esculenta L. *Turkey Journal of Biology*, *37*, 81–91.

Chatchada, S., Jintanaporn, W., & Wipawee, T. (2013). Moringa oleifera Mitigates memory impairment and neurodegenration in animal model of Age-Related Dementia. *Journal of Oxidative Medicine and Cellular Longevity*, *6*, 95–96.

Cuevas-Reyes, P., Pérez-López, G., Maldonado-López, Y., & GonzálezRodríguez, A. (2017). Effects of herbivory and mistletoe infection by Psittacanthus calyculatus on nutritional quality and chemical defense of Quercus deserticola along Mexican forest fragments. *Plant Ecology*, *218*, 687–697. https://doi.org/10.1007/s11258-017-0721-2

Dajas, F., Rivera, F., Blasina, F., Arredondo, F., Echeverry, C., Lafon, L., Morquio, A., & Heinzen, H. (2003). Cell culture protection and in vivo neuroprotective capacity of flavonoids. *Neurotoxicity Research*, *5*, 425–432.

Ecker, A., Gonzaga, T. K. S., Seeger, R. L., dos Santos, M. M., Loreto, J. S., Boligon, A. A., Meinerz, D. F., Lugokenski, T. H., Rocha, J. B. T. D., & Barbosa, N. V. (2017). High-sucrose diet induces diabetic-like phenotypes and oxidative stress in Drosophila melanogaster: Protective role of Syzygium cumini and Bauhinia forficata. *Biomedicine and Pharmacotherapy*, *89*, 605–616.

Edagbo, D. E., Ajiboye, T. O., Borokini, T. I., Ighere, D. A., Alowonle, A. A., & Michael, C. (2012). The influence of African Mistletoe (Tapinanthus bangwensis) on the conservation status of Citrus sinensis in moor plantation area of Ibadan. *Nigeria. International Journal of Current Research*, *4* (12), 484–487.

Ellman, G. E., Courtney, K. D., & Anderson, V. (1961). A new calorimetric determination of acetylcholinesterase activity. *Biochemistry and Pharmacology*, *7*, 88–95.

Ellman, G. L. (1959). Tissue sulfhydryl groups. *Archive of Biochemistry and Biophysics*, *82*, 70–77.

Farombi, E. O., Abolaji, A. O., Farombi, T. H., Oropo, A. S., Owoje, O. A., & Awunah, M. T. (2018). Garcinia kola seeds bioflavonoid fraction (Kolaviron), increases longevity and attenuates rotenone induced toxicity in Drosophila melanogaster. *Pesticide Biochemistry and Physiology*, *145*, 39–45.

Ferreira, P. S., Victorelli, F. D., Fonseca-Santos, B., & Chorilli, M. (2019). A review of analytical methods for p-coumaric acid in plant-based products, beverages, and biological matrices. *Critical Reviews in Analytical Chemistry*, *49*, 21–31.

Folmer, V., Soares, J. C. M., & Rocha, J. B. T. (2002). Oxidative stress in mice is dependent on the free glucose content of the diet. *International Journal of Biochemistry and Cellular Biology*, *34*, 1279–1285. https://doi.org/10.1016/S1357-2725(02)00065-1

Fyhrquist, P., Mwasumbi, L., Haeggström, C. A., Vuorela, H., Hiltunen, R., & Vuorela, P. (2002). Ethnobotanical and antimicrobial investigation on some species of Terminalia and Combretum (Combretaceae) growing in Tanzania. *Journal of Ethnopharmacology*, *79*(2), 169–177. https://doi.org/10.1016/S0378-8741(01)00375-0

Gyamfi, M. A., Yonamine, M., & Aniya, Y. (1999). Free-radical scavenging action of medicinal herbs from Ghana Thonningia sanguinea on experimentally-induced liver injuries. *General Pharmacology*, *32*(6), 661–667.

Habig, W. H., & Jakoby, W. B. (1981). Assays for differentiation of glutathione-S-transferases. *Methods in Enzymology*, *77*, 398–405.

Halliwell, B., & Gutteridge, J. M. C. (1981). Formation of thiobarbituric acid-reactive substance from deoxyribose in the presence of iron salts. *FEBS Letter*, *128*, 347–352.

Hashemipour, M. A., Lotfi, S., Torabi, M., Sharifi, F., Ansari, M., Ghassemi, A., & Sheikhshoaie, S. (2017). Evaluation of the effects of three plant species (Myrtus Communis L., Camellia Sinensis L., Zataria Multiflora Boiss.) on the healing process of intraoral ulcers in rats. *Journal of Dentistry*, *18*(2), 127–135.

Hayashi, I., Morishita, Y., Imai, K., Nakamura, M., Nakachi, K., & Hayashi, T. (2007). High-throughput spectrophotometric assay of reactive oxygen species in serum. *Mutation Research*, *631*, 5561.

Ikhwanuddin, M., Moh, J. H. Z., Hidayah, M., Noor-Hidayati, A. B., Aina-Lyana, N. M. A., & Juneta, A. S. N. (2014). Effect of Indian almond, Terminalia catappa leaves water extract on the survival rate and growth performance of black tiger shrimp, Penaeus monodon post larvae. *AACL International Journal of Bioflux Society*, *7*(2), 65–73.

Jewett, S. L., & Rocklin, A. M. (1993). Variation in one unit of activity with oxidation rate of organic substrate in indirect superoxide dismutase assays. *Annual Review of Biochemistry*, *212*, 55–59.

Kawada, M., Ohno, Y., Ri, Y., Ikoma, T., Yuugetu, H., Asai, T., Watanabe, M., Yasuda, N., Akao, S., Takemura, G., Minatoguchi, S., Gotoh, K., Fujiwara, H., & Fukuda, K. (2001). Anti-tumor effect of gallic acid on LL-2 lung cancer cells transplanted in mice. *Anticancer Drugs*, *12*, 847–852.

Kelley, W. T. D., Coffey, D. L., & Mueller, T. C. (1994). Lipid chromatographic determination of phenolic acids in soil. *Journal of Association of Analytical Community International*, *77*, 805–809.

Lakhanpal, P., & Rai, D. K. (2007). Quercetin: A versatile flavonoid. *Internet Journal of Medical Update*, *2*, 22–37.

Lee, K. P., Simpson, S. J., & Clissold, F. J. (2008). Lifespan and reproduction in Drosophila: New insights from nutritional geometry. *Proceedings of the National Academy of Science USA*, *105*, 2498–2503.

Lenz, S., Karsten, P., Schulz, J. B., & Voigt, A. (2013). Drosophila as a screening tool to study human neurodegenerative diseases. *Journal of Neurochemistry*, *127*, 453–460.

Manuja, R., Sachdeva, S., Jain, A., & Chaudhary, J. (2013). A comprehensive review on biological activities of p-hydroxy benzoic acid and its derivatives. *International Journal of Pharmaceutical Sciences Review and Research*, *20*, 109–115

Matsui, T. (2015). Condensed catechins and their potential health benefits. *European Journal of Pharmacology*, *765*, 495–502.

Meda, A., Lamien, C. E., Romito, M., Millogo, J., & Nacoulma, O. G. (2005). Determination of the total phenol, flavonoid and proline contents in Burkina Faso honey, as well as their radical scavenging activity. *Food Chemistry*, *91*(3), 571–577. https://doi.org/10.1016/j.foodchem.2004.10.006

Milius, S. (2000). Botany under the mistletoe. *Science News*, *158*, 411.

Musselman, L. P., Fink, J. L., Narzinski, K., Ramachandran, P. V., Hathiramani, S. S., Cagan, R. L., & Baranski, T. J. (2011). A high-sugar diet produces obesity and insulin resistance in wild-type Drosophila. *Disease Models and Mechanisms*, *4*(6), 842–849. https://doi.org/10.1242/dmm.007948

Musselman, L. P., Fink, J. L., Ramachandran, P. V., Patterson, B. W., Okunade, A. L., Maier, E., Brent, M. R., Turk, J., & Baranski, T. J. (2013). Role of fat body lipogenesis in protection against the effects of caloric overload in Drosophila. *Journal of Biology and Chemistry*, *288*(12), 8028–8042. https://doi.org/10.1074/jbc.M112.371047

Nichols, C. D. (2006). Drosophila melanogaster neurobiology, neuropharmacology, and how the fly can inform central nervous system drug discovery. *Pharmacology & Therapeutics*, *112*, 677–700. https://doi.org/10.1016/j.pharmthera.2006.05.012

Obatomi, D. K., Bikomo, E. O., & Temple, V. J. (1994). Anti-diabetic properties of the African mistletoe in streptozotocin-induced diabetic rats. *Journal of Ethnopharmacology*, *43* (1), 13–17.

Oboh, G., Ademosun, A. O., Ademiluyi, A. O., Omojokun, O. S., Nwanna, E. E., & Longe, K. O. (2014). In vitro studies on the antioxidant property and inhibition of α-amylase, α-glucosidase and angiotensin I-converting enzyme by polyphenol-rich extracts from cocoa (Theobroma cacao) bean. *Pathology Research International*, *2014*, 1–6. http://doi.org/10.1155/2014/549287

Ohkawa, H., Ohishi, N., & Yagi, K. (1979). Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. *Analytical Biochemistry*, *95*(2), 351–358.

Ohno, T., Inoue, M., & Ogihara, Y. (2001). Cytotoxic activity of gallic acid against liver metastasis of mastocytoma cells P-815. *Anticancer Research*, *21*, 3875–3880.

Oyaizu, M. (1986). Studies on products of browning reaction: Antioxidative activity of products of browning reaction prepared from glucosamine. *Japanese Journal of Nutrition*, *44*, 307–315.

Oyeniran, O. H., Ademiluyi, A. O., & Oboh, G. (2020a). Modulatory effects of moringa (Moringa oleifera L.) leaves infested with African mistletoe (Tapinanthus bangwensis L.) on the antioxidant, antidiabetic, and neurochemical indices in high sucrose diet-induced diabetic-like phenotype in fruit flies (Drosophila melanogaster M.). *Journal of Food Biochemistry*, e13318. https://doi.org/10.1111/jfbc.13318

Oyeniran, O. H., Ademiluyi, A. O., & Oboh, G. (2020b). Comparative study of the phenolic profile, antioxidant properties and inhibitory effects of Moringa (Moringa oleifera L.) and Almond (Terminalia catappa L.) leaves on acetylcholinesterase and monoamine oxidase activities in the head region of Fruit fly (Drosophila melanogaster Meigen) *in vitro*. *Journal of Food Biochemistry*, e13401. https://doi.org/10.1111/jfbc.13401

Pasco, M. Y., & Leopold, P. (2012). High sugar-induced insulin resistance in Drosophila relies on the lipocalin neural lazarillo. *PloS One*, *7*, e36583.

Prakash, M., Shetty, M. S., Tilak, P., & Anwar, N. (2009). Total thiols: Biomedical importance and their alteration in various disorders. *Online Journal of Health Allied Science*, *8*(2), 2.

Provan, G. L., Scobbie, L., & Chesson, A. (1994). Determination of phenolic acids in plant cell walls by microwave digestion. *Journal of Science and Food Agriculture*, *64*, 63–65.

Puntel, R. L., Nogueira, C. W., & Rocha, J. B. T. (2005). Krebs cycle intermediates modulate thiobarbituric reactive species (TBARS) production in rat brain *in vitro*. *Neurochemical Research*, *30*, 225–235.

Re, R., Pellegrini, N., Proteggente, A., Pannala, A., Yang, M., & Rice-Evans, C. (1999). Antioxidant activity applying an improved ABTS radical cation decolorisation assay. *Free Radical Biology and Medicine*, *26*, 1231–1237.

Rulifson, E. J., Kim, S. K., & Nusse, R. (2002). Ablation of insulin-producing neurons in flies: Growth and diabetic phenotypes. *Science*, *296*(5570), 1118–1120.

Singleton, V. L., Orthofer, R., & Lamuela-Raventos, R. M. (1999). Analysis of total phenols and other oxidation substrates and antioxidants by means of folin-ciocalteu reagent. *Methods in Enzymology*, *299*, 152–178. https://doi.org/10.1016/S0076-6879(99)99017-1

Souravh, B., Guru, S. S., & Ramaica, S. (2014). Antiobesity and hypolipidemic activity of Moringa oleifera leaves against high-fat diet induced obesity. *Advances in Biology*, *2014*, 1–9. https://doi.org/10.1155/2014/162914

Subashinee, S. K. W., & Siriwardhana, S. F. (2002). Anti-radical activity of extracts of almond and its by-products. *Journal of America Oil and Chemical Society*, *79*, 903–908.

Szwajgier, D., Borowiec, K., & Pustelniak, K. (2017). The neuroprotective effects of phenolic acids: Molecular mechanism of action. *Nutrients*, *9*(477), 1–21. https://doi.org/10.3390/nu9050477

Thielecke, F., & Boschmann, M. (2009). The potential role of green tea catechins in the prevention of the metabolic syndrome: A review. *Phytochemistry*, *70*, 11–24.

Wolf, M. J., & Rockman, H. A. (2011). Drosophila, genetic screens, and cardiac function. *Circulation Research*, *109*(7), 794–806. https://doi.org/10.1161/CIRCRESAHA.111.244897

Zaku, S. G., Emmanuel, S., Tukur, A. A., & Kabir, A. (2015). Moringa oleifera: An underutilized tree in Nigeria with amazing versatility: A review. *African Journal of Food Science*, *9*(9), 456–461.

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