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Effects of hydroethanolic garlic extract on oxidative stress, lipolysis, and glycogenesis in high-fat diet-fed *Drosophila melanogaster*

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ABSTRACT

Introduction: Metabolic syndrome is multifaceted health condition associated with metabolism, which leads to the accumulation of oxidation products and impairment of the antioxidant system. Medicinal, edible plant alternatives are being sought to prevent the predisposing factors of metabolic syndrome, one of which is obesity. We studied the effects of hydroethanolic garlic extract (HGE) on oxidative stress, lipolysis, and glycogenesis in high-fat diet-fed *Drosophila melanogaster.*

Methods: The HGE was processed and antioxidant assays (in vitro and *in vivo*) coupled with metabolic biomarkers were assayed following standard procedures.

Results: HGE inhibited ABTS (2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid), hydroxyl, and hydrogen peroxide radicals in high-fat-fed *D. melanogaster.* The HGE also reduced the activity of superoxide dismutase and increased catalase activity significantly. The HGE promoted lipolysis and glycogenesis by reducing cholesterol, triglycerides, and total sugar concentrations while glycogen content was increased.

Conclusion: Our results revealed that HGE improved the antioxidant defense system and promoted lipolysis and glycogenesis in metabolic deranged *D. melanogaster.* This suggests the therapeutic usefulness of garlic in the management of metabolic diseases.

Implication for health policy/practice/research/medical education:
Hydroethanolic garlic extract improved the antioxidant defense system in metabolic deranged *Drosophila melanogaster* by scavenging of free radicals and modulation of antioxidant enzymes. The garlic extract also promoted lipolysis and glycogenesis, thereby ameliorating obesity. This indicates that garlic or its compo-nents might be used for obesity.

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Introduction

Metabolic syndrome is a cluster of conditions that co-occur and increase the risk of developing heart disease, stroke, and type 2 diabetes. This condition includes a variety of risk factors, such as increased blood pressure, high blood sugar, excess body fat around the waist, and abnormal cholesterol or triglyceride levels (1,2). The prevalence of metabolic syndrome is increasing globally, affecting approximately 20%-30% of adults worldwide (3). Chronic inflammatory conditions in the event of metabolic syndrome give rise to oxidative stress, which in turn links to associated diseases. The formation of oxidizing species is known to increase the incidences of metabolic syndrome, and this is considered a primary mechanism behind mitochondrial dysfunction, as well as the accumulation of oxidation products of proteins and lipids and impairment of antioxidant systems (4,5).

Oxidative stress is an imbalance between the oxidative

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and anti-oxidative systems of the cells and tissues, resulting in the overproduction of free radicals and reactive oxygen species (ROS) (6). Stress as a result of ROS has been reported to be linked to different diseases, such as cancer, diabetes mellitus, and other cardiometabolic diseases (7). Metabolic diseases that are mediated by oxidative stress can be promoted by nutritional stress, such as that caused by excessive high-fat and/or carbohydrate diets (8). Antioxidants help scavenge these free radicals/ROS before damaging vital molecules in the body.

One such plant with a high profile of antioxidants is *Allium sativum* (garlic), which is a plant with a bulb belonging to the family of the Amaryllidaceae. Garlic has a long history of traditional medicine in different cultures worldwide (9). The phytoconstituents in garlic are primarily grouped into polyphenols, fructan carbohydrates e.g., inulin and organo-sulphur compounds e.g., allicin and alliin. It possesses antimicrobial, antithrombotic, anticoagulant, antihypertensive, immunomodulation, antidiabetic, and antioxidant activities (10). These phytoconstituents regulate energy metabolism and homeostasis, with the metabolites of the fructan polysaccharides, allicin, and polyphenols eliciting these effects (11).

Majorly, the polyphenolic content of garlic is responsible for its medicinal properties. Some of the phenolic compounds present in garlic are phenolic acids (12) and anthocyanins (13). Polyphenols have been shown to be effective antioxidants in various biological systems, as they can scavenge reactive oxygen or nitrogen species such as hydroxyl, peroxyl, superoxide, hydrogen peroxide (H_2O_2), or singlet oxygen (14,15).

Drosophila melanogaster (Fruit fly) belongs to the family "Drosophilidae", and its life cycle lasts approximately 10 days at 25 °C. More than 75% of human disease-causing genes have their homologs in *D. melanogaster* (16). When fed with high-fat or high-sugar diets, *Drosophila* stores lipids in its fat body, which might reveal a phenotypic model for diabetes and obesity (17). Drosophila fed with high fat diet (HFD; 30% coconut oil-enriched food) for many days showed symptoms of obesity, such as increased levels of triglyceride, glucose, and insulin, a hallmark of insulin resistance (18). The HFD-induced elevation of triglyceride levels was accompanied by increased signaling via the target of rapamycin (TOR) pathway, as well as reduction in the expression of adipose triglyceride lipase and a corresponding rise in fatty acid synthase expression (18). The current study investigated how a hydroethanolic garlic extract (HGE) affects oxidative stress, lipolysis, and glycogenesis in HFD-fed *Drosophila melanogaster*.

Materials and Methods

Material

Hydrogen peroxide (Roche), 5, 5'-dithiobis (2-nitro-benzoic acid) (Ellman's reagent), sulfuric acid (Sigma-Aldrich), N-(1-naphthyl) ethylenediamine dihydrochloride (Sigma-Aldrich), sulfanilamide (Sigma-Aldrich), phosphoric acid (Sigma-Aldrich), Trichloroacetic acid (Sigma-Aldrich), thiobarbituric acid (Sigma-Aldrich), hydrochloric acid (BGI), Potassium chloride (Roche), tris base (Sigma-Aldrich), dinitrophenyl hydrazine (Sigma-Aldrich), quercetin (Sigma-Aldrich), trehalose (Swanson) used, including the commercially available Randox kits for lipid profile procured from a local supplier in Osun State, Nigeria, were of analytical grade

Plant materials and extract preparation

The garlic bulb (purchased at Oje-Olobi Market in Ede, Nigeria) was peeled, washed, chopped, air dried, and pulverized using a sterile procedure. 200 g of the pulverized sample was added to 50:50 (v/v) ethanol: distilled water and left for 72 hours extraction time, and filtered thereafter. The hydroethanolic solvent was considered for better extraction and the solubility of the plant bioactive compounds in water (polar) and ethanol (non-polar) (19). The HGE was concentrated from the filtrate using rotary evaporator and freeze drier to remove the aqueous portion of the extract, with percentage yield of 22.8%. The sample of garlic was identified and confirmed with voucher number 21/17735 at the Herbarium, Department of Botany, Obafemi Awolowo University, Ile-Ife. Nigeria.

In vitro assay

Total phenolic content

Folin-Ciocalteu reagent (20) was employed where the extracted plant sample (0.5 mL of 1 mg/mL concentration) was added to10% Folin-Ciocalteu reagent (2.5 mL) together with Na_2CO_3 (2% w/v) (2 mL) in triplicate. This was followed by incubation at 45 °C with shaking for 15 minutes. The optical density of resulting solution was measured at a wavelength of 765 nm with the use of spectrophotometer. The amount of Gallic acid present in the solution was expressed as micrograms per milliliter following the varied gallic acid concentrations (0–0.2 mg/mL).

Total flavonoid content

This was measured as reported by AsokKumar et al (21). To determine the flavonoid content, the colorimetric method involving aluminum chloride was utilized, dissolving the extracted plant sample (1 mL of 1 mg/mL concentration) in methanol (3 mL), 10% aluminum chloride (0.2 mL), 1 M potassium acetate (0.2 mL), and distilled water (5.6 mL). The reaction mixture was left at 25 °C for 30 minutes. The resulting solution's optical density was measured at a wavelength of 420 nm with the use of a spectrophotometer. The content of flavonoid content was calculated by extrapolating the calibration curve obtained by a varied quercetin concentration (0–0.2 mg/mL). The flavonoid content was adjusted as µg/mL QE equivalent.

Percentage inhibition of hydroxy radical

This was determined according to the procedure described by Ozyürek et al (22). The prepared sample solution (1-4 mg/mL) was achieved by dissolving the plant sample in absolute ethanol. Then, an aliquot of the sample solution was mixed with 400 µL of phosphate buffer (0.2 M, pH 7.4). This was followed by the addition of 50 µL each of deoxyribose (50 mM), Na_2EDTA (1 mM), $FeCl_3$ (3.2 mM), and H_2O_2 (50 mM). To initiate the reaction, 50 µL volume of 1.8 mM vitamin A was mixed in buffer to adjust the total volume of the mixture to 800 µL. This was followed by 20 minutes incubation at 50 °C. 250 µL of 10% trichloroacetic acid was added to the mixture to stop the reaction. The resulting mixture passed through oven heating at 105 °C for 15 minutes which was followed by the addition of 150 µL of TBA (5%, in 1.25% NaOH aqueous solution) to develop color. After cooling, the absorbance of plant extract was measured at a wavelength of 530 nm against the buffer (as blank). The percentage hydroxyl (OH) radical scavenging of the plant extract was compared with quercetin, used as standard. The plant extract percentage scavenging value was calculated by:

$$Percentage\ OH\ scavenging\ (\%) = \frac{Abs_{control} - Abs_{sample}}{Abs_{control}} \times 100$$

where Abs stands for absorption.

Percentage inhibition of 2,2'-azino-bis-(3-ethylbenzothiazoline-6-sulfonic) acid (ABTS) radical

Re et al (23) procedure was followed to perform the experiment. Two stock solutions of 7 mM ABTS and 2.4 mM potassium persulfate in equal amounts were mixed to prepare the working solution. The mixture was left to react for 12 hours at room temperature in the dark. The resulting solution was diluted by combining 1 mL of freshly prepared $ABTS^+$ solution, and 7 minutes after, the measurement of absorbance was performed at a wavelength of 734 nm. Butylated hydroxytoluene (BHT) was employed as control. The measured absorbance of the HGE and control was used to calculate the percentage of scavenging capacity of $ABTS^+$ of the plant extract, using equation below. Quercetin, a known antioxidant, was used as standard to compare ABTS scavenging activity of the plant extract.

$$Percentage\ ABTS\ radical\ scavenging\ (\%) = \frac{Abs_{control} - Abs_{sample}}{Abs_{control}} \times 100$$

where Abs stands for absorption.

Percentage inhibition of hydrogen peroxide radical

The procedure described by Ruch et al (24) and Oktay et al (25) with little adjustment was followed to carry out this experiment. The plant extract at different concentrations was added to H_2O_2 solution (0.6 mL of 4 mM) and phosphate buffer (0.1 M, pH 7.4) followed by 10 minutes incubation. The resulting mixture's absorbance was determined at 230 nm wavelength alongside plant extract without H_2O_2 (as blank). Ascorbic acid was employed as control. The absorbance of HGE and control was used to calculate the percentage inhibition of H_2O_2 of the plant extract, using equation below. Percentage scavenging of H_2O_2 for both HGE and quercetin (standard) was calculated.

$$Percentage\ H_2O_2\ radical\ scavenging\ (\%) = \frac{Abs_{control} - Abs_{sample}}{Abs_{control}} \times 100$$

where Abs stands for absorption.

In vivo assay

Drosophila melanogaster (Harwich strain) new breeds were collected and acclimatized for 2 days in an incubator 20-23 °C following standard procedures (26). The flies were fed with standard corn diet *ad libitum*.

Treatment grouping

Doses of coconut oil and plant extract were selected following preliminary investigations in our laboratory using some biophysical assays.

- Group A: Normal diet (ND) (normal control)
- Group B: ND + HFD (70% Coconut oil for 3 days)
- Group C: ND + HGE (1 mg/5 g diet for 3 days)
- Group D: ND + HFD (70% Coconut oil for 3 days) + 1 mg/5 g HGE (for the next 3 days)

A total of 250 flies were assigned per treatment group. Ten flies each were homogenized independently for each assay and conducted in triplicate (n=3).

Hydrogen peroxide stress

This was performed according to a previously described method (27). The flies were allowed to age for 3 days then moved into 5% sucrose-containing vials. They were kept on the sucrose diet for 24 hours before being exposed to H_2O_2. For pretreatment (to initiate adaptation), flies were placed for 8 hours in vials without H_2O_2 or in vials that contained 10µM or 100µM H_2O_2. After the 8-hour pretreatment period, the flies were moved to vials containing different concentrations of H_2O_2 (ranging from 0M to 8M). The flies were monitored for survival, and they were considered dead when they were completely immobile. The purpose of measuring survival was to evaluate their adaptation to the toxic doses of H_2O_2.

Superoxide dismutase (SOD) activity

As reported by Misra and Fridovich (28), the prepared 0.1 mL homogenized flies were made up to 1:10 dilution with distilled water. This was followed by adding 0.2 mL of homogenate initially diluted to carbonate buffer (2.5 mL of 0.05 M pH 10.2) in a cuvette, which was left to equilibrate. Reaction initiation was achieved by adding

adrenaline (0.3 mL of 0.3 M), which was followed by addition of carbonate buffer (2.5 mL), adrenaline (0.3 mL), and distilled water (0.2 mL) into same cuvette. The absorbance was determined at a 480 nm wavelength every 30 seconds for 150 seconds to monitor the increase in absorbance.

Catalase activity

According to Claiborne (29), with a little modification, 50 µL homogenate of the flies was added to 500 µL of 59mM H_2O_2 and 950 µL of 50mM phosphate buffer (pH 7.0) to develop a reaction mixture at 25 °C. The absorbance was determined at 570 nm wavelength at 0, 60 and 120 seconds. Enzyme activity was measured in units, which expresses enzyme amount needed to catalyze decomposition of 1 µmol of H_2O_2/minute at 25 °C and pH of 7.0 under the specified conditions.

Glycogen and total sugar concentrations

According to Van Handel's (30) method, 10 *D. melanogaster* were homogenized in 2% sodium sulfate solution. The homogenate was then mixed with 1 mL of methanol and centrifuged at 90 G for 1 minute. The supernatant was separated by decanting into another test tube and followed by air drying to reduce it to a volume of 100-200 µL. The separated residue contained the glycogen and fly tissue as precipitate. The two samples were made into mixture with anthrone reagent to give a final 5 mL reaction volume each. After thoroughly mixing, the reaction mixtures were incubated at 90 °C for 17 minutes. The absorbances were measured at a dual optical densities of 625 and 555 nm, followed by extrapolating the concentrations of glycogen and total sugar as triplicate using glucose standard curve.

Total carbohydrate concentration

Van Handel's (30) methods were utilized to determine carbohydrate concentrations. Initially, 25% ethanol was used to prepared various concentrations of glucose (25 to 200 µg/mL). This was used to create calibration curve for glucose. The reaction volume was made up of H_2O, conc H_2SO_4, dissolved anthrone reagent, and heated in water bath at 90 °C for 17 minutes before cooling to room temperature. The absorbance of each standard was measured at a dual wavelength of 625 and 555 nm using a microplate, and subsequently the concentrations were used to plot the glucose standard curve.

Carbohydrate concentration was measured by homogenizing 10 *D. melanogaster* in 1 mL of anthrone reagent as described by Van Handel (30). Thereafter, anthrone reagent was added to make up 5 mL total reaction volume. This was followed by heating the reaction mixture in regulated water-bath at 90 °C for 17 minutes before cooling, and optical density was checked at dual wavelengths of 625 and 555 nm using a microplate reader. Carbohydrate concentrations for each of the triplicate were determined by extrapolating measured absorbance using the standard curve of glucose.

Trehalose concentrations

To determine trehalose concentrations, the method described by Van Handel (30) was employed. Briefly, 2% sodium sulfate was used to homogenize ten flies, which was followed by centrifugation at 90 G for 1 minute to collect the supernatant and resuspend the pellet in 300 µL H_2O and 1 mL methanol. The resuspended pellet was centrifuged for 1 minute. The two supernatants were pulled together, and the sugar fraction was concentrated to 500 µL. The sugar fraction (100 µL) was mixed with HCl (50 µL of 1 N) and heated at 90 °C in water bath for 7 minutes; after cooling NaOH (150 µL) was added to the reaction mixture and again heated at 90 °C in water bath for another 7 minutes. Anthrone reagent was added to make a 5 mL mixture volume and heated at 90 °C in water bath for 17 minutes. The measurement of absorbance was performed at dual wavelengths of 555 and 625 nm in triplicate using a microplate reader. The concentration of trehalose was calculated using the standard curve of trehalose.

Triglyceride concentration

The principle was based on the hydrolysis with lipase coupled with reaction, which produced quinone-imine (an indicator) formed from hydrogen-peroxide, 4-aminophenazone, and 4-chlorophenol as catalyzed by peroxidase (31). Firstly, 10 µL distilled water and 1 mL reagent (reagent blank), 10 µL standard, 1000 µL reagent (standard solution), 10 µL sample, and 1 mL reagent (sample solution) were pipetted into the cuvette. They were stirred together, followed by incubation for 10 minutes at 25 °C or 5 minutes at 37 °C. The measurement of absorbance was performed at a wavelength of 500 nm relative to the blank.

$$\text{Triglyceride concentration} = \frac{\text{Abs}_{\text{sample}}}{\text{A}_{\text{standard}}} \times \text{concentration of standard (743 mg/dL)}$$

where Abs stands for absorption.

Cholesterol concentration

This was determined after enzymatic hydrolysis and oxidation. The indicator quinone-imine is formed from H_2O_2 and 4-aminoantipyrine in the presence of phenol and peroxidase. Firstly, 10 µL distilled water and 1000 µL reagent (reagent blank), 10 µL standard, 1000 µL reagent (standard solution), 10 µL sample, and 1000 µL reagent (sample solution) were pipetted into the cuvette. They were stirred together, followed by incubation for 10 minutes at 25 °C or 5 minutes at 37 °C. The measurement of absorbance was performed at a wavelength of 500 nm

relative to the blank.

$$\text{Total Cholesterol concentration} = \frac{\text{Abs}_{\text{sample}}}{\text{A}_{\text{standard}}} \times \text{concentration of standard (553 mg/dL)}$$

where Abs stands for absorption.

Statistical analysis

Data were presented as means ± SEM (n=3) and analyzed using GraphPad Prism 6.0 software and Microsoft Excel 2016. One-way ANOVA followed by Tukey's post hoc test was used to compare the groups. Significant differences were considered significant at $P < 0.05$.

Results

Total flavonoid and total phenolic contents of hydroethanolic garlic extract

HGE contained phenols (49.36 µg GAE/mg) and flavonoid (15.54 µg QE/mg) extrapolating from the gallic acid (GAE) and quercetin (QE) standard curve.

Hydroxyl radical inhibition of hydroethanolic garlic extract

HGE and quercetin inhibited hydroxyl radical in an increasing order. There was no significant difference in the quercetin and HGE percentage in hydroxyl radical inhibition. Quercetin and HGE at 500 µg/mL inhibited 100% and 93.24% of the hydroxyl radicals, respectively, while at 15 µg/mL, quercetin and HGE inhibited 7.64% and 11.26 % of the hydroxyl radical (Figure 1A).

ABTS inhibition of hydroethanolic garlic extract

The inhibitory power of HGE and quercetin against ABTS radical was revealed in an increasing order. There was no significant difference in the quercetin and HGE percentage in ABTS radical scavenging activity. Quercetin and HGE scavenged 93.5% and 95.34% ABTS radicals, respectively at 250 µg/mL. 31.62 and 38.98% of ABTS radicals were scavenged by quercetin and HGE, respectively at 15 µg/mL concentration (Figure 1B). The calculated ABTS IC_{50} for the quercetin and HGE were 26.15 µg/mL and 25.35 µg/mL, respectively.

Hydrogen peroxide radical inhibition of hydroethanolic garlic extract

Figure 1C shows the *in vitro* percentage H_2O_2 radical scavenging of HGE. The scavenging power of HGE and quercetin against H_2O_2 radical was revealed in an increasing order. There was no significant difference in the quercetin and the HGE percentage in H_2O_2 radical scavenging activity. Quercetin and HGE scavenged 97.37% and 87.36% H_2O_2 radicals, respectively at 500 µg/mL, while at 15 µg/mL concentration quercetin and HGE scavenged 4.59% and 5.04% of H_2O_2 radicals, respectively (Figure 1C). The calculated H_2O_2 IC_{50} for the quercetin and HGE were 65.49 µg/mL and 67.39 µg/mL, respectively.

Hydroethanolic garlic extract effects on hydrogen peroxide resistance

The flies fed with HFD had 20% survival after 96 hours of exposure to H_2O_2 stress. There was hundred percent (100%) improved survival rate in the HFD-fed flies that were treated with HGE after 96 hours exposure to H_2O_2 stress (Figure 2).

Hydroethanolic garlic extract effect on superoxide dismutase and catalase activities

HFD significantly ($P < 0.05$) increased SOD activity in *D. melanogaster* when matched with the control. There was a significantly reduced SOD activity in the HFD-fed flies treated with HGE when compared with the flies that received only HFD. HFD significantly increased

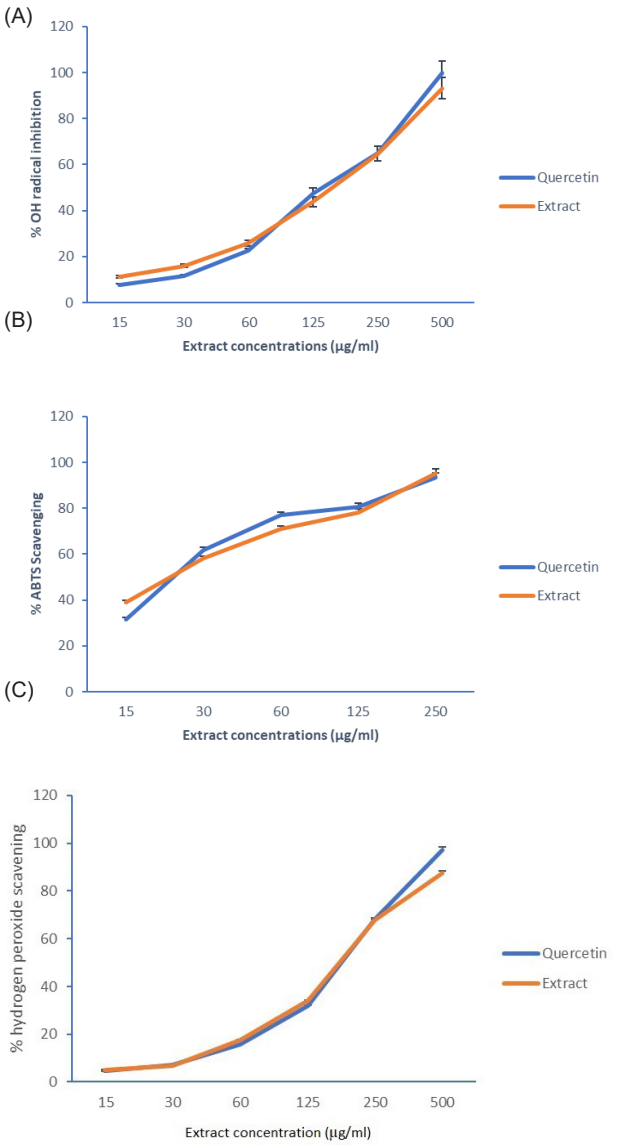

Figure 1. Percentage inhibition of hydroxyl (A), ABTS (2,2′-azino-bis-(3-ethylbenzothiazoline-6-sulfonic) acid (B), and hydrogen peroxide (C) radicals by hydroethanolic garlic extract (HGE).

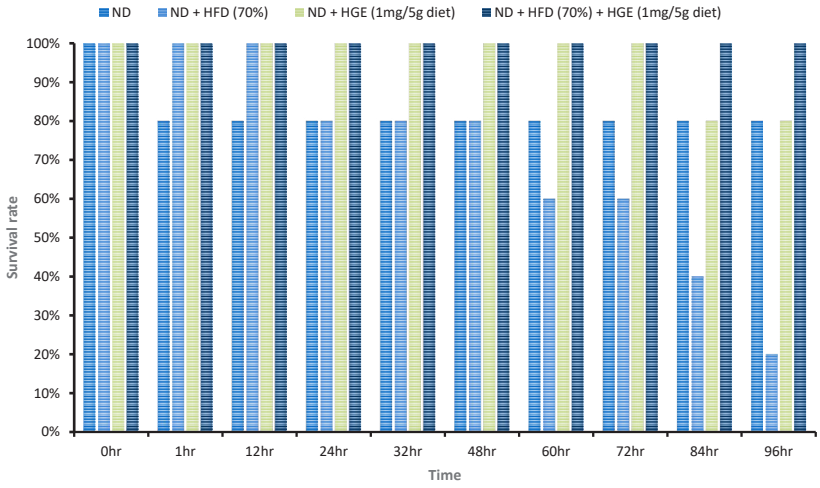

Figure 2. Hydrogen peroxide stress resistance in high fat diet (HFD)-fed *Drosophila melanogaster* supplemented with hydroethanolic garlic extract (HGE). ND: Normal diet.

catalase activity in *D. melanogaster*, when compared to the control group. Also, HFD-fed flies that received HGE had significantly increased catalase activity (Table 1).

Hydroethanolic garlic extract effects on carbohydrate metabolism

Drosophila melanogaster exposed to HFD significantly ($P < 0.05$) increased total sugar, glycogen, trehalose, and total carbohydrate concentrations (Table 2). Furthermore, HFD-fed flies that received HGE had significantly reduced total sugar and total carbohydrate concentrations. However, the glycogen and trehalose concentrations were increased in the HFD-fed flies that received HGE.

Table 1. Effect of hydroethanolic extract on the activities of superoxide dismutase and catalase

Groups	SOD (µmol/min/mg protein)	CAT (µmol/min/mg protein)
ND	13.33 ± 0.00	1.10 ± 0.22
ND + HFD	36.67 ± 1.67^{a}	9.70 ± 1.47^{a}
ND + HGE	16.67 ± 1.67^{b}	7.43 ± 0.69^{a,b}
ND + HFD + HGE	26.67 ± 0.00^{a,b,c}	11.42 ± 4.10^{a,c}

Data are represented as mean ± SEM, n=3, $^{a,b,\text{ and }c}$ $P < 0.05$ as compared with control diet (ND), ND + HGE, and ND + HFD + HGE groups.
SOD: Superoxide dismutase; CAT: Catalase; HFD: High fat diet; HGE: Hydroethanolic garlic extract; ND: Normal diet.

Hydroethanolic garlic extract effects on cholesterol and triglyceride metabolism

Drosophila melanogaster exposed to HFD significantly increased the concentration of total cholesterol when compared to the control group (Table 3). Supplementation of HFD-fed flies with HGE significantly decreased the concentration of total cholesterol when compared to the HFD and control groups. Triglyceride concentration in the HFD group was significantly increased as compared to the control group. Upon supplementation of HFD-fed flies with HGE, triglyceride concentration decreased significantly compared with the HFD-fed flies.

Discussion

High fat diet intake predominates westernized diet with increasing predisposition to a metabolic disorder like obesity with established complications, including movement disorder, diabetes and dyslipidemia (32,33). Complementing or total replacement of this HFD with medicinal food plants, rich in beneficial phytocompounds can help reduce the severity or prevent the incidence of the risk factors and complications of obesity. *Allium sativum* is an example of these medicinal food herbs with proven biological activities against dyslipidemia, hyperglycemia,

Table 2. Effect of hydroethanolic garlic extract on the concentrations of carbohydrate parameters

Groups	Total sugar (µg/mL)	Glycogen (µg/mL)	Trehalose (µg/mL)	Total carbohydrate (µg/mL)
ND	5.62 ± 0.32	11.40 ± 0.37	11.18 ± 0.68	17.02 ± 3.29
ND + HFD	9.76 ± 0.91^{a}	16.77 ± 1.25^{a}	14.11 ± 0.09^{a}	26.53 ± 4.86^{a}
ND + HGE	15.04 ± 0.10^{a,b}	15.45 ± 0.42^{a}	10.45 ± 0.12^{b}	30.49 ± 5.08^{a}
ND + HFD + HGE	5.99 ± 0.52^{b,c}	18.28 ± 0.12^{a,b,c}	17.17 ± 0.54^{a,b,c}	24.27 ± 5.38^{a,c}

Data are represented as mean ± SEM, n=3, $^{a,b,\text{ and }c}$ $P < 0.05$ as compared with control diet (ND), ND + HGE, and ND + HFD + HGE groups.
HFD: High fat diet; HGE: Hydroethanolic garlic extract; ND: Normal diet.

Table 3. Effect of hydroethanolic garlic extract on total cholesterol and triglyceride concentrations

Groups	Total cholesterol (mg/dL)	Triglyceride (mg/dL)
ND	16.31 ± 0.49	13.54 ± 0.52
ND + HFD	25.76 ± 8.58[a]	16.65 ± 0.92[a]
ND + HGE	12.95 ± 1.20[b]	8.42 ± 0.07[a,b]
ND + HFD + HGE	10.87 ± 0.33[a,b]	13.78 ± 0.67[b,c]

Data are represented as mean ± SEM, n=3, [a,b, and c] $P < 0.05$ as compared with control diet (ND), ND + HGE, and ND + HFD + HGE groups.
SOD: Superoxide dismutase; CAT: Catalase; HFD: High fat diet; HGE: Hydroethanolic garlic extract; ND: Normal diet.

oxidative stress, and the ability to reduce body mass index and waist circumference in clinical research (34-36). This study addresses how HGE impacts oxidative stress and lipolysis and glycogenesis in HFD-fed *D. melanogaster.*

Garlic phytoconstituents include nutritional and non-nutritional constituents. The constituents with medicinal potentials include the polyphenols, alkaloids, tannins, saponins, cardiac glycosides etc. Polyphenols are compounds with aromatic ring that bears one or more hydroxyl groups, of which flavonoids are a subclass of the polyphenols. In the present study, *A. sativum* total phenolics and total flavonoids were quantified in gallic acid and quercetin equivalents, respectively. Phenolics have been established to be of medicinal importance as they possess antioxidant, anticancer, anti-inflammatory, neuroprotective, and cardio-protective potentials (37). Some of the quantified polyphenols in *A. sativum* include gallic acid, catechin, protocatechuic acid, isovanillic acid, vitexin, kaempferol, rutin, isoorientin, orientin, quercetin, and catechin.

The redox imbalance can be consequential on various diseases, like metabolic syndrome, obesity, diabetes, cancer, etc (38,39). The potency of exogenous antioxidants is assessed biochemically by measuring the reducing power and percentage inhibition of oxidative stressors (i.e., free radicals). The percentage inhibition of radicals also shows the antioxidant activities of medicinal plants. In this study, the percentage inhibition of ABTS, hydroxyl, and H_2O_2 radicals showed that HGE inhibited these radicals in a dose-dependent manner. The 250 µg/mL concentration had the highest percentage inhibition, closely related to the percentage inhibition of the quercetin standard that was employed. The IC_{50} of the extract (25.35 µg/mL) was close to the standard, quercetin (26.15 µg/mL) IC_{50}. This shows that HGE is a potent antioxidant *in vitro*. This supports Jang et al (40) report that garlic extract had greater scavenging activities for ABTS (40,41). HGE also scavenged H_2O_2, which was similar to the study reported by Ide et al (42) who evaluated the H_2O_2 scavenging activity of s-allyl cysteine and other polyphenols. The high H_2O_2 and hydroxyl radical scavenging activities offer protection against oxidative stress.

Antioxidant enzymes can protect the biological system from radical generating cellular activities. Antioxidant enzymes like catalase and superoxide dismutase defend cellular component against oxidative damage during metabolic conditions. In this study, HFD-fed flies significantly increased the SOD activity of the *D. melanogaster*, and treatment with garlic extract significantly decreased the SOD activity. This showed that exposure to HFD triggered a state of oxidative stress which was ameliorated by the treatment of the HGE. Kayode et al (43) reported a significant increase in SOD activity in sucrose-induced insulin resistant drosophila exposed to garlic. This agrees with the results of present study, where garlic extract reduced the increasing activity of SOD when compared with the control, and in contrast with a study by Sohal et al (44) who reported a decrease in SOD activity in ageing drosophila, stating that antioxidant defenses are selective and are quite variable in different species and tissues. The reduction of H_2O_2 is catalyzed by the enzyme catalase, which then nullify the damaging effects of H_2O_2 radicals. In this study, exposure of *D. melanogaster* to HFD-fed flies significantly increased the catalase activity, which is a reflective of an overburden of ROS. This supports Kayode et al (43,45), who reported a significant decrease in catalase activity in drosophila exposed to a high sucrose diet. However, garlic extract supplementation of the HFD-fed flies modulated catalase activity, which could result from an overburden of the flies' peroxide stress. Catalase enzyme metabolizes H_2O_2 into water and oxygen. Albeit increased catalase activity is directly linked to an elevated H_2O_2 production. Hydrogen peroxide radicals cause oxidative damage and stress the antioxidant-defense system. In this study, HFD-fed flies had the least percentage survival in post-exposure to H_2O_2. This was ameliorated post-treatment with HGE, which showed 100% survival and resistance to H_2O_2 stress, which agrees with Niedzwiecki et al (46), who reported an increase in prooxidant burden with an increase in H_2O_2 concentration and mortality in *D. melanogaster*.

The metabolic paths of macromolecules like carbohydrates and lipids determine the bioaccumulation and degradation of their end-products and energy homeostasis. The imbalance between energy intake and expended results in energy accumulation, which can cause obesity or other metabolic derangements. Diet rich in carbohydrates and lipids has been linked to metabolic diseases, as they cause the accumulation of excessive energy from carbohydrate and lipid metabolism (47). These carbohydrates in *D. melanogaster* are of different classes (total sugar, glycogen, trehalose, sucrose, and total carbohydrate), which their assessment reveals the state of metabolic and energy imbalance. From this study, the total sugar concentration was significantly increased in the HFD-fed flies, which was ameliorated after treatment

with HGE. This reveals that HFD causes an increased total sugar concentration, which directly results in an excessive energy accumulation. The same trend was observed as glycogen concentration was increased significantly in flies fed with HFD when compared to the control group. The treatment with HGE further increased glycogen concentration in Drosophila body. The glycogen storage in Drosophila muscles serves as a source of energy (48).

Trehalose, a disaccharide which is a primary energy source in drosophila, showed a significantly increased concentration in HFD-fed flies. Trehalose concentration was also increased when treated with HGE, and this explains the report by Tang et al (49) on trehalose, a non-reducing sugar which becomes elevated in the hemolymph and fat body of flies to meet the energy demands of flight muscles. Trehalose is synthesized in the fat body by the enzyme trehalose-6-phosphate synthase (TPS1) and is degraded into glucose in the hemolymph by trehalase (50). In this study, total carbohydrate concentration in the flies fed with HFD increased significantly and treatment with HGE ameliorated the total carbohydrate concentration. This agrees with Schwasinger-Schmidt et al (51) who reported that starvation had a significant effect on carbohydrate metabolism. Total carbohydrates affect energy metabolism, which is essential for life with profound implications on growth, reproduction, and cellular maintenance (52).

Excess energy in *D. melanogaster* is stored in the lipid droplet of the cytoplasm in the form of triglyceride, which is a high calorie and hydrophobic metabolite (53). *D. melanogaster* also uses triglyceride as energy storage in the lipid droplet of the gut, brain, fat body, oocytes, and ovaries (54,55). This study reveals that HFD significantly increased the triglyceride concentration in *D. melanogaster,* which agrees with Heinrichsen et al (56), who reported that HFD significantly increased triglyceride level in *D. melanogaster*. Lipolysis, which is an anabolic process, hydrolyzes triglycerides into fatty acids and glycerol. Chauhan et al (57) reported a decreased level of triglyceride and lipase activity during the starvation of flies. Total cholesterol is also stored in the fat body of the fruit flies, which is profiled for a better understanding of lipid metabolism in *D. melanogaster* (58). Cholesterol is required as for steroidal hormone synthesis and also needed as the cell membrane structural component in *D. melanogaster* (59). This study reported a significant increase in the concentration of total cholesterol of flies fed with HFD. Also, the treatment of these HFD fed flies with HGE significantly reduced total cholesterol. Cholesterol overburdens contribute to an increase in the incidence of diseases like metabolic dysfunction, cardiovascular, and neurodegenerative diseases (59,60).

Conclusion

This study showed that HGE possessed strong antioxidative activity by scavenging free radicals and modulation of the activities of antioxidant enzymes. The hydroethanolic extract also showed both lipolytic and glycogenic effects. This suggests that garlic might be useful in the management of metabolic disorders, most especially obesity.

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Conflict of interests

The authors declare no conflict of interest.

Ethical considerations

The research protocols employed in the present study was sought, approved, and issued by the Ethics Committee of the Adeleke University, Ede with reference no AUERC/FOS/BCH/03

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References

1. Ali AH. Prevalence and predictors of metabolic syndrome among patients with bronchial asthma: a cross sectional study. Open Respir Med J. 2021;15:14-8. doi: 10.2174/1874306402115010014.
2. Ando K, Fujita T. Metabolic syndrome and oxidative stress. Free Radic Biol Med. 2009;47(3):213-8. doi: 10.1016/j.freeradbiomed.2009.04.030.
3. Bonomini F, Rodella LF, Rezzani R. Metabolic syndrome, aging and involvement of oxidative stress. Aging Dis. 2015;6(2):109-20. doi: 10.14336/ad.2014.0305.
4. Haas JT, Staels B. An oxidative stress paradox: time for a conceptual change? Diabetologia. 2016;59(12):2514-7. doi: 10.1007/s00125-016-4117-y.
5. Grattagliano I, Palmieri VO, Portincasa P, Moschetta A, Palasciano G. Oxidative stress-induced risk factors associated with the metabolic syndrome: a unifying

hypothesis. J Nutr Biochem. 2008;19(8):491-504. doi: 10.1016/j.jnutbio.2007.06.011.
6. Sies H, Berndt C, Jones DP. Oxidative stress. Annu Rev Biochem. 2017;86:715-48. doi: 10.1146/annurev-biochem-061516-045037.
7. Liguori I, Russo G, Curcio F, Bulli G, Aran L, Della-Morte D, et al. Oxidative stress, aging, and diseases. Clin Interv Aging. 2018;13:757-72. doi: 10.2147/cia.s158513.
8. Trochine C, Díaz Villanueva V, Balseiro E, Modenutti B. Nutritional stress by means of high C:N ratios in the diet and starvation affects nitrogen isotope ratios and trophic fractionation of omnivorous copepods. Oecologia. 2019;190(3):547-57. doi: 10.1007/s00442-019-04438-5.
9. Ansary J, Forbes-Hernández TY, Gil E, Cianciosi D, Zhang J, Elexpuru-Zabaleta M, et al. Potential health benefit of garlic based on human intervention studies: a brief overview. Antioxidants (Basel). 2020;9(7):619. doi: 10.3390/antiox9070619.
10. Atazadegan MA, Bagherniya M, Askari G, Tasbandi A, Sahebkar A. The effects of medicinal plants and bioactive natural compounds on homocysteine. Molecules. 2021;26(11):3081. doi: 10.3390/molecules26113081.
11. Adepoju AE, Joseph G, Babarinde SO, Ayeleso AO. Role of phytochemicals in *Allium sativum* on obesity: a review. In: Curative and Preventive Properties of Medicinal Plants. Taylor & Francis, Apple Academic Press, 2023; doi: 10.1201/9781003328544
12. Vlase L, Parvu M, Parvu EA, Toiu A. Chemical constituents of three *Allium* species from Romania. Molecules. 2012;18(1):114-27. doi: 10.3390/molecules18010114.
13. Andersen ØM. Anthocyanins. In: eLS. John Wiley & Sons; 2001.
14. Ross JA, Kasum CM. Dietary flavonoids: bioavailability, metabolic effects, and safety. Annu Rev Nutr. 2002;22:19-34. doi: 10.1146/annurev.nutr.22.111401.144957.
15. Sandoval-Acuña C, Ferreira J, Speisky H. Polyphenols and mitochondria: an update on their increasingly emerging ROS-scavenging independent actions. Arch Biochem Biophys. 2014;559:75-90. doi: 10.1016/j.abb.2014.05.017.
16. Trinh I, Boulianne GL. Modeling obesity and its associated disorders in *Drosophila*. Physiology (Bethesda). 2013;28(2):117-24. doi: 10.1152/physiol.00025.2012.
17. Baenas N, Wagner AE. *Drosophila melanogaster* as a model organism for obesity and type-2 diabetes mellitus by applying high-sugar and high-fat diets. Biomolecules. 2022;12(2):307. doi: 10.3390/biom12020307.
18. Diop SB, Bodmer R. *Drosophila* as a model to study the genetic mechanisms of obesity-associated heart dysfunction. J Cell Mol Med. 2012;16(5):966-71. doi: 10.1111/j.1582-4934.2012.01522.x.
19. Khan MI, Sharma M, Sonkar P, Komre P, Kumar S, Barik P. Documentation of phytochemicals, bio-toxicity and haemato-physiological responses at sub-lethal concentrations of *Parthenium hysterophorus* hydroethanolic extract in *Cyprinus carpio*: a concept of waste valorization as a phyto-ichthyotoxin. Environ Sci Pollut Res Int. 2023. doi: 10.1007/s11356-023-27444-w.
20. Aiyegoro OA, Okoh AI. Preliminary phytochemical screening and in vitro antioxidant activities of the aqueous extract of *Helichrysum longifolium* DC. BMC Complement Altern Med. 2010;10:21. doi: 10.1186/1472-6882-10-21.
21. AsokKumar K, UmaMaheswari M, Sivashanmugam AT, SubhadraDevi V, Subhashini N, Ravi TK. Free radical scavenging and antioxidant activities of *Glinus oppositifolius* (carpet weed) using different *in vitro* assay systems. Pharml Biol. 2009;47(6)474-482. doi: 10.1080/13880200902817901.
22. Ozyürek M, Bektaşoğlu B, Güçlü K, Apak R. Hydroxyl radical scavenging assay of phenolics and flavonoids with a modified cupric reducing antioxidant capacity (CUPRAC) method using catalase for hydrogen peroxide degradation. Anal Chim Acta. 2008;616(2):196-206. doi: 10.1016/j.aca.2008.04.033.
23. Re R, Pellegrini N, Proteggente A, Pannala A, Yang M, Rice-Evans C. Antioxidant activity applying an improved ABTS radical cation decolorization assay. Free Radic Biol Med. 1999;26(9-10):1231-7. doi: 10.1016/s0891-5849(98)00315-3.
24. Ruch RJ, Cheng SJ, Klaunig JE. Prevention of cytotoxicity and inhibition of intercellular communication by antioxidant catechins isolated from Chinese green tea. Carcinogenesis. 1989;10(6):1003-8. doi: 10.1093/carcin/10.6.1003.
25. Oktay M, Gülçin İ, Küfrevioğlu Öİ. Determination of in vitro antioxidant activity of fennel (*Foeniculum vulgare*) seed extracts. LWT Food Sci Technol. 2003;36(2):263-71. doi: 10.1016/s0023-6438(02)00226-8.
26. Abolaji AO, Adedara AO, Adie MA, Vicente-Crespo M, Farombi EO. Resveratrol prolongs lifespan and improves 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine-induced oxidative damage and behavioural deficits in *Drosophila melanogaster*. Biochem Biophys Res Commun. 2018;503(2):1042-8. doi: 10.1016/j.bbrc.2018.06.114.
27. Pomatto LCD, Davies KJA. The role of declining adaptive homeostasis in ageing. J Physiol. 2017;595(24):7275-309. doi: 10.1113/jp275072.
28. Misra HP, Fridovich I. The role of superoxide anion in the autoxidation of epinephrine and a simple assay for superoxide dismutase. J Biol Chem. 1972;247(10):3170-5. doi: 10.1016/s0021-9258(19)45228-9.
29. Claiborne AI. Catalase activity. In: Handbook Methods for Oxygen Radical Research. 1st ed. CRC Press; 1985. p. 2.
30. Van Handel E. Rapid determination of total lipids in mosquitoes. J Am Mosq Control Assoc. 1985;1(3):302-4.
31. Bucolo G, David H. Quantitative determination of serum triglycerides by the use of enzymes. Clin Chem. 1973;19(5):476-82.
32. Wang GH, Berdy BM, Velasquez O, Jovanovic N, Alkhalifa S, Minbiole KPC, et al. Changes in microbiome confer multigenerational host resistance after sub-toxic pesticide exposure. Cell Host Microbe. 2020;27(2):213-24.e7. doi: 10.1016/j.chom.2020.01.009.
33. Andres-Hernando A, Lanaspa MA, Kuwabara M, Orlicky DJ, Cicerchi C, Bales E, et al. Obesity causes renal mitochondrial dysfunction and energy imbalance and accelerates chronic kidney disease in mice. Am J Physiol Renal Physiol. 2019;317(4):F941-8. doi: 10.1152/ajprenal.00203.2019.
34. Aslani N, Entezari MH, Askari G, Maghsoudi Z, Maracy MR. Effect of garlic and lemon juice mixture on lipid

profile and some cardiovascular risk factors in people 30-60 years old with moderate hyperlipidaemia: a randomized clinical trial. Int J Prev Med. 2016;7:95. doi: 10.4103/2008-7802.187248.
35. Seo YJ, Gweon OC, Im JE, Lee YM, Kang MJ, Kim JI. Effect of garlic and aged black garlic on hyperglycemia and dyslipidemia in animal model of type 2 diabetes mellitus. J Food Sci Nutr. 2009;14(1):1-7. doi: 10.3746/jfn.2009.14.1.001.
36. Sobenin IA, Nedosugova LV, Filatova LV, Balabolkin MI, Gorchakova TV, Orekhov AN. Metabolic effects of time-released garlic powder tablets in type 2 diabetes mellitus: the results of double-blinded placebo-controlled study. Acta Diabetol. 2008;45(1):1-6. doi: 10.1007/s00592-007-0011-x.
37. Tungmunnithum D, Thongboonyou A, Pholboon A, Yangsabai A. Flavonoids and other phenolic compounds from medicinal plants for pharmaceutical and medical aspects: an overview. Medicines (Basel). 2018;5(3):93. doi: 10.3390/medicines5030093.
38. Demaison L. Oxidative stress and obesity-and type 2 diabetes-induced heart failure. Antioxidants (Basel). 2020;9(8):653. doi: 10.3390/antiox9080653.
39. Găman MA, Epîngeac ME, Diaconu CC, Găman AM. Evaluation of oxidative stress levels in obesity and diabetes by the free oxygen radical test and free oxygen radical defence assays and correlations with anthropometric and laboratory parameters. World J Diabetes. 2020;11(5):193-201. doi: 10.4239/wjd.v11.i5.193.
40. Jang HJ, Lee HJ, Yoon DK, Ji DS, Kim JH, Lee CH. Antioxidant and antimicrobial activities of fresh garlic and aged garlic by-products extracted with different solvents. Food Sci Biotechnol. 2018;27(1):219-25. doi: 10.1007/s10068-017-0246-4.
41. Martysiak-Żurowska D, Wenta W. A comparison of ABTS and DPPH methods for assessing the total antioxidant capacity of human milk. Acta Sci Pol Technol Aliment. 2012;11(1):83-9.
42. Ide N, Matsuura H, Itakura Y. Scavenging effect of aged garlic extract and its constituents on active oxygen species. Phytother Res. 1996;10(4):340-1. doi: 10.1002/(sici)1099-1573(199606)10:4<340::aid-ptr831>3.0.co;2-4.
43. Kayode OT, Rotimi D, Okoh E, Iyobhebhe M, Kayode AA, Ojo OA. Novel ketogenic diet formulation improves sucrose-induced insulin resistance in canton strain *Drosophila melanogaster*. J Food Biochem. 2021;45(9):e13907. doi: 10.1111/jfbc.13907.
44. Sohal RS, Allen RG. Oxidative stress as a causal factor in differentiation and aging: a unifying hypothesis. Exp Gerontol. 1990;25(6):499-522. doi: 10.1016/0531-5565(90)90017-v.
45. Kayode OT, Rotimi D, Emmanuel F, Iyobhebhe M, Kayode AA, Adeleke Ojo O. Contraceptive and biochemical effect of juice extract of *Allium cepa*, *Allium sativum*, and their combination in Canton S fruit flies. J Food Biochem. 2021:e13821. doi: 10.1111/jfbc.13821.
46. Niedzwiecki A, Kongpachith AM, Fleming JE. Aging affects expression of 70-kDa heat shock proteins in *Drosophila*. J Biol Chem. 1991;266(14):9332-8. doi: 10.1016/s0021-9258(18)31590-4.
47. Na J, Musselman LP, Pendse J, Baranski TJ, Bodmer R, Ocorr K, et al. A *Drosophila* model of high sugar diet-induced cardiomyopathy. PLoS Genet. 2013;9(1):e1003175. doi: 10.1371/journal.pgen.1003175.
48. Boukhatmi H. *Drosophila*, an integrative model to study the features of muscle stem cells in development and regeneration. Cells. 2021;10(8):2112. doi: 10.3390/cells10082112.
49. Tang Q, Zheng G, Feng Z, Chen Y, Lou Y, Wang C, et al. Trehalose ameliorates oxidative stress-mediated mitochondrial dysfunction and ER stress via selective autophagy stimulation and autophagic flux restoration in osteoarthritis development. Cell Death Dis. 2017;8(10):e3081. doi: 10.1038/cddis.2017.453.
50. Yasugi T, Yamada T, Nishimura T. Adaptation to dietary conditions by trehalose metabolism in *Drosophila*. Sci Rep. 2017;7(1):1619. doi: 10.1038/s41598-017-01754-9.
51. Schwasinger-Schmidt TE, Kachman SD, Harshman LG. Evolution of starvation resistance in *Drosophila melanogaster*: measurement of direct and correlated responses to artificial selection. J Evol Biol. 2012;25(2):378-87. doi: 10.1111/j.1420-9101.2011.02428.x.
52. Mattila J, Hietakangas V. Regulation of carbohydrate energy metabolism in *Drosophila melanogaster*. Genetics. 2017;207(4):1231-53. doi: 10.1534/genetics.117.199885.
53. Klepsatel P, Wildridge D, Gáliková M. Temperature induces changes in *Drosophila* energy stores. Sci Rep. 2019;9(1):5239. doi: 10.1038/s41598-019-41754-5.
54. Singh SR, Zeng X, Zhao J, Liu Y, Hou G, Liu H, et al. The lipolysis pathway sustains normal and transformed stem cells in adult *Drosophila*. Nature. 2016;538(7623):109-13. doi: 10.1038/nature19788.
55. Heier C, Knittelfelder O, Hofbauer HF, Mende W, Pörnbacher I, Schiller L, et al. Hormone-sensitive lipase couples intergenerational sterol metabolism to reproductive success. Elife. 2021;10:e63252. doi: 10.7554/eLife.63252.
56. Heinrichsen ET, Zhang H, Robinson JE, Ngo J, Diop S, Bodmer R, et al. Metabolic and transcriptional response to a high-fat diet in *Drosophila melanogaster*. Mol Metab. 2014;3(1):42-54. doi: 10.1016/j.molmet.2013.10.003.
57. Chauhan V, Anis A, Chauhan A. Effects of starvation on the levels of triglycerides, diacylglycerol, and activity of lipase in male and female *Drosophila melanogaster*. J Lipids. 2021;2021:5583114. doi: 10.1155/2021/5583114.
58. Parisi F, Riccardo S, Daniel M, Saqcena M, Kundu N, Pession A, et al. *Drosophila* insulin and target of rapamycin (TOR) pathways regulate GSK3 beta activity to control Myc stability and determine Myc expression in vivo. BMC Biol. 2011;9:65. doi: 10.1186/1741-7007-9-65.
59. Niwa R, Niwa YS. The fruit fly *Drosophila melanogaster* as a model system to study cholesterol metabolism and homeostasis. Cholesterol. 2011;2011:176802. doi: 10.1155/2011/176802.
60. Saini SG, Liu C, Zhang P, Lee TH. Membrane tethering by the atlastin GTPase depends on GTP hydrolysis but not on forming the cross-over configuration. Mol Biol Cell. 2014;25(24):3942-53. doi: 10.1091/mbc.E14-08-1284.