pISSN 2302-1616, eISSN 2580-2909
Vol 9, No. 1, June 2021, pp. 66-72
Available online http://journal.uin-alauddin.ac.id/index.php/biogenesis
DOI https://doi.org/10.24252/bio.v9i1.18108

Effect of *Chlorella vulgaris* extract on high sucrose diet-induced diabetes in *Drosophila melanogaster*

Mohamad Agus Salim[1*], Muhammad Subandi[2], Yeni Yuniarti[3]
[1]Department of Biology, Faculty of Science and Technology, Universitas Islam Negeri Sunan Gunung Djati
Jl. AH. Nasution No. 105A Bandung, West Java, Indonesia. 40614
*Email: agus.salim@uinsgd.ac.id
[2]Department of Agrotechnology, Faculty of Science and Technology, Universitas Islam Negeri Sunan Gunung Djati
Jl. AH. Nasution No. 105A Bandung, West Java, Indonesia. 40614
[3]Department of Math Education, Faculty of Mathematics and Science Education, Universitas Pendidikan Indonesia
Jl. Dr. Setiabudi No. 229, Bandung, West Java, Indonesia. 40154

ABSTRACT. Microalgae *Chlorella vulgaris* is a microscopic green alga known to have potential as a source of food and medicine (functional food). However, the pharmacological capacity of these microalgae species against disease due to metabolic disorders is not understood. Therefore, this study aims to evaluate the effect of *C. vulgaris* extract on animal models of *Drosophila melanogaster*, which are fed with a high-sucrose diet (HSD). In this study, the wild-type *Drosophila* used for seven days of observation was divided into four groups. The first group was used as a control without treatment, the second was treated with HSD (30%), the third was treated with *C. vulgaris* extract (120 g/L), and the fourth group had double treatment (HSD and *C. vulgaris* extract). Meanwhile, the parameters observed included fecundity, hatchability, hemolymph glucose, and triglyceride levels. The results showed that the *C. vulgaris* extract was able to reduce the negative effects of administering HSD. In addition, the single and double treatment of *C. vulgaris* extract and HSD significantly decreased the levels of hemolymph glucose as well as triglycerides of *Drosophila*. At the same time, the fecundity parameters and hatchability significantly increased compared to *Drosophila* that received only HSD treatment. Based on these results, *C. vulgaris* extract the has potential to be used as an antihyperglycemic agent. However, further study is recommended to prove it.

Keywords: antihyperglycemic; fecundity; green alga; hatchability; hemolymph glucose

Article History: Received 17 December 2020; Received in revised form 15 March 2021; Accepted 30 May 2021; Available online 30 June 2021
How to Cite This Article: Salim MA, Subandi M, Yuniarti Y. 2021. Effect of *Chlorella vulgaris* extract on high sucrose diet-induced diabetes in *Drosophila melanogaster*. *Biogenesis: Jurnal Ilmiah Biologi*. vol 9(1): 66–72. doi: https://doi.org/10.24252/bio.v9i1.18108.

INTRODUCTION

Currently, people often consume foods and drinks with high sugar levels which leads to the emergence of various deadly diseases (Kumar *et al*., 2014; Bai *et al*. 2018; Kandinasti & Farapti, 2018). Meanwhile, sugar in the body is digested, absorbed, and converted into monomer compounds, monosaccharides, then transported by the blood for target cells and tissues to be metabolized (Dashty, 2013; de Aquino Silva *et al*., 2020). The long-term consumption of foods with high sugar levels disrupts the homeostasis of glucose metabolism in the body. This metabolic disorder is in the form of hypertension, coronary heart disease, stroke, cancer, and especially, type 2 diabetes mellitus (T2DM) (Rizos & Elisaf, 2014; Fernández-Real *et al*., 2015; Harshavardhana & Krishna, 2019).

As a disease with metabolic disorders, T2DM has distinctive symptoms such as high blood glucose and triglyceride levels (Musselman *et al*., 2019). When the development of T2DM becomes uncontrollable, it leads to the emergence of various serious problems such as ocular morbidity and blindness, kidney failure, amputation, and permanent disability (Bertram *et al*., 2013; Manu *et al*., 2018). Meanwhile, new cases of the disease are on the increase, characterized by its occurrence in children which was previously found in adults only. Moreover, the treatment of T2DM uses insulin as a synthetic hypoglycemic agent which is administered orally or by injection (Peyrot *et al*., 2012; Wong *et al*., 2016). This treatment is very risky with more severe side effects such as damage to the eyes, kidneys, heart, and others (Oyeniran *et al*., 2020).

Microalgae have been known for various therapies against degenerative disease. Meanwhile, *C. vulgaris* is a microscopic green

alga that is used as a supplement and functional food (Gui *et al*., 2019). The content of phenolic compounds from this species strengthens its function as a source of antioxidant compounds which improve body metabolism (Tugcu *et al.*, 2017). Furthermore, the antioxidant ability of *C. vulgaris* extract is high enough to stabilize free radicals that damage DNA, mitochondria, and other organelles (Zakaria *et al*., 2017). However, the regulation of *C. vulgaris* against metabolic disorders due to high sugar levels consumption is not fully understood.

Drosophila or fruit flies have been known as animal models that are used to study diabetes. *Drosophila* has 74% homologous genes with human genes that control various diseases such as diabetes mellitus (Westfall *et al*., 2018). In addition, the mechanism of glucose homeostasis is fully observed through these fruit flies. Based on the observations of Wang *et al*. (2020), *Drosophila* shows the characteristics of T2DM patients such as symptoms of hyperglycemia.

In this study, the effect of microalgae *C. vulgaris* extracts was observed on the appearance of T2DM symptoms in animal models of HSD-induced *Drosophila*. The observations conducted include fecundity, hatchability, hemolymph glucose, and triglyceride levels. Moreover, *C. vulgaris* extracts were observed for total phenolic level and antioxidant activities using the diphenyl picryl hydrazyl (DPPH) method. The results of this study serve as the basis for preclinical drug discovery using microalgae *C. vulgaris.*

MATERIALS AND METHODS

Culture and treatment of *Drosophila*. *Drosophila* used was the wild type from the collection of Laboratory of Plant Physiology, Department of Biology, UIN SGD Bandung. *Drosophila* were reared in a culture vial containing 10 ml of solid medium with the following composition: 1% weight/volume (w/v) of yeast, 2% w/v sucrose, 1% w/v powdered milk, 1% w/v agar, and 0.08% w/v nipagine (Brookheart *et al*., 2017). The treatment started from *Drosophila* in the 3rd instar phase and observations were carried out for seven days of treatment. Furthermore, the four treatment groups were prepared as follows: the first group was used as a control without treatment, the second group received 30% of HSD treatment, the third group was treated with 120 g of *C. vulgaris* extract in 1 L medium, and the fourth group received HSD and *C. vulgaris* extract treatments. The environmental conditions around the *Drosophila* culture site were temperature of 26 ± 1°C, relative humidity of 60 ± 5%, and light intensity of 2000 lux while photoperiod was 12 h of light and 12 h of darkness.

Extraction of *C. vulgaris*. A total of 5 g dry biomass from *C. vulgaris* was dissolved in 25 ml of methanol p.a and stored for approximately 24 h at room temperature. The solution was centrifuged at 4000 rpm for 15 min and the supernatant obtained was concentrated using a rotary evaporator at a temperature of 40-50°C to form a concentrated extract (Chen *et al.*, 2018).

Measurement of total phenolic levels. Measurement of total phenolic levels in *C. vulgaris* extract was conducted following the procedures from McCann *et al.* (2007), with slight modifications. The extract of *C. vulgaris* was added with 35 µL of Folin Ciocalteu 1 N reagent. After leaving for 3 min, 70 ml of 15% Na_2CO_3 solution and 284 µL of aquadest were added to the solution. The mixture was stored in a dark room for 2 h and measured on a UV-vis spectrophotometer 760 nm. Gallic acid was used as a standard with a concentration range of 10 to 300 µg/mL. Meanwhile, the data obtained were expressed as mg gallic acid equivalents (GAE) per 100 g of extract.

DPPH test. The test for antioxidant activity of *C. vulgaris* extract was carried out according to the procedure of Von Stockum *et al.* (2019). The stable DPPH solution has a purple color that immediately changes to a light yellow color when it reacts with antioxidant compounds derived from *C. vulgaris* extract. Meanwhile, 500 µL of *C. vulgaris* extract was mixed with 250 µL of 0.3 mM DPPH solution. This mixture was shaken until homogeneous and stored in a dark room for 30 minutes. Furthermore, the OD resistance of *C. vulgaris* extracts against DPPH free radicals was measured by a spectrophotometer at a

wavelength of 520 nm. The inhibition was calculated using the following equation:

$$\begin{pmatrix}\text{Inhibition radical}\\ \text{scavenging (\%)}\end{pmatrix} = \frac{\text{Control Absorbance} - \text{Sample absorbance}}{\text{Control absorbance}} \times 100$$

Fecundity and hatchability test. A total of 20 female *Drosophila* aged three days from each group were placed in a culture vial that contains a standard medium and yeast as the only food source. The *drosophila* was transferred every 3 h to a new culture bottle that contains standard medium and yeast. This was followed by the calculation of the fecundity of total eggs produced by 20 *Drosophila* per h. After 22 h, all culture bottles were examined and the 1st instar larvae were counted. The hatching rate was calculated from the total number of larvae divided by the total number of eggs produced (Gáliková *et al.*, 2017).

Measurement of hemolymph glucose levels. This test used 35 individuals (one male and female *Drosophila*) for each group after seven days of treatment. A total of 2 µL of colorless hemolymph was placed on a 96-well microplate (Thermo-Scientific) which contained 0.1% N-phenylthiourea in 50 µL of PBS (Phosphate-Buffered Saline). Furthermore, 150 µL of Glucose Autokit (Wako) reagent was added to each well and incubated at room temperature for 20 minutes before measuring the absorbance at a wavelength of 505 nm. The calculation of glucose concentrations was conducted using standard curves (Navrotskaya *et al*., 2016).

Measurement of hemolymph triglyceride levels. About 35 male and female *Drosophila* from each treatment group were crushed to obtain their homogenate. At the initial stage, homogenate in PBS was mixed with Tween 0.1%. The mixture was later heated to 65°C for 5 min to inactivate the lipase. After the mixture cools down, the vortex was added to the triglyceride infinity test reagent (ThermoFisher TR22421) and the mixture was incubated at 37°C for 5 min. The mixture absorbance was measured on a spectrophotometer with a wavelength of 540 nm and the results were compared against a standard curve (Ecker *et al*., 2017).

Data analysis. The data were analyzed statistically using one-way analysis of variance (ANOVA) and when the test showed a significant difference at a 95% confidence level, further tested was conducted using Duncan's Multiple Range Test (DMRT). Moreover, all data were presented as mean ± standard deviation while various letter labels on each accompanying data showed significant differences in the 95% confidence interval ($P < 0.05$) (Steel & Torrie, 1984).

RESULTS AND DISCUSSION

Drosophila is a model animal that is often used for research on metabolic diseases such as diabetes due to its advantages which include a short life cycle, ease of food treatment manipulation, and a well-known methodology of metabolic observation. Meanwhile, HSD consumption stimulates metabolic homeostasis disharmony and causes uncontrolled inflammation that damages organelles associated with T2DM. Our study showed for the first time that *C. vulgaris* extract has a protective effect against HSD-induced diabetes symptoms in *Drosophila* by preventing oxidative stress and restoring its endogenous antioxidant defenses. The effect of *C. vulgaris* extract includes increased satiety from *Drosophila* due to the intake of organic compounds. Meanwhile, a previous study by Bortolatto *et al.* (2015), stated that adding organic compounds to the diet of *Drosophila* increased their satiety process. Furthermore, the extracts of *C. vulgaris* act by regulating appetite in the hunger center or through certain mechanisms that correlate with food entry pathways in *Drosophila*. There is sufficient evidence that the inductors causing hyperglycemia have induced oxidative stress followed by the auto-oxidation process of glucose which triggers oxidative damage.

Previous studies showed that antioxidant compounds such as phenolic acid have the potential to delay and prevent complications associated with diabetes. Similarly, antioxidant compounds as phytochemical substances reduce oxidative damage by free radicals (Forni *et al*., 2019). Moreover, oxidative stress

damages vital organs and compounds such as lipids, proteins, DNA, and cell membranes (Oyeniran *et al*., 2020). The potential of *C. vulgaris* extract includes inhibiting glucose absorption in *Drosophila*'s body, followed by utilizing existing glucose through the increased activity of enzymes involved in glucose metabolism. Meanwhile, the consumption of HSD is correlated with the development of a hyperglycemic state in *Drosophila* which is responsible for the increased oxidative stress (Ecker *et al.*, 2017). The extract of *C. vulgaris* is a compound with highly relevant functions in the antioxidant defense system. There is a correlation between hyperglycemia and oxidative stress in *Drosophila* through the emergence of an imbalance between free radical production and endogenous antioxidant physiological mechanisms (Robson *et al*., 2018). These results showed the defensive role of antioxidant compounds in the extract of *C. vulgaris* against the adverse effects of oxidative stress on *Drosophila.*

Table 1. The analysis results of the biomarker of HSD-induced Diabetes in *Drosophila.*

Paramaters	Sex	Treatments			
		C	S	V	S+V
Fecundity (number/h)	♀	40.60 ± 1.20^{c}	9.60 ± 0.80^{a}	41.60 ± 1.02^{c}	25.40 ± 0.49^{b}
Hatchability (%)	♀	98.60 ± 0.80^{b}	78.20 ± 0.75^{a}	96.40 ± 1.02^{b}	87.80 ± 1.17^{b}
Hemolymph glucose	♂	22.48 ± 0.32^{a}	77.44 ± 0.34^{c}	26.52 ± 0.43^{a}	50.72 ± 0.79^{b}
(mg/dL)	♀	24.18 ± 0.53^{a}	88.06 ± 0.40^{c}	26.02 ± 0.70^{a}	55.22 ± 0.54^{b}
Hemolymph triglyceride	♂	42.78 ± 0.68^{a}	86.34 ± 0.83^{c}	56.34 ± 0.56^{b}	76.48 ± 0.85^{c}
(µg/mg)	♀	45.9 ± 0.55^{a}	96.56 ± 0.45^{c}	62.88 ± 0.74^{b}	81.16 ± 0.57^{c}

Notes: C= Control; S= HSD; V= *C. vulgaris* extract; S + V= HSD + *C. vulgaris* extract. The different alphabets for each data value shows significantly different at DMRT test with $\alpha = 0.05$).

According to DMRT, *Drosophila* group treated with *C. vulgaris* extract produced the number of eggs was not significantly different from the control *Drosophila* group. Similarly, the number of eggs hatched in the *Drosophila* group treated with *C. vulgaris* extract was not insignificantly different from the group with *C. vulgaris* extract and HSD treatments (Table 1). Moreover, the ovary of *Drosophila* is the largest among the organs, while observation of the organ showed that various treatments affect reproductive ability, including the number of eggs produced (Diop *et al*., 2015). Furthermore, it is acceptable to use female *Drosophila* affected by HSD due to the positive correlation between ovarian size and the number of eggs (Mendes & Mirth, 2016). Disorders of the reproductive organs of *Drosophila* due to HSD treatment are shown from decreased egg production and fertility as well as the accumulation of triglycerides (Buescher *et al.*, 2013). In this study, the data correlated with Werthebach *et al.* (2019), which showed that HSD treatment of *Drosophila* successfully decreased egg production. Moreover, oxidative stress is caused by the entry of dietary components such as carbohydrates which continues with changes in metabolite structure and affects *Drosophila* reproduction. The female *Drosophila* with HSD treatment produced few mature eggs, which lead to decreased egg hatching. Furthermore, the consumption of HSD in female *Drosophila* cause decreased viability of eggs to hatch. The HSD treatment also decreased the hatching of *Drosophila* eggs, while excess glucose led to increased cellular degeneration and decreased fertility associated with low hatchability of eggs.

Based on DMRT calculations for hemolymph glucose and triglyceride levels, the female and male *Drosophila* groups in the *C. vulgaris* extract treatment were insignificantly different from the *Drosophila* control group. Meanwhile, the female group generally showed higher hemolymph glucose and triglyceride levels than the male group (Table 1). The intolerance mechanism of glucose and T2DM in mammals is caused by metabolic changes such as enzymatic dysregulation and increased glucose as well as triglycerides (Ecker *et al*., 2017). Meanwhile, *Drosophila* administered a double treatment of *C. vulgaris* extract and HSD showed improvements in glucose and

triglyceride levels due to its work in regulating appetite and reducing HSD intake. Furthermore, the results showed a close relationship between increased glucose and triglycerides as well as increased oxidative stress in *Drosophila*. Excessive sucrose consumption inhibits the formation of satiety signals in the brain, leading to the release of more glucose into the body (Rovenko *et al*., 2015). The researchers observed that the effect of dietary manipulation such as HSD in *Drosophila* response according to gender differences (Castellanos *et al*., 2013). Therefore, HSD treatment of female *Drosophila* clearly shows an increase in hemolymph glucose and triglyceride levels (Buescher *et al.*, 2013).

CONCLUSION

C. vulgaris extract has various actions in *Drosophila* metabolism. It acts mainly by increasing satiety, responding to the decrease in glucose and triglyceride levels in HSD-induced hyperglycemic *Drosophila*. The results also showed the pharmacological potential of *C. vulgaris* extracts against metabolic disorders such as T2DM. Therefore, further studies are recommended to analyze the cellular and physiological mechanism of *C. vulgaris* extract against HSD, which causes T2DM disease.

ACKNOWLEDGEMENTS

The authors would like to thank Department of Biology, Faculty of Science and Technology, Universitas Islam Negeri Sunan Gunung Djati for material and immaterial support.

REFERENCES

Bai Y, Li K, Shao J, Luo Q, Jin LH. 2018. Flos *Chrysanthemi indici* extract improves a high sucrose diet induced metabolic disorder in *Drosophila*. *Experimental and Therapeutic Medicine*. vol 16(3): 2564–2572. doi: https://doi.org/10.3892/etm.2018.6470.

Bertram MY, Jaswal AV, Van Wyk VP, Levitt NS, Hofman KJ. 2013. The non-fatal disease burden caused by type 2 diabetes in South Africa, 2009. *Global Health Action*. vol 6(1): 206–212. doi: https://doi.org/10.3402/gha.v6i0.19244.

Bortolatto CF, Heck SO, Gai BM, Zborowski VA, Neto JSS, Nogueira CW. 2015. Effects of diphenyl and p-chloro-diphenyl diselenides on feeding behavior of rats. *Psychopharmacology*. vol 232(13): 2239–2249. doi: https://doi.org/10.1007/s00213-014-3856-z.

Brookheart RT, Swearingen AR, Collins CA, Cline LM, Duncan JG. 2017. High-sucrose-induced maternal obesity disrupts ovarian function and decreases fertility in *Drosophila melanogaster*. *Biochimica et Biophysica Acta (BBA)-Molecular Basis of Disease*. vol 1863(6): 1255–1263. doi: https://doi.org/10.1016/j.bbadis.2017.03.014.

Buescher JL, Musselman LP, Wilson CA, Lang T, Keleher M, Baranski TJ, Duncan JG. 2013. Evidence for transgenerational metabolic programming in *Drosophila*. *Disease Models & Mechanisms*. vol 6(5): 1123–1132. doi: https://doi.org/10.1242/dmm.011924.

Castellanos MC, Tang JCY, Allan DW. 2013. Female-biased dimorphism underlies a female-specific role for post-embryonic Ilp7 neurons in Drosophila fertility. *Development*. vol 140(18): 3915–3926. doi: https://doi.org/10.1242/dev.094714.

Chen Y, Liu X, Wu L, Tong A, Zhao L, Liu B, Zhao C. 2018. Physicochemical characterization of polysaccharides from *Chlorella pyrenoidosa* and its anti-ageing effects in *Drosophila melanogaster*. *Carbohydrate Polymers*. vol 185: 120–126. doi: https://doi.org/10.1016/j.carbpol. 2017.12.077.

Dashty M. 2013. A quick look at biochemistry: carbohydrate metabolism. *Clinical Biochemistry*. vol 46(15): 1339–1352. doi: https://doi.org/10.1016/j.clinbiochem.2013.04.027.

de Aquino Silva D, Silva MRP, Guerra GP, do Sacramento M, Alves D, Prigol M. 2020. 7-chloro-4-(phenylselanyl) quinoline co-treatment prevent oxidative stress in diabetic-like phenotype induced by hyperglycidic diet in Drosophila melanogaster. *Comparative Biochemistry and Physiology Part C: Toxicology & Pharmacology*. vol 239: 1–10. doi: https://doi.org/10.1016/j.cbpc.2020.108892.

Diop SB, Bisharat-Kernizan J, Birse RT, Oldham S, Ocorr K, Bodmer R. 2015. PGC-1/spargel counteracts high-fat-diet-induced obesity and cardiac lipotoxicity downstream of TOR and brummer ATGL lipase. *Cell Reports*. vol 10(9): 1572–1584. doi: https://doi.org/10.1016/j.celrep.2015.02.022.

Ecker A, Gonzaga TKSDN, Seeger RL, Santos MMD, Loreto JS, Boligon AA, Meinerz DF, Lugokenski TH, Rocha JBTD, Barbosa NV. 2017. High-sucrose diet induces diabetic-like phenotypes and oxidative stress in *Drosophila melanogaster*: Protective role of *Syzygium cumini* and *Bauhinia forficata*. *Biomedicine & Pharmacotherapy*. vol 89: 605–616. doi: https://doi.org/10.1016/j.biopha.2017.02.076.

Fernández-Real JM, McClain D, Manco M. 2015. Mechanisms linking glucose homeostasis and iron metabolism toward the onset and progression of type 2 diabetes. *Diabetes Care*. vol 38(11): 2169–2176. doi: https://doi.org/10.2337/dc14-3082.

Forni C, Facchiano F, Bartoli M, Pieretti S, Facchiano A,

D'Arcangelo D, Norelli S, Valle G, Nisini R, Beninati S, Tabolacci C, Jadeja RN. 2019. Beneficial role of phytochemicals on oxidative stress and age-related diseases. *BioMed Research International*. vol 2019: 1–17. doi: https://doi.org/10.1155/2019/8748253.

Gáliková M, Klepsatel P, Münch J, Kühnlein RP. 2017. Spastic paraplegia-linked phospholipase PAPLA1 is necessary for development, reproduction, and energy metabolism in Drosophila. *Scientific Reports*. vol 7: 1–14. doi: https://doi.org/10.1038/srep46516.

Gui J, Tong W, Huang S, Liang X, Fang Z, Wang W, Zhang Y. 2019. Effects of Chlorella vulgaris polysaccharides accumulation on growth characteristics of Trachemys scripta elegans. *International Journal of Biological Macromolecules*. vol 141: 1304–1313. doi: https://doi.org/10.1016/j.ijbiomac.2019.08.248.

Harshavardhana HR, Krishna MS. 2019. Protective role of leaf extract on high sucrose diet-induced diabetic Gymnema sylvestre like phenotype, oxidative stress, reproductive fitness and longevity in *Drosophila melanogaster*. *Asian Journal of Pharmacy and Pharmacology*. vol 5(3): 535–546. doi: https://doi.org/10.31024/ajpp.2019.5.3.15.

Kandinasti S, Farapti F. 2018. Obesitas: pentingkah memperhatikan konsumsi makanan di akhir pekan?. *Amerta Nutrition*. vol 2(4): 307–316. doi: http://dx.doi.org/10.20473/amnt.v2i4.2018.307-316.

Kumar GS, Pan L, Park S, Lee-Kwan SH, Onufrak S, Blanck HM. 2014. Sugar-sweetened beverage consumption among adults—18 states, 2012. MMWR. *Morbidity and Mortality Weekly Report*. vol 63(32): 686–690.

Lobo V, Patil A, Phatak A, Chandra N. 2010. Free radicals, antioxidants and functional foods: Impact on human health. *Pharmacognosy Reviews*. vol 4(8): 118–126. doi: https://dx.doi.org/10.4103%2F0973-7847.70902.

Manu AS, Davalgi SB, Aithal SS, Dilip B. 2018. Awareness of diabetic retinopathy and barriers for eye screening among adults with type 2 diabetes mellitus attending tertiary care teaching hospital, Davanagere, Karnataka. *International Journal of Medical Science and Public Health*. vol 7(9): 686–691. doi: 10.5455/ijmsph.2018.0514717052018.

McCann MJ, Gill CIR, O'brien G, Rao JR, McRoberts WC, Hughes P, Rowland IR. 2007. Anti-cancer properties of phenolics from apple waste on colon carcinogenesis in vitro. *Food and Chemical Toxicology*. vol 45(7): 1224–1230. doi: https://doi.org/10.1016/j.fct.2007.01.003.

Mendes CC, Mirth CK. 2016. Stage-specific plasticity in ovary size is regulated by insulin/insulin-like growth factor and ecdysone signaling in Drosophila. *Genetics*. vol 202(2): 703–719. doi: https://doi.org/10.1534/genetics.115.179960.

Musselman LP, Fink JL, Baranski TJ. 2019. Similar effects of high-fructose and high-glucose feeding in a *Drosophila* model of obesity and diabetes. *PloS One*. vol 14(5): 1–13. doi: https://doi.org/10.1371/journal.pone.0217096.

Navrotskaya V, Oxenkrug G, Vorobyova L, Summergrad P. 2016. Attenuation of high sucrose diet–induced insulin resistance in ABC transporter deficient white mutant of *Drosophila melanogaster*. *Integrative Obesity and Diabetes*. vol 2(2): 187–190.

Oyeniran OH, Ademiluyi AO, Oboh G. 2020. 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*. vol 45(3): 1–14. doi: https://doi.org/10.1111/jfbc.13318.

Peyrot M, Barnett AH, Meneghini LF, Schumm-Draeger PM. 2012. Factors associated with injection omission/non-adherence in the Global Attitudes of Patients and Physicians in Insulin Therapy study. *Diabetes, Obesity and Metabolism*. vol 14(12): 1081–1087. doi: https://doi.org/10.1111/j.1463-1326.2012.01636.x.

Rani L, Saini S, Shukla N, Chowdhuri DK, Gautam NK. 2020. High sucrose diet induces morphological, structural and functional impairments in the renal tubules of *Drosophila melanogaster*: A model for studying type-2 diabetes mediated renal tubular dysfunction. *Insect Biochemistry and Molecular Biology*. vol 125: 1–17. doi: https://doi.org/10.1016/j.ibmb.2020.103441.

Rizos CV, Elisaf MS. 2014. Antihypertensive drugs and glucose metabolism. *World Journal of Cardiology*. vol 6(7): 517–530. doi: https://dx.doi.org/10.4330%2Fwjc.v6.i7.517.

Robson R, Kundur AR, Singh I. 2018. Oxidative stress biomarkers in type 2 diabetes mellitus for assessment of cardiovascular disease risk. *Diabetes & Metabolic Syndrome: Clinical Research & Reviews*. vol 12(3): 455–462. doi: https://doi.org/10.1016/j.dsx.2017.12.029.

Rovenko BM, Kubrak OI, Gospodaryov DV, Perkhulyn NV, Yurkevych IS, Sanz A, Lushchak OV, Lushchak VI. 2015. High sucrose consumption promotes obesity whereas its low consumption induces oxidative stress in *Drosophila* melanogaster. *Journal of Insect Physiology*. vol 79: 42–54. doi: https://doi.org/10.1016/j.jinsphys.2015.05.007.

Steel RGD, Torrie JH. 1984. Principles and procedures of statistics. Singapore: Mc Graw Hill Book C., Inc. pp 172–177.

Tugcu G, Ertürk MD, Saçan MT. 2017. On the aquatic toxicity of substituted phenols to Chlorella vulgaris: QSTR with an extended novel data set and interspecies models. *Journal of Hazardous Materials*. vol 339: 122–130. doi:

https://doi.org/10.1016/j.jhazmat.2017.06.027.

Von Stockum S, Sanchez-Martinez A, Corrà S, Chakraborty J, Marchesan E, Locatello L, Costa R, Bubacco L, Rasotto MB, Szabo I, Whitworth AJ, Scorrano L, Ziviani E. 2019. Inhibition of the deubiquitinase USP8 corrects a *Drosophila* PINK1 model of mitochondria dysfunction. *Life Science Alliance*. vol 2(2): 1–16. doi: http://doi.org/10.26508/lsa.201900392.

Wang Q P, Lin YQ, Lai ML, Su Z, Oyston LJ, Clark T, Shi YC, James DE, Ja WW, Herzog H, Simpson SJ, Neely GG. 2020. PGC1α controls sucrose taste sensitization in *Drosophila*. *Cell Reports*. vol 31(1): 10–15. doi: https://doi.org/10.1016/j.celrep. 2020.03.044.

Werthebach M, Stewart FA, Gahlen A, Mettler-Altmann T, Akhtar I, Maas-Enriquez K, Droste A, Eichmann TO, Poschmann G, Stühler K, Beller M. 2019. Control of *Drosophila* growth and survival by the lipid droplet-associated protein CG9186/Sturkopf. *Cell Reports*. vol 26(13): 3726–3740. doi: https://doi.org/10.1016/j.celrep.2019.02.110.

Westfall S, Lomis N, Prakash S. 2018. A polyphenol-rich prebiotic in combination with a novel probiotic formulation alleviates markers of obesity and diabetes in *Drosophila*. *Journal of Functional Foods*. vol 48: 374–386. https://doi.org/10.1016/j.jff.2018.07.012.

Wong CY, Martinez J, Dass CR. 2016. Oral delivery of insulin for treatment of diabetes: status quo, challenges and opportunities. *Journal of Pharmacy and Pharmacology*. vol 68(9): 1093–1108. doi: https://doi.org/10.1111/jphp.12607.

Zakaria SM, Kamal SMM, Harun MR, Omar R, Siajam SI. 2017. Subcritical water technology for extraction of phenolic compounds from *Chlorella* sp. microalgae and assessment on its antioxidant activity. *Molecules*. vol 22(7): 1–9. doi: https://doi.org/10.3390/molecules22071105.