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EVALUATION OF THE EFFECT OF AQUEOUS SEED EXTRACT OF *Carica papaya* ON SUCROSE-INDUCED DIABETES IN *Drosophila melanogaster*

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Abstract
Diabetes mellitus is a group of metabolic disorder in which there is high blood sugar level in the body over a prolonged period of time. *Carica papaya* is one of the medicinal plant that help in the treatment of metabolic syndrome. This study was carried out to evaluate the effects of *Carica papaya* seed on sucrose-induced diabetes in *Drosophila melanogaster.* The flies were grouped into four; the first were the flies treated with normal diet with water, the second with sucrose supplemented diet, the third with aqueous pawpaw seed extract supplemented diet while the fourth group were treated with sucrose and pawpaw seed extract in their diet. After the intake of daily supplemented diet for seven days the flies were homogenized and enzyme activities were determined. The results showed that glucose, triglycerides levels and acetylcholinesterase activity were significantly increased in a sucrose-induced or high sucrose diet group when compared to the control while it was observed that total thiol level was reduced significantly when compared to the control group. The reduction in glucose and triglyceride levels, alteration in enzyme activity and enhancement of the total thiol level of aqueous extract of *Carica papaya* seed on sucrose-induced diabetes in *Drosophila melanogaster* might be considered as the mechanisms of its antidiabetic action.
Keywords: *Carica papaya,* acetylcholinesterase, *Drosophila melanogaster,* sucrose, diabetes

Introduction

Diabetes is a chronic metabolic malaise that affects and is forecast to affect many millions of people in the world (Ripsin *et al.*, 2013). It is a disease caused by insulin deficiency or loss of insulin action. In addition to genetic factors, certain lifestyles such as high dietary fat content and physical inactivity are risk factors for the development of diabetes. It has outpaced many other diseases and is predicted to become one of the major health concerns in the future. In human diabetic patients where the condition has existed for some time, there are several comorbidities. It courses with macrovascular complications, leading to heart disease and stroke, and increased cardiovascular morbidity and mortality. In addition, microvascular complications lead to nephropathy, retinopathy, and neuropathy (Pasquel *et al.*, 2014). Sucrose, as a pure carbohydrate, has an energy content of 3.94 kilocalories per gram (or 17 kilojoules per gram). If consumed excessively, sucrose may contribute to the development of metabolic syndrome, including increased risk for type 2 diabetes, weight gain and obesity in adults and children. (Nicholson, 2017). High-sucrose diet (HSD) has been stated to induce diabetes-like genetic, biochemical, and phenotypic alterations in D. melanogaster (Musselman *et al.*,2011; Navrotskaya *et al.*, 2015; Ecker *et al.*, 2017). New hypoglycemic agents derived from plants have shown both hypoglycemic action and the ability to improve some of the secondary complications of diabetes such as kidney damage, fatty liver, and oxidative stress (Tricco *et al.,* 2012).

Carica papaya also known as pawpaw is a plant belonging to family *Caricaceae* with potential medicinal values and has been cultivated in most of the tropical countries. *Carica papaya* is a large tree like herbaceous perennial plant with soft single stem growing up to 5-10m height, with sparely arranged leaves at the top of the trunk, lower trunk is scarred where leaves and fruits are born (Heywood., *et al.,* 2007). *Carica papaya* seed is well known for its exceptional nutritional and medicinal properties throughout the world. *Carica papaya* seed is commonly known for its food and nutritional values throughout the world. The genus *Carica papaya* Linn is the most widely cultivated and best known species of the four genera that belongs to a small family *Caricaceae* (Omonkhua and Onoagbe, 2011). The seeds are numerous, small, black, round and covered with gelatinous aril. Medical research in animals has confirmed the contraceptive and abortifacient capability of *papaya*. Its seeds also have contraceptive effects in adult male Langur Monkeys, possibly in adult male humans (Oderinde *et al.*, 2002). It has been reported that both leaves and seeds of *Carica papaya* exhibited antioxidant, anti-hypertensive, hypoglycemic, and hypolipidemic actions, which, in turn, could contribute to the prevention and treatment of obesity and accompanying metabolic syndromes (Santana et al., 2019).Recent study also revealed the *in vitro* and *in vivo* inhibitory effects of *Carica papaya* seed on α-amylase and α-glucosidase enzymes (Agada *et al.*, 2020).

Drosophila melanogaster has been used as a very versatile and potent model in the past few years for studies in metabolism and metabolic disorders, including diabetes types 1 and 2 (McGurk*et al.,*2015). *Drosophila* insulin signaling, despite having seven insulin-like peptides with partially redundant functions, is very similar to the human insulin pathway and has served to study many different aspects of diabetes and the diabetic state.Most studies on antidiabetic potentials of *Carica papaya* seeds have been on animal models. However, there is dearth of scientific information on the effects of the seed on Drosophila model and likely mechanism of action therefore this study sought to evaluate the biochemical effect of pawpaw seed in sucrose-induced diabetes mellitus in *Drosophila melanogaster*.

Materials and Methods

Materials

Plant Collection

resh *Carica papaya* was bought from the Main market (Oja-Oba) in Akure, Nigeria and identified at the Crop Soil and Pest Management Department of the University. The samples were washed under running tap water to remove the dirt. The leaves were chopped into pieces with the use of a table knife, and then air dried until constant weight was achieved. Thereafter, the samples were pulverized using a Marlex blender. The powdered samples were stored in air-tight containers and kept in the refrigerator for further analysis.

Preparation of Aqueous Extract of Pawpaw Seed

Mature, unripe *Carica papaya* fruits were cut into pieces and the wet seeds were removedand rinsed in tap water two times. The seeds were air-dried at room temperature for 4 weeks and pulverized into fine powder using a grinder. Ten grams of the powdered samples of pawpaw seed were weighed and extracted with 50 ml distilled water in extraction bottle and shaken vigorously for 2 h. The extract was filtered using Muslin cloth and further filtered with Whatman filter paper and further centrifuged to obtain clear supernatant. The supernatant was weighed and stored in air and water proof container, kept in a refrigerator.

Drosophila melanogaster Stock Culture

Wild type*D. melanogaster* (Oregon strain) stock culture was obtained from Department of Biochemistry, Fedral University Of Technology Akure, Ondo State. The flies were maintained and reared on normal diet made up of corn meal medium containing 1% w/v brewer's yeast and 0.08% v/w nipagin at constant temperature and humidity (25 ± 1°C; 60% relative humidity respectively) under 12 hours dark/light cycle conditions. All the experiments were carried out with the same *D. melanogaster* strain.

Reagents

Chemical reagents such as phosphate buffer (pH 6.9), p-nitrophenyl-α-D-glucopyranoside solution, sodium phosphate buffer (pH 6.9 with 0.006 M NaCl), Porcine pancreatic α-amylase (EC3.2.1.1)(0.5mg/mL), 1% starch solution, dinitrosalicylic acid colour reagent, Pipes reagent (40 mM phosphate buffer, 5.5 mM 4-chlorophenol and 17.5 mM Mg^{2+}) and 1mL of enzyme reagent (4-aminophenazone, adenosine triphosphate, lipase, glycerolkinase, glycerol-3-phosphate oxidase and peroxidase) and the water was glass distilled.

Methods

Preliminary Survival Study

A preliminary study was conducted to assess the effect of dietary inclusion of pawpaw leaf and pawpaw seed extract on survival rate of flies after seven days of exposure. Flies (both gender, 3–5 days old) were divided into four groups containing 40 flies each. Each group was exposed to different dietary inclusions of pawpaw seed extract(1ml of 0.1g/ml) each. The flies were observed daily for the incidence of mortality and the survival rate were determined by counting the number of dead flies for the first 7 days.

Experimental Design

The flies (both gender, 3–5 days old) were divided into 4 groups containing 40 flies each. Group I was placed on normal diet alone, group II were placed on sucrose diet alone, group III were placed on normal diet containing pawpaw seed extract, group IV were placed on diet containing sucrose and pawpaw seed extract as shown thus;

Groups

I	Normal Diet + 1ml of distilled water
II	Normal Diet + 1.5g of sucrose
III	Normal Diet + (1ml of 0.1g/ml) of pawpaw seed extract
lV	Normal Diet + 1.5g of sucrose + (1ml of 0.1g/ml) of pawpaw seed

The flies were exposed to these treatments for 7 days and the vials containing flies were maintained at room temperature before being homogenized in 0.1 M phosphate buffer (pH 7.4), and used for different assays (Sudati *et al.,*., 2013). All experiments were carried out in duplicate (n=2).

Lethality Response

The control and treated flies were observed daily for the incidence of mortality throughout their life span. The survival rate was determined by counting the number of dead flies, while the survivors were transferred to freshly prepared diet after 7 days. The data were subsequently analyzed and plotted as cumulative mortality and percentage of live flies after the treatment period.

Preparation of Tissue Homogenate

The flies were anesthetized in ice and homogenized in 0.1M phosphate buffer, pH 7.4, using a Teflon homogenizer. The resulting homogenates were centrifuged at 10,000 X g, 4°C for 10 minutes in a Kenxin refrigerated centrifuge Model KX3400C (KENXIN Intl. Co., Hong Kong).Subsequently, the supernatant was separated from the pellet into labelled Eppendorf tubes and used for the various biochemical assays.

Biochemical Assays

Determination of triglyceride concentration

The triglyceride concentration was determined using the colorimetric method as described by Tietz (1986). Briefly, 10 µL of the sample was mixed with 1 mL of Pipes reagent (40 mM phosphate buffer, 5.5 mM 4-chlorophenol and 17.5 mM Mg^{2+}) and 1mL of enzyme reagent (4-aminophenazone, adenosine triphosphate, lipase, glycerolkinase, glycerol-3-phosphate oxidase and peroxidase). Thereafter the mixture was incubated for 5 min at 37°C and the absorbance at 546 nm was taken against reagent blank within 60 min. The triglyceride concentration was subsequently calculated against the standard.

Acetylcholinesterase (AChE) Activity Assay

Acetylcholinesterase activity was assayed according to the method described by Ellman *et al.,* (1959). The reaction mixture was made up of 50 µL of distilled water, 50 µL of 0.1M potassium phosphate buffer (pH 7.4), 30 µL of 10 mM 5,5-dithio-bis(2-nitrobenzoic) acid (DTNB), 15 µL of tissue homogenate, and 30 µL of 8 mM acetylthiocholine. Thereafter, reaction was monitored for 5 minutes at 412 nm in a spectrophotometer. The AChE activity was thereafter calculated and expressed as mmolAcSch/h/mg protein.

Determination of the Total Thiol Content

Determination of the level of total thiol content in tissue homogenate was done by the method of Ellman *et al.*, (1959). The reaction mixture was made up of 200 µL of 0.1 M potassium phosphate buffer (pH 7.4), 25 µL of homogenate, 35 µL volume of water and 10 µL of 10 mM DTNB. This was followed by 30-minute incubation at room temperature, and the absorbance was measured at 412 nm. The total thiol content was subsequently calculated and expressed as (µmol/mg protein).

Data Analysis

The results of replicate readings were pooled and expressed as mean ± standard deviation (S.D). One-way Analysis of Variance (ANOVA) was used to analyze the results followed by Dunnelt's post hoc test, with levels of significance accepted. All statistical analysis was carried out using the software Graph pad PRISM (V.5.0).

Result

The effect of dietary inclusion of pawpaw seed on the survival rate or life span of sucrose-induced *D. melanogaster* was shown in Figure 1. The results revealed that there was no significant reduction of life span when compared to the control group.

The effect of dietary inclusion of *Carica papaya* seed extract on glucose activity in *D. melanogaster* was presented in Figure 2. This result revealed that flies fed with diet supplemented with 1ml of pawpaw seed exhibited significantly lower ($p<0.05$) glucose activity when compared with the negative control group (Sucrose). However, glucose activity in flies raised on diet supplemented with 1.5g of sucrose was significantly higher ($p<0.05$) when compared to the control. I

n Figure 3, the effect of dietary inclusion of*Carica papaya* seed extract on the triglycerides concentration in *D. melanogaster*fed on high sucrose diet was observed. The result revealed that there was a significant increase in the triglyceride concentration in the group fed with sucrose only when compared to the normal control. Nevertheless, a significant decrease was observed in the flies fed with diet supplemented-pawpaw seed when compared to the group II (sucrose only).

Figures 4 showed the effect of dietary inclusion of 1ml of *Carica papaya* seedextract on the acetylcholinesterase activity of *D. melanogaster*fed with high sucrose. This result revealed that there was significant increase in the activity of acetylcholinesterase in the group fed with sucrose only when compared to the control at $p<0.05$. Though, this rise was reversed by the supplementation of pawpaw seed compared to the control.

In addition, the effect of dietary inclusion of *Carica papaya* seedextracton total thiol concentration in *D. melanogaster*fed on high sucrose diet was represented in Figure 5. The result demonstrated that there was a significant diminution in total thiol concentration in the group fed with sucrose only when compared to the control. However, flies fed with pawpaw seed supplemented diet enhanced total thiol concentration in comparison with the group fed with sucrose only.

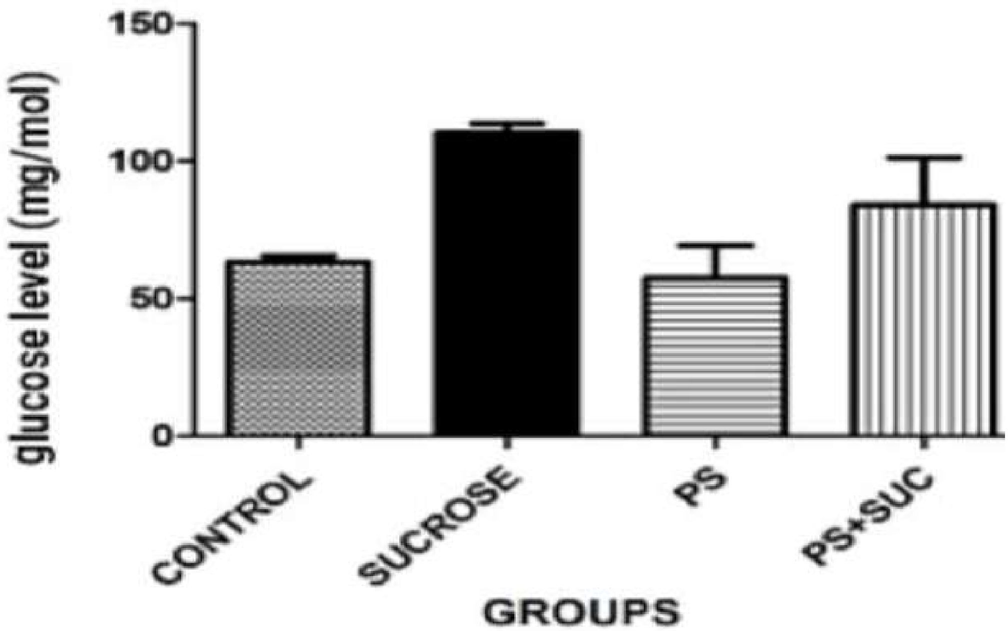

Figure 1 Effect of dietary inclusions of pawpaw seed on survival rate of sucrose-induced diabetes in *D. melanogaster* Bars represent mean ± SD

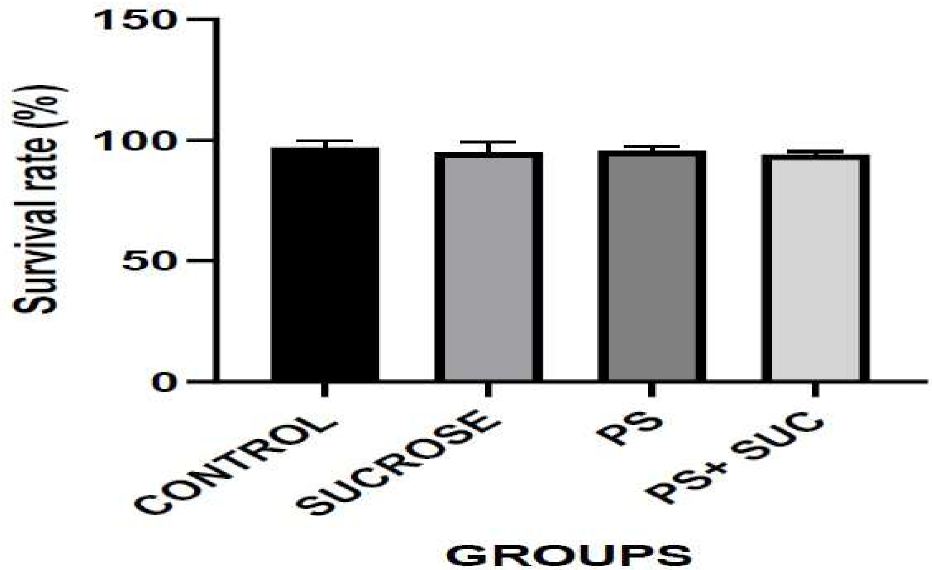

Figure 2 Effect of dietary inclusions of pawpaw Seed and sucrose on the glucose concentration in *D. melanogaster* Bars represent mean ± SD

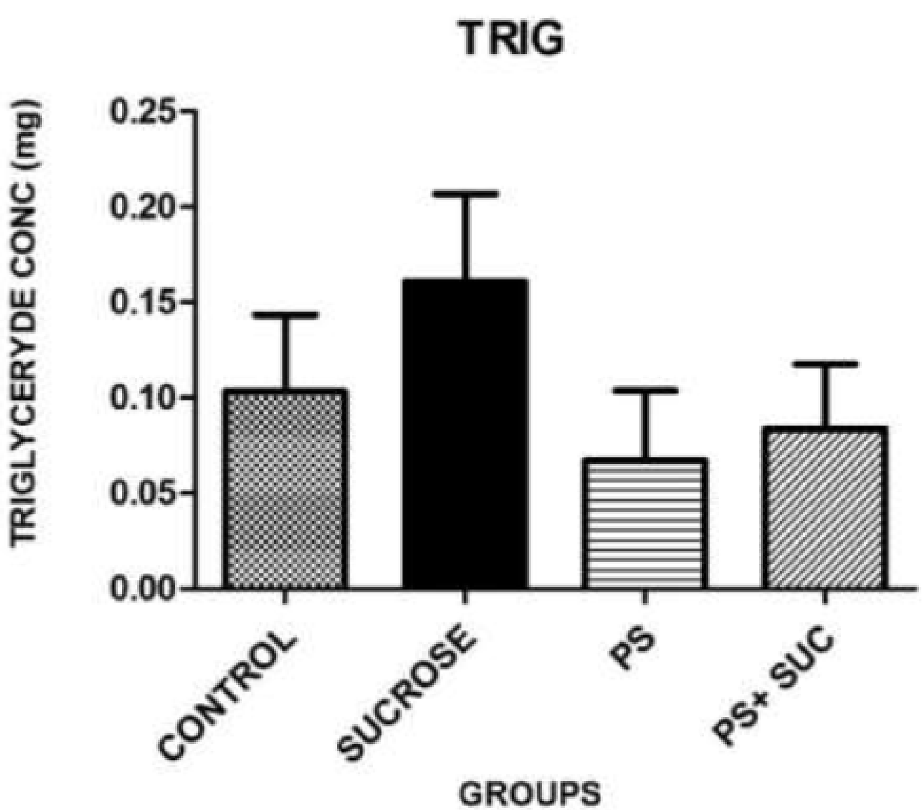

Figure 3 Effect of dietary inclusions ofpawpaw Seed and sucrose on triglyceride concentration in *D. melanogaster* Bars represent mean ± SD

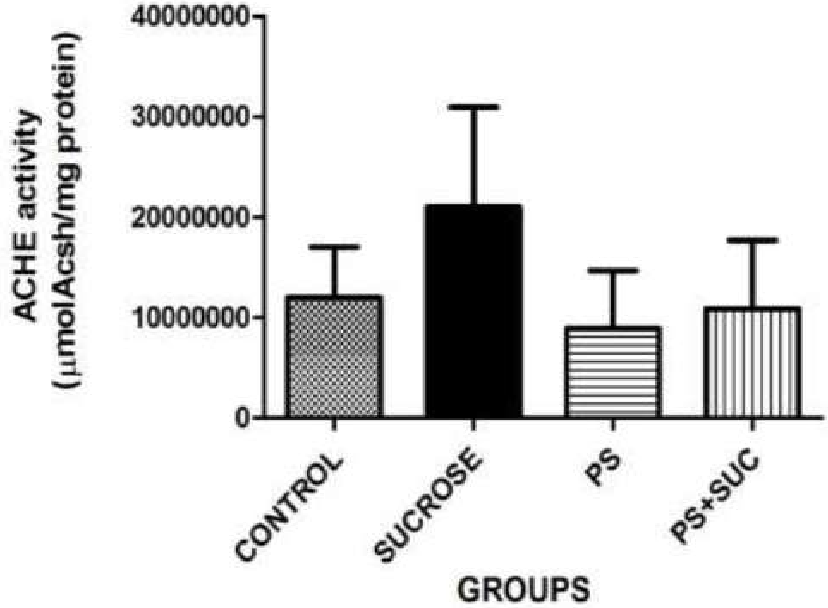

Figure 4 Effect of dietary inclusions of pawpaw Seed and sucrose on acetycholinesterase activity in *D. melanogaster*
Bars represent mean ± SD

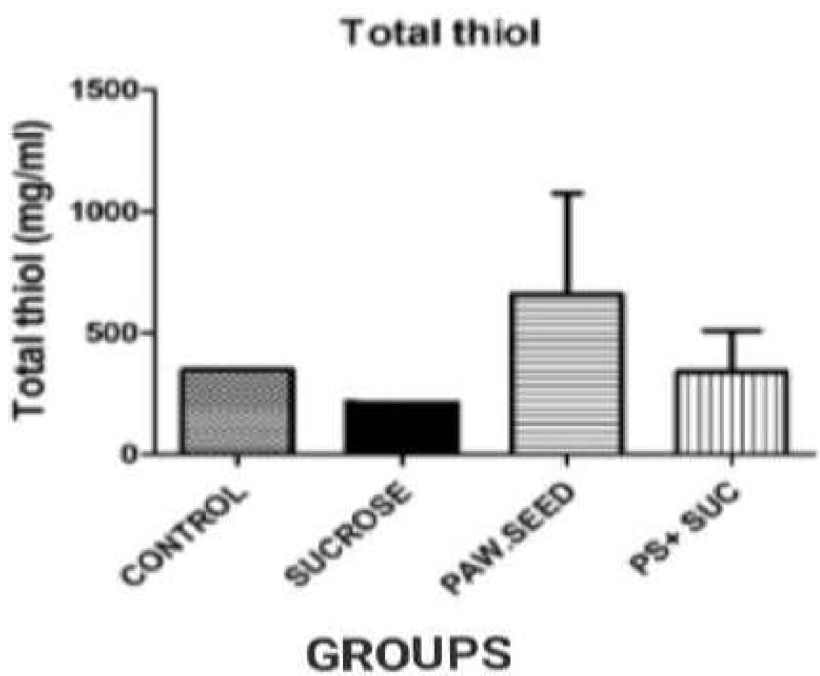

Figure 5 Effect of dietary inclusions of pawpaw Seed and sucrose on the Total thiol concentration in *D. melanogaster*
Bars represent mean ± SD

Discussion

Medicinal plants are gradually gaining global acceptability given their potential as bioactive agents to be used as pharmaceuticals. New hypoglycemic agents derived from plants have shown both hypoglycemic action and the ability to improve some of the secondary complications of diabetes such as kidney damage, fatty liver, and oxidative stress (Tricco., *et al.* 2012). *Carica papaya* seed used in indigenous medicines for the management of diabetes mellitus contain both organic and inorganic constituents. Some of these inorganic trace elements possess antidiabetic properties (Rivera-Pastrana *et al.* 2010).

This study sought to investigate the effects of aqueous seed extract of *Carica papaya* on sucrose-induced diabetes in *Drosophila melanogaster.* Fruitfly has become a veritable and extensive option of non-mammalian experimental model (Bharucha, 2009; Morris et al., 2012). Glucose has been described to be a pro-aging factor and to interact with several age-associated processes in the organism. The results from this study showed that there was no significant difference in the survival rate in both treated and untreated groups which was contrary to the previous studies that postulated that high glucose availability has been shown to shorten life span whereas a glucose restriction increased life span in the model organism (Piper *et al.*,2005; Partridge *et al.*, 2005; Mair *et al.*,2005, Ecker *et al.*, 2017).

HSD-fed Drosophila adults had significantly higher circulating glucose concentration levels than the pawpaw seed-fed *Drosophila* adults. The reversal of the high glucose concentration may be due to the ability of pawpaw seed to reduce hyperglycemia as reported by Adeneye and Olagunju, 2009).

The capacity of dietary addition of sucrose to elicit increased glucose and triglyceride levels in this research is in concordance with former reports on the utilization of High sucrose diet (HSD) in demonstrating diabetes-like phenotype in fruit flies at diverse evolving phases (Musselman *et al.*, 2011; Navrotskaya *et al.*,2015; Ecker *et al.*, 2017). Different biochemical parameters of glucose metabolic imbalances which have been stimulated by high sucrose diet. Examples include high glucose and triglyceride levels, insulin resistance, oxidative stress, impaired metabolic and redox gene expression, and impaired development and aging (Navrotskaya *et al.*, 2015; Ecker *et al.*, 2017; Harshavardhana and Krishna, 2019).

A triglyceride is an ester derived from glycerol and three fatty acids. Triglycerides are the main constituents of body fat in humans and other animals, as well as vegetable fat. They are also present in the blood to enable the bidirectional transference of adipose fat and blood glucose from the liver, and are a major component of human skin oils. (Ivanova., *et al.* 2017). Circulating triglyceride levels represent a balance between triglyceride synthesis and utilization. These are greatly affected by insulin sensitivity. Consistently, an increasing triglyceride level, particularly when accompanied by low HDL, was shown to be a surrogate marker of insulin resistance, a strong predisposing condition for type 2 diabetes (Berglund *et al.* 2012). Furthermore, high free fatty acids potentially derived from triglyceride further deteriorate insulin sensitivity, creating a vicious cycle between triglyceride level and insulin resistance. This process have operated to acutely increase diabetes risk, when triglyceride levels progressed during follow-up from the lowest to the highest tertile, potentially surpassing the excessive risk associated with persistently elevated triglyceride levels. (Drummond *et al.,* 2012).

A significant (p < .05) increase in the triglyceride level of flies fed with sucrose with a simultaneous significant ($p < .05$) decrease in the treated fruit flies is connected to the fact that high sugar diets usually give rise to elevated levels of glucose and triacylglycerides. This is symbolic of metabolic disorders and biochemical signals of diabetes mellitus. This result was in consonance with the work of Pasco and Leopold (2012) where high sugar diet was stated to bring about the noticeable attributes of type-2 diabetes in flies. This effect was mitigated by the supplementation of pawpaw seed which reduced the triglyceride level. The ameliorative effect observed might be attributed to the presence of phenolic compounds such as benzyl isothiocyanate, glucosinolates, tocopherols (α and δ), β-cryptoxanthin, β-carotene and carotenoids (Kermanshai *et al*., 2001; Santana *et al*., 2019).

Acetylcholinesterase also known as AChE is the primary cholinesterase in the body. It is an enzyme that catalyzes the breakdown of acetylcholine and of some other choline esters that function as neurotransmitters (Day *et al*., 1991). The effects of pawpaw seed in AChE activity on cells were also evaluated.

The significant ($p < .05$) accentuated AChE activity achieved in the sucrose control might connote a continuing neurodegenerative state arising from hyperglycemia in the flies (Oyeniran *et al*., 2020). However, the significant ($p < .05$) decrease found in the cholinesterase activity of the treated flies proposesthe promising modulatory properties of this pawpaw seed which might ensue through decline in oxidative stress and thus improving the cholinergic system (Chatchada *et al*., 2013). Earlier research has demonstrated that neurodegeneration could be improved by flavonoids,so, the enhancement of cholinergic system by pawpaw seed may probably be accredited to its reported phenolics and flavonoid content (Ademiluyi *et al*., 2018; Youdim and Joseph, 2001).

Adaptive reaction, which is the ability of an organism to efficiently thwart cellular injuries prompted by cytotoxic mediators such as free radicals, has been recognized in *D. melanogaster* (Abolaji *et al*., 2017). Such boost in antioxidant enzymes' actions as an adaptive reaction to cytotoxic agents has been earlier described in *D. melanogaster*, and they are always attended by damages in cellular thiol levels (Abolaji *et al*., 2017; Bayliak *et al*., 2018; Perkhulyn *et al*., 2017). Thiols signify the principal portion of cellular antioxidant systems, and they play a vibrant role in protection against ROS (Mungli et al., 2009). Nevertheless, the total response of thiols to oxidative cellular attacks is frequently a creation of connections between thiol oxidation and synthesis (Osburn *et al*., 2006). Therefore, the insignificant difference in thiol contents among the treatmentgroups in this study could be as a result of correlation betweenthiol consumption in response to the presence of ROS and replenishing as a result of adaptive response under acute exposure.

Thiols are the organic compounds that contain a sulphydryl group. Among all the antioxidants that are available in the body, thiols constitute the major portion of the total body antioxidants and they play a significant role in defense against reactive oxygen species and regulations of glucose concentration (Lee *et al.,* 2012). Total thiols composed of both intracellular and extracellular thiols either in the free form as oxidized or reduced glutathione, or thiols bound to proteins. There was a decreased plasma total thiol levels in *Drosophila* that are induced with sucrose (diabetic). The result revealed a decrease in thiol activity in fruit fly with high sucrose diet which was similar to the work of Ivashchenko *et al.,* (2011).

Conclusion

Based on the findings from this study, High sucrose diet consumed by D. melanogaster exhibited significantly elevated glucose and triglyceride levels as well as increased activity of acetylcholinesterase however reduced total thiol level. Conversely, pawpaw seed demonstrateddiminution of biochemicals investigated but enhanced total thiol levels which is an index of antioxidant status. This may be part of mechanism of action. The phytochemicals present in the pawpaw seed such as phenolic acids, tocopherols might be involved in the ameliorative and curative effects. Nonetheless, more studies can be carried out experimental modelsare encouraged to further justify these findings. It further suggests that *Carica papaya* seed could be used as potent antidiabetic agent and functional food for protecting against insulin insensitivity and cells from oxidative damage.

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