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Original Research Article

Anti-Diabetic Activity of Aqueous Leaves Extract of *Acacia nilotica* in High Sucrose Diet-Induced Diabetic *Drosophila melanogaster* Model

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Abstract

Diabetes mellitus remains a global public health problem associated with many complications. *Acacia nilotica* has been used in local management of diabetes and demonstrated anti-diabetic activities in animal model. *Drosophila melanogaster* (fruit-fly) has been used as a model for investigating pharmacological activities of natural products because of its genetic resemblance to human genes. This study aims at evaluating the anti-diabetic activities of aqueous leaves extract of *Acacia nilotica* in HSD-induced diabetic *Drosophila melanogaster* model. The results showed that flies treated with 0.250 g/mL and 0.500 g/mL of the extract demostrated significant (p < 0.05) increase in locomotor performance compared with diabetic control. The aqueous leaves extract of *Acacia nilotica* demonstrated significant (p < 0.05) decrease in glucose, glycogen, trehalose and triglycerides levels in the treated flies compared with diabetic control. In comparison with diabetic control, the extract exhibited significant (p < 0.05) decrease in MDA level and increase in SOD, CAT and GSH levels in treated flies. The aqueous leaves extract of *Acacia nilotica* demonstrates significant anti-diabetic activity in *Drosophila melanogaster* model validating its anti-diabetic effect in animal model and its use in the local management of diabetes. **Keywords:** Acacia nilotica, Antioxidants enzymes, Diabetes, Drosophila melanogaster, High sucrose diet.

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INTRODUCTION

Diabetes remains a public health problem with high morbidity and mortality rate. Diabetes mellitus is a metabolic disorder characterized by persistence hyperglycemia and glucose intolerance due to absolute or relative deficiency in insulin secretion or/and insulin action (Arora et al., 2019; Abdulkadir et al., 2017). About 463 million people have been suffering with diabetes and is predicted to reach up to 578 million by the year 2030 and 700 million by 2045 (Shubham et al., 2021). According to the World Health Organization global report, almost 422 million people are suffering with diabetes worldwide (WHO, 2016). Report showed that the prevalence rate of diabetes is higher in low and middle-income countries (Sunmonu and Lewu, 2019). In Nigeria, about 4 million people are suffering with diabetes, representing a fifth of all diabetes cases in subSaharan Africa (Sunmonu and Lewu, 2019). Diabetes is associated with many complications including vascular dysfunction and high risk of coronary artery and peripheral vascular diseases (Paari and Pari, 2019).

Management of diabetes mellitus relies on maintaining normal blood glucose levels without causing unwarranted patient risks (Isah *et al.*, 2013). Conventional treatment of diabetes involves dietary management, exercise, and the use of appropriate medications (Isah *et al.*, 2013). Anti-diabetic conventional drugs are expensive thus, cannot be afforded by many patients. The drugs produce many side effects such as severe weight loss and increased risk of cardiovascular risk (Tzoulaki *et al.*, 2009). More numbers of communities in the world have depending on medicinal plants for remedies (Abubakar *et al.*, 2022).

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Plants and herbs demonstrate pharmacological activities due to their various phytoconstituents or bioactive compounds that have therapeutic applications (Abubakar et al., 2022). Medicinal plants have been used in almost all African countries for the treatment of many diseases. Plants and herbs are important sources of bioactive compounds that have been used in research and pharmaceutical industries for development phytomedicine (Abubakar et al., 2021). Plants and herbs have demonstrated anti-diabetic activities and have been used in the management of diabetes and its complications. Plants and herbs are widely available in local communities and are easy to access by majority of the people in the world. Medicinal plants are less expensive and produce no or little side effects than conventional therapy (Abubakar et al., 2021).

Acacia nilotica is a tropical and subtropical plant that is belongs to the family Fabaceae (Raj et al., 2015). The plant is widely found throughout Asia, Africa, and America. Acacia nilotica has been used in local treatment of many diseases. The demonstrated several pharmacological including anti-hypertensive, anti-spasmodic, microbial, anti-hypertensive, hypoglycemic, inflammatory, anti-cancer activities (Kaur et al., 2022; Goronyo et al., 2022). Anti-diabetic activity of Acacia nilotica has been reported in animal models. However, no any study reported on the medicinal properties and pharmacological activities of the plant using fruit flies' model. Drosophila melanogaster (Fruit flies) which belongs to the family Drosophilidae is a two-winged insect (Dipteran insect) that is widely used in research as a standard research model (Baenas and Wagner, 2019). The use of organism model in research was because the organisms contain about 60-75% human disease genes and possess a mammalian-like intestinal system with a fat body resembling the adipose tissue (Baenas and Wagner, 2019). This aims at evaluating the anti-diabetic activity of aqueous leaves extract of Acacia nilotica in high sucroce diet-induced diabetic Drosophila melanogaster model.

MATERIALS AND METHODS

Study Site

The research work was conducted at the Fly Laboratory, Centre for Advance Medical Research and Training (CAMRET), Usmanu Danfodiyo University, Sokoto, Nigeria. The experiments were performed in accordance to the principles and protocols for using the organisms (Fruit flies) set by the laboratory.

Drug and Chemicals

Metformin manufactured by Salud Care (I) Pvt Ltd, India was purchased from Pharmacy Unit, Usmanu Danfodiyo University Teaching Hospital, Sokoto. Glucose, triglycerides, glycogen, and trehalose kits manufactured by Spinreact (Spain) were used in this study. Catalase, SOD and GSH kits were purchased from

Elabscience Biotechnology (Inc, USA). All the chemicals used in this study were of analytical grade.

Collection and Identification of Plant Sample

Fresh Leaves of *Acacia nilotica* were collected from Kalambaina, Wamakko Local Government Area, Sokoto State, Nigeria. The plant sample was identified and authenticated (voucher number: UDUH/ANS/0158) at the Herbarium Unit, Department of Biological Sciences, Usmanu Danfodiyo University, Sokoto, Nigeria.

Sample Preparation and Extraction

The fresh leaves of *Acacia nilotica* were thoroughly washed with distilled water and shed dried for two weeks. The dried leaves were pulverized to fine powder using an electric grinding machine. The extract was prepared using the method of Aliyu *et al.*, (2024) and Abubakar *et al.*, (2020). Two hundred and fifty grams (250 g) of the powdered sample were extracted in three liters (3 L) of distilled water for 72 hours with intermittent stirring. The extract was filtered using Whitman No.1 filter paper. The filtrate was concentrated in using rotary evaporator (RV 8, 001000217+, IKA, Germany) at 40 °C under reduced pressure for 5 hours. The weight (15.4 g) and percentage yield (6.2 %) of the extract was recorded and the extract was stored in desiccator until further analysis.

Fly Stock and Maintenance

Drosophila melanogaster (Harwich strain) was obtained from Fly Laboratory, Centre for Advance Medical Research and Training in Usmanu Danfodiyo University Teaching Hospital, Sokoto. The flies were reared in 2.5 x 6.5 cm² glass bottles containing 100 mL of standard medium (corn flour 10 g, brewer's yeast 1 g, agar 1 g, methyl paraben 0.1 g, distilled water 160 mL) at constant temperature 24 °C under 12 hours light-dark cycle. The orifices of the bottles were covered with a piece of clean polyethane by means of rubber bands. The bottles were kept upright in the lab. The emerging flies were collected and separated accordingly. All experiments were performed with the same strain at 24 °C under 12 hours light/dark cycle.

Anti-diabetic Activity Experimental Design

The flies were randomly distributed into six groups of 30-50 flies each. Group I served as normal control in which no diabetes was induced and no treatments were given. Group II served as diabetic control in which the flies were induced with diabetes and no treatments were given. Group III was treated as positive control in which the flies were induced with diabetes and then treated with standard drug, Metformin (0.16 mg/mL fly diet). Group IV; the flies in this group were induced with diabetes and then treated with 0.125 g/mL fly diet of *A. nilotica* leaves aqueous extract. Group V in which the flies were induced with diabetes and then treated with 0.250 g/mL fly diet of *A. nilotica* leaves

aqueous extract. Group VI; the flies in this group were induced with diabetes and then treated with 0.500 g/mL fly diet of *A. nilotica* leaves aqueous extract.

Induction of Diabetes

Diabetes was induced in *D. melanogaster* using high sucrose content diet according to the method described by Musselman *et al.*, (2018) and Morris *et al.*, (2012). The flies were subjected to high sucrose content diet (2.5 g sucrose/10 g diet) for ten (10) days. The flies were observed daily for diabetes characterized symptoms such as delayed egg production, delayed emergence of first instar Larvae (L3), decreased body size for both larvae (L3) and adult flies, as well as decreased locomotor activity. The glucose level of the fly homogenate was determined to ascertain hyperglycemia by glucosidase method using standard kits according to the manufacturer's instruction.

Assessment of Locomotor Performance

Negative geotaxis technique was employed in the analysis of locomotor performance of the diabetic and treated flies using the method of Adedara et al., (2016) and Ali et al., (2011). The vertical glass columns were labeled to 15 cm length and 1.5 cm diameter. A total of ten flies were anesthetized under mild ice and separately placed in the columns. About twenty minutes after anesthesia the flies were recovered from the ice exposure and gently tapped to the bottom of the column. The number of flies that climbed up to the 6 cm mark of the column in 6 seconds, as well as those that remained below the mark after 6 seconds was recorded. The mean number of flies at the top was obtained and expressed as a percentage of the total number of flies. The experiment was performed in triplicate at 60 seconds interval and the mean score was calculated.

Preparation of Sample Homogenate

After the treatment, the diabetic and treated flies were anaesthetized on ice. The anaesthetized flies were treated for many times with cold phosphate buffer saline (PBS) (pH 7.4, 0.1 M) to remove all traces of diet materials. The flies were respectively deposited into labeled pre-weighed eppendorf tubes at different concentrations. The flies in the respective tubes were weighed and recorded and then 10 uL of the phosphate buffer saline (PBS) was added into the tubes per mg body weight of the flies. The flies were homogenized on ice chips and then centrifuged at 3000 rpm and 48 °C for 10 minutes. The supernatant was separated and transferred into the respective pre-labelled eppendorf tubes. The tubes were stored at -18 °C until further use.

Biochemical Analysis

The levels of glucose, glycogen, triglyceride, trehalose in the sample homogenate were determined using standard kits according to the manufacturer's instructions.

Determination of Membrane Lipid Peroxidation

The level of membrane lipid peroxidation marker (MDA) in the sample homogenate was estimated using Thiobarbituric acid Reactive Substances (TBARS) method as described by Abubakar *et al.*, (2021). The sample homogenate (0.1 mL) was treated with 0.5 mL of 10 % TCA and 75% TBA. The contents were incubated at 80 °C for 45 minutes using water bath. The mixture was allowed to cool in ice and then centrifuged at 4000 rpm for 5 minutes. The clear supernatant was separated and the absorbance of the sample against the blank was measured at 260 nm wavelength. The level of MDA was obtained using the following equation:

MDA level = Absorbance of sample \div Molar extinction

Determination of Anti-oxidant Enzymes

The levels of SOD, CAT, and GSH in the sample homogenate were determined using standard ELISA kits according to the manufacturer's instructions.

Statistical analysis

The results were expressed as mean \pm standard error of the mean (SEM). All the parameters were analyzed using one-way analysis of variance (ANOVA) followed by Duncan multiple comparison tests using Statistical Package for Social Sciences (SPSS) version 20.0. Limit of statistical significance (p-value) was set as p < 0.05.

RESULTS

Effect of Aqueous Leaves Extract of Acacia nilotica on Locomotor Activity of Drosophila melanogaster

Figure 1 shows the Effect of aqueous leaves extract of *Acacia nilotica* on locomotor activity (negative geotaxis) of *Drosophila melanogaster*. Exposure of the flies to all the doses for three days changed the locomotion activity of the flies in the first, second, and third trial when compared to control (Figure 1). The mean number of flies exposed to all the doses in the first, second, and third trial at the top of the column was reduced by 1 when compared to the control group (Table 4.5). However, the mean number of flies exposed to 1g/9mls/diet in the third trial at the top of the column was reduced by 2 when compared to the control group (Figure 1).

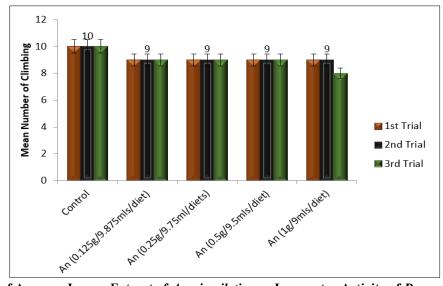


Figure 1: Effect of Aqueous Leaves Extract of *Acacia nilotica* on Locomotor Activity of *Drosophila melanogaster* Values are Mean ± SEM. (n = 10 flies/group), Negative geotaxis (Mean number of flies that crossed after 6 sec).

Acacia nilotica (An)

Effect of Aqueous Leaves Extract of Acacia nilotica on Glucose Level of the Drosophila melanogaster

The effect of aqueous leaves extract of *Acacia* nilotica on glucose level of *Drosophila melanogaster* is shown in Figure 2. The result revealed a significant (p < 0.05) decrease in glucose level of the flies treated with

0.125 g/mL, 0.250 g/mL and 0.500 g/mL of the extract in dose dependant manner compared with diabetic control. However, glucose level of the flies treated with 0.500 g/mL of the extract was decreased compared to the flies treated with the standard drug, metformin (Figure 2).

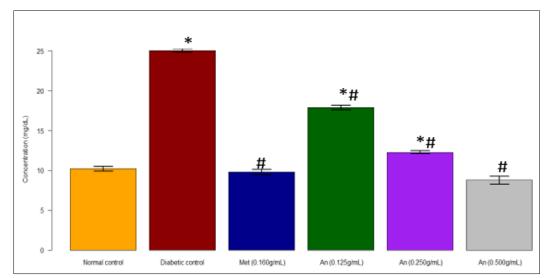


Figure 2: Effect of Aqueous Leaves Extract of *Acacia nilotica* on Glucose Level of the *Drosophila melanogaster* Values are Mean ± SEM. (n = 10 flies/group)

*p < 0.05 statistically significant compared with normal controls, #p < 0.05 statistically significant compared with diabetic controls. An (Acacia nilotica), Met (Metformin).

Effect of Aqueous Leaves Extract of Acacia nilotica on Triglycerides, Trehalose, and Glycogen Levels of the Drosophila melanogaster

Table 1 shows the effect of aqueous leaves extract of *acacia nilotica* on triglycerides, trehalose, and glycogen levels of the *Drosophila melanogaster*. The extract (0.125 g/mL, 0.250 g/mL and 0.500 g/mL)

exhibited significant (p < 0.05) decrease in triglycerides, trehalose, and glycogen level in the treated flies compared with diabetic control. However, the level of triglycerides, trehalose, and glycogen in the flies treated with 0.500 g/mL of the extract was comparable to that of the flies treated with the standard drug, metformin (Table 1).

Table 1: Effect of Aqueous Leaves Extract of Acacia nilotica on Triglycerides, Trehalose, and Glycogen Levels of the Drosophila melanogaster

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Treatment Group	Triglyceride (mg/dL)	Trehalose (mg/dL)	Glycogen (mg/dL)	
Normal Control	5.17 ± 0.136	39.93 ± 0.078	8.54 ± 0.195	
Diabetic Control	$15.27 \pm 0.108^*$	$60.28 \pm 0.212^*$	$12.25 \pm 0.259^*$	
Met (0.160g/mL)	$5.08 \pm 0.145^{\#}$	$39.44 \pm 0.263^{\#}$	$7.08 \pm 0.245^{\#}$	
An (0.125g/mL)	$12.90 \pm 0.064^{*\#}$	$58.04 \pm 0.164^{*\#}$	$10.99 \pm 0.066^{*\#}$	
An (0.250g/mL)	$8.05 \pm 0.082^{*\#}$	$47.92 \pm 0.177^{*\#}$	$9.28 \pm 0.142^{\#}$	
An (0.500g/mL)	$5.14 \pm 0.090^{\#}$	$40.04 \pm 0.060^{\#}$	$6.55 \pm 0.220^{*\#}$	

Values are Mean \pm SEM. (n = 10 flies/group)

*p < 0.05 statistically significant compared with normal controls, "p < 0.05 statistically significant compared with diabetic controls. An (*Acacia nilotica*), Met (Metformin).

Effect of Aqueous Leaves Extracts of Acacia nilotica on Lipid Peroxidation Marker (MDA) Level of the Drosophila melanogaster

The effect of aqueous leaves extracts of *acacia nilotica* on lipid peroxidation marker (MDA) level of the *Drosophila melanogaster* is shown in Figure 3. The level of MDA in the tissue homogenates of the diabetic flies

increased significantly (p < 0.05) compared with the normal control. Treatment of the flies with the extract decreased the levels of MDA in a dose-dependent manner. The result shows a significant (p < 0.05) decrease in MDA level in the flies treated with 0.125 g/mL, 0.250 g/mL and 0.500 g/mL of the extract compared with the diabetic control (Figure 3).

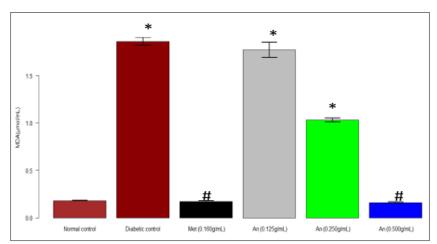


Figure 3: Effect of Aqueous Leaves Extracts of *Acacia nilotica* on Lipid Peroxidation Marker (MDA) Level of the *Drosophila melanogaster*

Effect of Aqueous Leaves Extracts of Acacia nilotica on Antioxidants Enzymes Levels of the Drosophila melanogaster

The effect of aqueous leaves extracts of *acacia nilotica* on antioxidants enzymes levels of the *Drosophila melanogaster* is shown in Table 2. In comparison with the normal control, the SOD, CAT, and

GSH level of the tissue homogenates of the diabetic flies significantly (p<0.001 decreased. Treatments of the flies with 0.125 g/mL, 0.250 g/mL and 0.500 g/mL of the extract significantly (p<0.05) elevated the level of SOD, CAT, and GSH in the tissue homogenates of the respective groups (Table 2).

Table 2: Effect of Aqueous Leaves Extracts of *Acacia nilotica* on Antioxidants Enzymes Levels of the *Drosophila melanogaster*

Treatment Group	SOD (µmol/mg)	CAT (µmol/mg)	GSH (μmol/mg)	
Normal Control	1.73 ± 0.003	0.29 ± 0.003	3.45 ± 0.002	
Diabetic Control	$0.15 \pm 0.002^*$	$0.17 \pm 0.002^*$	$1.22 \pm 0.002^*$	
Met (0.160g/mL)	$1.60 \pm 0.093^{\#}$	$0.34 \pm 0.002^{\#}$	$3.49 \pm 0.002^{\#}$	
An (0.125 g/mL)	$0.25 \pm 0.001^*$	$0.19 \pm 0.002^*$	$1.57 \pm 0.029^*$	
An (0.250g/mL)	$0.99 \pm 0.010^{*\#}$	0.23 ± 0.002	$2.76 \pm 0.002^{\#}$	
An (0.500g/mL)	$1.79 \pm 0.003^{\#}$	$0.34 \pm 0.008^{\#}$	$3.45 \pm 0.001^{\#}$	

Values are Mean \pm SEM. (n = 10 flies/group)

^{*}p < 0.05 statistically significant compared with normal controls, *p < 0.05 statistically significant compared with diabetic controls. An (*Acacia nilotica*), Met (Metformin).

DISCUSSION

The anti-diabetic activity of Acacia nilotica has been reported by several literatures in animals (rats, rabbits) models. Acacia nilotica has been used in the management of diabetes mellitus caused by free radicals (Pareek and Choudhry, 2013). It has been reported that stem bark extract of A. nilotica exhibited anti-diabetic activity in rats model (Abdirahman et al., 2015). Study by Ahmad et al., (2008) that A. nilotica pods and barks demonstrated anti-diabetic activities alloxan-induced diabetic rabbits. However, no study reported on the antidiabetic activity of Acacia nilotica in Drosophila model. Drosophila is an established model organism for evaluating the anti-diabetic activity of natural products (Palanker et al., 2011). Drosophila genome consist homologues of almost 77% of the disease-related loci in humans (Reiter et al., 2001). Drosophila model is easy and relatively inexpensive and have a mammalian resemble genetic homology (Mackay and Anholt, 2006). Drosophila has been used as standard experimental model in nutrition and diabetes research (Luersen et al., 2019; Staats et al., 2018). Drosophila is characterized by insulin producing cells in the brain that resemble mammalian pancreatic beta cells and which secret eight insulin-like peptides (Nässel et al., 2013; Broughton et al., 2005; Garelli et al., 2012). It has been documented that high-sugar diet causes high blood sugar, insulin resistance, elevated fat storage, and decreased life expectancy in Drosophila (Na et al., 2013; Teleman, 2010; Murillo-Maldonado et al., 2011).

Locomotion is an important behavior that indicates the health condition of an organism. Negative geotaxis is a common technique used to determine the locomotory behaviour of flies that is the ability of flies to move vertically when startled (Rhodenizer et al., 2008). In the present study, exposure of Drosophila melanogaster on different concentrations of the extract changed the locomotor activity of the flies and displayed 10% mortality of the flies. Increase in reactive oxygen and nitrogen species (RONS) and the decrease in antioxidants activity causes decrease in climbing activity of D. melanogaster (Abolaji et al., 2014). Type 2 diabetes is characterized by increased muscle weakness, loss of reflexes, and loss of balance and coordination (Bansal et al., 2006). The decrease locomotory activities of the diabetic flies could be attributed to these symptoms of Type 2 diabetes. In the current study, the aqueous leaves extract of Acacia nilotica demonstrated significant increase in the body weight of the treated flies. The finding is in line with the results of the relevant study by Musselman et al., (2011) showed that the weight of diabetic larvae decreased compared with the normal control and treated groups.

Result of this study showed that the aqueous leaves extract of *Acacia nilotica* demonstrated significant decrease in glucose, triglycerides, trehalose, and glycogen levels in diabetes induced *Drosophila melanogaster* model. The result is in agreement with the

findings of many researchers who reported that aqueous leaves extracts of *A. nilotica* significantly reduce the glucose, triglycerides, trehalose, and glycogen levels in alloxan-induced diabetic rats (Ahmad *et al.*, 2008). Drosophila utilizes glucose and trehalose as the major hemolymph sugars (Thompson, 2003). The present findings indicated that the high sugar diet elevated the levels of glucose and trehalose in the tissue homogenate of diabetic flies. The present findings are in agreement with the findings of Musselman *et al.*, (2011) who reported elevated levels of glucose and trehalose in the HSD-fed Drosophila. High level of trehalose has been attributed to insulin deficiency and insulin resistance (Song *et al.*, 2010).

Reactive oxygen species (ROS) produce adverse effects in many physiological, biological, and biochemical processes as well as pathological processes (Sies et al., 2022). It has been documented that free radicals produce many adverse effects in biological system including damage of membrane lipids, proteins and DNA molecules leading to oxidative stress (Ibrahim et al., 2024). Imbalance between ROS generation and neutralization by the antioxidant system results to oxidative stress (Sies, 2023). Oxidative stress plays a vital role in the development of diabetes mellitus and its complications (Giacco, 2010). Prolong hyperglycemia generates reactive oxygen species leading to diabetes mellitus. An increase in free radical activity coupled with decrease in antioxidant level has been observed in diabetes induced rats (Hajizadeh et al., 2014; Quilliot et al., 2005).

In this study, the aqueous leaves extract of Acacia nilotica exhibited a significant decrease in MDA level and elevated level of SOD, CAT, and GSH in the tissue homogenates of the flies treated with different doses of the extract. The most important antioxidant enzymes include superoxide dismutase (SOD), catalase (CAT), and peroxiredoxin (Prx) (Ighodaro and Akinloye 2018). These enzymes play an important role in the first line antioxidant defense system against elevated oxidative stress (Forman and Zhang, 2021). Antioxidant enzymes suppress the level of oxidative stress thereby alleviating oxidative-induced damage to biomolecules (Gulcin 2020). Superoxide dismutase (SOD) is a vital antioxidant enzyme that plays an important role in preventing cells from the harmful effects of the highly reactive superoxide ion and peroxynitrite (Younus, 2018). It simultaneously catalyzed the oxidation and reduction of superoxide ion into oxygen and hydrogen peroxide (Younus, 2018; Wang et al., 2018). Study showed that optimizing the level of SOD is one of the therapeutic strategies for treating oxidative stressinduced pathologies (Prasad et al., 2018). SOD mimics are supplemented with catalase mimics to alleviate vascular complications caused by hydrogen peroxideinduced oxidative damage (Prasad et al., 2018). GSH prevents normal cells against oxidative injury by acting on free radical chain reactions and by function as a cofactor in certain enzymatic activities including glutathione transferase, GPX, and glutathione reductase. Reactive oxygen species can cause damage to proteins, DNA, and membrane lipids. GSH protects these biomolecules against the reactive oxygen species including superoxide anion, hydroxyl radical, nitric oxide, and carbon radicals (Ighodaro and Akinloye, 2018). Catalase catalyzes the conversion of hydrogen peroxide (H₂O₂) to oxygen and water. It has been observed that the activity of catalase (CAT) decreased in type 2 diabetes (Goth *et al.*, 2008).

CONCLUSION

Aqueous leaves extract of *Acacia nilotica* demonstrates significant anti-diabetic activity in *Drosophila melanogaster* model. This validated the anti-diabetic effect of *Acacia nilotica* in animal model and its use in the local management of diabetes. Further studies should be done to isolate and characterize the bioactive compound in the plant extract and to evaluate the anti-diabetic effect of the active compound.

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