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The Phytochemical Constituents, Hypoglycemic, and Antioxidant Activities of *Senna occidentalis* (L.) Ethanolic Leaf Extract in High Sucrose Diet Fed *Drosophila melanogaster*

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Authors' contributions

This work was carried out in collaboration among all authors. Author OCT designed the study, performed the statistical analysis, wrote the protocol and the first draft of the manuscript. Author OA supervised the work. Authors EMA and SPU managed the analysis and literature reviews of the study. All authors read and approved the final manuscript.

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ABSTRACT

Aim: This study was aimed at evaluating the phytochemical constituents, hypoglycemic, and antioxidant activities of *Senna occidentalis* ethanolic leaf extract in diabetic *Drosophila melanogaster* with emphasis on survival, phytochemical, biochemical and fecundity assays as well as locomotor activities.
Methodology: *S. occidentalis* leaves was collected and prepared for extraction using 70% ethanol as solvent. The extract was subjected to phytochemical screening and the lethal dose (LD_{50}) was

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carried out on *D. melanogaster* for 7 days. Survival study was conducted by treating fruit flies with different concentration of the extracts for 28 days. Hyperglycemia was induced by feeding the flies with food containing 30% of sucrose for 10 days, and thereafter treated with different concentration of the extract and metformin (positive control) for 7 days. Diabetic flies were used for the negative geotaxis and fecundity assays. The homogenates of flies from the different groups of the treatment and control were prepared and used to quantify the glucose content and the antioxidant activities which included the thiol content, glutathione-s-transferase and catalase activities.

Results: The phytochemical screening revealed the presence 8 different phytochemicals. The LD_{50} was determined to be 277.8 mg/10 g fly food of *S. occidentalis*. Supplementation with *S. occidentalis* ethanolic extracts showed a non-significant increase ($P > 0.05$) in the survival of *D melanogaster* when compared to the baseline group. Diabetic flies treated with the extract showed a dose-dependent decrease in serum glucose which was significant ($P < 0.05$) at 100 mg when compared to the negative control group(untreated). Negative geotaxis, fecundity and catalase activities of treated flies showed no significant difference ($P > 0.05$) when compared to flies in the negative control group. *S. occidentalis* ethanolic leaf extract significantly elevated ($P < 0.05$) the total thiol content and glutathione-s-transferase activities at certain concentration in a non-linear manner.

Conclusion: From the findings, *S. occidentalis* ethanolic leaf extract contained several phytochemicals and it is relatively safe; possessing hypoglycemic and antioxidant properties when administered to diabetic *D. melanogaster*.

Keywords: Diabetes; drosophila melanogaster; senna occidentalis; antioxidant; and hypoglycemic.

1. INTRODUCTION

Medicinal plants have been useful for therapeutical purposes and they have been used in place or alongside conventional drugs for treatments/prevention of diseases in man and animals [1,2]. Since medicinal plants are more easily sourced and contains varieties of organic/natural products or molecules, researchers primarily focus on these plants for their active components [3]. Native to Asia, Africa, and America, *Senna occidentalis* (L.) seeds and leaves are used for treatment of several ailments [4]. Belonging to the family "Fabaceae", *S.occidentalis* leaves are used traditionally in the treatment of diabetes and other ailments [5]. The plant leaves have been used by several people to prepare certain dishes despite the claims that it is poisonous for consumption [6]. *S. occidentalis* Leaf extracts have been confirmed to have antidiabetic activities in albino mice and an *In-vitro* study validates the presence of antioxidant properties in the plant [7,8].

Diabetes mellitus, simply known as Diabetes occurs as a result of inadequate control of blood sugar level characterized by chronic hyperglycemia that is caused by the defect in insulin action and secretion [9]. In humans, diabetes causes a lot of complications like stroke, kidney disease and cardiovascular disease [10]. *S. occidentalis* and a number of medicinal plants have been proven to possess anti-diabetic properties, as these plants are considered to be one of the good sources for production of new drugs [11]. A study published in 2011 shows the regeneration of Pancreatic β-cells in diabetic wistar rats, when the rats were administered aqueous *S. occidentalis* extracts, hence, useful for diabetes treatments [12]. More studies showed that despite the antidiabetic properties of *S. occidentalis*, there is a probable potential to induce hepatotoxicity due to its high content of active secondary metabolite that contain potent insulin mimetic and β-cell regenerating potentials [4].

The cells of aerobic organisms produce free radicals that affects several cellular organelles and components that could even lead to death of the cells [13]. These free radicals in excess, lead to Oxidative Stress and this stress could result to certain types of neurodegenerative diseases, heart disease, cancer, and ageing [13]. Highly reactive free radicals like the Reactive Oxygen and Nitrogen Species (RONS) damage the cell biomolecules at high concentration [14]. There are several enzymatic and nonenzymatic cellular defense systems which acts on these free radicals and neutralize them [15]. Example of these complex defense system (Antioxidants) include catalase, glutathione-S-transferase, superoxide dismutase. It is of importance that researchers access the plant-based antioxidants to obtain useful information on the efficacy of

several medicinal plants [2]. Oxidative Stress-induced cell death and the cell functions are defended by antioxidants [16]. High concentration of sugar in the blood promotes oxidation of glucose to form free radicals that could results to dysfunction when not scavenged by the endogenous antioxidants [17]. A study has shown the increment of free radicals and defects of antioxidants in cells from the onset of diabetes [18]. Enzymes like Nitric Oxide Synthase, Xanthine Oxidase, and NADPH Oxidase serve as sources of free radical generation in cells that could lead to diabetes and the mechanisms behind the free radicals-antioxidant activities leading to Diabetes include auto-oxidation of glucose, polyol pathway, hexosamine pathway, and mitochondrial respiratory chain [19].

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 model organism because it contains about 60-75% human disease genes and it possess a mammalian-like intestinal system with a fat body resembling the adipose tissue [18,20]. Just like mammals, *D. melanogaster* possess a specified antioxidant defense system; for this, they have been used to study several antioxidant properties of plants or conventional drugs and how they influence the body's oxidative stress [19]. Furthermore, this insect is favorable in carrying out research because of its short developmental cycle, the ease-of-use, and little space occupation, short fecundity, a well characterized genome, and availability of several mutant fly lines [21,22,20]. Studies like the Locomotor behavior (Negative geotaxis), the ability to produce offspring (Fecundity), and biochemical assays can provide several information on how *Drosophila* responds to external factors [23]. The greatly developed genetic composition, simplified insulin pathway, and other aforementioned advantages possessed by *D. melanogaster* makes it an excellent model organism for diabetes research [24]. Since *S. occidentalis* leaves has been used in treatment of diabetes [5], it is important to establish the safety and efficacy of this plant in terms of its hypoglycemic and antioxidant activities using *D. melanogaster* as a model organism.

This study is aimed at investigating the phytochemical content, hypoglycemic, and antioxidant activities of *Senna occidentalis* ethanolic leaf extract in *Drosophila melanogaster* (model organism) fed with high sucrose fly food. In regards to this research, the survival, biochemical and fecundity assay, as well as locomotor activities of treated *D. melanogaster* were studied.

2. MATERIALS AND METHODS

2.1 Plant Collection and Extraction

S. occidentalis leaves were collected from Fudawa Village Jos North Local government area, Plateau State, Nigeria. The preparation and extraction process were carried out according to the method described by Etuh et al. [25]. The leaves were rinsed off of sand and some particles under running tap water. The rinsed plant materials were air dried for 7 days at room temperature. The surface areas of these leaves were increased by pulverizing using a mortar and pestle. 70g of pulverized leaves of *S. occidentalis* was extracted using 70% ethanol and this was done by macerating for 72 hours. The solution was filtered and the filtrate was dried using a water bath at 30^0C as described by Etuh et al. [25]. Percentage total yield was calculated using the formular below:

$$\% \text{ extraction yield} = \frac{\text{Final weight of extract}}{\text{Original weight of crude powder}} \text{ x } 100$$

2.2 Phytochemical Screening of *S. occidentalis* Ethanolic Leaf Extracts

Qualitative phytochemical screening of the extract was carried out using a standard procedure described by Sofowara [26]. The ethanolic leaf extract of *S. occidentalis* was screened for the presence of various phytochemicals including alkaloids, phenols, tannins, flavonoids, saponins, glycosides, terpenoids, anthraquinones, and steroids.

2.3 *D. melanogaster* Strain and Culture

Wild type *D. melanogaster* (Harwich strain) was obtained from the Drosophila Research Laboratory of the Africa Centre of Excellence in Phytomedicine Research and Development (ACEPRD), University of Jos, Plateau state, Nigeria. All flies were maintained at a constant temperature of 23°C±2 and 60% relative humidity with about 12-hour light-dark clock cycle in vials containing standard fly food [18]. These flies are fed with corn meal food containing 100g of yellow-corn which serves as a source of carbohydrate, 10g of yeast as a source of protein, 10g of agar to help solidify the

food, 1g of methylparaben(dissolved with 10ml of absolute ethanol) which serves as a preservative to give the food a longer shelf life. Young flies of 1-3 days old were collected under mild ice anesthesia from stock vials and used for the study.

2.4 Determination of Median Lethal Dose (LD_{50}) and Survival Assay

According to Mohammed and Singh [27], the lethal dose is the amount of substance contained in a standard fly food that kills 50% of flies in seven days. In this experiment, fifty (50) flies each (1-3 day old), were anaesthetized using ice and collected into a transparent vial. These flies were exposed to series of different concentration of *S. occidentalis* ethanolic leaf extracts;5mg, 10mg, 50mg 100mg, 500mg and 1000mg each per 10g of fly food, and a baseline group of 1ml of distilled water mixed with 10g of fly food only, for 7 days, to determine the LD_{50} as described by Etuh et al. [25]. Each treatment was replicated three (3) times. Every 24 hours after exposure, the number of deaths on each treatment and their replicates are counted by observation. The $LD_{50}$was calculated by using the cumulative number of dead flies that was recorded after the 7^{th} day and this data was subjected to a dose-response simulation in a Graphpad prism 8.0.2.

The survival assay was carried out to observe the number of flies that could survive a treatment for a 28-dayperiod. It was done according to the method described by Abolaji et al. [28]. 50 flies each, of both genders, were exposed to;

1. 10 g of fly food mixed with 1 ml of water (Baseline group)
2. 1 mg of extract/10 g fly food
3. 10 mg of extract/10 g fly food
4. 100 mg of extract/10 g fly food

For 28 days. The experiment was replicated three (3) times. The number of live and dead flies was scored daily till the end of the experiment and the survival rate was expressed as percentage of live flies.

2.5 Induction of Diabetes in *D. melanogaster*

The method employed in this study to induce type 2 diabetes in *D. melanogaster* was described by Tennessen et al. [29], with a little modification. Fifty (50) young flies of 6 different groups were starved by transferring them into an empty vial for 24 hours. The flies were then fed with high-sucrose fly food containing 30% Sucrose, 8.3% cornmeal, 3.4% Yeast, 1% agar, methyl paraben as a preservative, and water (after starvation). The flies were observed for 10 days for symptoms of diabetes, which include delayed egg production, delayed emergence of larvae, decreased body size for both larvae and adult flies and decreased locomotor activities. All the flies in one of the groups were homogenized and their blood glucose concentration was ascertained using the RANDOX GOD-PAP assay kit according to the manufacturer's protocol to confirm the presence of high blood sugar (diabetes). The remaining five (5) groups were used for carrying out the next assay.

2.6 Hypoglycemic Activities of *S. occidentalis* leaf Extract in *D. melanogaster*

The Coogan [30] method with a little modification, was used to treat diabetic flies with different concentrations of the leaf extract (1-100 mg) of *S. occidentalis* and 16mg metformin (standard) respectively, for 7 days, after which their glucose levels were determined. Metformin is a drug used as a first-line therapy for treatment of type 2 diabetes and 16 mg is used as a standard control in *Drosophila* diabetes research work [31,32]. Six (6) groups of 50 flies (both gender) each, replicated three (3) times were exposed to fly food containing various treatments, including the control groups. The six (6) groups include;

1. Healthy flies that fed on 10 g fly food mixed with 1ml of distilled water (baseline group)
2. Untreated diabetic flies (negative control)
3. Diabetic flies treated with 16 mg metformin/10 g fly food (positive control)
4. Diabetic flies treated with 1 mg extract /10 g fly food
5. Diabetic flies treated with 10 mg extract /10 g fly food
6. Diabetic flies treated with 100 mg extract/10 g fly food.

2.7 Negative Geotaxis (Climbing Activity) and Fecundity (Emergence) Assay

The negative geotaxis assay is the climbing performance of *D. melanogaster* when exposed to the *S. occidentalis* leaf extract and this was done according to the method described by Abolaji et al. [28]. Ten (10) diabetic flies each,

were treated with various concentration of *S.occidentalis* leaf extract(1 mg, 10 mg, and 100 mg) and metformin (16 mg), for 5 days. After the 5th day, the flies (treated and untreated) were immobilized under mild ice anesthesia and placed separately in labeled vertical glass vials of 15 cm in length and1.5cm in diameter. The height of the vials was marked at 6cm. After the recovery period (about 20 min), the flies were gently tapped to the bottom of the vial. Following 6 seconds, the numbers of flies that climb up to the 6 cm mark of the vial, as well as those that remain below this mark were recorded. Data were expressed as the percentage of flies that moved beyond the 6cm mark in 6seconds. The score of each group was an average of three trials for each group of healthy, diabetic (untreated), and treated flies.

The fecundity assay was carried out to determine the reproductive effects of *S. occidentalis* leaf extract on *D. melanogaster r*and was carried out according to Bergland et al. [33]. Under ice anesthesia, ten (10) virgin diabetic fruit flies each, were collected to feed only food containing the several concentration of *S. occidentalis* leaf extract (1 mg, 10 mg, and 100 mg) and metformin (16 mg) for 5 days. Males and female flies were treated separately (separate vials) for the exposure period of 5 days. After the 5th day, flies of both sex (5 male, 5 female) from their respective treatment's groups were transferred into labelled vials (based on the treatment/control groups they were from) containing freshly prepared fly food (untreated) and allowed to mate for 24 hours. Thereafter, the adult flies were transferred off the food. The empty Vials were maintained at constant temperature and humidity (23±2 °C; 60% relative humidity, respectively) under 12-hour dark/light cycles for the development of eggs laid, for 12 days. This was observed and number of emerged flies were recorded every 24 hours up to the 12th day. The average number of flies that emerged from the treatment, negative and positive control, and baseline groups were computed and recorded as Mean ± SEM.

2.8 Sample Preparation for Biochemical Assays

The flies were prepared for biochemical assay as described by Abolaji et al. [28]. From the baseline, negative control(untreated), metformin, and *S. occidentalis* extract-treated groups, flies were anaesthetized under ice, weighed, and homogenized in 0.1 M phosphate buffer saline (PBS), pH 7.0 (1mg: 10µL). The homogenates were centrifuge for 10 minutes at 4000 rpm (temperature, 4°C). The supernatants obtained were used to quantify the Circulating glucose and total thiol content, and to determine the activities of Glutathione-S-transferase (GST) and Catalase (CAT) in the fruit flies.

2.8.1 Determination of circulating glucose

RANDOX GOD-PAP assay kit with glucose oxidase reagent was used in the determination of Glucose concentrations in the samples. The assay was carried out according to the manufacturer's protocol. The samples were diluted in 1:6 with Phosphate buffer saline (PBS) at pH 7.4 and mixed with the glucose oxidase reagents and incubated for 25minutes at 25℃ and read at 540 nm using a spectrophotometer (Jenway).

2.8.2 Total thiol determination

The total thiols level was assayed according to the method described by Ellman [34] with little modification. The total reaction mixture of 600 µl containing 25µl of sample, 510µl potassium phosphate buffer (0.1 M, pH 7.4), 35 µl of 5,5'-dithiobis-2-nitrobenzoic acid (DTNB) and 30 µl glutathione (GSH) was used. This was incubated for 30 minutes at 25°C and the absorbance was read at 412 nm. A glutathione (GSH) standard curve was prepared to calculate the total thiol content and the results were calculated and represented in mmol/mg protein.

2.8.3 Glutathione-S-transferase (GST) activity

Determination of the Glutathione-S-Transferase(GST) content of the sample was carried out according to the method described by Habig and Jakoby [35],with little modifications. Briefly, the assay reaction mixture was made up of 600 µl of a solution (20 µl of 0.25 M potassium phosphate buffer, pH 7.0, with 2.5 mM of ethylene diaminetetraacetic acid (EDTA)), 10 µl of distilled water and 500 µl of 0.1 M glutathione (GSH) at 25°C, 60 µl of sample (1:4 dilution), and 10 µl of 25 mM 1-chloro-2,4-dinitrobenzene (CDNB). The reaction was monitored for 2 min (10 s intervals) at 340 nm using a Spectrophotometer (Jenway). The data were expressed as mmol/min/mg protein.

2.8.4 Catalase (CAT) activity

Catalase activity was determined according to the method described by Aebi [36] and Abolaji [37] (with slight modifications). The reaction mixture was prepared and it contained 100μl of potassium phosphate buffer (pH 7.4) and 194 μl of 300 mM hydrogen peroxide (H_2O_2). The total reaction mixture was made up of 590 $\mu l$of H_2O_2 and 10 μl of the sample (1:60 dilution PBS) which were mixed together. The rate of dissociation of H_2O_2 was read for 2 minutes (10 seconds interval) at 240 nm at room temperature using a spectrophotometer (Jenway). Data was represented as mmol of H_2O_2 consumed/min/mg of protein.

2.9 Statistical Analysis

Using a Graph pad Prism version 7, the data were statistically analyzed with the help of a one-way ANOVA followed by Turkey's post-hoc test. Results were presented as mean ± SEM. At P<0.05, the results were considered statistically significant.

3.RESULTS

3.1 Percentage Yield and Phytochemical Screening

The percentage total of the extraction carried out on *S. occidentalis* with 70% ethanol was determined to be 14.5%. About eight (8) of the phytochemicals assayed for were present in *S. occidentalis* ethanolic leaf extracts (Table 1). The phytochemicals included tannins, alkaloids, glycosides, flavonoids, steroids, saponins, anthraquinones, and terpenoids. Phenols were completely absent.

3.3 Lethal Dose (LD_{50}) and Survival Assay

The calculated Lethal Dose (LD_{50}) result revealed a significant difference (P< 0.05) in fruit flies' mortality when exposed to500 mg and 1000mg concentration of *S. occidentalis* ethanolic leaf extract per 10 g food, compared to the baseline group, but there was no significant difference (P> 0.05) in mortality when exposed to 5 mg, 10 mg, 50 mg, and 100 mg *S. occidentalis* ethanolic leaf extract per 10 g food.TheLD_{50} was calculated to be 277.8 mg (Fig. 1).

The survival of fruit flies that were exposed to *S. occidentalis* ethanolic leaf extract of the different treatment showed a non-significant increase (P>0.05) in the survival of the flies, when compared to the baseline group (Fig. 2). 52.4% Survival was recorded in the baseline group, while, 56.3%, 60.1%, and 56.4% was recorded in 1 mg/10 g, 10 mg/10 g, and 100 mg/10 g per fly food of *S. occidentalis* ethanolic leaf extract group, respectively.

3.4 Antidiabetic Activities of *S. occidentalis* ethanolic Leaf Extracts in *D. melanogaster*

The result revealed a significant decrease (P <0.05) of free circulating glucose in 100 mg/10 g (0.223 ± 0.013) treated flies compared to negative control (0.571 ± 0.126) but there was a non-significant decrease (P>0.05) of free circulating glucose in the positive control (0.502 ± 0.029), 1 mg/10 g (0.475 ± 0.043), and 10 mg/10 g (0.384 ± 0.036) group when compared to the negative control group (Fig. 3). All treated groups showed lower circulating glucose when compared to the baseline group.

Table 1. Observations of phytochemicals assayed for in *S. occidentalis* ethanolic leaf extract

S/N	Photochemical	Observations
1	Tannins	+
2	Alkaloids	+
3	Glycoside	+
4	Flavonoids	+
5	Steroids	+
6	Saponins	+
7	Anthraquinones	+
8	Terpenoids	+
9	Phenols	-

+ Present- Absent

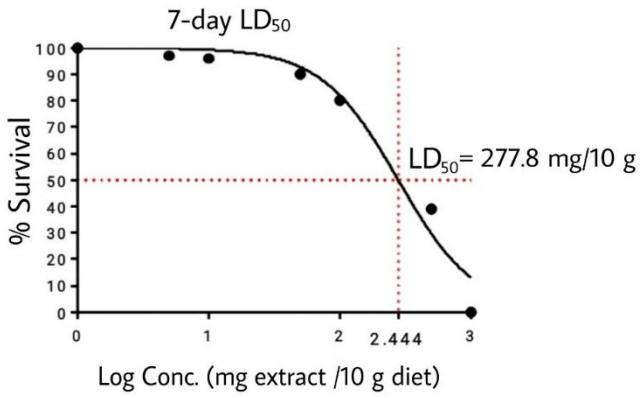

Fig. 1. 7-days LD50 of *S. occidentalis* ethanolic leaf extract in *D. melanogaster* (LD_{50}=277.8 mg/10 g fly food)

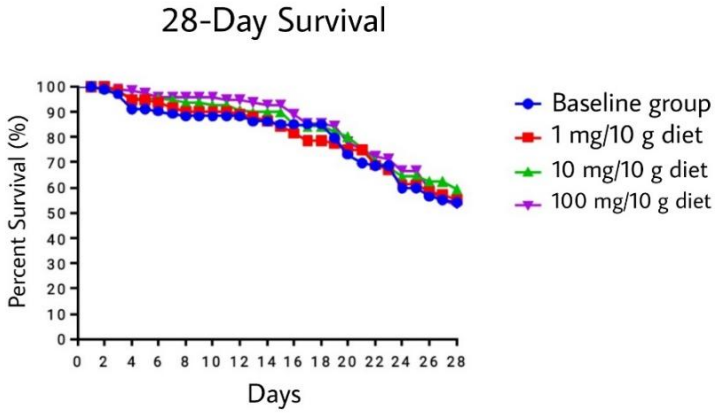

Fig. 2. 28-days survival curve of *D. melanogaster* administered *S. occidentalis* ethanolic leaf extract

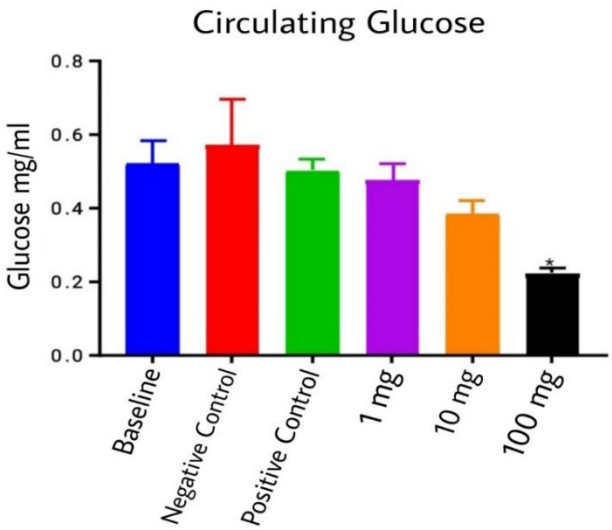

Fig. 3. Circulating Glucose in*D. melanogaster* administered *S. occidentalis* Ethanolic leaf extract

*(Data are presented as mean ± Standard error of means of three independent biological replicates. *P < 0.05 vs. control)*

3.5 Negative Geotaxis and Fecundity Assay

The negative geotaxis result showed no significant difference (*P*> 0.05) in climbing activities in the groups exposed to 1 mg/10 g (61.11 ± 5.879), 10mg/10 g (70 ± 5.092), and 100 mg/10 g (62.22 ± 12.370) *S. occidentalis* Ethanolic leaf extracts, and positive (54.443 ± 8.678) control (Fig. 4), compared to the negative control (67.781 ± 4.843).

The result of the fecundity assay (Fig. 5) showed that the emergence of *D. melanogaster* fed with high sucrose fly food (untreated)was significantly decreased (*P*<0.05) when compared to the emergence of healthy flies in the baseline group (20.5 ± 0.5). Supplementation with Metformin (14 ± 1.0), 1 mg/10 g (17 ± 1.0), 10 mg/10 g (15 ± 1.0), 100 mg/10 g (16 ± 3.0) *S. occidentalis* ethanolic leaf extract in *D. melanogaster* showed no significant difference (*P*> 0.05) in emergence when compared to the negative control group (untreated).

3.6 Total Thiol

The total thiol content (Fig. 7) was significantly higher (*P*<0.05) in *D. melanogaster* groups treated with 1 mg/10 g (0.028± 0.001), 10 mg/10 g (0.020 ± 0.003), 100 mg/10 g (0.021 ± 0.003) *S. occidentalis* ethanolic extract and metformin (0.022± 0.002) when compared to the negative control group (0.007 ± 0.001).

3.7 Glutathione-S-transferase (GST)

The result showed a significantly elevated (*P*< 0.05) Glutathione-S-transferase (GST) in 10 mg/10 g (77.03 ± 15.59) fly food treated fruit flies compared to the negative control group (18.97 ± 2.673). There was a non-significant increase (*P* > 0.05) of GST in flies fed with 1 mg/10 g (53.72 ± 9.732), 100 mg/10 g (63.34 ± 10.234) *S. occidentalis* ethanolic extract, and metformin (60.17 ± 13.151) when compared to the untreated diabetic flies (Fig. 7).

3.8 Catalase

There was a non-significant increase (*P*>0.05) of catalase activities in fruit flies fed with metformin (0.056 ± 0.006), 1 mg/10 g (0.066 ± 0.015), and 10 mg/10 g (0.044 ± 0.018) fly food when compared to the negative control group (0.020 ± 0.003). However, there was a non-significant decrease at 100 mg /10 g (0.017 ± 0.009) fly food compared to the negative control group. Result is shown in Fig. 8.

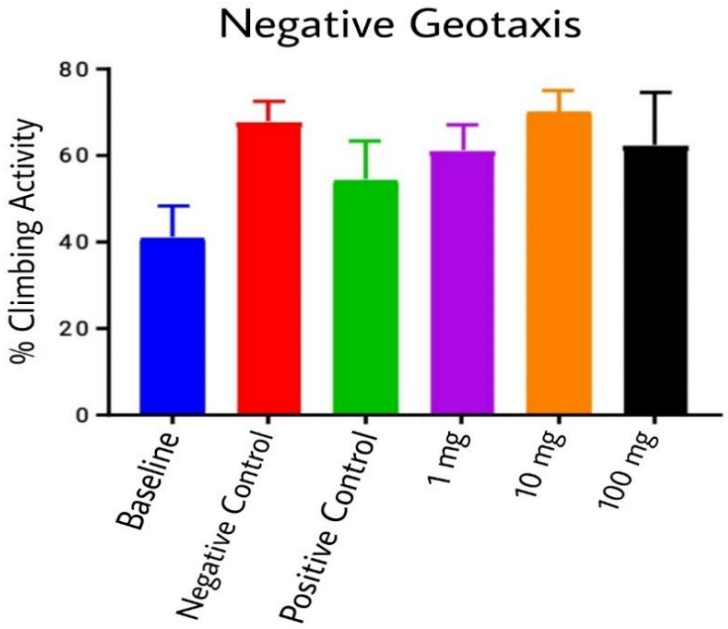

Fig. 4. Negative geotaxis (climbing activities) of *D. melanogaster* administered *S. occidentalis* ethanolic leaf extract

(*Data are presented as mean ± Standard error of means of three independent biological replicates. P> 0.05 vs. negative control)*

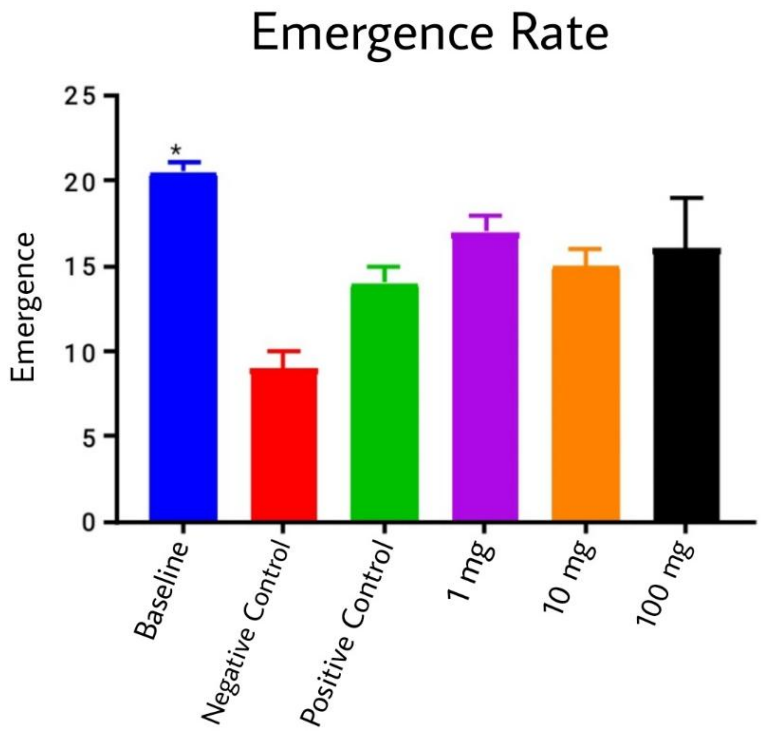

Fig. 5. Emergence rate of *D. melanogaster* administered *S. occidentalis* ethanolic leaf extract
*(Data are presented as mean ± Standard error of means of three independent biological replicates. *P < 0.05 vs. basal control and P>0.05 vs. negative control)*

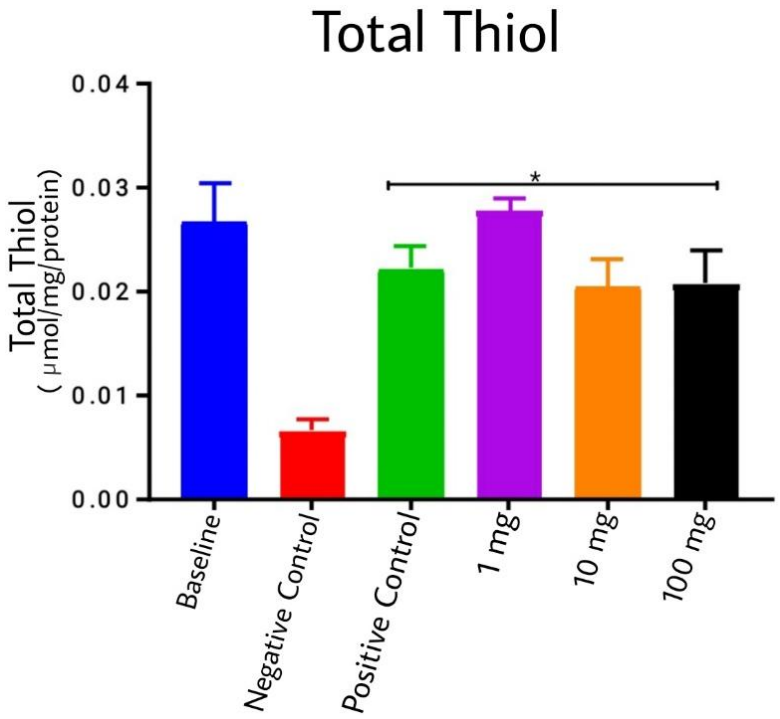

Fig. 6. Total thiol contents of *D. melanogaster* administered *S. occidentalis* ethanolic leaf extract
(*Data are presented as mean ± Standard error of means of three independent biological replicates. *P < 0.05 vs. negative control)*

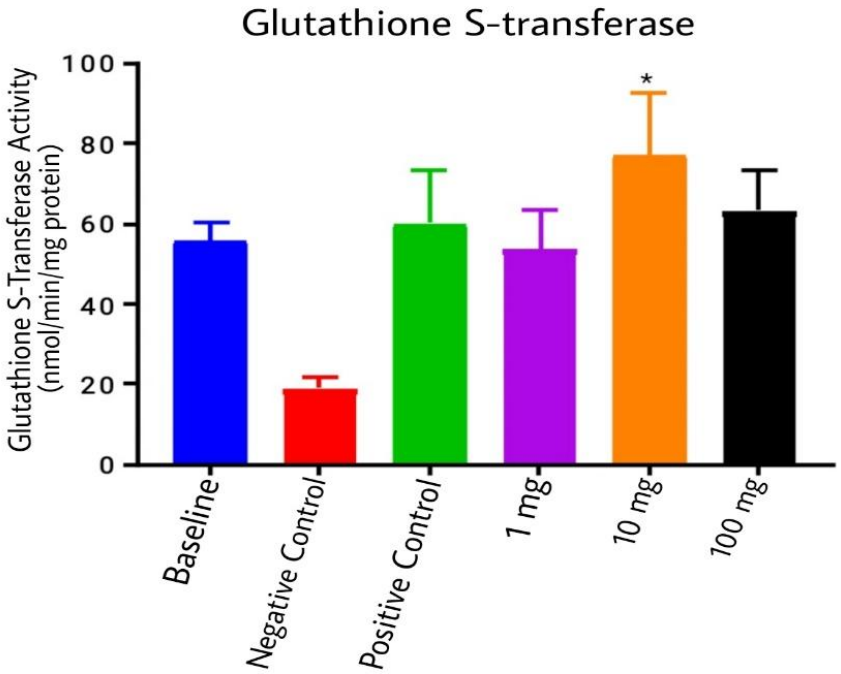

Fig. 7. GST Activities of *D. melanogaster* administered *S. occidentalis* ethanolic leaf extract
*(Data are presented as mean ± Standard error of means of three independent biological replicates. *P < 0.05 vs. negative control)*

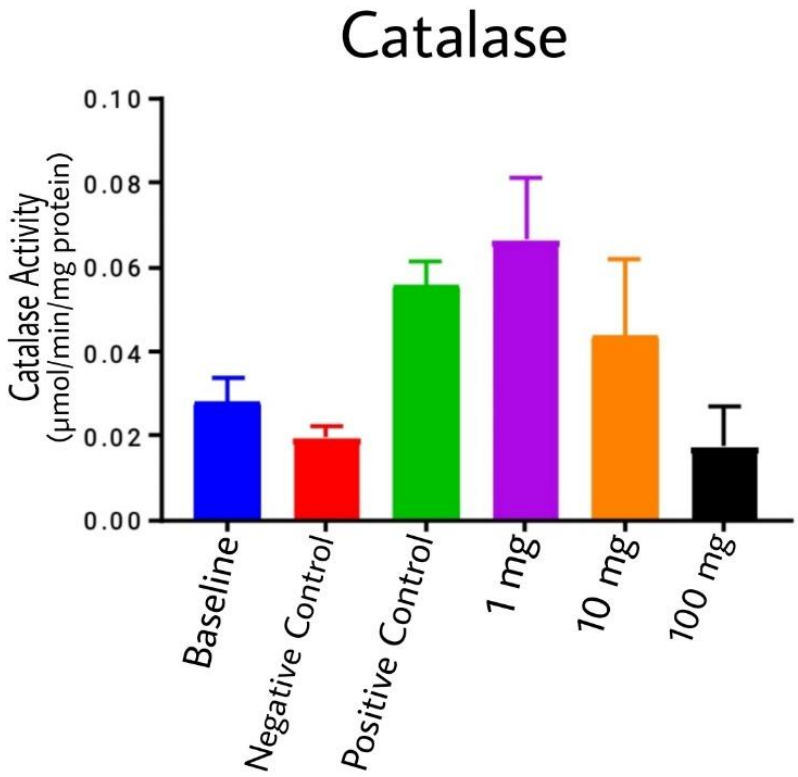

Fig. 8. Catalase activities of *D. melanogaster* administered *S. occidentalis* ethanolic leaf extract
(*Data are presented as mean ± Standard error of means of three independent biological replicates. P> 0.05 vs negative control)*

4. DISCUSSION

According to Orgi et al. [38], the medicinal properties of plants are attributed to the plant phytochemical contents. Phytochemical screening of *S. occidentalis* ethanolic leaf extract revealed the presence of tannins, glycosides, flavonoids, alkaloids, steroids, saponins, anthraquinones, and terpenoids while phenols were absent. This findings on the phytochemical screening agreed with Odeja et al. [39] who carried out Phytochemical screening, antioxidant and antimicrobial activities of *S. occidentalis*(L) leaves. Plants that exhibit hypoglycemic and antioxidant activities in animals (mice and fruit flies) mostly contain alkaloids and flavonoids as reported by Tiong et al. [40] who researched on the antidiabetic and antioxidant properties of alkaloids from *Catharanthus roseus* (L). Saponins naturally ward off microorganisms, and also protect against hyperglycemia, hypercholesterolemia, hypertension [38,41]. Terpenoids are active against bacteria and also as anti-diarrheal agent [41]. Tannins serve as healing and anti-inflammatory agents [38].

The LD50 was determined to be277.8 mg/10 fly food. Isah et al. [42] evaluated the LD50 of *S. occidentalis* in wistar rats and it resulted to be 5000 mg/ kg. Since the lethal dose is the amount of substance contained in a standard fly food that kills 50% of flies in seven days, it is shown that more than 50% of flies exposed to 500 mg/10 g and 1000 mg/10 g of *S. occidentalis* ethanolic leaf extract resulted to rapid mortality and there was a significant difference ($P< 0.05$) between these concentrations when compared to the untreated baseline group. Lower concentrations of extracts (5 mg, 10 mg/10 g. 50 mg/10 g, and 100 mg/10 g fly food) showed no significant difference ($P> 0.05$) in the mortality of flies, when compared to the baseline group.

From the findings, it was revealed that the treated flies survived more than those from the baseline group, although, there was no significant difference ($P> 0.05$) between their survival. This result suggests that *S. occidentalis* ethanolic leaf extract is relatively safe for *D.melanogaster* consumption. The acute or subacute administration of *S. occidentalis* extract to male and female wistar rats also showed no toxicity [43]. Since treated flies survived more than those from the baseline group, it can also be suggested that this extract have protective activities in *D. melanogaster.* Based on the survival percentage, the best concentration is 10 mg/10 g fly food.

The hypoglycemic activities revealed that 100 mg /10 g fly food of *S. occidentalis* ethanolic leaf extracts exhibited a significant reduction($P<0.05$) in the serum circulating glucose of *D. melanogaster* compared to negative control(untreated) group. This finding agrees with Gidado et al. [4] who reported that several dose of *S. occidentalis* leaf supplement significantly reduce the blood glucose level in alloxan-induced diabetic wistar rats. Verma et al. [12] also reported a significant hypoglycemic activity in wistar rats when treated with aqueous extract of *S. occidentalis* leaf extract. This hypoglycemic activity may be attributed to the presence of alkaloids and saponins in higher concentration [38,40]. Compared to the negative control, there was a slight decrease, although not significant ($P>0.05$), of free circulating glucose in the metformin-fed flies (positive control). This is as expected because Metformin is a drug used as a first-line therapy for treatment of type 2 diabetes, according to Maruthur et al. [31], hence, there could be a significant decrease of free circulating glucose if the concentration of metformin were to be increased per 10g of fly food. 1 mg and 10 mg-treated flies also revealed a non-significant decrease ($P>0.05$) in circulating glucose, when compared to the negative control. It is observed from the result that doses of the extract decreased free circulating glucose in a dose-dependent manner, with the highest dose (100 mg) having flies with the lowest free circulating glucose content. Hence, the result suggests that increasing the dosage of the extract will increase the hypoglycemic activities in diabetic fruit flies.

The result of the negative geotaxis showed a non-significantly difference ($P> 0.05$) in climbing activities of the treatments groups when compared to the negative control group(untreated), with some of the treatments (metformin, 1 mg, and 100 mg) having slight decrease in climbing activities while 10 mg-treated flies had a slight increase. Decrease of *D. melanogaster* climbing activities has been attributed to increased oxidative stress [42]. According to Abolaji et al. [28], increase in reactive oxygen and nitrogen species (RONS) and the *decrease* in acetylcholinesterase could lead to the decrease in climbing activity of *D. melanogaster*. Hence, it is suggested that *S. occidentalis* ethanolic leaf extract at certain concentration did not affect the climbing activities of *D. melanogaster.* The negative geotaxis

activities of *S. occidentalis* ethanolic extract and metformin-treated flies ranged from 54.44 ± 8.678 – 62.22 ± 12.37 with metformin treated-flies having the lowest climbing activity and 100 mg-treated flies having the highest.

The fecundity assay showed that the emergence of *D. melanogaster* fed with high sucrose food (untreated) was significantly decreased (P<0.05) when compared to the emergence of flies in the baseline group. This decrease in reproduction can be attributed to increase in oxidative stress as suggested by Adedara et al. [44]. According to Perkins et al. [45] and Haghnazari et al. [46], this oxidative stress could lead to the decrease in amount and function of the reproductive cells of the male *and* the decrease in the female oocyte, hence, affecting fertilization. Bajaj & khan [17] illustrated that high concentration of sugar in the blood promotes the oxidation of glucose to form free radicals that could be detrimental to the cells, hence, affecting reproduction. Furthermore, when diabetic flies were treated with food containing metformin, 1 mg/10 g, 10 mg/10 g, 100 mg/10 g *S. occidentalis* ethanolic leaf extract, there was a non-significant increase (P> 0.05) in emergence compared to the negative control group. This increase was in a non-linear manner and may be attributed to the antioxidant properties of metformin and *S. occidentalis* ethanolic leaf extract [47,48].

The antioxidant activities of *S. occidentalis e*thanolic leaf extract and metformin in *D. melanogaster* revealed a significant elevation in total thiol content in the treated flies compared to the flies in the negative control (untreated) group. The total thiol content of the treated flies ranged from 0.020 ± 0.003 – 0.028 ± 0.001 µmol/mgprotein with 1mg-treated flies having the highest total thiol content and 10mg-treated flies having the lowest. All concentrations were observed to increase the total thiol content significantly, but 1 mg/10 g of the extract is observed to be the best concentration to increase the total thiol content in diabetic *D. melanogaster*. By enzymatic and non-enzymatic processes, total thiol has been proven to destroy reactive oxygen species (ROS) and free radicals, hence, acting as a marker of oxidative stress [49]. This result suggests that metformin and *S. occidentalis* ethanolic leaf extract (at certain concentrations) increases total thiol content (significantly) in hyperglycemic *D. melanogaster,* hence, increasing the antioxidant activities.

Glutathione-S-transferase (GST) activities were significantly elevated in 10 mg-treated flies compared to the flies in the negative control group (untreated), and there was a non-significant increase (P> 0.05) of GST in flies fed with 1 mg/10 g, 100 mg/10 g *S. occidentalis* ethanolic extract, and metformin when compared to the GST of untreated diabetic flies. This result agrees with that of Lapshina et al. [50] who tested the glutathione-S-transferase activities in diabetic rats and the result showed a significant increase in GST content compared the control. The GST content of the treated flies ranged from 53.72 ± 9.73 –77.03 ± 15.59 nmol/min/mgprotein with 10 mg-treated flies having the highest GST content and 1 mg-treated flies having the lowest (not dose-dependent). Hence, 10 mg/10 g of the extract is shown to be the best concentration to increase GST activities in diabetic flies. Since GST is an enzymatic antioxidant that destroys electrophilic pro-oxidants [43], the increase in its content in diabetic *D. melanogaster* may be due to the antioxidant activities possessed by the different concentration *S. occidentalis* ethanolic extract and metformin. Study has shown that the present of flavonoids influences the GST potentials in cells [51].

The catalase activities of *S. occidentalis* ethanolic extract and metformin-treated flies ranged from 0.02 ± 0.009 – 0.07 ± 0.015 µmol/min/mgprotein with 1 mg-treated flies having the highest CAT content and 100 mg-treated flies having the lowest. This was ranged in a dose dependent manner and the result suggests that increase in concentration of the extract leads to the decrease in catalase activities. So, lower concentration of the extract per 10 g fly food is ideal for improving the catalase activities in diabetic fruit flies. Compared to negative control group, the catalase activities of treated flies were elevated (non-significantly) except in 100 mg/10 g-treated flies, where catalase activities were reduced(non-significantly).Catalase help protect the cells from the deleterious effect of $H_2O_2$by reducing it to H_2O and O_2 [52], hence, this result suggests that certain concentrations of *S. occidentalis* ethanolic leaf extract and metformin improves the antioxidant activities in hyperglycemic *D.* melanogaster by improving the production of catalase to scavenge free radicals, but at certain concentration, reduces the production.

5. CONCLUSION

In conclusion, the results suggests that *S. occidentalis* ethanolic leaf extracts possess

hypoglycemic and antioxidant properties, and it can be used alone or in combination with conventional drugs for the treatment of diabetes. Results also showed that the extract is relatively safe and it contains several therapeutic phytochemicals, making it an ideal alternative in the treatment of diabetes.

7. RECCOMENDATION

It is recommended that more advanced studies be carried out on thehypoglycemic and antioxidant properties of *Senna occidentalis* at molecular level, to ascertain a more detailed knowledge on this study.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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