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RESEARCH ARTICLE

Potential anti-diabetic effect of certain plant extracts from the Egyptian flora on type II diabetes using *Drosophila melanogaster* as an animal model

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ABSTRACT

Background: Type 2 diabetes (T2D) remains one of the most important diseases that threaten human health worldwide. Hence, there is a great demand for a safe and effective treatment. **Aim:** This study aimed to evaluate the effects of extracts of the aerial parts of four Egyptian flora namely *Moringa oleifera* Lam*, Morus nigra* L.*, Tagetes patula* L. leaves and *Atriplex halimus* L on T2D using *Drosophila melanogaster* as a model system. **Material and Methods:** A high sugar diet (HSD) was used to induce T2D in *Drosophila* larvae. The four plants were subjected to phytochemical screening. Plant extracts were dissolved with the control diet at a concentration of $1x10^4$ µg/ml. Glimepiride (Amaryl®) drug at concertation of 80 µg/ml was used in the diet as a marker. Titers of glucose and trehalose were measured in the larval whole body and hemolymph in HSD-induced and plant extract-treated larvae along with the controls. The weight of larvae was also recorded. **Results:** The results revealed that HSD significantly increased the titer of glucose and trehalose in the larval whole body compared with the control. The larvae fed on HSD lost an amount of their weight compared with the control. The plant extracts of *M. oleifera, A. halimus*, and *T. patula* induced a significant decline in the whole-body glucose and trehalose. Regarding the larval weight, *M. nigra*, *A. halimus,* and *T. patula* extracts induced larvae to regain their weight. Phytochemical screening of the tested plants revealed the presence of several constituents such as carbohydrates, glycosides, sterols and/or triterpenes, catechol tannins and flavonoids. **Conclusion:** The tested plant extracts, particularly *A. halimus* can recover and improve the symptoms of T2D in *Drosophila*.

Keywords: *Atriplex halimus;* Blood sugar; Fruit fly; Metabolic Disorder; *Tagetes patula*

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INTRODUCTION

Diabetes mellitus (DM) is a complex chronic systemic disease associated with metabolic disorders, including hyperglycemia, hyperinsulinemia, and hypertriglyceridemia (Xu et al., 2018). Diabetes mellitus is categorized into type 1 diabetes (T1D) and type 2 diabetes (T2D); T2D accounts for almost 95% of individuals worldwide (Thomas and Philipson, 2015). Type 2 diabetes is primarily manifested by insulin resistance or deficiency in insulin secretion (DeFronzo, 2004). Mammals, basically depend on insulin secretion to metabolize the blood sugar for energy and survival (Murillo-Maldonado and Riesgo-Escovar, 2017). Insulin is secreted from pancreatic Langerhans islets ß-type cells in response to elevated glucose levels (Álvarez-Rendón et al., 2018). In the bloodstream, it binds to plasma membrane receptors particularly in muscle cells and activates a signalling cascade that leads the glucose transporter GLUT4 to allow glucose to enter the cell (Murillo-Maldonado and Riesgo-Escovar, 2017; Álvarez-Rendón et al., 2018). Insulin also induces glucose uptake in skeletal muscles, liver, and adipose tissue (main target tissues of insulin action), promotes the synthesis of glycogen and prevents glycogen breakdown (LeRoith and Accili, 2008; Murillo-Maldonado and Riesgo-Escovar, 2017). Type 2

diabetes can cause numerous organ-lesions and complications (Forbes and Cooper, 2013). High blood glucose causes acute and chronic complications. Some acute complications are mainly associated with coma, hypoglycemia and ketoacidosis, while chronic complications are most destructive to the body organs (Xu et al., 2018). The microvascular complications can lead to diabetic retinopathy, diabetic nephropathy and diabetic neuropathy (Labazi and Trask, 2017). In addition, macrovascular complications involve cardiovascular and cerebrovascular diseases (Konig et al., 2013).

Currently, many hypoglycemic drugs are used in the treatment of diabetes including sulfonylureas (such as chlorpropamide, tolazamide, glipizide, and glimepiride), biguanides, thiazolidinediones, glinide, and glyburide. However, some cases do not respond positively to the treatment (Khunti et al., 2018; Kazemian et al., 2019). In addition, synthetic drugs have several defects including drug resistance, adverse effects and toxicity (Dey et al., 2002). Thus, it is vital to develop new anti-diabetic treatments (Xu et al., 2018).

Several reports recommended the use of herbal drugs to treat DM and its complications. Moreover, the majority in developing countries still depends on medicinal plants (Mahmoud and Gairola, 2013; Hosseinzadeh et al., 2015; Nazarian-Samani et al., 2018)**.** Natural products are considered the best choice for improving diabetes complications for their availability, low cost, lack of side effects, and powerful pharmacological actions (Sharma and Arya, 2011; Mishra et al., 2018). Plant extracts are a rich source of many metabolites like alkaloids, glycosides, flavonoids, steroids, polyphenols, tannins, saponins, and terpenoids which are responsible for the antidiabetic action (Satyavati et al., 1989; Savithramma et al., 2011). Earlier studies reported the beneficial use of *Moringa oleifera* Lam*, Morus nigra* L., *Atriplex halimus* L., and *Tagetes patula* L. as anti-diabetic, anti-inflammatory, antioxidant, and hepatoprotective agents (Day, 1998; Wong et al., 2006; Volpato et al., 2011; Chen et al., 2016; Kushwaha and Verma, 2017; Vergara-Jimenez et al., 2017; Khaoula and Ali, 2020).

Different model organisms were used to identify the pharmacological properties of different plant extracts such as mice (Adhikarimayum et al., 2007), Zebrafish (Giacomotto and Ségalat, 2010), *Caenorhabditis elegans* (Kumarasingha et al., 2016), and *Drosophila melanogaster* (Mezzoug et al., 2006). Each model organism possesses special advantages and limitations (Panchal and Tiwari, 2017). Many features make a fruit fly the ideal model for experimentation as it is easy to handle, produces a large number of flies, and the life cycle is short (Va et al., 2009; Panchal and Tiwari, 2017). This model has approximately 75% similarity with disease-causing genes in humans (Reiter et al., 2001; Nass and Przedborski, 2011; Sengupta et al., 2016). Many studies have revealed that most biological signaling pathways are conserved between the fruit fly and human (Apidianakis and Rahme, 2011; O'Kane, 2011) Additionally, this model can simply feed on a diet to cause the disease and then transferred to another diet mixed with the plant extract to observe the recovery (changes of phenotypes and behaviour) (Panchal and Tiwari, 2017). In recent years, *Drosophila* has become a valid model to study metabolism, physiology, and the development of metabolic diseases, such as obesity, diabetes, etc… (Haselton and Fridell, 2010; Morris et al., 2012).

Therefore, the present study aimed to evaluate the effect of four plant extracts, *M. oleifera, M. nigra, A. halimus,* and *T. patula* from Egyptian flora compared to Glimepiride (Amaryl®) drug on the T2D in *D. melanogaster*.

MATERIAL AND METHODS

Fly strain and diet

Egyptian wild type of *Drosophila melanogaster* was obtained from the Department of Entomology, Faculty of Science, Ain Shams University. It is used in the current study as a model for T2D. In all experiments, insects were kept in the laboratory at 25 ± 2 ˚C, 70-80 % RH and 12D:12L hr photo cycle. The fly diet was prepared with a recipe of semi-defined medium with slight modifications (14 gm agar, 80 gm yeast, 20 gm peptone, 20 gm yeast extract, and 51 gm sucrose dissolved in 1 L distilled water for control diet). To induce T2D, high sugar diet

(HSD) was used by increasing the sucrose to 342 gm according to Musselman et al. (2011). Flies were maintained in transparent sterilized vials (100 ml size) containing about 25 ml semi-defined medium and plugged with a piece of sponge for ventilation (BDSC, 2019). At least five biological replicates were used.

Plant materials

Aerial parts of *Atriplex halimus* L. (Family Amaranthaceae) were collected from Balteem, Kafr El-Sheikh Governorate in February 2014. Leaves of *Moringa oleifera* Lam. (Family Moringaceae) and *Tagetes patula* L. (Family Asteraceae) were collected in November 2018 from the plants cultivated in the Experimental Station of Medicinal Plants, Faculty of Pharmacy, Tanta University. Leaves *of Morus nigra* L. (Family Moraceae) were collected from Dessouk, Kafr El-Sheikh Governorate in May 2019. *Atriplex halimus* L. and *Morus nigra* L. were identified according to Huxley et al. (1999) at the Department of Botany, Faculty of Sciences, Tanta University. Voucher specimens were deposited in the Herbarium of the Faculty of Pharmacy, Tanta University. The air-dried collected plant organs were separately reduced to fine powder by an electrical grinder, then packed in tightly closed containers.

Preliminary phytochemical screening of plants under investigation

The dried powdered leaves of *M. oleifera* Lam., *T. patula* L., *M. nigra* L., and the dried powdered aerial parts of *A. halimus* L. (50 gm of each) were separately subjected to preliminary phytochemical screening for various constituents by using qualitative chemical tests described in previous reports (Shellard, 1957; Gonzalez and Delgado, 1962; Robinson, 1964; Harborne, 1973; Harborne, 1998; Parthasarathy et al., 2009) as the following:

- Molisch's test for carbohydrates/ glycosides.
- Keller-Killiani, Kedde and Baljet tests for cardiac glycosides (cardenolides).
- Salkowski and Liberman-Burchard tests for unsaturated sterols and triterpenes.
- Mayer's test for alkaloids/basic nitrogenous compounds.
- Ferric chloride test for tannins and phenolic compounds.
- Aluminium chloride test and 10% sodium hydroxide test for flavonoids.
- Froth test for saponins.

Plant material preparation and Amaryl

Plant powdered leaves of *M. oleifera* Lam., *T. patula* L. and *M. nigra* L. (50 gm of each) were separately extracted trice with methanol by sonication for 6 hr (30 min × 12) at room temperature. The extracts were concentrated under reduced pressure to give residues (3.5 gm) for *M. oleifera*, (5.1 gm) for *T. patula*, and (4.6 gm) for *M. nigra*. Aerial parts powder of *A. halimus* (50 gm) was extracted trice with methanol: dichloromethane (1:1) by sonication for 6 hr (30 min × 12) at room temperature. The extract was concentrated under reduced pressure to afford a residue (5.8 gm). Glimepiride (Amaryl®) (Sanofi, Egypt) was used as a food additive to recover T2D symptoms, as it is commonly used in Egypt.

Experimental setting

Fifty inseminated adult females (7-day old) were transferred to a new vial and allowed to lay eggs on a control diet overnight. After two days, about 50 early 2nd instar larvae were transferred to develop on HSD for 48 hours (to induce T2D). Then, the early 3rd instar larvae were taken from HSD and were transferred into two vials; one contains a control diet, and the other contains a diet with 1x10^4 µg/ml of each plant extract. Regarding Amaryl, the vial contains 80 µg/ml of concentration of Glimepiride mixed with the control diet. The larvae were let to develop for 24 hours in all treatments. This procedure was repeated five times for each determination.

Glucose and trehalose measurements in *Drosophila* larval homogenate

To check T2D in *Drosophila* larvae, the main sugars (Trehalose and Glucose) were determined in the body homogenate. Eight to fifteen 3rd instar larvae (equal 10 mg) were weighed from control, high sugar, and plant extract-treated diets on the fifth day after egg-laying. The samples were immediately cooled in ice and homogenized in 100 µl cold trehalase buffer (TB) (5 mM Tris pH 6.6, 137 mM NaCl, and 2.7 mM KCl). The homogenates were heated in a thermomixer at 70 ˚C for 10 minutes. Then, all samples were centrifuged at 4 ˚C, 5000 *xg* for 5 minutes.

A twenty µl of supernatant was mixed with 20 µl trehalase buffer to measure free glucose and another amount mixed with 20 µl porcine trehalase (Sigma; T8778-1UN, Germany) to measure digested trehalose. Two standards, Glucose (spectrum©, Egypt) and trehalose (T9531, Sigma-Aldrich, Germany) were prepared at a concentration of 25 mg/ml. These standards were treated as samples for glucose or trehalose determination. Blank was prepared using 40 µl of TB. All samples were incubated at 37 ˚C overnight. Thereafter, samples were centrifuged at 4 ˚C, 5000 *xg*, for 5 minutes. The amount of glucose was determined using a glucose assay kit (spectrum©, Egypt), by adding 1 ml of glucose reagent to 20 µl from each sample. Finally, all samples were measured on a spectrophotometer (UNICO S1200, USA) at 546 nm (Tennessen et al., 2014). The trehalose concentration in the sample was determined by subtracting the free glucose concentration from the total glucose concentration. This procedure was replicated five times from each treatment.

Effect of plant extracts and Amaryl drug on larval weight

As diabetes may be reflected in the weight of patients, 10 (L3) *Drosophila* larvae were collected from control, high sugar, and plant extract-treated diets as done earlier. The larvae were washed in Phosphate-buffered saline (PBS) (0.1 M, pH 7.2) then dried on Whatman filter paper and were weighed. This was replicated five times from each treatment.

Effect of plant extracts and Amaryl drug on glucose and trehalose measurements in larval hemolymph

For this experiment, we determined the trehalose and glucose in the larval hemolymph following treatment with *A. halimus* extract (new candidate). Twenty 3rd instar *D. melanogaster* larvae were picked from control, high sugar, and plant extract-treated diets. Then, the larvae were disrupted from the posterior cuticle gently with forceps under a dissecting microscope on ice (Hiroyasu et al., 2018). The hemolymph oozed from larvae (approximately 10 µl) is diluted with 100 µl of TB. These samples are treated as reported previously in the larval homogenate experiment. This procedure was replicated five times for each treatment.

Statistical analysis

Data were expressed as mean ± standard deviation (M ± SD). The response variables were checked for normality using Anderson-Darling test and homogeneity of variances using Bartlett test. The difference in the diet on larval weight, glucose, trehalose was examined by using one-way ANOVA accompanied by Bonferroni test for multiple comparisons. Non-normal data were analysed using Kruskal-Wallis test accompanied by Dunn test for multiple comparisons. All tests were done in GraphPad Prism version 8.0.2 for Windows, GraphPad Software, San Diego, California USA, www.graphpad.com.

RESULTS

Preliminary phytochemical screening of plants under investigation

All investigated plants contain carbohydrates and/or glycosides, sterols and/or triterpenes, catechol tannins and flavonoids. *M. oleifera, T. patula* leaves and *A. halimus* aerial parts contain alkaloids. Saponins are present in both *M. oleifera* Leaves and *A. halimus* aerial parts. Cardenolides are absent in all investigated plants (Table 1).

Effect of plant extracts and Amaryl drug on total body glucose of diabetic third instar larvae of *Drosophila melanogaster*

As shown in Figures 1a-d, there are significant ($P < 0.01$) differences in the whole body glucose among the different treatments (ANOVA and Kruskal-Wallis test). The total body glucose of HSD-fed larvae was significantly ($P < 0.033$) higher than that in the control (Bonferroni and Dunn tests). However, when the diabetic larvae fed on a diet treated with the plant extract or Amaryl drug, the total body glucose significantly ($P < 0.033$) decreased compared with those in HSD-fed larvae. All plant extracts used were able to recover the total body glucose near the control level. Meanwhile, the Amaryl drug significantly ($P < 0.033$) decreased the body glucose lower than the control.

Effect of plant extracts and Amaryl drug on the total body trehalose of diabetic third instar larvae of *Drosophila melanogaster*

There are significant ($P < 0.05$) differences in the whole body trehalose among the different treatments (Figures 2a-d) (ANOVA). The high sugar diet induced a significant ($P < 0.033$) increase in the total body trehalose of *D. melanogaster* larvae compared with the control (Bonferroni test). *Atriplex halimus* and *T. patula* extracts and Amaryl drug were able to significantly ($P < 0.033$) decrease the body trehalose compared with HSD. The levels of body trehalose following treatment with *A. halimus* and *T. patula* extracts reached the control level.

Effect of plant extracts and Amaryl drug on the total body weight of diabetic third instar larvae of *Drosophila melanogaster*

Data in Figures 3a-d demonstrated significant ($P < 0.001$) differences in *Drosophila* body weight among treatments (ANOVA). Bonferroni Multiple comparisons indicated that HSD significantly ($P < 0.033$) decreased the total body weight of third instar larvae of *D. melanogaster* compared to the control. Bonferroni multiple comparisons indicated that all plants extracts and Amaryl drug significantly ($P < 0.033$) increased the weight of treated larvae compared with the diabetic larvae. In particular, *M. nigra* was able to recover the larval weight to the control level (un-significant difference $P \geq 0.033$).

Effect of total extract of *Atriplex halimus* on the hemolymph glucose and trehalose of diabetic third instar larvae of *Drosophila melanogaster*

ANOVA results showed significant ($P < 0.01$) differences in the hemolymph glucose and trehalose among the different treatments (Figures 4a, b). The HSD induced higher ($P < 0.033$) levels in hemolymph glucose and trehalose in *Drosophila* larvae compared with the controls (Bonferroni test). Bonferroni test showed that the methanolic extract of *A. halimus* was able to significantly ($P < 0.033$) decrease the titers of hemolymph glucose and trehalose ($P < 0.033$) compared with those in HSD. These levels observed were near the control level.

DISCUSSION

Many drugs such as biguanides, thiazolidinediones and sulfonylureas are widely used for T2D treatment (Salehi et al., 2019). However, these drugs could recover the glucose levels, but they fail to achieve complete treatment of its complications (Rao et al., 2010). Moreover, several side effects have been reported on the use of those synthetic agents. These effects include drug resistance, physiological disturbance, and toxicity (Dey et al., 2002). Accordingly, the search for effective and safe alternatives from a natural source is needed (Wadkar et al., 2008).

Natural products especially of plant origin play an important role in the treatment of diabetes in traditional medicine all over the world (Al-Snafi et al., 2019). Therefore, in the current study, certain medicinal plant extracts from the Egyptian flora were tested compared with glimepiride (Amaryl) against the diabetic fruit fly. Many studies have shown that most of the biological signaling pathways are conserved between fly fruit and human (Apidianakis and Rahme, 2011; O'Kane, 2011). Thus, it has been used as a model organism for diabetes. *Drosophila* possesses specialized insulin-producing cells in the brain which secret eight insulin-like peptides (Nässel et al., 2013). These cells resemble mammalian pancreatic beta cells (Broughton et al., 2005; Garelli et al., 2012). It has been reported that HSD induces clear features of insulin resistance in *Drosophila* which is the principal pathology of T2D in humans (Teleman, 2010; Murillo-Maldonado et al., 2011). *Drosophila* like most organisms such as fungi, yeast, plants, and invertebrates (Elbein et al., 2003), have two main forms of circulating sugars: glucose and trehalose (Thompson, 2003). However, trehalose is the primary hemolymph sugar in the fruit fly (Wyatt and Kalf, 1957). It is synthesized from glucose in the fat body by trehalose-6-phosphate synthase (Matsuda et al., 2015). The results of the current study showed that HSD increased the levels of glucose and trehalose in the whole body and hemolymph of *Drosophila* compared with the controls.

Table 1. Phytochemical screening of the leaves and aerial parts of the plants tested

Constituent	*Moringa oleifera* leaves	*Tagetes patula* leaves	*Morus nigra* leaves	*Atriplex halimus* aerial parts
Carbohydrate	+	+	+	+
Cardenolides	_	_	_	_
Unsaturated Sterols and/ or Triterpenes	+	+	+	+
Alkaloids	+	+	_	+
Tannins (catechol)	+	+	+	+
Flavonoids	+	+	+	+
Saponins	+	_	_	+

(+) refers to the present constituents, while (-) refers to the absent ones.

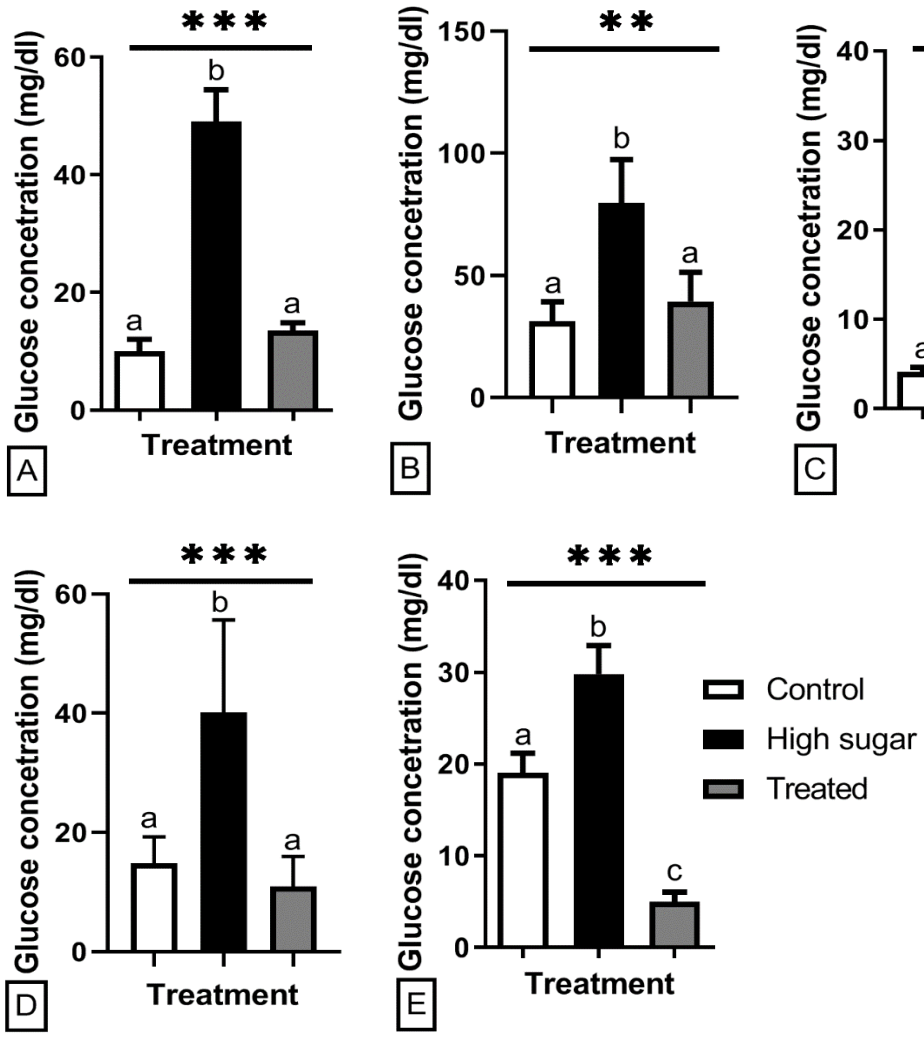

Figure 1. Total body glucose (Mean ± SD) of in *Drosophila melanogaster* larvae fed on *Moringa oleifera* (A), *Morus nigra* (B), *Atriplex halimus* (C)*, Tagetes patula* (D) methanolic extracts and Amaryl drug (E) in diets. *n* = 5 replicates (15 pooled samples). ** & *** refer to significant differences among means when $P < 0.01$ and < 0.001, respectively (One-way ANOVA or Kruskal-Wallis test). The bars with the same letters are not significantly different at $P \geq 0.033$ (Bonferroni or Dunn test for multiple comparisons).

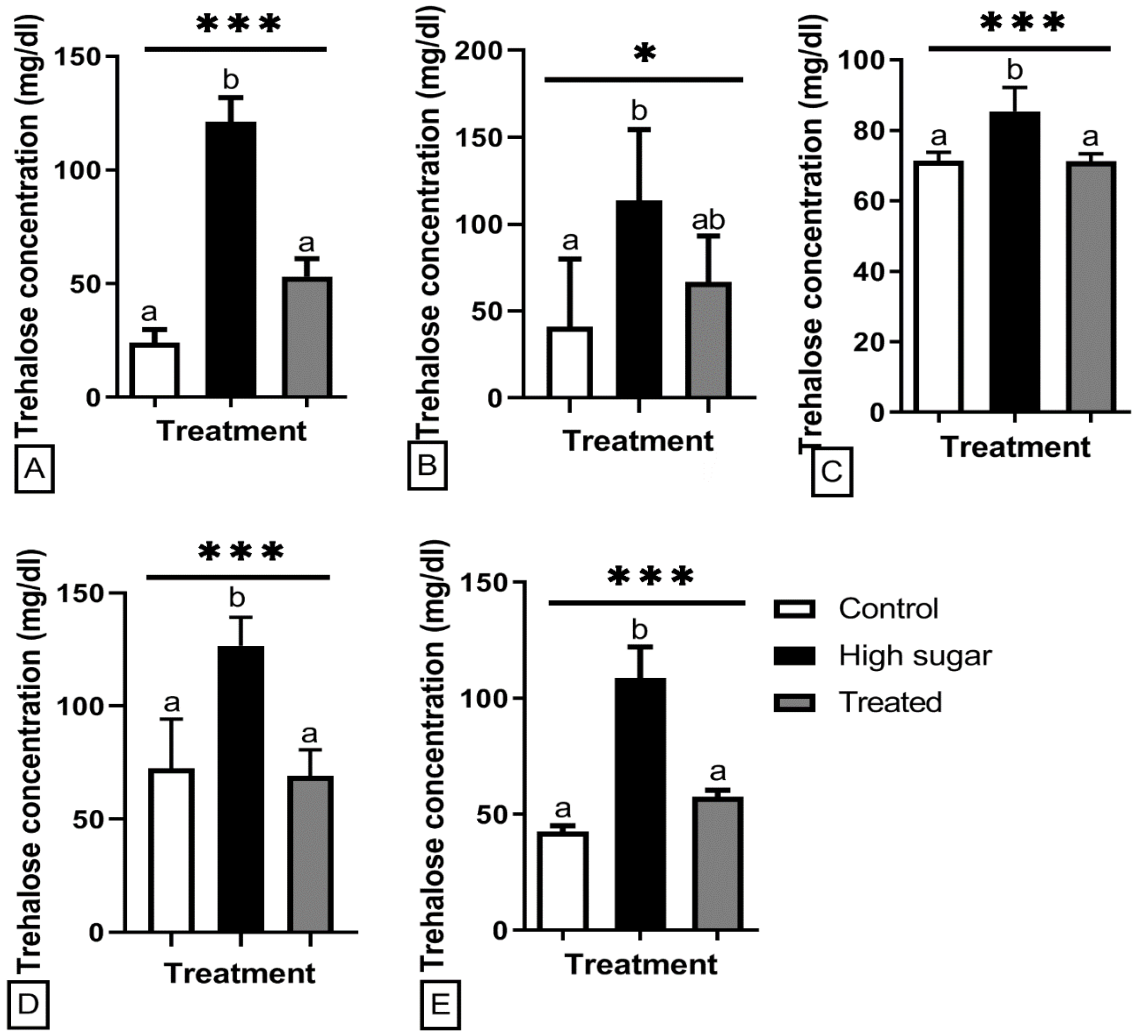

Figure 2. Total body trehalose (Mean ± SD) in *Drosophila melanogaster* larvae fed on *Moringa oleifera* (A), *Morus nigra* (B), *Atriplex halimus* (C), *Tagetes patula* (D) methanolic extracts and *Amaryl drug* (E) in diets. *n* = 5 replicates (15 pooled samples). * & *** refer to significant differences among means when $P < 0.05$ and < 0.001, respectively (One-way ANOVA). The bars with the same letters are not significantly different at $P \geq 0.033$ (Bonferroni multiple comparisons).

These results are consistent with the findings of Musselman et al. (2011) who observed an increase in glucose and trehalose levels in the blood of HSD-fed *Drosophila*. Rulifson et al. (2002) and Song et al. (2010) attributed the elevation in trehalose level to insulin deficiency and insulin resistance.

Our results also revealed that the weight of diabetic larvae decreased compared with the controls. Similar results were reported by Musselman et al. (2011). This reduction in body weight was similar to that observed in insulin receptor mutant (Brogiolo et al., 2001). The reduction in the weight of the larvae may be attributed to a deficiency in the insect physiological functions like digestion and absorption. Chen et al. (2021) reported that the diabetic flies had difficulty in absorbing and utilizing carbohydrates from the diet.

The above-mentioned results concluded that HSD successfully induced the features of T2D in *Drosophila*. So that, the antidiabetic effect of *M. oleifera, M. nigra, A. halimus, and T. patula* were tested in *Drosophila* larvae.

The plant extracts of *M. oleifera, A. halimus, and T. patula* could recover the glucose and trehalose levels to the control level. Previous studies reported the role of these plants in the diabetes recovery in insects and vertebrates. Oyeniran et al. (2020) reported a significant reduction of glucose in adult *Drosophila* due to the treatment with *M. oleifera*.

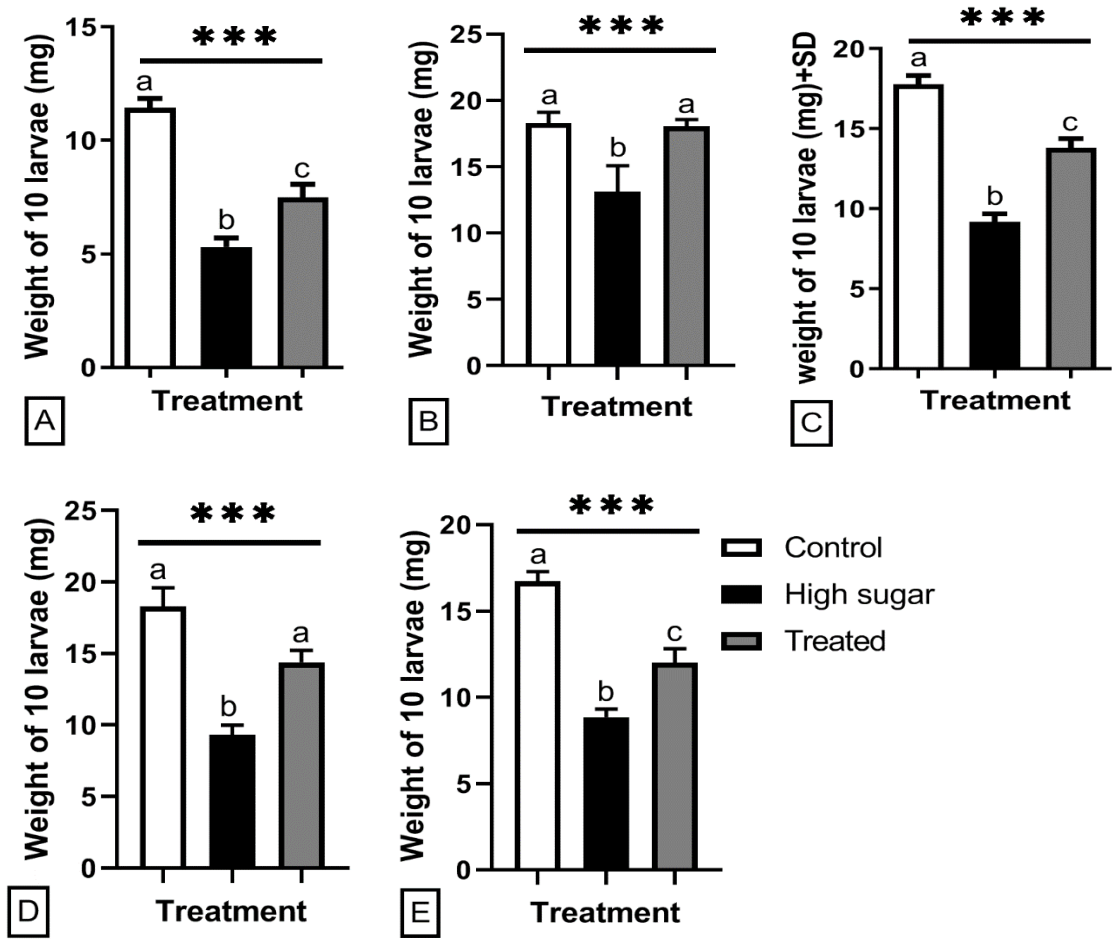

Figure 3. Total body weight (Mean ± SD) in *Drosophila melanogaster* larvae fed on *Moringa oleifera* (A), *Morus nigra* (B), *Atriplex halimus* (C), *Tagetes patula* (D) methanolic extracts and Amaryl drug (E) diets. *n* = 5 replicates (15 pooled samples). *** refers to a significant difference among means when $P < 0.05$ (One-way ANOVA). The bars with the same letters are not significantly different at $P \geq 0.033$ (Bonferroni multiple comparisons).

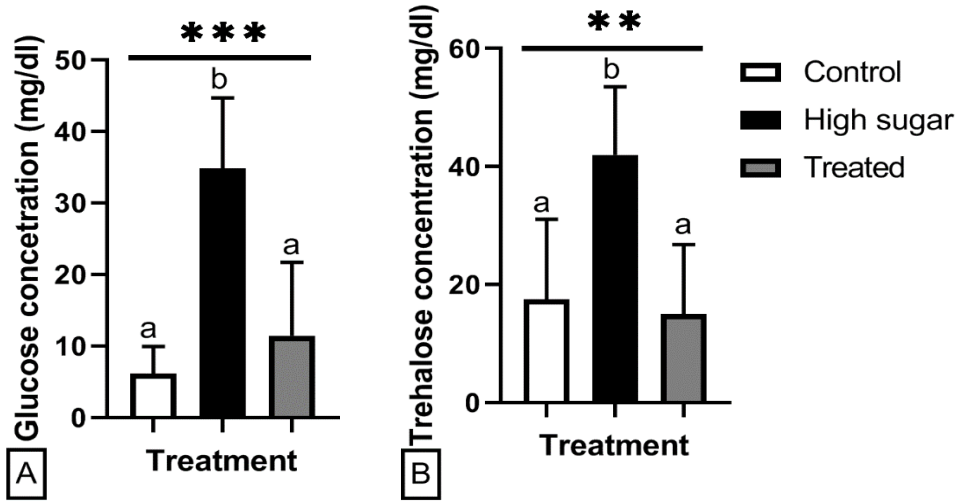

Figure 4: Mean ± SD of hemolymph glucose (A) and trehalose (B) in *Drosophila melanogaster* larvae fed on *Atriplex halimus* diet. *n* = 5 replicates (20 pooled samples). **, *** refer to significant differences among means when $P < 0.01$ and < 0.001, respectively (One-way ANOVA). The bars with the same letters are not significantly different at $P \geq 0.033$ (Bonferroni multiple comparisons).

Additionally, Zabin et al. (2013) and Raju and Gayathri (2021) reported that *T. patula* has hypoglycemic activity on STZ-induced diabetic mice. Chikhi et al. (2014) reported that *A. halimus* has hypoglycemic activity by enhancement of glucose tolerance in normal rats and by reducing the blood glucose levels in diabetic rats.

The results here also showed that the tested plant extracts significantly increased the weight of diabetic larvae near the control level. Oriabi (2016) demonstrated the effect of *M. oleifera* on the weight of STZ-induced diabetic mice. Other researchers pointed out the role of different plant extracts in the recovery of the weight of diabetic animals. For example, Júnior et al. (2017) reported that the bodyweight of diabetic mice increased after administration of ethanol extract of mulberry. Oliveira et al. (2008) attributed the recovery of weight to the ability of the plant extract to improve glucose utilization by cells and thereby preserve adipose and muscular tissues. Recently, Khaoula and Ali (2020) showed the beneficial effect of *A. halimus* on body weight gain in hepatic injury in rats.

The anti-diabetic effect of the tested plant extracts may be attributed to their bioactive components. Phytochemical analysis of the four tested plants revealed the presence of several constituents such as saponins, carbohydrates, alkaloids, unsaturated sterols, triterpenes, flavonoids, and catechol tannins. Other studies also documented the role of similar components as anti-diabetic agents (Bailey and Day, 1989; Stankovic, 2011). The antioxidants compounds play an important role in ameliorating diabetes symptoms (Livingstone et al., 1995). Chuffa et al. (2014) reported that flavonoid compounds improve glycemic levels and lipid profile in rats fed with a hyper-caloric diet. Alkaloids and triterpenoids could reduce blood sugar by increasing insulin secretion from pancreatic beta cells (Murali and Saravanan, 2012; Ponnulakshmi et al., 2019). On the other hand, flavonoids help in glucose uptake by peripheral tissues (Cazarolli et al., 2008). Olayaki et al. (2015) recorded that the active components of *M. oleifera* inhibit soluble epoxide hydrolase. The suppression of this enzyme has been reported to improve Diabetes (Minaz et al., 2018).

From the above-mentioned results, we concluded that most tested plant extracts succeed to recover the symptoms of type2 diabetes. The effect of these plants was similar to the antidiabetic effect of the commercial drug, Amaryl. However, Amaryl should be taken under caution as it reduced the level of glucose lower than normal. As *M. oleifera* and *M. nigra* are already in use by patients, we recommend further studies on *A. halimus* and its fractions to develop a new hypoglycemic drug.

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CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

DATA AVAILABILITY

The data that support the findings of this study are available from the corresponding author, upon reasonable request.

AUTHORS' CONTRIBUTION

MA & WSM suggested the study idea and designed the experiments. HE, OM and MA collected the results. WSM did the statistical analysis. All authors wrote and approved the manuscript.

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