



Evaluation of antidiabetic activities of *Etlingera elatior* flower aqueous extract *in vitro* and *in vivo*

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ABSTRACT

Diabetes mellitus (DM) is a global health problem for millions of people worldwide. Nowadays, medicinal plants have started gaining more attention in diabetes management due to the minimal side effects and cost-effectiveness. This study aims to ascertain the antidiabetic potential of the *Etlingera elatior* flowers aqueous extract on the *in vitro* inhibition of carbohydrate digestive enzymes and *in vivo* type 2 DM rat model. The findings demonstrate that *E. elatior* flower has a high percentage of inhibitory activity against α -amylase ($99.70 \pm 2.88\%$) and moderate activity for the α -glucosidase enzyme ($52.39 \pm 1.50\%$). In addition, *E. elatior* flower has also exhibited an antihyperglycemic effect that is dose-dependent and has significantly decreased the elevated total cholesterol, triglycerides, and low-density lipoprotein levels in the *in vivo* study. Furthermore, serum creatinine, aspartate aminotransferase, and alkaline phosphatase also were significantly decreased when compared to untreated DM. The high contents of total phenolic (39.06 ± 1.59 mg GAE/g), total flavonoid (39.00 ± 2.42 mg QE/g extract), and potent antioxidant activity were suggested to contribute to the antidiabetic activity of the extract. In conclusion, *E. elatior* flower could potentially be developed as a natural source of antioxidants that can be used for the prevention or treatment of diabetes.

INTRODUCTION

Diabetes mellitus (DM) is a chronic condition that occurs when there is an increase in the blood glucose level, which leads to a condition called hyperglycemia (International Diabetes Federation, 2017). Hyperglycemia is one of the crucial factors that plays a role in the development of oxidative stress and actively contributes to the impairment of insulin action and insulin secretion. A large amount of evidence suggests that oxidative stress contributes to the pathogenesis of DM and its complications (Evans *et al.*, 2002; Tiwari *et al.*, 2013). In addition, antioxidant mechanisms are plummeting in diabetic patients, which may

further enhance the oxidative stress (Lazo-de-la-Vega-Monroy and Fernandez-Mej, 2013).

Oxidative stress occurs when there is an imbalance in the production of free radicals and antioxidant protection in our body, thus, acting as the primary mediator of cellular damage. The medicinal plant has a variety of chemical families, and the amounts of antioxidants can retard or prevent the oxidation process by stabilizing the free radicals (Patel *et al.*, 2013). In recent years, medicinal plants have started gaining more attention in disease management due to its minimal side effects, and they are relatively low cost with various beneficial health effects. Traditionally, medicinal plants are widely used as a functional food to prevent diseases such as cancer, cardiovascular disease, and diabetes (Yuan *et al.*, 2016).

Etlingera elatior is one of the medicinal plants in Asia that belongs to the ginger family, Zingiberaceae. This plant is widely cultivated in many places throughout Southeast Asia, and it is commonly known as “Kantan” among Malaysians, and

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known as “Kecombrang” and “Kaala” in Indonesia and Thailand, respectively. (Habsah *et al.*, 2005; Lachumy *et al.*, 2010). It is an essential ingredient in Malaysian dishes that is widely used as a spice and freshly consumed as a vegetable (Haleagrahara *et al.*, 2010; Jackie *et al.*, 2011). Interestingly, this plant is potentially used as a source of alternative medicine and has been consumed traditionally to reduce diabetes and hypertension (Chan *et al.*, 2007; Jeevani *et al.*, 2011; Wai Mai *et al.*, 2009). Furthermore, *E. elatior* has been described to exhibit antimicrobial, antioxidant, anticancer, antidiabetic, antiinflammation, antiageing as well as wound-healing properties (Ghasemzadeh *et al.*, 2015; Habsah *et al.*, 2005; Hueh Zan *et al.*, 2011; Lachumy *et al.*, 2010; Nor Asiah *et al.*, 2019; Srey *et al.*, 2014).

Recently, extensive research has been carried out to study the phytochemical and pharmacological activities of *E. elatior* through various methods. Furthermore, it consists of high levels of secondary metabolites, such as phenols, flavonoids, glycosides, saponins, tannins, steroids and terpenoids, which contribute to its bioactivity (Chan *et al.*, 2011). Total phenolic and flavonoids contents within *E. elatior* are found in high amounts in the leaves, flowers, stems, and rhizome (Wai Mai *et al.*, 2009). Moreover, it also exhibits diverse antioxidant activity levels (Chan *et al.*, 2011; Habsah *et al.*, 2005; Ghasemzadeh *et al.*, 2015; Ramasamy *et al.*, 2016). A study by Jackie *et al.* (2011) reveals that the flower extract of *E. elatior* has exhibited potent antioxidant activity by increasing the antioxidant enzyme levels, such as superoxide dismutase, glutathione peroxidase, total antioxidants and lowering lipid peroxidation, and protein carbonyl content levels.

Based on the potent antioxidant and anticancer activities of the flower extract, it appears that *E. elatior* can be found in the northeast of Malaysia (Kelantan) and is a possible source of therapeutic compounds (Ghasemzadeh *et al.*, 2015). Despite numerous pharmacological activities of *E. elatior* that have been previously studied, there is limited research carried out to evaluate the antidiabetic activity of this plant. Thus, this study aims to determine both *in vitro* and *in vivo* antidiabetic activities of *E. elatior* flower's aqueous extract through a sonication extraction method.

MATERIALS AND METHODS

Materials

Folin–Ciocalteu reagent, gallic acid, butylhydroxytoluene, ferric chloride, 1,1-Diphenyl-2-picrylhydrazyl (DPPH) (D 9132), acarbose (PHR 1253), p-nitrophenyl- α -D-glucopyranoside (pNPG) (N0877), quercetin high performance liquid chromatography (HPLC) grade (Q4951), potassium chloride, 3,5-dinitrosalicylic acid (DNS) (D0550), starch, α -amylase from porcine pancreas (A 3176), and α -glucosidase (G5003) (Sigma-Aldrich Germany). For the animal study, streptozotocin (S0130) was purchased from Sigma Aldrich, Germany; trisodium citrate (1064481000) and citric acid monohydrate (1002441000) from Merck, Germany; metformin from Tokyo Chemical Industry; and sodium pentobarbitone from Alfasan Woerden, Holland.

Sample collection and extraction procedure

Etilingera elatior flowers were purchased from a local farmer in Kelantan and were authenticated at the International

Islamic University of Malaysia (IIUM), Kuantan, Pahang, with plant voucher PIIUM 0275. Fresh, unopened, uniform-coloured, and no apparent physical defect flowers were selected for this study. Before extraction, the petals were detached from the stalks and then washed to remove foreign particles. Then, it was cut into small pieces and oven-dried at 50°C until the weight was constant. The dried sample was ground into powder using a standard laboratory grinder and stored at 4°C for the extraction procedure.

Etilingera elatior flower aqueous extract (EEAE) was prepared at the School of Health Sciences (PPSK), Universiti Sains Malaysia (USM), by an ultrasonic-assisted extraction (UAE) method by using a sonicator (SC-221). In this study, water was used as a solvent to emulate the herbal decoction by traditional practitioners. Additionally, when testing on animals, especially when taken orally, water is the best solvent for safety reasons. Initially, 200 g of the sample powder was sonicated three times with 2,000, 1,800, and 1,400 ml of preboiled distilled water (80°C), respectively, for 30 minutes (Annegowda *et al.*, 2012). The resultant extract was filtered using grade 1 Whatman filter paper. The combined, filtered extract was frozen overnight at –20°C before freeze-drying. The resulting EEAE powder was then stored in the amber vial at –20°C until further analysis.

Determination of the total phenolic content

The total phenolic content (TPC) was determined by the Folin–Ciocalteu reagent assay adapted from Singleton and Rossi (1965). The EEAE (1 mg/ml) was diluted in distilled water. 200 μ l of sample/standard was pipetted into the cuvette, and then 800 μ l of distilled water and 500 μ l of Folin's reagent were added into the solution. The samples were prepared in triplicates. The reaction mixtures were allowed to stand in the dark for 5 minutes. Then, 1.5 ml sodium carbonate solution (20% w/v) was added, and the mixtures were incubated again in the dark at room temperature for 2 hours. Concurrently, the absorbance was measured at the wavelength of 760 nm against a reagent blank using a spectrophotometer (Secomam Prim, France). Different concentrations of gallic acid were used to prepare a calibration curve and the concentration of the gallic acid equivalent in the samples was calculated as follows:

$$\text{“ TPC} = (C \times V) / M.\text{”}$$

where TPC is total phenolic content in mg gallic acid equivalent per gram extract (GAE/ g); *C* is the concentration of gallic acid established from the calibration curve in mg/ml; *V* is the volume of the extract in ml; and *M* is the weight of the sample in g.

Determination of the total flavonoid content

The total flavonoid content (TFC) was determined by using the aluminum chloride colourimetric method described by Wijekoon *et al.* (2011). Quercetin was used as a standard and the flavonoid content of the EEAE was expressed as mg of quercetin equivalent to per g of dried extract. The EEAE was dissolved in distilled water (1 mg/ml). Briefly, 1 ml of test items (sample and standard) were transferred into the test tubes, and 4 ml of distilled water were added. Then, 0.3 ml of sodium nitrite solution (5% w/v) was added into the mixture and was allowed to stand for about 5 minutes. After that, 0.3 ml of aluminium chloride solution (10%

w/v) was added and incubated for 1 minute before the addition of 2 ml of sodium hydroxide (1 M). Subsequently, the reaction mixture was transferred into the cuvette, and the absorbance was measured at the wavelength of 420 nm against a reagent blank using a spectrophotometer (Secomam Prim, France). Different concentrations of quercetin were used to prepare a calibration curve and the concentration of quercetin equivalent in the samples was calculated as follows:

$$\text{“ TFC} = (C \times V) / M \text{”}$$

where TFC is total flavonoid content in mg quercetin equivalent per gram extract (QE/ g); *C* is the concentration of quercetin established from the calibration curve in mg/ml; *V* is the volume of the extract in ml; and *M* is the weight of the sample in g.

1,1-Diphenyl-2-picrylhydrazyl (DPPH) assay

The DPPH assay was used to determine the free radical scavenging activity of the EEAE based on the method described by Ghasemzadeh *et al.* (2015). 2 ml of test items (0–200 µg/ml of sample and positive control) were transferred into the test tubes. Then, 2 ml of DPPH solution (0.1 mM) in methanol was added. These tests were prepared in triplicates. The solution was mixed well, wrapped in aluminium foil, and incubated for about 30 minutes at room temperature in the dark. After the incubation period, the reaction mixture was transferred into the cuvette, and the absorbance was measured at the wavelength of 517 nm against a reagent blank using a spectrophotometer (Secomam Prim, France). In this test, butylhydroxytoluene (BHT) was used as a positive control. The scavenging activity was calculated using the following formula:

$$\text{“ DPPH radical scavenging (\%)} = (\text{control OD} - \text{sample OD} / \text{control OD}) \times 100 \text{”}$$

Ferric reducing antioxidant power (FRAP) assay

The ferric reducing power of the extracts was determined by the method described by Benzie and Strain (1996) and Ojha *et al.* (2018). The FRAP reagent consisted of 10 volumes of 300 mM acetate buffer (pH = 3.6), 1 volume of 10 mM 2,4,6-tripyridyl-S-triazine (TPTZ) solution in 40 mM hydrochloric acid (HCl), and 1 volume of 20 mM ferric chloride (FeCl₃) solution. The EEAE was dissolved in distilled water (1 mg/ml). 50 µl of sample/standard was pipetted into the cuvette, followed by 150 µl of distilled water and 1.5 ml of FRAP reagent. All tests were prepared in triplicates. The mixture was mixed well and incubated at room temperature in the dark for 30 minutes. Then, the absorbance of the mixtures was measured at the wavelength of 593 nm against a reagent blank using a spectrophotometer (Secomam Prim, France). The reagent blank comprised of the same reagents without the test items (sample/standard). A standard curve was prepared using various concentrations of Iron(II) sulphate heptahydrate (FeSO₄·7H₂O). The FRAP activity was expressed as millimoles of Fe(II) per gram of sample, based on the calibration curve prepared with FeSO₄·7H₂O.

In vitro antidiabetic activity of EEAE

α-amylase inhibition assay

The *α*-amylase inhibition activity was determined by using previous methods (Md Norodin *et al.*, 2018). In this test, a well-known amylase enzyme inhibitor, acarbose, was used as

a positive control. Ten µl of EEAE (100 µg/ml) and acarbose (100 µg/ml) were transferred into the test and blank well in a 96-well plate (Greiner Bio-one). Then, 50 µl of *α*-amylase enzyme solution (4.0 U/ml) and 40 µl of distilled water were added into each well (a total 90 µl of distilled water at blank wells) and the plate was incubated at 25°C for 5 minutes by using a thermo shaker incubator. After that, 100 µl of starch (0.5 % w/v) solution was added into each well and incubated again at 25°C for 7 minutes. After the incubation process, 100 µl of DNS colour solution was added into each well in the dark and incubated at 85°C for 30 minutes. The plate was let to cool down to room temperature, and the absorbance was measured at 540 nm. The inhibition of *α*-amylase (%) was calculated by using the following equation:

$$\text{“ Inhibition (\%)} = \frac{(A_{\text{Control}} - A_{\text{Sample}})}{A_{\text{Control}}} \times 100 \text{”}$$

where *A* is the absorbance of a test mixture of a wavelength of 540 nm.

α-glucosidase inhibition assay

The *α*-glucosidase inhibition activity was determined through the methods described by Md Norodin *et al.* (2018). 10 µl of sample (100 µg/ml), acarbose (100 µg/ml), and quercetin (100 µg/ml) (positive control) were transferred into a 96-well plate. 20 µl of the *α*-glucosidase enzyme, 40 µl of phosphate buffer saline (pH 6.5), and 20 µl of distilled water were added into each well and incubated at 37°C for 10 minutes by using a thermo shaker incubator. Next, 10 µl of pNPG was added into all wells in the dark. Then, the plate was incubated again at 37°C for 30 minutes, and the absorbance was read at 405 nm (BMG, FLUOstar Omega). The inhibition of *α*-glucosidase (%) was calculated using the following equation:

$$\text{“ Inhibition (\%)} = \frac{(A_{\text{Control}} - A_{\text{Sample}})}{A_{\text{Control}}} \times 100 \text{”}$$

where *A* is the absorbance of a test mixture of a wavelength of 405 nm.

In vivo antidiabetic activity of EEAE

Experimental animal

Forty-two male Sprague Dawley rats (SD) weighing 200–250 g were purchased from the Animal Research and Service Centre (ARASC), USM, and were placed in a temperature-controlled room at 23±1°C and a 12-hour dark/ light cycle. All the animals were treated according to the guidelines of animal care from ARASC, USM, and the protocols were approved by the Institutional Animal Care and Use Committee (IACUC; USM/ Animal Ethics Approval/2016(760)).

Induction of type 2 DM rat model

After 1 week of the adaptation period, the rats were randomly divided into two groups: the control group (*n* = 7) and high-fat diet (HFD) group (*n* = 35). Control group was given a standard diet, while HFD group was given high fat diet pellet consisted of 32% animal fat prepared based on the method described by Nursyuhana *et al.* (2016). Rats were fed according to their corresponding diets for six weeks to induce obesity. Consequently, the rats with a body mass index of more than 0.68 g/cm² were considered obese (Novelli *et al.*, 2007).

At the end of week six, the HFD-feeding rats were divided into two groups, healthy obese ($n = 7$) and diabetic obese (Obese-DM, $n = 28$). The induction of type 2 DM was carried out on the Obese-DM group by using a single low dose of STZ (35 mg/kg). Before the injection, the rats were fasted overnight and were given a single intraperitoneal injection of STZ in a citrate buffer (pH 4.5), while the rats in the standard group and the obese group received just the citrate buffer. After the induction, the blood glucose level was measured on day seven to confirm the diabetic condition in the rats. The level of blood glucose more than 11.1 mmol/l was included in this study. Overall, the groups were assigned as Group 1: normal; Group 2: obese; Group 3: untreated DM; Group 4: DM treated with 250 mg/kg of metformin; Group 5: DM treated with 500 mg/kg of EEAE; and Group 6: DM treated with 1000 mg/kg of EEAE, respectively. The normal (Group 1) and obese (Group 2) groups acted as standard diet control and obese control without DM, respectively. All rats were treated equally for 6 weeks and the weekly fasting blood glucose level was measured throughout the study period by using portable Accu-Check Advantage glucometer (One Touch Ultra). At the end of the study, all rats were euthanized, and the blood was collected by a cardiac puncture, and the samples were sent to a local laboratory service for the measurement of biochemical parameters by using an Architect chemistry analyser (Abott Ci 8200).

Statistical analysis

All measurements were carried out in triplicate ($n = 3$). All the results were presented in the form of mean \pm standard deviation (SD). Data for α -amylase was statistically analyzed by Student's *t*-test, while data for α -glucosidase and biochemical parameters were analyzed by one-way analysis of variance (ANOVA). Random blood glucose level and body weight were analyzed by two-way ANOVA by using GraphPad Prism version 8.0.

RESULTS

Total phenolic, total flavonoid content, and antioxidant activity of *E. elatior* extract

TPC of the EEAE was expressed in mg GAE/g extract by using the gallic acid standard curve ($R^2 = 0.9913$), and the TFC was expressed in mg QE/g extract by using the quercetin standard curve ($R^2 = 0.9999$). Meanwhile, the antioxidant activity was evaluated by the DPPH and FRAP assays through a synthetic antioxidant BHT and ascorbic acid as a controlled drug, respectively. The results demonstrate that the EEAE exhibited a high amount of total phenolic (39.06 ± 1.59 mg GAE/g) and total flavonoid (39.00 ± 2.42 mg QE/g extract) contents. The percentage of the DPPH inhibition at 200 μ g/ml was 88.7% for EEAE and 96.9% for BHT, with the IC_{50} value of 47.55 ± 7.88 μ g/ml ($n = 3$) and 18.45 ± 2.52 μ g/ml ($n = 3$), respectively. For the FRAP assay, the reducing power of the extract was determined as

the reduction of Fe^{3+} -TPTZ complex to Fe^{2+} -TPTZ. The intensity of the reduced form was measured spectrophotometrically in comparison with the standard curve of Fe^{2+} ($R^2 = 0.9999$). The FRAP value of the EEAE obtained was 0.627 ± 0.02 mM Fe^{2+} equivalent/g of extract ($n = 3$), while the FRAP value for ascorbic acid was 10.77 ± 0.42 mM Fe^{2+} equivalent/g of ascorbic acid. The overall values are summarized in Table 1.

In vitro α -amylase and α -glucosidase inhibitory activities

Preliminary screening of EEAE for *in vitro* antidiabetic activity was carried out at 100 μ g/ml extract. The inhibitory activity against the α -amylase and the α -glucosidase enzymes are shown in Figure 1a and b. In this study, acarbose, an established inhibitor for α -amylase, and quercetin, a well-known compound to control postprandial hyperglycemia, were used as the positive control drugs. Acarbose blocks the pancreatic α -amylase and membrane-bound α -glucosidase, reducing the rate of carbohydrate digestion. Meanwhile, quercetin was used as a positive control for the α -glucosidase assay based on reports claiming that the phenolic compounds have a stronger inhibitory activity on α -glucosidase compared to acarbose (Indrianingsih *et al.*, 2015). As shown in Figure 1a, the EEAE had a strong inhibitory activity against the α -amylase enzyme, and no significant difference was observed when compared with the positive control, acarbose. The results also demonstrate that the EEAE exhibited $99.7 \pm 4.97\%$ of enzyme inhibition, while $100 \pm 1.92\%$ inhibition was achieved for acarbose. For the α -glucosidase assay, the EEAE showed moderate inhibition ($52.39 \pm 2.12\%$) and was statistically significant with the quercetin compound ($97.62 \pm 1.23\%$) that was used as a positive control ($p < 0.001$). Note that in this test, acarbose ($10.62 \pm 11.03\%$) displayed a weak inhibition for α -glucosidase enzyme, and the inhibitory activity of EEAE was significantly higher than acarbose but lower than quercetin (Fig. 1b).

In vivo antihyperglycemic activity of EEAE and its effect on body weight

The efficacy of the study of EEAE was evaluated in the SD rats induced type 2 DM model. Figure 2 shows that the EEAE significantly decreased the fasting blood glucose level in a dose-dependent manner with a blood glucose level of 13.77 ± 3.59 mmol/l for 500 mg/kg of EEAE and 9.63 ± 2.47 mmol/l for 1,000 mg/kg of EEAE when compared to untreated DM (22.43 ± 1.30 mmol/l) after 6 weeks of the experimental period ($p < 0.001$). Interestingly, no significant difference in blood glucose level was observed between 1,000 mg/kg EEAE and control drug metformin.

As shown in Figure 3, the near average level of body weight was achieved for metformin and 1,000 mg/kg of EEAE at the end of the study period. Note that the body weight dropped after DM induction and remained constant starting from week 3 of the treatment. On the other hand, there was a pattern of consistent weight loss in untreated DM throughout the study period, but

Table 1. TPC, TFC, DPPH scavenging activity, and FRAP of *E. elatior* flower aqueous extract. Values are means \pm SD ($n = 3$).

Samples	TPC (mg GAE/g extract)	TFC (mg QE/g extract)	DPPH assay		FRAP assay (mM Fe^{2+} /mg extract)
			% inhibition	(IC_{50} μ g/ml)	
EEAE	39.06 ± 1.59	39.00 ± 2.42	88.3	47.55 ± 7.8	0.627 ± 0.02

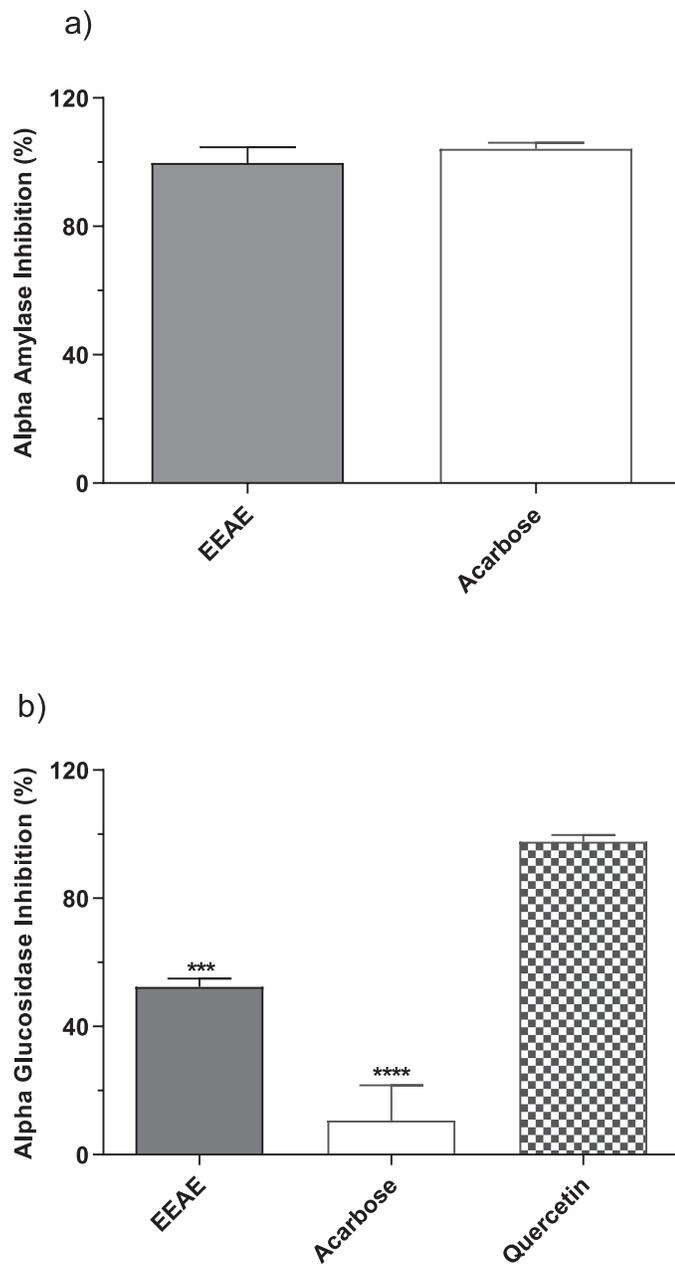


Figure 1. Inhibitory activity of EEAE against (a) α -amylase and (b) α -glucosidase enzyme at a concentration of 100 μ g/ml. Values are expressed as mean inhibition (%) \pm SD of triplicate measurements from three independent experiments (** $p < 0.001$ and **** $p < 0.0001$ when compared to quercetin).

no significant difference was observed when compared with all treatment groups.

The effect of EEAE on biochemical profiling

Biochemical parameters were evaluated at the end of the study to determine the effect of the extract on biochemical profiles related to DM. The findings revealed that EEAE improved hyperlipidemia in the type 2 DM rat model. Figure 4 shows that EEAE significantly reduced the elevated total cholesterol ($p < 0.01$), triglycerides ($p < 0.001$), and low-density lipoprotein (LDL; $p < 0.01$) levels when compared with untreated DM. However, no significant difference was detected for high-density lipoprotein

(HDL) levels. The EEAE treatment also significantly decreased the plasma creatinine level ($p < 0.01$), alanine aminotransferase (ALT) ($p < 0.01$), and alkaline phosphatase (ALP) ($p < 0.001$) when compared with untreated DM, and no significant difference was observed for urea and aspartate aminotransferase (AST) levels (Table 2).

DISCUSSION

Diabetes is characterized by high blood glucose levels, which lead to severe complications if left untreated for a long time. The prevalence of type 2 DM was increasing worldwide and accounted for approximately 95% of all diabetes cases (International Diabetes Federation, 2017; Zheng *et al.*, 2018). Obesity is the most common risk factor for the development of type 2 DM, which leads to the elevation of serum triglycerides, hypertension, and insulin resistance (Gheibi *et al.*, 2017). Moreover, various complications are also related to the disease, which include retinopathy, nephropathy, and neuropathy, as well as atherosclerosis and coronary heart disease.

Nowadays, the management of diabetes without any severe side effects remains a challenge as most of the glucose-lowering drugs may come with side effects, including severe hypoglycemia, lactic acidosis, idiosyncratic liver cell injury, digestive discomfort, headache, and dizziness (Prabhakar and Doble, 2011). Therefore, medicinal plants with potential antidiabetic properties are sought after in search of safer and cost-effective antidiabetic drugs. This study has revealed that *E. elatior* flower exhibited an *in vitro* inhibitory activity for both the α -amylase and the α -glucosidase enzymes. Theoretically, a rise in blood glucose levels, which causes hyperglycemia conditions in type 2 DM, occurs due to the hydrolysis of starch by pancreatic α -amylase and uptake of glucose by intestinal α -glucosidases (Kwon *et al.*, 2006). The inhibition of these enzymes is recognized as a therapeutic target for modulation of postprandial hyperglycemia. Therefore, it is suggested to be a useful approach for the management and prevention of type 2 diabetes (Lordan *et al.*, 2013; Nair *et al.*, 2013; Oboh, 2013).

The efficacy of this extract was further evaluated in the *in vivo* study by using the type 2 DM rat model. In this study, we used a combination of HFD feeding to induce obesity and a low dose of STZ that caused minimal dysfunction of the β -cells in the pancreas for the development of type 2 DM. It was proposed that obese animals tend to develop insulin resistance and exhibited metabolic characteristics as well as developed a natural history of disease progression similar to those seen in humans with type 2 DM (Fang *et al.*, 2019). Moreover, this model develops a stable and long-lasting hyperglycemia which becomes a valuable research tool in a diabetic study (Guo *et al.*, 2018). Consistent with *in vitro* inhibition, the EEAE was shown to decrease fasting blood glucose levels in a dose-dependent manner in the *in vivo* study. The results clearly indicated that EEAE was able to regulate a positive outcome toward reducing hyperglycemia where no significant difference was observed when compared with control drug, metformin, after 6 weeks.

Concurrently, the body weight was reduced to near normal in metformin and EEAE at a dose of 1000 mg/kg groups, therefore, indicating that the EEAE at this dose was able to reduce obesity when compared with the obese control group at the end

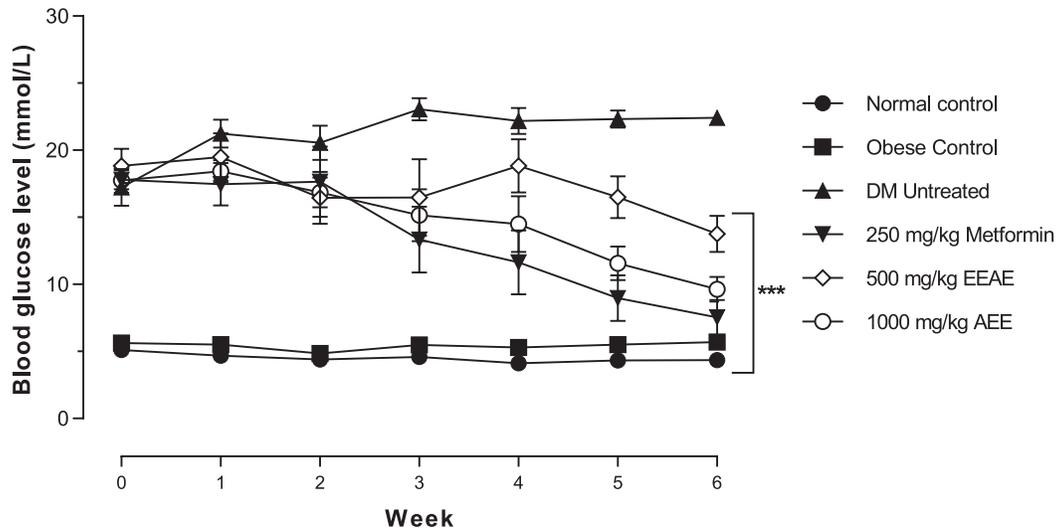


Figure 2. The effects of the different treatments on fasting blood glucose levels in experimental rats (** $p < 0.001$, main effects when compared to untreated diabetic rats).

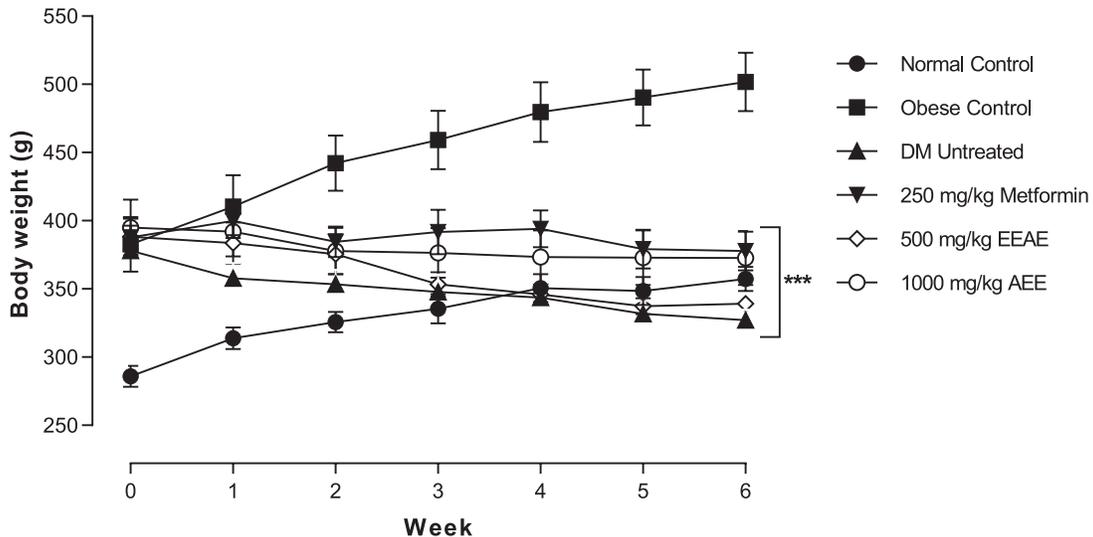


Figure 3. The effects of the different treatments on body weight in experimental rats throughout the study period (** $p < 0.001$, main effects when compared to the obese control group).

of the study period. There was a pattern of weight loss which occurred in untreated DM, but no significant difference in body weight was detected in all the treatment groups after six weeks of treatment. This could be because there was no massive weight loss in type 2 DM rats used this model. In contrast to type 1 DM, a clear and sudden weight loss is not observed as in type 2 diabetes. However, significant weight loss was observed with prolonged disease exposure (Skovso, 2014).

Type 2 DM is always associated with changes in the lipid profile, hepatic enzyme, and kidney function (Khadke *et al.*, 2019). In this study, *E. elatior* extract was observed to significantly lower the elevation of total cholesterol, triglycerides, and LDL levels. However, the mechanism involved in this effect was not evaluated in this study. A systematic review on various herbal extracts suggested that antihyperlipidemia activity was related to

several mechanisms, such as modification of lipid peroxidation by reducing glutathione peroxidase activity, increased excretion of faecal bile acids, upregulation of LDL receptor as well as the presence of bioactive compound high in antioxidant properties (Rouhi *et al.*, 2015). At the molecular level, a decreased expression of adipogenic factors, such as peroxisome proliferators activated-receptor-gamma and sterol regulatory element-binding protein, were also suggested as a possible mechanism involved in hypolipidemic effect of herbal plant *in vitro* (Vijayakumar *et al.*, 2010). In this study, the levels of serum creatinine, ALT, and ALP were also significantly reduced when compared to the untreated ones, which suggested that this extract exhibited a hepatoprotective effect and improved renal function in type 2 DM rats. Controlled hyperglycemic levels could be one of the possible reasons for the improvement of biochemical parameters in all treatment groups.

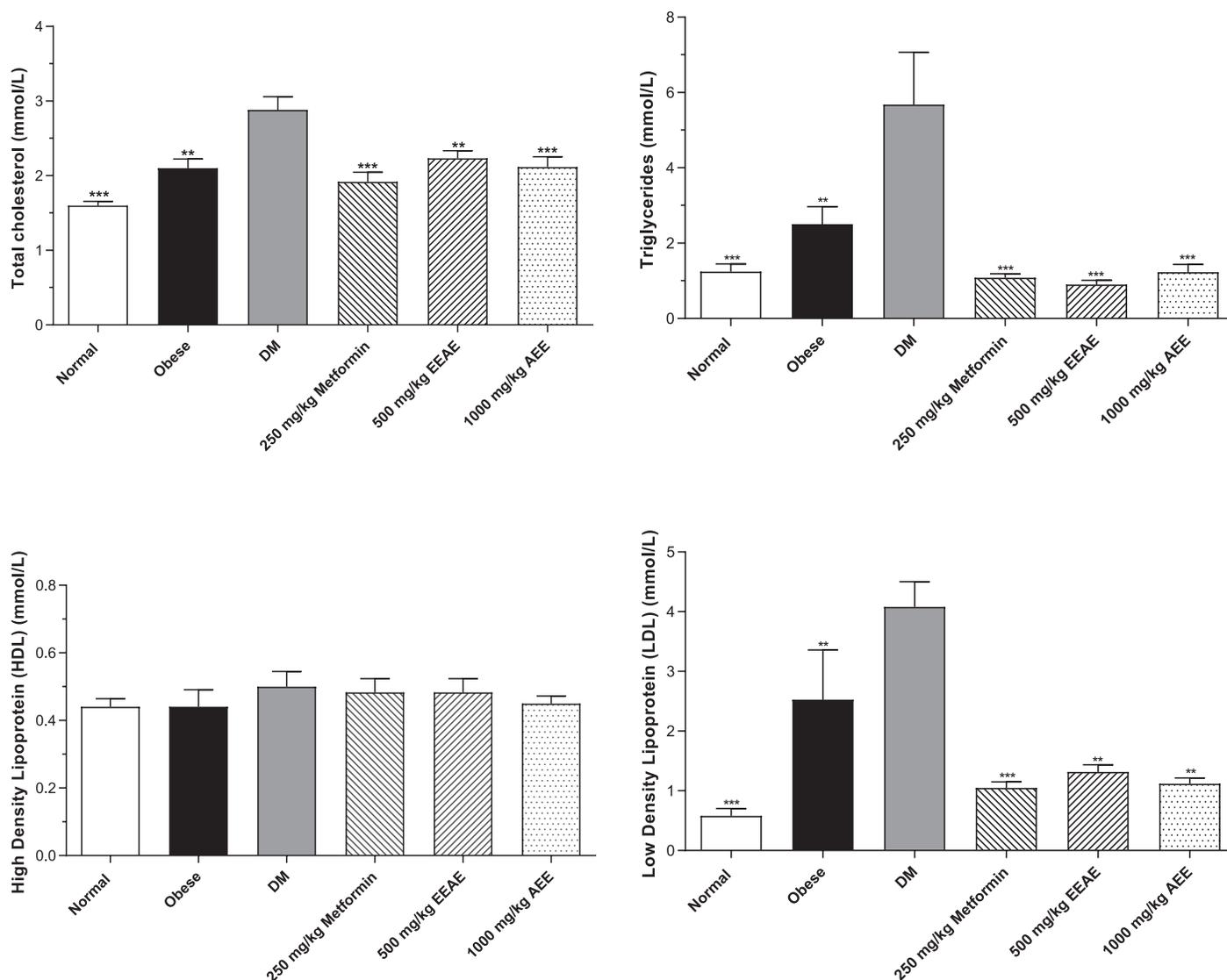


Figure 4. Lipid profile for all treatment groups after six weeks. Data were analysed by using one-way ANOVA (** $p < 0.01$ and *** $p < 0.001$ when compared to untreated DM).

Table 2. Biochemical parameters for particular groups after 6 weeks. Values are mean \pm SD of seven animals in each group. Data were analysed by using one-way ANOVA, respectively (* $p < 0.05$, ** $p < 0.01$ and *** $p < 0.001$ when compared to the untreated DM group).

Biochemical parameters	Normal	Obese	Diabetes Mellitus	250 mg/kg metformin	500 mg/kg EEAE	1000 mg/kg EEAE
Urea	7.36 \pm 0.27	6.46 \pm 0.28**	9.04 \pm 0.53	7.09 \pm 0.53*	8.74 \pm 0.59	8.31 \pm 0.43
Creatinine	47.03 \pm 1.34***	52.25 \pm 1.87***	69.07 \pm 1.35	54.67 \pm 1.61***	60.02 \pm 2.11**	57.44 \pm 1.72***
SGOT/AST	123.6 \pm 7.87	145.0 \pm 9.40	151.4 \pm 21.80	128.4 \pm 12.69	107.3 \pm 17.83	116.1 \pm 8.63
SGPT/ALT	68.56 \pm 3.02**	70.63 \pm 7.14**	108.6 \pm 12.65	75.25 \pm 5.65*	66.13 \pm 8.35**	61.75 \pm 5.9***
ALP	269.0 \pm 14.95***	454.5 \pm 34.22***	1459 \pm 98.38	507.9 \pm 130.8***	579.3 \pm 106.5***	403.3 \pm 84.38***

The findings suggested that the antidiabetic activity could be due to the high antioxidant properties and the presence of active compounds in the extract. A study by Omari *et al.* (2019) on *in vitro* antioxidant and antidiabetic activities of *Aristolochia longa* extracts revealed that the presence of phenols and flavonoids in the extract contributed to its multiple biological properties. In another study, they claimed that the phenolic compounds could regulate the glucose uptake by inhibiting carbohydrate digestion

and absorption (Shobana *et al.*, 2009). Likewise, the presence of flavonoids may also account for the inhibitory activity observed. Indeed, flavonoids are known to possess high inhibitory potential toward *in vitro* and *in vivo* studies (Fang *et al.*, 2019; Kidane *et al.*, 2018; Li *et al.*, 2018). Notably, some researchers have indicated that there is a positive correlation between polyphenolic composition and the ability of the extract to inhibit both α -amylase and α -glucosidase enzymes (Ramkumar *et al.*, 2010). By

inhibiting those enzymes concerning its antioxidant activity, the hyperglycemia as well as biochemical changes toward diabetes disease are improving.

CONCLUSION

This study revealed that *E. elatior* flower's extract had the ability to inhibit both α -amylase and α -glucosidase enzyme *in vitro* and possessed antihyperglycemic activity in the type 2 DM rat model. A potent antioxidant capacity with a high content of TPC and TFC in the extract may have a great influence on its antidiabetic effect. The findings from this study provide the scientific data on potential antioxidant compounds present in the extract as well as the efficacy assessment toward the hypoglycemic effect of the EEAE. Thus, *E. elatior* can be an ideal natural source of antioxidants and antidiabetic compounds that can be used for the prevention or treatment of DM. Furthermore, research should be conducted to investigate the bioactive compounds of antidiabetic activity.

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CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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