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Evaluation of antioxidant and antihyperglycemic effects *Dovyalis Abyssinica (A. Rich)*

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ABSTRACT

Background: The leaves of *Dovyalis Abyssinica* have been used traditionally for the management of diabetes mellitus. Thus, this study aimed to evaluate the Antioxidant and Antihyperglycemic Effects of *Dovyalis Abyssinica* leaves crude extract in streptozotocin-induced diabetic mice.

Methods: To Evaluate the Antihyperglycemic, and Antioxidant Effects of Dovyalis Abyssinica Leaves Extract in Streptozotocin-Induced Diabetic Mice. Male Swiss albino mice were induced into diabetes using 100 mg/kg of streptozotocin. Mice were allocated randomly into six groups, six mice per group. The body weight and FBG measurements were done on days 0, 7th, 14th and 21st of treatment. Additionally, in vitro Antioxidant Activity of the Extract was determined using a DPPH assay. The data were entered into Epi-Data version 4.6, exported to SPSS version 26.0, and analysed by using a one-way ANOVA followed by a Tukey post hoc test, and $P < 0.05$ was considered statistically significant.

Results: Dovyalis Abyssinica leaves crude extract showed significant ($P < 0.05$-$P < 0.001$) blood-glucose-lowering activity. Moreover, the crude extract of *D. abyssinica* reduced the fasting blood glucose level by 45.13 %, 52.51 %, 54.85 %, and 56.38 %, respectively, for DA 100, DA 200, DA 400, and GLC 5 mg/kg on the 21st day of treatment. After diabetic mice were treated with Dovyalis Abyssinica (100, 200, and 400 mg/kg) for 21 days, there was a significant increase in body weight as compared to diabetic control. Antioxidant activities of the leaf extract was found to be comparable to ascorbic acid with an IC50 of 140.04 μg/ml.

Conclusion: The present findings revealed that *D. abyssinica* leaves could be useful for the management of diabetes mellitus and other abnormalities related to this metabolic disorder. Thus, the present study may support the traditional use of *D. abyssinica* for diabetes mellitus treatment.

1. Introduction

Diabetes mellitus (DM) is a heterogeneous complex metabolic disorder resulting from defective insulin secretion, resistance to insulin action or both. It is one of the most prevalent metabolic disorders affect 537 million people (ages 20 to 79) worldwide and is predicted to affect 783 million people by the year 2045. In Africa 24 million adults aged 20–79 years are living with diabetes [1]. The prevalence of diabetes mellitus in Ethiopia is 2–6.5 %. It became the top 5 listed Sub-Saharan African countries, and 1 % of deaths in the country are caused by hyperglycemia and its complication [2]. The most frequent types of DM are type 1 and type 2, with type 1 caused by the inability of the body to produce adequate insulin, whereas type II diabetes mellitus is characterized by fasting hyperglycemia due to insulin resistance [3] (see Tables 1 and 2).

The World Health Organization estimated that approximately 80 percent of the population in developing countries relies on herbal products, which are considered to be affordable, easily available, and less toxic [4]. Currently, nearly 25 % of drugs in modern pharmacopeia are descended from natural products that were first used in traditional medicine. Taking metformin as an example, it was originally isolated from the medicinal plant Galega officinalis and is now prescribed in modern medicine to treat diabetes [5]. Diabetes management without side effects remains a problem. Apart from the currently known

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Table 1
Effect of *Dovyalis Abyssinica* leaves extract on body weight in diabetic mice.

Groups	Body weight (g)				
	Before Induction 0 day (Baseline)	7th day	14th day	21st day	
NC	32.18 ± 0.70	32.80 ± 0.73	33.86 ± 0.80	35.30 ± 1.03c1	36.19 ± 1.06^{c1}
DC	31.20 ± 0.73	29.92 ± 0.64^{a1}	27.74 ± 0.44^{a3c1}	26.16 ± 0.32^{a3c2}	25.05 ± 0.24^{a3c3}
DALE 100	31.98 ± 0.83	29.74 ± 0.45^{a1}	30.44 ± 0.73^{a2}	30.88 ± 0.33^{a2}	31.77 ± 0.37^{a2b2}
DALE 200	32.40 ± 0.60	29.86 ± 0.65^{a1}	30.66 ± 0.52^{a1}	31.08 ± 0.26^{a2b1}	31.89 ± 0.75^{a2b2}
DALE 400	30.10 ± 0.20	29.66 ± 0.46^{a1}	31.30 ± 0.41^{b1}	32.30 ± 0.28^{b2c2}	33.19 ± 0.22^{b3c3}
GLC 5 (PC)	32.36 ± 0.34	29.76 ± 0.65^{a1}	31.96 ± 0.59^{b1}	33.28 ± 0.62^{b2c2}	34.17 ± 0.21^{b3c3}

The values indicate mean ± S.E.M (n = 6) for each treatment; Analysis was performed by one way ANOVA followed by Tukey's post hoc test; a, compared to normal control; b, compared to diabetic negative control and c, compared to baseline (0 day) body weight in their respective group; number followed by DALE and GLC indicates dose in mg/kg; NC, normal control treated with 2 % tween 80; DC, diabetic negative control treated with 2 % tween 80; PC, diabetic positive control treated with glibenclamide; GLC, glibenclamide; DALE, *Dovyalis Abyssinica* leaves extract; 1P < 0.05; 2p < 0.01; 3P < 0.001.

therapeutic alternatives for diabetes, such as oral hypoglycemic medications and insulin, both of which have drawbacks, various medicinal plants have been advocated for diabetic treatment. Medicinal plants serve an essential role in diabetes therapy, particularly in developing nations with limited resources [6]. Antidiabetic drug development has switched to medicinal plants in order to provide new promising, efficient treatments with fewer side effects and lower costs. Medicinal plants store many bioactive compounds like antihyperglycemic [7]. In Ethiopia, DM is treated locally using a variety of medicinal plants. One of the plant used to manage diabetes is *Dovyalis Abyssinica* roots [8].

Dovyalis Abyssinica is commonly called African gooseberry which is native to Africa [9] and locally known as "Koshim" in Amharic and" koshommii'' in Afan Oromo, belongs to the small genus Dovyalis and family Flacourtiaceae. It occurs naturally from Ethiopia, Eritrea and Somalia in the North through Kenya and Tanzania to Malawi in the South. Grows in upland rain forest, dry evergreen forest, on river banks and sometimes in more open wood land. The plant has been commonly used for antiepileptic, anti-microbial, anti-obesity, anti-viral, anticholinergic, and bronchodilator activities [10].

These plants have been in use for traditional management of dysentery, diarrheic diseases, flatulence, and general debility and mosquito repellent activities, trypanosomes in humans and animals [11]. The roots also have medical properties with alleged effect on gonorrhea, bilharzias, stomach-ache and fever. The roots and thorns are used in African traditional medicine to treat amenorrhea and chest pain. Leaves are used traditionally to treat gonorrhea, brucellosis and teeth problems in humans and mastitis in animals [12].

Previous studies on the aqueous extracts of the barks of Salix tetrasperma Roxburgh (The same family of experimental plants) hypoglycaemic activity [13]. Leaves, twigs, root's part of *D. Abyssinica* used traditionally to treat diabetics mellites in Ethiopia and Kenya [14]. Hence, this study is aimed to investigate the antioxidant activity in vitro and antihyperglycemic effects of *Dovyalis Abyssinica* leaves in a diabetic mice model.

2. Material and methods

2.1. Study area

The study was conducted at the Department of Biochemistry, School of Medicine, College of Health Sciences, University of Gondar.

2.2. Drugs, chemicals, and instruments

The drugs, chemical reagents, and instruments used in the experiment were: DPPH (Sigma Aldrich, Germany), ascorbic acid (Blulux Labratories, India), citric acid (Lab tech chemicals, India), STZ (Fisco Research laboratories, India), glibenclamide (Sanofi aventis, France), 5 % glucose solution (Reyoung Pharmaceuticals, Shandong, China), distilled water, tween 80 (Avishkar Lab Tech chemicals, India), diethyl ether. Filter paper, gauze, digital electronic balance, pH meter, oral gavages, refrigerator, oven (Medit- Medizin Technik, Germany), lyophilizer (Labfreez, China), rotary evaporator (Hamato, Japan), centrifuge, spectrophotometer (Agilent Technologies, Malaysia), caresens glucometer (Seochu-gu, seoul 06646, Korea), auto lab clinical chemistry analyzer (Beckman coulter, Germany).

2.3. Experimental animals

Healthy Swiss albino mice (body weight, 30–35 gm; age 10–12 weeks old) were obtained from University of Gondar Department of Pharmacology. Male mice were used for STZ induced diabetic model. All the mice were acclimatized to the laboratory condition for one week before commencing the experiments and fed with pellet and tap water ad libitum. The animals were housed in 12 h light/dark cycle at room temperature.

2.4. Collection and identification of the plant

The leaves of *Dovyalis Abyssinica* were collected from Gondar town (located in the Central Gondar zone of Amhara region, northwest Ethiopia). Taxonomic identification of the plant was done by a botanist at the University of Gondar Biology Department. A specimen was deposited in the Herbarium of Biology Department, College of Natural and Computational Science, University of Gondar.

Table 2
Effect of *Dovyalis Abyssinica* Leaves on fasting blood glucose in diabetic mice.

Groups	Fasting blood glucose level (mg/dL)				% reduction in baseline BGL	
	Baseline (0 day)	7th day	14th day	21st day	14th day	21st day
NC	92.40 ± 1.32	101.60 ± 5.59	99.38 ± 5.08	98.20 ± 4.50	−7.55 %	−6.27 %
DC	295.18 ± 6.88^{a3}	308.40 ± 5.85^{a3}	318.20 ± 4.60^{a3}	318.40 ± 4.60^{a3}	−7.79 %	−7.86 %
DALE 100	297.90 ± 8.52^{a3}	273.20 ± 72.83^{a3d2}	187.40 ± 13.66^{a3d3}	163.44 ± 13.46^{a2b2c2d3}	37.09 %	45.13 %
DALE 200	302.65 ± 10.08^{a3}	199.20 ± 16.76^{a3}	165.50 ± 16.37^{a2b2c2d1}	143.70 ± 16.80^{a2b3c3d1}	45.31 %	52.51 %
DALE 400	303.02 ± 3.04^{a3}	190.80 ± 16.36^{a3}	151.80 ± 5.50^{a2b2c2}	136.80 ± 5.90^{a1b3c3}	49.90 %	54.85 %
GLC5 (PC)	302.20 ± 5.28^{a3}	188.00 ± 9.54^{a3}	145.80 ± 5.31^{a2b2c3}	131.80 ± 5.31^{a1b3c3}	51.75 %	56.38 %

The values indicate mean ± S.E.M (n = 6) for each treatment; Analysis was performed by one-way ANOVA followed by Tukey's post hoc test; a, compared to normal control; b, compared to diabetic negative control; c, compared to baseline FBG level in their respective group; d, compared to positive control; number followed by DALE and GLC indicates dose in mg/kg; NC, normal control treated with 2 % tween 80; DC, diabetic negative control treated with 2 % tween 80; PC, diabetic positive control treated with glibenclamide; GLC, glibenclamide; DALE, *Dovyalis Abyssinica* leaves extract; 1P < 0.05; 2P < 0.01; 3P < 0.001.

2.5. Preparation of crude extract

The leaves of *D. Abyssinica* were washed with distilled water and dried under shade at room temperature. The plant material was coarsely grounded into powder using a mortar and pestle. The coarse powder (400 g) of *D. Abyssinica* leaves was macerated in hydroethanolic (1:6 leaves powder to solvent ratio) for 72 h with mechanical shaking frequently [15]. This was repeated 3 times until the extract gave discoloration. Then the plant material was filtered with gauze and then by filter paper. The filtrate of hydroethanolic *D. Abyssinica* leaves extract was concentrated using a rotary evaporator under reduced pressure at a temperature not exceeding 40 °C. Then it was dried by using lyophlizer then after it is stored at −40 °C. The percentage yield of extraction was calculated by the formula:

$$\text{Yield\%} = \frac{\text{weight of extract obtained}}{\text{Weight of plant sample}} \text{X } 100$$

It was then powdered, packed into a glass vial, properly labelled, and stored in a desiccator until use.

2.6. Determinations of antioxidant activity

The free radical scavenging activity of *D. Abyssinica* leaves was examined using a DPPH assay [16]. Four mg of DPPH was dissolved in 100 mL methanol in the dark, and 4 mL of a 0.1 mM methanolic solution of DPPH was mixed with a 100 μL methanolic solution of different concentrations (5, 10, 20, 40, and 80 μg/mL) of the leaves extract. Ascorbic acid was used as a positive control at the same concentration. It was allowed to mix well and incubated in the dark for 30 min at room temperature. A methanolic solution of DPPH without the tested samples was used as a negative control. After 30 min, the absorbance of the mixture and the control at 517 nm was measured using a UV spectrophotometer. The test was repeated with the same concentration of each sample in triplicate and the average value was taken. The percentage scavenging of DPPH free radical was calculated using the equation below, and the IC_{50} value of each sample, which is the concentration of sample required to inhibit 50 % of the DPPH free radical, was obtained from the concentration vs. inhibition curve.

$$\text{\% Free radical scavenging} = \frac{\text{Ao} - \text{As}}{\text{Ao}} \times 100$$

Ao is the absorbance of the negative control, and As is the absorbance of the solution in the presence of sample extract or ascorbic acid.

2.7. Acute oral toxicity test

An acute oral toxicity test on the leaves extract of *D. Abyssinica* was carried out based on the limit test recommendations of the Organization for Economic Co-operation and Development guideline-425 (OECD) [17]. Five female mice aged 10–12 weeks old were used for this study. A dose of 2 g/kg was given to the first mouse. Since no death was observed within 24 h, the crude extract of 2 g/kg was administered to 4 additional mice. The mice were kept separately and then observed for behavioural and physical changes with special attention during the first 4 h. The observation was performed for the general signs and symptoms of toxicity, such as unusual skin and fur color, tremors, convulsions, salivation, diarrhoea, food and water consumption, coma, and mortality. The observation was continued for 14 days.

2.8. Induction of diabetes mellitus in experimental mice

Diabetes mellitus was induced in fasting mice (16 h) by a single IP injection of STZ at a concentration of 100 mg/kg body weight in 0.1 M citrate buffer (pH 4.5). It was then given to each mouse immediately. To prevent death from hypoglycemic shock, the mice were given free access to a 5 % glucose solution for the next 24 h after 6 h. Blood samples were taken from the tails of mice three days following STZ injection and measured using a CareSens glucometer. Diabetic mice were included in this study if their FBG levels were greater than 200 mg/dL [18].

2.9. Grouping and dosing of animals

These experimental mice were randomly divided into six groups consisting of 5 male mice in each group. The first group was normal mice, and the next five were diabetic mice.

- Group I (Normal control) was given only 2 % tween 80, 10 ml/kg distilled water.
- Group II STZ-induced diabetic mice that served as diabetic negative control was given 2 % tween 80, 10 ml/kg distilled water.
- Group III STZ-induced diabetic mice were treated with 100 mg/kg of *Dovyalis Abyssinica* Leaves Extract.
- Group IV STZ-induced diabetic mice were treated with 200 mg/kg of *Dovyalis Abyssinica* Leaves Extract.
- Group V STZ-induced diabetic mice were treated with 400 mg/kg of *Dovyalis Abyssinica* Leaves Extract. ’
- Group VI STZ-induced diabetic mice that served as diabetic positive control were treated with 5 mg/kg of glibenclamide.

2.10. Blood sample collection

Blood sample was collected from the tail vein of the mice using insulin syringe, and fasting blood glucose was estimated using a CareSens glucometer after overnight fasting just before starting the treatment (3rd days after STZ injection) as baseline (0), 7th, 14th, and 21st day of treatment.

2.11. Data analysis

Data were entered into Epi-data software version 4.6 and then exported to SPSS version 26.0 software for analysis and Microsoft Excel. Results were presented by tables and figures and expressed as mean ± standard error of the mean (SEM). Statistical data analyses were done using one-way ANOVA followed by the Tukey post hoc test. $P < 0.05$ was considered statistically significant.

3. Results

3.1. Percentage yield of crude extract

The crude extract obtained from 400-g coarse Powder *D. Abyssinica* leaves was 68 g. Therefore, the percentage yield of these extracts by using hydroethanolic (80/20 v/v) was calculated and given as follows:

$$\text{Percentage yield} = \frac{\text{weight of extract obtained}}{\text{Weight of plant sample}} \text{X } 100$$

$$\text{Percentage yield} = \frac{68\text{ g}}{400\text{g}} \text{X } 100 = 17\%(\text{w / w})$$

Therefore, 17 % w/w of crude extract was obtained from dried powder.

3.2. Acute toxicity test

Acute toxicity test results revealed no mortality at a dose of 2 g/kg in female mice within 21 days of observation. The mice did not show any toxic effects like changes in physical and behavioural activities such as unusual skin and fur color, tremors, convulsions, salivation, diarrhoea, and decreased food and water consumption.

3.3. Anti-oxidant activity of Dovyalis Abyssinica leaves

The IC_{50} values were obtained from the equations represented by a function of Y (DA) and Y (A.A) for extract and ascorbic acid, respectively (Fig. 1). The result from Fig. 1 indicates that the IC_{50} of *D. Abyssinica* Leaves is 140.04 μg/ml and ascorbic acid is 62.60 μg/ml. The percentage inhibition between extract and ascorbic acid did not produce a significant difference. As the concentration of the sample increased, the percentage inhibition of DPPH radical also increased.

3.4. Effect of Dovyalis Abyssinica leaves on body weight

The body weight of diabetic negative control mice was significantly reduced compared with the normal control mice on the 0, 7th, 14th, and 21st days of treatment. DALE at doses of 100 mg/kg showed significant ($P < 0.01$) improvement in body weight of the diabetic mice on the 21st day, whereas DALE 400 mg/kg, and GLC 5 mg/kg ($P < 0.05$, $P < 0.01$ and $P < 0.001$) showed significant improvement in body weight on 7th, 14th and 21st day respectively compared to the diabetes negative control. The body weight of normal control mice was significantly ($P < 0.05$) increased on the 14th and 21st day, while the diabetic control mice significantly decreased on the 7th, 14th and 21st days compared to baseline body weight in their respective group. Similarly, compared with baseline body weight, the diabetic mice treated with DALE 400 mg/kg and GLC 5 mg/kg significantly increased on the 14th and 21st days. However, the body weight of diabetic mice treated with DALE 100 and 200 mg/kg was not significantly changed from the baseline.

3.5. Effect of Dovyalis Abyssinica leaves on FBG level in diabetic mice

The effect of different doses of DALE on the FBG level in diabetic mice is given in (Fig. 2). The FBG levels of diabetic negative control mice were significantly ($P < 0.001$) higher than those of normal control mice on the 0, 7th, and 21st days. However, there were no significant differences in baseline FBG levels across all diabetic mice groups. All doses of extract-treated groups had significantly higher FBG levels at all-time points compared to normal control ($P < 0.001$). On the 7th day, treatment doses of DALE 200 mg/kg ($P < 0.01$), DALE 400 mg/kg, and GLC 5 mg/kg ($P < 0.001$) produced statistically significant reductions in FBG levels as compared to diabetes negative control. Similarly, as compared to diabetes negative control, DALE 100 mg/kg ($P < 0.01$) and all other treatment groups (DALE 200 mg/kg, DALE 400 mg/kg, and GLC 5 mg/kg) cause statistically significant ($P < 0.001$) reductions in FBG levels on the 21st day. There was no significant difference between DALE 400 mg/kg treated group and the group treated with 5 mg/kg GLC on 0, 7th, and 21st days.

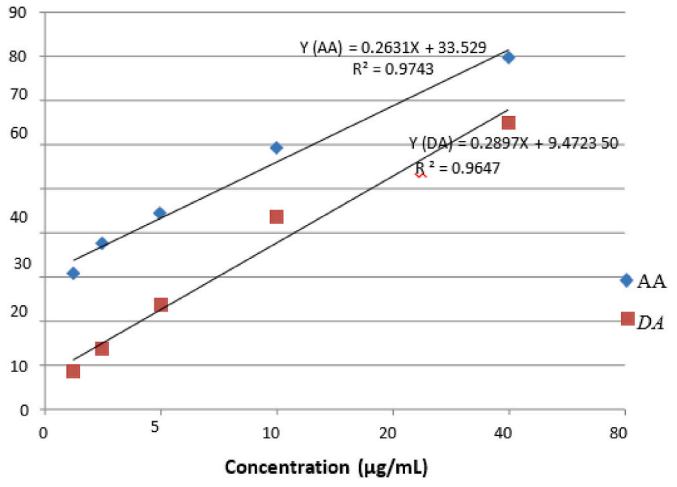

Fig. 1. Percentage inhibition of DPPH free radical by ascorbic acid (AA) and methanolic leaves extract of *Dovyalis Abyssinica* (DA).

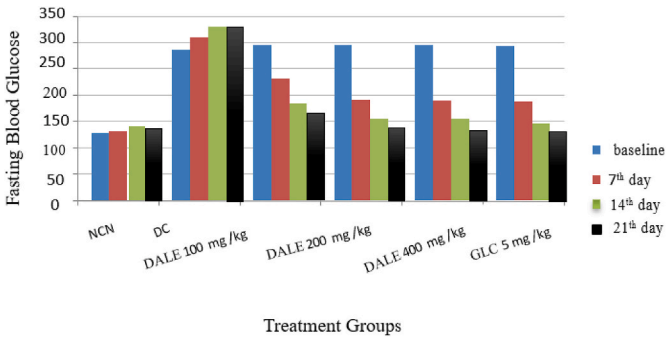

Fig. 2. The effects of *Dovyalis Abyssinica* leaf extracts on fasting blood glucose in STZ induced diabetic mice.

4. Discussion

Streptozotocin has been used to induce DM in experimental mice. A Single IP administration of 100 mg/kg STZ effectively induced DM in mice, which was confirmed by an elevated level of FBG after 3 days of injection. Mice fasted before STZ injection. This is because glucose can compete with STZ in the fed state, and thus, fasted animals tend to be more susceptible to this chemical [19]. This drug is a glucose analogue that is rapidly transported into the β-cells via GLUT2. The major pathways associated with cell death are (i) activation of the poly ADP-ribose synthetase as part of the cell repair mechanism, (ii) free radical generation, and (iii) nitric oxide production [20]. Streptozotocin sensitivity is highly variable in mice, and a dose that causes severe hyperglycemia in most animals may produce mild or no hyperglycemia in other mice [21]. Therefore, expecting to get a similar result is difficult. In this research, 78.06 % of mice receiving STZ were found to have FBG ≥200 mg/dl, which was an acceptable diabetic range for this research. Another study reported that 72.37 % of animals developed hyperglycemia with FBS ≥200 mg/dl by using 150 mg/kg of STZ [18]. This gap may be due to animal species, route of administration, nutritional status, and GLUT 2 expression difference [21].

Administering DALE at a single dose of 2 g/kg did not cause mortality within the first day as well as for the following 14 days of observation. Physical and behavioural observations of the mice also revealed no visible signs of toxicity like unusual skin and fur colour, diarrhoea, salivation, tremors, convulsions, decreased food and water consumption, and the like. This finding revealed that the median lethal dose (LD_{50}) of the plant extract is greater than 2 g/kg, which is similar to previous study done on the same plant leaves [22].

Oxidative stress plays a central role in the impairment of insulin action and exacerbating DM complications. Therefore, antioxidants and antidiabetic drugs are frequently recommended to avoid diabetic complications [23]. IC_{50} of the DALE was found to be 140.04 μg/ml, and IC_{50} of ascorbic acid was 62.60 μg/ml there was no significant difference between the two in DPPH scavenging activity. DALE

concentration-dependent antioxidant activity in this study, comparable with ascorbic acid. This finding is in line with antioxidant activity of the fruits extract of *D. Abyssinica* [24]; the fruits extract of *D. caffra* and *D. hebecarpa* [25]. Antioxidant activities of DALE might be due to the presence of phenolic compounds that can to donate hydrogen atoms or electrons and capture the free radical [26]. DALE might be exerting antioxidant activity through the enhancement of endogenous free radical scavenging enzymes. This is supported by the administration of fruits of D. caffra increased levels of superoxide dismutase [27].

The body weight of daily treated diabetic mice was measured because it is the best indicator of good health and an effective metabolic balance. The body weight of the normal control group significantly increased on the 14th and 21st day, while the diabetic negative control significantly decreased on the 14th and 7th and 21st days compared to the baseline body weight in their respective group. Diabetic negative control group, significant body weight loss was observed compared with normal control. This is consistent with a study of dichloromethane and methanol Extract of *D. Abyssinica* in parasitemia of mice [28]. In diabetic mice, this loss of body weight may be due to tissue protein breakdown and muscle wasting due to unavailability of carbohydrates as an energy source, catabolism of fats (catabolic effects of insulin deficiency), and volume depletion associated with osmotic diuresis [29].

However, DALE treatment at doses of 100 mg/kg showed significant improvement in body weight of the diabetic mice on the 21st day and DALE 200 mg/kg showed significant improvement in body weight on 14th and 21st days, whereas DALE 400 mg/kg, and GLC 5 mg/kg showed significant improvement in body weight on 7th, 14th and 21st days compared to the diabetes negative control. This finding is similar to dichloromethane and methanol Extract of D. Abyssinica [28]. The protective effect of DALE on body weight loss may be due to its capability to decrease hyperglycemia which was an indication of proper glucose utilization. Here, the bioactive compounds of DALE may help suppress the free radicals generated due to hyperglycemia, and control over muscle wasting or sparing protein catabolism resulting from glycemic control in treated diabetic mice ultimately leading to normalizing the level of body weight.

The increase in FBG level is an important characteristic feature of DM. Studies showed that a single IP injection of a high dose of STZ could produce sustained hyperglycemia in mice at least for a period of 8 weeks [30]. Likewise, STZ induced continuous hyperglycemia in this study with no substantial change in BGL during the study period of 21 days, as observed in the diabetic negative control. The FBG levels of the diabetic negative control group were significantly higher than those of the normal control. However, all doses of DALE reduced FBG levels compared to the diabetic negative control. This result agrees with the treatment of photosynthesized gold nanoparticles from *D. caffra* [31]. Hence, when the doses of DALE increased, the percentage of FBG level reduction also increased. The difference among the three DALE doses (100, 200 and 400 mg/kg) might be attributed to the latter containing a higher concentration of the active component responsible for more fall of FBG levels than the former.

The glycemic control was no significant difference between GLC 5 mg/kg and 400 mg/kg DALE in all days of treatment. Therefore, the increment of the DALE doses may further provide a similar result as the GLC. This might be due to similar mechanisms of action. Glibenclamide produces its effect via blockage of ATP-sensitive K^+ channels in the plasma membrane. This leads to membrane depolarization, activates voltage-gated $Ca2^+$ channels, a rise in cytosolic ($Ca2^+$) and release of endogenous insulin in β-cells of the pancreas [32]. This suggests that STZ at 100 mg/kg IP might not be sufficient for complete destruction of β-cells and/or few cells remaining capable of regenerating and secret insulin.

Pancreas β-cells are sensitive to damage by free radicals, which can be generated by STZ and hyperglycemia [33]. Previous studies revealed that different plant extracts had shown pancreas β cell-protective activity due to their antioxidant activities [34,35]. In the same way, DALE showed anti-oxidant activity in the present study. This suggests that the β-cell protecting effect secondary to the antioxidant activity of DALE might contribute to its antihyperglycemic activity in STZ-induced diabetic mice. Another study suggested the antidiabetic action of *D. Abyssinica* leaves through inhibiting pancreatic α-amylase [36].

The antihyperglycemic activity of DALE may be due to phytochemicals like alkaloids, phenolic, glycosides, saponins, tannins, flavonoids, and terpenoids. The majority of active compounds of alkaloids delay carbohydrate digestion and absorption, promote glucose uptake; glycosides promote insulin secretion, glycogen synthesis, increase glycolysis and lower gluconeogenesis; saponins increase regeneration of pancreatic β-cells [37]. Flavonoids insulin-mimetic, diminish glucose absorption, increasing expression and translocation of GLUT-4, and promote proliferation of pancreatic β-cells [38]. Tannins inducing glucose transport [39]; Terpenoids inhibiting gluconeogenesis and glycogenolysis [40]. Thus, the blood glucose-lowering effect of the DALE may be due to the presence of different secondary metabolites with possible independent or synergistic effects.

5. Conclusion

In this study, DALE revealed a significant reduction of FBG levels in STZ-induced diabetic mice. In addition, the in vitro study of DPPH suggested that DALE works by inhibiting free radicals activity. These effects might be through different mechanisms attributed by secondary metabolites present in the extract either synergistically or independently. Generally, the overall findings of this study suggested that DALE has the effect of normalizing metabolic disturbances of carbohydrate, lipid, and free radicals caused by diabetes and has antihyperglycemic as well as antioxidant activities.

Data availability

All the data that were used during the process of the experiment are available from the corresponding author and proved upon reasonable request.

Ethical approval

The research was conducted after getting an ethical approval letter from the School of Medicine Ethical Review Committee, College of Medicine and Health Sciences, University of Gondar. All procedures on mice were carried out in accordance with Guidelines for Care and Use of Laboratory Animals [41].

CRediT authorship contribution statement

Temesgen Baylie: Supervision, Software, Resources, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Wuhabie Tsega:** Methodology, Formal analysis, Data curation, Conceptualization. **Mamaru Getinet:** Supervision, Funding acquisition, Data curation, Conceptualization. **Desalegn Abebaw:** Writing – original draft, Visualization, Validation, Data curation, Conceptualization. **Gashaw Azanaw:** Writing – review & editing, Formal analysis, Data curation, Conceptualization. **Adane Adugna:** Methodology, Funding acquisition, Formal analysis, Data curation, Conceptualization. **Mohammed Jemal:** Writing – original draft, Methodology, Data curation, Conceptualization.

Declaration of competing interest

They have no conflict interests.

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Abbreviations

BGL	Blood Glucose Level
CE	Cholesterol Ester
DM	Diabetes Mellitus
D.S	*Datura stramonium*
DALE	*Dovyalis Abyssinica* Leaves Extract
FBG	Fasting Blood Glucose
IC_{50}	Inhibition Concentrations
LD_{50}	Median lethal dose
OECD	Organization for Economic Cooperation and Development
STZ	Streptozotocin
WHO	World Health Organization

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