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Effect of *Justicia schimperiana* (Acanthaceae) roots extract on blood glucose level and lipid profiles in streptozotocin-induced diabetic mice

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ABSTRACT

Background: *Justicia schimperiana* has been used traditionally for the treatment of different diseases, including, diabetes. Yet, no *in vivo* study was conducted to substantiate these claims. This study aimed to evaluate the effect of *Justicia schimperiana* roots extract on blood glucose levels and lipid profiles in streptozotocin-induced diabetic mice.

Methods: Male Swiss albino mice weighing 25–35 g were induced diabetes with 150 mg/kg of STZ. Animals were randomly grouped into six groups of five each. Group I was a normal control, Group II was a Diabetic control, Group III–V were Diabetic Mice treated with the extract (100, 200, and 400 mg/kg) respectively, and Group VI was standard control. The treatments were followed for 14 days. The FBG measurements were done on 0, 7th, and 14th days of treatment. On the 15th day, the mice were anesthetized with diethyl ether; blood samples were collected for the assessment of serum lipid profiles. The antioxidant and α-amylase inhibitory activities of the extract were also investigated *in vitro* using the DPPH and DNSA assay methods, respectively. The data were entered into EPI DATA version 4.6, exported to IBM, SPSS version 26.0, and analyzed using a one-way ANOVA followed by Tukey's post hoc test. $P < 0.05$ was considered statistically significant.

Results: The hydromethanolic extract of *J. schimperiana* roots exhibited no toxicity up to a dose of 2000 mg/kg body weight. In the STZ-induced diabetic mice, the extract reduced blood glucose levels at all tested doses: 100, 200, and 400 mg/kg on the 14th day as compared to diabetic control. The higher dose showed maximum reduction (29.73 %, $p < 0.001$) on the 14th day of treatment compared to the baseline. There were significant reductions in serum TG, TC, LDL, and a significant increase in body weight and HDL compared to the diabetic control. Besides, good antioxidant and α-amylase inhibitory activity were obtained from the *in vitro* laboratory tests.

Conclusions: Evidence from our study revealed that the root extract of *J. schimperiana* has antihyperglycemic and antihyperlipidemic effects in STZ-induced diabetic mice.

1. Introduction

Diabetes mellitus is a group of metabolic disorders characterized by chronic hyperglycemia with disturbances of carbohydrates, fat, and protein metabolism resulting from defects in insulin secretion, insulin action, or both [1]. Nowadays, it is becoming a worldwide public health issue with an alarmingly high prevalence and mortality rate. It affects 536 million people worldwide, and this number is expected to exceed 783.2 million by the year 2045 [2]. In Africa, 24 million adults (ages 20–79 years) are living with diabetes. The prevalence of diabetes mellitus (DM) in Ethiopia is 2 %–6.5 % and 76 % of them were unaware of their disease until complications [3]. The chronic hyperglycemia of diabetes is linked with long-term damage, dysfunction, and failure of various organs including the eyes, kidneys, nerves, heart, and blood vessels [4]. In addition, hypertriglyceridemia, and hypercholesterolemia are common complications of diabetic mellitus. Diabetic patients

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have high serum triglyceride, total cholesterol levels, and low high-density lipoprotein cholesterol compared to non-diabetic individuals [5,6].

The primary targets of diabetes management are alleviating hyperglycemia symptoms, lowering blood glucose levels, and avoiding or postponing the development of diabetic complications [7]. Insulin and oral antihyperglycemic medications are pharmacological treatments for diabetes mellitus in addition to diet and exercise. The management of diabetes without adverse effects is still a challenge. Even though insulin therapy and oral hypoglycemic medications are the first line of treatment for diabetes mellitus, they have various limitations including side effects, a high cost, and low effectiveness, and fail to significantly alter diabetic problems. These limitations of oral anti-diabetic medications now on the market, concerning efficacy and safety, as well as the general global epidemic of the illness, have prompted the development of alternative therapies that can control diabetes more effectively and safely. Many bioactive substances, such as antihyperglycemic and antihyperlipidemic drugs, are found in medicinal plants [8].

Many people around the world use medicinal plants for a variety of purposes, the most common of which is to treat multiple diseases because they contain a wide range of bioactive components, making them a potential source for various types of drugs. Polysaccharides, amino acids, flavonoids, saponins, tannins, phenols, alkaloids, glycosides, terpenoids, steroids, and other phytochemical constituents derived from these medicinal plants have anti-diabetic properties. These molecules can communicate with a variety of metabolic cascades in the human body, affecting blood glucose levels either directly or indirectly [9]. Because of the presence of bioactive compounds in medicinal plants' leaves, roots, flowers, seeds, stems, and barks, are responsible for medicinal plants' ability to lower blood glucose [10]. Many medicinal plants are used locally in Ethiopia to treat diabetes mellitus. *Justicia schimperiana* plant are one of the plants used to treat diabetes [11].

Justicia schimperiana is a plant in the genus of *Justicia* and the Acanthaceae family [12]. The plant is widely distributed in Ethiopia and Eretria. It has been commonly used for the treatment of various health problems such as malaria, hepatitis B, common cold, diarrhea, constipation, and leishmaniasis. A past study on the leaf of *Justicia schimperiana* revealed hypoglycemic and antihyperglycemic activity [13]. Plants having potent phytochemicals including phenolic, tannin, terpenoid, and flavonoid compounds have strong antioxidant properties [14]. The extract *Justicia schimperiana* roots also contain secondary metabolites such as phenols, flavonoids, tannins, and terpenoids, which are known to have blood glucose-lowering activity according to previous phytochemical studies [15,16]. In addition, another plant (the root of *Acanthus ilicifolius)* with specious had been shown to have good antidiabetic activity [17]. According to an ethnobotanical survey conducted around Wolayta, Ethiopia, *Justicia schimperiana* has traditionally been used to treat diabetes [11] and the root extract has not been scientifically studied. Thus, the present study aimed to evaluate the effects of *Justicia schimperiana* root extracts on blood glucose levels (BGL) and lipid profiles in STZ-induced diabetic mice.

2. Materials and methods

2.1. Experimental animals

Swiss albino mice were obtained from the animal house of the Department of Pharmacology, School of Pharmacy, College of Medicine and Health Sciences, University of Gondar. Male and female mice weighing 25–35 g and aged 8–12 weeks were used in the study. In the experiment, male mice were used for the STZ-induced diabetic model and female mice for the acute toxicity test [18]. The animals were housed in polypropylene cages under standard conditions (at a temperature of 22 ± 2 °C, with a 12 h light-dark cycle) and provided with free access to a pellet diet and water *ad libitum* up to the date of experimentation. The mice were acclimatized to laboratory conditions for a week before the experiment began. At the end of the experiment, the mice were sacrificed after being anesthetized with diethyl ether, and blood was drawn via cardiac puncture and then buried in the college's disposal area.

2.2. Drugs, reagents and instruments

To conduct this research, different types of chemicals and reagents were used. These were Streptozotocin (Fisco Research Laboratories Pvt. Ltd, India), glibenclamide (Sanofi aventis, France), DPPH (Sigma-Aldrich, Germany), alpha amylase (Blulux Laboratories Pvt. Ltd., Faridaban, India), acarbose (Bayer, Germany), DNSA (Sisco Research Laboratories Pvt. Ltd. Mumbai, India), ascorbic acid (Blulux Laboratories, India), citric acid (Lab tech chemicals, India), 5 % glucose solution (Reyoung Pharmaceuticals, Shandong, China), tween-80 (Avishkar Lab Tech chemicals, India), diethyl ether (BDH chemical pool), distilled water, sodium hydroxide and sodium citrate (Blulux Laboratories, India), caresence glucometer (Secho-gu, Soul 06646. Korea), rotary evaporator (Yamato, Japan), lyophilizer (Labfreez, China), spectrophotometer (Jenway, model 6500), refrigerator, oven (MeditMedizin Technik, Germany), auto lab clinical chemistry analyzer (Beckman coulter, Germany), whatman no-1 filter paper, gavages (oral feeding syringes), Whatman filter paper No.1 (Maidstone, UK), digital analytical balance (EPH-400 Abron Exports), pH meter, oral gavages, refrigerator, desiccator, centrifuge scientific LTD (made in West Sussex U.K).

2.3. Collection and extraction of J. schimperiana roots

The fresh roots of *J. schimperiana* were collected from Gondar town, Amhara region, Ethiopia (Alt.: 2699 m, Lat.: 12° 35′ N and Long.: 37° 28′ E). The plant material was authenticated by Mr. Zelalem Getnet, a botanist at the University of Gondar Department of Biology with a voucher number Biol/872/11/2022 has been given and deposited in the herbarium of the department.

The collected roots of the plant were transported to the University of Gondar Department of Biochemistry laboratory and the fresh plant roots were washed with distilled water to remove dirt and soil, and dried under shade and optimal ventilation. The dried plant materials were powdered by an electrical mill. Then, the coarse powdered plant materials (600 g) were macerated with hydromethanol for 72 h with an automatic shaker. The resulting liquid extract was filtered with gauze and then on Whatman No. 1 filter paper and the remaining residue (marc) was re-extracted for the second and third time by adding another fresh solvent. The filtrates were concentrated in a rotary evaporator under reduced pressure at 40 °C and then, it was dried in a lyophilizer and kept in a refrigerator (4 °C) with brown bottle [19].

2.4. Determinations of in vitro α-amylase inhibitory activity

In vitro, the α-amylase inhibitory activity was conducted through 3, 5-dinitrosalicylic acid (DNSA) method with minor modifications [20]. The crude extract of *J. schimperiana* was diluted in buffer (Na_2HPO_4/-NaH_2PO_4 (0.02 M), NaCl (0.006 M) at pH 6.9) to obtain concentrations ranging from 10 to 500 μg/mL. Similarly, Acarbose solution (a positive standard drug) with the same concentration was prepared. A volume of 200 μL of pancreatic α-amylase solution was mixed with the same volume of each plant extract or Acarbose concentration and incubated at 30 °C for 10 min. Thereafter, 200 μL of starch solution (1 % in water (w/v)) was added to each test tube and incubated for 3 min. The reaction was stopped by the addition of 200 μL of DNSA color reagent (prepared from 12 g of sodium potassium tartrate tetrahydrate ($KNaC_4H_4O_6{\cdot}4H_2O$) in 8.0 mL of 2 M NaOH and 20 mL of 96 mM of DNSA solution) and boiled for 10 min in water bath at 85 °C. The mixture was cooled at room temperature and diluted with 5 mL of distilled water. Then the absorbance was measured at 540 nm by using UV spectrophotometer. The blank with 100 % enzyme activity was prepared by replacing the plant

extract or Acarbose with 200 μL of the buffer. A control blank solution, and sample blank (extract or Acarbose) without enzyme were performed similarly. The α-amylase inhibitory activity was expressed as percent inhibition and calculated using the equation below.

$$\%\ \alpha\text{amylase inhibitio} = \frac{(Ac - Acb) - (As - Asb)}{Ac - Acb} \times 100 \quad (1)$$

Where Ac- absorbance of control (enzyme and buffer); Acb - absorbance of control blank (buffer without enzyme); As - absorbance of sample (enzyme and inhibitor); and Asb - absorbance of sample blank (inhibitor without enzyme).

Finally, the percentage of α-amylase inhibition was plotted against the extract and Acarbose concentration. The IC50 value, the sample concentration required to inhibit 50 % α-amylase activity, was obtained from the graph for both extract and Acarbose. The measurement was done three times, and the average of the IC50 was taken.

2.5. Determination of in vitro antioxidant activity

The Free radical scavenging activity of *J. schimperiana* roots extract was measured by using DPPH assay [21]. The plant extract was dissolved in methanol and samples with different concentrations ranging from 12.5 to 600 μg/mL were prepared. Ascorbic acid was used as a standard antioxidant and examined under the same conditions as the extract. A methanolic solution (0.1 mM) of DPPH was prepared and stored in a dark and cool place until use. A methanolic DPPH solution (3 ml of 0.004 % methanol solution) was mixed with 1 mL of each extract and ascorbic acid solution. The blank solution was prepared by replacing the plant extract or ascorbic acid with 1 mL of methanol mixed with 3 mL of DPPH solution. After keeping the reaction solution in the dark for 30 min at room temperature, the absorbance of each solution was read at 517 nm by UV spectrophotometer. Absorbance was measured triplicate for each sample and the average value was taken. The antioxidant activity in terms percent inhibition was calculated (Equation (2)). IC50 is the amount of sample required to scavenge 50 % DPPH free radicals and was obtained from dose vs. inhibition graph.

$$\%\ \text{DPPH inhibition} = \frac{Ac - As}{Ac} \times 100 \quad (2)$$

Ac is the absorbance of the control in the absence of the test sample and As is the absorbance of the plant extract or standard drug (ascorbic acid).

2.6. Acute toxicity test

Before beginning the entire study, an acute oral toxicity test was performed on the crude extract, based on the limit test recommendations of the Organization for Economic Cooperation and Development (OECD) No 425 Guideline [18]. Five female Swiss albino mice (aged 8–12 weeks) were used in the study. Initially, one mouse was fasted for 4 h (except for water), and a single dose of 2000 mg/kg body weight of the root extracts was subsequently administered orally through oral gavage. The mouse was then kept under strict observation for physical and behavioral changes such as, hair erection, convulsions, lacrimation, restlessness, salivation, diarrhea, and coma, as well as mortality, for 24 h with special attention paid during the first 4 h. The remaining four female mice were chosen based on the results of the first mouse. Based on the acute toxicity test result, 100 mg/kg, 200 mg/kg, and 400 mg/kg doses of the root extracts were selected for this study. The follow-up was continued for a total of 14 days for any sign of toxicity or mortality.

2.7. Induction of diabetes mellitus in experimental mice

Diabetes was induced in overnight fasting mice (for 16 h) by a single intraperitoneal injection of STZ at a dosage of 150 mg/kg body weight in 0.1 M citrate buffer (pH 4.5) in a volume of 10 mL/kg body weight [22]. Then it was provided right away to every mouse. After 6 h, the mice were allowed free access to a 5 % glucose solution for the following 24 h in order to prevent hypoglycemia shock-related death. Three days after STZ injection, each animal's blood glucose concentration was measured with a CareSence glucometer. Mice with diabetes were enrolled in this study if their FBG levels exceeded 200 mg/dL [23].

2.8. Grouping and dosing of animals

The experimental mice were randomly divided into six groups comprising five male mice in each group. The first group was normal mice (randomly selected before STZ injection), and the next five were diabetic mice. They were assigned as follows:

A. Mice in Group I (Normal control group) were treated with 2 % Tween 80, 10 mL/kg distilled water.
B. Mice in Group II STZ-induced diabetic mice that served as diabetic control was given 2 % tween 80, 10 mL/kg distilled water.
C. Mice in Group III (Experimental group) STZ-induced diabetic mice were treated with 100 mg/kg of *Justicia schimperiana* roots extract.
D. Mice in Group IV (Experimental group) STZ-induced diabetic mice were treated with 200 mg/kg of *Justicia schimperiana* roots extract.
E. Mice in Group V (Experimental group) STZ-induced diabetic mice were treated with 400 mg/kg of *Justicia schimperiana* roots extract.
F. Mice in Group VI STZ-induced diabetic mice that served as standard control were treated with 5 mg/kg of glibenclamide.

The doses of the extract to be administered were determined based on acute toxicity test results, and the volume of administration was 10 mL/kg (1 mL/100 g) of body weight [24]. The middle dose (200 mg/kg) is 1/10th of the limit dose (2000 mg/kg), the higher dose (400 mg/kg) is twice the middle dose, and the lower dose (100 mg/kg) was calculated as half of the middle dose [18].

2.9. Measurement of blood glucose level

Blood sample was withdrawn from the tail vein of the mice, and fasting blood glucose was measured with a CareSens glucometer after an overnight fast just prior to treatment (3 days after STZ injection) as baseline (0) and then on the 7th and 14th day of treatment.

2.10. Determination of serum lipid profiles

At the end of the experiment, animals were fasted overnight and anesthetized by inhaled diethyl ether and 1 mL blood was collected via cardiac puncture to a serum separator tube (SST) using a sterile 3 mL syringe. After the blood was coagulated for 30 min at room temperature, it was centrifuged for 10 min at 3000 rpm. The serum sample was transferred to a Nunc tube and maintained in a deep freezer until analysis was performed. Finally, serum lipid profiles (total cholesterol, triglycerides, high-density lipoprotein cholesterol, and low-density lipoprotein were determined with an automated chemistry analyzer [25].

2.11. Statistical analysis

The data were expressed as mean ± standard error of mean (SEM). Mean differences among groups were analyzed using one-way analysis of variance (ANOVA) followed by post hoc Tukey's test. Statistical analysis was processed using the International Business Machine of Statistical Package for the Social Sciences, (IBM SPSS), version 26 software, and Microsoft Excel. The results with p value < 0.05 were considered statistically significant.

3. Results

3.1. Percent yield of J. schimperiana roots extract

In the preparation of crude hydro methanol (20/80 v/v) extracts of *J. schimperiana* from 600 g of coarse powder roots, 16.5 % w/w yield of dark brown gummy residue was obtained.

3.2. In vitro α-amylase inhibitory activity of root extracts

The α-amylase inhibitory activity of the crude extract and Acarbose (the standard α-amylase inhibitory drug) are shown in Fig. 1. Concentration-dependent inhibition was seen for various concentrations of the tested extract and the standard drug. Hydromethanolic crude root extracts of *J. schimperiana* revealed a maximum α-amylase enzyme inhibitory activity of 76.27 % at the highest concentration with IC50 of 41.534 μg/mL. At the same concentration, the maximum α-amylase inhibition of standard drug, acarbose was 88.25 % with IC50 of 19.002 μg/mL. All tests were independently performed in triplicate.

3.3. In vitro antioxidant activity of root extracts

The DPPH radical scavenging activity of hydro methanolic extract *J. schimperiana* roots and standard antioxidant, ascorbic acid by using UV spectrophotometer indicated by Fig. 2. The IC50 values of ascorbic acid and the root extract were obtained from the equations represented by a function of Y (AA) and Y (JS) for ascorbic acid and *J. schimperiana* respectively (Fig. 2). The maximum radical scavenging activity of the standard drug, ascorbic acid was 79.78 % with IC50 value of 76.13 μg/mL and the root extract was 65.10 % with IC50 value of 139.03 μg/mL. The percent inhibition of the DPPH radical increased as the sample concentration increased. All tests were independently performed in triplicate See Fig. 2).

3.4. Acute toxicity test

The acute toxicity test of *J. schimperiana* root extracts did not show mortality in the animals at the limit dose of 2000 mg/kg within the first 24 h and 14 days of follow-up. The experimental mice did not show any physical or behavioral changes such as tremor, diarrhea, restlessness, salivation, or hair erection, showing no overt toxicity. This indicated that the median lethal dose (LD50) of roots extract, a dose required to kill 50 % of the experimental animal, was greater than 2000 mg/kg.

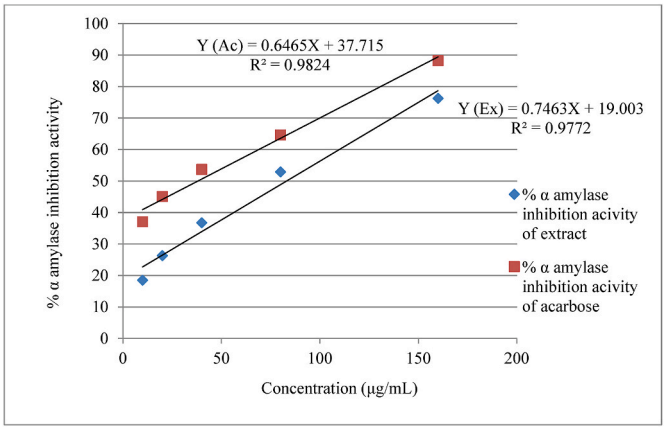

Fig. 1. α-Amylase inhibitory activity of crude root extracts of *J. schimperiana* and Acarbose
Abbreviation: IC50; median half of maximum inhibition concentration.

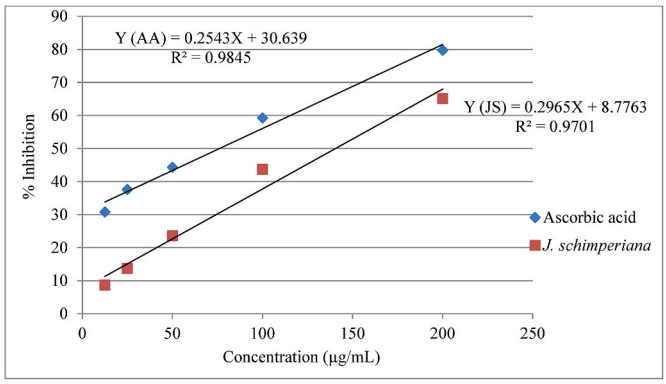

Fig. 2. Radical scavenging activity of root extracts of *J. schimperiana* and ascorbic acid.

3.5. Effects of J. schimeriana root extract on fasting blood glucose level

In this study, group analysis showed that initially (on day 0) there was no significance difference in fasting blood glucose among diabetic groups, but a marked increase was recorded as compared to the normal control group. The fasting BGL of the diabetic control group showed significant ($p < 0.001$) increase as compared to the normal control group on day 0, 7th and 14th. However, in diabetic mice treated with the 200 mg/kg, 400 mg/kg of the root extracts and GLC 5 mg/kg on day 7th ($p < 0.01$, $p < 0.001$, $p < 0.001$ respectively) and 14th ($p < 0.001$) the fasting BGL was significantly reduced as compared to diabetic control group. The lower dose treated group significantly decreased ($p < 0.05$) fasting BGL on day 14th as compared with diabetic control group. There were also statistically significant difference in fasting BGL of the extract at lower and middle dose compared to glibenclamide, but insignificance difference with higher dose. Besides, at all the tested dose of root extract and GLC showed significant reduction of BGL on 14th day compared to the baseline. In contrast, diabetic control and the normal control group showed statistically insignificant reduction of BGL on the 14th day compared to the respective baseline level. The maximum reduction in fasting BGL was attained on the 14th day 29.73 %, and 39.09 %, respectively, for JSRE 400 mg/kg, and GLC 5 mg/kg compared to respective baseline (Table 1).

Table 1
Effect of hydro methanol extract of *J. schimperiana* on fasting BGL in diabetic mice.

Groups	Fasting Blood Glucose Level (mg/dL)			Percent reduction	
	Baseline (0 day)	7th day	14th day	7th day	14th day
Normal control	94.40 ± 2.657	92.80 ± 0.860	93.60 ± 2.293	1.69 %	0.85 %
Diabetic control	275.20 ± 16.257^{a3}	279.80 ± 16.390^{a3}	282.60 ± 16.798^{a3}	−1.67 %	−2.69 %
JSRE 100 mg/kg	274.80 ± 11.110^{a3}	260.20 ± 7.774^{a3c1d3}	243.00 ± 2.864^{a3b1c1d3}	5.31 %	11.57 %
JSRE 200 mg/kg	276.40 ± 13.688^{a3}	242.20 ± 0.58^{a3b2c1d1}	232.80 ± 1.356^{a3b3c1d3}	12.37 %	15.77 %
JSRE 400 mg/kg	277.20 ± 19.353^{a3}	222.40 ± 5.59^{a3b3c1}	194.80 ± 10.307^{a3b3c2}	19.77 %	29.73 %
Glc 5 mg/kg	276.80 ± 18.258^{a3}	192.80 ± 9.008^{a3b3c2}	168.60 ± 9.196^{a3b3c3}	30.35 %	39.09 %

Each value represents mean ± SEM; (n = 5 mice in each group); Analysis was performed by one-way ANOVA followed by Post Hoc Tukey's test; **a**, compared to the normal control; **b**, compared to the diabetic control; **c**, compared to baseline; **d**, compared to glibenclamide; $^{1}p < 0.05$; $^{2}p < 0.01$; $^{3}p < 0.001$.
Abbreviations: Glc, glibenclamide; JSRE, *Justicia schimperiana* root extract.

3.6. Effects of J. schimperiana root extracts on body weight

Before root extract administration (day 0), there was no significant difference between diabetic groups, but there was a considerable weight loss when compared to the normal control group. Group analysis revealed that diabetic control experienced significant ($p < 0.001$) body weight loss on the 14th day compared to the normal control group. However, on the same day all the three doses of the extract (lower, middle and higher) showed significant ($p < 0.01$, $p < 0.01$, $p < 0.001$ respectively) improvement in body weight compared to the diabetic control group. In addition, there was no statistically significant difference in body weight change between the standard drug glibenclamide and extract doses as well as among the three doses of extract (Fig. 3).

3.7. Effect of J. schimperiana root extracts on serum lipid profiles

There was a significant ($p < 0.001$) elevation of serum TC, TG, and LDL-c level with significant ($p < 0.001$) reduction of HDL-c in diabetic control as compared to normal control, confirming the induction of diabetic dyslipidemia. However, the diabetic mice treated with the lower, middle and higher doses of the extracts and glibenclamide with the duration of 14 days showed a significant reduction of serum TC, TG, and LDL-c levels with a significant elevation of serum HDL-c level as compared to the diabetic control group. The higher dose of the root extract has no significance difference as compared to glibenclamide in all lipid metabolites (Table 2).

4. Discussion

The present study aimed to investigate the effects of *Justicia schimperiana* root extract on blood glucose levels and lipid profiles in STZ-induced diabetic mice and *in vitro* antioxidant activity.

In this study, before proceeding to an *in vivo* experiment, the alpha-amylase inhibition and antioxidant activity of *J. schimperiana* root extracts were determined *in vitro*. Pancreatic alpha amylase is found in the brush border of the intestine and hydrolyzes complex polysaccharides into disaccharides and monosaccharides with the help of *α*-glucosidase that can be easily absorbed in the intestine [26]. Alpha amylase inhibitors prevent or delay this step to reduce hyperglycemia. It also plays a role in the managing diabetic complications [27]. The findings on *J. schimperiana* root extracts revealed concentration-dependent inhibition of α-amylase activity and inhibitory potential; as a result, the plant could have antidiabetic properties because there was no statistically

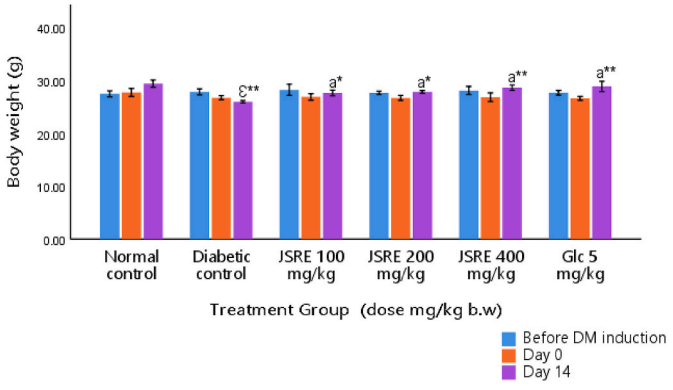

Fig. 3. Effect of *J. schimperiana* root extracts on body weight in STZ induced diabetic mice

The results are expressed as mean ± SEM (n = 5 mice in each group); **α**, compared to diabetic control; **Ɛ**, compared to normal control: *p < 0.01, **p < 0.001

Abbreviations: DM, diabetes mellitus; Glc, glibenclamide; JSRE, *Justicia schimperiana* root extracts.

Table 2
Effect of the crude root extracts on serum lipid profiles in STZ induced diabetic mice.

Groups	Serum lipid profiles (mg/dL)			
	TC	TG	HDL C	LDL C
Normal control	87.00 ± 3.12	84.00 ± 4.74	38.80 ± 2.22	31.40 ± 1.33
Diabetic control	176.00 ± 3.9^{A3}	151.00 ± 2.59^{A3}	23.80 ± 0.58^{A3}	122.00 ± 4.03^{A3}
JSRE 100 mg/kg	149.80 ± 8.73^{A3B2C3}	132.40 ± 2.71^{A3B2C3}	29.40 ± 1.29^{A3B1C2}	93.92 ± 9.59^{A3B2C3}
JSRE 200 mg/kg	131.20 ± 0.58^{A3B3C2}	127.60 ± 3.06^{A3B3C2}	31.60 ± 1.21^{A1B2}	74.08 ± 1.12^{A3B3C2}
JSRE 400 mg/kg	120.00 ± 1.59^{A3B3}	116.00 ± 1.82^{A3B3}	34.40 ± 1.08^{B3}	63.20 ± 2.29^{A2B3}
Glc 5 mg/kg	92.60 ± 3.33^{B3}	89.00 ± 1.95^{B3}	36.60 ± 0.51^{B3}	38.20 ± 3.21^{B3}

The results are expressed as mean ± SEM (n = 5 for each group) and analyzed by one-way ANOVA followed by post hoc Tukey's test. **A** compared to the normal control; **B**, compared to the diabetic control; **C**, compared to glibenclamide, $^{1}p < 0.05$, $^{2}p < 0.01$ and $^{3}p < 0.001$.

Abbreviations; Glc, glibenclamide, JSRE, *J. schimperiana* root extract, HDL-C, High density lipoprotein cholesterol, LDL-C, Low-density lipoprotein cholesterol, TC, Total cholesterol, TG, Triglyceride.

significant difference in IC50 values of the standard drug, Acarbose and the root extract. Flavonoids, phenols, and tannins are a major class of polyphenolic compounds that have been shown to inhibit α-amylase activity [27]. The phytochemical analysis revealed that the root extracts are rich in polyphenolic components, suggesting the bioactive components that inhibit α-amylase could be present at varying concentrations in all plant extracts.

Antioxidants protect the body by scavenging free radicals, inhibiting lipid peroxidation, and preventing other free radical-mediated processes. It also reduces oxidative stress and alleviates diabetic complications [28]. Plants having potent phytochemicals, such as phenolic and flavonoid compounds, have high antioxidant activity [14]. The presence of these compounds in *Justica schimperiana* root extracts may contribute to antioxidant activity by donating hydrogen atoms or electrons and capturing free radicals [29]. The extract of *J. schimperiana* root had no significant difference in radical scavenging activity as compared to the standard drug, ascorbic acid. The result of *J. schimperiana* root extract showed dose-dependent antioxidant activity. This finding is consistent with a previous study reported by Wakuma et al. [30].

The *in vitro* results of the extract establish its candidacy for further investigations of its anti-diabetic activity *in vivo* models. In the acute oral toxicity test, a single dose of 2000 mg/kg body weight of a crude extract of *J. schimperiana* roots administered orally remained non-toxic throughout the study period. The lethal dose (LD50) was greater than 2000 mg/kg, indicating that the extract is well tolerated and safe when administered orally. This safety profile is consistent with a report on a toxicity study of the same plant's leaf extract by Tesfaye et al. [13] and the same family root parts extract conducted by Manjula et al. [31].

The effect of the plant extracts on BGL was investigated using the STZ-induced diabetic mice model [32]. STZ has a selective cytotoxic effect on pancreatic β-cells, resulting in hyperglycemia since it increases the production of ROS, which reduce the synthesis and release of insulin [33]. In this study, the vehicle-treated groups did not show a significant reduction in BGL compared to the baseline level. However, the diabetic mice treated with the root extracts and the standard drug showed a significant reduction in fasting BGL. It is in line with a study reported by Wakene et al. [30]. Furthermore, on day 14th, diabetic mice treated with all tested doses of the root extracts and GLC showed a significant reduction in fasting blood glucose compared to their respective baselines. However, there was a significant difference between the extract at the lower and middle doses compared to the standard drug, glibenclamide, and insignificance at the higher dose. The dose of 400 mg/kg

resulted in the maximum percent reduction (29.73 %) in fasting BGL. It was comparable with the standard drug glibenclamide (5 mg/kg) (39.09 %) on the same day. This result could be due to a higher dose containing more bioactive constituents of the crude extract of the root. This finding is in line with the works of Muthulingam et al. [34] and Manjula et al. [31].

The blood glucose-lowering effects of *J. schimperiana* root extracts in STZ-induced diabetic mice could be due to insulin effect potentiation, most likely by increasing insulin secretion from pancreatic β-cells or increasing peripheral glucose uptake via expressing and translocating GLUT 4, and inhibit the activity of α-amylase enzymes in the intestine. Hence, a single intraperitoneal administration of 150 mg/kg STZ did not completely destroy β-cells and only a few cells retained the ability to regenerate and secrete insulin. Additionally, it might be due to secondary metabolites with possible synergistic effects, like flavonoids, which are known to have insulinogenic and pancreatic beta cell regenerating activities [35], and tannins, which have excellent antioxidant activity [36]. However, detailed pharmacological and biochemical researches are required to identify the exact mechanism for the anti-hyperglycemic effects observed in the study.

In this study, slight body weight loss was observed in STZ-induced diabetic mice and was almost normalized by treatment with *J. schimperiana* root crude extract. Dehydration and body weight loss have been related to DM [37]. A decrease in body weight and increased water intake was observed in diabetic mice. This indicates a polydipsia condition and loss of body weight due to the excessive breakdown of tissue proteins. The decrease in body weight in diabetic mice could be due to dehydration and the catabolism of proteins or fats, which might lead to muscle wasting [38]. Oral administration of *J. schimperiana* roots crude extract for 14 successive days to diabetic mice decreased their water intake and improved body weight. These effects could be due to its ability to reduce hyperglycemia. The bioactive compounds of the root extracts may help suppress the free radicals generated due to hyperglycemia and control muscle wasting resulting from glycemic control in treated diabetic mice and, eventually, body weight normalization [39]. It is consistent with the study conducted by Amare et al. [40].

In diabetes, elevated TC, TG, LDL, and VLDL levels and decreased HDL cholesterol levels, characterizes hyperlipidemia. These changes are a major contributor to cardiovascular issues [41]. The abnormal concentration of serum lipids in diabetes is mainly due to the activation of hormone-sensitive lipase, which leads to increased lipolysis (an increase in free fatty acid mobilization from the peripheral depots) and increased secretion of VLDL from the liver. Lipoprotein lipase is less active in diabetic patients due to insulin deficiency, leading to diabetic dyslipidemia [42]. *J. schimperiana* root extract-treated groups of mice demonstrated significant effects on abnormal lipid profiles compared to the diabetic control group.

In this study, diabetic mice were given root extracts and GLC for two weeks, significantly reducing serum TC, TG, and LDL-C levels while increasing HDL-C levels compared to the diabetic control group. After 14 days of treatment, the higher dose of the plant extract was able to reverse the values of TC, TG, HDL-C, and LDL-C closer to the standard drug than the lower dose. It could be due to the dose-dependent effect of the plant extract, indicating that efficacy was proportional to the dose of *J. schimperiana* root extracts. It is also possible that the higher dose contains more bioactive constituents. This finding agrees with the previous study by Amare et al. [40].

The lowering of serum TG levels in the root extract-treated groups could be due to inhibiting endogenous TG synthesis in the liver. Alternatively, improvement in insulin level and the presence of active constituent (s) in *J. schimperiana* root extracts that suppressed the activity of hormone sensitive lipase in adipose tissue, or increased the activity of lipoprotein lipase or hepatic lipase responsible for the hydrolysis of excess lipoprotein bound TG into fatty acids. Furthermore, the increase in HDL-C levels in the root extract-treated groups, on the other hand, could be due to the rise in lecithin cholesterol acyltransferase (LCAT), which is essential in incorporating free cholesterol into HDL and transporting it back to the liver. The LDL-C lowering effect of the plant extract is likely due to increased expression of low-density lipoprotein receptor (LDLR), which raises LDL particle uptake in the liver from circulation by depleting intracellular cholesterol [43]. The lowering serum TC property of the plant extract also might be due to the presence of hypocholesterolemia compounds in the root extracts acting as an inhibitor for hepatic hydroxyl methyl glutaryl CoA (HMG CoA) reductase in cholesterol synthesis [44]. Isolated tannin, a phytochemical constituent of *J. schimperiana* extract from *Justicia* species, has shown antioxidant activity [12]. Hence, the reduction of TC in JSRE-treated diabetics might be due to this phytochemical constituent reducing lipid peroxidation via scavenging free radicals. Besides, flavonoids and lignin have lowering properties [12].

5. Recommendation

The result presented in this study regarding the root part of *Justicia schimperiana* should be taken as the basis for further investigation. Therefore, we recommend for future researchers, further study is needed to isolate and purify the active constituents present in the root of *Justicia schimperiana*. It also needs to identify the exact biochemical mechanism, which are responsible for the plant's antidiabetic activity. In addition, in this study, we didn't measure insulin.

6. Conclusion and future outlook

In this study, hydro methanol extracts of *Justicia schimperiana* roots showed a reduction in fasting blood glucose levels in STZ-induced diabetic mice. This could be due to the insulin-like and insulin-releasing activities of the extract. Similarly, the root extract also caused a decrease in TC, TG, and LDL-C and increased HDL-C, implying that it has an anti-hyperlipidemia effect on STZ-induced diabetic mice. The results obtained from the *in vitro* studies suggested that the extract works by inhibiting free radicals and ameliorating oxidative stress via antioxidant and alpha-amylase inhibitory activity. The higher dose the root extract showed a significant blood glucose lowering activity. Generally, the findings suggest that JSRE has antihyperglycemic and anti-hyperlipidemic effects in STZ-induced diabetic mice and have antioxidant activity.

Availability of materials and data

The data sets used and/or analyzed during the current study are available from the corresponding author upon reasonable request.

Ethical approval

Ethical clearance was requested and approved by the Ethical Review Committee of the School of Medicine, University of Gondar with a reference number SOM/1758/2022. All procedures on mice were performed per guidelines for the care and use of laboratory animals [45].

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CRediT authorship contribution statement

Mulugeta Kasaw Feleke: Writing – review & editing, Writing – original draft, Visualization, Resources, Methodology, Investigation, Formal analysis, Conceptualization. **Tesfahun Bekele:** Visualization, Supervision. **Gashaw Dessie:** Visualization, Supervision. **Tiget Ayelgn:** Visualization, Supervision. **Amare Nigatu:** Visualization, Supervision. **Tezera Jemere:** Visualization, Supervision. **Adugna Nigatu Alene:**

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Declaration of competing interest

The authors declare that they have no competing interests.

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Abbreviations

DM	Diabetes Mellitus
FBG	Fasting Blood Glucose
HDL	High-Density Lipoprotein
IC_{50}	Inhibition Concentrations
JSRE	*Justicia schimeriana* root Extract
LD_{50}	Median lethal dose
LDL	Low-Density Lipoprotein
OECD	Organization for Economic Cooperation and Development
STZ	Streptozotocin
TC	Total Cholesterol
TG	Triglyceride
VLDL	Very Low-Density Lipoprotein
WHO	World Health Organization

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