




# Antidiabetic Activity of Extracts of *Terminalia brownii* Fresen. Stem Bark in Mice

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**Background:** Diabetes mellitus is a chronic metabolic disorder that imposes a huge health and economic burden on societies. Because the currently available medications have many drawbacks, it is important to search for alternative therapies. Medicinal plants used in traditional medicines are ideal candidates. Hence, this study was undertaken to investigate the antidiabetic activity of crude extract and solvent fractions from the stem bark of *Terminalia brownii* Fresen. (Combretaceae) in mice.

**Materials and Methods:** The in vitro  $\alpha$ -amylase inhibition assay was performed using the chromogenic 3, 5-dinitrosalicylic acid (DNSA) method while the antihyperglycemic activity was assessed using three mouse models: streptozotocin-induced diabetic mice, normoglycemic mice, and oral glucose challenged mice. Experimental diabetes was induced by a single intraperitoneal injection of streptozotocin at a dose of 150 mg/kg and animals with fasting blood glucose level (BGL) >200 mg/dL were considered diabetic. Glibenclamide (5 mg/kg) was used as a standard drug. Fasting BGL and body weight were used to assess the antidiabetic activity. The result was analyzed using GraphPad Prism software version 8 and one-way ANOVA followed by Tukey's post hoc test with  $p < 0.05$  considered as statistically significant.

**Results:** The crude extract of *T. brownii* at all tested dose levels (250, 500 and 750 mg/kg) showed a significant BGL reduction in all the three animal models. Moreover, the ethyl acetate and aqueous fractions (at 500 mg/kg) significantly ( $p < 0.01$ ) reduced the BGL in the streptozotocin induced diabetic model. The crude extract and different solvent fractions also showed a dose-dependent in vitro  $\alpha$ -amylase inhibitory activity and improvement of body weight.

**Conclusion:** *T. brownii* crude extract and its solvent fractions showed a significant anti-hyperglycemic activity in STZ induced diabetic mice, hypoglycemic activity and improvement of oral glucose tolerance in normal mice.

**Keywords:** diabetes mellitus, streptozotocin,  $\alpha$ -amylase, medicinal plant

## Introduction

Diabetes mellitus (DM) is a chronic metabolic disorder characterized by chronic hyperglycemia resulting from defects in insulin secretion, insulin action, or both.<sup>1</sup> Abnormalities in carbohydrate, fat, and protein metabolism are common feature of DM, which are caused by inefficient action of insulin on target tissues primarily on skeletal muscle, body fat and liver.<sup>2</sup> It is associated with acute and chronic complications, which are accountable for the majority of DM-related morbidity and mortality, financial burden and poor quality of life. Moreover, the persistently elevated sugar level induced diabetic complications result in damage of various organs, mainly the eyes, kidneys, nerves, and blood vessels.<sup>3</sup> The global social,

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economic and health burden of diabetes is rising at alarming rate with its devastating complications.<sup>4</sup> DM has become one of the leading causes of morbidity and mortality worldwide. The number of patients is predicted to grow to 642 million in the year 2040, with the greatest increase expected in low and middle-income countries.<sup>5</sup>

In spite of the fact that several antidiabetic agents have been introduced to the market from natural and synthetic sources, diabetes, as well as its micro and macro complications, continues to be a major medical problem worldwide.<sup>6</sup> The currently available modern drugs used for the treatment of diabetes are often associated with limitations such as inadequate efficacy, high cost, and various side effects.<sup>7</sup> In view of the aforesaid drawbacks of conventional medicines, medicinal plants with claimed antidiabetic activity can be used as an alternative approach in the management of diabetes especially in developing countries due to their cost effectiveness, accessibility, far-reaching cultural acceptability, and lower side effects.<sup>8</sup> More than 1200 species of medicinal plants are used throughout the world by different ethnic people in traditional medicine for their supposed antidiabetic activity.<sup>6</sup> Some of these traditional medicines may be advocated to be formulated as active ingredients that can potentially exert health benefits including antidiabetic effects; yet, considerations of possible variations as a result of difference of geographic, climatic and extraction techniques should be taken into account.<sup>9,10</sup>

*Terminalia brownii* Fresen., which belongs to the Combretaceae family, is one of the medicinal plants that are traditionally used for management of diabetes and which require scientific validation.<sup>11,12</sup> *T. brownii* is native to Eritrea, Ethiopia, Somalia, Kenya, Democratic Republic of Congo, Sudan, Tanzania, and Uganda. In Ethiopia it is locally known as “Weiba” in Tigrigna and “Abalo” in Amharic.<sup>13</sup> *T. brownii* is one of the medicinal plants extensively used for treatment of various ailments in Eastern, Southern, and Western Africa. Traditional healers in Ethiopia use the various parts of the plant for treatment of diabetes, jaundice, hepatitis, liver cirrhosis, and yellow fever.<sup>14</sup> In Eritrea, the stem bark or leaves of *T. brownii* are used for treatment of infectious hepatitis, diabetes, wounds, malaria, dermatitis, tuberculosis, abdominal pain, burns, gynecological problems, dandruff, conjunctivitis, and other eye ailments.<sup>12,15</sup> Traditional Health Practitioners in Kenya use the decoction of *T. brownii* stem bark for the management of diabetes, heartburn, amoebiasis, eye problems, jaundice, cancer, and arthritis.<sup>11,16</sup>

Previous studies indicated that different parts of *T. brownii* have been shown to exhibit varieties of biological activities including anti-inflammatory, antinociceptive and antipyretic, in vitro antimicrobial and antiplasmodial, central analgesic, and radical scavenging activities.<sup>13,14,17-20</sup> Although *T. brownii* has been traditionally used for management of diabetes and its complication in the Ethiopian folklore medicine, scientific study that supports its traditional claim has not been carried out yet. Therefore, this study aimed to investigate the antidiabetic effect of the crude extract and solvent fractions of *T. brownii* stem bark.

## Materials and Methods

### Chemicals and Equipments

The chemicals, solvents and drugs used to conduct this experiment were analytical grade. Streptozotocin (Thermo Fisher Scientific, Waltham, MA, USA), Glibenclamide active powder (Thermo Fisher Scientific), citric acid (Loba Chemie Ltd, India), methanol (Carlo Erba Reagents, Val-de-Reuil, France), ethyl acetate (Carlo Erba Reagents), n-butanol (Carlo Erba Reagents), dimethyl sulfoxide (UNI-CHEM chemical reagents, UK), chloroform (Labort Fine Chem Pvt. Ltd, Surat, Gujarat, India), n-hexane (Blulux Laboratories Ltd, Faridabad, Haryana, India), glucometer, 3,5-dinitrosalicylic acid (DNSA) (Sigma-Aldrich Co., St Louis, MO, USA), and Accu-Check<sup>®</sup> Active glucometer test strips (Hoffman-La Roche Ltd., Basel, Switzerland) were used to carry out the experiment.

### Collection, Processing, and Authentication

The stem bark of *T. brownii* was collected from around Abiy Addi, Northwest Tigray of Ethiopia. The plant was authenticated by Mr Shambel Alemu, at the National Herbarium of Addis Ababa University, where a sample specimen with voucher number (NM-001) was deposited for future reference. The cleaned stem bark was air dried under shade and ground to a coarse powder using an electric grinder and kept in an airtight container at room temperature until used for extraction.

### Experimental Animals

Healthy, adult Swiss albino mice of either sex weighing 20–30 grams and 6–8 weeks of age were used. The experimental animals were acclimatized to the laboratory conditions for one week as per OECD guidelines.<sup>21</sup> The animals were housed in polypropylene cages with standard

condition (at room temperature, and 12 h light-dark cycle) with free access to commercial pellet laboratory diet and water ad libitum throughout the experimental period.<sup>22</sup> All the procedures in the experiment were carried out according to the Guide for the Care and Use of Laboratory Animals.<sup>23</sup> Ethical clearance was obtained from Mekelle University, College of Health Sciences, Health Research Ethics Review Committee (HRERC) with reference number 1040/2017. At the end of the experiment animals were sacrificed under anesthesia.

## Preparation of Crude Extract and Solvent Fractions

The dried and powdered stem bark of *T. brownii* (1 kg) was extracted by maceration using 80% methanol (1:5 plant material to solvent ratio) for 72 h. The mixture was stirred using an orbital shaker at 120 rotations per minute to facilitate the extraction process. After 72 h of maceration, the mixture was filtered using ordinary cloth and then by Whatman filter paper No. 1. The marc was re-extracted following the same procedure as the initial extraction so as to exhaustively extract the components in the plant material. The filtrates were combined and solvent was evaporated and dried in an oven at 40°C. The dried extract was transferred into a vial and kept in a refrigerator until used.<sup>13</sup> Solvent fractions were prepared by liquid-liquid partitioning method. Thirty grams of the crude extract was suspended in 200 mL of distilled water in separatory funnel and it was then partitioned successively with 200 mL of different solvents of increasing polarity, starting from n-hexane, followed by chloroform, ethyl acetate, and n-butanol, each three times. The different solvent fractions were collected in flasks and then dried in an oven at 40°C.<sup>20</sup> The dried fractions were transferred into vials and kept in a refrigerator until used.

## Acute Oral Toxicity Study

An acute toxicity test was performed according to the limit test recommended by the Organization for Economic Cooperation and Development (OECD) guideline 425.<sup>21</sup> Five female Swiss albino mice (nulliparous, non-pregnant) aged 6–8 weeks were used. On the first day a single female mouse that had fasted for four hours but with water ad libitum; received 2000 mg/kg of the crude extract orally. The mouse was kept under strict observation for any behavioral or physical changes within 24 h. After 24 h, the other four female mice were treated in the same

manner as the first mouse. The mice were observed for gross behavioral changes such as loss of appetite, hair erection, lacrimation, tremors, diarrhea, mortality, and other signs of toxicity manifestation for a period of 14 days.<sup>21</sup>

## In Vitro $\alpha$ -Amylase Inhibition Assay

The  $\alpha$ -amylase inhibitory effect of the plant extract and its solvent fractions was measured employing the chromogenic DNSA method described by Miller.<sup>24</sup> The total reaction mixture containing 1.4 mL of 0.05M sodium phosphate buffer (pH 6.9), 50  $\mu$ L of  $\alpha$ -amylase and acarbose (used as a positive control) or test samples (the crude extract and its solvent partitions) at concentrations of 10, 50 and 100  $\mu$ g/mL were incubated at 37°C for 10 min. After pre-incubation, 500  $\mu$ L of 1% (w/v) starch solution dissolved in the aforementioned buffer was added to each tube and incubated for 15 min at 37°C. One milliliter of DNSA reagent was added to the reaction tube and it was boiled in water bath for 5 min to stop the reaction. Then it was cooled at an ambient temperature and its absorbance was measured at 540 nm using ultraviolet-visible spectrophotometer (PG Instrument Ltd, Beijing, China). Absorbance of the control (100% enzyme activity) was performed in similar way as described above in the presence of the enzyme but in the absence of any test sample. Besides, a blank assay using the test samples in their respective concentration in the absence of the enzyme was performed to consider any possible intrinsic absorbance produced by the test samples. The inhibitory activity of the samples and the standard drug (acarbose) was calculated in comparison with the negative control (100% enzyme activity) using the equation given below.<sup>25</sup>

$$\% \text{ Inhibition} = \frac{\text{Absorbance of the control} - \text{Absorbance of the test sample}}{\text{Absorbance of the control}} \times 100$$

## Induction of Experimental Diabetes

Diabetes was induced by a single intraperitoneal injection of freshly prepared streptozotocin (STZ) at a dose of 150 mg/kg (dissolved in 0.1 M of cold citrate buffer; pH=4.5) to overnight fasted male Swiss albino mice.<sup>26,27</sup> All the animals were provided with free access to water and pellet diet after 30 min of administration of STZ.<sup>28</sup> The animals were kept under strict observation, and seven days after STZ injection the fasting BGL was determined using a glucometer.<sup>29</sup> Mice with fasting BGL of greater

than 200 mg/dL were selected and included in the study.<sup>28,30</sup>

## Grouping and Dosing of Animal Hypoglycemic Test on Normal Mice

Male Swiss albino mice were fasted for 6 h, but with free access to water, and then randomly divided into five groups (n=6). Group I served as vehicle control and received 5% dimethyl sulfoxide (DMSO); group II served as positive control and received glibenclamide (5 mg/kg) while the other three groups (Group III, IV and V) served as treatment groups and received 250 mg/kg, 500 mg/kg and 750 mg/kg of the crude extract of *T. brownii*, respectively. Glibenclamide and the crude extracts were dissolved in 5% DMSO. All groups were administered a single dose via oral gavage in a volume of 10 mL/kg. Blood sample was collected aseptically from the tail tip of each animal and the BGL was determined at 0, 1, 2, 3 and 4 h of post treatment using electronic glucometer.<sup>30,31</sup>

## Oral Glucose Tolerance Test (OGTT) in Mice

Mice were fasted for 6 h, but with water ad libitum, and randomly assigned into five groups (n=6). Group I (normal control) mice were treated with vehicle (5% DMSO); group II were treated with standard drug glibenclamide (5 mg/kg); groups III, IV and V were treated with 250 mg/kg, 500 mg/kg and 750 mg/kg of the crude extract, respectively. Glibenclamide and the crude extracts were dissolved in 5% DMSO and all the animals were orally administered in a single dose. Thirty minutes after treatment all the animals received 2 g/kg of glucose solution. Blood was collected from the tail tip of each mouse and BGL was determined immediately prior to treatment (at 0 min) as baseline and then after 30, 60, 120 and 180 min of glucose administration.<sup>32,33</sup>

## Antidiabetic Activity of *T. brownii* in STZ-Induced Diabetic Mice

Male mice were used for evaluation of antidiabetic activity of the crude methanolic extract and solvent fractions because rodents show a substantial gender difference in STZ sensitivity, male mice being more susceptible to the STZ induced diabetes mellitus than female mice.<sup>34,35</sup> Animals with confirmed diabetes (BGL >200 mg/dL) were randomly assigned into five groups (n=6). One additional normal control (nondiabetic control) group was also used. Group I (nondiabetic control) and Group II (diabetic control) group were treated with vehicle (5% DMSO) while Group III (positive control) were treated with standard drug

glibenclamide (5 mg/kg).<sup>30</sup> Groups IV, V and VI were administered with 250, 500 and 750 mg/kg of the crude extract, respectively. In the case of solvent fractions, the first three control groups were treated similarly as described above while Groups IV, V and VI received 500 mg/kg of the aqueous residue, ethyl acetate and n-butanol fractions respectively. All groups were administered daily for 15 days via oral gavage in a volume of 10 mL/kg. Blood was collected aseptically from the tail tip and BGL (6 h fasting) was measured at 0, 5, 10 and 15 days of the experiment with glucose reactive strips using Accu-Check®. Active electronic glucometer. Body weight of each mouse was also determined at the same time using electronic scales.<sup>36</sup>

## Preliminary Phytochemical Screening

The hydromethanolic extract of *T. brownii* was qualitatively screened for the presence or absence of bioactive phytochemicals such as alkaloids, polyphenols, flavonoids, tannins, saponins, terpenoids, and steroids.<sup>37</sup>

## Statistical Analysis

Statistical package for social sciences (SPSS) version 20 (IBM Corporation, Armonk, NY, USA) was used to enter and analyze the data. Data were expressed as mean  $\pm$  standard error of the mean. Statistical analysis was carried out using one-way analysis of variance (ANOVA) followed by Tukey's post-hoc test with multiple comparisons to determine the source of significant difference between the groups. The result was considered statistically significant when  $p < 0.05$ . GraphPad Prism software version 8 (GraphPad Software, Inc., La Jolla, CA, USA) was also employed to analyze the in vitro  $\alpha$ -amylase inhibition assay.

## Results

A 179 g of reddish brown powder was obtained from the 1 kg of the stem bark at the end of extraction process which resulted in 17.9% percentage yield.

## Acute Oral Toxicity Study

Oral administration of a single dose (2 g/kg) of the hydro-methanolic extract of *T. brownii* stem bark and fractions to female Swiss albino mice did not produce any signs of toxicity such as lacrimation, hair erection, convulsion, coma, and death during 24 h as well as during the 14 observation days of follow-up.

## Preliminary Phytochemical Screening

Preliminary phytochemical screening of the 80% methanolic stem bark extract of *T. brownii* revealed the presence of tannins, saponins, polyphenols, flavonoids, terpenoids, and steroids while alkaloids were not detected.

## In vitro $\alpha$ -Amylase Inhibitory Activities

As illustrated in Figure 1, the crude extract and the different solvent fractions showed concentration-dependent in vitro  $\alpha$ -amylase inhibitory activities with the highest percentage inhibition exhibited by the chloroform and butanol fractions. Similarly, the standard drug, acarbose, at the medium and highest doses showed roughly 100% enzyme inhibition. The chloroform fraction exhibited the highest  $\alpha$ -amylase inhibitory potential with the lowest  $IC_{50}$  value of 63.41  $\mu$ g/mL. The positive control, acarbose, revealed  $IC_{50}$  value of 12.5  $\mu$ g/mL (Table 1).

## Hypoglycemic Activity of Crude Extract of *T. brownii* Stem Bark in Normoglycemic Mice

The effect of the crude extract of *T. brownii* stem bark on fasting BGL of normoglycemic mice is summarized in Table 2. The crude extract treated groups showed significant reduction in BGL at all dose levels after 3 and 4 h of treatment compared to normal mice. Intragroup analysis of the crude extract at dose of 250, 500 and 750 mg/kg and glibenclamide 5 mg/kg showed significant reduction ( $p < 0.05$ ) in BGL at the fourth hour compared to the base line (0 h) of extract

**Table 1**  $IC_{50}$  Value of the Crude Extract and Solvent Fractions Against  $\alpha$ -Amylase

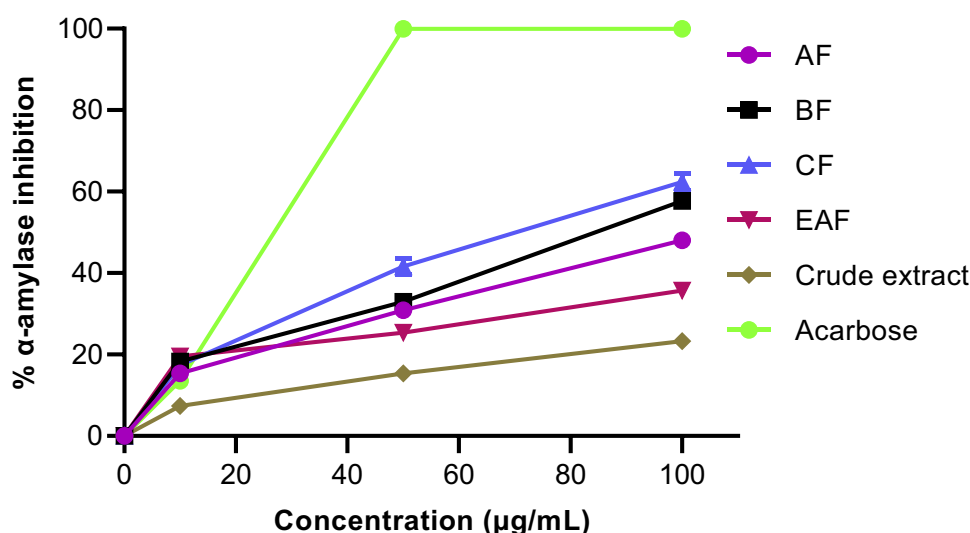
	AF	BF	CF	EAF	Crude Extract	Acarbose
$IC_{50}$ ( $\mu$ g/mL)	>100	84.69	63.41	>100	>100	~12.50

**Abbreviations:** AF, aqueous fraction; BF, butanol fraction; CF, chloroform fraction; EAF, ethyl acetate fraction.

administration. Moreover, comparing the fourth hour to first hour, the extract at all tested dose and glibenclamide produced significant ( $p < 0.05$ ) reduction in BGL. But, the BGL did not significantly change with in the different time interval after oral administration of the vehicle at a dose of 10 mL/kg. After 4 h of treatment the glibenclamide and crude extract at dose of 250, 500 and 750 mg/kg resulted in 43.2, 20.8, 28.2 and 32.6% reduction in BGL respectively compared to the baseline.

## Effect of the Crude Extract of *T. brownii* on OGTT in Normal Mice

As indicated in Table 3, there were no significant differences in BGL among all the groups ( $p > 0.05$ ) before glucose loading. But, all groups showed significant increase in BGL 30 min following glucose challenge, confirming the induction of hyperglycemia. The hyperglycemia with glucose challenge was significantly brought down by the crude extract at a dose of 500 mg/kg ( $p < 0.01$ ), 750 ( $p < 0.05$ ) after 60 min and 750 mg/kg ( $p < 0.01$ ) after 120 min of administration compared to normal control. Likewise, glibenclamide



**Figure 1** Plot of %  $\alpha$ -amylase inhibition of the crude extract and solvent fractions vs concentration.

**Abbreviations:** AF, aqueous fraction; BF, butanol fraction; CF, chloroform fraction; EAF, ethyl acetate fraction.



**Table 2** Effect of 80% Methanolic Stems Bark Extract of *T. brownii* on BGL of Normoglycemic Mice

Groups	Fasting Blood Glucose Level (mg/dL)				
	0 h	1 h	2 h	3 h	4 h
NC	146.16±7.58	147.66±10.87	150.16±9.35	145.33±6.48	144.00±5.85
GL 5 mg/kg	125.00±4.40	119.33±17.88	85.50±6.23 <sup>c***,a*</sup>	75.00±7.41 <sup>c***,a*</sup>	71.33±4.83 <sup>c***,a*</sup>
TB 250 mg/kg	144.83±6.22	153.66±4.40	135.16±7.30	112.50±4.66 <sup>c**,a***,b***</sup>	114.83±3.91 <sup>c*,a***,b***</sup>
TB 500 mg/kg	156.50±16.54	158.33±12.65	154.16±9.23	115.83±6.24 <sup>c*</sup>	112.33±5.23 <sup>c**,a*,b*</sup>
TB 750 mg/kg	153.00±10.12	160.00±13.24	153.50±13.32	116.66±6.30 <sup>c*</sup>	103.83±8.03 <sup>c***,a*,b**</sup>

**Notes:** Results are expressed as mean ±SEM, n=6 in each group. <sup>a</sup>Compared to 0 h; <sup>b</sup>compared to 1 h; <sup>c</sup>compared to normal control. \**p* <0.05, \*\**p* <0.01, \*\*\**p* <0.001. **Abbreviations:** TB, *Terminalia brownii*; NC, normal control; GL, Glibenclamide.

**Table 3** Effect of the Crude Hydromethanolic Stem Bark Extract of *T. brownii* on BGL of Mice Loaded with Oral Glucose

Groups	Blood Glucose Level (mg/dL)				
	0 min	30 min	60 min	120 min	180 min
NC	127.16±3.01	278.16±15.95	230.83±10.14	177.66±5.87	133.83±4.58
GL 5 mg/kg	144.16±8.60	174.83±22.73**	102.33±11.51***	89.50±2.72***	81.66±4.87***
TB 250 mg/kg	134.00±4.22	230.50±19.82	187.00±16.95	162.33±9.60	121.66±5.45
TB 500 mg/kg	136.50±8.88	276.33±14.24	183.83±9.10	139.33±5.21**	128.16±5.92
TB 750 mg/kg	132.00±6.77	276.83±10.81	179.33±12.33*	137.16±6.75**	126.33±6.83

**Notes:** Values are expressed as mean ±SEM, n=6 per group. \**p* <0.05, \*\**p* <0.01, \*\*\**p* <0.001 compared to normal control. **Abbreviations:** TB, *Terminalia brownii*; NC, normal control; GL, Glibenclamide.

at a dose of 5 mg/kg resulted in a very significant (*p* <0.001) reduction in BGL starting from 30 min of glucose challenge relative to normal control group.

### Effect of the Crude Extract of *T. brownii* on BGL of STZ-Induced Diabetic Mice

As shown in Table 4, the crude extract and the standard drug demonstrated a significant blood glucose lowering effect in diabetic treated group as compared to the diabetic control. The crude extract at 250 mg/kg significantly (*p* <0.05) lowered the fasting BGL at day 15 whereas, administration of 500 mg/kg and 750 mg/kg of the crude extract daily lowered the BGL significantly starting from day 10 (*p* <0.01) of the

experiment. Similarly, a significant reduction in BGL was observed with the standard drug, glibenclamide, at day 5 (*p* <0.05), day 10 (*p* <0.001) and day 15 (*p* <0.001) compared to the diabetic control. After treatment with *T. brownii* (250, 500, 750 mg/kg) and glibenclamide (5 mg/kg) for 15 days, the BGL were lowered by 39.09%, 49.1%, 66.75% and 69.32% respectively.

### Effect of the Solvent Fractions of *T. brownii* on BGL of STZ-Induced Diabetic Mice

As shown in Table 5, administration of the aqueous residue lowered the BGL significantly at day 5 (*p* <0.01), day 10 and day 15 (*p* <0.001) of treatment.

**Table 4** Effect of the Hydromethanolic Stem Bark Extract of *T. brownii* on BGL of STZ -induced Diabetic Mice

Groups	Fasting Blood Glucose Level (mg/dL)			
	Day 0	Day 5	Day 10	Day 15
Normal control	160.00±14.40	147.66±9.1	187.50±6.93	157.50±4.73
Diabetic control	386.16±45.83	422.33±37.12	405.33±29.27	394.83±49.40
GL 5 mg/kg	388.50±45.60	288.83±28.47*	214.66±38.80***	119.66±28.07***
TB 250 mg/kg	399.33±48.78	346.33±40.29	306.33±27.17	243.33±32.64*
TB 500 mg/kg	389.83±30.74	371.83±30.74	266.83±24.20*	198.33±37.22**
TB 750 mg/kg	391.16±39.02	374.83±29.58	226.66±30.36**	130.50±20.63***

**Notes:** The values indicate mean ±SEM (n=6). \**p* <0.05; \*\**p* <0.01; \*\*\**p* <0.001 compared to diabetic control. **Abbreviations:** TB, *Terminalia brownii*; GL, Glibenclamide.

**Table 5** Effect of the Different Solvent Fractions of *T. brownii* on BGL of STZ-induced Diabetic Mice

Treatment Groups	Fasting Blood Glucose Level (mg/dL)			
	Day 0	Day 5	Day 10	Day 15
Normal control	160.66±7.07	154.00±9.02	144.16±17.43	134.50±5.32
Diabetic control	361.00±39.02	400.83±29.80	403.16±49.22	469.00±39.40
GL 5 mg/kg	367.66±36.02	234.33±30.91*	209.33±39.04*	133.00±17.79***
AQF 500 mg/kg	320.66±49.92	191.50±42.34**	154.83±24.58***	144.16±24.43***
ETAF 500 mg/kg	320.33±46.57	253.83±39.88	244.16±45.27	190.50±33.41***
BF 500 mg/kg	396.00±44.34	421.33±48.52	417.00±40.47	400.33±48.79

**Notes:** The result are expressed as mean ±SEM (n=6). \* $p < 0.05$ ; \*\* $p < 0.01$ ; \*\*\* $p < 0.001$  compared to diabetic control.

**Abbreviations:** GL, glibenclamide; AQF, aqueous residue; ETAF, ethyl acetate fraction; BF, butanol fraction.

Ethyl acetate fraction also reduced the BGL very significantly ( $p < 0.001$ ) at the fifteenth day of treatment. Similarly glibenclamide at a dose of 5 mg/kg significantly reduced the fasting BGL on the days 5 and 10 of treatment ( $p < 0.05$ ) and very significantly at day 15 ( $p < 0.001$ ). However, administration of the n-butanol fraction did not significantly diminish the BGL. The aqueous residue, ethyl acetate fraction at a dose of 500 mg/kg and glibenclamide at a dose of 5 mg/kg diminished the BGL by 55%, 40.6% and 63.76% after 15 days of treatment respectively.

### Effect of the Crude Extract of *T. brownii* on Body Weight of STZ-Induced Diabetic Mice

STZ-caused weight reduction was partially reversed after 15 days of treatment by the crude extract of *T. brownii* at all dose levels Administration of glibenclamide to diabetic mice resulted in increase in body weight compared to diabetic control mice even though the body weight gain was not statistically significant. However, diabetic mice treated with plant extract, at doses of 250, 500 and 750 mg/kg decreased the body

weight by 4.7%, 0.13%, and 2.91%, respectively at the end of the experimental period, which was much lower than the decrease in body weight of diabetic control group (21%). On the contrary, normal animals increased their body weight by 21% at the end of treatment (Table 6).

### Effect of the Different Solvent Fractions of *T. brownii* on Body Weight of STZ-Induced Diabetic Mice

Initially, there were no significant differences in body weights among the groups ( $p > 0.05$ ). After induction of diabetes, statistically significant differences in body weight of normal and diabetic mice were observed ( $p < 0.05$ ). The body weight of normal control group was significantly increased by 19.0%, while that of diabetic control decreased by 10.3% at the end of the experiment. Administration of glibenclamide showed an increase in the body weight by 3.4%. However, there was a decrease in body weight in the aqueous, ethyl acetate, and n-butanol fractions treated mice the decrease was 3.7%, 2.99% and 6.22%, respectively; although the decrease was lower than that in the diabetic control group (Table 7).

**Table 6** Effect of the Hydromethanolic Stem Bark Extract of *T. brownii* on Body Weight of STZ Induced Diabetic Mice

Treatment Groups	Body Weight in Grams			
	Day 0	Day 5	Day 10	Day 15
Normal control	25.43±0.85	28.35±0.88	29.66±0.81	30.78±0.86
Diabetic control	28.50±1.81	27.70±2.00	27.70±2.00	25.03±1.80*
GL 5 mg/kg	27.28±1.63	26.03±1.76	27.03±1.92	27.30±1.75
TB 250 mg/kg	26.43±1.25	23.96±1.15	24.11±1.02	25.17±1.08
TB 500 mg/kg	27.06±1.25	24.51±0.88	24.83±0.95	26.07±0.80
TB 750 mg/kg	26.40±1.41	25.08±1.34	24.86±1.52	25.63±1.14

**Notes:** The values indicate mean ±SEM (n=6). \* $p < 0.05$  compared to normal control.

**Abbreviations:** GL, Glibenclamide; TB, *Terminalia brownii*.

**Table 7** Effect of the Different Solvent Fractions of *T. brownii* on Body Weight of STZ Induced Diabetic Mice

Treatment Groups	Body Weight in Grams			
	Day 0	Day 5	Day 10	Day 15
Normal control	27.23±1.23	28.75±1.13	31.15±1.11	32.41±1.08
Diabetic control	26.35±0.61	24.83±0.58	24.13±0.89**	23.63±0.86***
GL 5 mg/kg	24.21±1.39	23.65±1.52	24.13±1.54	24.95±1.66
AQF 500 mg/kg	27.00±1.82	25.66±2.28	24.96±1.81	26.00±1.65
ETAF 500 mg/kg	26.41±1.16	24.86±1.58	25.00±1.34	25.62±1.20
BF 500 mg/kg	25.23±0.39	24.76±0.99	23.75±0.56	23.66±0.63

**Notes:** The result are expressed as mean ± S.E.M (n=6). \*\*P < 0.01; \*\*\*P < 0.001 compared to normal control.

**Abbreviations:** GL, Glibenclamide; AQF, Aqueous residue; ETAF, Ethyl acetate fraction; BF, butanol fraction.

## Discussion

In this study, the potential antidiabetic activity of the methanolic crude extract and solvent fractions of *T. brownii* was investigated using normoglycemic and diabetic mice. The test substances significantly reduced the BGL in STZ induced diabetic model, in normoglycemic mice and in oral glucose challenged mice. In addition, the crude extract and the solvent fractions showed in vitro  $\alpha$ -amylase inhibition activities.

Pancreatic  $\alpha$ -amylase is a vital enzyme of the digestive system which hydrolyses starch in to a mixture of smaller oligosaccharides which are further broken down by  $\alpha$ -glucosidase into glucose. The resulting glucose enters the bloodstream upon absorption and leads to a raised level of postprandial hyperglycemia (PPHG). Hence, plant extract that have the potential to inhibit the aforementioned two enzymes may be valuable in lowering the PPHG associated complications in type 2 diabetes.<sup>25</sup> In the present experiment, the crude extract and its different (aqueous, butanol, chloroform, and ethyl acetate) fractions showed a significant  $\alpha$ -amylase inhibitory activities in a dose dependent manner with the highest inhibition observed by the chloroform fraction (Figure 1). These results indicate that the study plant could demonstrate hypoglycemic activity possibly by inhibition of pancreatic  $\alpha$ -amylase.

Similarly, oral administration of *T. brownii* stem bark extract at all tested doses (250, 500 and 750 mg/kg) and glibenclamide at a dose of 5 mg/kg produced a significant reduction in BGL of normoglycemic mice. This result indicates that the crude extract can exhibit hypoglycemic activity in normal mice which is in agreement with other studies performed on similar species. It has been reported that methanolic leaf extracts of *Terminalia bellerica*, *Terminalia chebula*, and *Terminalia arjuna* had a significant hypoglycemic activity in normal rats.<sup>38</sup>

Besides, oral administration of *T. brownii* stem bark extract at doses of 500 mg/kg and 750 mg/kg to glucose loaded mice showed a significant reduction ( $p < 0.01$ ) in BGL at 60 and 120 min post treatment compared to glucose loaded untreated groups. Glibenclamide also exhibited significant reduction starting from the 30 min (Table 3). This signifies that the extract may improve glucose tolerance in normal mice, reflecting its potential benefit to lower PPHG associated complication of diabetes. Control of PPHG is one of the strategies for management of type 2 diabetes and OGTT is the measure of the body's ability to utilize glucose that serves as standard procedure for diagnosis of border line of diabetic patients in clinical set-up.<sup>26</sup> The observed effect may partly be due attenuation of glucose absorption in to blood streams via inhibition of  $\alpha$ -amylase as this plant extract has demonstrated  $\alpha$ -amylase inhibitory activity. Moreover, the ability to improve glucose tolerance might be due to other possible mechanism like stimulation of glycogenesis in liver, enhanced tissue glucose utilization, and decreased gluconeogenesis.<sup>39</sup>

Streptozotocin is by far the most common and well established chemical model used for induction of experimental diabetes. It is a better diabetogenic agent than alloxan as it is linked with wider species effectiveness and greater reproducibility. The diabetogenic action of STZ is mainly, due to the DNA alkylating activity of its methylnitrosourea moiety, release of nitric oxide from the nitroso group in its further course of action and generation of reactive oxygen species.<sup>28,34</sup> In the present study, STZ at a dose of 150 mg/kg body weight was used to induce experimental diabetes and it led to an elevated level of plasma BGL.

An upsurge in fasting BGL is an important characteristic feature of DM.<sup>12</sup> *T. brownii* stem bark extract at doses of 250, 500 and 750 mg/kg and glibenclamide at a dose of



5 mg/kg produced a significant reduction ( $p < 0.05$  or  $p < 0.01$ ) in BGL of STZ induced diabetic mice compared to diabetic control proving its potential antihyperglycemic effect. The reduction in BGL was dose and time dependent with the maximum reduction in BGL achieved at the highest dose (66.75%), which was comparable to glibenclamide (69.32%). Results of the present study coincides with the findings of other investigators on similar *Terminalia* species, which reported that *Terminalia superba*, *T. arjuna* and *Terminalia paniculata* exhibited significant antidiabetic activity in STZ induced diabetic rodents.<sup>40-42</sup> In the case of solvent fractions, the aqueous residue was the most active fraction that showed a significant reduction ( $p < 0.001$ , 55%) of BGL, nearly comparable with the standard drug ( $p < 0.001$ , 63.76%). The ethyl acetate fraction also showed significant reduction ( $p < 0.001$ , 40.6%) of BGL (Table 5). Thus, active constituents with BGL lowering potential may be expected in the aqueous and ethyl acetate fractions.<sup>43</sup> However, the butanol fraction produced no significant alteration in BGL throughout the treatment period indicating that active antidiabetic constituent(s) may be quite less in the butanol fraction.

STZ-induced DM is characterized by loss of body weight possibly due to the inability of cells to utilize glucose, lipolysis in adipose tissue and protein break down which leads to skeletal muscle wasting.<sup>44</sup> Likewise, in the present study, STZ caused a massive percentage of weight loss in the diabetic control groups. Such weight loss was ameliorated by the crude extract at all dose levels (250, 500 and 750 mg/kg) and the aqueous and ethyl acetate fractions at a dose of 500 mg/kg as compared to the diabetic control group even though the effect was not statistically significant (Tables 6 and 7). The protective effect of the *T. brownii* crude extract and the solvent fractions on body weight loss could be explained due to their ability to reduce hyperglycemia.<sup>35</sup>

Briefly, results of the present study show that the crude extract of *T. brownii* stem bark and some of its solvent fractions exhibited hypoglycemic activity in normoglycemic mice, suppression of postprandial hyperglycemia in oral glucose loaded mice and antihyperglycemic activity in STZ induced diabetic mice in a similar manner as that of glibenclamide. Therefore, it is possible to assume that the plant extract may possess hypoglycemic and antidiabetic effect possibly by similar mechanism as glibenclamide that boost the release of insulin from the pancreatic beta cells. In this context, a number of medicinal plants such as, *T. chebula*, *T. arjuna*, *Calyusea abyssinica* and *Croton*

*macrostachys* have been reported to have a similar mode of action with glibenclamide, providing support to our work.<sup>22,30,45,46</sup>

Phytochemical screening of methanolic leaf and stem bark extract of *T. brownii* revealed the presence of tannins, saponins, flavonoids, polyphenols, terpenoids, steroids, phytosterols, and coumarins<sup>17,19</sup> Flavonoids, polyphenols, tannins, saponins, steroids, alkaloids, terpenoids, glycosides, carbohydrates, and polysaccharides have been demonstrated to have antidiabetic activity.<sup>46,47</sup> Thus, the potential antidiabetic activity of the crude extract and solvent fractions of *T. brownii* could be attributed to the aforementioned bioactive phytochemical implicated for their potential antihyperglycemic activity which might exert their effects individually or in synergy with each other.

## Conclusion

In conclusion, the present study demonstrated that 80% methanolic extract of *T. brownii* stem bark and its aqueous and ethyl acetate fractions exhibited significant antihyperglycemic activity in STZ induced diabetic mice, hypoglycemic activity and suppression of postprandial hyperglycemia in normoglycemic mice providing justification to the traditional use of this plant for treatment of diabetes mellitus. The crude extract and solvent fractions also exhibited a significant in vitro  $\alpha$ -amylase inhibition. Thus, it is indispensable to conduct further studies including safety and the isolation and characterization of bioactive compound(s) responsible for its antidiabetic activity and postulate the possible mechanism of action.

## Abbreviations

*T. brownii*, *Terminalia brownii*; STZ, streptozotocin; BGL, blood glucose level; OGTT, oral glucose tolerance test; DM, diabetes mellitus; OECD, Organization for Economic Cooperation and Development; DNSA, 3,5-dinitrosalicylic acid; DMSO, dimethyl sulfoxide; ANOVA, analysis of variance; IC<sub>50</sub>, half maximal inhibitory concentration.

## Ethical Approval

Ethical clearance were secured from Mekelle University, College of Health Sciences, Health Research Ethics Review Committee (HRERC) with reference number 1040/2017. Animals were used and sacrificed in accordance with the Guide for Use and Care of Laboratory Animals.

## Data Sharing Statement

All the datasets used and analyzed during the present study will be available from the corresponding author on reasonable request.

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## Author Contributions

All authors contributed to the data analysis, drafting and revising the article, gave final approval of the version to be published, and agree to be accountable for all aspects of the work. NMA conducted the actual experiment and statistical analysis. GG and GP reviewed the proposal and involved at all implementation stages of the study and write up. GHT and MGH critically reviewed the manuscript for important intellectual content. All authors reviewed and approved the final version of the manuscript.

## Disclosure

The authors report no conflicts of interest in this work.

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