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Effects of Solvent Fractions of Caylusea abyssinica (Fresen.) Fisch. & Mey. on Blood Glucose Levels of Normoglycemic, Glucose Loaded and Streptozotocin-induced Diabetic Rodents

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Abstract

In Ethiopia the leaves of *Caylusea abyssinica* (Fresen.) Fisch. & Mey. (Family: Resedaceae) are used as a cooked vegetable and also for the management of diabetes mellitus in folk medicine. The present study was carried out to investigate effects of solvent fractions of the leaves of *C. abyssinica* on Blood Glucose Levels (BGLs) of normoglycemic, glucose loaded and streptozotocin-induced diabetic rodents. Solvent fractions were prepared by successive extraction of the leaves in a Soxhlet apparatus with chloroform and methanol, and then further maceration of the marc left with distilled water. Phytochemical analysis indicated presence of alkaloids, terpenes, flavonoids, tannins, reducing sugars and steroids. Acute toxicity test showed that all the fractions were safe at doses of up to 2 g/kg. Whilst only the methanol fraction at a dose of 300 mg/kg produced significant hypoglycemic effect 4 h after treatment, both the methanol and aqueous fractions significantly decreased BGL of glucose loaded and streptozotocin-induced diabetic rodents. A reduction of 55.37% ($p<0.05$) and 59.54% ($p<0.001$) in BGL was observed within 2 h of oral glucose tolerance test by 200 and 300 mg/kg of the methanol fraction, respectively. The same fraction at doses of 200 and 300 mg/kg reduced BGL by 80.24% ($p<0.001$) and 81.05% ($p<0.001$), respectively, whereas the aqueous fraction at a dose of 300 mg/kg lowered BGL by 55.33%. It was therefore concluded that the reputed application of *C. abyssinica* leaves for the management of diabetes in traditional medical practice is well founded.

Keywords: Antidiabetic activity, *Caylusea abyssinica* leaves, Streptozotocin-induced diabetes, Hypoglycemia, Blood glucose level, Rodents

1. Introduction

Diabetes Mellitus (DM) is an endocrinal disorder associated with depleted insulin secretions, damaged pancreatic β-cells with altered carbohydrate, lipid and protein metabolism and additionally increased risk of complications of various vascular diseases. The vast majority of cases of DM fall into two broad etiopathogenetic categories: In type 1 DM, the pancreas cannot synthesize enough amounts of insulin as required by the body, whilst in type 2 DM, the insulin hormone secreted by the β-cells is normal or slightly lower than the ideal amount but, the body cells are not responding to insulin as they do in a healthy person [1]. Women who develop diabetes because of the stress of pregnancy are classified as having gestational diabetes.

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Other types of diabetes result from specific conditions such as medications, infections, pancreatic disease, and other illnesses [2]. The incidence of diabetes has soared worldwide in recent years and is expected to keep growing, with the greatest increase seen in metabolic forms of diabetes, notably type 2. A recent report estimates that 6.4% of the world population is currently diabetic, and that by the year 2030, that estimate will rise to 7.7% [3, 4].

DM is probably the fastest growing metabolic disease in the world and as knowledge of the multifactorial/ heterogeneous nature of the disease increases so does the need for more challenging and appropriate therapies. Traditional plant remedies have been used for centuries in the treatment of diabetes, but only a few have been scientifically evaluated [5]. The search for new agents with lower cost and better efficiency has therefore become a matter of major priority. The great number of plants used to manage diabetic patients in Africa might provide a useful source for the discovery of new compounds that can be used as pharmaceutical entities or simple dietary adjuncts to existing therapies [6]. Since time immemorial, patients with non-insulin dependent diabetes have been treated orally in folk medicine, with a variety of plant extracts [7]. One such plant is *Caylusea abyssinica* (Fresen.) Fisch. & Mey. (Resedaceae), the leaves of which are widely used for management of DM in Ethiopian folk medicine [8]. Moreover, in Tanzania tender leaves and stems of the plant are collected from the wild, washed, chopped, cooked and eaten mixed with other vegetables as are in Ethiopia where the leaves are also used as a cooked vegetable [9].

Earlier studies on the crude leaf extract of the *C. abyssinica* proved the promising antidiabetic activity [10]. In this study different solvent fractions were prepared and tested for their hypoglycemic, antihyperglycemic and antidiabetic activities in normal and STZ-induced diabetic rodents.

2. Materials and Methods

2.1 Drugs and Chemicals

The following drugs and chemicals were used in the experiment: streptozotocin (Sigma Chemicals Co., St. Louis, MO, USA.), One touch glucometer and glucose standard strip/kits (Prodigy Autocode, Diagnostic Divices , USA), glibenclamide (Sanofi–Aventis, USA), Tween–80 (BDH Laboratory Supplies Ltd, England), absolute methanol acetone free (ReAgent Chemical Services Ltd, UK), hydrochloric acid (BDH Ltd, England,) chloroform (ACS, ISO, Merck), sulfuric acid (Farm Italia Carrloerba, Italy), acetic anhydride (Techno Pharmchem, India), ferric chloride (Fischer Scientific Company, New Jersey), potassium ferrocyanide, ferric sulfate, lead acetate, and ethyl acetate (ACS, Merck).

2.2 Plant Material

C. abyssinica leaves were collected from Dirre, 55 km south east of Addis Ababa, Ethiopia (10 km on the road from Bishoftu to Mount Ziquala) in October 2011. Taxonomic identification was done and a voucher specimen (collection number WT/001) was deposited at the National Herbarium, College of Natural Sciences, Addis Ababa University.

2.3 Experimental Animals

Healthy male Swiss albino mice (weighing 20–30 g and age of 8–12 weeks) and Wistar rats (Weighing 150–200 g and age of 3 months) were used for the study. The experimental animals were housed separately in polypropylene cages (6–10 rodents per cage), maintained under standard condition of 12 h light / dark cycle, and temperature 2025°C and allowed free access to pellet diet and water *ad libitum*. After randomized grouping and before initiation of the experiment, animals were acclimatized to the laboratory conditions. All procedures complied with The Guide for the Care and Use of Laboratory Animals [11] and approved by the Research and ethics committee of Department Pharmacology and Clinical Pharmacy, Addis Ababa University.

2.4 Preparation of Extract

Leaves of *C. abyssinica* were thoroughly washed with water and shade dried at room temperature. The dried leaves were then coarsely powder by using dry grinder, passed through sieve and packed into soxhlet apparatus and extracted successively with chloroform and methanol. The marc left after methanol extraction was further extracted with distilled water by maceration. The chloroform and methanol fractions were concentrated under reduced pressure, while the aqueous extract was lyophilized and stored in airtight containers in refrigerator below 4°C. Suspensions of chloroform and methanol extracts

were prepared by using 2% Tween 80 in distilled water, and solution of aqueous extract was prepared by using distilled water as solvent for the experiment.

2.5 Preliminary Phytochemical Screening

Standard screening tests of the fractions was carried out for secondary metabolites such as phenolic compounds, tannins, saponins, flavonoids, cardiac glycosides, and anthraquinones according to the methods discussed in the literature [12, 13].

2.6 Acute Toxicity Study

Acute toxicity test was done on the limit test recommendations of OECD 425 Guideline. Three female Swiss albino mice were fasted for 3–4 h, and chloroform, methanol and aqueous fractions were given to each animal at a dose of 2000 mg/kg. The mice were kept for 24 h under strict observation. Based on the result from the first three mice, twelve mice (6 males and 6 females), four for each extract, were fasted for 3–4 h, and a single dose of 2000 mg/kg of each of fraction was given to each mouse. The animals were then observed for 14 days for any sign of toxicity [14].

2.7 Experimental Designs

Male mice were used for the hypoglycemic and anti-diabetic study where two dosage levels (200 mg/kg and 300 mg/kg) were employed for each fraction. These doses were selected based on the result of limit test and preliminary tests conducted. Sixteen groups (eight groups for each normoglycemic and diabetic models) each containing six mice, were used for the experiments. Similarly, Oral Glucose Tolerance Test (OGTT) was carried out on forty eight healthy male Wistar rats in eight groups, each containing six animals. In all three models Groups A and B, were treated with 2% Tween 80 (negative control) and 5 mg/kg glibenclamide (standard drug), respectively. Groups C, D, E, F, G and H, were treated with the solvent fractions of the plant (in two doses for each extract) of the chloroform, methanol, and aqueous fractions, respectively.

2.8 Induction of Experimental Diabetes

Induction of diabetes was conducted according to Animal Models of Diabetic Complications Consortium (AMDCC). Streptozotocin (STZ) was dissolved in freshly prepared 0.1M citrate buffer whose pH was adjusted to 4.5. The solution was then administered intraperitoneally at 150 mg/kg single dose to male mice that were fasted for 4–6 h (with free access to water) prior to administration. The mice were then kept for the next 24 h on 10% glucose solution bottles, in their cages, to prevent hypoglycemia. After 72 h, fasting BGL was assayed and animals with BGL ≥ 11.1 mmol/l (≥ 200 mg/dl) were considered diabetic. Those animals with BGL ≥ 27.78 mmol/l or 500 mg/dl were excluded from the study [15].

2.9 Assessment of Hypoglycemic Activity in Normal Mice

Hypoglycemic effect of the solvent fractions was assessed on normoglycemic mice. Mice were fasted for 4–6 h with free access to water, and randomly divided into eight groups each comprising six animals. Blood samples were collected from animal tail veins at 0 h (just before administration of extracts, control or standard drug), 1, 2, 3, and 4 h after treatment [10, 16].

2.10 Oral Glucose Tolerance Test (OGTT) in Normal Rats

Wistar rats were fasted for 14–16 h, and divided randomly into eight groups each having six animals. Thirty min before extract treatment, a single dose of glucose solution (2 g/kg) was given to each rat. Blood was collected from tail veins of the animal to determine BGL immediately before treatment, and 30, 60, 120 min after treatment [17, 18].

2.11 Assessment of Anti-hyperglycemic Activity in Diabetic Mice

Anti-hyperglycemic activity of the chloroform, methanol, and aqueous fractions was carried out on STZ-induced diabetic mice. The diabetic mice were randomly divided in to eight groups each having six animals. Blood was collected at 0 h (just before treatment), 1, 2, 3, and 4 h after treatment [10, 16].

2.12 Statistical Analysis

The data were expressed as mean ± standard error of the mean (SEM), and analyzed by using SPSS Version 19.0. Homogeneity of variance and normality of data was assessed using levene test and Shapiro-Wilks test,

respectively. Between and within group, analysis was carried out using one way analysis of variance ANOVA followed by Tukey post hoc test (for homogenous variances and normally distributed data) and Dunnett's T3 post hock test (for data that are normally distributed and having heteroscedastic variance) [19]. The level of significance was set at $p < 0.05$.

3. Results

Phytochemical screening of the fractions showed the presence of various chemical constituents. Of all the fractions tested, the methanol fraction was found to contain the highest number of phytochemicals such as alkaloids, tannins, terpinoids, saponins, steroids and flavonoids. On the other hand tannins, cardiac glycosides and reducing sugars were found in aqueous fraction. The chloroform fraction was found to contain alkaloids and steroidal compounds. Acute toxicity studies revealed the non-toxic nature of all the three fractions of *C. abyssinica*. There were no lethality or toxic reactions observed with any of the fractions until the end of study.

Administration of STZ (150 mg/kg, i.p.) led to elevation of fasting BGLs. Only the methanol fraction at a dose of 300 mg/kg produced significant hypoglycemic effect 4 h after treatment when compared with glibenclamide (Table 1). The mice treated with glibenclamide and both doses of the methanol fraction produced highly significant ($p > 0.001$) reduction in BGL starting from the 2nd h onwards when compared with the initial values. At a dose of 300 mg/kg, the methanol fraction brought about a significant reduction 2, 3, and 4 h after treatment as compared to the negative control group. By contrast administration of the same fraction at a dose 200 mg/kg could only produced a significant reduction in BGL at the 3rd and 4th h after treatment when compared to the control.

Administration of glucose to the experimental animals produced significant hyperglycemia (results not shown). Methanol fraction (both 200 and 300 mg/kg) and glibenclamide treated animals showed a significant reduction in BGLs from 30 min onwards (Table 2) in OGTT when compared to the control and initial blood glucose values (Table 2). Table 3 shows the changes in the levels of blood glucose in control, glibenclamide, chloroform, methanol and aqueous fractions treated diabetic mice. At doses of 200 and 300 mg/kg, the methanol fraction reduced BGL by 80.24% and 81.05%, respectively, whereas the aqueous fraction at 300 mg/kg lowered it by 55.33%. Glibenclamide at 5 mg/kg reduced BGL by 51.85%.

Only the 300 mg/kg dose of the chloroform fraction administered to diabetic mice showed a significant decline in BGL when compared with the initial BGL.

Table 1: The effect of the chloroform, methanol and aqueous fractions of *Caylusea abyssinica* on Blood Glucose Levels (BGLs) in normoglycemic mice

Treatment	BGL in mg/dl				
	0 h	1 h	2 h	3 h	4 h
Chlor200	97.33 ± 0.84	93.00 ± 2.67	96.17 ± 1.90	92.00 ± 2.18	100.00 ± 5.21
Chlor300	97.00 ± 1.39	95.17 ± 2.18	99.33 ± 2.95	97.00 ± 6.07	78.50 ± 5.40I1
Meth200	94.00 ± 2.39	89.83 ± 1.70	80.00 ± 2.37^{h3,I3}	63.83 ± 1.70$^{a1, c2,d3 ,h3, I3}$	55.33 ± 2.43^{a2,c3,d1,g1,h3,,I3}
Meth300	98.83 ± 0.70	90.33 ± 2.78	71.50 ± 2.31$^{a2,c2,d2,g2,h3, I3}$	61.83 ± 4.09$^{a1,c2,d3 ,h3, I3}$	45.17 ± 1.78^{a3,b1,c3,d3,g3,h3,I3}
Aqu200	96.67 ± 1.94	93.17 ± 2.75	98.50 ± 9.71	82.83 ± 9.13^{h3}	80.67 ± 9.40
Aqu300	98.67 ± 1.38	94.57 ± 2.96	112.50 ± 4.54	106.00 ± 5.47	93.83 ± 4.32
Gl5	96.33 ± 1.92	86.83 ± 3.33	73.17±3.53$^{a2,c1,d2,g2,h3, I3}$	64.00 ± 2.49$^{a1,c2,d3,h3, I3}$	62.6 ± 1.26^{a1,c3,h3,I3}
Tw80	92.67 ± 2.28	89.83 ± 2.09	88.81 ± 2.96	87.17 ± 5.00	81.67 ± 3.32

Data are: Mean ± SEM; n= 6; Chlo200 = Chloroform fraction 200 mg/kg, Chlo300 = Chloroform fraction 300 mg/kg, Meth200 = Methanol fraction 200 mg/kg, Meth300 = Methanol fraction 300 mg/kg, Aqu200 = Aqueous fraction 200 mg/kg, Aqu300 = Aqueous fraction 300 mg/kg, Gl5= Glibenclamide 5 mg/kg, Tw80 = 2% Tween-80; a = Compared with control (2% Tween-80); b= Compared with Glibenclamide 5 mg/kg; c = Compared with Chlo200; d = Compared with Chlo300; e = Compared with Meth200; f = Compared with Meth300; g = Compared with Aqu200; h = Compared with Aqu300; I = Compared with initials; 1 = $p > 0.05$; 2 = $p > 0.01$; 3 = $p > 0.001$

Table 2: The effect of chloroform, methanol and aqueous fractions of *Caylusea abyssinica* on oral glucose tolerance

Treatment	Blood glucose level in mg/dl			
	0 h	30 min	60 min	120 min
Chlor200	108.00 ± 2.71	101.50 ± 2.08	87.17 ± 4.59^{I3}	73.33± 1.61^{a3,I3}
Chlor300	108.33 ± 2.40	103.83 ± 2.51	80.00 ± 1.03^{a1,I3}	72.67± 2.16^{a3,I3}
Meth200	108.67 ± 2.44	83.17 ± 2.30^{a1,c1,d1,g1,I3}	75.50 ± 2.53^{a1,g3,I3}	48.50 ± 2.50$^{a3, b1,c3,d3,g3,h1,I3}$
Meth300	109.50 ± 3.17	85.33 ± 2.01^{a1,d1,g2,I3}	60.00 ± 2.16^{a3,c3,d1,g3,h3,I3}	44.33 ± 1.71 $^{a3, b3,c3,d3,g3,h3,I3}$
Aqu200	109.6 ± 2.78	112.16 ± 6.72	112.50 ± 5.63	83.33 ± 2.91^{I2}
Aqu300	107.17 ± 1.51	89.50 ± 5.94^{g2,I1}	88.50 ± 4.94^{g2,I2}	61.33 ± 3.49^{a3,I3}
Gl5	105.50 ± 1.96	84.17 ± 1.49^{a1,d1,g3,I3}	67.16 ± 2.71^{a3,g3,h2,I3}	60.33 ± 1.20^{a3,c1,d1,g3,I3}
Tw80	106.83 ± 2.24	103.66 ± 4.67	97.67 ± 4.31	90.50 ± 3.34

Data are: Mean ± SEM; n= 6; Chlo200 = Chloroform fraction 200 mg/kg, Chlo300 = Chloroform fraction 300 mg/kg, Meth200 = Methanol fraction 200 mg/kg, Meth300 = Methanol fraction 300 mg/kg, Aqu200 = Aqueous fraction 200 mg/kg, Aqu300 = Aqueous fraction 300 mg/kg, Gl5= Glibenclamide 5 mg/kg, Tw80 = 2% Tween-80; a = Compared with control (2% Tween-80); b= Compared with Glibenclamide 5 mg/kg; c = Compared with Chlo200; d = Compared with Chlo300; e = Compared with Meth200; f = Compared with Meth300; g = Compared with Aqu200; h = Compared with Aqu300; I = Compared with initials; 1 = p > 0.05; 2 = p > 0.01; 3 = p > 0.001

Table 3: The effect of the chloroform, methanol and aqueous fractions of *Caylusea abyssinica* on Blood Glucose Levels (BGLs) in streptozotocin-induced diabetic mice

Treatment	BGL in mg/dl				
	0 h	1 h	2 h	3 h	4 h
Chlor200	377.83 ± 32.96	343.83 ± 39.95	319.17 ± 44.56	310.67 ± 51.41	335.00 ± 51.30
Chlor300	388.67 ± 30.99	339.50 ± 39.74	300.67 ± 36.99	300.67 ± 35.15	307.33 ± 40.82^{I1}
Meth200	350.83 ± 9.99	286.00 ± 5.59^{I3}	142.67 ± 6.57 $^{a3, b2,g3,h2,I3}$	108.50 ± 3.10 $^{a3, b2,g2,h2,d1,I3}$	69.33 ± 5.11 $^{a3, b3,g1,h2, c1,d1,I3}$
Meth300	360.67 ± 14.00	294.00 ± 11.21^{I3}	132.67 ± 4.71 $^{a3, b2, g3, h2, I3}$	101.83 ± 2.76 $^{a3, b2, g2, h3, d1, I3}$	68.33 ± 4.32 $^{a3, b3,g1,h2, c1,d1,I3}$
Aqu200	356.33 ± 14.55	300.00 ± 10.41	243.50 ± 9.77^{a2,I3}	220.33 ± 12.29^{a2,I3}	206.17 ± 21.89^{I3}
Aqu300	353.33 ± 12.41	306.50 ± 9.59^{I1}	250.00 ± 12.54^{a2,I3}	225.16 ± 10.02^{a2,I3}	157.83 ± 10.10^{a3,I3}
Gl5	325.00 ± 11.82	298.17 ± 12.30	250.33 ± 14.85^{a1,I3}	219.00 ± 10.39^{a1,I3}	156.50 ± 6.44^{a3,I3}
Tw80	341.00 ± 13.61	329.83 ± 16.31	368.67 ± 17.92	331.33 ± 12.74	300.17 ± 12.57

Data are: Mean ± SEM; n= 6; Chlo200 = Chloroform fraction 200 mg/kg, Chlo300 = Chloroform fraction 300 mg/kg, Meth200 = Methanol fraction 200 mg/kg, Meth300 = Methanol fraction 300 mg/kg, Aqu200 = Aqueous fraction 200 mg/kg, Aqu300 = Aqueous fraction 300 mg/kg, Gl5= Glibenclamide 5 mg/kg, Tw80 = 2% Tween-80; a = Compared with control (2% Tween-80); b= Compared with Glibenclamide 5 mg/kg; c = Compared with Chlo200; d = Compared with Chlo300; e = Compared with Meth200; f = Compared with Meth300; g = Compared with Aqu200; h = Compared with Aqu300; I = Compared with initials; 1 = p > 0.05; 2 = p > 0.01; 3 = p > 0.001

4. Discussion

Most plants with antidiabetic properties have been found to contain secondary metabolites such as tannins, saponins, alkaloids, and flavonoids [20]. These constituents may in part be responsible for the observed significant activity of these fractions either singly or in synergy with one another. A significant hypoglycemic activity was observed in healthy mice after the oral administration of higher doses of the methanol fraction obtained from the *C. abyssinica* leaves. The results of this investigation are consistent with the previously observed hypoglycemic effect of the total extract in healthy mice [10]. It has been reported that sulphonylureas produce hypoglycemia in

normal animals by stimulating the pancreatic β-cells to produce more insulin and increasing glycogen deposition in the liver [21]. Flavonoids, terpenes, alkaloids, saponins and tannins have been shown to possess hypoglycemic activity [6, 22]. Flavonoid and tannins isolated from other antidiabetic medicinal plants has been found to stimulate insulin secretion from pancreatic β-cells or possess insulin like effect [23]. It is, therefore, conceivable that hypoglycemic effect produced by the methanol fraction of *C. abyssinica* leaves may be due to the presence of any of these active ingredients. Moreover, the methanol fraction may possibly exert its hypoglycemic action by stimulating insulin secretion from pancreatic β-cells or possess insulin like effect, but further studies should be performed to confirm this hypothesis.

Compared to glibenclamide no significant hypoglycemic activity was observed but higher percentage of reduction in BGL was produced by lower doses of the methanol fraction starting from the 2nd h onwards. The non-hypoglycemic effect of lower doses of the methanol fraction could be attributed to inability of the dose to overcome counter regulatory physiological mechan isms, lesser concentration of the active principles to induce hypoglycemia or the small sample size employed that precluded statistical significance. The observation that the lower dose of methanol fraction did not significantly (compared to glibenclimide) cause a change in the glucose level of normoglycemic mice throughout the study implies that the fraction at lower dose is safe and does not cause hypoglycemia in a normal subject guaranteeing the safety of the plant when consumed as food or used as medicine for other diseases.

On the other hand, the chloroform and aqueous fractions showed no hypoglycemic effects at all doses used in normal mice unlike the reference oral hypoglycemic agent (glibenclamide) which significantly lowered BGL starting from 2 h after treatment. This finding is consistent with the results reported for other plant extracts [24]. However, in some other plants chloroform and aqueous extracts have been shown to have significant hypoglycemic activity [25, 26].

Effective blood glucose control is the key for preventing or reversing diabetic complications and improving quality of life in patients with diabetes. Thus sustained reduction in hyperglycemia will decrease the risk of developing microvascular complications and most likely reduce the risk of macrovascular complications. Thus glucose-induced hyperglycemic model was selected to screen the antihyperglycemic activity of the plant extracts. Any drug that is effective in diabetes will have the ability to control the rise in glucose level by various mechanisms and the ability of the extracts to prevent hyperglycemia could be determined by glucose-loaded hyperglycemic model [27].

In the glucose loaded hyperglycemic model used in the present study, the methanol fraction exhibited significant antihyperglycemic activity at both dose levels 30 min onwards. It is well established that after glucose loading in OGTT, excessive amount of glucose in the blood induces insulin secretion. The secreted insulin will then stimulate peripheral glucose consumption and controls the production of glucose through different mechanisms. Insulin brings down blood glucose in about 2 to 3 h to bring back the glucose level to normal [28]. The present results with the fraction and glibenclamide are in support of this. The antihyperglycemic activity of the methanol fraction of *C. abyssinica* could be due to a beneficial effect of the active constituents on carbohydrate metabolism in glucose loaded rats. The effect of glibenclamide, the standard drug used in this study, on glucose tolerance has been attributed to enhanced activity of β-cells of the pancreas resulting in secretion of larger amounts of insulin. So the mechanism behind this anti-hyperglycemic activity of the methanol fraction, at both doses, involves an insulin-like effect, probably, through peripheral glucose consumption or enhancing the sensitivity of β-cells to glucose, resulting in increased insulin release.

These results suggest that the methanol fraction has antihyperglycemic activity by improving the glucose tolerance in the treated animals, which are in agreement with those reported by Tamiru *et al.* [10] and comparable with the results obtained for the roots of *Spondias mangifera* Willd., stem bark of *Elaeodendron glaucum* Pers. and leaves of *Actinodaphne hookeri* Meissn [18, 19, 29]. However, the aqueous fraction at a dose of 300 mg/kg, and both doses of the chloroform fraction of *C. abyssinica* leaves produced plasma glucose levels significantly lower than those of the initial BGL and control (2% Tween 80) group starting from 30 and 60 min after treatment, respectively. The results were found to be comparable with the studies done on *Ocimum gratissimum* L. and *Cassia kleinii* Wight & Arn. leaf extracts [30, 31]. Phytochemical screening of the

chloroform and aqueous fractions of *C. abyssinica* has demonstrated that they contain alkaloids, and glycosides and tannins, respectively (Table 2). It has been reported that some alkaloids possess antihyperglycemic activity which is mediated by the inhibition of α-glucosidase or stimulation of insulin secretion [6, 22]. Glycosides and tannins are also known to stimulate insulin secretion from β-cells [22, 33].

In the present study, administration of STZ to mice resulted in hyperglycemia. The results of these study showed that both doses of the methanol fraction significantly reduced BGLs in high-dose STZ-treated mice starting from 1 h after treatment, with a significantly high glucose disposal rate as compared with initial BGL. The reduction in glucose levels after single oral administration of the methanol fraction was time dependent (maximal effect was achieved at the 4th h) and was much faster and better than that seen in glibenclamide treated diabetic mice, which was in agreement with those reported by Tamiru *et al.* [10] Furthermore, the methanol fraction at lower dose exhibited significant antihyperglycemic activity in SZT–induced diabetic mice (Table 3) without causing significant hypoglycemia when compared to glibenclamide (Table 1).

There are a lot of reports implicating some phyto-chemicals as being responsible for the antidiabetic activities of plants.The mechanism behind the glucose lowering effect of *C. abyssinica* is unclear, but the reduction in plasma glucose levels in the methanol fraction treated diabetic mice could be due to the presence of substances in this fraction that stimulate insulin secretion or protect the intact functional β-cells from further deterioration. It has been well documented that protection of the β-cells could be at least, in part, a result of the reduction in blood glucose, eliminating glucotoxicity to the β-cells [29, 33, 34]. Flavonoids are well known to regenerate the damaged b-cells in diabetic rodents, while phenolics are found to be effective antihyperglycemic agents [35]. Apart from the effect of the fraction on β-cell, the methanol fraction may have extra-pancreatic effect like inhibition of gluconeogenesis in liver and enhancing insulin sensitivity in the tissues. It has been reported that saponins and alkaloids enhance glucose uptake, whereas flavonoids protect various cell types from oxidative stress-mediated cell injury [32]. Phenolic compounds and flavonoids can also prevent lipoperoxidation and induce favorable changes in the lipid profile [36].

The present findings have demonstrated that administration of the aqueous fraction to STZ-induced diabetic mice significantly lowers plasma glucose levels from 2nd h onwards as compared to the diabetic control and initial BGL. Similar antidiabetic activity has been reported previously for aqueous extracts of other plants [31, 37, 38]. Compared to glibenclamide the glucose-lowering effect of the aqueous fraction at higher dose was found to be non-significant, but its action was similar to glibenclamide. The effect observed indicated the similarity of mechanism of action between this fraction and sulfonylureas plus extra pancreatic mechanism of action of the fraction such as enhancing the ability of insulin to stimulate glucose disposal. Previous report by Ali *et al.* [39] indicated that tannins, flavonoids and terpenoids exert their antidiabetic actions via α-glucosidase modulation, a typical extra-pancreatic mechanism.

The present study indicates that the chloroform fraction at doses of 200 and 300 mg/kg does not have any antidiabetic activities when compared with 2% Tween 80 and glibenclamide. However, this fraction at a dose 300 mg/kg showed significant blood glucose reduction when compared to initial BGL. The results also showed that blood glucose lowering activity of almost all doses of the fractions increased with time, as maximal effect was achieved at the 4th h (Tables 1, 2, 3). These may be an indication that the active agents in these fractions exist in very small amounts, hence may require an initial duration of bioaccumulation for cumulative and concerted effect. Such a trend has been observed previously by fruit pericarp extracts *Phaleria macrocarpa* (Scheff.) Boerl [39].

The present study clearly demonstrated that the methanol fraction of *C. abyssinica* possesses significant inter-group reduction in BGLs in hypoglycemic model in normal mice, OGT in normal rats and also in streptozotocin-induced diabetic mice as compared to the other fractions, implying that this fraction contains bioactive secondary metabolites (which may act individually or synergistically) with blood glucose lowering effect that is likely to be mediated through various mechanisms. However, further studies would be required to elucidate the exact mechanism(s) of the hypoglycemic, antihyperglycemic and antidiabetic activities of the methanol and aqueous fractions of *C. abyssinica* leaves and to establish their efficacy and safety for long term treatment of DM.

6. Conclusion

The present study has established that oral administration of the methanol and aqueous fractions of *C. abyssinica* leaves has a beneficial effect in reducing BGLs in normal and streptozotocin-induced diabetic rodents. The promising antidiabetic effect along with the relative safety of the extracts further indicate that the leaves of *C. abyssinica* could be used as simple dietary adjunct to existing therapies or as a source for novel antidiabetic compounds, which can be useful in the continuing fight against DM. Although the results seem to justify the traditional uses of the plant for the management of diabetes, further investigations need to be carried out in order to confirm the actual benefit of the leaves of *C. abyssinica* in diabetic patients.

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