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Research Article

Pharmacological Evaluation of the Antidiabetic Effect of the Leaf Crude Extract of Hagenia abyssinica on Normoglycemic, Oral Glucose Loaded, and Streptozotocin-Induced Diabetic Mice; A Single Dose Study

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Abstract

Background: In Ethiopian traditional medicine, the leaves of *H. abyssinica* (Rosaceae) have been used to treat diabetes mellitus. However, the anti-diabetic efficacy of *H. abyssinica* leaves crude extract has not been properly examined. The goal of this study was to see if the crude extract of *H. abyssinica* on might prevent diabetes in normoglycemic, oral glucose-loaded, and STZ-induced diabetic mice.

Methods: Successive maceration was used as a method of extraction using solvents of increasing polarity: methanol and water. After extraction of the leaves with 80% hydro methanol, the crude extract was evaluated for its anti diabetic activities using oral glucose loaded, normoglycemic, and single dose-treated diabetic mice model. The extract was evaluated at 100, 200 and 400 mg/kg doses. One-way ANOVA followed by Tukey's post hoc test was used for data analysis, and $p < 0.05$ was considered as statistically significant.

Results: The acute toxicity study of *H. abyssinica* leaf extract did not show mortality in the animals at the limit dose of 2000 mg/kg during the observation period. In normoglycemic model, the percentage reduction of baseline blood glucose level (BGL) was 26.08%, 32.90%, 35.24%, and 53.61% for *Hagenia abyssinica* crude extract (HAC) 100 mg/kg, HAC 200 mg/kg, 400 mg/kg, and glibenclamide (GLC) 5 mg/kg, respectively when compared to the negative control. The extract at the dose of 200 mg/kg, 400 mg/kg, and GLC treated groups demonstrated significant BGL reduction from peak levels in the OGTT model. In STZ-induced diabetic mice, all doses of the crude extract showed a significant reduction in the fasting BGL. At the 8th hour, the highest percent reductions in blood glucose level (BGL) were recorded in HAC100, HAC200, HAC400 and GL5 with 26.78%, 31.27%, 43.53% and 51.72% reduction respectively compared to their respective baseline fasting BGL levels.

Conclusion: *H. abyssinica* leaf extract displays antidiabetic activity in normal and STZ-induced diabetic mice, which justifies the claimed traditional use of *H. abyssinica* in managing DM in Ethiopian folk medicine.

Keywords: *Antidiabetic; Diabetes Mellitus; Hagenia abyssinica; Hypoglycemia and Streptozotocin*

Abbreviations

BGL: Blood Glucose Level; DM: Diabetes Mellitus; OECD: Organization for Economic Cooperation and Development; OGTT: Oral Glucose Tolerance Test; STZ: Streptozotocin

Introduction

Diabetes mellitus (DM) a metabolic condition characterized by insulin secretion, action, or a combination of the two [1]. Diabetes mellitus is one of the leading chronic health problems in both children and adults to date. It is mainly caused by lifestyle, obesity, genetic factors, and a unhealthy diet [2]. Regardless of the availability of sufficient insulin preparations and oral hypoglycemic drugs, DM complications remain the most challenging and devastating health problems which affect both adults and children worldwide. Globally, the prevalence of DM for all age groups is estimated at 2.8% in 2000, which is expected to increase to 4.4% by 2030 [4]. In 2017, more than 451 million people (18 - 99 years) were lived with DM, and the number was projected to rise to 693 million by 2045 [3,4].

Despite the fact that a range of insulin formulations and oral hypoglycemic medicines are available to treat DM, none of them can eliminate the disease's core causes and chronic consequences [5,6]. The currently available standard anti diabetic drugs remains the mainstay for managing and treating the disease. However, they are associated with many side effects and toxicities, such as increase in the risk of cardiovascular disease [7,8], increased body weight, gastrointestinal reactions, and BGL fluctuations [9,10], as well as an inability to correct diabetic complications and serious biochemical disorders. Antidiabetic medications have a different mechanism of action for glycemic regulation such as stimulation of pancreatic insulin secretion [7], inhibiting liver gluconeogenesis [8], sensitizing insulin receptors [9], and inhibiting intestinal absorption [10].

Globally, more than 1000 plant species are traditionally used in the treatment of DM [11]. Herbal treatments should also be researched further, according to the WHO expert committee on diabetes, as they are frequently regarded to be less toxic and have less untoward effects [12]. Traditional healers in various parts of Ethiopia treat and control DM and its complications with a variety of traditional medicinal plants. However, several of these medicinal plants' therapeutic potential has not yet to be clinically tested and proven. *H. abyssinica* is the sole species of the genus *Hagenia* which belongs to the family Rosaceae and widely used as hypoglycemic agents by traditional healers in Ethiopian folk medicine [13]. It is found in, Kenya, Ethiopia Uganda, Tanzania, Congo, Malawi, Burundi, Sudan, and Rwanda [14]. In Ethiopia, the leaves part of *H. abyssinica* is traditionally used to manage different illnesses[15,16]. Specifically, *H. abyssinica* has been used for the treatment of DM. Ethnobotanical surveys which were carried out in Ethiopia reported that the leaf of the plant is taken orally to treat DM [17-19],but the effect of the crude leaf extract on BGL has not been scientifically studied.

Rosaceae plant family have been found to possess insulinomimetic and anti-diabetic properties, according to pharmacological studies conducted on different plant species under this family [20,21]. *H. abyssinica* is one of the plant species in the family, Rosaceae [13], suggesting it may have an anti-diabetic property. Furthermore, the crude extract and solvent fractions of *H. abyssinica* leaves have been proved to own *in vitro* anti-amylase and anti-glucosidase inhibitory and antioxidant activities, according to a prior study conducted by [22]. Likewise, the repeated doses of crude extract of *H. abyssinica* leaves showed significant antidiabetic activity in STZ-induced diabetic mice [23].

The presence of different active secondary phenolic substances (2-hydroxy-3-methyl-anthraquinone, C-glycosylated anthrones, physcion), terpenoids, glycosides, flavonoids, anthraquinones, steroid, lipids, alkaloids, peptides, and other phytoconstituents elements is contribute for medicinal plants' antidiabetic effect [24-26]. A prior investigation found the presence of tannins, saponins, terpenoids, flavonoids, steroids, and phenols in the methanol leaf extract of *H. abyssinica* [27], which are known to have antidiabetic activity. Accordingly,

this study was conducted to investigate the antidiabetic effect of the crude leaf extract of *H. abyssinica*on normoglycemic, oral glucose loaded, and STZ-induced diabetic mice.

Methods

Plant materials

Fresh *H. abyssinica* (Rosaceae) leaves were collected in Kosoye, a town 15 kilometers from Gondar, in March 2019. (The Amhara region of northwest Ethiopia). The plant material was then botanically identified and authenticated by botanist Mr. Abiyu Enyew, and a voucher specimen (002ZDK/2019) was deposited at the Biology Department's Herbarium for further reference.

Experimental animals

The swish albino mice of either sex (weighing 20 - 28 g and aged 6 - 10 weeks) were obtained from the Ethiopian Public Health Institute (EPHI) in Addis. The animals were housed in polypropylene cages and given unrestricted access to a pellet food and water ad libitum under conventional conditions (12-hour light/dark cycle, room temperature). Before beginning the experiment, the animals were acclimatized to the laboratory conditions for two weeks. The study was approved by Wollo University's research and ethics committee with the reference number WU Phar/116/11, and all procedures followed The Guide for the Care and Use of Laboratory Animals.

Preparation of plant crude extract

After collection, the plant's leaves were properly cleaned with distilled water to eliminate dirt before being dried under shade area at room temperature (25 - 27°C) with enough ventilation. After that, the dried leaves were ground into a coarse powder using an electrical grinder. And then, the coarsely crushed leaves were macerated in 80 percent methanol for 72 hours before being filtered through Whatman filter paper No. 1. The residue was re-macerated twice with fresh hydromethanol solvent for 72 hours each time, and the filtrates obtained from the successive macerations were concentrated under reduced pressure using a rotary evaporator (Hamato, Japan), followed by a hot air oven (Medit-Medizin Technik, Germany) set at 40°C. The semi-dried leftovers were then frozen overnight in the refrigerator before being dried using a lyophilizer (Labfreez, China). The desiccator was employed to keep the dried leaf extract until it was used in the experiment. Finally, a total of 153 grams of dried *H. abyssinica* leaf crude extract was obtained at the end of the extraction process with a percentage yield of about 14.6% (w/w).

Acute toxicity study

An acute oral toxicity test was performed on a crude extract of *H. abyssinica* leaves using the OECD No 425 Guideline limit test instructions. On the first day of the test, one female Swiss albino mouse was fed 2g/kg of the extract orally and observed for one day for any physical or behavioral abnormalities. The remaining four female mice were recruited the next day and starved for four hours because the first animal showed no signs of toxicity. The mice were then given a single dose of 2g/kg extract orally, and they were monitored in the same way. The observation was continued for a total of 2 weeks for any sign of toxicity [28].

Grouping and dosing of animals

Because male mice are more susceptible to STZ and insulin than female mice, they were employed in all in-vivo experimental studies (normoglycemic, oral glucose loading, and STZ-induced diabetes animal models) [29-31]. In all cases, mice were assigned randomly into different groups of 6 mice each (n = 6).

In the normoglycemic and oral glucose tolerance test models, distilled water (DW) and glibenclamide 5 mg/kg (GLC 5 mg/kg) were used as negative and positive controls, respectively; test groups (groups III to V) received 100 mg/kg, 200 mg/kg, and 400 mg/kg of *H. abyssinica* crude extract, respectively.

There were also three test groups (groups III-V) in the diabetic animal model: a negative control group (groups I) DW; a positive control group (group II) that received the standard drug, (GLC 5 mg/kg); and test groups (group III - V) that received 100 mg/kg, 200 mg/kg, and 400 mg/kg of *H. abyssinica* crude extract respectively.

Plant extract doses to be administered were determined based on the result from the acute toxicity study and the volume of administration was 1 ml/100 g of body weight of the mouse [28]. The middle dose was one-tenth of the limit dose, the higher dose was twice the middle dose, and the lower dose was calculated as half of the middle dose. Glibenclamide was selected as a standard drug for the study based on earlier studies [32].The study was conducted using the oral route of administration because the plant leaves are traditionally used by people via the oral route [17-19].

Measurement of BGL

Blood samples were taken from the tails of each mouse by cutting the tip of the tail aseptically for BGL determination in all animal models. The BGL was tested using a DS-W® blood glucose meter in triplicates so that the average value could be calculated.

Evaluation of the effect of the crude extract on BGL of normoglycemic mice

Healthy normal Swiss albino mice were fasted for 14 hours but were permitted to drink freely to assess the effect of the crude extract on BGL in normoglycemic mice. Fasted mice were then divided into five groups of six mice each, and each group was treated separately. Blood glucose level (BGL) was measured in blood samples obtained from the ends of each mouse's tails under aseptic conditions directly before treatment (at 0 hour) as a baseline, and then at 1-, 2-, 4-, and 6-hours following treatment [33].

Evaluation of the effect of the crude extract on oral glucose tolerance test (OGTT)

The baseline BGL was taken just before the drugs were provided in this model to investigate the effect of crude extract on the OGTT. Then, 30 minutes after receiving the extract, the mice were given 2g/kg of glucose solution orally. The BGL of each animal was measured at zero minutes before treatment and again at 30, 60, and 120 minutes following glucose injection [34].

Induction of experimental diabetes

Diabetes was induced with STZ in the current investigation. This compound was first dissolved in a citrate buffer of 0.1 M (pH = 4.5). After that, mice who had fasted for 14 hours the night before were administered the solution intraperitoneally at a dose of 150 mg/kg. Animals were given a 5% glucose solution to drink for the next 24 hours after receiving STZ to avoid death due to hypoglycemia shock. Diabetes was detected 72 hours later in the animals. After that, diabetic mice were added to the trial if their fasting BGL was greater than 200 mg/dl [35-37].

Evaluation of the anti-hyperglycemic activity of the single dose of crude extract in STZ-induced diabetic mice

After fasting for 14 hours, diabetic mice were randomly divided into 5 groups (n = 6) in a STZ-induced diabetes model. The mice were administered distilled water, glibenclamide, and 80 percent methanolic leaf crude extract depending on their respective grouping. The

BGL was then measured as a baseline right before treatment (at 0 hour) and then at 2-, 4-, 6-, and 8-hours after treatment. Mice were euthanized by administering 150 mg/kg sodium pentobarbitone intraperitoneally at the end of the experiment [38].

Statistical analysis

Analysis of results was done using Statistical Package for Social Sciences (SPSS) software version 24. All results obtained were expressed as mean ± standard error of mean (SEM) of responses. The statistical significance was determined by using One-way Analysis of Variance (ANOVA) followed by a Tukey post hoc test to compare variations among groups and the results were considered significant at $p < 0.05$. The analyzed data were then presented using tables and graphs where necessary.

Result

Acute toxicity test

The acute toxicity study of *H. abyssinica* did not exhibit mortality in animals at the limit dose of 2g/kg during the observation period, nor did it reveal any signs of toxicity: neurological, autonomic, physical changes, or behavioral. Therefore, the LD_{50} of the leaf extract is greater than 2g/kg.

Hypoglycemic activity of the extract in normoglycemic mice

Table 1 summarizes the effect of crude leaf extract on fasting BGL in normal mice. There was no statistically significant variation in baseline BGL among groups before starting treatment ($P > 0.05$). Significant BGL reduction was observed with HAC100 at the 4th ($p < 0.05$) and 6th ($p < 0.001$) hours; HAC200 at the 2nd ($p < 0.05$), 4th ($P < 0.001$), and 6th ($P < 0.001$) hours; and HAC400 at the 1st, 2nd, 4th, and 6th hours ($p <0.001$) compared to the negative control. Likewise, BGL was significantly reduced by GLC5 at the 1st, 2nd, 4th, and 6th hours ($p < 0.001$) compared to the negative control. Comparing GLC5 treated group with extract-treated groups, it was revealed that GLC5 significantly reduced the BGL at all time points except for the higher dose (HAC400). Within group analysis showed that treatment with the standard drug reduced the BGL significantly ($p < 0.001$) at the 1st, 2nd, 4th, and 6th ($p < 0.001$) hours compared to the baseline BGL with a percentage reduction of 28.06%, 42.54%, 47.71%, and 53.62%, respectively. Similarly, a significant reduction in baseline BGL was observed with HAC100 at the 4th ($p < 0.05$) and 6th ($p < 0.001$) hours with percentage reduction, 14.22%, and 26.07%, respectively; HAC200 at the 2nd ($p < 0.01$), 4th ($p < 0.001$) and 6th ($p < 0.001$) hours with a percentage reduction of 20.90%, 26.69%, and 32.91%, respectively; and HAC 400 at the 2nd, 4th, and 6th hours ($p < 0.001$) with a percentage reduction of 26.93%, 33.74%, and 35.25%, respectively.

Group	BGL (mg/dl)				
	0hr	1hr	2hr	4hr	6hr
DW10	117.00 ± 5.01	118.83 ± 4.00	115.17 ± 4.97	117.83 ± 2.32	114.67 ± 3.06
GLC5	112.83 ± 3.30	$81.17 \pm 2.71^{a3,\beta3}$	$64.83 \pm 3.12^{a3,\beta3}$	$59.00 \pm 3.39^{a3,\beta3}$	$52.33 \pm 2.57^{a3,\beta3}$
HAC100	111.83 ± 4.50	107.17 ± 4.01^{n3}	102.17 ± 2.73^{n3}	$93.50 \pm 2.88^{a1,n3,\beta1}$	$82.67 \pm 4.74^{a3,n2,\beta3}$
HAC200	118.00 ± 3.34	108.33 ± 2.91^{n3}	$93.33 \pm 5.01^{a1,n3,\beta2}$	$86.50 \pm 5.39^{a3,n2,\beta3}$	$79.17 \pm 4.62^{a3,n1,\beta3}$
HAC400	110.17 ± 3.05	$91.50 \pm 2.99^{\beta3}$	$80.50 \pm 1.96^{a3,\beta3}$	$73.00 \pm 1.59^{a3,\beta3}$	$71.33 \pm 1.56^{a3,\beta3}$

Table 1: *Hypoglycemic effect of the extract in normoglycemic mice.*

Each value represents mean ± SEM; n = 6 for each treatment. [a]compared to the negative control, [b]compared to HAC100, [c]compared to HAC200, [d]compared to HAC400, and [β]compared to baseline BGL, [n]compared to GLC5. [1]p < 0.05, [2]p < 0.01, and [3]p < 0.001. HAC100 = *H. abyssinica* crude extract 100 mg/kg, HAC200 = *H. abyssinica* crude extract 200 mg/kg, HAC400 = *H. abyssinica* crude extract 400 mg/kg, DW10 = distilled water 10 ml/kg, and GLC = glibenclamide 5 mg/kg.

Antihyperglycemic activity of the extract on OGTT

The effects of *H. abyssinica* leaf crude extract on OGTT are indicated in table 2. When comparing BGL of all groups before extract administration (0 min), there was no discernible difference. Regardless of the medications administered, all groups demonstrated a significant (p < 0.001) increase in BGL at 30 minutes compared to the baseline fasting BGL, demonstrating the induction of hyperglycemia. When compared to the negative control, HAC100 mg/kg did not significantly reduce hyperglycemia after a glucose challenge at 60 and 120 minutes. However, as compared to the negative control group, HAC200 mg/kg and HAC400 mg/kg significantly (p 0.01 and p 0.001, respectively) improved hyperglycemia at 60 minutes. When compared to the negative control group, HAC200 mg/kg and HAC400 mg/kg significantly (p < 0.01 and p < 0.001, respectively) improved hyperglycemia at 120 minutes. Similarly, GLC5 improved hyperglycemia significantly (p < 0.001) after 60 and 120 minutes as compared to the negative control group. A statistically significant (p < 0.001) drop in BGL after 60 and 120 minutes was seen in all groups when compared to the corresponding BGL at 30 minutes after glucose administration.

Group	BGL (mg/dl)			
	0min	30min	60min	120min
DW10	97.50 ± 2.22	192.33 ± 11.20	154.17 ± 9.68	108.67 ± 12.64
GLC5	93.33 ± 4.01	149.33 ± 8.68$^{a3,\beta3}$	70.83 ± 12.36$^{a3,\beta1,\mu3}$	61.50 ± 9.87$^{a3,\beta2,\mu3}$
HAC100	88.67 ± 1.98	178.33 ± 9.54$^{n1,\beta3}$	128.50 ± 15.37$^{n3,\beta3,\mu3}$	86.33 ± 16.35$^{\mu3}$
HAC200	94.67 ± 4.10	173.67 ± 12.65$^{\beta3}$	118.17 ± 7.98$^{a2,\beta2,n3,\mu3}$	77.00 ± 14.51$^{a2,\mu3}$
HAC400	91.17 ± 2.89	161.33 ± 15.34$^{a1,\beta3}$	106.17 ± 10.37$^{a3,n2,\mu3}$	68.83 ± 6.89$^{a3,\beta2,\mu3}$

Table 2: *Antihyperglycemic effect of the crude hydromethanol extract on oral glucose tolerance test.*

Each value represents mean ± SEM; n = 6 for each treatment.[a]compared to the negative control, [b]compared to HAC100, [c]compared to HAC200, [d]compared to HAC400, [β]compared to the BGL at 30 minute and [β]compared to baseline BGL, [n]compared to GLC5. [1]p < 0.05, [2]p < 0.01, and [3]p < 0.001. HAC100 = *H. abyssinica* crude extract 100 mg/kg, HAC200 = *H. abyssinica* crude extract 200 mg/kg, HAC 400 = *H. abyssinica* crude extract 400 mg/kg, DW 10 = distilled water 10 ml/kg, and GLC5 = glibenclamide 5 mg/kg.

Antihyperglycemic activity of the single dose of the crude hydromethanol extract of *Hagenia abysinica* leaves on STZ-induced diabetic mice

Table 3 summarizes the effects of *H. abyssinica* crude extract on BGL of STZ-induced diabetic mice. BGL differences were examined using both between-group and within-group analysis. There were no significant differences in baseline fasting BGL across the groups, according to the between-group analysis. When groups treated with plant extract were compared at all time points, there was no significant difference in BGL. The standard drug (GLC5) produced a significant BGL reduction at the 4th (p < 0.05), 6th (p < 0.01), and 8th (p < 0.001) hours compared to the negative control. Similarly, a significant reduction in BGL was observed with HAC100 at the 4th (p < 0.05), 6th (p < 0.001), and 8th (p < 0.001) hours; HAC 200 at the 4th (p < 0.01), 6th (p < 0.001), and 8th (p < 0.001) hours compared to the negative control. The greatest percenta reductions in BGL were recorded as 26.78% in HAC 100, 31.27% in HAC 200, 43.53% in HAC 400, and 51.72% in GLC5 treated groups at the 8th hour compared to the respective baseline fasting BGL level.

Group	BGL (mg/dl)				
	0hr	2hr	4hr	6hr	8hr
DW10	320.33 ± 6.94	318.00 ± 9.54	326.67 ± 5.67	327.33 ± 8.65	322.67 ± 5.94
GLC5	343.83 ± 11.25	268.50 ± 16.54$^{\beta 3}$	205.00 ± 6.89$^{\beta 3,n3}$	180.33 ± 16.54$^{\beta 3,n3}$	166.00 ± 11.25$^{\beta 3,n3}$
HAC100	297.50 ± 10.25	277.50 ± 9.87	253.33 ± 13.24^{n1}	226.33 ± 16.35$^{\beta 1,n}$3	217.83 ± 13.25$^{\beta 2,n3}$
HAC200	309.67 ± 13.35	260.00 ± 11.52	255.00 ± 10.65^{n1}	236.00 ± 13.25$^{\beta 2,n3}$	212.83 ± 11.24$^{\beta 3,n3}$
HAC400	314.33 ± 15.64	258.67 ± 8.67	241.83 ± 9.58$^{\beta 2,n2}$	226.83 ± 11.54$^{\beta 3,n3}$	177.50 ± 8.67$^{\beta 3,n3}$

Table 3: *Antihyperglycemic effect of the single dose of crude hydromethanol on STZ-induced diabetic mice.*

The standard drug, GLC5 also produced a significant BGL reduction at the 2nd ($p < 0.001$), 4th ($p < 0.001$), 6th ($p < 0.001$), and 8th ($p < 0.001$) hour compared to baseline BGL. Similarly, all doses of the crude extract produced a significant ($p > 0.05$) BGL reduction at different time points compared to baseline BGL.

Each value represents mean ± SEM; n=6 for each treatment. ⁿCompared to the negative control, ᵝcompared to baseline BGL. ¹$p < 0.05$, ²$p < 0.01$, and ³$p < 0.001$, HAC100 = *H. abyssinica* crude extract 100 mg/kg, HAC200 = *H. abyssinica* crude extract 200 mg/kg, HAC400 = *H. abyssinica* crude extract 400 mg/kg, DW10=Distilled water 10 ml/kg and GLC5 = glibenclamide 5 mg/kg.

Discussion

Considering the socioeconomic impacts of DM and its complications and having the knowledge of potential herbal medicines from traditionally claimed plants, the need for searching effective hypoglycemic drugs with minimal untoward effects and high efficacies from traditional medicinal plants seems reasonable. Thus, searching for medicinal plants which have been widely used in the community to treat DM with anti oxidant activities are essential concern in this regard. *H. abyssinica* on is among the widely used traditional medicinal plants in Ethiopian folk medicine for treating DM [39]. However, the anti diabetic effects of this plant have not been evaluated on normoglycemic, oral glucose loaded, and streptozotocin-induced diabetic models**.** It may therefore be worthwhile scientifically investigating the *in vivo* antidiabetic effect of the crude extract of *H. abyssinica* on normoglycemic, oral glucose loaded, and STZ-induced diabetic mice to substantiate its traditionally claimed uses.

There was no mortality or evidence of behavioral abnormalities or toxicity in mice after oral administration of the 80 percent methanolic crude leaf extract of *H. abyssinica* at a dose level of 2g/kg in an acute oral toxicity trial. This shows that the plant is relatively safe.

In the normoglycemic mouse model, the negative control group did not exhibit a significant drop in BGL when compared to the baseline BGL. When compared to the baseline BGL, all doses of the crude extract and the standard drug-treated groups showed a significant decrease in BGL at a different time in point. The standard drug's hypoglycemic effect was related to the suppression of glucagon secretion and promotion of insulin release from pancreatic β -cells [40]. Due to the presence of many phytoconstituents, the crude leaf extract of *H. abyssinica* may stimulate insulin production from pancreatic β-cells or have an insulin-like effect. Tannins and flavonoids, among other phytoconstituents, have been shown to promote insulin release from pancreatic β-cells [41].

OGTT is a measure of the body's ability to utilize glucose and it is seen as the "gold standard" in diagnosing DM [42]. At one hour, the conventional treatment exhibited a significant decrease in BGL as compared to the negative control. Furthermore, at two hours, the standard antidiabetic drug, HAC 200 mg/kg, and HAC 400 mg/kg all exhibited a substantial reduction in BGL when compared to the negative control. As a consequence, from one hour onwards, the conventional medication and higher doses of the crude extract (200 mg/kg

and 400 mg/kg) showed substantial improvements in glucose utilization as compared to the BGL at 30 minutes. These findings could be explained by the crude extract of *H. abyssinica's* ability to increase glycolysis and peripheral glucose consumption while lowering glucose absorption, gluconeogenesis, and glycogenolysis [34].

The *H. abyssinica* leaf crude extract had a later onset of BGL-lowering action than the usual medicine. This could be owing to the presence of phytoconstituents with a higher glycemic index, which could boost free glucose after digestion and BGL absorption [32,43].

STZ (N-methylnitro carbamoyl-D-glucosamine) is a well-known diabetogenic drug that has been utilized as a screening model for assessing the antidiabetic effects of medicinal plants in several animal models [44,45]. This substance functions as a nitric oxide donor in pancreatic cells and is a powerful DNA methylating agent. Because of their low amounts of free radical scavenging enzymes, pancreatic cells are particularly vulnerable to nitric oxide (through suppression of aconitase activity) and free radical damage [46]. STZ causes diabetes like symptoms by killing insulin-secreting pancreatic β- cells, resulting in lower insulin secretion. The current study found that STZ treatment (i.p.) at 150 mg/kg efficiently produced DM in physiologically normal mice as evidenced by hyperglycemia. The crude extract of *H. abyssinica* showed considerable glucose-lowering efficacy as compared to the negative control group in a single-dose STZ-induced diabetic mouse model. This finding is in line with earlier research reports [36,47,48].

In the previous study, leaves extract of *H. abyssinica* exhibited significant *in vitro* *α*-glucosidase and *α*-amylase inhibitory activities [22]. The inhibition of the *α*-amylase and *α*-glucosidase enzymes, in the small intestine, which catabolizes complex carbohydrates into small carbohydrates and modulates weight gain and BGL [28]. By delaying stomach emptying and the absorption of fructose and glucose in the gastrointestinal tract, changing insulin secretion, and lowering the rate of sucrose and starch metabolism, -amylase and -glucosidase inhibitors decrease BGL, particularly postprandial BGL [49]. Furthermore, slowing starch metabolism may promote weight loss by reducing the availability of carbohydrate-derived calories [50].

Previous preliminary phytochemical analysis revealed that the leaf crude extract of *H. abyssinica* is rich with active preliminary phytoconstituents including tannins, terpenoids, saponins, flavonoids, phenols, anthraquinones, and glycosides [27]. Several phytoconstituents isolated from different plant species have been reported to have potent antidiabetic activities. These secondary metabolites include sterols/triterpenoids [51], flavonoids [52,53], alkaloids, and phenols [54]. The antidiabetic effects might be achieved by inhibiting glucose absorption in the gut, facilitating insulin release from pancreatic ß-cells, stimulating glycogenesis in the liver, and/ or increasing glucose utilization by the body [55]. Additionally, phytochemicals are known to induce regeneration of the damaged beta cells and inhibit oxidative stress in beta cells of experimental diabetic rats [56].

Conclusions

This study revealed that the crude leaf extract of *H. abyssinica* possess *in vivo* blood glucose reducing activity which is comparable to the standard clinically established antidiabetic medication, Glibenclamide.

Declarations

Ethics Approval and Consent to Participate

Ethical clearance was obtained from the research and ethics committee of the department of pharmacy, Wollo University to conduct the experiment.

Availability of Data and Materials

The data used to support the findings of this study are included in the article.

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Competing Interests

The authors declare that they have no competing interests.

Authors' Contributions

All authors were involved in the design, analysis and write up of the study. All authors read and approved the final draft of the manuscript.

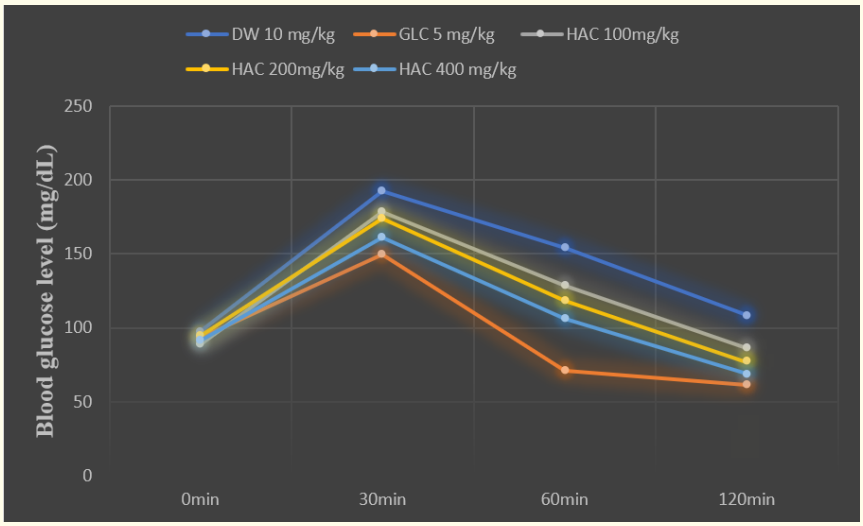

Figure 1: *Effect of Hagenia abyssinica crude extract on oral glucose tolerance test.*

Abbreviations*: HAC= crude extract, GLC = glibenclamide, DW= distilled water, and min= minutes.*

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