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Glinus lotoides linn. Seed extract as antidiabetic agent: *In vitro* and *in vivo* anti-glucolipotoxicity efficacy in Type-II diabetes mellitus

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ABSTRACT

Background: Diabetes, especially type-II, prevailed despite recent medical advances. An edible *G. lotoides* (GL) seed is sold in Ethiopian traditional market such as 'Merkato' and used in folkloric medicine to treat diabetes. But to date not scientifically proven in this optic. As a result, this study set out to validate this claim.
Methods: Following *G. lotoides* seed has been extracted, its antidiabetic efficacy was initially validated *in vitro* before *in vivo* investigation. The *in vitro* activity was probed by employing carbohydrate and lipid metabolizing enzymes inhibition assay. Based on this fact, the *in vivo* antidiabetic efficacy was conducted in normoglycemic, oral glucose-loaded and streptozotocin (150 mg/kg)-nicotinamide (65 mg/kg)-elicited type II diabetic rats.
Results: The extract's LD_{50} was found to be greater than 2 g/kg. In vitro tests pill up evidence that seed extract foils carbohydrate and lipid metabolizing enzyme activities ($p < 0.001$). On the other hand, seed extract significantly abridged blood glucose in normoglycaemic rats markedly ($p < 0.05$–0.001). The highest dose exhibited the strongest glucose tolerance effect, with a maximum slaying (41.1%) in glucose-loaded rats' plasma glucose ($p < 0.001$). All doses of the extract ameliorate blood glucose levels significantly in diabetic rats after 4 weeks of therapy ($p < 0.05$–0.001). Likewise, all test doses tempered harmful lipides in diabetic rats markedly ($p < 0.05$–0.001). But HDL ($p < 0.01$–0.001) and body weight losses ($p < 0.05$–0.001) were rectified.
Conclusion: In consequence, our data unveils the safety and glucolipotoxicity inhibition potential of *G. lotoides* seed extract, authenticating the traditional standpoint that it might be converted into a viable anti-diabetic lead upon subsequent investigations.

1. Introduction

A partial or complete lack of insulin hormone secretion and resistance culminate in diabetes mellitus (DM). It is a protracted metabolic syndrome accompanied by high level of glucose, lipid, and amino acid in blood. Type-I, type-II, and gestational diabetes mellitus are the three major subtypes of DM considering its etiology. DM patients are most likely to have type-II DM (TIIDM), the most frequent variant (>90%). On account of insulin resistance and insulin inadequacy, the insulin discharge flaw in TIIDM evolves with time [1]. Chronic hyperglycaemia has a significant role in the emergence and evolution of DM.

Around 537 million adults (20–79 years) were afflicted by it in the world by 2021 and is expected to climb to 643 million by 2030 and to 783 million by 2045 [2]. Despite the advent of contemporary medications, DM related complications still persists. Moreover, the majority of modern drugs are plugged with side effects, drug-resistance, and high-cost [3]. In order to improve public health by managing diabetes and lowering systemic consequences, novel therapeutic techniques for TIIDM and complementary, natural agents targeting molecules in relevant cell signaling pathways must be investigated.

Over 25% of modern medicines come from medicinal plants [4]. Metformin, an important anti-diabetic medicine, was discovered from the medicinal herb *Galega officinalis*, for instance Ref. [5]. In Ethiopia diabetic patient used medicinal plants at a higher rate in complement to modern therapy [6]. To cure diabetes, Ethiopian traditional healers employs several plants, including the roots of *Dorstenia barnimiana* [7], flower and leaf of *Hagenia Abyssinica* [8,9], seed of *Glinus lotoides* [10] and leaf latex of *Aloe megalacantha* [11].

Glinus lotoides L. is a member of the Molluginaceae family and of the genus *Glinus*. Molluginaceae is a family comprised of sixteen genera and over one hundred species. Although the family Molluginaceae was

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Abbreviations

HDL	High-density lipoprotein
LD_{50}	Median Lethal Dose
LDL	Low-density lipoprotein

formerly lumped in with the family Aizoaceae, it is now widely understood that the 16 genera of Molluginaceae are unrelated to the other Aizoaceae genera [12]. *Glinus lotoides* a dietary vegetable in Africa and Asia is also known as "Metter" by its vernacular name [13]. It is widespread throughout the world's tropical and subtropical climates. It is common in dense populations and is found in damp areas, particularly along the Nile banks following the flood season [14]. Ethiopians have traditionally utilized the seeds of this plant as an anthelmintic, laxative, wound healing [15], anti-bacterial [16], nutritional, and anti-diabetic agents [10,17–19]. It is sold in Ethiopian local markets such as 'Merkato, for nutritional and medicinal purpose. Previous researches have confirmed the pharmacological effects *G. lotoides*, such as anti-tumor [20], chemo-preventive [13], wound healing [15], *anti*--cholesterolemic, hepatoprotective, and anti-oxidant properties [21]. Likewise, plants such as *Glinus oppositifolius* (L)., Mollugo *pentaphylla* [22], *Mollugo oppositifolia* Linn. And *Mollugo nudicaulis* Lamk [23]. Found within the same family exhibited a proven efficacy of anti-diabetic activity.

As a result, it is necessary to verify this conventional knowledge based on empirical data with a scientific basis. As a result, the current study was designed to examine the hypoglycaemic, *anti*-hyperglycaemic, and *anti*-dyslipidaemic benefits of *G. lotoides* seed extract. Furthermore, this work was accomplished to substantiate the hunt for innovative pharmacophores that preferentially inhibit pancreatic α-amylase, lipase (PL), and cholesterol esterase (ChEase), as well as intestinal maltase and sucrase enzymes.

2. Methods

2.1. Animal housing and husbandry

Wistar albino rats (180–210 g, 8–10 weeks, either sex) acquired from the Ethiopian Public Health Institute (EPHI), Addis Ababa, Ethiopia were involved in the present investigation. Ventilated polypropylene cages with (25–30 °C, 12-h photoperiod and 60–65% relative humidity) water and standard rat pellated ration (EPHI, Ethiopia) *ad libitum* was employed to house the animals. Sawdust wood shavings were offered as a form of bedding and changed every other day. At most, six rats could be housed in each cage. The animals were acclimated for two weeks after being randomly grouped and before the trial began. During this time, they were able to adjust to a new setting while also promoting reproducible results. The experimental approaches were undertaken in concordance with the National Academy of Sciences' Guideline [24], and the European Union directive [25].

2.2. Collection and authentication

G. lotoides L. seeds were harvested in March 2021 on Zegie Peninsula, Northwestern Ethiopia, at 11° 43' N, 37° 20' E and an elevation of 1800 m above sea level, approximately 600 km northwest of Ethiopia's capital city, Addis Ababa. Indigenous practitioners identified the plants first, and subsequently Getinet Masresha, a taxonomist at the University of Gondar, authenticated them, and a voucher specimen, Wor27/2021, was stored there.

2.3. Extraction

Freshly collected matured seeds of *G. lotoides* were thoroughly washed with tap water to eliminate any contaminants. After complete drying of the sample sunshade, it was pulverized by an electrical grinder. The dry powdered seeds of *G. lotoides* were subjected to extraction employing**.** One kg sample was soaked in a total of 5.7 litter of 80% hydromethanol with continuous shaking employing automatic shaker (Stuart Scientific) at 120 rpm at room temperature for 72 h. Then, the solution was primarily filtered employing muslin cloth and then refiltered with Whatman filters paper No.1. The marc was re-soaked with the same volume of solvents twice. Then subsequent filtrates were dried in to solid mass in a rotary evaporator (YAMATO rotary evaporator RE301, Japan, 40 °C, 60 rpm). The concentrated filtrate was subjected to deep freezer (DN-86W258) and then freeze-dried in a lyophilizer (Labfreeze Instruments Group Co., Ltd., Japan) at −50 °C. Ultimately, the %yield of extract was computed and kept at −4 °C using sealed container for experimentation [26].

The percentage yield of the rude extract

$$\%\text{Yield} = \frac{\text{Seed extract weight obtained(g)}}{\text{Seed powder employed as stock}} \times 100 \quad (1)$$

2.4. Phytochemistry

To detect the existence or dearth of major classes of phytochemicals in the hydroalcoholic seed extract of *G. lotoides viz.*, anthraquinones, glycosides, flavonoids, terpenoids, tannins, steroids, phenols, alkaloids, and saponins we employed recognized methodologies to conduct qualitative preliminary phytochemical profiling studies [27].

2.5. In vitro studies and experimental procedures

Involves evaluation of the extract on its carbohydrate and lipid-hydrolyzing enzyme inhibitory potential. All experiments were carried out thrice and the mean was undertaken. The half maximal inhibitory concentration values (IC_{50}) were derived from standard graphs of inhibitor (seed extract/standard) concentration vs % enzyme inhibition employing a simple regression equation.

2.5.1. α-amylase inhibition assay

We employed the 3, 5-dinitrosalicylic acid (DNSA) chromogenic technique [28]. To begin, a sodium phosphate buffer (pH 6.9) was applied to dissolve the seed extract and acarbose (Bayer, Germany). The optimal pH for its peak enzymatic performance is this one [29]. Following that, specimens with concentrations ranging from 2 to 400 μg/ml were developed. Each extract and acarbose was poured to 200 μl of porcine pancreatic α-amylase (2 units/ml, Molychem, India) solution and incubated for 10 min. Afterwards, each test tube received an identical amount of 1% w/v starch solution (i-QAREDS-W Alliance International CO., Ltd Taiwan). Then 3 min later, DNSA (200 μl, Sigma-Aldrich, USA) was poured to halt catalysis. After that, the combination was brought to a boil in a water bath at 85 °C for 10 min. After chilling to ambient temperature, 5 ml of distilled water was poured into the mixture. The absorbance intensity of specimens was established employing UV–Visible spectrophotometer (Agilent Technologies, Malaysia) at 540 nm. The control was prepared by mixing 200 μl of α-amylase with 200 μl of sodium phosphate buffer. Moreover, the control blank (sodium phosphate buffer only) and test compound blank solutions (seed extract/acarbose only) have been established in a similar fashion. The %inhibitory potential of different preparations were computed employing Equation (2). Ultimately, the percent inhibition of an enzyme was graphed versus the corresponding strength of the seed extract and acarbose.

$$\text{Rate of Enzyme Inhibition}(\%) = \frac{\varepsilon_c - \varepsilon_{cb} - \varepsilon_{tc} - \varepsilon_{tcb}}{\varepsilon_c - \varepsilon_{cb}} \times 100 \quad (2)$$

Note: ε_c-denotes absorbance intensity of the control (α-amylase + buffer alone).

ε_{cb}-denotes absorbance capacity of a control blank (buffer only)
ε_{tc}-denotes absorbance capacity of the test compound (α-amylase + seed extract/acarbose)
ε_{tcb}-denotes absorbance capacity of test compound blank (seed extract/acarbose alone). Acarbose act as a standard inhibitor control.

2.5.2. Intestinal α-glucosidases inhibition assay

Appraisal of intestinal α-glucosidases inhibitory potential were determined based on the modified glucose oxidase approach early documented [30]. For the sake of simplicity, 3 ml of sterile saline solution (0.9%) was homogenized with 100 mg of powder of acetone (Sigma-Aldrich Co. St. Louis, Missouri, USA) derived from rat intestine tissue employed as source of α-glucosidases. After 30 min of centrifugation at 12,000 rpm, the sample (the supernatant considered as a crude enzyme solution) was assayed.

The crude enzyme solutions such as 10 μl in maltase assay and 30 μl in sucrase assay were treated with maltose (Wako Chemical Co., Japan, 30 μl, 86 mM) or sucrose (Wako Chemical Co., Japan, 40 μl, 400 mM), respectively. To make an ultimate 100 μl combinations, 10 μl of seed extract (2–400 μg/ml) and phosphate buffer (0.1 M, pH = 6.9) were added. The biochemical process was left to run for half hrs. In maltase or 1 h In sucrase assays, respectively at 37 °C prior to getting halted by suspending the mixture in scalding water. The glucose-oxidase approach was employed to determine the quantity of glucose freed from reaction medium at 450 nm. Enzyme inhibitory potential were presented as % inhibition and computed using Equation (3).

$$\%\ \text{Inhibition} = \frac{A_{\text{Control}} - A_{\text{Sample}}}{A_{\text{Control}}} \times 100 \quad (3)$$

Note: $A_{Control}$- Denotes absorbance of control (untreated crude enzyme solution).

A_{sample}- Denotes absorbance of tested sample. In line with prior investigations, acarbose was utilized as an inhibitor control.

2.5.3. Pancreatic lipase inhibition assay

This assay was carried out spectrophotometrically according to Lewis and Liu's protocol (2012) [31]. Taurodeoxycholicacid (5 mM) and p-nitrophenyl palmitate (0.2 mM) were incubated with the extract (25–800 μg/ml) in Na_2HPO_4/NaH_2PO_4 (pH 8.0, 50 mM). To catalyze the enzymatic reaction, porcine pancreatic lipase (10 mg/ml, Sigma-Aldrich Co. St. Louis, MO, USA), was introduced. After 5 min of incubation at 37 °C, the mixtures' absorbance at 450 nm was recorded.

$$\%\ \text{Inhibition} = \frac{A_{\text{Control}} - A_{\text{Sample}}}{A_{\text{Control}}} \times 100 \quad (4)$$

Note: $A_{Control}$- Denotes absorbance of control (An untreated enzyme solution without tested sample).

A_{sample}-Denotes absorbance of tested sample.

A prescription drug orlistat (Nanjing Zelang Pharmaceutical Technology Co., Ltd.) was used as a standard inhibitor control.

2.5.4. Pancreatic cholesterol esterase inhibition assay

In accordance with earlier research, this experiment was carried out spectrophotometrically [32]. In a nutshell, different concentrations of the extract (25–800 μg/ml) were incubated with chromogenic substrate mixtures containing taurocholic acid (5.16 mM) and p-nitrophenylbutyrate (0.2 mM) in Na_2HPO_4/NaH_2PO_4 (100 mM) diluted with NaCl (100 mM, pH 7.0). Porcine ChEase (1 μg/ml, Sigma-Aldrich Co. St. Louis, MO, USA), was used to catalyze the reaction. To this end, after 5 min (25 °C). Of incubation their absorbance intensity was determined at 405 nm.

$$\%\ \text{Inhibition} = \frac{A_{\text{Control}} - A_{\text{Sample}}}{A_{\text{Control}}} \times 100 \quad (5)$$

Note: $A_{Control}$- Denotes absorbance of control.

A_{sample}-Denotes absorbance of tested sample. This study's standard drug was the cholesterol-lowering agent simvastatin (Nanjing Zelang Pharmaceutical Technology Co., Ltd.).

2.6. In vivo studies

2.6.1. Acute toxicity test

The LD_{50} was anticipated in female Wistar albino rats employing a limit test of 2008 OECD guideline 425 [33]. In brief, five animals (8–10 weeks old) were employed. For four and 2 h prior to and following the extract administration, all rats were denied feed but not drink. The dose was established for each animal based on their fasting body weight. To begin, a limit dose of 2 g/kg of seed extract was orally administered to a single animal, and 24-h risk of death was recorded. Following that, four additional rats medicated and handled independently and examined consistently for 4 h With a half hr. Interval, followed by 14 days in a row with a 24 h Interval for the general toxicity patterns [34].

2.6.2. In vivo anti-diabetic study design

This study used solely male Wistar albino rats (10-week-old, 180–210 g) in all animal models save the acute oral toxicity investigation, in which female rats were employed. This is based on the notion that female are less responsive to STZ and insulin than male rats [35]. The overnight fasting normal animals were randomized into 5 classes (G_I-G_V, n = 6) in the normoglycaemic and OGTT paradigms as shown below.

Group	Treatment
G_I	Negative control received 2% Tween 80 (TW80), (10 ml/kg).
G_{II}	Normal rat GL100, (100 mg/kg).
G_{III}	Normal rat GL200, (200 mg/kg)
G_{IV}	Normal rat GL400, (400 mg/kg)
G_V	Positive control rat GLC5, (5 mg/kg).

In the STZ-NA-elicited TIIDM rat paradigm, diabetic rats abstained feed for 18 h Were arbitrarily allocated into six treatment groups (n = 6) and treated as depicted in Scheme 1.

G_I (Disease control): The diabetic rats were received 2% TW80, 10 ml/kg b.wt./day.

G_{II}: DM-induced animals that took 100 mg/kg b.wt./day of *G. lotoides* seed extract.

G_{III}: DM-induced animals that took 200 mg/kg b.wt./day of *G. lotoides* seed extract.

G_{IV}: DM-induced animals that took 400 mg/kg b.wt./day of *G. lotoides* seed extract.

G_V (Positive control): DM-induced animals that took 5 mg/kg b.wt./day of glibenclamide (Cadila Pharmaceuticals, Bengaluru, India).

G_{VI} (Normal control): Healthy rats received 2% TW80 10 ml/kg b.wt./day. To simulate the indigenous community's traditional practice of treating DM at the claimed site, all remedies were administered orally via oral gavage for 4 consecutive weeks.

2.6.3. Determination of parametric outcome variables

Fasting blood glucose levels were determined employing a one-touch automated glucometer and a test strip (Alliance international, Taiwan) after blood specimen was retrieved from tail vein. After triplicate observations of parametric variables, the average value was established. Body weight was measured using a digital analytical weighing balance (adventurer OHAUS, China).

2.6.4. Induction of experimental type-II diabetes mellitus

Streptozotocin (Sigma, St. Louis, MO, USA, 65 mg/kg), dissolved in citrate buffer (0.1 M, PH = 4.5) immediately before use was introduced intraperitoneally (i.p.) to feed deprived (6–8 h, water *ad libitum*) rats, after a 15-min i. p. Injection of NA (230 mg/kg, Sigma, St. Louis, MO, USA) prepared in normal saline. A total 40 Wistar albino male rats have been treated with STZ and NA solutions to impart TIIDM. Normal rat pellated diet intake was permitted 30 min after STZ delivery. After 6 h, the animals were allowed easy access to a 5% glucose solution for 24 h In order to avert death from hypoglycaemic incidents. After 3rd days of STZ treatment, the rats were tested for DM and those with FBG levels ≥ 250 mg/dl were declared diabetic upon repeated measurements of FBG by the 7th and 14th days and enrolled in the following investigation [36].

2.6.5. Validation of hypoglycaemic effect in normoglycaemic rats

After an overnight fasting (18 h, water *ad libitum*), 30 healthy rats were randomized into five distinct classes as depicted in Scheme 2. Following that, as specified in section 2.6.2, the rats were treated accordingly. The FBG was taken prior to therapy as a benchmark, and again at 1, 2, 4, and 6 h Post-treatment [37].

2.6.6. Appraisal of anti-hyperglycaemic efficacy in healthy rats

In order to investigate the effect of extract on glucose metabolism 30 overnight fasting rats were arbitrarily assigned into five categories and treated in accordance with section 2.6.2. Each rat was provided with an oral 40% w/v glucose solution (3 g/kg, Aristar, England) after 30 min of treatment. The FBG was taken at pre-oral glucose loading benchmark, and then again at 30, 60, 90, and 120 min post-glucose treatment [37].

2.6.7. Effect on hyperglycaemia, body weight, and lipogram of TIIDM rats

Six groups (n = 6) of male STZ-NA-induced diabetic (30 diabetic surviving rats) and normal rats (6 healthy rats) were involved. Following that, rats received daily treatment for four successive weeks. Rats' FBG and weight were measured on day 0, 7, 14, 21, and 28. Pre-initiation of DM, the weight of each rat was first determined. In order to compute the percentage change in the FBG and body weight level, Equation (6) and Equation (7) were used, respectively.

$$\%\ \text{Reduction in FBG} = \frac{\text{FBG D0} - \text{FBG Dt}}{\text{FBG D0}} \text{X100} \quad (6)$$

Note: FBG D0 = day 0 fasting blood glucose level.

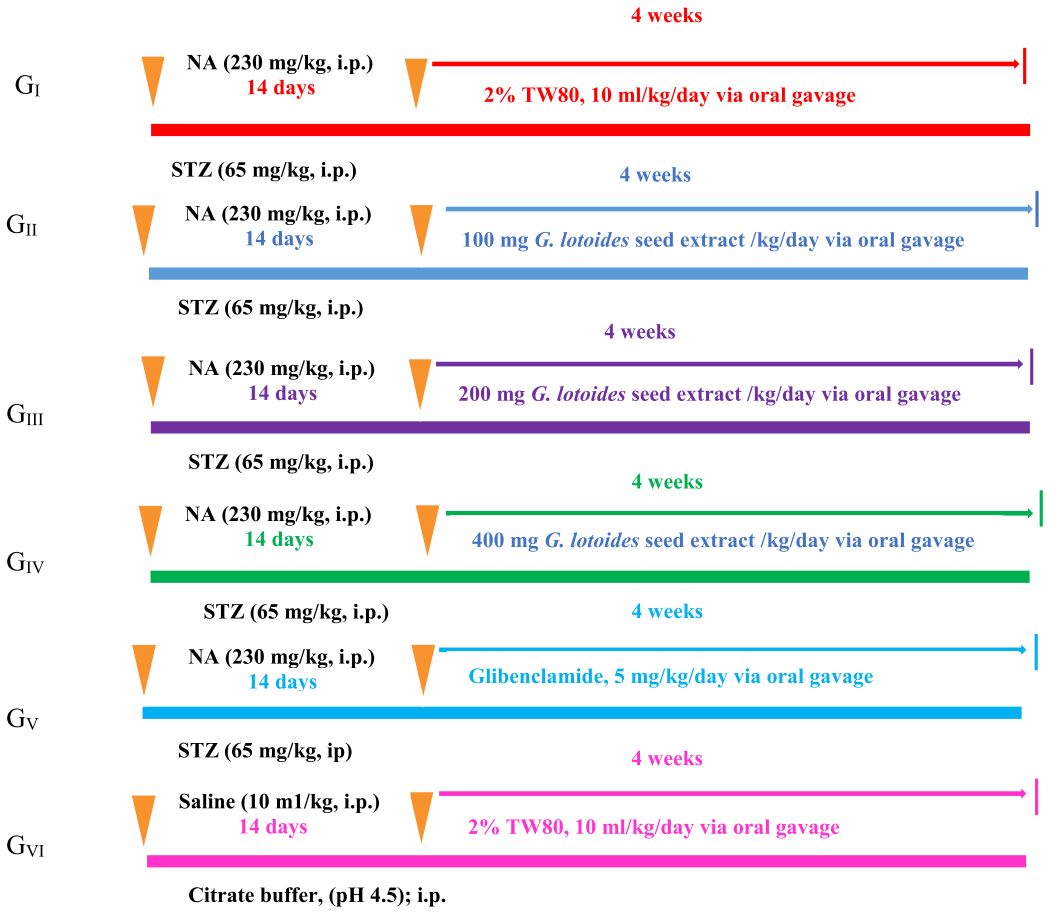
FBG Dt = blood glucose level at time t treatment days.

$$\%\text{Change in b.wt} = \frac{\text{b.wt. Dt} - \text{b.wt. Do}}{\text{b.wt. Do}} \times 100 \quad (7)$$

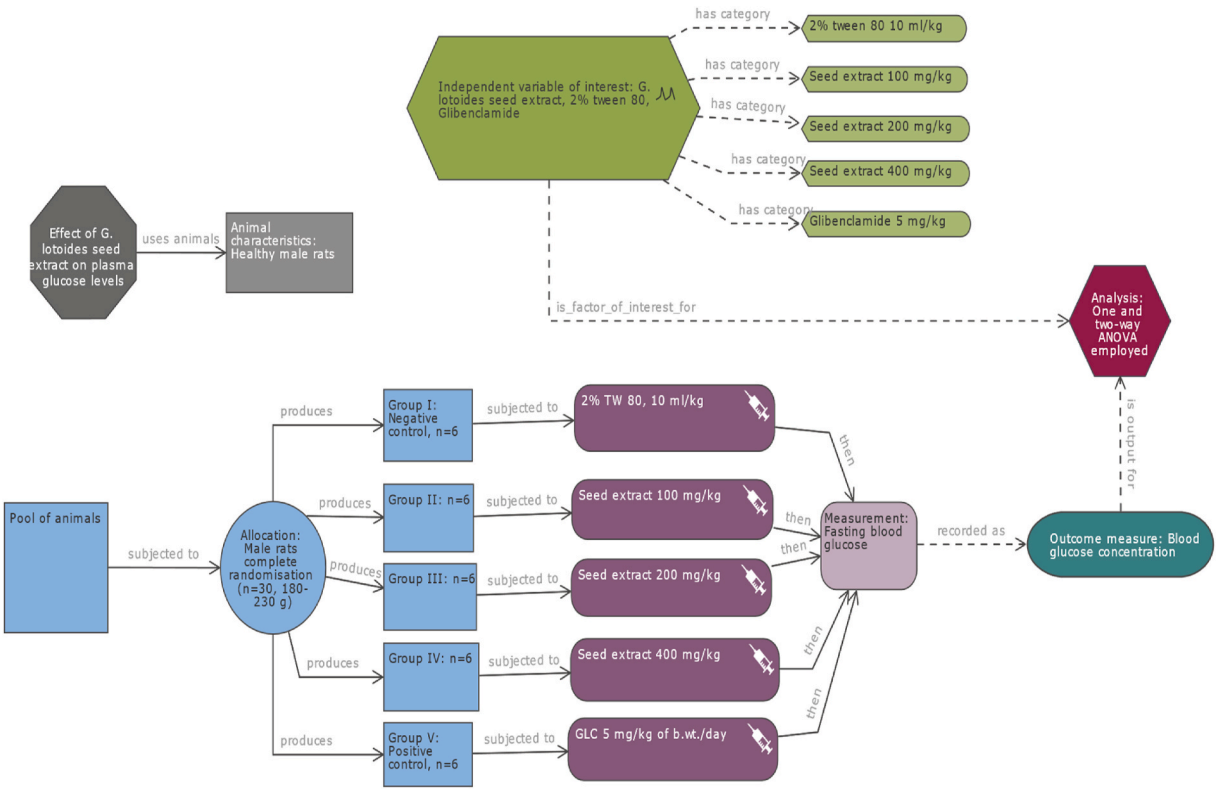
Note: b. wt. D0 = Body weight on Day t of treatment.

b.wt. Dt = Body weight on Day t of treatment.

Decapitation was used to collect blood samples from the rats after the experiment. An automated biochemical analyzer apparatus (humostar 80, Germany) was applied to find out the concentrations of triglycerides (TG), and high-density lipoprotein cholesterol (HDL-c), total cholesterol (TC) in the blood specimens. Low-density lipoprotein cholesterol (LDL-



Scheme 1. Schematic representation of experimental design with timeline and treatment allocation: Abbrevations-NA, Nicotinamide; STZ, Streptozotocin; i. p., Intraperitoneal.



Scheme 2. Schematic representation of experimental design and treatment allocation in normoglycaemic study.

c), and very-low-density lipoprotein cholesterol (VLDL-c) levels were discovered utilizing Friedewald formula as shown in Equation (7) [38].

$$\text{LDL cholesterol} = \text{TC} - (\text{HDL}) - \frac{\text{TG}}{5} \tag{8}$$

VLDL-cholesterol i.e., 20% TG was calculated employing Norbert's formula [39]:

$$\text{VLDL cholesterol} = \frac{\text{TG}}{5} \tag{9}$$

2.7. Statistical analysis

Windows International Business Machine (IBM) Statistical Package for Social Sciences (SPSS) version 24 (Chicago, IL, USA) was applied to investigate the data gathered and displayed as mean ± standard error of the mean (SEM). ANOVA with one-way effects preceded by HSD *Post hoc* Tukey's multiple comparison tests were utilized to compare both within and between-group. For multiple comparisons between tests, we used ANOVA with two-way effects preceded by an HSD *post hoc* Bonferroni's test to compare outcome variables prior and following treatment. An independent sample *t*-test was implemented for *in vitro* experiments. With a 95% CI, p-values lower than 0.05 were deemed meaningful statistically. To generate the bar and line graphs, we employed Excel 2016 software.

2.8. Data quality assurance

Unrandomized group and treatment allocation unconcealment do not support deductive approach [40]. Based on this fact, the experimental study was done by employing a simple random sampling approach to assign the laboratory animals to groups and their respective treatment. During the duration of the experiment, all personnel involved in the care and treatment of the animals, as well as those who collected data and analysed it, were blinded. Scientifically labeled and calibrated instruments and chemicals were employed. The experimental procedures were carried out in adherence to approved protocols. Animals were cared in humane and meticulous way to ablate stress induction through poor handling techniques and data derangement thereof.

3. Results

3.1. Extraction yield

We obtained 134.93 g (13.493%) of dried extract from 1000 g seed powder of the herb which is in line with previous studies [41].

3.2. Phytochemistry

The types of tests conducted, their appearance when determined to

Table 1
G. lotoides seed extract preliminary qualitative phytochemical characterization.

Phytochemical	Type tests undertaken	Appearance	Inference
Flavonoids	Lead acetate	Yellow precipitate seen	+
Alkaloids	Wagner's	Reddish precipitate seen	+
Anthraquinones	Borntrager's	Pink violet lower layer seen	+
Phenolics	Ferric chloride	Bluish black colour indicated	+
Glycosides	Keller–Killiani	A brown ring at the interface was seen	+
Tannins	Braemer's	A blue-green colour was indicate	-
Steroids	Liebermann–Burchard	Pink to red colour not formed	-
Terpenoids	Salkowski's	Black coloration was observed	+
Saponins	Froth	Frothing was seen markedly	+

Note: (+): discerned, (−): not discerned.

be positive, and the ultimate inferences are listed in Table 1.

3.3. In-vitro studies

3.3.1. α-Amylase inhibitory test

The inhibitory efficacy of acarbose and seed extract on α-amylase is illustrated in Table 2. For varied strengths of the tested extract and reference, strength-dependent inhibition was observed. The extract exhibited the maximum inhibitory activity at 400 μg/ml (IC_{50} = 5.22 μg/ml). IC_{50} value of inhibitor control (acarbose) was found to be 2.99 μg/ml.

3.3.2. α-Glucosidases inhibition assay

Despite *G. lotoides* seed extracts inhibited both enzymes, they were more effective against sucrase (IC_{50} = 4.67 μg/ml, 78.21 ± 0.47%) than maltase (IC_{50} = 5.84 μg/ml, 60.21 ± 0.61%), as illustrated in Fig. 1(A) and (B) respectively. In comparison to the negative control, the extract revealed markedly ($p < 0.001$) higher % inhibition concentration-dependently.

3.3.3. Pancreatic lipase inhibition assay

G. lotoides seed extracts patterned inhibition of PL was in a concentration-dependent fashion as depicted in Fig. 2. At an extract concentration of 800 μg/ml, the best lipase inhibitory activity (56.83 ± 0.27%) was observed (y = 10.618x-5.9853, R^2 = 0.9956, IC_{50} = 5.273 μg/ml).

Orlistat, which was employed as an inhibitor control, produced a maximal inhibition of 99.97 ± 0.38% with an IC_{50} value of (y = 9.8049x+18.891, R^2 = 0.9956) 3.173 μg/ml.

3.3.4. Cholesterol esterase inhibitory test

G. lotoides had considerably ($p < 0.001$) lower ChEase inhibitory activity than the inhibitor control, simvastatin. The highest ChEase inhibitory activity in seed extract was 67.82 ± 0.47% while 85.23 ± 0.51% in simvastatin (see Fig. 3). Simvastatin had an IC_{50} of 2.422 μg/ml (y = 10.274x+25.117, R^2 = 0.9941), while seed extract had an IC_{50} of 4.251 μg/ml (y = 11.177x+2.4853, R^2 = 0.99).

3.4. In-vivo studies

3.4.1. Acute oral toxicity test

At 2000 mg/kg, no mortality was detected, indicating that the extract's half maximum fatal dose (LD_{50}) was greater than 2 g/kg.

3.4.2. Hypoglycaemic effect in normal rat

Prior to initiating treatments, there were no meaningful discrepancy in FBG levels between groups ($P > 0.05$) (see Table 3). Starting 2 h Later, both extract and glibenclamide considerably ($p < 0.05$–0.001) lowered FBG levels matched to the negative control. Following 6 h Of therapy, both seed extract and GLC5 were able to drastically diminish FBG levels in comparison to 2 h.

Table 2
Inhibitory efficacy of the hydromethanolic seed extract of Glinus lotoids on α-amylase.

Concentration (μg/ml)	α-amylase inhibition (%)	
	Seed Extract	Acarbose
2	3.55 ± 0.09	24.36 ± 0.22
5	17.65 ± 0.27	35.30 ± 0.07
25	19.18 ± 0.64	52.25 ± 1.20
50	32.65 ± 0.41	64.32 ± 0.83
100	35.87 ± 0.83	78.31 ± 0.61
200	64.61 ± 1.05	85.32 ± 1.75
400	76.28 ± 1.09	95.76 ± 1.60

Notes: Data are displayed as means ± SEM, n = 3.

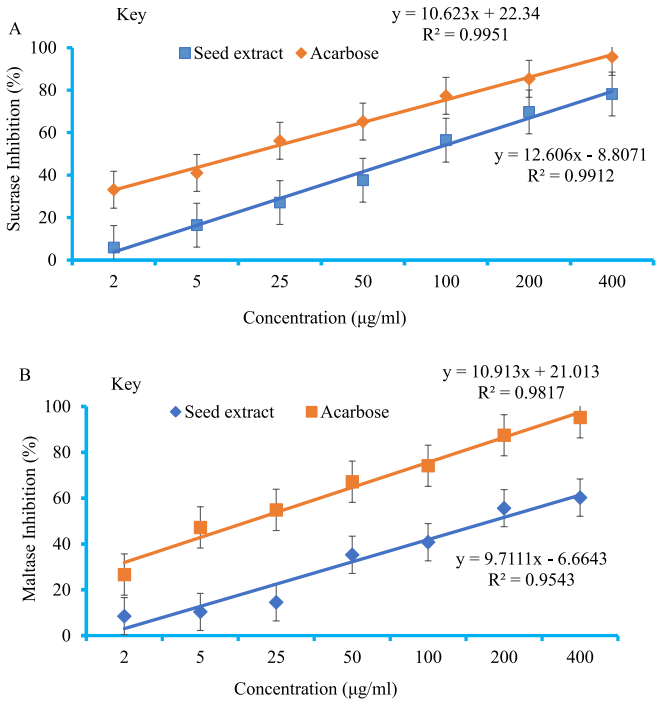


Fig. 1. Inhibitory potential of G. lotoides seed extract on intestinal α-glucosidases. (A) Sucrase enzyme activity; (B) Maltase enzyme activity. Notes: Data is displayed as means ± SEM, n = 3.

3.4.3. Oral glucose challenge model

Apparently, there was no substantial difference in the FBG levels of rats across groups at ahead of glucose loading (0 min) ($p > 0.05$). OGTT with 40% glucose culminated in peak hyperglycemia after 30 min, replicating the production of genuine post prandial hyperglycemia. Treatment begins to demonstrate a meaningful effect in FBG level at 30 min onwards ($p < 0.05$–0.001) (see Table 4). When matching with its 30 min value, the dose GL400 displayed a highest fall (41.1%) that was comparable to GLC5 (44.18%). Additionally, within-group analysis demonstrated that therapy of animals with the test extract and GLC leads in a statistically relevant drop in FBG levels at 60 min, 90 min, and 120 min following glucose delivery compared to 30 min FBG (peak level). At all-time intervals, no substantial and statistically meaningful change in FBG levels was detected between GLC5 and GL400, indicating that GL400 is comparable to GLC5 though unequieffective (Table 4).

3.4.4. Anti-diabetic efficacy in STZ-nicotinamide-elicited diabetic rat

After induction, diabetic rats exhibited considerably higher baseline FBG levels than sham rats (non-diabetic rats), but no meaningful difference existed across any of the diabetic rat groups. FBG levels were drastically decreased in a dose-dependent approach when seed extract and glibenclamide were employed (see Table 5). Rats receiving GL100, 200 and 400 saw a considerable improvement in their FBG by 61.41, 68.60, and 72.37% following a four-week intervention period, according to the results of an in-group analysis matching with baseline. Likewise, glibenclamide reduce FBG by 74.72% which is comparable to the test extract GL400.

3.4.5. Effect on body weight of diabetic rat

There was no statistically relevant change in weight among diabetic and healthy rats prior to the affliction of DM and at baseline. Following ten days of injection onwards, STZ produces a considerable fall of body weight in diabetes controls when matched to normal controls and baseline values ($p < 0.05$–0.001). Neither seed extract nor glibenclamide could rectify the weight loss during the first two weeks of

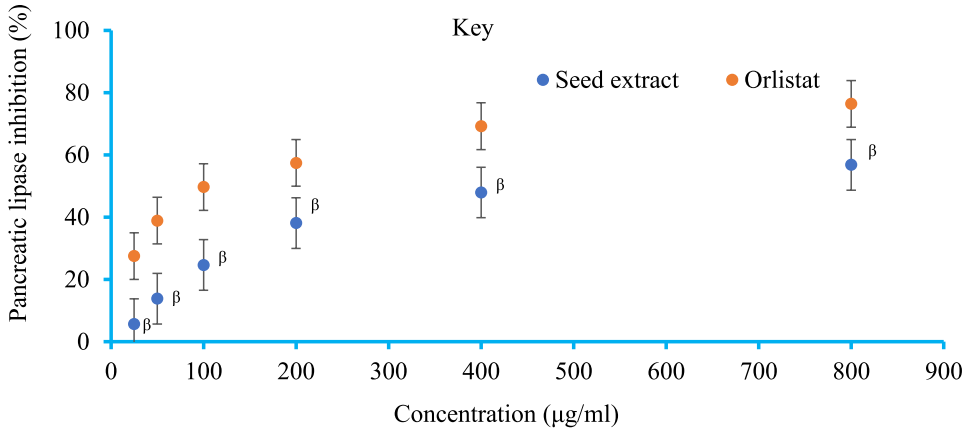


Fig. 2. Inhibitory potential of *G. lotoides* seed extract on pancreatic lipase. Data are displayed as means ± SEM (n = 3). $^{\beta}p<0.001$ versus orlistat.

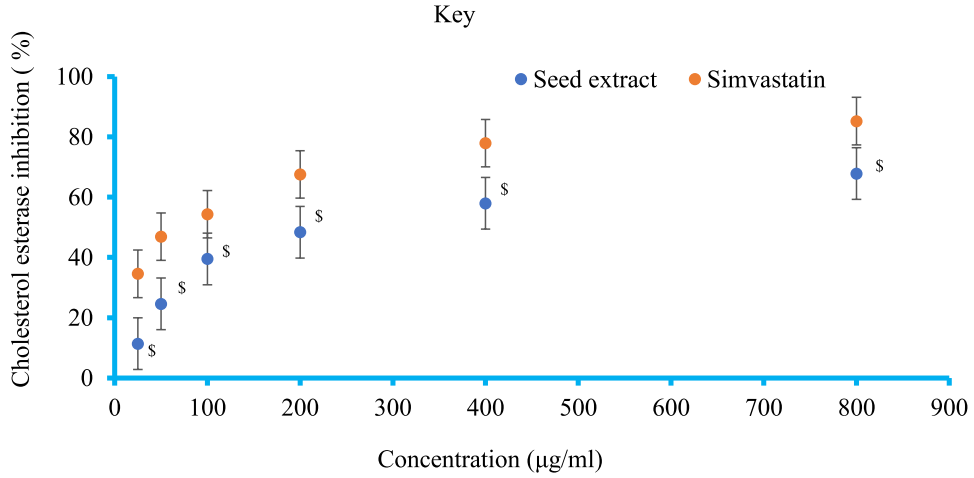


Fig. 3. Inhibitory potential of G. lotoides seed extract on pancreatic cholesterol esterase. Data are displayed as means ± SEM (n = 3). $^{\$}p < 0.001$, compared to inhibitor controls. Abbreviations: ChEase, cholesterol esterase.

Table 3
The activity of G. lotoides seed extract on normoglycaemic rats.

Group	FBG Level (mm/l)				
	0 h.	1 h.	2 h.	4 h.	6 h.
DC	7.24 ± 0.17	7.21 ± 0.08	7.16 ± 0.02	7.09 ± 0.11	7.02 ± 0.31
GL100	7.00 ± 0.11	6.97 ± 0.17	6.26 ± 0.28 $^{\alpha1\beta1\delta1\$1}$	6.08 ± 0.31 $^{\alpha2\beta1\delta1\$1}$	5.21 ± 0.78 $^{\alpha3\delta2\$2\varepsilon1}$
GL200	7.46 ± 0.83	7.41 ± 0.43	6.54 ± 0.12 $^{\alpha2\ \beta1\delta1\$1}$	6.05 ± 0.05 $^{\alpha3\beta1\delta1\$1}$	5.00 ± 0.19 $^{\alpha3\delta2\$2\varepsilon2}$
GL400	7.37 ± 0.08	7.31 ± 0.56	6.14 ± 0.82 $^{\alpha3\delta1\$1}$	5.48 ± 0.09 $^{\alpha3\delta2\$2}$	5.13 ± 0.03 $^{\alpha3\delta2\$3\varepsilon3}$
GLC5	7.24 ± 0.17	7.04 ± 0.21	6.04 ± 0.06 $^{\alpha3\delta1\$1}$	5.37 ± 0.07 $^{\alpha3\delta2\$2}$	4.73 ± 0.05 $^{\alpha3\delta3\$3\varepsilon3}$

Notes: Data are examined by employing one and two-way ANOVA preceded by *Post hoc* Tuckey's test and provided as mean ± SEM (n = 6); Matched to the $^{\alpha}$disease control, $^{\beta}$GLC, $^{\delta}$reference FBG (0 h), $^{\$}$1 h. FBG, $^{\varepsilon}$2 hrs. FBG, $^{1}P < 0.05$, $^{2}P < 0.01$, $^{3}P < 0.001$, Abbreviations: GL, *G. lotoides*; GLC, glibenclamide.

Table 4
Effect of the seed extract of G. lotoides on glucose-challenged healthy rats.

Group	FBG profile (mm/l)				
	0 min	30 min	60 min	90 min	120 min
DC	7.58 ± 0.16	11.03 ± 0.05	10.23 ± 0.02	9.58 ± 0.19	9.05 ± 0.01
GL100	7.41 ± 0.98	10.94 ± 0.41	9.02 ± 0.46 $^{\alpha1\beta1\delta1}$	8.73 ± 0.11 $^{\alpha1\beta1\delta1}$	7.09 ± 0.21 $^{\alpha1\beta1\delta1\mu1}$
GL200	7.06 ± 0.54	10.42 ± 0.73	8.05 ± 0.14 $^{\alpha1\beta1\delta1}$	7.33 ± 0.16 $^{\alpha2\beta1\delta1}$	6.42 ± 0.43 $^{\alpha2\beta1\delta1\mu2}$
GL400	7.51 ± 0.08	8.03 ± 0.44 $^{\alpha1}$	7.01 ± 0.32 $^{\alpha2\delta1}$	5.14 ± 0.04 $^{\alpha3\delta2}$	4.73 ± 0.17 $^{\alpha3\delta3\mu2}$
GLC5	7.09 ± 0.15	8.08 ± 0.03 $^{\alpha1}$	6.04 ± 0.11 $^{\alpha3\delta2}$	5.32 ± 0.20 $^{\alpha3\delta2}$	4.51 ± 0.19 $^{\alpha3\delta3\mu2}$

Notes: Data are examined by employing one and two-way ANOVA preceded by *Post hoc* Tuckey's test and provided as mean ± SEM (n = 6); matched to the $^{\alpha}$negative control, $^{\beta}$GLC5, $^{\delta}$30 min, $^{\mu}$60 min, $^{1}P < 0.05$, $^{2}P < 0.01$, $^{3}< 0.001$. Abbreviations: *GL: G. lotoides;* GLC, Glibenclamide.

intervention. It took around 28 days for GL100 and GL200 to demonstrate their considerable weight loss ablation potential as compared to the DC ($p < 0.05$), however GL400 and GLC5 demonstrated just after 21 intervention days (see Table 6). After 4 weeks of treatment body weight was decreased by 28.22%, 1.78%, and 0.54% in rats treated with vehicle, GL100 and GL200 mg/kg when matched with their baseline (day 0).

3.4.6. *Effect on serum lipogram*

Both the seed extract and glibenclamide demonstrated a notable (P

Table 5
Anti-hyperglycaemic efficacy of *G. lotoides* seed extract on diabetic rats.

Group	FBG profile (mm/l)				
	Day 0	Day 7	Day 14	Day 21	Day 28
DC	22.81 ± 0.02$^{\mu 3}$	22.60 ± 0.37$^{\mu 3}$	22.53 ± 0.17$^{\mu 3}$	21.43 ± 0.28$^{\mu 3}$	21.20 ± 0.21$^{\mu 3}$
GL100	21.43 ± 0.15$^{\mu 3}$	20.45 ± 0.63$^{\beta 1 \mu 3}$	15.23 ± 0.02$^{\alpha 1 \delta 1 \beta 1 \mu 3}$	10.72 ± 0.08$^{\alpha 1 \delta 1 \mu 3}$	8.27 ± 0.06$^{\alpha 3 \delta 1 \mu 3}$
GL200	25.03 ± 1.07$^{\mu 3}$	19.11 ± 1.02$^{\alpha 1 \mu 3}$	14.05 ± 0.12$^{\alpha 1 \delta 1 \mu 3}$	9.21 ± 0.04$^{\alpha 2 \delta 1 \mu 3}$	7.86 ± 0.03$^{\alpha 3 \delta 1 \mu 3}$
GL400	24.86 ± 0.0 $^{\mu 3}$	13.81 ± 0.12$^{\alpha 1 \mu 3}$	10.17 ± 0.09$^{\alpha 2 \delta 1 \mu 3}$	8.23 ± 0.11$^{\alpha 3 \delta 2 \mu 3}$	6.87 ± 0.42$^{\alpha 3 \delta 2 \mu 3}$
GLC5	25.36 ± 1.07$^{\mu 3}$	14.02 ± 0.61$^{\alpha 3 \mu 3}$	9.91 ± 0.05$^{\alpha 3 \delta 1 \mu 3}$	8.08 ± 0.43$^{\alpha 3 \delta 2 \mu 3}$	6.41 ± 0.11$^{\alpha 3 \delta 2 \mu 3}$
NC	7.49 ± 0.18$^{\alpha 3}$	7.39 ± 0.12$^{\alpha 3}$	7.27 ± 0.04$^{\alpha 3}$	7.14 ± 0.12$^{\alpha 3}$	7.03 ± 0.22$^{\alpha 3}$

Notes: Data are examined by employing one and two-way ANOVA preceded by *Post hoc* Tuckey's test and provided as mean ± SEM (n = 6); matched to the $^{\alpha}$DC, $^{\beta}$GLC5, $^{\delta}$to reference (Day 0), $^{\mu}$to NC ^{1}p < 0.05, ^{2}p < 0.01, ^{3}p < 0.001. Abbreviations: GL, *G. lotoides*; GLC5, Glibenclamide; NC, Normal control.

< 0.05–0.001) shrinkage in serum TC, TG, LDL-c, and VLDL-c levels, but a rise in serum HDL-c, in comparison to the DC (see Table 7).

4. Discussion

Despite tremendous breakthroughs in the discovery of typical anti-diabetic medicines, these drugs are typically deemed ineffectual due to their failure to avoid DM related secondary complications, expensiveness and side effects. Hence, modern research examining medicinal herbs and natural items as therapeutic alternatives is welcomed.

Prior to conducting animal studies, the anti-diabetic efficacy of seed extract *G. lotoides* was examined *in vitro*. Lopsided carbohydrate and lipid breakdown in the tissue results in an upsurge in postprandial hyperglycaemia levels, which inevitably lead in the establishment and evolution of TIIDM. Pancreatic α-glucosidases and α-amylase suppression is a promising technique for lowering such postprandial hyperglycaemia by restricting starch hydrolysis, which is particularly important in TIIDM patients and prediabetics [42]. Nevertheless, neither of the currently approved α-glucosidases nor α-amylase inhibitors are effective and devoid of serious side effects [43]. As a result, this *in vitro* study was designed to meticulously determining *G. lotoides* seed extract's pancreatic alpha-amylase, PL, ChEase and intestinal maltase, and sucrases inhibitory potential in detail. This study demonstrates that the plant possesses good suppressive potential on pancreatic (alpha-amylase and α-glucosidases) enzymes thereby delaying carbohydrate metabolism. Hindrance in starch metabolism may play its role in controlling insulin resistance and glycemic index in diabetic patients [44]. Our findings corroborate prior research showing modest α-amylase and α-glucosidases inhibition is sufficient to avoid aberrant bacterial fermentation caused by undigested carbohydrates in the colon, which causes bloating and diarrhoea [43]. But its α-amylase inhibition potential was lower than *Pleurotus pulmonarius* [45].

Unrelenting elevated blood TG and cholesterol levels are features of TIIDM. Pancreatic lipases and ChEases involved in the metabolism and assimilation of lipids. Suppressing those pancreatic enzymes thereof ablating TIIDM-related dyslipidaemia [46]. Obese patients with TIIDM benefit with orlistat, which works by blocking pancreatic lipase, a major lipolytic enzyme. Unfortunately, it is generally characterized by severe adverse effects including gastrointestinal intolerances, which can harm up to 40% of patients [47]. Multiple potent ChEase inhibitors, such as phosphonates and carbamates, have lately emerged, but lack selectivity [48]. As a result, patients would benefit tremendously from a medicine that suppresses PL and ChEase while reducing or eliminating negative effects. The fact that in this study *G. lotoides* seed extract abridged PL and ChEase activity moderately. However, these inhibitions were at a lower rate than the positive controls orlistat and simvastatin, respectively which might be owe to its crude nature.

This could be attributed to secondary metabolites such as flavonoids [49], terpenoids [50], saponins [51], alkaloids [52], and even polysaccharides [53,54]. Crocin, a carotenoid ameliorates PL activity utilized as *anti*-hyperlipidaemic medication [55]. ChEase and PL enzymes are known to be inhibited with flavonoids including gallic acid, catechin, chalcone, and quercetin [52,56]. Polysaccharides discovered in *Cordyceps sinensis* such as PS-A suppresses ChEase enzyme activity substantially (IC50 = 12.7 μg/ml) which may treat DM descended-dyslipidaemia [53]. To this end, one explanation for *G. lotoides' anti*-dyslipidaemic effects is that due to its inhibitory effect on PL and ChEase enzymes in the body. By doing so *G. lotoides* seed extract ameliorate threats of DM-related cardiac complications.

The above mentioned *in vitro* finding gave us impetus for further *in vivo* studies on its benefits by ablating TIIDM-related glucolipotoxicity. The LD_{50} in naive rats was predicted to be over 2 g/kg based on the acute toxicity test result implying a wide safety margin concordant to previously reported safety profiles (LD_{50} > 5 g/kg) [41].

Table 7
Effect of seed extract on the serum lipogram of STZ-NA-afflicted rats.

Group	Lipid profile (mg/dl)				
	TC	TG	LDL-c	VLDL-c	HDL-c
DC	185.75 ± 3.10$^{\theta 3 \mu 3}$	188.15 ± 4.09$^{\theta 3 \mu 3}$	124.03 ± 1.07$^{\theta 3 \mu 3}$	37.63 ± 0.22$^{\theta 3 \mu 3}$	24.09 ± 1.17$^{\theta 3 \mu 3}$
GL100	167.76 ± 5.03$^{\pi 1 \theta 1}$	150.35 ± 2.03$^{\pi 2 \theta 1}$	106.41 ± 1.13$^{\pi 2 \theta 1}$	30.07 ± 1.31$^{\pi 1 \theta 1}$	31.28 ± 2.00$^{\pi 2 \theta 1}$
GL200	163.69 ± 1.53$^{\pi 1 \theta 1}$	135.20 ± 5.31$^{\pi 2}$	97.69 ± 1.08$^{\pi 3}$	27.04 ± 1.13$^{\pi 2}$	38.96 ± 0.04$^{\pi 2}$
GL400	145.76 ± 4.17$^{\pi 2 \theta 1}$	113.25 ± 1.29$^{\pi 3}$	81.05 ± 2.09$^{\pi 3}$	22.65 ± 1.09$^{\pi 3}$	42.06 ± 0.15$^{\pi 3}$
GLC5	102.58 ± 0.11$^{\pi 3}$	100.41 ± 3.71$^{\pi 3}$	36.41 ± 1.44$^{\pi 3}$	20.08 ± 0.11$^{\pi 3}$	46.09 ± 0.71$^{\pi 3}$
NC	97.87 ± 4.01$^{\pi 3}$	92.35 ± 1.43$^{\pi 3}$	34.21 ± 0.06$^{\pi 3}$	18.47 ± 1.81$^{\pi 3}$	45.12 ± 1.30$^{\pi 3}$

Notes: Data are examined by employing one-way ANOVA preceded by *Post Hoc* Tuckey's test and provided as mean ± SEM (n = 6); matched to $^{\pi}$DC, $^{\theta}$NC, $^{\mu}$GLC5, ^{1}P < 0.05, ^{2}P < 0.01, ^{3}P < 0.001. Abbreviations: DC, Diabetic control; GL, *Glinus lotoides*; GLC, glibenclamide; NC, Normal control.

Table 6
Effect of seed extract on body weight of diabetic rats.

Group	Body weight (g)					
	Before DM	Day 0	Day 7	Day 14	Day 21	Day 28
DC	197.11 ± 0.80	191.03 ± 1.43	176.90 ± 0.40$^{\theta 1 \rho 2}$	153.35 ± 0.67$^{\mu 2 \rho 303}$	142.31 ± 0.56$^{\mu 3 \rho 303}$	137.12 ± 0.58$^{\mu 303 \rho 3}$
GL100	202.94 ± 0.85	194.11 ± 2.01	181.58 ± 0.71$^{\theta 1}$	177.78 ± 0.53$^{\rho 1 \theta 1}$	170.26 ± 0.68$^{\mu 2 \rho 1 \theta 1}$	190.66 ± 0.67$^{\pi 1}$
GL200	186.84 ± 1.21	180.60 ± 4.11	175.72 ± 0.47$^{\theta 1}$	172.60 ± 0.53$^{\rho 1 \theta 1}$	161.59 ± 0.55$^{\mu 1 \rho 1 \theta 1}$	179.62 ± 0.66$^{\pi 1}$
GL400	198.61 ± 0.08	186.35 ± 1.41	177.96 ± 0.37$^{\theta 1}$	165.59 ± 0.50$^{\rho 1 \theta 1}$	174.07 ± 0.43$^{\pi 1 \theta 1}$	186.85 ± 0.69$^{\pi 2}$
GLC5	187.94 ± 0.15	182.83 ± 5.49	179.67 ± 0.13$^{\theta 1}$	175.38 ± 0.86$^{\theta 1}$	176.56 ± 0.73$^{\pi 2 \theta 1}$	183.70 ± 0.88$^{\pi 3}$
NC	196.00 ± 0.46	197.09 ± 3.21	199.54 ± 1.19$^{\pi 1}$	200.98 ± 1.20$^{\pi 2}$	201.47 ± 0.88$^{\pi 3}$	203.57 ± 0.51$^{\pi 3}$

Notes: Data are examined by employing one and two-way ANOVA preceded by *Post Hoc* Tuckey's test and provided as mean ± SEM (n = 6); matched to $^{\pi}$DC, $^{\mu}$GLC5, $^{\theta}$NC, $^{\rho}$day 0 (baseline), ^{1}P < 0.05, ^{2}P < 0.01, ^{3}P < 0.001. Abbreviations: GL, *Glinus lotoides*; GLC, glibenclamide; NC, Normal control.

A study in normoglycaemic rats revealed its hypoglycaemic effect in a dose and time dependent fashion. It is probable that the extract has glibenclamide-mimetic properties, as glibenclamide is proven to provoke insulin release while decreasing glucagon secretion, hypoglycemia thereof. These observations were concurred with the effect of the arial part of *Mollugo pentaphylla* and *Glinus oppositifolius* existed in the same family [22].

Both Crude extract and glibenclamide-treated rats demonstrated good glucose tolerance in this OGTT investigation. Confirmation of its ability to alter glucose regulation systems shows that seed extract has a potential advantage in reducing snack-related hyperglycemia and diabetic complications. These observations were consistent with the effect of the ethanolic extract of *M. pentaphylla, and G. Oppositifolius* which enables to maintain glucose homeostasis [22]. Its antihyperglycaemic effect may be ascribed to the extract's ability to defer the assimilation of glucose by suppressing pancreatic alpha-amylase, maltase, and sucrase enzymes, as well as to an increase in peripheral glucose uptake, hindrance of glycogenolysis, and gluconeogenesis [8].

TIIDM in rats developed with STZ–NA is a well-established and well-characterized model of experimental diabetes. STZ is routinely used to generate chemical-induced DM in mammals due to its faster induction rate and focused cytotoxicity on pancreatic β-cells, which replicates T1DM. Nonetheless, it was shown that when it was primed by cytoprotective NA, it produced substantial and persistent hyperglycemia mimicking TIIDM [57].

Oral administration of *G. lotoides* seed extract in anyway studied dosages dramatically improved diabetic rats' glucose homeostasis (see Table 5). The maximal fall in FBG level (71.68%) obtained with the highest dose (GL400) is consistent with previous investigations on the antihyperglycaemic properties of related species such as *Mollugo pentaphylla* [58] and *Mollugo nudicaulis* [23]. Because of their antioxidant properties, many secondary metabolites safeguard pancreatic β-cells from STZ-NA-induced attack [59]. Hypoglycaemic effect of *G. lotoides* may be attributed by its previously proven strong antioxidant effect in part. Moreover, it may mimic metformin's action by enhancing the responsiveness of hepatic insulin receptors and increasing uptake of glucose from muscles via facilitation of GLUT-1 and GLUT-4 translocation to the plasma membrane [60].

Diabetes mellitus is ascribed to its ability to induce weight loss. In the present study when disease control animals were matched to sham rats, a significant weight loss was seen ($p < 0.001$). Nevertheless, body weight lost was restored following intervention with *G. lotoides* seed extract. It could be because the seed extract contains bioactive substances that alleviate oxidative stress induced by free radicals produced by unrelenting chronic hyperglycemia. Furthermore, it may protect against protein catabolism and muscle atrophy, and osmotic diuresis [44] presumably as a result of enhanced insulin secretion and/or activity [21].

Another differentiating hallmark of diabetes mellitus pathogenesis is hyperlipidemia. In this investigation, the serum lipogram of the disease control group revealed a significant increase in circulating TGs, VLDL-c, LDL-c, and TCs, but a drop in HDL-c, relative to sham rats. Fortunately, repetitive administration of the extract to rats for 28 days tempered TCs, TGs, VLDL-c, and LDL-c levels while improving HDL-c. These noteworthy discoveries were concurred with an earlier study on the same plant, which established the plant's efficacy in preventing hypertriglyceridaemia and hypercholesterolaemia. Corroborating its efficacy in averting known treats of diabetes mellitus-related cardiac complications [21,61]. Flavonoids [48] and anthraquinones [62] detected in *G. lotoides* have been attributed to blood TG, TC, LDL-c, and VLDL-c reduction. Hence, its antidyslipidaemic effect may be due to those bioactive constituents. Its possible mechanism of hypolipidemic efficacy could be due to diminished cholesterol and fatty acid genesis by inhibition of cholesterol anabolic factors, pancreatic ChEase and PL, respectively. Apart from inhibiting dietary lipid digestion and assimilation, ChEase also hinders the conversion of anti-atherogenic HDL-c to the atherogenic LDL-c subtype [63,64]. Furthermore, it may be directly attributable to the increase in insulin status is associated with seed extract therapy.

According to a qualitative preliminary study in the current work, *G. lotoides* seed extract comprises of secondary metabolites *vz*. Alkaloids, terpenoids, phenolic compounds, anthraquinones, saponins, cardiac glycosides, and flavonoids. This qualitative result was in agreement with previous studies [16]. Furthermore, quantitative evaluation of *G. lotoides* seed extract divulges that it contains total saponins of 25.4% and flavonoids 21.3% [65]. Its antidiabetic potential may be owed to those phytochemicals which have been revealed to ameliorate hyperglycaemia and hyperlipidaemia [8].

In difference owing to its nutritive value [13], *G. lotoides* may be used as an ingredient of nutraceuticals to avert obesity, TIIDM-related dyslipidemia and anti-decrepitude thereof, as demonstrated by its inhibitory potential of lipolytic enzymes in our study and its previously demonstrated *anti*-cholesterolemic efficacy [21]. Furthermore, it is equipped with hepatoprotective [21], gastroprotective [66], wound healing (epithelization or cell regeneration) [15], free radical scavenging, and antioxidative [21] features. Oxidative stress has been tied to the genesis of DM and its consequences [67]. Ultimately, the probable way by which *G. lotoides* brings about its antidiabetic effect may be by ameliorating insulin resistance and insufficiency through increasing pancreatic β-cell regeneration and attenuating β-cell slaying caused by chronic hyperglycaemia induced oxidant burden. Those finding was also in agreement with other aforesaid studies [68,69].

5. Conclusion

Altogether, these findings indicate that *G. lotoides* has dual inhibitory activity against carbohydrate and lipid absorption and ameliorates diabetic hyperglycemia and hyperlipidemia, rendering it a promising nutraceutical and novel candidate for alternative management of type-II diabetes. Presumptive hypoglycemic use of *G. lotoides* has been verified.

Limitations

- This was a preliminary study that was designed to validate only the antidiabetic potential of the crude extracts of *G. lotoides.*
- A direct extrapolation of this study 's discoveries to humans would be hindered by species heterogeneity.
- Pharmacological STZ-NA-induced TII DM model does not allow to replicate TII DM caused by insulin receptor resistance.

Recommendations

Although this study is preliminary, we urge pioneering scholars to:

— Isolate the responsible bioactive principle for further mechanistic studies.
— Evaluate it in a high-fat diet-STZ-induced TIIDM model to validate its efficacy in averting insulin resistance.
— Histopathological analysis is worthwhile to reveal the regeneration or status of pancreatic β-cell density.

Data access

Upon legitimate inquiry, the study's principal investigator will make any evidence used to corroborate its findings available to anyone.

Ethical approval

The scientific and ethical review board at Debre Tabor University's College of Health Sciences authorised this project on December 3, 2021, through reference code SOP39/173/22.

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CRediT authorship contribution statement

Woretaw Sisay: Conceptualization, Formal analysis, Funding acquisition, Investigation, Methodology, Data curation, Project administration, Resources, Validation, Visualization, Writing – original draft, Writing – review & editing. **Yared Andargie:** Methodology, Project administration, Investigation, Data curation, Resources. **Mulugeta Molla:** Conceptualization, Investigation, Methodology, Project administration, Formal analysis, Writing – original draft. **Getaye Tessema:** Conceptualization, Methodology, Investigation, Writing – review & editing. **Pradeep Singh:** Conceptualization, Methodology, Supervision, Resources, Investigation.

Declaration of competing interest

The authors have no potential conflicts of interests that affects the research objective to declare.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.metop.2022.100189.

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