Clinical Phytoscience

ORIGINAL CONTRIBUTION

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Antidiabetic properties of an Ethanolic leaf extract of *Launaea taraxacifolia* (Willd.) Amin ex C. Jeffrey (Asteraceae) in SD rats

De-Graft Gyamfi Adjei[1], Nana Ama Mireku-Gyimah[2], Joseph Adusei Sarkodie[2*], Benoit Banga Nguessan[1], Emmanuel Kodua[3], Jonathan Komla Amedior[1], Irene Asare Lartey[1], Ofosua Adi-Dako[4], Isaac Julius Asiedu-Gyekye[1] and Alexander Kwadwo Nyarko[1]

Abstract

Background: Diabetes Mellitus (DM) is a major health problem, with a global prevalence of 9.3%, 4.7% in Africa, and 2.5% in Ghana. Despite the availability of the classic anti-diabetic medications, many patients have not benefited from them due to their poor glycemic controls, high costs, inability to halt disease progression, and untoward side effects. Some patients thus resort to plant-based medicines such as those obtained from *L. taraxacifolia* etc., which have little empirical evidence of efficacy. Therefore, this study investigated the possible antidiabetic effects of the leaf extracts of *L. taraxacifolia* and some potential mechanistic targets involved.

Methodology: Ethanolic extract of *L. taraxacifolia* leaves (LTE) was screened for phytoconstituents and tested for blood glucose-lowering properties in both non-diabetic and streptozotocin-nicotinamide-induced (STZ-NAD) type-2 model diabetic rats for 4 weeks at doses of 500 mg/kg, 750 mg/kg, and 1000 mg/kg. Metformin (200 mg/kg) and glibenclamide (5 mg/kg) were used as positive controls. Effects of LTE on blood glucose, serum lipids, hepatic gluconeogenesis, intestinal glucose absorption, liver enzymes, oral glucose tolerance, and rat organ weights were all studied. Pancreatic Islet histology was also conducted.

Results: The ethanolic extract of *L. taraxacifolia* leaves reduced fasting blood glucose levels and suppressed hyperglycemia during the oral glucose tolerance test. In addition, hepatic gluconeogenesis and intestinal glucose absorption were inhibited. The extract lowered levels of liver enzymes, total cholesterol, and LDL cholesterol while increasing HDL cholesterol levels. Again, it reversed STZ-induced weight changes to the liver, kidneys, and pancreas as well as restored the morphology of the pancreatic Islet of Langerhans.

Conclusion: *Launaea taraxacifolia* leaves extract (LTE) possesses anti-diabetic constituents and has the potential to repair diabetes-induced damages to the liver, kidney, and pancreatic Islets in SD rats.

Keywords: Diabetes, *Launaea taraxacifolia*, Streptozotocin-nicotinamide, Gluconeogenesis, Dyslipidemia, Fasting glucose

Background

Diabetes mellitus (DM) is an autoimmune, hormonal, and metabolic disease [1]. There are two main types: Type 1 (insulin dependent) and Type 2 (non - insulin dependent), the most common form being the latter [2]. Type 2 diabetes, which affects 90–95% of diabetics, is characterized by inadequate glucose accessibility and utilization

*Correspondence: jasarkodie@ug.edu.gh

[2] Department of Pharmacognosy and Herbal Medicine, University of Ghana School of Pharmacy, Legon, Accra, Ghana
Full list of author information is available at the end of the article

by target tissues due to β-cell dysfunction and insulin receptor resistance [3]. Poor management can result in retinopathies, nephropathies, neuropathies, cardiovascular challenges, and mortalities [4]. Non-conventional complications like dementia, hepatic abnormalities, cancers, and adverse geriatric conditions have recently been linked to poorly controlled diabetes [5]. Diabetes has become a public health problem globally. The WHO has warned that death due to diabetes in Africa will increase by 40% in the next ten years [3, 6]. However, the direct costs in case management in many developing countries is dominated by the relatively high costs of insulin and oral hypoglycaemic medicines at public health centres [7]. The populace rather depend on less expensive and readily available medicinal plant products for the management of diabetes and many other disease conditions which afflict them. Generally it is estimated that 70–80% of the world's population use medicinal plants as the first line of treatment in their Primary Health Care system [6]. These medicinal plants are frequently considered to be less toxic and free from side effects than synthetic drugs [8–12]. However, most of these medicinal plants used in the treatment of diseases in these developing countries have no scientific documented information. It is therefore imperative to investigate such medicinal plants in order to provide enough scientific data which may form the basis for the formulation of herbal hypoglycaemic products for clinical use after their safety has been ascertained.

African lettuce (*Launaea taraxacifolia*) is native to Africa, America, Europe, and Asia [13]. It is an annual herb that grows up to about 1 m tall and has a single glabrous stem that branches at the plant's distal end. The leaves are linked to the aerial stem without a stalk. When studied microscopically, *L. taraxacifolia* is the only Asteraceae species without trichomes [14]. In many West African households, leaves of *L. taraxacifolia* are consumed raw in salads or cooked in stews and soups [15]. The leaves have also been used in traditional medicine to strengthen children's bones [13] and to treat excessive blood pressure [16]. Adinortey and colleagues [12] revealed that *L. taraxacifolia* contains useful amounts of minerals and phytochemicals such as iron, magnesium, potassium, calcium, flavonoids, terpenoids, tannins, and saponins among others. It has been shown to possess antioxidant [17], anti-inflammatory [18], antimicrobial [18], anticancer [19], neuro-nephroprotective [20], and cardioprotective properties [21, 22]. A few investigators have reported on the blood glucose studies of *L. taraxacifolia* [16, 23, 24]. These studies generally used lower doses, and the reports are inconsistent. However, doses up to 5 g/kg are reportedly very safe and tolerable, with zero animal mortalities recorded in those studies [25, 26]. Due to the paucity and inconsistencies of such information on *L. taraxacifolia*, it was thus necessary to use higher doses to assess the antidiabetic properties of the leaf extract on glycemic control [27] and to further investigate the effects of the extract on pancreatic morphology [28], gluconeogenesis [29], intestinal glucose absorption [30], bodyweight [31] and dyslipidemia [32] that affect the status of diabetes. Therefore, the current study considered the potential antidiabetic activities of *L. taraxacifolia* and the possible mechanistic targets involved.

Materials and methods

Chemicals and reagents

All chemicals and reagents used in this study were of high pharmaceutical and analytical grades. Streptozotocin, nicotinamide, sodium citrate dihydrate, and citric acid powder were obtained from Sigma-Aldrich (Germany). The standard rat chow (70.23% carbohydrate, 22.77% protein, and 7.00% fat) was purchased from Agro-Food Company Limited (Ghana).

Metformin (glucophage®, Merck Pharmaceuticals, Germany), glibenclamide (daonil®, Sanofi Aventis pharmaceuticals, France), biochemical autoanalyzer (Shenzhen Mindray biochemical electronics Co. Ltd., China), and OneTouch select plus® glucometer & strips (LifeScan Inc., Switzerland), etc. were all used in this study.

Plant collection, extract preparation, and phytochemical screening

L. taraxacifolia leaves, collected from the University of Ghana Botanical Gardens in August 2019, were authenticated by Dr. (Mrs.) Cindy Kitcher at the Department of Pharmacognosy & Herbal Medicine, where a voucher specimen (PH/LT/2019/001) was deposited. The leaves were air-dried at room temperature for 21 days to achieve a constant weight and then pulverized into a coarse powder. Using cold maceration techniques, a total of 500 g was weighed into 5 l of 70% v/v ethanol in a stoppered container for 72 hours with periodic shaking [11]. The supernatant was decanted. The residue (marc) was then squeezed to remove all the supernatant and double filtered. The ethanol was evaporated using a rotary evaporator at 40 °C, and the aqueous leaf extract was concentrated and freeze-dried [33] to yield 8.5% w/w residue. It was kept desiccated until required [17]. Phytochemical analyses were performed using standard protocols [34, 35].

Animal husbandry

One hundred male Sprague-Dawley rats (10 weeks old) weighing between 150 g and 200 g were used after approval by the University of Ghana Institutional Animal Care and Use Committee (number UG-IACUC

005/19–20). The rats were housed in stainless-steel cages in a room at a temperature of 22 ± 2 °C under a constant 12-hour light/12-hour dark cycle [36] at the Department of Animal Experimentation of the Noguchi Memorial Institute for Medical Research (NMIMR). All animals were fed with standard rat chow and had unlimited access to water. The rats were allowed to acclimatize for one (1) week before the commencement of the various studies.

Induction of diabetes and treatment groups

In rats fasted for 12 hours, type 2 diabetes was induced by intraperitoneal administration of nicotinamide (NAD, 120 mg/kg) in saline, followed 15 minutes later by a single intraperitoneal administration of streptozotocin (STZ, 55 mg/kg), freshly prepared in citrate buffer (0.1 M at pH 4.5) [11]. To avoid hypoglycemic shock, each rat was given 5% glucose overnight on the first day [36]. Fasting blood glucose (FBG) levels were recorded using a standard glucometer (OneTouch Select Plus®) on blood samples collected from each rat's tail vein [24, 36], 72 hours after diabetes induction.

Rats with FBG levels of more than 11.1 mmol/l [11] were randomly assigned to one of six treatment groups (5 rats per group) and given the following treatments for 28 days: Group 1 was the diabetic control (water/vehicle); Group 2 was the diabetic positive control 1 (metformin, 200 mg/kg); Group 3 was the diabetic positive control 2 (glibenclamide, 5 mg/kg); Group 4 was the diabetic - LTE low dose (500 mg/kg); Group 5 was the diabetic -LTE medium dose (750 mg/kg); and Group 6 was the diabetic -LTE high dose (1000 mg/kg). A 7th group comprising non-diabetic rats that were not given STZ-NAD was set as overall control and administered water over the experimental period.

In another set of experiments for studies on intestinal glucose absorption, non-diabetic normoglycemic rats were placed in similar treatment groups as above. FBG levels were recorded on blood samples collected from the tail veins before commencement and on weekly basis during the 4-week treatment period, as previously described. All the extracts and controls were administered via oral gavage.

Measurement of fasting blood glucose (FBG) levels

Fasting blood samples were taken from the tail veins of all the rats before treatment, weekly for 4 weeks, and at termination, as described by Sarkodie et al. [11]. Blood samples were analyzed for glucose levels using a One-Touch Select Plus® glucometer. Before sample collection, the rats fasted for 8-hours but were given water ad libitum. Each rat was controlled in a rat restrainer and the tail was massaged to allow enough blood at the tip of the tail. The end of the tail was cleaned with an alcohol swab and pricked using a lancet. The first drop of blood was discarded, the tail cleaned, and the subsequent drop was collected with a strip inserted into a glucometer. Each reading was then recorded accordingly.

Two (2)-hour Oral glucose tolerance test (OGTT)

A 2-hour OGTT was conducted on the diabetic rats following the 4-week treatments [36]. Each rat was administered a glucose dose of 2.5 g/kg following an 8-hour fast. Blood samples were obtained at times 0, 30, 60, 90, and 120 minutes for glucose determination using a OneTouch Select Plus® glucometer and strips [24].

Determination of serum liver enzymes and lipid profile

At the termination of the experiments, rats were euthanized and their blood samples were collected via cardiac puncture. The whole-blood samples were allowed to clot and centrifuged at 10,000 rpm for 10-minutes. Sera, obtained from blood samples from the groups, were stored at − 80 °C and later analyzed for the levels of Total Cholesterol (TC), High-Density Lipoprotein Cholesterol (HDL-C), Low-Density Lipoprotein Cholesterol (LDL-C), and Triglycerides (TG), using a biochemical autoanalyzer according to the manufacturer's manual [24, 37].

Similarly, sera from the treated rats were processed for the determination of levels of Aspartate Aminotransferase (AST), Alanine Aminotransferase (ALT), and Alkaline Phosphatase (ALP) [24, 36].

Determination of rat body weights and organ-to-body weight ratios

The body weight of each rat was recorded before the commencement of the experiments, weekly, and at the end of the treatments. Target organs such as the brain, pancreas, liver, kidneys, heart, and lungs were also removed, weighed, and paired for determination of organ-to-body weight ratios at the termination of the study [38].

Preparation of pancreas for histology of islet cells

The pancreas of each rat was extracted, washed in normal saline, blotted with filter paper, fixed in 10% buffered formalin, and processed for histological analysis as described by Lai and Lu [39]. They were stained with hematoxylin and counter-stained with eosin. The slides were then mounted and examined under a light microscope at a magnification of × 40.

Measurement of hepatic gluconeogenesis

First, a time-course study was conducted to determine the optimal time for glucose synthesis ($\mathbf{T_{max}}$). The livers of five non-diabetic rats were harvested and utilized to study hepatic glucose production, as described by

Andrade-Cetto and Vasquez [40]. The livers of rats that had been fasted overnight and euthanized were quickly dissected and rinsed in ice-cold saline. Slices (2 mm) of liver were prepared and gently shaken in saline for one (1) minute to wash off any preformed glucose. Slices weighing 200–300 mg (wet weight) were quickly incubated with 0.01 M pyruvate in 4 ml glucose-free Krebs-Henseleit Buffer (KHB) at 37 °C. The glucose content of the incubation medium was recorded every 30 minutes for 180 minutes. Slices were removed after 180 minutes, blotted to remove the excess medium, weighed, and used to normalize the glucose produced by the slices. Following the determination of the $\mathbf{T_{max}}$ using the non-diabetic rats, livers harvested from the diabetic rats previously treated with the different concentrations of LTE were processed for determination of hepatic gluconeogenesis at the $\mathbf{T_{max}}$, as described.

Measurement of intestinal glucose absorption in normoglycemic rats

Effects of LTE on intestinal glucose absorption were assessed as described by Hannan [33]. First, five (5) non-diabetic rats were used for a time-course study to identify the time for maximal glucose absorption ($\mathbf{T_{max}}$) in the intestines. A catheter was inserted into the pylorus and another into the ileum of each rat that had been fasted for 24 hours and anesthetized with sodium pentobarbital (50 mg/kg). Perfusates were collected from the ileal catheter after passing a glucose solution (of 7 mmol/L in Krebs-Henseleit Buffer) through the pylorus at a rate of 0.5 ml/minute at 37 °C. The $\mathbf{T_{max}}$ for glucose absorption was determined after sampling perfusates at time intervals of 10 minutes for 60 minutes [33]. Following that, intestinal glucose absorption was assessed in non-diabetic normoglycemic rats already treated with the different dose levels of LTE for 4-weeks.

An alternative experiment was conducted using 5 cm intestinal segments ligated and loaded with glucose as originally described by Wilson and Wiseman [41] with modifications [42–44]. Intestinal segments were ligated at both ends and loaded with 0.3 mL (7 mmol/L) of glucose (in KHB) in 25 mL Erlenmeyer flasks containing 5 mL of glucose-free KHB and oxygenated with carbogen (5% CO_2 and 95% O_2). $\mathbf{T_{max}}$ was determined by collecting samples of the contents of the intestinal sacs at 30-minute intervals and analyzing them. Following that, intestinal segments loaded with both glucose and the different concentrations of LTE were incubated at the $\mathbf{T_{max}}$.

Statistical analyses

Statistical analyses were performed using GraphPad Prism Version 8.0.2 for windows. Data for the various treatments were expressed as Mean ± Standard Error of Mean (SEM). Differences in means were analyzed with Analysis of Variance (ANOVA) followed by Dunnett's multiple analyses post hoc test or Sidak's multiple analyses test. Differences were considered statistically significant at $p < 0.05$. Photomicrographs (× 40 magnifications) were used in the histological analyses.

Results

Phytochemical screening of LTE

The results indicate that alkaloids, saponins, flavonoids, phenols, tannins, phytosterols, diterpenes, and glycosides were detected in the ethanolic leaf extract of *L. taraxacifolia*. Table 1

Induction of diabetes

Fasting blood glucose levels increased 4-fold, from about 5 mmol/l pre-treatment levels to about 22 mmol/l after STZ-NAD administration. The increase was statistically significant ($p < 0.05$). Figure 1.

Effects of LTE on fasting blood glucose

A general lowering of fasting blood glucose was observed in all the treated animals during the study period. There was no clear dose-related LTE-mediated glucose reduction as the glucose-lowering effect at 750 mg/kg was higher than both 500 mg/kg and 1000 mg/kg. Metformin and glibenclamide showed relatively greater FBG reduction than observed in the LTE-treated groups.

Figure 2B corroborated the findings in 2A. LTE 750 mg/kg had the highest blood-glucose-lowering potential, followed closely by LTE 1000 mg/kg and then LTE 500 mg/kg [albeit statistically insignificant ($p > 0.05$)].

Findings on Oral glucose tolerance test

The treatment groups exhibited a substantial lowering of blood glucose levels during the glucose tolerance test (3A). The medium dose extract (750 mg/kg) and metformin suppressed the elevation of blood glucose in the OGTT more than all the other treatments. The AUCs

Table 1 Phytochemical analyses of LTE

Secondary metabolite	Observation	Inference
Alkaloids	Red ppt	+
Saponins	Foam lasted over 10 min	+
Glycosides (General)	Reddish-brown ppt	+
Tannins	Yellowish coloration	+
Phytosterols	Yellow ppt	+
Diterpenes	Emerald green coloration	+
Flavonoids	Intense yellow coloration	+
Phenols	Bluish black coloration	+

KEY: "ppt" means precipitate, and "+" means detected

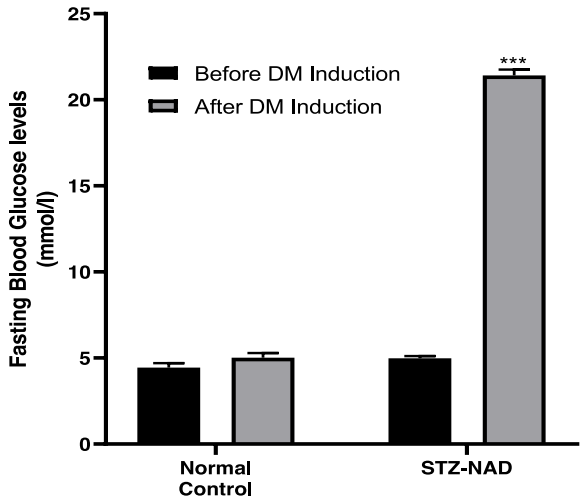

Fig. 1 Fasting Blood Glucose levels (mmol/l) of SD rats before and after induction of diabetes. Values were expressed as mean ± SEM ($n = 30$). Data were analyzed using 2-way ANOVA followed by Sidak's multiple comparisons test. * represents statistical significance at $p < 0.05$ vs. FBG before DM Induction

presented in Fig. 3B corroborate the observation in Fig. 3A.

Results of LTE on serum lipids

Serum lipid indices were elevated upon STZ-NAD administration. Total cholesterol (4A) and triglyceride (4B) levels significantly dropped upon treatment with LTE. Similarly, low-density lipoprotein levels (4D) were lowered in all treatment groups, except LTE medium dose and glibenclamide-treated groups. High-density lipoprotein levels (4C) in all the treatment groups were elevated. Figure 4

Results of LTE on liver enzymes

Figures 5A-C show that the liver enzymes were elevated following the induction of diabetes. However, treatments with LTE significantly lowered the enzymes. All the LTE dose levels and the positive controls showed similar reductions in the levels of the liver enzymes.

Effects of LTE on rat body weight

Diabetes induction resulted in massive weight loss in the rats as compared to the non-diabetic rats (normal controls). Treatments appeared to reverse the observed weight loss as compared to the diabetic untreated (negative control) group. However, none was statistically significant except for the group treated with glibenclamide. Figure 6.

Findings of LTE on organ-to-body weight ratios

Treatment-related changes in organ-to-body weight ratios for the pancreas, liver, kidney, brain, heart, and lung are presented in Table 2. First, compared to the non-diabetic rats, induction of diabetes resulted in a significant reduction in pancreas weight. Liver and kidney weight on the other hand were increased. There were no substantial changes in brain, heart, and lung weights.

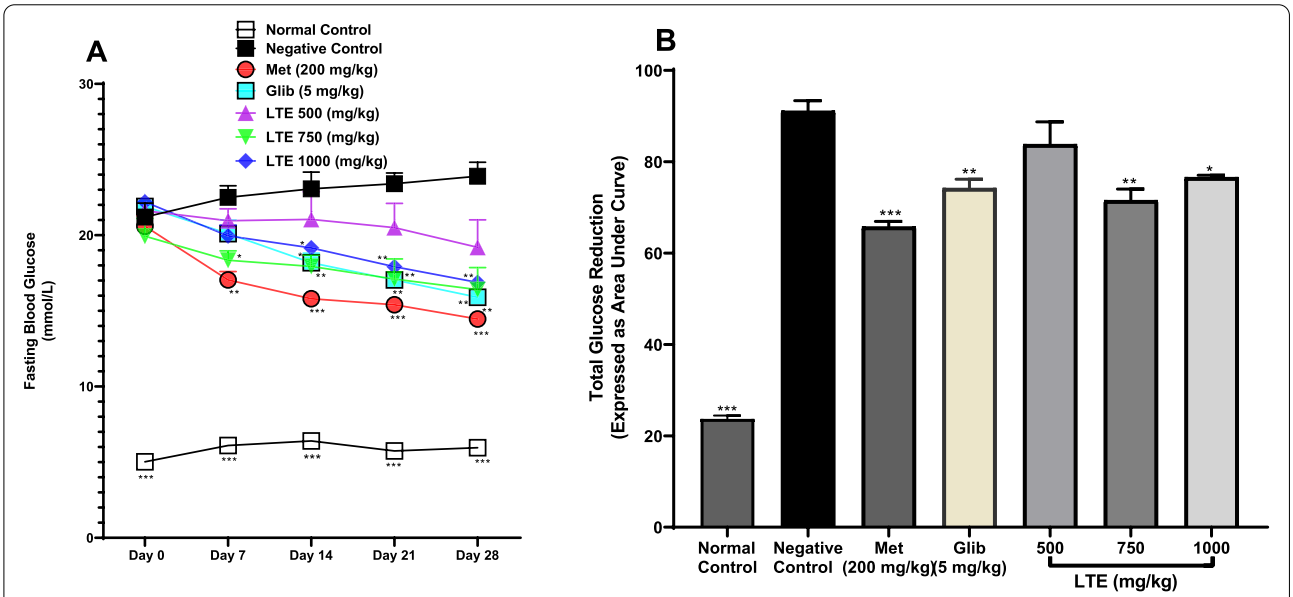

Fig. 2 Effects of LTE on Fasting Blood Glucose levels during the 28-day treatments (**A**) vs. Total AUCs of the effects of the different treatments (**B**). Values were expressed as mean ± SEM ($n = 5$). Data were analyzed using two-way ANOVA for 2A and one-way ANOVA for 2B, followed by Dunnett's multiple comparisons test. * represents statistical significance at $p < 0.05$ vs. the negative control group

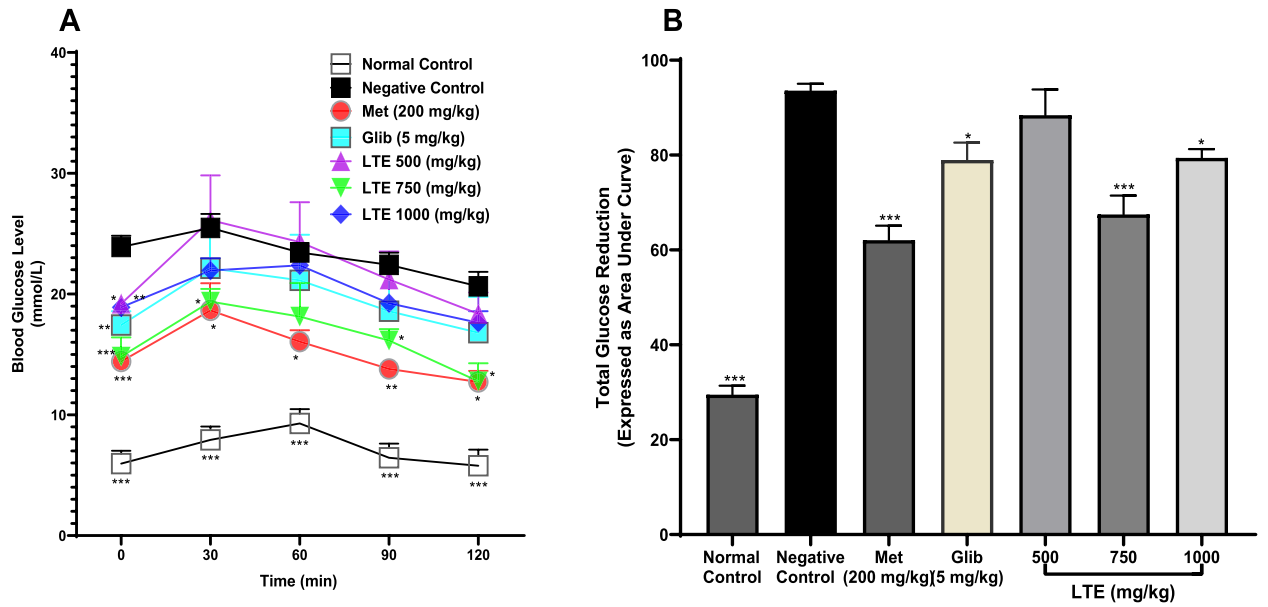

Fig. 3 A 2-hour OGTT of the various treatments (**A**) vs. Total AUCs of the 2-hour OGTT study (**B**). All values were expressed as mean ± SEM ($n = 5$). Data were analyzed using two-way ANOVA for 3A and one-way ANOVA for 3B, followed by Dunnett's multiple comparisons test. * represents statistical significance at $p < 0.05$ vs. the negative control group

Compared to the untreated diabetic group, the pancreas-to-body weight ratio significantly improved in all the LTE doses levels. Same observations were made for the groups treated with metformin and glibenclamide. Liver-to-body weight ratios were lowered in all the LTE, metformin and glibenclamide treated groups. Except for the glibenclamide treated group, kidney-to-body weight ratios were also lowered in the LTE and metformin-treated groups. None of the treatments altered the organ-to-body weight ratios of the brain, heart, and lung.

Modulation of pancreatic islet cells morphology

Figure 7 presents photomicrographs showing morphological changes in the pancreas following induction of diabetes and subsequent treatments with LTE. Figure 7A shows an Islet cell from a non-diabetic control rat. The image shows a normal Islet cell morphology with the usually spherical, ellipsoid, or elongated shape of an intact pancreatic Islet of Langerhans. The figure shows a well-defined boundary with the Islet cells proportionally distributed. The β-cells (pointed with black double arrows) occupy the core of the Islets, sandwiched by the non-β-cells (α-cells, δ-cells, and PP-cells, pointed with orange-colored single arrows) which dominate most portions of the peripheries. In contrast, Fig. 7B, a photomicrograph of a pancreas from an untreated diabetic rat, exhibits distortion and pathological changes to the general architecture of the Islet of Langerhans. The boundaries are adversely affected, with a markedly reduced number of β-cells and randomly scattered non-β-cells. Figure 7C, D, and E, representing Islet cells from diabetic rats pretreated with LTE 500, 750, and 1000 mg/kg respectively, demonstrate recoveries from the damages caused by the induction of diabetes. Compared to the negative control (7B), these micrographs show regeneration and restoration into near-intact Islets, similar to the normal control (7A). Figure 7F and G from diabetic rats treated with metformin (200 mg/kg) and glibenclamide (5 mg/kg) respectively, also demonstrate extensive recoveries.

Inhibitory effects on hepatic gluconeogenesis

The time for maximum production of glucose by the liver slices (T_{max}) was found to be 120 minutes (8A1). Hepatic glucose production was significantly lowered in all the treated animals (8A). Figure 8.

Modulation of rat intestinal glucose absorption

Figure 9A represents glucose absorption by whole intestines following a 28-day pretreatment with LTE. The time for maximum absorption of glucose ($\mathbf{T_{max}}$) was noted at 30 minutes (9A1). Glucose absorption by the intestines from LTE-treated rats was significantly lowered, relative to control levels. Inhibition of absorption in rats pretreated with metformin was also statistically significant, unlike that of glibenclamide.

Figure 9B with time-course insert (9B1) is a result of LTE modulation of glucose absorption by rat intestinal

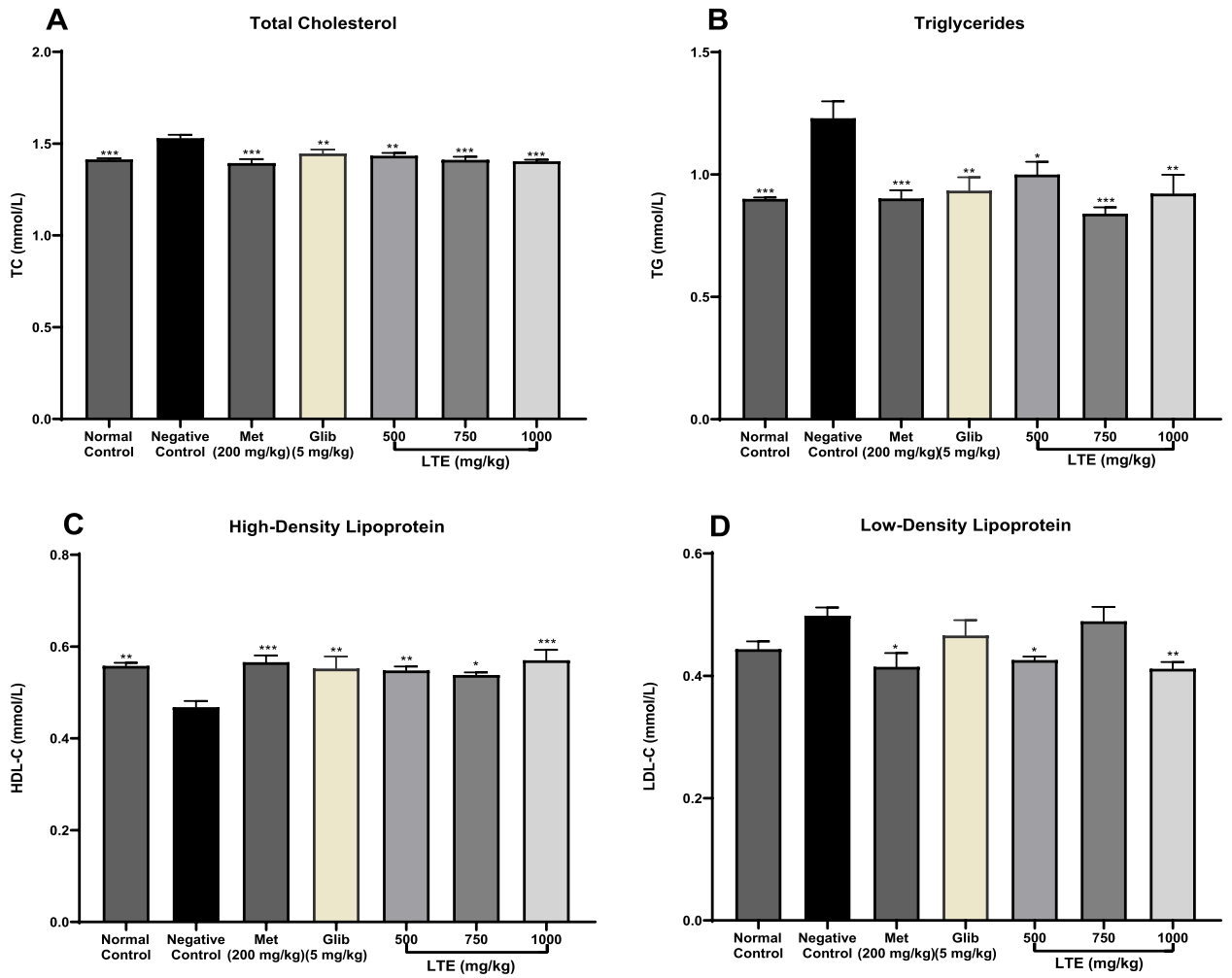

Fig. 4 Effects of LTE on serum lipid profile. Values were expressed as mean ± SEM ($n = 5$). Data were analyzed using one-way ANOVA followed by Dunnett's multiple comparisons test. * represents statistical significance at $p < 0.05$ vs. the negative control

sacs. The peak time (T_{max}) for glucose absorption ex-vivo was 60-minutes. Glucose absorption was significantly reduced by direct application of equivalents of LTE medium and high concentrations to the intestinal sacs (9B). LTE low concentration did not seem to alter glucose absorption by the intestinal sacs. Unlike glibenclamide, glucose absorption was significantly lowered for intestinal sacs containing metformin.

Discussion

Type-2 diabetes continues to pose adverse microvascular and macrovascular challenges to patients, despite the availability of several orthodox antidiabetic medicines. Patients suffer from complications and adverse side effects, eventually requiring insulin shots, due to inadequate glycemic controls with the oral hypoglycemics [6, 7]. Plant-based therapies serve as alternatives but usually with a paucity of scientific evidence of efficacy. Given the scantiness and inconsistencies in the scientific evidence on the antidiabetic properties of *L. taraxacifolia*, the STZ-NAD model of type-2 diabetes [45] was used in this study to examine the effects of *L. taraxacifolia* extract on blood glucose. This study was also designed to further investigate the potential mechanistic targets of LTE in blood glucose regulation as well as its effects at the histological level.

Phytochemicals act as plant fingerprints [35] and offer lead compounds to develop conventional medicines [29]. The phytochemical analyses revealed the presence of alkaloids, saponins, glycosides, tannins, phytosterols, diterpenes, and flavonoids. These are secondary metabolites which are believed to be responsible for the observed effects in this study. The phytochemical results matched with previous research findings [12]. Other researchers

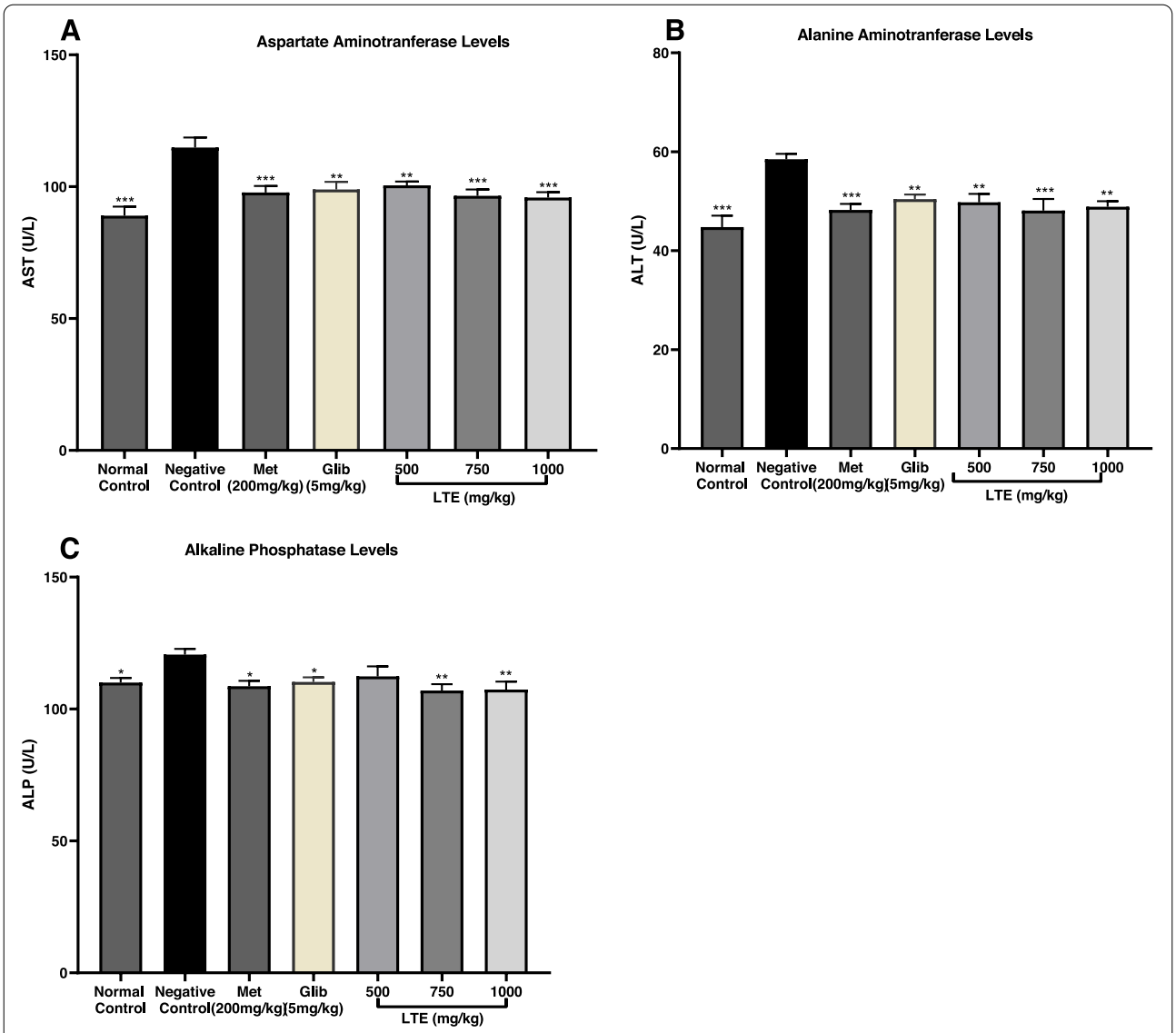

Fig. 5 Effects of LTE on some liver enzymes. Values were expressed as mean ± SEM ($n = 5$). Data were analyzed using one-way ANOVA followed by Dunnett's multiple comparisons test. * represents statistical significance at $p < 0.05$ vs. the negative control group

have further identified compounds such as trans-9-tetradecenoic acid, cis-11-hexadecenoic acid, trans-2-octadecadecen-1-ol, oleic acid, palmitic acid, margaric acid, and stearic acid in the secondary metabolites of LTE using GC-MS analysis [16]. Catechin, caffeic acid, ellagic acid, quercetin and rutin have also been identified in LTE [46].

In the present study, the administration of STZ-NAD significantly increased plasma glucose levels sharply from 5 mmol/l to about 22 mmol/l. Streptozotocin, a known cytotoxic antibiotic with diabetogenic properties, is a glucose derivative that is selectively transported to pancreatic β-cells by GLUT-2 transporters [45]. In addition to alkylating and fragmenting DNA, STZ also generates reactive oxygen species (ROS) and nitric oxide and suppresses the activity of the enzyme aconitase [28]. On the other hand, Nicotinamide is a water-soluble B-vitamin with antioxidant and neuroprotective effects that offers partial protection to β-cells [28]. STZ-NAD is thus widely accepted as an experimental model of type-2 diabetes because it induces a near-60% decline in β-cell functions, accompanied by moderately high blood glucose levels [45].

A general reduction in blood glucose was noted following the administration of the different doses of LTE. The findings also revealed that LTE possesses

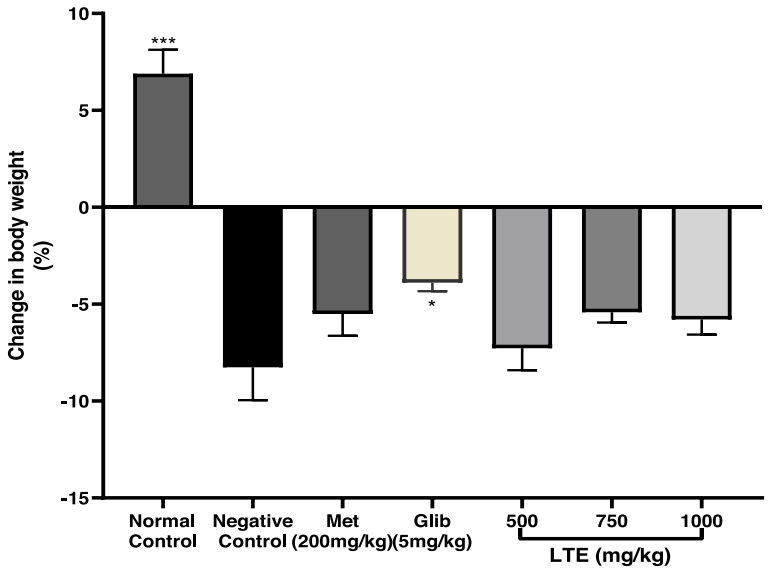

Fig. 6 Effects of LTE on rat body weight. Values were expressed as mean ± SEM ($n = 5$). Data were analyzed using one-way ANOVA followed by Dunnett's multiple comparisons test. * represents statistical significance at $p < 0.05$ vs. the negative control

Table 2 Organ-to-body weight ratio (%)

Groups	Pancreas	Liver	Kidney	Brain	Heart	Lung
Normal Control	0.39 ± 0.01**	3.93 ± 0.20*	0.71 ± 0.02***	0.65 ± 0.01	0.48 ± 0.04	0.79 ± 0.03
Negative Control	0.24 ± 0.01	4.49 ± 0.35	0.98 ± 0.07	0.70 ± 0.08	0.48 ± 0.04	0.80 ± 0.06
LTE 500 (mg/kg)	0.37 ± 0.02*	3.93 ± 0.10*	0.80 ± 0.04*	0.76 ± 0.04	0.49 ± 0.03	0.80 ± 0.07
LTE 750 (mg/kg)	0.39 ± 0.01**	3.70 ± 0.10**	0.78 ± 0.02**	0.88 ± 0.04	0.62 ± 0.03	0.83 ± 0.03
LTE 1000 (mg/kg)	0.39 ± 0.02**	3.73 ± 0.12**	0.77 ± 0.02**	0.76 ± 0.03	0.44 ± 0.03	0.80 ± 0.05
Metformin (200 mg/kg)	0.40 ± 0.05**	3.79 ± 0.06**	0.77 ± 0.02**	0.69 ± 0.03	0.54 ± 0.04	0.82 ± 0.05
Glibenclamide(5 mg/kg)	0.43 ± 0.04***	4.2 ± 0.16*	0.89 ± 0.05	0.71 ± 0.03	0.43 ± 0.05	0.80 ± 0.12

KEY: Values in the table represent the means ± SEM ($n = 5$). Data from each column (organ) were analyzed using one-way ANOVA followed by Dunnett's multiple comparisons test. * represents statistical significance vs. the negative control group at $p^{\circ}0.05$

anti-hyperglycemic potentials, as observed in the oral glucose tolerance test. These findings supported that of Gbadamosi et al. [47], who reported on the glucose-lowering and ameliorative potentials of some ethanolic fractions of LTE against diabetes complications. The authors attributed these effects to the extract's ability to activate antioxidant enzymes. Again, using relatively lower doses of LTE in type-1 diabetic rats, Kuyoro et al. [24] noticed some anti-diabetic effects, but only after 2 weeks of treatment. However, Isehunwa et al. [23] and Koukoui et al. [16] found no antihyperglycemic effects with LTE treatment, using non-obese non-diabetic rats in a similar blood glucose study.

Anti-diabetic medications regulate blood glucose levels through various mechanisms [31]. The reduction in blood glucose levels seen in the rats given LTE might have happened via a single or multiple processes, including facilitation of insulin secretion, obstruction of intestinal glucose absorption, and inhibition of hepatic gluconeogenesis, among others. Several investigators have reported that certain plant medicines containing polyphenols improve insulin release [9, 11, 46]. As a result, the polyphenol phytochemicals in LTE, like glibenclamide (a long-acting sulfonylurea and an insulin secretagogue), may have worked on the residual β-cells to release insulin, facilitating glucose absorption [48]. Again, LTE and metformin were proven to lower fasting blood glucose levels. LTE phytoconstituents like flavonoids and tannins, as reported in other studies [9], may also have functioned similarly to metformin to lower glycemia by boosting peripheral glucose utilization [49].

Diabetes and cardiovascular diseases are strongly linked to dyslipidemia [27]. Other studies have found that type-2 diabetics have a high prevalence of abnormalities in plasma lipids such as total cholesterol (TC), triglycerides (TG), high-density lipoprotein cholesterols (HDL-C),

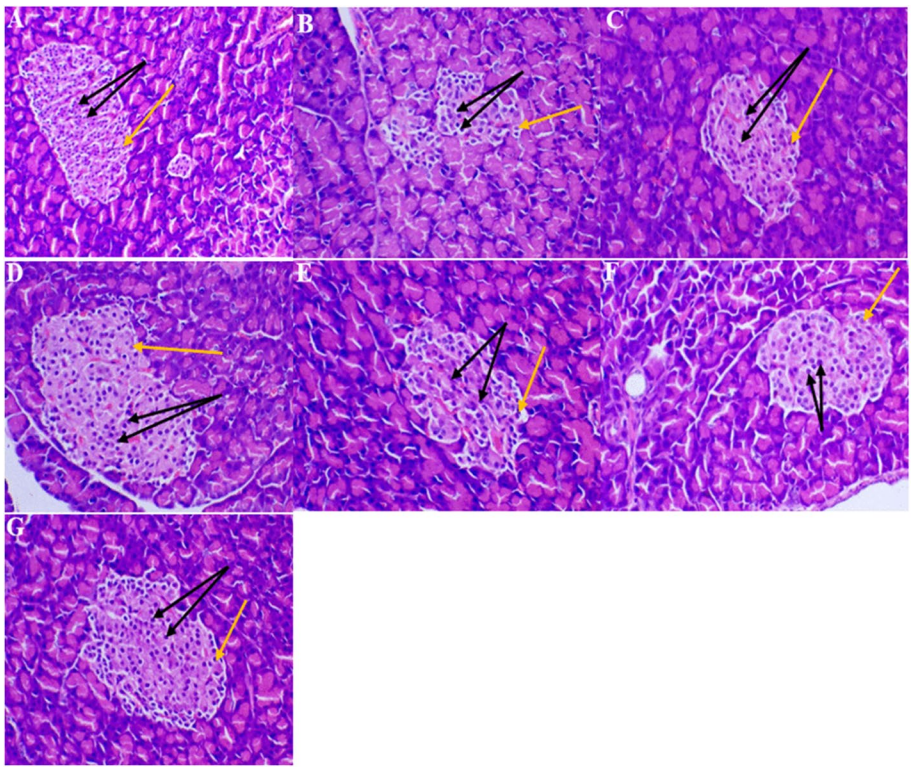

Fig. 7 Hematoxylin and Eosin (H&E) Photomicrographs (× 40 magnification) representing pancreatic Islet cells from the various treatment groups. The double-arrowed lines point to the β-cells while the single-arrowed lines point to the non-β cells

and low-density lipoprotein cholesterols (LDL-C) [50]. Alterations in serum lipids were noted following diabetes induction, which is consistent with earlier studies that diabetes promotes dyslipidemia [27]. The pathogenesis of diabetes has been strongly linked to the production of free fatty acids and low-grade inflammatory reactions as a result of dyslipidemia [51]. The improvements in lipid indices seen in the LTE-treated groups are comparable to what has previously been reported by Koukoui et al. [17].

Diabetes-related hepatic anomalies such as fatty liver disease, hepatic cirrhosis, and hepatocellular carcinomas [52] as well as STZ-induced liver damages [53] manifest as elevations in levels of liver enzymes such as AST, ALT, and ALP [52, 53]. These increments, as noted in this study, were attenuated with LTE administration. In addition, unlike the liver and kidney, the pancreas sizes shrank in the diabetic rats as has been found in certain people with type-2 diabetes [54]. Streptozotocin (STZ) is known to cause inflammatory reactions, lipid peroxidation, and severe antioxidant depletion in the Islet of Langerhans, which leads to apoptosis and necrosis [55, 56]. These findings are congruent with those shown in the photomicrographs of the pancreas from untreated diabetic rats, which showed severe pathological alterations in the Islet of Langerhans. LTE was able to moderate these developments. The observed beneficial qualities may be attributed to its antioxidant properties [17]. This is also consistent with recent findings that reducing oxidative stress enhances β-cell function regeneration [57].

Substantial weight loss was observed in all the diabetic rats. Even though weight loss was reversed in all treatment groups, the reversal was statistically significant only in diabetic rats fed with glibenclamide. This finding is consistent with previous studies, as glibenclamide has been linked to weight gain [48]. The poor reversal of weight loss noticed in diabetic rats treated with the extracts may also be a favorable feature of LTE because more than 80% of people with type-2 diabetes are already overweight or obese and require weight loss as part of their treatment plan [58]. Organ enlargements (hypertrophies) were noted in the livers and kidneys of the diabetic rats relative to their body weights. Hepatomegaly has been linked to about 70% of improperly treated type-2 diabetes cases [52]. Similarly, renal hypertrophy, which may be irreversible, is linked to diabetes [59]. The reduction in liver-to-body weight ratio and kidney-to-body weight ratio in the LTE treated groups suggests that the extract may be useful in the management of

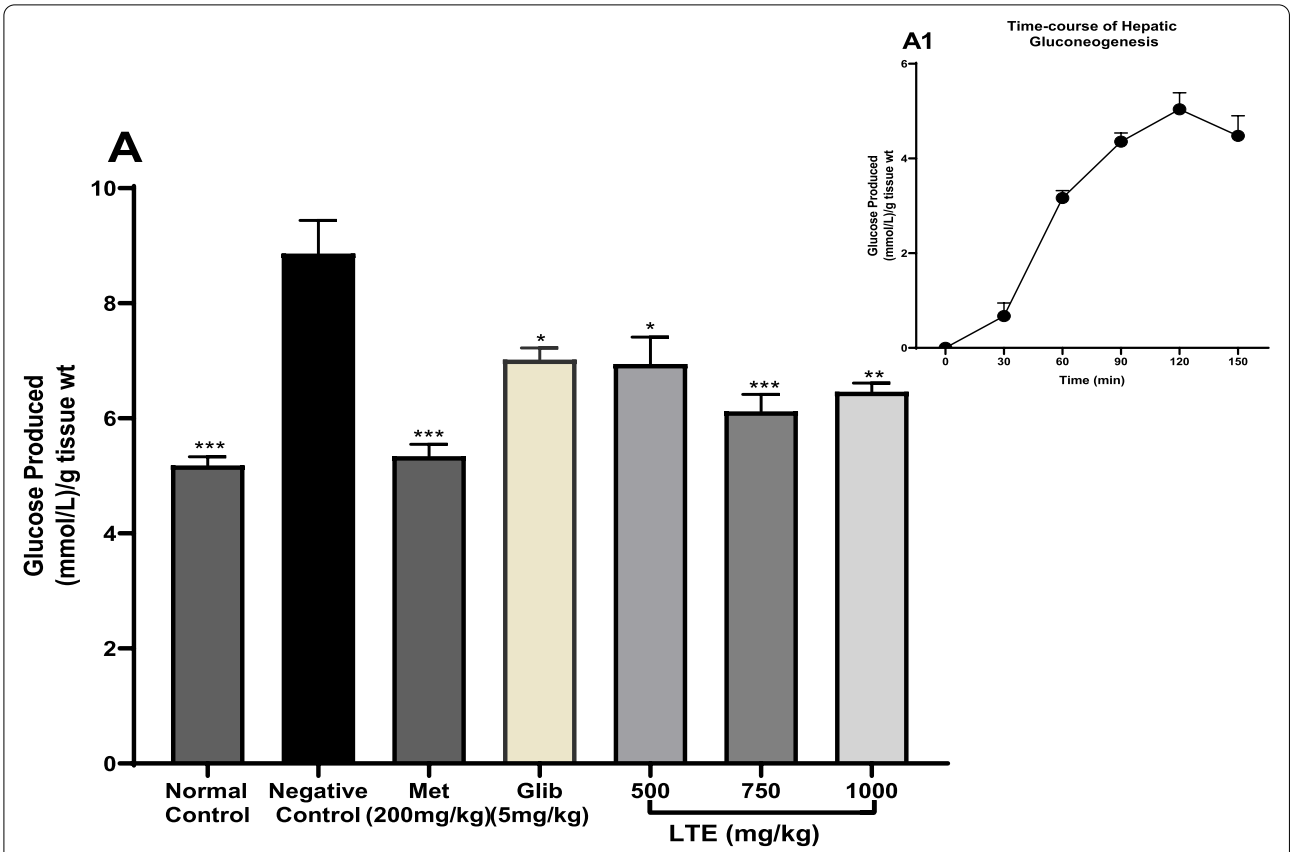

Fig. 8 Time-course of hepatic glucose production (8A1) vs. gluconeogenesis at time 120 minutes (**A**). Values were expressed as mean ± SEM ($n = 5$). Data were analyzed using one-way ANOVA followed by Dunnett's multiple comparisons test. * represents statistical significance at $p < 0.05$ vs. the negative control group

complications associated with diabetes, such as hepatomegaly and renal hypertrophy [52, 59].

Hepatic gluconeogenesis, which is increased in type-2 diabetes, tends to aggravate the disease [14]. Gluconeogenesis is mediated by enzymes such as pyruvate carboxylase, phosphoenolpyruvate (PEP) carboxykinase, fructose-1,6-bisphosphatase, and glucose-6-phosphatase [60]. The extracts decreased hepatic glucose synthesis, although in a concentration-independent manner. Phytochemicals like glycosides and triterpenes [9] in LTE, like metformin, may have activated the AMP Kinase, causing a switch from lipogenesis and gluconeogenesis to lipid oxidation and glucose absorption by muscle and liver cells [61]. In addition, LTE, like metformin, may have suppressed cAMP accumulation and hence lowered adenylate cyclase activity [9, 62].

While it is still unclear which of the phytoconstituents in this study were responsible for the anti-diabetic effects of LTE in the intestines, some investigators have reported on the ability of some flavonoids to inhibit the actions of glucose transporters, GLUT-2 and SGLT-1 [63, 64]. The LTE-mediated inhibition of glucose absorption by the whole intestines (in-vivo) and intestinal sacs (ex-vivo) may therefore have been similar to that of metformin, which is known to be deposited many folds into the brush borders of the intestines rather than plasma [65] and thus interferes with the actions of glucose transporters SGLT-1 and GLUT-2 to reduce blood glucose spikes [66]. Furthermore, Adinortey et al. [69] and Adedayo et al. [46] have also demonstrated that phytoconstituents in LTE inhibit gut enzymes including alpha-glucosidase, an enzyme found at the intestinal brush borders that plays a key role in converting complex sugars into glucose for easy absorption.

The similarities in results obtained using both the whole intestines and intestinal sacs suggest that the intestinal sac method may be a useful model for high throughput rapid screening of phytomedicines that may inhibit intestinal glucose absorption. Furthermore, the similarities suggest that the phytoconstituents in LTE may not be prodrugs, which require bioactivation to exert their pharmacological effects.

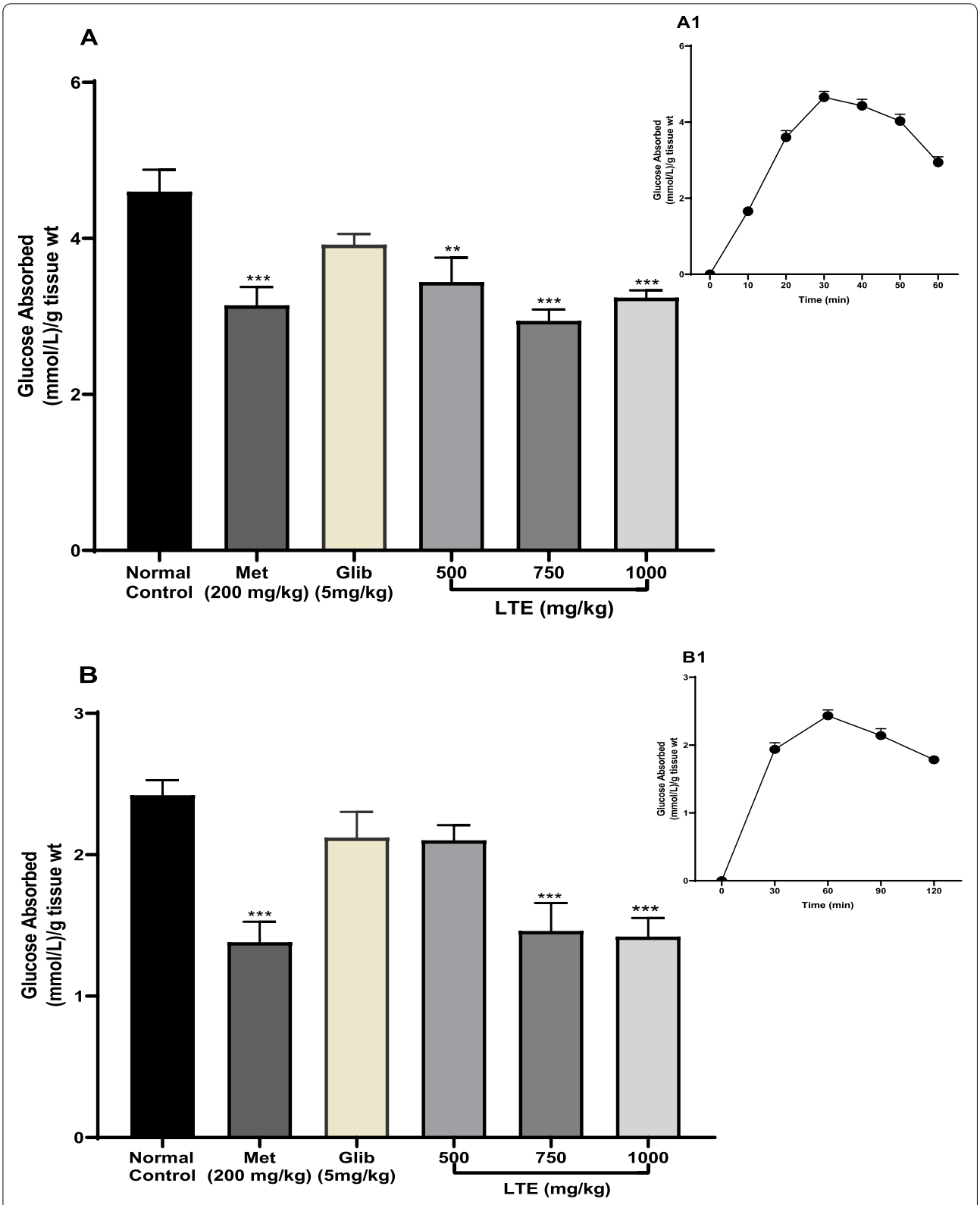

Fig. 9 Effects of LTE on (in-vivo) intestinal glucose absorption (**A**) with time-course insert, 9A1. Figure 9**B** shows glucose absorption by intestinal sacs with a time-course insert, 9B1. Values were expressed as mean ± SEM ($n = 5$). Data were analyzed using one-way ANOVA followed by Dunnett's multiple comparisons test. * represents statistical significance at $p < 0.05$ vs. the normal control group

Summary and conclusion

The extract lowered FBG levels and also suppressed hyperglycemia during the oral glucose tolerance test. LTE inhibited hepatic gluconeogenesis and suppressed intestinal glucose absorption. It also positively modulated levels of plasma lipids and liver enzymes. Although it did not improve body weight considerably, it reversed STZ-induced liver, kidney, and pancreatic damage and restored the morphology of the pancreatic Islet of Langerhans.

These findings suggest that the ethanolic leaf extract of *Launaea taraxacifolia* has blood glucose-lowering properties achieved through multiple processes. LTE may therefore be beneficial in the management of diabetes.

Future studies would consider a bioassay-guided fractionation to isolate and investigate the compounds responsible for the observed anti-diabetic and anti-lipidemic effects. The specific targets, enzymes and stages of gluconeogenesis and intestinal glucose transport interfered with by phytochemicals at the molecular level would also be investigated.

Abbreviations

DM: Diabetes Mellitus; FBG: Fasting Blood Glucose; GC-MS: Gas Chromatography-Mass Spectrometry; GLUT-2: Glucose Transporter-2; IDF: International Diabetes Federation; LTE: *Launaea taraxacifolia* Extract; OGTT: Oral Glucose Tolerance Test; ROS: Reactive Oxygen Species; SGLT-1: Sodium-Glucose Linked Transporter-1; STZ-NAD: Streptozotocin-Nicotinamide.

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Authors' contributions

ADG designed the study, analyzed the data, and drafted the manuscript. MGNA reviewed the manuscript and provided technical support. SJA designed, reviewed, and drafted the manuscript. SJA is also the corresponding author. BBN reviewed the manuscript and provided technical assistance. KE provided technical expertise in the experimental procedures. JKA assisted in the experimental procedures. IAL provided support in the experimental procedures. OAD designed, reviewed the manuscript and provided technical support. IJAG provided technical assistance and reviewed the manuscript. NAK designed, reviewed, and drafted the manuscript. All authors read and approved the final manuscript.

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Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Declarations

Ethics approval and consent to participate

Ethical approval was given by the University of Ghana Institutional Animal Care and Use Committee with reference number UG-IACUC 005/19–20.

Again, ***L. taraxacifolia*** leaves were approved to be collected from the University of Ghana Botanical Gardens in August 2019. The authentication was done by Dr. (Mrs.) Cindy Kitcher at the Department of Pharmacognosy & Herbal Medicine, where a voucher specimen (PH/LT/2019/001) was deposited.

Consent for publication

Not applicable.

Competing interests

There were no competing interests.

Author details

[1]Department of Pharmacology and Toxicology, University of Ghana School of Pharmacy, Legon, Accra, Ghana. [2]Department of Pharmacognosy and Herbal Medicine, University of Ghana School of Pharmacy, Legon, Accra, Ghana. [3]Department of Animal Experimentation, Noguchi Memorial Institute for Medical Research, University of Ghana, Legon, Accra, Ghana. [4]Department of Pharmaceutics and Microbiology, University of Ghana School of Pharmacy, Legon, Accra, Ghana.

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